

DNA purification

1. Resuspend the pellet sample in step 12 in 180 ul of Buffer ATL and add 40 ul of proteinase K. Mix by vortexing (A cocktail of Buffer ATL and proteinase K can be prepared and 220ul of the cocktail can be added to each sample). Repeat this step for all of the samples.
2. Incubate the samples at 56 degrees Celsius for 1 hour.
3. After incubation, transfer the samples to 90 degrees Celsius and incubate for 2 hours.
4. Briefly centrifuge the samples to remove drops from the sides.
5. Add 200 ul of Buffer AL and 200 ul of ethanol to each sample and mix by vortexing after each addition of reagent.
6. Transfer entire sample to a QIAamp MinElute spin column placed in a 2 ml collection tube. Close the lid and centrifuge for 1 minute at 8000 x g. Discard the collection tube with flow through.
7. Place the QIAamp MinElute spin column in a new 2 ml collection tube. Add 700 ul of Buffer AW1 to the spin column, close the lid and centrifuge for 15 seconds at 8000 x g to wash the spin column. Discard the flow through.
8. Add 700 ul of Buffer AW2 to the spin column, close the lid and centrifuge for 15 seconds at 8000 x g to wash the spin column. Discard the flow through.
9. Add 700 ul of ethanol (100%) to the spin column, close the lid and centrifuge for 15 seconds at 8000 x g to wash the spin column. Discard the collection tube with flow through.
10. Place the spin column in a new 2 ml collection tube. Open the lid and spin the column by orienting the caps in the opposite direction of the spin direction and keeping spaces open between the columns. Spin the samples at full speed for 5 minutes. Discard the collection tube with flow-through.
11. Place the spin column in a new sterile 1.5 eppendorf tube and add 50 ul of RNase-free water to the spin column membrane. Close lid of the column and let it incubate at room temperature for 1 minute. Centrifuge the columns at full speed for 1 minute to elute the DNA.
12. Take the OD of both the RNA and DNA samples and measure the concentration at 260 nm on the nanodrop.

If the concentration is lower than 50 ng/ul, concentrate the RNA/DNA samples by vacuum centrifugation.