Protocol

New Viral Tattoos Recipe (modified for Cryobooks Archives) For visible expression of transfected cells on epidermis

Determine Multiplicity of infection (MOI) based on amount of viral vector to cell culture ratio. We used 1.0x 10 4 Transducing particles (TU) per ml and then divided accordingly to test for best MOI rate.

1. Suspend cells grown in flasks with 5 ml Trypsin.
2. Add DMEM medium with 10% FBS
3. Count cells
4. Add 1x 10 (power of 4) cells to 1 ml cell culture in each 24 well dish. (Optional – add Poly-L-Lysine for stronger adhesion of cells on plates).
5. Put dish in incubator overnight (12 hours)
6. Check that cells are semi-confluent (60 % is best).
7. Put fresh medium (1 ml) in the dishes. Put 2ml of medium in first dish (Nat)
8. Take 10 microliters of lentivirus and put in first dish.
9. Pipette 1ml into second dish—1ml into the third to the sixth well (to dilute the viral nate. (1/2, 1/4, 1/8, 1/16, 1/33)
10. Put 1.1 ml in the second dish. Take another 10 microliters of lentivirus. Take 100 microliters into each of the other six dishes. (1/10, 1/100, 1/000, etc)
11. Add polybrane (8 ug/ml)
12. Seal dish with parafilm and put in incubator for 4 to 12 hours.
13. Replace medium (without FBS)
14. Place in incubator for 12 hours.

Option: Skip the cell count and hope for the best. Add as much or as little quantity of virus as you feel like to the cell culture.

Prepare skin- Wash in PBS. Cut sections with sterilized scissors prior to suspending viral cells. Place skin samples into dishes and place in -20 freezer for 15 minutes (varies depending on thickness of skin and skin type). Take out and stamp.

15. Remove medium from dishes
16. Suspend virus and cells with trypsin (fill bottom of dish) for approx 2 mins or until you see cells lifting.
17. Add medium

Pipette cells onto the surface. (Separately each skin sample with one cell dish to determine MOI with microscopy, if desired).Try to keep the concentration of cells in one area for dense expression of immunostains. (Ie. Bluer colour concentration).

Immunostaining pig and human skin (Stuart Hodgetts)

1. “Seed” skin with cells and virus in about 1ml of medium – leave for 30 minutes to two hrs. Check to insure the stamp design is still visible.
2. Fix tissue with 1:1 acetone:methanol (volume:volume or v/v) for 5-10 mins 2:1 PBS

Then block in 10% FBS (V/V) and 1% BSA (V/V or in 2:3 1AB diluted in Block). (The block was added into the primary antibodies).
3. Incubate with primary antibodies for 30 mins at 37°C
4. Rinse 3x with phosphate buffered saline (PBS) or medium (serum free)
5. Incubate with secondary antibodies for 30 mins at 37°C
6. Rinse 3x with L-15 (or medium without FBS)
7. Rinse 3x with PBS or medium (serum free)
8. Develop with 4-chloro-1-naphthol (4CN) chromagen solution until desired staining is achieved
9. If required, rinse and leave submerged in tap water to intensify 4CN staining

Primary antibodies:
Thy1.1 (Mouse) diluted 1/400 (10 microliters: 4 millliliters)
Fibronectin (Rabbit)
Laminin (Rat)
=together mixed with PBS to 22 milliters.

Secondary Antibodies:
Goat anti-Rabbit Ig – horse radish peroxidase conjugated at 1/2000 in PBS
Donkey anti-mouse Ig – horse radish peroxidase conjugated at 1/2000 in PBS

4CN Chromagen:
20mg 4CN dissolved in 20ml methanol = Solution A
50mM NaCl/Tris-HCl solution = Solution B
Mix 1:5 solutions A:B then add 0.1ml hydrogen peroxide (few drops)mix again
(.2m NaCl/50 mM Tris)
Use 50ml

Additional notes on skin:
Pig skin is tough and the cells need to be pipetted in one area in order to render colour. The cells expresses more easily in the fibreblasts found in the dermal tissue of both human and pig skin.
It is recommended to submerge both types of skin in tap water for at least two days for more intensity of staining