**PureLink Genomic DNA Extraction Protocol**

Choose the appropriate lysate preparation protocol. For this kit (Invitrogen PureLink Genomic DNA Mini Kit), the closest match to corals is the option for “Mammalian Tissue and Mouse/Rat Tail Lysate.” This can be found on page 16 of the manual.

**Overview of Steps**

1. Prepare the lysate using the *Digestion Buffer* and the *Proteinase K*
2. Add *Lysis/Binding Buffer* and ethanol to the lysate
3. Apply sample to a *PureLink Spin Column*
4. Wash the column with *Wash Buffer 1*
5. Wash the column with *Wash Buffer 2*
6. Elute the DNA with MilliQ water
7. **PREPARE THE LYSATE**

**This portion of the protocol mirrors directions from p. 16 of the Invitrogen manual.**

* 1. Make sure that the water bath is at temperature (55˚C)
  2. Put on gloves and get a bead blast tube and cap. Be careful not to touch other tubes to minimize cumulative contaminant exposure. Open the DNA kit. Make sure that ethanol has been added to all wash buffers and check when the kit was opened to make sure it’s not expired
  3. Get your samples. For DNA, it is not crucial to keep them on ice. Put the cryotubes, which contain the samples, in a tube rack at your workstation. Start out with ~2 samples at a time if you are new to extractions
  4. Sterilize your workspace, including your tweezers and the area of the workbench where you will be handling the samples. You can do this by squirting 50% ethanol onto a Kimwipe and rubbing down the surfaces
  5. Lay down a new Kimwipe onto the dry workbench in case you need to set down a piece of coral
  6. **Label the bead mill tubes with the sample ID found on the cryotubes;** ***labeling is critical to the correct identification of samples and care should be taken at all steps during extraction to appropriately label all intermediate and final tubes***
  7. Locate the acid washed glass lysis beads (150-212µm) in the molecular cabinet along with the bead scoop. Open the bead mill tubes and scoop a small amount of the beads into each tube. There should be enough to fill the only the bottom, conically-shaped portion of the tube
  8. For coral samples, use tweezers to get a small piece of skeleton out of the tube and transfer it to the correspondingly labeled bead mill tube, which now has the glass beads in it. Make sure that the bit of sample you get has polyps and is not just a stray chunk of sub-polyp skeleton
  9. Add *Digestion Buffer* and *Proteinase K*. Use 360 µl of *Digestion Buffer* and 40 µl *Proteinase K* for each sample*.* This is double the amount recommended in the kit manual. Pipette slowly to avoid bubbles and eject only to the first stop. Screw on the bead blaster caps
  10. Lyse your sample: take your tubes over to the bead mill. Lift the cover and carefully unscrew the lid plate. Evenly distribute your samples in the bead mill with a minimum of 4 tubes, counterweighting as needed. Make sure that the lid cover is locked before turning on the machine. Set the mill to 5 m/s (labeled as “S=5” on the machine) for 30 seconds. At the end of the 30 seconds, spin down the tubes in the centrifuge (~1 minute at max speed) so that you can see if the skeleton has been ruptured. Repeat up to 2 times to ensure sufficient lysis of skeleton
      1. NK update 6/6: Have been doing 2.6 m/s for 1 minute, twice
  11. Incubate at 55˚C for 30 minutes by floating the tubes in the bubble wrap that is in the water bath
  12. After 30 minutes, dry the tubes and return them to the bead mill for another 5 m/s for 30 seconds run
  13. Centrifuge again at max speed for 1 minute
  14. Incubate at 55˚C for another 30 minutes in the water bath
  15. Take out the tubes and use tweezers to remove any remaining large pieces of skeleton (dispose of them)
  16. Centrifuge the samples at max speed for 3 minutes so that any remaining particulates will pellet at the bottom of the tube
  17. Transfer the supernatant (liquid in upper portion of bead mill tube) into a new sterile 1.5 ml tube. Be careful not to place the pipette tip all the way down into the bottom, as you want to avoid picking up the particulate material
  18. Add 20 µl *RNase A* to the lysate; mix the solution briefly by vortexing and spin down the sample briefly using the mini centrifuge to get any lysate off the interior of the cap and down into the tube. Incubate at room temperature for 2 minutes. *RNase A* will minimize RNA contamination by digesting any RNA nucleotide.
  19. Estimate the amount of lysate solution you have; you will be using a 1:1:1 mix of supernatant:binding buffer:ethanol. If you have 350 µl of supernatant, add 350 µl of *Lysis/Binding Buffer* and then add 350 µl of 100% ethanol. **NOTE:** the maximum amount of liquid that you can have in total is 1500 µl due to the volume of microtube, so do not use more than 500 µl of supernatant. If you have more than 500 µl of supernatant, you are using too large of a coral tissue sample.
  20. Mix well by vortexing for 5 seconds. Spin down briefly on mini centrifuge to keep sample in the bottom of the tube

1. **BINDING DNA USING THE SPIN COLUMN (PURIFICATION)**

**This portion of the protocol mirrors directions from p. 23 of the Invitrogen manual.**

* 1. Carefully remove a spin column in a collection tube from the provided Invitrogen packages
  2. Add about 640 µl of the lysate mixture to the Spin Column, backpipetting (only go to the first stop) in the lysate tube as needed to mix the lysate thoroughly. Centrifuge at 10,000 x *g* for 1 minute. After centrifugation, empty the contents of the collection tube from beneath the spin column into the trash. Repeat this process in 650 µl increments until you have filtered all of the lysate supernatant through the spin column. At this point, all of your DNA is resting on the filter in the spin column. Discard the collection tube and place the spin column into a clean collection tube

1. **WASHING THE DNA**

**This part of the protocol mirrors directions from p. 24 in the Invitrogen manual.**

* 1. Add 500 µl of *Wash Buffer 1* prepared with ethanol to the column
  2. Centrifuge the column at room temperature at 10,000 x *g* for 1 minute
  3. Empty the collection tube
  4. Add 500 µl of *Wash Buffer 2* prepared with ethanol to the column
  5. Centrifuge the column at room temperature at 10,000 x *g* for 1 minute
  6. Empty the collection tube
  7. Centrifuge the column at maximum speed for 3 minutes at room temperature. This is important to make sure that no ethanol remains in the sample, which can interfere with electrophoresis
  8. Label a new, final, sterile 1.5 ml microcentrifuge tube with the appropriate ID. Also include the date of extraction and initials of the extractor
  9. Discard the collection tube used with the spin column

1. **ELUTE THE DNA**

**This part of the protocol mirrors directions from p. 24 in the Invitrogen manual.**

* 1. Place the spin column in the new labeled 1.5 ml microcentrifuge tube
  2. Add 50 µl of MilliQ molecular grade water to the column (instead of elution buffer); ensure that the pipette tip is touching the filter -- do not ram it into it, but be sure to make contact
  3. Incubate at room temperature for 1 minute
  4. Centrifuge the column at max speed for 1 minute
  5. To recover the rest of the DNA, perform a second elution step with the same elution buffer (MilliQ) volume as the first elution, through the same column and into the same labeled tube
  6. Centrifuge for 2 minutes at max speed
  7. Throw away the spin column and close the final DNA sample tube
  8. Use the nanodrop microvolume spectrophotometer to ensure that DNA was successfully extracted and to determine the concentration of the DNA.

OR skip this step and put the purified DNA in the freezer