

Nassellaria single cell rDNA amplification

Two different enzymes:

GoTaq (Promega, Lyon, France):

	Concentration	Volume (μ l)
GoTaq PCR buffer	5x	5.9
MgCl ₂	25 mM	2.96
dNTP	10 mM	1
Forward primer	10 μ M	0.5
Reverse primer	10 μ M	0.5
GoTaq	5 u/ μ l	0.25
miliQ sterile water	-	16.9
DNA template	-	0.5
Final volume		28.5

		Time	Temperature ($^{\circ}$ C)	39 cycles
Denaturation		2 min	95	
Amplification	Denaturation	30 s	95	
	Annealing	45 s	*	
	Elongation	1 min	72	
Final elongation		10 min	72	

* See Table at the end of the protocol for specific annealing temperature of each primer

Phusion[®] High-Fidelity PCR Master Mix (Finnzymes):

	Concentration	Volume (μ l)
miliQ sterile water	-	8.75
DMSO	100%	0.75
Forward primer	10 μ M	1
Reverse primer	10 μ M	1
Mix Phusion	2x	12.5
DNA template	-	1
Final volume		25

		Time	Temperature ($^{\circ}$ C)	35 cycles
Denaturation		30 s	98	
Amplification	Denaturation	10 s	98	
	Annealing	30 s	*	
	Elongation	30 s	72	
Final elongation		10 min	72	

* See Table at the end of the protocol for specific annealing temperature of each primer

PCR amplicons visualized on 1% agarose gel stained with ethidium bromide.

Targeted gene	Primer	Specificity	Sequence 5'-3'	Direction	Tm °C	Reference
18S (1st part)	SA	Eukaryotic	AAC CTG GTT GAT CCT GCC AGT	Forward	56-60	Medlin et al., 1988
	18S NasIb R	Nassellaria	GAG ACT ACG ACG GTA TCT GAT C	Reverse	60	Sandin et al. (in prep.)
	S879	Radiolaria	CCA ACT GTC CCT ATC AAT CAT	Reverse	56	Decelle et al., 2012
18S (2nd part)	S32_TASN	Radiolaria	CCA GCT CCA ATA GCG TAT RC	Forward	52	Ishitani et al. 2012
	V9R	Eukaryotic	CCT TCY GCA GGT TCA CCT AC	Reverse	52	Romac (unpub.)
	18S NassII F	Nassellaria	AGC ATG GAA TAA TAA CTG ATG A	Forward	57	Sandin et al. (in prep.)
	18S NassII R	Nassellaria	CAC CAR TTC ATC CAA TCG GTA G	Reverse	57	Sandin et al. (in prep.)
28S (D1+D2)	28S Nas F	Nassellaria	AGT AAC GGC GAG TGA AGC	Forward	56	Sandin et al. (in prep.)
	28S Nas R	Nassellaria	CCA ACA TAC DTG CTC TTG T	Reverse	56	Sandin et al. (in prep.)
	28S Rad2	Radiolaria	TAA GCG GAG GAA AAG AAA	Forward	50	Ando et al., 2009
	ITSa3	Radiolaria	TCA CCA TCT TTC GGG TCC CAA CA	Reverse	50	Ando et al., 2009