CASE STUDY

The Cystic Fibrosis (CF) Canada Program in Individualized Therapy (CFIT)

Establishing a bank of 100 patient-derived iPSC lines and isogenic controls

CHALLENGE
Workflow optimization to enable efficient generation of new iPSC lines.

PROCESS DEVELOPED
Standardized processes for reprogramming and CRISPR-Cas9 correction of CF-causing mutations.

OUTCOMES
• 100 patient-derived iPSC lines
• Platform to generate gene-corrected controls from iPCs

INTRODUCTION

Cystic fibrosis (CF) is a complex disease with symptoms that vary widely between patients. Researchers at The Hospital for Sick Children in Toronto, Canada, believe that a personalized medicine approach will facilitate the prediction of therapeutic outcomes of next-generation treatments for CF. CFIT was established to create a resource of patient-derived tissues and genetic data that can be accessed by CF researchers around the world. CFIT engaged CCRM to generate a bank of induced pluripotent stem cell (iPSC) lines from 100 CF patients and to correct the point mutations in the *CFTR* gene in a subset of those lines.

METHODS

Characteristic	Assay	Release Criteria
General Characterization		
Pluripotency	Flow Cytometry	>80% SSEA4, TRA160, OCT4 and SOX2
Pluripotency	qRT-PCR	>80% expression OCT4, NANOG, DNMT3B
Germ Layer Differentiation	Directed Differentiation	Upregulation of lineage specific gene expression
Cell Bank Quality	Post thaw viability	Viability >80%
Cell Bank Quality	Lonza MycoAlert	Mycoplasma -ve
Cell Line Identity	Short Tandem Repeat	Consistent with parental
Karyotype	G-Banding	19/20 Normal chromosomes
Sendai Clearance	qRT-PCR	Undetectable by P20
CFIT-specific characterization		
Definitive Endoderm Differentiation	qRT-PCR	Upregulation of DE gene expression
Definitive Endoderm Differentiation	Flow Cytometry	>80% cKIT, CXCR4 double positive

Table 1: *Quality control and release criteria.*

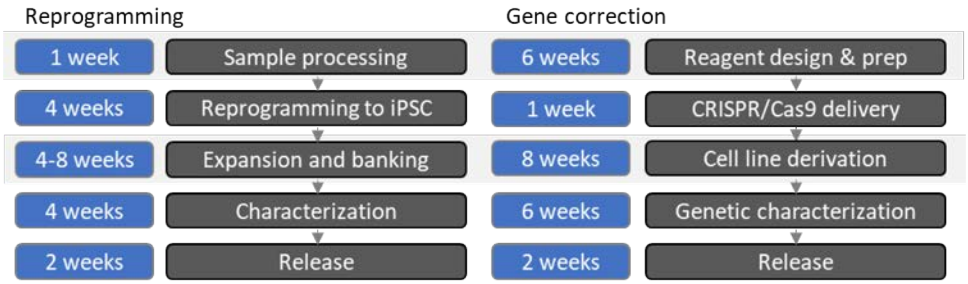

Figure 1: *Workflow and timeline for iPSC reprogramming and gene editing.*

RESULTS & DISCUSSION

Now in the third year of a five-year program, CCRM has reprogrammed over 60 patient samples and banked over 100 characterized iPSC lines. The iPSCs are generated from peripheral blood using a non-integrative reprogramming method. Once established, a standardized panel of quality control assays are performed, including confirmation of chromosomal stability, cell line identity, cell bank viability, expression of pluripotency-associated proteins, and the ability to differentiate to all three germ layers (Table 1 and Figure 2). Since these cells will be used in downstream applications requiring robust differentiation to lung epithelium cells, each cell line is additionally assessed for its efficiency in forming definitive endoderm (Figure 2B(iii)).

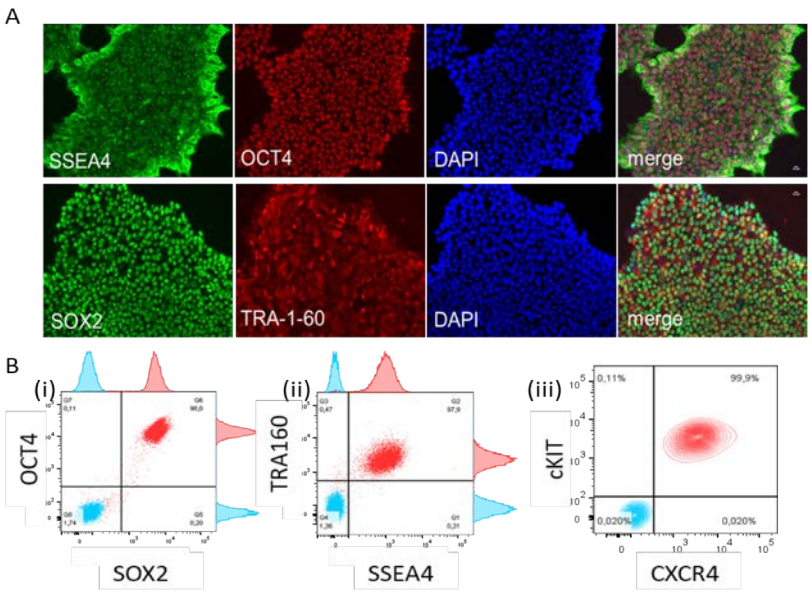

Figure 2: *(A) Immunofluorescent staining for markers of pluripotency. (B) Flow cytometry to assess pluripotency (i & ii) and directed differentiation to definitive endoderm (DE) (ii).*

Gene correction has been performed on a subset of the iPSC lines, including multiple lines from patients with the F508del and the W128X *CFTR* mutations (Figure 3). Experiments performed by CFIT-associated researchers using the isogenic control lines demonstrate that the gene correction performed at CCRM restores CFTR function in iPSC-derived lung epithelium cells.

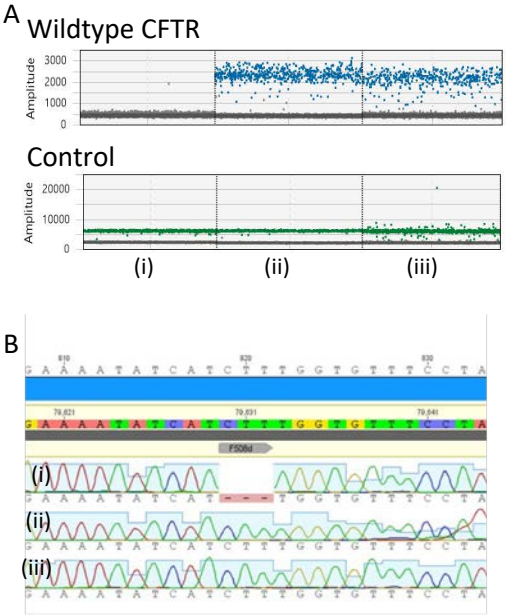

Figure 3: *Screening of gene correction (A) ddPCR results for (i) CFTR F508del patient iPSC (ii) CFTR F508del patient iPSC after gene correction (iii) wild-type iPSC control. (B) Sanger sequencing results of (i), (ii) and (iii).*

SUMMARY

iPSC lines and isogenic control cell lines are being derived at CCRM for CFIT. This study showcases:

1. High throughput capabilities in iPSC reprogramming and gene correction
2. Standardized characterization of banked iPSC lines
3. Successful recovery of CFTR function in gene corrected isogenic controls

REFERENCES

1. Eckford et al. The CF Canada-SickKids Program in individual CF therapy: A resource for the advancement of personalized medicine in CF. *J Cyst Fibros.* 2018. pii: S1569-1993(18)30086-9.

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