DNA Extraction Protocol using DNAzol Reagent[i]
Optimized for Swab Sampling Method

Materials Needed [See end of protocol for all recipes]

- 1x TBS [ii]
- 0.010 M EDTA [iii]
- 1 L Autoclaved NanoPure H_2O
- Tween-20 [iv]
- DNAzol reagent [v] (stored in chemical hood)
- 100% ethanol (Cold; -20°C)
- 75% ethanol (Cold; -20°C)
- 3M NaOAc (Sodium Acetate) [vi]
- 8 mM NaOH
- 1 M HEPES

Equipment Needed

- Trimming Utensil
- 2 mL screw cap tubes for homogenization
- 1 mm zirconia beads for homogenization
- 2 mL microcentrifuge tubes
- Test Tube Rack
- Microcentrifuge Tube Rack
- Microcentrifuge Tube Rack (Cooled to -20°C)
- Refrigerated Microcentrifuge (FastCool to 4°C)
- Microcentrifuge (Room Temperature)

Swab Preparation

1. Remove swabs from -80°C freezer and place in test tube rack at RT
    a. This will allow the swabs to defrost while the TEt Buffer Solution is prepared

Extraction Medium Preparation (TEt Buffer Solution)

2. Extraction medium components are as follows (per swab sample):
    a. 202 µL 1x TBS
    b. 202 µL 0.01 M EDTA
    c. 2 µL of Tween-20
3. Sample Calculations

# of Samples	**6**	**8**	**10**	**12**
Total Vol. 1x TBS	1212 µL	1616 µL	2020 µL	2424 µL
Total Vol. 0.01 M EDTA	1212 µL	1212 µL	2020 µL	2424 µL
Total Vol. Tween-20	12 µL	16 µL	20 µL	24 µL
Total Volume of TEt Buffer Solution	**2436 µL**	**3248 µL**	**4060 µL**	**4872 µL**
Container	**5 mL Conical Centrifuge Tube**			

4. Combine total volumes of 1x TBS, 0.01 M EDTA, and Tween-20 into the appropriate size container
    a. Vortex on high setting until the solution mixes together (approx. 30 seconds)
    b. Set aside for use in the Sample Homogenization process

Sample Preparation

5. Fill 2 ml screw cap tubes quarter full with **1mm zirconia beads**[vii]
6. Label tubes (on cap & sides)

7. Insert the swab tip into the bottom of the beads
8. Trim the swab so that it is the same height as the screw cap homogenization tube
    a. Clean the trimming utensil in between samples with 70% ethanol and KimWipes
9. Add **400 µL of TEt extraction medium** into each tube
    a. Vortex each capped screw cap tube on setting 6 for 10 seconds to allow the TE extraction medium and the swab to be fully saturated with the buffer
    b. Verify that the entire swab tip is submerged in TEt extraction buffer
10. Let the swabs sit in the extraction medium for 10 minutes at room temperature

<u>Sample Homogenization</u>

11. Add **1 ml DNAzol reagent** to tubes
12. Place tubes in homogenizer (BeadBeater 96), ensure layout is balanced
13. Homogenize swab samples for 60 sec at 1500 rpm
    a. The solution will be very foamy (full of air bubbles)
14. Wait 10 minutes
15. Remove and discard the swab tip from the screw cap tube using forceps
    a. Clean forceps in between each sample with 70% Ethanol and KimWipes
16. Transfer supernatant and foam to a new tube
17. Centrifuge at 10,000 x g for 10 minutes at 4°C
18. Transfer supernatant to a new tube

<u>DNA Pellet Precipitation</u>

19. Add **0.5 ml 100% COLD ethanol**
20. Invert the tubes several times to mix the ethanol into the solution (do not vortex!)
21. Keep tubes on ice until step 43
22. Incubate 3 minutes on ice
    a. DNA precipitate will most likely not be visible at this step
23. Centrifuge at 10,000 x g for 15 minutes at 4°C
    a. Ensure the microcentrifuge tube stem is pointing outward, this will help standardize where the DNA precipitate accumulates in the tube
24. Pipet off the supernatant and place in DNAzol/NaOAc extraction waste bottle (stored in chemical hood)
    a. Be careful! The DNA precipitate will be very small, stringy, and in line with the tube stem.
    b. When removing the supernatant, be careful around the tip of the microcentrifuge tube to not pipette off the DNA precipitate

<u>Primary DNA Pellet Wash</u>

25. Add **1 ml 75% COLD ethanol** to DNA pellet
26. Wash DNA by gently inverting tube 5-6 times
27. Let tubes stand upright for 1 min to allow DNA pellet to settle to bottom of tube
28. Centrifuge at 7,000 x g for 6 minutes at 4°C
29. Pipet off the supernatant and place in DNAzol/NaOAc extraction waste bottle
30. Repeat wash step once more (steps 25 through 29)
31. Suspend the DNA pellet in 300 µL of 8mM Sodium Hydroxide (NaOH)

Purification of DNA by Sodium Acetate and Ethanol Precipitation[viii]

32. Add 600 µL of 100% cold ethanol to the suspended DNA solution
33. Add 30 µL of 3M Sodium Acetate (NaOAc)
34. Mix by quickly inverting several times until well combined.
    a. You may see a "cloudy" or "cotton-ball-like" precipitate begin to form at this point
35. Put the tubes at -20°C for 60 minutes to overnight to facilitate precipitation
36. Centrifuge at 13,000 x g for 15 minutes at 4°C
37. Pipet off the supernatant and discard in DNAzol/NaOAc extraction waste bottle
    a. Be careful! The DNA pellet may be very small, stringy, and in line with the tube stem.
    b. If DNA pellet is not visible, assume it is where you expect it to be

Secondary DNA Pellet Wash

38. Add 500 µL of 70% cold ethanol
    a. You may wish to try and dislodge the pellet by aiming directly above the pellet, and expelling forcefully
39. Invert several times to thoroughly wash
40. Centrifuge at 13,000 x g for 5 minutes at 4°C
41. Pipet off the supernatant and discard in DNAzol/NaOAc extraction waste bottle
42. Air dry DNA by inverting tube and tapping out the ethanol drops
    a. Do not air dry for more than 1 min, since it will be difficult to resuspend if exposed to air any longer

DNA Resuspension

43. Resuspend DNA pellet in **0.1 ml to 0.3 ml (100 µl to 300 µl) 8 mM NaOH** [ix]
44. Dissolve the DNA pellet by pipetting up and down
    a. If the DNA pellet does not dissolve, place tubes in refrigerator overnight. The next day, mix DNA solution by pipetting up and down, ensuring that everything is dissolved, before proceeding to step 45
45. Centrifuge at 12,000 x g for 10 minutes at RT to remove insoluble materials
46. Transfer supernatant to a new tube
47. Adjust the pH to neutral range (pH 7-8) by adding **1 M HEPES** (free acid) solution at the appropriate volume (0.023 µl 1 M HEPES per 1 µl sample)
    a. Stored at 4C

Sample Volume	1 M HEPES Volume
100 µl	2.3 µl
200 µl	4.6 µl
300 µl	7 µl
400 µl	9 µl
500 µl	11.5 µl

48. Vortex briefly to mix
49. Measure DNA concentration on the NanoDrop

Solutions

Autoclaved H_2O (1 L, Stock Liquid)

- Add 1 L NanoPure Water to clean 1 L bottle
- Autoclave on Liquid Cycle
- Store at RT; store indefinitely

1x TBS Solution (1 Liter, Stock Solution)

- Autoclave empty 1 L bottle
- 8 g NaCl
- 0.2 g KCl
- 3 g Tris Base
- 800 mL NanoPure water
- After the solution becomes clear, adjust pH to 7.4 with HCl.
- Fill to final volume of 1 liter with NanoPure water
- Autoclave
- Store at RT; store indefinitely

0.5 M EDTA, pH 8.0 (1 Liter, Stock Solution)

- Autoclave empty 1 L bottle
- 186.12 g EDTA (EDTA disodium salt dehydrate; MW = 372.24)
- 700 mL NanoPure water
- Adjust pH to 8.0 (ca. 20 g NaOH pellets & 5 mL 5N NaOH solution)
- Adjust volume to 1000 mL with NanoPure water
- Autoclave
- Store at RT; stable for 1 year

0.01 M EDTA (0.5 Liter, Working Solution)

- Autoclave empty 0.5 L bottle
- (0.010 M / 0.5 M) x 500 mL = 10 mL
- Add 10 mL of 0.5 M EDTA solution to 490 mL autoclaved NanoPure water
- Store at RT
- Stable until 0.5 M EDTA Stock Solution expires

100% Ethanol (45 mL, Stock Liquid)

- 45 mL of 100% ethanol
- store at -20°C; store indefinitely

75% Ethanol (45 mL, Working Solution)

- (75% / 100%) x 45 ml = 33.75 ml of 100% ethanol
- Add 33.75 mL of 100% ethanol to 11.25 ml NF-water
- store at -20°C; store indefinitely

3 M NaOAc (50 mL, Stock Solution)

- 20.42 g NaOAc in 35 mL nuclease-free water (NF-water)
  - FW of NaOAc: 136.1 g/mole
- Adjust the pH to 5.2 by adding glacial acetic acid
- Bring the final volume to 50 mL with nuclease-free water (NF-water)
- store at 4°C; store indefinitely

2 M NaOH (50 mL, Stock Solution)

- 4 g NaOH in 40 mL nuclease-free water (NF-water)
  - FW of NaOH: 40 g/mole
- Adjust volume to 50 mL with nuclease-free water (NF-water)
- store at RT; stable for 6 months

8 mM NaOH (50 mL, Working Solution)

- 8 mM / 2000 mM x 50 ml = 0.2 ml or 200 μl
- 200 μl of 2 M NaOH solution in 49.8 ml NF-water
- store at RT; stable for 1 month

1 M HEPES (50 mL, Stock Solution)

- Dissolve 11.92 g HEPES (free acid) in 35 ml deionized water (in beaker with stir bar)
  - FW: 238 g/mole
- Adjust the pH to 7.0 using NaOH pellets and/or concentrated NaOH solution
- Fill to final volume of 50 ml with deionized water
- Filter sterilize
- Store at 4°C; stable for up to six months

---

[i] Protocol adapted from DNAzol Reagent product sheet (Invitrogen)

[ii] TBS recipe from Cold Springs Harbor Laboratory Press – Molecular Cloning A Laboratory Manual On The Web (PDF) on page 552

[iii] 0.5 M EDTA recipe from how to make home-made RNA Later Solution on Igloo

[iv] Tween-20 (CAS #: 9005-64-5; Sigma-Aldrich; purchased through BioBio)

[v] DNAzol (Catalog #: 10503-027 – Invitrogen, Life Technologies, Thermo Fisher Scientific)

[vi] 3M Sodium Acetate recipe from Cold Springs Harbor Laboratory Press – Molecular Cloning A Laboratory Manual On The Web (PDF) on page 62

[vii] BioSpec Products (Cat # 11079124zx bought through Fisher Scientific)

[viii] DNAzol Protocol modified by addition of Sodium Acetate Purification. Protocol from following link:https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwjW44fN5KfWAhWhv1QKHQr-B44QFggzMAA&url=http%3A%2F%2Fbio.classes.ucsc.edu%2Fbio121l%2Fprotocols%2520ForBio121L-2011%2FPurification%2520of%2520Genomic%2520DNA%2520by%2520Ethanol%2520Precipitation.doc&usg=AFQjCNFTrFc5pK9o7ZyqQC1CQUh09tNOvg

[ix] Weak alkaline solutions are neutralized by CO2 from the air. The 8 mM NaOH should be prepared fresh once a month, from a 2-4 M NaOH stock solution that is less than six months old.