Preparation of Retroviral Supernatant (PEIMax) Ver.4

Preparation of the packaging cells (PLAT-E)

Aspirate the culture medium, rinse the PLAT-E cells with 10mL PBS, add 1mL Trypsin.

↓
↓ Incubate at 37 oC for 1min
↓

Add 10mL -P/S 10%FCS DMEM and harvest the cells gently (without bubbles).

↓

Seed PLAT-E to 6-well plates.

PLAT-E	2.5-3.0x10^6/well
-P/S 10%FCS DMEM	1.5 ml/well

↓
↓ Incubate at 37 oC for 3hrs
↓

Lipofection with PEIMax

After 3h-culture, PLAT-E will be in 80% confluent

↓

Prepare (A) and (B).

(A)

(pME18S-VSVG	1 μg[1])
Plasmid	4 μg
Serum-free OPTI-MEM	250 μl

If an amphotropic retroviral receptor is required, add 1μg/well of pME18S-VSVG (Plasmid #2453) to (A).

(B)

PEIMax[2]	10 μl
Serum-free OPTI-MEM	250 μl

↓
↓ Incubate at RT for 5 min.
↓

Mix (A) and (B) and Vortex.

↓
↓ Incubate at RT for 20 min
↓

Drop the mixture (A) and (B) to the dish gently with P1000-Pipetman.
Final volume will be 2ml/well in a 6-well plate.

↓
↓ Incubate at 37 oC for more than 12h or o/n
↓

Collect the retroviral supernatants

Aspirate the medium and add 8-12 ml of antibiotics-free assay media required by the cells to be infected.

↓
↓ Incubate at 37 oC for 24 h.
↓

Collect the supernatant containing virus (day1 viral sup.) and add more 8-12 ml antibiotics-free assay media.

↓
↓ Incubate at 37 oC for 24 h.
↓

Collect the supernatant (day2 viral sup.).

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[1] 必要に応じてパントロピック受容体である VSVG を加える。

[2] Polyethyleneimie “MAX” (MW 40,000) Polysciences Inc. Cat# 24765
PEI MAX is soleved by DDW (2mg/ml), filtrate, and stored at 4oC.

Concentration of the viral supernatants

Centrifuge the supernatants containing virus at 8000g 4oC for 12-16 h

Remove 90 % of the upper layer of the supernatant.

Stir the bottom 10% supernatant and use it to the infection.

If the viral sup is frozen, the infection efficiency will be decreased by 30 %.

If the viral sup is kept on ice, the infection efficiency will be decreased by 30 %.

The packaging cells produce the same amount of virus at least for 4 days.