APPENDIX

LIST OF SOLUTION AND BUFFER

1X Phosphate buffer saline (PBS)

8.0 g of NaCl, 0.2 g of KCl, 1.15 g of $Na_2HPO_4.2H_2O$, and 0.2 g of KH_2PO_4 were dissolved in distilled water in final volume of 1000 ml. The solution was adjusted to pH 7.4 and stored at room temperature.

Versene solution

0.1 g of EDTA was dissolved in 1XPBS in final volume of 500 ml. The volume was filtrated by sterile filter (pore size 0.20 µm) then preserved at 4 °C.

0.25% trypsin

0.25 g of trypsin was dissolved in versene solution in final volume of 100 ml. The volume was filtrated by sterile filter (pore size 0.20 µm) then preserved at 4 °C.

Freezing medium

Formula 1: 10% DMSO

- DMSO	1 ml
- DMEM containing 20% FBS	9 ml
Total volume	10 ml

Formula 2: 25% Glycerol, 25% Propylene glycol

- Glycerol	2.5 ml
- Propylene glycol	2.5 ml
- DMEM containing 20% FBS	5 ml
Total volume	10 ml

Formula 3: 25% DMSO, 25% Propylene glycol, 20% FBS in DMEM

- DMSO	2.5 ml
- Propylene glycol	2.5 ml
- DMEM containing 20% FBS	5 ml
Total volume	10 ml

The solutions were filtrated by sterile filter (pore size 0.20 µm) and aliquot into microcentrifuge tubes. The solution was preserved at -20 °C.

Sodium citrate buffer

2.94 g of Na3C6H5O7.2H2O was dissolved in distilled water in final volume of 1000 ml. The solution was adjusted to pH 6.0.

4 µg/ml mitomycin C

2 mg Mitomycin C was dissolved in 5 ml distilled water and kept this stock solution 400 µg/ml Mitomycin C at 4 °C. Working solution was done by dissolving 0.5 ml stock solution in 50 ml DMEM then filtrated by sterile filter (pore size 0.20 µm). The solution was preserved at 4 °C.

1N NaOH

0.4 g of NaOH was dissolved in distilled water in final volume of 10 ml.

0.9% NaCl

0.9 g of NaCl was dissolved in distilled water in final volume of 100 ml.

2 µg/ml epidermal growth factor (EGF)

100 µg EGF was dissolved in 50 ml distilled water. The volume was filtrated by sterile filter (pore size 0.20 µm) to discard the microorganism then aliquot into microcentrifuge tubes. The solution was preserved at 4 °C. This stock solution was diluted to 10 ng/ml final concentration in cultured medium.

1 mg/ml insulin

10 mg of insulin was dissolved in 10 ml DMEM. The volume was filtrated by sterile filter (pore size 0.20 µm) and preserved at 4 °C. This stock solution was diluted to 20 µg/ml final concentration in cultured medium.

5 mg/ml streptomycin

50 mg of streptomycin was dissolved in 10 ml DMEM. The volume was filtrated by sterile filter (pore size 0.20 µm) and preserved at 4 °C. This stock solution was diluted to 100 µg/ml final concentration in cultured medium.

40 mg/ml gentamicin

80 mg of gentamicin was dissolved in 2 ml DMEM. The volume was filtrated by sterile filter (pore size 0.20 µm) and preserved at 4 °C. This stock solution was diluted to 50 µg/ml final concentration in cultured medium.

1.5 mg/ml ampicillin

15 mg ampicillin was dissolved in 10 ml DMEM. The volume was filtrated by sterile filter (pore size 0.20 µm) and preserved at 4 °C. This stock solution was diluted to 3 µg/ml final concentration in cultured medium.

25 ml Culture medium

Culture medium composed of 10% FBS, 10 ng/ml epidermal growth factor, 20 µg/ml insulin, 100 µg/ml streptomycin, 3 µg/ml ampicillin in DMEM/Ham's F12 (1:1).

FBS	2.5 ml
2 µg/ml epidermal growth factor	125 µl
1 mg/ml insulin	0.5 ml
5 mg/ml streptomycin	0.5 ml
1.5 mg/ml ampicillin	50 µl

All reagents were dissolved in DMEM/Ham's F12 (1:1) in final volume of 25 ml.

4% paraformaldehyde

16 g of paraformaldehyde was added in erlenmeyer flask then dissolved in 200 ml distilled water. The flask was placed on a stir plate and heated while stirring to

approximately 60 °C. 1N NaOH was slowly added to raise the pH by drop from a pipette until the solution clears. Then, 200 ml 1XPBS was added and adjusted to pH 7.4.

Artificial cerebrospinal fluid (aCSF)

3.633 g of NaCl, 0.093 g of KCl, 0.075 g of NaH_2PO_4, 0.056 g of $CaCl_2$, 0.102 g of $MgCl_2$, 1.050 g of $NaHCO_3$ and 0.450 g of D-glucose were dissolved in deionized water (18 mΩ) in final volume of 500 ml. The solution was adjusted to pH 7.4 and further filtrated by sterile filter (pore size 0.20 µm) and kept at 4 °C.

SCORING FOR IMMUNOHISTOCHEMICAL STAINING

Number of p63 positive cells along the thickness of epithelial sheet within 50 µm length was counted.

≥ 5 cells indicate strongly positive

< 5 cells indicate weakly positive

Number of K3 positive cell layers in epithelial sheet within 50 µm length was counted.

≥ 2 layers with heavy staining indicate strongly positive

≥ 2 layers with light staining indicate weakly positive

< 2 layers indicate weakly positive

CURRICULUM VITAE

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Education Background

2003	Dara Academy, Chiang Mai, Thailand
2007	First class honors, Bachelor of Science (Zoology), Faculty of science, Chiang Mai University

Work Experience

2007-2009	Technician at Chiangmai Genetic Center

Grant

2004-2007	Human Resource Development in Science Project (Science Achievement Scholarship of Thailand, SAST)
2007	Industrial and Research Projects for Undergraduate Student (IRPUS)