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

a, ca... (yellow, lowercase) heterogeneity spacers

AGATGTGTATAAGAGACAG Illumina pad sequence

TCGTCGGCAGCGTC Illumina overhang adapter (5-direction)

GTCTCGTGGGCTCGG Illumina overhang adapter (7-direction)

CCTACGGGAGGCAGCAG, GGACTACHVGGGTWTCTAAT Specific portions of V3-forward and V4-reverse primers for 16S rDNA profiling, according to **Caporaso et al**, Proc Natl Acad Sci U S A 2011; 108(Suppl):4516 – 4522. http://dx.doi.org/10.1073/pnas.1000080107.

GGAGGTAGTGACAATAAATC, TGCTTTCGCACTTGTTCATC Specific portions of F and R primers for subtyping of *Blastocystis* sp. according to **Maloney et al**, Infect. Genet. Evol. 2019;73:119–125. doi: 10.1016/j.meegid.2019.04.013.