Diary of LIU Jintan

6.20

HEK293T wild type cell was obtained ,start cultivating HEK293T cell

6.21-6.22

Both HEK 293T cell and Hela cell is in a good condition, but still adequate for viability assay

6.23

100 microlitre E.coli strain BL21 was inoculated in Hela cell culture, incubated in 37 celsius degrees for 16hrs, Hela cell was dead. HEK293T cell is sick and not appropriate for viability assay. a new group of Hela cell was cultivated.

6.24-6.27

Plasmid pVSVG,pLentiCMV-Firefly luciferase and pR8.74 was obtained successively,start design primers for an unknown vector which contains Renilla luciferase gene.

another viability assay was done, Hela cell is still alive and in a relative good condition with in 7 hrs

6.28-6.30
HEK293T was inappropriate for experiments and get discarded. a new cell line was obtained from another laboratory

7.1
Transformation E.coli with plasmid pVSVG,pLentiCMV-Firefly luciferase,pR8.74,pLentiCMV backbone vector

Analysis on restriction enzyme site

7.2
Mini-Prep for plasmid pLentiCMV backbone vector

PCR for Renilla sequence,digest PCR product with Enzyme XbaI and BamHI,digest pLentiCMV backbone with the same enzymes

Gel-purification for double digested backbone

7.3
Ligate digested PCR product and backbone with T4 ligase

Transformation E.coli with ligation product

7.4-7.7
Mini-prep for pLentiCMV-Renilla plasmid (recombined clone) ,Sequencing for check if its sequence is correct

7.8
The sequence is correct, inoculate E.coli which contains plasmid pVSVG,pLentiCMV-Firefly luciferase,pR8.74 and pLentiCMV-Renilla, shaking for 15 hrs in 37 Celsius degree

Adjusting HEK293T state for transfection

7.9
Mini-prep plasmids in a way that free of endotoxin

7.10-7.17

Transfect HEK293T cell with plasmid pVSVG,pLentiCMV-Renilla,pR8.74

7.18
Collect HEK293T cell culture, centrifuge in 1000 rpms for 3 minutes, collect the supernatant (Lenti-virus inside)and discard the precipitate

7.19-7.25
Test the titer of lenti-virus, the titer was poor but still suitable for infection

Adjusting Hela cell state for transfection

7.26-7.27
Infect wild type Hela cell with collected supernatant

7.28
Start screening for infected Hela cell with blasticidin in a concentration of 6μg/mL

Set out for Peking-OUC iGEM workshop in Tsingdao,Shandong Province in China

7.29-7.30
Peking-OUC iGEM workshop

7.31-8.8

Screening for infected Hela in further step and higher concentration of blasticidin, back home for a little vocation for 3 days

8.9-8.13
Start photo printing device design, test the best condition of photo printing

8.14
First try of photo printing, failed due to unparallel light source and reflection of light inside the petri dish

8.15
Screening for Hela cell finished. A very small population of Hela cell obtained,start enlarge the population

Agarase gene obtained.

8.16
Another try of photo printing, distinct differences between control group and experimental group,but still cannot form sharp and clear images
Device optimized

8.17
Hela cell was not healthy due to a very low population , no enlargement of population was observed.
Succeed in photo printing with distinct and sharp image!
Start repeat photo printing experiment to test robustness

8.18
Hela cell was still sick , change DMEM medium into 100% pure fetal bovine serum for richer nutrition

8.19-8.22
Enlargement of cell population was observed.
A mature protocol of 2D photo printing was accomplished

8.23
Digest cell and change fetal bovine serum into DMEM medium. Further step of enlarging cell population, inoculating cell into 96 well plate

8.24
Participating in iPad printing device design, handle prating part to other team member

8.25-9.1
Make a new clone with ColE promoter and agarase gene

9.2-9.5

Test substrate of Renilla luciferase, measure in microplate reader,significant differences between control group but still not visible with bare eyes