

## **How to obtain adult human epidermal keratinocytes to transduce with viral oncogenes**

1. Wash the skin biopsy with PBS
2. Cut skin into 3-5 mm<sup>2</sup> pieces
3. Digest skin pieces with Dispase (10 U/mL) for 4 hours at 37 °C
4. Peel the epidermis from dermis
5. Transfer epidermis in Trypsin (500 mg/L)
6. Pipette vigorously, every 10 minutes, 3 times
7. Discard the epidermis
8. Transfer the Trypsin containing cells into a tube
9. Add medium with serum to inactivate Trypsin
10. Centrifuge for 5 minutes at 1200 rpm at room temperature
11. Resuspend the pellet in Keratinocyte Serum Free Medium containing bovine pituitary extract (BPE, 30 µg/mL) and epidermal growth factor (EGF, 0.2 ng/mL)
12. Grow cells at 37°C in 5% CO<sub>2</sub> buffered incubator.