Title	Isolating Dopaminergic Progenitors
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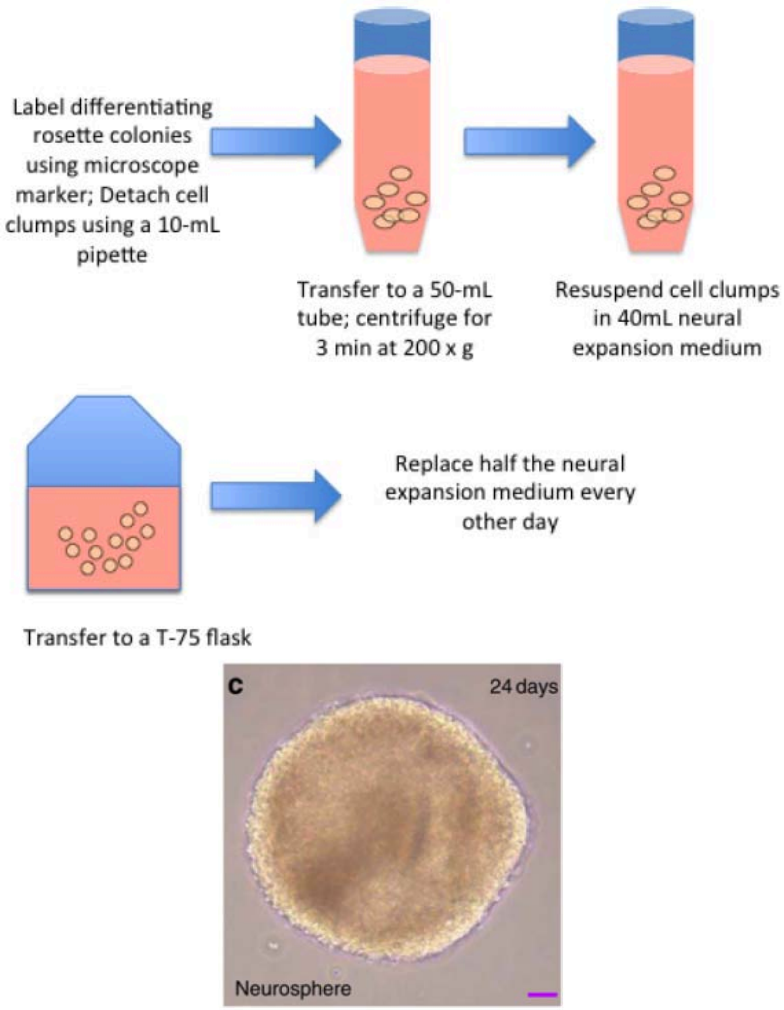
Submitted by -	Efthymiou, Anastasia - anastasia.efthymiou@nih.gov
Adapted from -	Gibco Protocol
Contributors -	Efthymiou, Anastasia
Affiliation(s) -	NIH CRM - NIAMS – Laboratory of Stem Cell Biology

❖ Introduction:



Neurosphere[1]

❖ Protocol:

1. Label all differentiating colonies containing rosettes using a microscope marker.

2. Using a 200- uL pipette tip pointing to the center of each marked colony, blow off the cells in rosettes.
3. Use a 10- mL pipette to transfer the detached cell clumps into a 50- mL centrifuge tube.
   Note: You can combine the cell clumps from five 100- mm dishes into one 50- mL tube.
4. Centrifuge the cells for 3 minutes at 200 × g.
5. Aspirate the supernatant and resuspend the cell clumps in 40 mL of neural expansion medium containing 100 ng/mL FGF- 8b and 200 ng/mL SHH.
6. Transfer the cell clumps to a T- 75 flask and place the flask in a 37 C incubator with a humidified atmosphere of 5% CO2. The rosettes will roll up to form neurospheres after about 1 day in the incubator.
7. Replace half of the neural expansion medium containing 100 ng/mL FGF- 8b and 200 ng/mL SHH with fresh medium every other day.
   Note: Contaminating non- neural cells tend to attach to the flask. When changing the medium, set the flask down at a tilted angle to allow the neurospheres to settle in one corner of the flask. Aspirate half of the neural expansion medium and use a 10- mL pipette to transfer the neurospheres with the rest of the spent neural expansion medium to a fresh T- 75 flask. Add 20 mL of pre- warmed fresh neural expansion medium to the flask and incubate in a 37 C incubator with a humidified atmosphere of 5% CO2.

❖ Materials:

Neural expansion medium	
FGF-8b	
SHH	
Neural Expansion Medium	
Component	Amount
D-MEM/F-12	96 mL
N-2 Supplement	1 mL
B-27® Supplement	2 mL
NEAA	1 mL
Basic FGF Solution	200 µL
Heparin Solution	100 µL

❖ Troubleshooting:

❖ References:

1. Houbo Jiang, Yong Ren, Eunice Y. Yuen, et al. Parkin controls dopamine utilization in human midbrain dopaminergic neurons derived from induced pluripotent stem cells. Nature Communications 3, Article number: 668 (2011).