Transfection protocol for shRNA transfection in K562 cells

By using Ingenio® Electroporation Kit from Mirus Bio LLC

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Preparation

1. Subculture cells 1or 2 days before transfection. Use cell passage number 8-11 only.
2. Each cuvette will need 1X10^6 cells, 5 ug plasmid DNA, 100 100 µl Ingenio electroporation solution.

Transfection

1. Prepare 12-well plates by filling 1.5ml of culture media and pre-incubate at 37˚C
2. Determine cell density and spin down cells.
3. Wash once in PBS to remove media and serum.
4. While washing, turn on nucleofector and open and label cuvettes.
5. Resuspend washed cells in 100 µl Ingenio Electroporation solution.
6. Combine 100 µl of cell suspension with 5 µg plasmid DNA
7. Transfer cell/DNA suspension into cuvette (sample must cover the bottom of the cuvette without air bubbles)
8. Nucleofect program T-016
9. Immediately add 0.5 ml of K562 media and transfer to 12-well plate
10. Isolate total RNA after 3days post transfection.