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

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A new metabarcoding approach to survey diversity at the species level of Arcellinida (Amoebozoa: Tubulinea)

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ABSTRACT

PCR protocol for the paper:" A needle in a haystack: a new metabarcoding approach to survey diversity at the species level of Arcellinida (Amoebozoa: Tubulinea)"

Polymerase Chain Reaction protocol

1 First PCR

Primers:
Primer forward: LCO 1490 (5´ GGTCAACAAATCATAAAGATATTGG 3´)
Primer reverse: HCO 2198 (5´ TAAACTTCAGGGTGACCAAAAAATCA 3´)

Reagents for the PCR:

	A	B
	Polymerase	6 µL
	BSA	1 µL
	Primer forward	1 µL (10 µmol)
	Primer reverse	1 µL (10 µmol)
	Destilated water	1 µL
	Sample (eDNA)	1 µL

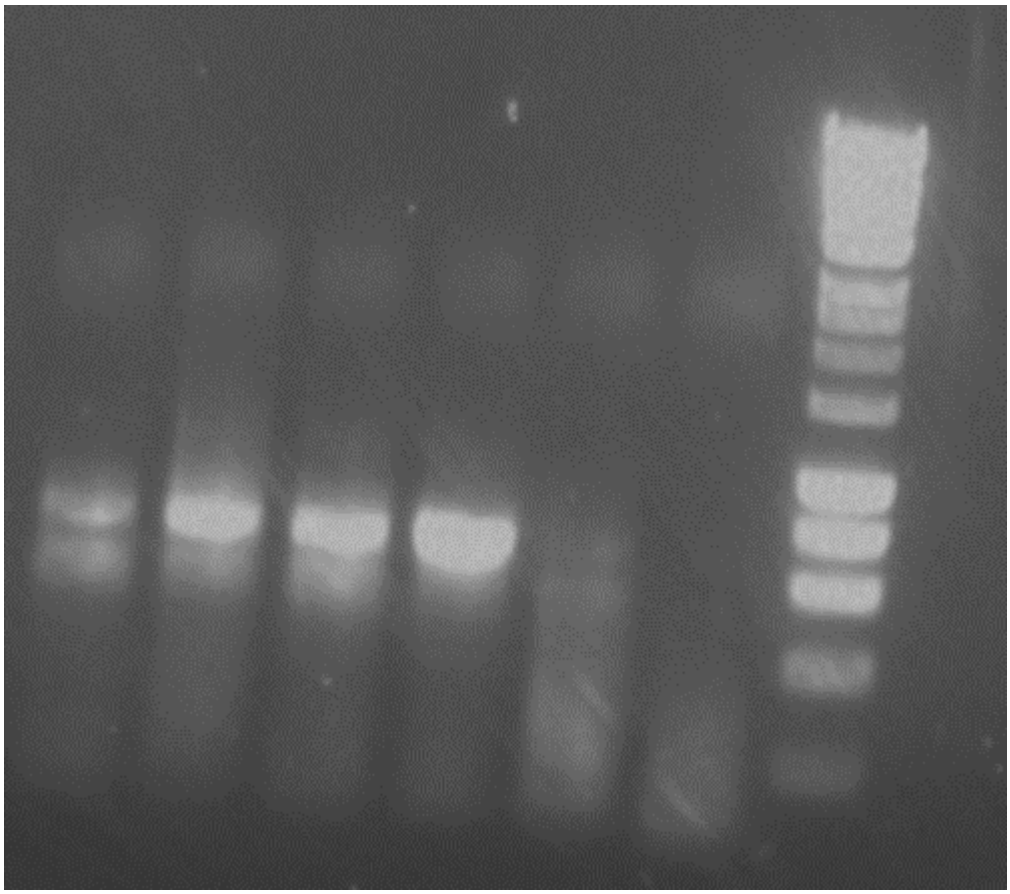
Total reaction mix per sample= 10 µL

Thermocycler program:

	A	B	C	D
		Temperature	Time	cycles
	Initial denaturation	96 °C	5 min	1
	Denaturation	94 °C	15 s	40
	Annealing	40 °C	15 s	
	Extension	72 °C	90 s	
	Final extension	72 °C	10 min	1

Denaturation temperatures and times may vary depending on the polymerase used. This protocol was tested with the MyTaq™ Red Mix™ polymerase.

Gel electrophoresis:
Analyze the results of your PCR reaction via gel electrophoresis. The resultant fragment was 692 bp long:



example of four PCR products with one negative control on the right. HyperLadder™ 1kb as the molecular weight marker.

2 Second PCR

Primers:
Primer forward: LCO 1490 (5´ GGTCAACAAATCATAAAGATATTGG 3´)
Primer reverse: ArCOIR (5´ CCACYNGAATGWGCTARAATACC 3´)

Reagents for the PCR:

	A	B
	Polymerase	12 µL
	Primer forward	1 µL (10 µmol)
	Primer reverse	1 µL (10 µmol)
	Destilated water	6 µL

	A	B
	Sample (first PCR product)	1 µL

Total reaction mix per sample= 20 µL

Thermocycler program:

	A	B	C	D
		Temperature	Time	cycles
	Initial denaturation	96 °C	5 min	1
	Denaturation	94 °C	15 s	40
	Annealing	55 °C	15 s	
	Extension	72 °C	90 s	
	Final extension	72 °C	10 min	1

Denaturation temperatures and times may vary depending on the polymerase used. This protocol was tested with the MyTaq™ Red Mix™ polymerase.

Gel electrophoresis:

Analyze the results of your PCR reaction via gel electrophoresis. The resultant fragment was 407 bp long