Suggested PCR primers useful for parasitic helminths

Genetic marker	Target helminth group	Primer name	Primer sequence (5'-3')	Reference
18S rRNA	Platyhelminths	WormA WormB	GCGAATGGCTCATTAAATCAG CTTGTTACGACTTTTACTTCC	Waeschenbach & Littlewood (2017)
	Nematodes	988F 1912R 1813F 2646R	CTCAAAGATTAAGCCATGC TTTACGGTCAGAACTAGGG CTGCGTGAGAGGTGAAAT GCTACCTTGTTACGACTTTT	Holterman et al. (2006)
28S rRNA	Platyhelminths	digl2* LO	AAGCATATCACTAAGCGG GCTATCCTGAG(AG)GAAACTTCG	Tkach et al. (2001)
	Nematodes	D2A** D3B	ACAAGTACCGTGAGGGAAAGTTG TCGGAAGGAACCAGCTACTA	De Ley et al. (2005)
COI	Helminths	JB3 JB4.5	TTTTTTGGGCATCCTGAGGTTTAT TAAAGAAAGAACATAATGAAAATG	Bowles et al. (1992)
ND1	Platyhelminthes	JB11 JB12	AGATTCGTAAGGGGCCTAATA ACCACTAACTAATTCACTTTC	Bowles & McManus (1993)
12S rRNA	Platyhelminths	12STreF 12STreR	GTGCCAGCADYYGCGGTTA AGCAGCAYATHGACCTG	Chan et al. (2022)
	Nematodes (Trichocephalida)	12S.C1.F 12S.C1.R	GTGCCAGCTAYCGCGGTTA GRTGACGGGCRATATGTG	Chan et al. (2020)
	Nematodes (Ascaridida, Spirurida, Strongylida)	12S.C345.F 12S.C345.R	GTWCCAGAATAATCGGMTA ATTGAYGGATGRTTTGTRC	
16S rRNA	Platyhelminths	16SCesTreF 16SCesTreR	GTGYDAAGGTAGSATAAT CCGGTYTYAACTCARCTCAT	Chan et al. (2022)
	Nematodes (Trichocephalida)	16S.C1.F 16S.C1.R	ACGAGAAGACCCTRGRAAYT GRTYTAAACTCAAATCACG	Chan et al. (2020)
	Nematodes (Ascaridida, Spirurida, Strongylida)	16S.C345.F 16S.C345.R	AAGATAAGTCTTYGGAARYT GAAYTAAACTAATATCAMG	

*Primers targeting the D1-D3 region of the 28S rRNA gene

**Primers targeting the D2-D3 region of the 28S rRNA gene

Note:

- The primers provided here are popular for targeting broad species range in helminths. PCR conditions can be found at the respective references.
- Primers for the ITS regions, *COII*, and *cytB* genes were not included as the primers depends on the target helminth species.
- The full-length mitochondrial sequences (*COI*, *COII*, *cytB*, *NAD1*, 12S, 16S) obtained for use in ABIapp were mined from the complete mitochondrial genomes available in GenBank database.
- The nuclear sequences (18S, 28S, ITS1, ITS2) obtained for use in ABIapp were mined from almost complete sequences available in GenBank database.

References:

Bowles J, Blair D, McManus DP. (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Molecular and Biochemical Parasitology. 54(2):165-173

Bowles J, McManus DP. (1993) NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. International Journal for Parasitology. 23(7):969-972

Chan AHE, Chaisiri K, Morand S, Saralamba N, Thaenkham U. (2020) Evaluation and utility of mitochondrial ribosomal genes for molecular systematics of parasitic nematodes. Parasites & Vectors. 13(364)

Chan AHE, Saralamba N, Saralamba S, Ruangsittichai J, Thaenkham U. (2022) The potential use of mitochondrial ribosomal genes (12S and 16S) in DNA barcoding and phylogenetic analysis of trematodes. BMC Genomics.

De Ley P, De Ley IT, Morris K, Abebe E, Mundo-Ocampo M, Yoder M, Heras J, Waumann D, Rocha-Olivares A, Burr AHJ, Baldwin JG, Thomas WK. (2005) An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences. 360(1462):1945-1958

Holterman M, van der Wurff A, van den Elsen Sven, van Megen H, Bongers T, Holovachov O, Bakker J, Helder J. (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Molecular Biology and Evolution. 23(9):1792-1800

Tkach VV, Pawlowski J, Mariaux J, Swiderski Z. (2001) Molecular phylogeny of the suborder Plagiochiata and its position in the system of Digenea. In Interrelationships of the Platyhelminthes. Littlewood DTJ, Bray RA (eds.). Taylor and Francis, London, pp. 186-193

Waeschenbach A, Littlewood DTJ. (2017) A molecular framework for the Cestoda. In Planetary Biodiversity Inventory (2008-2017): Tapeworms from Vertebrate Bowels of the Earth. Caira JN,

Jensen K (eds.). University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, KS, USA, pp. 431-451