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'-TCGTCGCGCACGCGTCAGATGTGTATAAGAGACAGTTTGATCMTGGCTCAG-3'

Hyb338R_rRNA: 3'-TGAGGATGCCCTCCGTGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5'

So the Oligos for ORDERING are...

Hyb8F_rRNA: 5'-TCGTCGCGCACGCGTCAGATGTGTATAAGAGACAGTTTGATCMTGGCTCAG-3'

Hyb338R_rRNA: 5'-GTCTCGTGGGCGAGATGTGTATAAGAGACAGTGCCCTCCGTAGGAGT-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'-TCGTCGCGCACGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA-3'

Hyb806R_rRNA: 3'-TAATCTWTGGGVHCACTCAGGGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5'

So the Oligos for ORDERING are...

Hyb8F_rRNA: 5'-TCGTCGCGCACGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA-3'

Hyb338R_rRNA: 5'-GTCTCGTGGGCGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-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":"GTGYCAGCMGCCGCGGTA", *reverse_primer_sequence":"TAATCTWTGGGVHCACTCAGG"
Bacterial 16S rRNA V4/V5 primers (version 1) - these amplify Eukaryotic 18S rRNA!!!:

Inner (first) round:

Hyb515F_rRNA: 5’-TCGTCGCGACGCAGTCAGATGTATAAGAGACAGGTGYCACAGCMGCCGCGGTA-3’

Hyb909R_rRNA: 3’-TGARTTTMCTTAACYGCCCGACAGAGAATATGTGTAGAGGGCTCGGTGCTCTG-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

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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’-TCGTCGCGACGCAGTCAGATGTATAAGAGACAGCAGTTAGAGGAAGTAA-3’

HybITS2_rRNA, 3’-CGTAGCTACTTTCTTGCGTCGACAGAGAATATGTGTAGAGGGCTCGGTGCTCTG-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’-TCGTCGCGACGCAGTCAGATGTATAAGAGACAGGTGAATTGCAAGAATCTCGTG

ITS2alg-R, 3’-TTCTTGGTATATTTGCTCCGACTCAGAGAATATGTGTAGAGGGCTCGGTGCTCTG-5’

Barcoding primers, common to all designs:

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

Hyb_F01_i5, AATGATACGGCGACGCTACAC ATCACG TCGTCGCGACGCAGACAG

Hyb_R21_i7, CAAGCAGAAGACGCATACAGAT 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.