BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

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Protocol: Isolation of genomic DNA – Mini kit

Purpose:
This protocol is used to extract gDNA from a frozen cell pellet of less than 5e6 cells using the QIAamp DNA Blood Mini Kit (Qiagen Cat# 51104).

Procedure

1. Clean bench area. Set the water bath to 70^{o}C and thaw cell pellets on ice. (Do not use DYMO labels on tubes as they fade in the hot water bath)
2. After thawing and before incubating the samples, add 1 ul of carrier nucleic acid (yeast RNA 10mg/ml, Thermo Fisher Cat.# AM7118 preferred) for samples consisting of less than 200,000 cells. Carrier RNA is added as a co-precipitant to improve the amount of DNA recovered.
3. Spin down each sample in a bench top centrifuge at the highest speed for 2 minutes, remove supernatant and resuspend the sample in PBS to attain a volume of 270 ul.
4. Add 30 ul QIAGEN Protease to each sample. The final volume should be 300 ul. Vortex completely.
5. If cells remain clumped, incubate at 70^{o}C for 10 to 20 minutes, vortexing every few minutes, otherwise move on to step 6.
6. Add 300 ul Buffer AL once cells have completely dissolved. Mix thoroughly by inverting the tube 10 times, followed by additional vigorous shaking for at least 1 minute.
7. Incubate at 70^{o}C until no longer cloudy, about 10 to 30 min.
8. Place a closed Buffer AE bottle on top of a heat block set at 50-60^{o}C for approximately 1-2 hours.
9. Open tubes and add 300 ul ethanol (96-100%) to each sample and mix by inverting the tube 10 times, followed by additional vigorous shaking (10-20 seconds). Let the foam settle before opening the tube.
10. Add the solution from step 9 onto a QIAamp Mini column. Close the cap and centrifuge at 3,750 rpm for 30 seconds.
11. Discard the filtrate and add 750 ul Buffer AW1 to the column. Centrifuge at 3,750 rpm for 30 seconds.
12. Discard the filtrate and add 750 uL Buffer AW2 to the column. Centrifuge at 3,750 rpm for 30 seconds.

13. Dry the columns (removes all residual EtOH) on the bench top (about 30 minutes).
14. If necessary, dry the outside of the column with KimWipe to remove residual EtOH. Place the column in a clean eppendorf, and discard the tube containing the filtrate.
15. Pipet 75 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 2 minutes.
16. Pipet an additional 75 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 2 minutes, measure DNA concentration.