

- I. DNeasy protocol
 - A. Preparation of kit
 1. Add EtOH to AW1 and AW2 if needed
 2. Heat ATL and AL if they have precipitates
 3. Equipment
 - a. 55C water bath
 - b. 70C heat block
 - B. Sample digest
 1. put <25 mg tissue in 1.5 ml tube
 2. Add
 - a. 180 ul buffer ATL
 - b. 20 ul proteinase K
 3. Fin clips seem to take only 1 hour
 - C. Binding
 1. Vortex sample for 15s
 2. Add 200 ul buffer AL
 - a. Mix again by vortex
 - b. See white coagulant but it dissolves after heat
 - c. Incubate at 70 C for 10 min
 3. Add 200 ul ethanol
 - a. Mix by vortex
 4. Apply all to DNeasy spin column
 - a. Spin 1 min >6000 g (9000 rpm in Eppendorf)
 - b. Discard flow thru and tube
 - c. Place in new tube
 - D. Washes
 1. Wash #1
 - a. Apply 500 ul buffer AW1
 - b. Spin 1 min, >6000g
 - c. Discard flow thru and tube
 - d. Place in new tube
 2. Wash #2
 - a. Apply 500 ul buffer AW2
 - b. Spin 2 min >20000g
 - c. Discard flow thru and spin another minute
 - d. Put column in clean 1.5 ml tube
 - E. Elution
 1. Elute #1
 - a. Apply 100 ul buffer AE
 - b. Incubate 1 min
 - c. Spin 1 min in biorad; > 6000g (8500 rpm in biofuge)
 2. Elute #2
 - a. Apply 100 ul buffer AE
 - b. Incubate 1 min
 - c. Spin 1 min in biorad; > 6000g (8500 rpm in biofuge)