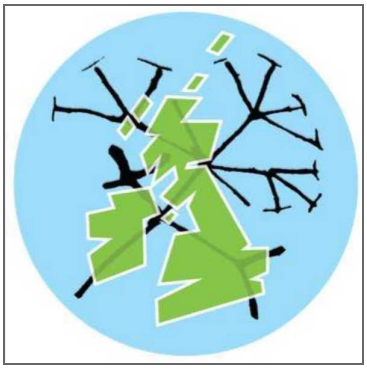

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Protocol status: Working
We use this protocol and it's working

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Taxon Group Protists: Barcoding

In 1 collection

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Darwin Tree of Life

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ABSTRACT

This protocol describes the DNA barcoding process for protists in the Darwin Tree of Life project.

PROTOCOL integer ID:
83343

DNA extraction

1 Extract genomic DNA from protists cultures using e.g. the **DNeasy Plant** or **MagAttract** kit (Qiagen) or equivalent and follow the manufacturer's protocol/instructions. Depending on the taxa, DNA extraction can require an alternative pre-protocol approach, e.g. including a Plant DNAzol Reagent (Gibco BRL Life Technologies) or bead-beating step (10 ms for 3 min at 1 min intervals with 5 min rest on ice to avoid overheating).

DNeasy (Qiagen; 69204)
MagAttract (Qiagen; 67563)

Barcoding

2 Amplify appropriate marker gene(s) (e.g. V4 or V9 region of 18S rRNA gene) using primer pairs as appropriate for group/taxa using a Taq Master Mix (Qiagen), GoTaq polymerase (Promega), or equivalent with a final volume of 50 µL (see example below). For each marker region two primer combinations are available. For difficult templates, it might help to use high fidelity DNA Polymerase (e.g. Deep Vent DNA polymerase, New England BioLabs) to the mix.

Taq Master Mix (Qiagen; 201445)
GoTaq polymerase (Promega; M5001)
Deep Vent DNA polymerase (New England BioLabs; M0258S)

Mastermix example	Volume per reaction (50 µl)
Molecular Grade H_20	up to 50 µl
5x Buffer	10 µl
$MgCl_2$	4 µl
dNTP Mix	1 µl
Forward Primer (0.25 µM)	2 µl
Reverse Primer (0.25 µM)	2 µl
GoTaq Polymerase	0.25 µl
BSA	1 µl
Template	1 µl

Example for PCR master mix.

2.1 25-30 cyclesSpin down the tubes and run the PCR using an appropriately programmed thermal cycler.

	TAReuk454FWD1/ TAReukREV3	E572/E1009R	1389F/1510R	1391F/EukBr
Initial denaturation	98C|30 sec	98C|30 sec	98C|30 sec	98C|30 sec
Denaturation*	98C|10 sec	98|10 sec	98C | 10sec	98C | 10 sec
Primer annealing*	50C | 30 sec	55C | 30 sec	57C | 30sec	62C | 30 sec
Elongation*	72C | 30 sec	72C | 30 sec	72C | 30sec	72C | 30 sec
Final elongation	72C | 10 min	72C | 5 min	72C |10 min	72C |10 min

* repeat for 25 to 35 cycles

Name	Sequence 5'-3'	Target gene	Target group	Direction	TM [°C]	Amplicon size [bp]	Reference
1389F	TTGTACACACCGCCC	V9	Euk.	forward	55	~170	Amaral-Zettler et al 2009
1510R	CCTTCYGCAGGTTCACCTAC	V9	Euk.	reverse	58-61	~170	Amaral-Zettler et al 2009
1391f	GTACACACCGCCCGTC	V9	Euk	Forward	59	~170	Stoeck et al 2010
EukBr	TGATCCTTCTGCAGGTTCACCTAC	V9	Euk	reverse	61	~170	Stoeck et al 2010
TAReuk454FWD1	CCAGCASCYGCGGTAATTCC	V4	Euk	forward	62-65	417	Stoeck et al 2010
TAReukREV3	ACTTTCGTTCTTGATYRA	V4	Euk	reverse	44-55	417	Stoeck et al 2010
E572F	CYGCGGTAATTCCAGCTC	V4	Euk	forward	56-59	438	Comeau et al 2011
E1009R	CRAAGAYGATYAGATACCRT	V4	Euk	reverse	45-56	438	Comeau et al 2011

Primer information

Gel electrophoresis

3 Check the PCR products by gel electrophoresis against positive and negative controls and a 1kb ladder. Load 4 µl PCR product with 1 µl loading dye on a 1% (w/v) agarose gel (SYBRSafe). Run the gel at 110 mV for ~40 minutes.

SYBRSafe (Invitrogen; S33102)

PCR purification

4 If one product is visible purify the PCR product using the QIAquick PCR purificastion kit or equivalent commercial PCR purification kits. If multiple products are visible on the gel, purify the PCR product using the QiaQick gel extraction kit (Qiagen) or equivalent commercial gel purification kits If no products are visible, repeat PCR using the second primer pair for the marker gene region and follow the steps.

QIAquick PCR purification kit (Qiagen; 28104)
QiaQick gel extraction kit (Qiagen; 28704)

DNA quantification

5 Determine the DNA concentration using a Qubit dsDNA HS Assay Kit (or equivalent). Ensure the sample is 10-20 ng µl -1 for submission for sequencing.

Qubit dsDNA HS Assay Kit (Invitrogen; Q32851)

Sanger sequencing

6 Submit for Sanger sequencing, do not clone the products prior to sequencing, sequence directly and inspect the chromatogram for evidence of dual amplicon sampling that would be indicative of contamination. For submitting to Eurofins (TubeSeq), mix 15ul of your purified PCR product (minimum concentration of 5ng/ul) and add 2ul of your primer (10uM). Ensure that the total volume is not more or less than 17 ul.