PCR using DNA from single tintinnid cells

PCR for nuclear Small Subunit rDNA (SSU)

Primers: Universal nuclear SSU rDNA Primers (Zhang et al., 2005) 18ScomF1 and 18ScomR1.

Alternative: Universal nuclear SSU rDNA Primers (Zhang et al., 2005) Tintinnid SSU rDNA Primers (Santoferrara et al., 2012a) = 18ScomF1 and Tin18SR1 Tin18SF and 18ScomR1. Program TIN0HZ0. *This option gives higher amount of PCR product, but you need to prepare two different master mixes (one for each pair of primers). You can use this protocol after the universal primers (nested PCR) if the amount of PCR product is low (dilute 1:1000 before you re-amplify). PCR products for sequencing can be purified in the same column.*

STOCK SOLUTION: 20 µM in 10 mM Tris HCL pH = 8 (pre-filtered × 0.2 µm)

WORKING SOLUTION: µM in 10 mM Tris HCL pH = 8 (pre-filtered × 0.2 µm) (2 µl STOCK + 78 µl Tris)

FINAL CONCENTRATION IN PCR REACTION: 0.2 µM

Procedure:

1. Prepare master mix based on number of samples being processed (include extraction, positive and negative controls, and one extra). Keep on ice and add TaKara Taq u/µl (0.125 µl per sample) or Phusion Pol u/µl (0.125 µl per sample).

2. Dispense 2 µl into PCR tubes.

3. Add µl of template DNA.

4. Run PCR.

5. Prepare checking gel 1.2%: 0.6 g agarose in 55 ml TAE 1x + 2 µl ethidium bromide (or 3 µl GelRed).

6. Load µl of PCR product 0.5 µl of loading dye (f.c. 1X). Load appropriate ladder (e.g., 1 kb) at the last time.

7. Run at 50 V for 30 min, then 100 V for 10 min. Photograph under UV light.

8. Store PCR products at -20º C.

Master Mix TaKara:

Reagent	**Vol 1× (µl)**	**Final cc**	**Vol 20× (µl) (-20°C)**
ddH_2O	17	-	340
10× buffer (Cl_2Mg 2 mM)	2.5	Mg 2 mM	50
DNTPs (2.5 mM each)	2	0.2 mM	40
primer (5 µM)	1	0.2 µM	20
primer (5 µM)	1	0.2 µM	20
Cl_2Mg (25 mM)	0.5	0.5 mM	10

Master Mix Phusion:

Reagent	**Vol 1× (µl)**	**Final cc**	**Vol 20× (µl) (-20°C)**
ddH_2O	14	-	280
5× buffer (Cl_2Mg 7.5 mM)	5	Mg 1.5 mM	100
DNTPs (2.5 mM each)	2	0.2 mM	40
primer (5 µM)	1	0.2 µM	20
primer (5 µM)	1	0.2 µM	20
Cl_2Mg (50 mM)	0.75	1.5 mM	10

Thermocycler conditions:

FOR Takara, Universal primers: Program SSU0HZ4

LID = 105º C WAIT AUTO

1. 95°C min

2. 94°C 1 seg

3. 56°C 3 seg

4. 72°C 4 seg

5. GOTO 2 REP 34

6. 72°C 1 min

HOLD 4°C

FOR Takara, alternative primers: Program TIN0HZ0

FOR PHUSION POL, Universal primers: Program 9859724

LID = 105º C WAIT AUTO

1. 95°C min

2. 98°C 1 seg

3. 59°C 1 seg

4. 72°C 4 seg

5. GOTO 2 REP 29

6. 72°C 1 min

HOLD 4°C

PCR for nuclear Large Subunit rDNA (LSU) or nuclear ITS1-5.8S rDNA-ITS2 (ITS)

Primers: -Universal nuclear Large Subunit rDNA primers (Ortman, 2008): LSU F, LSU R.

- Nuclear ITS1-5.8S rDNA-ITS2 (Snoeyenbos-West et al., 2002): ITS F, ITS R.

STOCK SOLUTION: 20 µM in 10 mM Tris HCL pH = 8 (pre-filtered × 0.2 µm)

WORK SOLUTION: 5 µM in 10 mM Tris HCL pH = (pre-filtered × 0.2 µm) (2 µl STOCK + 78 µl Tris)

FINAL CONCENTRATION IN PCR REACTION: 0.2 µM

Procedure and Master Mix: the same that SSU rDNA

Thermocycler conditions:

FOR Takara: Program 9450724

LID = 105º C WAIT AUTO

1. 95°C	1 min
2. 94°C	15 seg
3. 50°C	30 seg
4. 72°C	40 seg
5. GOTO 2	REP 34
6. 72°C	1 min
HOLD	4°C

FOR PHUSION POL: Program 9853724

LID = 105º C WAIT AUTO

1. 95°C	min
2. 98°C	1 seg
3. 53°C	1 seg
4. 72°C	4 seg
5. GOTO 2	REP 29
6. 72°C	1 min
HOLD	4°C

References

Ortman B.D. (2008). DNA barcoding the Medusozoa and Ctenophora. PhD tesis. Groton, CT, University of Connecticut.

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Snoeyenbos-West O., Salcedo T., et al. (2002). Insights into the diversity of choreotrich and oligotrich ciliates (Class: Spirotrichea) based on genealogical analyses of multiple loci. International Journal of Systematic and Evolutionary Microbiology 52: 1901-1913.

Zhang H., Bhattacharya D., et al. (2005). Phylogeny of dinoflagellates based on mitochondrial cytochrome *b* and nuclear small subunit rDNA sequence comparisons. Journal of Phycology 41: 411-420.