SBI System Biosciences
265 North Whisman Road, Mountain View, CA 94043
Tel: (650) 968-2200
Fax: (650) 968-2277
www.systembio.com

mRNAExpress and RNAFection Protocol

Transfection of mRNA Transcripts for Reprogramming

The following protocol is optimized for transfecting human fibroblast cells in 6-well plate. Other cell types or other formats should be adjusted to achieve optimal cell density for transfections.

1. The day before transfection, seed human fibroblasts in 6-well plate at the density of 2×10^4 per well in fibroblast medium. Incubate cells overnight in 37°C incubator.
2. 2 hour before transfection, aspirate old medium and add 2 mL fresh fibroblast medium with B18R at 200ng/mL.
3. To prepare reprogramming cocktail, thaw the individual vials of modified mRNA transcripts on ice and dilute them with sterile RNase-free buffer. The mRNA reprogramming cocktail is recommended at a molar stoichiometry of 3:1:1:1:1:1 for Oct4, Sox2, Klf4, c-Myc, Lin28 and GFP mRNAs. (The volumetric ratio is 42:13:17:16:9:12 for Oct4, Sox2, Klf4, c-Myc, Lin28 and GFP.)* Mix the cocktail solution well and aliquot them to the amount of a single tranfection event. Store the aliquots in -20°C freezer and avoid repeated freeze/thaw cycles. (Gloves should always be worn when handling mRNA to keep it away from RNase.)
4. Warm RNAfection and OptiMEM to room temperature.
5. For each well of 6-well plate, dilute 2 µg of mRNA cocktail to 100 µl in a sterile RNase-free Eppendoff tube with OptiMEM, and mix by tapping the bottom of the tube gently.
6. Briefly vortex RNAFection immediately before use and centrifuge shortly. Dilute 4 µl of RNAFection reagent to 100 µl in a sterile RNase-free Eppendorff tube with OptiMEM, and mix by tapping the bottom of the tube gently.
7. Add the RNAFection/OptiMEM solution to the mRNA/OptiMEM solution. Vortex for 5-10 seconds and spin down briefly to bring the mixed solution to the bottom of the tube.
8. Incubate at room temperature for 15 minutes to allow RNAFection/mRNA complexes to form.
9. Add the 200 µl RNAFection/mRNA/OptiMEM mix drop-wise to human fibroblasts and gently rock the plate back and forth.
10. 5 hours after transfection, aspirate the culture medium containing the mRNA transfection complex. Add 2mL fresh medium with B18R at 200ng/mL. Incubate in 37°C incubator overnight.
11. Repeat the transfection daily for up to 18 times. Switch to MEF-conditioned human ES cell medium after the 6^{th} transfection.

12. After up to 18 daily transfections, incubate the cells for 3 or more days to allow the colonies to form. Replace medium daily without supplementing B18R.

13. After 21 days, pick colonies for expansion and analysis.

    *Different combinations of reprogramming factors at different ratios can be used for different source cells.

Fibroblast Medium: DMEM containing 10% FBS

Human ES Cell Medium: Knockout DMEM/F12 containing 20% Knockout Serum Replacement, 2mM Glutamine, 0.1 M Nonessential Amino Acids, 0.1 M 2-Mercaptoethanol, 10 ng/mL bFGF, 50 U/mL and 50 µg/mL Penicillin and Streptomycin.