Screening of defined medium for the adherent expansion of an induced pluripotent stem cell line: process assessment and analytical signatures

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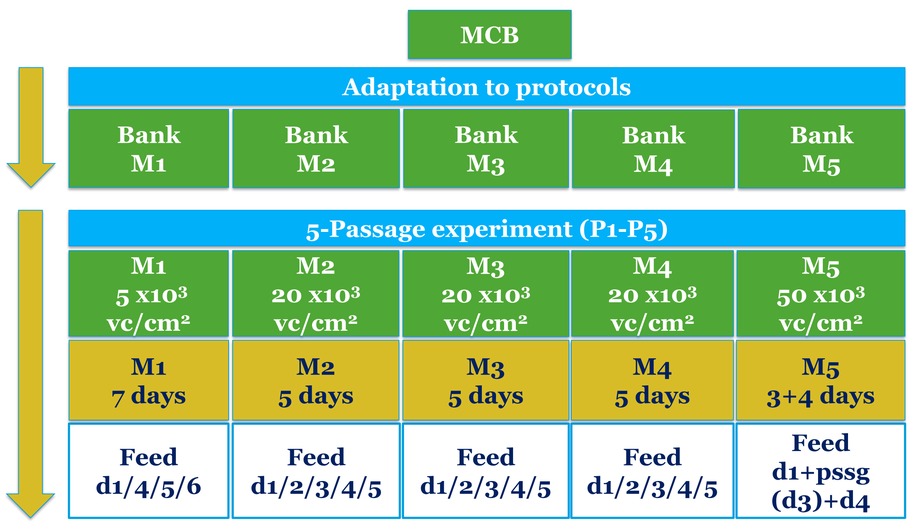
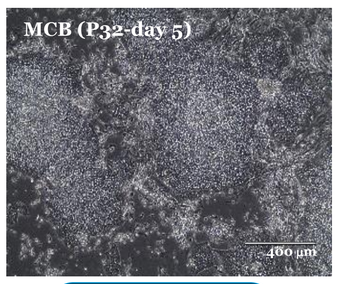
Background and Objectives: Adherent culture is widely accepted as the established method to derive and expand induced pluripotent stem cells (iPSC). The Cell and Gene Therapy Catapult (CGT Catapult) aims to develop cost effective processes for the industrial manufacture of iPSC-derived products in 2D and 3D culture systems. In this study, we showcase the abilities of our analytical framework to identify signatures of an iPSC line cultured in different medium and protocols for 2D-expansion.

Methods



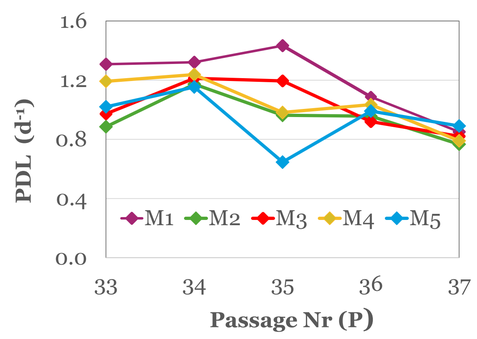


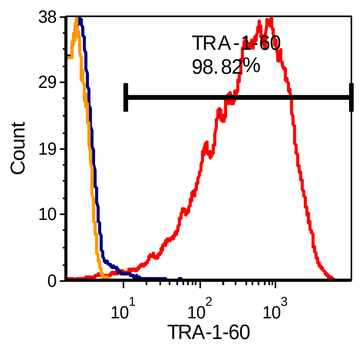
Analytics P1/P3/P5

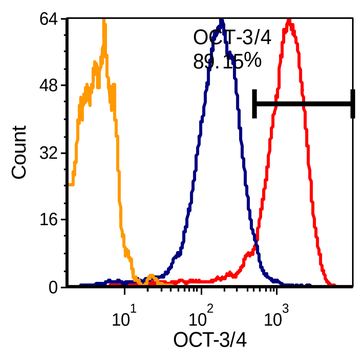
- Morphology
- Cell growth
- Metabolism
- FACS
- ICC
- qPCR
- Karyotype

CGT-RCiB10 is a research-grade iPSC line established from a cGMP pre-seed lot. Cells from a Master Cell Bank (MCB) were adapted to medium and expansion protocols (M) using single-cell seeding over 5 passages, and characterised prior to banking. Vials from the new banks were thawed and cells expanded for 3 passages before a 5-consecutive passage study. In this study, two consecutive passages were considered as one passage when using the protocol M5. Cell counts and viability were assessed by nuclear stain discrimination (NC-200), expression of pluripotency-related proteins by flow-cytometry, and gene expression using the TaqMan® ScoreCard™ assay, across the 5 passages. Levels of glucose, lactate, glutamine, and ammonia in culture were also quantified (Cubian HT-270). Cell potency was assessed by immunocytochemistry (ICC) upon differentiation into the 3-germ layers of embryoid bodies (EBs) cultured in serum-containing medium during 15-18 days. Karyotype stability was assessed by comparative genomics hybridisation (CGH) analysis and G-banding. The Luminex™ platform was employed to monitor the concentration of 2 factors present in the media.

Results – Cell adaptation and banking







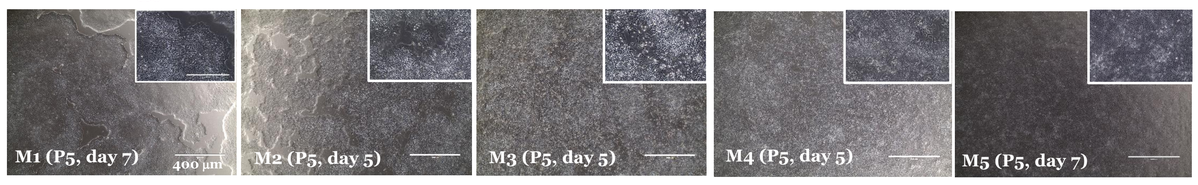
Marker	M1	M2	M3	M4	M5
TRA1-60⁺	99 %	99 %	98 %	96 %	96 %
SSEA-3⁺	94 %	93 %	98 %	94 %	98 %
SSEA-4⁺	99 %	99 %	99 %	99 %	99 %
OCT-4⁺	89 %	96 %	93 %	94 %	96 %

Protocol	CGH
M1	Normal
M2	Normal
M3	Normal
M4	Normal
M5	Normal

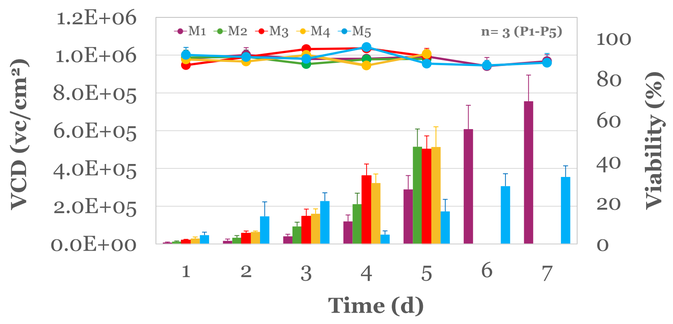
Cells were banked at a stage where the growth rate was found to be similar for all protocols tested. This is shown by the number of population doublings per day observed at P37 (PDL ≅ 0.8 d^{-1}). Representative FACS histograms are presented (unstained, isotype, stained). Expression of pluripotency-related proteins was > 80% for all banks and cell potency was confirmed by ICC after differentiation into the 3-germ layers (data not shown). No clonal abnormalities were detected in all banks.

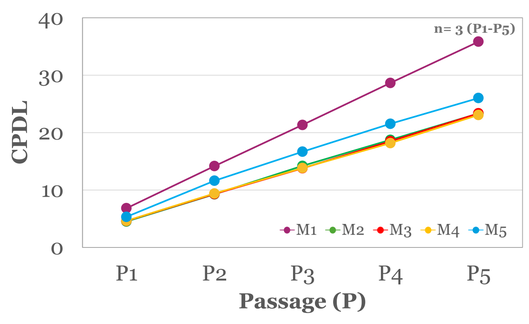
Results – 5-Passage experiment

Morphology



Cell growth





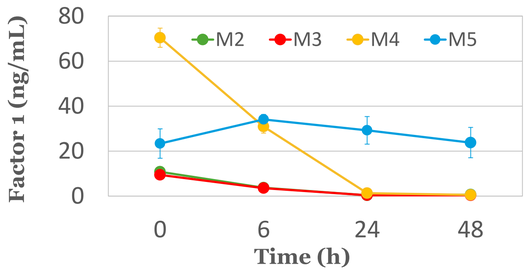
Pluripotency

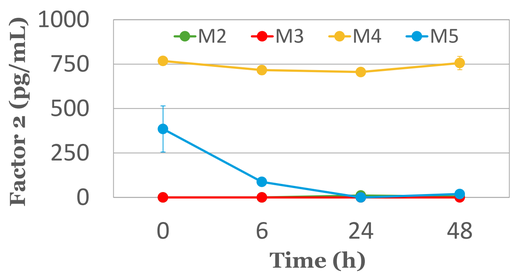
Marker	M1	M2	M3	M4	M5
TRA1-60⁺	97 ±1.6	98 ±1.7	95 ±1.0	96 ±1.5	94 ±6.4
SSEA-4⁺	99 ±2.3	99 ±0.0	99 ±0.2	99 ±0.2	89 ±23
OCT-4⁺	98 ±2.0	95 ±1.9	87 ±3.0	91 ±4.6	91 ±6.4

Metabolism

Medium	$Y_{lac/gluc}$	$Y_{amn/L\text{-}gln}$
M1	1.4 ±0.67	0.59 ±0.21
M2	1.6 ±0.51	0.45 ±0.37
M3	1.8 ±0.51	n.a.
M4	1.6 ±0.45	0.37 ±0.10
M5	1.6 ±0.37	0.66± 0.23

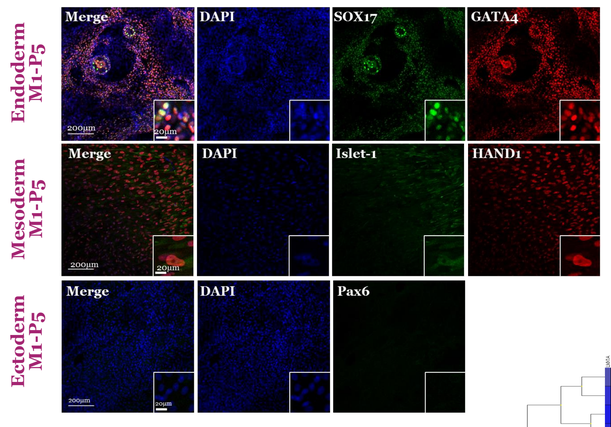
Soluble factors stability

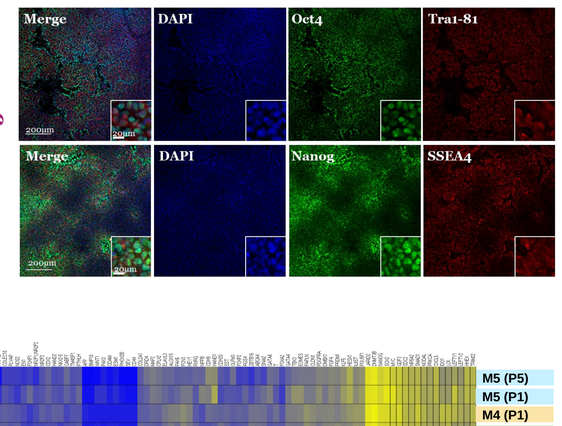




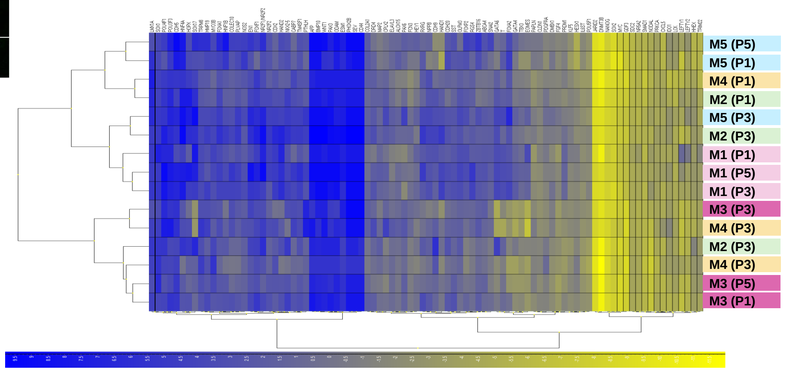
Cell confluence on the day of passage was variable between the protocols tested (80-100%). Densities of approx. 7x10⁵ viable cells/cm² (vc/cm²) were achieved with protocol M1 after a 7-day culture cycle. The number of doublings per day was found to be higher in M1 than that observed with other protocols tested ($PD_{M1} \cong 1.1\ d^{-1}$). Profiles of cell expansion in M2, M3 and M4 protocols were similar. All protocols were able to maintain cell pluripotency and viability >90%. A slight reduction in glycolytic metabolism was observed when cells were cultured with protocol M1 ($Y_{lac/glu}$=1.4). This fact would justify the higher growth rates observed due to reduced accumulation of lactate in the medium M1. The concentration of Factor 1 in most media decreased to undetectable levels after 48 h at 37 °C and in the absence of cells. Interestingly, the thermal stability of Factor 1 and Factor 2 was retained in M5 and in M4, respectively. Correlation between Luminex™ measurements and stability/activity of the soluble factors could be discussed.

Self-renewal





Maintenance of pluripotency was confirmed by ICC. The ability of RCiB10 iPSC line to differentiate into all 3 germ layers after 5 consecutive passages was also assessed for all protocols tested (data not shown).



Gene expression data was normalised to house keeping genes, and analysed using Genex 6 PCR analysis software from MultID. Clustering observed in the heat map suggests a consistent gene expression signature of RCiB10 cells across 5 consecutive passages in M1.

Relevance and On-going work

- ❑ CGT analytical framework was able to identify gene expression signatures of the RCiB10 iPSC line cultured in different media, and to monitor the thermal stability of 2 medium components.
- ❑ Maintenance of cell pluripotency and viability was sustained by all protocols tested. Protocol M1 led to higher cell yields in 2D-culture.
- ❑ The process of adapting iPSCs to culture in different media and protocols can impact the stability of cell karyotype. Development of sensitive methods for rapid testing would benefit process and product quality control.
- ❑ CGT are currently employing a proprietary Quality-by-Design tool kit for a cost-quality assessment of iPSC-manufacturing processes in 2D and 3D culture systems.

We work with Innovate UK

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