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Protocol: Isolation of genomic DNA - **Midi/Maxi** kits

Purpose:

This protocol is used to extract gDNA from a frozen cell pellet using the **QIAamp DNA Blood Midi Kit** (Qiagen Cat.# 51183) for 5e6 to 3e7 cells or **QIAamp DNA Blood Maxi Kit** (Qiagen Cat.# 51192) for 3e7 to 1e8 cells

Procedure

1. Clean bench area. Set the water bath to 70°C and thaw cell pellets on ice.
2. Label **15/50** mL conical tubes – one for each sample (Do not use DYMO labels as they fade in the hot waterbath). Add **300/1000** ul of QIAGEN Protease to each **15/50** ml tube and add the resuspended, thawed cell pellet.
3. Add PBS to bring the final volume to a total of **3.0/10.0** ml. Vortex completely.
4. Incubate at 70°C for 15-30 minutes, vortexing each conical briefly and shaking vigorously every 10 minutes.
5. Remove the tubes from the bath, vortex and shake the tubes until no pellet traces are visible. Continue to incubate at 70°C if the cells have not dissolved.
6. Add **3.6/12.0** ml Buffer AL to each sample. Mix thoroughly by inverting the tubes 15 times, followed by additional vigorous shaking for at least 1 minute.
7. Incubate at 70°C for 30 minutes, vortexing every 10 minutes.
8. Place a closed Buffer AE bottle on top of a heat block set at 50-60°C for approximately 1-2 hours.
9. Open tubes and add **3.0/10.0** ml ethanol (96-100%) to each sample and mix by inverting the tube 10 times, followed by additional vigorous shaking for 10-20 seconds. Let the foam settle before opening the tube.
10. Pour half of the solution from step 9 onto a QIAamp **Midi/Maxi** column. Close the cap and centrifuge at 3,750 rpm for 3 minutes.
11. Discard the filtrate, and load the remainder of the solution from step 9 onto the column. Close the cap and centrifuge again at 3,750 rpm for 3 minutes.
12. Discard the filtrate, and add **3.0/5.0** mL Buffer AW1 to the column. Centrifuge at 3,750 rpm for 2 minutes.
13. Discard the filtrate, and add **3.0/5.0** ml Buffer AW2 to the column. Centrifuge at 3,750 rpm for 10 minutes and dry the columns (removes all residual EtOH) on the bench top for approximately 30 minutes.
14. Place the column in a QIAamp collection tube, and discard the tube containing the filtrate.

- 15.** Pipet 200/700 ul Buffer AE onto the membrane of the column and close the cap. Incubate at room temperature for 5 minutes, and centrifuge at 3,750 rpm for 5 minutes.
- 16.** Pipet an additional 200/700 ul Buffer AE onto the membrane of the column and close the cap. Incubate at room temperature for 5 minutes, and centrifuge at 3,750 rpm for 10 minutes.
- 17.** Transfer the eluate to a clean eppendorf tube and measure DNA concentration.