gDNA extraction from Sterivex filters

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Abstract

Contact Dr. Alison Buchan (abuchan@utk.edu) with any questions.

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Protocol

Preparation

Step 1.

Plug the outflow port of Sterivex cartridge using cha-seal clay.

Ensure that incubator is set to 65°C.

Place rotator in the incubator.

REAGENTS

Cha-seal tube sealing compound CSX43510 by Medline

Sterivex™ filter unit without filling bell SVGP01050 by Emd Millipore

Labquake™ Rotisserie Hybridization Rotator M90615Q by Thermo Fisher Scientific

Lysis

Step 2.

Add CTAB extraction solution to each cartridge using a needle and syringe.

AMOUNT

1.7 ml Additional info: CTAB extraction solution

REAGENTS

CTAB extraction solution C2190 by Teknova

3ml syringe BD 309586 by BD Biosciences

22 gauge needle Z192473 by Sigma Aldrich

NOTES

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Dispense liquid slowly and be careful to avoid air bubbles that will clog the port.

Lysis

Step 3.

Add 65µL of Proteinase K (10mg/mL) and 65µL of Lysozyme (10mg/mL) to each cartridge using a needle and syringe.

REAGENTS

Proteinase K EO0491 by Thermo Fisher Scientific

Lysozyme 12671-19-1 by Sigma Aldrich

NOTES

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These may be combined and added to each tube in a single aliquot.

Lysis

Step 4.

Add 162µL of filter-sterilized SDS (10%) to each tube. Invert to mix.

REAGENTS

SDS, 10% Solution AM9822 by Life Technologies

Lysis

Step 5.

Cap open end of each cartridge with a luer lock cap and incubate in rotary agitator at 65°C for 2 hours.

Lysis

Step 6.

Label 2 sets of 2mL centrifuge tubes with sample name. One set will be kept and frozen (-20°C) while the other set will be used for further extraction.

Lysis

Step 7.

Using 3mL luer-lock syringe, attach to Sterivex cartridge and draw out fluid.

Depress 2mL of sample material into the first storage 2mL centrifuge tube, then pipette out 800µL of this solution into other labeled 2mL tube for further extraction.

Place the 1st 2mL tube into the freezer.

Start the cool-down sequence in the microcentrifuge (set to 4°C).

Precipitation

Step 8.

Add 800µL of phenol:chloroform:isoamyl alcohol (PCI, 25:24:1, pH 8.0) to each tube and vortex to mix.

AMOUNT

800 µl Additional info: P:C:I

REAGENTS

UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) 15593031 by Thermo Fisher Scientific

NOTES

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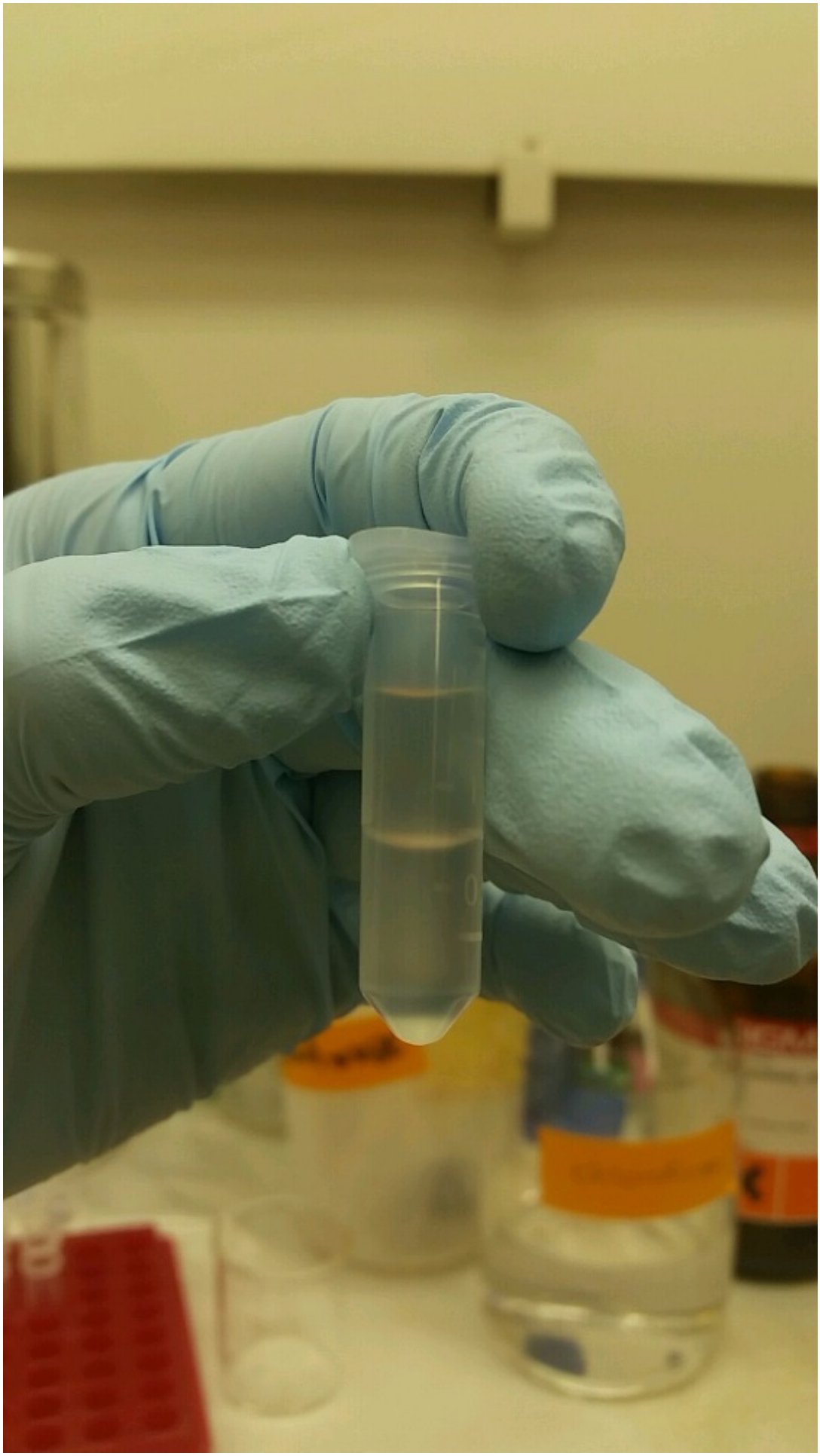
Do this step in a fume hood.

Precipitation

Step 9.

Centrifuge at 4°C, 10,000rpm for 5 minutes.

Transfer upper aqueous phase to a new 2mL centrifuge tube.



NOTES

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Be careful to not aspirate the interface between the aqueous phases. Do not attempt to remove all of the upper aqueous phase.

Precipitation

Step 10.

Add 800µL of chloroform:isoamyl alcohol (CI, 24:1) to each tube.

Invert and vortex to mix thoroughly, then centrifuge at 15,000rpm for 5 minutes.

Transfer upper aqueous phase to a new 2mL centrifuge tube.

AMOUNT

800 µl Additional info: C:I

REAGENTS

Chloroform:Isoamyl alcohol 24:1 C0549 by Sigma Aldrich

NOTES

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Repeat this step again. The second time, transfer the upper aqueous phase to a 1.5mL centrifuge tube.

Precipitation

Step 11.

Add 450µL of room temperature isopropanol (100%) to each tube, inverting gently to mix.

Incubate at room temperature for 2 hours to overnight.

AMOUNT

450 µl Additional info: Isopropanol

REAGENTS

Isopropanol by Contributed by users

Precipitation

Step 12.

Centrifuge the tubes at 10,000rpm for 20 minutes.

Carefully decant by pouring liquid out of the tube and into a small clean petri dish, then blot tubes dry using a paper towel on the benchtop. You can also aspirate the liquid using a pipette.

NOTES

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The petri dish serves to catch the DNA pellet if it is accidentally poured out during this step.

Purification

Step 13.

Add 1.4mL of 70% ethanol to each tube and gently invert several times to mix.

Centrifuge at 10,000rpm for 5 minutes.

Pour off ethanol.

AMOUNT

1.4 ml Additional info: ethanol

REAGENTS

Ethanol by Contributed by users

Purification

Step 14.

Dry tubes in laminar hood for 10-15 minutes or until completely dry. Note that pellets may become dislodged from sides of tube, so take care not to invert tubes.

Purification

Step 15.

Add 50µL of sterile Nuclease free ultra-pure water to each tube and gently pipet to dissolve DNA.

AMOUNT

50 µl Additional info: nuclease free water

REAGENTS

Ultrapure Distilled, Nuclease Free Water by Contributed by users

NOTES

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It may be helpful to either pre-heat the water to 50°C or to incubate the tubes at 37°C for 1-2 hrs to facilitate dissolution.

Purification

Step 16.

Measure the DNA concentration using NanoDrop and freeze samples at -80°C. Record 260/280 ratio as well as DNA concentration.

REAGENTS

NanoDrop spectrophotometer ND-1000 by Thermo Fisher Scientific

NOTES

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A 260/280 ratio of 1.8-2 is considered "pure" DNA.