Standard operating procedure to isolate genomic DNA from blastocysts

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DNA isolation

Samples

- frozen blastocysts

Equipment

- Centrifuge and benchtop centrifuge
- Qubit
- Pipets
- Stand
- Styrofoam box with ice
- Heating block

Plastics

- RNase/DNase-free tubes
- Pipet tips

Kits & Chemicals

- Quick-DNA Microprep Plus Kit chemicals
- Proteinase K from Quick-DNA kit.
- Qubit dsDNA HS Assay Kit

Preparation

- Pre-heat the heating block to 55 °C
- Dissolve the neophylised 5 mg Proteinase K in 260 µl Proteinase K Buffer from the kit to a concentration of 20 mg/ml
- Transfer some DNA Elution Buffer into a 1.5 ml tube for better preheating in the heating block

Procedure

1. Resuspend the blastocysts with 50 µl DNA Elution Buffer and transfer to a new 1.5 ml tube.
2. Add 50 µl BioFluid & Cell Buffer (Red) to the suspension. Also flush the 0.2 ml cell storage tube with the buffer.
3. Add 5 µl Proteinase K and vortex the tube for 10 to 15 seconds.
4. Incubate the mix at 55 °C in the heating block for 10 minutes.
5. Preheat the DNA Elution Buffer to 65 °C in the heating block.
6. Add one volume 105 µl Genomic Binding Buffer and vortex 10 to 15 seconds.
7. Centrifuge briefly.
8. Transfer the lysat to the Zymo Spin IC-XM Column and centrifuge at 12.000 g for 5 minutes at room temperature.
9. Discard the flowthrough and transfer the column in a new collection tube.

10. Add 200 µl DNA Pre-Wash Buffer to the column and centrifuge at 12.000 x g for 1 minute at room temperature.
11. Discard the flowthrough and transfer the column in a new collection tube.
12. Add 700 µl g-DNA Wash Buffer and centrifuge at 12.000 x g for 1 minute at room temperature.
13. Discard the flowthrough and transfer the column in a new collection tube.
14. Add 200 µl g-DNA Wash Buffer and centrifuge at 12.000 x g for 1 minute at room temperature.
15. Discard the flowthrough and transfer the column in a clean 1.5 ml tube.
16. Pipette 11 µl of 65 °C warm elution buffer directly onto the column matrix without touching the matrix.
17. Incubate for 5 minutes at room temperature.
18. Centrifuge at full speed for 1 minute.
19. Tansfer the eluate one more time to the column matrix.
20. Incubate for 3 minutes at room temperature.
21. Centrifuge at full speed for 1 minute at room temperature.

For storage keep the samples at – 20 °C. Use the Qubit dsDNA HS Assay Kit to determine the DNA concentration with Qubit.