RAD Extractions

For every tissue sample, combine the following reagents in a tube and store on ice until use:

Digestion Solution	
TENSII	250µL
Prot. K, 20mg/ml	20µL
RNase A Solution	5µL
Total Volume	**275µL**

TENSII	
4mL	5M NaCl
50mL	1M Tris ph8
2mL	0.5M EDTA ph8
844mL	dH2O
autoclave	
add 100mL	10% SDS

1. Dissect up to 20mg of tissue sample. Cut tissue sample into two equally sized pieces and place them in a 1.5mL microcentrifuge tube.

2. Add 275µL of the prepared Digestion Solution Master Mix (above) to each sample tube. Be sure that the sample is completely covered with the Digestion Solution Master Mix. If the tissue sample is not covered by the Digestion Solution, cut the tissue into smaller pieces.

3. Incubate the sample tubes overnight (16–18 hours) in a 55°C heat block or water bath.

4. Transfer entire solution to a pre-spun (15,000 g for 1 to 2 minutes) PLG 2 ml Heavy tube. Add 0.5 mL Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1) to the sample in the PLG 2 ml tube and mix well by repeated inversion. Do not vortex. Centrifuge at full speed (12,000 g or greater) for 5 minutes in a microcentrifuge, then carefully transfer the resultant aqueous phase to a fresh pre-spun PLG 2 mL Heavy tube.

5. Add 0.5 mL Chloroform:Isoamyl Alcohol (CI, 24:1) to the sample in the PLG2 ml tube and mix well by repeated inversion. Do not vortex. Centrifuge at full speed (12,000 g or greater) for 5 minutes in a microcentrifuge, then carefully transfer resultant aqueous phase to a fresh microcentrifuge tube.

6. Add 30µL Sodium Acetate (3M, pH 5.2)

7. Add 1.25 mL of 95% ethanol.

8. Incubate in -20˚C overnight.

9. Centrifuge at > 14,000 g for 20 minutes at room temperature

10. Discard supernatant being careful not to throw out DNA pellet, which may or may not be visible.

11. Rinse with 1 mL 70% Ethanol

12. Centrifuge again for 10 minutes.

13. Discard supernatant and air dry.

14. Resuspend in 200µL water.