GENERATION OF RETROVIRAL VECTOR PACKAGING CELL LINES BY SHUTTLE VECTORS

Francesco Marangoni January 13, 2010

The gist of this protocol is to generate a small batch of VSVg-pseudotyped retroviral vector by transient transfection. This small batch, henceforth called "shuttling vector", can infect human cells because it is VSVg pseudotyped, and will be used to infect human-derived Platinum-E cells to make them stably produce retroviruses. NOTE: the final packaging cell lines will produce retroviruses pseudotyped with ecotropic envelope that will be thus only able to infect mouse cells.

MATERIALS

DMEM + 10% FCS
Platinum-E cells – these cells are derivatives of 293T, stably expressing retroviral gag/pol and ecotropic env. ATTENTION: never let them overgrow, as they change phenotype
Lipofectamine 2000
OptiMem
Plasmids:
- Retroviral construct
- VSVg encoding plasmid

METHOD

Day 1:

1. Target cell culture.
    a. Per each packaging cell line to be generated, seed Plat-E cells $5x10^4$ per well of a 6-well plate in 2ml DMEM + 10% FCS.
2. Shuttling vector preparation.
    a. Per each packaging cell line to be generated, seed Plat-E cells $1.5x10^6$ per well of a 6-well plate in 1.2ml DMEM + 10% FCS.
    b. 8 hours later, transfected by Lipofectamine:
        i. Mixed 57.6ul OptiMem + 3.5ul Lipofectamine (dropwise)
        ii. Diluted 1.6ug vector plasmid and 300ng VsVg plasmid in 61.1ul OptiMem;
        iii. Mixed 61.1ul of both mixtures dropwise and incubated RT 5';
        iv. Added onto cells.

Day 2

Shuttling vector preparation.
    a. Change medium using 0.9ml DMEM + 10% FCS per well

Day 3

1. Harvest 0.9ml of shuttling vector from producer cells and add new 0.9ml of medium on the cells.
2. Substitute the medium of one well of target cells with the pure shuttling vector.
3. **PRO TIP** Spinfection greatly improves transduction rates. Centrifuge at 1000g for 90 minutes at 32C
4. Add 2ml fresh medium on top and put in the incubator.

Day 4

Repeat day 3

Day 5 or 6
Before the cells reach confluence, split them and sort the ones expressing the retroviral construct. Sorting may occur by FACS (e.g. GFP) or MACS (e.g. DLNGFR). ATTENTION: selection by puromycin or blasticydin does not work because Plat-E are resistant.

After a few days, repeat selection as needed to reach retroviral construct expression in 100% of cells. You now have your Plat-E based packaging cell line.