PCI EXTRACTION OF GENOMIC DNA FROM TISSUE OR BLOOD

NOTE: Protocol must be carried out in a fume hood. Read the phenol and chloroform SOP documents before working with these hazardous chemicals. Protocol must not be undertaken without proper training in the handling and disposal of these chemicals in the Wayne Lab.

Necessary Reagents and Materials:

Reagent	Amount per Sample
1x TNE buffer (recipe below)	500 µl
10% sodium dodecyl sulfate solution (SDS) (recipe below)	50 µl
Proteinase K solution (20 mg/ml) (stored in -20°C freezer)	40 µl
100% ethanol (EtOH)	1 ml
70% ethanol (must be made fresh)	1 ml
Buffered phenol, pH 8 (stored in Cold Room)	750 µl
Chloroform (stored in chemical fume hood)	~ 750 µl
Iso-amyl alcohol (stored in chemical fume hood)	~ 30 µl
3M sodium acetate (NaOAc)	100 µl
1x TE buffer	100-200 µl (varies)
For tissue extraction: PBS buffer	Varies

Consumables:
2 ml low-retention tubes with attached caps
Pipet tips (particularly 1000 µl)
Petri dish, razors, tweezers, PBS buffer, etc. for obtaining and mincing tissue
15 and 50 ml polypropylene Falcon tubes with screw caps
Gloves (nitrile is not advised)
Bench diaper
Glass container with screw cap for liquid waste
Large re-sealable plastic bag for solid waste

Equipment:
Centrifuge
Refrigerated centrifuge
Incubator
Tube rotator
Pipets
Tube rack

Digestion of Tissue

1. Turn on incubator and set for 56°C.
2. Finely mince ~25 mg (0.5-1.0 cm^3) of tissue, or add 200 µl of blood to a 2.0 ml low-retention tube.
3. Add the following reagents to the tube:

   500 µl of 1x TNE buffer
   50 µl of 10% SDS
   40 µl of Proteinase K (20 mg/ml)

4. Mix by pulse vortexing for 15 seconds.
5. For tissue extraction: incubate at 56°C overnight on a rocking platform. For blood extraction: incubate at 56°C for 1 hour on a rocking platform.

PCI Extraction

Preparation:

Put 100% Ethanol in freezer to chill.

If necessary, obtain a new LABELED hazardous waste container for the solid and/or liquid waste. All consumable solid materials used in this protocol must be disposed as solid hazardous waste. All liquid waste must be disposed as hazardous liquid waste.

Always work with phenol and/or chloroform in a fume hood. Lay down a bench diaper to catch any errant drops or spills in the fume hood working area.

Make the chloroform-iso-amyl alcohol mixture (recipe at the bottom). Be sure to prepare this in a polypropylene tube with a screw cap. Use serological pipets to transfer the chloroform. This mixture must be made up immediately before it is to be used, and any remainder must be discarded in the hazardous liquid waste container at the end of use that same day. Chloroform dissolves plastic, so it can only be contained in a disposable plastic tube for a short time. It is recommended to keep the 15ml tube in a secondary 50 ml tube in case the plastic dissolves. Do not put chloroform in a polystyrene tube, only use polypropylene.

NOTE: Phenol can dissolve nitrile and latex gloves. Change gloves often during protocol (dispose of gloves as solid waste), and change immediately if any phenol and/or chloroform comes into contact with gloves.

Procedure:

1. Remove tubes from incubator, and add 500 µl of buffered phenol (bottom layer in the container).
2. Shake tubes and invert for 5 mins. Spin tubes in centrifuge at max speed (14,000 rpms) for 5 mins.
3. Observe 2 layers. The top layer contains the DNA/RNA and the bottom layer contains the organic waste.
4. Remove the top layer (avoid the bottom layer entirely) and transfer into a new labeled tube.
5. Discard the bottom layer in the liquid waste container.
6. Add 500 µl of PCI (250 µl phenol + 250 µl CI) to the samples in the new tubes, shake tubes and invert for 5 mins. Spin tubes in centrifuge at max speed (14,000 rpms) for 5 mins.
7. As in steps 3-5, Remove the top layer and transfer into a new labeled tube, and discard the bottom layer in the liquid waste container.
8. Add 500 µl of CI to these new tubes, and shake tubes and invert for 5 mins. Spin tubes in centrifuge at max speed (14,000 rpms) for 5 mins.
9. Pipette off the top layer and transfer it to a new tube.
10. To this tube add the following:

    100 µl of 3M sodium acetate (NaOAc)
    1 ml of cold 100% ethanol (EtOH)

11. Invert tubes several times and place in a -20 freezer for 1 hour (or longer, if desired – can be left overnight if necessary).

<u>Ethanol Precipitation</u>

1. Turn on refrigerated centrifuge and set to 4°C.
2. When centrifuge has cooled to 4°C, remove tubes from the freezer, and centrifuge for 30 mins at max speed (14,000 rpms) to pellet the DNA.
3. Observe a white or brownish pellet (may be absent in low concentration samples).
4. Decant the EtOH-NaOAc into liquid waste container (or a secondary tube in case pellet is dislodged), being careful not to lose the pellet.
5. Add 1ml of fresh 70% EtOH to the tube containing the pellet. Vortex briefly to re-suspend the pellet.
6. Centrifuge in cooled centrifuge for 15 mins at max speed (14,000 rpms) to pellet the DNA.
7. Decant the EtOH, being carefµl not to lose the pellet.
8. Remove the remaining 70% EtOH by vacuum centrifuging in the tubes in the Savant Speed-Vac for ~10 mins or until the pellet is dry (do not over-heat or over-dry the pellet as this will prevent DNA from going into solution)
9. Re-suspend DNA in 100-200 µl 1x TE (or Qiagen's AE) buffer – amount can be adjusted as needed for higher/lower concentration.

Reagent Recipes

1X TNE Buffer (50 ml)

0.5M EDTA	100 µl
5M NaCl	1000 µl
1M TRIS	500 µl
ddH_2O	48.4 ml

10% SDS (10ml)

1g Sodium Dodecyl Sulfate

Add distilled H_2O to bring volume up to 10ml

CI (15 ml)

Chloroform	14.4 ml
Iso-Amyl Alcohol	600 µl