Published in final edited form as:

Methods Mol Biol. 2020 ; 2081: 107–126. doi:10.1007/978-1-4939-9940-8_8.

Multiplex Imaging of Polymicrobial Communities—Murine Models to Study Oral Microbiome Interactions

Jens Kreth¹, Yasser M. Abdelrahman^{1,2}, Justin Merritt^{3,4}

¹Department of Restorative Dentistry, School of Dentistry, Oregon Health and Science University, Portland, OR, USA.

²Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

³Department of Restorative Dentistry, School of Dentistry, Oregon Health and Science University, Portland, OR, USA.

⁴Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, OR, USA.

Abstract

Similar to other mucosal surfaces of the body, the oral cavity hosts a diverse microbial flora that live in polymicrobial biofilm communities. It is the ecology of these communities that are the primary determinants of oral health (symbiosis) or disease (dysbiosis). As such, both symbiosis and dysbiosis are inherently polymicrobial phenomena. In an effort to facilitate studies of polymicrobial communities within rodent models, we developed a suite of synthetic luciferases suitable for multiplexed in situ analyses of microbial ecology and specific gene expression. Using this approach, it is feasible to noninvasively measure multiple luciferase signals in vivo with both spatial and temporal resolution. In the following chapter, we describe the relevant details and protocols used to establish a biophotonic imaging platform for the study of experimental polymicrobial oral biofilms and abscesses in mice. The protocols described here are specifically tailored for use with oral streptococci, but the general strategies are adaptable for a wide range of polymicrobial infection studies using other species.

Keywords

Microbiome; Streptococcus; Animal model; Luciferase; Abscess; Biofilm; Dental plaque; Polymicrobial infection; Biophotonic; Bioluminescent imaging

1 Introduction

The oral cavity is a unique host environment that supports host–flora interactions on a variety of mucosal surfaces like the gingiva, hard palate, and tongue as well as on the mineralized nonshedding surfaces of the teeth [1-3]. The oral cavity is also the site of the

two most prevalent human dysbiotic diseases, caries (tooth decay) and periodontitis (gum disease).

Symbiosis between the host and microbiota supports oral and systemic health and is achieved through a complex array of microbe–microbe and host–microbe interactions [1, 4, 5]. During a symbiotic relationship with the host, the microbial ecology of the flora favors a high proportion of health-supporting commensal species that prevent the overgrowth of pathobionts via various antagonistic abilities like the production of hydrogen peroxide (H₂O₂) [6, 7]. Pathobionts are potentially pathogenic members of the flora community that remain relatively benign provided they are maintained in a low overall abundance [2, 8]. Furthermore, the oral commensal microbiota are comprised of many pioneer colonizer species that are particularly adept at attaching to and inhabiting unoccupied oral surfaces [2, 6]. As such, they play an essential role in preventing niche occupation by less specialized species that are far more likely to trigger pathology to the host.

Oral diseases such as caries and periodontitis are the result of a dysbiotic flora that favors the overgrowth of pathobionts. Like the commensal flora, there are no specific pathobionts known to be singularly responsible for controlling the health or disease status of the host [2, 8]. Thus, both symbiosis and dysbiosis are inherently polymicrobial phenomena controlled by the composition of diverse communities of microorganisms. Modeling the ecological changes associated with polymicrobial diseases is a considerable challenge, as the complex ecology of the flora is itself influenced by a myriad of environmental pressures derived from both host behaviors and host genetics [2, 3]. Therefore, any physiologically relevant experimental model system would need to account for the many complexities of the human oral environment.

Another significant disease associated with the oral cavity is the odontogenic abscess, which similarly exhibits a complex polymicrobial etiology [9, 10]. Dental abscesses develop when oral bacteria breach the integrity of the soft tissues, invading and destroying deeper parts of the oral mucosa. These painful abscesses can cause severe complications requiring surgical intervention and are among the top sources of nontraumatic dental emergencies, especially among children [11, 12]. Currently, there is a limited molecular understanding of odontogenic abscess formation, which is further exacerbated by a dearth of suitable experimental model systems.

In an effort to address some of the major limitations of existing rodent oral disease models, we developed a suite of synthetic luciferases suitable for multiplexing as a biophotonic imaging platform for in situ analyses of oral biofilm ecology [13]. The rodent oral cavity shares many key aspects with the human oral cavity, including innate/adaptive immunity, salivary flow, mineralized teeth, and a resident microbial flora [14, 15]. Thus, it provides a suitable environment to model oral disease development and the resulting clinical aspects of human disease. However, previous animal model systems offer limited options to assess the oral ecology of experimental infections, since the typical PCR- or culture-based approaches used to measure the bacteria are both invasive and often require euthanasia of the host. Consequently, limited information can be gleaned from the spatiotemporal aspects of experimental in vivo oral biofilms. Using multiplexed luciferases for biophotonic imaging,

we have demonstrated the feasibility of noninvasively measuring multiple species and specific gene expression in vivo with both spatial and temporal resolution [13]. Our successes with the polymicrobial oral biofilm model subsequently led to an adaptation of this technology for the study of experimental polymicrobial abscesses formed by oral microbes. Our currently employed luciferases have been optimized specifically for expression in oral streptococci and support multiplexing between separate luciferase-expressing species as well as multiplexing within individual organisms for the study of gene expression [13]. To facilitate multiplexed biophotonic imaging, we compared luciferases with easily distinguishable characteristics (i.e., unique enzyme substrates and emission spectra), such as firefly, Cypridina, Renilla, Green Renilla, click beetle green, and Luciola red luciferases (for more information see Note 1).

Oral streptococci are highly abundant and crucial components of oral biofilms, constituting the vast majority of initial colonizers [3]. A recent metatranscriptomic study determined that > 50% of all transcripts detected in supragingival plaques are derived from oral streptococci, which is nearly 5× greater than the next most prevalent genus [16]. Not surprisingly, the ecological significance of oral streptococci for both oral health and disease has been well established by a plethora of clinical and experimental studies [2, 6, 7]. For our model system, we created luciferase reporter strains of the following species: *Streptococcus mutans* (major caries pathobiont), *Streptococcus sanguinis* (pioneer colonizing commensal), *Streptococcus gordonii* (pioneer colonizing commensal), and *Streptococcus anginosus* (periodontitis pathobiont and major component of odontogenic abscesses). In the following chapter, we describe the relevant details and protocols used to establish a biophotonic imaging platform for the study of experimental oral biofilms and abscesses in mice. Furthermore, we have successfully employed these protocols using both of the most commonly employed small animal imaging systems: IVIS (Perkin Elmer) and In-Vivo Xtreme (Bruker). The techniques, reagents, and other materials used in both the oral biofilm and abscess models are grouped when possible to avoid repetition. The following protocols should also be easily adaptable for use in other multiplexed infection models.

2 Materials

2.1 Bacterial Strains

Oral streptococcal bioluminescent reporter strains are constructed in the following wild-type strain backgrounds:

1. *S. mutans* UA159 (genome reference strain) [17].
2. *S. sanguinis* SK36 (genome reference strain) [18].
3. *S. gordonii* DL1 (genome reference strain) [19].
4. *S. anginosus* OUP10 (invasive disease clinical isolate). For more information about *S. anginosus* genomes, see [20, 21].

¹Table 1 provides a comparison of luciferases (click beetle green, Cypridina, firefly, Green Renilla, Luciola red, and renilla) that have been assayed in oral streptococci.

For the polymicrobial abscess model, the following wild-type abscess clinical isolates of *Fusobacterium nucleatum* and *Prevotella nigrescens* are coinfecting together with *S. anginosus*:

1. *Fusobacterium nucleatum* ssp. *nucleatum* OHSU59Fn (oral abscess clinical isolate).
2. *Prevotella nigrescens* OHSU79Pn (oral abscess clinical isolate).

2.2 Bacterial Growth Conditions

1. Todd-Hewitt +0.3% (wt/vol) yeast extract (THY) agar plates: 30 g/L TH base (Difco) + 3 g/L yeast extract +15 g/L bacteriological agar. Autoclave at 121 °C for 15 min prior to usage. If antibiotics are supplemented in the agar plates, these should be added after the autoclaved medium has cooled to 55 °C.
2. THY liquid medium: Prepare as for #1 above but without the addition of bacteriological agar.
3. Chemically defined medium (CDM): see ref. 22, supplement with 0.5% (wt/vol) fructose and 1% (wt/vol) sucrose [13].
4. Erythromycin: 5 µg/ml; used for antibiotic selection of *S. gordonii*, *S. sanguinis*, and *S. anginosus* in liquid cultures and on solid media. For *S. mutans* antibiotic selection, 800 µg/ml kanamycin or 12 µg/ml erythromycin are added.
5. *p*-Chlorophenylalanine (4-CP, Sigma): 0.02 M, For the construction of markerless mutants of *S. mutans* and *S. anginosus*, agar plates are supplemented with 0.02 M 4-CP for counterselection.

2.3 DNA Purification and Manipulation

1. Wizard Genomic DNA purification kit (Promega), DNA Clean and Concentrator 25 kit (Zymo Research), or other similar commercially available DNA extraction kit.
2. Phusion DNA polymerase (New England Biolabs) or Accuprime DNA polymerase (ThermoFisher Scientific) for polymerase chain reaction (PCR) and overlap extension PCR (OE-PCR).
3. PCR and OE-PCR reactions can be performed in any standard thermal cycler. In the described examples, the reactions are performed using a 2720 GeneAmp thermal cycler (Applied Biosystems).

2.4 Luciferase Assays

1. D-Luciferin: 2 mM stock solutions of D-luciferin are created by diluting in 0.1 M citrate buffer (0.04 M sodium citrate dihydrate +0.06 M citric acid dissolved in ddH₂O) adjusted to pH 6.0. Use as substrate for *Luciola* red luciferase (as well as other beetle luciferases).
2. Coelenterazine or its synthetic analog coelenterazine-h (NanoLight Technologies): Stock solutions are created by diluting to 0.75 mg/ml in 100%

ethanol. Both are suitable substrates for Green Renilla luciferase (*see* Notes 2 and 3).

3. Black or white 96-well microplates (*see* Note 4).
4. Microplate reader: in vitro bioluminescence measurements can be obtained in any plate reader capable of measuring luminescence. However, the greatest sensitivity is typically achieved with a dedicated luminometer. In the described examples, samples are measured in a GloMax Discoverer (Promega).

2.5 Animal Housing and Handling

1. BALB/cByJ or C57BL/6J mice (The Jackson Laboratory): House in a maximum of 5 mice/cage. Line cages with standard bedding and provide a source of rodent enrichment.
2. Laboratory Rodent Diet 5001.
3. Isoflurane: 3% (vol/vol), for anesthetization of mice, when required.

2.6 Oral Biofilm Model

1. Weanling 3-week-old BALB/cByJ mice (*see* Notes 5 and 6).
2. Mouse drinking water supplements: Depending upon the stage and/or type of experiment, mouse drinking water is supplemented with either 100 µg/ml

²Both coelenterazine and coelenterazine-h are suitable substrates for Green Renilla reporter strains. However, we have observed up to tenfold higher bioluminescence values with coelenterazine-h. Therefore, it is typically employed as the preferred substrate.

³We have demonstrated exceptionally strong bioluminescence from Cypridina luciferase reporter strains of oral streptococci. In fact, its signal output is quite similar to that of Green Renilla [13]. Unfortunately, its substrate vargulin is also apparently not freely diffusible across the bacterial cell membrane when in aqueous solutions, unlike both D-luciferin and coelenterazine. It was possible to circumvent this limitation by resuspending reporter bacteria in 100% dimethyl sulfoxide (DMSO, Sigma) immediately prior to vargulin addition. However, this requirement also precludes the use of Cypridina luciferase for in vivo studies, due to the potential toxicity of DMSO. To measure Cypridina luciferase activity in vitro, we centrifuge 100 µl of culture, resuspend the bacteria in 100% (vol/vol) DMSO, and then add 10 µl of vargulin stock solution (1.4 mg ml⁻¹ diluted in ethanol) [13]. Vargulin was purchased from NanoLight Technologies.

⁴For in vitro bioluminescence measurements, either black or white 96-well microplates can be used. Signal intensities for white plates can be up to tenfold greater than the same samples assayed in black plates. Therefore, luciferase data should be generated using only black or white plates, as luminescence values generated using different colored plates will not be directly comparable. While 96-well plate color can impact the absolute values of luminescence readings, it does not impact the ratios (i.e., fold-differences) observed between samples, provided all samples are assayed in plates of the same color. Clear 96-well plates are not recommended for bioluminescence measurements because light will leak into adjacent wells and greatly reduce the accuracy of RLU measurements.

⁵Successful inoculation of the murine oral cavity with nonresident bioluminescent bacteria is greatly enhanced by reducing competition from the resident mouse oral flora prior to infection. The following are key components of our infection protocol. (1) We infect 3-week-old weanling mice, since weanlings are likely to have a poorly developed natural oral flora. (2) Twenty-four hours before inoculation, the mouse drinking water is supplemented with 100 µg/ml ampicillin to reduce the abundance of live bacteria in the oral cavity, especially grampositive organisms. (3) We employ a mouse teeth cleaning protocol to remove any preexisting oral biofilm and to reduce the resident oral flora. Cotton swabs soaked in 4% (vol/vol) H₂O₂ proved to be the most effective for this task. H₂O₂ was freshly diluted from a 30% stock solution immediately prior to use. Preferably, two people should be present during teeth cleaning procedures. One person can restrain the mice, while the other cleans the teeth. We found it particularly useful for both people to each use one free hand to place the round end of a paperclip over the top and bottom incisors to hold open the mouse mouth during the teeth cleaning (Fig. 2a).

⁶Saliva flow is an important factor to consider for the stable establishment of nonresident foreign bacteria, as low saliva flow is likely to improve the chances of successful colonization. Therefore, we previously employed pilocarpine assays to measure the salivary output of multiple mouse strains to identify the preferred host for our oral biofilm model. Surprisingly, we found the commonly used BALB/CyJ strain to be among the best options, as its salivary output was even lower than that of the murine Sjögren's disease model strain NOD.B10.H2^b [30]. Salivary flow was assayed by injecting 0.2 mg isoproterenol and 0.05 mg pilocarpine per 100 g mouse weight into the peritoneal cavity to induce hypersalivation. Saliva was collected for 3 min immediately after the first signs of salivation appeared postinjection (typically 1–2 min after injection).

ampicillin, 5% (wt/vol) sucrose + 5% (wt/vol) fructose, or 5% (wt/vol) sucrose + 5% (wt/vol) fructose + 10% (wt/vol) xylose.

3. Glucose: 20% (wt/vol).
4. Phosphate buffered saline (PBS): 8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na₂HPO₄, 0.24 g/L KH₂PO₄, pH 7.4.
5. H₂O₂: 4% (vol/vol).
6. Cotton swabs: Prior to inoculation, mice teeth are cleaned with 2 mm head-width cotton swabs soaked in H₂O₂ (see Note 5).
7. Small animal imaging system: IVIS (Perkin Elmer), In-Vivo Xtreme (Bruker), or similar instrument.

2.7 Abscess Model

1. 6-Week-old C57BL/6J mice.
2. Depilatory cream: A small patch of fur on the dorsal side of the mice (corresponding to the planned bacteria injection site) is removed using any over-the-counter depilatory cream commonly sold at most drug stores.
3. Insulin syringes: For bacterial injection, Monoject 0.5 ml insulin syringes are used with 28 gauge needles.
4. Modified CDC medium supplemented with laked sheep blood (5%) (see ref. 26).
5. Meloxicam: If an analgesic is required, mice are given a daily dose of an oral suspension of meloxicam at 5 mg/kg body weight.

3 Methods

The following section describes the procedures utilized to create synthetic luciferases suitable for multiplexing, the steps required to construct bioluminescent reporter strains of oral streptococci, and the protocols used to measure bacterial growth and gene expression in vivo. Two different polymicrobial infection models are presented, a multispecies oral biofilm model as well as a multispecies abscess model. The abscess model consists of a mixture of *S. anginosus*, *Fusobacterium nucleatum*, and *Prevotella nigrescens*. For the abscess-related methods described here, we will focus specifically upon the *S. anginosus* component of the polymicrobial infection.

3.1 Creation of Synthetic Luciferases

1. We have previously demonstrated the utility of a variety of synthetic codon optimized luciferases for use in oral streptococci, including click beetle green, Luciola red, Green Renilla, and Cypridina luciferases [13]. By combining each of these, it is possible to multiplex at least four distinct bioluminescent signals. For most applications, one or two luciferase signals will suffice. Therefore, we will focus upon our two most commonly utilized luciferases, Luciola red and Green Renilla luciferases (see Note 7). Since all of these luciferase genes

originate from eukaryotic organisms, it is necessary to create codon optimized versions of the luciferase open reading frames (ORFs) to ensure their maximal expression in oral streptococci. The sequences of each luciferase ORF were first codon optimized using the online optimization tool provided by Integrated DNA Technologies (IDT) (www.idtdna.com). *S. mutans* strain UA159 was selected as the reference genome for codon optimization. After the initial optimization, sequences were inspected for the presence of any remaining rare codons and then manually adjusted as needed.

2. Codon optimized luciferase ORFs were synthesized by IDT and then later used as DNA templates for the construction of bioluminescent reporter strains (*see* Note 8).

⁷.Wavelengths in the red and far-red region of the spectrum have the greatest ability to penetrate mammalian tissues [31]. Therefore, when multiplexing in vivo, we prefer to combine Luciola red with Green Renilla luciferase. Green Renilla yields much stronger bioluminescence compared to Luciola red [13], which compensates for the greater tissue absorption of green light. Conversely, Luciola red yields less absolute bioluminescence relative to Green Renilla [13], but its inherent red color exhibits far less absorption by pigments within mouse tissues (i.e., a greater percentage of the signal is available for detection). It is also noteworthy that the bioluminescence emitted by these two enzymes can be distinguished by both substrate choice and signal wavelength.

⁸.DNA sequences of the codon optimized Luciola red and Green Renilla luciferase ORFs are as follows:

Luciola Red

```
ATGGAAACAGAACGTGAA GAAAATGTTGTCTATGGTCCATTACATTTTATCC
TATTGAAGAAGGTAGTGCAGGAATCCAGCTTCATAAG TATATGCAACAGTATGCTAAGCTTGGTGC
TATTGCTTTTAGTAATGCTCTTACAGGTGTTGA T A T T T C T T A T C A G C A A T A T T T T G A T A T
TACTTGTGCTTTGGTGAAGCTAT GAATGAAACCTGAAGGACATATTGCTTTGTGCAGT G A A A T T G T G A A G A A
T T T T T T A T T C C A G T T T T G G C T G G T C T T TATATTGGAGT
TACTGTTGCTCCTACAAACGAGATTTATACATTGAGA GAATTAATCATTTCTTTAGGTATTGCTCAACCAAC
TATTGTATTAGTTCACGTAAAGGTCTTCCAAAAGTTT TAGAGGTTCAAAAGACAGTTACATGTATCAAAAC
TATCGTTATTTTAGACTCTAAAGTTAATTTTGGT
GGTTACGATTGTGTTGAAACATTTATATAAAAG CATGTTGAATTGGGTTTCCAGCTACTAGCTTTGTTC C A T A T G A T G
T T A A G A T C G T A A C A T C A C A T T G C T T T G C T T A T G A A T A G T T C T G G C A G T A
CAGGTTTACCTAAGGGGTAGAAATTACTCATGAAG CACTTGTTACAGGTTTTTCACATGCTAAAGATC
CAATTTATGGTAATCAAGTTGCACCTGGTACAGC TATTCTTACAGTGGTTCCTTTTCATCACGGTTTTGG
TATGTTTACTACTTTAGGTTATTTTGCTTGTGGT TATCGTATCGTTATGTTAACTAAATTTGATGAA
GAGTTGTTTTTGCCTACATTACAGGATTATAAATGTAC CACTGTTATTTTAGTTCCAACACTTTTTGCTATTCT
TAATCGTAGTAATTAGTATGATAAGTTTGAAGTTGAG CAATCTTACAGAAATGCTTCTGGTGGTGCACCT
C T T G C A A A G A A A T T G G A G A G C A G T T G C T C G T C G T T T A A T T T G C C T G G A G T T C G T
CAAGGTTATGGTTAACTGAACTACATCTGCTTTTAT TATTACTCCTAAAGGAGACGATAAGCCTGGTGGCTT
CAGGTAAAGTTGTTCTCTTTTAAAGGTTAAATTTATT GATCTTGACACAAAAAGACTTTTAGGAGT
TAATCGTCGTGGTGAAATCTGTGTTAAAGGACC TAGTCTTATGCTTGGTTATACTAACAATCCAGAACG
TACGCGTGAAACTATTGATGAGGAAGGTTGGCTTCA TACAGGTGATATTGGATATTATGACGAAGATGAA
CATTTTTTTATCGTTGATCGTTGAAATCTTTGAT TAAATATAAAGGATATCAGGTTCCACCTGCTGAATTA
GAAAGTGATTATTGCAACATCCTAATATTCGTGATG CAGGTGTTGCAGGTGTTCCAGATTCTGAAGCTGGA
GAGTTACCTGGAGCTGTTGTAGTCATGGAAGG CAAGACTATGACTGAAAAAGAAATGTTGATTATGT
TAATAGTCAAGTCGTTAATCATAAACGTTTGC GTGGTGGAGTTTCGTTTGTAGATGAAGTTCC
TAAGGGTTTAAACAGGAAAAATTGATGCTAAAGT TATCCGTGAAATTTTGAAAAACCTCAAGCTGGTGGATAA
```

Green Renilla

```
ATGGCTAGTAAAGTTTATGATCCTGAACAACG T A A A C G T A T G A T T A C A G G T C C A
CAATGGTGGGCTCGTTGTAAGCAAATGAATGTTTTG GATAGTTTTATTAATTATTATGATAGTGAAAAACATG
CAGAAAATGCTGTTATTTTCCTTCATGGTAATGCTAC TAGTAGTTACTTATGGCGTCATGTTGTCCACACATT
GAACCAAGTTGCTCGTTGATTTATTCCTGATCT TATTGGTATGGGTAAGTGGCAAAAGTG
GAAATTTGCTTCTTATGTTTATTGGATCATTATAAG TATTAACTGCTTGGTTTGAATTGTTAAATCTTC
CAAAAAAATTTATTTTTGTTGGTTCATGATTGGGG TAGTGCCTTTAGCTTTCCATTATGCTTATGAACATCAA G A T C G T A T
T A A A G C T A T T G T T C A T A T G GAATCTGTTGTTGATGTTATTGAATCTTG GATGGGTTGGCCAGATATTGAA
GAAGAATTGGCTTTAATTAATCAGAAGAAGGA GAAAAAATGGTTCCTTGAAAAATACTTTTTTGTGTTGAAA
CAGTTTGGCCATCTAAATTTATGGCTTAAATTTGGAAC CAGAAGAATTGCTGCTTATTTGGAACCATTTAAG
GAAAAAGGTGAAGTTTCGTCGTCCTCAACTTTGT CATGGCCTCGTGAAATTCCTTTAGTTAAAGGTG
GAAAGCCTGATGTTGTGCTATTGTTTCGTAATTA TAATGCTTATTTGCGTGCTAGCGATGATCTTC
CAAAGTTATTCATTGAAAGCGATCCTGGATTCTTTT CAAATGCTATTGTTGAAGGTGCTAAAAAATTTCCAAA
TACGGAATTTGTTAAAGTTAAAGGCTTCAATTTCTT CAAGAAGATGCTCCTGATGAAATGGGTAAAGTATAT
TAAATCATTTGTTGAACGTGTTTTGAAGAATGAG CAACGTTCTATCTAA
```


3.2 Construction of Bioluminescent Reporter Strains

1. To yield the greatest sensitivity and accuracy for in vivo biophotonic imaging, luciferase genes should be highly expressed and constitutive. Furthermore, luciferase gene expression should be largely invariant to avoid significant changes in reporter activity due to variable growth conditions. For studies of oral streptococci, we have had the greatest successes with luciferase transcriptional fusions to the following: enolase (*eno*), phosphocarrier protein HPr (*ptsH*), ribosomal protein S21 (*rpsU*), and lactate dehydrogenase (*ldh*).
2. Our constructs are all created using overlap extension PCR (OE-PCR) protocols. For more information on the design and creation of OE-PCR constructs, see [23, 24].
3. Two mutagenesis strategies were employed for the construction of constitutive luciferase reporter strains. For *S. mutans* and *S. anginosus*, luciferase ORFs were inserted markerlessly downstream of target genes, whereas for *S. sanguinis* and *S. gordonii*, allelic exchange mutagenesis was employed using antibiotic resistance cassettes. Illustrations of both approaches are shown in Fig. 1. For additional information regarding the use of the IFDC2 cassette for streptococcal markerless mutagenesis, see [25].

3.3 Measurement of Bioluminescence in Planktonic and Biofilm Cells

1. Planktonic and biofilm cultures are assayed using a GloMax Discoverer (Promega). Cells are cultured directly within the wells of opaque black or white multiwell plates. Generally, in vitro bioluminescence assays are first conducted to confirm the performance of reporter strains prior to beginning animal imaging studies. It is advisable to test at least five separate clones for each reporter strain constructed. The specific activities for each reporter should be nearly identical. If an outlier is identified among the clones, it should not be used in subsequent studies. Specific activity is determined by normalizing bioluminescence Relative Light Units (RLU) with optical density or colony forming unit (CFU) values (i.e., RLU/OD₆₀₀ or RLU/CFU). Ideally, the specific activity of the reporter strain should also be relatively insensitive to different growth media compositions and culture conditions.
2. *S. mutans* and *S. anginosus* planktonic cells are grown statically at 37 °C to mid-logarithmic phase in an anaerobic chamber (85% N₂, 10% CO₂, and 5% H₂), while *S. sanguinis* and *S. gordonii* are cultured in a CO₂ incubator (5% CO₂).
3. *S. mutans*, *S. sanguinis*, and *S. gordonii* biofilms are grown similarly as described for their respective planktonic cultures, except that cells are diluted 500-fold in medium containing 1% (wt/vol) sucrose prior to 16 h incubation at 37 °C. After the incubation period, the spent overnight medium is aspirated and replaced with an equal volume of prewarmed fresh medium and then further incubated for an additional hour to re-energize cells depleted of ATP and other cellular cofactors (see Note 9). Afterward, luciferase substrate solutions are added directly into the wells before measuring luciferase activity.

4. For both planktonic and biofilm luciferase assays, stock solutions of luciferase substrates are added in the following ratios: *Luciola* red, D-luciferin (25 µl per 100 µl culture) and Green Renilla, coelenterazine-h (1.5 µl per 100 µl culture).
5. Stock luciferase substrate solutions are added directly into the sample wells, then pipetted vigorously for several seconds to briefly aerate the samples, and finally incubated at room temperature for ~30 s before measuring luciferase activity.

3.4 Animal Handling and Procedures

For instruction on the proper use of the IVIS and In-Vivo Xtreme imagers as well as their system software, please consult the relevant literature provided by the manufacturers. For the oral biofilm and abscess models, optimal image exposure times will vary depending upon the strength of bioluminescence emitted from the reporter bacteria. However, a 3–5 min exposure is usually an effective start point when scouting for the appropriate exposure times.

3.4.1 Oral Biofilm Model

1. For the oral infection of mice using human oral streptococci, the best results are obtained using mice soon after they have been weaned, typically at 21 days (*see* Note 5). Prior to experimentation, the mouse drinking water is supplemented with ampicillin (100 µg/ml) for 24 h to suppress the growth of the Gram positive oral flora. The following day, mice are anaesthetized with 3% (vol/vol) isoflurane and then their teeth are manually cleaned using 2 mm head-width cotton swabs soaked in 4% (vol/vol) H₂O₂ (Fig. 2a). Teeth cleaning is performed twice with a brief recovery period given between cleanings. After the second round of cleaning, mice are left in their respective cages for 1 h prior to inoculation with bacteria.
2. To prepare reporter bacteria for inoculation, overnight stationary phase cultures grown in chemically defined medium (CDM) are diluted 1:40 in 10 ml CDM supplemented with 5% (wt/vol) fructose (*see* Note 10). Cultures are incubated

⁹. Most beetle luciferases use D-luciferin as a substrate according to the following reaction:

Due to the requirement for ATP in this reaction, the physiological state of beetle luciferase-expressing reporter bacteria can influence the output of bioluminescence if ATP is limiting in the cell. Renilla luciferase uses coelenterazine as a substrate according to the following reaction:

Since Renilla luciferase does not require ATP as a cofactor, its activity is much less sensitive to the physiological state of the cell. However, both beetle and Renilla luciferases function through an oxidation reaction mechanism. Therefore, strict anaerobic conditions may impact signal output. It is worth noting that we routinely grow reporter bacteria in an anaerobic chamber and can still accurately measure luciferase activity. In this case, samples are given a brief aeration simply by vigorously pipetting the samples several times before measuring their reporter activity. If samples are not cultured in strictly anaerobic conditions, it is unnecessary to aerate the samples prior to measurement, even if the bacteria are grown in biofilms. Thus, while O₂ is required for light production, its required concentration for luciferase activity is apparently quite low. We have not observed O₂ concentration as a limiting factor for in vivo studies of the oral biofilm or abscess models.

until early logarithmic phase ($OD_{600} \approx 0.3$) before adding 1% (wt/vol) sucrose and then incubating for an additional hr. Afterward, cells are concentrated by centrifugation and resuspended in 1 ml CDM (i.e., concentrated tenfold).

3. To inoculate mice oral cavities, mice are first anesthetized with 3% (vol/vol) isoflurane. Next, an individual mouse is held horizontally with its left side facing down. 30 μ l of the concentrated reporter bacteria is pipetted directly into the mouse oral cavity. Immediately afterward, the mouse is returned to the isoflurane anesthesia chamber for an additional 2 min. The mouse should be placed with its inoculation side facing down (i.e., on its left side). The goal is to let the bacterial solution remain in contact with half of the mouth for 2 min. Next, the mouse is returned to its cage for a brief recovery period. During this time, other mice can be inoculated using the same procedure. After all of the mice have been similarly inoculated, the procedure is repeated, except that the mice are inoculated with their right sides facing downward to infect the right sides of their mouths. Once both sides of the mouths have been inoculated, the mice are kept without food or water for 1 h. Afterward, mice are fed a standard Laboratory Rodent Diet 5001 for the remainder of the experiment. At this stage, the mice should also be provided a continuous supply of drinking water supplemented with 5% sucrose and 5% fructose until the experiment has reached its conclusion.
4. When the mice are ready for imaging, they are first anesthetized using 3% (vol/vol) isoflurane and then 20% (wt/vol) glucose is pipetted into the mouse oral cavities to re-energize the reporter bacteria. After a 15 min incubation period in their cages, the mice are ready for imaging. To activate bioluminescence from the *Luciola* red reporter, 25 μ l of 2 mM D-luciferin stock solution is pipetted directly into the mouse oral cavity. To activate Green Renilla bioluminescence, 1.5 μ l of coelenterazine stock solution is added to 25 μ l of phosphate buffered saline (PBS) and then the entire 26.5 μ l is pipetted directly into the mouse oral cavity. After luciferase substrate(s) have been administered, the mice should be immediately placed into the animal imaging system and analyzed (Fig. 2b, c).

3.4.2 Abscess Model

1. For the polymicrobial abscess infection model, 6-week-old C57BL/6J mice are injected subcutaneously with a suspension of *S. anginosus*, *F. nucleatum*, and *P. nigrescens*.
2. 24 h before infection, the site of injection is treated with a commercially available chemical depilatory cream to remove a small patch of fur (Fig. 3a). The cream should be spread deeply into the fur so that hair removal is as complete as possible. Depilatory cream is generally left on the animal for 3–4 min before removing it using tissue paper or a rubber spatula.

¹⁰We have observed enhanced oral colonization of reporter bacteria if 25% (vol/vol) sterile human saliva is added to the CDM growth medium. This step is not essential, but in our experience, it improves the outcome.

3. The bacterial consortium is grown 24–48 h inside an anaerobic chamber (85% N₂, 10% CO₂ and 5% H₂) prior to infection. Bacteria are grown separately on either THY agar (*S. anginosus*) or Modified CDC medium with 5% laked sheep blood [26] (*F. nucleatum* and *P. nigrescens*). Isolated colonies of all 3 species are scraped directly from the agar plates, resuspended in PBS, adjusted to an optical density OD₆₀₀ ≈ 1.0, and then mixed in a 1:1:1 ratio.
4. Animal injection (*see* Note 11): each syringe is resuspended by vigorous shaking to disperse bacterial coaggregates before injection. The injection dose is 0.2 ml. Subcutaneous injections tend to cause superficial abscesses, while intramuscular injections trigger deeper abscesses that are less noticeable from the skin surface. However, intramuscular injections also tend to yield abscesses containing a greater quantity of pus compared to subcutaneous injections.
5. Animal imaging: the coelenterazine-h stock solution is diluted 1:10 in PBS and then delivered via intraperitoneal (IP) injection at a final substrate concentration of 4 mg/kg body weight. Total ethanol in the injection from the original coelenterazine stock solution should not exceed 10% (vol/vol). After luciferase substrate(s) have been administered, the mice should be immediately placed into the animal imaging system and analyzed (Fig. 3b).
6. Following euthanasia, abscess pus is assayed to confirm the organisms present within abscesses (*see* Note 12).

3.5 Measurement of Specific Gene Expression

1. Multiplexing luciferase signals provides a straightforward mechanism to simultaneously measure the growth and/or decline of multiple species within experimental infections. However, it also allows for the in situ measurement of changes in gene expression as well. In this case, it is necessary to create a dual luciferase reporter strain. Theoretically, any two easily discernable luciferase signals could be employed. We typically combine *Luciola* red and Green *Renilla* luciferases (*see* Note 7).
2. To measure specific gene expression, it is necessary to transcriptionally fuse one luciferase ORF to an internal housekeeping control and the other to the target gene of interest. Housekeeping control fusions can be made to any highly expressed constitutive genes, such as those previously described (*eno*, *ptsH*, *rpsU*, *ldh*, etc.). Typically, we fuse these to *Luciola* red. Since its signal is weaker than that of Green *Renilla*, the high expression of the housekeeping control gene compensates to yield a robust signal. Conversely, the strong signal output of

¹¹The abscess bacterial consortium will typically coaggregate shortly after mixing. Thus, repeated resuspension by vigorous shaking is required prior to injection. The best results are obtained when single dose injections are prepared inside an anaerobic chamber to maintain strict anaerobic conditions. Every syringe is labeled and filled with 300 µl of the consortium bacterial suspension. Syringes are prepared immediately before use and stored in double sealed ziplock bags containing anaerobic GasPaks (Becton Dickinson) to maintain anaerobic conditions.

¹²As an infection control, abscess pus should be collected immediately following euthanasia and then assayed for both the abundance of *S. anginosus*, *F. nucleatum*, and *P. nigrescens* as well as for contamination with exogenous microorganisms. Pus samples are resuspended in PBS, serially diluted tenfold, spotted onto duplicate modified CDC medium agar plates, and incubated both aerobically and anaerobically at 37 °C.

Green Renilla luciferase simplifies the detection of target genes with much lower expression relative to the housekeeping gene controls.

3. To calculate specific gene expression, RLU values measured for the gene of interest are normalized using the RLU values measured for the internal housekeeping control (i.e., $RLU_{\text{Target Gene}}/RLU_{\text{Housekeeping Control}}$). This approach accounts for the inevitable day to day changes in bacterial population size that occur during experimental infections. Consequently, specific gene expression can be compared between different days during the assay period or even between disparate experimental conditions that impact the growth or viability of the infecting bacteria.

3.6 Exogenous Control of Bacterial Gene Expression During Experimental Infections

1. In many cases, such as virulence gene studies, it can be highly beneficial to exogenously modulate bacterial gene expression within experimental infections. One of the inevitable challenges is determining whether the desired target gene expression pattern is actually occurring in vivo. Using biophotonic imaging, it is possible to make such a determination in situ with both spatial and temporal resolution. In this case, it is again necessary to employ a dual luciferase strain. However, the target gene of interest should be placed under the transcriptional control of an inducible promoter. We previously developed a xylose-inducible gene regulation cassette referred to as Xyl-S1 [27] and recently demonstrated its utility for the exogenous control of gene expression in vivo [13]. Since xylose is nontoxic to mice [28, 29] and is not metabolized by oral streptococci (and many other bacteria) [13], it is a convenient chemical to use in rodent models.
2. For the oral biofilm model, we were able to trigger target gene expression simply by adding 10% (wt/vol) xylose to the mouse drinking water. Furthermore, it was possible to demonstrate using biophotonic imaging that target gene expression could either be induced or repressed depending upon the presence of xylose in the drinking water [13].
3. The calculation of specific gene expression is performed as described in Subheading 3.5.

Acknowledgments

This work was supported by an NIH/NIDCR grant R35DE0282252 to J.M. and NIH/NIDCR grants R01DE021726 and R56DE021726 to J.K.

References

1. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F (2018) Oral microbiomes: more and more importance in oral cavity and whole body. *Protein Cell* 9(5):488–500. 10.1007/s13238-018-0548-1 [PubMed: 29736705]
2. Lamont RJ, Koo H, Hajishengallis G (2018) The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol* 16 (12):745–759. 10.1038/s41579-018-0089-x [PubMed: 30301974]

3. Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H, Ellwood R, Giacaman RA, Herrera D, Herzberg MC, Kononen E, Marsh PD, Meyle J, Mira A, Molina A, Mombelli A, Quirynen M, Reynolds EC, Shapira L, Zaura E (2017) Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 44(Suppl 18):S5–S11. 10.1111/jcpe.12682 [PubMed: 28266109]
4. Sudhakara P, Gupta A, Bhardwaj A, Wilson A (2018) Oral dysbiotic communities and their implications in systemic diseases. Dent J (Basel) 6(2):E10 10.3390/dj6020010 [PubMed: 29659479]
5. Kane SF (2017) The effects of oral health on systemic health. Gen Dent 65(6):30–34
6. Kreth J, Giacaman RA, Raghavan R, Merritt J (2017) The road less traveled - defining molecular commensalism with *Streptococcus sanguinis*. Mol Oral Microbiol 32(3):181–196. 10.1111/omi.12170 [PubMed: 27476770]
7. Redanz S, Cheng X, Giacaman RA, Pfeifer CS, Merritt J, Kreth J (2018) Live and let die: hydrogen peroxide production by the commensal flora and its role in maintaining a symbiotic microbiome. Mol Oral Microbiol 33 (5):337–352. 10.1111/omi.12231 [PubMed: 29897662]
8. Hajishengallis G, Lamont RJ (2016) Dancing with the stars: how choreographed bacterial interactions dictate nososymbiosis and give rise to keystone pathogens, accessory pathogens, and pathobionts. Trends Microbiol 24 (6):477–489. 10.1016/j.tim.2016.02.010 [PubMed: 26968354]
9. Robertson D, Smith AJ (2009) The microbiology of the acute dental abscess. J Med Microbiol 58(Pt 2):155–162. 10.1099/jmm.0.003517-0 [PubMed: 19141730]
10. Siqueira JF Jr, Rocas IN (2013) Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev 26(2):255–273. 10.1128/CMR.00082-12 [PubMed: 23554416]
11. Graham DB, Webb MD, Seale NS (2000) Pediatric emergency room visits for nontraumatic dental disease. Pediatr Dent 22 (2):134–140 [PubMed: 10769858]
12. Jundt JS, Gutta R (2012) Characteristics and cost impact of severe odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol 114(5):558–566. 10.1016/j.oooo.2011.10.044 [PubMed: 22819453]
13. Merritt J, Senpuku H, Kreth J (2016) Let there be bioluminescence: development of a biophotonic imaging platform for in situ analyses of oral biofilms in animal models. Environ Microbiol 18(1):174–190. 10.1111/1462-2920.12953 [PubMed: 26119252]
14. Abe T, Hajishengallis G (2013) Optimization of the ligature-induced periodontitis model in mice. J Immunol Methods 394(1-2):49–54. 10.1016/j.jim.2013.05.002 [PubMed: 23672778]
15. Bowen WH (2013) Rodent model in caries research. Odontology 101(1):9–14. 10.1007/s10266-012-0091-0 [PubMed: 23129523]
16. Peterson SN, Meissner T, Su AI, Snesrud E, Ong AC, Schork NJ, Bretz WA (2014) Functional expression of dental plaque microbiota. Front Cell Infect Microbiol 4:108 10.3389/fcimb.2014.00108 [PubMed: 25177549]
17. Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, Primeaux C, Tian R, Kenton S, Jia H, Lin S, Qian Y, Li S, Zhu H, Najjar F, Lai H, White J, Roe BA, Ferretti JJ (2002) Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. Proc Natl Acad Sci U S A 99 (22):14434–14439. 10.1073/pnas.172501299 [PubMed: 12397186]
18. Xu P, Alves JM, Kitten T, Brown A, Chen Z, Ozaki LS, Manque P, Ge X, Serrano MG, Puiu D, Hendricks S, Wang Y, Chaplin MD, Akan D, Paik S, Peterson DL, Macrina FL, Buck GA (2007) Genome of the opportunistic pathogen *Streptococcus sanguinis*. J Bacteriol 189(8):3166–3175. 10.1128/JB.01808-06 [PubMed: 17277061]
19. Vickerman MM, Iobst S, Jesionowski AM, Gill SR (2007) Genome-wide transcriptional changes in *Streptococcus gordonii* in response to competence signaling peptide. J Bacteriol 189(21):7799–7807. 10.1128/JB.01023-07 [PubMed: 17720781]
20. Olson AB, Kent H, Sibley CD, Grinwis ME, Mabon P, Ouellette C, Tyson S, Graham M, Tyler SD, Van Domselaar G, Surette MG, Corbett CR (2013) Phylogenetic relationship and virulence inference of *Streptococcus Anginosus* group: curated annotation and whole-genome comparative analysis support distinct species designation. BMC Genomics 14:895 10.1186/1471-2164-14-895 [PubMed: 24341328]

21. Rahman M, Nguyen SV, McCullor KA, King CJ, Jorgensen JH, McShan WM (2015) Complete genome sequence of *Streptococcus anginosus* J4211, a clinical isolate. *Genome Announc* 3(6):e01440–15. 10.1128/genomeA.01440-15
22. Merritt J, Niu G, Okinaga T, Qi F (2009) Autoaggregation response of *Fusobacterium nucleatum*. *Appl Environ Microbiol* 75 (24):7725–7733. 10.1128/AEM.00916-09 [PubMed: 19837836]
23. Aiyar A, Xiang Y, Leis J (1996) Site-directed mutagenesis using overlap extension PCR. *Methods Mol Biol* 57:177–191. 10.1385/0-89603-332-5:177 [PubMed: 8850005]
24. Pogulis RJ, Vallejo AN, Pease LR (1996) In vitro recombination and mutagenesis by overlap extension PCR. *Methods Mol Biol* 57:167–176. 10.1385/0-89603-332-5:167 [PubMed: 8850004]
25. Xie Z, Okinaga T, Qi F, Zhang Z, Merritt J (2011) Cloning-independent and counterselectable markerless mutagenesis system in *Streptococcus mutans*. *Appl Environ Microbiol* 77(22):8025–8033. 10.1128/AEM.06362-11 [PubMed: 21948849]
26. Yoneda S, Loeser B, Feng J, Dmytryk J, Qi F, Merritt J (2014) Ubiquitous sialometabolism present among oral fusobacteria. *PLoS One* 9 (6):e99263 10.1371/journal.pone.0099263 [PubMed: 24896093]
27. Xie Z, Qi F, Merritt J (2013) Development of a tunable wide-range gene induction system useful for the study of streptococcal toxinantitoxin systems. *Appl Environ Microbiol* 79 (20):6375–6384. 10.1128/AEM.02320-13 [PubMed: 23934493]
28. Johnson SA, van Tets IG, Nicolson SW (1999) Sugar preferences and xylose metabolism of a mammal pollinator, the Namaqua rock mouse (*Aethomys namaquensis*). *Physiol Biochem Zool* 72(4):438–444. 10.1086/316685 [PubMed: 10438681]
29. Zimmer JP, Lewis SM, Moyer JL (1993) Comparison of gavage, water bottle, and a highmoisture diet bolus as dosing methods for quantitative D-xylose administration to B6D2F1 (*Mus musculus*) mice. *Lab Anim* 27 (2):164–170. 10.1258/002367793780810423 [PubMed: 8501899]
30. Robinson CP, Yamachika S, Bounous DI, Brayer J, Jonsson R, Holmdahl R, Peck AB, Humphreys-Beher MG (1998) A novel NOD-derived murine model of primary Sjogren's syndrome. *Arthritis Rheum* 41 (1):150–156. 10.1002/1529-0131(199801)41:1<150::AID-ART18>3.0.CO;2-T [PubMed: 9433880]
31. Rice BW, Cable MD, Nelson MB (2001) In vivo imaging of light-emitting probes. *J Biomed Opt* 6(4):432–440. 10.1117/1.1413210 [PubMed: 11728202]

Fig. 1.

Construction of markerless and marked luciferase reporter strains. **(a)** Markerless reporter strains are created via a two-step procedure. The first step is to insert the IFDC2 counterselection cassette immediately downstream of the stop codon of a target housekeeping gene (*eno*, *ptsH*, *rpsU*, *Idh*, etc.). The IFDC2 cassette contains markers for both positive selection (erythromycin resistance) and negative selection (4-CP sensitivity). Individual homologous fragments and the IFDC2 cassette are amplified via PCR using primers that contain 5' reverse complementarity to the adjacent DNA fragments. The three PCR amplicons are then mixed in a single OE-PCR reaction to amplify the assembled construct using the primers shown in green. The resulting OE-PCR amplicon is then transformed into a wild-type background and selected on agar plates supplemented with erythromycin. The second step is to use the same OE-PCR assembly procedure to insert a luciferase ORF immediately downstream of the same target housekeeping gene. This amplicon is transformed into the IFDC2 strain created in step 1 and finally selected on agar plates supplemented with 4-CP. The resulting strain will contain a promoterless luciferase ORF transcriptionally fused to the upstream housekeeping gene in a single polycistronic transcript. **(b)** Marked reporter strains are created by transforming a single OE-PCR product into a wild-type background. Individual homologous fragments, the luciferase ORF, and the erythromycin resistance cassette are amplified via PCR using primers that contain 5' reverse complementarity to the adjacent DNA fragments. The four PCR amplicons are then mixed in a single OE-PCR reaction to amplify the assembled construct using the primers shown in green. The resulting OE-PCR amplicon is transformed into a wild-type background and selected on agar plates supplemented with erythromycin. The resulting strain will contain a promoterless luciferase ORF and antibiotic cassette transcriptionally fused to the upstream housekeeping gene in a single polycistronic transcript.

Fig. 2.

Infection and imaging for the oral biofilm model. (a) To manually clean the mouse teeth, paperclips are used to grasp the mouse incisors and open the mouth. A cotton swab soaked in 4% (vol/vol) H_2O_2 is then used to clean the teeth prior to inoculating with bioluminescent bacteria. The process requires two people. One person holds the mouse and restrains the lower incisors, while the other person restrains the upper incisors and uses the cotton swab to clean the teeth. (b) A single mouse was simultaneously infected with bioluminescent reporter strains of both *S. mutans* (left image, Green Renilla luciferase) and *S. gordonii* (right image, Luciola red luciferase). Signal intensity scale bars are located to the right of their respective images. (c) A single mouse was orally infected with bioluminescent *S. mutans* and then imaged from two separate angles to localize the sites of colonization in the mouse oral cavity. In the left image, a top-down view of the mouse indicates that *S. mutans* has colonized both halves of the oral cavity, but most of the reporter bacteria are located in the right half (i.e., stronger signal on the right side). In the right image, the mouse is imaged with its left side facing down. Signal localization suggests that *S. mutans* is primarily located

on the bottom half of the oral cavity (i.e., mandibular molars). Bioluminescence images in panels B and C were captured using an In-Vivo Xtreme (Bruker) immediately after pipetting the respective luciferase substrates directly into the mouse oral cavity

Fig. 3. Infection and imaging for the abscess model. **(a)** 6-day postinjection C57BL/6J mouse previously treated with a depilatory cream. **(b)** IVIS image of the same C57BL/6J mouse 6 days postinjection with a consortium of *S. anginosus*, *F. nucleatum*, and *P. nigrescens*. In this image, only *S. anginosus* emits bioluminescence due to its expression of the Green Renilla luciferase. Coelenterazine-h solution was injected intraperitoneally immediately before imaging. An IVIS (Perkin Elmer) was used to capture bioluminescence from the abscess

Table 1

Comparison of luciferases assayed in oral streptococci

Luciferase	Substrate	Emission (nm)	ORF (bp)	ATP	Source organism	Comments
Click beetle green ^a	D-Luciferin	537	1629	Yes	<i>Pyrophorus plagiophthalmus</i>	Less bright compared to Luciola red
Cypridina ^a	Vargulin	460	1611	No	<i>Vargula hilgendorfii</i>	Very bright, currently suitable for in vitro use only
Firefly	D-Luciferin	557	1653	Yes	<i>Photinus pyralis</i>	Yellow-green color is not suitable for multiplexing
Green Renilla ^a	Coelenterazine	527	945	No	<i>Renilla reniformis</i>	Very bright, suitable for multiplexing
Luciola red ^a	D-Luciferin	609	1647	Yes	<i>Luciola italica</i>	Suitable for multiplexing
Renilla	Coelenterazine	420	936	No	<i>Renilla reniformis</i>	Very bright, poorer tissue penetration than Green Renilla

^aNCBI GenBank Accession numbers: Click Beetle Green (MK215076), Cypridina (MK215073), Green Renilla (MK215074), and Luciola Red (MK215075)