rRNA bacterial gene and fungal ITS metagenomics samples

Introduction

The GSAF uses a design based on this tech note from Illumina 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”.

rRNA 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’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGTGATCMTGGCTCAG-3’
Hyb338R_rRNA: 3’-TGAGGATGCCCTCCGTGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5’

So the OLIGOS for ORDERING are...
Hyb8F_rRNA: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGTGATCMTGGCTCAG-3’
Hyb338R_rRNA: 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGCTCTCCGCTAGAGT-3’

Hyb8F is a 3 bp truncation of the classic 27F primer and has been used in many NGS studies such as this one. 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":"GTTTGATCMTGGCTCAG", "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’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCTCGACGGGCGGTCTCAG-3’
Hyb806R_rRNA: 3’-TAATCTWTTGGGVCATCAGGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5’

So the OLIGOS for ORDERING are...
Hyb8F_rRNA: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCTCGACGGGCGGTCTCAG-3’
Hyb338R_rRNA: 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAATT-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
Bacterial 16S rRNA V4/V5 primers (version 1) - these amplify Eukaryotic 18S rRNA!!!:

Inner (first) round:

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

**Hyb909R_rRNA**: 3' - TGARTTTMCTTAACYGCCCACAGACAGAATATGTTAGAGGTTCGGGTAGCTCTG - 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": "TGARTTTMCTTAACYGCCCACAGACAGAATATGTTAGAGGTTCGGGTAGCTCTG"

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' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAGTAA - 3'

**HybITS2_rRNA**, 3' - CGTAGCCTACTTCTTGCGTCCGACACAGAGAGAATAGTTGAGAGGCTTCGGGTAGCTCTG - 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' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTG - 3'

**ITS2alg-R**, 3' - TTCGTATATTTCTTCGCCTCCGACAGAGAGAATATGTTGAGAGGCTTCGGGTAGCTCTG - 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
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.