CATAPULT
Cell and Gene Therapy

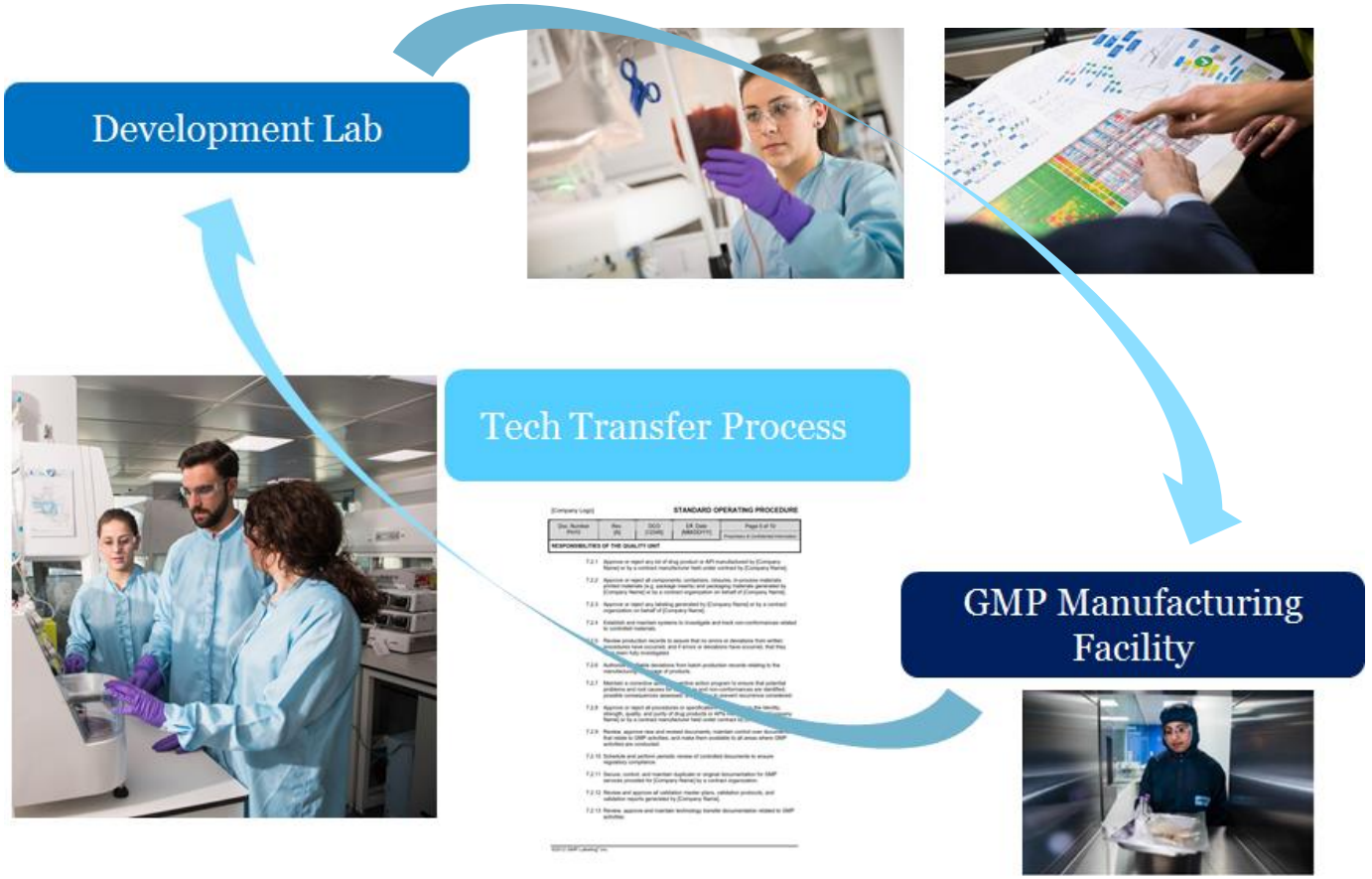
# Transfer into GMP environment and CGTC iPSC line

CATAPULT
Cell and Gene Therapy

# Technical transfer to GMP manufacturing facility

# Technical transfer master plan

A high level document which describes the approach and strategy outlining all of the requirements for TT of the manufacturing process and analytical assays in-line with current GMP standards and associated regulations. The document captures responsibilities, communications plan and project timelines.



# People

Forming an effective team with good communication channels is essential. Interactions include:

- Regular (weekly) meetings
- Process and assay observation at Dev. Lab
- Training- Gap analysis & training runs (process simulations)
- Person In Plant- Dev Lab scientist supporting manufacturing team on site.
- Subject matter experts- support engineering run design, validation runs and deviation investigations.

# Documents

Accurate, very detailed documents generated by the Development Laboratory that are version controlled and ensure all of the information transfer is performed successfully.

Documents that are required include:

- **Equipment** specification and SOPs
- **Materials**- Source, composition, purchasing information, storage, stability and specification with example CoCs or CoAs
- **Process**- Development and validation reports including design space knowledge, flow diagrams, detailed process description, critical steps, SOPs, Batch Manufacturing Records (BMRs)
- **Analytical assays** and quality assessments (Development tests, In-process and Release)- Development and validation reports, flow diagrams, detailed process description, SOPs, specification, test requests for CROs.
- Process **Risk assessment**- FMEA style

# Documents

- **Training records** for manufacturing team training at Dev Lab including test results to assess qualitative judgements.
- **Health and Safety-** MSDS; Risk Assessments
- **Supply chain requirements** for Starting Material, samples, Drug Product including temperature, packaging, monitoring and 3rd party service providers.
- **Storage and Stability** for Drug Product
- **Labelling**- Samples and Drug Product.
- **Data management** – sensible and proprietary data

# Technical transfer report

Generated by the manufacturing facility and summarises the TT batches / assay transfer performance together with any deviations/failures encountered.

Acceptance criteria is reviewed against the results and recommendations made.

CATAPULT
Cell and Gene Therapy

# CGT Catapult iPSC line

## *CGTC-RCiB10*

# Cell line generation and banking

## Donor Selection

- Female (55) from New Zealand ("Clean Country")
- Leukapheresis material procured in compliance with EUTCD (2004/23/EC)

## Reprogramming method

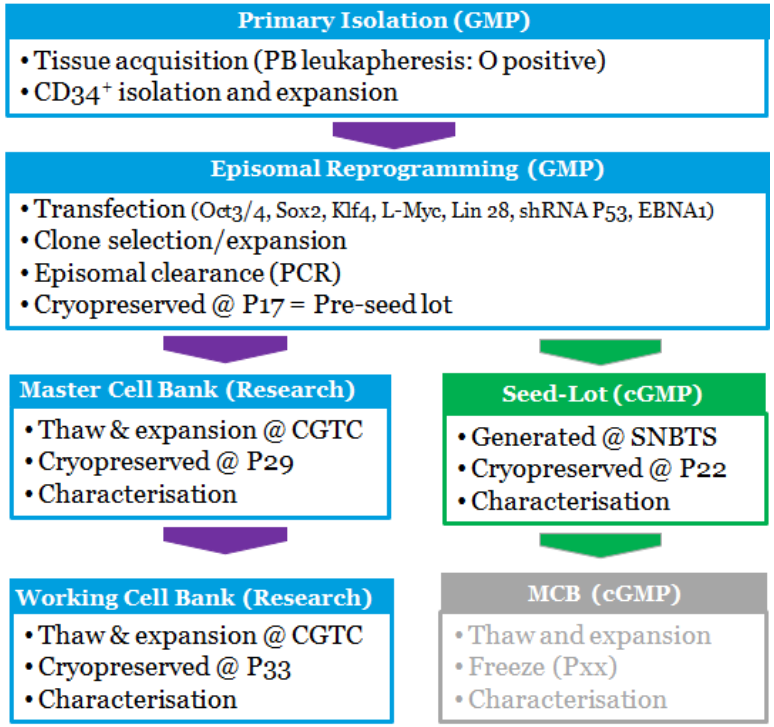
- Yamanaka (Y5) episomal reprogramming plasmids (GMP-grade)

## Early access lot (Research-grade line)

- Available at CGT Catapult
- Manufactured from GMP cells at P17
- Reagents of defined composition
- Single-cell passaging (process robustness)

## Clinical grade line

- On-going manufacturing from GMP cells at P17 (same clone as the Research-grade line)

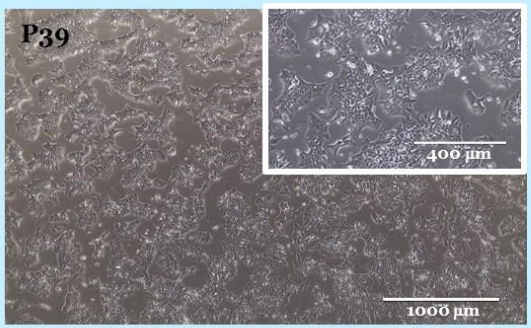


# Research-grade Working Cell Bank

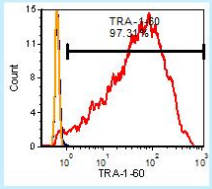
CATAPULT Cell and Gene Therapy

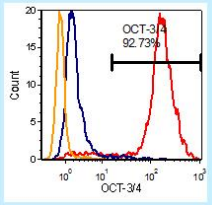
## Identity:

'Typical' pluripotent cell morphology

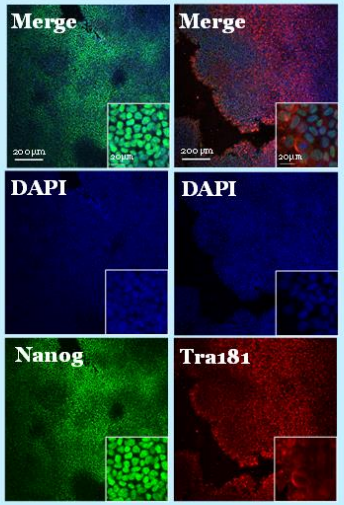



Pluripotency markers




| | |
|---|---|
| SSEA-1 + | 2.3 % |
| SSEA-4 + | 98 % |
| SSEA-3 + | 92 % |
| TRA1-60 + | 93 % |
| OCT4 + | 93 % |
| NANOG + | 90 % |
| SOX2 + | 95 % |




Gene expression signature

| Sample Name | Self-renewal | Ectoderm | Mesoderm | Endoderm |
|---|---|---|---|---|
| Seed Lot | | -1.20 | -1.02 | -1.91 |
| MCB01 | -0.77 | -0.70 | -0.75 | -1.33 |
| MCB02 | -0.75 | -0.82 | -0.84 | -1.34 |
| WCB01 | -0.61 | -1.15 | -1.00 | -1.78 |
| WCB02 | -1.23 | -0.79 | -1.32 | -1.55 |

Taq® hPSC Scorecard™

*Score*

| | |
|---|---|
| x>1.5 | Upregulated |
| 1.0<x<=1.5 | |
| 0.5<x<=1.0 | |
| -0.5<=x<=0.5 | Comparable |
| -1.0<=x<-0.5 | |
| -1.5<=x<-1.0 | |
| x<-1.5 | Downregulated |

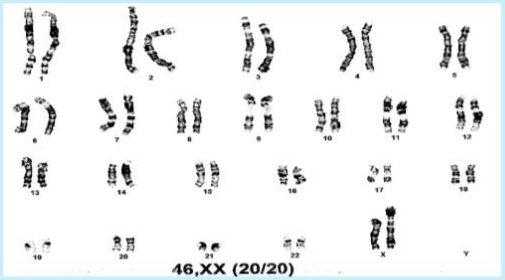
## Culture Methods:

- Compatible with commercial defined substrates (Laminin-521/511; Vitronectin; Synthemax®)
- Compatible with commercial medium (mTESR1; E8; E8 Flex; Nutristem; StemFit)
- 2D expansion in Quantum™ hollow-fibre bioreactor
- 3D expansion as aggregates up to 1.5-L STR

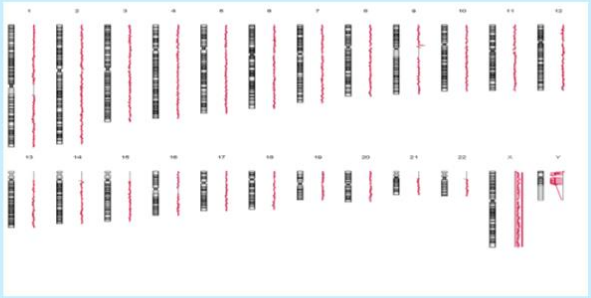
# Research-grade Working Cell Bank

**Safety:**

G-band

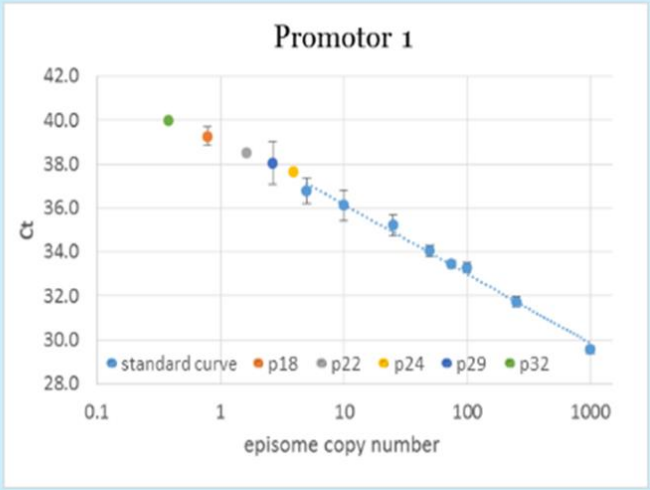



CGH-Comparative Genomic Hybridization



**Episomal Integration:**

qPCR

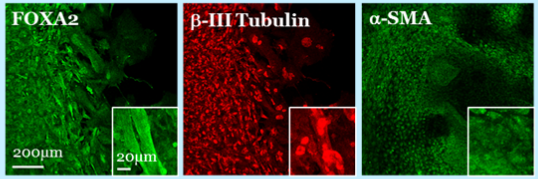


# Research-grade Working Cell Bank

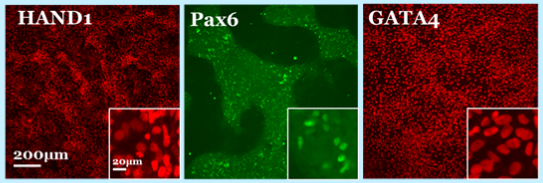
CATAPULT Cell and Gene Therapy

## Potency:

3 Germ line spontaneuous differentiation from EBs




3 Germ line directed differentiation in 2D




| Sample Name | Self-renewal | Ectoderm | Mesoderm | Endoderm |
|---|---|---|---|---|
| Undifferentiated | -0.99 | -1.27 | -1.02 | -1.76 |
| Cardiac | -9.07 | -1.43 | 7.10 | -1.15 |
| Neural | -4.39 | 0.61 | 1.21 | -1.19 |
| Pancreatic | -7.05 | 0.13 | -0.42 | 1.59 |

Taq® hPSC Scorecard™

| | |
|---|---|
| x>1.5 | Upregulated |
| 1.0<x<=1.5 | |
| 0.5<x<=1.0 | |
| -0.5<=x<=0.5 | Comparable |
| -1.0<=x<-0.5 | |
| -1.5<=x<-1.0 | |
| x<-1.5 | Downregulated |

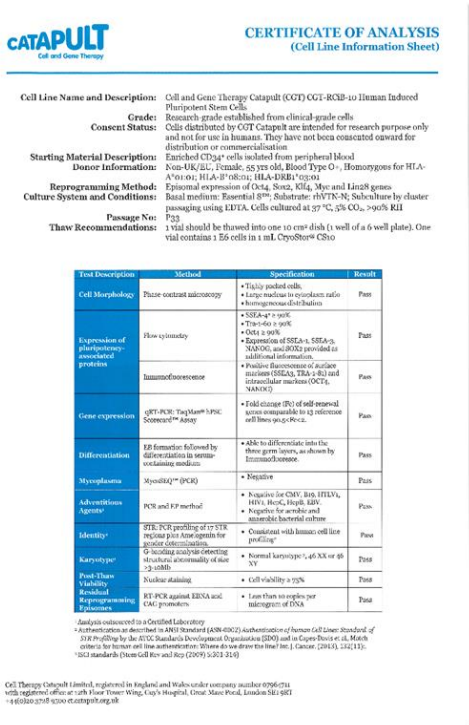
## Differentiation Protocols Tested:

- Dopaminergic neurons
- Hepatocytes
- Platelets
- Beta cells
- Retinal pigment epithelial cells
- Cardiomyocytes
- Hepatocytes

# Distribution

**Acquisition Package:**

- Research-grade Working Cell Bank RCiB10 WCB01-01
- 1 x10$^6$ cells/cryovial frozen at passage P33
- Certificate of Analysis
- SOP for adherent culture on Vitronectin substrate, Essential 8™ medium, and passage as aggregates with EDTA



CATAPULT Cell and Gene Therapy

CERTIFICATE OF ANALYSIS
(Cell Line Information Sheet)

**Cell Line Name and Description:** Cell and Gene Therapy Catapult (CGT) CGT-RCiB-10 Human Induced Pluripotent Stem Cells

**Grade:** Research grade established from clinical-grade cells

**Consent Status:** Cells distributed by CGT Catapult are intended for research purpose only and not for use in humans. They have not been consented onward for distribution or commercialisation

**Starting Material Description:** Enriched CD34$^+$ cells isolated from peripheral blood

**Donor Information:** Non-UK/EU, Female, 55 yrs old, Blood Type O+, Homozygous for HLA-A*01:01; HLA-B*08:01; HLA-DRB1*03:01

**Reprogramming Method:** Episomal expression of Oct4, Sox2, Klf4, Myc and Lin28 genes

**Culture System and Conditions:** Basal medium: Essential 8™; Substrate: rhVTN-N; Subculture by cluster passaging using EDTA. Cells cultured at 37 °C, 5% $CO_2$, >90% RH

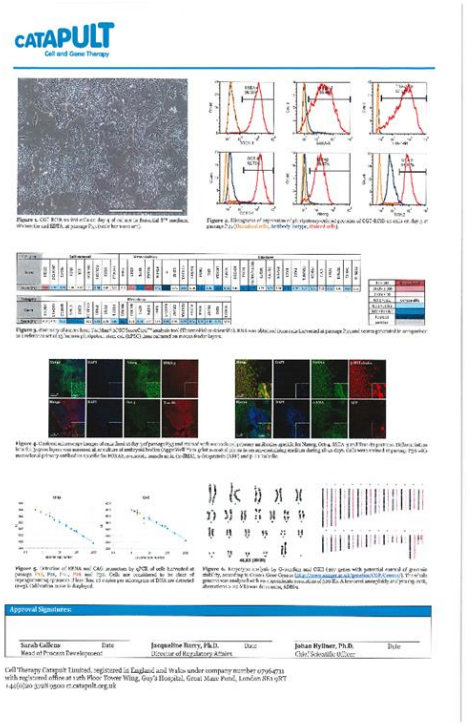
**Passage No:** P33

**Thaw Recommendations:** 1 vial should be thawed into one 10 cm² dish (1 well of a 6 well plate). One vial contains 1 E6 cells in 1 mL CryoStor® CS10

| Test Description | Method | Specification | Result |
|---|---|---|---|
| Cell Morphology | Phase-contrast microscopy | • Tightly packed cells.<br>• Large nucleus to cytoplasm ratio<br>• Homogeneous distribution | Pass |
| Expression of pluripotency-associated proteins | Flow cytometry | • SSEA-4$^+$ ≥ 90%<br>• Tra-1-60 ≥ 90%<br>• Oct4 ≥ 90%<br>• Expression of SSEA-4, SSEA-3, NANOG, and SOX2 provided as additional information. | Pass |
| | Immunofluorescence | • Positive fluorescence of surface markers (SSEA3, TRA-1-81) and intracellular markers (OCT3, NANOG) | Pass |
| Gene expression | qRT-PCR: TaqMan® hPSC Scorecard™ Assay | • Fold change (Fc) of self-renewal genes comparable to 13 reference cell lines 90.5<Fc<2. | Pass |
| Differentiation | EB formation followed by differentiation in serum-containing medium | • Able to differentiate into the three germ layers, as shown by Immunofluorescence. | Pass |
| Mycoplasma | MycoSEQ™ (PCR) | • Negative | Pass |
| Adventitious Agents[1] | PCR and EP method | • Negative for CMV, B19, HTLVs, HIVs, HepC, HepB, EBV.<br>• Negative for aerobic and anaerobic bacterial culture | Pass |
| Identity[2] | STR: PCR profiling of 17 STR regions plus Amelogenin for gender determination. | • Consistent with human cell line profiling[3] | Pass |
| Karyotype[1] | G-banding analysis detecting structural abnormality of size >5-10Mb | • Normal karyotype 46 XX or 46 XY | Pass |
| Post-Thaw Viability | Nuclear staining | • Cell viability ≥ 75% | Pass |
| Residual Reprogramming Episomes | RT-PCR against EBNA and OAC promoters | • Less than 10 copies per microgram of DNA | Pass |

[1] Analysis outsourced to a Certified Laboratory
[2] Authentication as described in ANSI Standard (ASN-0002) *Authentication of human Cell Lines: Standard of STR Profiling* by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? Int. J. Cancer. (2015), 132(11).
[3] SIGI standards (Stem Cell Rev and Rep (2009) 5:303-314)

Cell Therapy Catapult Limited, registered in England and Wales under company number 07964711 with registered office at 12th Floor Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
+44(0)20 3748 9100 ct.catapult.org.uk



CATAPULT Cell and Gene Therapy

Approval Signatures:

| Sarah Gilkes | Date | Jacqueline Barry, Ph.D. | Date | Johan Hyllner, Ph.D. | Date |
|---|---|---|---|---|---|
| Head of Process Development | | Director of Regulatory Affairs | | Chief Scientific Officer | |

Cell Therapy Catapult Limited, registered in England and Wales under company number 07964711 with registered office at 12th Floor Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
+44(0)20 3748 9100 ct.catapult.org.uk