High Standards and Quality Approach for banking Human induced Pluripotent Stem Cells (iPSCs)

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INTRODUCTION

ECACC is the official global distributor of iPSC lines from the European Bank of Induced Pluripotent Stem Cells (EBiSC) consortium and the Human Induced Pluripotent Stem Cell Initiative (HipSci). EBiSC is a centralised, not-for-profit iPSC bank providing researchers across academia and industry with access to scalable, cost-efficient and consistent high quality tools for new medicines development. EBiSC was established to address the increasing demand by iPSC researchers for quality-controlled, disease-relevant research grade iPSC lines, data and cell services. EBiSC currently holds > 800 iPSC lines in its catalogue deriving from both affected (by 26 different genetic disorders) and control donors (summarised in Table 1) achieved by various reprogramming strategies. ECACC represents EBISC's banking entity who coordinates cell line distribution worldwide. Production and maintenance of iPSCs banks require the highest level of competency in cell line production and handling supported by quality control for the maintenance and provision of large cell stocks for distribution to the international community.

Table 1. List of diseases represented in EBISC collection

Diseases represented in EBiSC Collection	Number of iPSC lines
Age-related macular degeneration	12
Alzheimers disease	85
Amyotrophic lateral sclerosis	4
Anemia	1
Anti-social behavior	2
Aplastic Anemia	2
Bardet-Biedl syndrome	22
Bipolar disorder	29
Brugada syndrome	6
Catecholaminergic polymorphic ventricular tachycardia	2
Corticobasal degeneration	4
Diabetes mellitus	62
DMD	5
Dravet syndrome	1
Drug-induced liver injury	4
Facioscapulohumeral dystrophy	5
Familial long QT syndrome	10
Frontotemporal dementia	15

Diseases represented in EBiSC Collection	Number of iPSC lines
Gaucher disease	1
Huntington disease	2
Hypertrophic cardiomyopathy	9
Migraine disorder	27
MODY	3
Monogenic diabetes	13
Myotonic dystrophy type 1	1
Neuropathy	33
Normal	328
Pain agnosia	2
Parkinson's disease	113
Progressive supranuclear palsy	4
Prolonged QT interval	6
Retinitis pigmentosa	5
Spinocerebellar ataxia type 3	4
X-linked creatine transporter deficiency	1
Unipolar depression	5

RESULTS

Banking operations. ECACC operates with a robust and standardised approach to iPSC lines banking to guarantee high quality of frozen iPSC lines from a range of genetic backgrounds and reprograming methods. The banking procedure adheres to international best practice standards covering all stages from culturing to the distribution of the lines. The representative diagram below summarises the main steps of the process (Fig. 1).

During the banking procedure of iPSC lines, an appropriate clearance and segregation regime is used and reagent traceability ensured.

Cellular growth rate, morphology and the absence of differentiation are daily checked and scored as explained in Fig. 2. The critical cryopreservation process is performed using rate-controlled freezers ensuring there is strict control on temperature variations

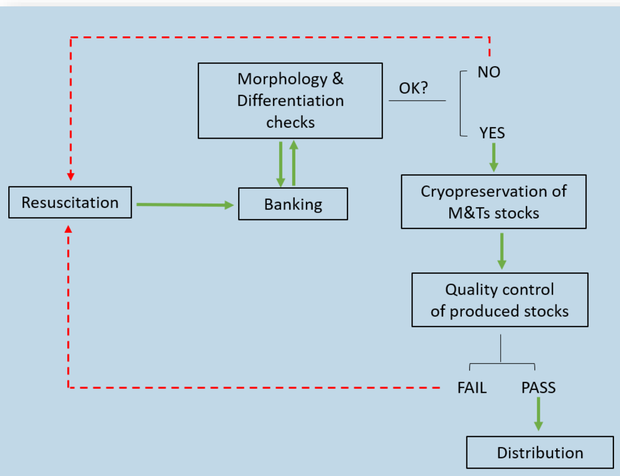


Figure 1. General outline for the production and verification of quality controlled batches of iPSCs at ECACC

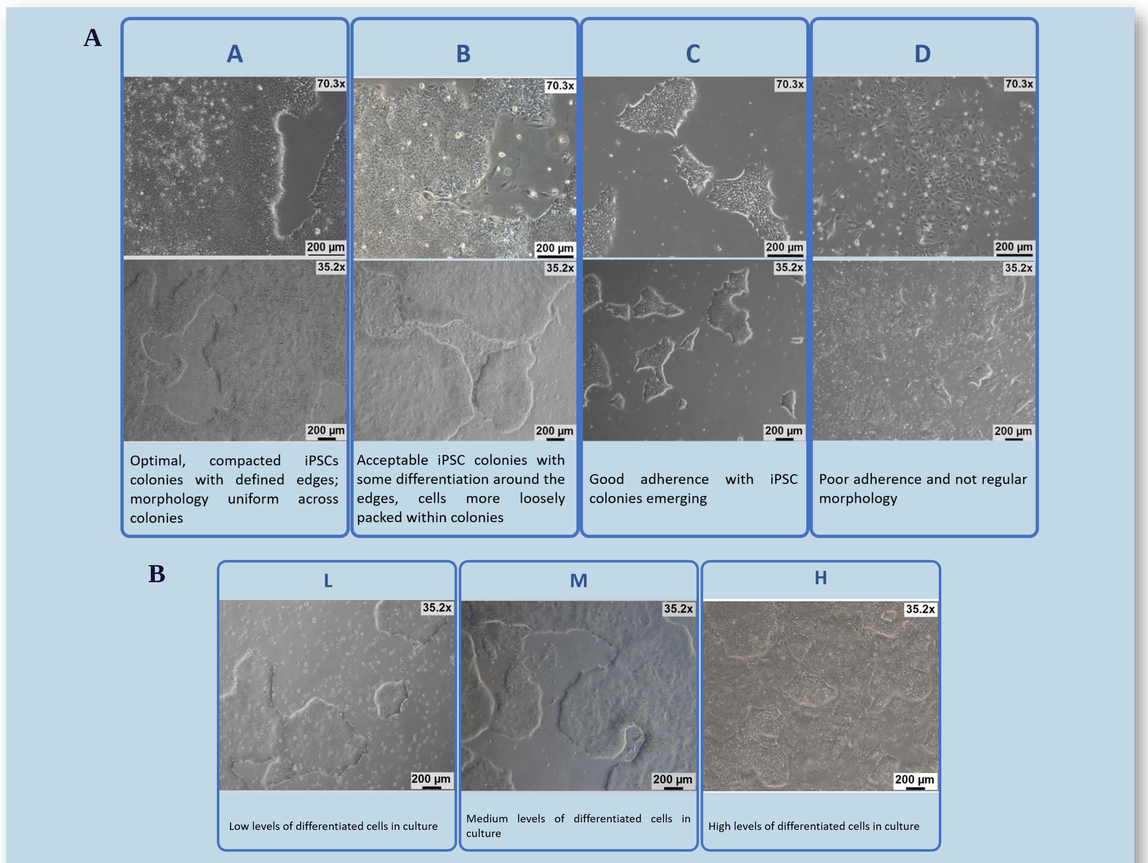


Figure 2. Score method used in ECACC to describe iPSCs morphology (A) and differentiation levels (B).

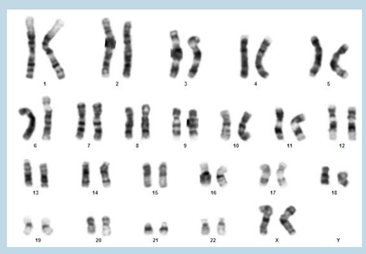
Critical quality attributes. After banking and cryopreservation all batches are submitted to the Quality Control facility which ensures the critical quality of such lines. Certificates of Analysis for produced iPSC batches are released containing results of the cell line identity, microbiological sterility, absence of viruses, genetic stability, viability and potency of produced banks.

Identity. Single tandem repeat (STR) genotyping of master banks is performed to demonstrate the genetic match of produced banks against the source material determining whether a cell line profile has changed over banking, for example, as a result of cross-contamination with another cell line or of inadvertent switching of lines.

Microbiological sterility. An overall microbiological control strategy is used to ensure banked cells are bacteria, mycoplasma and human pathogenic viruses-free. In particular, bacterial and fungal contamination is investigated using standard biochemical techniques. In parallel batches are examined for mycoplasma presence through PCR, Indirect Hoechst and Culture Isolation tests assuring high sensitivity and sensibility of the detection. All master banks are then tested for a panel of human pathogenic viruses by PCR i.e. HIV1, HIV2, Hepatitis B and Hepatitis C. These investigations are critical to assess the risk of handling iPSCs batches produced as derivations from human samples.

Karyotype. Genetic integrity is analysed in all batches (Master and Working stocks) as an informative test. Long-term cultures could accumulate culture-driven mutations affecting the genomic integrity of the cell line (1). Karyotype analysis of 20 G-banded metaphase spreads from a single-cell culture sample are provided and analysed to ensure cells diploidy, this is important for the reproducibility of experimental data of downstream usage (Fig. 3). However recurrent abnormalities have been reported in iPSC cultures as an adaptive response to passaging (2).

Cell viability and growth. All batches are tested for cell viability and growth after cryopreservation. This assessment gives an estimate of the number of viable cells after thaw and the preservation of colony integrity. The cell line is kept in culture for at least two passages to assess iPSC-like morphology and good recovery after splits. During culture, the presence of differentiated cells is visually assessed and scored, as indicated in Figure 2, to ensure the absence of atypical or spontaneously differentiating cells.



Karyotype: XX, 46

Figure 3. G-Band Karyotype analysis example of Master bank of an iPSC line. It is required to demonstrate stable karyotype to validate downstream usage.

Pluripotency characterization. Working and Master stocks are tested for the expression of self-renewal markers. Upon at least two culture passages, the assessment of pluripotency of iPSC lines is carried out to evaluate the positive expression of SSEA-4, OCT-4 and TRA1-60 antigens and the absence of the expression of SSEA-1 antigen through antibody-staining and flow cytometry analyses (3). This panel is a combination of intra- and extra-cellular pluripotency markers and differentiation markers. In Figure 4 the expression of the above markers are evaluated using flow cytometry to assess the self-renewal capacity of the tested iPSC line as biological triplicates.

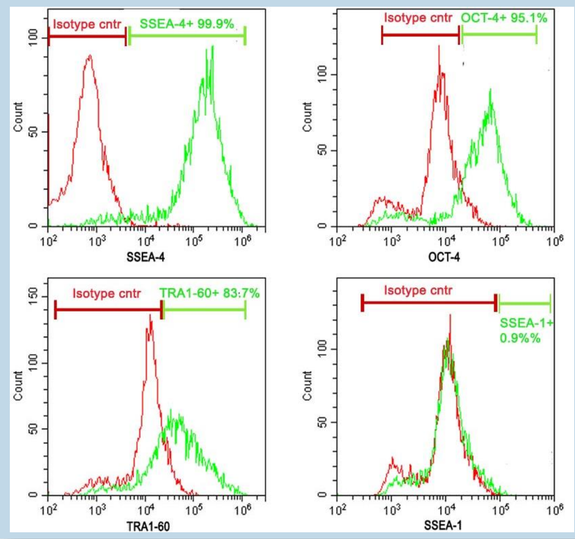


Figure 4. Assessment of expression of self-renewal markers. SSEA-4, OCT-4 and TRA1-60 and the absence of the differentiation marker SSEA-1 is assessed by flow cytometry analyses. Human iPSCs show SSEA-4, OCT-4 and TRA1-60 positive expression in >70% of cell population while SSEA-1 in <10% as it is a differentiation marker.

Master banks of iPSC lines are further functionally validated for their ability to differentiate into the three germ layers: ectoderm, mesoderm, and endoderm. Using germ layer specific media, iPSCs are cultured and induced to be committed towards the three germ layers. The trilineage differentiation potential is assessed by evaluating the expression of specific downstream markers of differentiation of iPSC lines, performed in biological triplicates. Fig. 5 shows results from a tested iPSC bank demonstrating the absence of expression of the differentiation markers in the pluripotent control and the up-regulation of Endoderm markers SOX17, GATA4 and CXCR4, Mesoderm markers VIMENTIN, DCN and MLX1 and Ectoderm markers PAX6, NEUROD1 and HES5.

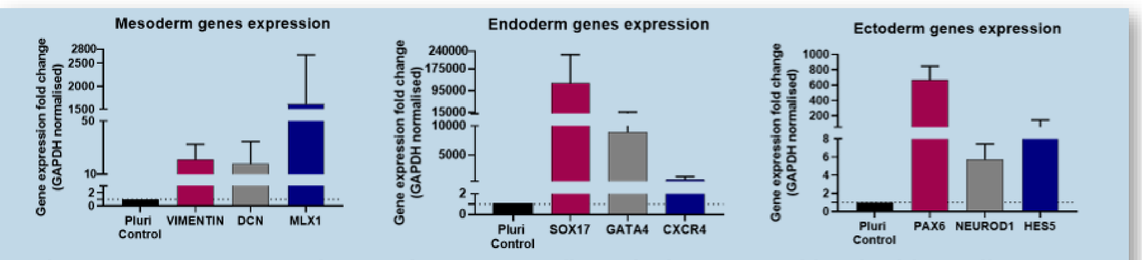


Figure 5. Gene expression analyses to confirm pluripotency of banked iPSC lines. Up-regulation of specific germ layers markers is observed in committed cells via qPCR. A cell line can be concluded to have significantly up-regulated gene expression for a specific germ layer if at least 2 markers out of 3, have a ΔΔCt > 3 fold change, compared to the pluripotent state.

CONCLUSIONS

In this study, we demonstrate the importance of producing well characterised and quality controlled batches of iPSCs at ECACC, an official global distributor of iPSC lines from the European Bank of Induced Pluripotent Stem Cells (EBISC). Large-scale iPSC banking is performed with standardization and traceability to provide clarity on how cells have been banked. Moreover, banked iPSC lines are validated through critical quality control tests to confirm their suitability for downstream uses. A high standard of banking and quality control are critical requirements for high quality iPSC lines to ensure the suitability of cell lines for streamline applications as models for the study of diseases and screening therapeutic drugs.

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