Preparation

一、Prepared a large number of 50 ml centrifuge tubes,with the following reagents respectively:

1、A tube of serum：for the cell culture medium.

2、A tube of trypsin: for digesting cells from the bottom and wall of the culture dish(cells are adherent growth)

3、A tube of PS antibiotic (added to the culture medium).

4、Pure medium substrate:DMEM and 1640.

5、DMEM complete memum:40mlDMEM+5ml serum +1mlPS antibiotic,for subculturing.

6、1640 complete memum:40ml1640+5ml serum +1mlPS antibiotics，for subculturing.

7、Prepared：40mlDMEM+5ml serum in a 50ml tube,for cell culturing.

8、Prepared：40ml1640+5ml serum in a 50ml tube,for cell culturing.

9、pure DMEM (for transfection)

10、pure 1640(for transfection)

二、Before opening the large bottle of DMEM and 1640,it needed to burn the bottleneck or wiped the bottle with alcohol cotton balls.

Other medium also should be paid attention to,they can not be polluted.

三、Before the experiment, the reagents needed to 37℃ water preheat.

四、Cell lysis solution (in a 15ml centrifuge tube) and Firefly luciferase substrate(in a 1.5mlEP, protecting from light with silver paper) for detecting the result.

Cells subculture

(According to the types of cell for choosing the corresponding medium,and before the medium using,wiped the bottle with alcohol cotton balls.)

1、Put a new culture dishes, 15ml centrifuge tubes into the operating table, ultraviolet irradiated for 30 minutes,preheated DMEM and 1640 complete culture medium and trypsin in 37℃ water.

2、Took out the culture dish had been fully covered with cells from the constant temperature incubator,and put it into the operating table.Took out the preheated reagents,wiped away the outer water,and then put it into the operating table after spraying alcohol.

3、Absorbed the culture medium in the culture dish with vacuum pump.

4、Added trypsin into the dish, which can digest cells from the bottom and wall of culture dish. (2ml for big dish,1ml for medium dish,1min for T24 and Hela, 2min for HFC,5min 5637 in the incubator.)

5、Added twice volume of trypsin as much complete culture medium into the dish to end the reaction of the trypsin (4ml big dish,2ml for medium dish) (If can be observed many small dots ofcells under the microscope, indicated the digestion is complete.Digestion time can not be too long to avoid cell death)

6、Tilted the dish,blew the solution which semi exposed in the air, and blew down the cells from the bottom of the dish.

7、Added the mixed cell culture medium into the 15ml centrifugal tube which was marked, and centrifugated with 300XG 5min. You must balance the quality of the centrifugal.

8、At the same time,marked on the new culture dish and added

complete culture medium(4ml for medium dish, 7ml for big dish).

9、Took out the centrifugal tube,and then removed the upper liquid.Next,added 2ml complete culture medium and blew the cell sediment at the bottom to mix.

10、According to the situation, add 1/2 to 1/6 volume of cells solution in a new culture dish (usually 1/4 volume is 500ul or 300ul).When added,should drop in different position drop by drop,to ensure that it was well-distributed in the new dish,but it is not allow to drop onto the dish wall.

11、After finishing drop cells solution,shook 20 times back and forth.

12、Put the dish in the incubator,and then cleaned operation table,wiped the operation table with alcohol cotton balls.

Transfer a part of cells into 12-well plates and subculture at the same time.

(According to the types of cell for choosing the corresponding medium,and before the medium using,wiped the bottle with alcohol cotton balls.)

1、Put the new culture dishes, a 12-well plate,15ml centrifuge tubes into the operating table, ultraviolet irradiated for 30 minutes,preheated culture medium without PS antibiotic and trypsin in 37℃ water.

2、Took out the culture dish had been fully covered with cells from the constant temperature incubator,and put it into the operating table.Took out the preheated reagents,wiped away the outer water,and then put it into the operating table after spraying alcohol.

3、absorbed the culture medium in the culture dish with vacuum pump.

4、Added trypsin into the dish, which can digest cells from the bottom and wall of culture dish. (2ml for big dish,1ml for medium dish,1min for T24 and Hela, 2min for HFC,5min 5637 in the incubator.)

5、Added twice volume of trypsin as much complete culture medium into the dish to end the reaction of the trypsin (4ml big dish,2ml for medium dish) (If can be observed many small dots ofcells under the microscope, indicated the digestion is complete.Digestion time can not be too long to avoid cell death)

6、Tilted the dish,blew the solution which semi exposed in the air, and blew down the cells from the bottom of the dish.

7、Added the mixed cell culture medium into the 15ml centrifugal tube which was marked, and centrifugated with 300XG 5min. You must balance the quality of the centrifugal.

8、At the same time,marked on the new culture dish and added complete culture medium(4ml for medium dish, 7ml for big dish),added culture medium without PS antibiotic into 12-well plate,1ml each well.

9、Took out the centrifugal tube,and then removed the upper liquid.Next,added 2ml culture medium without PS antibiotic and blew the cell sediment at the bottom to mix.

10、Added 400ul mix solution which includes cells into new culture dish,100ul into each well of 12-well plate.When added the mix solution,each drop of solution added at different positions to guarantee it uniform.

11、Shake the new culture dish and the 12-well plate back and forward 20 times to ensure the cells are evenly distributed in the container.

12、Put the dish in the incubator,and then cleaned operation table,wiped the operation table with

alcohol cotton balls.Use the vacuun pump to absorbed waste liquor and put the waste into the garbage can.

Cell transfection experiment

(According to the types of cell for choosing the corresponding medium,and before the medium using,wiped the bottle with alcohol cotton balls.)

When the culture dish had been fully covered with cells,we started Cell transfection experiment.We use 6 wells for each kind of cell line.In the first well,we transfected with GFP gene to confirm whether the plasmid was successfully transferred into the cells.In the second well,we transfected with our system but we didn't add the Ack unnatural amino acid to verify the function of the orthogonal system.The remaining four wells and cells in them are experimental group.The reagents we also needed to use are:Lipo reagent,plasmids containing our system,the plasmid containing GFP gene and various medium solution.

We use"transfected our system into cells in 6 wells of a 12-well plate"as an example.

1、Put 4 EP tubes into the operating table, ultraviolet irradiated for 30 minutes,preheated culture medium without PS antibiotic,culture medium without PS antibiotic and blood serum in 37℃ water.

2、Put 12-well plate into the operating table.Took out the preheated reagents,wiped away the outer water,and then put it into the operating table after spraying alcohol.

3、Absorbed the mix medium solution in each well,added 900ul medium without blood serum and PS antibiotic into each well,then put the 12-well plate into the constant temperature incubator.

4、Marked EP tubes,added 2.5ul and 50ul pure medium into tube1,1ug GFP plasmid and 50ul pure medium into tube2,12.5ul Lipo and 250ul pure medium into tube3,the same amount of three kinds of plasmids(hTERT,hUPII,RLU),a total of 1ug and 250ul pure medium into tube4.

5、Waiting for 15 minutes,then mix the solution in tube1 and tube2(mixture1),tube3 and tube4(mixture2).Waiting for 15 minutes again.

6、Took out the 12-well plate from the constant temperature incubator,and put it into the operating table.Added 100ul mixture1 into well1,100ul mixture2 into each well from well2 to well6.

7、Put the 12-well plate into the constant temperature incubator.Then wait 5 hours.

8、Took out the 12-well plate from the constant temperature incubator,absorbed the mix solution in each well,added 1000ul culture medium without PS antibiotic into each well.

9、Added 7.5ul Ack unnatural amino acid in well2-6.

10、Put the 12-well plate into the constant temperature incubator.We can detect result after 36 hours.

Detected the results of GFP(with amber mutation)

Used fluorescence microscope to see the expression result of GFP gene.

Luciferase

1、Prepared 13 1.5ml EP tubes,marked them,added 20ul water into tube1.

2、Get the cell lysis solution and luciferase substrate out and thaw.

3、Sucked the medium from 12-well plate and added 150ul lysis solution into each well,put 12-well

plate on the table concentrator,shaked for 20 minutes.

4、Added the solution from wells into tube2-7, centrifugated with 12000rpm 10min 4℃.

5、Absorbeb 20ul upper liquid from tube2-7 to tube8-13.

6、Turn off the light and turn on the microplate reader.

7、Added 80ul luciferase substrate into each EP tube,and put each EP tube into microplate reader to detect the data.

Matters need attention

1、Except detect the result,other experiments must be done in sterile operating table.

2、If we needs new culture dishes,new centrifuge tubes,new EP tubes or new 12-well plates in an experiment,we should put them into the operating table, ultraviolet irradiated for 30 minutes.

3、One should not open the lid of a new tube or a tube with reagent.

4、When doing an experiment,the hands of lab assistant or other things can’t go through the open container.

5、Containers containing cells need to be specified some information like the date,the type of cells and medium.

7、Use the vacuun pump to absorbed waste liquor,waste liquor shouldn’t be poured into garbage can.