DNA EXTRACTION PROTOCOLS

DNA ISOLATION FROM BLOOD OR TISSUE USING PHENOL/CHLOROFORM

SUPER PROTOCOL FOR EXTREMELY HIGH DNA YIELDS!
INEXPENSIVE!
TRAINING REQUIRED!

- **USE PHENOL/CHLOROFORM IN FUMEHOOD AT ALL TIMES.**
- **DOUBLE GLOVE-PHENOL CAN SEVERELY BURN YOUR SKIN.**
- **DISPOSE OF LIQUID AND SOLID PHENOL/CHLOROFORM IN PROPER WASTE DISPOSAL CONTAINERS. DO NOT POUR DOWN SINK!**

DAY 1:
Turn on 60˚C and 37˚C waterbaths

STEP 1: Remove Ethanol (EtOH):
Take a small piece of blood or tissue, place in a 1.5ml tube and chop into small pieces (using sterile technique). Centrifuge 5-10minutes at maximum speed. Remove and dispose of liquid by pipette. Air-dry by leaving tube caps opened until there is no moisture present when tube is flicked and there is no EtOH odor present (usually takes approximately half an hour).
OR use a speed-vac instead of air-drying by spinning for 5-10min. at 50˚C.
OR use the below PBS method instead of air drying (recommended for samples stored in chemicals other than EtOH, especially formalin):

Add twice the volume of PBS buffer, vortex (or mix by pipette) and centrifuge at max speed for 2-5 min. Extract and dispose of PBS by pipette and repeat two more times.

- It's important to remove EtOH and other tissue storage chemicals that may destabilize your extracted DNA or interfere with DNA applications such as PCR and DNA sequencing.
- **sterile technique**: performed by using a sterile instrument for each sample when transferring and chopping samples (a sterilized scalpel is often used: carefully flame scalpel blade between each sample with a Bunsen burner and EtOH).

STEP 2: Add 330ul of STE buffer and 40ul of 20% SDS solution (STE volume can be modified depending on how much Proteinase K is added in the next step. The final volume should be 400ul).

STEP 3: Add 20ul of 20mg/ml ProK (this volume may vary depending on tissue type). Mix by vortex or by pipette and incubate at 60˚C for 4hrs (mix occasionally to aid in digesting).
Add another 5-10ul of ProK (20mg/ml) and incubate overnight at 37˚C.

- 20ul is used for the first addition of ProK and 10ul for the second addition for most tissues (ie. Muscle, frog toes, lizard tails)
- Some tissues require less or more ProK:
  Liver (20ul/5ul)
  Blood (20ul/20ul)
- The two additions of ProK can be reversed for convenience (add 5-10ul and digest overnight at 37˚C, and then add 20ul and digest 4 hrs. at 60˚C).

DAY 2:

STEP 4: Centrifuge samples at max speed for 15-30min. to pellet cellular debris. Pour supernatant into a clean 1.5ml tube.

- For steps 4-9, you will need 4 sets of clean 1.5ml tubes.

STEP 5: Add 400ul of phenol. Mix by hand (do not vortex). Leave samples on ice for 10 min. (to precipitate SDS).

- Do not leave phenol for longer than 10min. It will degrade your DNA.

STEP 6: Centrifuge at max. speed for 2 min. Remove ONLY the top layer (containing DNA) by cutting a 200ul pipette tip (this will help to pipette viscous fluid). Expel layer into a clean 1.5ml tube. DO NOT include bottom (phenol) or interface (protein) layers.

- It's better to leave a bit of the top layer behind than to contaminate with the bottom or interface layers.

STEP 7: Repeat steps 5-6. Leave on ice for ONLY 5min.
STEP 8: Add 400ul of chloroform/isoamyl alcohol (24:1). Mix by hand.

- This step with remove traces of phenol.

STEP 9: Centrifuge at max. speed for 2 min. Remove top layer (containing DNA) by cutting a 200ul pipette tip. Expel layer into a clean 1.5ml tube. DO NOT include any of the bottom or interface layers.

STEP 10: Add 2 volumes of cold 95% ethanol and invert. DNA should be visible.
STEP 11: Add 4% 3M sodium acetate (NaAc) and invert.
STEP 12: place tubes in at -20˚C freezer overnight to precipitate DNA.

DAY 3:

STEP13: Centrifuge for 15-30min. at max speed to pellet DNA.
STEP 14: Discard supernatant and add 500ul of 70% EtOH to wash the DNA pellet, invert and centrifuge for 2min. at max speed.

STEP 15: Discard supernatant and dry DNA pellet by air or speed-vac as in step 1.

- DO NOT over-dry! It will be very difficult to resuspend your DNA if over-dried.

STEP 16: Resuspend DNA in 50-200ul of sterile distilled water. Add water and mix gently by pipette. Let incubate at 37˚C for 1-12hrs. Mix gently by pipette again and incubate at 4˚C overnight. Mix and store at -20 to -80˚C.

DNA EXRACTING FROM TOE PADS OR FEATHERS USING GENECLEAN II

GREAT FOR EXTRACTING DNA SMALL SCALE AND FROM DEGRADED SAMPLES!
EXPENSIVE!
TRAINING REQUIRED!

This protocol has been modified from the manufacturer's original protocol. Please review the GeneClean II Kit manual before proceeding with this protocol. It may contain important details and troubleshooting tips that are not mentioned below.

DAY 1:
Turn on 55˚C and 37˚C water baths
Make sure you have prepared New Wash (included in kit)

STEP 1: Remove EtOH or other storage chemicals by air-drying, speed-vac or PBS methods (see step 1 in the above protocol "DNA ISOLATION FROM BLOOD OR TISSUE USING PHENOL/CHLOROFORM")

STEP 2: Tissue Preparation:
Toe Pads: Chop into small pieces (10-20) using sterile technique. Chop tissue inside the tube to avoid losing any pieces.
Feather Tips: Cut quill from feather. Chop quill into small pieces (10-20) using sterile technique. Chop tissue inside the tube.

- **Static is often a problem when using such small sample specimens (your sample may fly away!). Add 10-20ul of the AB lysis buffer to the sample before chopping to eliminate static.**

STEP 3: Add 180ul of AB lysis buffer and 20ul of ProK (20mg/ml). Mix carefully by vortex or pipette. **Make sure all pieces of tissue are in the buffer!**

STEP 4: Incubate at 55˚C for 4 hrs. Mix by pipette occasionally to aid in digestion.

STEP 5: Add 10ul of ProK and incubate overnight at 37˚C.

- Steps 3-4 and step 5 can be reversed, if more convenient.

- Some tissues (ie. Insects) are difficult to digest. In this case, repeat step 3 and 4 (additional lysis buffer is NOT necessary).
- It is important that tissues are completely digested (no visible particles) to ensure highest DNA yield possible. **Hard outer layers of feathers and insects or very old outer layers of toe pads will not digest! They will be removed in the next step.**

DAY 2:
Turn on 55˚C waterbath

STEP 6: Centrifuge samples for 5-15min. at maximum speed to pellet cellular debris and/or indigestible tissue.

STEP 7: Carefully pipette liquid into a clean tube (do not disrupt pellet).

- Feather debris will NOT pellet making it difficult to pipette out liquid. If debris is left in the new tube, repeat step 6-7.

STEP 8: Add 3 volumes of Sodium Iodide (NaI), mix by vortex.
STEP 9: Resuspend glass-milk for 1 min. by vortex. Add 10ul of glass-milk to sample and vortex.
STEP 10: Incubate at room temperature for at least 10 min. (no more than 30min.) while mixing on a rocker or by hand. This step will bind DNA to the glass-milk.

STEP 11: Centrifuge for 5min. at max speed to pellet glass-milk. Pour off and discard supernatant.

- when extracting from feathers, a waxy film is often present. Pour as much off as possible. Remaining film will be removed in the following wash steps.

STEP 12: Wash pellet **3 times** with prepared New Wash: Add 700ul of New Wash, resuspend pellet by vortex, centrifuge at max speed for 30sec. Remove and discard supernatant. **Do not disturb pellet when pipetting!**

STEP 13: Centrifuge at max speed for 10sec. and carefully remove the last bit of New Wash by pipette (10ul tip works best).

STEP 14: Dry pellet by air (usually takes ~10min.) or speed-vac. (5 min. at 50˚C).

- pellets will lose their shine when they are dry.
- **Do not over-dry!**

STEP 15: Using a 10ul tip, resuspend pellet with 10ul of stertile distilled water or 1XTE (add DIRECTLY to pellet). Gently mix by pipette. Incubate at 55˚C for 2min, centrifuge at 13,000rpm for 30sec. Elute DNA solution into a clean tube. Repeat with another 10ul of sterile distilled water or 1xTE.

- **During the elution, it is important to minimize the amount of glass-milk that ends up in your DNA solution. It can potentially interfere with some molecular techniques! Repeat step 15 a third time if necessary .**
- the first elution should recover ~80% of the DNA.
- the second elution should recover ~20% of the DNA.
- it is **highly recommended** to centrifuge your samples before any application for 1min. at max speed.
- **Store DNA at -20**˚C to -80˚C.

AGAROSE GEL ELECTROPHORESIS:

1. Make gel following recommendations below;

Recommended Agarose gel Percentages for Best Resolution of DNA:

DNA percentage	DNA size range
0.5%	**1000-30 000bp**
0.7%	**800-12 000bp**
1.0%	**500-10 000bp**
1.2%	**400-7000bp**
1.5%	**200-2000bp**
2.0%	**50-2000bp**

note: This is a general guideline, some DNA fragment are run on a particular percentages of gel depending on the application it is going to be used for.

1% AGAROSE GEL/1x TBE (100ml)

1g	agarose
100ml	1x TBE

- Microwave on high for ~2min. or until clear and slightly bubbling.
- Cool slightly (so not boiling).
- Add Etidium Bromide (3ul/100ml).(**DANGER-MUTAGEN!/CARCINOGEN**)
- Cool under cold water or let sit @ room temperature until cool enough to touch flask through gloves.
- Pour into a gel tray and remove any bubbles.
- Let solidify 20min. to 1hr. depending on gel concentration and size.
- Adjust agarose and 1x TBE volumes to make various percentages of gels.
- **To make a higher or lower % gel, simply modify the amount of agarose you add.**

2. Place gel in rig with TBE buffer (use the same concentrstion of buffer that you used to make the gel above).

*make sure that the wells are covered with buffer.

3. Close lid and plug into powerpack (be sure that **black** is plugged into **black** and **red** is plugged into **red**).
4. turn on power. Run at appropriate voltage for that rig and type of gel.

Small rigs maximum 70volts
Large rig maximum 130volts

FEEZE AND SQUEEZE METHOD:

1. Harvest gel piece after gel electrophoresis using a sterile scalpel and UV box.
2. Freeze gel slice for at least 1 hour (can be frozen longer-max. 2 weeks).
3. Thaw gel slice.
4. Place into a cut filter-tip/0.6ml tube using sterile tweezers.
5. Centrifuge at 9000rpms for 1.5min.
6. Use liquid elution in manual DNA applications.

*this method is not clean enough for automated applications! Your DNA may require further clean-up.

MANUAL MICROSATELLIE GENOTYPING:

1. ENDLABEL your primer:

	Per sample
10uM forward or reverse primer	0.04ul
Buffer A (fermentas)	0.03ul
ddH20	0.13ul
PNK (fermentas)	0.05ul
Gamma-p33	0.05ul
total	0.3ul

*Sign –up on wall for isotope used.

2. Follow attached(E) protocol with modifications to suit your locus. **This will have to be modified.**
3. Follow "making/running/putting on film/developing protocols for sequencing"

***Do not forget to sign up for your gel .See example sheet in sequencing section-D.**