**XtremeGeneHP Transfection for Lentivirus Production**

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**Day 0:**

1. Seed the HEK293 cells 12-16hr later before transfection in antibiotic-free media (“Transfection media” = DMEM + 5.5mL 100x HEPES + 10% FBS)
   1. 6-well plate: 1.5-2 million cells/well
   2. 15-cm plate: split ½ from a confluent 15-cm plate

**Day 1:**

1. Prepare *two* tubes for *each* transfection:

- Tube A: 4 uL of XtremeGene HP reagent in 200 uL of pre-warmed Opti-Mem

- Tube B: 4.1 ug DNA

In this case, the DNA:XtremeGene ratio = 1:1 (ug/uL).

2) Preparing tube A:  
🡪 Using dropwise technique to add XtremeGene into OptiMEM (not vice versa!)  
🡪 Wait at RT for 5 minutes (not more than 25 mins before mixing with tube B)

3) Preparing tube B:

|  |  |  |
| --- | --- | --- |
| **Tube B** | **%vol** | **6-well plate well** |
| DNA |  | 4.1 ug |
| - Tranfection plasmid | 50% | 2 ug |
| - Lentivirus env+enz | 50% |  |
| - psPAX2 | 75% | 1.5 ng |
| - pMD2.G | 25% | 0.6 ng |
|  |  |  |

1. Slowly add the DNA (tube B) into the XtremeGene+OptiMEM (Tube A) drop by drop, then gently mix immediately
2. Incubate the mixture for exactly 30 minutes at room temp
3. Add mixture (200 uL total/well into 6-well plate), mix by shaking the plate forward and backward, then side to side (not swirling!)
4. Put the plates/dishes back to 37°C incubator with 5% CO2, in the lenti room O/N
5. If you need high titer, leave until 48 hours to collect.
6. Pool Day 1 and 2 collections.
7. Filter .45 um PVDF or PES filter
8. Storage
   * Temporary storage at 4C (stable up to 2 weeks) (drop 10% per week)
   * Long-term storage at -80C Eppendorf 1.5mL (RECOMMENDED)

**TRANSDUCTION into target cell**

Day 0 (24 hours before transduction):

1) Seed cancer cells in 6-well at 1,000,000 cells/well

Day 1:

1) Aspirate old media from seeded 6-well cancer cell plate

- Seed 1,000,000 cells at 24 hours beforehand

2) Add in 500-750 uL filtered virus media

+ Polybrene (1: 1000 dilution in the media at final concentration of 8 ug/mL) at 1.50-1.75-mL per well in a 6-well plate

\*Polybrene stock 8 mg/mL

3) Incubate in lenti room for 24 hours

Day 2:

1) Aspirate virus media

2) Replenish new media without puro

Day 3:

1) Add in puromycin-containing media (Stock 10 mg/ mL 🡪 5 ug/mL) to start selection

Equation: 10000 ug/mL \* Vi (mL)= (5 ug/mL) \* Vf (mL)

e.g., 5 uL taken from stock to make 10 mL media

Day 6:

1) Change old media with new puromycin-containing media (same puro concentration 5 ug/mL)

a) HT29: wait 3 Days

b) HT115: wait

Day 10+:

1) Change into 6-cm dish with no-puro media to let them grow 🡪 This becomes a stable cell line

2) If enough cells, split cells for flow, RT-qPCR, Western and frozen cell stock