DNA ISOLATION FROM NASAL WASHES

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BACKGROUND

- This protocol is used to isolate the DNA from samples such as nasal washes from mice, sputum, BAL fluid or other viscous samples. It is important to avoid any external DNA from entering the samples.

NOTES

- Be sure to keep all contents closed when they are not in use
- Use filter tips throughout the entire procedure
- Complete the procedure by a flame to create a zone of sterility
- For all steps involving water: use ultra pure distilled water (sterile DNase/ RNase free water)
- All steps involving PCl should be completed under a fumehood

EQUIPMENT

- Equipment:
  - 2ml plastic screw top tube with 0.2g of 0.1mm beads
  - Bead beater
  - 37°C and 65°C water bath
  - 1.5ml and 2ml eppendorf tubes
  - Vortex
  - Zymo DNA clean and concentrator 25-kit
  - Nanodrop
- Reagents:
  - GES
  - 200mM $NaPO_4$ (pH8)
  - Lysozyme (100mg/ml in H20)
  - Mutanolysin (10U/ul)
  - RNase A (10mg/ml in H20)
  - 25% SDS
  - Proteinase K
  - 5M NaCl
  - Phenol-chloroform-isoamyl alcohol (PCl)

PROTOCOLS

Pre-work:

A. Make GES (recipe per 100ml)
  - 60g of guanidine thiocyanat
  - 20ml 0.5M EDTA (pH8)
  - 20ml sterile ddH20
  - Heat to 65°C, cool to room temperature
  - Add 1g N-lauroyl sarkosine
  - Adjust to 100ml and filter sterilize
  - Store at room temperature

B. Make PCl
    - Make one day in advance in order to allow the layers to settle out
    - Add 25:24:1 phenol-chloroform-isoamyl alcohol
    - Use only the bottom layer of this solution
    - Keep PCl in a fumehood covered in foil
    - PCl should be discarded if it turns into a dark pink colour or appears to have stringy substances floating in it

Extractions:

Part 1: Bead Beating

1. Add 800ul of $NaPO_4$ and 100ul of GES to the plastic screw top tube.
2. Add up to 300ul of sample. (You can add less than 300ul but if you add more, it will overflow during the later steps of the extraction)
3. Homogenize samples for 3min at 2500rpm using a bead beater.

Part 2: Enzymatic Lysis- Part I

*Make a master mix of the following reagents and add 110ul to each sample.

4. Add 50ul of lysozyme.
5. Add 50ul of mutanolysin.
6. Add 10ul of RNase A.
7. Mix by vortexing for five seconds.
8. Incubate in a 37°C waterbath for 1-1.5 hours.

During wait time:

- Label a set of 2ml eppendorf tubes for step 15 and add 900ul of PCl (under fumehood)
- Label a set of 1.5ml tubes for step 18 and add 200ul of DNA binding buffer (found in Zymo kit)
- Label a set of 1.5ml tubes for step 21 and leave empty (this will contain the final DNA product)

Part 3: Enzymatic Lysis- Part II

*Make a master mix of SDS and proteinase K. Add in the NaCl separately to prevent it from precipitating.

9. Add 25ul of SDS.
10. Add 25ul of Proteinase K.
11. Add 100ul of NaCl.
12. Vortex samples for five seconds
13. Incubate in a 65°C waterbath for 0.5-1.5 hours.

Part 4:

14. Spin screwcap tubes at max speed for 5 minutes
15. Remove 900ul of supernatant and transfer to a 2ml tube (containing the PCl). (under fumehood)
16. Vortex for 10 seconds to shear DNA.
17. Vortex and spin at max speed for 10 minutes.
18. Carefully transfer the top layer into a 1.5ml tube containing the DNA binding buffer (under fumehood).
19. Transfer sample to a DNA column (found in the Zymo kit) 500-600ul at a time. Spin at high speed for 1min and discard flow-through.
20. Wash sample 2-3 times.
    a. Add 200ul of wash buffer to the column.
    b. Spin at high speed for 1min.
    c. Discard flow through.
21. Elute DNA in 50ul of sterile DNase/ RNase free ddH20.

a. Remove filter column and place in the previously labeled empty 1.5ml tube.
b. Add 50ul of ddH20 and leave to set for ≈10mins.
c. Spin at max for 1min.
d. Do NOT decant this. This is your DNA product.

22. Use a nanodrop to quantify DNA.

Using the nanodrop:

- Set to nucleic acid
- Type: DNA
- Blank before starting samples
- Graph produced should have a peak around 260 and 280
- 260/280 value should be around 1.7 (the higher the value, the higher the purity is of your template)