



# Leveraging iPSC Technology for Disease Modeling and Drug Screening in Neurological Disorders

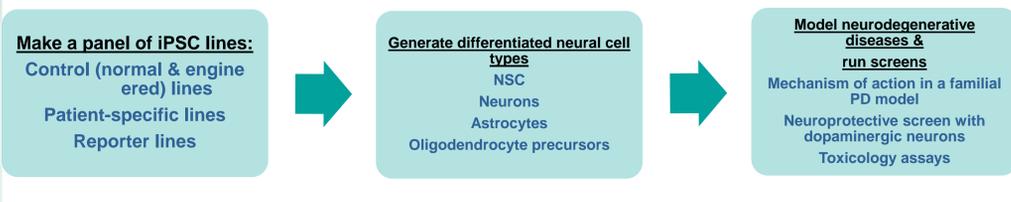
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## Introduction

- Human iPSC technology offers the benefits of a cell line, coupled with the advantages of using human primary cells.
  - Human disease mutations can be captured in a stable cell population
  - iPSCs can be terminally differentiated into multiple cell lineages and genetically engineered generating cell line models with the same allelic background
- iPSC technology and its differentiation into neuronal lineage cells has benefited research in neuroscience and neurological disorders (Ex. Parkinson's disease (PD)).
- To aid in furthering drug development and screening for PD, we have generated a panel of iPSC lines & terminally differentiated them into neural lineage cells<sup>1</sup> for neurotoxicity assays and disease modeling applications
  - Control (normal and engineered-isogenic) lines
  - Patient-specific lines: integration-free iPSC lines from PD patients carrying various mutations<sup>2</sup>
  - Reporter lines (with lineage specific promoters/ ubiquitous; locus-specific KI or safe-harbor KI)
- We describe the utility of these lines for neurotoxicity assays, including assays to determine the specificity of different neural cell types for a small range of chemicals and drugs from the Tox21 library, as well as for neuroprotective assays with dopaminergic neurons.

## Experimental Design



## Parkinson's Disease Modeling and Drug Screening With Three Panels of iPSCs

- Control Lines:** Well-characterized, integration-free control iPSC lines generated from male and female CD34+ cells (cord blood) using episomal vectors. These lines were also used for engineering isogenic lines with disease mutations.
- iPSC Lines from PD Patients<sup>2</sup>:** Extensively characterized integration-free lines from multiple donors of various genetic backgrounds. Their characterization includes whole genome analyses at various stages of differentiation (A).
- Engineered isogenic lines<sup>3</sup>:** From parental control iPSCs with knockout mutations of genes associated with PD (B); and reporter lines with knock-in of lineage-specific reporters and in safe-harbor locus (C).

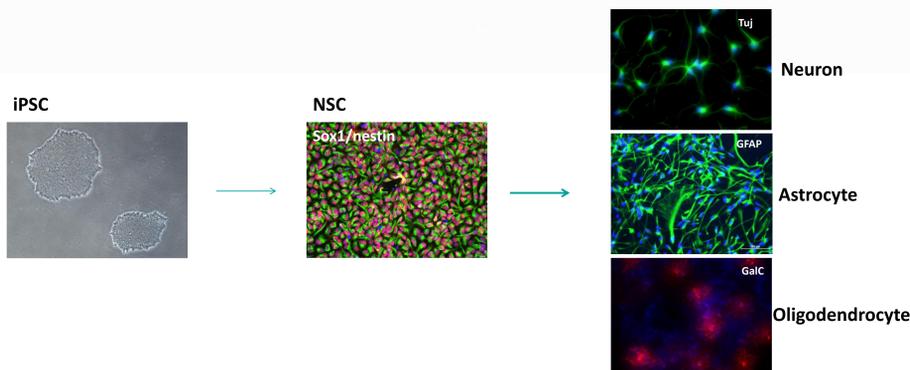
Gene	Mutation	Gender	Race	Age of PD	Age of sample	Family history
SNCA	SNCA triplication	Female	Caucasian	50	55	Yes
PARK2	PARK2: R42P	Male	Caucasian	42	54	No
	PARK2: EX3DEL					
	PARK2: EX3-4DEL	Male	Hispanic	16	50	No
	PARK2: 1-BP DEL, 255A					
	PARK2: R275W	Female	Hispanic	43	61	No
	PARK2: R42P	Female	Caucasian	44	63	N/A
LRRK2	LRRK2: G2019S	Male	Caucasian	40	52	No
	LRRK2: G2019S	Male	Caucasian	58	72	Yes
	LRRK2: G2019S	Male	Caucasian	34	57	No
GBA	GBA: N370S	Male	Caucasian	63	69	No
	GBA: N370S	Female	Caucasian	46	59	Yes
PINK1	PINK1: ILE368ASN	Male	Caucasian	66	64	Yes
	PINK1: ILE368ASN	Female	Caucasian	60	60	Yes
Control	Population control	Female	Caucasian	n/a	60	Yes

Isogenic knock-out lines	Disease
PARK2 -/-	PD
PARK7 -/-	PD
PINK1 -/-	PD
LRRK2 -/-	PD
Park2-/-; Park7-/-	PD
Park2-/-; Pink1-/-	PD
APOE -/-	Alzheimer's disease
SOD1 -/-	ALS
DICS1 -/-	Schizophrenia
CNTNAP2 -/-	Autism
BDNF -/-	CNS

Knock-in neural lineage-specific reporters	Description
MAP2-Nanoluc-Halotag KI	Neuron reporter
GFAP-Nanoluc-Halotag KI	Astrocyte reporter
MBP-Nanoluc-Halotag KI	Oligodendrocyte reporter

Safe-harbor knock-in lines	Description
CAG-GFP, AAVS/Chr19	Ubiquitous reporter
DCX-GFP	Neuron reporter

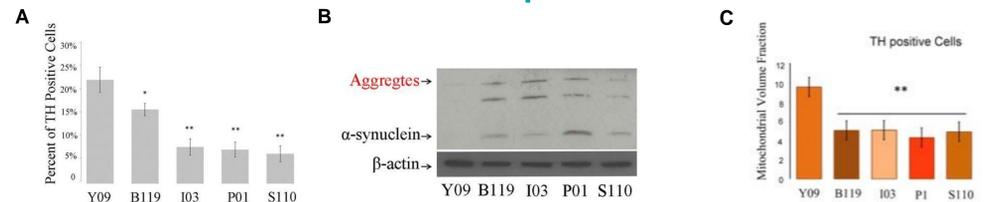
## Generation of Isogenic Panels of Neurons & Glia Using Neural Stem Cells as a Stable Intermediate



## Familial PD Model Using a Combination of Patient-specific & Isogenic Parkin (Park2) Lines

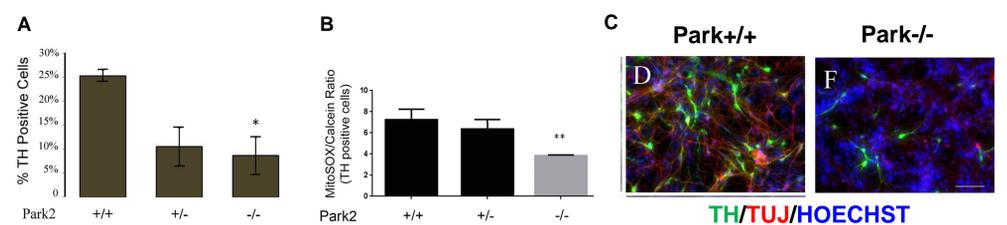
Line	NINDS Catalog ID	Mutation	Gender	Race	Age of onset	Age of sample
Park2	ND30171 (P)	Park2: R42P Park2: EX3DEL	Male	Caucasian	42	54
	ND29543 (I)	Park2: EX3-4DEL Park2: 1-BP DEL, 255A	Male	Hispanic	16	50
	ND29369 (B)	Park2: R275W	Female	Hispanic	43	61
	ND31618 (S)	Park2: R42P	Female	Caucasian	44	63
	Control	ND34791 (Y)	Population control	Female	Caucasian	n/a

## Impaired dopaminergic differentiation and accumulation of SNCA in Park2 patient lines



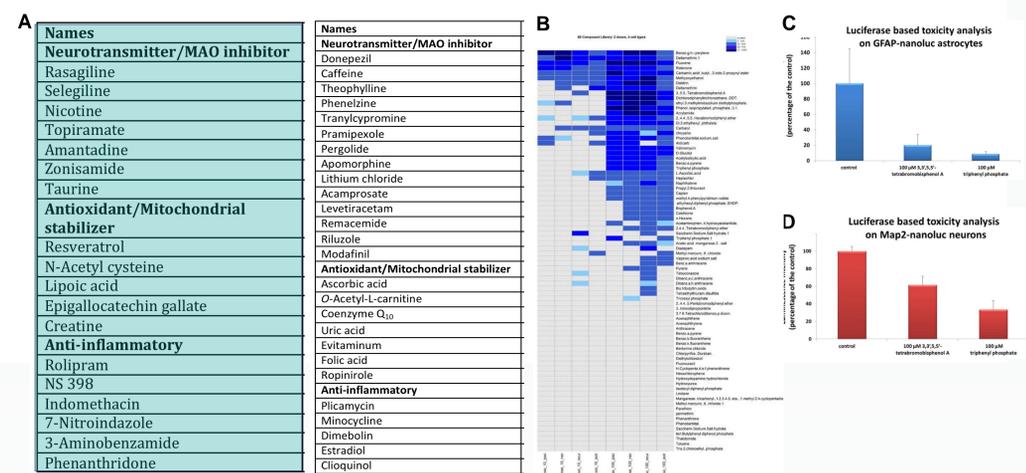
(A) The number of TH+ neurons derived from patient-derived iPSCs is significantly lower as compared to control iPSC (Y09); (B) Western Blot for  $\alpha$ -synuclein identified aggregates in patient iPSC-derived neurons and none in control line, Y09; (C) Mitochondrial volume fraction in TH+ dopamine neurons was significantly lower in patient lines.

## Park2-/- Lines Recapitulate Abnormal DA Neuron Phenotypes



(A) Decreased TH+ cells in derived neurons from Park2 -/- knockout iPSC lines; (B) Decreased mitochondrial volume fraction in TH+ dopamine neurons in Park2 -/- lines; (C) Immunostaining for TH (green) and neuronal marker, Tuj1 (red) shows decreased TH+ neurons in Park -/- lines

## Screening Drugs for Neuroprotection & Neurotoxicity



(A) 18 out of 44 compounds screened were found to be neuroprotective in derived-DA neurons, most of which have been used in human trials; (B) Screening for 80 compounds in the Tox21 library showed differential toxicity in isogenic iPSCs, NSCs, Neurons, and Astrocytes, using MTT assay in 96-well plates and at two doses for each compound<sup>4</sup>; (C-D) Luciferase-based toxicity analysis in astrocytes and neurons derived from lineage-specific reporter iPSC lines<sup>4</sup> for high throughput screening of compounds.

## Conclusions

- We have developed a panel of lines including control, patient-specific, isogenic and reporter lines, which provide a unique advantage for disease modeling.
- We have established robust methods for generating neurons and glia from iPSC using a NSC gateway concept. Our strategy enables us to derive neurons and glia from virtually all lines.
- We have shown that iPSC-derived neuronal and glial cells can be used for modeling neurodegenerative diseases as well as for neurotoxicological and neuroprotective screens.
- Human neural cultures may better mimic human neurodegenerative disorders.

## References

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