Generating Midbrain Dopaminergic Neurons from hPSCs

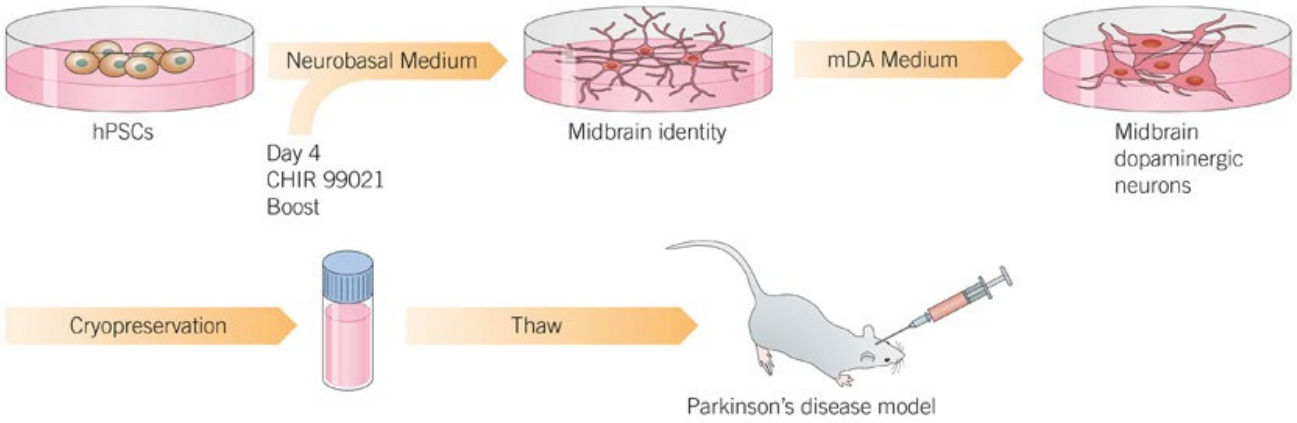
This is intended as a guide only; for full experimental details please read the reference provided.

In Brief

Kim *et al.* describe a scalable and reproducible protocol for the generation of midbrain dopaminergic (mDA) neurons from human embryonic stem cells, followed by their cryopreservation, and transplantation in mice and rats. Neurons generated using this protocol have potential for use in the treatment of Parkinson's disease.

On day 0 hESCs (WA09) were plated on basement membrane extract (BME e.g. Cultrex™) in Neurobasal medium supplemented with N2, B27 and L-glutamine, containing SB 431542, LDN 193189, CHIR 99021, SHH and Y-27632. Media was changed daily. On day 1 Y-27632 was withdrawn. On day 4 the concentration of CHIR 99021 was increased to optimize midbrain specification. On day 7 LDN 193189, SB 431542 and SHH were withdrawn. On day 10 media was changed to Neurobasal medium with B27 and L-glutamine supplemented with BDNF, Ascorbic Acid, GDNF, TGF-ß3, dibutyryl cAMP, CHIR 99021 (mDA medium). On day 11 cells were dissociated and replated in mDA medium with the addition of DAPT from day 12. On day 16 cells were dissociated and replated for further study or cryopreservation.

In vitro electrophysiology studies of resulting mDA neurons were conducted from day 25. Cryopreserved cells were thawed and when transplanted into the right striatum of 6-OHDA lesioned rats reduced motor abnormalities.



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Cocktails

Neurobasal medium supplemented with N2/B27/L-Glutamine						mDA differentiation medium (Neurobasal medium supplemented with B27/L-Glutamine			
(Days 1-3)		(Days 4-6)		(Days 7-9)		Days 10-12		Days 12-16)	
LDN 193189 (Cat No 6053)	250 nM	LDN 193189 (Cat No 6053)	250 nM	CHIR 99021 (Cat No 4423)	7.5 µM	BDNF (Cat No 248-BDB)	20 ng/ml	BDNF (Cat No 248-BDB)	20 ng/ml
SB 431542 (Cat No 1614)	10 µM	SB 431542 (Cat No 1614)	10 µM			GDNF (Cat No 212-GD)	20 ng/ml	GDNF (Cat No 212-GD)	20 ng/ml
CHIR 99021 (Cat No 4423)	0.7 µM	CHIR 99021 (Cat No 4423)	7.5 µM			Ascorbic Acid (Cat No 4055)	200 µM	Ascorbic Acid (Cat No 4055)	200 µM
Y-27632 (Cat.No 1254)	10 µM (Day 1 only)					Dibutyryl-cAMP (Cat No 1141)	500 µM	Dibutyryl-cAMP (Cat No 1141)	500 µM
SHH25II (Cat No 64-SH)	500 ng/ml	SHH25II (Cat No 64-SH)	500 ng/ml			TGF-β3 (Cat No 8420-B3)	1 ng/ml	TGF-β3 (Cat No 8420-B3)	1 ng/ml
						CHIR 99021 (Cat No 4423)	3 µM	DAPT (Cat No 2634)	10 µM

Reference

Kim *et al.* (2021) Biphasic activation of WNT signaling facilitates the derivation of midbrain dopamine neurons from hESCs for translational use. Cell Stem Cell ***28*** 343. PMID: 33545081