Standard Operating Procedure
for
LENTIVIRUS EXPRESSION

1.0 PURPOSE

This procedure describes how to produce Lentivirus.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who wish to generate Lentivirus.

3.0 PROCEDURE

1. Seed 293 FT cells (P3<passages<P16) to a 10-cm dishes (5-6 $\times 10^6$ /10 cm dish), culture them overnight in **DMEM Medium I** (10% FBS (not heat- inactivated) ; 6 mM L-glutamin; 0.1 mM MEM nonessential amino acids; DMEM medium, 1 mM MEM Sodium Pyruvate, 1% Pen-Strp).
2. Change the medium with 6 ml **DMEM Medium II** (10% FBS (not heat- inactivated) ; 6 mM L-glutamin; 0.1 mM MEM nonessential amino acids; DMEM medium, 1 mM MEM Sodium Pyruvate) 2 hours before infection.
3. FUW- Plasmid 10 µg
   PspAX 7.5 µg
   PMD2.G 2.5 µg
   Dilute these DNA into 1.5 ml Opti-MEM medium without serum in a 50-ml tube.
4. Dilute 45 µl Lipofectamine™ 2000 into 1.5 ml Opti-MEM medium without serum in a 50-ml tube, incubate for 5 min at room temperature.
5. After the 5 minute incubation, transfer the diluted Lipofectamine™ 2000 from step 4 into the diluted DNA from step 3. Mix gently, incubate for 20 min at room temperature to allow DNA-Lipofectamine™ 2000 complexes to form.
6. Add the DNA- Lipofectamine™ 2000 complexes directly into the plates of cells and mix gently by rocking the plate back and forth.
7. Culture the cells overnight and change the medium with 10 ml **DMEM Medium I**. if the virus need concentration, 1% FBS can be used in **DMEM Medium I**, but 10% FBS is better.
8. After 12 hours after infection, collect virus and do it again at 24 and 36 hours after infection (3 times in total). Put virus on ice after collection and mix all the viral supernatants.
9. Centrifuge supernatants at 3000 rpm for 15 minutes at 4 °C, filter the viral supernatants through 0.45 µM PVDF filter.
10. Pipet viral supernatants into cryovial in 1-2 ml aliquots. Store viral stocks at -80 °C.

Note: all the used things should be put into 10% bleach overnight and sterilize them in an autoclave.

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