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# C Localised axotomy of human Cortical Neurons (CNs) from induced pluripotent stem cells (iPSCs)

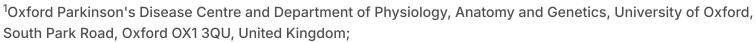


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We use this protocol and it's working

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#### Abstract

This protocol describes the process followed to perform localised axotomy of iPSC-derived human cortical axons cultured within fluid-walled dumbbells in 6 cm TCT-treated Petri dishes. A similar protocol was first described by **Soitu et al., 2020** to perform wounds assays in monolayer cell cultures.



### **Materials**

### Reagents:

- B-27<sup>™</sup> Supplement (50X), serum free (ThermoFisher Scientific, CAT# 17504044)
- FC40 (iotaSciences Ltd, CAS# 51142-49-5)
- Neurobasal (ThermoFisher Scientific, CAT#2113049)
- Phosphate-buffered saline, pH 7.4 (PBS) (Life Technologies, CAT# 10010056)

### **Equipment:**

■ In-house Fluid Printer (iotaSciences Ltd.)

## **Preparation of coating-prep medium:**

- Neurobasal
- 1x B-27 supplement

# **Troubleshooting**



# **Localised axotomy assay**

- On Day 20, remove the FC40 overlay from the Petri dish where human Cortical Neurons (CNs) were cultured inside fluid-walled dumbbells.
- Gently add ~2 ml of coating-prep medium (see **Materials**) to destroy every fluid wall without peeling cells off the substrate.
- Remove medium and wash with PBS twice. **Be careful with cells peeling.**
- 4 After last wash, add ~5 ml of fresh medium (Neurobasal supplemented with B27).

Note

Cells can now be stored in incubator for several minutes if needed.

- 5 Equip the fluid printer with a 1 mL glass syringe filled with medium.
- 6 Place dish into the fluid printer.
- Fluid printer automatically perform axotomy by means of a submerged medium jet. Such jet is held at a fixed height above cells and it is moved around the dish by the printer traverse to cross perpendicularly across axon bundles at the midpoint.

#### **Key parameters:**

- jet height =300 µm
- jet flow rate = 480 μl/min
- traverse speed = 960 mm/min

#### Note

Parameters must be finely tuned, depending on cells maturation and concentration.

Fluid printer is controlled by scripts written in G-code.

8 Remove medium and overlay fresh FC40.



9 New fluid-walled dumbbells can be fabricated around axotomized cultures following steps 1.5 and 1.6 in Protocol: Fabrication of fluid-walled dumbbells and generation of the human corticostriatal pathway

# **Live-imaging of axonal regeneration**

- 10 On DIV 0, fluorescent live images of all dumbbell conduits were taken just prior to CNs replating to serve as initial normalising timepoint.
- 11 Images were taken on a digital SLR camera (Nikon D7100 DSLR) connected to an epifluorescence microscope (Olympus IX53; 1.25×, 4×, 10×, 25× objectives) equipped with a translation stage and an overhead illuminator (Olympus IX3 with filters).
- 12 Medium was changed every other day from now on for the next 20 days.
- 13 Similar live images were taken again on day 20 prior to axotomy, and subsequently 36, 60, and 84 hours post-axotomy.