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Fugene 6 transfection of P19 cells

1. Plate out 1x105 cells per well of a 12-well plate the day before transfection. Plate the cells such that you have 1 ml of media covering the cells.

2. Prepare the following mixes:

DNA:

mix 1.5 μg of DNA of interest (can be a mix of several plasmids) in TE to a final volume of no more than 20 μl. You can do this at your bench-top, but try to use clean plastics.

Fugene 6:

Mix 3 μl of Fugene into 47 μl of serum-free media (I have been using α-MEM for P19 cells). I mix this in the TC hood in eppendorf tubes. Note that the Fugene is suspended in ethanol and stored at -20°C. You should let it equilibrate to room temperature and briefly mix and spin it before using. Also be aware that concentrated Fugene will stick to plastics, so the only plastic that you want it to come in contact with is the pipet tip used to add it to the serum-free media.

3. Add the DNA mix to the Fugene mix, tap gently and let sit at room temperature for 15-45 minutes.

4. Add the DNA/Fugene mix to your cells dropwise, trying to put it on in several different places in the well. Shake the plate to spread it some more.

5. Incubate cells for 1-2 days in the original media that you were growing the cells in. No changes are necessary.

P19 cells (ATCC 1825) culture media	Per 100 ml
Alpha minimum essential medium	87 ml
1x nucleosides	1 ml of 100x stock
7.5% bovine calf serum	7.5 ml
2.5% fetal bovine serum	2.5 ml
1xP/S	1 of 100x stock
1xQ	1 of 100x stock

Freeze medium:
Complete growth medium, 95%; DMSO, 5%

Subculturing:
Do not allow the cells to become confluent.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation ratio: A subcultivation ratio of 1:10 every 2 to 3 days is recommended

Medium renewal: Add fresh medium at least every 48 hours