**General Lipofectamine2000 Transfection**

Edited by Pornlada & Paul

**Day 0:**

1. Seed the HEK293 cells to be 70-90% confluency 24hr later before transfection in antibiotic-free media (“Transfection media”)
   1. 6-well plate: 1.5 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: 3 uL of XtremeGene HP reagent in 100 uL of pre-warmed Opti-Mem

Tube B: 1.5 ug DNA

In this case, the DNA:XtremeGene ratio = 1:2 (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 |  | 6-well plate well |
| Opti-MEM |  | 100 uL |
| DNA |  | 1.5 ug |
| - Tranfection plasmid | 50% | 0.75 ug |
| - Lentivirus env+enz | 50% |  |
| - psPAX2 | 75% | 562.5 ng |
| - pMD2.G | 25% | 187.5 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 20 minutes at room temp
3. Add mixture (100 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 filter
8. Storage
   * Temporary storage at 4C (stable up to 2 weeks) (drop 10% per week)
   * Long-term storage at -80C Eppendorf 1.5mL

**TRANSDUCTION into target cell**

Day 0 (24 hours before transduction):

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

2) Split

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 48 hours

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 2-3? 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

**OLD PROTOCOL**

**Day 1 XTREMEGENE**

MM per rxn: 3 uL of XtremeGene in 100 uL of Opti-Mem

PCR Tube A: 1 ug DNA in 20 ul of Opti-Mem

In this case, the DNA/XTREMEGENE ratio is 1:3 (ug/uL).

|  |  |  |
| --- | --- | --- |
| DNA |  | 1 ug |
| - Tranfection plasmid | 50% | 0.5 ug |
| - Lentivirus env+enz | 50% |  |
| - psPAX2 | 75% | 0.375 ug |
| - pMD2.G | 25% | 0.125 ug |

Put the plates/dishes back to 37°C incubator with 5% CO2, in the lenti room

Do not replace media after addition of XtremeGene+DNA.

**Day 2,3:**

1. Incubate 24 hr in Lenti room before first harvest. Incubate 36-40 hr before second harvest.
   1. Pool
   2. Temporary storage at 4C.
   3. Long-term storage at -80C Eppendorf 1.5mL
2. Optional steps to remove cell debris:
   1. Centrifuge viral supernatant at 500 g x 5 mins to pellet any packaging cells
   2. Filter supernatant through a 0.45 um PES filter (optional)
3. Viral supernatant can be stored at 4\*C for a few days but should be snap frozen in liquid nitrogen and stored at -80\*C

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