

Development and Validation of qPCR Targets for *Porphyromonas gingivalis* Abundance and the Expression Levels of Genes in the Human Oral Microbiome

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Introduction

- Porphyromonas gingivalis* is a keystone pathogen found in patients with periodontitis and produces toxic proteases called gingipains.¹
- This bacterium has also been found in the brains of patients with Alzheimer's disease.¹
- In mouse models, inhibiting gingipain (Kgp) production decreased the colonization of *P. gingivalis* in the brain.¹
- Kgp inhibitors block the acquisition of host heme by *P. gingivalis* – a process mediated by HmuY, a unique hemophore.²
- The nitrate-nitrite-nitric oxide pathway relies on bacteria within the oral microbiome to convert nitrate to nitrite, and this pathway may be important in overall health.³
- Supplementing the diet with nitrate could alter the oral microbial community composition and reduce *P. gingivalis* abundance.

Methods

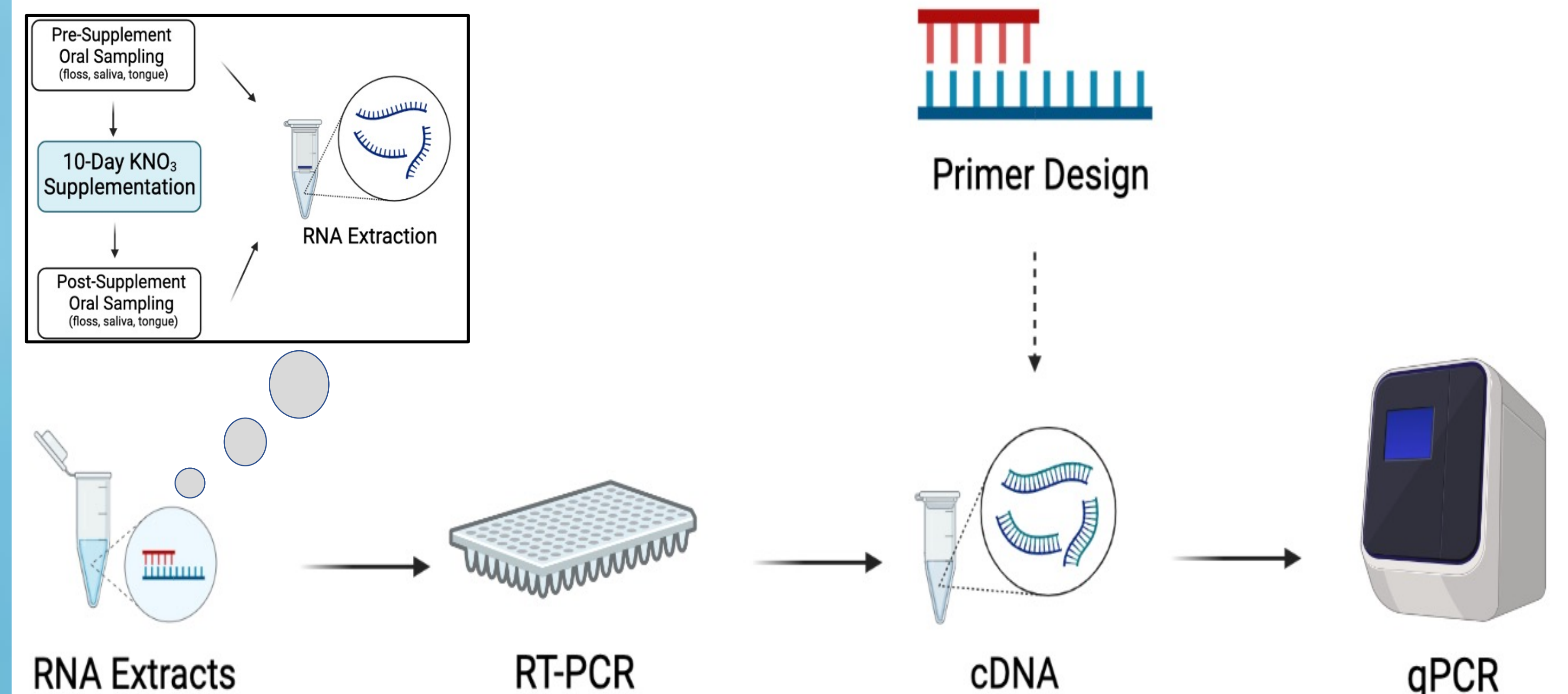
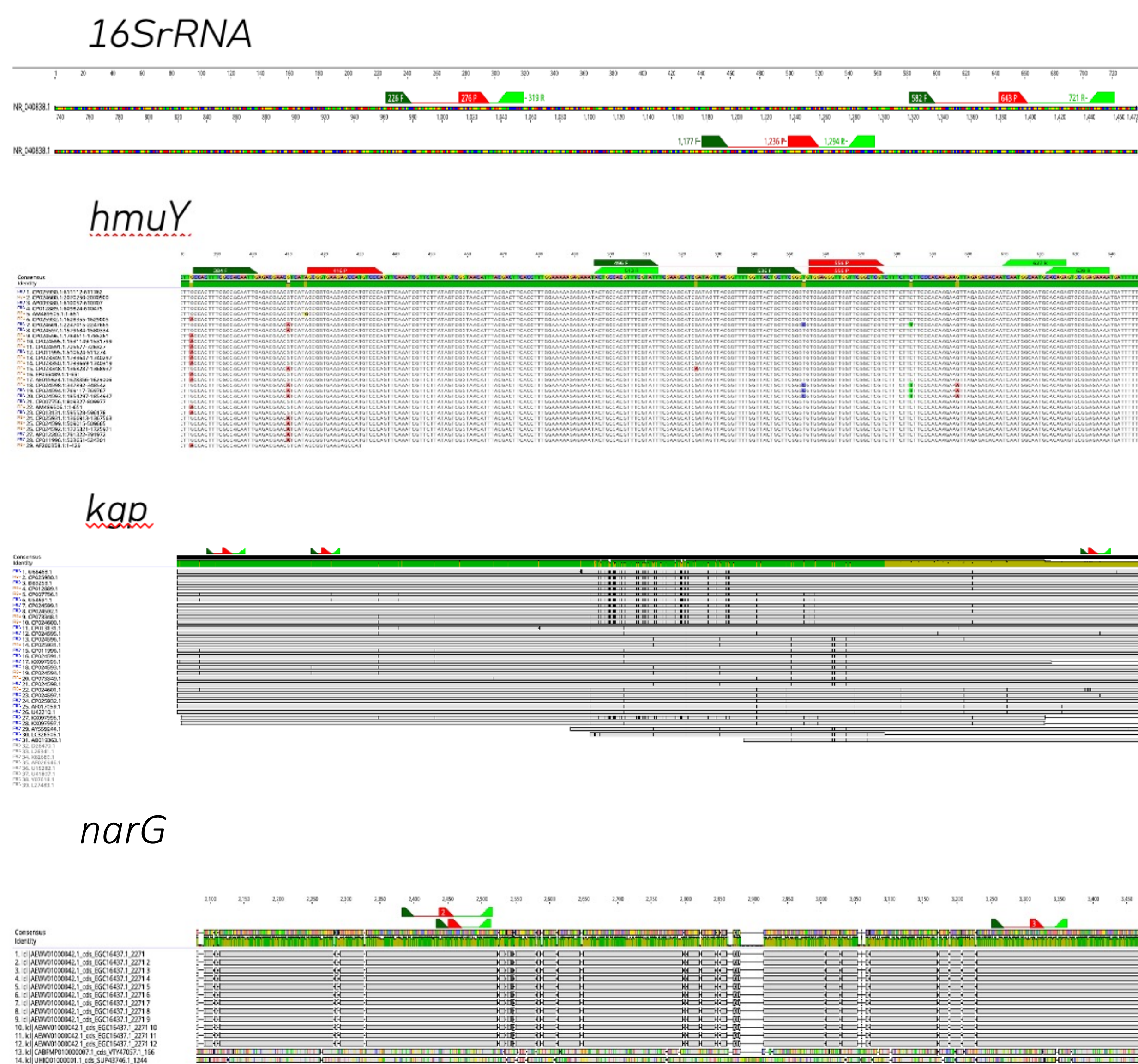


Figure 1. RNA extracts were prepared for cDNA synthesis using reverse transcriptase polymerase chain reaction (RT-PCR). Primers and probes were designed to target regions of the cDNA for subsequent quantitative polymerase chain reaction (qPCR).

Discussion & Future Analysis

- Primer combinations were identified that amplify the target region for *hmuY*, *kgp*, and *narG*; however, they were not identified for the 16S rRNA gene
- Future Work:
- Sanger sequencing to confirm the identity of amplified RT-PCR products
 - qPCR to compare gene expression levels and absolute abundance of *P. gingivalis* pre- and post-nitrate supplementation

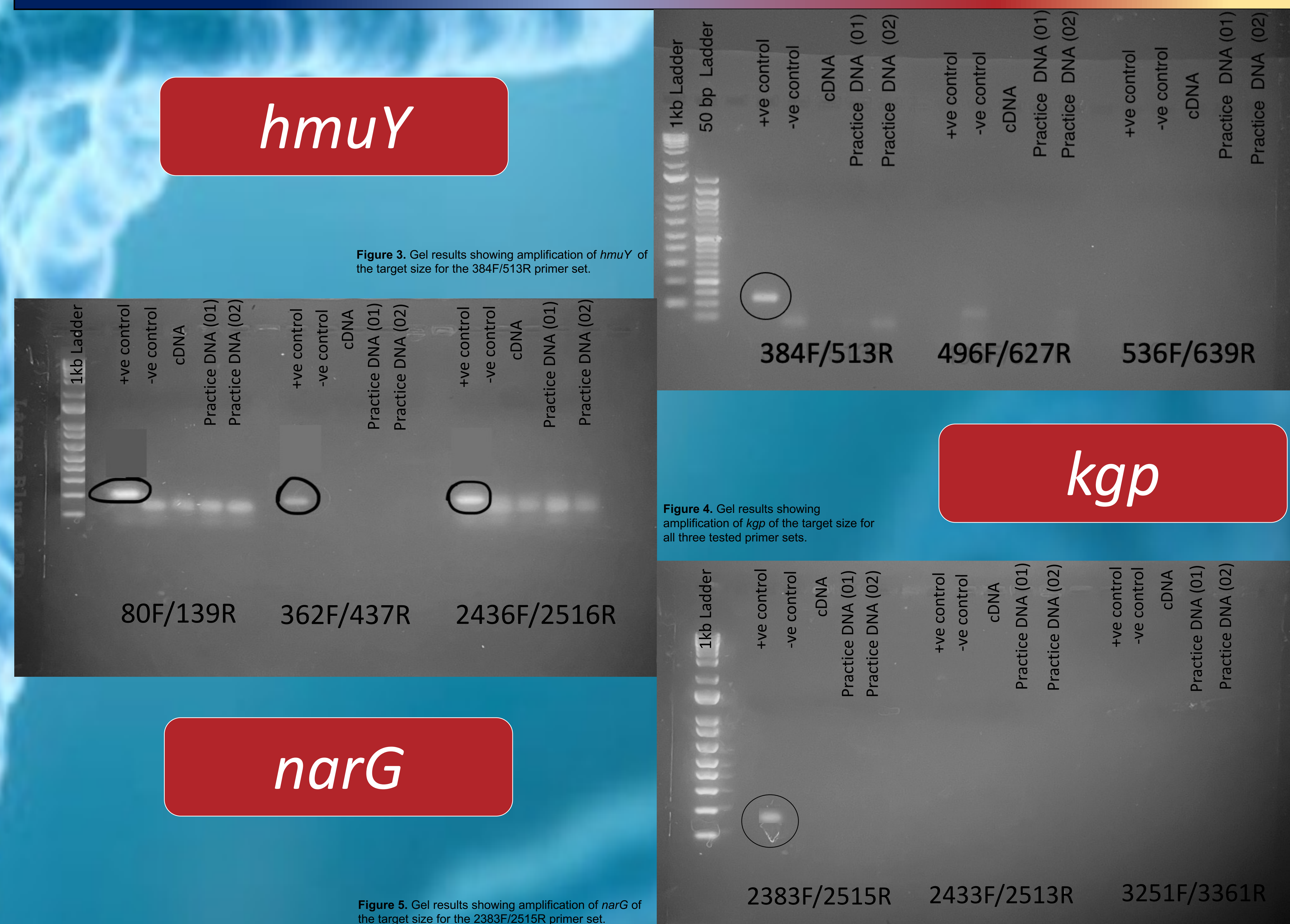
Primer Design



- Geneious Prime software
- 16SrRNA, *hmuY*, *kgp*:
 - Compiled sequences for each gene, specific to *P. gingivalis*
- narG*:
 - Compiled sequences for nitrate reducing bacteria: *Kingella*, *Streptomyces*, *Actinomyces*, *Veillonella*, *Rothia*
- MUSCLE alignment
- Designed primers and probes for the conserved regions of the aligned sequences

Figure 2. Direct output from Geneious Prime software showing primers and probes selected for qPCR for each of the four genes, 16SrRNA, *hmuY*, *kgp*, and *narG*.

Results



hmuY

Figure 3. Gel results showing amplification of *hmuY* of the target size for the 384F/513R primer set.

kgp

Figure 4. Gel results showing amplification of *kgp* of the target size for all three tested primer sets.

narG

Figure 5. Gel results showing amplification of *narG* of the target size for the 2383F/2515R primer set.

References

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