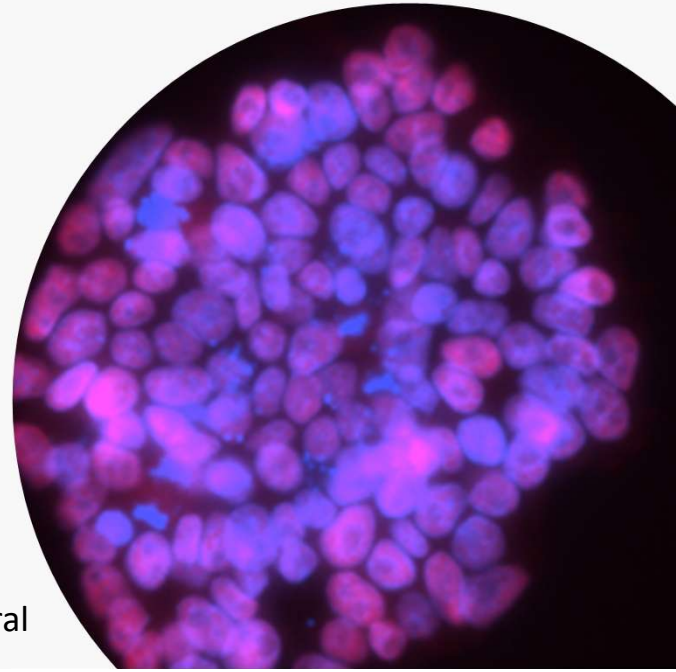
Generation of Cellular Models for Fabry Disease: Unlocking the Potential of iPSCs and Gene Editing

29th Annual Meeting
Sociedade Portuguesa de Genética Humana

20th November 2025



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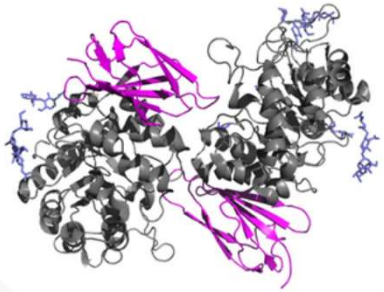
Fabry Disease

Lysosomal Storage Disorder

Genetic Defect

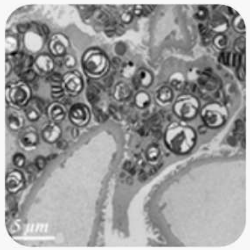
α-Galactosidase A
(*GLA*)

Biochemical Defect

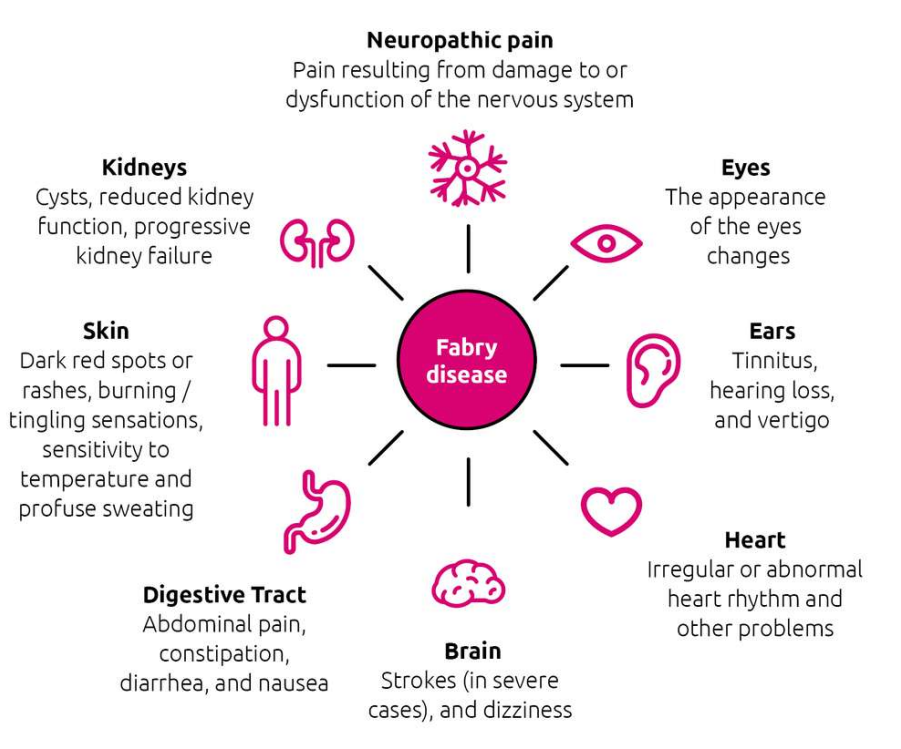


α-Galactosidase A
(α-Gal A)

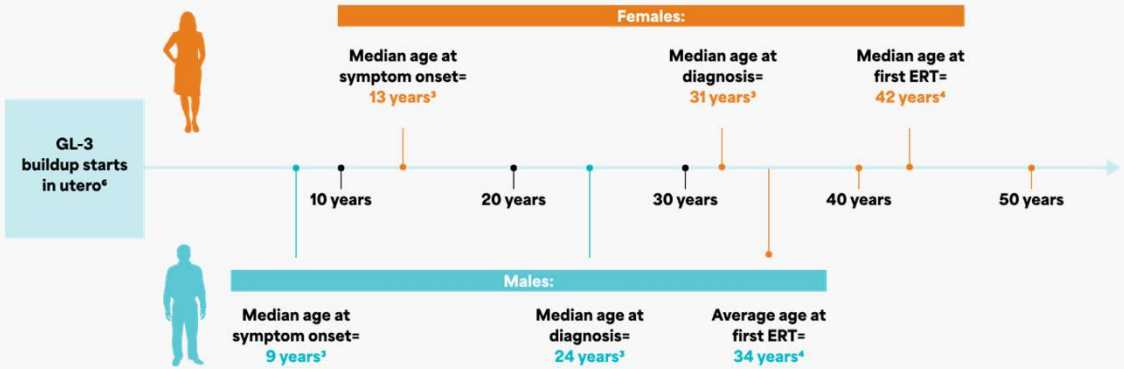
Gb3 and Lyso-Gb3 accumulation



Impact of the Accumulation



https://www.idorsia.com/our-innovation/target-diseases/fabry-disease.html



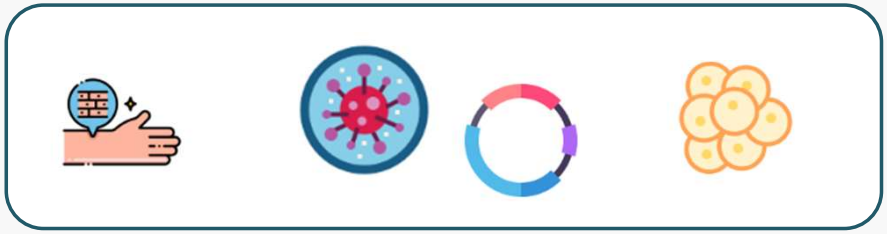
X-linked disease

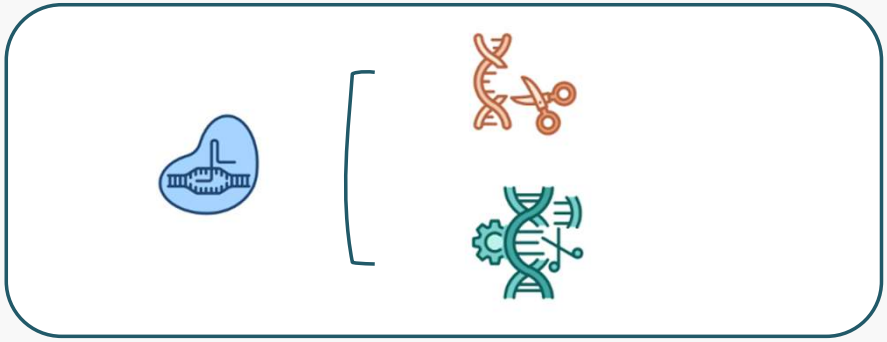
https://www.campus.sanofi/dk/article/rare-disease/fabry/ar/how-to-diagnose-fabry

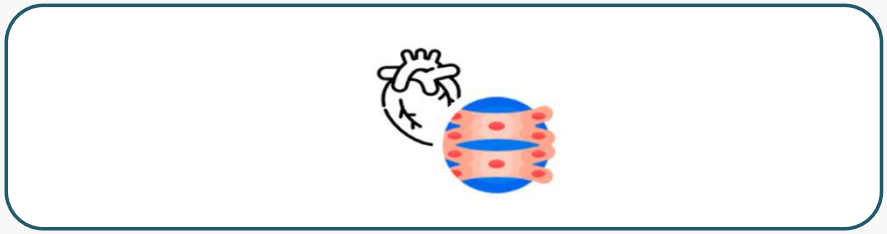
Classical Form 1:50,000 - 1:117,000
(< 1% - 5% α-Gal A activity)

Available Therapies: - Enzyme Replacement Therapy
- Chaperone (Migalastat)

Aim - Cell Models







induced Pluripotent Stem Cells (iPSCs)

- Fabry Disease patient's fibroblasts
- Control fibroblasts cell line (HDFa)

⇩

Gene Editing - CRISPR/Cas9

- Correcting the c.860G>A mutation (p.W287X) (KI)
- Generate a control *GLA* knockout (KO)

⇩

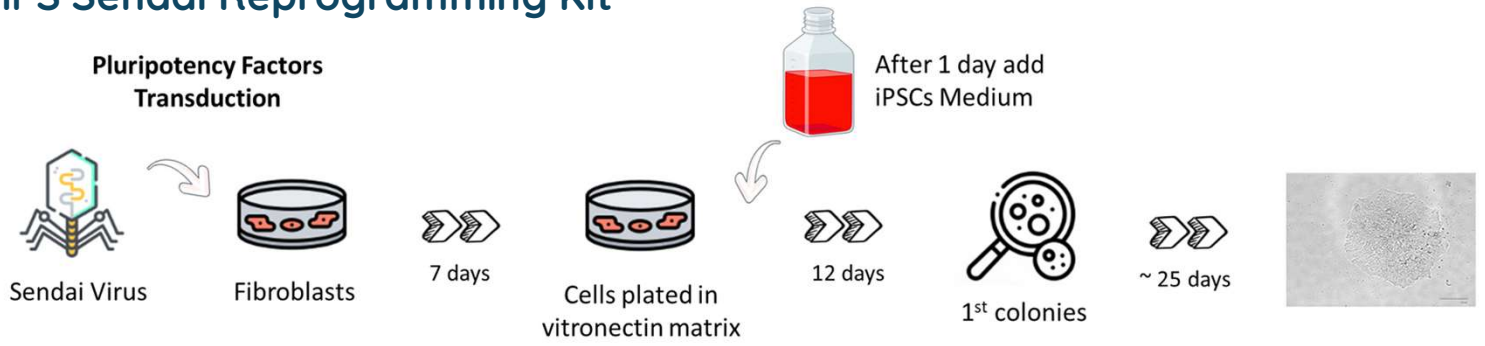
iPSCs-derived Cardiomyocytes (iPSC-CMs)

- A cell type specifically affected by the disease

Methods - iPSCs generation

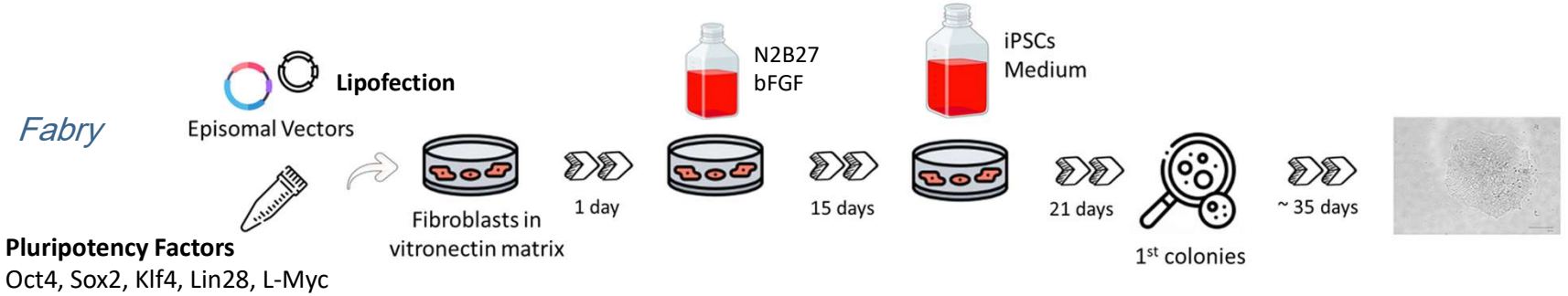
CytoTune™-iPS Sendai Reprogramming Kit

HDFa



Epi5™ - Episomal iPSC Reprogramming Kit

Fabry



Pluripotency Factors
Oct4, Sox2, Klf4, Lin28, L-Myc

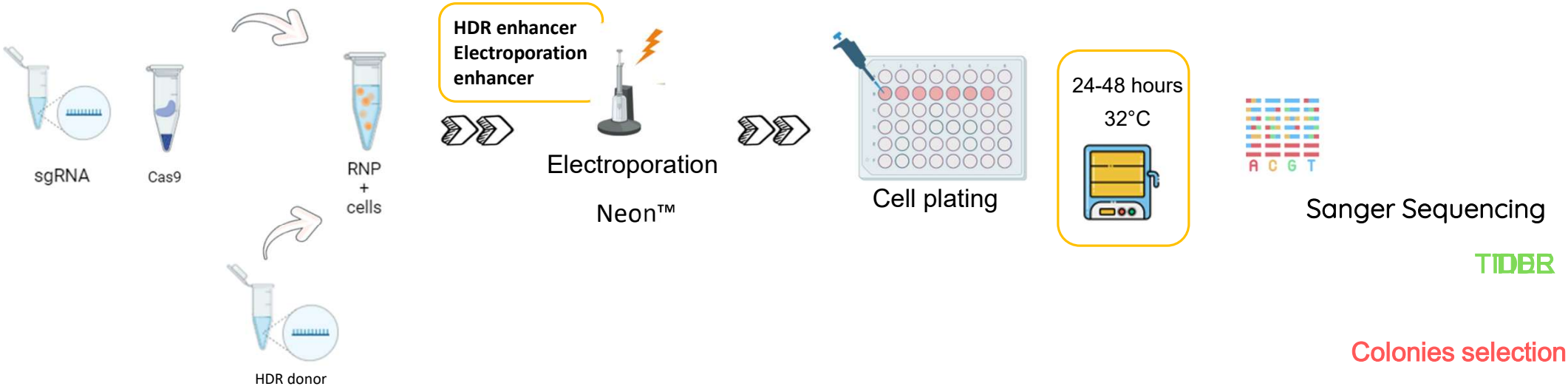
Methods – CRISPR/Cas9 KO and KI

- **sgRNAs chosen in Benchling**
- **Cas9 (IDT™)**
- **HDR donor** c.860G>A para c.860A>G
  p.W287X

HDFa iPSCs *GLA* exon 2 KO: 5'- GCTAGCTGGCGAATCCCATG-3'

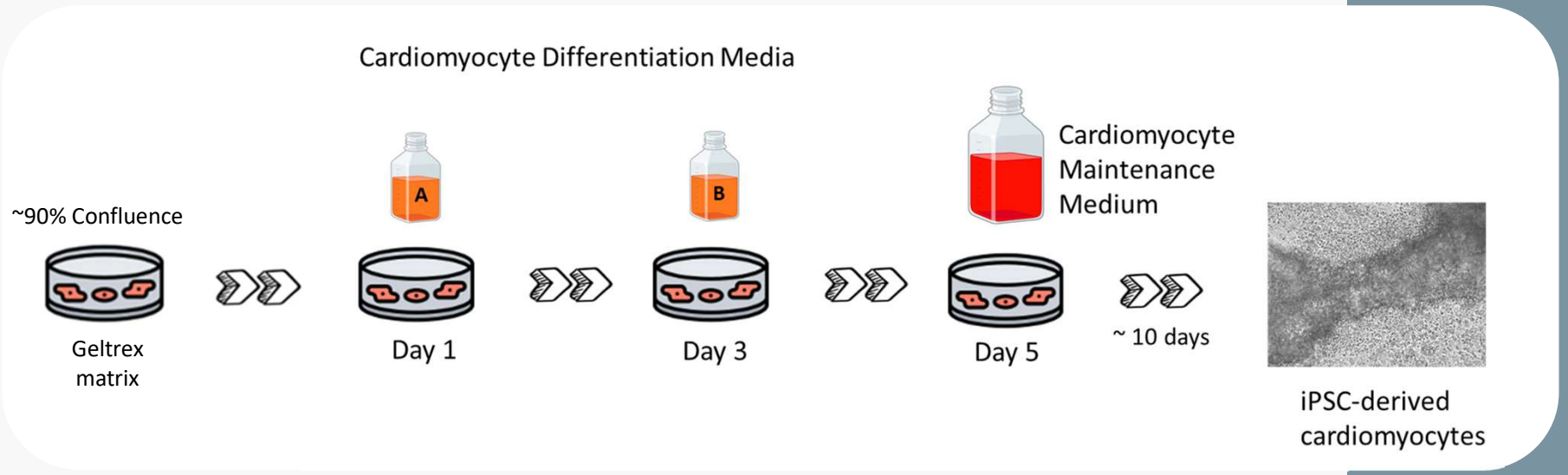
FD iPSCs *GLA* KI: 5'-GAGCAGCCATGATAGCCTAG-3'

5'GCCTCAGCTGGAATCAGCAAGTAACTCAGATGGC***A***CTCT**g**GGCTATCATGGCTG
CTCCTTTATTCATGTCTAATGACCTCGGCCTCAGCTGGAATCAGCAAGTAACTCAGAT
GGCCCTCTGGGCTATCATGGCTGCTCCTTTATTCATGTCTAATGACCTC-**3'**

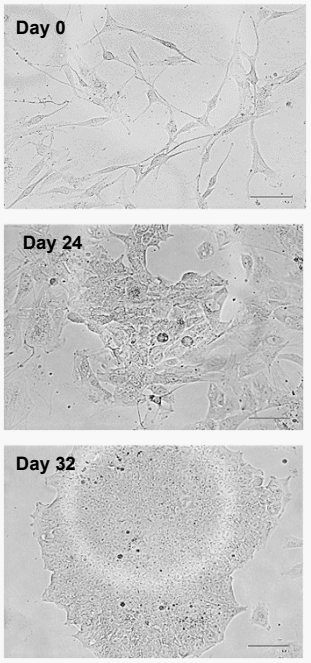


Methods – iPSC-derived CMs

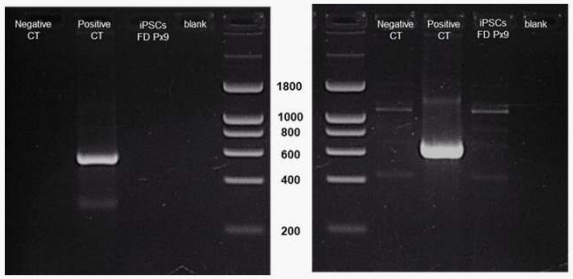
PSC Cardiomyocyte Differentiation Kit (Gibco™)



Results – FD fibroblast Reprogramming

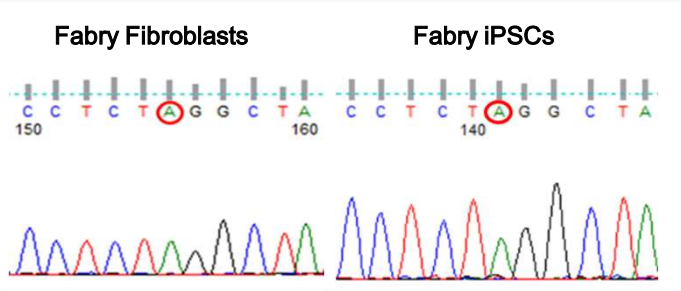


Absence of the Episomal Vectors

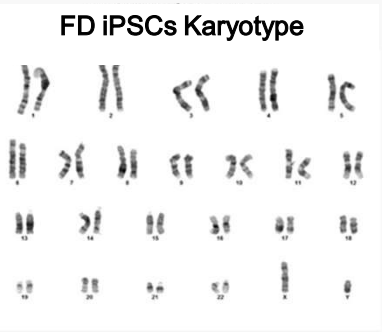


P9 oriP primers EBNA-1 primers

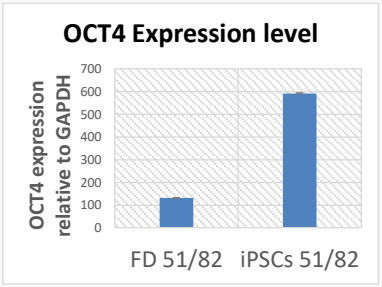
c.860G>A (p.W287X) Mutational Analysis

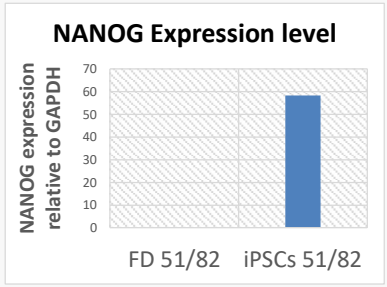


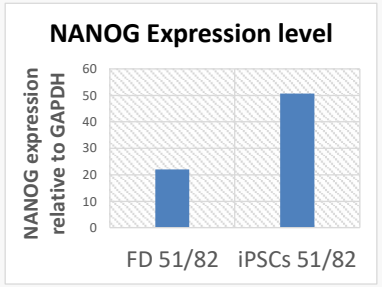
FD iPSCs Karyotype



RT-qPCR Pluripotency Markers

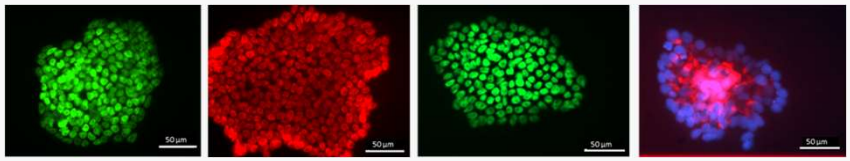




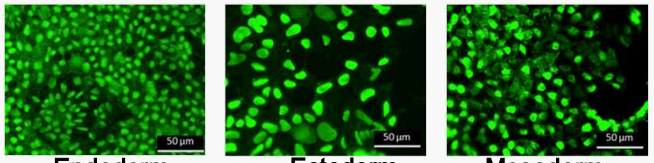


- ✓ Accurate phenotypic development
- ✓ Non-integrative method
- ✓ Expression of the pluripotency markers
- ✓ Presence of the mutation
- ✓ Ability to differentiate into the 3 germ layers
- ✓ Normal Karyotype

IFF Pluripotency Markers



Differentiation Markers



The FD iPSC line was successfully developed