Material and Method - Transfection

【Purpose】

Plasmid which was increased by mass culturing of the transformed E. coli is introduced into 293 T cells by lipofection. As a result, we aim to harvest virus-like bodies from 293 T cells.

【Reagents】

10 cm dish (the scaffold used by Tagawa Lab), replicon plasmid, capsid plasmid,
preME plasmid, Polyethylenimine (PEI), 1 M HEPES, FBS, DMEM, opti-MEM
MEM non-essential amino acid solution

【Day 0】

Put 293T cells in 10 cm dish. Because many virus-like bodies appear at about 80% conflict, at this time adjust to 20% confluent and 80% at Day 3. However, if it is too small, it may be annihilated, so be careful. Then incubate at 37 degrees.

* Media Composition DMEM 500 ml, FBS 50 ml, L-glutamine 5 ml

【Day 1】

1. Mix the reagents in Table 1.

Table 1. Reagent and Preparation

Reagent	Concentration	Amount
Opti-MEM	-	1ml
Replicon plasmid	1μg/μl	2.5μl
Capsid plasmid	1μg/μl	1.25μl
preME plasmid	1μg/μl	1.25μl
PEI	-	25μl

2. Standing for 15 minutes
3. Mix it to Dish of Day 0.
4. Incubate 5-6 hours at 37 degrees
5. Change of medium
6. Incubate at 37 degrees

【Day 3】

Media exchange. (Add 100 × 1 M HEPES and MEM non-essential amino acid solution to the culture medium used to date)

【Day 4】

1. Take culture supernatant containing virus like body.
2. Filter the resulting supernatant through a 0.45 μm filter.
3. Save at -80 degrees