Cell Experiment Record

SYSU-China 2019

19.6.6. 19:00

The first day of culturing cells began!

Passage a bottle of HEK293T cells

19.6.14 20:21

Transfecte cells

The transfection experiment was set up in 5 groups, and each group had four sets of parallel experiments;

1.endo-pcDNA3.1-L7Ae+DMEM

2.endo-pcDNA3.1-L7AeMUT+DMEM

3.pcDNA3.1+DMEM

4.DMEM

5.DC- (no cells)

The systems are as follows (unit μL):

pcDNA3.1-L7Ae	1.5
HD	1.2
DMEM	97.3
Total	100

pcDNA3.1-L7AemuT	1.5
HD	1.2
DMEM	97.3
Total	100

pcDNA3.1	1.5
HD	1.2
DMEM	97.3
Total	100

HD	1.2
DMEM	98.8
Total	100

Leave at room temperature for 10min. Take a 96-well plate, when the cells account for about 80%, add the corresponding 5 μL transfection agent to each well, blow and mix well, add 100 μL DC- to each well in the rightmost row, and mark the group on the 96-well plates. Culture at 37℃ for cytotoxicity test.

19.6.15

Cytotoxicity Test of L7Ae and L7Ae-MUT

Add 20 uL of MTS to each well and measure the absorbance with a microplate reader.

19.7.14

First attempt: Virus packaging

Conditions: $9x10^5$ cell, 3mL /well; 4ug PacI-pAdEasy

19.7.18

Transfect 293T to detect the efficiency of Tet-on system and L7Ae/ L7Ae-Mut-Kturn system.

The systems are as follows:

Dox 浓度 ng/ml	0	10	30	50	100	200	300	400	600
Dox 加量 μl	0	0.1	0.3	0.5	1	2	3	4	5
phEF1a-rtTA3	1μg								
pTRE-EBFP2	1μg								
Fugene	6μl								
HEK 293	200,000 cells								

	1xK-turn			2xK-turn		
	+L7AeMUT	+L7Ae	-L7Ae	+L7AeMUT	+L7Ae	-L7Ae
pcDNA-L7Ae		1μg			1μg	
pcDNA-L7AeMUT	1μg			1μg		
pcDNA-2Kt-eGFP				1μg	1μg	1μg
pcDNA-K1-eGFP	1μg	1μg	1μg			
pcDNA3.1			1μg			1μg
Fugene	6μl					
HEK-293	200,000 cells					

19.8.9

Adenovirus tilter test and detection of the efficiency of Tet-on system.

19.9

Second attemp: Virus Packaging

19.10.9

1. Resuscitate cryopreservated HeLa

The storage tube was taken out in the liquid nitrogen tank No. 1. Wrap in plastic gloves and melt at 37 ° C for 4 min (do not shake). Transfer to a 15mL centrifuge tube, centrifuge at 1000rpm for 3min, remove the supernatant (note that it is removed to prevent DMSO residue and affect the subsequent growth of the cells). Resuspend with 2 mL of DC-, add 1 mL to a culture flask containing 5 mL of medium, shake well, and incubate at 37 °C. Need to change the medium the next day.

19.10.10

Lipo2000 transfection.

The systems are as follows:

pTRE-EBFP 6ug
phEF1a-rtTA 6ug
DMEM to 300uL.....(1)

pTRE-EBFP 6ug
pcDNA-rtTA 6ug
DMEM to 300uL.....(2)

Lipo2000 24uL
DMEM 576uL
Total 600uL.....(3)

(1) (2) are mixed with 300uL of (3), separately, and place at room temperature for more than 10min.

Dox gradient: 0-100-200-...-700 ng/uL

Add a Tet Positive group (working concentration: 125ng/uL) and a Negative group (nothing is added in.)

19.10.18

Lipo2000 transfect HEK293 to test the system efficiency

phEF1α-rtTA3 +pTRE-L7Ae-miR592(different concentration to set experiment group)+pTRE-2Kt-EGFP