TECHNISCHE UNIVERSITÄT MÜNCHEN

Lehrstuhl für Technische Mikrobiologie

Enterococcus faecalis in the gut: dissipation, destination and interaction with pathogens

Marina Carina Lamparter

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. D. Haller

Prüfer der Dissertation:

1. Univ.-Prof. Dr. R. F. Vogel

2. Univ.-Prof. Dr. S. Scherer

Die Dissertation wurde am 29.03.2012 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 24.09.2012 angenommen.

Abbreviations

amp	ampicillin
BHI	brain heart infusion broth
BLAST	basic local alignment search tool
bp	base pair(s)
cam	chloramphenicol
cam^R	chloramphenicol resistance
Cbr (Cbred)	click beetle red
dNTP	desoxyribonucleotid triphosphate
Ec-p	empty transposon cassette without promoter
Ecwp	empty transposon cassette with promoter
EDTA	ethylenediaminetetraacetic acid
erm	erythromycin
erm^R	erythromycin resistance
Fluc	firefly luciferase
GIT	gastrointestinal tract
i.p.	intraperitoneally
LAB	lactic acid bacteria
LB	lysogeny broth
LEE	locus of enterocyte effacement
MCS	multiple cloning site
MIC	minimal inhibitory concentration
NGM	nematode growth medium
OD	optical density
OD_{600}	optical density at 600 nm wavelength
ori	origin of replication
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
rpm	rounds per minute
str^R	streptomycin resistance
TAE	tris-acetate-EDTA
TD50	“time to death” for 50% of the individuals
TBE	tris-borat-EDTA
TMW	Technische Mikrobiologie Weihenstephan
Tris	2-amino-2-hydroxymethyl-propane-1,3-diol
TTSS	type three secretion system
UV	ultra violet
wt	wild-type

1 Introduction

1.1 *Enterococcus faecalis*

1.1.1 Taxonomy

The first definition of enterococci dates back to 1899 when they were described as "entérocoque", cocci found in the intestine (Thiercelin 1899). Later they were renamed as *Streptococcus faecalis* because of their shared cell shape, staining characteristics, and their lack of catalase with other streptococci and their association with feces (Andrewes et al. 1906). Sherman (1937) classified a streptococci subgroup according to its physiological criteria as enterococci if they grow at 10°C to 45°C, at pH 9.6 in 6.5% (w/v) NaCl, and survive for at least 30 min at 60°C. In 1984 Schleifer and Kilpper-Bälz officially proposed the genus *Enterococcus* for the formerly named group fecal streptococci or enterococci (Schleifer et al. 1984, Sherman 1937).

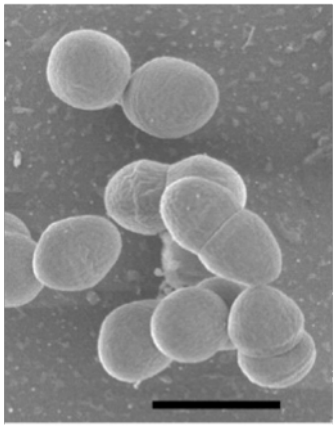


Fig. 1: *E. faecalis* OG1X, bar: 1.2 µm (from (Weaver et al. 2003)).

E. faecalis are ovoid, often chain forming Gram-positive bacteria, belonging to the low GC content, and homo-fermentative lactic acid bacteria. They can grow under aerobic and anaerobic conditions. They are ubiquitous, commonly found in mammals, birds, and insects in the intestinal tract as part of the microbiota or less frequently in other sites such as the oral cavity (Benno et al. 1986, Enzensberger et al. 1985, Noble 1978, Smyth et al. 1987). They are further detected on plants, soil or in milk products, and are long being used as an indicator of vertebrate fecal contamination (Ator et al. 1976, Cai 1999, Gelsomino et al. 2001, Lueoend et al. 1964).

1.1.2 The dualistic nature of *E. faecalis*

Because of its dualistic nature *E. faecalis* is a highly disputed species within the LAB (Fisher et al. 2009, Franz et al. 1999, Franz et al. 2003). Some strains are found in a variety of fermented foods, or are even sold as probiotics whereas other strains are described as opportunistic pathogens, which can cause severe illnesses to humans (Domann et al. 2007, Gelsomino et al. 2001, Pillar et al. 2004, Weber et al. 2003).

Food isolates, microbiota and probiotics

Enterococci are widely found in traditional Mediterranean cheese produced from raw or pasteurized milk (Del Pozo et al. 1988, Litopoulou-Tzanetaki 1990, Macedo et al. 1995). Their presence is mainly ascribed to human or animal feces, contaminated water, exterior of animals or any production tools, and devices. Due to their robustness under critical growth conditions i.e. pasteurization, enterococci can survive or even grow during milk procession or cheese ripening. This can lead to numbers of enterococci of 10^5 to 10^7 CFU/g in fully ripened cheese (Franz et al. 1999). In Cebreiro, a Spanish raw cow milk cheese, *E. faecalis* represents the predominant bacterium (more than 40%) after ripening and plays an important role in aroma development and ripening (Centeno et al. 1996, Franz et al. 1999). This is also true for Spanish Manchego cheese, in which *E. faecalis* is used as a starter culture (Eaton et al. 1993, Nieto-Arribas et al. 2011). *E. faecalis* is paid much attention in food production (Franz et al. 1999, Gelsomino et al. 2001). It has been tested as an adjunct culture to produce desirable flavor or prohibit growth of pathogens by bacteriocin production (Centeno et al. 1999, Oumer et al. 2001, Sulzer et al. 1991). Bacteriocins are peptides produced by bacteria that exhibit a toxic effect on closely related species (Farkas-Himsley 1980). Bacteriocins produced by enterococci are called enterocins and are active against various foodborne pathogens like *Staphylococcus aureus*, *Clostridium* spp., *Vibrio cholera,* and *Listeria monocytogenes* (Brock et al. 1963, Franz et al. 1996, Lauková et al. 1999, Maisnier-Patin et al. 1996, Nunez et al. 1997, Simonetta et al. 1997). *E faecalis* is also found in meat products as a result of contamination during slaughtering. It is isolated from chicken samples, beef, pig carcasses, but also from processed meats or fermented sausages (Aarestrup et al. 2002, Davies et al. 1999, Klein et al. 1998, Turtura et al. 1994). Numbers of enterococci in carcass surfaces ranges from 10^2 to 10^6 per cm^2 with *E. faecalis* being a

predominant specie (Knudtson et al. 1993). Such fermented or contaminated food poses the dispute (depending on the standpoint), if these food-borne bacteria only pass the human gastrointestinal tract (GIT) upon consumption, or if they are able to become a (transient) member of the intestinal microbiome. Different studies aimed at answering of this question, indicate that enterococci from food may be able to colonize the human GIT (Descheemaeker et al. 1999, Gelsomino et al. 2002, van den Bogaard et al. 1997). The administration of antibiotics supported the colonization potential of enterococci from food in mice (Dever et al. 1996, Donskey et al. 1999).

Some *E. faecalis* strains are part of the normal intestinal microbiota of animals and humans. The microbiome influences health upon a balanced interaction of different microorganisms with each other and the host. These microbiota comprise a very diverse, competitive, and dynamic ecosystem containing more than 10^{14} bacterial cells in total, which is about ten times more cells than the human body consists of (Savage 1977). The bacterial phyla represented in human adults at the highest ratio are *Firmicutes* (including LAB) and *Bacteroidetes*, most other belong to Proteobacteria, Actinobacteria (including *Mycobacterium*), and Verrucomicrobia (Palmer et al. 2007). Changes in the proportion are associated with severe metabolic disorders of the host, such as an unbalanced Firmicutes/Bacteroidetes ratio is related to obesity (Bäckhed et al. 2004, Ley et al. 2006b). More than 1000 different species were detected in the human GIT and were distinguished by 16S rDNA analysis, more than 60% were unknown and more than 80% have not been cultured before (Eckburg et al. 2005, Rajilic-Stojanovic et al. 2007). Further findings suggest that certain species colonize distinctive compartments of the GIT (Swidsinski et al. 2005a, Swidsinski et al. 2005b, Zoetendal et al. 2002). So far, *E. faecalis* has basically been detected in the colon of intestinal bowel disease (IBD) patients, where the bacterium is associated with the mucosa (Fyderek et al. 2009, Macfarlane et al. 2007). In early childhood, upon initial colonization of the GIT, enterococci are present in a relatively high number (up to 10^9 CFU/g of feces), but decrease with the increasing competition of other bacterial species to less than 1 % in adults (10^4 - 10^7 CFU/g of feces) (Tannock et al. 2002). Still, they represent the most prominent group among the Gram-positive detected in stool (Jett et al. 1994). Composition of the microbiota depends on various factors, like food intake, microbial competition, drug administration, developmental stage of the host, motility or host influences (Ley et al. 2006a). The bacterial commensals support their host by synthesizing essential amino acids and vitamins, absorbing nutrients,

and digest otherwise indigestible supplies (Bäckhed et al. 2005, Mazmanian et al. 2005). In addition, they help to prohibit pathogens colonization, and translocation to the GIT, they profoundly influence the development of the host intestinal immune system in early life, which seems critical for disease including allergy susceptibility later in life (Bäckhed et al. 2004, Kalliomäki et al. 2001, Mowat 2003, Penders et al. 2006, Renz-Polster et al. 2005, Round et al. 2009, Wang et al. 2008). Interestingly, human breast milk seems to be an important source of LAB for the initial infant microbiota, apart from the natural infection with LAB during delivery (Martin et al. 2003). Human breast milk was shown to be preloaded with LAB, among them *E. faecalis* (Albesharat et al. 2011, Heikkila et al. 2003). It is speculated that they might originate from the GIT and migrate to the mammary glands via the "enteromammary pathway" with the help of different immune cells (Man et al. 2008, Martin et al. 2003, Owen 1999, Perez et al. 2007). Apart from colonization via the mammary glands, the fetus itself is suggested to be reached by certain LAB through the placental barrier even before birth (Martin et al. 2004).

E. faecalis Symbioflor® 1® (Symbiopharm, Herborn, Germany) is sold as therapeutic probiotic and indicated according to the manufacturer for relapsing inflammations of the paranasal, bronchia, and pharyngeal tonsils. Probiotics are defined as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (Salminen et al. 1998). Specific strains are used for prevention and treatment of various diseases in animals and humans like lactose intolerance, acute diarrhea, allergies, respiratory infections, general blood pressure reduction or IBD (Allen et al. 2004, Castagliuolo et al. 2005, Gluck et al. 2003, Kalliomäki et al. 2001, Kim et al. 1983, Sanders 1998, Tannock 1999, Venturi et al. 1999). With rising antibiotic resistance problems, probiotics gained more and more attention as prevention and even treatment of infections in animals as well as in humans (Huovinen 2001, Strauss 2000, Uehara et al. 2001). To select new probiotics, certain criteria are recommended: They have to be identified taxonomically, they must be nontoxic for the host, they need to be technological suitable (e.g., viability in high population, genetically stable in mass production) as well as competitive (e.g., survival and proliferation *in vivo* at target site), and they must perform and function in the host as intended (e.g., exhibit clinically documented health benefit) (Klaenhammer et al. 1999). The mode of action of probiotics is still under investigation, but generally include the competitive exclusion of pathogens, the positive modulation of the microbiota, immune-stimulation or modu-

lation of the immune system, and benefit mucosal integrity. (Mackie et al. 1999, Perdigon et al. 2001, Sanz et al. 2009). Probiotic *E. faecalis* S1/X/00 was originally isolated in the 1950s from a healthy volunteer and since 1954 ten clones (S1/01/00 to S1/10/10) were applied without any known side effects. The German federal office of consumer protection and food safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit) did not record a pathogenic potential, though virulence associated genes and traits such as *ace* coding for adhesion, *agg*, an encoded aggregation substance (in S1/02/00 and S1/05/00), and capsule formation were detected (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit 2004, Domann et al. 2007, Huebner et al. 1999). Acute and sub-acute toxicity studies did not show any effects on growth, blood parameters, postnatal toxicity or allergic reactions in mice and guinea pigs. An antibiotic resistance against clinically relevant antibiotics of any of the Symbioflor® clones was not detected, neither the formation of biofilms (Domann et al. 2007, O'Toole et al. 1999).

Pathogenicity

In Canada enterococci are completely banned as components of natural health products (Fitzpatrick 2005). Enterococci isolated from farm animals and dairy products were shown to be susceptible to various antibiotics such as erythromycin or vancomycin (Bates et al. 1994, Giraffa et al. 1997, Teuber et al. 1996). *E. faecalis* is suspected to cause foodborne illnesses by the production of biogenic amines, which can lead to intoxication symptoms (Fisher et al. 2009, Giraffa 2002). This aspect may point to the other side of the Janus-faced characteristics of this controversial bacterial species.

E. faecalis has been isolated from patient suffering from severe illnesses, such as sepsis and endocarditis. Nowadays, *E. faecalis* is recognized as a major opportunistic pathogen, being one of the predominant pathogens in nosocomial infections (Fisher et al. 2009, Hidron et al. 2008, Murray 1990). *E. faecalis* is also often isolated from dental root canal systems, accounting for up to 77% of human endodontic infections (Stuart et al. 2006). Enterococcal infections are accompanied by high numbers of antibiotic resistances among these strains. Not only are they intrinsically resistant against many clinically used antibiotics, but they were also shown to often acquire resistance genes by genetic exchange with other resistant bacteria (Moellering 1992). E. g., pheromone responsive plasmids carrying antibiotic re-

sistance genes are easily transferred in the GIT of mini-pigs to other *E. faecalis* strains under non-selective conditions (Licht et al. 2002). This indicates the possible risk that the assumedly safe food or probiotic strains become turntables for the transfer of antibiotic resistances in the GIT (Teuber et al. 1996). Horizontal transfer of resistance genes like *vanA* from *E. faecalis* to *S. aureus in vivo* was verified (Noble et al. 1992, Weigel et al. 2003). Even a "safe" *E. faecalis* might display a risk in acquiring antibiotic resistances. E.g., in case of antibiotic treatment of mice, the colonization by resistant strains was found (Dever et al. 1996, Donskey et al. 1999). At worst, antibiotic selection leads to local bacterial overgrowth, leakage of the mucosal barrier, bacterial translocation of resistant (commensal) strains into the bloodstream, and systemic infections (Deitch 1990, Van Leeuwen et al. 1994, Wells et al. 1990). Further effects of antibiotic therapy are visible in the genome of *E. faecalis*. Antibiotic pressure is speculated to lead to a more dynamic genome structure, by the disruption of the bacterial genome integrity featuring CRISPR (clustered, regularly short palindromic repeats) (Palmer et al. 2010). Mobile elements transferring resistance genes to the acceptor strains, as well as other features (e.g., virulence genes) allow for an adaption to the selective environment. This scenario might explain the genomic structures development of strain V583, a vancomycin resistant isolate from a blood infection patient. This strain contains a large pathogenicity island, three plasmids, 7 putative phage integration regions, and 38 IS elements (Paulsen et al. 2003a) which together make up 25% of the genome.

Early on, enterococci have been recognized as pathogens. Several virulence factors indicate the pathogenic potential of *E. faecalis*, though they are not as distinctive as for other species containing pathogenic and harmless strains. Virulence factors include: aggregation substance (*as*), collagen adhesion protein (*ace*), enterococcal surface protein (*esp*), *E. faecalis* antigen A (*efaA*), gelatinase (*gelE*), serine protease (*sprE*), *E. faecalis* regulator (*fsrB*), cytolysin (*cyl operon*), capsular polysaccharides (*cps* locus), and biofilm formation (*epa* locus) (Day et al. 2003, Hancock et al. 2002, Hufnagel et al. 2004, Mylonakis et al. 2002, Olmsted et al. 1991, Qin et al. 2000, Rich et al. 1999, Shankar et al. 2001, Singh et al. 1998, Xu et al. 2000). These genes provide regulation to other virulence genes and factors for colonization, adhesion, invasion, translocation, induction of pathological characteristics, and resistance against hosts' defense capabilities to *E. faecalis*. Some of these virulence factors, such as *as* and *ace* are found in Symbioflor®, possibly contributing to the probiotic effect by contributing to proliferation and colonization of the strain in the GIT (Domann et al. 2007). How-

ever, the source of strain-isolation (food, environment, patients) is not clearly correlated with the distribution of these virulence factors. Their average prevalence is somewhat higher in clinical isolates, but they are widely found in environmental or food isolates (Lindenstrauß et al. 2011). In general this indicates that genetic features of *E. faecalis* add on its pathological potential, but hosts factors' seem to be crucial in disease development. Disease and mortality have been associated primarily with predisposed hosts that, e.g., underwent surgeries or were catheterized, were immunosuppressed or were already sick from another illness (Joyanes et al. 2000, Sandoe et al. 2002, Venditti et al. 1993). This is also, albeit rarely, found for other LAB, even widely used probiotics, which can cause an illness and even mortality in susceptible hosts (Alvarez-Olmos et al. 2001, Besselink et al. 2008, Rautio et al. 1999). In impaired hosts, *E. faecalis* disseminates to extraintestinal sites. The specific localization from the intestine to other body sites of these commensal and/or pathogenic strains is thought to be an indicator for the outcome of the bacterium-host interaction, e.g., commensal or pathogenic. Contrarily, the *E. faecalis* presence in babies and the mammary glands in healthy breast-feeding women shows that it should not be automatically associated with fatal outcome (Albesharat et al. 2011, Martin et al. 2004).

1.2 Enterohemorrhagic *Escherichia coli*

Escherichia coli was originally considered a common inhabitant of the lower intestinal (*colon*) tract in humans. It was described in 1885 by Theodor Escherich and later named after him (Escherich 1885, Judicial Commission of the International Committee on Bacteriological Nomenclature 1958). The Gram-negative, rod-shaped, non-spore-forming bacterium is facultatively anaerobic and able to gain energy by mixed-acid fermentation. It is used as an indicator for fecal contamination (United States Environmental Protection Agency 1986). Commensal strains are found in the mucus layer of the mammalian colon, being part of the microbiota and representing the biggest group of the facultatively anaer-

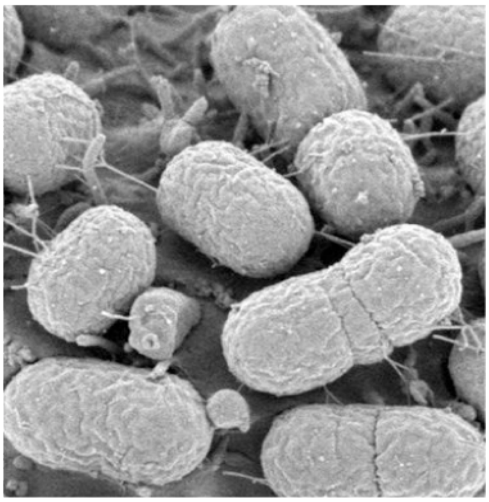


Fig. 2: EHEC EDL933 adhering to Hep-2 cells, from (Xicohtencatl-Cortes et al. 2007).

obes in this niche (Sweeney et al. 1996). However, new clones have emerged, which are highly pathogenic in healthy animals and humans generally due to the acquisition of new virulence genes. Shiga toxin-producing *E. colis* (STEC) are the pathotypes, which can cause severe enterohemorrhagic symptoms in humans (diarrhea, abdominal pain, vomiting). In 5 to 10% of the cases a hemolytic uremic syndrome (HUS, hemolytic anemia, thrombotic thrombocytopenic purpura TTP, kidney failure) develops. These strains are named for this enterohemorrhagic *E. coli* or EHEC, according to the current German Infection Protection Act. (Mellmann et al. 2005, Robert Koch-Institut 2007, Robert Koch-Institut 2011a, Tarr et al. 2005). Sporadic outbreaks of EHEC and related strains have become more frequent and severe, like the last one emanating from Germany in 2011 with in total 4397 recorded EHEC infected patients, including 901 HUS cases and 51 fatalities (Robert Koch-Institut 2011b). So far, therapy of EHEC infections focuses on treatment of the symptoms and prevention of infection. Some specific antibiotic administration is contraindicated as it stimulates Shiga toxin production and release (Zhang et al. 2000). Therefore, identification of the source of infection is fundamental for the control and prevention of an EHEC outbreak. EHEC is transmitted via the fecal oral route and only a few bacteria are needed for human infection (Griffin et al. 1991). This accounts for the high potential for person-to-person spread of infection. Other, main vehicles are contaminated food (e.g., raw sausages, ground beef, raw milk, leafy greens such as sprouts) or water and contact to ruminants. Cattle, as well as other livestock are thought to be the natural reservoir of EHEC, rarely showing symptoms of disease (Ferens et al. 2011).

1.2.1 Pathogenicity mechanisms

This work focuses on the EHEC EDL933, which belongs to the best-studied serotype O157:H7. This pathogen was recognized and isolated first in 1982 at an outbreak linked to contaminated, undercooked hamburgers in the USA (Karmali et al. 1983, Riley et al. 1983). The genome sequence of *E. coli* O157:H7 EDL933 revealed 5453 genes (protein coding and RNA) on the chromosome and 101 genes on the plasmid pO157 (Perna et al. 2001). The non-pathogenic *E. coli* K12 genome has more than 1000 genes less. The additional genes in EHEC compared to the K12 strain, mainly code for virulence factors, (cryptic) prophages or additional metabolic traits (Burland et al. 1998, Perna et al. 2001).

Pathogenic interactions with humans

In the human intestine, EHEC causes typical attaching and effacing (A/E) lesions upon contact to the epithelium, characterized by the rearrangement of the cytoskeleton and the destruction of microvilli. Thereby, a pedestal-like structure is formed, which encloses single EHEC cells tightly (Knutton et al. 1987, Moon et al. 1983). The main components responsible for this phenotype are chromosomally encoded on the multi-operon pathogenicity island (PAI) called Locus of Enterocyte Effacement (LEE) (Jarvis et al. 1995, Jerse et al. 1990). The LEE consists of 41 genes, most of them characterized and organized in five operons LEE1 - LEE5 (Fig. 3) (Pallen et al. 2005).

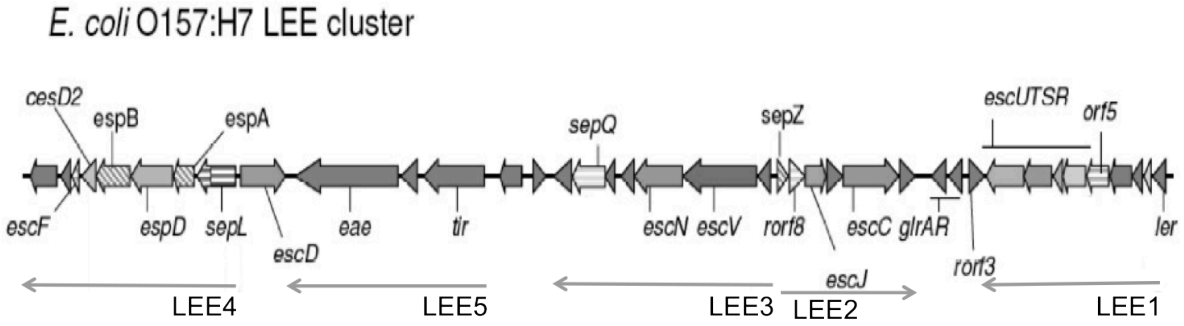


Fig. 3: Genomic organization of the *E. coli* O157:H7 LEE operon. *espG* and *rorf1* are not shown. Arrows below indicate the operon structures. Modified from (Pallen et al. 2005).

The LEE-island encodes, beside other virulence factors, a type III secretion system (TTSS), which is a multi-protein complex needle that spans the inner and outer membrane of the bacterium and is injected or inserted into host cells to translocate effector proteins (Fig. 4). The LEE-encoded structural proteins EscC, EscD, EscR, EscS, EscT, EscU, EscV, and SeqQ form the membrane spanning complex, EscA and EscF are the building blocks of the extracellular needle structure and are capped by EscB and EscD. A cytoplasmatic ATPase (EscN), associated in the cytoplasm with the complex and drives the excretion of the translocated effectors.

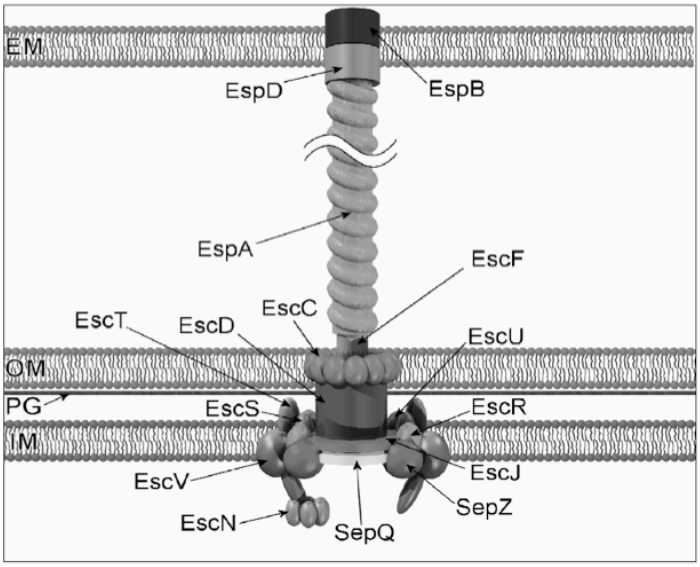


Fig. 4: Schematic overview of the LEE encoded type III secretion system and its structural components. IM: Inner membrane, PG: peptidoglycan layer, OM: Outer membrane, EM: Eukaryotic membrane, from (Pallen et al. 2005).

Several LEE-encoded chaperons (CesAB, CesD, CesD2, CesF, and CesT) are needed for the folding and secretion of LEE's structural end effector proteins (Creasey et al. 2003, Wainwright et al. 1998). An important effector is the translocated intimin receptor (Tir), which is one such protein, which is injected into the host cell. There it integrates in the host cell membrane and forms a receptor for EHEC intimate adherence. Intimin (encoded by *eae)* is the corresponding EHEC outer membrane protein on EHEC bound by Tir. Both genes are mainly responsible for the attaching and effacing (*E. coli* attaching and effacing) lesions (Garmendia et al. 2005, Kenny et al. 1997). Additional effector proteins, involved in attachment mechanisms or disrupting the host's cells signal transduction, are encoded on the LEE (EspF, EspG, EspH, Map). Additional non-LEE encoded TTSS secreted effector proteins are found chromosomally encoded (Creuzburg et al. 2011). Regulation of the LEE-genes is conduced by Ler (positive LEE regulator), GrlR (global regulator LEE-encoded repressor), and GrlA (global regulator LEE activator) (Barba et al. 2005, Mellies et al. 1999). GrlA also down regulates flagellar gene expression (Iyoda et al. 2006).

The other major virulence determinant, responsible for the hemorrhagic-uremic syndrome (HUS), which includes hemorrhagic colitis, thrombotic thrombocytopenic purpura (TTP), neurotoxic effect, is shigatoxin (Boerlin et al. 1999). Different variants of shigatoxins exist, EHEC EDL933 produces Stx1 and Stx2. All shigatoxins are AB toxins, Stx1- and Stx2-B-subunits bind Gb3-receptors on human cells to traffic the A subunit into host cells (Nataro et al. 1998, Obrig et al. 1993). Cells displaying the glycolipid Gb3-receptor include monocytes

platelets, renal endothelial cells, but also cerebral endothelial cells are targets for Stx. Upon internalization into the host cells, the A subunit enzymatically inactivates protein synthesis, causing cell death, which in turn causes severe EHEC infection symptoms. Stx2 is often associated with a more severe outcome of the disease (Ostroff et al. 1989). Stx1 and Stx2 are encoded on the prophages CP-933V and BP-933W respectively. Induction of the prophage into lytic cycle, e.g., initiated by antibiotics, causes Stx expression and release (Kimmitt et al. 1999).

Further chromosomally encoded toxins and virulence genes include *efa1*, *astA)*, fimbrial adhesion proteins (*lpf*), flagellar components (e.g., *mot*, *flhCD*) and fitness factors (e.g., conveying acid resistance) (Deacon et al. 2010, Doughty et al. 2002, Paiva de Sousa et al. 2001). The plasmid pO157:H7 also encodes virulence factors, including a type II secretion system, a hemolysin or a serine-protease (Burland et al. 1998).

Sensing and regulation

The expression of the EHEC virulence genes requires highly complex, timed, and coordinated regulation referring to internal and external conditions. EHEC can sense its surroundings by various sensor mechanisms. Different levels of regulation within EHEC process the integrated signals, which enable the bacterium to respond and establish itself by adapting to its challenging environment (Pauling et al. 2012).

The two-component systems QseBC and QseEF allow EHEC to sense abiotic as well as biotic environmental signals, such as human hormone (nor)epinephrine (Clarke et al. 2006, Reading et al. 2009). In addition, quorum sensing (QS) is an important mechanism to sense bacterial cell density by cell-to-cell autoinducer (AI) signaling. In EHEC AI-2 is produced by LuxS and assumed to play an important role in interspecies communication (e.g., by interfering with the LuxS system in *E. faecalis*), but also as a chemoattractant in biofilm formation (Bassler 2002, Surette et al. 1999). Autoinducer A3 acts synergistically to epinephrine and is sensed by the same unknown receptor, activating LEE expression via QseEF and Ler (Clarke 2005). Regulation of the expression of the LEE-island is still not completely understood, but various positive or negative regulators have been identified to date (Fig. 5). Some of them include global regulators, e.g., acid-resistance regulator RpoS, found also in

non-pathogenic *E. coli*, whereas others are only present in pathogenic *E. coli* (e.g., Pch, EtrA,) (Iyoda et al. 2006, Sharma et al. 2004, Sperandio et al. 2002, Zhang et al. 2004).

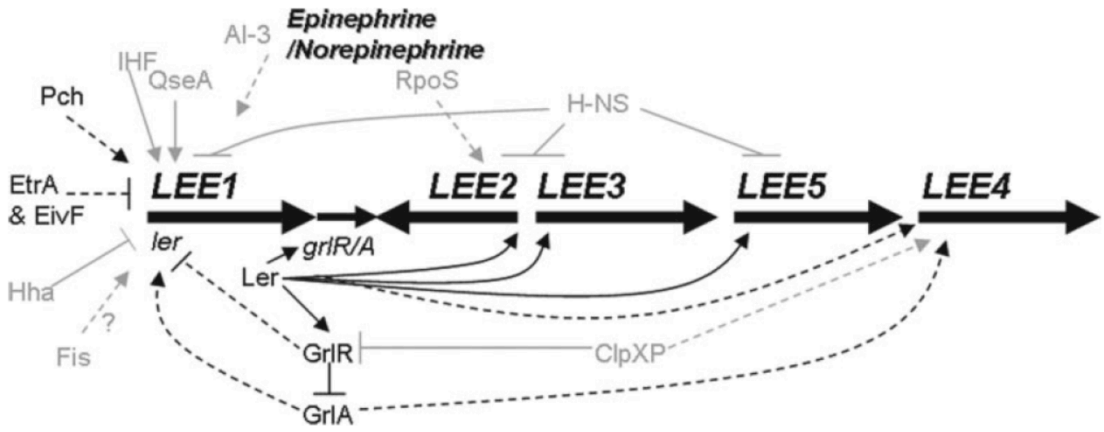


Fig. 5: Schematic overview of LEE and its regulation, taken from (Walters et al. 2006). Gray regulators are present in both *E. coli* K12 and EHEC, black, regulators are only found in EHEC. The arrows point to the respective regulatory target. Direct interactions are displayed by solid lines, indirect by dashed lines.

The Qse-regulatory system activates the expression of the flagellar genes, too, but in a different temporal pattern. The flagellum is activated in the beginning of the infection, enabling the bacterium to reach to the colonic epithelium. Subsequently, motility is basically switched off to allow LEE mediated attachment. The expression of the *stx*-genes has not been associated with Qse or any other pathogen-specific regulators, thus it seems to be regulated by phage mechanisms and global controls (Habdas et al. 2010). Interestingly, commensal *E. coli* in the gut are "abused" by EHEC, which transfer the Stx-encoding prophages causing the commensal bacteria to produce and release Stx upon cell lysis as well (Brussow et al. 2004).

C. elegans as infection model system

C. elegans is a nematode, which is used as a simple and inexpensive host system to study various human pathogens (Darby 2005). The food source for the nematodes is bacteria. Thus, to test the pathogenic potential of a bacterial strain in *C. elegans*, synchronized L4 larva are fed with the respective bacteria. Next, the mortality is assessed by determination of the time to death of 50% of the worms, TD50. As a standard reference food, *E. coli* OP50 grown on NGM agar and not colonizing its host upon ingestion are used. However, the same

bacteria grown on BHI agar reduce the worm's lifespan and seem to be mildly pathogenic (Garsin et al. 2001). *C. elegans* response to pathogens is characterized by several evolutionary conserved innate immune system signaling pathways (e.g., p38 MAP kinase or TGF-β related pathway) and effector molecules (e.g., lysozymes), also found in many other organisms (Nicholas et al. 2004). This allows, to some extend to interrelate the pathogenicity in nematodes with higher organisms. E.g., virulence factors of other pathogenic bacteria found by screening *C. elegans* were shown to affect pathogenicity in mammalians, too (Mahajan-Miklos et al. 1999, Sifri et al. 2005). Still not all facts of the worm's immune response are known, in particular pathogen recognition and immune reaction to microbes need further investigation.

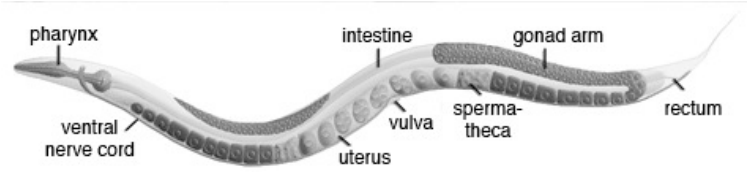


Fig. 6: *C. elegans* young adult anatomy. Modified from wormatlas (Altun et al. 2002 - 2010).

The mode of killing of various pathogens in this host system depends on different factors. Death occurs by colonization and / or due to other bacterial effectors (Darby 2005). Pathogenicity of *E. faecalis* clinical isolates and human-pathogenic *E. coli* serotypes was shown to caused a nematode a gut infection (Darby 2005, Garsin et al. 2001, Lee et al. 2008, Sifri et al. 2002). *E. faecalis* grown on BHI colonized the gut quickly upon ingestion, but so did an *E. faecium* strain without causing significant mortality. Additional *E. faecalis* genes involved in *C. elegans* killing, but also in human infection, *cyl*, *fsrB, gelE, and sprE* were identified and the killing kinetics of the respective virulence gene deletion mutants were determined in a *C. elegans* killing assay (Garsin et al. 2001, Sifri et al. 2002). Enteropathogenic (EPEC), enteroaggregative (EAEC) *E. coli* or, and EHEC, all causing severe human infections, were assessed in the nematode host system (Frankel et al. 1998). *E. coli* O157:H7 was pathogenic to the nematodes in relation to the degree of gut colonization. A *dsbA*-deletion mutant revealed reduced colonization capability and attenuated killing (Lee et al. 2008). Another study correlated Stx2 with *C. elegans* mortality (Kim et al. 2006). Genes of EPEC and EAEC-strains involved in the nematode pathogenicity include *ler*, *tnaB*, *csrA*, and *cadAB,* of which homologues are found in EHEC EDL933 (Bhatt et al. 2011, Hwang et al. 2010, Mellies et al. 2006).

The *C. elegans* model system was also used to assess the modulation of *E. coli* pathogenicity by various other bacterial species or other food ingredients. E.g., broccoli extract or *Lactobacillus acidophilus* lysate reduce EHEC pathogenicity (Kim et al. 2006, Lee et al. 2011). On the other hand a virulent synergism between *E. faecalis* and uropathogenic *E. coli* has been revealed (Lavigne et al. 2008).

1.2.2 Modulation of EHEC pathogenicity by LAB

Both, LAB (like *E. faecalis*) and EHEC strains are found in similar environmental niches. They are isolated from processed meat or dairy products, are found in the human colon, associated with the mucus and are, indicators of fecal contamination (Ferens et al. 2011, Franz et al. 1999, Fyderek et al. 2009, Giraffa 2002, Macfarlane et al. 2007). Especially in the human GIT, in which only few EHECs can cause severe damage to the host, a huge number and variety of LAB live closely to the enteric pathogen. Therefore, interaction of LAB and EHEC are interesting in terms of revealing LAB mechanisms that modulate the fitness and virulence of pathogens like EHEC the LAB. This might help to understand the disease's severity and clinical symptoms variations among different EHEC patients. Further, it might also unravel new targets or alternative ways to treat or prevent fatal EHEC infections. Different (transient) members of the human microbiota or factors, secreted by them, were shown to reduce or even inhibit *E. coli* O157:H7 virulence *in vitro* and *in vivo*. E.g., human microbiota secreted factors were shown to inhibit Stx2 synthesis on a transcriptional level (de Sablet et al. 2009). Similarly, *Lactobacillus acidophilus* A4 and secreted factors of strain La-5 were shown to interfere with EHEC quorum sensing, thereby reducing the expression of virulence factors (Kim et al. 2008, Medellin-Peña et al. 2007). The production of organic acids by LAB was shown to be related to the reduction of virulence gene expression, but the precise mechanisms are to be clarified.

1.3 Optical imaging techniques

Imaging techniques based on luminescence are widely applied in microbiology. Two different marker protein systems were used in this work. Generally bioluminescence reporter systems are e.g., used in *in vivo* imaging or gene promoter activity approaches. They allow the

noninvasive monitoring of light-emitting bacteria in live small animal models. Meanwhile fluorescent reporter proteins are applied in microscopy for imaging, which approaches a cellular level monitoring single bacterial cells in tissues or cell cultural samples.

Luminescence is defined as the "spontaneous emission of radiation from an electronically excited species or from a vibrationally excited species not in thermal equilibrium with its environment" (Braslavsky 2007). Subtypes like bioluminescence and fluorescence are defined according to their mode of action.

1.3.1 Bioluminescence

Bioluminescence has been used as a versatile tool to study the route of metabolically active bacteria in mice. E.g., *Listeria monocytogenes* was shown to colonize the gall bladder upon infection (Hardy et al. 2004), and several bacterial species were monitored in tumor targeting studies, including *Salmonella* and *Bifidobacterium* (Cronin et al. 2012, Hoffman 2011). The effect of antibiotic therapy on infections was investigated by using luminescent pathogens (Francis et al. 2000, Kadurugamuwa et al. 2003, Rocchetta et al. 2001).

Bioluminescence is defined as luminescence produced by living systems (Braslavsky 2007). It occurs naturally in deep-sea vertebrates and invertebrates (mainly in a symbiosis with luminescent bacteria), as well as insects, worms fungi, centipedes and various other animals. Its basic function is communication, attraction of lures or mates and repulsion of enemies (Viviani 2002, Widder 2010). The light is generated by a luciferase-driven enzyme reaction. Two commonly used luciferases, also applied in this work, are the bacterial luciferase Lux (from *Photorhabdus luminesces*) (Francis et al. 2000) and the click beetle luciferase Click beetle red (Cbred, engineered from *Pyrophorus plagiophthalamus*) (Miloud et al. 2007). Both systems need the presence of oxygen for light production. The bacterial *lux* operon consists of two genes (*luxAB*) coding for the luciferase enzyme and three genes coding for the fatty acid reductase complex (*luxCDE*) to regenerate the aldehyde substrate of the light reaction (Fig. 7). The other substrate, reduced flavin mononucleotide FMNH is regenerated by flavin reductase P (Frp) homologous or LuxG type flavin reductases, enzymes widely found in eubacteria (also in *E. faecalis* OG1RF) involved in riboflavin metabolism (Campbell et al. 2009, Vitreschak et al. 2002).

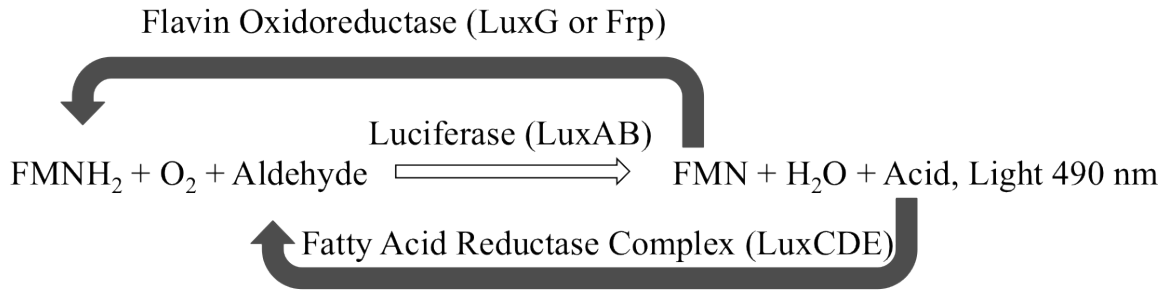


Fig. 7: Schematic illustration of bioluminescence reaction by bacterial luciferase. Luciferase catalyzes the oxidation of reduced flavinmononucletide and aldehyde, which eventually yields blue/green light (490 nm). The products flavin mononucleotide and the fatty acid are regenerated by flavin oxidoreductase and fatty acid reductase, respectively (Miloud et al. 2007).

To render bacteria luminescent, only the luciferase genes *luxAB* and the fatty acid reductase genes *luxCDE* are essential. Maybe, a flavin oxidoreductase gene may be included to increase light intensity via more efficient substrate regeneration and to add on the host's flavin reductase activity. Additionally, an exogenous substrate, like the volatile aldehyde nonanal can be added to additionally boost the signal. However, the latter method will ultimately kill the bacteria disallowing time series of the same culture.

For Cbred luciferase addition of D-luciferin is mandatory to drive the light reaction. The substrate is applied 10 – 20 min before imaging. Instead of $FMNH_2$, as the bacterial system, ATP is necessary for the Cbred light reaction (Fig. 8.). The maximum wavelength of the emitted light is in the red spectrum, which facilitates the tissue penetration of the bioluminescence signal by minimal absorption.

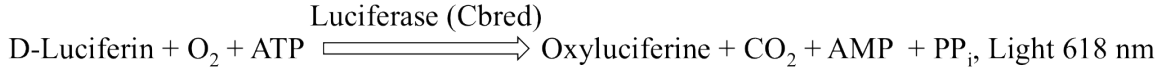


Fig. 8: Schematic illustration of bioluminescence reaction by Click beetle luciferase. Cbred luciferase catalyzes the oxidation of D-luciferin utilizing ATP. A light signal at a wavelength of 618 nm is generated by this reaction.

1.3.2 Fluorescence

Fluorescence is the spontaneous emission of radiation (luminescence) from an excited molecular entity with retention of spin multiplicity (Braslavsky 2007). Fluorescent proteins like GFP and similar homologs are excited by a light of a specific wavelength and subsequently

emit light of a typical emission wavelength. The proteins have a beta barrel fold and a central chromophore, composed of a typical tripepetide configuration. Fluorescence emission takes place upon transition from the excited state of the chromophore to the initial state (Yang et al. 1996, Youvan et al. 1996). GFP was the first green fluorescent protein isolated from an medusozoan (Shimomura et al. 1962). Homologs from antozoans emit light of different colors, followed later on. In corals, the fluorescent proteins seems to be the specific for the attraction of reef fishes, which in turn banishes predators and the photoprotection of symbiotic algae (Matz et al. 2006, Salih et al. 2000). The biological function in medusozoan is not fully clear. Light is produced by specific jellyfishes, but the effects are unclear. The fluorescent proteins have been engineered to be adapted to various experimental setups. E.g., mCherry and tdTomato are derived from DsRed, a red fluorescent protein. The monomer mCherry has an extinction maximum of 587 nm and a emission maximum of 610 nm, which offers a long emission wavelength, a high photo-stability, fast maturation at 37°C and a high pH resistance (Shaner et al. 2004). mCherry is immediately detected upon expression. A high brightness is found in the tandem-dimer tdTomato, also having a high photostability, good acid resistance, and short maturation time at 37°C. However, results in confocal laser scanning microscopy are poor. Its maximum extinction is at 554 nm, maximum emission at 581 nm. In this work, fluorescent proteins were applied in this work to be able to follow bacterial cells on a cellular level in cell culture or tissue experiment with epifluorescence or confocal laser scanning microscopy.

1.4 Aim of the study

While *E. faecalis* is found in food products or used as probiotic, it has also caught increasing attention in the past years as the causative agents of severe nosocomial infections. These ambiguous findings may reside in difference of host condition and strain-dependent properties, or both, defining the interaction of *E. faecalis* with the host. Therefore, the aim of the study was to establish methods and provide proof of concept for the analysis of dissipation and destination of different strains of *E. faecalis* in the host. On the other hand, the modulation of gene expression in pathogens by *E. faecalis* should be investigated to probe it as a contribution to its probiotic function.

To achieve this goal, a toolkit comprising various light-emitting and fluorescent reporter systems for *E. faecalis* should be established to track different (virulent, colitogenic *versus* food, probiotic) strains *in vivo* and compare their dissipation and destination in a host. This should provide a means to get insight in the route and the mechanisms by which *E. faecalis* strains travel to extraintestinal sites, such as e.g., the heart (clinical isolates) or the mammary glands (non-clinical isolate).

As a member of the intestinal microbiota, *E. faecalis* might also indirectly contribute to the host's health by modulating the pathogenicity of other enteric pathogens, apart from competitive niche exclusion. In this work the influence of the probiotic *E. faecalis* Symbioflor® on the EHEC pathogenicity in the *C. elegans* model system and its impact on EHEC gene expression should were evaluated.

2 Material and methods

2.1 Materials

2.1.1 Devices

Tab. 1: Devices used in this study.

Device	Model	Manufacturer
96-well pin	Aluminum	TU München, Werkstatt, Germany
Agarose gel chamber 25 x 20 cm	Easy Cast electrophoresis systems	Owl Separation Systems, Portsmouth NH, USA
Autoclaves	2540 ELV	Systec GmbH, Wettenberg, Germany
Breeding/incubation	Certomat BS-1 Hereaus B5042E Memmert INB series WiseCube®WIS-ML02	B. Braun Biotech, Germany International, Melsungen, Germany Hereaus Instruments, Hanau, Germany Memmert GmbH & Co. KG, Schwabach, Germany Witeg Labortechnik GmbH, Wertheim, Germany
Centrifuges	Sigma 1 K 15 Sigma 6-16K J-6 J-2 Hermle Z383 K	Sigma Labortechnik, Osterrode am Harz, Germany Sigma Labortechnik, Osterrode am Harz, Germany Beckman, Palo Alto, CA, USA Beckman, Palo Alto, CA, USA Hermle, Labortechnik, Wehningen, Germany
Confocal microscope	Ti inverted research confocal laser-scanning microscope	Nikon, Düsseldorf, Germany
Electroporation system	Bio-Rad Gene pulser device	Bio-Rad Laboratories Hercules CA, USA
Homogenizer (stomacher)	FastPrep® 24 instrument	MP Biomedicals, Solon OH, USA
Fluorescence microscopy	Stereo Discovery Stereomicroscope HBO50 Microscope Illumination device	Carl Zeiss, Jena, Germany

	Axciocam ICc1	
Fluorescent filter set Carl Zeiss 31	Excitation: BP 565/30, beam splitter: FT 585, Emission: BP 620/60	Carl Zeiss, Jena, Germany
Incubation hood	Certomat H	B. Braun Biotech International, Melsungen, Germany
Laminar flow sterile work bench	HERA safe	Hereaus Instruments, Hanau, Germany
Microscope	Axiolab	Carl Zeiss, Jena, Germany
Nanodrop	Nanodrop1000	Peqlab Biotechnologie GmbH, Erlangen, Germany
PCR cycler	Primus 96 plus Mastercycler gradient	MWG Biotech, AG, Ebersberg, Germany Eppendorf AG, Hamburg, Germany
pH determination	InLab 412, pH 0-14	Mettler-Toledo, Gießen, Germany
Photometer	Novaspellq	Pharmacia Biotech, Germany
Pipettes	Pipetman	Gilson-Abomed, Lanenfeld, Germany
Power supplies	MPP 2 x 3000 power supply Electrophoresis power supply EPS 3000 2197 supply PPS 200-1D	MWG Biotech AG, Ebersberg, Germany Pharmacia Biotech, Germany MWG Biotech AG, Ebersberg, Germany
Pure water	Euro 25 and RS 90-4/UF pure water system	SG Wasseraufbereitung GmbH, Barsbüttel, Germany
Shaking	Certomat R Vortex 2 Genie	B. Braun Biotech International, Melsungen, Germany Scientific Industries Inc., Bohemia, NY, USA
Stirring	RCT-Basic	Mettler-Toledo, Gießen, Germany
Lab-Blender 400	Model #BA6021	A.J. Seward, Edmunds, U.K.
Thermo block	Techno DRI-Block DB3	Thermo-Dux Gesellschaft für Laborgeräte mbH, Wertheim, Germany
UV table	Herolab UVT 28M	Herlab, GmbH Laborgeräte, Wiesloch, Germany
Water bath	Lauda BD	LAUDA Dr. D. Wobser GmbH&Co, Lauda-Königshofen, Germany
Gas anesthesia system	XGI-8 gas anesthesia system	CaliperLS, Hopkinton, MA, USA

2.1.2 Specific software

Name	Provider	Reference
BLAST	NCBI	http://blast.ncbi.nlm.nih.gov/Blast.cgi (McGinnis et al. 2004)
Galaxy software collection	Galaxy Project	http://galaxy.psu.edu/ (Blankenberg et al. 2010, Giardine et al. 2005, Goecks et al. 2010a)
Living Image software	CaliperLS, Hopkinton, MA, USA	
Picard tools 1.53	Sourceforge	http://picard.sourceforge.net/
VectorNTI	Invitrogen, Carlsbad CA, USA	
R version 2.13.2	GNU project	http://www.r-project.org/ (R Development Core Team 2010)

2.1.3 Chemicals

Tab. 2: Chemicals used in this study.

Chemicals (purity)	Manufacturer
6 x DNA Loading dye	Fermentas GmbH, St. Leon-Rot, Germany
Acetic acid, (99- 100%, glacial)	Merck, Darmstadt, Germany
Agar (European agar)	Difco, BD Sciences, Heidelberg, Germany
Agarose (for electrophoresis)	Biozym Scientific GmbH, Oldendorf, Germany
Ampicillin sodium salt (93,3%)	Gerbu Biotechnik GmbH, Gaiberg, Germany
Boric acid (≥ 99.5% purity)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Brain heart broth, BHI (for microbiology)	SIGMA-Aldrich, Steinheim, Germany
$CaCl_2$ * $2H_2O$ (p.a.)	Merck, Darmstadt, Germany
Dimidium bromide (≥ 98%)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
DNA marker GeneRuler™ 100 bp, 1 kb DNA ladder Lambda DNA/*EcoRI*+*HindIII* Marker, 3	Fermentas GmbH, St. Leon-Roth, Germany
EDTA (for molecular biology)	Sigma-Aldrich Steinheim, Germany
Elongase ®	Invitrogen, Carlsbad CA, USA
Erythromycin	Sigma-Aldrich, Steinheim, Germany
Ethanol (absolute, ≥ 99,8%)	VWR, Prolabo, Foutenay-sous-Bois, France
Fast-AP	Fermentas GmbH, St. Leon-Roth, Germany
FD restriction buffer	Fermentas GmbH, St. Leon-Roth, Germany
FD restriction enzymes	Fermentas GmbH, St. Leon-Roth, Germany
Glucose (for biochemical use)	Merck, Darmstadt, Germany
Glycerol (99,5%, high purity)	GERBU Biotechnik, GmbH, Gaiberg, Germany
Glycine (p.a.)	Merck, Darmstadt, Germany

HCl (37%, p.a.)	Merck, Darmstadt, Germany
Isoflurane	Abbott Laboratories, Abbott Park IL, USA
Isopropanol (p.a.)	Scharlau Chemi S.A., Sentmenat, Spain
KH_2PO_4	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
K_2HPO_4	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
KCl (p.a.)	Merck, Darmstadt, Germany
LongAmp® *Taq* 2x Master mix	New England Biolabs, Ipswich, MA, USA
Luciferin	CaliperLS, CA, USA
Lysozyme	SERVA, Heidelberg, Germany
$MgCl_2$*6 H_2O (p.a.)	Merck, Darmstadt, Germany
$MgSO_4$*7 H_2O (p.a.)	Merck, Darmstadt, Germany
NaCl (p.a.)	Merck, Darmstadt, Germany
Nalidixin	Merck, Darmstadt, Germany
NaOH (p.a.)	Merck, Darmstadt, Germany
Nonanal	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Peptone from Casein (for microbiology)	Merck, Darmstadt, Germany
Phusion® Hot Start High-Fidelity DNA Polymerase	Finnzymes, Vantaa, Finland
Primer	MWG-BiotechAG, Ebersberg, Germany
Restriction enzymes	Fermentas, GmbH, St. Leon-Roth, Germany
Restriction enzyme (USA)	New England Biolabs, Ipswich, MA, USA
Shrimp Alkaline Phosphatase	Fermentas GmbH, St. Leon-Roth, Germany
T4 DNA ligase	Fermentas GmbH, St- Leon-Roth, Germany
T4 DNA polymerase	Fermentas GmbH, St- Leon-Roth, Germany
Terrific broth complete	Teknova, Hollister CA, USA
Tris (ultra pure)	MP Biomedicals Solon, OH, USA
Tris-HCl (p.a.)	Merck, Darmstadt, Germany
TRIZol® Reagent	Invitrogen, Carlsbad CA, USA
Vectashield®	Vectorlabs, Burlingame CA, USA
Yeast extract (for microbiology)	Merck Darmstadt, Germany

2.1.4 Consumables

Tab. 3: Consumables used in this work.

Material	**Manufacturer**
Coating (Nail polish, clear)	P2 cosmetics, Karlsruhe, Germany
Electroporation cuvettes	Biozym Scientific GmbH, Oldendorf, Germany
Microtiter plates 96-well Microfluor Black	Nunc, Langenseibold, Germany
Reaction tubes 200µl, 1.5 ml, 2 ml	Eppendorf, Hamburg, Germany
Sterile 15 and 50 ml tubes	Sarstedt, Nümbrecht, Germany

Sterile filter Filtropur S 0.2 (0.2µm)	Sarstedt, Nümbrecht, Germany
Gavage feeding needle 22 mm	Braintree Scientific, Inc., Braintree MA, USA

2.1.5 Kits

Tab. 4: Kits used in this study.

Kit	Type	Manufacturer
E.Z.N.A. Bacterial DNA Kit	DNA Isolation	Omega Bio-Tek Inc., Norcross GA, USA
peqGOLD Gelextraction Kit	Gel extraction	PEQLAB Biotechnologie GmbH, Erlangen, Germany
GeneAmp® Fast PCR Master Mix (2X)	PCR master mix	Invitrogen, Carlsbad CA, USA
GeneTailor Site-Directed Mutagenesis System	Site directed mutagenesis	Invitrogen, Carlsbad CA, USA
peqGOLD Plasmid Miniprep Kit	Plasmid miniprep kit	PEQLAB Biotechnologie GmbH, Erlangen, Germany
pSTBlue1 Acceptor™ Vector Cloning Kit	Cloning kit	Merck, Darmstadt, Germany
Pure Yield Plasmid Midiprep System	Plasmid midiprep system	Promega, Madison, WI, USA
QIAquick PCR Purification Kit	PCR purification kit	Qiagen GmbH, Hilden, Germany
Ribominus^{TM} Transcriptome Isolation kit (Bacteria)	Ribo depletion for bacterial RNA	Invitrogen, Carlsbad CA, USA
Taq Core Kit	DNA Polymerase	MP Biomedicals Solon, OH, USA
TOPO® TA Cloning® Kit	pCRII® vector , TA cloning	Invitrogen, Carlsbad CA, USA
TOPO® XL Cloning Kit	pCR®-XL-TOPO® vector, blunt cloning	Invitrogen, Carlsbad CA, USA
TurboDNA-free^{TM}Kit	DNAse treatment for RNA	Applied Biosystems, Carlsbad CA, USA
AccuPrep Plasmid MiniPrep DNA Extraction Kit	Plasmid miniprep system	Bioneer, Daejeon, South Korea
Quick Ligation Kit	Ligation	New England Biolabs, Ipswich, MA, USA

2.1.6 Bacterial strains

Tab. 5: Bacterial strains used in this study. TMW strain collection numbers are indicated if available.

Bacterial strain	Relevant genotype/purpose	Reference/supplier
Escherichia coli DHα	F⁻ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 deoR *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-/ standard cloning	(Hanahan 1983)
Escherichia coli TOP10	F⁻mcrAΔ(mrr-hsdRMS-mcrBC) ϕ80lacZΔM15ΔlacX74 recA1 araΔ139Δ(ara-leu)7697 galUgalK rpsL (Str^R) endA1 nupG/standard cloning	Invitrogen, Carslbad CA, USA
Escherichia coli GM2163 TMW 2.1014	F⁻*dam*-13::Tn9(Cam^R) *dcm*-6 *hsd*R2 ($r_k^-m_k^+$) *leu*B6 *his*G4 *thi*-1 araC14 *lac*Y1 *gal*K2 *gal*T22 *xyl*A5 *mtl*-1 *rps*L136 (Str^R) *fhu*A31 *tsx*-78 *gln*V44 *mcr*A *mcr*B1/amplifying unmethylated plasmids	Fermentas GmbH, St-Leon-Roth, Germany
Escherichia coli TZ101a TMW 1.1730	F'/ *end*A1 *hsd*R17 *gln*V44 *thi*-1 *rec*A1 *gry*A *rel*A1 Δ(*lac*IZYA-*arg*F)U169 *deo*R (ϕ80d*lac*Δ(λαχZ)M15)	Trenzyme GmbH, Konstanz, Germany
Escherichia coli XL-1 Blue TMW 2.428	*end*A1 *gyr*A96(*nal*R) *thi*-1 *rec*A1 *rel*A1 *lac gln*V44 F'[::Tn10 *pro*AB+ *lac*Iq Δ(*lac*Z)M15] *hsd*R17(rK-mK+)/standard cloning	Stratagene, Santa Clara CA, USA
Escherichia coli XL 10-Gold	*end*A1 *gln*V44 recA1*gyr*A96(*nal*R) *thi*-1 *rel*A1 *lac Hte* Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 tet^R F'(*pro*AB *lac*I^qZΔM15 Tn10(TetR Amy Cm^R)/standard cloning	Stratagene, Santa Clara CA, USA
Escherichia coli INV110 TMW 2.1021	F´ (*tra*Δ36 *pro*AB *lac*Iq *lac*ZΔM15) *rps*L (StrR) *thr leu end*A *thi*-1 *lac*Y *gal*K *gal*T *ara ton*A *tsx dam dcm sup*E44 Δ(*lac-pro*AB) Δ(*mcr*C-*mrr*)102::Tn10 (TetR)/amplifying unmethylated plasmids	Invitrogen, Carlsbad CA, USA
Escherichia coli OP50	nematode feeding strain	Molecular Nutrition Unit, TUM, originally from *Caenorhabditis* Genetics Center, University of Minnesota, MN USA (Stiernagle 2006)
Escherichia coli EHEC O157:H7 EDL933	LEE, *stx1*, *stx2*, plasmid pO157	Weihenstephaner Sammlung, Abt. Mikrobiologie ZIEL-TUM WS4202 (originally from Collection de l'Intitute Pasteur, Paris, France, Collection Number CIP106327)
Enterococcus faecalis OG1RF TMW 2.645	*sprE, ace, as, esp*	(Dunny et al. 1979)

Enterococcus faecalis Symbioflor® TMW 2.777	*(ace), as*	(Domann et al. 2007)
Enterococcus faecalis V583 TMW 2.852	*gelE, fsrB, sprE, ace, as, cob*	(Paulsen et al. 2003b)
Enterococcus faecalis A/F_2	*gelE, sprE, ace, as*	TMW 2.647 (Lindenstrauß et al. 2011)
Lactococcus lactis subsp. cremoris TMW 1.1085		(Orberg et al. 1985)

2.1.7 Primers

All primers used in this work are listed (Table 6).

Tab. 6: Primers used in this study.

#	Primer name	Primer sequence: 5' -> 3'	Application
1	luxA_fwd	GGTCGCATCTCTGAGGAGTGT	pXen5 detection
2	luxA_rev	CGCTAAGGCGCGACTGTTATT	pXen5 detection
3	luxE_fwd	GTGTGGTCTTACGACGAGCAG	pXen5 detection
4	luxE_rev	CGCCCACCTGACTTTTTAAACC	pXen5 detection
5	pMG36e_fwd	CAAGGGTAAAATGGCCTTTTCCTG	pMG36e detection
6	pMG36e_rev	GAGCCAGTTGGGATAGAGCG	pMG36e detection
7	ori_tfwd	GCGAATTTCCCTGGGTTTGA	pXen5 detection
8	ori_rev	GTGTGATGCGCTGCGTCC	pXen5 detection
19	EcoRV-Cam_fwd	ATGATATCAGGAGGCATATCAAATGAAC	cloning of *cam*
20	EcoRV-Cam_rev	ATGATATCTTATCTCATATTATAAAAGCCAGTC	cloning of *cam*
21	SacIluxAB_fwd	ATGAGCTCAGGAGGAGAAAGAATTGAAATTT	cloning of *luxAB*
23	SacI_cherrry_fwd	ATGAGCTCAGGAGGGAATTCATGGTGTC	cloning of *mcherry*
25	SacI-tomato_fwd	ATGAGCTCAGGAGGGAATTCATGGTG	cloning of *tdtomato*
27	luxAB_KpnI_rev	ATGGTACCTTATGGGACAAATACAAGGAACT	cloning of *luxAB*
28	tomato_Hind3_rev	CATAAGCTTTTACTTGTACAGCTCGTCC	cloning of *tdtomato*
29	cherry_Hind3_rev	CATAAGCTTTTACTTGTACAGTTCGTCCATAC	cloning of *mcherry*
30	M13/puc_fwd	GTAAAACGACGGCCAGT	sequencing of pUC57 MCS
31	M13/puc_rev	CAGGAAACAGCTATGAC	sequencing of pUC57 MCS
32	pMG36e_fwd	CGATTCATTATAACCACT	sequencing of pMG36e
35	pmg36e	GGCAATCTGCCTCCTCATCC	sequencing of pMG36e
36	luxABSacI_fwd2	TATGAGCTCAGGAGGAGAAA-GAAATGAAATTTGG	cloning of *luxAB*

37	luxABKpnI-rev2	TATGGTACCTTATGG-GACAAATACAAGGAACTTATC	cloning of *luxAB*
38	cherrySalIXho_fw	TATGTCGACAGGAGGGAATTCATGGTGTCGAAG	cloning of *mcherry*
39	cherrySalXho_re	TATCTCGAGTTACTTGTACAG-TTCGTCCATACCGCC	cloning of *mcherry*
42	luxCDENotSal_fw	TATGCGGCCGCAGGAGGAG-TAAAAGTATGGAAAATGAATCA	cloning of *luxCDE*
43	luxCDENotSal_re	TATGTCGACTTAGACATCTAAATCTAGGTACTA-AAACAATTCATCC	cloning of *luxCDE*
44	CBRNot_fwd	TATGCGGCCGCAGGAGGGAATTCATGGTCAA-GCGCG	cloning of *cbr*
45	CBRSal_rev	TATGTCGACTTAGCCGCCGGCCTTCACGAGGAG	cloning of *cbr*
46	InPCRrev1	GTCATACGTATCCTCCAAGCCT	TAIL PCR
47	NesPCRrev2	CTGCAGGTCGACTCTAGAGG	TAIL PCR
48	InPCRSeq1	GAGCTCGAATTCGCGC	TAIL PCR
49	NesPCRfwd1	GCACTGCTATGCTTACTGGNNNNNN	TAIL PCR
50	NesPCRfwd2	GCACTGCTATGCTTACTGG	TAIL PCR
	609R	ACTACYYGGGTATCTAAK	16S rDNA (Müller et al. 2000)
	616V	AGAGTTTGATYMTGGCTCAG	16S rDNA (Müller et al. 2000)
U9	pMG36e_MCS_fwd	GAAAATTCGTAATTCGAGCTCG	insertion check of pMG36e
U10	pMG36e_MCS-rev	TTCAGACTTTGCAAGCTTGC	insertion check of pMG36e
U11	pMG36e_1kb_rev	TTTATCTTGCTCTTTTGTCAGAGA	insertion check of pMG36e
U12	pMG36e_XhoI_fwd	GGCGCTCGATATTTGGACTCGAGTTCAAG	insertion of *XhoI* in pMG36e
U13	pMG36e_XhoI_rev	TCCAAATATCGTAGCGCCGGGGTACCTG	insertion of *XhoI* in pMG36e
U29	ApaI Kan fwd	AGGGCCCAGGAGGGAAATAATAAAT	cloning of *cam*
U30	Kan XbaI rev	ATTCTAGAAAACAATTCATCCAGTAAAATATAA-TA	cloning of *cam*
U31	Apa Cam fwd	AGGGCCCAGGAGGCATATCAAATGAACTTTAA-TAAA	cloning of *kan*
U32	Cam XbaI rev	AATTCTAGATTATCCTCATATTATAAAAGCCAG-TCA	cloning of *kan*
U47	BamHI lux	AGGATCCAAGAGGAGGACTCTCTATGA	cloning of *lux* cassette
U48	lux KasI	ATGGCGCCTTAACTATCAAACGCTT	cloning of *lux* cassette
U49	EcoRV FMNH	ATGATATCAGGAGGATAACATAGAATAATCAAT	cloning of *frp*
U50	FMNH NotI	ATGCGGCCGCTTAACGTTTGCTAAACCTTTT-GAAT	cloning of *frp*
U55	PstI Transposase	AACTGCAGAAAAAGGCCATATAACAGTCCT	cloning of *transposase*
U56	Transposase PSTI	ATCTGCAGAAGATGCGAATAATCTTTTCTCT	cloning of *tranpsosase*
U57	SalI Tomato	ATGTCGACAGGAGGGAATTCATGGTG	cloning of *tdtomato*

U58	Tomato XhoI	ATCTCGAGTTACTTGTACAGCTCGTCCATACC	cloning of *tdtomato*
U59	BamHI luxAB fwd	ATGGATCCAGGAGGAGAAAGAAATGAAATTT	cloning of *luxAB*
U60	luxAB KasI rev	ATGGCGCCTTATGGGACAAATACAAGGAAC	cloning of *luxAB*
U61	BamHI luxCDEfwd	ATGGATCCAGGAGGATGGCAAATATGAC	cloning of *luxCDE*
U62	luxCDE KasI rev	ATGGCGCCTTAACTATCAAACGCTTCGG	cloning of *luxCDE*
U63	pMG36e_empty cassette_	CGATTCATTATAACCACTTATTTTTTG	insertion check pMG36e
U64	empty cassette_fwd	ATCGATGAATTCAGTCAAGTC	insertion check of transposon
U65	empty cassette_rev	CTGCAGAATTCGATAAAGTCC	insertion check of transposon
U66	EcoRV_Cam_fwd	ATGATATCAGGAGGCATATCAAATGAAC	cloning of *cam*
U67	Cam_EcoRV_rev	ATGATATCTTATCTCATATTATAAAAGCCAGTC	cloning of *cam*

2.1.8 Plasmids

Cloning plasmids

Descriptions of acceptor vectors, used for the amplification of toolkit genes, are displayed in the appendix (p. 116).

Tab. 7: Plasmids used in this study. TMW strain collection numbers are indicated.

Plasmid	**Maintenance in**	**Reference**	**# TMW**
pUC57 *cbr*	*E. coli* Top10	CaliperLS	2.1006
pCR XL Topo Xen10	*E. coli* XL-1Blue	this study CaliperLS	2.1007
pCRII *cam*	*E. coli* Top10	this study, CaliperLS	2.1008
pCRII *kan*	*E. coli* Top10	this study, CaliperLS	2.1009
pUC57 *fmnh*	*E. coli* Top10	CaliperLS	2.1010
pCRII transposase	*E. coli* Top10	this study, CaliperLS	2.1011
pCRII tdtomato	*E. coli* Top10	this study, CaliperLS	2.1012
pUC57 mcherry	*E. coli* Top10	CaliperLS	2.1013
pCRII *cam* (*EcoRV*)	*E. coli* Top10	this study, CaliperLS	2.1015
pUC57 *lux*G	*E. coli* Top10	this study, CaliperLS	2.1016
pUC57 Ecwp	*E. coli* Top10	this study, CaliperLS	2.1017
pUC57 Ec-p	*E. coli* Top10	this study, CaliperLS	2.1018
pUC57Ecwp	*E. coli* Tz101a	this study, CaliperLS	2.1077
pUC57Ec-p	*E. coli* Tz101a	this study, CaliperLS	2.1078

Expression plasmids

pMG36e

pMG36e is a Gram-positive, Gram-negative shuttle vector with an erythromycin resistance gene and the pWVO1 origin of replication. The p32 promoter from *L. lactis* subsp. cremoris Wg2 is located in front of the multiple cloning site.

(van de Guchte et al. 1989)

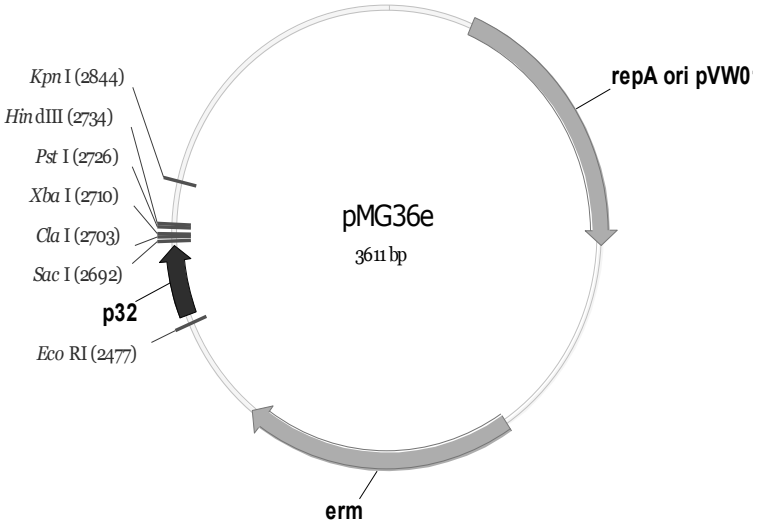


Fig. 9: Schematic overview of pMG36e. The selection marker *erm* gene, the *repA* gene, and the MCS driven by p32 promoter are displayed.

pXen5

pXen5 is a shuttle vector that replicates in Gram-negative and Gram-positive bacteria like *E. coli* and *E. faecalis*. It is recommended to be used directly form the supplier (CaliperLS) for transformation in Gram-positive because of its instability upon amplification in other bacteria. Its origin is temperature sensitive and it contains a transposable element (Tn4001 transposase) with the *luxABCDE* operon, a transposon, and a kan resistance gene. It has an additional *erm* resistance gene encoded on its plasmid backbone.

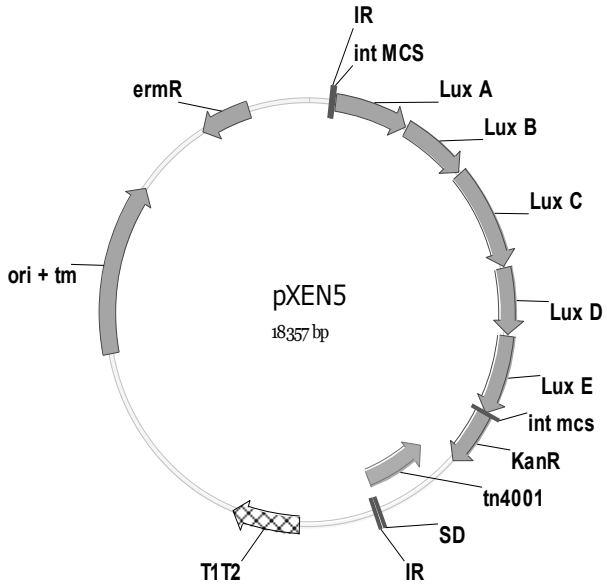


Fig. 10: Schematic overview of pXen5. The Tn4001 transposon, consisting of inverted repeats (IR) and the tn4001 transposase (tn4001), containing the *lux* operon are marked, as well as the *erm* selection marker gene and the temperature sensitive origin.

Tab. 8: Plasmids used in this study. TMW strain collection numbers are indicated.

Plasmid	in	Reference	# TMW
pMG36e*XhoI*	*E. coli* Top10	this study, CaliperLS	2.1002
pMG36e-p32	*E. coli* Tz101a	this study, CaliperLS	2.1003
pMG36e *cbr*	*E. coli* Top10	this study, CaliperLS	2.1005
pMG36e *cbr*	*E. faecalis* OG1RF	this study, CaliperLS	2.1019
pMG36e *cbr*	*E. faecalis* Symbioflor®	this study, CaliperLS	2.1020
pMG36eluxABCDE	*E. coli* Top10	this study, CaliperLS	2.1361
pMG36e-p32EcwpCam	*E. coli* Tz101a	this study	2.1065
pMG36e mcherry	*E. coli* DH5α	this study	2.0179
pMG36e tdtomato	*E. coli* Tz101a	this study	2.1357

2.1.9 Media and buffers

Bacterial media

Agar was prepared from liquid media by supplementing with 15 g agar per liter to the other ingredients. Antibiotics were added to the agar at 50°C after autoclaving.

Lysogeny broth (LB)

Peptone from casein (enzymatically digested)	10.0 g
Yeast extract	5.0 g
NaCl	5.0 g
H_2O dest.	add 1000 ml
pH	7.2

Brain heart infusion

Brain heart infusion powder	37.0 g
H_2O dest.	add 1000 ml
pH	7.2

M17

M17 medium	37.25 g/l
pH	7,0

NGM agar

Peptone from casein (enzymatically digested)	2,5 g
NaCl	3.0 g
H_2O dest.	add 970 ml
autoclave	
prepare separately, add at 42°C, mix well:	
Cholesterol (sterile filtered) 5 mg/ml in ethanol	1 ml
KPO_4 buffer, 1 M 132 mM K_2HPO_4, 868 mM KH_2PO, pH 6.0	25 ml
Nystatin solution (sterile filtered)	5 ml
MgSO4, 1 M	1 ml
CaCl2, 1 M	1 ml
Dry at room temperature	

Antibiotics

Tab. 9: Antibiotic concentrations.

Antibiotic	Dissolved in	Stock concentration	Final concentration
Ampicillin	Deionized H_2O	100 mg/ml	100 µg/ml
Chloramphenicol	Ethanol	34 mg/ml	68 µg/ml
Erythromycin	70% Ethanol	25 mg/ml	Gram negatives: 200 µg/ml Gram positives: 5 µg/ml mice *in vivo*: 10 µg/g bodyweight
Kanamycin	Deionized H_2O	25 mg/ml	*E. coli*: 50 µg/ml *E. faecalis:* 200 µg/ml
Nalidixin	70% Ethanol	12,5 mg/ml	50 µg/ml
Nystatin	DMSO	10 000 units/ml	10 units/ml

Competent cells

Washing buffer ($CaCl_2$ method)	100 mM	$CaCl_2$

Store buffer ($CaCl_2$ method)	100 mM	$CaCal_2$
	15%	Glycerol

Electroporation buffer *(E. coli)*	15%	Glycerol

SGBHI medium	37g	BHI
	0,5M	Saccharose
	3%	Glycine
	add 1l	dest. H_2O
	pH	7,2

SBHIMC medium	37 g	BHI
	0,5 M	Saccharose
autoclaved separately and added	10 mM	$MgCl_2$
to a final concentration of	10 mM	$CaCl_2$
	add 1 l	H_2O
	pH	7,2

Electroporation buffer	0,5 M	Saccharose
(*E. faecalis*)	10%	Glycerol

Agarose gel electrophoresis

TAE buffer, pH 8,0:	40 mM	Tris-acetate
	1 mM	EDTA

TBE- buffer, pH 8,0:	90 mM	Tris
	90 mM	borate
	2 mM	EDTA

DNA-agarose gel (0,8%, 1,0%, 1,8%) :	0,8 g, 1 g, or 1,8 g	agarose

ethidium- or dimidium bromide H_2O solution	0.5 µg / ml	ethidium- or dimidium bromide

destaining solution	H_2O

Imaging chemicals

D-Luciferin potassium salt (sterile filtered)	15 mg / ml in DPBS

2.2 Methods

2.2.1 Bacterial growth

Cultivation parameters

E. coli was cultivated in liquid LB medium aerobically at 37°C shaking at 180 rpm or on agar plates at 37°C for 16 h, if not indicated otherwise. Antibiotics were added if appropriate (Tab. 9).

E. faecalis cultivated in BHI and sometimes LB with shaking as above. Common incubation time was 16 h, but some transformants needed 30 h to growth time.

L. lactis subsp. *cremoris* was cultivated in M17 medium at 30°C for 24 h, with shaking as above.

Strain identification

E. faecalis Symbioflor® and OG1RF were verified by PCR-amplifying the 16S rDNA using Primer 616V and 609R and subsequent sequencing using primer 609R (Müller et al. 2000).

Stock culture preparation

Seven hundred-fifty µl overnight culture were mixed with 150 µl sterile 50% Glycerol and stored at -80°C.

Cell density measurement

OD_{600} was measured for growth curves.

Colony-forming units

Bacterial cultures were diluted serially and plated. Counts were determined as cfu. For counting of *E. faecalis* Symbioflor® 90 ml LB were mixed with 10 g LB Symbioflor® agar and homogenized by the stomacher. Of the mixture, 1 ml was diluted 10-fold repeatedly. Two hundred µl of each dilution were plated and incubated as detailed above. Count number between 25 and 200 per plate were used for calculating cfu.

Bacterial growth curves

For growth curve measurements of a bacterial strain 500 µl of an overnight culture were inoculated into 50 ml fresh medium. At defined time-points the OD_{600} was measured and usually 100 µl of the culture were used to determine the cfu in parallel.

Antibiotic sensitivity tests

Ten µl of the different *E. faecalis* strains were used to inoculate 5 ml BHI medium containing antibiotics (erm: 0 µg/ml, 2,5 µg/ml, 5 µg/ml, and 10 µg/ml; Kan: 0 µg/ml, 50 µg/ml, 400 µg/ml, and 800 µg/ml). The culture was grown at 37°C for 16 h. Visible turbidity was taken as evidence for resistance.

For the MIC-disc diffusion tests, *E. faecalis* V583 was used a positive control and *L. lactis* subsp. cremoris was used as a negative control, respectively. Antibiotic discs were soaked with 15 µg/20 µl erythromycin or 30 µg / 20 µl kanamycin. Three hundred µl bacterial overnight culture were plated on the appropriate agar plate. Three antibiotic discs used on each plate and the plates were incubated 24 h at the appropriate temperature, after which the zones of inhibition was measured after 24 h.

Plasmid stability test

To test the stability of a plasmid under non-selective conditions, an overnight culture of the bacteria containing the plasmid was diluted 100x in the appropriate medium without any specific antibiotic and cultured at standard conditions. At times, the culture was diluted 1:20 and diluted further serially at 1:10. Dilutions were plated and fractions of bacteria with and without plasmid were compared.

Competent cells and transformation

E. coli

For transformation of plasmids DNA in *E. coli* Top10, DH5α, Tz101a or SURE a heat-shock transformation protocol with $CaCl_2$ competent cells was used (Cohen et al. 1972, Morrison 1977). For more delicate transformation (e.g., large plasmids) electro-competent *E. coli* were prepared as follows: One ml bacterial overnight culture was inoculated into 50 ml LB medium until OD_{600} reached 0.4 to 0.5. The cells were pelleted at 4 000 rpm for 5 min at 4°C. The pellet was washed twice with electroporation buffer and then resuspended into 2 ml of this buffer. Aliquots of 100 ml were immediately frozen in liquid nitrogen and kept on -80°C.

For transformation of the $CaCl_2$-competent cells, an aliquot was thawed on ice and 1 µl ligation mix or plasmid was added and carefully mixed. After 20 min on ice, the cells were heat-shocked for 45 sec at 42°C and cooled on ice for 2 min. Next 500 µl LB medium was added to the tube and the mixture was incubated at 37°C for 1 h at shaking. In case of subsequent ampicillin selection, 30 min shaking were used.

For preparation of electro-competent *E. coli,* 1 ml bacterial overnight culture was inoculated into 50 ml LB medium in an Erlenmeyer flask. This was incubated at 37°C shaking until OD_{600} of 0.4 to 0.5. A pre-cooled Sarstedt-tube was filled with 50 ml culture and centrifuged at 4 000 rpm for 5 min at 4°C. The pellet was washed twice with electroporation buffer and then resuspended into 2 ml of this buffer. Aliquots were made, containing 100 µl of this solution, immediately frozen in dry ice and kept on -80°C.

For electroporation, electro-competent *E. coli* cells were thawed on ice and 1 µl ligation mix or plasmid solution, which had to be de-salted before, was added and carefully mixed. The mixture was pipetted into a 100 µl-electroporation cuvette on ice. The lid was closed and the ice was wiped from the cuvette before placing it into the electroporation chamber. The pulse was set on 25 mF, 400 Ω, 2,5 kV/cm. Immediately after, 500 µl LB was added. The cells were pipetted into a 1,5 ml Eppendorf tube and kept on ice for 2 min. Then the tube was incubated at the ideal conditions for up to 48h.

E. faecalis

For transformation of plasmid DNA into *E. faecalis* electro-competent cells were prepared (modified from (Shepard et al. 1995). Briefly, 100 ml SGBHI medium in a 500 ml Erlenmeyer flask was inoculated 1:100 using an overnight culture. After 24 h at 37°C with shaking, the cells were pelleted at 1 000 rpm for 10 min at 4°C and washed with ice-cold electroporation buffer twice. Subsequently, the cells were resuspended in 1 ml electroporation buffer and kept on ice and 40 µl aliquots were stored at -80°C.

For electroporation the aliquots were thawed on ice and max. 1 µl of the salt-free plasmid preparation was added and carefully mixed. Electroporation was conduced as described above, but using a resistance of 200 Ω. Next, 500 µl SBHI17MC was added immediately to the cuvette and the suspension was transferred to a 1,5 ml Eppendorf tube. This was kept on ice for 5 min and incubated shaking at 37°C for 2 h. Finally, 200 µl each were plated on BHI plates (with the appropriate antibiotic) and incubated at 37°C until colonies were visible.

2.2.2 Microscopy

Standard microscopy was performed using a 100 x oil immersion objective according to the manufacturer's instructions.

For fluorescence microscopy, to stabilize the fluorescence one drop (about 50 µl) of Vectashield® mounting medium was added to a 10 µl bacterial culture on glass slides. The cover slip was sealed onto the slide with coating. The fluorescence was observed with the epi-fluorescence microscope using filter set #31. The suitability of the fluorescent reporter system for laser scanning microscopy was tested using a 60 x oil immersion objective. The fluorescent image was monitored at λex= 543 nm, λem=590/50 nm in 512 x 512 pixel resolution and a constant z position.

To easily assess fluorescence of overnight-grown transformants the IVIS Lumina Camera system was used.

2.2.3 DNA / RNA methods

TBE or TAE agarose gels were prepared according to (Sambrook et al. 1989). The Agarose was dissolved either in TAE buffer for preparative gels or in TBE buffer for analytical gels. DNA samples were mixed either with loading dye (5x) or with FD Green buffer (10x). Size dependent separation was performed in electrophoresis chambers at 100 V to 130 V, max. 200 mA, max. 20 W for about 45 min to 60 min. Gels were stained with either ethidium bromide or Dimidium bromide and DNA visualized under the UV.

DNA from bacterial culture was isolated using the E.Z.N.A. Bacterial DNA kit according to the manufacturers instruction.

Plasmids were isolated from *E. coli* using a plasmid prep mini kit or, for low-copy vectors a midi kit. DNA or RNA solutions were precipitated using 1/10 volume of 3 M Na-acetate at pH 5.2 and 3 volumes of ice-cold ethanol was added to the DNA. After 20 min incubation at -20°C the DNA was centrifuged at 13 000 rpm at 4°C for 10 min. The pellet was washed with 70% ice-cold ethanol and the air-dried DNA was resuspended into an appropriate amount of H_2O. The concentration and quality of DNA or RNA was also estimated using a Nanodrop 1000 device according to the manufacturer's instructions.

PCR, cleanup and sequencing

DNA was amplified using either PCR Taq Core kit or GeneAmp® Fast PCR Master Mix and a Thermo cycler (PRIMUS 96 plus or Eppendorf gradient cycler). For the amplification of templates longer than 5 kb LongAmp® *Taq* or Elongase® was used according to the manufacturer's instructions. PCR products were either purified directly using a PCR clean-up kit or by gel extraction kit, if necessary. PCR products or plasmids were sequenced by GATC Biotech GmbH (Konstanz, Germany).

Tab. 10: Standard PCR reaction mix.

Buffer	1x
MgCl2 (in buffer)	1.5 mM
dNTPs (fresh)	0.2 mM
Primer	0.5 µM
Taq polymerase	1.5 U
Final volume with template	50 µl

Tab. 11: Standard PCR conditions.

Initial denaturation	95°C	2.00 min
Denaturation	95°C	30 sec
Annealing	melting temperature of primer -3°C	20 sec
Elongation	72°C	1 min per kb fragment
	amplification cycle 30 x	
Final elongation	72°C	7 min
Store	4°C	∞

Restriction and ligation

Restrictions enzymes were used either from Fermentas or New England Biolabs according to the manufacturer's instructions. Double digestion was favored above subsequent digestion. For overnight digestions the reaction mix indicated in Tab. 12 was prepared and incubated for 16 h in a 37°C. SAP was added to plasmid backbone restrictions.

Tab. 12: Overnight restriction digestion mix.

DNA	max. 10 µg
enzyme 1	2 µl
(enzyme 2)	2 µl

SAP	1 µl
Buffer	10 µl
dest. H_2O	add to 100 µl

Resulting fragments were cleaned as PCR-products above and ligated using T4 DNA Ligase from Fermentas of Quick Ligation™ kit from NEB according to the manufacturer's instructions. Ligation were verified by restriction analysis, PCR or sequencing.

2.2.4 Plasmid construction

Construction of pMG36eXen10

The *luxABCDE* operon + promoter region + Kan resistance gene (Xen10 fragment) from *Streptococcus pneumonia* Xen10 was cloned into pMG36e. For this, an *XhoI* restriction site was inserted into pMG36e (position: 2865 – 2870 bp) using the GeneTailor™ Site-Directed Mutagenesis kit according to the manufacturer's instructions (Primer# U12 and U13). The mutagenesis was verified by restriction analysis. Afterwards, XLTopo vector containing the Xen10 and pMG36eXhoI were digested with *KpnI* and *XhoI*. The pMG36eXhoI backbone was ligated with the Xen10 fragment and transformed into *E. coli* XL10-gold electro-competent cells following standard manufacturer's protocol. Transformants were selected for kan resistance. Positive luminescent colonies were re-streaked onto erm agar and the colony showing the highest luminescence was used for transformation in *E. faecalis* OG1RF and Symbioflor® following standard protocol. The bacteria were plated on LB containing, erm, kan or both antibiotics and incubated up to 48 h. Hundred µl nonanal were added to the lid of the petri dish to boost the signal.

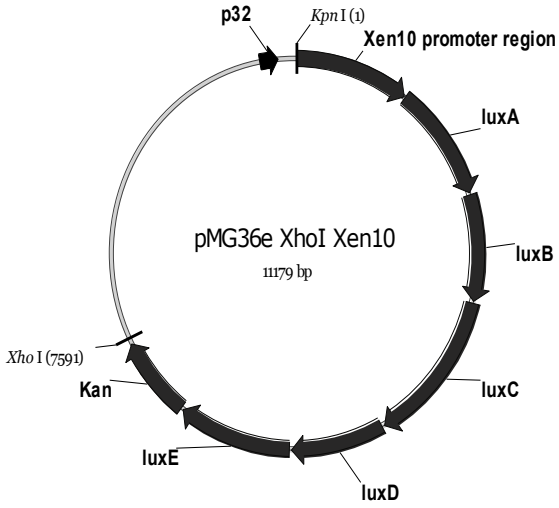


Fig. 11: Xen10 fragment in pMG36eXhoI. pMG36e contains the *luxABCDE* from *Streptococcus pneumonia* Xen10, a kan resistance and a promoter region.

Plasmid backbone for the reporter system toolkit

For the plasmid backbone of the transposon reporter system the backbones of pXen5 and pLS210 were chosen, but PCR amplification was not possible (data not shown), therefore pMG36e was used eventually (McCormick et al. 1996, van de Guchte et al. 1989, Venema et al. 1995). pMG36e is a shuttle vector, which was successfully tested for replication in *E. coli* and *E. faecalis* OG1RF and Symbioflor®. To minimize interfering promoter activity of the plasmid-borne p32 promoter, which is located upstream of the MCS, it was initially removed from pMG36e. pMG36e was digested with *EcoRI* and *SacI* and the fragment was purified on preparative agarose gel with subsequent ethanol precipitation. The DNA-ends were blunted by T4 Polymerase (Fermentas) according to the manufacturer's instructions. The mix was purified with a PCR purification kit, ethanol precipitated and checked on an agarose gel. Then, the fragment was re-ligated using T4 Ligase (Fermentas). PEG4000 was added to the ligation mix according to the manufacturer's instructions. The plasmid was transformed applying the standard transformation protocol. Positive clones were verified by sequencing. The deletion of the p32 promoter was verified by PCR with primer# 6 and 35 and subsequent gel analysis of the PCR product. The product was expected to be 868 bp (compared to 1048 bp for the fragment with the promoter). Additionally, the plasmid was restricted with *SacI* and *EcoRI* and the fragment was compared with the undigested plasmid

on an agarose gel. The plasmid digested with *SacI* looked similar to the undigested plasmid, whereas the plasmid digested with *EcoRI* seems to be bigger (digested) on the gel (Fig. 24).

The reporter system toolkit

To build up a reporter system toolkit, the genes or components were selected or designed and synthesized. The selection and preparation of the toolkit was done in close cooperation with CaliperLS (Ali Akin, Kevin Francis). All reporter genes (*cbr, mcherry, tdtomato*) and flavin oxidoreductase genes (*frp, luxG*) were provided by CaliperLS, adapted to expression in Gram-positives (codon usage and Shine Dalgarno sequence) and inserted into pUC57. The reporter genes were optimized for expression in *Mycobacterium spp*., the flavin oxidoreductase genes for expression in *Staphylococcus aureus*.

Tab. 13: The reporter system toolkit.

Name of the element/ gen	Function	DNA from
luxAB	luciferase enzyme	pXen5
luxCDE	fatty acid reductase complex	pXen5
luxG	flavin oxidoreductase	synthesized (Genscript) delivered in pUC57
frp	flavin oxidoreductase	synthesized (Genscript) delivered in pu57
cbr	luciferase enzyme	synthesized (Genscript) delivered in pu57
mcherry	monomeric red-fluorescent protein	synthesized (Genscript) delivered in pUC57
tdtomato	tandem-dimeric red fluorescent protein	synthesized (Genscript) delivered in pUC57
transposase	mobilizes the transposon	pXen5
cam	chloramphenicol resistance (for use in OG1RF and Symbioflor)	pXen1
kan	kan resistance (for other bacteria)	pXen5
Ecwp	transposon cassette with two inverted repeats flanking a multiple cloning site containing the p32 promoter	synthesized (Genscript) delivered in pUC57
Ec-p	transposon cassette with two inverted repeats flanking a multiple cloning site (no promoter)	synthesized (Genscript) delivered in pUC57
pMG36e-p32	plasmid backbone	-

The reporter genes *cbr, mcherry, and tdtomato* were inserted into the shuttle vector pMG36e to verify their function in *E. faecalis*. *cbr* was directly inserted from pUC57 into pMG36e after *SacI, HindIII* standard restriction and ligation of the heat-inactivated mix. To start the

bioluminescent reaction, the substrate luciferin was given to a separate aliquot of the culture, never to the culture used for subsequent applications. *tdtomato* and *mcherry* were amplified with primer #25 + #28 and #23 + #29 respectively, having *SacI* and *HindIII* restriction sites added. The PCR product was inserted into pSTBlue-1 by TA-cloning. Using *SacI* and *HindIII tdtomato* was cloned into pMG36e (A similar construct with *mcherry* was prepared by Stefanie Böllner/Simone Freiding). Integration of the transposase and upstream promoter PCR product (with primer U55 + 56) into pCRII was verified by PCR with primer U55 (annealing within the transposon fragment) and primer# 30 (annealing within the acceptor plasmid backbone).

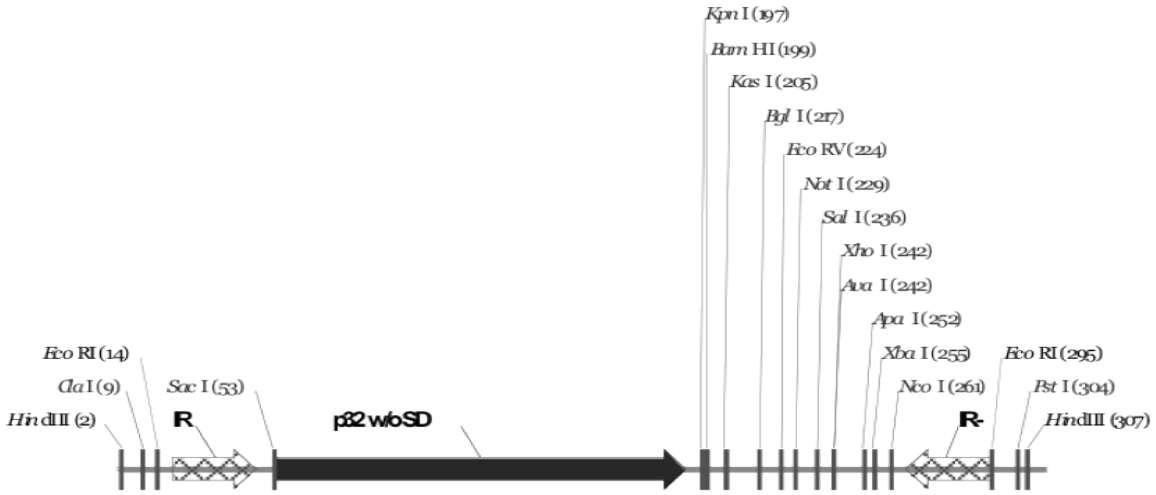


Fig. 12: Schematic design of the Ecwp, transposon cassette with the p32 promoter (without Shine-Dalgarno sequence) within the MCS. *HindIII*, *ClaI*, *EcoRI*, and *PstI* restriction sites can be used to insert the transposon cassettes into the plasmid backbone, whereas (*SacI in Ec-p), KpnI, BamHI, KasI, BglI, EcoRV, SalI, XhoI, AvaI ApaI XbaI*, and *NcoI* can be used to insert toolkit components into the transposon's MCS.

Construction of the reporter system

All components of the toolkit were initially prepared as or inserted into a(n acceptor) plasmid to be readily available for the cloning construction (Tab. 7). Restriction sites for each gene were assigned.

Tab. 14: Assigned restriction sites for reporter system components and primer sets used for attaching the assigned restriction sites to the toolkit components ends. Conflicting restrictions sites within the

components sequence that are used for the integration of other genes into the plasmid systems are also listed.

Insert	Assigned restriction site	Relevant restriction sites in gene	Primer#
transposon cassette	*ClaI, PstI*		*
transposase	*PstI*	*ClaI, HindIII*	U55, U56
kan	*XbaI, ApaI*	*EcoRV*	U31, U32
cam	*EcoRV (or XbaI, ApaI)*		19, 20 (U29, U30)
cbr	*NotI, SalI*	*KasI, XhoI, ApaI*	44, 45
luxAB	*BamHI, KasI*	*XbaI*	U47, U48
frp or luxG	*EcoRV, NotI*		U49, U50 (*frp*), * (*luxG)*
mcherry	*SalI, XhoI*	*KasI*	38, 39
tdtomato	*SalI, XhoI*		U57, U58

* synthesized with the restriction sites attached

Several toolkit components (Ecwp, Ec-p and *luxG*) were already attached to their respective restriction sites. The transposon cassettes were synthesized and inserted into the acceptor vector pUC57. The plasmids pUC57Ecwp (Fig. 12) and pUC57Ec-p were received and used for subsequent cloning. *luxG* is flanked by its assigned restriction sites *EcoRV* and *NotI* in pUC57. For others, the assigned restriction sites were attached by PCR amplification using the appropriate primers (Tab. 14). The restriction sites *SalI* and *XhoI* were attached to *tdtomato* by PCR amplification (primer #U57 + #U58) and insertion of the product into pCRII. This was verified by kan selection and detection of fluorescence. The chloramphenicol selection marker gene *cam* was amplified, attaching the assigned restriction (*EcoRV, XbaI, and ApaI*) sites to its ends into pCRII. The integration of the *cam* PCR product (primer #U31 + #U32, #U66 + #U67 respectively) into pCRII was verified by the PCR product with primer #U31/#U66 (annealing within *cam* sequence) + primer #30 (annealing within pCRII). *kan* was amplified, attaching the assigned restriction sites (*XbaI, ApaI*) to its ends and inserting it into pCRII. The integration of *kan* PCR product (primer #U29 + #U30) into pCRII was verified by PCR with primer #U29 (aligns within *kan* sequence) + primer #30 (aligns within pCRII). And the transposase gene was amplified using primer #U55 and #U56 attaching *PstI* restriction sites to the gene, which was inserted into pCRII. However, *cbr, mcherry, and frp* do not have the respective restriction sites attached to their gene and are inserted in pUC57.

The core of the reporter system was meant to consist of a shuttle plasmid containing the transposon and the transposase. Within the transposon different combinations of reporter genes, Fmn reductase genes, and selection markers may be possible to adjust the system to the experimental approach and the organism to be tagged. A cloning order considering the conflicting restriction sites is displayed in Tab. 15.

Tab. 15: Cloning strategy for the reporter system. Insertion order into pMG36e-p32 considers conflicting restriction sites.

1.	transposon cassette with *cam*		
2.	*transposase*		
3.	*frp/luxG*	*frp/luxG*	*mcherry/tdtomato*
4.	*luxAB*	*tdtomato*	*cbr*
5.	*mcherry*	*luxAB*	

The Cloning scheme of pMG36e-p32traEcwpcamcbr is displayed as an example in Fig. 13.

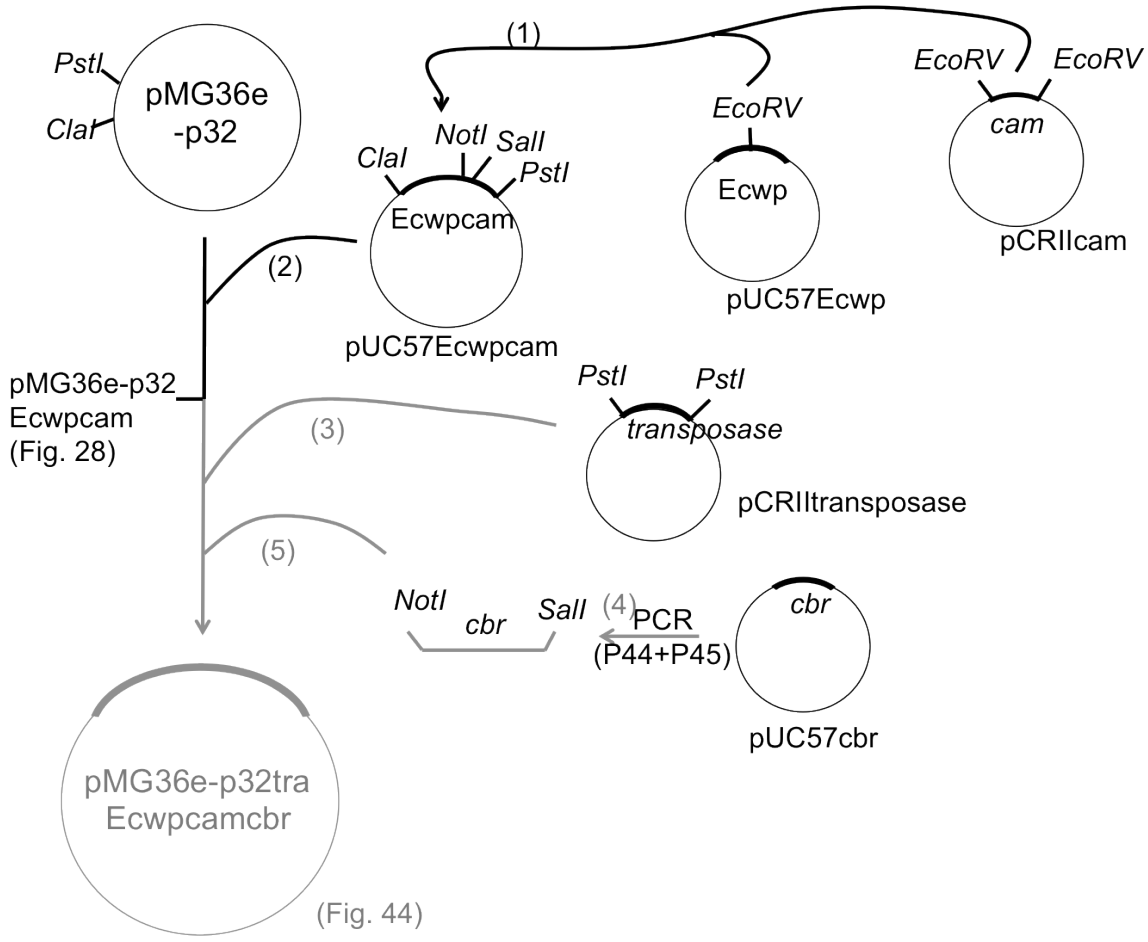


Fig. 13: Cloning scheme of transposon reporter system, based on pMG36e-p32tra Ecwpcamcbr. Black figures display finished cloning steps, gray figures planned cloning steps. Lines represent restriction,

ligation and transformation steps via indicated restriction enzymes. The PCR step is complemented by the respective primers to be used for amplification. Detailed figures of intermediate and end product are referred. Numbers refer to cloning steps indicated in the text below.

First, *cam* was inserted into the transposon cassettes as a spacer. The insertion of *cam* into pUC57 Ecwp (Fig. 13 (1)) and pUC57 Ec-p was verified by selection on LB amp and LB cam agar and PCR (primer #30 and #31). Ecwpcam was inserted by *ClaI* and *PstI* restriction into pMG36e-p32. First, *ClaI* did not cut pMG36e-p32 isolated from *E. coli* Tz101a (Fig. 26). Therefore pMG36e was introduced into *E. coli* GM2163 and isolated from this strain for *ClaI* restriction and subsequent cloning. The construction of pMG36e-p32 Ecwpcam (Fig. 13 (2)) was confirmed by PCR with the primers #35 + #6 (expected product size: 1799 bp, Fig. 27) and sequencing with primer #35 and #6. The next steps include the insertion of the transposase (Fig. 13 (3)) to have the core of the reporter system and the insertion of a reporter gene, e.g., cbr (Fig. 13 (5)). This has to be amplified by PCR (Fig. 13 (4)) to attach the restriction sites *NotI* and *SalI* needed for insertion.

2.2.5 Bioluminescence imaging

Bioluminescent imaging of bacteria (*in vitro*)

The expression of *luxABCDE* in bacterial cultures was evaluated using the IVIS imaging system. For this, a 100 µl overnight culture or an overnight colony was monitored for 1 min with the IVIS imaging system.

To evaluate expression of *cbr,* the substrate (1 µl Luciferin) was suspended in 100 µl overnight or sprayed onto the colonies of an agar plate and monitored immediately.

In vivo imaging

For the *in vivo* studies *E. faecalis* OG1RF pMG36ecbr and *E. faecalis* Symbioflor® pMG36ecbr overnight culture (Terrific broth complete + 5 µg/ml erm) were adjusted. The bioluminescence of the bacteria was assessed by adding 1 µl D-Luciferin (33 mg/ml in PBS) to 100 µl bacterial culture (in a 1,5 ml Eppendorf tube) and imaging culture with the IVIS system at default settings for luminescence measurement. For each strain 10^{10} cfu in 1 ml were set (OD_{600}: 0.7). 100 µl of this culture were used for testing the luminescence and

choosing the culture with the highest intensity for the *in vivo* study (Fig. 29). One hundred µl (10^9 cfu) were later used for oral inoculation of one mouse. Fifteen female Swiss-Webster mice (Jackson lab) 6 to 8 weeks old, and visually judged in a healthy condition, were provided by the animal facility of CaliperLS Alameda CA, USA. They were kept at 22°C in a conventional animal colony with standard laboratory food and water *ad libitum*. The mice were numbered on the tail, weighed, and divided into 5 groups with 3 mice each.

Tab. 16: Mice used in *in vivo study*.

Mouse#	E. faecalis strain	Bacterial dosage	Erythromycin*	Weight (g)
1	OG1RF	1 x 10^9	-	26
2	OG1RF	1 x 10^9	-	23.8
3	OG1RF	1 x 10^9	-	30.1
4	Symbioflor®	1 x 10^9	-	23.4
5	Symbioflor®	1 x 10^9	-	27.6
6	Symbioflor®	1 x 10^9	-	23.8
7	OG1RF	1 x 10^9	+	28.9
8	OG1RF	1 x 10^9	+	28.9
9	OG1RF	1 x 10^9	+	25.1
10	Symbioflor®	1 x 10^9	+	21.7
11	Symbioflor®	1 x 10^9	+	26.3
12	Symbioflor®	1 x 10^9	+	23.7
13	control	-	-	27.1
14	control	-	-	29.6
15	control	-	-	23.7

*200 µl erm was added to 5 ml drinking water for each mouse per day (Harkness et al. 1995).

Protocols provided by CaliperLS were followed (Kong et al. 2011a, Kong et al. 2011b). Briefly, the bacterial overnight culture was washed twice with PBS and then adjusted to the requested OD_{600}. The mice (one group at a time) were anesthetized in a gas anesthesia manifold using isoflurane (2.5%) following the manufacture's instructions. The bacteria (100 µl, 10^9 cells) were orally inoculated into the stomach using a gavage device with a curved feeding needle with a ball tip. The needle was inserted into the mouth down to the esophagus and the bacteria were injected slowly. D-Luciferin (150 ng/g body weight) was instantly injected intraperitoneally (i.p.) The mice were rested in the cage and after 10 min they were anesthetized again until imaging with IVIS Lumina at default/auto exposure settings was finished. If necessary (e.g., saturation), exposure time was reduced. Afterwards the mice

were put back into their cage. At subsequent time-points mice were anesthetized again and injected D-luciferin 10 min before imaging.

For the *in vivo* study setup, mice were orally inoculated with *E. faecalis* OG1RF or *E. faecalis* Symbioflor®. An IVIS Lumina (CaliperLS) was used for whole body imaging of the mice. The bacteria were administered by gavage into the stomach and bacteria monitored as above. Images were taken at 0 h, 2 h, 4 h, 19 h, 24 h, 48 h, and 72 h after infection.

2.2.6 *C. elegans* killing assays

To evaluate the effect of *E. faecalis* Symbioflor® on EHEC pathogenesis, *C. elegans* killing assays were conducted. *C. elegans* wild-type N2 worms (var. Bristol, kindly provided by Dr. Britta Spanier, TUM, Germany) were cultivated on NGM agar plates (92 mm diameter) with OP50 at 22°C (Stiernagle 2006).

For the killing assay (Rasmussen et al. 2005, Tan et al. 1999) NGM plates were prepared using different bacterial cultures (Tab. 17).

Tab. 17: Conditions of the bacterial cultures used for the killing assay.

Bacteria	Ratio	Volume seeded on NGM agar	Pre-incubation
EHEC	-	50 µl	16 h at 25°C
EHEC + *E. faecalis* Symbioflor®	1:10	50 µl	16 h at 25°C
EHEC + *E. faecalis* Symbioflor® lysate*	1:10	50 µl	16 h at 25°C
E. faecalis Symbioflor® 10x	-	100 µl	1 h at 25°C

* The lysate was prepared by standard fast prep of 1 ml (10 x concentrated) *E. faecalis* Symbioflor® culture. This did not completely kill the cells. About 10^4 to 10^5 cells could be recovered per ml on LB plates (standard conditions) whereas on NGM plates no growth was detected even after extended incubation (up to 72 h at 37°C).

Three plates of each type of bacterial culture were seeded with 35 – 50 L4 worms. Plates were incubated at 22°C and scored for live worms every 24 h, which were transferred to fresh plates. Worms were considered dead when they did not respond to touching by the platinum needle used for transfer. Worms, which died as a result of sticking to the petri-dish wall or while handling were excluded from the analysis. The survival rate was analyzed using GraphPad Prism Software as Kaplan-Meier plot, pairwise compared using the log

rank test and determine the median survival kindly conducted by Britta Spanier TUM. In addition, TD50 was determined using *R* (version 2.13.2).

2.2.7 EHEClux transposon database screening

An EHEC EDL933 collection containing 9408 random *lux* insertion mutants (EHEClux) was used to study the impact of *E. faecalis* Symbioflor® co-culture. The collection was kindly provided by Klaus Neuhaus, TUM and built to study the promoter activity of EHEC genes by luminescence. The mutants are stored at -80°C in glycerol.

LB Symbioflor® agar was prepared as follows: 15 ml *E. faecalis* Symbioflor® OD_{600} 0.1 was added to 600 ml agar at 42°C before pouring it into square petri dishes (30 ml). Plain LB agar plates containing no Symbioflor® were prepared as a control. All plates were incubated overnight at 37°C and pH value was measured with indicator paper. Symbioflor®-cfu in the agar were counted by serial plating. The cfu of LB agar + *E. faecalis* was determined to 2.2 x 10^8 cfu in 1 g agar. The EHEClux clones were transferred to the agar plates by a 96-well pin array. After incubation overnight at 37°C, the luminescence of the colonies was visualized in the IVIS® Lumina system. Luminescence intensity of colonies grown on LB Symbioflor® agar and on LB agar was compared at the same luminescence intensity color display. EHEClux clones, which showed a visible difference in luminescence intensities were further investigated. Positive clones were re-tested and, if positive again, streaked into LB-nal. A sub-collection of 84 EHEClux mutants was prepared and stored at -80°C in glycerol.

Next, the luminescence of the colonies was measured quantitatively. For this, 10 µl of the respective EHEClux clones' overnight culture was transferred to the agar plates with and without *E. faecalis*. After 18 h incubation at 37°C, the luminescence was measured using the IVIS® Lumina quantitatively and the mean value and standard deviation was calculated. The luminescence (measured in p/sec/cm2/sr) generated from one EHEClux mutant grown on LB Symbioflor® agar was compared to the respective mutant grown on LB using a mean of the least three experiments.

PCR for transposon integration location in EHEC

To identify the locus of integration of a *lux* transposon cassette in EHEClux a modified TAIL (thermal asymmetric interlaced) PCR with nested modified primer strategy (Jacobs et al. 2003, Lewenza et al. 2005, Liu et al. 1995, Tan et al. 2005) was applied using the PCR Taq Core kit and the PRIMUS 96 plus cycler. In the first TAIL PCR a forward N_6-whobble primer with a defined 5' attached sequence (not specific for EHEC EDL933) and a reverse 5' *lux* cassette annealing primer (Fig. 54, appendix) were applied. During the first five cycles of the TAIL PCR the annealing temperature was adjusted to the reverse primer to specifically amplify from its annealing start point (asymmetric amplification). Afterwards, lower annealing temperatures (symmetric amplification by primers) were alternated with higher annealing temperatures to enrich for a specific *lux* cassette - EHEC sequence product. This product was further amplified in a second nested PCR using a forward primer annealing to the defined sequence of the TAIL PCR whobble primer and a reverse primer, annealing upstream in the *lux* cassette sequence (Fig. 54, p. 121). Unspecific PCR products (based on amplification from one primer only) generated during the PCR procedures (mainly by non-specific whobble primer amplification) were identified by single primer nested PCR, whereas specific PCR products were only amplified by double primer (#47 an #50) nested PCR.

Tab. 18: PCR reaction mix for TAIL PCR.

Buffer (with $MgCl_2$)	5 µl
dNTPs	1.6 µl
Primer # 49	3 µl
Primer # 46	0.5 µl
Genomic DNA	1 µl
Taq Polymerase	0.4 µl
H_2O	38.5 µl

Tab. 19: TAIL PCR conditions.

1	95°C	2 min
2	95°C	20 sec
3	58°C	1 min
4	72°C	2 min
		repeat step 2 – 4 5x
5	95°C	20 sec
6	30°C	3 min

		ramp 0,3°C/sec
7	72°C	2 min
8	95°C	20 sec
9	42°C	1 min
10	72°C	2 min
		repeat step 9 – 11 10x
11	95°C	20 sec
12	58°C	1 min
13	72°C	2 min
		repeat step 11 – 13 2x
14	95°C	20 sec
15	42°C	1 min
16	72°C	2 min
		repeat step 11 – 16 15x
17	72°C	7 min
	4°C	∞

The product of the first PCR was diluted 100fold and used for the nested PCR. The second PCR was conducted at standard conditions using primer #47 and #50 and, as a control, each primer alone. DNA only visible in the PCR with both primers, were extracted and sequenced using primer #47. If sequence could be aligned to the 5' end of the *lux*-transposon cassette, the adjacent part of the sequence was blasted against the EDL933 genome.

2.2.8 Transcriptome sequencing of EHEC EDL933

The transcriptome of EHEC grown on LB supplemented with Symbioflor® versus LB alone was analyzed by strand-specific next generation transcriptome sequencing. For this, a workflow was established by Richard Landstorfer (TUM, personal communication) for EHEC as follows:

A single cell streak of EHEC EDL933 was grown on LB Symbioflor® and LB agar for 18 h at 37°C. From both plates, several colonies were picked and mixed with Trizol according to the manufacturer's instruction. About 200 µl 0.1 mm zirconia beads were used for homogenization using a FastPrep® instrument. The samples were shaken 3 times at 6.5 m/s for 45 sec and cooled between the runs. After the Trizol-RNA isolation procedure the RNA was resolved in 20 µl RNase free water. Concentration and 260/280 ratios were determined by Nanodrop analysis and RNA samples were visualized on an agarose gels.

Ten µg RNA were used to deplete ribosomal RNA by Ribominus kit. This was conduced according to the manufacturer's protocol except the hybridization step. The sample was denatured at 70°C and, subsequently it kept at room temperature for 30 min. For precipitation two volumes ethanol, 0.1 volume 1M Na-acetate pH5 and Glycogen was added. The final RNA samples were resuspended in 28 µl RNAse free water.

The TURBO DNA-free kit was used to remove DNA according to the manufacturer's instructions. The precipitated RNA was resuspended in 15 µl RNAse free water. 1 µl was used for Nanodrop analysis. Fourteen µl of each sample were sent to CEGAT for further preparation and sequencing (p. 126, appendix).

For each sample the QUAL file and the CSFASTA file, which was received from CEGAT was converted using the bioinformatics toolkit Galaxy Project (Blankenberg et al. 2010, Giardine et al. 2005, Goecks et al. 2010a, Goecks et al. 2010b) into a FASTQ files. Using Bowtie (Langmead et al. 2009) the FASTQ file was mapped (standard settings) onto the genome of EHEC EDL933 and the EHEC EDL933 plasmid pO157. Then, the reads were filtered for mappable reads using Filter SAM converted to BAM files. To omit duplicates, duplicate reads were removed by toolmark (version 0.01). Using Picard Tools 1.53, the reads were indexed to produce BAM.BAI files, which were loaded into Artemis 13.2.0 for visualization.

To assess the expression level of a gene under a given conditions, the data were further analyzed by Svenja Simon (Department Data Analysis and Visualization, Universität Konstanz, Germany) and converted to rpkm values. Briefly, counts were normalized (number of reads covering a gene, sequencing depth, for the length of a given gene) (Mortazavi et al. 2008) and displayed as rpkm. For this, BAM files were imported into *R* (R Development Core Team 2010) using *R*samtools and analyzed using the *Bioconductor* packages *GenomicRanges* and *iRanges* (Aboyoun et al. 2010, Gentleman et al. 2004, Morgan et al. 2011, Pages et al. 2011). Gene locations were given in a PTT file from refseq (Pages et al. 2011). Locations of 16S rRNA and 23S rRNA are given by in a RNT file from refseq. Reads mapping to RNA-genes were discarded using *countOverlaps* (Pages et al. 2011). However, 5S rRNAs were kept, as they were not deleted by the Ribominus kit. *countOverlaps* was also applied to determine the number of reads mapping a gene in the same strand. Further, counts were normalized by length in kilobase and the number of mapped reads in millions. Loga-

rithmic fold-change values, including statistical significance parameters were determined by Richard Landstorfer (Microbial Ecology, TU München) using the *Bioconductor* packages *edgeR* (commondispersion: 0.1) (Gentleman et al. 2004).

Gene function information (from refseq or genbank) was supplemented by information from the Cluster of Orthologous Groups (COG) database (Tatusov et al. 2000).

3 Results

3.1 *E. faecalis* characterization

3.1.1 16S rDNA sequencing

To verify *E. faecalis* identity, the 16S rDNA of four strains, Symbioflor®, OG1RF, V583 and TMW 2.629, was sequenced using Primer 609rev (in collaboration with Angela Lindenstrauß, TUM).

3.1.2 Growth curve

For subsequent *in vivo* studies an OD_{600} - cfu standard curve was needed. Therefore growth curve measurements and cfu counting were performed.

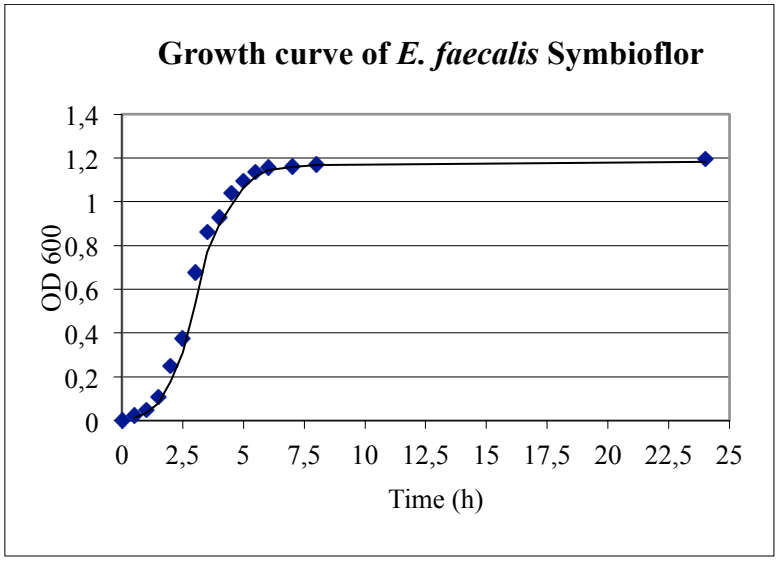


Fig. 14: Growth curve of *E. faecalis* Symbioflor® in BHI.

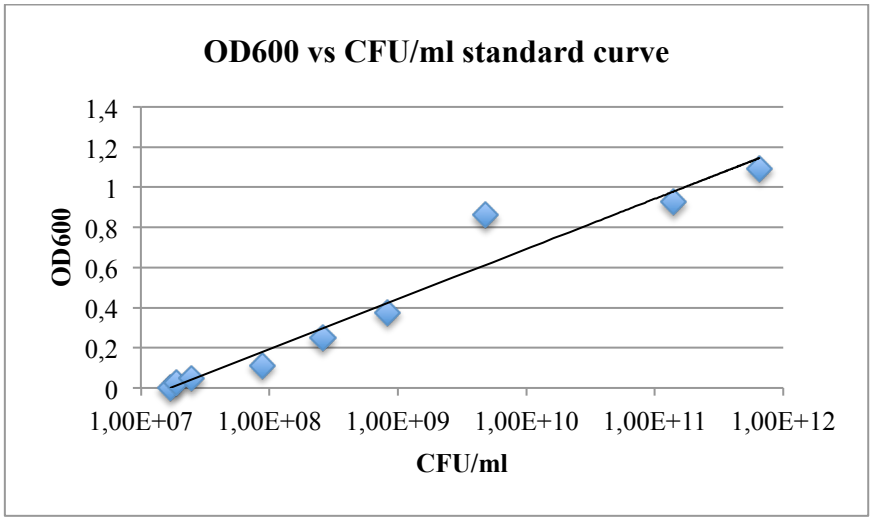


Fig. 15: OD_{600} vs. Cfu/ml standard curve for *E. faecalis* Symbioflor® cultivated in BHI at 37°C shaking.

3.1.3 Antibiotic sensitivity test

To test the intrinsic resistance against erm and kan, *E. faecalis* strains OG1RF, Symbioflor®, A/F2, and V583 were grown in LB at different concentration of the antibiotics and incubated shaking overnight at 37°C.

Tab. 20:Erythromycin resistance of different *E. faecalis* strains.

Erm / Strain	0 µg/ml	2.5 µg/ml	5 µg/ml	7.5 µg/ml	10 µg/ml
OG1RF	growth	no growth	no growth	no growth	no growth
Symbioflor®	growth	no growth	no growth	no growth	no growth
A/F_2	growth	no growth	no growth	no growth	no growth
V583	growth	growth	growth	growth	growth

Tab. 21: Kanamycin resistance of different *E. faecalis* strains in liquid culture.

Kan / Strain	0 µg/ml	50 µg/ml	100 µg/ml	400 µg/ml	800 µg/ml
OG1RF	growth	growth	growth	growth	no growth
Symbioflor®	growth	growth	growth	growth	no growth

A/F_2	growth	growth	growth	growth	growth
V583	growth	growth	growth	growth	growth

At standard culture conditions *E. faecalis* OG1RF, Symbioflor®, and A/F_2 are sensitive against erm, whereas *E. faecalis* V583 grows at up to 10 µg/ml erm (Tab. 20). V583 and A/F_2 are resistant against kan. For OG1RF and Symbioflor® growth was detected up to 600 µg/ml kan (Tab. 21).

Tab. 22: MIC test results.

Zones of inhibition (in cm) / Strain	Erm	Kan
OG1RF	2	-
Symbioflor®	2	-
A/F_2	2.1	-
V583	-	-
L. lactis subsp. cremoris TMW 2.644	2	1

The MIC test also showed the sensitivity of strain OG1RF, Symbioflor®, A/F_2, and *L. lactis* subsp. cremoris TMW 2.644 (positive control) and the resistance of V583 against Erm. Kan only inhibited growth of *L. lactis* subsp. *cremoris* (positive control), OG1RF, Symbioflor®, A/F_2 and V583 showed no inhibition.

3.1.4 Plasmid stability test

The recovery rate of pMG36eCBR from *E. faecalis* Symbioflor® and *E. faecalis* OG1RF was evaluated under non-selective culture conditions up to 60 h, 72 h respectively.

Tab. 23 Plasmid recovery from *E. faecalis* OG1RF.

Time	Cfu on LB agar	Cfu on LB erm agar	% (cfu erm/cfu)
0 h	$2{,}4 \times 10^6$	$2{,}4 \times 10^6$	100
12 h	1.2×10^7	9.9×10^6	82.5
24 h	1.6×10^7	5.4×10^6	33.8
36 h	2.3×10^8	5.2×10^7	22.6
48 h	2.6×10^8	3.2×10^7	12.3
60 h	2.4×10^8	8.8×10^6	3.6

Tab. 24 Plasmid recovery from *E. faecalis* Symbioflor®.

Time	Cfu on LB agar	Cfu on LB erm agar	% (cfu erm/cfu)
0 h	2.2×10^6	$2{,}2 \times 10^6$	100
12 h	9.4×10^6	7.0×10^6	75
24 h	3.9×10^7	7.4×10^6	19
36 h	1.6×10^7	1.0×10^6	6.2
48 h	3.1×10^7	2.1×10^6	6.7
60 h	7.1×10^6	3.0×10^5	4.2
72 h	8.4×10^8	1.4×10^6	1.6

The plasmid recovery rate of pMG36ecbr is not persistent in *E. faecalis* OG1RF and Symbioflor® under non-selective conditions. After 24 h, 34% of OG1RF and 19% of Symbioflor® are erm resistant and, thus, contain the plasmid. After 60 h at non-selective conditions less than 5% of the bacteria are erm resistant. Hence, more than 95% do not harbor the plasmid any more.

3.2 Reporter vector systems for *E. faecalis*

3.2.1 Reporter gene test in *E. faecalis*

To establish a plasmid reporter system for monitoring different *E. faecalis in vivo* in mouse models, various systems, established for closely related species were tested first. The plasmid pLS210 (with the *luxABCDE* operon), which was used to tag *Lactobacillus* spec. (Fang et al. 2008a), did not replicate in *E. faecalis* OG1RF or Symbioflor® (data not shown).

luxABCDE in pMG36e

As template for the *lux* operon the luminescence *Streptococcus pneumonia* (Xen10), which has a Tn4001 *lux* operon + kan resistance transposon insertion, was used. Ali Akin (CaliperLS, USA) performed the PCR amplification and ligation into Topo XL. The ligation was transformed into *E. coli* TOP10 and XL Blue ultra-competent cells. Only for the ultra-competent cells, kan resistant colonies grew after 24h. The functional integration of the *lux* operon was verified by standard luminescence detection. Nonanal was added to boost the luminescent signal.

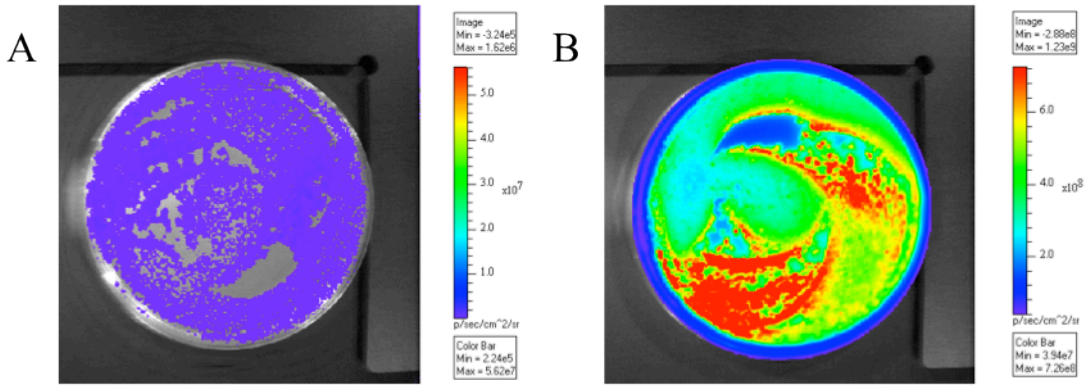


Fig. 16: Luminescence of *E. coli XL* Blue with TopoXL Xen10. A: Luminescence of transformants after overnight incubation. B: Luminescence after nonanal administration.

To insert the *lux* operon into pMG36e, an *XhoI* restriction site (CTC GAG) was inserted by site directed mutagenesis at position 2865 – 2870 in pMG36e (original sequence: GTT CAA). The insertion was verified by restriction analysis.

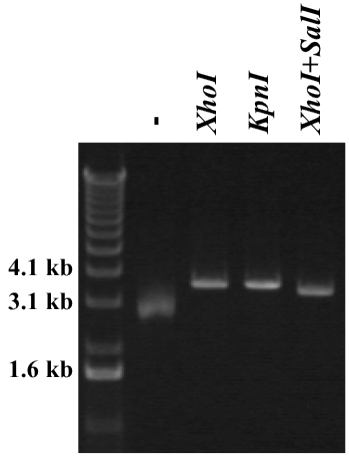


Fig. 17: Restriction analysis of pMG36e*XhoI*. Marker: 1 kb Plus DNA Ladder, pMG36e*XhoI* control (-), single restriction with *XhoI, KpnI*, and double digestion with *XhoI+SalI* (fragment size: 150 bp).

After restriction of pMG36e*XhoI* and Topo XL Xen10 using *KpnI* and *XhoI*, the respective fragments were ligated and transformed into *E. coli* XL Blue. After 24 h, more than 100 colonies grown at kan-selection showed luminescence and were re-streaked onto LB agar containing erm. After 24 h, eleven colonies, which showed the highest luminescence were chosen from the erm plates and inoculated into LB erm liquid culture.

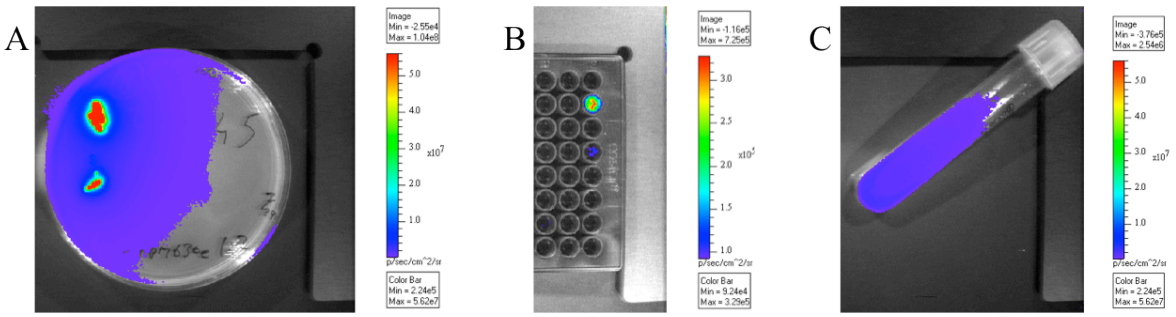


Fig. 18 Luminescence of *E. coli* XL10-gold with pMG36eXen10. A: 2 colonies of *E. coli* XL10-gold with pMG36eXen10 on LB erm after adding nonanal. B: 22 colonies of *E. coli* XL10-gold with pMG36eXen10 on LB erm. C: *E. coli* XL10-gold with pMG36eXen10 in LB broth (erm) used for plasmid isolation.

pMG36e*XhoI* Xen10 isolated from this transformants was used for transformation of *E. faecalis* OG1RF and Symbioflor®. More than 100 colonies grew for each strain, but none of them was luminescent, even after addition of nonanal no signal was detectable.

Click-Beetle-Red in pMG36e

Click beetle red luciferase gene (adapted to expression in *Mycobacteria spp.*) inserted in pUC57 (from Genscript) and pMG36e were digested with *SacI* and *HindIII,* following ligation and transformation. Positive clones were selected by growth on LB erm agar and detection of luminescence. Ten luminescent colonies were inoculated into LB erm broth to compare luminescence intensity. The clone with the highest luminescence intensity was chosen for stock culture preparation and plasmid isolation. This plasmid was electroporated into *E. faecalis* OG1RF and Symbioflor®. Positive clones were selected by growth on LB erm agar and detection of luminescence.

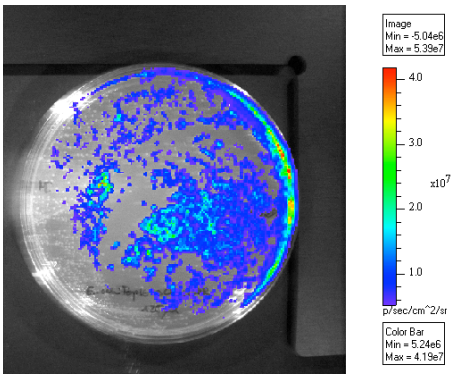


Fig. 19: *E. coli* Top10 pUC57cbr.

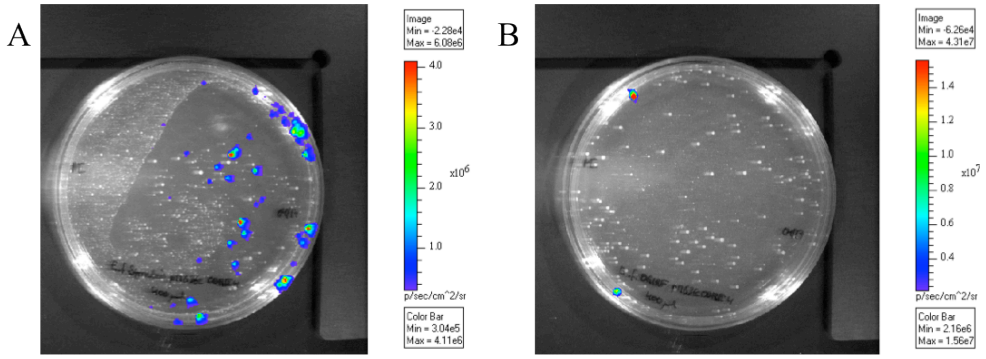


Fig. 20: Transformation of pMG36ecbr in *E. faecalis*. A: *E. faecalis* Symbioflor® after transformation with pMG36ecbr. B: *E faecalis* OG1RF after transformation with pMG36ecbr.

Positive clones (Symbioflor®: 22, OG1RF: 6) were re-streaked onto LB erm agar. Three *E. faecalis* Symbioflor® pMG36ecbr and one *E. faecalis* OG1RF pMG36ecbr transformant were inoculated into LB erm broth and screened for luminescence intensity. One transformant for each strain was used for stock culture preparation.

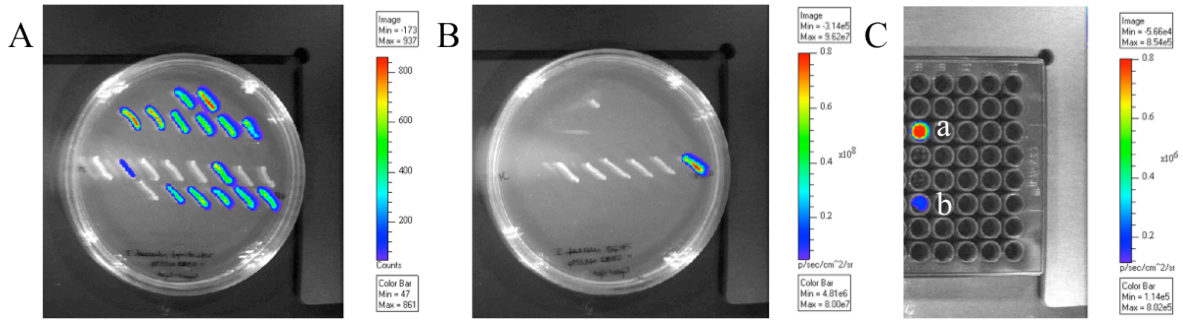


Fig. 21: Screening of *E. faecalis* Symbioflor® and OG1RF for *in vivo* application. A: 22 *E. faecalis* Symbioflor® pMG36ecbr transformants re-streaked onto LB erm agar. B: 6 *E. faecalis* OG1RF pMG36ecbr transformants re-streaked onto LB erm agar. C: Bacterial culture of *E. faecalis* OG1RF (a) and *E. faecalis* Symbioflor® (b) with pMG36ecbr used for stock culture preparation and further application in *in vivo* studies.

Red-fluorescence genes in pMG36e

The genes *tdtomato* and *mcherry* were synthesized with optimized codon usage for expression in Gram-positive bacteria and inserted into pUC57 by Genscript. In *E. coli* these plasmids yielded high fluorescence above autofluorescence. Even the bacterial colonies after transformation were fluorescent after 24h at 37°C. The insertion into pSTBlue-1 was verified by screening for kan resistance and fluorescence. The ligation of the genes flanked by *SacI HindIII* restriction sites was verified by screening for erm resistance, fluorescence, and

sequencing of pMG36e. The insertion into pMG36e resulted for both red fluorescence proteins in a lower fluorescence signal compared to the pUC57 construct. But the signal was still above autofluorescence. pMG36emcherry showed one point mutation in the *mcherry* sequence, at 399 bp, cytosine was replaced by thymine. This mutation did not result in an amino acid change of Asn_{132}. After electroporation into *E. faecalis* OG1RF and Symbioflor®, positive transformants of pMG36emcherry and pMG36etdtomato, respectively were screened for erm resistance and fluorescence. In contrast to *E. coli*, *E. faecalis* colonies (even after incubation for 48h) with pMG36emcherry did not turn visibly red. Colonies of *E. faecalis* with pMG36etdtomato became slightly visibly red after 48h. However, fluorescence was visible with the fluorescence microscope already after 16 hours of incubation. The fluorescence bleached out within seconds after excitation, using Vectashield® used for sample preparation did not improve this significantly. Fluorescent *E. faecalis* OG1RF and Symbioflor® expressing *mcherry* and *tdtomato* were used for stock culture preparation. Stability of the fluorescent proteins in laser scanning confocal microscopy was successfully tested with samples of *E. faecalis* OG1RF and Symbioflor® expressing *mcherry* and *tdtomato* (Fig. 23).

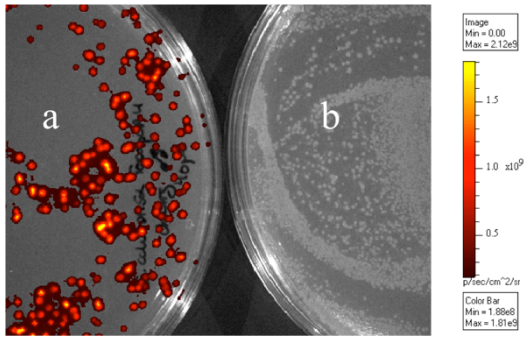


Fig. 22: *E. coli* DH6α pUC57mcherry (a). *E. coli* DH5α negative control (b).

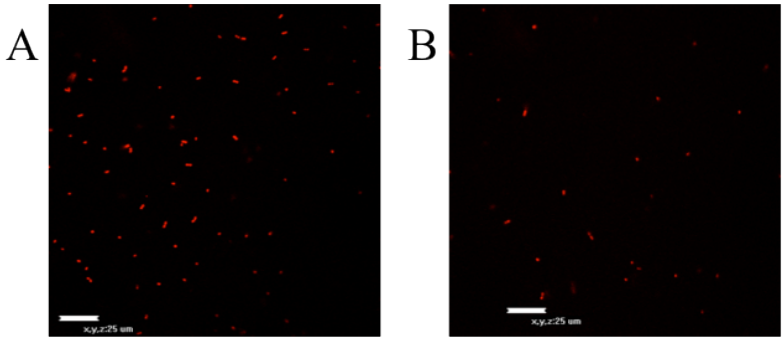


Fig. 23: A: *E. faecalis* Symbioflor® and B: *E. faecalis* OG1RF both with pMG36e tdtomato displayed by laser scanning confocal microscopy (pictures were taken by Zhen Peng M.Sc.).

3.2.2 Construction of a transposon reporter vector system

Preparation of toolkit components

To construct a transposon-based reporter system a toolkit consisting of a plasmid backbone (pMG36e-p32), a transposon element with a multiple cloning site plus transposase (Tn4001), luminescent (*cbr*), fluorescent reporter genes (*mcherry, tdtomato*), selection markers (*kan, cam*), and Fmn reductase genes (*frp, luxG*) was used. pMG36e-p32 was chosen as a backbone, because it is a shuttle vector and its origin of replication pVW01 makes it moderately temperature sensitive >42°C (Russell et al. 2001). The Tn4001 based transposon system was successfully tested in closely related species (Francis et al. 2001). The reporter genes *mcherry*, *tdtomato*, and *cbr* were chosen because they are functionally active in *E. faecalis*. *luxAB(CDE)* could not be amplified from different templates, yet (data not shown). However, the *fmn* reductase genes were added to the toolkit, even though they could not be tested in *E. faecalis*.

Plasmid backbone

The p32 promoter upstream of the pMG36e MCS was deleted to use this plasmid as a transposon shuttle, inserted in the MCS and to avoid plasmid expression interference with transposon genes. The sequencing of pMG36e-p32 revealed that the promoter p32 and the restriction site *SacI* (by T4 Polymerase exonuclease activity) were deleted as expected. The plasmid was used for further cloning.

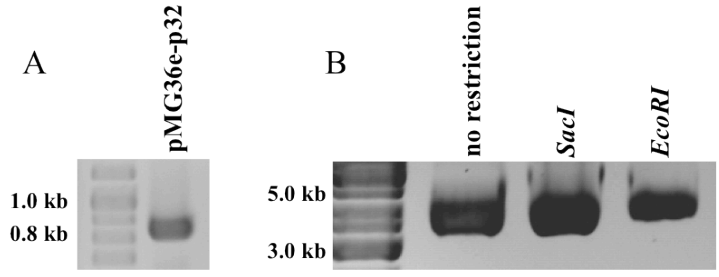


Fig. 24: A: PCR product of pMG36e-p32 with primer #6 and 35 of about 900 bp. B: pMG36e-p32, pMG36e-p32 after *SacI* restriction and pMG36e-p32 after *EcoRI* restriction on an agarose gel.

Transposon cassette and transposase

The composite-type Tn4001 transposon system was chosen because of its broad applicability in various Gram-positives. The transposase was amplified from pXen5 with the upstream promoter region, *PstI* restriction sites were attached to the fragment's ends.

Two transposon cassettes were designed based on the inverted repeats from pXen5 (Tn4001). One consisted of a MCS flanked by Tn4001 inverted repeats (Ec-p) and the other one additionally contained the p32 promoter in the MCS (Ecwp, Fig. 12, p. 41). The restriction sites to insert the cassettes into the pMG36e-p32 backbone and for the MCS were chosen to be unique and coordinated with the restriction sites on the plasmid backbone and the gene sequences.

Reporter genes and flavin oxidoreductase genes

The reporter genes were chosen upon functionality in *E. faecalis*. So far, none of them was integrated in the transposon reporter system.

Selection markers

The *E. faecalis* strains tested in the study, were sensitive to cam, therefore it was included in the toolkit. The kanamycin selection marker gene was included into the toolkit to allow for the adaption to a broader host range (apart from enterococci).

Insertion of transposon cassette in pMG36e-p32

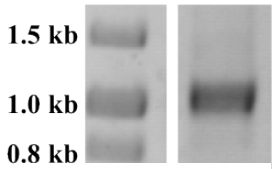


Fig. 25: 1 kb PCR product of Ecwpcam amplification from pUC57Ecwpcam.

The first step to build up the core of the transposon reporter system was the insertion of the transposon cassettes (Ecwp and Ec-p) into the plasmid backbone pMG36e-p32. Because attempts to insert the transposon cassette directly into pMG36e-p32 failed, a *cam* gene was first inserted into the empty cassette (with and without promoter) as a spacer and to simplify further cloning. Initial *ClaI* restriction of pMG36e-p32 (from *E. coli* TZ101a) failed. After isolation from *dam*$^{-}$ *E. coli* strain GM2163 and additional eluation cleanup, *ClaI* restriction and subsequent cloning was possible.

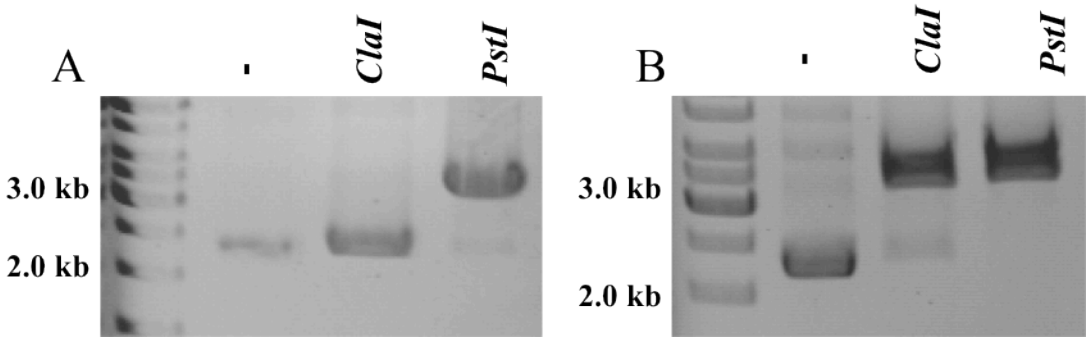
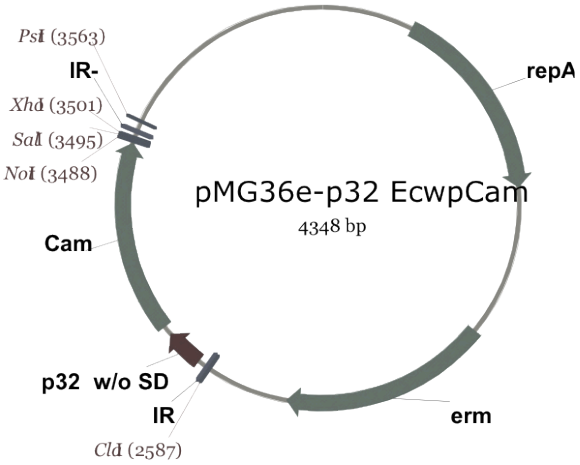


Fig. 26: A: pMG36e-p32 (from *E. coli* Tz101a) after no (-), *ClaI* or *PstI* restriction; B: pMG36e-p32 (from *E. coli* GM2163) after no (-), *ClaI* or *PstI* restriction.

The scheme of the intermediate of the transposon reporter system constructed within this work so far, is displayed in Fig. 28.



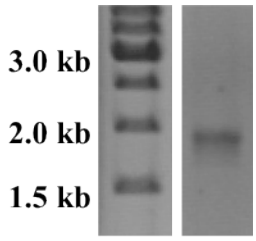


Fig. 27: 1.8 kb PCR product (primer#35+6) verifies integration of Ecwpcam into pMG36e-p32.

Fig. 28: pMG36e-p32 Ecwpcam construct. The transposon flanked by the inverted repeats (IR) is inserted into the pMG36e-p32 backbone, which contains the *repA* replication gen and *erm* resistance gene. The transposon contains the p32 promoter, the *cam* resistance gene, and the MCS.

3.3 *In vivo* experiments

The luminescence of the OG1RF culture used subsequently for the *in vivo* study shows a higher maximal luminescence level (>5.0e^7 photons/sec/cm^2/sr) than the Symbioflor® culture (<2.0e^7 photons/sec/cm^2/sr).

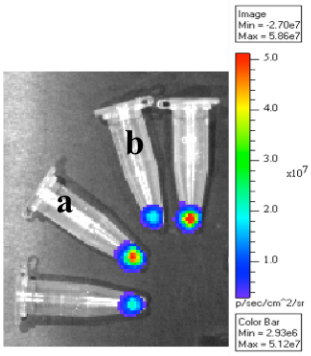


Fig. 29: Luminescence test of a) *E. faecalis* OG1RF and b) Symbioflor® with pMG36ecbr used for *in vivo* study.

Five groups of three mice each recived 1) *E. faecalis* OG1RF pMG36ecbr, 2) *E. faecalis* OG1RF pMG36ecbr + erm, 3) *E. faeaclis* Symbioflor® pMG36ecbr, 4) *E. faeaclis* Symbioflor® pMG36ecbr + erm 5) control group (no inoculation). The luminescenct signal of *E. faecalis* OG1RF pMG36ecbr and *E. faecalis* Symbioflor® pMG36ecbr was sufficient to be monitored *in vivo* in real time experiment. The negative control animals showed background signals of about 1.0e^4 (Fig. 30). Thus, the minimum of photons visualized in the pictures was set (more than) 1.5e^5.

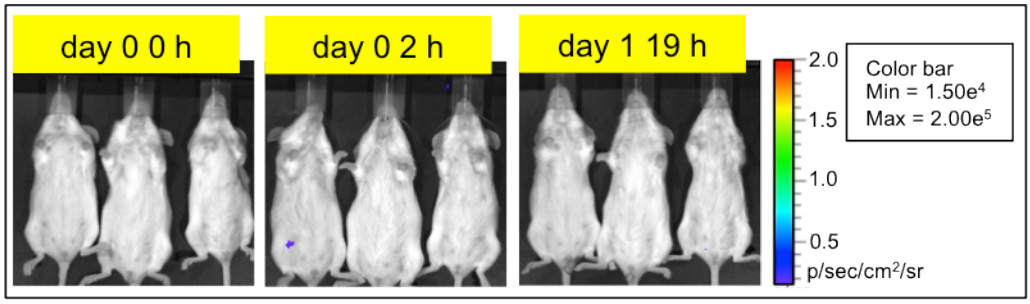


Fig. 30: Images of control group. The color scale was set for each image close to the luminescence detection threshold (minimum 1.50e^4, maximum 2.00e^5 color scale).

During whole body imaging of the mice the luminescence is basically traceable along the gastrointestinal tract. Among the individual animals of one group, especially the non-antibiotic treated, the luminescence signal intensity and location varies (Fig. 31, Fig. 33). In this respect, luminescence in the antibiotic treated groups is in general more consistent (Fig. 32, Fig. 34.). The time span of luminescence detection under these conditions ranged from 2 h after inoculation (Symbioflor® group) up to 24 h (Symbioflor® + erm group). In Fig. 31, the time course of OG1RF inoculated mice is shown. The bioluminescence of mice #1 and

#2 with about 1.0e5 maximum in a small area is lower than the signal intensity of mouse #3 especially 2 h after inoculation (ca. $5.1e^5$ / $5.4e^7$). No bioluminescence (above the minimum of 3.5 e^5 units) is seen after 4 h for mouse #2, while mouse #1 still shows a small spot and #3 still shows a signal of maximum $3.6e^6$ units. No signal is detectable after 19 h for mouse #1 and 3.

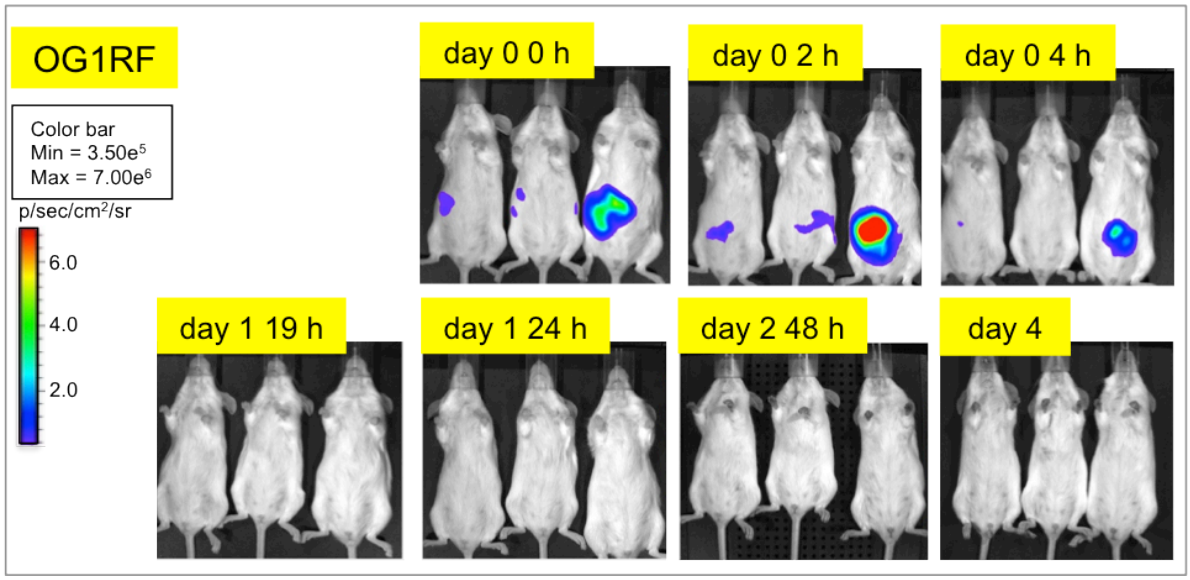


Fig. 31: Whole body imaging of mouse #1, #2, and #3 inoculated with *E. faecalis* OG1RF pMG36ecbr. Images were acquired at 0 h, 2 h, 4 h, 19 h, 24 h, 48 h, and 4 days after inoculation. The color scale represents minimum $3.5e^5$ and maximum $7.00e^6$ photon/sec/cm^2/sr.

In Fig. 32 the time course of OG1RF+erm inoculated in mice is shown. The bioluminescence among the individual animals is more uniform concerning the size of the area and signal intensity with maxima at about $9.7e^6$ (0 h) $3.3e^7$ (2 h), and $1.4e^7$ (4 h) units. Biolumi-

nescence is detected up to 19 h after inoculation (mouse #7 and #9).

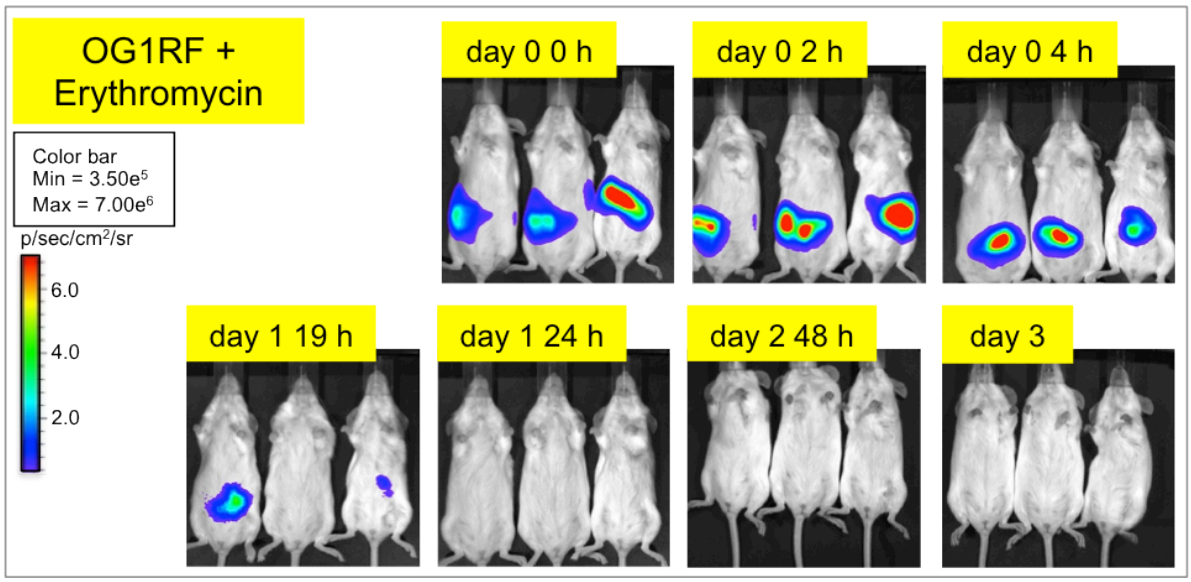


Fig. 32: Whole body imaging of mouse #7, #8, and #9 inoculated with *E. faecalis* OG1RF pMG36ecbr and erm administration Images were acquired at 0 h, 2 h, 4 h, 19 h, 24 h, 48 h, and 3 days after inoculation. The color scale represents the minimum 3.5e^5 and maximum 7.00e^6 photon intensity.

The time course of Symbioflor® inoculation is shown in Fig. 33. Mouse #4 shows maximal luminescence of 8.8e^6 units, while the signal intensity of #5 and 6 is below 2e^6 at 0 h. After 2 h the maximum luminescence signal detected from the image is 9.9e^5 units. After 4 h the highest luminescent signal is at 2.9e^5 below the display threshold and therefore not displayed. The time course of Symbioflor® + erm inoculation is displayed in Fig. 34. The maximal luminescence signals units detected are 5.5e^6 (0 h), 4.9e^6 (2 h), 9.5e^5 (4 h), 1.8e^5 (19 h), 5.2e^5 (24 h), and 3.5e^4 (48 h). The size of the luminescent area and the signal intensity are rather consistent among the animals among the individual animals of one group at a time.

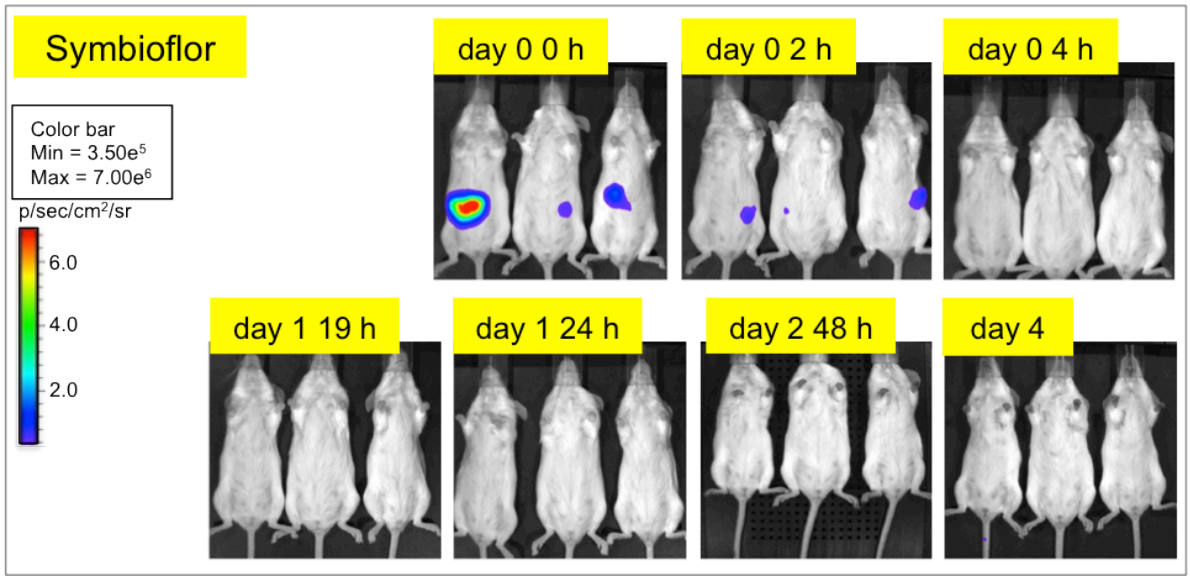


Fig. 33: Whole body imaging of mouse #4, #5, and #6 inoculated with *E. faecalis* Symbioflor® pMG36ecbr. Images were acquired at 0 h, 2 h, 4 h, 19 h, 24 h, 48 h, and 4 days after inoculation. The color scale represents the minimum 3.5e^5 and maximum 7.00e^6 photon intensity.

Mouse #11 shows luminescence down the right body side at 0 h. A luminescent spot is detected below the front paw of mouse #12 at 2 h and 4 h. Mouse #10 and 11 show a luminescent signal, potentially descending from the rectum. Feces of this group at day 4 and from *E. faecalis* OG1RF + erm were collected and imaged. Luminescence with a maximal signal of 1.6e^4 was detected from them.

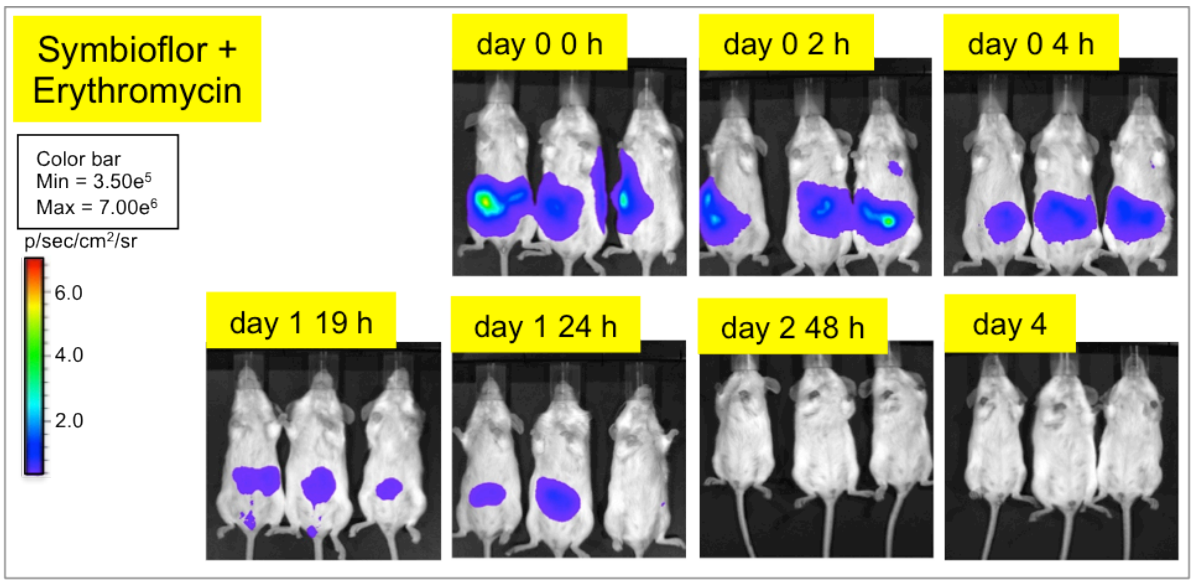


Fig. 34: Whole body imaging of mouse #10, #11, and #12 inoculated with *E. faecalis* Symbioflor® pMG36ecbr erm administration. Images were acquired at 0 h, 2 h, 4 h, 19 h, 24 h, 48 h, and 3 days after inoculation. The color scale represents minimum 3.5e^5 and maximum 7.00e^6 photon intensity.

In Fig. 35 the mice are grouped according to the time of imaging. The minimal and maximal luminescence display threshold was lowered and adjusted to the threshold of the negative control groups (Fig. 30), this better allows to display luminescence right above the background level.

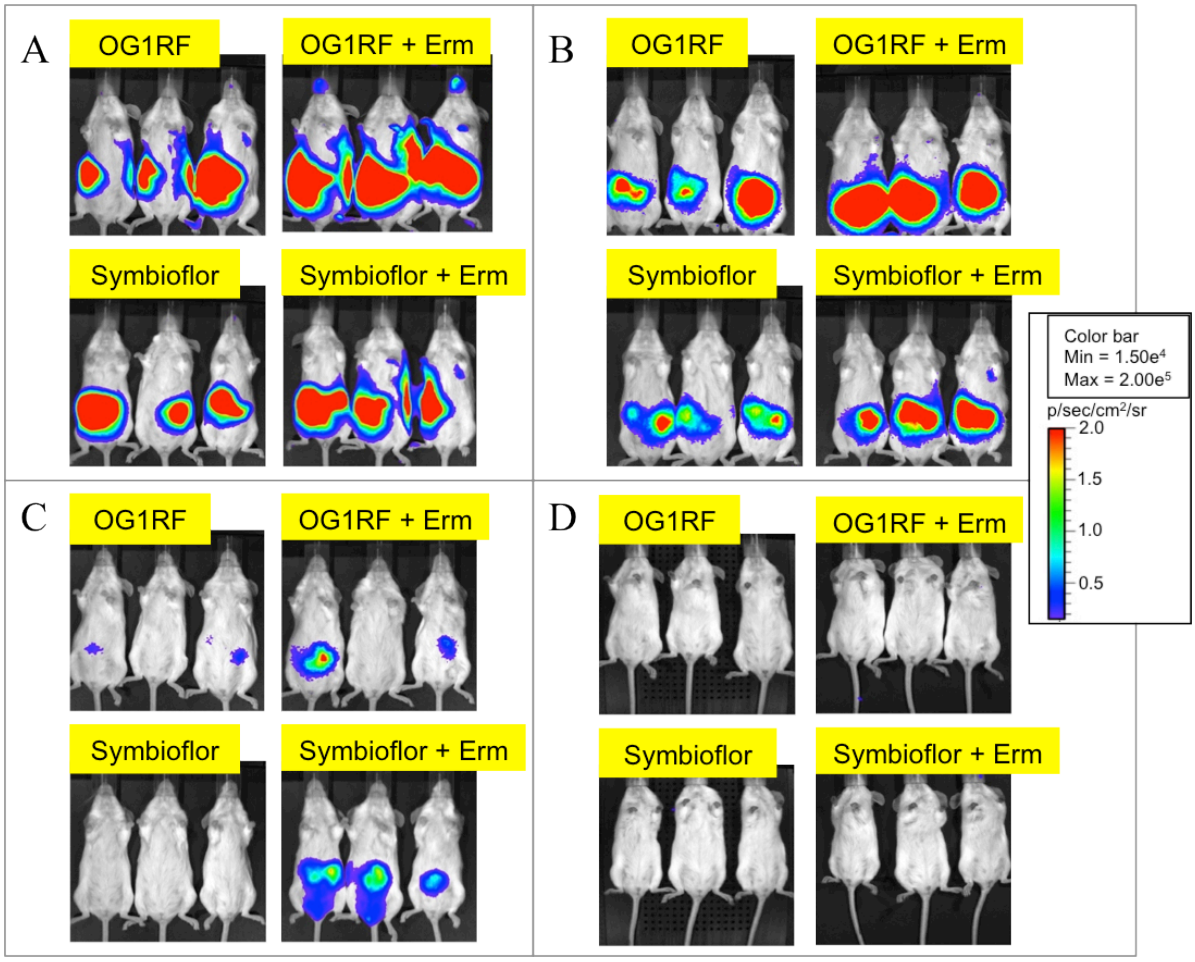


Fig. 35: Images taken at the same time-point were grouped together. SW mice administered with *E. faecalis* OG1RF or Symbioflor® and treated with erm. Images were acquired at A) 0 h, B) 4 h C) 19 h D) 48 h after administration. The color scale represents the same luminescent intensity for all images (minimum 1.50e^4, maximum 2.00e^5). The scale was set according to the minimal threshold value of the control group (Fig. 30).

Generally the erm groups show a higher luminescence compared to the respective non-treated group at a time. Mice inoculated with *E. faecalis* OG1RF showed higher maximal luminescence peaks, compared to Symbioflor®.

3.4 *E. faecalis* versus EHEC

3.4.1 *C. elegans* killing assay

C. elegans L4 larva were fed with *E. coli* OP50, *E. faecalis* Symbioflor®, *E. faecalis* Symbioflor® + EHEC, EHEC + *E faecalis* Symbioflor® lysate or EHEC. Growth of *E. faecalis* Symbioflor® was strongly attenuated on NGM agar, whereas a bacterial lawn was visible after EHEC overnight incubation at 20°C.

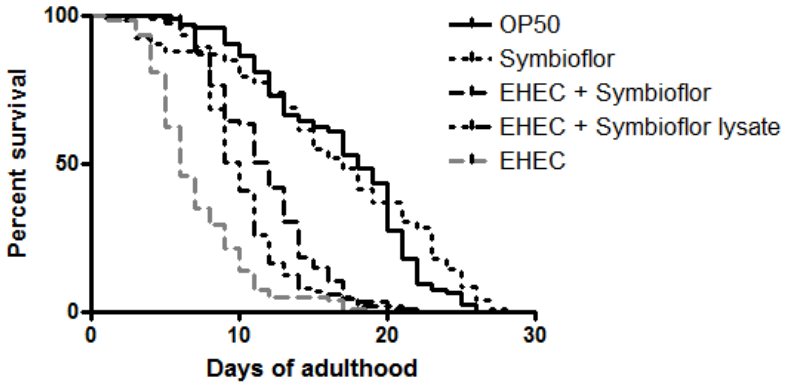


Fig. 36: Kaplan-Meier survival plot of *C. elegans* fed on different bacterial lawns on NGM agar. Except OP50 and Symbioflor® all curves are significantly different ($p < 0.0001$).

Tab. 25: TD50 and standard error calculated with *R*.

Bacterial lawn on NGM agar	Number of nematodes	TD_{50}	Standard deviation
E: coli OP50	95	16.2	0.3
E. faecalis Symbioflor®	106	15.9	0.3
E. faecalis Symbioflor® + EHEC	115	10.8	0.1
EHEC + *E. faecalis* Symbioflor® lysate	105	9.3	0.06
EHEC	109	5.3	0.07

Tab. 25 and Fig. 36 show that EHEC feeding leads to premature death of *C. elegans*. TD50 of feeding EHEC was about 5.8 days. Incubation with EHEC and *E. faecalis* Symbioflor®, or its lysate, delayed killing to 9.3 or 10.8 days, respectively. TD50 values of *E. faecalis* Symbioflor® (15.9 days) was similar to the standard worm food *E. coli* OP50 (16.2 days) in this assay.

3.4.2 EHEClux transposon database

Screening of EHEClux mutants

Of 9408 mutants inoculated onto LB agar and LB Symbioflor® agar (pH 6), 228 EHEClux mutants showed different luminescence intensities by visual inspection. Of those, about 90% showed higher luminescence on control plates.

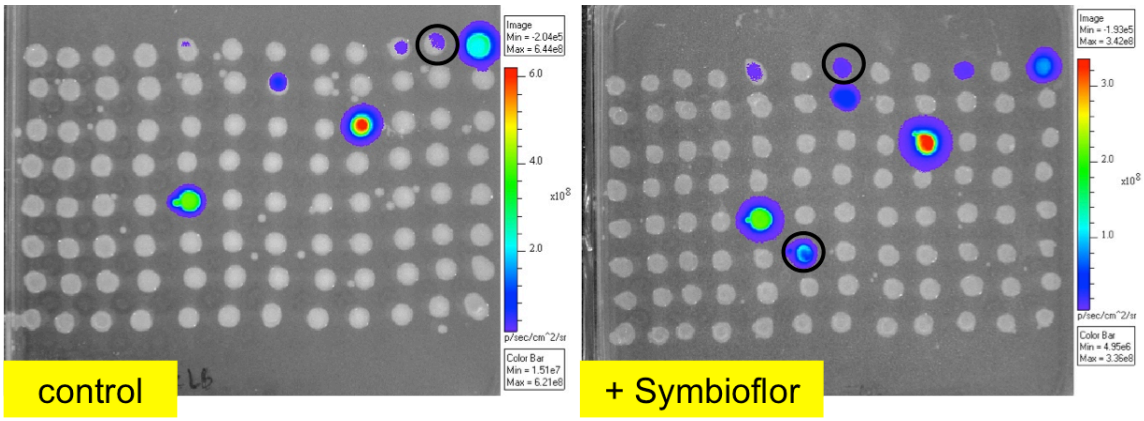


Fig. 37: 96 EHEClux clones from one 96 well plate were stamped from the EHEClux transposon database stock onto LB agar (control) and onto LB agar + Symbioflor®. Clones marked with a black circle were further analyzed.

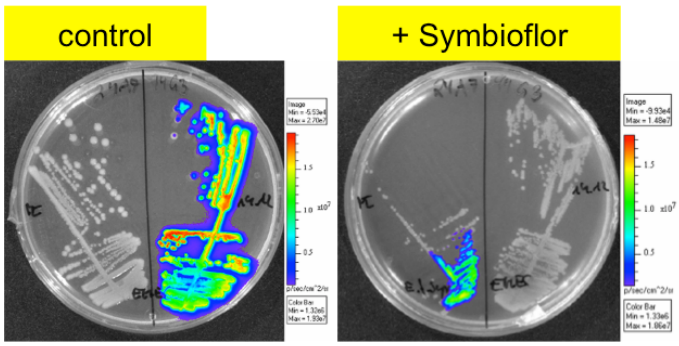


Fig. 38: Two EHEClux clones (left and right) were streaked out onto one LB agar and one LB Symbioflor® agar. Both clones showed different luminescence intensity comparing the two different plates.

After a second analysis, of re-streaked mutants, luminescence of 84 EHEClux clones differed significantly again and these strains were selected for further analysis. 69 clones showed higher luminescence on LB agar, 15 showed higher intensity on LB Symbioflor® agar. These 84 EHEClux clones were used for stock culture preparation, subsequent quantitative luminescence and *lux* transposon insertion analysis. Interestingly, EHEClux colonies grown on LB were bigger as compared to the respective colonies grown on LB Symbioflor® agar (see Fig. 38), which is possibly due to nutrient limitations.

Quantitative analysis of luminescence differences

To further assess differences in luminescence of EHEClux in the presence or absence of *E. faecalis* Symbioflor®, luminescence and deduced promoter activity were quantitatively compared after incubation at 37°C for 18 h using the Living Image software. Both luminescence mean values and corresponding standard deviation of each EHEClux clone culture are displayed in Fig. 53 (appendix), the fold change of each EHEClux clone is displayed in Fig. 39. Comparison of the relative luminescence for some EHEClux mutants of both independent measurements revealed contradictory results. Clones, showing higher luminescence intensity on LB Symbioflor® agar during selection screening, emitted less light during quantitative measurement grown on LB Symbioflor® (e.g., EHEClux #23, #26, #31, and #38). EHEClux #81 did not grow on LB Symbioflor® agar, but on LB agar.

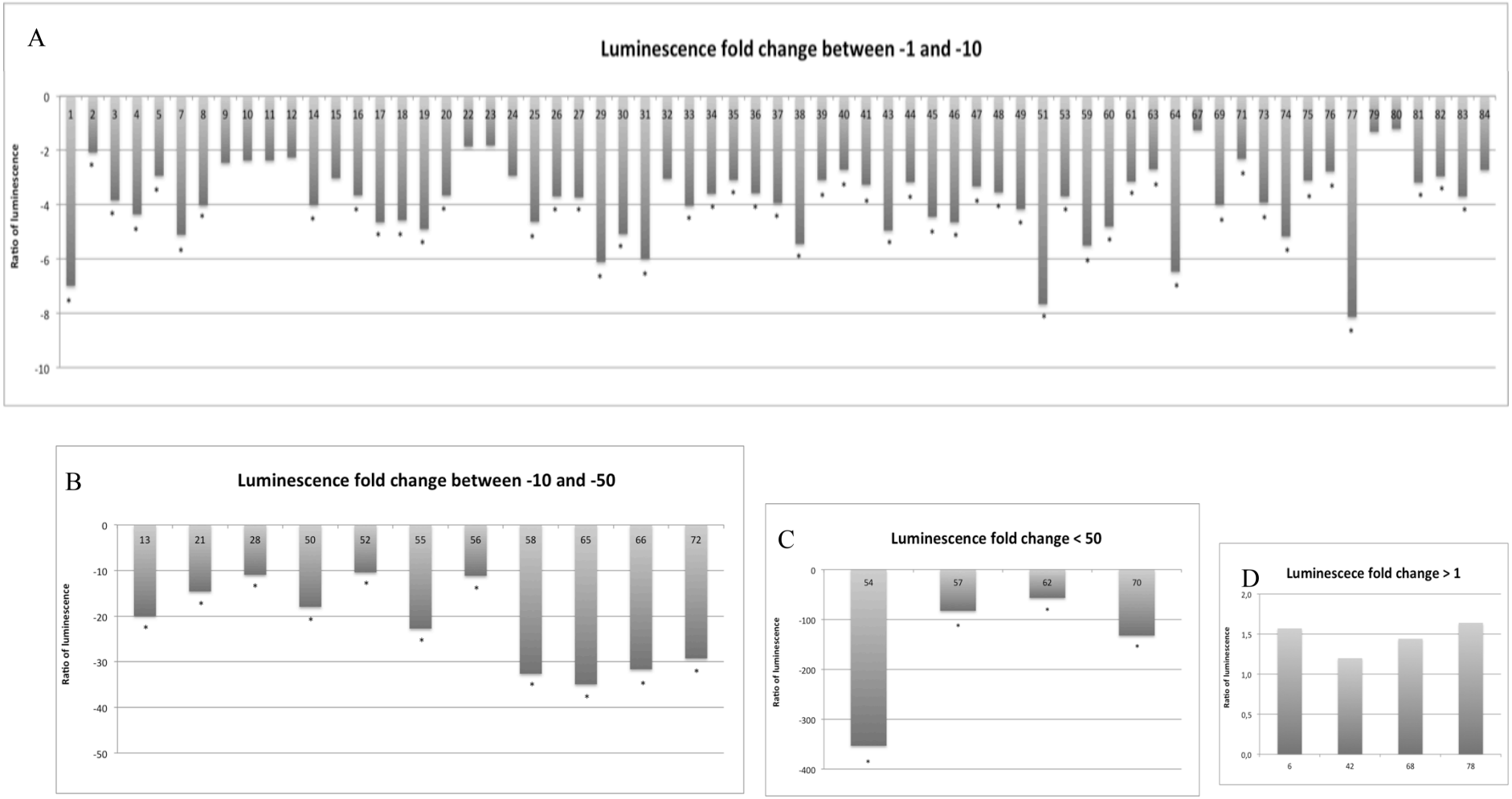


Fig. 39: **Luminescence fold change of 84 EHEClux clones from LB Symbioflor versus LB reference agar.** EHEClux clones are numbered and clustered according to the fold change level. Negative values indicate downregulation on LB Symbioflor agar, positive values indicate upregulation on Symbioflor agar. * indicates no overlapping standard deviation for the two respective initial quantitative luminescence measurements, see appendix Fig. 53

EHEC transposon insertion site sequencing

To identify the locus of transposon integration in the EHEC genome an adapted TAIL PCR program with nested primer strategy was applied. All 84 EHEClux mutants (numbered #1-#84) were analyzed using the TAIL and nested PCR method. In total, the upstream EHEC sequence from the *lux* cassette of 47 EHEClux clones was obtained, 40 sequences being non-redundant in the EHEC genome (Tab. 26). Results of EHEClux mutant #41 are displayed as an example (Fig. 40, Fig. 41, and Fig. 42). Generally a big primer-dimer band (below 100 bp) and other bands (below 1 kb) could be detected as illustrated in Fig. 40A for EHEClux #41 and #81. In Fig. 40B a representative nested PCR pattern is shown. Still, unspecific bands, which are recognized in the single primer control PCRs, are present. Unique bands detected in the double-primer PCR were extracted for subsequent sequencing analysis.

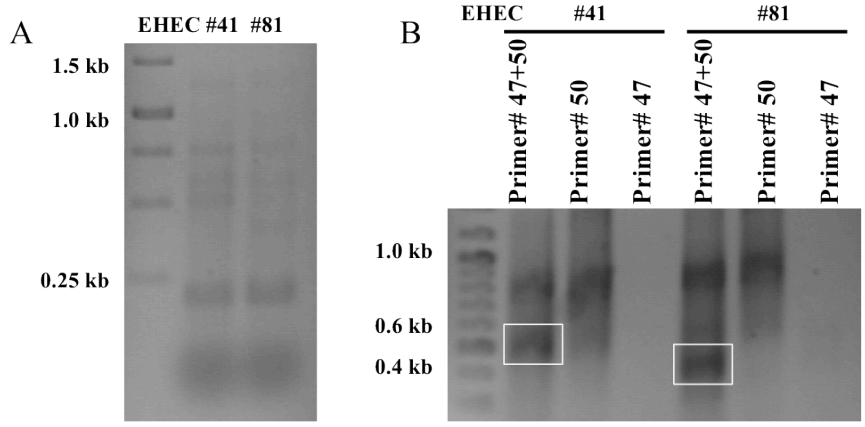


Fig. 40: TAIL (A) and nested (B) PCR agarose gel pattern from EHEClux #41 and #81 analysis. In B nested PCR with different primer combinations is shown for EHEClux #41 and #81. Unique bands showing up for EHEClux#41 (500 bp) and #81 (400 bp) from the nested double-primer PCR, are marked with a white rectangle and were subsequently extracted.

Sequences were determined and those sequences, whose 3' end aligned to the 5' end of the *lux* cassette were further blasted (without the respective 3' *lux* span). All sequencing results are listed in the appendix p. 121 f.

GGGGGGGCACCATACACACTACCCGATGTCTGTTCTTTGCAGCTTCCAATTGGATGGG
CGCTGATAATGGCAGGATACTTTTTTTCACTATTAAAATCGTCAGGGAAATAGATATCA
GCAGCTATATCCCAGTAAGTGTGTTTTATCGAAACAGTTTTCATTTCAAGCTCCGGGG
ATTTGCCTAAACAGATTTAAATATCATTTCTATTATCCGCAACTGGCATTTGTGTTCATA
TCGAGAACGGCGTAATAGTATGACTTGTATTGGTTATCATCAATGGTCTAATATCTATC
ACAGTGTTAAGTTAAGGTTTACAATGATGAAAATAGAGCCTTCAATTTTGCCTTCTCT
TGCCTGGTTTGCGCCTGACTCTTATACACAAGTGCGGCCGCGCG

Fig. 41: The sequence of EHEClux #41. The underlined bases align to the 5' end of the *lux* cassette. The other part of the sequence was aligned using nucleotide blast database search tool (NCBI).

Fig. 42: Nucleotide blast result of sequence inquiry EHEClux #41. The sequence shows 99% identity to a genome region in EHEC EDL933, from 350714 bp to 351078 bp.

In Tab. 26 all EHEClux are listed, whose precise unique insertion site could be identified. The gene *escV* was identified to be the target of transposon insertion redundantly in EHEClux clone #52, #54, #62, #63, and #70.

Tab. 26: Identification of insertion site of EHEClux mutants (unique insertion site). The feature and the locus tag, which are located in the reading direction of the insertion site, are indicated. The results from quantitative luminescence measurement are displayed as fold change. Negative values indicate luminescence reduction by *E. faecalis* Symbioflor®, positive values indicate increase of luminescence by *E. faecalis* Symbioflor®. [1] indicates no overlapping luminescence variation comparing the respective LB and LB Symbioflor® EHEClux mutant luminescence mean value (see Fig. 53, appendix).

#	Feature in the insertion site of the EHEC genome	Locus tag	Start bp	Fold change
1	sucrose permease	Z3623	3272583	-8[1]
2	putative malate dehydrogenase	Z0672	637619	-2[1]

3*	evolved beta-D-galactosidase, alpha subunit; cryptic gene*	Z4429	4030863	-4[1]
4	xylose binding protein transport system	Z4991	4545179	-4[1]
5	orf, hypothetical protein	Z4897	4444528	-3[1]
6	orf, hypothetical protein	Z2880	2596785	2
8	putative sensor for regulator EvgA	Z3632	3283541	-4[1]
10	putative outer membrane receptor for iron transporter	Z2268	2050449	-3
11	D-alanyl-D-alanine carboxypeptidase, fraction B	Z4544	4139884	-2
13	ATP-sulfurylase (ATP:sulphate adenylyltransferase)	Z4059	3662981	-20[1]
18	hypothetical protein	Z5102	4659139	-5[1]
19	processing of HyaB protein	Z1392	1299756	-5
20	putative helicase	Z5901	5419795	-4[1]
23	unknown protein encoded by bacteriophage BP-933W	Z1487	1374511	-2
26	putative tail fiber protein of bacteriophage BP-933W	Z1483	1369252	-4[1]
28	hypothetical protein	Z2553	2270193	-11[1]
31	orf, hypothetical protein	Z2320	2097213	-7[1]
38	methyl-directed mismatch repair	Z4043	3650740	-6[1]
39	ATP-binding component of 3rd arginine transport system	Z1094	1033914	-3[1]
40	plasmid pO157 espP	L7020	14229	-3[1]
41	putative LysR-like transcriptional regulator	Z0371	351078	-4[1]
43	326 bp at 5' side: glutathione oxidoreductase	Z4900	4449649	-5[1]
46	putative transcriptional repressor	Z2510	2234886	-5[1]
49	orf, hypothetical protein	Z5513	5025490	-4
51	intimin adherence protein	Z5110	4667862	-8[1]
52	escV	Z5120	4676303	-11[1]
54	escV	Z5120	4676658	-398[1]
55	hypothetical protein	Z5143	4692066	-24[1]
56	escC	Z5126	4681315	-12[1]
58	cesD	Z5127	4682174	-36[1]
61	thymidin phosphorylase	Z5984	5506709	-3[1]
62	escV	Z5120	4676083	-62[1]
63	escV	Z5120	4676649	-3[1]
66	orf, hypothetical protein	Z2754	2482759	-34[1]
68	hypothetical protein	Z0702	666022	1
69	putative translocated intimin receptor protein	Z5112	4669325	-4[1]
70	escV	Z5120	4676648	-132[1]
73	hypothetical protein	Z2213	1990092	-4[1]
76	bacteriophage N4 receptor, outer membrane protein	Z0699	662473	-3[1]
81**	putative reductase**	Z3063	2740077	-3[1]

EHEClux clones, which showed a relative higher luminescence on LB Symbioflor® agar when preparing the stock (p. 68) are shaded gray, the others showed higher signal intensity on LB agar. * Growth was attenuated on LB during stock preparation; ** no growth detected on LB Symbioflor® agar (during stock preparation).

Gene insertion loci identified are eleven hypothetical proteins including both identified genes up-regulated on LB Symbioflor®. The other ones are down-regulated. They includes six different genes involved in pathogenicity, four genes involved in carbohydrate transport and metabolism, three genes associated with phages, two genes involved in inorganic ion transport / metabolism, cell wall/membrane biogenesis, or transcription and one gene related to signal transduction mechanisms, repair, energy production/conversion, amino acid transport / metabolism or nucleotide transport. One transposon was identified in an intergenic region.

In Tab. 27 EHEClux clones are listed, whose sequence aligns to multiple locations on the genome. The insertion site of the remaining EHEClux clones could not be identified.

Tab. 27: Identification of insertion site of EHEClux mutants (redundant insertion site within the genome). The feature and the locus tag, which are located in the reading direction of the insertion site, are indicated. Negative values indicate luminescence reduction by *E. faecalis* Symbioflor®, positive values indicate increase of luminescence by *E. faecalis* Symbioflor®. [1] indicates no overlapping luminescence variation comparing the respective LB and LB Symbioflor® EHEClux mutant luminescence mean values (see Fig. 53, appendix).

#	Feature in the insertion site of the EHEC genome	Locus tag	Start bp	Fold change
9	142 bp at 3' side: hypothetical protein	Z1181	1109937	-3
9	142 bp at 3' side: hypothetical protein	Z1620	1505544	-3
14	putative exonuclease VIII, ds DNA exonuclease, 5'à3'	Z0173	185322	-4[1]
14	putative exodeoxyreductase encoded by cryptic prophage	Z6080	2326809	-4[1]
22	hypothetical protein	Z1200	1122705	-2
22	hypothetical protein	Z1640	1518312	-2
45	putative secreted protein	Z3026	2711445	-5[1]
45	putative secreted protein	Z3023	2711445	-5[1]
57	putative single-stranded DNA binding protein	Z1440	1339019	-93[1]
57	putative single-stranded DNA binding protein of prophage CP-933V	Z3363	3007464	-93[1]
71	hypothetical protein	Z0509	494335	-3[1]
71	trehalase, periplasmic	Z1968	1787012	-3[1]
78	hypothetical protein	Z1131	1071069	2
78	hypothetical protein	Z1570	1466676	2
78	unknown protein encoded by ISEc8 in prophage CP-933X	Z1929	1750021	2
78	putative IS encoded protein within CP-933O	Z2080	1876081	2
78	IS encoded protein encoded within CP-933O	Z2130	1907935	2
78	hypothetical protein	Z6016	2278996	2
78	hypothetical protein	Z3156	2813296	2
78	hypothetical protein	Z4337	3939051	2
78	prophage associated protein	Z5098	4656399	2

3.4.3 EHEC transcriptome sequencing

The transcriptome of EHEC cultures grown on LB agar was compared to EHEC cultures grown on LB Symbioflor® agar. Therefore, RNA was isolated from 2x ca. 5 µl cell mass taken from the colony on the agar surface for each condition (I and II: LB, III and IV: LB Symbioflor®). The RNA quality was assessed using Nanodrop analysis. The 260:280 value indicates good RNA quality in the range between 1.80 and 2.00.

Tab. 28: Nanodrop analysis of RNA after isolation.

Sample	Concentration (µg/ml)	260:280
I	711.2	1.99
II	808.0	1.92
III	796.0	1.99
IV	1111.8	1.98

RNA was dissolved in 20 µl RNAse-free water.

Sample I and III were used for ribo-depletion because they revealed optimal RNA quality. To start with 10 µg RNA 14.1 µl of sample I and 12.6 µl of sample III were used. After precipitation the pellet was dissolved in 25 µl RNAse free water. The ribo-depletion was confirmed by agarose gel electrophoresis analysis.

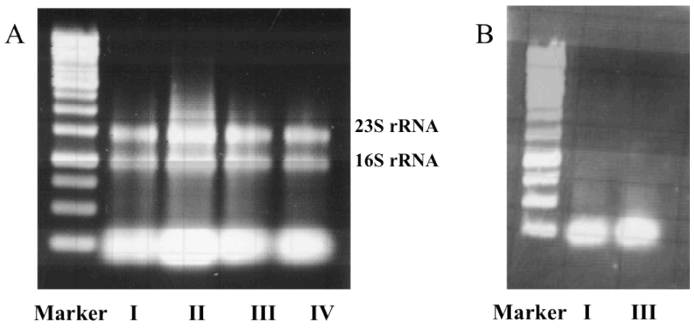


Fig. 43: RNA purification samples before (A) and after (B) 23S and 16S rRNA removal. 5S rRNA band is still clearly visible.

After DNAse treatment and precipitation, the RNA samples were eluted into 14 µl RNAse free water and concentration as well as quality was analyzed using Nanodrop.

Tab. 29: Nanodrop analysis of RNA after DNAse treatment.

Sample	Concentration (µg/ml)	260:280
I	222	2.00
III	223.7	1.97

The subsequent preparation of RNA and sequencing was performed by Cegat GmbH (Tübingen, Germany). The RNA quality and the 16S and 23S RNA depletion was assessed by Bioanalyzer analysis of RNA samples before RNA depletion (sample EI and EIII) and after DNAse (sample I and III; CEGAT protocol and results p. 126 appendix). After ribo-depletion the 16S and 23S RNA peak could not be detected.

The cs fasta and qual file, which were received from CEGAT for each sample were mapped onto the EHEC EDL933 genome and the pO157 plasmid. They were processed to bam files and the mapping was viewed in Artemis. This revealed a high number of multiple reads compared to other EHEC transcriptome data (Richard Landstorfer, Department of Microbial Ecology, TUM). Therefore an additional bioinformatic tool (Mark Duplicate Reads by Galaxyproject) was used to eliminate multiple identical reads. The Bam files were then further processed by Svenja Simon (Department of Computer and Information Science, University of Konstanz) rpkm values, which were generated by her, were evaluated.

Tab. 30: Number of processed reads.

	Reference LB condition		Sample LB Symbioflor® condition	
	EDL933 chromosome	pO157	EDL933 chromosome	pO157
Sequence data reads	59 000 000		48 000 000	
Mapped reads	7 198 911	57 924	5 764 549	49 351

An rpkm threshold of 5 was set, values below are not reliable (sequencing errors, background noise). Genes with an rpkm value above 5 were considered as transcribed. This value was chosen according to the rpkm value of minimally expressed essential genes (*lacI, rpoS*). In total 201 genes were significantly down-regulated in the presence of *E. faecalis* Symbioflor®, whereas 291 were significantly up-regulated under this condition. 2789 annotated genes were expressed, but not differentially regulated under both tested conditions. The rpkm data (LB condition: $rpkm_{ref}$, LB Symbioflor® condition: $rpkm_{Sym}$) and the log fold change (LogFC) of selected genes are listed in tables below. In Tab 32 and Tab 33 genes are illustrated, which are down-regulated on Symbioflor® agar. In Tab 34 and Tab 35

genes are listed, which are up-regulated on LB Symbioflor®. In both cases, genes with a count / rpkm value of 0 in one condition were listed in a separated table without log fold change. In Tab 36 and Tab 37 the transcriptome log fold change of the genes, which were identified in the EHEClux clones, is displayed. Tab 38 the expression pattern of several EHEC EDL933 housekeeping genes is displayed. Tab 39 Tab 40 genes involved in regulation, which are significantly up or down-regulated are grouped. Tab 41 – Tab 49 rpkm values and log fold change of genes involved in pathogenicity mechanisms of EHEC are listed. Genes in each table are ordered by increasing locus tag. Significant log fold change ($p < 0.05$) is displayed, insignificant log fold change is shaded and genes considered not expressed at all are marked by "–" log fold change. Only rpkm values are shown for silenced genes, no log fold change. In Tab 32 – Tab 35 COG functional categories are indicated.

Tab. 31: Description of COG functional categories.

Code	Description
K	Transcription
L	Replication, recombination and repair
V	Defense mechanism
M	Cell wall/membrane biogenesis
N	Cell motility
U	Intracellular trafficking, secretion and vesicular transport
O	Posttranslational modification, protein turnover, chaperons
C	Energy metabolism and conversion
G	Carbohydrate transport and metabolism
E	Amino acid transport and metabolism
F	Nucleotide transport and metabolism
H	Coenzyme transport and metabolism
P	Inorganic ion transport and metabolism
R / S	Poorly characterized

Tab. 32: Genes > 3 fold significantly (p value < 0.05) down-regulated by LB Symbioflor® (EDL933 chromosome and pO157 plasmid).

Gene	Locus tag	Product (COG functional category)	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
yaaF	Z0035	ribonucleoside hydrolase RihC (F)	11.7	0.6	-4.3
thiQ	Z0075	thiamine transporter ATP-binding subunit (H)	5.2	0.5	-3.3
-	Z0395	hypothetical protein (COG: site-specific recombinase XerD, L)	19.2	0.9	-4.3

-	Z0656	hypothetical protein	30.0	3.0	-3.3
dmsB	Z1241	anaerobic dimethyl sulfoxide reductase subunit B (R)	9.2	0.8	-3.4
-	Z1480	hypothetical protein	22.1	1.8	-3.6
-	Z1880	hypothetical protein	27.5	2.5	-3.4
-	Z2368	hypothetical protein	22.7	1.2	-4.1
-	Z2979	stability/partitioning protein encoded within prophage CP-933T	7.8	0.5	-3.8
fliD	Z3014	flagellar capping protein (N)	11.1	0.6	-4.1
-	Z3934	hypothetical protein	9.7	0.9	-3.4
-	Z4071	hypothetical protein	21.8	2.3	-3.2
-	Z4271	ATP-binding protein of ABC transport system (P)	5.1	0.5	-3.3
(efa1/lifA)	Z4332	cytotoxin	5.7	0.7	-3.0
espB	Z5105	hypothetical protein (LEE encoded secreted protein)	11.7	1.1	-3.3
gldA	Z5500	glycerol dehydrogenase (C)	7.2	0.3	-4.4
treC	Z5849	trehalose-6-phosphate hydrolase (G)	10.7	0.5	-4.3
treB	Z5850	PTS system trehalose(maltose)-specific transporter subunits IIBC (G)	34.9	2.3	-3.8
-	Z5943	conserved hypothetical protein	5.0	0.4	-3.6
-	Z6071	hypothetical protein	6.5	0.4	-3.9
-	L7070	hypothetical protein	5 222.3	414.4	-3.4

Tab. 33: Genes (rpkm LB Symbioflor® ≥ 5, rpkm LB Symbioflor® =0) significantly (p value < 0.05) down-regulated and silenced by LB Symbioflor® (EDL933 chromosome and pO157 plasmid).

Gene	**Locus tag**	**Product (COG functional category)**	**$rpkm_{ref}$**	**$rpkm_{Sym}$**
-	Z0110	hypothetical protein	11.3	0.0
pinH	Z0318	DNA invertase from prophage CP-933H (L)	6.8	0.0
-	Z0899	hypothetical protein	46.3	0.0
-	Z0950	hypothetical protein (COG: hypothetical ABC-type Fe transport system, P)	6.5	0.0
-	Z1334	hypothetical protein (COG: Phage DNA pack protein, L)	13.7	0.0
-	Z1966	hypothetical protein (COG: ABC-type Fe3+-hydroxamate transport system, periplasmic component, L)	7.9	0.0
-	Z2124	hypothetical protein (COG: Phage DNA packaging protein, Nu1 subunit of terminase, S)	5.0	0.0
-	Z2254	H repeat-containing Rhs element protein	14.7	0.0
-	Z2255	Rhs element protein	23.2	0.0
-	Z2353	tail component of prophage CP-933R	6.7	0.0
-	Z2374	holin protein of prophage CP-933R	7.4	0.0
-	Z2558	hypothetical protein	32.2	0.0

-	Z2717	hypothetical protein	10.1	0.0
-	Z3136	hypothetical protein	5.1	0.0
-	Z3388	hypothetical protein	20.9	0.0
napG	Z3462	quinol dehydrogenase periplasmic component (R)	5.2	0.0
-	Z3962	hypothetical protein	11.9	0.0
hycH	Z4026	processing of large subunit (HycE) of hydrogenase 3 (part of the FHL complex)	5.1	0.0
ygcE	Z4087	kinase (G)	21.4	0.0
-	Z4104	hypothetical protein (COG: L-alanine-DL-glutamate epimerase and related enzymes of enolase superfamily, MR)	10.6	0.0
-	Z4352	hypothetical protein	23.2	0.0
-	Z4757	hypothetical protein	6.3	0.0
-	Z5201	hypothetical protein	15.9	0.0
-	Z5490	hypothetical protein	15.4	0.0
phnO	Z5696	aminoalkylphosphonic acid N-acetyltransferase (R)	10.1	0.0
nrdG	Z5847	anaerobic ribonucleotide reductase-activating protein (O)	5.7	0.0
-	Z5891	hypothetical protein	15.8	0.0
yjjY	Z6005	hypothetical protein	10.8	0.0
-	Z6011	hypothetical protein	15.7	0.0
	Z6023	unknown protein encoded by cryptic prophage CP-933P	18.2	0.0
-	Z6048	hypothetical protein (encoded by cryptic prophage CP-933P)	5.5	0.0
-	L7003	hypothetical protein (COG: Micrococcal nuclease (thermonuclease) homologs, L)	275.5	0.0
-	L7052	hypothetical protein (COG: Transcriptional regulators, K)	3 394.5	0.0
-	L7057	replication protein	1 234.4	0.0

Tab. 34: Genes > 3 fold significantly (p value < 0.05) up-regulated by LB Symbioflor® (EDL933 chromosome and pO157 plasmid).

Gene	**Locus tag**	**Product (COG functional category)**	**$rpkm_{ref}$**	**$rpkm_{Sym}$**	**LogFC**
-	Z0475	hypothetical protein	9.9	86.5	3.2
ybdK	Z0720	carboxylate-amine ligase (S)	1.6	15.3	3.3
-	Z0948	hypothetical protein	0.7	9.2	3.8
-	Z0952	Bet recombination protein of prophage CP-933K	0.2	5.1	4.6
ybiO	Z1030	hypothetical protein (COG: Small-conductance mechanosensitive channel, M)	0.9	8.4	3.3
ssbW	Z1440	single-stranded DNA binding protein	10.9	113.3	3.4
-	Z1442	antitermination protein N of bacteriophage BP-933W	2.9	83.6	4.9
-	Z1563	prophage regulatory protein (K)	1.4	14.0	3.4
-	Z1632	IS1 protein InsB (L)	0.8	7.2	3.2
-	Z1782	hypothetical protein	1.3	13.2	3.4

-	Z1924	hypothetical protein	38.0	423.7	3.6
-	Z2086	division inhibition protein DicB within CP-933O	0.5	5.3	3.6
-	Z2312	hypothetical protein	1.0	11.4	3.6
sapF	Z2500	ATP-binding protein of peptide transport system (V)	0.3	8.2	4.6
ynhE	Z2711	cysteine desulfurase activator complex subunit SufB (O)	1.4	17.3	3.7
pheM	Z2744	phenylalanyl-tRNA synthetase (pheST) operon leader peptide	9.3	158.1	4.2
-	Z2974	hypothetical protein	1.0	20.0	4.4
-	Z2984	serine acetlyltransferase of prophage CP-933T (E)	0.5	8.0	4.2
-	Z3262	hypothetical protein (COG: ADP-ribosylglycohydrolase, E)	0.6	5.2	3.3
-	Z3361	transcription antitermination protein N of prophage CP-933V	3.6	41.3	3.6
-	Z3362	superinfection exclusion protein B of prophage CP-933V	1.8	21.4	3.6
-	Z3371	hypothetical protein	0.4	12.1	5.0
-	Z3917	hypothetical protein	1.6	65.8	5.4
argA	Z4135	N-acetylglutamate synthase (E)	0.3	7.7	4.7
ppdC	Z4140	hypothetical protein (COG: Tfp pilus assembly protein PilV, NU)	3.0	26.8	3.2
yqeH	Z4166	hypothetical protein (COG: DNA-binding HTH domain-containing proteins, K)	0.4	6.0	4.0
hypA	Z4345	hydrogenase nickel incorporation protein HybF (R)	0.4	7.6	4.3
-	Z4855	hypothetical protein (COG: Predicted membrane protein, S)	1.9	16.7	3.2
yhjR	Z4951	hypothetical protein	5.1	56.9	3.5
yibI	Z5022	hypothetical protein	0.8	9.6	3.7
kdgT	Z5454	2-keto-3-deoxygluconate permease	0.7	6.7	3.3
phnB	Z5709	hypothetical protein (COG: Uncharacterized protein conserved in bacteria, S)	4.7	36.7	3.0
yjfF	Z5841	inner membrane ABC transporter permease protein YjfF (G)	0.6	6.4	3.6
yjiE	Z5926	DNA-binding transcriptional regulator (R)	0.6	5.7	3.4
-	Z6060	Q antiterminator encoded by prophage CP-933P	0.6	5.5	3.3
-	L7002	hypothetical protein	101.0	1010.2	3.6
etpJ	L7039	type II secretion protein (U)	42.4	707.1	4.3

Tab. 35: Genes (rpkm LB Symbioflor® ≥ 5, rpkm LB =0) significantly (p value < 0.05) down-regulated and silenced by LB (EDL933 chromosome and pO157 plasmid).

Gene	**Locus tag**	**Product**	**$rpkm_{ref}$**	**$rpkm_{Sym}$**
fruL	Z0089	fruR leader peptide	0.0	6.0
-	Z0261	hypothetical protein (COG: Uncharacterized protein conserved in bacteria)	0.0	8.4

yafO	Z0294	toxin YafO	0.0	6.1
ykgL	Z0363	hypothetical protein	0.0	5.4
-	Z0387	hypothetical protein	0.0	5.7
yahN	Z0424	cytochrome subunit of dehydrogenase (E)	0.0	8.8
-	Z0855	hypothetical protein	0.0	25.1
-	Z0884	hypothetical protein (COG: Histidine ammonia-lyase, E)	0.0	9.1
-	Z1122	hypothetical protein (COG: Transposase and inactivated derivatives, L)	0.0	10.2
-	Z1218	hypothetical protein	0.0	5.5
-	Z1219	hypothetical protein	0.0	10.7
-	Z1347	hypothetical protein (COG: Tellurite resistance protein, P)	0.0	5.1
-	Z1348	hypothetical protein	0.0	7.2
-	Z1426	hypothetical protein	0.0	12.2
kilW	Z1439	Kil protein of bacteriphage BP-933W	0.0	7.1
-	Z1459	antitermination protein Q of bacteriophage BP-933W	0.0	6.0
-	Z1786	Q antiterminator of prophage CP-933N	0.0	7.5
-	Z1838	hypothetical protein	0.0	6.1
-	Z1840	hypothetical protein	0.0	7.5
-	Z2087	hypothetical protein	0.0	13.1
-	Z2199	hypothetical protein (COG: AraC-type DNA-binding domain-containing proteins, K)	0.0	6.8
-	Z2282	hypothetical protein	0.0	5.3
-	Z2562	transposase (partial)	0.0	5.5
yoaG	Z2838	hypothetical protein	0.0	26.5
-	Z2988	tail fiber protein component of prophage CP-933T	0.0	7.8
-	Z2989	hypothetical protein	0.0	5.2
molR_C	Z3285	regulator (fragment) (S)	0.0	5.4
yfhL	Z3842	hypothetical protein (COG: Formate hydrogenlyase subunit 6/NADH:ubiquinone oxidoreductase 23 kD subunit (chain I), C)	0.0	25.3
-	Z3951	hypothetical protein	0.0	13.6
-	Z4199	hypothetical protein	0.0	7.7
yqgD	Z4286	hypothetical protein	0.0	6.9
yhaK	Z4460	hypothetical protein (COG: Pirin-related protein, R)	0.0	5.2
-	Z5095	hypothetical protein	0.0	13.0
-	Z5118	hypothetical protein	0.0	6.4
ilvM	Z5280	acetolactate synthase 2 regulatory subunit (R)	0.0	7.9
-	Z5339	hypothetical protein	0.0	6.7
yigK	Z5345	hypothetical protein (COG: Putative threonine efflux protein, E)	0.0	5.4
yjeT	Z5783	hypothetical protein (COG: Uncharacterized protein	0.0	15.8

		conserved in bacteria, S)		
-	Z5889	hypothetical protein	0.0	15.3
-	Z5949	hypothetical protein (COG: Uncharacterized conserved protein, S)	0.0	12.8
-	L7016	hypothetical protein	0.0	471.4
-	L7023	hypothetical protein	0.0	2 640.0
-	L7025	hypothetical protein	0.0	2 275.8
-	L7051	hypothetical protein	0.0	3 481.3
-	L7053	putative serine-threonine protein kinase	0.0	979.6
-	L7058	hypothetical protein	0.0	1 909.6
-	L7061	hypothetical protein	0.0	1 160.4
-	L7081	hypothetical protein	0.0	166.1
traI	L7098	DNA helicase	0.0	87.1

A function is known or can be presumed according to phylogenetic classification of about 45% / 50% of the genes more than 3% up or down-regulated, while the function of the remaining is unknown.

Tab. 36: Fold change of rpkm values versus luminescence of selected genes. Significantly regulated genes from transcriptome analysis are marked by *. [1] indicates no overlapping luminescence variation comparing the respective LB and LB Symbioflor® EHEClux mutant luminescence mean values (see Fig. 53, p. 120).

Gene	locus tag	Product	Fold change (rpkm max)	Log fold change lux	EHEC lux#
-	Z0371	LysR-like transcriptional regulator	0.19 (8)	-4[1]	41
ylbC	Z0672	malate dehydrogenase	- (0)	-2[1]	2
nfrB	Z0699	bacteriophage N4 adsorption protein B	-0.02 (7)	-3[1]	76
-	Z0702	hypothetical protein (COG: Rhs family protein)	-0.04 (12)	1	68
artP	Z1094	arginine transporter ATP-binding subunit	1.94 (93)*	-3[1]	39
hyaD	Z1392	hydrogenase 1 maturation protease	- (0)	-5[1]	19
-	Z1483	tail fiber protein of bacteriophage BP-933W	-0.2 (5)	-4[1]	26
-	Z1487	hypothetical protein (COG: Phage related protein, tail component; encoded within bacteriophage BP-933W)	- (0)	-2	23
yddB	Z2213	hypothetical protein (COG: Outer membrane receptor proteins, mostly Fe transport)	- (0)	-4[1]	73
-	Z2268	outer membrane receptor for iron transport	0.87 (15)	-3	10
ydbD	Z2320	hypothetical protein	-0.12 (13)	-7[1]	31
-	Z2510	transcriptional repressor	-0.40 (8)	-5[1]	46
yciG	Z2553	hypothetical protein	2.20 (161)*	-11[1]	28
-	Z2754	hypothetical protein (COG: Fructosamine-3-kinase)	0.38 (58)	-34[1]	66

-	Z2880	hypothetical protein (COG: Uncharacterized paraquat-inducible protein A)	0.64 (22)	2	6
-	Z3063	sulfite oxidase subunit YedY	1.40 (5)	-3[1]	81
lacY	Z3623	galactoside permease	- (0)	-8[1]	1
evgS	Z3632	hybrid sensory histidine kinase in two-component regulatory system with EvgA	0.13 (17)	-4[1]	8
mutS	Z4043	DNA mismatch repair protein MutS	-0.30 (10)	-6[1]	38
cysN	Z4059	sulfate adenylyltransferase subunit 1	-1.76 (10)*	-20[1]	13
ebgA	Z4429	cryptic beta-D-galactosidase subunit alpha	- (0)	-4[1]	3
dacB	Z4544	D-alanyl-D-alanine carboxypepti-dase/endopeptidase	-0.51 (11)	-2	11
yhiQ	Z4897	methyltransferase	0.88 (6)	-3[1]	5
gor	Z4900	glutathione reductase	0.03 (22)	-5[1]	43
xylF	Z4991	D-xylose transporter subunit XylF	-0.16 (11)	-4[1]	4
-	Z5102	hypothetical protein (encoded within LEE4)	- (0)	-5[1]	18
eae	Z5110	intimin adherence protein	-1.39 (6)*	-8[1]	51
tir	Z5112	translocated intimin receptor protein	- (0)	-4[1]	69
escC	Z5126	hypothetical protein (COG: Type III secretory pathway, structure protein)	- (0)	-12[1]	56
cesD	Z5127	hypothetical protein (COG: Type III secretory pathway, chaperon)	- (0)	-36[1]	58
-	Z5143	hypothetical protein (COG: Uncharacterized protein, conserved in bacteria)	- (0)	-24[1]	55
yijP	Z5513	hypothetical protein (COG: Predicted mem-brane-associated, metal-dependent hydrolase)	-0.84 (31)	-4[1]	49
-	Z5901	helicase	-0.56 (5)	-4[1]	20
deoA	Z5984	thymidine phosphorylase	0.87 (5)	-3[1]	61
espP	L7020	putative exoprotein-precursor	-0.16 (8721)	-3[1]	40

Additionally five EHEClux clones were identified to have the lux transposon inserted at distinct positions within the TTSS translocator gene *escV* (located in EHEC genome from 4 678 028 bp > 4 676 001 bp, minus strand).

Tab. 37: Fold change of rpkm values versus luminescence intensity of EHEClux *escV* insertion mutants. The EHEClux mutants are listed according to the insertion site bp. [1] indicates no overlapping luminescence variation comparing the respective LB and LB Symbioflor® EHEClux mutant luminescence mean values.

Gene	locus tag	Log fold change (rpkm max)	Fold change luminescence	EHEClux# (bp)
escV	Z5120	- (0)	-62[1]	62 (4 676 083)
escV	Z5120	- (0)	-11[1]	52 (4 676 303)
escV	Z5120	- (0)	-148[1]	70 (4 676 648)
escV	Z5120	- (0)	-3[1]	63 (4 676 649)
escV	Z5120	- (0)	-398[1]	54 (4 676 658)

The transcriptome data of 16 genes corresponds with the luminescence data, whereas 24 data sets do not go together.

Tab. 38: EHEC EDL933 housekeeping genes expression pattern. Insignificant log fold (p value > 0.05) change is shaded.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
hnr	Z2011	response regulator of RpoS	9.2	22.1	1.3
mdh	Z4595	malate dehydrogenase	607.6	609.5	0.1
gapA	Z2818	glyceraldehyde-3-phosphate dehydrogenase	793.2	541.5	-0.7
arcA	Z4094	ArcA	215.0	162.0	-0.5

Tab. 39: Genes involved in regulation, significantly up-regulated by LB Symbioflor® condition (EDL933 chromosome and pO157 plasmid). Rpkm value ≥ 5 is considered as transcribed.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
yabN	Z0079	transcriptional regulator SgrR	1.7	5.1	1.7
-	Z1124	prophage regulatory protein	4.2	12.8	1.7
-	Z1563	prophage regulatory protein	1.4	14.0	3.4
-	Z2269	DNA-binding transcriptional regulator	6.3	16.8	1.5
molR_C	Z3285	regulator (fragment)	0.0	5.4	log(5.4/0)
yrbA	Z4553	hypothetical protein (COG: Predicted transcriptional regulator, BolA superfamily)	15.4	41.1	1.5
yhiX	Z4929	DNA-binding transcriptional regulator GadX	57.6	191.8	1.8
yiaG	Z4980	transcriptional regulator	139.4	354.1	1.4
asnC	Z5244	DNA-binding transcriptional regulator AsnC	4.2	20.0	2.3
ilvM	Z5280	acetolactate synthase 2 regulatory subunit	0.0	7.9	log(7.9/0)
yjiE	Z5926	DNA-binding transcriptional regulator	0.6	5.7	3.4
-	L7024	regulatory protein	2263.0	9428.4	2.3

Tab. 40: Genes involved in regulation, significantly down-regulated by LB Symbioflor® condition (EDL933 chromosome and pO157 plasmid). Rpkm value ≥ 5 is considered as transcribed.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
araC	Z0073	DNA-binding transcriptional regulator AraC	9.3	3.0	-1.6
-	Z0342	LysR-like transcriptional regulator	8.8	1.7	-2.3
ykgD	Z0382	AraC-type regulatory protein	12.0	2.2	-2.4
-	Z0442	AraC-like transcriptional regulator	7.7	1.5	-2.3
yaiN	Z0457	regulator protein FrmR	44.4	15.2	-1.5

-	Z1333	DicA, regulator of DicB; encoded within cryptic prophage CP-933M	397.2	125.6	-1.6
putA	Z1513	trifunctional transcriptional regulator/proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	6.4	1.4	-2.1
csgD	Z1673	DNA-binding transcriptional regulator CsgD	25.4	8.5	-1.5
lsrB	Z2189	LacI-type transcriptional regulator	33.9	12.4	-1.4
cheY	Z2936	chemotaxis regulatory protein CheY	19.2	2.7	-2.8
yidL	Z5175	AraC-type regulatory protein	6.0	1.7	-1.8
pyrI	Z5855	aspartate carbamoyltransferase regulatory subunit	11.7	2.6	-2.1

Tab. 41: Genes regulated within the LEE pathogenic island. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	**Locus tag**	**Product**	**$rpkm_{ref}$**	**$rpkm_{Sym}$**	**LogFC**
espF	Z5100	secreted effector protein	0.9	0.5	-
orf29	Z5102	unknown function	0.0	0.0	-
escF	Z5103	LEE structural element with effector activity	0.0	1.6	-
cesD2	Z5104	chaperon	3.7	3.0	-
espB	Z5105	LEE structural element	11.7	1.1	-3.33
espD	Z5106	LEE structural element	8.4	2.2	-1.89
espA	Z5107	LEE structural element	17.8	4.8	-1.82
sepL	Z5108	regulation secretion hierarchy	6.7	4.1	-0.64
escD	Z5109	LEE structural element	1.3	1.1	-
eae	Z5110	intimin adherence protein	6.3	2.3	-1.39
cesT	Z5111	chaperon	0.0	4.4	-
tir	Z5112	translocated intimin receptor protein	2.9	3.5	-
map	Z5113	effector, mitochondrial associated type III regulation	23.4	6.8	-1.71
cesF	Z5114	chaperon	3.6	2.7	-
espH	Z5115	putative cytoskeleton modulating factor	20.8	31.8	0.62
sepQ	Z5116	pore forming protein	9.5	1.7	-2.41
orf16	Z5117	unknown function	0.0	0.0	-
orf15	Z5118	unknown function	0.0	6.4	log(6.4/0)
escN	Z5119	ATPase component	3.9	4.3	-
escV	Z5120	LEE structural element	2.2	1.6	-
orf12	Z5121	hypothetical protein	9.8	6.9	-0.44
sepZ	Z5122	secreted effector protein	121.3	21.4	-2.43
rorf8	Z5123	unknown function	13.6	5.3	-1.36
escJ	Z5124	LEE structural element	6.8	2.7	-1.24
sepD	Z5125	regulation secretion hierarchy	5.5	1.5	-1.78

escC	Z5126	LEE structural element	0.5	2.1	-
cesD	Z5127	chaperon	0.0	1.5	-
grlA	Z5128	positive regulator GrlA	10.1	4.6	-1.05
grlR	Z5129	negative regulator GrlR	7.8	15.9	1.09
rorf3	Z5131	unknown function	0.0	0.0	-
escU	Z5132	secretion system apparatus protein SsaU	1.2	0.8	-
escT	Z5133	LEE structural element	3.8	0.0	-
escS	Z5134	LEE structural element	3.6	0.0	-
escR	Z5135	type III secretion system protein	0.4	1.6	-
orf5	Z5136	unknown function	3.2	1.4	-
orf4	Z5137	unknown function	10.9	2.3	-2.16
cesAB	Z5138	chaperon	3.0	12.8	2.17
orf2	Z5139	hypothetical protein	20.9	7.1	-1.48
ler	Z5140	LEE-encoded positive LEE regulator	15.0	16.0	0.17
espG	Z5142	secreted effector protein	5.2	2.9	-0.78
rorf1	Z5143	unknown function	0.0	1.3	-

Tab. 42: Transcription pattern of non-LEE encoded type-III secreted effector genes. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
espX1	Z0025	hypothetical protein	0.0	1.1	-
espY1	Z0065	hypothetical protein	12.7	15.9	0.39
espY2	Z0078	hypothetical protein	45.3	63.9	0.57
espY3	Z0521	hypothetical protein	0.0	1.5	-
nleB2-1	Z0985	hypothetical protein	7.8	13.6	0.88
nleC	Z0986	hypothetical protein	12.9	14.2	0.21
nleH1-1	Z0989	hypothetical protein	5.7	3.5	-0.61
nleD	Z0990	hypothetical protein	14.9	22.3	0.66
espX2	Z1019	hypothetical protein	0.0	0.4	-
espF2-1	Z1385	hypothetical protein	1.5	2.8	-
espV	Z1387	hypothetical protein	38.0	60.7	0.75
nleG2-4	Z2075	hypothetical protein	14.3	10.9	-0.33
nleGG7	Z2077	hypothetical protein	6.4	6.4	0.07
nleG2-3	Z2149	hypothetical protein	13.7	11.7	-0.15
nleG6-2	Z2150	hypothetical protein	15.7	22.3	0.58
nleG5-2	Z2151	hypothetical protein	3.2	4.1	-
espR2	Z2242	hypothetical protein	0.0	0.8	-
espL1	Z2749	hypothetical protein	4.0	4.7	-

nleG5-1	Z2337	hypothetical protein	6.7	8.1	0.33
nleG6-1	Z2338	hypothetical protein	35.5	18.2	-0.89
nleG2-2	Z2339	hypothetical protein	31.4	68.7	1.20
nleG9	Z2560	hypothetical protein	5.7	6.1	0.17
espM1	Z2565	chaperone protein	893.9	744.1	-0.19
espJ	Z3071	hypothetical protein	5.1	8.5	0.81
tccp	Z3072	hypothetical protein (KEGG: Tir-cytoskeleton coupling protein)	1.4	0.3	-
espM2	Z3918	chaperone protein	17.9	25.2	0.57
nleG8-2	Z3919	hypothetical protein	1.9	8.8	2.27
espW	Z3920	hypothetical protein	2.1	5.1	1.33
nleG6-3	Z3921	hypothetical protein	22.0	22.0	0.02
espL2	Z4326	enterotoxin	5.2	5.2	0.05
nleB	Z4328	hypothetical protein	14.2	8.4	-0.68
nleE	Z4329	hypothetical protein	3.7	0.8	-
espY4	Z5211	hypothetical protein	1.3	0.1	-
espY5	Z5214	hypothetical protein	0.3	1.0	-
espL4	Z5608	regulator of acetyl CoA synthetase	3.7	1.6	-
espX4	Z5636	hypothetical protein	1.3	2.5	-
espX5	Z5665	hypothetical protein	2.0	1.3	-
espX6	Z5935	hypothetical protein	1.6	1.9	-
nleF	Z6020	hypothetical protein	109.4	113.5	0.13
nleH1-2	Z6021	hypothetical protein	135.9	126.3	-0.03
-	Z6024	hypothetical protein	14.6	10.1	-0.46
nleG2-1	Z6025	hypothetical protein	11.0	16.7	0.67

Tab. 43: Transcription profiles of toxin genes of EHEC. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
stx2A	Z1464	shiga-like toxin II A subunit encoded by bacterio-phage BP-933W	14.3	11.7	-0.21
stx2B	Z1465	shiga-like toxin II B subunit encoded by bacterio-phage BP-933W	15.9	37.3	1.30
stx1B	Z3343	shiga-like toxin 1 subunit B encoded within pro-phage CP-933V	102.9	142.0	0.54
stx1A	Z3344	shiga-like toxin 1 subunit A encoded within pro-phage CP-933V	111.2	114.9	0.12
astA	Z2779	arginine succinyltransferase (heat stable enterotoxin)	14.6	27.0	0.96
efa1/lifA	Z4332	cytotoxin	5.7	0.7	-3.01

efa1/lifA	Z4333	cytotoxin	0.0	1.9	-

Tab. 44: Regulated plasmid pO157 virulence factors genes. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
katP	L7017	EHEC-catalase/peroxidase	2 187.8	2 699.3	0.54
espP	L7020	putative exoprotein-precursor	8 721.1	6 565.9	-0.17
(ecf1)	L7026	hypothetical protein	1 920.3	1 789.3	-0.14
(ecf2)	L7027	hypothetical protein	2 167.7	1 665.4	-0.14
(ecf3)	L7028	hypothetical protein	3 518.7	2 217.5	-0.43
(ecf4)	L7029	lipid A biosynthesis (KDO)2-(lauroyl)-lipid IVA acyltransferase	33 920.5	29 546.1	0.04
(steC)	L7031	hypothetical protein	5 201.3	4 226.5	-0.06
etpC	L7032	type II secretion protein	668.4	1 065.5	0.91
etpD	L7033	type II secretion protein	2 085.3	2 404.8	0.45
etpE	L7034	type II secretion protein	1 420.0	2 385.3	0.99
etpF	L7035	type II secretion protein	852.8	993.7	0.46
etpG	L7036	type II secretion protein	2 458.1	3 251.2	0.64
etpH	L7037	type II secretion protein	229.4	1 121.2	2.53
etpI	L7038	type II secretion protein	347.8	231.8	-0.34
etpJ	L7039	type II secretion protein	42.4	707.1	4.30
etpK	L7040	type II secretion protein	3 670.5	3 702.0	0.25
etpL	L7041	type II secretion protein	956.2	2 102.6	1.38
etpM	L7042	type II secretion protein	1 439.2	827.1	-0.56
etpN	L7043	type II secretion protein	1 098.9	678.3	-0.46
etpO	L7044	type II secretion protein	6 839.7	3 588.4	-0.69
EHEC-hlyC	L7047	hemolysin transport protein	5 575.3	3 398.6	-0.47
EHEC-hlyA	L7048	hemolysin toxin protein	7 832.2	4 303.7	-0.62
EHEC-hlyB	L7049	hemolysin transport protein	3 589.0	1 960.4	-0.63
EHEC-hlyD	L7050	hemolysin transport protein	1 626.5	1 571.4	-0.19
toxB	L7095	putative cytotoxin	928.9	550.2	-0.51

Tab. 45: Transcription pattern of genes related to acid resistance. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
dps	Z1034	DNA starvation/stationary phase protection protein Dps	2 016.9	2 046.9	0.09
gadB	Z2215	glutamate decarboxylase isozyme	44.4	135.3	1.68
xasA	Z2216	acid sensitivity protein, transporter	95.1	212.9	1.23
cysB	Z2535	transcriptional regulator CysB	160.6	128.8	-0.32
rpoS	Z4049	RNA polymerase sigma factor RpoS	18.9	14.0	-0.36
sspA	Z4587	stringent starvation protein A	286.5	283.7	-0.06
hdeB	Z4921	acid-resistance protein	42.6	144.8	1.84
hdeA	Z4922	acid-resistance protein	1 816.7	3 737.2	1.11
gadA	Z4930	glutamate decarboxylase isozyme	32.6	70.1	1.18
adiA	Z5719	biodegradative arginine decarboxylase	0.1	0.0	-

Tab. 46: Transcription pattern of genes related to EHEC adhesion. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
-	Z1178	bifunctional enterobactin receptor/adhesin protein	1.1	2.2	-
espF2-1	Z1385	hypothetical protein	1.5	2.8	-
-	Z2200	major fimbrial subunit	90.1	57.2	-0.58
lpfE	Z4965	fimbrial subunit	12.6	2.9	-2.02
lpfD	Z4966	fimbrial protein	1.6	7.7	2.36
lpfC	Z4968m	PapC-like porin protein involved in fimbrial biogenesis	1.2	0.1	-
lpfA	Z4971	major fimbrial subunit	11.4	1.6	-2.74
lpfA2	Z5225	major fimbrial subuit	0.9	0.0	-

Tab. 47: Transcription pattern of additional EHEC colonization and fitness factors genes. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
kdpE	Z0841	DNA-binding transcriptional activator KdpE	1.6	0.0	-
pfs	Z0170	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase	43.5	68.5	0.73
ompA	Z1307	outer membrane protein A	314.2	356.3	0.25
csgG	Z1670	curli production assembly/transport component, 2nd curli operon	6.8	3.3	-0.96
csgD	Z1673	DNA-binding transcriptional regulator CsgD	25.4	8.5	-1.50

csgA	Z1676	cryptic curlin major subunit	8.5	1.5	-2.41
motB	Z2943	flagellar motor protein MotB	1.8	2.6	-
motA	Z2944	flagellar motor protein MotA	8.0	6.2	-0.28
flhC	Z2945	transcriptional activator FlhC	33.4	33.0	-0.06
flhD	Z2946	transcriptional activator FlhD	8.9	33.3	1.98
lrhA	Z3549	LysR family NADH dehydrogenase transcriptional regulator	84.5	95.0	0.24
yfhK	Z3833	2-component sensor protein	15.0	15.4	0.11
chuA	Z4911	outer membrane heme/hemoglobin receptor	3.6	1.2	-
dsbA	Z5392	periplasmic protein disulfide isomerase I	197.6	262.3	0.48
tolC	Z4392	outer membrane channel protein	115.4	86.6	-0.34

Tab. 48: Transcription pattern of EHEC genes related to quorum sensing. Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
lsrB	Z2189	LacI-type transcriptional regulator (AI-2 receptor)	33.9	12.4	-1.4
sdiA	Z3004	DNA-binding transcriptional activator SdiA	29.8	31.7	0.16
qseF	Z3830	2-component transcriptional regulator	9.3	6.6	-0.41
qseE	Z3833	2-component sensor protein	15.0	15.4	0.11
ygaG	Z3988	S-ribosylhomocysteinase (luxS)	187.6	150.9	-0.24
qseB	Z4377	DNA-binding transcriptional regulator QseB	4.2	7.4	0.88
qseC	Z4378	sensor protein QseC	6.5	5.3	-0.23
yfhA (qseF)	Z3830	2-component transcriptional regulator (QseF)	9.26	6.63	-0.41
yfhK (qseE)	Z3833	2-component sensor protein (QseE)	15.00	15.36	0.11

Tab. 49: Transcription pattern of homologues genes of EPEC and EAEC virulence factors for *C. elegans.* Insignificant (p value > 0.05) log fold change is shaded. Rpkm value ≥ 5 is considered as transcribed. "-" LogFC indicates the gene is not transcribed in both conditions.

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
csrA	Z3998	carbon storage regulator	3 208.4	2 604.0	-0.33
tnaA	Z5203	tryptophanase	239.6	330.9	0.54
cadA	Z5734	lysine decarboxylase 1	1.2	1.7	-
cadC	Z5736	DNA-binding transcriptional activator CadC	8.0	10.6	0.47

4 Discussion

The dualistic nature of *E. faecalis* causes it to be a highly controversially discussed bacterial species. In this work, a toolkit was established and proof of concept was provided for *in vivo* imaging of the dissipation and destination of different *E. faecalis* strains in the mouse. The modulation of EHEC pathogenicity by *E. faecalis* Symbioflor® was evaluated in another host system, namely in the nematode *C. elegans* revealing clear reduction of EHEC-induced lethality in this model. The capacity to modulate the EHEC transcription and down regulate pathogenicity determinants was further demonstrated by using *lux*-transposon mutants and strand specific NGS transcriptome analysis.

4.1 Dissipation and destination of *E. faecalis* in the gut

4.1.1 Construction of a plasmid reporter system for *E. faecalis*

Established plasmid reporter systems for other bacteria

The vector pXen5 used to insert a *luxABCDE* containing transposon into *Streptococcus pneumonia* (Francis et al. 2001*)* was inserted into *E. faecalis* OG1RF and Symbioflor®. But, eventually, no luminescent *E. faecalis* was produced. Sequencing analysis of re-isolated pXen5 revealed deletion or point mutations in the *luxAB* region (Taptigyna, T. Ehrmann M., TUM, personal communication). A reason for this might be deletion mutation induced by *E. coli,* which was used to amplify the plasmid. pXen5 transformation into *E. faecalis* without previous amplification in *E. coli* (directly from CaliperLS) did not yield any luminescence either. For the bacteria to emit luminescence, it is necessary that the *lux*

transposon integrates into the chromosome downstream of an active promoter. This might not take place under the tested conditions in *E. faecalis*. The temperature shift stopping the temperature sensitive plasmid from replicating might be set up to 45°C or higher, to adapt it to the growth characteristics of *Enterococcus* spec (Sherman 1937). Since *E. faecalis* strains are intrinsically resistant against high concentrations of aminoglycosides like kanamycin (Moellering et al. 1971), this selection marker used in the pXen5 transposon is not appropriate to select for transposition.

Toolkit for *E. faecalis* reporter system

Since the established reporter systems were not available for *E. faecalis,* appropriate plasmids and transposon systems, selection markers, and reporter systems were chosen for a toolkit, to design construction an adapted system. This was done in close cooperation with CaliperLS, Alameda USA. The high number of toolkit components was needed to find at least one strategy to success.

Bioluminescence reporter genes

The functionality of the *luxABCDE* operon in *E. faecalis* was tested in a plasmid system. While the operon was cloned from a luminescent *S. pneumonia* Xen10, cloning steps in *E. coli* could not be avoided. A similar approach of *luxCDABE* in pMG36e was successfully tested in *L. lactis* earlier (Jiang et al. 2006), but the pMG36e *luxABCDE* construct did not yield luminescence in *E. faecalis* upon successful transformation. Instability of the plasmid, the *lux operon* or an inactive promoter in *E. faecalis* might account for this. It is also possible that the luminescence light reaction of this *lux* system does not take place within *E. faecalis,* the substrates cannot be regenerated adequately (Eaton et al. 1993), or the luminescence is quenched by any present factor (Klerk et al. 2007, Troy et al. 2004). But, the external addition of substrate did not yield any luminescence, inefficient regeneration of the products does not seem to be the critical point. Despite these results, the *lux* operon is planned in the toolkit for further adaption, e.g., like the separation of the luciferase genes from those of the substrate producing genes, which improved luminescence performance in the past (Yagur-Kroll et al. 2011). Accessory helper genes, like *luxG* or *frp*, whose products help to furnish the substrates, were included into the toolkit as well.

Click beetle red luciferase (Branchini et al. 2004, Zhao et al. 2005) was tested as an alternative luminescence reporter gene. Similar to the widely used firefly luciferase (Fluc) Cbr needs external addition of D-luciferin for the light reaction. Cbr was used, since it was proposed to be superior in *in vivo* bioluminescence imaging concerning photon yield (Miloud et al. 2007). Further, emission of click beetle red luciferase in the red to infrared spectrum (> 600 nm) may lead to better transmission through mammalian tissue than green or blue light (Rice et al. 2001). The bacterial luciferase reveals an emission peak at about 490 nm.

The luciferase gene *cbr* was integrated into a plasmid expression system. This simple and straight strategy was successfully tested in *E. faecalis*. The absence of the substrate and hence the luminescence reaction during the cloning process in *E coli*, might have help to avoid the selection of deletion mutants by *E. coli* (Schweder et al. 2002). Disadvantages are that the external application of D-luciferin cannot be available for all experimental setups (e.g., food matrix) and it is more expensive than using the *lux* operon due to substrate necessity.

Fluorescent reporter genes

Fluorescent reporter genes were used to investigate *E. faecalis* by available fluorescence or laser scanning confocal microscopy. Green fluorescent protein has been successfully expressed in *E. faecalis (Nieto et al. 2003, Scott et al. 2000)*. Recently engineered red-fluorescent proteins mCherry and tdTomato promised high photostability, brightness, fast maturation and a sufficient acid resistance (Shaner et al. 2004). Both genes were available in an optimized form for expression in Gram-positive bacteria, the codon usage bias and GC content had been adjusted to *Mycobacterium spp.*. Despite that, *mcherry* and *tdtomato* expression in *E. coli* yielded a high fluorescence signal above autofluorescence when provided in trans on pUC57 or pMG36e. But the signal was remarkably less intense in pMG36e and incubation of transformants had to be 24 h longer to yield fluorescence. It could be that maturation is delayed, e.g., a suboptimal folding environment in the cell might account for that. The lower signal obtained from using, pMG36e is possible due to its low copy nature, or the strong viral promoters T7 and SP6 driving the expression in the acceptor vectors compared to the constitutive p32 promoter from a Gram-positive organism (Dunn et al. 1983, Kassavetis et al. 1982). However, poor performance of the red fluorescence proteins in *E. faecalis* OG1RF and Symbioflor® in fluorescent microscopy was mainly due to insuf-

ficient photostability, even after the use of Vectashield® mounting medium. Especially mCherry, whose original version is described as superior concerning photostability (Shaner et al. 2005), suffered from fast photobleaching and, thus, is not appropriate for further use in fluorescence microscopy. tdTomato bleached out at a slightly slower rate, and optimal filter set might improve the photostability, since less excitation light intensity would be necessary. Surprisingly, the performance of both proteins in laser scanning confocal microscopy improved and was comparable, though tdtomato was described as to rapidly bleach under laser scanning confocal illumination (Shaner et al. 2008). For another red fluorescence protein (mRFP1) it has been shown that photobleaching effects seen with fluorescent microscopy improve drastically using laser scanning confocal microscopy (Shaner et al. 2005).

Transposon cassette

A random transposon insertion strategy of reporter genes into *E. faecalis* was preferred to a homologous or site-specific recombination system (e.g., using p3TET (Hancock et al. 2004)). This system has been shown to work in closely related species, allowing selection for mutants with a constitutive expression of the reporter genes. Contrarily, random insertion is a disadvantage, because of lethal knockout transposition. Furthermore, a strong and constitutive upstream promoter is needed to produce sufficient amounts of luciferase. The selected transposon IS256 derived from the Tn4001 transposon of *S. aureus* (Byrne et al. 1989). Variants of Tn4001 have been identified in *E. faecalis* (Hodel-Christian et al. 1991, Paulsen et al. 2003a). One transposon cassette was designed without a promoter upstream of the reporter genes according to other systems like pXen5 (Francis et al. 2001). A version with p32 as promoter has been used to drive the expression of the reporter gene. For promoter studies, promoter regions with a terminator region upstream can be used. In contrast to other plasmid transposon systems like pXen5, the transposase is outside of the transposed genetic region. This omits the possibility of secondary transpositions after integration into the chromosome or any degradation of the plasmid during experiments.

Plasmid backbone and selection marker

The failed recovery of the plasmid backbones pXen5 and pLS210 might be due to instability of the rather large (> 10 kb) plasmids. Agarose gel analysis indicated rearrangements and deletion mutations of the plasmids (data not shown). Thus, plasmid pMG36e was used as a

backbone. It replicates in *E. faecalis*, but in a thermosensitive fashion (Russell et al. 2001). Further, the erm selection of this plasmid is applicable for most *E. faecalis* strains (among them OG1RF and Symbioflor®).

According to the analysis of the genomic sequences and investigation on antibiotic resistances of *E. faecalis* OG1RF and Symbioflor®, both are susceptible to cam (Bourgogne et al. 2008, Domann et al. 2007), therefore it was chosen as the transposon selection marker.

Construction of the plasmid transposon system

The insertion of the empty transposon cassette into pMG36e-p32 failed initially. Typical problems in cloning inverted repeats, like secondary structure formation might account for this. Hence, the selection marker *cam* was inserted as a spacer (Svoboda 2009). Another issue was the restriction enzyme *ClaI,* which did not cut the plasmid under standard restriction conditions. The inhibition might be due to a *dam* methylation pattern (Kessler et al. 1985) or reaction inhibitors. A *dam* deletion mutant strain and subsequent purifying the plasmid resolved the problems.

The insertion of *cbr* into the construct was not possible. Analytical gel electrophoresis indicated correct fragments sizes after restriction and ligation, but clones could not be detected. Reasons might include transformation problems for this rather large plasmid construct.

4.1.2 *In vivo* monitoring of *E. faecalis*

In vivo monitoring of *E. faecalis* in mouse models may help to evaluate the pathogenic potential of different strains and to unraveled mechanism of translocation from the gut.

Initial tests using *cbr* expressed on a plasmid in *E. faecalis* in mice performed by Ali Akin (CaliperLS, USA), confirmed basic functionality of the plasmid system *in vivo*. Next, the suitability of the pMG36ecbr plasmid reporter system for *in vivo* monitoring of *E. faecalis* in wild type mouse models in terms of stability and intensity of bioluminescence in a mouse model was assessed. *E. faecalis* OG1RF and Symbioflor® were chosen as representative colitogenic and probiotic strains, respectively of this species to study opposed behavior *in vivo* (Balish et al. 2002, Habermann et al. 2001, Habermann et al. 2002, Rosenkranz et al. 1994). Both strains do not harbor a plasmid and are susceptible against the toolkit's selection markers (Bourgogne et al. 2008, Domann et al. 2007).

Suitability of the plasmid-based reporter system for *in vivo* studies

Luminescent plasmid systems have been used by other groups in bacteria for *in vivo* mouse studies before e.g., in sporulation studies or evaluation of antibiotic therapy (Jawhara et al. 2004, Sanz et al. 2008). While expression and thus sensitivity of detection is high, the main issue of plasmid systems is their instable inheritance without antibiotic selection. This is corroborated by a better signal detection in the antibiotic administered reference groups. The more intense signal in the antibiotic treated group might result from a decrease in competitors in the murine microbiome and, therefore higher colonization rates of *E. faecalis*. Other explanation for a more intense signal in the antibiotic treated group comes from the observation that antibiotic treated animals have increased fattening: A reduction in tissue connectivity, thinner gut walls and villus lamina propria, caused by antibiotic administration, leads to increased nutrient uptake by these animals (Abrams et al. 1963, Coates 1980, Gaskins et al. 2002). This, together with higher infiltration of immune cells, might also increase bacterial translocation and colonization (Wells et al. 1988). The *in vitro* plasmid stability test indicated higher growth rates for *E. faecalis*, which lost their plasmid within the *in vivo* experiment.

The bioluminescence signal intensity of the pMG36ecbr reporter system was shown to be sufficient for *in vivo* monitoring. Signals above the background bioluminescence were detectable. Background luminescence is caused by chemiluminescence during normal cellular oxidative metabolic reactions (Cilento 1988, Devaraj et al. 1997, Duran et al. 1987, Popp et al. 1984). These "biophotons" are detected by the highly sensitive IVIS Imaging system. The average background radiance level was determined at $1.6e^3$ photons/sec/cm^2/sr. This level increases about 20% higher upon luciferin-injection, which is oxidized in unspecific cellular reacting. Interestingly, signal intensity is also about 30% higher in female compared to male mice, probably due to a higher metabolism in general. White fur – compared to nude mice – is observed to increase autoluminescence, too (Troy et al. 2004). Thus, a general fixed threshold between background noise and (low intensity) signals is difficult to evaluate. Since a newly build constructs was used and because of the above mentioned background luminescent issues, comparative dissection and microbial analysis of the mouse are necessary to correlate the level of bioluminescence with the number of bacteria. Unfortunately, some experiments of the *in vivo* study, especially some animals treated with

OG1RF and Symbioflor® at 0 h (Fig. 31, Fig. 33), did not support such a correlation between light and bacterial numbers. Obviously, the *in vivo* location and tissue depth of the source of luminescence plays an important role. In a firefly luciferase-based reporter system, it has been shown that the minimal luminescent bacterial count needed for bioluminescence detection using an IVIS system varied between a few, 10^2 (subcutaneous location) and 10^6 cells (2 cm depth in tissue) (Rice et al. 2001). To have a comparable starting position, a defined volume of bacterial culture was used and administered by gavage instead of mixing it with mouse food. The latter has been conduced in the initial *in vivo* study testing the system. Despite this standardization, the signal intensity varied highly for the Cbr system (Hawes et al. 2008). However, the difference of the maximal signal peak detected in the inoculated culture between OG1RF and Symbioflor® (signal intensity of OG1RF was about tenfold higher than for Symbioflor®) is still detected in the average maximal bioluminescence peak, this was in particular true for the more consistent erm groups.

Scattering of the bioluminescence signal is another issue, since it reduces spatial resolution (Contag et al. 2002). This has been observed in experiments with intense signals e.g., the OG1RF+erm group in Fig. 35A. Scattering effects can be reduced by application of hyperosmotic clearing agent on the skin area to be imaged (Jansen et al. 2006).

In these first experiments with the newly build system, it is necessary to correlate the luminescence signal back to the conventional colony counts. Further, a possible loss of the luciferase function or the reporter plasmids had to be evaluated, since *in vitro* plasmid stability tests confirmed that in antibiotic treated mice the bioluminescence signal retained longer than in the control group.

In general, antibiotic treatment caused increased plasmid stability and bioluminescent intensity to be more consistent among the individual. The exact reason, has to be further investigated, e.g., individual animal microbiomes might also account for the inconsistencies.

However, in conclusion the newly developed system performed well in respect to sufficiently luminescent *E. faecalis*, detectable *in vivo* in mouse models. Thus, this system allows for further dissipation and destination studies of probiotic and pathogenic *E. faecalis*. Despite first results, further correlation between colony counts and bioluminescence are necessary, thus mice have to be sacrificed each time. However, on the long run, this system holds promises for lesser use of animals, once the progress of *E. faecalis* interactions has been

established. Furthermore, examinations in using probiotic strains (like *E. faecalis* Symbioflor®) in, e.g., antibiotic therapy caused diarrhea, are now possible (D'Souza et al. 2002, Hickson et al. 2007, Johnston et al. 2006, Ruszczynski et al. 2008). Also other outcomes of probiotic use in different therapeutic combinations of other antibiotics are now achievable in mice.

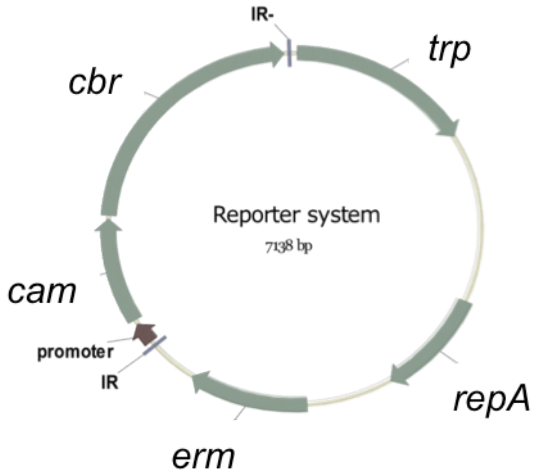


Fig. 44: Proposed transposon reporter system construct for genomic integration of reporter gene into the bacterial chromosome, omitting the necessity to administer antibiotics during experiments. Genes and plasmid elements: *cbr:* click beetle red luciferase, *cam*: chloramphenicol resistance gene, *erm*: erythromycin resistance gene, *repA*: plasmid replication protein, *trp*: transposase, IR: inverted repeat, promoter: p32 promoter.

Further steps in using our system should aim at the integration of the bioluminescent reporter genes into the chromosome enabling the use of the system without antibiotic administration. The latter, e.g., as in the case for erythromycin has been shown to modulate the host's metabolism and changes *E. faecalis* gene expression (Aakra et al. 2005). By replacing the bioluminescent reporter with a fluorescent protein, a higher spatial resolution, e.g., for translocation studies is achievable (Zeng et al. 2004). This will allow monitoring bacteria at a cellular level, e.g., in isolated tissues or in cell cultures. We started to use mCherry and tdTomato, but, despite results reported in literature, detected issues in photostability (Shaner et al. 2008). Other improved versions of fluorescent proteins might solve this problem.

Dissipation and destination observation

In our pilot study with the newly developed reporter system we could successfully show the dissipation and destination of *E. faecalis* strains in a mouse model. The signal was detectable along the lower part of the GIT in mice for up to 24 h, which is comparable to the GIT transit of other LAB (Kimoto et al. 2003). The GIT is passed by viable luminescent *E. faecalis* Symbioflor®. These bacteria were detected in the rectum and low luminescence of their feces after 48 h was found (data now shown). Next to Symbioflor®, we also used OG1RF, both strains possess genetic traits (e.g., *agg, ace; esp* and, only in OG1RF, *efaA*), which potentially enable them to proliferate in the intestine and colonize on mucosal surfaces (Bourgogne et al. 2008, Domann et al. 2007). However, colonization of the mouse by luminescent *E. faecalis* OG1RF or Symbioflor® was not observed after 24 h with this whole body monitoring system. Discrete luminescent signals are detected outside the lower intestinal tract. In Fig. 35A OG1RF + erm mouse #7 and #9 show luminescence in their mouth, which probably are bacterial remains from the inoculation. Discrete bioluminescent signals are also detected close to the front paw of several different animals at early time-points (Fig. 34, Fig. 35). This is the location of brachial and axillary lymph nodes (Fig. 45). It was shown before, that *E. faecalis* is able to translocate across the intact intestinal tract of antibiotic-treated mice and spreads within the lymph system (Wells et al. 1990). However, translocation and spread into lymph nodes at the front paw within minutes needs supportive data. For *E. coli* strain Nissle 1917, translocation was shown to take part 6 h after oral administration (Schultz et al. 2005). An *in vitro* study by Sartingen also indicates longer incubation time for enterococcal translocation to occur (Sartingen et al. 2000). This has to be further

investigated e.g., by dissection and imaging of the referred organs.

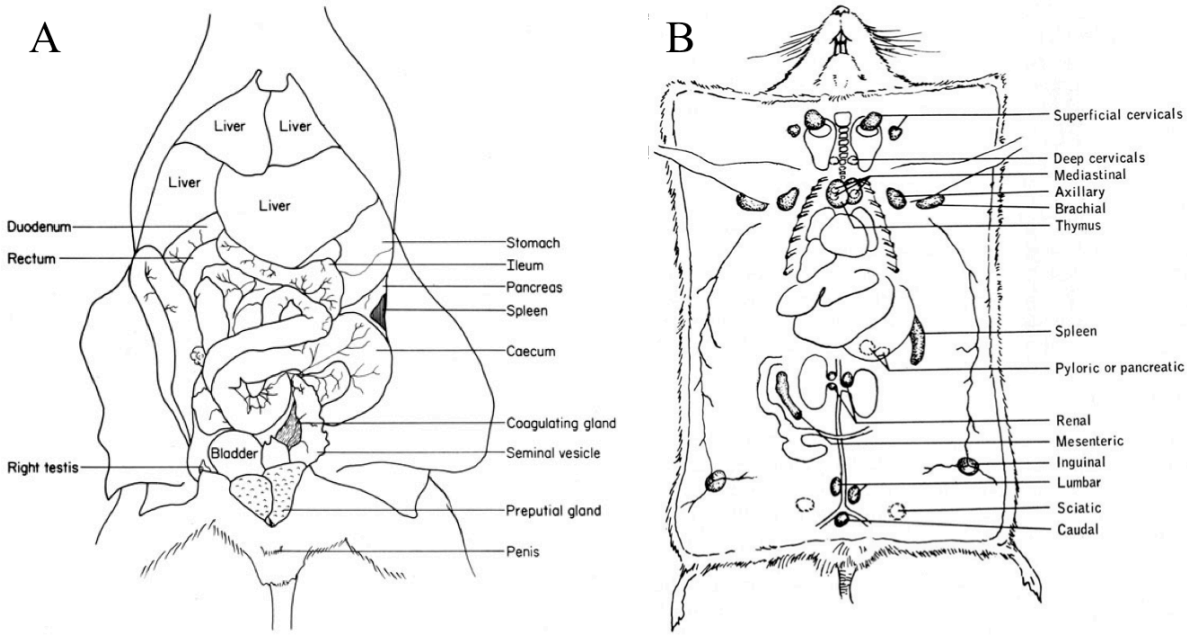


Fig. 45: The abdominal viscera of a male mouse are shown in A. The location of principal lymph nodes, spleen and thymus are shown in B (Cook 1965, Dunn 1954).

Despite success, two issues of this pilot study have to be solved in future: The correlation of bioluminescent intensity and bacterial counts was limited and the exact organ source in which the bacteria reside is hard to determine under given spatial resolution. A "standard progress time chart" has to be established first. However, once the course of bacterial infection, probiotic or pathogenic, has been established firmly, variation in various experimental parameters are conceivable. This then can help to understand the principles of pathogenic and probiotic behavior of bacteria.

4.2 *E. faecalis* modulation of enterohemorrhagic *E. coli* pathogenicity

EHEC, the causative agent of bloody diarrhea, has recently attracted media attention due to a large outbreak in Germany 2011 (Robert Koch-Institut 2011b). Treatment can be symptomatic, since Stx is produced upon antibiotic treatments (Goldwater et al. 2012). Several studies indicate an important influenced of the host's microbiome on the EHEC disease development. Human microbiota-secreted factors, probiotics, and LAB were shown to reduce

EHEC pathogenicity, mainly by reducing Shiga toxin synthesis (Carey et al. 2008, de Sablet et al. 2009, Kim et al. 2008, Medellin-Peña et al. 2007). The capability of *E. faecalis* Symbioflor® - a human probiotic used for more than 55 years – to modulate and reduce EHEC pathogenicity was assessed here in expression studies and a *C. elegans* killing assay. *C. elegans* is an established model even for human pathogens (Darby 2005). On food and produce, contaminating EHEC most likely appears in biofilm on surfaces. Thus, the natural model of food uptake in *C. elegans*, scavenging bacteria from surfaces, was advantageous to our study. The biofilm mode of growth confers among other advantages, to a higher persistence and stress tolerance, as well as enhanced host immune system protection to the pathogen (Hall-Stoodley et al. 2004).

4.2.1 Modulation of EHEC pathogenicity in *C. elegans* by *E. faecalis* Symbioflor®

To establish our *C. elegans* model system, the pathogenic potential of *E. faecalis* Symbioflor® was tested in this infection model because *E. faecalis* E002 (clinical isolate) tested previously by Garsin et al. (2001) was shown to colonize and kill *C. elegans* when provided as a sole food source (Garsin et al. 2001, Lavigne et al. 2008). In contrast, *E. faecalis* Symbioflor® fed to *C. elegans* showed no significant difference in survival as compared to the standard food source *E. coli* OP50. In this work, NGM agar plates were used to exclude a possible induction of pathogenicity when growing bacteria on BHI. This has been shown for the standard food strain, *E. coli*, causing premature worm death on BHI. However, an *E. faecium* strain tested on BHI, colonized *C. elegans*, but did not kill its host (Garsin et al. 2001). Growth of *E. faecalis* on NGM agar is reduced (Spanier, B., TUM, personal communication). Thus, a concentrated culture of *E. faecalis* Symbioflor® was spread on NGM agar and used without further incubation. Genes normally involved in *C. elegans* killing and human infection by *E. faecalis*, *cyl*, *fsrB, gelE, and sprE* are not found in the strain Symbioflor® (Domann et al. 2007, Garsin et al. 2001, Sifri et al. 2002). This could be the cause for *E. faecalis* Symbioflor® presented on NGM agar to *C. elegans* showed no pathogenicity compared to other *E. faecalis* strains.

C. elegans was used before as a model system for *E. coli* O157:H7 pathogenicity and to identify reducing factors (Kim et al. 2006, Lee et al. 2011). In this work *E. faecalis* Symbioflor® was shown to decrease significantly *C. elegans* mortality caused by cultivation on EHEC. This again highlights the two-faced nature of *E. faecalis*, since in another study, using clinical *E. faecalis* strains, a synergistic effects in pathogenicity was observed when feeding uropathogenic *E. coli* (Lavigne et al. 2008). Interestingly, not only live bacteria, but also Symbioflor® lysate decreased nematode mortality in our study. The significant effect of the lysate might indicate an important influence of effector substances, however, lysate preparation does not eliminate viable cells, but reduces cell counts about 10 000 fold. Thus, reduction in EHEC pathogenicity might still be due to Symbioflor® colonization and pathogen exclusion in the GIT of *C. elegans*. Especially, since a small inoculum of *E. faecalis* is enough to colonize the GIT of the worm (Garsin et al. 2001). *E. coli* O157:H7 was shown to colonize the nematode gut and colonization capability was related to virulence (Lee et al. 2008). For EPEC, colonization of *C. elegans* is not necessary for killing, instead a toxin is thought to be responsible (Kim et al. 2006). To further understand the mechanisms of pathogenicity towards *C. elegans* regulation and to identify the EHEC genes, expression patterns of EHEC genes influenced by *E. faecalis* Symbioflor® were investigated.

4.2.2 EHEC lux transposon database

An EHEC EDL933 mini-Tn5-*luxCDABE* transposon mutant library containing 9408 EHEClux clones was screened on *E. faecalis* Symbioflor®. The EHEC genome consists of a 5.5 Mbp chromosome with 5 416 annotated genes (including RNA-genes) and a 92 kb F-like plasmid with 100 open-reading frames (ORFs) (Burland et al. 1998, Perna et al. 2001). The transposon library consisting of more than 9000 transposon mutants, represent a sufficient number for high-throughput screening. The transposon system transfers a *lux* cassette into the chromosome, also conferring an antibiotic resistance. Thus, it may lead to knockout mutants. The activity of the next upstream promoter is visible by the luminescence signal, which allows gene expression studies. A possible drawback is that the knockout might lead to differential upstream promoter activity. The co-culture expression analysis was performed on solid medium as the worm assays have been conduced on solid medium as well. After two subsequent visual selections of differentially regulated genes, 84 EHEClux mutants were found and analyzed for quantitative luminescence intensity measurements. Of

those, the transposon insertion site was identified in 47 clones. The combination of nested primer and TAIL PCR yielded was successfully used and sequences between 44 bp and almost 1 kb were gained. Forty insertion mutations could be mapped to a definitive genomic site and seven insertions were redundant. Generally luminescence intensities were higher for EHEC mutants grown on LB was higher. One reason might be better growth (bigger colony size) on LB agar, possibly due to the absence of a nutritional competitor. Among the identified genes including the transposon, virulence genes active in humans were detected. Interestingly, luminescence was lower on LB Symbioflor® for all of those. Some genes were located in the LEE, e.g., *eae* and *tir* (located on LEE5) *escC* and *cesD* (LEE2), *escV* (LEE3), and *espP* (located on the plasmid pO157). All of the above mentioned genes are regulated by Ler, which is activated itself by quorum sensing mechanisms (Barba et al. 2005, Li et al. 2004). Similar results were found for *L. acidophilus*. Secreted factors of this bacterium were shown to interfere with EHEC quorum sensing, thereby reducing LEE expression and preventing EHEC colonization (Medellin-Peña et al. 2007). For future experiments, endpoint luminescence detection, continued luminescence measurement and normalization of the quantitative data are needed. Next, the transposon insertion site identification of more EHEClux mutants is advisable, notably to evaluate the assumed random distribution of the transposon cassette into the EHEC genome (Lewenza et al. 2005). The repeated transposition insertion into the *escV* gene does not support the assumed random insertion. An interesting finding is the highly diverse luminescence fold change among the *escV*-transposon mutants. The insertion site shifts of plus one or plus ten bp, compared to the first mutant (EHEClux mutant #54, #63, and #70). These shift in insertion resulted in 50 or 100-fold fold change in luminescence. This indicates the significance of the insertion site in a single gene. Quite unexpected, data from the transcriptome sequencing (see below) did not show expression of *escV* at all. Similar findings have been made for 19 other genes evaluated in both approaches, while the relative expression pattern of 16 genes matched transcriptome data.

Taken together, *lux*-transposon tagging displayed clear limits. Low promoter activities might go unnoticed, the insertion site seem to influence quantitative gene expression levels, and vital genes are not detected at all. However, this method can be used as search strategy for important genes in a specific niche, since light production is easily monitored without exogenous substrates. Thus, environmental niches can be monitored, not available for other

techniques, e.g., microbiome studies. Found suspects have to be corroborated by other methods, e.g., quantitative promoter activity analysis or, if possible, transcriptome sequencing.

4.2.3 Next generation transcriptome sequencing

Next generation sequencing is a promising sequencing technology, which allows characterization of bacterial transcriptome (Liu et al. 2011, Passalacqua et al. 2009). This innovative technique was applied here to evaluate the strand specific EHEC transcriptome modulation by *E. faecalis* Symbioflor®.

The modulation of the EHEC EDL933 transcriptome by *E. faecalis* Symbioflor® co-cultivation was analyzed focusing on especially, two groups of genes: Virulence associated genes and genes involved in regulation. Additional genes up- or down-regulated are mentioned if appropriate. Further genes identified in EHEClux mutant screen were compared to the transcription data. In total the analysis revealed the expression of 3452 of the annotated open reading frames. Of those, 201 genes were significantly down-regulated or completely silenced and 457 genes were up-regulated or switched on by the presence of *E. faecalis* Symbioflor®.

EHEC coordinates its virulence in an environment and temporal dependent manner, responding not only to host factors, but also to surrounding bacteria and their effectors. The shiga toxins *stx1* and *stx2* were expressed in both conditions at a consistent level, while the antitermination protein N and Q of BP-933W (Stx2 encoding) and the antitermination protein N of prophage CP-933V (Stx1) are significantly up-regulated. Their expression is regulated by the bacterial SOS stress response system. It activates the lytic growth of the Stx phage and leads to lysis of the bacterium and toxin release (Waldor et al. 2005). In a similar setup, *L. acidophilus* was not shown to modulate *stx* expression in EHEC, but the lysate was able to neutralize the Stx mediated cytotoxic effect (Kim et al. 2006, Medellin-Peña et al. 2007). The probiotic *Bifidobacterium breve* was demonstrated to reduce Stx concentration in the cecal content of EHEC infected mice by lowering of pH and acetate production (Asahara et al. 2004). *E. faecalis* Symbioflor® also decreased pH to 6 in the assay. pH as part of the stressors was confirmed by the significant upregulation of *hdeB* and *gadB,* both genes are involved in acid resistance of EHEC.

The locus of enterocyte effacement is organized in 5 major operons, which encode a type III secretion system, the adhesin intimin, and other effectors. Its expression confers the characteristic histopathology of EHEC infections, the A/E lesions in humans (Nataro et al. 1998). In total, 19 LEE-genes were transcribed in LB reference condition, coding for structural LEE elements, secreted effector proteins, regulatory proteins, and proteins of unknown function. In Symbioflor® co-culture, 10 LEE-genes were considered to be expressed, albeit at threshold levels only. Most of those genes are of unknown function. However, the transcription of a chaperon (*cesAB*) was turned on, while the transcription of the structural elements, which are folded by the chaperon, is turned off. Interestingly, only a minority of non-LEE encoded effector proteins was differentially expressed and, even of those, the expression level was only 1.5 to 2-fold above the levels found in LB (Matthews 2010). The detected mRNA level of some LEE-genes was different from its neighboring genes in the same operon (e.g., *orf4*, *orf15*). This might be explained by individual mRNA procession or stabilization, or even monocistronic instead of polycistronic expression, thus additional promoter, as it was proposed e.g., for *sepL* (Dahan et al. 2004, Kresse et al. 2000, Newbury et al. 1987). Eight genes were significantly down-regulated and two were significantly up-regulated by Symbioflor® conditions. The major regulator Ler was expressed consistently in both conditions. The ratio between GrlA and GrlR did not change significantly between both conditions.

An important positive regulator of biofilm formation CsgD was significantly down-regulated. The regulator is known be responsible for switching a bacterium from flagellar-expression and, thus, motility to biofilm-mode of growth. The protein binds to several spacer region located within flagellum associated operons and herby represses genes involved in the formation of flagellum (Ogasawara et al. 2011). Most of the genes were expressed but not significantly regulated in this study. However, *fliD*, part of the *fliCD* operon coding for a flagellar capping protein, was even significantly down-regulated and silenced (Ogasawara et al. 2011). CsgD is regulated by various external stresses relayed by other positive or negative regulatory systems (e.g., CpxR, FlhD, Hns, OmpR), whose transcription is not differentially regulated except significant upregulation of FlhD. The latter is the positive master regulator of flagellum formation, which activates *csgD* expression but also directly competes with CsgD for binding regulatory sites (Claret et al. 2002, Pruss et al. 1997). This expression pattern of the flagellar regulatory proteins and the flagellar traits points to so far

unidentified additional factors in this regulatory cascade. The transcription of *flhDC* itself is regulated by the quorum sensing (AI-3) dependent two-component system QseBC, but also inhibited by the LEE encoded GrlA (Iyoda et al. 2006). LsrB is the periplasmic of the AI-2 binding protein (Hegde et al. 2011). Its expression is significantly down-regulated by Symbioflor®. AI-2 serves as a chemoattractant, it is proposed to recruit bacteria for biofilm formation an recognized by Tsr/LsrB (Hegde et al. 2011). Efa1/LifA, which is involved in adherence, but also suggested to play a role in immunity modulation in the host, is also significantly down-regulated.

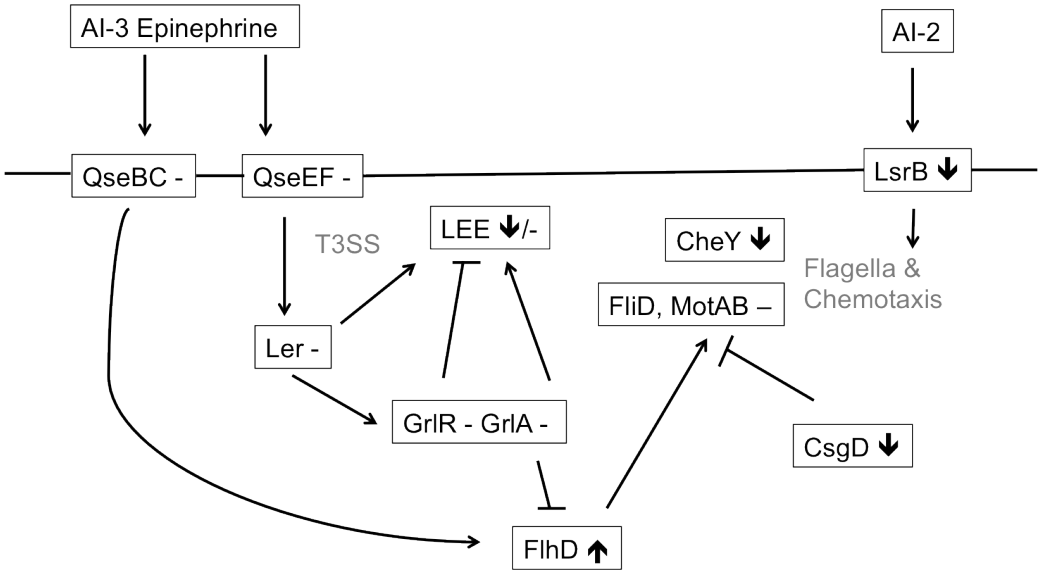


Fig. 46: Schematic regulatory signaling model of EHEC virulence associated genes differentially regulated by *E. faecalis* Symbioflor. An arrow indicates activation, a T indicates inhibition. ↑indicates significant up-regulated, ↓ indicates significant down-regulated in the presence of Symbioflor®. "–" indicates no differential regulation.

Genes, which are up-regulated by Symbioflor include several antitermination proteins encoded by phages. This might point to stress (nutritional, oxidative) stress exposure (Allen et al. 2012, Wang et al. 2010). Indeed, *E. faecalis* is capable of producing hydrogen peroxide through NADH oxidase and possibly other reactions. Further, significantly up-regulated genes also take part in the adaption to stress conditions. E.g., YjfF might be involved as an ABC transporter in secretion of siderophores, but also bacteriocins.

In conclusion, *E. faecalis* Symbioflor® was shown to be able to reduce EHEC virulence in a nematode host model system. To find out about the mechanisms of EHEC virulence reduction (e.g. direct inhibition of EHEC virulence or reduction of EHEC dose by *E. faecalis*

Symbioflor®), controls studies with EHEC + *E. faecalis* Symbioflor® supernatant/lysate or EHEC + OP50 might be used. Several virulence associated genes were shown to be significantly down-regulated by *E. faecalis* Symbioflor® in transcription analysis. EHEC gene-knockout nematode infection studies, especially of hypothetical proteins, which are significantly down-regulated by Symbioflor®, might help to unravel further virulence genes. Additionally, transcriptome pattern analyses of EHEC versus Symbioflor® in a host mimicking environment, which is known to activate transcription of respective virulence factors, are necessary. Further validation of the transcriptome sequencing results, using e.g. protein expression analysis or phenotypic analyses is needed. Generally, the virulence-gene associated transcription was reduced, not a single virulence gene was shown to be significantly up-regulated by Symbioflor. Interestingly, the transcriptome data also revealed the differential regulation of many so far uncharacterized proteins. Some of them might include further virulence genes. Knockout mutant and phenotype characterization will be necessary to unravel signaling pathways and regulators to fully understand the EHEC disease development and Symbioflor® protection.

5 Summary

E. faecalis is one of the highly discussed lactic acid bacteria due to its dualistic nature with respect to pathogenicity, commensalism and probiosis. On the one hand it is a harmless component of fermented food products, or part of the intestinal microbiota of healthy humans. Even probiotic therapeutics containing strains of *E. faecalis* are available. On the other hand *E. faecalis* strains, cause foodborne or even severe, life-threatening nosocomial infections. Another issue is the high number of antibiotic resistances among *E. faecalis* and the transfer of acquired resistances to other bacteria. The contrary ecotypes cannot strictly be ascribed to the presence of certain, so far identified virulence genes. Further factors, such as the immune status of the host determine the mode and outcome of interaction between humans and *E. faecalis*.

To unravel basic mechanisms causing the different behavior of *E. faecalis*, this work focused on studying its dissipation in host model systems and the modulation capability of enteric pathogens by *E. faecalis*. A luminescent reporter system was established to study the distribution and the route of ingested *E. faecalis* in a mouse model and to assess the connection between localization and behavior of different ecotypes. A probiotic and a colitogenic strain were successfully marked with this system and its functionality with and without the use of antibiotic selection, as well as the distribution of the luminescent bacteria *in vivo* in a mouse model was tested in a pilot study to demonstrate proof of concept. First results indicate a general boost of signal intensity and stability using antibiotic selection. Further, the signal was basically detected along the GI tract of the mice. Distinct signals from extraintestinal sites (especially below the front paw) from several different conditions were observed. This system, completed by future cell counting correlations, allows to conduce comparative dissipation studies between different *E. faecalis* strains in various mouse model systems.

The reduction of pathogenicity of the enteric pathogen EHEC by *E. faecalis* Symbioflor® was observed in a *C. elegans* killing assay. EHEC induced *C. elegans* killing was significantly decreased when grown with *E. faecalis* Symbioflor®. The modulation of the EHEC

expression pattern of certain genes, the whole transcriptome respectively, was evaluated using an EHEClux transposon collection and by next-generation transcriptome sequencing. EHEC, grown in the presence of *E. faecalis* Symbioflor® revealed down regulation of several virulence associated genes, including genes involved in T3S, adhesion and quorum sensing. Among the other significantly regulated EHEC genes, many so far uncharacterized genes were found possibly representing further virulence associated genes.

6 Zusammenfassung

E. faecalis ist auf Grund seines zwiespältigen Verhaltens hinsichtlich Pathogenität, Kommensalismus und Probiose eines der meist diskutierten Milchsäurebakterien. Auf der einen Seite ist es ein harmloser Bestandteil fermentierter Nahrungsmittel oder findet sich in der intestinalen Mikrobiota gesunder Menschen. Es gibt sogar Stämme, die als probiotisches Medikament verkauft werden. Auf der anderen Seite gibt es unter den Stämmen sowohl Lebensmittelpathogene, als auch solche, die schwere bis tödliche nosokomiale Infektionen verursachen. Eine weitere Problematik stellen Antibiotikaresistenzen in *E. faecalis* und deren Weitergabe an andere Bakterien dar. Prinzipiell lassen sich die unterschiedlichen Ecotypen nicht ausschließlich auf ihre Ausstattung mit bestimmten, bisher identifizierten Virulenzfaktoren zurückführen. Weitere Faktoren, wie der Immunzustand des Wirts bestimmen die Art und das Ergebnis einer Interaktion zwischen Mensch und *E. faecalis*.

In der vorliegenden Arbeit wurde zum einen die Lokalisation und Ausbreitung von *E. faecalis* Stämmen im Modellsystems und zum andern die Modulation der Pathogenität des enterischen Pathogens durch einen probiotischen *E. faecalis* Stamms untersucht, um Einsicht in den zugrundeliegenden Mechanismus zu erhalten. Ein Lumineszenz-basiertes Reportersystem wurde etabliert, um die Verteilung und die Route verdauter *E. faecalis* im Mausmodel zu untersuchen. Mit dem System wurden ein probiotischer und ein colitogener Stamm (Symbioflor® und OG1RF) erfolgreich markiert und sowohl seine Funktionalität ohne/mit Antibiotikagabe, als auch die Verteilung der lumineszenten Bakterien *in vivo* im Wildtyp Maus Modell untersucht. Vorläufige Ergebnisse zeigen eine generelle Verstärkung und Stabilität des Signals bei Antibiotikagabe. Das Signal wurde grundsätzlich entlang des Verdauungstrakts detektiert. In einigen Bedingungen können auch extraintestinale Signale (vor allem im Bereich unter den Vorderpfoten) festgestellt werden. Mit Hilfe dieses Systems in Verbindung mit der Bestimmung der Zellzahlen in unterschiedlichen Geweben können nun vergleichende *E. faecalis* Ausbreitungsstudien in unterschiedlichen prädispositionierten Modellsystemen durchgeführt werden.

Die Reduzierung der Pathogenität des enterischen Pathogens EHEC durch *E. faecalis* Symbioflor® wurde durch eine *C. elegans* Infektion festgestellt. Die Modulation der Expression bestimmter Gene bzw. des gesamten Transkritptoms wurde mit einer EHEC-Transposon-Lux-Datenbank und mittels Next-Generation-Transkriptomsequenzierung evaluiert. EHEC, das mit *E. faecalis* Symbioflor® kokultiviert wurde, wies eine verminderte Expression einiger Virulenzgene aus (u.a. LEE Gene, Adhäsions- und Quorum Sensing Gene). Unter den übrigen signifikant regulierten Genen befinden sich viele bisher uncharakterisierte, die möglicherweise weitere Virulenz-assoziierte Gene darstellen.

7 Appendix

Sequences of toolkit components

pMG36e-p32

ggtgatttcagaatcgaaaaaaagagttatgatttctctgacaaaagagcaagataaaaaattaacagatatggcgaaacaaaaaggt
ttttcaaaatctgcggttgcggcgttagctatagaagaatatgcaagaaaggaatcagaacaaaaaaaataagcgaaagctcgcgttt
ttagaaggatacgagttttcgctacttgtttttgataaggtaattatatcatggctattaaaaatactaaagctagaaattttggatttttattat
atcctgactcaattcctaatgattggaaagaaaaattagagagtttgggcgtatctatggctgtcagtcctttacacgatatggacgaaa
aaaaagataaagatacatggaatagtagtgatgttatacgaaatggaaagcactataaaaaaccacactatcacgttatatatattgcac
gaaatcctgtaacaatagaaagcgttaggaacaagattaagcgaaaattggggaatagttcagttgctcatgttgagatacttgattata
tcaaaggttcatatgaatatttgactcatgaatcaaaggacgctattgctaagaataaacatatatacgacaaaaaagatattttgaacatt
aatgattttgatattgaccgctatataacacttgatgaaagccaaaaaagagaattgaagaatttacttttagatatagtggatgactataa
tttggtaaatacaaaagatttaatggcttttattcgccttaggggagcggagtttggaattttaaatacgaatgatgtaaaagatattgtttc
aacaaactctagcgcctttagattatggtttgagggcaattatcagtgtggatatagagcaagttatgcaaaggttcttgatgctgaaac
gggggaaataaaatgacaaacaaagaaaaagagttatttgctgaaaatgaggaattaaaaaaagaaattaaggacttaaaagagcgt
attgaaagatacagagaaatggaagttgaattaagtacaacaatagatttattgagaggagggattattgaataaataaaagccccctg
acgaaagtcgaaggggttttttatttggtttgatgttgcgattaatagcaatacaattgcaataaacaaaatgatcgacctcgggaccc
ctatctagcgaacttttagaaaagatataaaacatcagagtatggacagttgcggatgtacttcagaaaagattagatgtctaaaaagct
agctttttagacatctaaatctaggtactaaaacaattcatccagtaaaatataatattttatttttctcccaatcaggcttgatccccagtaag
tcaaaaaatagctcgacatactgttcttccccgatcgacccgattcacaaaaaaataggcacacgaaaaacaagttaagggatgcagtt
tatgcatcccttaacttacttattaaataatttatagctattgaaaagagataagaattgttcaaagctaatattgtttaaatcgtcaattcctg
catgttttaaggaattgttaaattgatttttgtaaatattttcttgtattctttgttaacccatttcataacgaaataattatacttttgtttatctttg
tgtgatattcttgatttttttctacttaatctgataagtgagctattcactttaggtttaggatgaaaatattctcttggaaccatacttaatatag
aaatatcaacttctgccattaaaagtaatgccaatgagcgttttgtatttaataatcttttagcaaacccgtattccacgattaaataaatctc
attagctatactatcaaaaacaattttgcgtattatatccgtacttatgttataaggtatattaccatatattttataggattggtttttaggaaat
ttaaactgcaatatatccttgtttaaaacttggaaattatcgtgatcaacaagtttattttctgtagttttgcataatttatggtctatttcaatgg
cagttacgaaattacacctctttactaattcaagggtaaaatggccttttcctgagccgatttcaaagatattatcatgttcatttaatcttata
tttgtcattattttatctatattatgttttgaagtaataaagttttgactgtgttttatatttttctcgttcattataaccctctttaatttggttatatga
attttgcttattaacgattcattataaccacttatttttgtttggttgataatgaactgtgctgattacaaaaatactaaaaatgcccatatttttt
cctccttataaaattagtataattatagcacggtcgatcttctatataaaagatatattatcttatcagtattgtcaatatattcaaggcaatct
gcctcctcatcctcttcatcctcttcgtcttggtagctttttaaatatgggtcgatcgaattcgcccggggatcgatcctctagagtcgacc
tgcaggcatgcaagcttgcaaagtctgaaaacgaaggtggcagctgccgttgaagcggccaagacagttggtaaaggcgacggta
caaccggtactagcgacaaaggcggcggtcaaggtaccccggcgctacgatatttggagttcaagttcaaagtcaaatggtactgat
gaccggtaaaatttaatattttgaaccttgcttaggcagctgacttcacattgttgagatcagctgccttttgcttatagttcattgagtaga
aacggttctgttgcgaagtttgaaaatcaaacgcaagctcgatttttttattaaaacgtctcaaaatcgtttctgagacgttttagcgtttattt
cgtttagttatcggcataatcgttaaaacaggcgttatcgtagcgtaaaagcccttgagcgtagcgtggctttgcagcgaagatgttgtc
tgttagattatgaaagccgatgactgaatgaaataataagcgcagcgcccttctatttcggttggaggaggctcaagggagtatgagg
gaatgaaattccctcatgggtttgattttaaaaattgcttgcaattttgccgagcggtagcgctggaaaatttttgaaaaaaatttggaattt
ggaaaaaaatgggggggaaaggaagcgaattttgcttccgtactacgacccccccattaagtgccgagtgccaatttttgtgccaaaaa

cgctctatcccaactggctcaagggtttaaggggtttttcaatcgccaacgaatcgccaacgttttcgccaacgtttttataaatctatatt
taagtagctttattgttgtttttatgattacaaagtgatacactaactttataaaattatttgattggagtttttttaaat

Transposon cassette Ecwp (with p32 promoter)

aagcttatcgatgaattcagtcaagtccagactcctgtgtaaaatgtgagctcagattaatagttttagctattaatctttttttatttttatttaa
gaatggcttaataaagcggttactttggatttttgtgagcttggactagaaaaaaacttcacaaaatgctatactaggtaggtaaaaaaat
attggtaccggatccggcgccgccatgatggcgatatcgcggccgcgtcgacctcgaggggcccctctagaccatggacttttaca
caattatacggactttatcgaattctgcagcaagctt

Transposon cassette Ec-p

aagcttgctgcagaattcgataaagtccgtataattgtgtaaaagtccatggtctagaggggcccctcgaggtcgacgcggccgcga
tatcgccatcatggcggcgccggatccgagctcggtaccacattttacacaggagtctggacttgactgaattcatcgataagctt

Transposase

aagatgcgaataatcttttctcttctgcgtacttcttgattcagtcgttcaattagattggtactctttagtcgattgtgggaatttccttgtacg
gtatattgaaaggcgtcttcgaatccatcatccaatgatgcgcaagcttttgaatattttggttgatcgatataatcatgaatcaatcgatttt
tagcctcacgcgctaagttaatatctgtgaacttaaaaattcctttaacagcttctctgaaagattttgaatttttttttaggaatggtggtaaa
gatatttcttaggaagtgaacttggcatctttgccaacttacgttggtgaaggattttctaatggcagagactaatcctttgtgcgcatcag
aaataacgagttccgtaccttgtaaaccgcgttcttttaggtattcaaaaaatgttgtccaggtctcttcgctttcgccactttgaatcatga
agccgataatttcacggtcgccatctttggttattccaatcgctatatgacagctttttgagagtactcgattttcttctcgtacttttatatag
agtacatcggtcattaagtaaggataatttttttctgataataaacgattctgccactcgttaaccataggttctagctgttctgttaagctag
aaacgaaggacttagagacggatttaccacaaagttcttccacaatttttgatactttacgagttgaaacgcctgatacatacatttccaa
cattgaagccatgagggctttttcgtttcgttgataacgttcaaacactgtgggtgaaaaatggccatcacgtgttctgggtacttttaatt
ctagcgtgcctacacgtgtcgtaaagctgcgctcataatagccatttcgttgactttgtcggttttctgttcgttcatattcttttgcttgaata
tattctgttcgttgattttccattagttgattaaataccgttgttaaaatatttttagaaacgtcatcctttacagaatattcaataatgctttgaat
ctcttcgcttttcagtgtaaaatgtacttgggtcatgtaaaagtcctcctgggtatgtttttgtcgttaaaaacattgtaccgtaaaaggact
gttatatggccttttt

cbr

aggagggaattcatggtcaagcgcgagaagaacgtgatctacggcccggagccgctgcatccgctggaggacctgacggccgg
cgagatgctgttccgcgcgctccgcaagcactcgcacctgccgcaagccctggtggacgtcgtgggcgacgagtccctgtcgtac
aaggagttcttcgaggcgacggtgctgctggcgcaatcgctgcataactgcggctacaagatgaacgacgtggtgtccatctgtgc
ggaaaacaacacccgcttcttcatccccgtcatcgccgcctggtacatcggcatgatcgtcgcgcccgtgaacgaatcctacatccc
cgacgagctgtgtaaggtgatgggtatctccaagccgcagatcgtcttcacgaccaagaacatcctcaacaaggtcctcgaggtgca
gtcgcgcaccaacttcatcaagcgcatcatcatcctggacaccgtggaaaacatccatggctgcgagtccctgcccaacttcatctcg
cggtactcggacggtaacatcgccaacttcaagcccctccacttcgaccccgtcgagcaggtcgcggccatcctgtgttcgtcgggc
accacgggtctgccgaagggtgtcatgcagacgcatcagaacatctgtgtccggctgatccacgccctggacccgcggtacggta
cgcagctgatccccggcgtgaccgtgctggtgtacctgcccttcttccacgccttcggtttccatatcaccctcggttacttcatggtcg
gcctgcgggtgatcatgttccgccgcttcgaccaggaggccttcctgaaggcgatccaggactacgaggtccgcagcgtgatcaac
gtcccgtcggtcatcctgttcctgagcaagagcccgctggtggacaagtacgacctctcctcgctccgcgagctgtgctgcggcgcc
gccccgctggccaaggaagtggcggaggtggccgcgaagcgcctgaacctgccgggtatccgctgcggcttcggcctgacgga
gagcacctccgcgatcatccagaccctgggcgacgagttcaagtcgggttcgctgggccgggtcacccccctcatggcggcgaa
gatcgcggaccgcgaaacgggtaaggccctcggccccaaccaagtcggtgagctgtgcatcaagggccccatggtgagcaagg

gttacgtgaacaacgtcgaggccaccaaggaggcgatcgacgacgacggctggctgcattcgggcgacttcggctactacgacg
aagacgagcatttctacgtggtggaccggtacaaggaactgatcaagtacaagggcagccaggtcgcgcccgcggagctggagg
aaatcctgctgaagaacccgtgtatccgcgacgtcgccgtcgtcggcatccccgacctggaggcgggtgaactgccgtcggccttc
gtcgtgaagcagcccggcacggagatcaccgccaaggaggtctacgactacctggccgagcgcgtgtcccacaccaagtacctg
cgcggcggcgtccggttcgtggactcgatcccgcgcaacgtgaccggcaagatcacccggaaggaactcctgaagcaactcctc
gtgaaggccggcggctaa

mcherry

aggagggaattcatggtgtcgaagggcgaggaggacaacatggcgatcatcaaggagttcatgcgcttcaaggtgcacatggagg
gctcggtgaacggccacgagttcgagatcgagggcgagggcgagggtcggccgtacgagggcacgcagaccgccaagctgaa
ggtcaccaagggcggtccgctgccgttcgcctgggacatcctgtccccgcagttcatgtacggtagcaaggcctacgtcaagcacc
ccgccgacatccccgactacctgaagctctcgttcccggagggcttcaagtgggagcgcgtgatgaacttcgaggacggcggtgt
ggtcaccgtcacccaggactcgtccctccaggacggtgagttcatctacaaggtgaagctgcgcggcaccaacttcccgtccgacg
gtccggtcatgcagaagaagaccatgggctgggaggcctcgtcggagcgcatgtaccccgaggacggcgcgctgaagggcga
gatcaagcagcgcctgaagctgaaggacggcggccactacgacgccgaggtcaagacgacctacaaggcgaagaagccggtg
caactgccgggcgcctacaacgtgaacatcaagctggacatcacgagccacaacgaagactacaccatcgtcgagcagtacgaa
cgggccgagggccgccactcgaccggcggtatggacgaactgtacaagtaa

tdtomato

aggagggaattcatggtgagtaagggcgaggaggtcattaaggagttcatgcggttcaaggtcaggatggagggcagcatgaacg
gtcatgagttcgaaatcgaaggagaaggagagggccggccgtacgaggggacgcagaccgcgaagctgaaggtgacgaaggg
cggcccactccccttcgcatgggacatcctgtcgccgcagttcatgtatggttccaaggcgtacgtgaagcatccggcggacatacc
ggactacaagaagctgagcttcccggaggggttcaagtgggagcgggtcatgaactttgaggacggcggccttgtcacagtgacc
caggatagcagtttgcaggacggcacgctgatctataaggtgaagatgcgagggaccaacttcccgcccgacggccccgtgatgc
agaagaagaccatgggctgggaggcttcgacggagcgcctgtacccgcgtgacggtgtgctgaagggcgaaatccaccaggcc
ctgaagctgaaggatggaggtcactacctcgtcgagttcaagaccatctacatggccaaaaagcccgtgcagctccccggctacta
ctatgttgacaccaagctcgacattacctcgcataacgaggactataccatcgtggagcaatacgaacgctcggagggccggcacc
acctgttcctcggtcatgggaccggttcgactggcagcggtagttcaggaaccgcctcgtcggaggacaacaatatggccgtgatc
aaagagttcatgcgcttcaaggtgcgaatggaaggttcgatgaacggccacgagttcgagatcgaggggggaaggcgaaggtcgg
ccatacgaaggcacccagacggcgaagttgaaagtgaccaagggcggcccgctgccgttcgcgtgggacatcctgtcaccgcaa
ttcatgtatggctcgaaggcctacgtcaagcacccggccgacattccagactacaagaagctctcttttcccgagggcttcaagtggg
agcgcgtcatgaactttgaggatggtggcctggtgaccgtaacgcaggacagctccctccaggacgggaccctgatctataaagtg
aagatgcgcggcaccaacttcccacccgatggcccagtaatgcaaaagaaaacgatgggatgggaggcaagcaccgaacgtctg
tacccgcgcgacggagtcctgaagggtgaaatccaccaagccctaaaactgaaggacggtgggcactacctggttgagttcaaga
ccatctatatggcaaagaagcccgtgcaactccccggatattactacgtggacacgaagctggacatcaccagccacaacgaagac
tacacgatcgtcgagcaatacgaacggagtgagggtcggcaccatctcttcctctatggtatggacgagctgtacaagtaa

cam

aggaggcatatcaaatgaactttaataaaattgatttagacaattggaagagaaaagagatatttaatcattatttgaaccaacaaacga
cttttagtataaccacagaaattgatattagtgttttataccgaaacataaaacaagaaggatataaattttaccgtgcatttatttttcttagtg
acaagggtgataaactcaaatacagcttttagaactggttacaatagcgacggagagttaggttattgggataagttagagccactttat
acaatttttgatggtgtatctaaaacattctctggtatttggactcctgtaaagaatgacttcaaagagttttatgatttatacctttctgatgta
gagaaatataatggttcggggaaattgtttcccaaaacacctatacctgaaaatgctttttctctttctattattccatggacttcatttactg

ggtttaacttaaatatcaataataatagtaattaccttctacccattattacagcaggaaaattcattaataaaggtaattcaatatatttacc
gctatctttacaggtacatcattctgtttgtgatggttatcatgcaggattgtttatgaactctattcaggaattgtcagataggcctaatgac
tggcttttataatatgagataa

Plasmids for cloning of toolkit components

pCRII Vector(Invitrogen Life Technologies)

The pCRII cloning vector was used for directly inserting PCR products amplified with *Taq* polymerase. This polymerase adds a desoxyadenine to the 3' end of the product. The vector is linearized and bears a single 3' desoxythymidine as well as a Topoisomerase I, which is covalently bound to the vector (= activated vector). The multiple cloning site lies between P*lac* and *lac*Z, which allows blue/white screening.

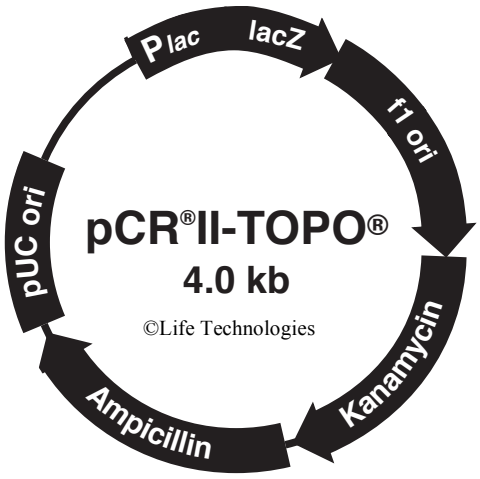


Fig. 47: Schematic diagram of pCRII-Topo (from (Moulton et al. 2009) **Invitrogen Life Technologies).**

pCR XL Topo Vector (Invitrogen Life Technologies)

The vector pCR XL Topo was used for cloning long (3-10 kb) PCR products. The vector is Topoisomerase I activated.

Fig. 48: Schematic diagram of pCR-XL-Topo (from Invitrogen Life Technologies).

pSTBlueScript-1 AccepTor™ Vector

pSTBlue-1 is a TA-vector for cloning and subsequent sequencing. The linearized plasmid contains a 3'desoxyuracil overhang, but no topoisomerase.

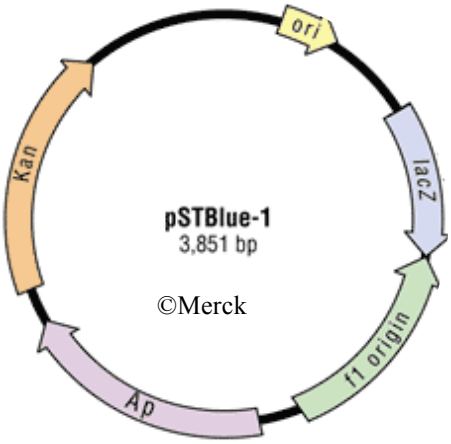


Fig. 49: Schematic diagram of pSTBlue-1 (from Merck).

pUC57

Genscript used this common cloning vector for inserting synthetized genes in the *EcoRV* restriction site.

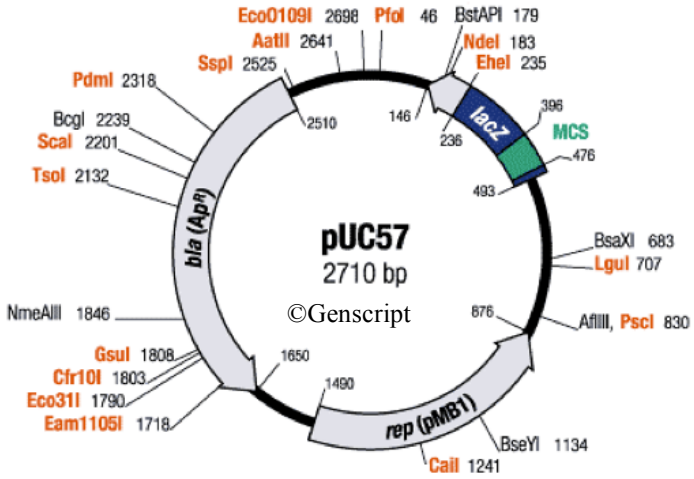


Fig. 50: Schematic diagram of pUC57 (Genscript). The amp selection marker (*bla*), the replication gene *rep*, and the MCS containing the *lacZ* gene are marked (from Genscript).

Template and expression plasmids containing the *lux* operon

pXen1

pXen1 (pMK4 *luxABCDE*) from CaliperLS (Francis et al. 2000) is a Gram-positive, Gram-negative shuttle vector (pMK4) containing the *luxABCDE* cassette without a promoter site as well as the *cam* (named Cml in Fig. 51) selection marker, which was used subsequently. A promoter can be added via either *SmaI* or *BamHI* restriction site and the plasmid can be inserted into bacteria, which drive the promoter and eventually emit light.

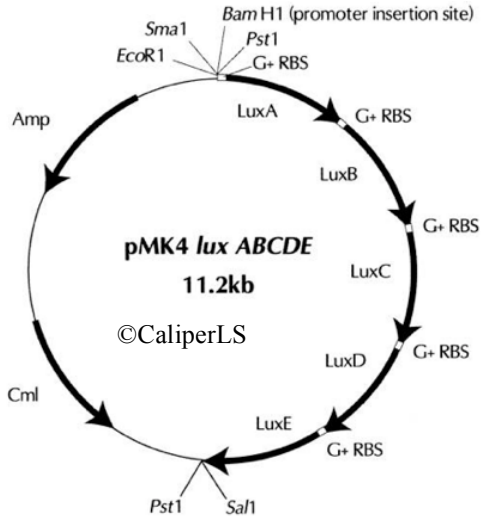


Fig. 51 Schematic diagram of pXen-1 (pMK4 *luxABCDE*, CaliperLS). The *lux operon* and the resistance genes (amp) and (cml) are marked (from Francis et al. 2000).

pLS210

pLS210 (Fang et al. 2008b) is an *E. coli Lactobacillus* shuttle vector, which is used for expressing of *luxABCDE* under the control of *cysK* promoter (a native promoter of *L. salivarius* UCC118) in Gram-positive.

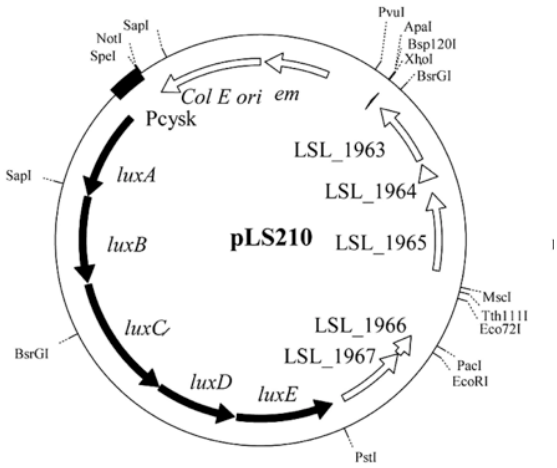


Fig. 52: Schematic overview of pLS210. The *lux* operon and the promoter P_{cysk} are displayed (from Fang et al. 2008).

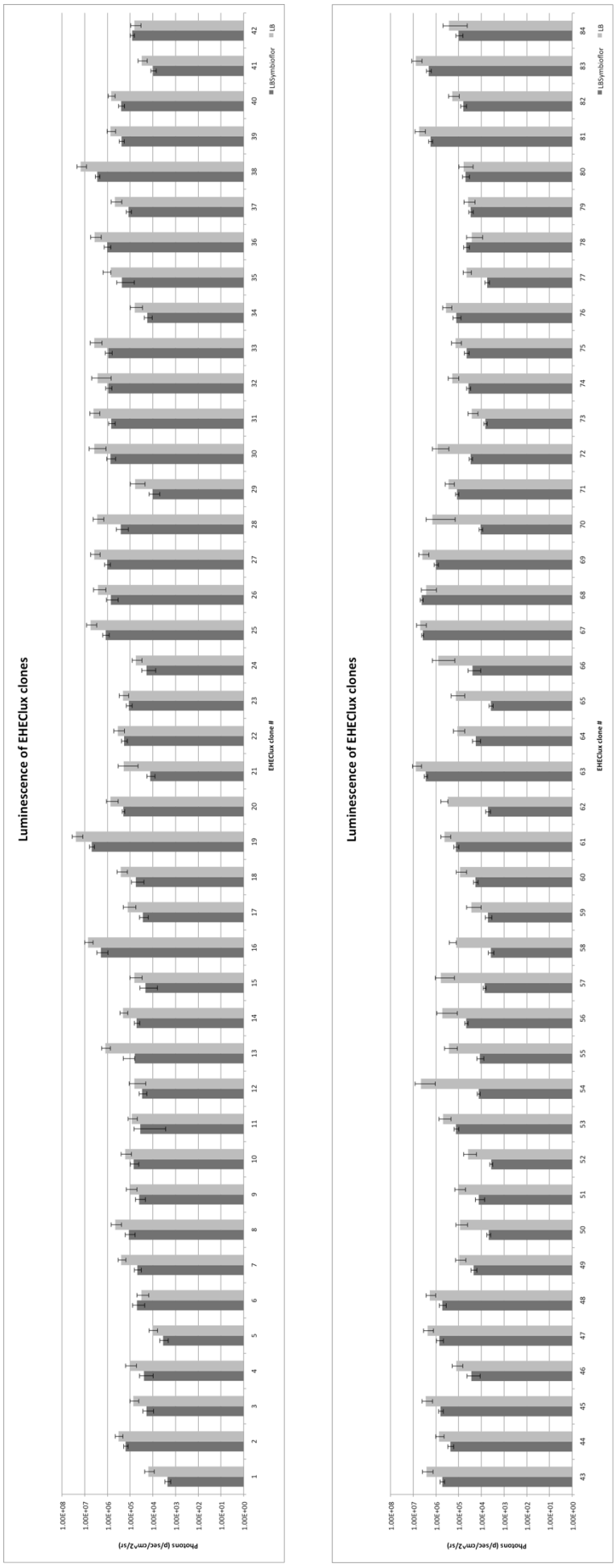


Fig. 53: Luminescence mean values from quantitative luminescence measurement of EHEClux clones grown on LB (light grey) and LB Symbioflor® agar (dark grey).

EHEC transposon sequencing

Primer #47

5‘ ctgactcttatacacaagtgcggccgcgcgaattcgagctcggtacccggggatcctctagagtcgacctgcaggtcgacg

gatccggggaattcaggcttggaggatacgtatgac

Primer #46

Fig. 54: 5’ end sequence of the lux transposon cassette. Primer annealing sites for primer #46 used for 1. TAIL PCR and primer #47 used for 2. nested PCR and sequencing are underlined.

The 3’ end of the sequence displayed in italics is the 5’ end of the lux transposon cassette. The remaining sequence was found to align to the EHEC EDL933 genome.

EHEClux#1

TGNNTTNNANAGNNNCGTTATTGGGNGTGTTCTTTGCCGGCATATTTTTTAGTATCAGTCCCCATATCAACTTCTGGTTGGT
CTCGCTATTTGGCGCTGTATTTATGATGATCAACATGTGTTTTAAAGATAAGGATCACCAGTGCGTAGC *CTGACTCTTATACA*
CAAGTGCGGCCGCG

EHEClux #2

NGGGGCCGAAAGGGTATGGCCTGATGATGATGATTGACGTCCTCTCAGGCGTCTTACTCGGCTTACCGTTCGGGCGACAGG
TTAGTTCGATGTATGACGATTTACACGCCGGGCGTAATTTGGGGCAATTACATGTAGTTATTAACCCGAACTTTTTCTCCTC
CAGCGAATTATTCCGTCAACATCTTAGCCAGACCATGCGCGAATTAAATGCCATTACCCCCGCGCCCGGTTTTAATCAGGTT
TATTATCCCGGACAGGATCAGGATATTAAACAACGCCAAGC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #3

TATGGGGGGGGATATCAGCCGTATTACCGACGATCCGCAGTGGGAAAAAGTCTACGTCGAGCGCATTGTTCGCCATATTCA
CGCGCAGAAAAACCATCCGTCGATCATCATCTGGTCGCTGGGCAATGAATCCGGCTATGGCTGTAACATCCGCGCGATGTA
CCACGC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #4

GGGGNGGGAGGGGACATCTTTCAGGCCATTATTTAGTGAAGTATCTGCTTTTGGCTCCTGACCATTGCCCAATTCAACGGC
AATTTCTGCGGCAGTATTTGCCAGCAATGTAATAGGTTTATATACCGTCATAGTTTGCGTACCGGCAGCAATACGTTTAATA
CCTGCGAGATCCGCATCCTGGCCAGAAATTGCTACTTTCCCTGATAAACCTTGCGCGCTTAATGCCTGAATTGCCCCACCTG
CGGTGGCATCGTTTGAGG *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #5

NNNTNNNNNTNTTTGCTTNNTGGGGGNTGGAGCACGATGAAGACAACCTGATGGCGCTGGTGTTAACGCCGGAACATCNN
NNATTGCGCAAGCGTGNTNNNNCAAAACNNNNCTTNTTATACA *CNAGTGCGGCCGCGCG*

EHEClux #6

GGGATGCCAATTCGCTATCTCGGTATTGATATCGGGCAAATCCAGACGCTGGATCTGATTACCGCGCGCAATGAAGTACAG
GCAAAGGCGGTGCTCTATCCGGAATATGTCCAGACCTTTGCTCGCGGTGGTACGCGCTTCTCAGTGGTCACACCGCAAATTT
CGGCAGCTGGCGTTGAGCATCTTGATACTATCCTCCAGCCGTATATCAACGTCGAACCAGGCCGTGGCAATCCTCGTCGCG
ACTTTGAATTACAAGAGGCCACCATTACTGATTCGCGTTACCTGGATG *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #8

NCCCCTATTCTNTTNCNGATGAAAATGGCTCGGTTCGGGGCGTTATGGGGGACATTCTTAATATTATTACCTTGCAAACAGG
TTTAAATTTTTCTCCGATCACCGTTTCACACAATATCCATGCTGGAACACAGC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #9

NNNNNNCTGCTNNGCTTACTGGGGGGGGAATTGTTACGTGCTGGCAAGCATGGCAGGGCTCTGTACGTATTGACAGGACAT
GTCAATGAATTTCCCCCCTACTGGTACGAGCTTGCTCTTTTACATTTTATGAATGAGGCATATATTAATGCTGCAACGGCAT
TTCGATATGGATTTTTAACAAGTATATATTGTTGAAATGCTTTGTTAGAATCTTTATCCATTCCCTAGGGCTGTAT *CTGACTC*
TTATACACAAGTGCGGCCGCGCG

EHEClux #10

NTGGGGGTACGCATTGATGACGCCAGCAAATTAAGCCTGATTTTCAATAGCGTGGATATCAAAGCAGATGACCCAGGTGG
GCTAACCAAAGCAGAATGGAAAGCGAATCCGCAACAAGCGCCTCGTGCAGAAC *CTGACTCTTATACACAAGTGCGGCCGCG*
CGA

EHEClux #11

GCCGGGTCGGACGCTGTGCGTCAGATCCTGCGCCAGCAAGCCGGTGTCGATATTGGAAACACCATTATTGCCGATGGTTCA
GGGCTTTCGCGGCATAACCTGATTGCCCCCGCCACCATGATGCAGGTGCTGCAATACATTGCCCAACACGACAATGAACTT
AACTTTATCTCCATGCTGCCGCTAGCGGGCTATGACGGCTCTTTGCAGTACCGTGC *CTGACTCTTATACACAAGTGCGGCCGC*
GCG

EHEClux #13

NGTAGCGTCGATGACGGCAAAAGTACCCTGATTGGTCGTCTGTTGCACGATACCCGCCAAATCTATGAAGATCAGCTCTCA
TCGCTGCATAATGACAGTAAGCGTCACGGCACCCAGGGCGAAAAGCTGGATCTGGCTCTGCTGGTGGACGGCCTGCAAGC
CTGACTCTTATACACAAGTGCGGCCGCGCG

EHEClux #14

TTGCNNNNNNNNTGNTANGNGNNNGGGTGATGAACGTCGCGCCCGCGCAAAATTTCACTGGCAATTCCTGGAACATTATC
CGGCTGCTCAGGAC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEC#18

GGGCGGCTGTAGCTGCGGCAAACCATAAGCTTCCCAAATATGCGGAAGCTATCCTGAATGTATTCCCACAAATTATACCTG
ATAAAAAAGATATCGCACATTTAGAATTTATTATCTTATTTGGATTAAATAGAAAAAATGATGCGGTAAAGGCTTTGGAGG
ACTGTATGGATG *CTGACTCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #19

GGGGATGTGGCTGATTGGCGCGTTTGTGATCGGTCATGTCTATATGGCGCTGCGTGAAGACATCATGTCCGACGACACGGT
GATCTCCACCATGGTCAACGGCTACCGTAGCCACAAATTTGGCAAAATAAGTAACAAGGAGCGTTCATGAGCGAGCAACG
CGTGGTGGTAATGGGGCTGGGCAACCTGCTGTGGGC *CTGACTCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #20

GTGATTTCTCCGGTATGCTTACTGGGGGGCGCCGGAATTGAGACATTTAATTTAGGCAATGATAGCGGTGACAGCGATGCA
CGCCCTGGCG *CTGACTCTTATACACAAGTGCGCCG* G *CGCGA*

EHEClux #22

ACTTATTNTTTCCCNTCCATTGTCCCCCCCCATTCCCCCCTTTTTTTTAAAAATNGTGNNGGAAAACGNCCTTCNGTTTGGGCN
NNGNTANTCTTACNNGCGGTCAGCGNGGGTATTCCGGGATGGCAGGTTTTGACACAGCCATCACCGGTGGAGCAGCATGTT
ACCTGGGGGAATGGTTGTAAAAAGCAGTGAAAACAGTAAATACAGAGTATCGTGATGAATTAGTGCTCAGGGGATACCAG
TATTCAATAACCAGCGTATCGGCATCGCATTCCTGTAATTCTGGTATTGACTGTTATGACTAAATTCAANGTATATAGGGCA
GGCAGAAATATGTTAACAGTCCCGGAG *CTGACTCTTATACACAAGTGCGGCCGCG*

EHEClux #23

GCGGAAGGTTGTGGCGGATGAGGTAAAAGCCGGGGTAAGTATCACTTCGCCAGTTATCCGGAGTGCCGTTATTCAGAACG
GAAACTTTCAGGTTGATTCTCAGGGTAACCTGAATATTGGAGGCCTTTTCAGTGTTACGTCACAAGGGCAACTGACAATTC
GTTACTCTAATCAGAATGTAGGACTGGTGATCCGCAATGATAAAATTGAGGTTTATGATCAGAATGGACGACTGGCTGTTC
GCATAGGCAGATTACGC *CTGACTCTTATACACAAGTGCGGCCGCGCGAAT*

EHEClux #26

NNNGNNNAANANNNTAANGTCTNNTATTCTTTTCCATTCATAGTTCCCCCCCANTTCCCCAGTGGGGGCGGTGGATCCGGGG
GAGTATGCGGTTTCGGTGACGGTGAAAGGGAAGACTGCTGTCTACGGACGTGTGCGTATTGAGGGGACCGAAAGTACGGT
GACGCTCAATATGCTGTTACGCCGCAGTCTTGTTGAGGTTAGCATACCCGGAGAACTGCTGACAGATTTCCGGCAGATACA
GAATAATGTGGCTGATGACCTTGCCACTATTTGTCGCCTGAATGAAGACACGGCGACAAAAAACACTCAGGCCACACAGTC
AAAAGAAAGTGCAGCAGCCAGTGCGAAGAGTGCATCTGACAGTGCAAAGA *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #28

ANTTGCNNTGCTATNNTTNCNGGGGGAAATAACATGGCCGAACATCGTGGTGGTTCAGGAAATTTCGCCGAAGACCGTGA
GAAGGCATCCGACGCAGGCCGTAAA *CNNNNTCTTATACACAAGTGCGGNCGC*

EHEClux # 31

GGGGCGGAACTTCGATGCGTTGCAAATGCAGTTGATATTGAACCTTTTTTTTCTGCGGCTACCGCTGAAGATAAACAACAA
GTTGAACAAGCTATCAACAGTAGCGTGAATCTTGTCCCCTTCGGTTTATCTGCATCGGACTGGAAAGTGCATCGTGGCGATT
TAGTGGTAGAAGGTAATATAGAGAGTAATCAAAAATTGATTGTTCTTGGCAATTTGACAG *CTGACTCTTATACACAAGTGCG*
GCCGCGCGA

EHEClux #36

ACTTCCCAATTGAGGTTTGACAATGAGGGCTTGAACACTATTCGCGCAATGGAGGGCGGGCCCGGCCTTTGCACTGCTATG
CTTACTGGGGGGGGCGATCGAGCGTGCGCAGGCCTATGTTGAAGCGGGTGCCGAGATGTTGTTCCCGGAGGCGATTACCGA
ACTCGCCATGTACCGCCAGTTTGCCGATGCGGTGCAGGTGCCAATCCTCGCCAACATTACCGAATTTGGCGCCACGCCGCT
GTTTACCACCGACGAATTACGCAGCGCTCATGTCGCGATGGCGCTGTACCCGCTTTCAGCGTTTCGCGCCATGAACCGCGCC
GCCGAACATGTCTACAACGTCCTGCGCCAGGAAGGCACGCAGAAAAGCGTCATCGACACCATGCAGACCCGCAACGAGCT
GTACGAAAGCATCAACTACTACCACTACGAAGAGAAGCTCGACGACCTGTTTGTCCGTAGCCAGGCGAAATAAAAAACGC
CCGTTGGTTGTATTCGACAACCGATGCCTGATGCGCCGCTGACGCGACTTATCAGGCCTACGGGGTGAACTGTAGGTCGGA
TAAGACGCATGGCGTCGCATCCGACAACAATCTCGACCCTACAAATGATAACAATGACGAGGACAATATGAGCGACACAA
CGATCCTGCAAAAGCAGTACCCCATGTCATTAAAACCGAAAAAATCC *CTGACTTCTTATACACAAGTGCGGCCCGCGCGAATT*
CGGAGTTCGGTCCCGGC

EHEClux #38

NTNNNNCTGCTNTGCTTACTGGGGGGCGTGCCAAAAGAGGTTATTAAGCGCGCACGGCAAAAACTGCGTGAGCTGGAAAG
CATTTCGCCGAACGCCGCCGCTACGCAAGTGGATGGTACGCAAATGTCTTTGCTGTCCGTACCGGAAGAAACCTCGCCTGC
AGTCGAGGCACTGGAAAACCTCGATCCGGATTCACTCACCCCGCGTCAGGCGCTGGAATGGATTTATCGCCTGAAGAGTCT
GGTGTAATAATAATTCCCGATAGTCTTTTGCTATCGGGAATATTAACGATAACTGACGAATCAAATAAAAATACCCTGTAT
AATAGGAAAGCTTATTTTACAGGGTAAAAC *CTGACTCTTATACACAAGTGCGGCCGCGC*

EHEClux #39

CGCCCCCGTTATTTTGTGCTATGTTTATTGAATAATGCGCTTTGCTTTTAACTTTTAAAGCAGAAATATTGCATAATTATTCT
GTCAAAGGTACTATCTTCGACCTCAATAGCTATCAGACTGCCAGTATACGAGTGTCAATGAGTATTCAATTAAACGGCATT
AATTGCTTCTACGGCGCGCATCAGGCGCTGTTCGATATCACGCTGGATTGCCC *CTGACTCTTATACACAAGTGCGGCCGCGCG*
A

EHEClux #40

TGGGGGGGGCGCCAAAAGACACCAATGAAAATGTCTTTAAAGCCAGTAAACAAACCATTGGTTTCAGTGATGTAACGCCG
GTCATTACAACCAGGGAAACCGATGACAAAATAACATGGTCACTGACAGGCTATAAC *CTGACTCCTATACACAAGTGCGGC*
CGCGCGAA

EHEClux #41

GGGGGGGCACCATACACACTACCCGATGTCTGTTCTTTGCAGCTTCCAATTGGATGGGCGCTGATAATGGCAGGATACTTTT
TTTCACTATTAAAATCGTCAGGGAAATAGATATCAGCAGCTATATCCCAGTAAGTGTGTTTTATCGAAACAGTTTTCATTTC
AAGCTCCGGGGATTTGCCTAAACAGATTTAAATATCATTTCTATTATCCGCAACTGGCATTTGTGTTCATATCGAGAACGGC
GTAATAGTATGACTTGTATTGGTTATCATCAATGGTCTAATATCTATCACAGTGTTAAGTTAAGGTTTACAATGATGAAAAT
AGAGCCTTCAATTTTGCCTTCTCTTGCCTGGTTTGCGC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #43

CCATTTCCTTGGTTTGGTTTGCGGGGGGGGGAATTGTTACGTGTTGGCAAGCATGGCAGGGTTCGGGGGGCGGCGGCAGCT
CAGGCCAACCATATCACTGCTATGCTTACTGGATAATCATACAGTATATTCAGGTTATAAAACCAGCATGTCCTTGCAATAG

TTTCAGTATGGTATTAGTATTGATGCGTTAGATGATGGCATTCTCACTCCAGTCAGAGCCACCAACTCAGGGCTGAAC *CTG ATTCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #45

CTGTCTGAATTTAAGTTATTGAGAGAGCAATGTTTCAGGTGGGGTAATTATACTCTATTGTTTGAAAATTATGAGTCTTATG ATAAGACGGGGTCTATCACGATAGAAAAAAATCAGGGGGAGGGGACTTTACCCATTCGGCATAAATTAGAGTTTATATCG ACTAATATTGCAGAGTTGCTGGACAAGTTAACGAAAATTACAGATGCCAGACTTTGCAAAGGCTTCAGTGACTGGGCTAGT TCAGTCAAAGAAGGCGGATCGAATGACCTCAAAGAAAATGTAGATAGAGCGTTGGTGAGAATGTTTAAATGTGTTAAGCT TCACAGTAATGAACTTGACTTATCATACCTATTTTTGGGTTCTGTGCCGCCTCTTCCAGATTGGATTGAAATGCTTAGC *CTG ACTCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #46

GCTATAGTTGNGCTTTCTGTACCGGAGAGAAGCTGCGGTACAGAGCATTTTGCGTTATCTACTCTTGACGCCTGCAAGGGGT AGGTTGGGGAAATATCTTATAGAATCTGACTATGAAACTATTNGTATTAGCTCCGATAAGTTTGATGATGAAAACAGAACT GGCGACAACAAGTAGCAGCCTTTCTGATGAATTGACCCCAACTTAATATTGTCAACTATCTTCACAGAATCTCCACAATCTC TTCATTATCAGAATATGCACCATGAATAAGATAAAAATACTTTCCATTACTTTGGAGAAACGTATAATCAGCAAAAATGAG AACGATCATTCTCACCAAGTGATAGGCCAGGAGACAGAAATGACCAGCAAGCTGGAAATACGCCACAAACAGCGTCAGGA TGAAATCATTAACGCCGCCCGTCGG *CTGACTCTTATACACAAGTGCGGCCGCGCGAA*

EHEClux #49

NNNNGGGGGGGCTGTGCTACTACGTCATCTACGGTCAGGAGTTCTCGCAGAGCGTTCTGTTTGTGATGTTCGAAACCAACA CCAACGAAGCCAGCGAGTATTTAAGCCAGTATTTCAGCCTGAAAATTGTGCTTATCGCGCTGGCCTATACGGCGGTGGCAG TTCTGCTGTGGACACGCC *CTGACTCTTATACACAAGTGCGGCCGCGC*

EHEClux #50

NNNNACTGCTATGCTTACTGGGTGTGCTGTCTGAATTTAAGTTATTGAGAGAGCAATGTTTCAGGTGGGGTAATTATACTCT ATTGTTTGAAAATTATGAGTCTTATGATAAGACGGGGTCTATCACGATAGAAAAAAATCAGGGGGAGGGGACTTTACCCAT TCGGCATAAATTAGAGTTTATATCGACTAATATTGCAGAGTTGCTGGACAAGTTAACGAAAATTACAGATGCCAGACTTTG CAAAGGCTTCAGTGACTGGGCTAGTTC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #51

NTTGCNNTGNTNTGCTTACTGGGGGGGCCTGGTCAGCAGATCATTTTGCCACTCAAAAAACTTCCCTTTGAATNCTGNGCAC TANCACTTTTAGGT *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #52

NTGGNACTGCTATGCTTACTGGGGTGGATGTTTATGCAACAACAAATGATAAGATAACGTTTTTGCTAAAGAAACTTGTAT TGTCTAATGCCAAAGAGTTCATCGGCGTACAAGAAACGCGTTATTTGATGGACATCATGGAGAGAAAATATAACGAGCTTG TGAAAGAGCTGCAGCGCCAGCTTGGTTTGAGCAAAATTGTTGACATCCTACAACGTCTCGTAGAGGAAAATGTCTCAATTA GAGACCTGAGAACTATCTTTGAGACGCTTATTTTTTGGTCAACAAAAGAAAAGGATGTGGTTATCTTGTGCGAATACGTTC GTATTGCCCTGCGTCGGCATATTTTAGGTCGCTATAGCGTTAGCGGTACAC *CTGACTCTTATACACAAGTGCGGCCGCGCGAA T*

EHEClux #54

ATNCAGAGATATCATTCGAATTTGTCCAGGAAAAGCTTTCGACTAACTCTATCGTATATAAGACGAATAAGACTAATCAAC AGCTCGCTCACCTTACANNG *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #55

GNNNNNNNCTGCACAGCTACTTAAAATAACCAAAAGTGGCGACATCATGGACAGAGCCCCACTGCCTTTTATCTCTGATGC CGAAACAATTGAACACATACAAGGGAACATTTTTGCTGCAGTAGACGAAAAGACTTCTGAAC *CTGACTCTTATACACAAGTG CGGCCGCGCG*

EHEClux #56

NTTCCNNTNNANGGNTNNNNGGGGGGGTACGTTAAATGCAGGACAAGGTACGATTGCCTTTAATAGCTCTACCGCACAGG CTAATATC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #57

TTTAGCACACCCGGAAAACTGTGTGTATGTTTTTCCCAAACCGTAGGCCTATTTAGGCGGCCAGGGTTACCAGGAAATTCA
AAACCAAACTGACGGGGGAGGTGGAATTTGTGTTTGGTCCGCCAGAAAAATGAAATTTGGGCAGCAAAACCACTTTTTTTG
AGGTGAGATATTGACAAAATTCATGGAGGCTACCTTTTTCCTGAATCAGAAACTGAACATGATGGAATGCCTGTTTTCTGG
AGATTCCAGGCGACAGTTGAAGAAGATGGGATAAAAATATTCGCACTTCAATATATAGCTTTTCATCAGACAGAGCATTAT
GCATGGTTGGTTCCTGCGCATTGGATTGTTAATTTTAAACCAGCACCAAATCAGTGGTTACAGGAATGGAAACAAAGGAGA
AATAGATATGCAATTAAGAAAGTAGCAAAAAATGCAGAAAGATCTTTTGCATTCCCAACGAAGAAACTTGCCATTGAAAG
TTTATTGCGCCGGAAGAAATACCATTTAATGAGAATCAAACAAGATTTGGCTGTTGTATCAACTCTTGTTGATGGTATGAAG
AATATTGATACATCAACACCAGATATTGAATATAACTTTGGACACAACCAAGAAACAGAAAATTGGGTATTTTATTAGTAC
GAATAAGCACTGTGTATTCATTCCAACGAGTGAATACACGGAGCAATGTCGCTCGTAACTAAACAGGAGCCGACTTGTTCT
GATTATTGGAAATCTTCTTTGCCCTCCAATGTGAGGGCGATTTTTTATCTGTGAGGATATGAACAGATGTCAAACATCAAAA
AATACATCATTGATTACGACTGGAAAGCATCAATAGAAATTGAAATCGACCATGACGTAATGACAGAGGAAAAACTTCAC
CAGATTAATAATTTCTGGTCAGACTCTGAATACCGACTAAATAAACACGGCTCTGTATTAAATGCTGTATTAATCATGCTGG
CGCAACCTGCTCTGCTT *CTGACTCTTATACACAAGTGCGGCCGCGGATGAGTTGACGAC*

EHEClux #58

GNCAGCAGCACCTTACCTGAAGTACAAAATTCAGAAATGACAACAGAAGCGACATTAAGTGCAGGAGAAAGTTTATTACT
TGGCGGATTTATCCAAGATAAAGAAAGTTCAAGTAAAGATGGTATACCGCTGTTAAGCGACATTCCTGTTATTGGTAGTTT
ATTCTCCAGTACGGTAAAACAAAAACATAGTGTTGTTCGACTCTTTTTAATTAAAGCAACCCCGATAAAATCTGCATCTAGC
GAATAATAAATGAGGCTTTATGAGCAGGAAATTTAGCTCTCTAGAGGATATTTATGATTTCTACCAGGATGGTGGCACATT
AGCGTCATTAACAAATCTGACACAACAAGATCTCAATGACCTTCATTCTTATGCCTATACAGCATATCAATCTGGTGATGTT
ATAACCGCAAGAAATCTATTCCATTTGCTCACATATCTGGAACACTGGAATTATGACTACACCTTATCTCTGGGCTTATGTC
ATCAGCGTTTATCAAATCATGAAGATGCACAACTGTGT *CTGACTCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #61

NCGGCGGATAAACGTTTCTACGCGACCCGTGATATTACCGCAACCGTGGACTCCATCCCGCTGATCACTGCCTCGATCCTG
GCGAAGAAACTGGCGGAAGGTCTGGACGCGCTGGTGATGGACGTGAAAGTGGGTAGCGGCGCGTTTATGCCGACCTACGA
AACCN *NNNNNTNNNNACNCNAAGTGCGGCCGCG*

EHEClux #62

ACTGAGCAGATAATTGGCTTCTTAAAAAATATCATGAATCCAACGGGGAACGGCGTCATTCTGACCGCTTTAGATATCAGG
CGCTATGTGAAGAAAATGATTGAAGGTTCGTTCCCGTC *CTGACTCTTATACACAAGTGCGGCCGCG*

EHEClux #63

NGCAGAGATATCATTNGAATTTGTCCAGGAAAAGCTTTCGACTAACTCTATCGTATATAAGACGAATAAGACTAATCAACA
GCTCGCTCACCTTACAGGTATGGAT CTGACTCTTATACACAAGTGCGGCCGCGCG

EHEClux #66

NTTTCNTTGCTATGCTTACTGGGGGGGCAGACAACATGGGAGAGACATCATGTGGCAGGCAATCAGTCGTCTTTTGAGCGA
GCAGTTAGGTGAAGGC *CTGACTNNTATACACAAGTGCGGCCGCGCGA*

EHEClux #68

GCACTGCTATGCTTACTGGATGGGGTTCGTTTATCACGTAGTCAAATAAACAATATTGCTAAAGAAATGGAGAAGCTAGGA
ATTAAAGTAATAAGGAAAGCAGATAAATATTTGCCACCAAATGCTAGGGCAGCTTTTGATTATGGTCTTCGCAATATTTAT
CTTAGGAAAAATGCTACCTTATATGAGGTGTATCATGAAGTGATTCATGCTAAGCAATTTGCGAAAATTGGACGAGAAGCA
TACGAAGCACTAGGACGTTTAT *CTCTGACTCTTATACACAAGTGCGGCCGCGCG*

EHEClux #69

GGGGGGGGAATTGGTGTTGCCGTCACCGCTGCGCTTCATCGAAAAAATCAGCCGGTAGAACAAACAACAACAACAACTAC
TACAACTACAACTACAAGCGCACGTACGGTAGAGAATAAGCCTGCAAATAATACACCTGCACAGGGCAATGT *CTGACTCTT*
ATACACAAGTGCGGCCGCGC

EHEClux #70

TTTTAAACTTAAGTAGTAATATTTATAGTTCAGACATTACACAGCAAATTGAGGTCATGCGTTGGAATTTCTTTGAGGAAAG
TGGAATTCCATTGCCTAAGATTATTGTTAATCCGGTTAAAAATAATGATAGCGCAATAGAATTTTTGCTCTATCAAGAGTCA
ATATACAAAGATACTCTTATAGATGATACTGTCTATTTTGAGGCTGGGCATGCAGAGATATCATTCGAATTTGTCCAGGAA

AAGCTTTCGACTAACTCTATCGTATATAAGACGAATAAGACTAATCAACAGCTCGCTCACCTTACAGGTATGGATG *CTGAC TCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #71

NNACTGCTATGCTTACTGGATGGTCCAGTCTGGTCGTTATCTGNAT *CTCTTATACACAAGTGCGNCCGCGCGA*

EHEClux #73

NNNNNCTGCTATGCTTACTGGGCNATGTGACGCTTTATGANNGTTTTGTGCCGGTTGAATTCGGTNCGCTNNCNATGG *CTG ACTCTTATACACAAGTGCGGCCGCGC*

EHEClux #76

NNNNNNNTGCTNTGCTTACTGGGGCGCGTTAAAACAGGTACTTCAACACGGCGATCCACGTCGCGTCGCGTGGGATAAAA CAACGCATGACTTCCCCAGCGTCACTGGCGATACCCGCTCGTTGCGCCCGTTAGGTCAAATTCTGCTGGAAAATCAGGTCA TCACTGAAGAACAGCTCGATACAGCACTGCGTAATCGCGTCGAAGGTCTACGCCTGGGC *CTGACTCTTATACACAAGTGCGG CCGCGCG*

EHEClux #78

NNGGGGGTATCATCGGCATGGAGTTTGCCGTCAGTCATGACATAGCCATGAAGCGCCTCTTCCAGCGGAGACAGCAGCCG GCAGCATGCATCCACCCAGCCCGACAGCAGTGAACGCCTCAGCTCCACACCTTGCCGGCCGTATATTTCTGACTGGCGATA CAGCGGGGTGTGCTCTGCATACTTCGAGG *CTGACTCTTATACACAAGTGCGGCCGCGCGA*

EHEClux #81

CTGCTNTGCTTACTGGGGGGGGGCTGAAATATCCTTATGTCGAAGGATTGCGTCTCGACGAAGCAATGCATCCGCTCACAC TGATGACCGTAGGTGTTTATGGCAAGGCGTTACCGCCACAAAATGGCGCGCCGGTGCGACTGATTGTGCCGTGGAAATATG GCTTTAAAGGGATTAAATCGATCGTCAGTATTAAGCTGACCCGCGAGCGTCCGCCAACCACCTGGAATCTGGCAGCGCCTG ACGAATACGGTTTTTACGCCAACGTTAATC *CTGACTCTTATACACAAGTGCGGCCGCGCG*

Transcriptome data

CEGAT procession protocol

S66_1_ProbeI: EHEC sample from LB agar

S66_2_ProbeIII: EHEC sample from LB Symbioflor® agar

a) QUBIT

Invitrogen

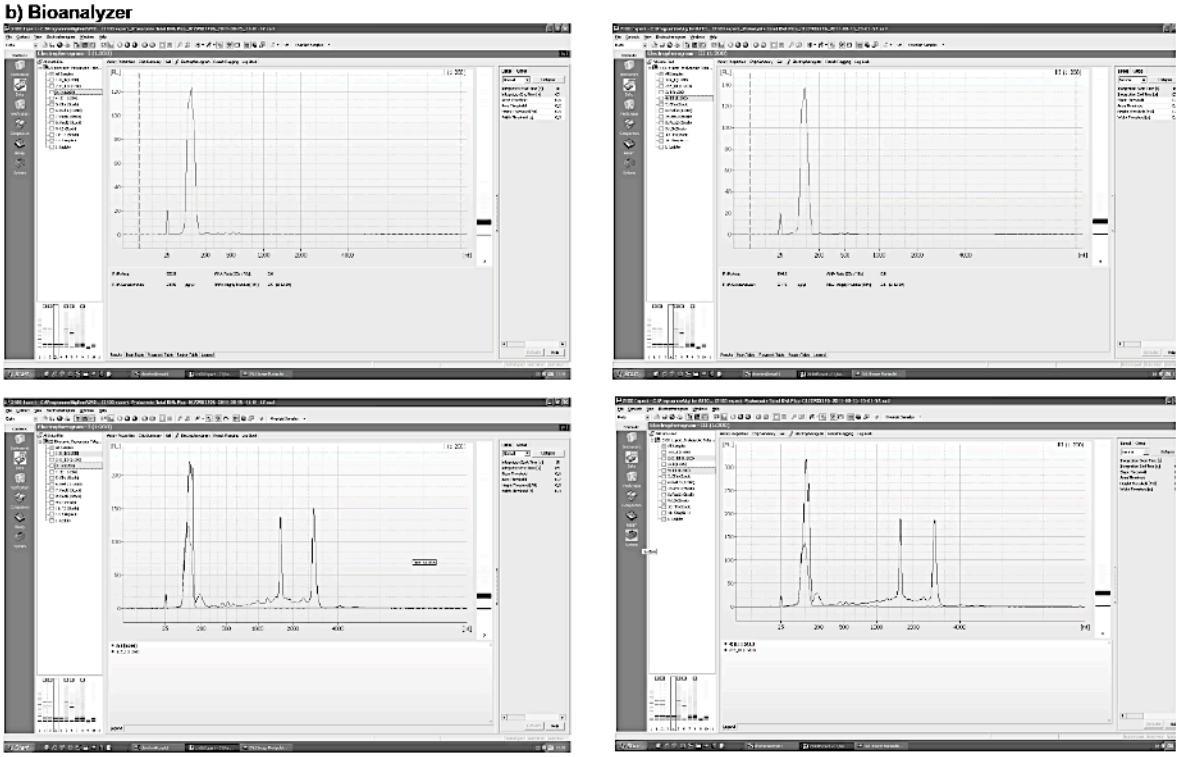
Qubit(TM) Fluorometer

15/08/11 13:11

Quant-iT RNA BR

Sample	Concentration in the Qubit	uL used	Dilution	Sample Concentration	'1:200 (ng/ul	Bioanalyzer	500 ng	H2O ad 8ul
EI	3.79 ug/mL	1	200	757 ng/ul	3.79 1 ul	**ng/ul**		
EIII	4.54 ug/mL	1	200	908 ng/ul	4.54 1 ul	**ng/ul**		
ProbeI				222 ng/ul	1.11 1 ul	415 **ng/ul**	2.25	5.75
ProbeIII				223.7 ng/ul	1.12 1 ul	429.2 **ng/ul**	2.24	5.76

b) Bioanalyzer



Fragmentation of RNA

RNA	8 ul
10 x RnaseIII - Buffer	1 ul
RnaseIII	1 ul

37 degr. Celsius 10 Minutes

Add 90 ul Nuclease-free Water

Clean up of RNA

On Ice

follow protocol RiboMinus Concentration Module

elute RNA in 12 ul Rnase-free Water

QUBIT

Invitrogen
Qubit(TM) Fluorometer
22.08.2011 12:54:23

Quant-iT RNA

Sample	Concentration in the Qubit	uL used	Dilution	Sample Concentration	Ng / 12 ul	Ng / 3 ul
SO066-1_I	306 ng/mL	1	200	61.2 ug/mL	734.4	183.6
SO066-2_III	264 ng/mL	1	200	52.9 ug/mL	634.8	158.7

Hybridize and Ligate RNA

SOLiD Adaptor Mix	2 ul

Hybridization Solution	3 ul

add to 3 ul Fragmented RNA

65 °C	**10 Min**
16 °C	**5 Min**

10 x Ligation Buffer	**10 ul**
Ligation Enzyme Mix	**2 ul**

16 °C	**16 h**

Perform Reverse transcription

Nuclease free H2O	**11 ul**
10 x RT – Buffer	**4 ul**
DNTP – Mix	**2 ul**
SOLiD RT Primer	**2 ul**
	∑ 19 ul

Add 19 ul mastermix to 20 ul Ligation Reaction

70°C	**5 Min**

snap cool on ice

add ArrayScript RT	**1 ul**
42°C	**30 Min**

Purify the cDNA and SizeSelect the cDNA

using Agencourt AMPure XP

1. round of bead capture binds cDNA > 100 bp using 1.8 x addition of Bead Volume

Add 72 ul of Agencourt AMPure XP to 40 ul

mix and incubate 5 Min RT in 1.5 ml tube

magnetic separation 2-5 Minutes

discard supernatant

Wash 2 x 200 ul 70 % EtOH in magnetic stand, remove supernatant

dry bead pellet 5 Min at RT

elute with 40 ul H2O in magnetic stand

2. round of bead capture binds cDNA > 150 bp using 1.6 x addition of Bead Volume

Add 64 ul of Agencourt AMPure XP to 40 ul

mix and incubate 5 Min RT in 1.5 ml tube

magnetic separation 2-5 Minutes

discard supernatant

Wash 2 x 200 ul 70 % EtOH in magnetic stand, remove supernatant

dry bead pellet 5 Min at RT

elute with 40 ul H2O in magnetic stand

Amplify cDNA

Use 5 ul size-selected ss-cDNA in PCR

add		2.5
Nuclease free H2O	33.4	83.5
10 x PCR Buffer	5	12.5
dNTP Mix	4	10
SOLiD 5' PCR primer	1	2.5
AmpliTaq DNA Polymerase	0.6	1.5
	∑ 44 ul	
S66-1_Probe_I	1 ul	3' Primer BC9
S66-2_Probe_III	1 ul	3' Primer BC10

Hold

Cycle (15 cycles)

	95 °C	5 Min
	95 °C	30 Sek.
	62 °C	30 Sek.
	72 °C	30 Sek.
	72 °C	7 Min.

Purification cDNA

using PureLink PCR Micro Kit

End QC

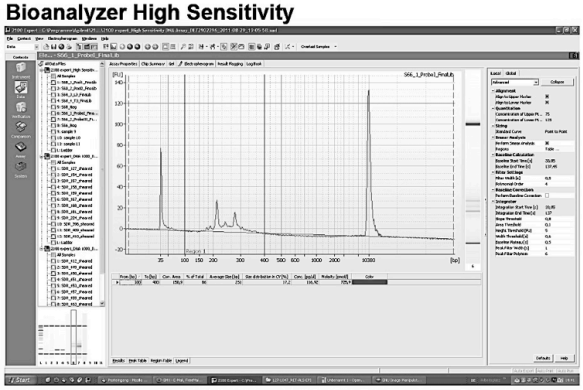
Invitrogen
Qubit(TM) Fluorometer
29.08.2011 18:47:38

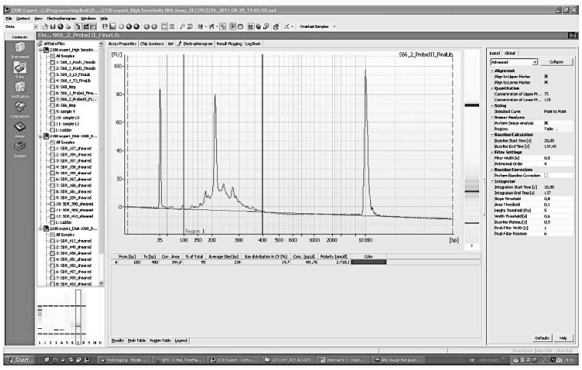
Quant-iT dsDNA	HS

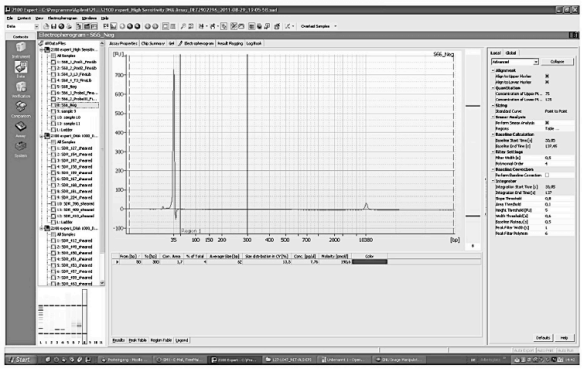
Library Prep

Sample	Concentration in the Qubit	uL used	Dilution	Sample Concentration	
S66-1_Probe_I	1.38 ng/mL	1	200	0.28 ug/mL	
S66-2_Probe_III	2.34 ng/mL	1	200	0.47 ug/mL	

Bioanalyzer High Sensitivity







S66-1_Probe_I

Average Size	251 bp	passed
Concentration	116.92 pg/ul	passed
Mol.-Concentration	725.9 pM	passed

S66-2_Probe_III

Average Size	234 bp	passed
Concentration	401.76 pg/ul	passed
Mol.-Concentration	2718.1 pM	passed

						ePCR			
Sample ID	Barcode	Average Size	Concentration [pg/ul]	Mol.-Concen-tration [pM]		percent from Full Slide (708 M)		calculated number reads	calculated number of mappable reads
S66-1_Probe_I	BC9	251	116.92	725.9	1 Oct	12.50%	6.25%	44,250,000	26,550,000
S66-2_Probe_III	BC10	234	401.76	2718.1			6.25%	44,250,000	26,550,000
S72-1_Efm	BC7	225	4486	36744	1 Oct	12.50%	6.25%	44,250,000	26,550,000
S72-2_Refil	BC8	275	11036	68500			6.25%	44,250,000	26,550,000
SO068_1: Human Pool1 (2. PCR +2)	BC11	117	26.04	356.7	1 Oct	12.50%	3.13%	22,125,000	13,275,000
SO068_2: Human Pool2 (1. PCR + 5)	BC12	113	365.35	4962.8			3.13%	22,125,000	13,275,000
SO068_3: Maus_L3	BC13	130	803.78	9702			3.13%	22,125,000	13,275,000
SO068_4: Maus_T3	BC16	125	1166.02	14220.1			3.13%	22,125,000	13,275,000
SO078_1_1190	BC1	264	799.1	4616	3 Oct	37.50%	9.38%	66,375,000	39,825,000
SO078_1_1190	BC2	262	923.84	5379.6			9.38%	66,375,000	39,825,000
SO078_1_1190	BC3	259	857.25	5022.1			9.38%	66,375,000	39,825,000
SO078_1_1190	BC4	258	799.75	4713.3			9.38%	66,375,000	39,825,000
S073_19_20000107	BC5	253	484.14	2908	1 Oct	12.50%	6.25%	44,250,000	26,550,000
S073_20_10-205	BC6	245	496.7	3072.7			6.25%	44,250,000	26,550,000
SDNMD_798	BC14	282	3209.49	17330.5	1 Oct	12.50%	6.25%	44,250,000	26,550,000
SDNMD_893	BC15	273	3039.86	16981			6.25%	44,250,000	26,550,000
						100.00%	100.00%	708,000,000	424,800,000

22 Berechnung (Bioanalyzer based)

Lib conc. (pM)	500
Volume (ul)	200

	Konzentration pg/ul	Konzentration pg/ul	pM (für 215 bp)	Molarity pM	pM (für Av Size bp)	Av. Size / bp		Final Molarity pM	ul fuer Final 500 pM				
Sample ID	OUBIT	Bioanalyzer	ABI	Bioanalyzer	berechnet	Bioanalyzer	Vorverdünnung	Bioanalyzer	DNA	1x low TE	Dil.Fact	part pM	pM/ Library
S66-1_Probe_I	-	116.92	823	725.9	706	251	1	725.9	8.6		23.2	31.3	31.3
S66-2_Probe_III	-	401.76	2829	2718.1	2601	234	1	2718.1	2.3		87.0	31.3	31.3
S72-1_Efm	-	4486	31592	36744	30209	225	10	3674.4	1.7		117.6	31.3	31.3
S72-2_Refil	-	11036	77718	68500	60804	275	10	6850	0.9		219.2	31.3	31.3
SO068_1: Human Pool1 (2. PCR +2)	-	26.04	183	356.7	337	117	1	356.7	8.8		22.8	15.6	15.6
SO068_2: Human Pool2 (1. PCR + 5)	-	365.35	2573	4962.8	4899	113	10	496.28	6.3		31.8	15.6	15.6
SO068_3: Maus_L3	-	803.78	5660	9702	9368	130	10	970.2	3.2		62.1	15.6	15.6
SO068_4: Maus_T3	-	1166.02	8211	14220.1	14134	125	10	1422.01	2.2		91.0	15.6	15.6
SO078_1_1190	-	799.1	5627	4616	4586	264	1	4616	2.0		98.5	46.9	46.9
SO078_1_1190	-	923.84	6506	5379.6	5343	262	1	5379.6	1.7		114.8	46.9	46.9
SO078_1_1190	-	857.25	6037	5022.1	5015	259	1	5022.1	1.9		107.1	46.9	46.9
SO078_1_1190	-	799.75	5632	4713.3	4697	258	1	4713.3	2.0		100.6	46.9	46.9
S073_19_20000107	-	484.14	3409	2908	2899	253	1	2908	2.1		93.1	31.3	31.3
S073_20_10-205	-	496.7	3498	3072.7	3072	245	1	3072.7	2.0		98.3	31.3	31.3
SDNMD_798	-	3209.49	22602	17330.5	17244	282	10	1733.05	3.6		55.5	31.3	31.3
SDNMD_893	-	3039.86	21407	16981	16871	273	10	1698.1	3.7	146.9	54.3	31.3	31.3
										200.00		500	500

22 ePCR

		Date
E120 pM =	0.4	

Aqueous Master Mix	(µl)	47950.6
1:10 diluted P1 Reagent	(µl)	200
undiluted P2 Reagent	(µl)	300
Library template	(µl)	49.4
P1 Beads	(µl)	1430
Vol. Ges.	(µl)	49930
Oil Master Mix	(g)	67.9
P1 Reagent	(µl)	21
1x Low TE Buffer	(µl)	189

BeadsPreEnrich

25 WFA

Sample ID	N2S	On Axis	P1 (median per panel)	Number P1 of beads	P2 (median per panel)	Number P2 of beads	P2/P1 – ratio	P2/P1 – ratio (calc.)	P1 beads/µl	P2 positive/per ul	842.4 ul P1 positive	M beads ul P2 positive	Millions P1	Millions P2
S66_S68_	7%	84%	34,702	###	34,652	###	100%	99.86%	739,153	738,088	1139.68	1141.33	842.4	841.19

FC1

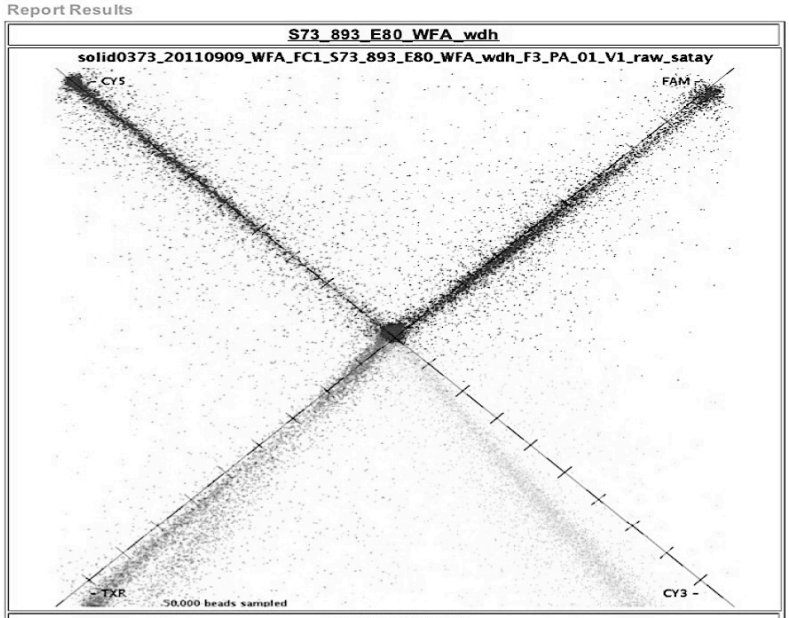
Report Summary

Name: Template Bead Titration and Selection Report for: solid0373_20110909_WFA_FC1

Date Created: Fri Sep 09 14:37:30 CEST 2011

Created By: corona

Report Results



Analysis Results	
P2_Rfu	694287
P2_Exp	22
P2_Gain	16
P1# (median per panel)	34702
P2# (median per panel)	34652
P2#/P1# ratio	100%
N2S	7%
On Axis	84%

BarcodeStatistic

##Library	Barcode	0 Mismatches	1 Mismatch	Total	**Count**
S66_1_Probe_I	Subtotals	27,745,157	1,510,400	29,255,557	**28,044,046**
S66_2_Probe_III	Subtotals	22,492,256	973,455	23,465,711	**22,641,378**

Expression pattern of EHEC EDL933 transcriptome NGS

Tab. 50: Genes ≤ 3-fold significantly down-regulated by LB Symbioflor® (EDL933 chromosome and pO157 plasmid).

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
yaaI	Z0012	hypothetical protein	8.1	1.1	-2.8
lspA	Z0031	lipoprotein signal peptidase	51.1	17.9	-1.4
yaaF	Z0035	ribonucleoside hydrolase RihC	11.7	0.6	-4.3
carA	Z0037	carbamoyl phosphate synthase small subunit	18.7	6.6	-1.4
carB	Z0038	carbamoyl phosphate synthase large subunit	12.1	3.4	-1.7
araC	Z0073	DNA-binding transcriptional regulator AraC	9.3	3.0	-1.6
ilvH	Z0088	acetolactate synthase 3 regulatory subunit	14.1	3.5	-1.9
yacG	Z0111	zinc-binding protein	43.5	12.3	-1.8
yadI	Z0140	PTS enzyme II B component	26.5	7.9	-1.7
-	Z0342	LysR-like transcriptional regulator	8.8	1.7	-2.3
ykgD	Z0382	ARAC-type regulatory protein	12.0	2.2	-2.4
-	Z0394	hypothetical protein	19.2	3.3	-2.5
-	Z0442	AraC-like transcriptional regulator	7.7	1.5	-2.3
yaiN	Z0457	regulator protein FrmR	44.4	15.2	-1.5
copA	Z0604	copper exporting ATPase	84.8	23.8	-1.8
ybbJ	Z0641	hypothetical protein	38.4	7.2	-2.3
ybdJ	Z0719	hypothetical protein	79.2	28.6	-1.4
ybfA	Z0846	hypothetical protein	391.2	123.2	-1.6
nadA	Z0919	quinolinate synthetase	7.6	1.0	-2.9
galE	Z0929	UDP-galactose-4-epimerase	9.2	2.0	-2.1
modA	Z0933	molybdate transporter periplasmic protein	28.0	10.3	-1.4
modB	Z0934	molybdate ABC transporter permease protein	36.0	4.8	-2.8
modC	Z0935	molybdate transporter ATP-binding protein	17.1	5.4	-1.6
-	Z0955	hypothetical protein	10.0	2.0	-2.3
ybhO	Z1008	cardiolipin synthase 2	5.4	0.7	-2.9
mipB	Z1048	fructose-6-phosphate aldolase	8.6	2.6	-1.6
-	Z1054	transport protein	5.5	0.8	-2.7
-	Z1071	hypothetical protein	19.4	5.9	-1.6
-	Z1333	DicA, regulator of DicB; encoded within cryptic prophage CP-933M	397.2	125.6	-1.6
etp	Z1399	phosphotyrosine-protein phosphatase	18.3	3.5	-2.3
putA	Z1513	trifunctional transcriptional regulator/proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	6.4	1.4	-2.1
-	Z1604	hypothetical protein	6.3	2.0	-1.6
-	Z1628	hypothetical protein	7.5	1.8	-2.0

-	Z1645	hypothetical protein	7.5	1.3	-2.4
csgD	Z1673	DNA-binding transcriptional regulator CsgD	25.4	8.5	-1.5
csgA	Z1676	cryptic curlin major subunit	8.5	1.5	-2.4
-	Z1687	hypothetical protein	35.1	7.0	-2.3
flgA	Z1710	flagellar basal body P-ring biosynthesis protein FlgA	55.4	20.5	-1.4
flgH	Z1717	flagellar basal body L-ring protein	5.4	1.0	-2.4
-	Z1766	hypothetical protein	5.2	1.0	-2.3
-	Z1846	hypothetical protein	32.5	4.6	-2.8
phoQ	Z1858	sensor protein PhoQ	23.1	6.8	-1.7
ycgK	Z1940	hypothetical protein	21.8	3.9	-2.4
yciD	Z2034	outer membrane protein W	78.7	25.0	-1.6
-	Z2189	LACI-type transcriptional regulator	33.9	12.4	-1.4
-	Z2251	hypothetical protein	9.4	1.2	-2.9
ycjM	Z2475	putative polysaccharide hydrolase	8.0	2.7	-1.5
trpD	Z2548	bifunctional glutamine amidotransferase/anthranilate phosphoribosyltransferase	6.4	1.0	-2.6
-	Z2557	outer membrane protein	25.7	6.4	-1.9
-	Z2576	oxidoreductase, major subunit	5.2	0.9	-2.5
tqsA	Z2595	transport protein	36.2	12.9	-1.4
-	Z2658	hypothetical protein	12.3	2.9	-2.0
ydhE	Z2690	multidrug efflux protein	47.7	13.5	-1.7
ydhU	Z2697	hypothetical protein	6.7	0.9	-2.9
-	Z2700	hypothetical protein	6.0	1.6	-1.8
-	Z2708	bifunctional cysteine desulfurase/selenocysteine lyase	6.3	2.0	-1.6
ydjC	Z2763	hypothetical protein	11.7	2.3	-2.3
celB	Z2767	PTS system N,N'-diacetylchitobiose-specific transporter subunit IIC	6.7	2.3	-1.5
-	Z2789	thiosulfate sulfur transferase	5.8	1.3	-2.1
topB	Z2796	DNA topoisomerase III	40.9	13.1	-1.6
yeaI	Z2825	hypothetical protein	8.0	1.3	-2.6
manY	Z2861	PTS enzyme IIC, mannose-specific	36.8	13.0	-1.4
-	Z2888	resistance protein	34.7	7.9	-2.1
flhA	Z2932	flagellar biosynthesis protein FlhA	8.2	2.2	-1.8
cheY	Z2936	chemotaxis regulatory protein CheY	19.2	2.7	-2.8
yecG	Z2948	universal stress protein UspC	19.8	4.4	-2.1
-	Z3065	hypothetical protein	148.9	40.0	-1.8
-	Z3120	hypothetical protein	24.0	7.2	-1.7
-	Z3374	hypothetical protein	10.3	1.3	-2.9
sanA	Z3399	hypothetical protein	41.1	12.3	-1.7
-	Z3508	hypothetical protein	9.1	2.5	-1.8
ais	Z3510	protein induced by aluminum	28.6	10.1	-1.4
-	Z3618	hypothetical protein	6.2	0.7	-3.0

	Z3673		5.6	1.0	-2.4
cysA	Z3687	sulfate/thiosulfate transporter subunit	28.1	8.2	-1.7
cysP	Z3690	thiosulfate transporter subunit	21.4	6.7	-1.6
yfeT	Z3692	hypothetical protein	8.7	2.8	-1.6
purN	Z3763	phosphoribosylglycinamide formyltransferase	16.7	4.3	-1.9
pbpC	Z3786	penicillin-binding protein 1C	29.4	10.9	-1.4
stpA	Z3968	DNA binding protein, nucleoid-associated	21.6	6.9	-1.6
ygaH	Z3984	hypothetical protein	31.0	10.3	-1.5
cysN	Z4059	sulfate adenylyltransferase subunit 1	10.4	2.9	-1.8
cysD	Z4060	sulfate adenylyltransferase subunit 2	19.1	5.3	-1.8
cysH	Z4072	phosphoadenosine phosphosulfate reductase	28.2	9.4	-1.5
cysJ	Z4074	sulfite reductase subunit alpha	12.7	3.4	-1.8
syd	Z4110	SecY interacting protein Syd	35.9	11.8	-1.5
ygdL	Z4129	enzyme	13.3	2.8	-2.2
mutH	Z4149	DNA mismatch repair protein	7.7	2.3	-1.7
-	Z4215	hypothetical protein	9.4	2.0	-2.2
bglA	Z4239	6-phospho-beta-glucosidase BglA	12.6	3.7	-1.7
yqgF	Z4294	Holliday junction resolvase-like protein	25.7	7.9	-1.6
(efa1/lifA)	Z4332	cytotoxin	5.7	0.7	-3.0
hybA	Z4350	hydrogenase 2 protein HybA	8.0	1.4	-2.4
-	Z4351	hydrogenase 2 small subunit	53.4	15.0	-1.8
-	Z4374	binding protein	14.6	5.0	-1.5
mdaB	Z4379	modulator of drug activity B	26.5	8.9	-1.5
tdcC	Z4468	threonine/serine transporter TdcC	6.5	1.8	-1.8
tdcA	Z4470	DNA-binding transcriptional activator TdcA	20.6	7.0	-1.5
yhaG	Z4480	hydrolase	5.2	1.7	-1.6
yheL	Z4701	sulfur transfer complex subunit TusB	128.3	34.3	-1.8
yheT	Z4714	hydrolase	11.4	3.7	-1.5
yheU	Z4715	hypothetical protein	34.9	8.7	-1.9
-	Z4734m	fructoselysine 6-kinase	6.0	1.5	-1.9
-	Z4867	holo-(acyl carrier protein) synthase 2	9.4	3.0	-1.6
-	Z4875	phosphotransferase system enzyme subunit	15.5	4.8	-1.6
pitA	Z4893	low-affinity phosphate transport	128.0	42.2	-1.5
chuW	Z4914	coproporphyrinogen III oxidase	5.6	1.2	-2.2
chuU	Z4918	permease of iron compound ABC transport system	6.2	1.9	-1.6
IpefE	Z4965	fimbrial subunit	12.6	2.9	-2.0
lpfA	Z4971	major fimbrial subunit	11.4	1.6	-2.7
yiaH	Z4986	hypothetical protein	26.5	4.9	-2.4
selA	Z5012	selenocysteine synthase	10.4	3.4	-1.6
yibQ	Z5041	hypothetical protein	22.8	5.8	-1.9
slmA	Z5065	nucleoid occlusion protein	33.3	12.2	-1.4

yicE	Z5082	transport protein	12.2	1.7	-2.7
espD	Z5106	hypothetical protein	8.4	2.2	-1.9
espA	Z5107	hypothetical protein	17.8	4.8	-1.8
eae	Z5110	intimin adherence protein	6.3	2.3	-1.4
-	Z5113	hypothetical protein	23.4	6.8	-1.7
sepQ	Z5116	hypothetical protein	9.5	1.7	-2.4
sepZ	Z5122	hypothetical protein	121.3	21.4	-2.4
-	Z5137	hypothetical protein	10.9	2.3	-2.2
-	Z5150	hypothetical protein	26.3	3.4	-2.9
uhpA	Z5159	DNA-binding transcriptional activator UhpA	6.3	0.9	-2.8
yidL	Z5175	ARAC-type regulatory protein	6.0	1.7	-1.8
ibpA	Z5183	heat shock protein IbpA	579.5	215.8	-1.4
yidR	Z5185	hypothetical protein	5.3	1.1	-2.2
yidS	Z5186	oxidoreductase	6.3	1.8	-1.7
-	Z5199	hypothetical protein	37.5	4.7	-2.9
yifB	Z5277	ATP-dependent protease	6.4	2.0	-1.6
ilvL	Z5278	ilvG operon leader peptide	934.5	329.4	-1.4
rffH	Z5300	glucose-1-phosphate thymidylyltransferase	12.0	3.7	-1.6
yigJ	Z5344	hypothetical protein	25.2	8.5	-1.5
yihD	Z5390	hypothetical protein	254.2	90.6	-1.4
yihN	Z5410	resistance protein (transport)	5.8	0.8	-2.8
hslU	Z5478	ATP-dependent protease ATP-binding subunit HslU	92.5	25.7	-1.8
htrC	Z5563	heat shock protein C	46.3	15.2	-1.5
malG	Z5630	maltose transporter permease	40.5	13.8	-1.5
malK	Z5633	maltose/maltodextrin transporter ATP-binding protein	65.7	24.4	-1.4
lamB	Z5634	maltoporin	63.9	19.4	-1.6
frdA	Z5762	fumarate reductase flavoprotein subunit	13.6	4.7	-1.5
nrdD	Z5848	anaerobic ribonucleoside triphosphate reductase	9.6	3.3	-1.5
-	Z5852	hypothetical protein	182.3	62.4	-1.5
pyrI	Z5855	aspartate carbamoyltransferase regulatory subunit	11.7	2.6	-2.1
fimB	Z5910	tyrosine recombinase	34.1	7.2	-2.2
iraD	Z5925	DNA replication/recombination/repair protein	14.5	1.7	-3.0
-	Z5945	endoribonuclease SymE	25.2	3.0	-3.0
-	Z6078	inhibitor of cell division encoded by cryptic prophage CP-933P	26.0	8.3	-1.6

Tab. 51: Genes ≤ 3 fold significantly up-regulated by LB Symbioflor® (EDL933 chromosome and pO157 plasmid).

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
yabN	Z0079	transcriptional regulator SgrR	1.7	5.1	1.7
yadN	Z0152	fimbrial protein	1.6	6.6	2.1
yadB	Z0155	glutamyl-Q tRNA(Asp) synthetase	7.3	23.4	1.8
yaeJ	Z0203	peptidyl-tRNA hydrolase domain protein	3.7	15.6	2.1
cutF	Z0204	lipoprotein involved with copper homeostasis and adhesion	1.8	10.7	2.7
-	Z0272	hypothetical protein	2.4	9.4	2.0
fadE	Z0278	acyl-CoA dehydrogenase	13.3	59.2	2.2
dinP	Z0292	DNA polymerase IV	2.2	10.3	2.3
yafN	Z0293	antitoxin of the YafO-YafN toxin-antitoxin system	6.1	24.2	2.0
intH	Z0307	integrase for prophage CP-933H	4.4	14.1	1.7
aroM	Z0486	hypothetical protein	12.1	60.1	2.4
-	Z0510	hypothetical protein	38.4	113.2	1.6
pgpA	Z0520	phosphatidylglycerophosphatase A	1.6	6.4	2.1
ybaA	Z0568	hypothetical protein	28.3	87.7	1.7
ybaK	Z0600	hypothetical protein	1.2	7.6	2.8
ybaS	Z0606	glutaminase	15.5	54.8	1.9
ybbA	Z0648	ABC transporter ATP-binding protein YbbA	2.0	6.1	1.7
entB	Z0737	2,3-dihydro-2,3-dihydroxybenzoate synthetase, isochroismatase	2.1	7.3	1.9
ybdB	Z0739	hypothetical protein	1.0	6.7	2.8
dsbG	Z0748	disulfide isomerase/thiol-disulfide oxidase	6.0	17.6	1.6
ybdR	Z0752	oxidoreductase	3.9	13.7	1.9
phpB	Z0785	alpha-ribazole phosphatase	3.6	11.6	1.8
ybgA	Z0858	hypothetical protein	7.1	35.4	2.4
pnuC	Z0920	required for NMN transport	9.6	24.6	1.4
-	Z0923	homeobox protein	84.2	306.9	1.9
-	Z0984	hypothetical protein	6.7	21.9	1.8
ybhB	Z0992	kinase inhibitor protein	23.3	61.8	1.5
	Z1051		2.0	11.0	2.5
rimO	Z1061	ribosomal protein S12 methylthiotransferase	6.9	17.7	1.4
grxA	Z1076	glutaredoxin 1	8.1	30.9	2.0
potF	Z1081	putrescine transporter subunit: periplasmic-binding component of ABC superfamily	6.2	18.2	1.6
potG	Z1082	putrescine transporter ATP-binding subunit	1.2	5.0	2.1
artJ	Z1090	arginine 3rd transport system periplasmic binding protein	13.8	40.8	1.6
artQ	Z1092	arginine transporter permease subunit ArtQ	7.0	26.1	2.0

artI	Z1093	arginine 3rd transport system periplasmic binding protein	49.9	183.9	2.0
artP	Z1094	arginine transporter ATP-binding subunit	25.5	93.0	1.9
poxB	Z1105	pyruvate dehydrogenase	3.1	9.4	1.7
-	Z1124	prophage regulatory protein	4.2	12.8	1.7
-	Z1150	hypothetical protein	2.4	12.2	2.4
-	Z1192	IS1 protein InsB	0.8	5.5	2.8
-	Z1195	hypothetical protein	2.7	8.9	1.8
focA	Z1250	formate transporter	21.2	68.1	1.8
ycaP	Z1252	hypothetical protein	6.2	16.3	1.5
-	Z1320	acylphosphatase	10.0	44.8	2.2
yccD	Z1417	chaperone-modulator protein CbpM	3.6	18.7	2.4
cbpA	Z1418	curved DNA-binding protein CbpA	7.8	33.3	2.2
-	Z1441	hypothetical protein	27.1	114.2	2.1
-	Z1498	hypothetical protein	33.0	88.0	1.5
ycdB	Z1521	hypothetical protein	2.0	10.6	2.5
-	Z1531	hypothetical protein	14.2	42.7	1.7
-	Z1534	chaperone	0.8	5.0	2.7
-	Z1535	hypothetical protein	1.6	5.2	1.8
-	Z1589	hypothetical protein	1.2	8.6	2.9
terB_2	Z1612	phage inhibition, colicin resistance and tellurite resistance protein	0.9	5.7	2.7
-	Z1635	hypothetical protein	2.7	11.1	2.1
-	Z1690	lipid A biosynthesis lauroyl acyltransferase	2.0	7.0	1.9
pabC	Z1735	4-amino-4-deoxychorismate lyase	8.4	20.8	1.4
ycfX	Z1760	N-acetyl-D-glucosamine kinase	5.9	15.2	1.4
pepT	Z1832	peptidase T	23.9	57.3	1.3
-	Z1863	phosphohydrolase	9.0	27.0	1.7
-	Z1922	hypothetical protein	10.7	27.0	1.4
-	Z1923	hypothetical protein	6.8	32.2	2.3
ycgB	Z1951	SpoVR family protein	41.6	98.6	1.3
lolB	Z1980	outer membrane lipoprotein LolB	4.5	25.0	2.6
-	Z2083	hypothetical protein	7.4	24.8	1.8
ydeI	Z2162	hypothetical protein	31.1	76.4	1.4
marB	Z2169	hypothetical protein	1.3	8.7	2.9
-	Z2180	hypothetical protein	1.5	10.1	2.8
-	Z2181	hypothetical protein	4.2	25.2	2.7
-	Z2182	hypothetical protein	8.5	29.8	1.9
-	Z2185	hypothetical protein	2.6	7.9	1.7
-	Z2186	trans-aconitate 2-methyltransferase	1.3	9.4	2.9
gadB	Z2215	glutamate decarboxylase isozyme	44.4	135.3	1.7
-	Z2223	hemin-binding lipoprotein	5.1	13.0	1.4

osmC	Z2228	osmotically inducible protein	131.8	407.6	1.7
-	Z2229	biofilm-dependent modulation protein	70.7	373.5	2.5
-	Z2269	DNA-binding transcriptional regulator	6.3	16.8	1.5
tehA	Z2289	potassium-tellurite ethidium and proflavin transporter	4.8	12.2	1.4
-	Z2297	hypothetical protein	5.9	22.8	2.0
gapC	Z2304	glyceraldehyde-3-phosphate dehydrogenase	10.4	25.6	1.4
-	Z2366	hypothetical protein	2.5	17.7	2.9
-	Z2442	hypothetical protein	2.4	11.5	2.3
pspD	Z2478	peripheral inner membrane phage-shock protein	16.9	57.8	1.8
puuD	Z2490	gamma-glutamyl-gamma-aminobutyrate hydrolase	15.7	45.5	1.6
yciG	Z2553	hypothetical protein	36.8	160.7	2.2
yciF	Z2554	structural proteins	21.9	72.4	1.8
-	Z2569	hypothetical protein	1.7	8.5	2.4
-	Z2593	multidrug efflux system protein MdtI	12.6	41.5	1.8
ydgA	Z2617	hypothetical protein	14.9	43.7	1.6
-	Z2631	oriC-binding nucleoid-associated protein	3.2	16.1	2.4
tyrS	Z2650	tyrosyl-tRNA synthetase	63.6	187.0	1.6
sodC	Z2661	superoxide dismutase	17.8	52.2	1.6
-	Z2691	hypothetical protein	5.2	23.5	2.2
sufA	Z2712	iron-sulfur cluster assembly scaffold protein	4.1	25.4	2.7
ydiE	Z2734	hypothetical protein	8.7	36.1	2.1
-	Z2735	hypothetical protein	8.8	30.5	1.9
btuD	Z2738	vitamin B12-transporter ATPase	1.1	7.6	2.9
btuE	Z2739	glutathione peroxidase	6.5	28.6	2.2
pfkB	Z2752	6-phosphofructokinase 2	3.1	9.5	1.7
astD	Z2778	succinylglutamic semialdehyde dehydrogenase	10.9	32.1	1.6
argD	Z2780	bifunctional succinylornithine transaminase/acetylornithine transaminase	21.7	59.4	1.5
yeaJ	Z2826	hypothetical protein	3.9	11.3	1.6
-	Z2853	hypothetical protein	6.9	20.2	1.6
-	Z2863	hypothetical protein	3.3	10.2	1.7
-	Z2873	hypothetical protein	12.2	90.4	3.0
-	Z2883	hypothetical protein	192.9	540.4	1.6
flhD	Z2946	transcriptional activator FlhD	8.9	33.2	2.0
otsA	Z2949	trehalose-6-phosphate synthase	17.1	52.7	1.7
otsB	Z2950	trehalose-6-phosphate phosphatase	19.1	82.5	2.2
tyrP	Z2963	tyrosine-specific transport system	5.3	34.4	2.8
intT	Z2966	integrase for prophage CP-933T	4.0	17.7	2.2
-	Z2994	hypothetical protein	8.8	39.7	2.2
amyA	Z3017	cytoplasmic alpha-amylase	9.6	25.6	1.5
-	Z3043	hypothetical protein	89.7	269.8	1.7

-	Z3062	hypothetical protein	3.7	19.7	2.5
wcaD	Z3220	colanic acid biosynthesis protein	0.9	5.4	2.6
-	Z3243	multidrug efflux system subunit MdtA	1.2	5.3	2.2
-	Z3260	fructose-bisphosphate aldolase	134.7	358.3	1.5
thiM	Z3268	hydroxyethylthiazole kinase	31.5	96.1	1.7
-	Z3306	hypothetical protein	7.2	23.8	1.8
-	Z3307	tail fiber protein encoded within prophage CP-933V	3.0	19.9	2.8
-	Z3363	single-stranded DNA binding protein of prophage CP-933V	6.4	32.0	2.4
yehW	Z3377	transport system permease protein	1.3	5.5	2.1
yehZ	Z3380	transport system permease protein	12.1	34.2	1.6
yohC	Z3384	hypothetical protein	12.3	35.7	1.6
-	Z3394	transporter	96.3	356.3	2.0
-	Z3400	hypothetical protein	4.1	22.4	2.5
napC	Z3459	cytochrome c-type protein NapC	2.8	15.2	2.5
elaB	Z3526	hypothetical protein	215.6	590.7	1.5
yfbM	Z3533	hypothetical protein	3.6	13.1	1.9
nuoK	Z3538	NADH dehydrogenase subunit K	16.5	51.5	1.7
-	Z3640	hypothetical protein	3.1	9.5	1.7
-	Z3658	manganese transport protein MntH	7.8	26.0	1.8
-	Z3672	hypothetical protein	4.5	14.7	1.8
ligA	Z3677	NAD-dependent DNA ligase LigA	3.3	17.0	2.4
pdxK	Z3684	pyridoxal kinase	8.8	23.0	1.5
-	Z3699	acetyltransferase	7.8	22.0	1.6
-	Z3731	hypothetical protein	11.7	44.9	2.0
-	Z3769	outer membrane lipoprotein	3.2	11.4	1.9
guaB	Z3772	inosine 5'-monophosphate dehydrogenase	21.3	51.6	1.3
yfhJ	Z3791	hypothetical protein	10.4	50.9	2.4
csiE	Z3804	stationary phase inducible protein CsiE	33.0	110.5	1.8
-	Z3866	hypothetical protein	8.2	26.4	1.8
rluD	Z3888	23S rRNA pseudouridine synthase D	8.4	31.1	2.0
aroF	Z3893	phospho-2-dehydro-3-deoxyheptonate aldolase	12.6	37.3	1.6
nleG8-2	Z3919	hypothetical protein	1.9	8.8	2.3
-	Z3966	hypothetical protein	22.7	57.8	1.4
emrB	Z3987	multidrug resistance protein B	1.2	5.9	2.4
fucK	Z4120	L-fuculokinase	1.1	7.5	2.9
yqeI	Z4167	sensory transducer	1.5	5.4	1.9
-	Z4201	hypothetical protein	1.6	5.4	1.8
dsbC	Z4231	thiol:disulfide interchange protein DsbC	16.4	40.7	1.4
yghA	Z4356	oxidoreductase	3.9	18.2	2.3
dkgA	Z4365	2,5-diketo-D-gluconate reductase A	29.7	79.0	1.5

ygiW	Z4376	hypothetical protein	20.5	54.7	1.5
ygjL	Z4434	NADPH dehydrogenase	5.6	22.3	2.1
yqjG	Z4456	transferase	15.1	41.1	1.5
yhbO	Z4512	hypothetical protein	16.6	117.8	2.9
yhbQ	Z4516	GIY-YIG nuclease superfamily protein	2.3	8.6	2.0
yrbA	Z4553	hypothetical protein	15.4	41.1	1.5
-	Z4803	ATP-dependent DNA helicase (together with adjacent 3 orfs)	6.6	17.1	1.4
yhhA	Z4815	hypothetical protein	16.7	57.0	1.8
ugpQ	Z4817	cytoplasmic glycerophosphodiester phosphodiesterase	1.7	6.3	2.0
yhiI	Z4886	hypothetical protein	2.0	11.0	2.6
yhiJ	Z4887	hypothetical protein	2.0	5.3	1.5
yhiM	Z4890	hypothetical protein	11.1	28.2	1.4
hdeB	Z4921	acid-resistance protein	42.6	144.8	1.8
hdeD	Z4923	acid-resistance membrane protein	52.1	260.7	2.4
yhiE	Z4925	hypothetical protein	271.5	1047.1	2.0
yhiU	Z4926	multidrug efflux system protein MdtE	11.6	31.6	1.5
yhiX	Z4929	DNA-binding transcriptional regulator GadX	57.6	191.8	1.8
yhjG	Z4937	hypothetical protein	1.5	9.3	2.7
IpfD	Z4966	fimbrial protein	1.6	7.7	2.4
yiaC	Z4975	hypothetical protein	1.9	7.9	2.1
yiaD	Z4977	outer membrane lipoprotein	1.7	5.5	1.8
yiaG	Z4980	transcriptional regulator	139.4	354.1	1.4
dinD	Z5070	DNA-damage-inducible protein D	9.9	34.3	1.9
-	Z5138	hypothetical protein	3.0	12.8	2.2
yicL	Z5146	permease transporter	5.4	13.7	1.4
yidB	Z5189	hypothetical protein	12.6	33.2	1.5
asnC	Z5244	DNA-binding transcriptional regulator AsnC	4.2	20.0	2.3
-	Z5294	hypothetical protein	3.2	17.4	2.5
fadA	Z5366	3-ketoacyl-CoA thiolase	18.0	42.8	1.3
yiiF	Z5432	hypothetical protein	1.7	8.6	2.4
yiiR	Z5466	hypothetical protein	12.0	31.9	1.5
yijE	Z5498	hypothetical protein	2.2	7.6	1.8
nrfA	Z5669	cytochrome c552	1.6	7.0	2.2
yjdJ	Z5729	hypothetical protein	14.2	46.4	1.8
sugE	Z5755	suppresses groEL, may be chaperone	33.5	94.2	1.6
aidB	Z5794	isovaleryl CoA dehydrogenase	3.3	8.7	1.5
chpS	Z5835	antitoxin ChpS	3.2	13.4	2.1
chpB	Z5836	toxin ChpB	13.9	37.1	1.5
pyrB	Z5856	aspartate carbamoyltransferase catalytic subunit	1.6	5.0	1.7
argI	Z5866	ornithine carbamoyltransferase subunit I	1.4	9.8	2.9

-	Z5954	hypothetical protein	2.8	10.2	1.9
osmY	Z5977	periplasmic protein	101.5	519.6	2.4
-	Z5978	hypothetical protein	118.8	416.3	1.9
gpmB	Z5997	phosphoglycerate mutase	8.4	26.5	1.7
-	Z6026	hypothetical protein	6.2	18.6	1.7
-	Z6064	hypothetical protein	23.8	87.6	2.0
-	Z6067	hypothetical protein	1.0	7.0	2.8
-	L7024	regulatory protein	2263.0	9428.4	2.3
etpH	L7037	type II secretion protein	229.4	1121.2	2.5
etpL	L7041	type II secretion protein	956.2	2102.6	1.4
ccdA	L7062	plasmid maintenance protein CcdA	7323.8	18211.1	1.6

Tab. 52: Genes involved in regulatory processes, which were not significantly differentially regulated under the tested conditions. Log fold change is either "-" (no expression at all), or not significant (shaded, p value ≥ 0.05).

Gene	Locus tag	Product	$rpkm_{ref}$	$rpkm_{Sym}$	LogFC
fruR	Z0090	DNA-binding transcriptional regulator FruR	85.1	79.9	0.0
yacA	Z0107	SecA regulator SecM	40.6	30.8	-0.3
ampE	Z0121	regulatory protein AmpE	15.6	6.1	-1.3
pdhR	Z0123	transcriptional regulator PdhR	61.9	32.7	-0.9
yaeG	Z0174	carbohydrate diacid transcriptional activator CdaR	26.8	13.2	-0.9
yafC	Z0230	transcriptional regulator LYSR-type	7.1	6.1	-0.2
crl	Z0301	DNA-binding transcriptional regulator Crl	1028.3	1066.3	0.1
-	Z0321	AraC-type regulatory protein encoded in prophage CP-933H	4.3	5.1	0.3
-	Z0337	regulator encoded in prophage CP-933I	24.1	19.6	-0.2
-	Z0346	LysR-like transcriptional regulator	7.4	4.5	-0.6
-	Z0348	transcriptional regulator	0.1	0.3	-
ykgK	Z0361	regulator	0.0	2.1	-
-	Z0371	LysR-like transcriptional regulator	7.4	8.1	0.2
ykgA	Z0376	AraC-like transcriptional regulator	10.3	12.8	0.4
betI	Z0400	transcriptional regulator BetI	38.5	54.0	0.6
prpR	Z0426	regulator for prp operon	7.4	6.1	-0.2
cynR	Z0434	DNA-binding transcriptional regulator CynR	2.8	2.7	0.0
-	Z0463	response regulator; hexosephosphate transport	45.6	44.9	0.0
phoB	Z0497	transcriptional regulator PhoB	8.7	10.1	0.3
-	Z0509	hypothetical protein (COG: Predicted transcriptional regulators containing the CopG/Arc/MetJ DNA-binding domain)	104.0	214.9	1.1
nrdR	Z0514	transcriptional regulator NrdR	53.7	32.0	-0.7

bolA	Z0539	transcriptional regulator BolA	946.2	1875.5	1.1
hupB	Z0547	transcriptional regulator HU subunit beta	619.2	592.9	0.0
ybaO	Z0555	transcriptional regulator	13.3	9.1	-0.5
ybbI	Z0636	transcriptional regulator	0.0	0.0	-
fimZ	Z0693	transcriptional regulator FimZ	0.0	1.5	-
ybdO	Z0747	transcriptional regulator LYSR-type	0.0	1.0	-
rnk	Z0754	nucleoside diphosphate kinase regulator	183.9	138.0	-0.3
dpiA	Z0765	two-component response regulator DpiA	8.4	5.6	-0.5
ybeF	Z0774	DNA-binding transcriptional regulator	0.9	0.2	-
ybeZ	Z0809	ATP-binding protein in pho regulon	33.7	16.5	-1.0
fur	Z0831	ferric uptake regulator	1085.2	876.3	-0.2
seqA	Z0836	replication initiation regulator SeqA	53.4	50.2	0.0
-	Z0885	LysR-like transcriptional regulator	3.2	2.9	-
modE	Z0931	DNA-binding transcriptional regulator ModE	6.9	9.9	0.6
ybhD	Z0939	transcriptional regulator LYSR-type	0.0	1.7	-
ybiH	Z1016	DNA-binding transcriptional regulator	7.7	8.4	0.2
-	Z1039	manganese transport regulator MntR	18.5	16.0	-0.1
bssR	Z1062	biofilm formation regulatory protein BssR	1308.1	815.9	-0.6
-	Z1072	DEOR-type transcriptional regulator	20.1	12.2	-0.6
-	Z1073	DEOR-type transcriptional regulator	22.5	24.6	0.2
ybjN	Z1080	sensory transduction regulator	73.1	60.0	-0.2
-	Z1100	regulator	7.9	5.2	-0.5
cspD	Z1117	stationary phase/starvation inducible regulatory protein CspD	1996.0	1481.9	-0.4
terW	Z1164	hypothetical protein (COG: Predicted transcriptional regulator)	2.7	5.2	1.0
lrp	Z1234	leucine-responsive transcriptional regulator	890.4	459.4	-0.9
-	Z1309	hypothetical protein (COG: Regulator of competence-specific genes)	22.7	21.8	0.0
ymcC	Z1403	regulator	44.8	36.3	-0.2
torR	Z1412	DNA-binding transcriptional regulator TorR	12.0	9.0	-0.3
-	Z1447	repressor protein CI of bacteriophage BP-933W	107.1	81.1	-0.3
-	Z1448	regulatory protein Cro of bacteriophage BP-933W	25.6	58.6	1.3
-	Z1449	regulatory protein CII of bacteriophage BP-933W	0.5	2.9	
ycdC	Z1512	tet operon regulator	12.0	19.0	0.7
terW_2	Z1603	hypothetical protein (COG: Predicted transcriptional regulator)	4.5	5.2	0.3
bssS	Z1697	biofilm formation regulatory protein BssS	1513.8	781.0	-0.9
flgM	Z1709	anti-sigma28 factor FlgM (COG: Negative regulator of flagellin synthesis)	18.0	8.3	-1.0
phoP	Z1859	DNA-binding transcriptional regulator PhoP	29.4	16.3	-0.8
-	Z1932	hypothetical protein (COG: predicted transcriptional regulator)	297.3	196.2	-0.5

fadR	Z1950	fatty acid metabolism regulator	104.8	160.7	0.7
-	Z1984	transcriptional regulator	10.3	23.0	1.2
ychA	Z1985	transcriptional regulator	30.7	35.1	0.3
chaB	Z1992	cation transport regulator	47.5	110.4	1.3
chaC	Z1993	cation transport regulator	22.0	52.3	1.3
narL	Z1996	transcriptional regulator NarL	9.6	7.5	-0.3
hnr	Z2011	response regulator of RpoS	9.2	22.1	1.3
hns	Z2013	global DNA-binding transcriptional dual regulator H-NS	2249.7	1755.3	-0.3
-	Z2039	regulator of cell division encoded by prophage CP-933O	26.5	36.6	0.5
-	Z2046	DNA-binding transcriptional regulator DicC	0.0	1.5	-
-	Z2104	ARAC-type regulatory protein of CP-933O	32.9	42.5	0.4
yneJ	Z2177	transcriptional regulator LYSR-type	4.9	10.8	1.2
ydeW	Z2193	SorC family transcriptional regulator	62.8	36.7	-0.7
hipA	Z2197	persistence to inhibition of murein or DNA biosynthesis, DNA-binding regulator	1.0	0.0	-
-	Z2209	transcriptional regulator YdeO	1.6	0.2	-
-	Z2280	multi modular; transcriptional regulator; also ATP-binding component of a transport system	7.2	7.9	0.2
ydcN	Z2285	hypothetical protein (COG: Predicted transcriptional regulator)	3.6	6.1	0.8
-	Z2299	transcriptional regulator LYSR-type	191.5	234.6	0.4
-	Z2399	regulatory protein Cro of prophage CP-933R	0.0	0.0	-
ydaK	Z2423	DNA-binding transcriptional regulator	27.4	24.6	-0.1
fnr	Z2433	fumarate/nitrate reduction transcriptional regulator	578.3	517.9	-0.1
ycjZ	Z2439	transcriptional regulator LYSR-type	12.2	11.4	0.0
tyrR	Z2454	DNA-binding transcriptional regulator TyrR	29.5	38.4	0.5
ycjW	Z2461	LACI-type transcriptional regulator	20.9	29.5	0.6
pspC	Z2479	DNA-binding transcriptional activator PspC	72.9	77.1	0.2
pspF	Z2484	phage shock protein operon transcriptional activator	11.8	10.3	-0.1
ycjC	Z2489	DNA-binding transcriptional repressor PuuR	60.7	114.4	1.0
-	Z2521	DEOR-type transcriptional regulator	29.4	37.9	0.4
cysB	Z2535	transcriptional regulator CysB	160.6	128.8	-0.2
mlc	Z2587	NAGC-like transcriptional regulator	19.7	10.8	-0.8
ynfL	Z2589	transcriptional regulator LYSR-type	0.0	1.6	-
rstA	Z2609	DNA-binding transcriptional regulator RstA	31.1	16.4	-0.8
slyA	Z2657	transcriptional regulator SlyA	259.0	333.0	0.4
ydhB	Z2682	DNA-binding transcriptional regulator	6.7	5.8	-0.1
-	Z2724	ARAC-type regulatory protein	1.1	1.1	-
celD	Z2765	DNA-binding transcriptional regulator ChbR	24.9	19.8	-0.3
-	Z2808	DEOR-type transcriptional regulator	25.8	13.0	-0.9
yeaM	Z2831	AraC-type regulatory protein	8.1	8.7	0.2
yeaT	Z2842	transcriptional regulator LYSR-type	12.5	7.5	-0.7

-	Z2874	regulator	84.7	65.1	-0.3
yebK	Z2905	DNA-binding transcriptional regulator HexR	21.9	21.7	-0.1
cheZ	Z2935	chemotaxis regulator CheZ	3.0	1.6	-
coxT	Z2970	regulator for prophage CP-933T	52.4	80.8	0.7
uvrY	Z3002	response regulator	135.5	125.7	-0.0
rcsA	Z3041	positive regulator for ctr capsule biosynthesis, positive transcription factor	51.9	66.7	0.4
yedW	Z3061	transcriptional regulatory protein YedW	14.7	15.5	0.1
cbl	Z3146	transcriptional regulator Cbl	0.0	0.0	-
nac	Z3147	nitrogen assimilation transcriptional regulator	0.0	0.0	-
yeeY	Z3177	transcriptional regulator LYSR-type	37.7	28.6	-0.3
wzzB	Z3189	regulator of length of O-antigen component of lipopoly-saccharide chains	632.2	727.8	0.3
baeR	Z3248	DNA-binding transcriptional regulator BaeR	34.2	49.9	0.6
yegW	Z3264	transcriptional regulator	18.4	13.7	-0.4
molR_A	Z3283	regulator (fragment)	4.6	1.7	-
molR_B	Z3284	regulator (fragment)	0.1	0.6	-
molR_D	Z3286	regulator (fragment)	0.2	0.5	-
yehI	Z3287	regulator	1.0	2.1	-
yehT	Z3302	two-component response-regulatory protein YehT	24.0	26.0	0.2
-	Z3357	regulatory protein CII of prophage CP-933V	9.8	16.4	0.8
yehV	Z3376	transcriptional regulator (mlrA homologus)	8.8	3.7	-1.2
-	Z3395	regulator	20.3	21.0	0.1
galS	Z3407	DNA-binding transcriptional regulator GalS	25.4	10.5	-1.3
yeiE	Z3414	DNA-binding transcriptional regulator	50.6	35.2	-0.4
narP	Z3450	transcriptional regulator NarP	50.2	29.4	-0.7
rcsB	Z3476	transcriptional regulator RcsB	285.3	268.9	-0.0
-	Z3495	regulator	3.3	0.6	-
-	Z3506	regulator	0.0	0.0	-
lrhA	Z3549	LysR family NADH dehydrogenase transcriptional regu-lator	84.5	95.0	0.2
-	Z3561	regulator	24.0	15.3	-0.6
flk	Z3583	flagella biosynthesis regulator	16.9	10.8	-0.6
-	Z3626	sucrose specific transcriptional regulator	95.0	75.8	-0.3
-	Z3646	2-component transcriptional regulator	19.8	10.6	-0.8
-	Z3647	ARAC-type regulatory protein	8.9	3.4	-1.3
yfeR	Z3673 m	transcriptional regulator	26.5	13.5	-0.9
yfeG	Z3702	transcriptional regulator EutR	0.0	1.3	-
gcvR	Z3738	glycine cleavage system transcriptional repressor	110.1	104.1	-0.0
hyfR	Z3751	2-component regulator, interaction with sigma 54	0.4	1.1	-
-	Z3798	DNA-binding transcriptional regulator IscR	57.4	67.0	0.3

hcaR	Z3808	DNA-binding transcriptional regulator HcaR	66.3	60.2	-0.1
yphF	Z3823	LACI-type transcriptional regulator	1.0	0.0	-
yphH	Z3826	NAGC-like transcriptional regulator	4.2	7.5	0.9
glnB	Z3829	nitrogen regulatory protein P-II 1	98.3	103.9	0.2
yfhA	Z3830	2-component transcriptional regulator	9.3	6.6	-0.4
yfhH	Z3841	DNA-binding transcriptional regulator	1.3	0.8	-
rseB	Z3853	periplasmic negative regulator of sigmaE	115.8	138.9	0.3
rseA	Z3854	anti-RNA polymerase sigma factor SigE	482.6	555.4	0.3
yfiE	Z3860	transcriptional regulator LYSR-type	7.6	5.4	-0.4
ygaE	Z3963	DNA-binding transcriptional regulator CsiR	17.0	6.3	-1.4
csrA	Z3998	carbon storage regulator	3208.4	2604.0	-0.2
recX	Z4001	recombination regulator RecX	4.7	2.8	-
ygaA	Z4017	anaerobic nitric oxide reductase transcription regulator	0.6	2.3	-
hypF	Z4020	transcriptional regulatory protein	0.6	2.3	-
hycA	Z4033	formate hydrogenlyase regulatory protein HycA	4.8	0.0	-
-	Z4048	regulator	86.0	101.1	0.2
ygcP	Z4078	anti-terminator regulatory protein	10.4	16.3	0.7
galR	Z4155	DNA-binding transcriptional regulator GalR	42.8	33.5	-0.3
lysR	Z4157	DNA-binding transcriptional regulator LysR	4.0	0.6	-
ygeH	Z4173	transcriptional regulator	0.2	1.3	-
-	Z4176	2-component transcriptional regulator	0.6	1.2	-
-	Z4198	regulatory protein for type III secretion apparatus (*eivF*)	0.6	1.4	-
ygeV	Z4208	transcriptional regulator	97.3	112.6	0.3
ygfZ	Z4236	global regulator	5.9	14.7	1.4
-	Z4258	transcriptional regulator LYSR-type	4.1	0.6	-
yggD	Z4274	DNA-binding transcriptional regulator	26.7	15.3	-0.7
yqgE	Z4293	hypothetical protein (COG: Putative transcriptional regulator)	48.8	34.1	-0.4
yqhC	Z4363	ARAC-type regulatory protein	3.8	4.3	-
qseB	Z4377	DNA-binding transcriptional regulator QseB	4.2	7.4	0.9
ygjM	Z4435	hypothetical protein (COG: Predicted transcription regulator containing HTH domain)	12.7	7.9	-0.6
yhaJ	Z4459	transcriptional regulator LYSR-type	4.2	2.7	-
sohA	Z4481	regulator PrlF	59.9	93.4	0.7
agaR	Z4483	DNA-binding transcriptional regulator AgaR	18.9	20.8	0.2
nlp	Z4551	DNA-binding transcriptional regulator Nlp	1.5	2.5	-
ptsN	Z4567	PTS system transporter subunit IIA-like nitrogen-regulatory protein PtsN	25.1	22.9	-0.1
ptsO	Z4569	phosphohistidinoprotein-hexose phosphotransferase component of N-regulated PTS system (Npr)	43.8	37.5	-0.2
yhcK	Z4584	transcriptional regulator NanR	19.1	32.2	0.8
qseA	Z4602	DNA-binding transcriptional regulator	1.2	3.7	-

yhdA	Z4611	regulatory protein CsrD	19.3	16.4	-0.2
envR	Z4624	DNA-binding transcriptional regulator EnvR	0.0	0.0	-
zntR	Z4662	zinc-responsive transcriptional regulator	87.7	57.8	-0.5
crp	Z4718	cAMP-regulatory protein	834.8	641.8	-0.3
yhfR	Z4736	DNA-binding transcriptional regulator FrlR	7.1	11.1	0.7
ompR	Z4760	osmolarity response regulator	21.6	20.0	0.0
malT	Z4774	transcriptional regulator MalT	47.8	43.5	-0.1
rtcR	Z4780	2-component regulator	16.1	15.5	0.0
gntR	Z4806	regulator of gluconate (gnt) operon	22.1	21.0	0.0
yhhG	Z4873	nickel responsive regulator	12.4	3.9	-1.6
-	Z4874	regulator	4.4	3.4	-
uspA	Z4895	universal stress protein; broad regulatory function?	359.2	287.5	-0.2
yhiW	Z4928	ARAC-type regulatory protein	13.3	30.5	1.3
yhjB	Z4933	regulator	3.0	5.2	0.9
yhjC	Z4934	transcriptional regulator LYSR-type	3.1	5.5	0.9
yhjN	Z4947	cellulose synthase regulator protein	0.1	0.1	-
xylR	Z4994	regulator of xyl operon	9.1	6.3	-0.4
-	Z5000	regulatory protein	5.6	4.7	-0.2
yiaU	Z5004	transcriptional regulator LYSR-type	47.7	18.9	-1.3
grlA	Z5128	hypothetical protein	10.1	4.6	-1.1
grlR	Z5129	negative regulator GrlR	7.8	15.9	1.1
ler	Z5140	hypothetical protein (LEE regulator)	15.0	16.0	0.2
uhpC	Z5157	regulatory protein UhpC	0.2	0.7	-
ilvN	Z5164	acetolactate synthase 1 regulatory subunit	43.4	17.9	-1.2
yidF	Z5169	transcriptional regulator	30.1	35.2	0.3
yidP	Z5179	transcriptional regulator	0.0	1.5	-
yidZ	Z5206	DNA-binding transcriptional regulator YidZ	3.0	7.8	1.4
phoU	Z5215	transcriptional regulator PhoU	21.8	24.6	0.2
yieN	Z5247	regulatory ATPase RavA	24.3	26.8	0.2
yifA	Z5275 m	transcriptional regulator HdfR	78.6	86.7	0.2
ilvY	Z5284	DNA-binding transcriptional regulator IlvY	9.2	4.7	-0.9
aslB	Z5313	arylsulfatase regulator	2.9	5.3	0.9
metR	Z5349	regulator for metE and metH	2.9	2.9	-
glnG	Z5404	nitrogen regulation protein NR(I)	2.7	2.8	-
glnL	Z5405	nitrogen regulation protein NR(II)	6.9	3.0	-1.1
yihL	Z5408	transcriptional regulator	11.7	5.4	-1.1
frvR	Z5440	frv operon regulatory protein	2.7	0.5	-
cpxR	Z5457	DNA-binding transcriptional regulator CpxR	214.2	167.5	-0.3
menG	Z5476	ribonuclease activity regulator protein RraA	189.2	133.5	-0.4
cytR	Z5481	DNA-binding transcriptional regulator CytR	49.3	44.5	-0.1

metJ	Z5493	transcriptional repressor protein MetJ	96.1	82.9	-0.1
yijO	Z5512	ARAC-type regulatory protein	2.0	4.1	-
oxyR	Z5519	DNA-binding transcriptional regulator OxyR	21.8	22.3	0.1
yjaE	Z5570	anti-RNA polymerase sigma 70 factor (COG: Regulator of sigma D)	466.2	578.2	0.4
hupA	Z5576	transcriptional regulator HU subunit alpha	954.6	802.6	-0.2
hydG	Z5580	transcriptional regulatory protein ZraR	1.6	0.8	-
espL4	Z5608	regulator of acetyl CoA synthetase	3.7	1.6	-
-	Z5619	transcriptional regulator of sorbose uptake and utilization genes	0.9	0.2	-
malM	Z5635	maltose regulon periplasmic protein	158.1	78.2	-0.9
soxS	Z5661	DNA-binding transcriptional regulator SoxS	17.6	25.2	0.6
-	Z5684	transcriptional regulator	12.4	9.6	-0.3
phnF	Z5705	phosphonate metabolism transcriptional regulator PhnF	0.0	0.0	-
basR	Z5715	DNA-binding transcriptional regulator BasR	16.8	10.9	-0.6
adiY	Z5718	ARAC-type regulatory protein	4.7	6.4	0.5
melR	Z5720	DNA-binding transcriptional regulator MelR	8.3	7.8	0.0
yjdC	Z5740	transcriptional regulator	25.2	43.7	0.9
hflK	Z5781	FtsH protease regulator HflK	39.5	24.6	-0.6
hflC	Z5782	FtsH protease regulator HflC	135.9	76.3	-0.8
ytfH	Z5823	hypothetical protein (COG: predicted transcriptional regulator)	7.1	11.0	0.7
ytfQ	Z5838	LACI-type transcriptional regulator	4.1	3.8	-
yjiR	Z5941	regulator	0.8	2.3	-
yjjQ	Z5966	regulator	0.0	1.4	-
creB	Z6001	DNA-binding response regulator CreB	10.1	5.0	-0.9
arcA	Z6004	two-component response regulator	669.6	721.0	0.2

8 Acknowledgments

This thesis was conduced within the research training group 1482 “Interface functions of the intestine between luminal factors and host signals”, at the Institute of Technical Microbiology, TUM and I would like to thank everyone, who made this possible:

First, I would like to thank my PI Prof. Rudi Vogel for the interesting topic, for guidance, support and patience throughout the duration of my graduation studies.

Thanks goes to Matthias Ehrmann for all the helpful advises and discussions.

I also want to thank Prof. Siegfried Scherer for supervision and giving me the opportunity to conduced the EHEC research. I thank Dr. Klaus Neuhaus for all practical ideas and feedback and all members of his group, for a nice, welcoming atmosphere. A special thank goes to Richard Landstorfer for all the NGS support. I thank Svenja Simon for bioinformatic analysis.

My sincere thanks goes to Kevin P. Francis PhD for offering me the internship opportunities at CaliperLS, for the support building up a reporter system for *E. faecalis* and insightful comments on imaging issues. I want to thank Ali Akin for technical support and all colleagues at CaliperLS for a great time.

I thank Dr. Roger Vogelmann and Viktoria Doll for the opportunity to conduce *E. faecalis* translocations studies.

I thank Britta Spanier for contributing to the *C. elegans* work.

A special thanks goes to all coworkers and members of the TMWbiota, for a nice atmosphere and inspiring discussions. I thank Angela Lindenstrauß for great *E. faecalis* collaboration, Simone Freiding for the “clonator” and Zhen Peng for the confocal microscopy pictures and all GRK fellows for a great time.

This work was financially supported by DFG, TUM and BaCaTec. I am grateful to the management of the GRK, Prof. Hannelore Daniel, Prof. Dirk Haller and Dorothea Wörner.

Of course, I thank all my friends for constant support and encouragement

And finally, I want to thank my family, who has been an important and indispensable source of support.

9 Bibliography

Aakra A., Vebo H., Snipen L., Hirt H., Aastveit A., Kapur V., Dunny G., Murray B. E., Nes I. F. 2005. Transcriptional response of *Enterococcus faecalis* V583 to erythromycin. *Antimicrob. Agents Chemother.*49:2246-2259

Aarestrup F. M., Hasman H., Jensen L. B., Moreno M., Herrero I. A., Dominguez L., Finn M., Franklin A. 2002. Antimicrobial resistance among enterococci from pigs in three European countries. *Appl. Environ. Microbiol.*68:4127-4129

Aboyoun P., Pages H., Lawrence M. 2010. GenomicRanges: Representation and manipulation of genomic intervals. R package version 1.0.9.

Abrams G. D., Bauer H., Sprinz H. 1963. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Lab. invest.*12:355-364

Albesharat R., Ehrmann M. A., Korakli M., Yazaji S., Vogel R. F. 2011. Phenotypic and genotypic analyses of lactic acid bacteria in local fermented food, breast milk and faeces of mothers and their babies. *Syst. Appl. Microbiol.*34:148-155

Allen K. J., Griffiths M. W. 2012. Impact of hydroxyl- and superoxide anion-based oxidative stress on logarithmic and stationary phase *Escherichia coli* O157:H7 stress and virulence gene expression. *Food Microbiol.*29:141-147

Allen S. J., Okoko B., Martinez E., Gregorio G., Dans L. F. 2004. Probiotics for treating infectious diarrhoea. *Cochrane Database Syst. Rev.*CD003048

Altun Z. F., Herndon L. A., Crocker C., Lints R., Hall D. H., (ed.s). 2002 - 2010. WormAtlas

Alvarez-Olmos M. I., Oberhelman R. A. 2001. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. *Clin. infect. dis.*32:1567-1576

Andrewes F. W., Horder T. J. 1906. A study of the Streptococci pathogenic for man. *Lancet.*168:852-855

Asahara T., Shimizu K., Nomoto K., Hamabata T., Ozawa A., Takeda Y. 2004. Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing *Escherichia coli O157:H7*. *Infect. Immun.*72:2240-2247

Ator L. L., Starzyk M. J. 1976. Distribution of group D streptococci in rivers and streams. *Microbios.*16:91-104

Bäckhed F., Ding H., Wang T., Hooper L. V., Koh G. Y., Nagy A., Semenkovich C. F., Gordon J. I. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad Sci. USA.*101:15718-15723

Bäckhed F., Ley R. E., Sonnenburg J. L., Peterson D. A., Gordon J. I. 2005. Host-bacterial mutualism in the human intestine. *Science.*307:1915-1920

Balish E., Warner T. 2002. *Enterococcus faecalis* induces inflammatory bowel disease in interleukin-10 knockout mice. *Am. J. Pathol.*160:2253-2257

Barba J., Bustamante V. H., Flores-Valdez M. A., Deng W., Finlay B. B., Puente J. L. 2005. A positive regulatory loop controls expression of the locus of enterocyte effacement-encoded regulators Ler and GrlA. *J. Bacteriol.*187:7918-7930

Bassler B. L. 2002. Small talk. Cell-to-cell communication in bacteria. *Cell.*109:421-424

Bates J., Jordens J. Z., Griffiths D. T. 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. *J. Antimicrob. Chemother.*34:507-514

Benno Y., Suzuki K., Narisawa K., Bruce W. R., Mitsuoka T. 1986. Comparison of the fecal microflora in rural Japanese and urban Canadians. *Microbiol. Immunol.*30:521-532

Besselink M. G., van Santvoort H. C., Buskens E., Boermeester M. A., van Goor H., Timmerman H. M., Nieuwenhuijs V. B., Bollen T. L., van Ramshorst B., Witteman B. J., Rosman C., Ploeg R. J., Brink M. A., Schaapherder A. F., Dejong C. H., Wahab P. J., van Laarhoven C. J., van der Harst E., van Eijck C. H., Cuesta M. A., Akkermans L. M., Gooszen H. G. 2008. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet.*371:651-659

Bhatt S., Anyanful A., Kalman D. 2011. CsrA and TnaB coregulate tryptophanase activity to promote exotoxin-induced killing of *Caenorhabditis elegans* by enteropathogenic *Escherichia coli*. *J. Bacteriol.*193:4516-4522

Blankenberg D., Kuster G. V., Coraor N., Ananda G., Lazarus R., Mangan M., Nekrutenko A., Taylor J. 2010. Galaxy: a web-based genome analysis tool for experimentalists. *Curr. Protoc. Mol. Biol.*Chapter 19:Unit 19.10.11-Unit 19.10.21

Boerlin P., McEwen S. A., Boerlin-Petzold F., Wilson J. B., Johnson R. P., Gyles C. L. 1999. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J. Clin. Microbiol.*37:497-503

Bourgogne A., Garsin D., Qin X., Singh K., Sillanpaa J., Yerrapragada S., Ding Y., Dugan-Rocha S., Buhay C., Shen H., Chen G., Williams G., Muzny D., Maadani A., Fox K., Gioia J., Chen L., Shang Y., Arias C., Nallapareddy S., Zhao M., Prakash V., Chowdhury S., Jiang H., Gibbs R., Murray B., Highlander S., Weinstock G. 2008. Large scale variation in *Enterococcus faecalis* illustrated by the genome analysis of strain OG1RF. *Genome Biol.*9:R110

Branchini B. R., Southworth T. L., Murtiashaw M. H., Magyar R. A., Gonzalez S. A., Ruggiero M. C., Stroh J. G. 2004. An alternative mechanism of bioluminescence color determination in firefly luciferase. *Biochemistry* 43: 7255-7262.

Braslavsky S. E. 2007. Glossary of Terms used in Photochemistry, 3rd edition (IUPAC recommendations 2006). *Pure Appl. Chem.*79:293-465

Brock T. D., Peacher B., Pierson D. 1963. Survey of the Bacteriocines of Enterococci. *J. Bacteriol.*86:702-707

Brussow H., Canchaya C., Hardt W. D. 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev.*68:560-602

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. 2004. Stellungsnahme der ZKBS zur Risikobewertung von Enterococcus faecalis Stämme S1/01/00 bis S1/10/00. *Az.: 6790-10-89*

Burland V., Shao Y., Perna N. T., Plunkett G., Sofia H. J., Blattner F. R. 1998. The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic Acids Res.*26:4196-4204

Byrne M. E., Rouch D. A., Skurray R. A. 1989. Nucleotide sequence analysis of IS256 from the *Staphylococcus aureus* gentamicin-tobramycin-kanamycin-resistance transposon Tn4001. *Gene.*81:361-367

Cai Y. 1999. Identification and characterization of *Enterococcus* species isolated from forage crops and their influence on silage fermentation. *J. Dairy Sci.*82:2466-2471

Campbell Z. T., Baldwin T. O. 2009. Fre Is the Major Flavin Reductase Supporting Bioluminescence from *Vibrio harveyi* Luciferase in *Escherichia coli*. *J. Biol. Chem.*284:8322-8328

Carey C. M., Kostrzynska M., Ojha S., Thompson S. 2008. The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* O157:H7. *J. Microbiol. Methods.*73:125-132

Castagliuolo I., Galeazzi F., Ferrari S., Elli M., Brun P., Cavaggioni A., Tormen D., Sturniolo G. C., Morelli L., Palu G. 2005. Beneficial effect of auto-aggregating *Lactobacillus crispatus* on experimentally induced colitis in mice. *FEMS Immunol. Med. Microbiol.*43:197-204

Centeno J. A., Menendez S., Rodriguez-Otero J. L. 1996. Main microbial flora present as natural starters in Cebreiro raw cow's-milk cheese (northwest Spain). *Int. J. Food Microbiol.*33:307-313

Centeno J. A., Menendez S., Hermida M., Rodriguez-Otero J. L. 1999. Effects of the addition of *Enterococcus faecalis* in Cebreiro cheese manufacture. *Int. J. Food Microbiol.*48:97-111

Cilento G. 1988. Photobiochemistry without light. *Experientia* 44: 572-576.

Claret L., Hughes C. 2002. Interaction of the atypical prokaryotic transcription activator FlhD2C2 with early promoters of the flagellar gene hierarchy. *J. Mol. Biol.*321:185-199

Clarke M. B. 2005. The functional characterization of quorum sensing *E. coli* regulators B and C in EHECUniversity of Texas Southwestern Medical Center, Dallas.

Clarke M. B., Hughes D. T., Zhu C., Boedeker E. C., Sperandio V. 2006. The QseC sensor kinase: a bacterial adrenergic receptor. *Proc. Natl. Acad. Sci. USA* 103:10420-10425

Coates M. E. 1980. The gut microflora and growth. Pages 175-188 in Lawrence T. L. J., ed. Growth in Animals. Boston: Butterworths.

Cohen S. N., Chang A. C., Hsu L. 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proc. Natl. Acad Sci. USA.*69:2110-2114

Contag C. H., Bachmann M. H. 2002. Advances in in vivo bioluminescence imaging of gene expression. *Annu. Rev. Biomed. Eng.*4:235-260

Cook M. J. 1965. The Anatomy of the Laboratory Mouse. London & New York: Academic Press.

Creasey E. A., Delahay R. M., Bishop A. A., Shaw R. K., Kenny B., Knutton S., Frankel G. 2003. CesT is a bivalent enteropathogenic *Escherichia coli* chaperone required for translocation of both Tir and Map. *Mol. Microbiol.*47:209-221

Creuzburg K., Middendorf B., Mellmann A., Martaler T., Holz C., Fruth A., Karch H., Schmidt H. 2011. Evolutionary analysis and distribution of type III effector genes in pathogenic *Escherichia coli* from human, animal and food sources. *Environ. Microbiol.*13:439-452

Cronin M., Akin A. R., Collins S. A., Meganck J., Kim J. B., Baban C. K., Joyce S. A., van Dam G. M., Zhang N., van Sinderen D., O'Sullivan G. C., Kasahara N., Gahan C. G., Francis K. P., Tangney M. 2012. High resolution in vivo bioluminescent imaging for the study of bacterial tumour targeting. *PLoS ONE.*7:e30940

D'Souza A. L., Rajkumar C., Cooke J., Bulpitt C. J. 2002. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *BMJ* 324: 1361.

Dahan S., Knutton S., Shaw R. K., Crepin V. F., Dougan G., Frankel G. 2004. Transcriptome of enterohemorrhagic *Escherichia coli* O157 adhering to eukaryotic plasma membranes. *Infect. Immun.*72:5452-5459

Darby C. 2005. Interactions with microbial pathogens: WormBook 10.1895/wormbook.1.21.1 http://www.wormbook.org.

Davies R., Roberts T. A. 1999. Antimicrobial susceptibility of enterococci recovered from commercial swine carcasses: effect of feed additives. *Lett. Appl. Microbiol.*29:327-333

Day A. M., Cove J. H., Phillips-Jones M. K. 2003. Cytolysin gene expression in *Enterococcus faecalis* is regulated in response to aerobiosis conditions. *Mol. Genet. Genomics.*269:31-39

de Sablet T., Chassard C., Bernalier-Donadille A., Vareille M., Gobert A. P., Martin C. 2009. Human microbiota-secreted factors inhibit shiga toxin synthesis by enterohemorrhagic *Escherichia coli* O157:H7. *Infect. Immun.*77:783-790

Deacon V., Dziva F., van Diemen P. M., Frankel G., Stevens M. P. 2010. Efa-1/LifA mediates intestinal colonization of calves by enterohaemorrhagic *Escherichia coli* O26: H- in a manner independent of glycosyltransferase and cysteine protease motifs or effects on type III secretion. *Microbiology.*156:2527-2536

Deitch E. A. 1990. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. *Arch. Surg.*125:403-404

Del Pozo B. F., Gaya P., Medina M., Rodriguez-Marin M. A., Nunez M. 1988. Changes in the microflora of La Serena ewes' milk cheese during ripening. *J. Dairy Re.*55:449-455

Descheemaeker P. R., Chapelle S., Devriese L. A., Butaye P., Vandamme P., Goossens H. 1999. Comparison of glycopeptide-resistant *Enterococcus faecium* isolates and glycopeptide resistance genes of human and animal origins. *Antimicrob. Agents Chemother.*43:2032-2037

Devaraj B., Usa M., Inaba H. 1997. Biophotons: ultraweak light emission from living systems. *Curr. Opin. Solid St. M.*2:188-193

Dever L. L., Handwerger S. 1996. Persistence of vancomycin-resistant *Enterococcus faecium* gastrointestinal tract colonization in antibiotic-treated mice. *Microb. Drug Resist.*2:415-421

Domann E., Hain T., Ghai R., Billion A., Kuenne C., Zimmermann K., Chakraborty T. 2007. Comparative genomic analysis for the presence of potential enterococcal virulence factors in the probiotic *Enterococcus faecalis* strain Symbioflor 1. *Int. J. Med. Microbiol.*297:533-539

Donskey C. J., Hanrahan J. A., Hutton R. A., Rice L. B. 1999. Effect of parenteral antibiotic administration on persistence of vancomycin-resistant *Enterococcus faecium* in the mouse gastrointestinal tract. *J. Infect. Dis.*180:384-390

Doughty S., Sloan J., Bennett-Wood V., Robertson M., Robins-Browne R. M., Hartland E. L. 2002. Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. *Infect. Immun.*70:6761-6769

Dunn J. J., Studier F. W. 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J. Mol. Biol.*166:477-535

Dunn T. B. 1954. Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. *J. Natl. Cancer Inst.*14:1281-1433

Dunny G. M., Craig R. A., Carron R. L., Clewell D. B. 1979. Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. *Plasmid.*2:454-465

Duran N., Cadenas E. 1987. The role of singlet oxygen and triplet carbonyls in biological systems. *Rev. Chem. Intermed.*8:147-187

Eaton T. J., Shearman C. A., Gasson M. J. 1993. The use of bacterial luciferase genes as reporter genes in *Lactococcus*: regulation of the *Lactococcus lactis* subsp. lactis lactose genes. *J. Gen. Microbiol.*139:1495-1501

Eckburg P. B., Bik E. M., Bernstein C. N., Purdom E., Dethlefsen L., Sargent M., Gill S. R., Nelson K. E., Relman D. A. 2005. Diversity of the human intestinal microbial flora. *Science.*308:1635-1638

Enzensberger R., Shah P. M., Knothe H. 1985. Impact of oral ciprofloxacin on the faecal flora of healthy volunteers. *Infection* 13: 273-275.

Escherich T. 1885. Die Darmbakterien des Neugeborenen und Säuglinge. *Fortschr. Med.*3:515-522

Fang F., Flynn S., Li Y., Claesson M. J., van Pijkeren J.-P., Collins J. K., van Sinderen D., O'Toole P. W. 2008a. Characterization of endogenous plasmids from *Lactobacillus salivarius* UCC118. *Appl. Environ. Microbiol.*74:3216-3228

Fang F., Flynn S., Li Y., Claesson M. J., van Pijkeren J.-P., Collins J. K., van Sinderen D., O'Toole P. W. 2008b. Characterization of endogenous plasmids from Lactobacillus salivarius UCC118. *Appl Environ Microbiol* 74: 3216-3228.

Farkas-Himsley H. 1980. Bacteriocins--are they broad-spectrum antibiotics? *J. Antimicrob. Chemother.*6:424-426

Ferens W. A., Hovde C. J. 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog. Dis.*8:465-487

Fisher K., Phillips C. 2009. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology.*155:1749-1757

Fitzpatrick K. C. 2005. Probiotics - Discussion Paper. *Natural Health Products Directorate*

Francis K. P., Joh D., Bellinger-Kawahara C., Hawkinson M. J., Purchio T. F., Contag P. R. 2000. Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel luxABCDE construct. *Infect. Immun.*68:3594-3600

Francis K. P., Yu J., Bellinger-Kawahara C., Joh D., Hawkinson M. J., Xiao G., Purchio T. F., Caparon M. G., Lipsitch M., Contag P. R. 2001. Visualizing pneumococcal infections in the lungs of live mice using bioluminescent *Streptococcus pneumoniae* transformed with a novel gram-positive lux transposon. *Infect. Immun.*69:3350-3358

Frankel G., Phillips A. D., Rosenshine I., Dougan G., Kaper J. B., Knutton S. 1998. Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. *Mol. Microbiol.*30:911-921

Franz C. M., Holzapfel W. H., Stiles M. E. 1999. Enterococci at the crossroads of food safety? *Int. J. Food Microbiol.*47:1-24

Franz C. M. A. P., Schillinger U., Holzapfel W. H. 1996. Production and characterization of enterocin 900, a bacteriocin produced by *Enterococcus faecium* BFE 900 from black olives. *Int. J. Food Microbiol.*29:255-270

Franz C. M. A. P., Stiles M. E., Schleifer K. H., Holzapfel W. H. 2003. Enterococci in foods--a conundrum for food safety. *Int. J. Food Microbiol.*88:105-122

Fyderek K., Strus M., Kowalska-Duplaga K., Gosiewski T., Wedrychowicz A., Jedynak-Wasowicz U., Sladek M., Pieczarkowski S., Adamski P., Kochan P., Heczko P. B. 2009. Mucosal bacterial microflora and mucus layer thickness in adolescents with inflammatory bowel disease. *World J. Gastroenterol.*15:5287-5294

Garmendia J., Frankel G., Crepin V. F. 2005. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. *Infect. Immun.*73:2573-2585

Garsin D. A., Sifri C. D., Mylonakis E., Qin X., Singh K. V., Murray B. E., Calderwood S. B., Ausubel F. M. 2001. A simple model host for identifying Gram-positive virulence factors. *Proc. Natl. Acad. Sci. USA* 98:10892-10897

Gaskins H. R., Collier C. T., Anderson D. B. 2002. Antibiotics as growth promotants: mode of action. *Anim. Biotechnol.*13:29-42

Gelsomino R., Vancanneyt M., Condon S., Swings J., Cogan T. M. 2001. Enterococcal diversity in the environment of an Irish Cheddar-type cheesemaking factory. *Int. J. Food Microbiol.*71:177-188

Gelsomino R., Vancanneyt M., Cogan T. M., Condon S., Swings J. 2002. Source of enterococci in a farmhouse raw-milk cheese. *Appl. Environ. Microbiol.*68:3560-3565

Gentleman R. C., Carey V. J., Bates D. M., Bolstad B., Dettling M., Dudoit S., Ellis B., Gautier L., Ge Y., Gentry J., Hornik K., Hothorn T., Huber W., Iacus S., Irizarry R., Leisch F., Li C., Maechler M., Rossini A. J., Sawitzki G., Smith C., Smyth G., Tierney L., Yang J. Y., Zhang J. 2004. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.*5:R80

Giardine B., Riemer C., Hardison R. C., Burhans R., Elnitski L., Shah P., Zhang Y., Blankenberg D., Albert I., Taylor J., Miller W., Kent W. J., Nekrutenko A. 2005. Galaxy: a platform for interactive large-scale genome analysis. *Genome Res.*15:1451-1455

Giraffa G., Sisto F. 1997. Susceptibility to vancomycin of enterococci isolated from dairy products. *Lett. Appl. Microbiol.*25:335-338

Giraffa G. 2002. Enterococci from foods. *FEMS Microbiol. Rev.*26:163-171

Gluck U., Gebbers J. O. 2003. Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphylococcus aureus, Streptococcus pneumoniae*, and beta-hemolytic streptococci). *Am. J. Clin. Nutrit.*77:517-520

Goecks J., Nekrutenko A., Taylor J. 2010a. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.*11:R86

Goecks J., Nekrutenko A., Taylor J., Team G. 2010b. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol* 11: R86.

Goldwater P. N., Bettelheim K. A. 2012. Treatment of enterohemorrhagic *Escherichia coli* (EHEC) infection and hemolytic uremic syndrome (HUS). Pages 12. BMC Med., vol. 10.

Griffin P. M., Tauxe R. V. 1991. The Epidemiology of Infections Caused by *Escherichia coli* O157: H7, Other Enterohemorrhagic *E. coli,* and the Associated Hemolytic Uremic Syndrome. *Epidemiol. Rev.*13:60-98

Habdas B. J., Smart J., Kaper J. B., Sperandio V. 2010. The LysR-type transcriptional regulator QseD alters type three secretion in enterohemorrhagic *Escherichia coli* and motility in K-12 *Escherichia coli*. *J. Bacteriol.*192:3699-3712

Habermann W., Zimmermann K., Skarabis H., Kunze R., Rusch V. 2001. The effect of a bacterial immunostimulant (human *Enterococcus faecalis* bacteria) on the occurrence of relapse in patients. *Arzneimittel-Forsch.*51:931-937

Habermann W., Zimmermann K., Skarabis H., Kunze R., Rusch V. 2002. Reduction of acute recurrence in patients with chronic recurrent hypertrophic sinusitis by treatment with a bacterial immunostimulant (*Enterococcus faecalis* Bacteriae of human origin. *Arzneimittel-Forsch.*52:622-627

Hall-Stoodley L., Costerton J. W., Stoodley P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.*2:95-108

Hanahan D. 1983. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Microbiol.*166:557-580

Hancock L. E., Gilmore M. S. 2002. The capsular polysaccharide of *Enterococcus faecalis* and its relationship to other polysaccharides in the cell wall. *Proc. Natl. Acad Sci. USA.*99:1574-1579

Hancock L. E., Perego M. 2004. Systematic inactivation and phenotypic characterization of two-component signal transduction systems of *Enterococcus faecalis* V583. *J. Bacteriol.*186:7951-7958

Hardy J., Francis K. P., DeBoer M., Chu P., Gibbs K., Contag C. H. 2004. Extracellular replication of *Listeria monocytogenes* in the murine gall bladder. *Science.*303:851-853

Harkness J. E., Wagner J. E. 1995. Biology and Medicine of Rabbits and Rodents. Philadelphia: Williams & Wilkins.

Hawes J. J., Nerva J. D., Reilly K. M. 2008. Novel dual-reporter preclinical screen for antiastrocytoma agents identifies cytostatic and cytotoxic compounds. *J. Biomol. Screen.*13:795-803

Hegde M., Englert D. L., Schrock S., Cohn W. B., Vogt C., Wood T. K., Manson M. D., Jayaraman A. 2011. Chemotaxis to the quorum-sensing signal AI-2 requires the Tsr chemoreceptor and the periplasmic LsrB AI-2-binding protein. *J. Bacteriol.*193:768-773

Heikkila M. P., Saris P. E. 2003. Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk. *J. Appl. Microbiol.*95:471-478

Hickson M., D'Souza A. L., Muthu N., Rogers T. R., Want S., Rajkumar C., Bulpitt C. J. 2007. Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ.*335:80

Hidron A. I., Jonathan E., Jean P., Teresa H., Dawn S., Daniel P., Scott F. 2008. NHSN Annual Update: Antimicrobial-Resistant Pathogens Associated with Healthcare Associated Infections: Annual Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect. Control Hosp. Epidemiol.*29:996-1011

Hodel-Christian S. L., Murray B. E. 1991. Characterization of the gentamicin resistance transposon Tn5281 from *Enterococcus faecalis* and comparison to staphylococcal transposons Tn4001 and Tn4031. *Antimicrob. Agents Chemother.*35:1147-1152

Hoffman R. M. 2011. Tumor-seeking Salmonella amino acid auxotrophs. *Curr. Opin. Biotechnol.*22:917-923

Huebner J., Wang Y., Krueger W. A., Madoff L. C., Martirosian G., Boisot S., Goldmann D. A., Kasper D. L., Tzianabos A. O., Pier G. B. 1999. Isolation and chemical characterization of a capsular polysaccharide antigen shared by clinical isolates of *Enterococcus faecalis* and vancomycin-resistant *Enterococcus faecium*. *Infect. Immun.*67:1213-1219

Hufnagel M., Hancock L. E., Koch S., Theilacker C., Gilmore M. S., Huebner J. 2004. Serological and genetic diversity of capsular polysaccharides in *Enterococcus faecalis*. *J. Clin. Microbiol.*42:2548-2557

Huovinen P. 2001. Bacteriotherapy: The time has come. *BMJ.*323:353-354

Hwang J., Mattei L. M., VanArendonk L. G., Meneely P. M., Okeke I. N. 2010. A pathoadaptive deletion in an enteroaggregative *Escherichia coli* outbreak strain enhances virulence in a *Caenorhabditis elegans* model. *Infect. Immun.*78:4068-4076

Iyoda S., Koizumi N., Satou H., Lu Y., Saitoh T., Ohnishi M., Watanabe H. 2006. The GrlR-GrlA regulatory system coordinately controls the expression of flagellar and LEE-encoded type III protein secretion systems in enterohemorrhagic *Escherichia coli*. *J. Bacteriol.*188:5682-5692

Jacobs M. A., Alwood A., Thaipisuttikul I., Spencer D., Haugen E., Ernst S., Will O., Kaul R., Raymond C., Levy R., Chun-Rong L., Guenthner D., Bovee D., Olson M. V., Manoil C. 2003. Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc. Natl. Acad Sci. USA.*100:14339-14344

Jansen E. D., Pickett P. M., Mackanos M. A., Virostko J. 2006. Effect of optical tissue clearing on spatial resolution and sensitivity of bioluminescence imaging. *J. Biomed. Opt.*11:041119

Jarvis K. G., Giron J. A., Jerse A. E., McDaniel T. K., Donnenberg M. S., Kaper J. B. 1995. Enteropathogenic *Escherichia coli* contains a putative type III secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. *Proc. Natl. Acad. Sci. U.S.A.*92:7996-8000

Jawhara S., Mordon S. 2004. In vivo imaging of bioluminescent *Escherichia coli* in a cutaneous wound infection model for evaluation of an antibiotic therapy. *Antimicrob. Agents Chemother.*48:3436-3441

Jerse A. E., Yu J., Tall B. D., Kaper J. B. 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc. Natl. Acad. Sci. U.S.A.*87:7839-7843

Jett B. D., Huycke M. M., Gilmore M. S. 1994. Virulence of enterococci. *Clin. Microbiol. Rev.*7:462-478

Jiang A., Wang H., Lee N., Yang G., Griffiths M. W. 2006. Biological characteristics of luminescent *Lactococcus lactis* transformed with lux genes. *Food Res. Int.*39:426-432

Johnston B. C., Supina A. L., Vohra S. 2006. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. *CMAJ* 175: 377-383.

Joyanes P., Pascual A., Martinez-Martinez L., Hevia A., Perea E. J. 2000. In vitro adherence of *Enterococcus faecalis* and *Enterococcus faecium* to urinary catheters. *Eur. J. Clin. Microbiol.*19:124-127

Judicial Commission of the International Committee on Bacteriological Nomenclature. 1958. Conservation of the family name Enterobacteriaceae, of the name of the type genus, and the designation of the type species.73-74

Kadurugamuwa J. L., Sin L. V., Yu J., Francis K. P., Kimura R., Purchio T., Contag P. R. 2003. Rapid direct method for monitoring antibiotics in a mouse model of bacterial biofilm infection. *Antimicrob. Agents Chemother.*47:3130-3137

Kalliomäki M., Salminen S., Arvilommi H., Kero P., Koskinen P., Isolauri E. 2001. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet.*357:1076-1079

Karmali M. A., Steele B. T., Petric M., Lim C. 1983. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet.*1:619-620

Kassavetis G. A., Butler E. T., Roulland D., Chamberlin M. J. 1982. Bacteriophage SP6-specific RNA polymerase. II. Mapping of SP6 DNA and selective in vitro transcription. *J. Biol. Chem.*257:5779-5788

Kenny B., DeVinney R., Stein M., Reinscheid D. J., Frey E. A., Finlay B. B. 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell.*91:511-520

Kessler C., Neumaier P. S., Wolf W. 1985. Recognition sequences of restriction endonucleases and methylases--a review. *Gene* 33: 1-102.

Kim H. S., Gilliland S. E. 1983. *Lactobacillus acidophilus* as a Dietary Adjunct for Milk to Aid Lactose Digestion in Humans. *J. Dairy Scr.*66:959-966

Kim Y., Han K. S., Imm J. Y., Oh S., You S., Park S., Kim S. H. 2006. Inhibitory effects of *Lactobacillus acidophilus* lysates on the cytotoxic activity of shiga-like toxin 2 produced from *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.*43:502-507

Kim Y., Oh S., Park S., Seo J. B., Kim S.-H. 2008. *Lactobacillus acidophilus* reduces expression of enterohemorrhagic *Escherichia coli* O157:H7 virulence factors by inhibiting autoinducer-2-like activity. *Food Control.*19:1042-1050

Kimmitt P. T., Harwood C. R., Barer M. R. 1999. Induction of type 2 Shiga toxin synthesis in *Escherichia coli* O157 by 4-quinolones. *Lancet.*353:1588-1589

Kimoto H., Nomura M., Kobayashi M., Mizumachi K., Okamoto T. 2003. Survival of lactococci during passage through mouse digestive tract. *Can. J. Microbiol.*49:707-711

Klaenhammer T. R., Kullen M. J. 1999. Selection and design of probiotics. *Int. J. Food Microbiol.*50:45-57

Klein G., Pack A., Reuter G. 1998. Antibiotic resistance patterns of enterococci and occurrence of vancomycin-resistant enterococci in raw minced beef and pork in Germany. *Appl. Environ. Microbiol.*64:1825-1830

Klerk C. P., Overmeer R. M., Niers T. M., Versteeg H. H., Richel D. J., Buckle T., Van Noorden C. J., van Tellingen O. 2007. Validity of bioluminescence measurements for noninvasive in vivo imaging of tumor load in small animals. *Biotechniques.*43:7-13, 30

Knudtson L. M., Hartman P. A. 1993. Comparison of fluorescent gentamicin-thallous-carbonate and KF streptococcal agars to enumerate enterococci and fecal streptococci in meats. *Appl. Environ. Microbiol.*59:936-938

Knutton S., Lloyd D. R., McNeish A. S. 1987. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. *Infect. Immun.*55:69-77

Kong Y., Akin A. R., Francis K. P., Zhang N., Troy T. L., Xie H., Rao J., Cirillo S. L. G., Cirillo J. D. 2011a. Whole-body imaging of infection using fluorescence. *Curr. Protoc. Microbiol.*Chapter 2:Unit 2C.3

Kong Y., Shi Y., Chang M., Akin A. R., Francis K. P., Zhang N., Troy T. L., Yao H., Rao J., Cirillo S. L. G., Cirillo J. D. 2011b. Whole-body imaging of infection using bioluminescence. *Curr. Protoc. Microbiol.*Chapter 2:Unit 2C.4

Kresse A. U., Beltrametti F., Muller A., Ebel F., Guzman C. A. 2000. Characterization of SepL of enterohemorrhagic *Escherichia coli*. *J. Bacteriol.*182:6490-6498

Langmead B., Trapnell C., Pop M., Salzberg S. L. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.*10:R25

Lauková A., Czikková S. 1999. The use of enterocin CCM 4231 in soy milk to control the growth of *Listeria monocytogenes* and *Staphylococcus aureus*. *J. Appl. Microbiol.*87:182-182

Lavigne J.-P., Nicolas-Chanoine M.-H., Bourg G., Moreau J., Sotto A. 2008. Virulent synergistic effect between *Enterococcus faecalis* and *Escherichia* coli assayed by using the *Caenorhabditis elegans* model. *PLoS ONE.*3:e3370

Lee K. M., Lim J., Nam S., Yoon M. Y., Kwon Y. K., Jung B. Y., Park Y., Park S., Yoon S. S. 2011. Inhibitory effects of broccoli extract on *Escherichia coli* O157:H7 quorum sensing and *in vivo* virulence. *FEMS Microbiol. Lett.*321:67-74

Lee Y., Kim Y., Yeom S., Kim S., Park S., Jeon C. O., Park W. 2008. The role of disulfide bond isomerase A (DsbA) of *Escherichia coli* O157:H7 in biofilm formation and virulence. *FEMS Microbiol. Lett.*278:213-222

Lewenza S., Falsafi R. K., Winsor G., Gooderham W. J., McPhee J. B., Brinkman F. S., Hancock R. E. 2005. Construction of a mini-Tn5-luxCDABE mutant library in *Pseudomonas aeruginosa* PAO1: a tool for identifying differentially regulated genes. *Genome Res.*15:583-589

Ley R. E., Peterson D. A., Gordon J. I. 2006a. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell.*124:837-848

Ley R. E., Turnbaugh P. J., Klein S., Gordon J. I. 2006b. Microbial ecology: human gut microbes associated with obesity. *Nature.*444:1022-1023

Li M., Rosenshine I., Tung S. L., Wang X. H., Friedberg D., Hew C. L., Leung K. Y. 2004. Comparative proteomic analysis of extracellular proteins of enterohemorrhagic and enteropathogenic *Escherichia coli* strains and their ihf and ler mutants. *Appl. Environ. Microbiol.*70:5274-5282

Licht T. R., Laugesen D., Jensen L. B., Jacobsen B. L. 2002. Transfer of the pheromone-inducible plasmid pCF10 among *Enterococcus faecalis* microorganisms colonizing the intestine of mini-pigs. *Appl. Environ. Microbiol.*68:187-193

Lindenstrauß A. G., Pavlovic M., Bringmann A., Behr J., Ehrmann M. A., Vogel R. F. 2011. Comparison of genotypic and phenotypic cluster analyses of virulence determinants and possible role of CRISPR elements towards their incidence in *Enterococcus faecalis* and *Enterococcus faecium*. *Syst. Appl. Microbiol.*34:553-560

Litopoulou-Tzanetaki E. 1990. Changes in Numbers and Kinds of Lactic Acid Bacteria During Ripening of Kefalotyri Cheese. *J. Food Science.*55:111-113

Liu Y.-G., Whittier R. F. 1995. Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. *Genomics* 25: 674-681.

Liu Z., Klatt C. G., Wood J. M., Rusch D. B., Ludwig M., Wittekindt N., Tomsho L. P., Schuster S. C., Ward D. M., Bryant D. A. 2011. Metatranscriptomic analyses of chlorophototrophs of a hot-spring microbial mat. *ISME J.*5:1279-1290

Lueoend H., Gasser H. 1964. Fecal streptococci as pathogens of food poisoning. *Mitt Geb Lebensmittelunters Hyg.*55:144-149

Macedo A. C., Malcata F. X., Hogg T. A. 1995. Microbiological profile in Serra ewes' cheese during ripening. *J. Appl. Microbiol.*79:1-11

Macfarlane S., Dillon J. F. 2007. Microbial biofilms in the human gastrointestinal tract. *J. Appl. Microbiol.*102:1187-1196

Mackie R. I., Sghir A., Gaskins H. R. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am. J. Clinc. Nutrit.*69:1035S-1045S

Mahajan-Miklos S., Tan M.-W., Rahme L. G., Ausubel F. M. 1999. Molecular Mechanisms of Bacterial Virulence Elucidated Using a *Pseudomonas aeruginosa* in a *Caenorhabditis elegans* Pathogenesis Model. *Cell.*96:47-56

Maisnier-Patin S., Forni E., Richard J. 1996. Purification, partial characterisation and mode of action of enterococcin EFS2, an antilisterial bacteriocin produced by a strain of *Enterococcus faecalis i*solated from a cheese. *Int. J. Food Microbiol.*30:255-270

Man A. L., Lodi F., Bertelli E., Regoli M., Pin C., Mulholland F., Satoskar A. R., Taussig M. J., Nicoletti C. 2008. Macrophage migration inhibitory factor plays a role in the regulation of microfold (M) cell-mediated transport in the gut. *J. Immunol.*181:5673-5680

Martin R., Langa S., Reviriego C., Jiminez E., Marin M. L., Xaus J., Fernandez L., Rodriguez J. M. 2003. Human milk is a source of lactic acid bacteria for the infant gut. *J. Pediatr.*143:754-758

Martin R., Langa S., Reviriego C., Jiminez E., Marin M. L., Olivares M., Boza J., Jimiez J., Fernandez L., Xaus J., Rodriguez J. M. 2004. The commensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. *Trends Food Sci.*15:121-127

Matthews S. A. 2010. Cellular subversion: Towards a complete repertoire of type-III secretion system effectors in enterohaemorrhagic *Escherichia coli* O157:H7. Ph.D. Thesis University of Birmingham.

Matz M. V., Marshall N. J., Vorobyev M. 2006. Are corals colorful? *Photochem. Photobiol.*82:345-350

Mazmanian S. K., Liu C. H., Tzianabos A. O., Kasper D. L. 2005. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122: 107-118.

McCormick J. K., Worobo R. W., Stiles M. E. 1996. Expression of the antimicrobial peptide carnobacteriocin B2 by a signal peptide-dependent general secretory pathway. *App. Environ. Microbiol.*62:4095-4099

McGinnis S., Madden T. L. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Res.*32:W20-W25

Medellin-Peña M. J., Wang H., Johnson R., Anand S., Griffiths M. W. 2007. Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*73:4259-4267

Mellies J. L., Elliott S. J., Sperandio V., Donnenberg M. S., Kaper J. B. 1999. The Per regulon of enteropathogenic *Escherichia coli*: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). *Mol. Microbiol.*33:296-306

Mellies J. L., Barron A. M. S., Haack K. R., Korson A. S., Oldridge D. A. 2006. The global regulator Ler is necessary for enteropathogenic *Escherichia coli* colonization of *Caenorhabditis elegans*. *Infect. Immun.*74:64-72

Mellmann A., Bielaszewska M., Zimmerhackl L. B., Prager R., Harmsen D., Tschape H., Karch H. 2005. Enterohemorrhagic Escherichia coli in human infection: In vivo evolution of a bacterial pathogen. Pages 785-792. Clinical infectious diseases : An official publication of the Infectious Diseases Society of America, vol. 41.

Miloud T., Henrich C., Hammerling G. J. 2007. Quantitative comparison of click beetle and firefly luciferases for in vivo bioluminescence imaging. *J Biomed. Opt.*12:054018

Moellering R. C., Wennersten C., Weinberg A. N. 1971. Studies on antibiotic synergism against enterococci. I. Bacteriologic studies. *J. Lab. Clin. Med.*77:821-828

Moellering R. C., Jr. 1992. Emergence of Enterococcus as a Significant Pathogen. *Clin. Infect. Dis.*14:1173-1176

Moon H. W., Whipp S. C., Argenzio R. A., Levine M. M., Giannella R. A. 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infect. Immun.*41:1340-1351

Morgan M., Page e. 2011. Rsamtools: Import aligned BAM file format sequences into R. *Bioconductor. R package version 1.0.8*

Morrison D. A. 1977. Transformation in *Escherichia coli*: cryogenic preservation of competent cells. *J Bacteriol.*132:349-351

Mortazavi A., Williams B. A., McCue K., Schaeffer L., Wold B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods.*5:621-628

Moulton K., Ryan P., Lay D., Willard S. 2009. Photonic plasmid stability of transformed *Salmonella typhimurium:* a comparison of three unique plasmids. *BMC Microbiol.*9:152

Mowat A. M. 2003. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat. Rev. Immunol.*3:331-341

Müller M. R. A., Ehrmann M. A., Vogel R. F. 2000. Multiplex PCR for the detection of *Lactobacillus pontis* and two related species in a sourdough fermentation. *Appl. Environ. Microbiol.*66:2113-2116

Murray B. E. 1990. The life and times of the Enterococcus. *Clin. Microbiol. Rev.*3:46-65

Mylonakis E., Engelbert M., Qin X., Sifri C. D., Murray B. E., Ausubel F. M., Gilmore M. S., Calderwood S. B. 2002. The *Enterococcus faecalis* fsrB gene, a key component of the fsr quorum-sensing system, is associated with virulence in the rabbit endophthalmitis model. *Infect. Immun.*70:4678-4681

Nataro J. P., Kaper J. B. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.*11:142-201

Newbury S. F., Smith N. H., Higgins C. F. 1987. Differential mRNA stability controls relative gene expression within a polycistronic operon. *Cell* 51: 1131-1143.

Nicholas H. R., Hodgkin J. 2004. Responses to infection and possible recognition strategies in the innate immune system of *Caenorhabditis elegans*. *Mol. Immunol.*41:479-493

Nieto C., Espinosa M. 2003. Construction of the mobilizable plasmid pMV158GFP, a derivative of pMV158 that carries the gene encoding the green fluorescent protein. *Plasmid* 49: 281-285.

Nieto-Arribas P., Sesena S., Poveda J. M., Chicon R., Cabezas L., Palop L. 2011. *Enterococcus* populations in artisanal Manchego cheese: Biodiversity, technological and safety aspects. *Food Microbiol.*28:891-899

Noble C. J. 1978. Carriage of group D streptococci in the human bowel. *J. Clin. Pathol.*31:1182-1186

Noble W. C., Virani Z., Cree R. G. 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol. Lett.*72:195-198

Nunez M., Rodriguez J. L., Garcia E., Gaya P., Medina M. 1997. Inhibition of *Listeria monocytogenes* by enterocin 4 during the manufacture and ripening of Manchego cheese. *J. Appl. Microbiol.*83:671-677

O'Toole G. A., Pratt L. A., Watnick P. I., Newman D. K., Weaver V. B., Kolter R. 1999. Genetic approaches to study of biofilms. *Method Enzymol.*310:91-109

Obrig T. G., Louise C. B., Lingwood C. A., Boyd B., Barley-Maloney L., Daniel T. O. 1993. Endothelial heterogeneity in Shiga toxin receptors and responses. *J. Biol. Chem.*268:15484-15488

Ogasawara H., Yamamoto K., Ishihama A. 2011. Role of the biofilm master regulator CsgD in cross-regulation between biofilm formation and flagellar synthesis. *J. Bacteriol.*193:2587-2597

Olmsted S. B., Kao S. M., van Putte L. J., Gallo J. C., Dunny G. M. 1991. Role of the pheromone-inducible surface protein Asc10 in mating aggregate formation and conjugal transfer of the *Enterococcus faecalis* plasmid pCF10. *J. Bacteriol.*173:7665-7672

Orberg P. K., Sandine W. E. 1985. Survey of antimicrobial resistance in lactic streptococci. *App.l Environ. Microbiol.*49:538-542

Ostroff S. M., Tarr P. I., Neill M. A., Lewis J. H., Hargrett-Bean N., Kobayashi J. M. 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J. Infect. Dis.*160:994-998

Oumer B. A., Gaya P., Fernandez-Garcia E., Marciaca R., Garde S., Medina M., Nunez M. 2001. Proteolysis and formation of volatile compounds in cheese manufactured with a bacteriocin-producing adjunct culture. *J. Diary Res.*68:117-129

Owen R. L. 1999. Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches‚Äî a personal and historical perspective. *Semin. Immunol.*11:157-163

Pages H., Aboyoun P., Lawrence M. 2011. iRanges: Infrastructure for manipulating intervals on sequences, R package version 1.6.8.

Paiva de Sousa C., Dubreuil J. D. 2001. Distribution and expression of the astA gene (EAST1 toxin) in Escherichia coli and Salmonella. *Int. J. Med. Micrbiol.*291:15-20

Pallen M. J., Beatson S. A., Bailey C. M. 2005. Bioinformatics analysis of the locus for enterocyte effacement provides novel insights into type-III secretion. *BMC Microbiol.5*:9

Palmer C., Bik E. M., DiGiulio D. B., Relman D. A., Brown P. O. 2007. Development of the human infant intestinal microbiota. *PLoS Biol.*5:e177

Palmer K. L., Gilmore M. S. 2010. Multidrug-resistant enterococci lack CRISPR-cas. *mBio.*1:

Passalacqua K. D., Varadarajan A., Ondov B. D., Okou D. T., Zwick M. E., Bergman N. H. 2009. Structure and complexity of a bacterial transcriptome. *J. Bacteriol.*191:3203-3211

Pauling J., Rottger R., Neuner A., Salgado H., Collado-Vides J., Kalaghatgi P., Azevedo V., Tauch A., Puhler A., Baumbach J. 2012. On the trail of EHEC/EAEC-unraveling the gene regulatory networks of human pathogenic *Escherichia coli* bacteria. *Integr. Biol.*

Paulsen I. T., Banerjei L., Myers G. S. A., Nelson K. E., Seshadri R., Read T. D., Fouts D. E., Eisen J. A., Gill S. R., Heidelberg J. F., Tettelin H., Dodson R. J., Umayam L., Brinkac L., Beanan M., Daugherty S., DeBoy R. T., Durkin S., Kolonay J., Madupu R., Nelson W., Vamathevan J., Tran B., Upton J., Hansen T., Shetty J., Khouri H., Utterback T., Radune D., Ketchum K. A., Dougherty B. A., Fraser C. M. 2003a. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. *Science.*299:2071-2074

Paulsen I. T., Banerjei L., Myers G. S. A., Nelson K. E., Seshadri R., Read T. D., Fouts D. E., Eisen J. A., Gill S. R., Heidelberg J. F., Tettelin H., Dodson R. J., Umayam L., Brinkac L., Beanan M., Daugherty S., DeBoy R. T., Durkin S., Kolonay J., Madupu R., Nelson W., Vamathevan J., Tran B., Upton J., Hansen T., Shetty J., Khouri H., Utterback T., Radune D., Ketchum K. A., Dougherty B. A., Fraser C. M. 2003b. Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. *Science* 299: 2071-2074.

Penders J., Thijs C., Vink C., Stelma F. F., Snijders B., Kummeling I., van den Brandt P. A., Stobberingh E. E. 2006. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics.*118:511-521

Perdigon G., Fuller R., Raya R. 2001. Lactic acid bacteria and their effect on the immune system. *Curr. Issues Intest. Microbiol.*2:27-42

Perez P. F., Dore J., Leclerc M., Levenez F., Benyacoub J., Serrant P., Segura-Roggero I., Schiffrin E. J., Donnet-Hughes A. 2007. Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics.*119:e724-732

Perna N. T., Plunkett G., 3rd, Burland V., Mau B., Glasner J. D., Rose D. J., Mayhew G. F., Evans P. S., Gregor J., Kirkpatrick H. A., Posfai G., Hackett J., Klink S., Boutin A., Shao Y., Miller L., Grotbeck E. J., Davis N. W., Lim A., Dimalanta E. T., Potamousis K. D., Apodaca J., Anantharaman T. S., Lin J., Yen G., Schwartz D. C., Welch R. A., Blattner F. R. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature.*409:529-533

Pillar C. M., Gilmore M. S. 2004. Enterococcal virulence--pathogenicity island of *E. faecalis*. *Front. Biosci.*9:2335-2346

Popp F. A., Nagl W., Li K. H., Scholz W., Weingartner O., Wolf R. 1984. Biophoton emission. New evidence for coherence and DNA as source. *Cell biophysics* 6: 33-52.

Pruss B. M., Matsumura P. 1997. Cell cycle regulation of flagellar genes. *J. Bacteriol.*179:5602-5604

Qin X., Singh K. V., Weinstock G. M., Murray B. E. 2000. Effects of *Enterococcus faecalis* fsr genes on production of gelatinase and a serine protease and virulence. *Infect Immun.*68:2579-2586

R Development Core Team. 2010. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rajilic-Stojanovic M., Smidt H., de Vos W. M. 2007. Diversity of the human gastrointestinal tract microbiota revisited. *Environ. Microbiol.*9:2125-2136

Rasmussen T. B., Bjarnsholt T., Skindersoe M. E., Hentzer M., Kristoffersen P., Kote M., Nielsen J., Eberl L., Givskov M. 2005. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J. Bacteriol.*187:1799-1814

Rautio M., Jousimies-Somer H., Kauma H., Pietarinen I., Saxelin M., Tynkkynen S., Koskela M. 1999. Liver Abscess Due to a *Lactobacillus rhamnosus* Strain Indistinguishable from *L. rhamnosus* Strain GG. *Clin. Infect. Dis.*28:1159-1160

Reading N. C., Rasko D. A., Torres A. G., Sperandio V. 2009. The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *Proc. Natl. Acad. Sci. USA* 106:5889-5894

Renz-Polster H., David M. R., Buist A. S., Vollmer W. M., O'Connor E. A., Frazier E. A., Wall M. A. 2005. Caesarean section delivery and the risk of allergic disorders in childhood. *Clin. Exp. Allergy.*35:1466-1472

Rice B. W., Cable M. D., Nelson M. B. 2001. In vivo imaging of light-emitting probes. *J. Biomed. Opt.*6:432-440

Rich R. L., Kreikemeyer B., Owens R. T., LaBrenz S., Narayana S. V., Weinstock G. M., Murray B. E., Höök M. 1999. Ace is a collagen-binding MSCRAMM from Enterococcus faecalis. *J. Biol. Chem.*274:26939-26945

Riley L. W., Remis R. S., Helgerson S. D., McGee H. B., Wells J. G., Davis B. R., Hebert R. J., Olcott E. S., Johnson L. M., Hargrett N. T., Blake P. A., Cohen M. L. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.*308:681-685

Robert Koch-Institut. 2007. Falldefinitionen des Robert Koch-Instituts zur Übermittlung von Erkrankungs- oder Todesfällen und Nachweis von Krankheitserregern. *gemäß §4 Abs. 2 des Gesetzes zur Verhütung und Bekämpfung von Infektionskrenkheiten beim Mensch (Infektionsschutzgesetzt)*

Robert Koch-Institut. 2011a. Erkrankungen durch Enterohämorrhagische Escherichia coli (EHEC), Erstveröffentlichung im Epidemiologischen Bulletin 31/1999, Aktualisierte Fassung vom Juni 2011

Robert Koch-Institut. 2011b. EHEC/HUS O104:H4 - Der Ausbruch wird als beendet betrachtet. Pressemitteilung des Robert Koch-Instituts. 26. Juli 2011

Rocchetta H. L., Boylan C. J., Foley J. W., Iversen P. W., LeTourneau D. L., McMillian C. L., Contag P. R., Jenkins D. E., Parr T. R. 2001. Validation of a noninvasive, real-time imaging technology using bioluminescent *Escherichia coli* in the neutropenic mouse thigh model of infection. *Antimicrob. Agents Chemother.*45:129-137

Rosenkranz W., Grundmann E. 1994. Immunomodulator action of living, nonpathogenic *Enterococcus faecalis* bacteria from humans. *Arzneimittel-Forschung.*44:691-695

Round J. L., Mazmanian S. K. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.*9:313-323

Russell W. M., Klaenhammer T. R. 2001. Efficient system for directed integration into the *Lactobacillus acidophilus* and *Lactobacillus gasseri* chromosomes via homologous recombination. *Appl. Environ. Microbiol.*67:4361-4364

Ruszczynski M., Radzikowski A., Szajewska H. 2008. Clinical trial: effectiveness of *Lactobacillus rhamnosus* (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Aliment. Pharmacol. Ther.*28:154-161

Salih A., Larkum A., Cox G., Kuhl M., Hoegh-Guldberg O. 2000. Fluorescent pigments in corals are photoprotective. *Nature* 408: 850-853.

Salminen S., Bouley C. B.-R., M-C. 1998. Functional food science and gastrointestinal physiology and function. *Brit. J. Nutr.*80:147-171

Sambrook J., Fritsch E. F., Maniatis T. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor, USA.

Sanders M. E. 1998. Overview of functional foods: emphasis on probiotic bacteria. *Int. Dairy J.*8:341-349

Sandoe J. A., Witherden I. R., Au-Yeung H. K., Kite P., Kerr K. G., Wilcox M. H. 2002. Enterococcal intravascular catheter-related bloodstream infection: management and outcome of 61 consecutive cases. *J Antimirob. Chermother.*50:577-582

Sanz P., Teel L. D., Alem F., Carvalho H. M., Darnell S. C., O'Brien A. D. 2008. Detection of *Bacillus anthracis* spore germination in vivo by bioluminescence imaging. *Infect. Immun.*76:1036-1047

Sanz Y., Palma G. D. 2009. Gut microbiota and probiotics in modulation of epithelium and gut-associated lymphoid tissue function. *Int. Rev. Immunol.*28:397-413

Sartingen S., Rozdzinski E., Muscholl-Silberhorn A., Marre R. 2000. Aggregation substance increases adherence and internalization, but not translocation, of *Enterococcus faecalis* through different intestinal epithelial cells in vitro. *Infect. Immun.*68:6044-6047

Savage D. C. 1977. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.*31:107-133

Schleifer K. H., Kilpper-Bälz R. 1984. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the Genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *IJSEM.*34:31-34

Schultz M., Watzl S., Oelschlaeger T. A., Rath H. C., Göttl C., Lehn N., Schälmerich J., Linde H.-J. 2005. Green fluorescent protein for detection of the probiotic microorganism *Escherichia coli* strain Nissle 1917 (EcN) in vivo. *J. Microbiol. Methods.*61:389-398

Schweder T., Lin H. Y., Jurgen B., Breitenstein A., Riemschneider S., Khalameyzer V., Gupta A., Buttner K., Neubauer P. 2002. Role of the general stress response during strong overexpression of a heterologous gene in *Escherichia coli*. *Appl. Microbiol. Biotech.*58:330-337

Scott K. P., Mercer D. K., Richardson A. J., Melville C. M., Glover L. A., Flint H. J. 2000. Chromosomal integration of the green fluorescent protein gene in lactic acid bacteria and the survival of marked strains in human gut simulations. *FEMS Microbiol. Lett.*182:23-27

Shaner N. C., Campbell R. E., Steinbach P. A., Giepmans B. N. G., Palmer A. E., Tsien R. Y. 2004. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.*22:1567-1572

Shaner N. C., Steinbach P. A., Tsien R. Y. 2005. A guide to choosing fluorescent proteins. *Nat. Methods.*2:905-909

Shaner N. C., Lin M. Z., McKeown M. R., Steinbach P. A., Hazelwood K. L., Davidson M. W., Tsien R. Y. 2008. Improving the photostability of bright monomeric orange and red fluorescent proteins. *Nat. Methods.*5:545-551

Shankar N., Lockatell C. V., Baghdayan A. S., Drachenberg C., Gilmore M. S., Johnson D. E. 2001. Role of Enterococcus faecalis surface protein Esp in the pathogenesis of ascending urinary tract infection. *Infection and immunity* 69: 4366-4372.

Sharma V. K., Zuerner R. L. 2004. Role of hha and ler in transcriptional regulation of the esp operon of enterohemorrhagic *Escherichia coli* O157:H7. *J. Bacteriol.*186:7290-7301

Shepard B. D., Gilmore M. S. 1995. Methods in Molecular Biology: Humana Press, Inc. Totowa, NJ.

Sherman J. M. 1937. The streptococci. *Bacteriology Reviews.*1:3-97

Shimomura O., Johnson F. H., Saiga Y. 1962. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J. Cell. Physio.*59:223-239

Sifri C. D., Mylonakis E., Singh K. V., Qin X., Garsin D. A., Murray B. E., Ausubel F. M., Calderwood S. B. 2002. Virulence effect of *Enterococcus faecalis* protease genes and the quorum-sensing locus fsr in *Caenorhabditis elegans* and mice. *Infect. Immun.*70:5647-5650

Sifri C. D., Begun J., Ausubel F. M. 2005. The worm has turned - microbial virulence modeled in *Caenorhabditis elegans*. *Trends Microbiol.*13:119-127

Simonetta A. C., Moragues de Velasco L. G., Frison L. N. 1997. Antibacterial activity of enterococci strains against Vibrio cholerae. *Lett. App. Microbiol.*24:139-143

Singh K. V., Qin X., Weinstock G. M., Murray B. E. 1998. Generation and testing of mutants of *Enterococcus faecalis* in a mouse peritonitis model. *J. Infect. Dis.*178:1416-1420

Smyth C. J., Halpenny M. K., Ballagh S. J. 1987. Carriage rates of enterococci in the dental plaque of haemodialysis patients in Dublin. *Brit. J. Oraal Surg.*25:21-33

Sperandio V., Torres A. G., Kaper J. B. 2002. Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol. Microbiol.*43:809-821

Stiernagle T. 2006. Maintenance of *C. elegans*: WormBook 10.1895/wormbook.1.101.1 http://www.wormbook.org.

Strauss E. 2000. Microbiology. Fighting bacterial fire with bacterial fire. *Science* 290: 2231-2233.

Stuart C. H., Schwartz S. A., Beeson T. J., Owatz C. B. 2006. *Enterococcus faecalis*: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment. *J. Endod.*32:93-98

Sulzer G., Busse M. 1991. Growth inhibition of Listeria spp. on Camembert cheese by bacteria producing inhibitory substances. *Int J. Food Microbiol.*14:287-296

Surette M. G., Miller M. B., Bassler B. L. 1999. Quorum sensing in *Escherichia coli, Salmonella typhimurium,* and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. U.S.A.*96:1639-1644

Svoboda P. 2009. Cloning and sequencing an inverted repeat. *Cold Spring Harb. Protoc.*2009:pdb.ip64

Sweeney N. J., Klemm P., McCormick B. A., Moller-Nielsen E., Utley M., Schembri M. A., Laux D. C., Cohen P. S. 1996. The Escherichia coli K-12 gntP gene allows E. coli F-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine. *Infection and immunity* 64: 3497-3503.

Swidsinski A., Loening-Baucke V., Lochs H., Hale L. P. 2005a. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *World J Gastroenterol.*11:1131-1140

Swidsinski A., Weber J., Loening-Baucke V., Hale L. P., Lochs H. 2005b. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J. Clin. Microbiol.*43:3380-3389

Tan G., Gao Y., Shi M., Zhang X., He S., Chen Z., An C. 2005. SiteFinding-PCR: a simple and efficient PCR method for chromosome walking. *Nucleic Acids Res.*33:e122

Tan M. W., Mahajan-Miklos S., Ausubel F. M. 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl. Acad. Sci. USA.*96:715-720

Tannock G. W. 1999. Introduction. *Probiotics: A critical review*1-4

Tannock G. W., Cook G. 2002. Enterococci as Members of the Intestinal Microflora of Humans. *The enterococci: pathogenesis, molecular biology and antibiotic resistances.*1:101-126

Tarr P. I., Gordon C. A., Chandler W. L. 2005. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet.*365:1073-1086

Tatusov R. L., Galperin M. Y., Natale D. A., Koonin E. V. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.*28:33-36

Teuber M., Perreten V., Wirsching F. 1996. Antibiotikumresistente Bakterien: eine neue Dimension in der Lebensmittelmikrobiologie: Institut für Lebensmittelwissenschaft, ETH Zürich.

Thiercelin M. E. 1899. Sur un diplocoque saprophyte de l'intestin susceptible de devenir pathogen. *C. R. Soc. Biol.*5:269-271

Troy T., Jekic-McMullen D., Sambucetti L., Rice B. 2004. Quantitative comparison of the sensitivity of detection of luorescent and bioluminescent reporters in animal models. *Molecular imaging.*3:9-23

Turtura G. C., Lorenzelli P. 1994. Gram-positive cocci isolated from slaughtered poultry. *Microbiol. Res.*149:203-213

Uehara Y., Kikuchi K., Nakamura T., Nakama H., Agematsu K., Kawakami Y., Maruchi N., Totsuka K. 2001. H(2)O(2) produced by viridans group streptococci may contribute to inhibition of methicillin-resistant *Staphylococcus aureus* colonization of oral cavities in newborns. *Clin. Infect. Dis.*32:1408-1413

United States Environmental Protection Agency. 1986. Ambient water quality criteria for bacteria. *EPA-440/5-84-002*

van de Guchte M., van der Vossen J. M., Kok J., Venema G. 1989. Construction of a lactococcal expression vector: expression of hen egg white lysozyme in *Lactococcus lactis* subsp. lactis. *Appl. Environ. Microbiol.*55:224-228

van den Bogaard A. E., Mertens P., London N. H., Stobberingh E. E. 1997. High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in The Netherlands: is the addition of antibiotics to animal feeds to blame? *J. Antimicrob. Chemother.*40:454-456

Van Leeuwen P. A., Boermeester M. A., Houdijk A. P., Ferwerda C. C., Cuesta M. A., Meyer S., Wesdorp R. I. 1994. Clinical significance of translocation. *Gut* 35: S28-34.

Venditti M., Biavasco F., Varaldo P. E., Macchiarelli A., De Biase L., Marino B., Serra P. 1993. Catheter-related endocarditis due to glycopeptide-resistant *Enterococcus faecalis* in a transplanted heart. *Clin. Infect. Dis.*17:524-525

Venema K., Kok J., Marugg J. D., Toonen M. Y., Ledeboer A. M., Venema G., Chikindas M. L. 1995. Functional analysis of the pediocin operon of *Pediococcus acidilactici* PAC1.0: PedB is the immunity protein and PedD is the precursor processing enzyme. *Mol. Microbiol.*17:515-522

Venturi A., Gionchetti P., Rizzello F., Johansson R., Zucconi E., Brigidi P., Matteuzzi D., Campieri M. 1999. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment. Pharmacol. Ther.*13:1103-1108

Vitreschak A. G., Rodionov D. A., Mironov A. A., Gelfand M. S. 2002. Regulation of riboflavin biosynthesis and transport genes in bacteria by transcriptional and translational attenuation. *Nucleic Acids Res.*30:3141-3151

Viviani V. R. 2002. The origin, diversity, and structure function relationships of insect luciferases. *Cell. Mol. Life Sci.*59:1833-1850

Wainwright L. A., Kaper J. B. 1998. EspB and EspD require a specific chaperone for proper secretion from enteropathogenic *Escherichia coli*. *Mol. Microbiol.*27:1247-1260

Waldor M. K., Friedman D. I. 2005. Phage regulatory circuits and virulence gene expression. *Curr. Opin. Microbiol.*8:459-465

Walters M., Sperandio V. 2006. Autoinducer 3 and epinephrine signaling in the kinetics of locus of enterocyte effacement gene expression in enterohemorrhagic *Escherichia coli*. *Infect. Immun.*74:5445-5455

Wang S., Ng L. H., Chow W. L., Lee Y. K. 2008. Infant intestinal Enterococcus faecalis down-regulates inflammatory responses in human intestinal cell lines. *World journal of gastroenterology : WJG* 14: 1067-1076.

Wang X., Kim Y., Ma Q., Hong S. H., Pokusaeva K., Sturino J. M., Wood T. K. 2010. Cryptic prophages help bacteria cope with adverse environments. *Nat. Commun.*1:147

Weaver K. E., Weaver D. M., Wells C. L., Waters C. M., Gardner M. E., Ehli E. A. 2003. *Enterococcus faecalis* plasmid pAD1-encoded Fst toxin affects membrane permeability and alters cellular responses to lantibiotics. *J. Bacteriol.*185:2169-2177

Weber S. G., Gold H. S. 2003. Enterococcus: an emerging pathogen in hospitals. *Sem. Resp. Crit. Care M.*24:49-60

Weigel L. M., Clewell D. B., Gill S. R., Clark N. C., McDougal L. K., Flannagan S. E., Kolonay J. F., Shetty J., Killgore G. E., Tenover F. C. 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science.*302:1569-1571

Wells C. L., Maddaus M. A., Simmons R. L. 1988. Proposed mechanisms for the translocation of intestinal bacteria. *Rev. Infect. Dis.*10:958-979

Wells C. L., Jechorek R. P., Erlandsen S. L. 1990. Evidence for the translocation of *Enterococcus faecalis* across the mouse intestinal tract. *J Infect. Dis.*162:82-90

Widder E. A. 2010. Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity. *Science.*328:704-708

Xicohtencatl-Cortes J., Monteiro-Neto V., Ledesma M. A., Jordan D. M., Francetic O., Kaper J. B., Puente J. L., Giron J. A. 2007. Intestinal adherence associated with type IV pili of enterohemorrhagic *Escherichia coli* O157:H7. *J. Clin. Invest.*117:3519-3529

Xu Y., Singh K. V., Qin X., Murray B. E., Weinstock G. M. 2000. Analysis of a gene cluster of *Enterococcus faecalis* involved in polysaccharide biosynthesis. *Infect. Immun.*68:815-823

Yagur-Kroll S., Belkin S. 2011. Upgrading bioluminescent bacterial bioreporter performance by splitting the lux operon. *Anual Bioanal. Chem.*

Yang F., Moss L. G., Phillips G. N., Jr. 1996. The molecular structure of green fluorescent protein. *Nature Biotech.*14:1246-1251

Youvan D. C., Michel-Beyerle M. E. 1996. Structure and fluorescence mechanism of GFP. *Nature Biotech.*14:1219-1220

Zeng J., Teng F., Weinstock G. M., Murray B. E. 2004. Translocation of *Enterococcus faecalis* strains across a monolayer of polarized human enterocyte-like T84 cells. *J. Clin. Microbiol.*42:1149-1154

Zhang L., Chaudhuri R. R., Constantinidou C., Hobman J. L., Patel M. D., Jones A. C., Sarti D., Roe A. J., Vlisidou I., Shaw R. K., Falciani F., Stevens M. P., Gally D. L., Knutton S., Frankel G., Penn C. W., Pallen M. J. 2004. Regulators encoded in the *Escherichia coli* type III secretion system 2 gene cluster influence expression of genes within the locus for enterocyte effacement in enterohemorrhagic *E. coli* O157:H7. *Infect. Immun.*72:7282-7293

Zhang X., McDaniel A. D., Wolf L. E., Keusch G. T., Waldor M. K., Acheson D. W. 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J. Infect. Dis.*181:664-670

Zhao H., Doyle T. C., Coquoz O., Kalish F., Rice B. W., Contag C. H. 2005. Emission spectra of bioluminescent reporters and interaction with mammalian tissue determine the sensitivity of detection in vivo. *J Biomed. Opt.*10:41210

Zoetendal E. G., von Wright A., Vilpponen-Salmela T., Ben-Amor K., Akkermans A. D., de Vos W. M. 2002. Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl. Environ. Microbiol.*68:3401-3407