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gDNA extraction from Sterivex filters

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Abstract

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

Attachments



DNAextractionfromSte...

14KB

Materials

STEP MATERIALS

-  Cha-seal tube sealing compound **Medline Catalog #CSX43510**
-  Sterivex™ filter unit without filling bell **Merck Millipore (EMD Millipore) Catalog #SVGP01050**
-  Labquake™ Rotisserie Hybridization Rotator **Thermo Fisher Scientific Catalog #M90615Q**
-  CTAB extraction solution **Teknova Catalog #C2190**
-  3ml syringe **Becton Dickinson (BD) Catalog #BD 309586**
-  22 gauge needle **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z192473**
-  Proteinase K **Thermo Fisher Scientific Catalog #EO0491**
-  Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**
-  SDS, 10% Solution **Life Technologies Catalog #AM9822**
-  UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) **Thermo Fisher Scientific Catalog #15593031**
-  Chloroform:Isoamyl alcohol 24:1 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0549**
-  Isopropanol
-  Ethanol
-  Ultrapure Distilled, Nuclease Free Water
-  NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #ND-1000**
-  Cha-seal tube sealing compound **Medline Catalog #CSX43510**
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-  Isopropanol
-  Ethanol
-  Ultrapure Distilled, Nuclease Free Water



 NanoDrop spectrophotometer Thermo Fisher Scientific Catalog #ND-1000

Protocol materials


- ✕ NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #ND-1000**
- ✕ Isopropanol
- ✕ Ethanol
- ✕ SDS, 10% Solution **Life Technologies Catalog #AM9822**
- ✕ Proteinase K **Thermo Fisher Scientific Catalog #EO0491**
- ✕ Ethanol
- ✕ Cha-seal tube sealing compound **Medline Catalog #CSX43510**
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- ✕ Cha-seal tube sealing compound **Medline Catalog #CSX43510**
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- ✕ Chloroform:Isoamyl alcohol 24:1 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0549**
- ✕ Isopropanol
- ✕ Ethanol
- ✕ Ultrapure Distilled, Nuclease Free Water
- ✕ NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #ND-1000**

Preparation

- 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.

 Cha-seal tube sealing compound **Medline Catalog #CSX43510**

 Sterivex™ filter unit without filling bell **Merck Millipore (EMD Millipore) Catalog #SVGP01050**

 Labquake™ Rotisserie Hybridization Rotator **Thermo Fisher Scientific Catalog #M90615Q**


Lysis

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

 1.7 mL CTAB extraction solution

Note

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

 CTAB extraction solution **Teknova Catalog #C2190**

 3ml syringe **Becton Dickinson (BD) Catalog #BD 309586**

 22 gauge needle **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z192473**

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

Note

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

 Proteinase K **Thermo Fisher Scientific Catalog #EO0491**

 Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**

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

 SDS, 10% Solution **Life Technologies Catalog #AM9822**

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



02:00:00 incubation

- 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.
- 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

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

800 µL P:C:I

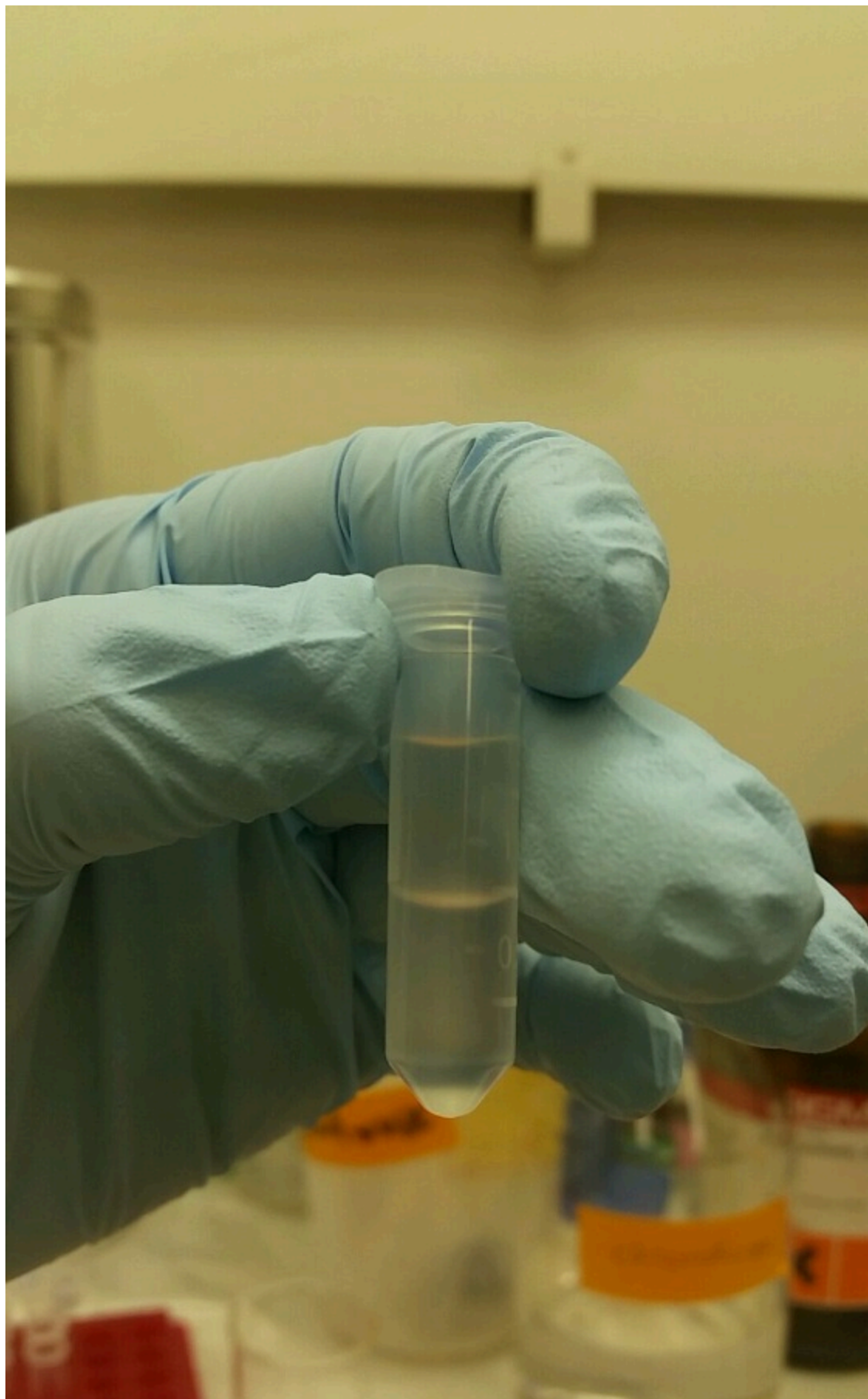
Note

Do this step in a fume hood.



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

- 9 Centrifuge at 4°C, 10,000rpm for 5 minutes. Transfer upper aqueous phase to a new 2mL centrifuge tube.






Note


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

- 10 Add 800 μ L 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.

 800 μ L C:I

Note


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

 Chloroform:Isoamyl alcohol 24:1 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0549**

- 11 Add 450 μ L of room temperature isopropanol (100%) to each tube, inverting gently to mix. Incubate at room temperature for 2 hours to overnight.

 450 μ L Isopropanol

 02:00:00 Incubation

 Isopropanol

- 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.

Note


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

Purification




- 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.

 1.4 mL ethanol

 Ethanol


- 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.

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

 50 μ L nuclease free water

Note

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.

 Ultrapure Distilled, Nuclease Free Water

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

Note

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

 NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #ND-1000**