Genomic DNA Preparation from Plants

1. Each student will be provided a special blue 1.5 ml extraction tube containing 750 µl of extraction buffer (EB). The tube is designed to fit the special blue plastic grinder (the tubes in your lab kit will not fit as well, and the homogenization will not work).

2. Go and get an ultra-frozen (-80 C) soybean embryo from the TA or instructor, taking your blue 1.5ml extraction tube. **Wear gloves** – the extraction buffer contains β-mercaptoethanol – toxic. The TA will put the embryo directly into your extraction buffer where it will quickly thaw. Now you need to *work quickly*.

3. ***Immediately*** after the embryo is placed in the extraction buffer, homogenize the tissue in the extraction buffer using the supplied plastic grinder until it is an even green suspension. Be careful to keep all the liquid in the tube. When finished, no particles should be visible and the homogenate should be even, cloudy and soupy-looking. This brings the chemicals in the buffer into contact with the DNA in the tissue.

4. Incubate the homogenate (the mixture of tissue and extraction buffer) at 65C for 10 minutes in a water bath.

5. Add 250 µl of potassium acetate (KOAc) to the extraction. Mix gently but thoroughly (invert 4-6 times but do not vortex. You should see the KOAc solution disperse and there should be no separate phases visible).

6. Incubate on ice for 15 minutes to precipitate the proteins.

7. Centrifuge the mixture for 10 minutes at high speed.

8. Transfer 900 µl of supernatant to a new tube containing 500 µl of cold isopropanol. The isopropanol will precipitate the DNA, which should become visible as fluffy white threads.

9. Centrifuge the precipitated DNA for 1 minute at full speed in the centrifuge, then remove the supernatant (leaving the DNA pellet in the bottom of the tube) and dissolve the DNA in 600 µl of TE.

10. Carefully add 600 µl of buffered Phenol:Chloroform:Isoamyl Alcohol mixture (labeled PC) to the dissolved DNA, mix vigorously by inversion. Phenol is a corrosive neurotoxin. **Be very careful, wear safety glasses and *nitrile* gloves (phenol will go through latex gloves!)**.

11. Centrifuge the tubes for 2 minutes at high speed.

12. Transfer the upper phase (the aqueous phase) containing the DNA (about 500 ul) to a new 1.5 ml tube (do not transfer the proteins at the interface).

13. Add 500 µl of chloroform (labeled CH) to the DNA solution and mix vigorously by inversion. **Careful: chloroform causes liver cancer, and is much denser than water. Do not inhale vapor.**

14. Centrifuge the tubes for 2 minutes at high speed.

15. Transfer the upper phase (the aqueous phase) to a new tube (about 450 µl) and add 45 ul of 3M sodium acetate (NaOAC), mix by inversion.

16. Add one volume (this means the same amount as you already have in your tube; should be about 500 µl) of cold isopropanol and invert well to mix. You should see a DNA precipitate within a few minutes. (place the tube on dry ice 10 minutes if the DNA does not precipitate).

17. Centrifuge for 1 minute to pellet the DNA, then remove and discard the supernatant.

18. Dry the pellet for five minutes in the speed-vac, then re-dissolve the DNA in 50 µl of TE, then incubate at 37C. BE SURE TO WRITE YOUR NAME/GROUP NUMBER ON YOUR TUBE!!! The TA will collect your samples from the incubator and store them for future use.

Buffer formulation

DNA CTAB extraction buffer
(100 mM Tris, 1.4M NaCl, 20 mM EDTA, 1% PEG, 2% CTAB, pH 9.5)

Ingredients	100 ml	Final conc
Tris Base (MW 121) 1M soln pH9.5	10ml 1M Tris pH9.5	100 mM
NaCI (MW 58.4) 5M soln	28ml 5M NaCl	500 mM
EDTA disodium (MW 372) 0.5M soln	4ml 0.5M EDTA	20 mM
PEG 6000 solid	1g	1%
CTAB solid	2g	2%
final volume	100ml	

Add liquid components to a beaker, followed by solids. Stir with magnetic stirrer until solids are dissolved. Make up volume to 100ml in graduated cylinder. Store in labeled Pyrex bottle and use quickly. Add beta-mercaptoethanol to 0.25% before use.

TE (10 mM Tris, 1 mM EDTA, pH 7.5)

Ingredients		Per 500 ml	Final conc
Tris Base (MW121)		0.605 grams	10 mM
EDTA		0.186 grams	1 mM
		500 ml	

Add H_2O to 500 ml in a beaker. Weigh out Tris and add to water with stirring. Then add EDTA. Begin pH adjustment with 6M HCl until pH 7.5 is reached. Bring final volume to 500 ml. Mix well and autoclave in a media bottle. Label container with initials and date.

TER (10 mM Tris, 1 mM EDTA, pH 7.5, with 20 µg/ml RNase)
Dissolve 10 mg of Rnase in 1 ml of H2O. Boil 95 C, 10 min before use to denature any contaminating Dnase. Store as aliquots at –20 C. Add 2 ul of 10 mg/ml RNA per each ml of TE.

5M KOAC (Potassium Acetate) 200 ml: Potassium acetate (MW 98). Weigh 98.0 g potassium acetate and bring to a final volume of 200 ml with deionized H_2O. Do not adjust pH. Autoclave and store in coldbox.