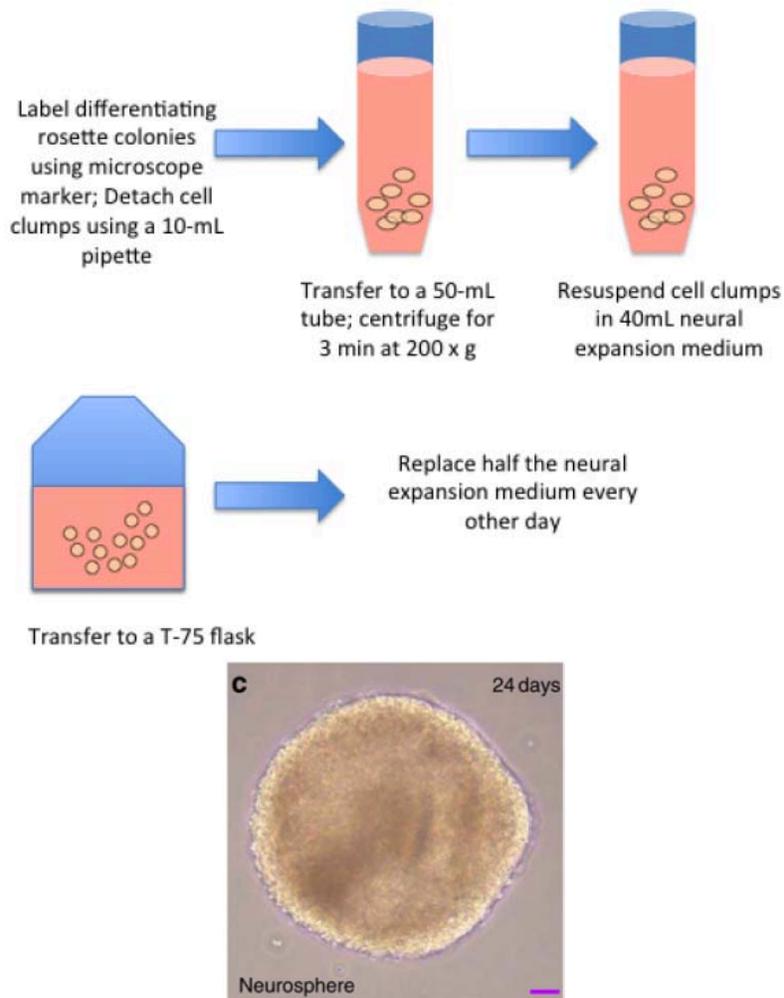


Title	Isolating Dopaminergic Progenitors
Date Submitted	May 5, 2012
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Adapted from -	Gibco Protocol
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❖ Introduction:



Neurosphere¹

❖ Protocol:

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

2. Using a 200- μ L 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 $200 \times g$.
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 μ L
Heparin Solution	100 μ L

❖ **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).