**SOP: Propagation of Human Olfactory Neurosphere-derived Cells**
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## **Source of material**: Olfactory mucosal biopsies from healthy volunteers.

Lineage of cells:
Volunteer donors were recruited from the general population. Nasal biopsies were collected as an outpatient procedure as described (Feron et al., 1998). This was done by a registered otolaryngologist via an endoscopic procedure to ensure biopsy samples were taken from the superior, posterior region of the nasal septum close to the cribriform plate. Small pieces of olfactory mucosa (about 4 $mm^2$) were obtained. Donor tissue was obtained with the informed consent of the participants prior to their involvement in the study and all procedures were in accordance with National Health and Medical Research Council Code of Practice for Human Experimentation, and approved by the Griffith University Human Experimentation Ethics Committee.

The olfactory mucosa contains stem cells in the olfactory epithelium that give rise to new olfactory sensory neurons and other cells of the olfactory epithelium (Mackay-Sim, 2011). The stem cell population derived from human olfactory mucosa has been described as ecto-mesenchymal (Delorme et al., 2010) because it has characteristics of both neural and mesenchymal stem cells (Delorme et al., 2010; Matigian et al., 2010), suggesting a neural crest origin. Olfactory mucosal stem cells are multipotent. In vitro or after transplantation into the chick embryo they give rise to many cell types of the body including neurons, astrocytes, oligodendrocytes, cardiac muscle, skeletal muscle, adipocytes, and chondrocytes (Murrell et al., 2005; Murrell et al., 2008; Murrell et al., 2009; Wetzig et al., 2012).

**Procedure**

**1) Process of converting biopsy to cell line**

The biopsies were initially received in cold Dulbecco's Modified Eagle Medium/Ham F-12 (DMEM/F12 Gibco) containing 10% FBS ( Bovogen) and 1% streptomycin-penicillin (GibcoBRL). Olfactory cell suspensions were generated by digestion with dispase II (Boehringer; 2.4 units/ml, 45 minutes, 37°C) and collagenase H (Sigma; 0.25 mg/ml, 10 minutes, 37°C) accompanied by mechanical trituration, as described previously (Murrell et al., 2005). The primary cultures were grown until confluent (~ 7 days) DMEM/F12 supplemented with 10% FBS before passaging into flasks (Nunclon) pretreated with 1 $\mu g/cm^2$ poly-L-lysine (Sigma, P6282) and culturing in DMEM/F12 containing ITS (Gibco), EGF (50 ng/ml, Chemicon) and FGF2 (25 ng/ml, Chemicon). Neurospheres formed initially from cell clusters attached to the culture dish surface but detached when they reached about 125-150 $\mu m$ in diameter. The free-floating neurospheres were harvested every second day from the medium change. These were dissociated with TrypLE, replated at 4000 cells/$cm^2$ into 75 $cm^2$ flasks and cultured in DMEM/F12 with 10% FBS. These neurosphere-derived (ONS) cells were then expanded by passage and banked down in aliquots after harvest by storage in liquid nitrogen with 90% FBS and 10% dimethyl sulfoxide (Sigma).

**2) Cell culture protocol (growth conditions, splitting, feeding, etc)**

Frozen aliquots of ONS cells were used as the starting point for all the experiments. All cultures were grown under standard conditions on tissue culture plastic in DMEM/F12 supplemented with 10% FBS at 37°C and 5% $CO_2$.

## Cell lines used for analysis

| Cell Line | Age (years) | Sex | Place of biopsy | Mycoplasma testing |
|---|---|---|---|---|
| **C1 100 08 0002 (Replicate B2)** | 59 | Male | Septum | Negative |
| **C1 100 08 0003 (Replicate B3)** | 66 | Female | Septum | Negative |
| **C1 100 08 0013 (Replicate B1)** | 64 | Male | Septum | Negative |

Reagents used

| REAGENT | CATALOGUE NO | LOT NO | COMMENTS |
|---|---|---|---|
| DMEM/F12 | GIBCO 11320-082 | 507089<br>774760 | |
| FBS | Bovogen SFBS | ED001<br>0511A | |
| Pen/Strep | GIBCO 15140-122 | 383044 | Only at the initial stages |
| Collagenase H | Sigma C8501-100mg | 066K8608 | |
| Dispase II | Roche 10295825001 | 12951100 | 2.4U/ml |
| TrypLE | GIBCO 12604 | 527167<br>786710 | |
| Poly-L-lysine | Sigma P6282 | 107K5107 | |
| $H_2O$ | GIBCO 10977-015 | 527182 | |
| EGF | Chemicon GF144 | R07070008 | |
| FGF2 | Chemicon GF003 | DAM1521351 | |

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|---|---|---|---|
| ITS | GIBCO 41400-045 | 488626 | |
| DMSO | Sigma D2650 | 078K2344<br>RNBB0641 | |
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