## Lab 3.4: DNA Extraction, Revised

#### Third Task: Prepare template DNA from plants

#### Materials:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Reagent** | **conc** | |  | **pH** | **also known as** |
| DEB  (DNA Extraction Buffer) | 100  50  25  1  10 | mM  mM  mM  %  mM | NaCl  Tris  EDTA  SDS  BME | 8.0 | Sodium chloride  trishydroxymethylaminomethane  Ethylene diamine tetra-acetid acid  Sodium dodecyl sulfate  β-mercaptoethanol |
| T10E5 | 10  5 | mM  mM | Tris  EDTA | 8.0 |  |
| T10E1 | 10  1 | mM  mM | Tris  EDTA | 8.0 |  |
| EtOH | 95 | % |  | - | Ethanol |
| EtOH | 70 | % |  | - | Ethanol |
| ISOP | 100 | % |  | - | Isopropanol |
| KOAc | 5 | M |  | - | Potassium acetate (CH3COOK) |
| NaOAc | 3 | M |  | 5.2 | Sodium acetate (CH3COONa) |

#### Methods

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.

1. ***Label*** 13 1.5 mL blue microfuge tubes:
   1. 1 for a WT (Cl0) plant grown on soil,
   2. 1 for a WT plant grown on a plate,
   3. 11 for the Salk seedlings you have chosen to sample
2. Put 600 600 L DEB (DNA Extraction Buffer) into each tube
3. Cut about a half a leaf per plant and immediately put it into its 1.5 mL tube.
4. Gently squish the leaf against the side of the tube, using about two turns of a little blue pestle..
5. Incubate the tubes at 65°C in a dry bath for 10 minutes.
6. Place the tubes on ICE and add 250L 5M KOAc. Mix well by inverting the tubes repeatedly. Incubate ON ICE for 20 minutes.
7. Centrifuge at maximum speed for 7 minutes 4°C.
8. Remove your tubes from the centrifuge and notice the pellet consisting of tissue debris and precipitated protein. REMEMBER, you are trying to GET RID of the pellet in this step!
9. Carefully transfer the supernatant (the liquid on top of the pellet), without disturbing the pellet, to a new, labeled 1.5 mL tube containing 600L of 100% isopropanol. Mix well by inverting the tube gently but repeatedly.
10. Centrifuge at maximum speed for 7 minutes 4°C.
11. Remove your tube from the centrifuge. REMEMBER, this time, YOU WANT YOUR PELLET—the pellet is the nucleic acids—including your DNA.
12. Remove supernatant by tipping over the tube, being sure the pellet remains at the bottom.
13. Rinse your pellet carefully with 300 L 70% EtOH. Use a pipette to remove as much liquid as humanly possible, then let the pellet air dry for a few minutes. Getting rid of the EtOH is essential before proceeding to the next step.
14. Add 225 L of T10E5 and resuspend the pellet.
15. Add 25 L of 3M NaOAc and mix gently by pipetting.
16. Add 500 L of 95% EtOH and mix gently by inverting the tube.
17. Centrifuge at maximum speed for 7 minutes 4°C.
18. Remove supernatant by tipping over the tube, being sure the pellet remains at the bottom.
19. Wash the pellet with 500 L of 70% EtOH[[1]](#footnote--1)
20. Centrifuge at maximum speed for 7 minutes 4°C, then remove as much EtOH as possible. Let the tube air dry for a few minutes.
21. Resuspend the pellet in 15 L of sterilized T10E1.
22. Store DNA at -20°C.

#### Third Task, part 2: Reconstitute your Salk genotyping primers

##### Make a 100 µM stock solution.

Find the number of nmol in the tube.

Multiply that number by 10 to get the number of µL of sterile water to add.

##### Make a 12.5 µM working solution.

Mix 12.5 µL of your 100 mmol solution with 87.5 µL sterile water.

1. If you’re going to be pressed for time, you don’t NEED to do these final purification steps, but I would recommend it. I have not tried skipping them, but other members of the lab have, and found the DNA was still good enough to be amplified in PCR. If so, I would add EtOH, mix, centrifuge, remove EtOH, and resuspend in T10E1. [↑](#footnote-ref--1)