Lab 1: *BRACHYPODIUM DISTACHYON* DNA

Lab 1.1: DNA Extraction

Goal for this lab:

Extract DNA from *B. distachyon*.

Introduction

In this lab, we will extract DNA from our model organism, *B. distachyon*. We will be using the DNA we make today throughout the rest of the semester, so this is an important activity!

In any DNA purification (or extraction) protocol, there are three important considerations:

1. We must break up the tissues and cells to release the DNA.
2. We must prevent the DNA from being degraded by cellular enzymes during the purification.
3. We must get rid of cellular components that are not desired—in this case, proteins, lipids, carbohydrates (cell walls), and RNA.

Break up tissues and cells.

We will cause tissue and cell disruption in three ways. The first is mechanical grinding. We will use a ball mill to pulverize leaf tissue. This breaks up the extracellular matrix in which the cells reside and allows better access of our chemicals to the cells themselves. The second method of disruption is chemical. The DNA extraction buffer that we use contains a high concentration of a detergent, sodium dodecyl sulfate (SDS). You will recall that detergents have both hydrophobic and hydrophilic moieties, and thus will bind both lipid and water. This detergent will solubilize the lipid bilayers in the cells, so that the plasma membrane and also the internal membranes are dissolved. In addition, we use the metal chelating compound ethylene diamine tetra-acetic acid (EDTA), which tightly binds calcium (Ca^{++}) and magnesium (Mg^{++}) effectively locking it away from cellular components. In the absence of Ca^{++} and Mg^{++}, proteins denature and membranes lose their integrity. The last disruption is high temperature. This encourages the detergent and EDTA to work efficiently and hastens cellular disintegration. This high temperature step is critical for efficient cellular disruption and release of DNA.

Prevent DNA degradation.

We prevent DNA degradation by inhibiting the enzymes (deoxyribonucleases, or 'DNases') that degrade DNA. Conveniently, DNAses require Mg^{++} as a co-factor, so, by chelating the Mg^{++} with EDTA, we inhibit the activity of DNases. Thus, it is imperative to get the EDTA containing DNA extraction buffer well mixed with the ground tissue as soon as possible.

Get rid of undesirable molecules.

We will use two methods to accomplish this. Both rely on differential solubility of specific macromolecules in particular solutions. Proteins and complex carbohydrates (such as those found in cell walls) are insoluble in solutions containing high concentrations of potassium acetate (KOAc). By adding KOAc, we force the proteins and carbohydrates to precipitate (become solid and fall out of solution) while the nucleic acids will remain in solution. Nucleic acids are insoluble in solutions containing high levels of sodium plus the alcohols ethanol (EtOH) or isopropanol (ISOP), while lipids are quite soluble in these conditions. Proteins and carbohydrates are somewhat soluble in alcohol. By adding alcohols to a salty solution, we can force the nucleic acids to precipitate, while leaving the lipids, and the remaining carbohydrates and proteins, in solution. The main problem with alcohol precipitation of nucleic acids is that the sodium precipitates along with the nucleic acids. This is undesirable, and so we always rinse nucleic acid pellets with 70% EtOH (containing no salts) to remove the sodium from the pellets prior to dissolving the nucleic acids in the next solvent.

Materials:

Reagent	conc			pH	also known as
DEB	100	mM	NaCl	8.0	Sodium chloride
(DNA Extraction Buffer)	50	mM	Tris		trishydroxymethylaminomethane
	25	mM	EDTA		Ethylene diamine tetra-acetid acid
	1	%	SDS		Sodium dodecyl sulfate
	10	mM	BME		β-mercaptoethanol
$T_{10}E_1$	10	mM	Tris	8.0	
	1	mM	EDTA		
EtOH	95	%		-	Ethanol
EtOH	70	%		-	Ethanol
ISOP	100	%		-	Isopropanol
KOAc	5	M			Potassium acetate (CH_3COOK)
LN_2					Liquid nitrogen

Method:

Before you begin, read through the directions and get everything out that you will need so you don't have to fumble for it in the middle of some critical step in the extraction procedure. Each student will do one extraction (*i.e.*, two extractions per pair).

1. Prepare about 10 cm of *B. distachyon* leaf tissue.
2. Add 1 mL DEB and 2 metal beads to a 2 mL tube.
3. Place the tube in the Verder ball grinder, to pulverize the leaf tissue. This essentially works in the same manner as disrupting the tissue with a mortar and pestle. Shake for 5 min at the maximum frequency. Please check that your mixture looks uniformly green.
4. When your sample is ready, transfer the tube to a 65°C hot block for 10 minutes.
5. Place the tube on ice.
6. Add 415 µL 5M KOAc. Mix well by inverting the tube repeatedly. Incubate ***ON ICE*** for 20 minutes.
7. Centrifuge at maximum speed for 10 minutes.
8. Remove your tube from the centrifuge and notice the very large pellet consisting of tissue debris and precipitated protein. ***REMEMBER***, you are trying to ***GET RID*** of the pellet in this step!
9. Transfer 1 mL of the supernatant (the liquid on top of the pellet) into a fresh round-bottomed 2 mL tube containing 1 mL of 100% isopropanol. Mix well by inverting the tube gently but repeatedly. ***Precipitating nucleic acids may be clearly visible at this point.***
10. Spin in the centrifuge at maximum speed for 15 minutes.
11. Remove your tube from the centrifuge. This time, ***YOU WANT YOUR PELLET***—the pellet is the nucleic acids—including your DNA. Remove as much of the supernate as possible.
12. Rinse your pellet carefully by adding ~500 µL 70% EtOH.
13. Spin the sample down at 5000 RPM for 10 minutes.
14. Pipet out as much liquid as possible.
15. Spin the samples at 500 RPM for 30 seconds, and pipet out any remaining ethanol. Let the pellet air dry for a few minutes. Getting rid of the EtOH is essential before proceeding to the next step. Leave the tube open to dry for at least 2 minutes.
16. Add 50 µL $T_{10}E_1$ to your tube. Incubate at 65°C for 10 minutes, then pipet up and down gently to mix.
17. If your pellets do not go into solution, discuss with your instructor whether you should add more $T_{10}E_1$.
18. Label your tube carefully with your group initials, the date, and At gDNA (for *Arabidopsis thaliana* genomic DNA). Put it in a rack in a freezer box with your group name on it in label tape.
19. Write in your lab notebook what you did, either "exactly as in manual", or "as in manual, except that …" Give the date, and what label you put on the tube. Later on in the

semester, when you will need this DNA again, all this will help you remember (or figure out) what's in the tube you just put in the freezer.

Clean-up:

Alcohols are safe to go down the drain ***only when they are less than 10%. Run plenty of water while discarding alcohols so that this dilution will be achieved.***

Save your reagents. If you have to make DNA again later, you'll be glad to have them. You will use your $T_{10}E_1$ again.

Put the tubes that contained any β-mercaptoethanol in the marked bucket in the hood.

Put plant debris and used potting soil in the bucket labeled "Compost".

Save the pot the plants were grown in.