Material and Method - Construction

Final Products

- pCAGGS_DV1_C
- pCAGGS_DV1_prME
- Replicon (Transferred from National Institute of Infectious Diseases)

Procedures

1. Transformation into DH5α (using competent cells of Takara Bio)
2. Incubate for 16 hours 37 degrees on LB agar medium with ampicillin
3. Select colonies and transfer to master plate. At the same time transfer to ampicillin-containing circle glow 3 ml, shake incubation for 16 h at 37 ° 135 rpm.
4. Shake cultured E. coli solution with Fujifilm QuickGene Plasmids Kit mini prep
5. Electrophorese the solution obtained by mini prep to confirm the presence of plasmid. * 1
6. Once the plasmid was confirmed, transfer the E. coli from the colony of the master plate to 200 ml of ampicillin-containing circle glow and shake cultured at 37 ° C and 135 rpm for 16 hours. * 2
7. Shake cultured E. coli solution with QIAGEN Plusmid plus Maxi Kit large prep

At this point, pCAGGS_DV1_C, pCAGGS_DV1_prME allowed transfection to HEK293T cells. The following work is done only on the replicon.

8. Concentrate the solution obtained with large prep by ethanol precipitation.
9. Since this replicon contains nano luc instead of fluorescent protein gene, it is cut out with two kinds of restriction enzymes. At the same time, DNA containing the fluorescent protein gene ordered to the IDT is cut with two kinds of the same restriction enzymes.
10. Electrophorese the cut plasmid and confirm that the plasmid is divided into two.
11. Among the two separated plasmids, only the vector is extracted from the gel using the Fast Gene Gel / PCR Exatraction Kit from NIPPON Genetics.
12. DNA containing the fluorescence protein gene cut with the gel extracted vector and restriction enzyme is ligated using Takara Bio's DNA Ligation Kit <Mighty Mix>.
13. The plasmid obtained by ligation is adjusted in large amount by the same operation as 1 to 7 and used for transfection to HEK293T cells.

* 1: Because the replicon was low copy, 400 ml of the E. coli solution was large preoed and after ethanol precipitation, the presence of the plasmid was confirmed by electrophoresis.

* 2: The replicon was cultured with shaking in 800 ml of medium.