Lentiviral Production and Transduction Procedure

I. Plate HEK cells

a. Coat plate with Poly-L-Lysine
    i. Remove excess and allow plate to dry
b. Seed plate with HEK cells (in D10)
    i. $18x10^6$ cells / 15cm dia. Dish
    ii. Incubate overnight, 37C
    iii. Plate should be 70%<x<90% confluent before transfection

II. Transfection

a. Prepare DNA in serum-free media
    i. 20ug/15cm plate: **packaging signal** (PCDNLBH) and 20ug/15cm **vector DNA**
    ii. 5ug/15cm plate: **packaging envelope** pMN-VSV-G (or 1.5-2ug/15cm plate EO1-Foamy) for lentivirus
b. Prepare Lipofectamine in serum-free media, incubate 5min @ RT
    i. Add Lipofectamine dropwise and swirl gently
        1. 80ul/15cm plate
c. Combine DNA and Lipofectamine solutions and incubate 30min @ RT
d. Remove media from HEK cells (just before end of incubation)
e. After incubation period, add mixed solution to HEK cells + 10ml serum-free media for 1-4hrs @ 37C
f. Add 15ml growth media and incubate overnight @37C
g. Change to growth media with 20ul .5M sodium butyrate/ml media, incubate 6-8hrs
h. Change to growth media, incubate 18-24hrs.

D10 (growth media)	DMEM, 10% FBS, 1% Pen/Strep, 1% Sodium Pyruvate, 1% Non Essential Amino Acids

III. Virus Harvest

a. Lenti Virus:
    i. Remove and filter media from HEK cell plates
        1. Spin down media prior to filtering to pellet dead cell debris
            a. 400 RCF
            b. 5 min
        2. Use .45 vacuum filter
    ii. Using *Centricons* spin down virus media and collect in filter
        1. Max 60 ml in *Centricons*
        2. 3000g for 30 minutes
        3. Repeat with any other media
    iii. Remove virus from filter
        1. remove *Centricons* filter apparatus
        2. turn upside down

3. attach to conical collection dish
4. re-spin 1000g for 15min(or 10min)

iv. Collect virus
1. Remove virus from conical collection tube
2. Determine M.O.I.

v. Freeze @ -80°C

b. Retro Virus:
i. Remove virus media from HEK cell plates.
ii. Centrifuge to remove cell debris
iii. Filter supernatant with .45 vacuum filter
iv. Determine M.O.I.
v. Flash freeze with Liquid N_2
vi. Store @ -80°C

IV. Retronectin Transduction

a. Dissolve Retronectin lyophilized powder in sterile water to a concentration of 1mg/ml by gentle swirling (do not vortex) and filtrate through 0.22µm filter (Millipore Millex GV).
b. Store solution in aliquots at –20°C (can be stored for one year).
c. Coating:
i. Use only non-tissue culture-treated six-well plates (Becton Dickinson 351146, polystyrene) otherwise it won't work.
d. Dilute the 1mg/ml stock solution of Retronectin to a concentration of 25-50µg/ml in sterile PBS.
e. Dispense 2ml of the diluted Retronectin into each well and incubate the covered plate for 2 hours at room temperature (or at 4°C overnight).
f. Remove Retronectin solution and block each plate with 2 ml PBS (containing 2% BSA) for 30 minutes at RT.
g. Remove PBS (+2% BSA) and wash the wells once with 2 ml PBS. (The coated plate can be stored at 4 °C for one week)
h. Remove the PBS and add virus supernatant (add growth media if needed to just cover surface of well)
i. Incubate virus on retronectin for 15 min
j. Add $1x10^6$ Cells /cm^2 in each well with growth medium and centrifuge for 30 min with 600xg at 32°C.
k. Incubate the cell for 2-days in the incubator (32-37°C).