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 control of 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: 1.1×10^6 Transfection Units (TU) / mL
    - *NGN1* vector: 0.9×10^6 TU/mL
    - Empty vector: 1.4×10^6 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 μL 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 μg/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 transient expression of BRN3A and NGN1 is sufficient for differentiation.

7. On day 10 post-induction, **replace the media** with 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.