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Bulk Astrocyte RNA Seq Extraction

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

Only draft form

Abstract

Bulk Astrocyte RNA Seq preparation

Cell Preparation

1 Assume microglia and OPCs have been removed prior to this procedure, if not refer to proper protocols to have pure astrocyte culture

2 Remove media and discard

3 Rinse twice with PBS, aspirate the PBS, add 5 ml trypsin-EDTA (.05%) and incubate in the CO_2 incubator at 37 °C. Check detachment of astrocytes every 5 min and enforce detachment of astrocytes by hitting the flask against the palm of your hand (2-3 times)

4 After astrocytes are detached from the culture flask, add 5 ml of astrocyte culture media to inactivate trypsin

5 Cell Counting

5.1 Take a previously prepared 1.5 mL microtube (with 10 µL of trypan blue) and combine with 10 µL of cell suspension

5.2 Load a hemacytometer with 10 µL of the cells and trypan blue solution and examine immediately under a microscope at low magnification.

5.3 Count the number of viable cells. Those will light up white and are not invaded with the blue dye

5.4 To calculate the number of viable cells per mL of culture, use the formula: Number of viable cells × 10^4 = cells/mL culture. Remember to correct for the dilution factor.

5.5 For Plasmidsaurus e cell couFor Plasmidsaurus bulk RNA seq, recommended cell count per well is $2*10^5$

6 Transfer proper amount of cell suspension to microtubes. Spin cells at 180 x g for 5 min, aspirate supernatant and add 50 µL of Zymo 1x DNA/RNA shield and resuspend

6.1 If the lysed cell solution appears highly viscous, vortex it vigorously for about 30s to shear chromosomal DNA.

7 Transfer prepared supernatant to properly labeled 200 µL strip tubes or 96 well plate according to https://plasmidsaurus.com/sample-prep/rna#section1 instructions