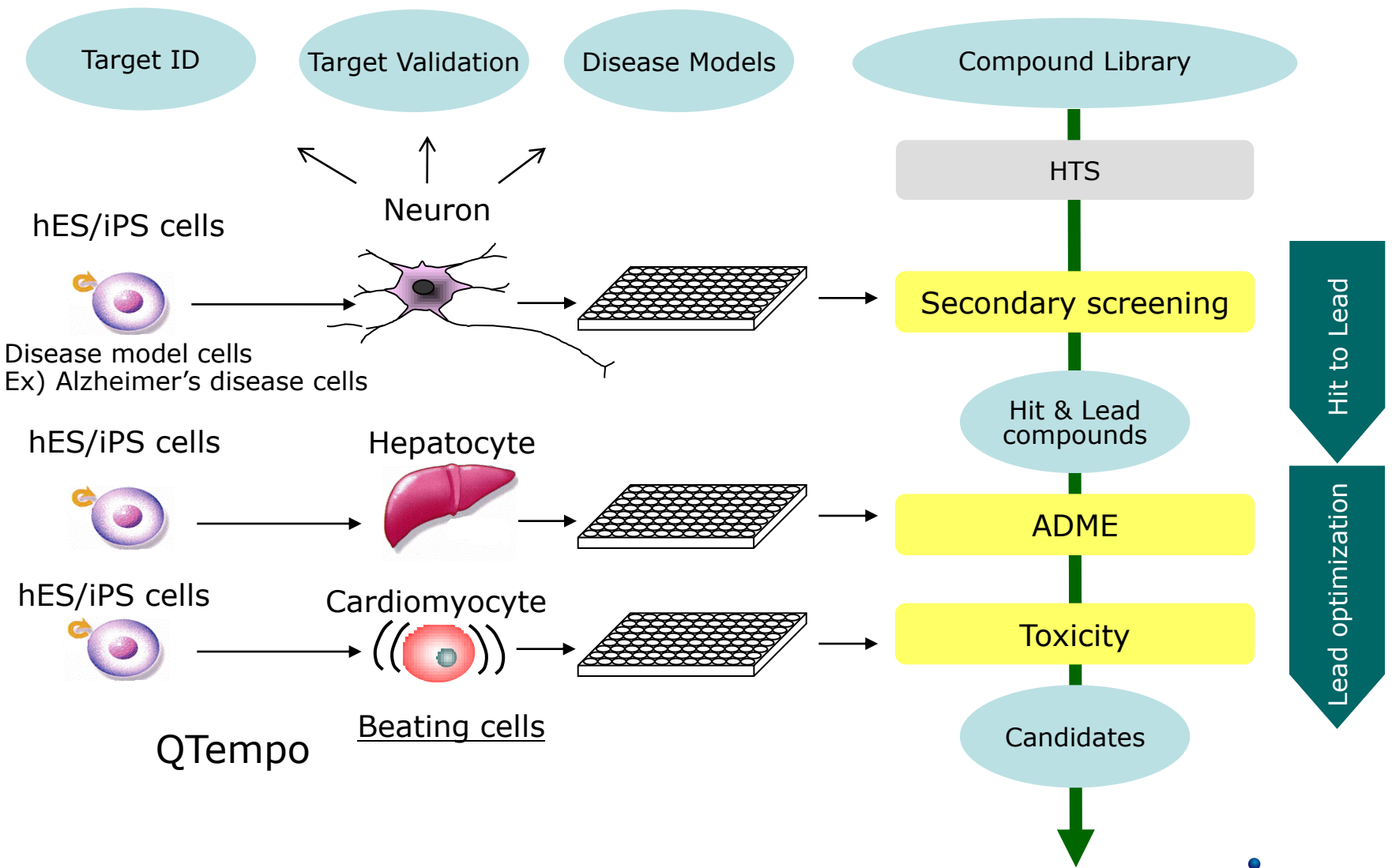
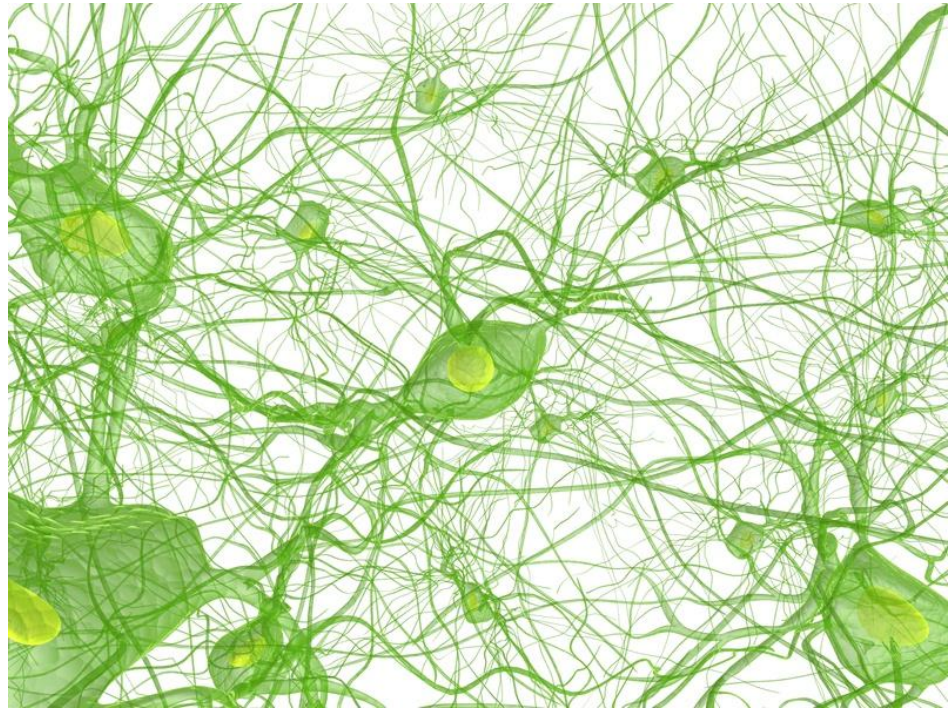




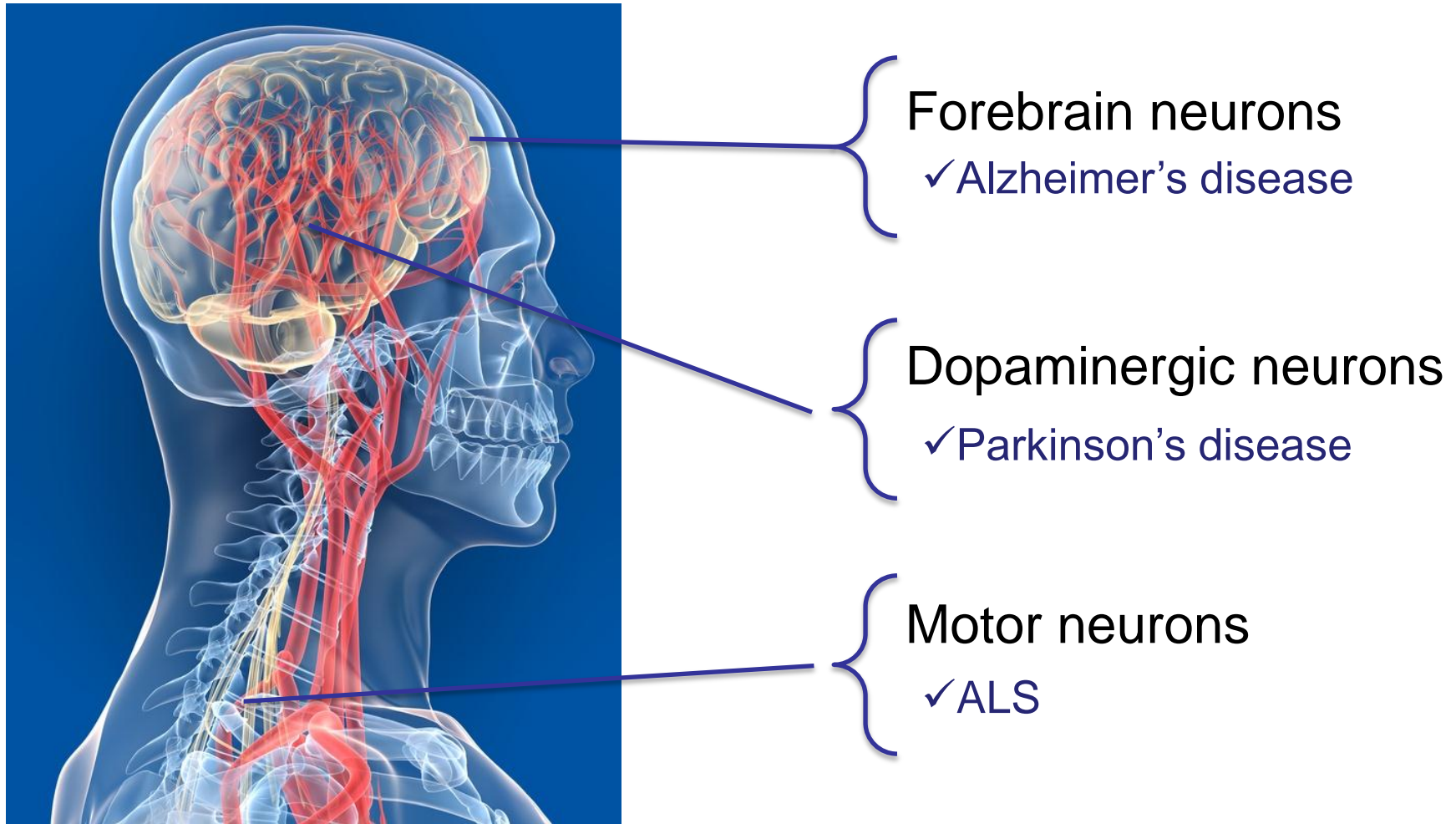
ReproCELL – Areas of Focus and Benefits



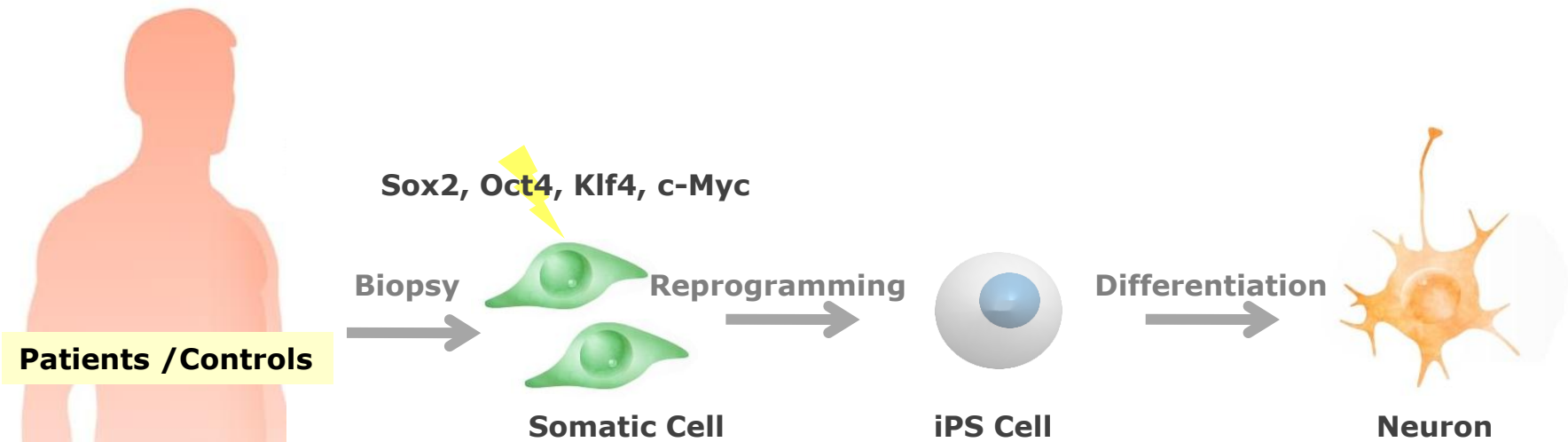
Neuronal Technologies



Cellular Models for Human Neurodegenerative Diseases



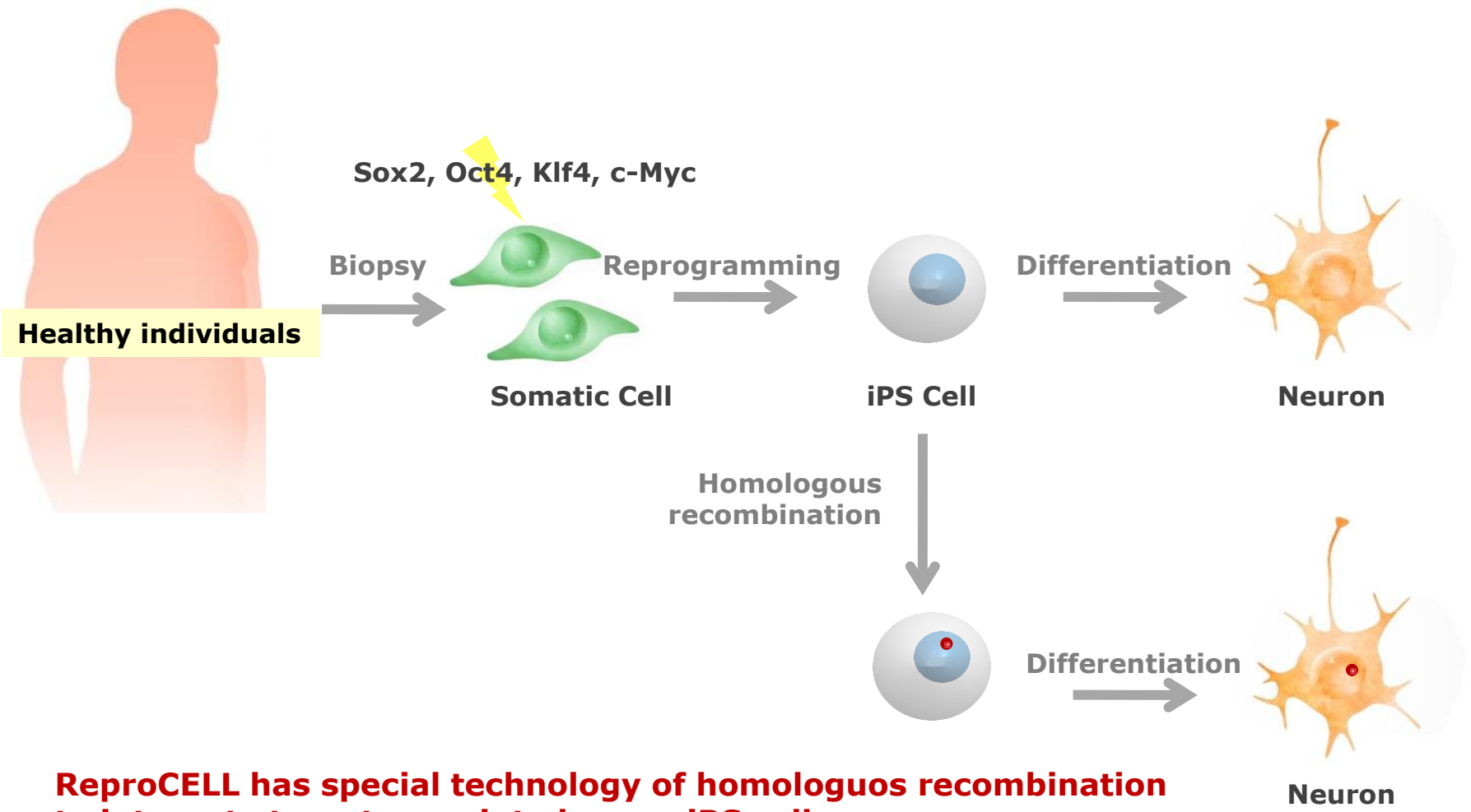
Patient-derived iPS Cells



ReproCELL is proceeding a joint research with a few cell providers

- Parkinsons disease**
- Alzheimers disease**

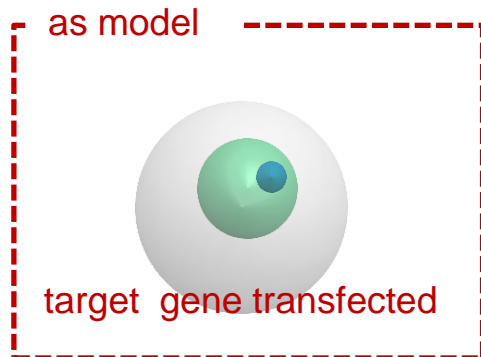
iPS Cells w/ or w/o genetic modification for Secondary Screening



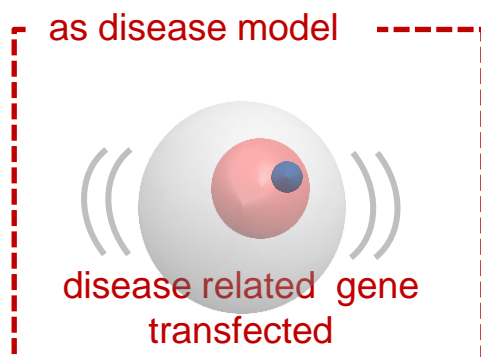
ReproCELL has special technology of homologous recombination to integrate target gene into human iPS cells

Next Generation Screening Assays

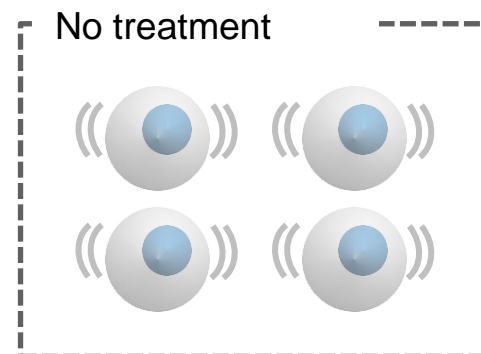
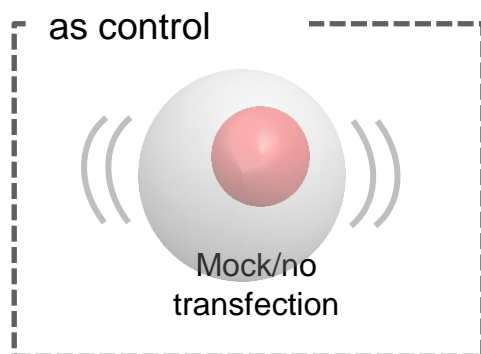
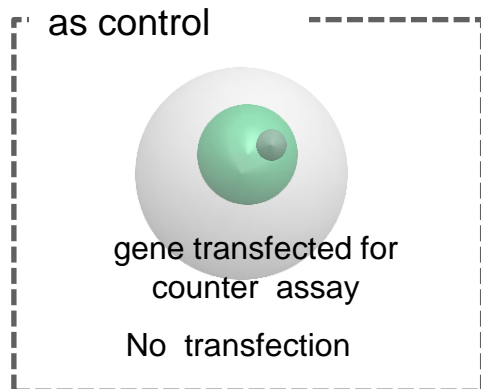
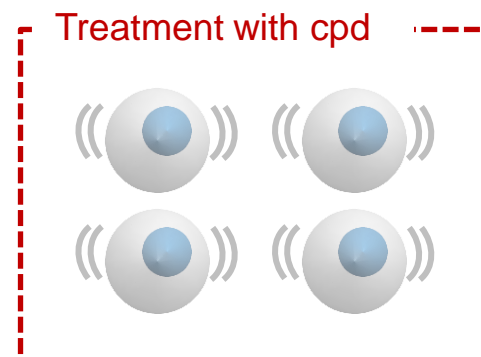
Primary assay on HTS
(for whole library)



Secondary assay on M/LTS
(for biased/focused library)



Third assay on LTS
(for hit compounds)



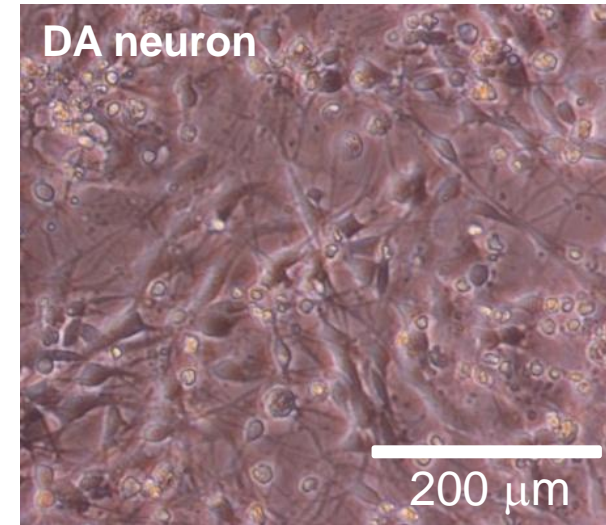
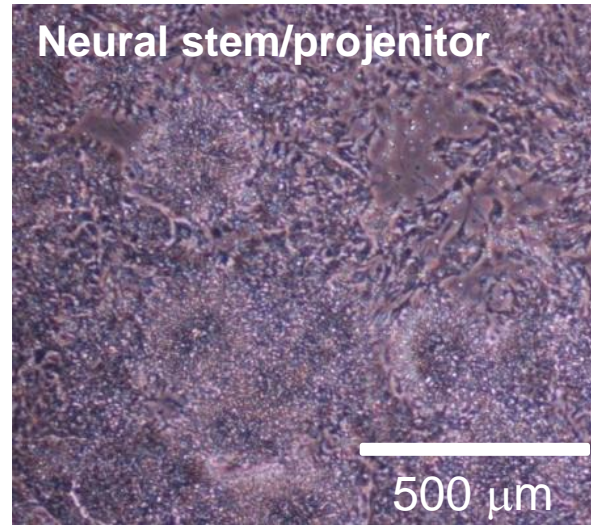
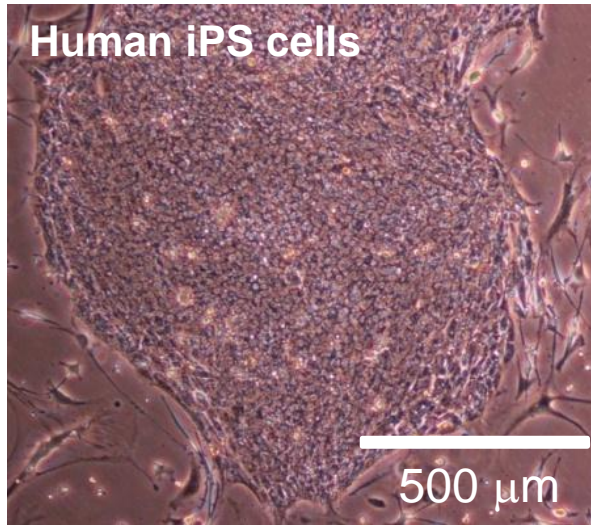
✓ **Cell line** with **target gene** integrated

✓ **iPS/ES cell** with **target gene** integrated

✓ **Paneled iPS cells** derived from various **patients**

Dopaminergic neurons

Differentiation of human iPS Cells into DA Neuron



- ReproNeuro DA kit**
- Frozen cells 3×10^6 cells
 - Maturation medium
 - Additive A
 - Coating material

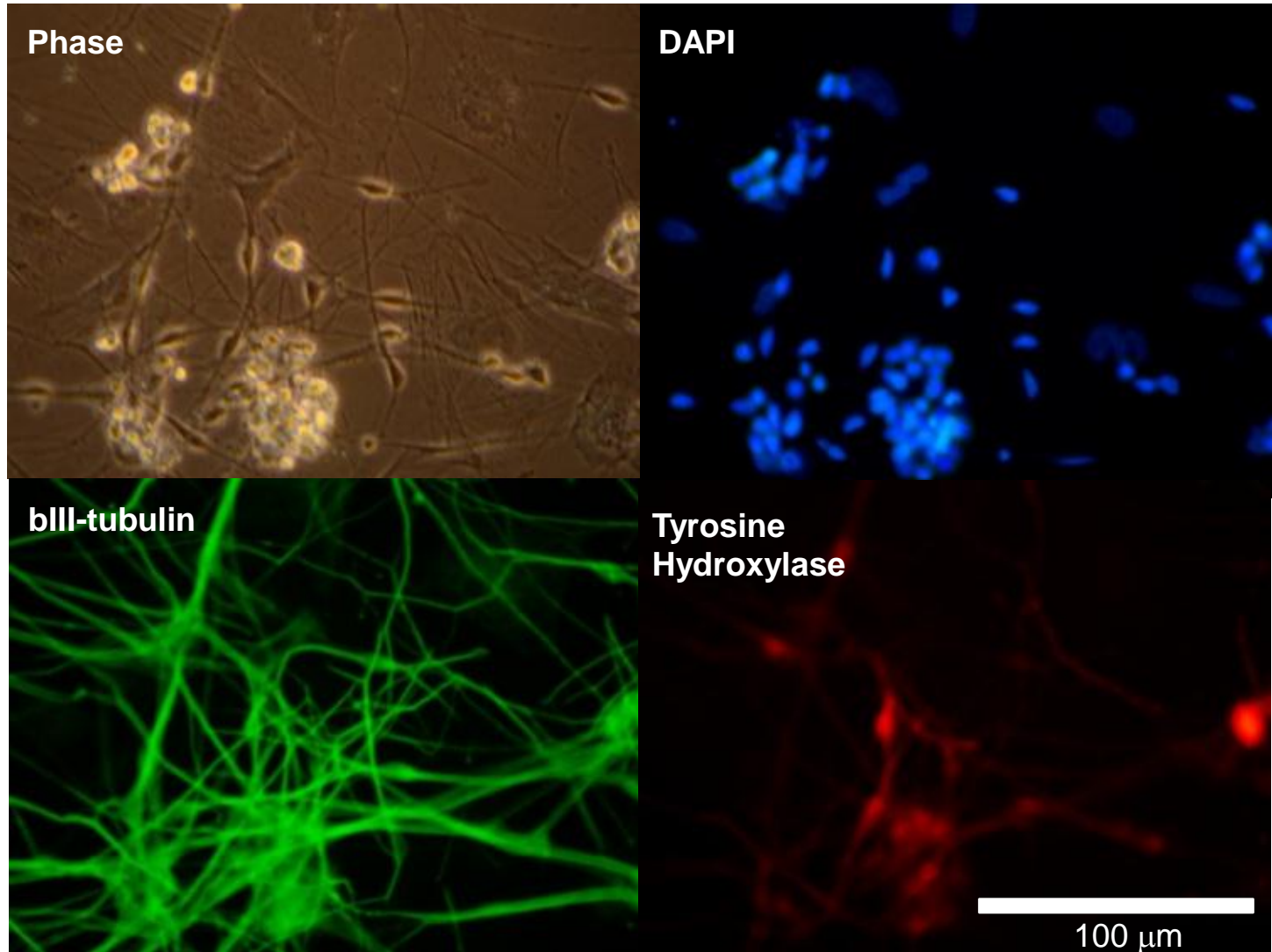
In customer site

- Thawing
- 2-week culture

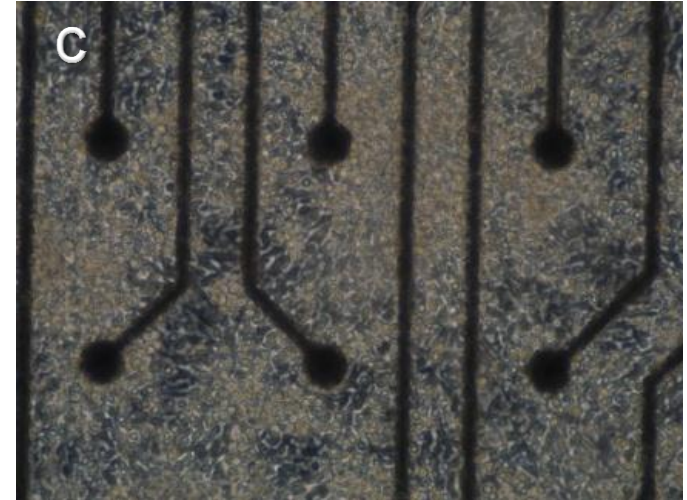
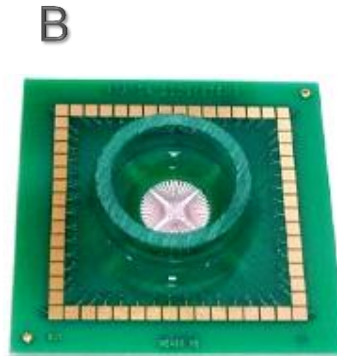
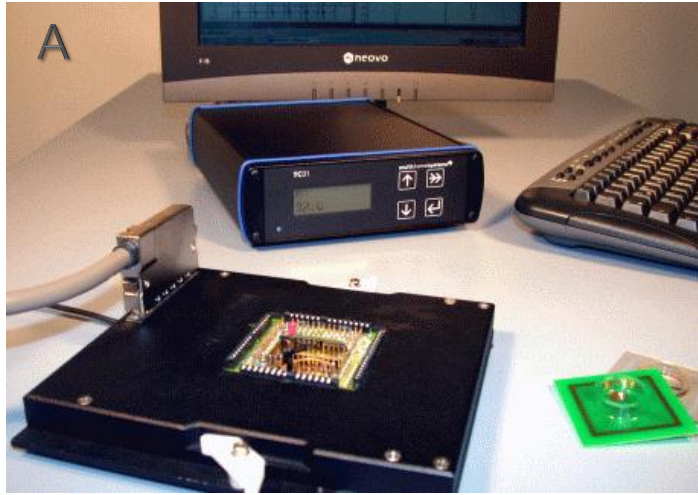
Understanding brings INNOVATION



Characterisation of Dopaminergic Neuron Derived from Human iPS cell



Electrophysiological Assay Device for Neuron



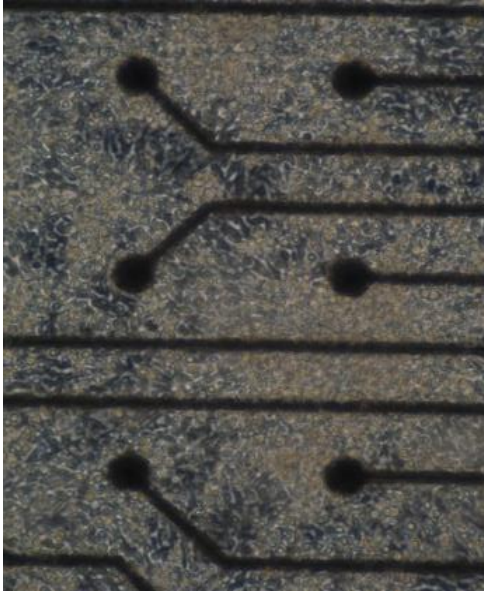
A: MEA system

B: multi electrodes dish

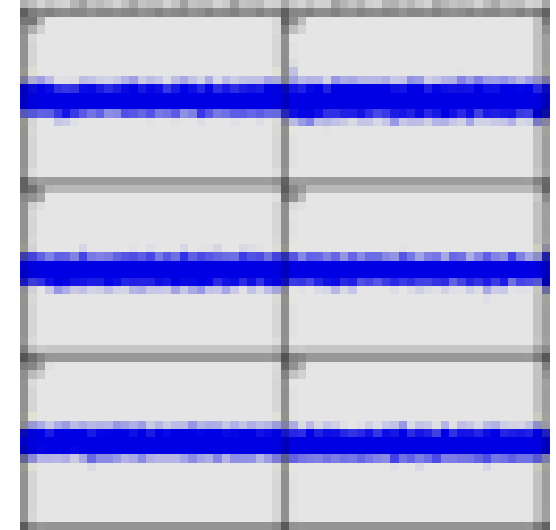
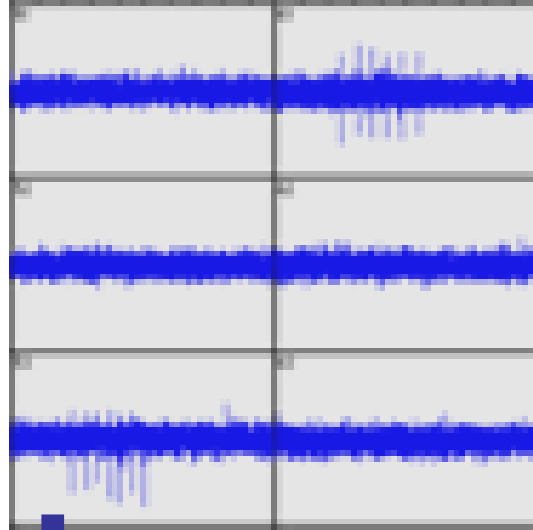
C: Neuron seeded on electrodes

For the electrophysiological characterization hiPSC-neuron were placed on the recording field of micro electrode arrays (MEA) (Multi Channel Systems, Reutlingen, Germany).

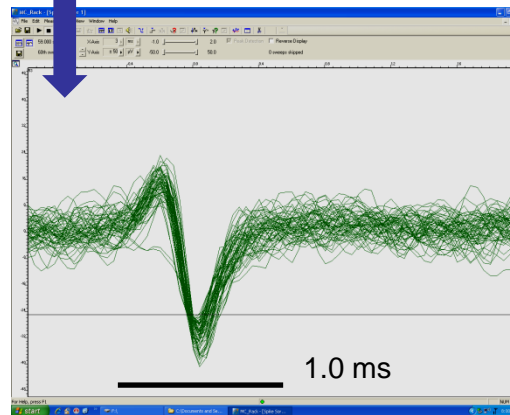
Spontaneous excitation from hiPSC derived Neuron detected by MEA system



hiPSC derived neuron on MEA

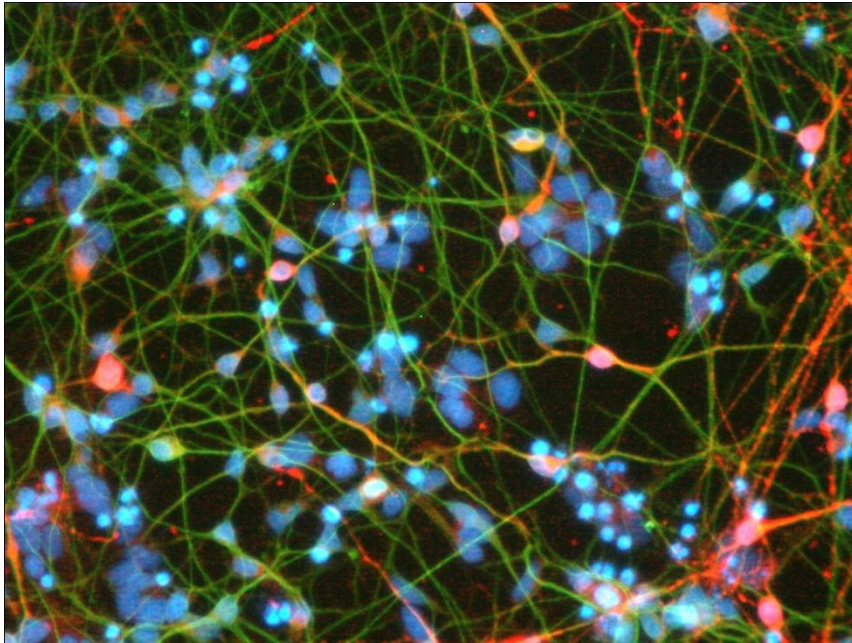


Treated with 100uM of TTX

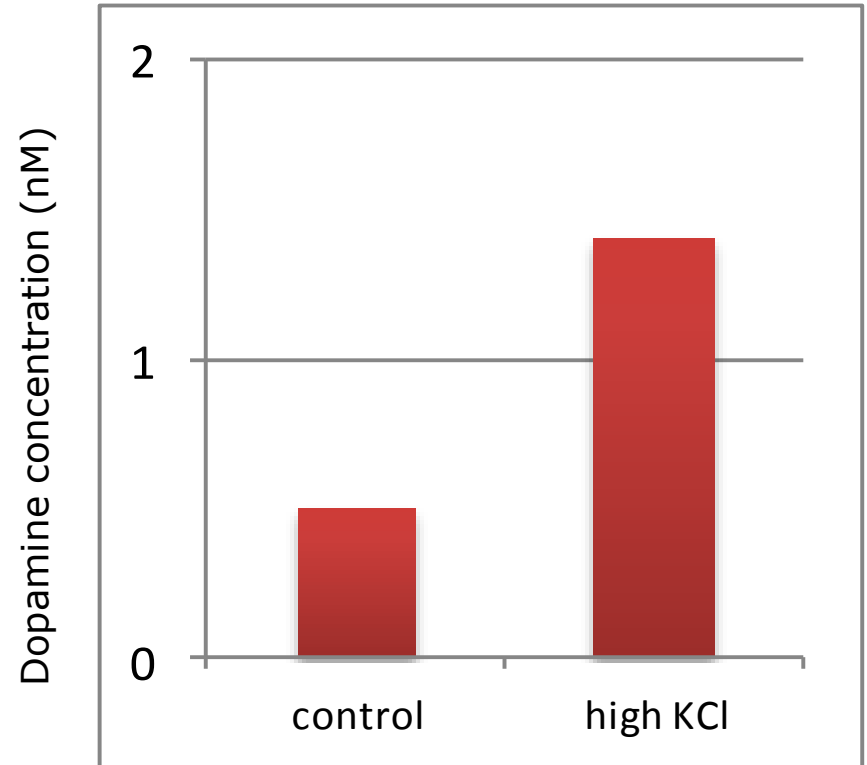


Overlaid each wave from neuron

Dopamine Release and Re-uptake Assay

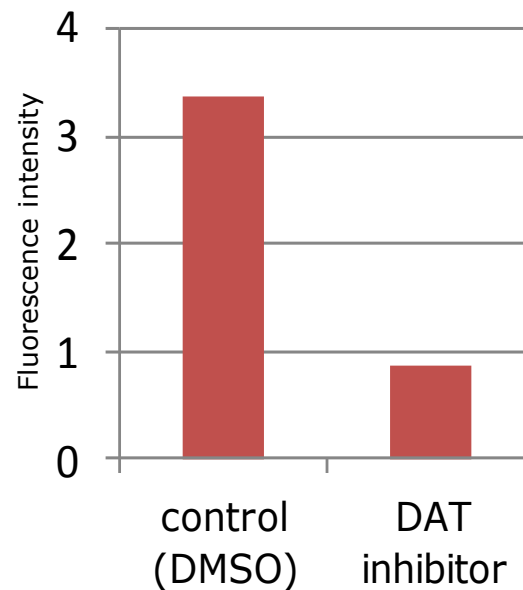
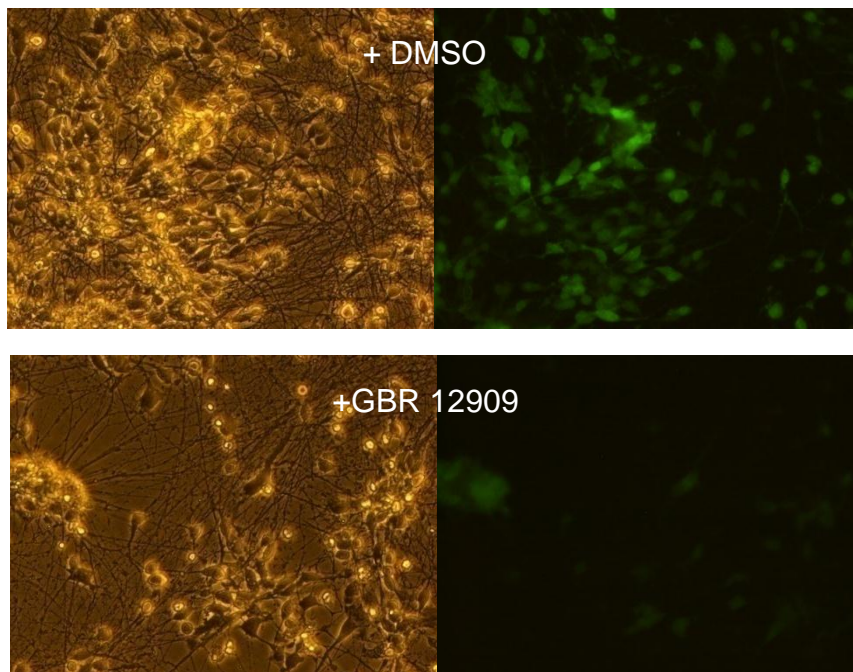


Blue: DAPI
Green: β III-tubulin (neuron)
Red: tyrosine hydroxylase (dopaminergic (DA) neuron)



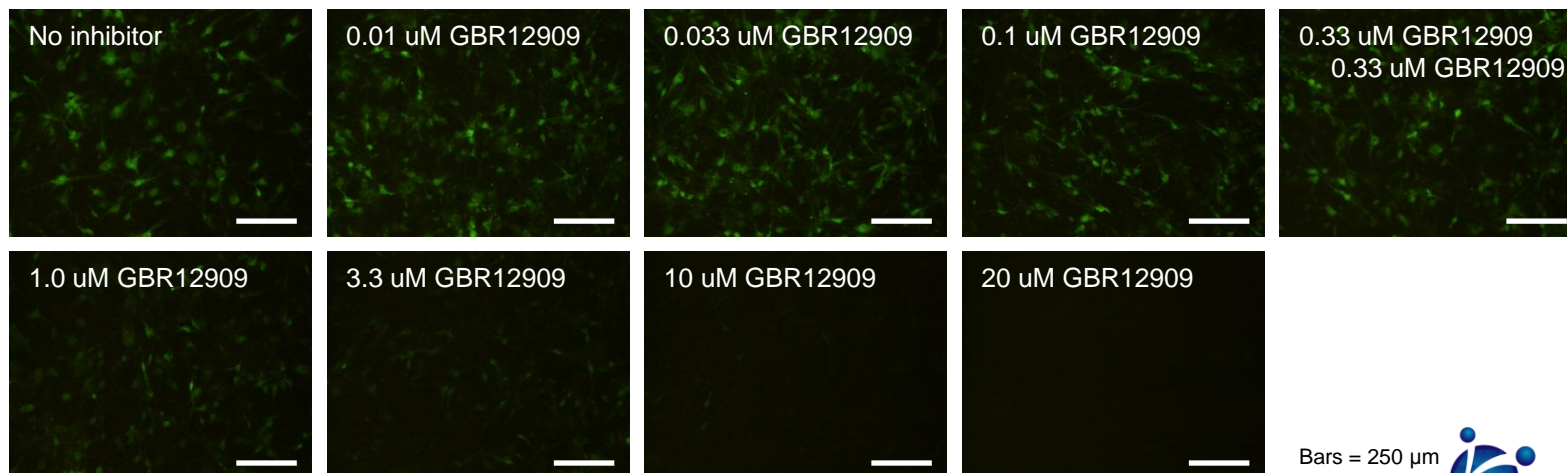
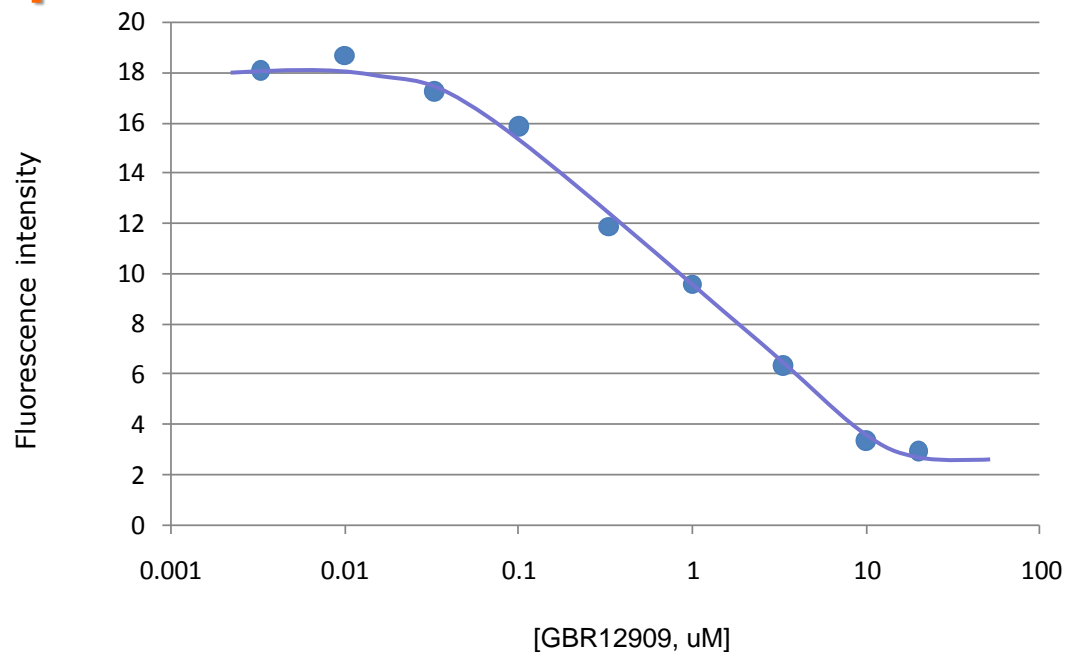
The dopamine release from the differentiated neuron derived from human iPS cells was demonstrated, since dopamine concentration in the culture medium was increased by depolarization (elevate the KCl concentration to 56 mM).

Dopamine Release and Re-uptake Assay (2)



The differentiated neuron derived from human iPS cells were treated with DMSO or DAT (Dopamine Transporter) inhibitor (GBR12909, 20 μ M). Dopamine uptake was measured by Neurotransmitter transporter uptake assay kit (Molecular Device) The fluorescence intensity in control was reduce by DAT inhibitor treatment. That results indicate functional expression of the neurotransmitter transporter.

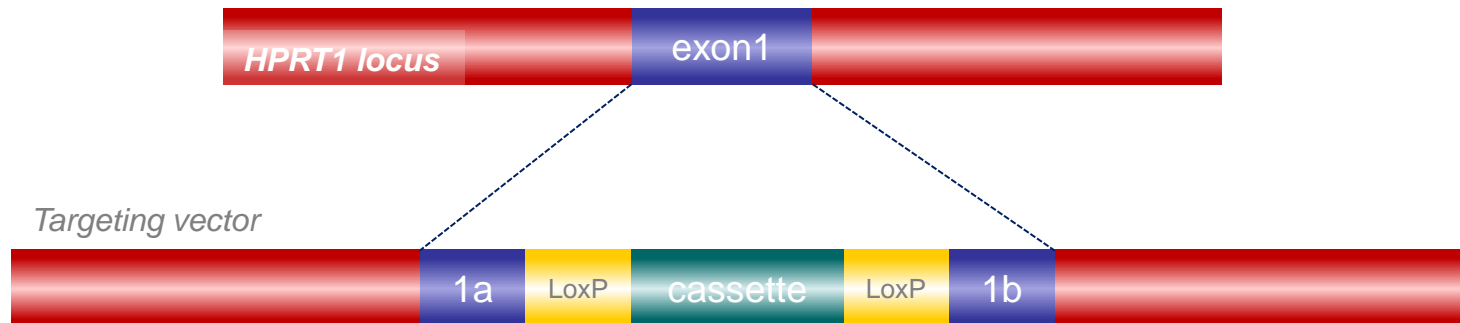
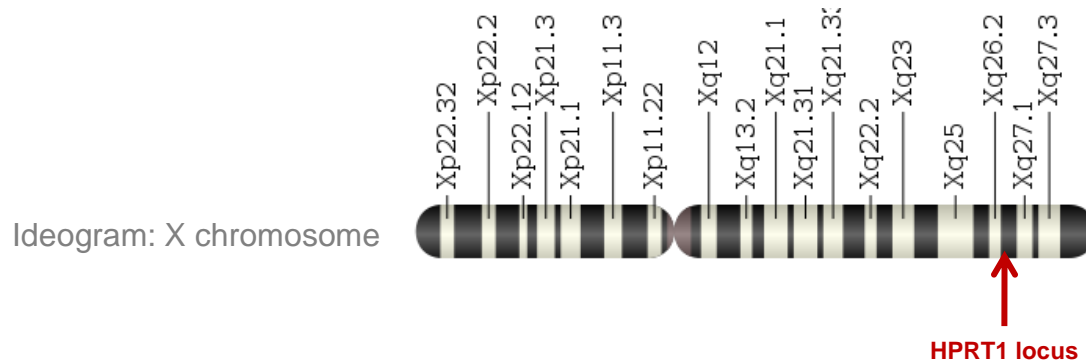
Dopamine Release and Re-uptake Assay - dose dependency -



Cholinergic neuron and Alzheimer's disease

Homologous recombination in hiPSC to generate disease model

Transfer the gene replacement cassette in HPRT1 locus by homologous recombination

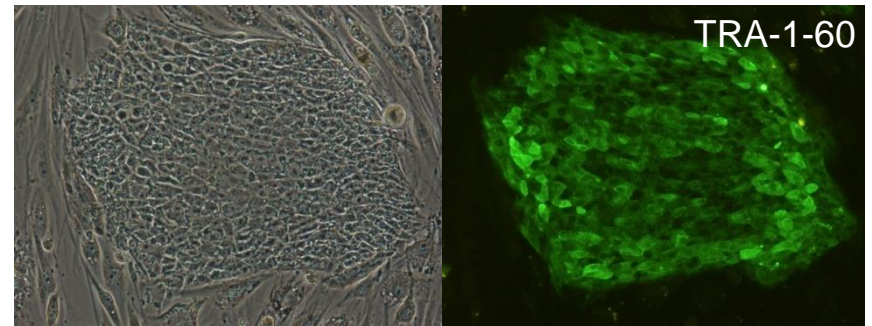
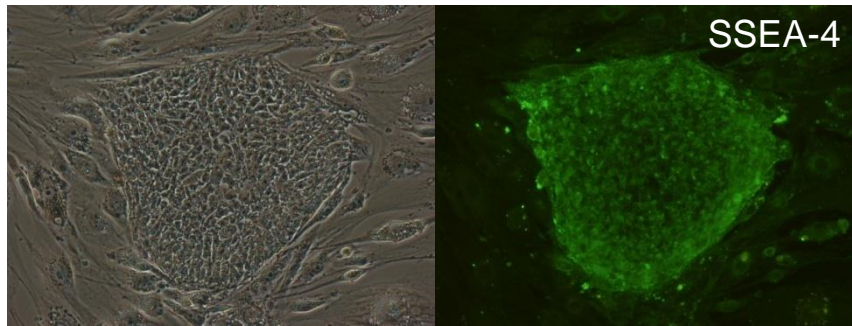
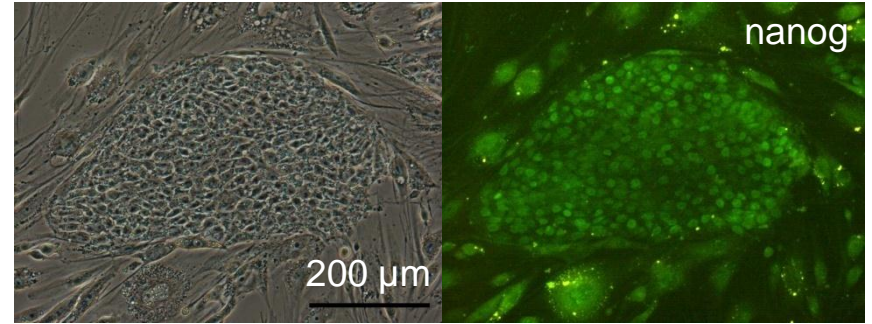
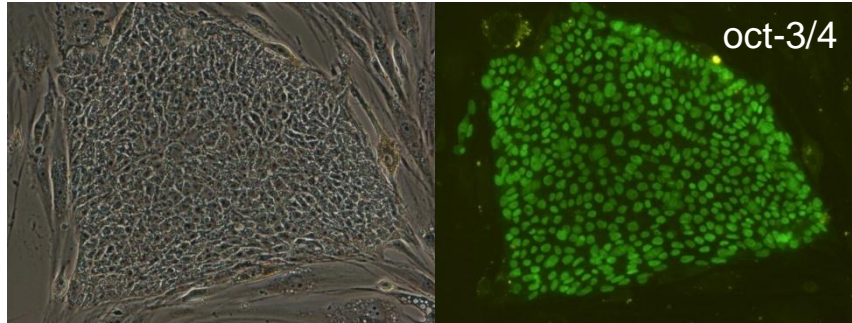


HPRT1 locus can be used as target site to transfer the gene according to this locus is constitutive active.

"Site-specific integration" has advantage in drug development screening-use rather than "random integration"

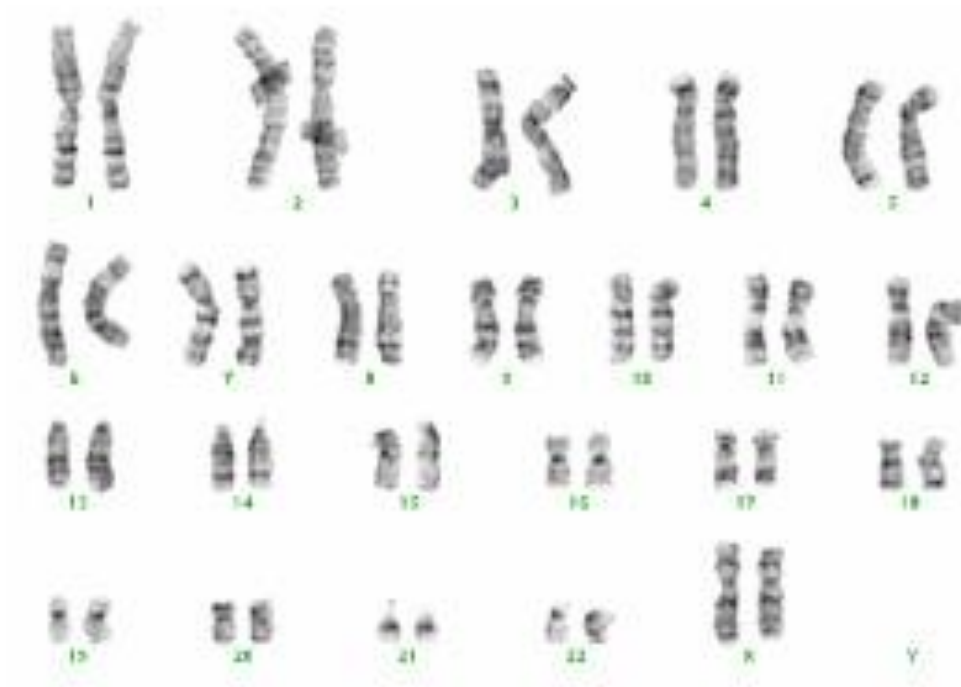
	Random integration	Site-specific integration with cassette
Possibility of gene silencing	HIGH	LOW
Possibility of undesired gene destruction	HIGH	LOW
Replacing to new desired gene	HARD	EASY

Human iPS Cell Line Having Gene Cassette Specific Marker Expression



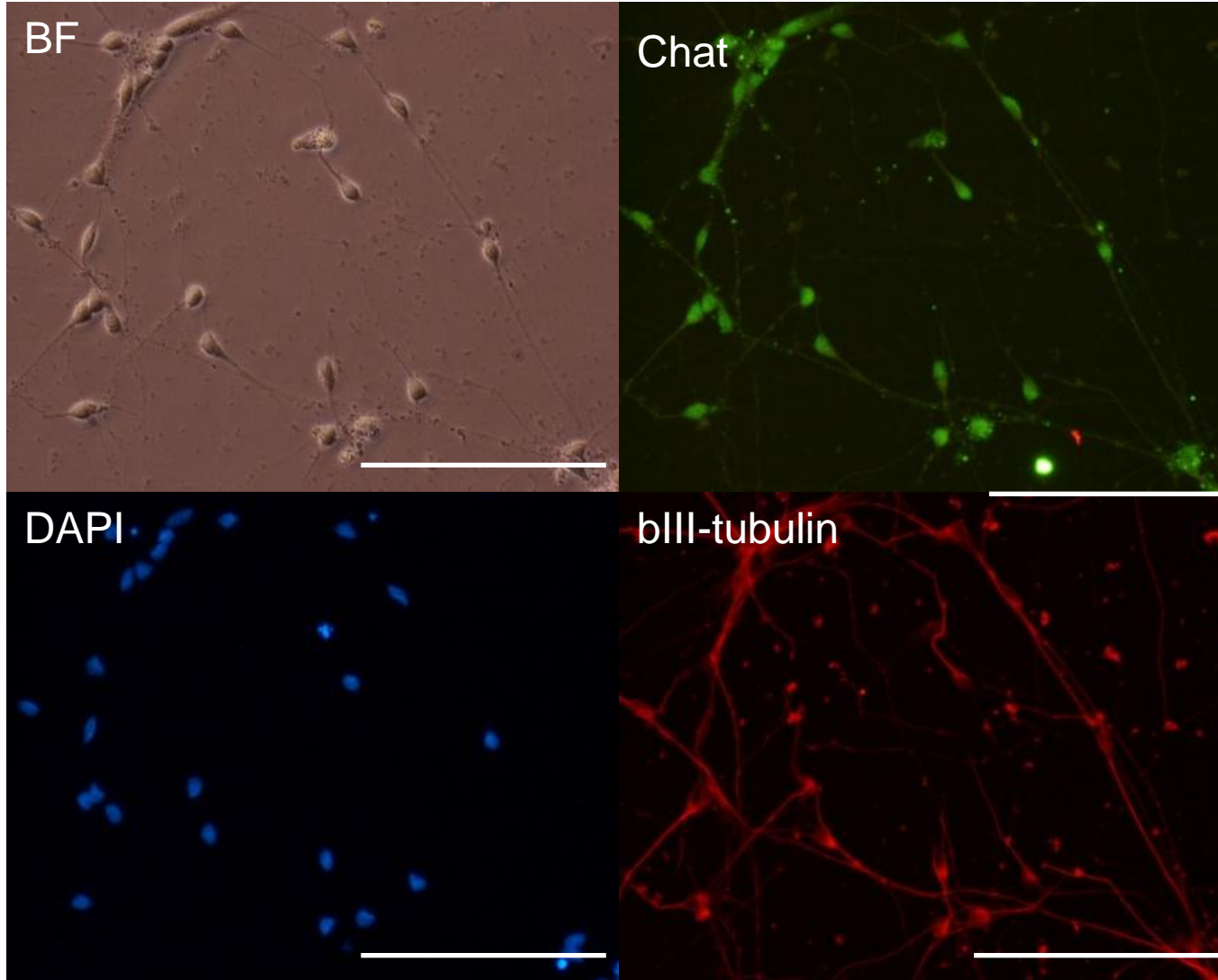
Oct3/4, Nanog, SSEA-4 and Tra1-60 are positive.
No abnormality was observed on growth and colony shape.

Human iPS Cell Line Having Gene Cassette Karyotyping



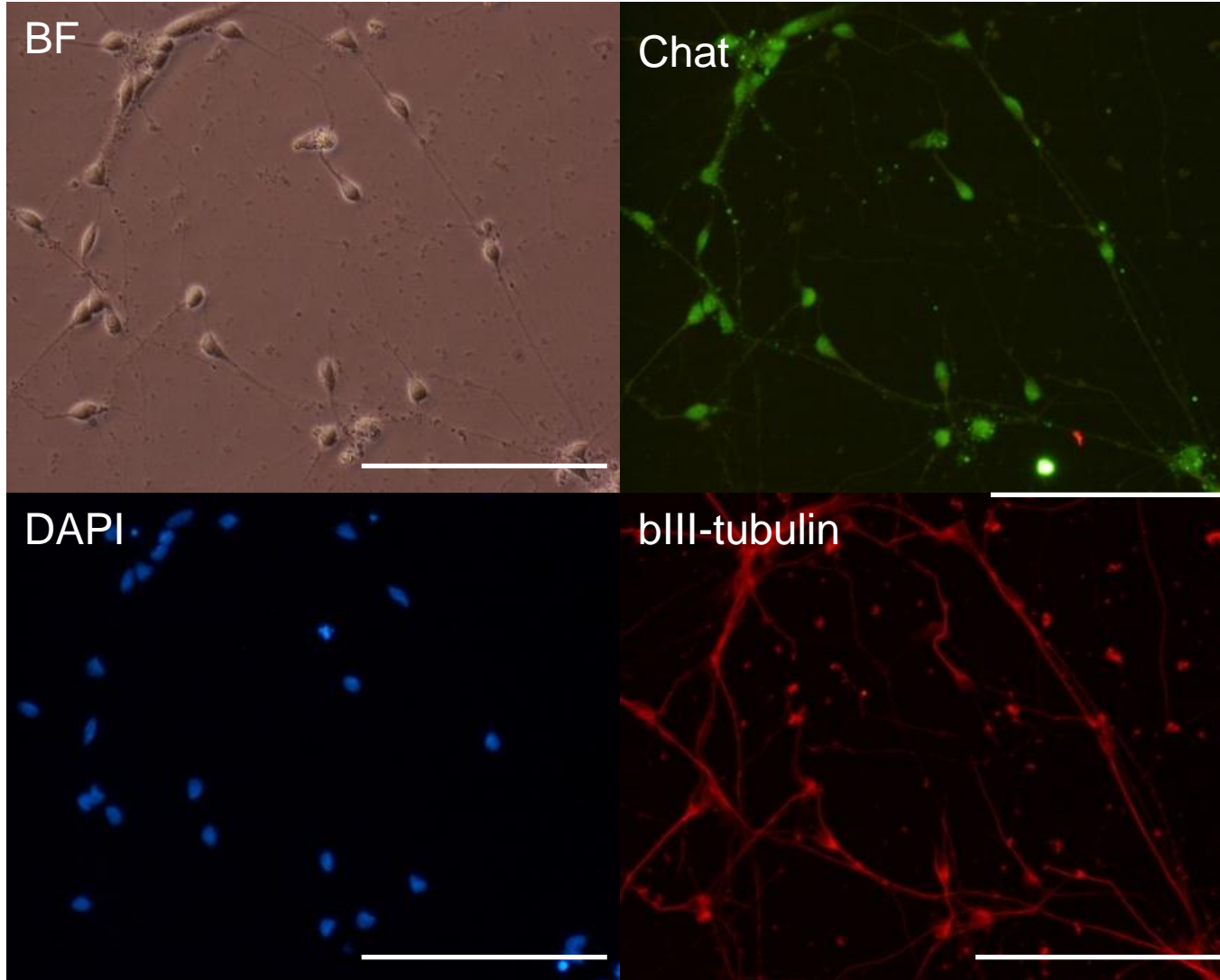
No abnormality was observed (18 passage).

Cholinergic neuron from Human iPS Cells for Alzheimer's disease model



Chat:Choline acetyltransferase
Chat positive cells are 90~% of beta III positive neuron

Cholinergic neuron from Human iPS Cells for Alzheimer's disease model



Chat:Choline acetyltransferase
Chat positive cells are 90~% of beta III positive neuron

Alzheimer's disease: Amyloid Hypothesis

Mutations in APP, **PS1** or PS2 genes

(Familial Alzheimer's disease)

↓
Increase of Ab42 production
and accumulation

↓
Oligomerization and aggregation

↓
Neuronal dysfunction and cell death

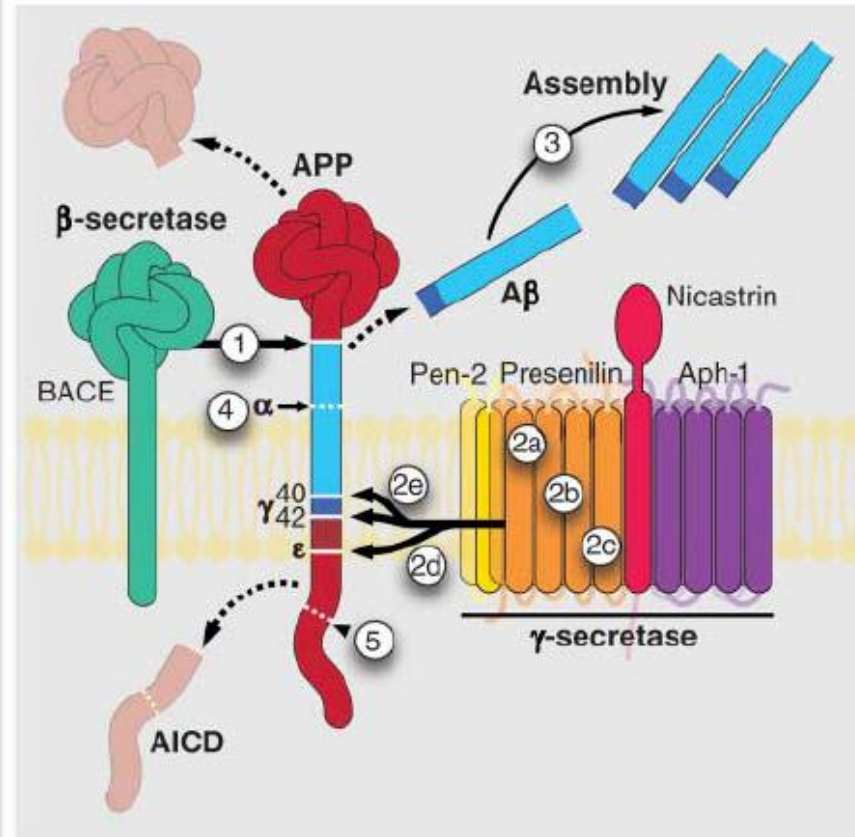
↓
Dementia

APP: amyloid precursor protein

PS1: Presenilin 1

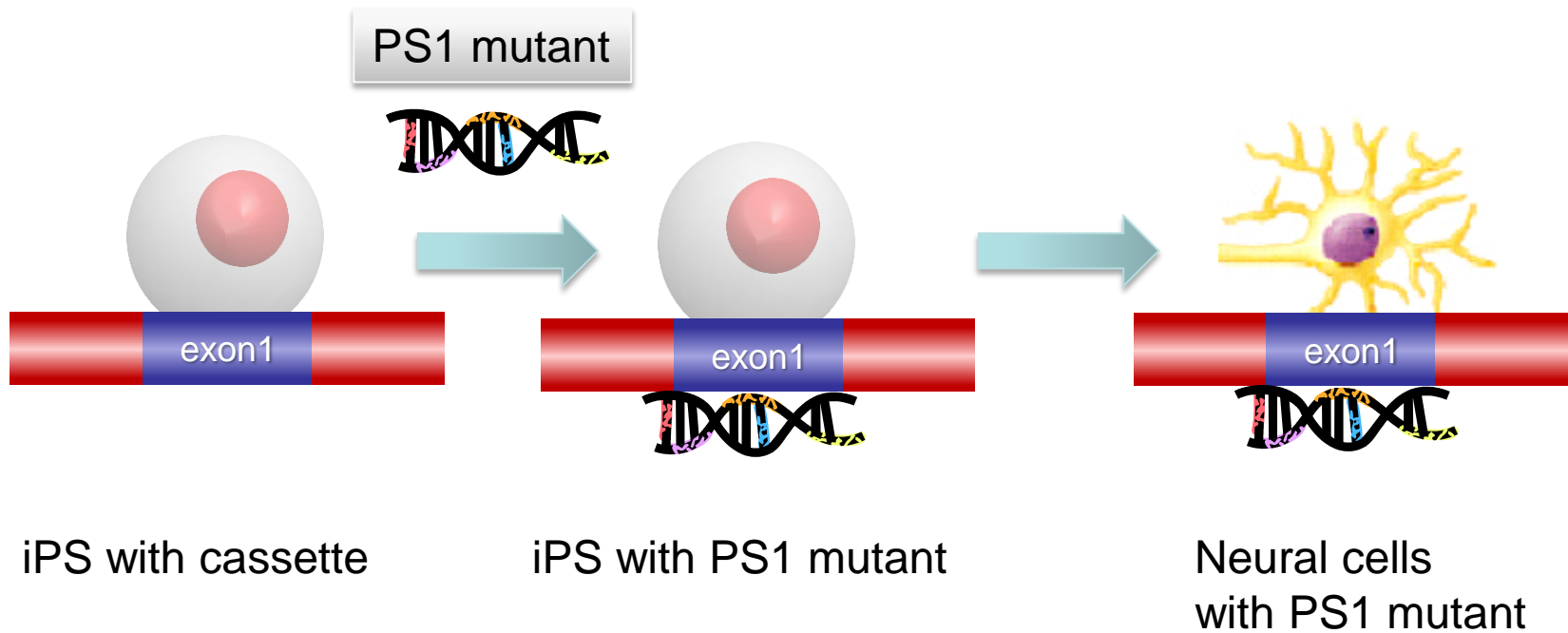
PS2: Presenilin 2

Ab: Amyloid b peptide



Roberson and Mucke, Science 2006

How to make Alzheimer's disease cells



Alzheimer's Disease Model Cells

Mutant PS1

Phenotype analysis

?

Morphology

?

A β 42 production

?

Expression of Synaptophysin

?

**Spontaneous excitatory
current frequency**

Alzheimer's Disease Model Cells

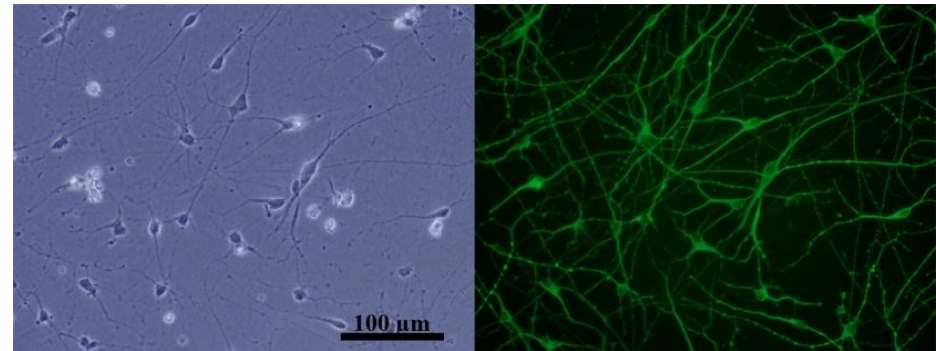
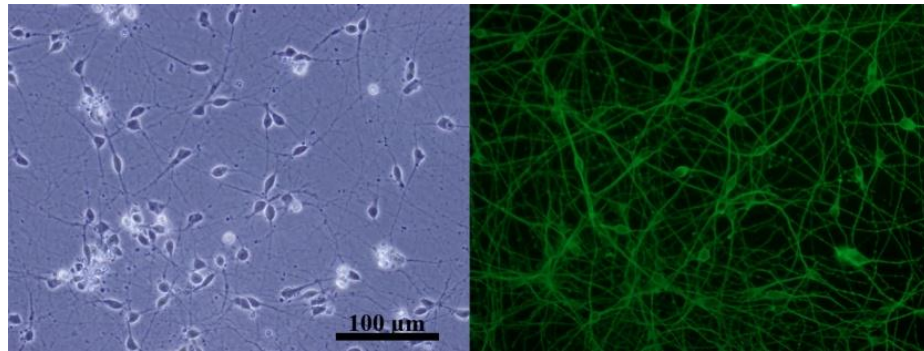
Phenotype analysis 1: Morphology of Differentiated Cells

Wild Type

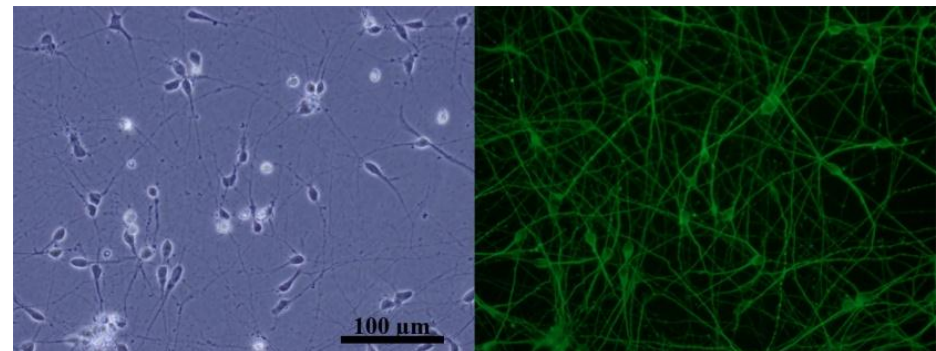
Mutant

P117L

β III-tubulin



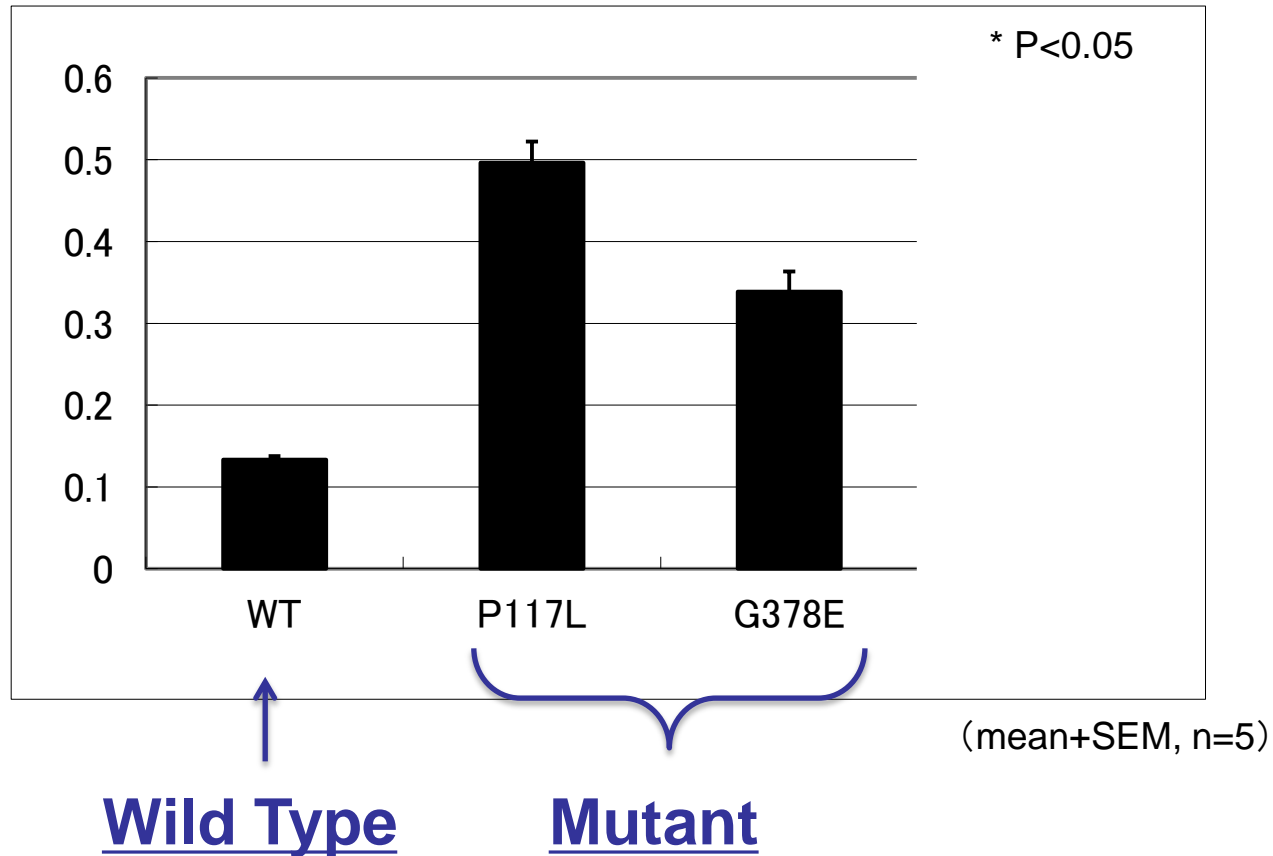
G378E



Alzheimer's Disease Model Cells

Phenotype analysis 2: A β 42 production

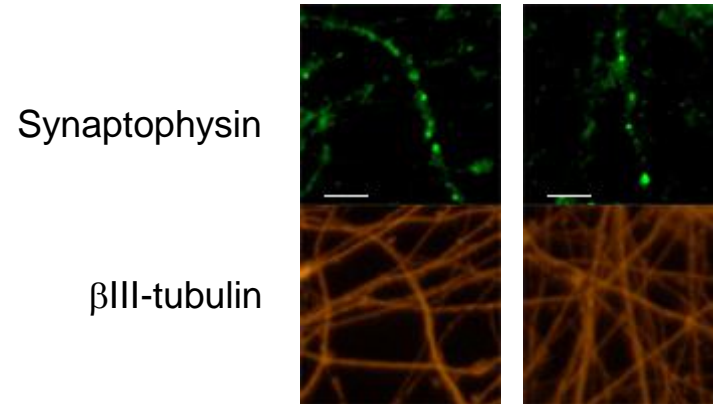
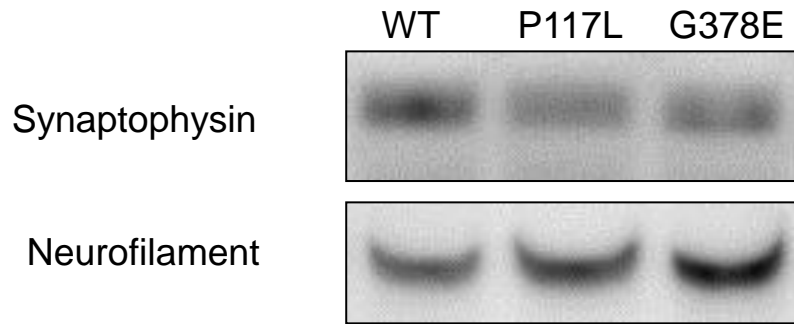
A β 42/A β 40



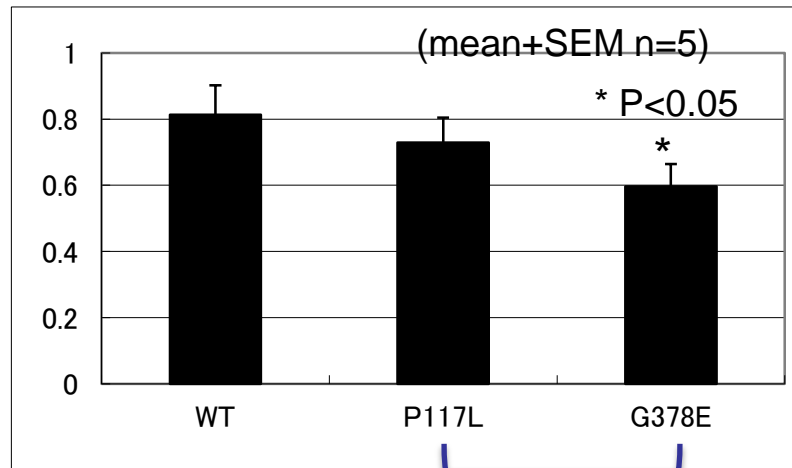
Alzheimer's Disease Model Cells

Phenotype analysis 3:

Expression of Synaptophysin

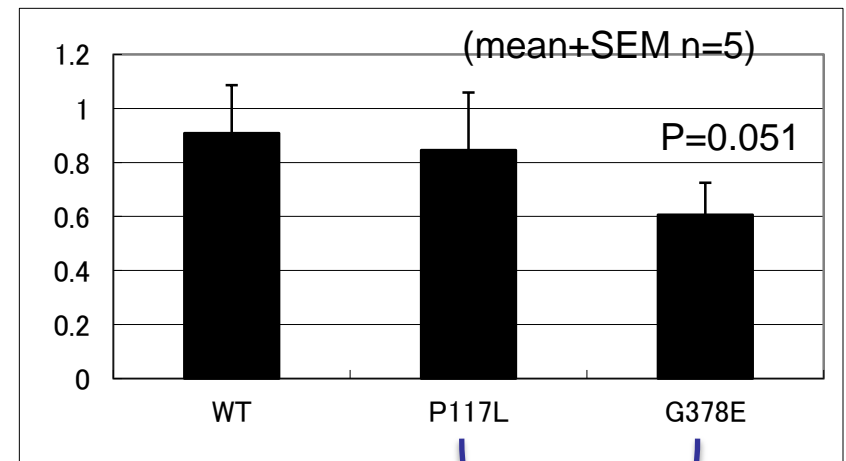


Synaptophysin



Mutant

Synaptophysin(+) dots



Mutant

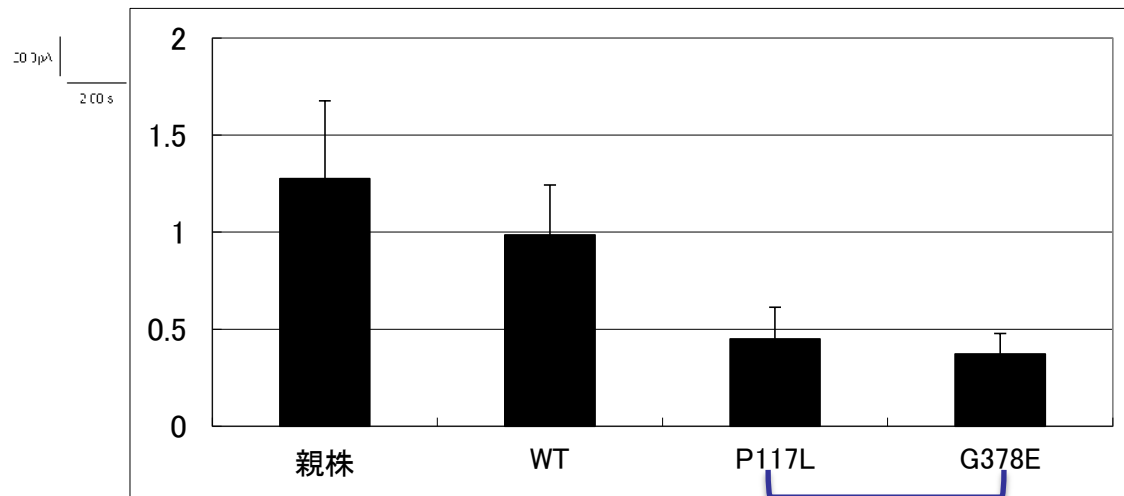
Alzheimer's Disease Model Cells

Phenotype analysis 4:

Spontaneous excitatory current frequency

Wild Type

Mutant



Mutant

Alzheimer's Disease Model Cells

Mutant PS1



Little change



Increase



Decrease



Decrease

Phenotype analysis

Morphology

A β 42 production

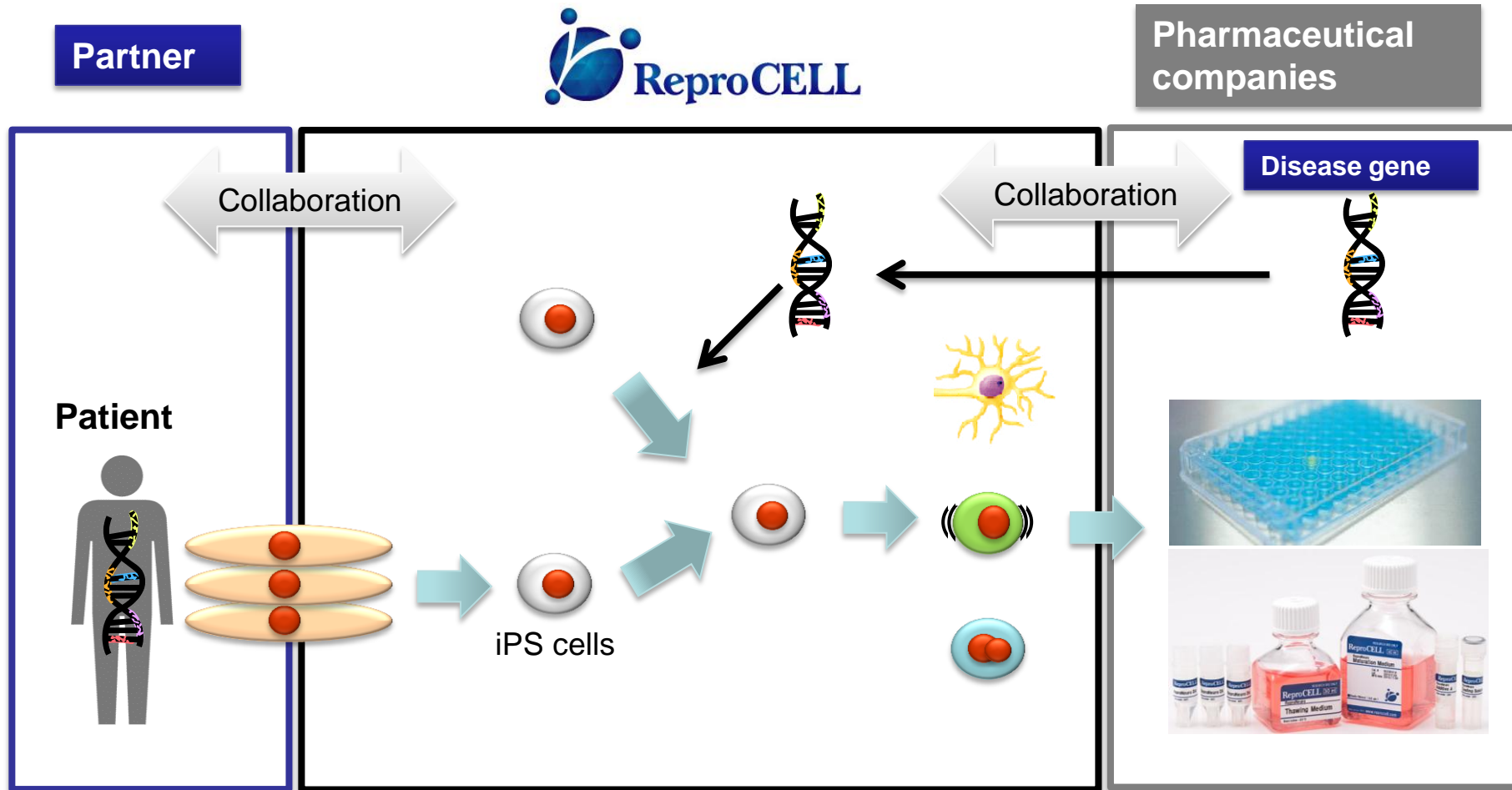
Expression of Synaptophysin

**Spontaneous excitatory
current frequency**



Alzheimer's Disease Model Cells

Business model





Thank you very much

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