Gozani Lab
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Retroviral generation of stable cell lines

I. Generate retrovirus

Day 1
1. Transfect 293T cells (40-60% confluent) with 3 plasmids: 9 μg of retroviral plasmid (e.g. pBABE-ING2), 4.5 μg of GAG-POL plasmid and 4.5 μg of VSV plasmid using Transit293 transfection reagent.

Day 2
2. (Optional) The next day, change the medium, but only replace 4-5 ml DMEM-10% FCS (to keep virus concentrated). The medium change is required to remove a growth inhibitory factor produced by 293 cells upon transfection.

Day 3
3. 2 days after tranfection, collect the supernatant (medium) with a 10 ml syringe, and add 4-5 ml fresh DMED.
4. Filter the medium through **0.45μm** filter.
5. Aliquote 2 ml each and freeze @ -80oC or use fresh.

Day 4
6. The next day, harvest the virus as day 3.

II. Transduction

Day 3
1. Plate of HT1080 cells should be around 40-50% confluent (MMLV based retrovirus cannot enter non-dividing cells).
2. For a 10-cm dish, remove media and add 6 ml of new medium containing 64 μg Polybrene (16 μl of stock solution 4 mg/ml at -20oC).
3. Thaw the virus in the medium at 37oC quickly, and add 2ml into each plate (final polybrene conc. is 8 μg/ml).
4. After 4-6 hrs, add 4 more mls media.

Day 4
5. After 24 hrs, change media/and split if necessary.

III. Stable line selection

Day 5
1. 24 hrs after the first media change (2 days after transduction), start selection with proper drugs (2 μg/ml puromycin (1 to 1000 dilution from 2 mg/ml stock) for HT1080, may vary for different cell types).
   (1) Split cells if necessary, cells need to be dividing for puro to be active.
   (2) Puro can be added 24 hrs or 48 hrs after transduction.
   (3) 1 mock tranfected plate can be used to ensure that the puro did its job.

Day 6-8
2. Change media every 1-2 days/split cells when necessary.
3. In 2-3 days all non-transduced cells should have been killed and you have your stable population of cells.