

MacManes Salt Extraction Protocol

- > **Digest Tissue:**
 - a. Tissue + 410 μ L extraction buffer + 2% SDS (80 μ L 10% SDS) + 20 μ L Proteinase K (10mg/ml)
 - b. Digest overnight (or until tissue dissolved) at temperature of preference (55° usually).
- > **Extraction Step 1- Get rid of excess protein.**
 - a. Centrifuge 3 minutes at 13,000rpm
 - b. Transfer supernatant to new eppi. Discard pellet.
- > **Extraction Step 2- Get rid of salts.**
 - a. Add 200 μ L NaCl to supernatant from step 1.
 - b. Vortex above mixture.
 - c. Centrifuge mixture (at 4°) from step one- 7 minutes, 13,000rpm
- > **Extraction Step 3- Precipitate DNA**
 - a. Transfer supernatant from step 2 quickly to new eppi.
 - b. Add 500 μ L chilled isopropanol.
 - c. Gently mix.
 - d. Cool on dry Ice x 10 minutes
 - e. Centrifuge mixture (at 4°) from step two- 10 minutes, 13,000+rpm
- > **Extraction Step 4- Wash DNA-1**
 - a. Carefully discard supernatant from step 3- (might see pellet at this point)
 - b. Add 500 μ L 70-80% ethanol
 - c. Mix gently.
 - d. Cool on dry Ice x few minutes
 - e. Centrifuge mixture (at 4°) from step three- 10 minutes, 13,000+rpm
- > **Extraction Step 5- Wash DNA-2**
 - a. Carefully discard supernatant from step 4.
 - b. Add 500 μ L 70-80% ethanol
 - c. Mix gently
 - d. Cool on dry Ice x few minutes
 - e. Centrifuge mixture (at 4°) from step three- 10 minutes, 13,000+rpm
- > **Extraction Step 6- Dry Pellet**
 - a. Discard Supernatant from step 5.
 - b. Dry Pellet via method of choice. (vacuum centrifuge is best)
- > **Extraction Step 7- rehydrate DNA**
 - a. Rehydrate DNA in 50 μ L TE buffer (or buffer of choice)
 - b. Let sit overnight to completely rehydrate.
 - c. Quantify DNA using nanodrop.

Extraction Buffer Mix		
Additive	Concentration	Amount
Tris	1M	.5ml
NaCl	5M	1ml
EDTA	0.5M	1ml
ddH2O	—	45ml