

Simplified DNA extraction and PCR

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PART 1 – LEAF COLLECTION AND PREPARATION

1. Collect 2 leaves of various plants per student. Collect 1 Garlic Mustard leaf (per group) as a positive control.
2. Acquire 2 (8) strip tubes per group.
3. Label one strip tube 'ITS2' and the other 'rbcl'. Label the side of each tube of the strip tube with the numbers 1-8 as well as the group # following the notation below:

Group#_ITS2_Tube#

e.g. 3 ITS2_5 or further abbreviated to 3_I_5

The top of the tube is domed and the tubes are small so using 'I' for ITS2 and 'r' for rbcl will suffice.

4. Using the micro-punch, press 2 leaf discs 1-2mm² from the 1st leaf and place 1 leaf disc into tube 1 of strip tube labelled ITS2 and the other disc into tube 1 of the strip tube labelled rbcl.
5. Wipe the micro-punch with 70% ethanol to sterilize in between samples.
6. Repeat steps 4 and 5 for all remaining leaf samples in tubes 2-6 of each strip tube.
7. Include garlic mustard in tube 7 of each strip tube as a positive control.
8. Leave tube 8 of each strip tube empty as a negative control.
9. Add 200uL FTA Purification Buffer to each PCR tube containing leaf disc and incubate at room temperature for 5 mins.
10. Remove and discard FTA Purification Buffer.
11. Repeat steps 9 and 10.
12. Add 200uL 0.1X TE Buffer to each PCR tube containing leaf disc and incubate at room temperature for 5 mins.
13. Remove and discard 0.1X TE Buffer.
14. Repeat steps 12 and 13.
15. Remove all remaining buffer and allow the leaf disc to dry for 30s - 1 minute. There should be no visible droplet in the tube.
16. Close tubes and set aside at room temperature.

Each group should now have 2 (8) strip tubes with 7 leaf discs each (6 collected and 1 garlic mustard control) and 1 empty tube to be used as a negative control.

PART 2 – PCR

1. Prepare a ITS2 Mastermix for all 8 samples by filling in the table below:

Component	Volume (ul) for 1 reaction	Volume (ul) for 8.5 reactions
2x PCR Buffer	12.5	
ITS2 Fw primer	1.25	
ITS Rv primer	1.25	
Nuclease-free water	10.0	

2. Calculate the volumes of each component required for 8.5 reactions.
3. Add the volumes of each component required for 8.5 reactions together in a 1.5mL tube. Pipette up and down several times to mix well.
4. Label the 1.5mL tube 'ITS2'.
5. Aliquot 25uL of mastermix into each of the tubes on the strip tube labelled 'ITS2' containing the washed leaf discs.
6. Centrifuge tubes briefly and place on ice until the 2nd mastermix for rbcl is ready.
7. Prepare a rbcl Mastermix for all 8 samples by filling in the table below:

Component	Volume (ul) for 1 reaction	Volume (ul) for 8.5 reactions
2x PCR Buffer	12.5	
rbcl Fw primer	1.25	
rbcl Rv primer	1.25	
Nuclease-free water	10.0	

8. Calculate the volumes of each component required for 8.5 reactions.
9. Add the volumes of each component required for 8.5 reactions together in a 1.5mL tube. Pipette up and down several times to mix well.
10. Label the 1.5mL tube 'rbcl'.
11. Aliquot 25uL of mastermix into each of the tubes on the strip tube labelled 'rbcl' containing the washed leaf discs.
12. Centrifuge all tubes briefly and place in PCR machine programmed with the following parameters:

Temperature	Time	Cycles
94°C	3 mins	1
94°C	30 secs	35
55°C	30 secs	
72°C	1 min	
72°C	10 min	1
4°C	∞	1



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18. Cover the top of the electrophoresis unit with the lid and plug into the power pack.
19. Turn the power pack on. Set voltage to ~110V. Look for bubbles.
20. Allow gel to run for ~45 mins- 1 hour or until the dye runs $\frac{3}{4}$ way down the top half of the gel.
Do not allow the samples in the top half of the gel to run into the 2nd half of the gel.

Visualize the gel under UV light and take note of the samples that showed amplification. Take a picture of the gel if possible.

21. Store remainder of samples in fridge overnight to be sent for Sanger Sequencing.