# The sequences of staggered primers

## Primers for profiling the V3-V4 region of 16S DNA

**Primers for reaction 1 (direct orientation of the amplicon)**

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGAGGCAGCAG

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGgaCCTACGGGAGGCAGCAG

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGtagCCTACGGGAGGCAGCAG

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGagcaattCCTACGGGAGGCAGCAG

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGcaGGACTACHVGGGTWTCTAAT

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGtctGGACTACHVGGGTWTCTAAT

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGatctGGACTACHVGGGTWTCTAAT

**Primers for reaction 2 (reverse orientation of the amplicon)**

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGcaGGACTACHVGGGTWTCTAAT

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGatctGGACTACHVGGGTWTCTAAT

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGtctactGGACTACHVGGGTWTCTAAT

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTACGGGAGGCAGCAG

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGgaCCTACGGGAGGCAGCAG

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGtagCCTACGGGAGGCAGCAG

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGagcaattCCTACGGGAGGCAGCAG

## Primers for subtyping *Blastocystis* sp.

Reaction 1 (direct orientation of the amplicon)

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAGGTAGTGACAATAAATC

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGaGGAGGTAGTGACAATAAATC

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGcaGGAGGTAGTGACAATAAATC

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGactGGAGGTAGTGACAATAAATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCTTTCGCACTTGTTCATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGaTGCTTTCGCACTTGTTCATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGcaTGCTTTCGCACTTGTTCATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGactTGCTTTCGCACTTGTTCATC

Reaction 2 (reverse orientation of the amplicon)

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCTTTCGCACTTGTTCATC

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGaTGCTTTCGCACTTGTTCATC

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGcaTGCTTTCGCACTTGTTCATC

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGactTGCTTTCGCACTTGTTCATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAGGTAGTGACAATAAATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGaGGAGGTAGTGACAATAAATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGcaGGAGGTAGTGACAATAAATC

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGactGGAGGTAGTGACAATAAATC

*Legend:*

## Illumina overhang tags:

plain uppercase

TCGTCGGCAGCGTC (P5-tag, a Read1 Nextera pre-adapter) and GTCTCGTGGGCTCGG (P7-tag, a Read 2 Nextera pre-adapter). The P5- and P7-tags are the sites where second-round indexing primers anneal.

## Illumina pad sequence:

underlined

AGATGTGTATAAGAGACAG; it is the annealing site of the Illumina sequencing primers. The read 1 and read 2 thus both start from the specific locus primer (or the heterogeneity spacer if it is present). The Illumina overhang tag along with the pad sequence make together the whole overhang sequence - these are the annealing sites for the read 1 sequencing primer (the P5-tag and the following pad) and the read 2 sequencing primer (the P7-tag and the sequencing primer).

## Heterogeneity spacers (primer staggering, heterogeneity spacers):

ga, agt... (lowercase, yellow)

## Specific locus primers:

bold uppercase

16S rDNA profiling: **CCTACGGGAGGCAGCAG, GGACTACHVGGGTWTCTAAT** (bold uppercase): specific portions of primers V3-forward (341-F, [1]) and V4-reverse (806-R, [2]).

*Blastocystis* subtyping: **GGAGGTAGTGACAATAAATC, TGCTTTCGCACTTGTTCATC** are specific portions of F (ILMN_Blast505_532F) and R (ILMN_Blast998_1017R) primers for subtyping of *Blastocystis* sp. according to Maloney et al [3]

**References:**

[1] Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59: 695-700

[2] Caporaso JG, Lauber CL, Walters WA, et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108 Suppl 1: 4516-4522

[3] Maloney JG, Molokin A, Santin M (2019) Next generation amplicon sequencing improves detection of Blastocystis mixed subtype infections. Infect Genet Evol 73: 119-125