

# DNA Extraction from Vaginal Swabs

Using QIAamp DNA stool mini kit (51504)

Modified protocol (A. McMillan)

## Prep:

- Mix buffers well before use
- If buffer ASL or AL have precipitate formation, incubate at 70°C to dissolve. (Jean's note: put in 55°C waterbath to dissolve)
- Prep 95°C sand
- All centrifugation should be at 20,000 *xg* or use 10,000 *xg* and double time (eg.)

## Caution

- Buffers ASL and AL contain guanidine hydrochloride. Do not bleach or add acidic solutions

## Protocol

1. Suspend swab(s) in 1mL ASL Buffer. Vortex 1min. Remove swab (?)
  - a. Modification: If swab already in 800uL PBS, add 200uL-600uL ASL.
2. Add liquid to 200mg sterile (autoclaved) beads in 2mL screw-cap tubes. Bead beat 2x 30s (max speed)
3. Heat suspension for 5min at 95°C.
4. Vortex 15s. Centrifuge 1min at 14000rpm.
5. Pipette supernatant into a new 2mL tube. Discard pellet.
6. Add HALF InhibitEX tablet to each sample. Vortex for 1min until tablet is suspended. Incubate 1min at room temperature.
7. Centrifuge 4min at 14000rpm to pellet inhibitors.
8. Pipette all supernatant into a new 1.5mL tube. Discard pellet. Centrifuge 3min at 14000rpm.
9. Pipette 15uL Proteinase K into a new 1.5mL tube.
10. Add 200uL from Step 8 to tube containing Proteinase K.
11. Add 200uL Buffer AL. Vortex 15s.
12. Incubate at 70°C for 10min.
13. Add 200uL 96-100% ethanol to lysate and vortex (centrifuge briefly to remove droplets from lid before opening tubes)
14. Apply lysate to QIAamp spin column membrane in a 2mL tube. Don't moisten rim of column.
15. Centrifuge 1min at 14000rpm.
16. Transfer column to new 2mL tube. Add 500uL Buffer AW1. Centrifuge 1min at 14000rpm.
17. Transfer column to new 2mL tube. Add 500uL Buffer AW2. Centrifuge 3min at 14000rpm.
18. Empty collection tube and put column back in. Centrifuge 1min at 14000rpm.
19. Transfer column to new 1.5mL tube. Add 200uL Buffer AE. Incubate at room temperature for 1min. Centrifuge 1min at 14000rpm.
20. Quantify and store at  $\leq -20^{\circ}\text{C}$  until further analysis.