# Comparison of molecular diagnostic approaches for the detection and differentiation of the intestinal protist *Blastocystis* sp. in humans

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## SUPPLEMENTARY DATA 1: Detailed description of the next-generation sequencing protocol for *Blastocystis.*

Positive samples from qPCR were subjected to NGS to determine *Blastocystis* subtypes according to the method by Maloney et al. (2019). Briefly, amplicons of an informative region (~450 bp) of the SSU rDNA gene were generated using overhang primers, purified, and provisioned with indices and sequencing adaptors using a limited number of PCR cycles with combinatorial indices (Nextera XT Index Kit v2 Set A and D, Illumina, San Diego, CA, USA). The amplicon libraries were purified and equalized using on the SequalPrep plates (Thermo, Waltham, MA, USA), pooled, supplemented with 20% PhiX control to balance the amplicon signal, and sequenced on a MiSeq instrument with the Reagent Kit v2, 2x250 bp (Illumina). The ensuing sequences were downloaded from BaseSpace as demultiplexed fastq files, and processed using the USEARCH10 program (Edgar et al. 2010): primers were trimmed, reads were filtered for quality, and unique sequences defined as zero-radius operational taxonomic units, denoised, their frequencies were tabulated, off-target amplicons were removed and subtypes of *Blastocystis* identified by clustering with a reference set of representative sequences as described in Cinek et al. (2021).

**References:**

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