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Astrocyte Viral Transduction

In 2 collections

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for transducing astrocytes with lentivirus

1 Coate desired number of wells of a 6 well plate in 0.1% gelatin

2 Wash astrocytes with 1x PBS

3 Dissociate astrocytes in a trypsin enzyme solution (TrypLE Select, Gibco 12563011). For a 10cm plate of astrocytes, use 3mL per well

4 Transfer dissociated cells to a falcon tube with 10mL astrocyte media (AM; ScienCell #1801)

5 Centrifuge at 300rcf for 3 minutes at room temperature

6 Resuspend cells in 1mL of AM and count

7 Thaw lentivirus on ice
See https://www.protocols.io/edit/lentiviral-production-dkdi4s4e for lentivirus protocol

8 Transfer between 1×10^5 and 3×10^5 cells into a 1.5mL microfuge tube, 1 tube per viral transduction

9 Add lentivirus to the microfuge tube, so that the final concentration once cells are plated with the total volume of media will be 1:50 (40uL of lentivirus when final volume will be 2mL in a well of a 6-well plate)
NOTE: Viral titration may need to be determined on a batch to batch basis, depending on the lentivirus plasmid and the cells being transduced.

10 Incubate cells in the microfuge tube with the higher lentivirus concentration for 5-10 minutes at room temperature

11 Add the cell/lentivirus mixture to the prepared well and bring the final volume of AM to 2mL

12 After 24hrs, change to fresh AM media

13 If antibiotic selection is necessary, start adding at least 3 days after transduction, and continue selecting for at least 2 days