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Feeder-Free Reprogramming of Human Fibroblasts

General Points

1. Use a low-oxygen tissue culture incubator set for physiological (5%) O_2 tension.
2. Equilibrate basal media to 37°C, 5% CO_2, 5% O_2 in the incubator for 2–24 hours before use.
3. Growth media may be supplemented with antibiotics (penicillin-streptomycin).
4. Add protein supplements and transfection cocktails to basal media immediately before use.
5. Store Pluriton Supplement and B18R aliquots at -70 degrees. Thaw aliquots in a fridge or on ice before addition to media. Do not re-freeze or re-use aliquots.

Protocol (6-Well Plate Format)

Day -1: Fibroblast Plating

Coat tissue culture plates with CELLStart substrate per the manufacturer's directions, then seed fibroblasts in 2 mL Pluriton basal media plus Pluriton Supplement and B18R (200 ng/ml) interferon inhibitor per well. See the notes section below for suggested fibroblast seeding densities.

Days 0-8: mRNA Transfection

Prepare 6-factor mRNA premix for transfection by diluting the 100 ng/uL stock 10X into the Stemfect buffer. Add 4 uL Stemfect reagent per ug of RNA. Mix gently and incubate the transfection cocktail for 15 minutes at room temperature. Dilute the transfection cocktail 25X into equilibrated culture media to give a final media RNA concentration of 400 ng/mL. Add Pluriton Supplement and B18R and replace old media on the cells with the freshly-supplemented media. Use 0.5 mL of mRNA-supplemented media per well on Day 0, 1 mL on Day 1, 1.5 mL on Day 2, and 2 mL per well on Days 3-8.

Day 9 et seq: iPSC Recovery

Continue to change media on the reprogramming cultures daily, but discontinue addition of the reprogramming cocktail and B18R interferon inhibitor. Pick iPSC colonies to fresh plates using your hESC culture system of choice when they are of sufficient size (typically, around Day 14).

Materials

6-Well Tissue Culture Plates (Becton Dickinson Falcon 3046)
CELLstart CTS Xeno-Free Substrate (Life Technologies A10142)
Pluriton Reprogramming Medium (Stemgent 00-0070)
B18R Recombinant Protein, Carrier-Free (eBioscience 34-8185)
6F mRNA Premix (Allele Biotechnology ABP-SC-6FMRNA)
Stemfect RNA Transfection Kit (Stemgent 00-0069)

Notes

1. Use of ambient (20%) oxygen tension instead of the recommended 5% O_2 condition will significantly reduce the performance and robustness of reprogramming.

2. The B18R product referenced above is supplied at a concentration of 0.5 mg/mL, or 2500X the desired final media concentration (200 ng/mL). When using this format, the master stock can be aliquoted directly without dilution or the use of carrier. Avoid unnecessary freeze-thaw cycles.

3. Reprogramming performance is sharply dependent on the starting cell density. We recommend plating three reprogramming wells with 50K, 75K and 100K cells as a good starting point. If cultures fail to reach confluence during reprogramming, the starting density is too low. If confluence is reached within the first few days, the density is too high. The highest productivity will be achieved when cells reach confluence around day 7 of the reprogramming regime.

4. Once assembled, Stemfect/RNA cocktails are stable enough that they can be refrigerated for ~48 hours before delivery to cells without noticeable performance degradation. It is therefore possible to prepare cocktail for three days of transfection at a time. Incubate Stemfect/RNA cocktails for 15 minutes at room temperature before and after refrigeration. The colloidal stability of RNA/vehicle complexes is specific to the Stemfect reagent. The transfection cocktails have limited stability once added to media, so RNA-supplemented media should be applied to cells immediately.

5. Addition of an extra 150 uL of media per well can be used to prevent the dehydration sometimes seen in the center of the wells when using the recommended 0.5 mL Day 0 media volume.

6. Keep the media replacement/transfection interval as close as possible to a constant 24 hours.

7. Transfection may be continued beyond 9 days if desired. This may increase the yield of colonies, particularly in moderately overgrown cultures. Colonies can often be obtained after as little as six days of transfection, but with much reduced yields.