High quality DNA extraction from Sycon capricorn for MinION long read sequencing

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Abstract

Long-read sequencing (e.g. Nanopore's MinION or PacBio) has the potential to dramatically improve genome assembly but the quality of the reads is critically dependent upon the quality of the input gDNA.

Here I describe the extraction of high molecular weight DNA from the calcareous sponge, *Sycon capricorn*. I choose to use Qiagen RLT buffer in the initial lysis step as this solution results in higher molecular weight DNA compared to alternatives. I suspect that RLT is deactivating endogenous DNases that would otherwise digest the gDNA.

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Before start

Equilibrate phenol:chloroform:isoamyl alcohol to pH 8.0 and allow enough time (several hours) for phases to separate.

Chill absolute and 70% ethanol at 4°C.

Fill the 20 mL syringe with vacuum grease and autoclave.

Materials

✓ Ultrapure Distilled, Nuclease Free Water by Contributed by users

✓ 1.5 mL Eppendorf tubes by Contributed by users

RLT Buffer by Qiagen

✓ Phenol:Chloroform:Isoamyl alcohol (25:24:1) Saturated with 10 mM Tris, pH 8.0 by Contributed by users

- ✓ 3 M Sodium acetate, pH 5.2 by Contributed by users
- ✓ Absolute Ethanol by Contributed by users
- ✓ 70% Ethanol by Contributed by users
- ✓ scalpel blades by Contributed by users
- TissueRuptor by Qiagen
- ✓ 15 mL Falcon tubes by Contributed by users
- ✓ 20 mL syringe by Contributed by users
- High Vacuum Grease by Corning
- ✓ 30 mL glass centrifuge tubes (Corex) by Contributed by users

Protocol

Dessection and (optional) Reproductive Status Assessment

Step 1.

With a scalpel, dissect a thin lengthwise axial slice and transfer to a screwtop vile containing your favorite fixation solution. Incubate the vile overnight at 4°C with shaking and proceed to Step 2. This section can be viewed under a microscope the following day to check for embryos.

Step 2.

If performing hybrid assembly, remove 30 mg of tissue from the apical end containing the osculum and transfer to a 1.5 ml Eppendorf tube. Extract this sample for Illumina sequencing using the Qiagen AllPrep RNA/DNA/protein kit following the kit's protocol and eluting in nuclease-free water.

gDNA Extraction

Step 3.

Weigh remaining tissue.

Step 4.
I make my own phase lock tubes. Feel free to use the pricey pre-made ones. To make your own, add 500 uL sterilised vacuum grease to a 15 mL Falcon tube and centrifuge at 1500 x g for 2 min to pellet the grease. Set aside.

Step 5.
Add 2.5 mL buffer RLT per 1 g of tissue to a separate 15 mL Falcon tube. If the amount of tissue is less than 1 g, add 2.5 mL so that the Tissue Ruptor probe will be fully emersed.

Step 6.
Disrupt the sample with a sterilised disposable TissueRuptor probe on setting 4 for 20-30 seconds or until homogenous and then pour the homogenate into the pre-spun tube containing the vacuum grease.

Step 7.
Add an equal volume equilibrated phenol:chloroform:isoamyl alcohol to the homogenised tissue and mix thoroughly by inversion. DO NOT VORTEX.

Step 8.
Centrifuge at 1500 x g for 5 minutes at room temperature to separate the phases. The grease should form a barrier between the organic and aqueous phases.

Step 9.
Carefully decant (or pipet with a wide bore tip) off the nucleic acid-containing aqueous upper phase to a 30 mL Corex tube.

Step 10.
Add 0.1 volume 3 M NaOAc, wrap with parafilm and mix well by inversion. DO NOT VORTEX.

Step 11.
Add 2 volumes ice cold 100% ethanol, wrap with parafilm, and mix well by inversion. DO NOT VORTEX.

Step 12.
Incubate on ice 30 min.

Step 13.
Centrifuge at 7700g for 30 min in a chilled rotor.

Step 14.
Carefully decant the supernatant and wash the pellet once with ice cold 70% ethanol.

Step 15.
Air dry the pellet just until dry and resuspend in 500 uL nuclease free water, heating gently at 50°C if

necessary.

Step 16.
Transfer to a 1.5 mL Eppendorf tube by pouring or using a wide-bore pipet tip.

Step 17.
Measure your gDNA concentration by Qubit, visualise 200 ng by PFGE, marvel at your high molecular weight DNA.

Step 18.
Happy Sequencing!

Warnings

Carefully read the MSDS for phenol:chloroform and perform all transfers of the solution in a fume hood.