

**An outline of the experimental protocol for deriving sensory neurons from human fibroblasts** (based on Blanchard et al., *Nature Neurosci* 2015).

*Note:* Some details of the protocol have been adapted for this Task, and therefore it should not be used in a realistic setting.

This protocol is based on transient ectopic expression of two transcription factors: *BRN3A* and *NGN1* in human fibroblasts, followed by culturing in specialised neural induction media with supplements.

1. **Prepare lentiviruses delivering the ectopic expression of *BRN3A* and *NGN1* genes**
  - a. Clone cDNAs of these genes into “transfer vector” plasmids that place them under the control of a tetracycline operator (TetO) that can be induced with doxycycline.
  - b. Transfect each plasmid into 293T cells alongside a “packaging vector” encoding additional lentivirus components that we don’t want to be produced in the target cells and an “envelope vector” encoding a surface protein from a different virus that promotes effective infection of target cells.
  - c. Harvest and purify the lentiviruses produced.

**Assume the above steps have already been performed by your colleague.**

- Your colleague has generated two virus stocks, driving the inducible expression of *BRN3A* and *NGN1*, respectively. In addition, they have generated a control virus bearing the empty lentiviral vector.
- Your colleague then measured the virus titres using ELISA (enzyme-linked immunosorbent assay) for the lentiviral capsid protein p24.

The titres were:

- *BRN3A* vector:  $1.1 \times 10^6$  Transduction Units (TU) / mL
- *NGN1* vector:  $0.9 \times 10^6$  TU/mL
- Empty vector:  $1.4 \times 10^6$  TU/mL

*Note:* p24 ELISA cannot distinguish between live and defective virus particles and therefore these values can be **overestimates**.

2. **Infect the fibroblasts** (cultured in DMEM media + 10% Fetal Bovine Serum) with a mix of lentivirus particles delivering *BRN3A* and *NGN1* (or the control virus). Aim for a multiplicity of infection with live virus (MOI) of 2-5 TU/cell.

*Note:* Assume the experiment is performed in 96-well plates, with ~20,000 cells per well in 150  $\mu$ L media.

3. After 12-16h of infection, replace the virus-containing media with fresh media.
4. After 48h, **induce the expression of *BRN3A* and *NGN1*** by adding 5  $\mu$ g/ml doxycycline.

5. On day 4 post-induction, **replace the media** with N3 media + 5 µg/ml doxycycline.

*Note: N3 is a neural induction medium based on DMEM but containing additional nutrients, the hormones insulin and progesterone; growth factor FGF2 and other components. N3 contains penicillin/streptomycin so there's no need to add them on top.*

6. On day 7 post-induction, **withdraw doxycycline**.

*Note: This is done because the transient expression of BRN3A and NGN1 is sufficient for differentiation.*

7. On day 10 post-induction, **replace the media** with a 1:1 mix of DMEM/F12 media and Neurobasal media supplemented with B27, NGF, BDNF and GDNF, all at 10 ng/mL.

8. Optional: add NT3 supplement at 25 ng/mL.

*Note: B27 is a mix of components including many antioxidants; NGF, BDNF, GDNF and NT3 are various growth factors.*