DNA extraction - open filter, full lysis with bead-beating

This protocol will cut open the filters, then follow our usual extraction method that we use with the Pall filters.

Preparation

- Prepare 2XS buffer
    - 0.2g xanthogenate
    - 2.0ml 1M Tris
    - 0.8ml 0.5M EDTA
    - 2.0ml 10% SDS
    - 2.0ml 7.5M AmAc
    - 3.2ml ddH2O
- Prepare lysozyme and protease mixes.
    - Lysozyme mix (per sample)
        - 215ul TE
        - 25ul lysozyme (10mg/L)
        - 10ul RNase A
    - Protease mix (per sample)
        - 225ul 2XS
        - 25ul proteinase (10mg/L)
- Place a bowl of ethanol, tweezers, razor blades, and a bunsen burner in a laminar flow hood.
- Sterilize one petri dish for each sample using UV box.

Part one Remove filter from cartridge

- Thaw Sterivex filters
- Sterilize PVC cutter over open flame.
- Cut open the filter cartridge with the PVC cutter and remove filter holder.
- Sterilize two tweezers and razor blade by ethanol flaming.
- Use razor blade to cut filter along ridge where it's held to the holder.
- Gently peel the filter off of the holder using the two tweezers.
- Place filter in the sterile petri dish, and cut into two pieces.
- Add one piece to a bead-beating tube, to be used for the subsequent extraction. The other piece will be added to a screw-top centrifuge tube and stored in the -80 freezer for later use.

Part two Cell lysis

- Add 250ul of lysozyme mix.
- Bead-beat for 60 sec on 2.5 intensity.
- Spin down tubes for 30 seconds.
- Incubate at room temp for 15 minutes.

- Add 250ul of protease mix.
- Bead-beat for 30 sec on 2.5 intensity.
- Spin down tubes for 30 seconds.
- Incubate at room temp for 10 minutes.
- Add 190ul of 5M NaCl. Vortex.
- Add 100ul of 10% CTAB and 10μl of 10% PVPP. Vortex and incubate at 65C for 20 minutes.

Part three DNA extraction

- Add 750ul phenol:chloroform:IAA. Invert repeatedly for 2-3 minutes.
- Spin down at 13000g for 5 minutes.
- Pipet off the aqueous layer, add to new Eppendorf tube.
- Add 750ul chloroform. Invert repeatedly for 2 minutes.
- Spin down at 13000g for 5 minutes.
- Pipet off the aqueous layer, add to new Eppendorf tube.

Part four DNA purification

- Add 750ul of 100% isopropanol. Invert and incubate at room temp for 2 hours.
- Spin down at 13000g for 15 minutes.
- Pour off supernatent.
- Add 1ml of room-temperature 70% ethanol. Invert several times.
- Spin down at 13000g for 15 minutes.
- Pour off supernatent. Try to remove as much of it as possible without disturbing the DNA pellet.
- Let the DNA pellet dry.
- Resuspend the pellet in 50μl nuclease-free water. Gently move the pellet off the walls and incubate at 50˚C for 5 minutes.

Part five Check extraction

- Quantify on the Qubit.
- Check purity on the Nanodrop
- Run a PCR test on the extracted DNA to make sure it works.