**Introduction**

The GSAF uses a design based on [this tech note from Illumina](https://www.illumina.com) for reading of the V4/V5 bacterial 16S rRNA gene region, and has extended it to other useful metagenomics primers. This design is a nested PCR in which the first (inner) PCR primes the gene-specific region and adds the Illumina sequencing primer sites, then the second (outer) PCR adds Illumina indexes and flow cell binding/amplification sites. The Illumina sequences are "Nextera" not "TruSeq".

RNA gene-specific primer sequences are shown in this teal color.

Illumina platform-specific sequences are shown in this green color.

Dual-barcode sequences are shown in this orange color.

**NOTE that all primer pairs are shown here FWD: 5'-3', REV: 3'-5'**

### Bacterial 16S rRNA V1/V2 primers (version 1):

**Inner (first) round:**

- **Hyb8F_rRNA**: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTTGATCGMTGGCTCAG-3'
- **Hyb338R_rRNA**: 3'-GTAGGATGCCCTCCGTGCAGATGTGTATAAGAGACAGTTTTGATCGMTGGCTCAG-5'

So the OLIGOS for ORDERING are...

- **Hyb8F_rRNA**: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA-3'
- **Hyb338R_rRNA**: 5'-GTCTCTGTCGGGAGAGAGACTACHVGGGTWTCTAAT-3'

Hyb8F is a 3 bp truncation of the classic 27F primer and has been used in many NGS studies such as [this one](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4635074/). Hyb338R is a 3 bp truncation of the classic 338R primer. These primers amplify a 343 bp region in E. coli.

**GSAF Metadata for this primer set**

*fragment_length*:343, "linker_primer_sequence":"GTTTTGATCGMTGGCTCAG", "reverse_primer_sequence"="TGAGGATGCCCTCCGT"

### Bacterial 16S V4 primers (version 1) - recommended to avoid amplification of Eukaryotic 18S, especially helpful in host/pathogen systems:

- **Hyb515F_rRNA**: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA-3'
- **Hyb806R_rRNA**: 3'-TAATCTWTGGGTVHACAGGGACAGAGAACATATGTAGAGGCTCGGGTGCTCTG-5'

So the OLIGOS for ORDERING are...

- **Hyb515F_rRNA**: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA-3'
- **Hyb806R_rRNA**: 5'-GTCTCTGTCGGGAGAGACTACHVGGGTWTCTAAT-3'

These are based on, "Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies" by Yong Wang and Pei-Yuan Qian, 2009. These primers amplify a 292 bp region in E. coli.

**GSAF Metadata for this primer set**

*fragment_length":292, "linker_primer_sequence":"GTGAGCAGCMGCCGCGGTA", "reverse_primer_sequence":"TAATCTWTGGGVHACAGGGACAGAGAACATATGTAGAGGCTCGGGTGCTCTG"
Bacterial 16S rRNA V4/V5 primers (version 1) - these amplify Eukaryotic 18S rRNA!!!:

Inner (first) round:

Hyb515F_rRNA: 5' - TCGTCGGCAGCGTCAGATGTATAAGAGACAGTGTYCAGCMGCCGCGGTA -3'

Hyb909R_rRNA: 3' - TGARTTTMCTTAACYGCCCCGACAGAGAATATGTGTAGAGGGCTCGGGTGCTCTG -5'

These are based on, "Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies" by Yong Wang and Pei-Yuan Qian, 2009.

In E. coli (O157:H7) these primers amplify a total region of 414 bp from the 16S gene with the final sequencing library being exactly 485 bp.

GSAF Metadata for this primer set

*fragment_length*:414, "linker_primer_sequence":"GTGYCAGCMGCCGCGGTA",
"reverse_primer_sequence":"TGARTTTMCTTAACYGCCCC"

Fungal genome ITS primers (version 1) (ITS-1F and ITS-2 as shown under "Fungal Internal Transcribed Spacer (ITS)" on this very helpful primer maps page from Matthew Nelsen) are also available - these should NOT be used for certain dinoflagellate genomes:

HybITS-1F_rRNA, 5' - TCGTCGGCAGCGTCAGATGTATAAGAGACAGCTTGGTCATTTAGAGGAAGTAA -3'

HybITS2_rRNA, 3' - TCTGGTCATTTAGAGGAAGTAA -5'

In Candida albicans SC5314 these primers create a 256 bp amplicon in ITS1 with the total amplicon with hyb regions being exactly 323 bp. The ITS-1F primer is rooted in the 18S locus while ITS2 is in 5.8S.

ITS primers (version 1) for dinoflagellate Symbiodinium genomes based on the work of Pochon et. al.:

ITS2alg-F, 5' - TCGTCGGCAGCGTCAGATGTATAAGAGACAGGTGAATTGGCAGAACTCCGTG -3'

ITS2alg-R, 3' - TCTGGTCATTTAGAGGAAGTAA -5'

Barcoding primers, common to all designs:

Outer (second) round primers (examples of two specific indexes are shown, both 5'->3'):

Hyb_F01_i5, AATGATACGGCGACCACCGAGATCTACAC ATCACG TCGTCGGCAGCGTC

Hyb_R21_i7, CAAGCAGAAGACGGCATACGAGAT CGAAAC GTCTCGTGGGCTCGG

This paper is an excellent resource from the SILVA group on Bacteria, Archaea, and Eukaryota. Here is a link directly to their main supplemental table of primer designs and coverage.