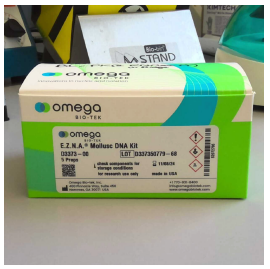
Aug 20, 2024

Sanger Tree of Life HMW DNA Extraction: Modified Omega Bio-Tek E.Z.N.A.®



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.36wgq3wj3lk5/v1

Amy Denton[1], Caroline Howard[1]

[1]Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA

Amy Denton
Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridges...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.36wgq3wj3lk5/v1

Protocol Citation: Amy Denton, Caroline Howard 2024. Sanger Tree of Life HMW DNA Extraction: Modified Omega Bio-Tek E.Z.N.A.®. **protocols.io https://dx.doi.org/10.17504/protocols.io.36wgq3wj3lk5/v1**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: February 13, 2024

Last Modified: August 20, 2024

Protocol Integer ID: 95166

Keywords: HMW DNA extraction, Omega Bio-Tek, E.Z.N.A, magnetic bead extraction, SpeedBeads, manual DNA extraction, jellyfish, ASG, solid phase reversible immobilisation, reference genome, long read sequencing

Funders Acknowledgement:
Wellcome Trust
Grant ID: 206194
Wellcome Trust
Grant ID: 218328
Gordon and Betty Moore Foundation
Grant ID: GBMF8897

Abstract

This protocol describes the manual extraction and SPRI of HMW DNA from fresh frozen jellyfish samples intended for long-read sequencing using a combination of the Omega Bio-Tek E.Z.N.A.® Mollusc & Insect DNA kit and Sera-Mag™ SpeedBeads. It is effective for approximately 50 to 60% of jellyfish species covered by the Tree of Life Programme for the Aquatic Symbiosis Genomics (ASG) project. This protocol has resulted in successful extractions for a number of species including *Mastigias papua, Mastigias albipunctatus, Rhopilema esculentum*, *Catostylus mosaicus, Cassiopea ornata, Cassiopea xamachana*, *Mertensia ovum, Bolinopsis mikado, Pseudorhiza haeckeli, Aurelia* spp. and *Pelagia noctiluca.*

The output of this protocol is HMW DNA, which depending upon yield and genome size of the species, can be directed towards either HMW DNA Fragmentation: Diagenode Megaruptor®3 for LI PacBio or HMW DNA Fragmentation: g-Tube for ULI PacBio.

This protocol was developed through R&D by the Tree of Life Core Laboratory; it was primarily adapted from the Omega Bio-Tek E.Z.N.A.® Mollusc & Insect DNA kit protocol by combining the initial lysis and precipitation (steps 1 to 10) with reduced incubation temperatures, omission of vortexing and the addition of a bead-based extraction using Sera-Mag™ SpeedBeads, as utilised in the Sanger Tree of Life HMW DNA Extraction: Automated Plant Organic HMW gDNA Extraction (POE) protocol.

Acronyms
HMW: high molecular weight
LI: low input
ULI: ultra-low input

Guidelines

- Input amounts of 100 to 200 mg of fresh frozen jellyfish tissue are required for this protocol. Smaller input amounts can be used, however the yields of DNA obtained may be lower.
- Ideally fresh frozen samples should be used for this protocol. Ethanol preserved samples can also be processed using this protocol, however the yields of DNA obtained may be lower.
- Keep samples on dry ice until the lysis buffer is ready to be added to them to maintain temperature and prevent nucleic acid degradation.
- An experienced operator can expect to comfortably process 8 samples, with approximately 3 to 4 hours handling time over a start to finish period of 4 to 5 hours. This estimation excludes subsequent QC checks.

Additional notes:

- FluidX tubes are used throughout the Tree of Life programme in order to track samples, therefore rather than the microcentrifuge tubes which have been mentioned in this protocol for DNA storage, all routine DNA extracts are stored in FluidX tubes.

Materials

- 1.5 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030108418)
- 2 mL DNA Lo-Bind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.078)
- 1.5 mL BioMasher II tubes and pestles (sterile) (Cat. no. 9791a)
- 15 mL or 50 mL centrifuge tubes
- Omega Bio-Tek EZNA® Mollusc & Insect DNA kit (Omega Bio-Tek Cat. no. D3373-00)
- Qiagen MagAttract HMW DNA extraction kit (Qiagen Cat. no. 67563)
- Sera-Mag™ magnetic carboxylate modified particles (Cat. no. GE24152105050250)
- AMPure PB beads (Pacific Biosciences Cat. no. 100-265-900)
- Buffer EB (Qiagen Cat. no. 19086)
- 100% absolute ethanol
- Chloroform:isoamyl alcohol (24:1, v/v)
- Nuclease free water
- PEG 8000
- Tris-HCl (1 M stock concentration, pH 8.0)
- EDTA (0.1 M stock concentration, pH 8.0)
- NaCl (5 M stock concentration)
- Tween-20
- Dry ice

Equipment:

- Pipettes for 0.5–1000 µL and filtered tips
- Wide-bore tips (200 µL, filtered if available)
- Diagnocine PowerMasher II tissue disruptor (Product no. 891300)
- Eppendorf SmartBlock 2.0 mL (Cat. no. 5362000035)
- Mini centrifuge (Cat. no SS-6050)
- Eppendorf Centrifuge 5425/5425 R (Cat. no. 5405000263)
- HulaMixer Sample Mixer (Cat. no. 15920D)
- DynaMag™-2 magnetic rack (Cat. no. 12321D)
- Timer
- Chemical Fume Hood

Reagent Recipe (taken directly from the Sanger Tree of Life HMW DNA Extraction: Automated Plant Organic HMW gDNA Extraction (POE) protocol):

50% PEG 8000

	Reagent	Target concentration	Molecular weight (g/mol)	Stock concentration	Input from stock (15 mL total)
	PEG 8000	50% (w/v)	8000	Powder	7.5 g
	Nuclease-free water	-	-	-	6 mL

	Reagent	Target concentration	Molecular weight (g/mol)	Stock concentration	Input from stock (15 mL total)
	Incubate for 60 minutes, 75°C at 600 rpm, routinely vortexing until fully dissolved.				
	Nuclease-free water	-	-	-	Up to 15 mL
	Should be prepared fresh and allowed to cool before use in the SpeedBead Binding solution				

10% Tween-20

	Reagent	Target concentration	Molecular weight (g/mol)	Stock concentration	Input from stock (50 mL total)
	Nuclease-free water	-	-	-	44 mL
	Tris-HCl, pH 8.0	20 mM	157.60	1 M	1 mL
	Tween-20	10% (v/v)	1,227.54	100% (v/v)	5 mL
	Place on a tube rotator for 30 minutes, 20 rpm, ensuring Tween is dissolved.				
	Store protected from the light at room temperature for up to 1 year (replace if solution becomes yellowed).				

SpeedBead Wash Suspension:

	Reagent	Target concentration	Molecular weight (g/mol)	Stock concentration	Input from stock
	Sera-Mag™ Speed Bead stock solution, 4°C	0.2% (w/v)	-	0.5% (w/v)	800 µL
	Wash beads 4 times with nuclease free water before use to remove sodium azide				
	Nuclease-free water	-	-	-	Up to 2.0 mL
	This should be prepared fresh before use in the Sera-Mag™ SpeedBead solution.				

1. Allow Sera-Mag™ SpeedBeads aliquot to reach room temperature (~30 mins).
2. Vortex thoroughly to resuspend the beads.
3. Pipette 800 µL of Sera-Mag™ SpeedBead stock solution into a 2 mL Lo-Bind tube on a magnetic stand and wait for the beads to migrate to the magnet.
4. When the supernatant is completely clear, remove and discard the supernatant from the tube without disturbing the beads.
5. Add 1000 µL nuclease-free water to the tube.
6. Vortex the tube to resuspend beads.
7. Centrifuge briefly to remove droplets from tube lid.
8. Place the tube on a magnetic stand until the supernatant is completely clear and beads are bound towards the magnet.
9. Remove and discard the supernatant without disturbing beads.
10. Repeat steps 5 to 9 three times.
11. Add nuclease-free water up to 2 mL.
12. Vortex tube to resuspend beads.
13. Centrifuge briefly to remove droplets from tube lid.

14. SpeedBead wash suspension can now be used for the Sera-Mag™ SpeedBead solution.

SpeedBead Binding Solution:

	Reagent	Target concentration	Molecular weight (g/mol)	Stock concentration	Input from stock (40 mL total)
	Tris-HCl, pH 8.0	10 mM	157.60	1 M	400 µL
	EDTA, pH 8.0	1 mM	292.24	0.1 M	400 µL
	NaCl	1.6 M	58.44	5 M	12.8 mL
	Tween-20	0.05% (v/v)	1,227.54	10% (v/v)	200 µL
	PEG 8000	18% (w/v)	8000	50 % (w/v)	14.4 mL
	Nuclease-free water	-	-	-	up to 40 mL
	Filter sterilise through a 0.45 µM filter into a fresh 50 mL falcon. SpeedBead binding solution should be prepared fresh before use in the Sera-Mag™ SpeedBead solution.				

Ensure that the exact volume of 50% PEG 8000 is added, as this is crucial for gDNA binding.

Sera-Mag™ SpeedBead Solution:

	A	B	C	D	E
	SpeedBead Binding Solution	-	-	-	38 mL
	SpeedBead Wash Suspension	0.01% (v/v)	-	0.2% (v/v)	2 mL
	Store at 4°C in the dark for up to 3 months.				

40 mL of Sera-Mag™ SpeedBead solution is enough for 50 - 65 samples.

Protocol PDF: Sanger Tree of Life HMW DNA Extra… 198KB

Safety warnings

- The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol. Cotton glove liners are strongly recommended when handling the samples on dry ice.
- Eye protection and silver shield/chemical resistant gloves should be worn when handling chloroform, with all handling performed in a chemical fume hood.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar or Biobin) and disposed of in accordance with local regulations.
- Liquid waste needs to be collected in a suitable container (e.g. glass screw-top jar) and disposed of in accordance with local regulations.

Before start

- Set a heat block to 25 °C.
- Ensure that an adequate amount of Sera-Mag™ SpeedBead solution has been prepared prior to initiating the protocol. A volume of 600 to 800 µL is needed per sample, and requires 50% PEG 8000, 10% Tween-20, SpeedBead wash suspension and SpeedBead binding solution to be prepared prior to initiating the protocol (details in the Materials section).
- Remove the prepared Sera-Mag™ SpeedBead solution from the fridge 30 minutes before starting the extraction step so they can be brought to room temperature.
- Remove the AMPure PB beads from the fridge 30 minutes before starting the 0.45X SPRI step to allow them to be brought to room temperature.

Sample Lysis & Precipitation

1 Set a heat block to 25 °C.

2 Label 1.5 mL BioMasher tubes for each sample being processed. Save the BioMasher pestles for later use in their sterile packet, also labelling them for each sample.

3 Transfer the samples into their corresponding 1.5 mL BioMasher tube.

4 Add 350 µL of ML1 buffer and 10 µL Proteinase K solution to each sample.

5 Use the BioMasher pestle to manually disrupt the sample within the buffer. Make sure to retain these pestles for further sample disruption at a later stage by returning them to their labelled sterile packets.

6 Incubate samples for 15 minutes at 25 °C.

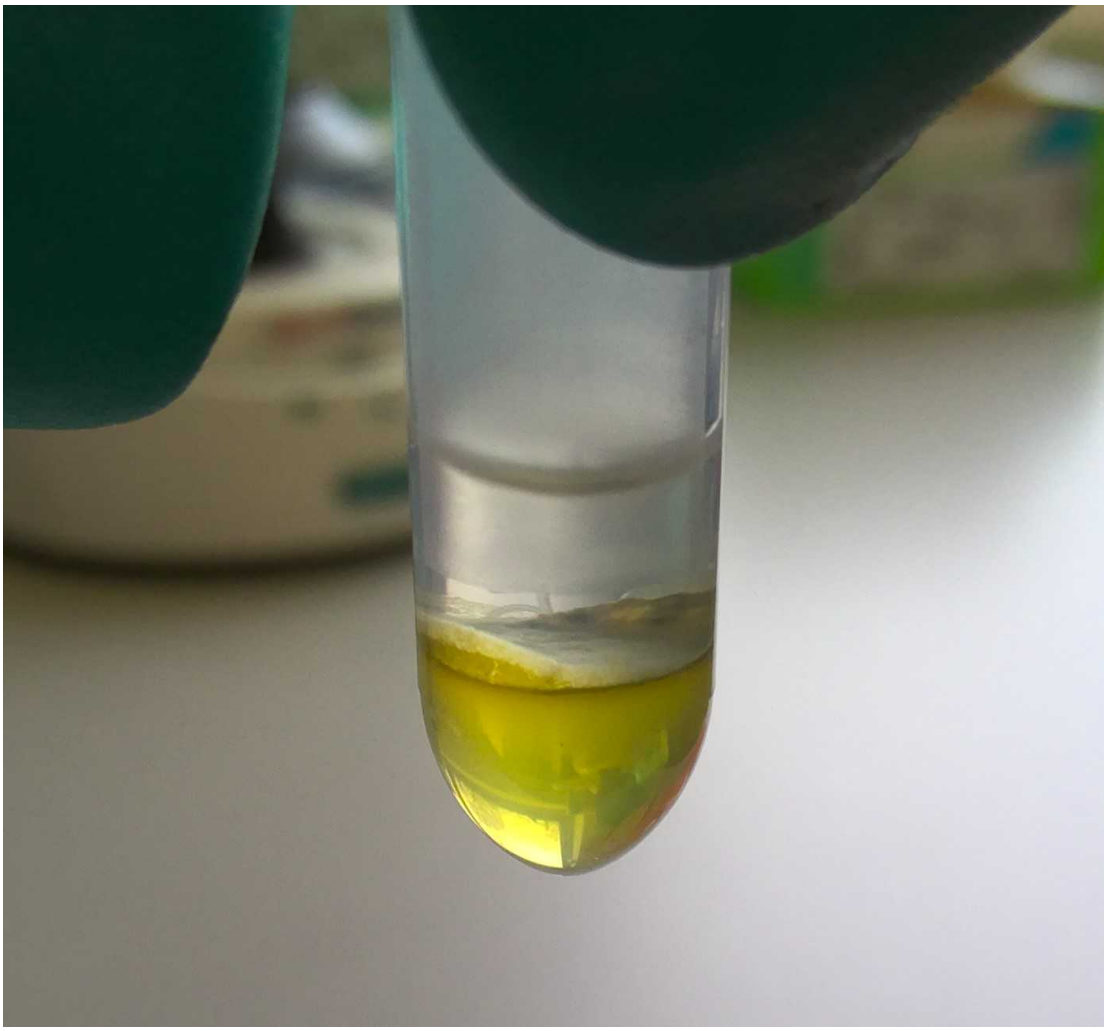
7 Halfway through incubation, return to the samples and manually disrupt any tissue using the BioMasher pestles.

8 After incubation, transfer samples from the 1.5 mL BioMasher tubes to fresh 2 mL microcentrifuge tubes using a wide bore pipette – label these 2 mL microcentrifuge tubes for each sample.

9 Transfer samples to the fume hood for chloroform separation, then add 350 µL of chloroform:isoamyl (24:1) to each sample.

10 Transfer samples to the HulaMixer™, rotating at 8 rpm for 5 minutes.

11 Centrifuge samples at 10,000 *g* for 2 minutes at room temperature. The samples will then have three distinct phases as seen below in Figure 1: an upper aqueous phase containing the gDNA, a milky interphase containing inhibitors and contaminants, and a lower organic phase containing denatured proteins.



Centrifuged sample with three distinct phases.

12 Return samples to the fume hood in order to transfer the upper aqueous phase to a clean 2 mL microcentrifuge tube using a wide bore pipette tip. Avoid transferring the milky interphase containing contaminants and inhibitors.

13 Add 1 X volume of BL buffer and 5 µL RNase A to each sample. For example, if 400 µL aqueous upper phase was recovered in Step 12, add 400 µL of BL buffer. Pipette mix 15 times with a wide bore pipette tip.

14 Incubate samples for 10 minutes at 25 °C.

15 Add 1 x volume 100% ethanol: for example, if 400 µL aqueous upper phase was recovered in Step 12, add 400 µL of 100% ethanol. Transfer samples to the HulaMixer, rotating at 8 rpm for 5 minutes.

Extraction

16 Add 600 to 800 µL Sera-Mag™ SpeedBead solution to each sample - the Sera-Mag™ SpeedBead solution should be vortexed before use to ensure that the beads are resuspended.

17 Invert the sample tubes 10 to 20 times to ensure that beads are suspended in the lysate, then allow at least 5 minutes for binding.

18 Briefly centrifuge the sample in a mini-centrifuge to collect at the bottom of the tubes.

19 Place the tubes on the magnetic rack and allow 2 to 5 minutes for the beads to migrate. Be aware that more viscous samples will take longer. Remove the supernatant and discard.

20 Add 1 mL 80% ethanol opposite the bead pellet, incubate for 30 seconds, then aspirate and discard.

21 Repeat for a total of two washes.

22 Remove the tubes from the magnetic rack and add 200 µL Buffer AE directly to the beads. Mix 15 times using a wide bore pipette tip in order to dislodge the beads from the side of the tube.

23 Incubate samples for 15 minutes at room temperature, with a gentle mix halfway through and again at the end.

24 Briefly centrifuge the samples in a mini-centrifuge and place on a magnetic rack, allowing 2 to 5 minutes for bead migration.

25 Using a 200 µL wide bore pipette tip, carefully transfer the supernatants containing purified gDNA to fresh 1.5 mL microcentrifuge tubes.

26 Remove samples from the magnetic rack and add 200 µL Buffer AE directly to the bead pellet. Incubate samples on the heat block for 3 minutes at 25 °C, shaking at 1,000 rpm.

27 Centrifuge samples briefly in a mini-centrifuge and place on a magnetic rack, allowing 2 to 5 minutes for bead migration.

28 Using a wide bore pipette tip, carefully transfer the supernatants containing purified gDNA to the same 1.5 mL microcentrifuge tubes as in Step 25. Pipette-mix 5 to 10 times to homogenise

the gDNA. The total eluate volume for each sample will now be 400 µL.

0.45X SPRI

29 Add 0.45X AMPure PB beads to the eluates (180 µL of beads for 400 µL eluate). Mix 15 times with a wide bore pipette tip.

30 Incubate samples for 5 minutes at room temperature.

31 Centrifuge samples in a mini-centrifuge for 1 to 2 seconds, then place on a magnetic rack. Allow 2 to 5 minutes for the beads to migrate.

32 Remove and discard the supernatant.

33 Keep samples on the magnetic rack and add 1 mL 80% ethanol. Incubate for 30 seconds and then remove and discard.

34 Repeat for a total of two 80% ethanol washes.

35 Remove samples from the magnetic rack and add 130 µL EB Buffer. Pipette mix 15 times with a wide bore pipette.

36 Incubate samples on a heat block for 15 minutes at 37 °C.

37 Return samples to the magnetic rack and transfer the supernatant containing the purified and SPRI'ed gDNA into a new 1.5 mL microcentrifuge tube.

38 Incubate the DNA at room temperature overnight and perform the required QC the following morning.

39 Store the DNA at 4 °C.

Protocol references

E.Z.N.A.® Mollusc & Insect DNA Kit Protocol: **E.Z.N.A.® Mollusc & Insect DNA Kit - (omegabiotek.com)**

Jackson, B. and Howard, C. (2024) Sanger Tree of Life HMW DNA Extraction: Automated Plant Organic HMW gDNA Extraction (POE)