Gentra® Puregene® (Qiagen®) DNA Isolation

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1. From Lymphoblast Cell Pellets

Note: To prevent degradation of DNA, avoid vigorous vortexing and heating. Vortex using light pulses (except in step 7 where thorough vortexing is key). Do not heat samples except when doing the RNAse A step.

1. Mix cell pellet from 10 ml actively growing cell culture (spun down) with 1.0 ml of Qiagen Cell Lysis Solution. Place the sample in a 2.0 ml microfuge tube.
2. Freeze sample at – 20 ºC, or leave on bench top (for up to 3 or 4 days) until ready to complete the isolation.
3. Add 4.5 µl RNase A solution.
4. Mix by inversion 25 times; incubate at 37ºC for 40 minutes.
5. Cool to room temperature.
6. Add 333 µl of the Qiagen Protein Precipitation Solution.
7. Vortex thoroughly for 20 seconds.
8. Place on ice for 10 minutes.
9. Spin 3 minutes at maximum speed in a 4ºC microfuge tube. Note: This is an important step. If sample is not well separated, vortex well and re-spin. Take care not to get precipitated protein in the DNA fraction.
10. Split supernatant into two separate labeled 1.5 ml tubes, about 750 µl each.
11. Add 750 µl isopropanol to each tube.
12. Invert 50 times to mix.
13. Spin 1 minute at maximum speed in a 4ºC microfuge tube.
14. Discard supernatant.
15. Add 666 µl 70% ethanol to one of the two tubes for each sample.
16. Vortex (lightly pulse).
17. With a pipette tip, transfer all the ethanol, PLUS the pellet, into the second tube for that sample.
18. Vortex (lightly pulse) again.
19. Spin 1 minute at maximum speed.
20. Wash with 666 µl 70% ethanol two more times: pour off EtOH, add new EtOH, briefly vortex until pellet floats up, then re-spin at maximum speed for 1 minute.
21. Last spin: pour off EtOH, re-spin and pipette off remaining EtOH to remove as much as possible. Air dry sample upside down at an angle for 15 minutes. Turn over and dry 10 more minutes, or until all EtOH is gone. Note: Do not allow sample to sit out longer than 30 minutes.
22. Re-suspend in Qiagen's DNA Hydration Solution (DHS). DO NOT VORTEX; simply add the DHS. Note: I usually use 110 µl for a normal 10 ml culture.
23. Store at 4ºC for two days before determining the concentration. Note: Don't heat, as this can contribute to degradation.
24. Pipette up and down a number of times and flick (don't vortex) to assure homogeneity, then measure 1.2 µl on a Nanodrop® spectrophotometer or 2 µl on a Qubit® system. Measure twice. If DNA is too concentrated for your use, or if it doesn't freely pipette, add H_20 to dilute to the range you want or so DNA can be pipetted.
25. The sample is ready for storage when you have two readings that are consistent (within about 25-30 ng/µl of each other). If not homogeneous, pipette a few more times, let sit another day and pipette to mix again.
26. If not processed right away, wait two days and perform Qubit measurement of sample in duplicate to test.
27. For each sample, record the sample ID, the concentration, and the ratio of RNA/DNA (taken from both Qubit and Nanodrop systems).

2. From Fibroblast Cell Pellets

Note: To prevent degradation of DNA, avoid vigorous vortexing and heating. Vortex using light pulses in all cases. Do not heat samples except when doing the RNAse A step.

1. It is best to harvest DNA when fibroblasts are 70-80% confluent, or about 3 X 106 cells. This is roughly equivalent to 8-10 ml of lymphoblasts. For example, CHM1H tert fibroblasts are about 3.8-5 X 10^6 cells per flask.
2. Take 1 or more flasks of fibroblasts, pipette off media and wash cell layer with 3 ml each of Phosphate-Buffered Saline (PBS).
3. Pipette off PBS and add 3 ml 0.25% trypsin-EDTA. Put in incubator at 37ºC for 5 minutes.
4. Check cells to make sure they are freely floating. Note: this is important; cells should not be in sheets. Add 8 ml media to each flask to deactivate trypsin, pipette well to mix, then pipette into 15 ml tube. If you are preparing multiple flasks, put each in a separate 15 ml tube.
5. Spin down at 1200 rpm for 5 minute.
6. Note: To count cells, bring back up in 2-5 ml media (amnio-max + complement + ab for tert mol) and count a 100-fold dilution of cells, or 20 µl in 1980 µl PBS.
7. Count cells and spin back down.
8. After spin, pour off supernatant, leaving 200 µl media in tube. Note: this is what will remain with a simple pour off.
9. Flick pellets well to resuspend.
10. Pulse vortex cells lightly using setting 7.
11. Add 1 ml cell lysis solution to each 15 ml tube.
12. Pipette up and down well to insure cells are lysed (light pulse vortex at setting 7 if necessary, but it is best to limit vortexing, which can damage DNA).
13. If not totally lysed, leave on bench for an hour (or longer until cells are no longer thick or chunky), pipette up and down. Do not heat. If still not totally lysed, you may try limited pulse vortexing at setting 7.
14. Transfer lysed cells to 2.0 ml centrifuge tubes.
15. Proceed from here with standard protocol (starting at step 3 above, copied below).
16. Add 7 µl RNase A solution and perform Lymphoblast procedure starting with step 4 on page 1.