

DNA Purification from Tissue Using the Gentra Puregene Tissue Kit

Day 1

1. Make sure the fly is in the correct type of tube (Denville 1.5 ml tubes)
2. Work quickly and make sure the fly remains frozen at all times
3. Place the tube on dry ice and wait **5** minutes until you are confident the fly has been frozen (or remains frozen) by the dry ice
 - a. Smashing the dry ice into a lot of small pieces makes these steps work better
4. Open the vial and grind the fly vigorously using a short, blue pestle
 - a. Hold the fly on the dry ice while you are grinding to prevent defrosting during this step
 - b. It is essential that you use a tube and pestle which fit together closely
 - c. The fly should be completely pulverized at the end of this step
5. Add **100 ul** of Cell Lysis Solution and swish the pestle around to make sure the tissue has been completely ground up and suspended in the fluid (this may take a lot of swishing)
6. Add **100 ul** of Cell Lysis Solution, using it to wash the pestle by pipetting over the head of the pestle while rotating it
7. repeat Step 6 with **100 ul** Cell Lysis Solution
8. Add **1.5 ul** of Proteinase K (concentration 20 mg/ul) and invert the tube 25 times to mix
9. Incubate 2 hours to overnight at 55 degrees on a shaking platform (the shaking is not absolutely essential)

10. Add **1.5 ul** of RNase A solution and mix by inverting the sample 25 times. Incubate at **37** degrees for **15 – 60 minutes**
11. Incubate for **3-5 minute** on ice to cool the sample.
12. Add **100 ul** Protein Precipitation Solution and vortex vigorously for **20 s** at high speed (the sample should turn whitish and milky). If it does not turn white and milky it is not cool enough. Return the sample to the ice for an additional 5-10 minutes.
13. Centrifuge for **3 min** at 13,000-16,000 g and transfer the tubes back to ice
 - a. The precipitate proteins should form a tight pellet. If the pellet is not tight, incubate on ice for 5 minutes and repeat the centrifugation

- 14.** Pipette **300 ul** of isopropanol into a clean 1.5 ml tube and transfer in the supernatant from the previous step (Do not dislodge the protein pellet while pouring)
 - a. You may either pipette the supernatant from one tube to the other or pour it, regardless take care not to transfer any of the protein pellet
 - b. At this step you may also put the samples at -20 for as long as necessary if need be
- 15.** Add **3 ul** Glycogen Solution
- 16.** Mix by inverting 50 times
- 17.** Centrifuge for **10 min** at 13,000 – 16, 000
- 18.** Carefully discard the supernatant, taking care that the pellet remains in the tube.
 - a. You may not be able to see the pellet. Centrifuge the samples in the same orientation so that all pellets will be in the same location, and avoid the area with your pipette tip
- 19.** Add **300 ul** of 70% ethanol softly, taking care not to dislodge the pellet and invert several times to wash the pellet
- 20.** Centrifuge for **1 min** at 13,000 – 16, 000
- 21.** Discard the supernatant carefully making sure to remove as much as possible without touching the pellet. Allow to air dry but be sure not to over dry the pellet either
 - a. If a large amount of ethanol gets on the walls of the tube you may centrifuge the sample again
 - b. The pellet is dry when it is either entirely clear or clear on the edges – don't over dry!
 - c. They dry quickly, if you are processing a large amount of samples the first may be dry before you finish the last, check back
- 22.** Add **21 ul** of DNA Hydration Solution and vortex for 5 s at medium speed to mix
- 23.** Incubate the samples for about a week at room temperature on a shaking platform. You can also put them at **65** for an hour.
- 24.** Vortex the samples briefly and spin them down
- 25.** Nanodrop and record the concentration

Store the samples in a labeled freezer box. You may leave them at -20 if need be until the box is full, then transfer to -80 freezer