Establishment of EUTCD-grade human embryonic stem cell banks for use as starting-material in clinical therapies

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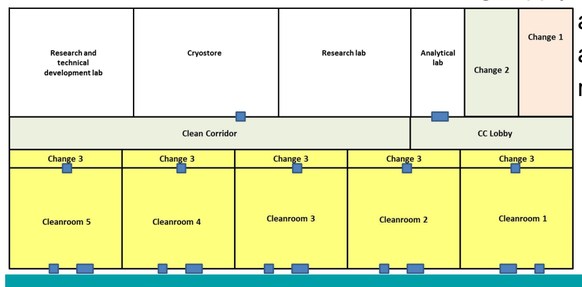
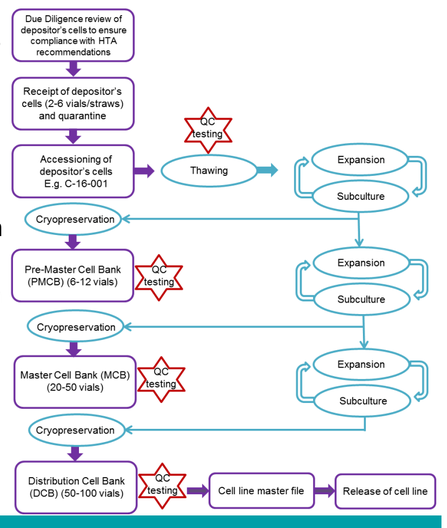
Overview of the Cell Banking Process

EUTCD-grade cell lines are cultured and banked at the UKSCB under the European Union Tissue and Cells Directives (2004/23/EC, (EU) 2015/565, (EU) 2015/566, 2006/17/EC and 2006/86/EC) which set a benchmark for the standards that must be met when carrying out any activity involving the use of human cells and tissues for therapeutic applications.

- Banking is an iterative process conducted in the UKSCB GMP compliant cleanrooms.
- Each cleanroom has its own air handling supply so acts as both a quarantine and production room.

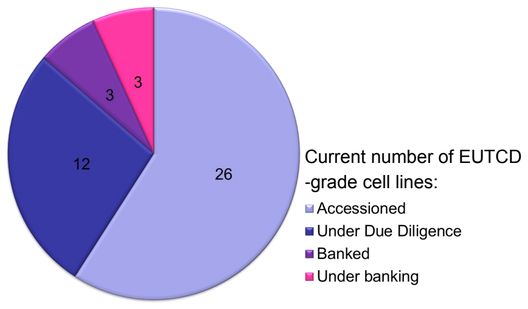




Due Diligence, Cell Receipt and Accessioning

Due Diligence Process

Cell lines proposed as EUTCD compliant undergo a due diligence process prior to Accession to ensure compliance of the cell line with the EUTCD as applied under HTA Directions. This is an important process, designed to assure that the cell line itself complies with the EU Tissue and Cell Directives required of cell lines used as starting materials in Advanced Therapy Medicinal Products (ATMPs)



Receipt of the cell line at the UKSCB

Cell sample integrity and depositor's documentation are checked upon receipt at the UKSCB to ensure the information matches the cells received.

Allocation of Accession Number

The accession number is a unique identifier used to track cells through the production process and is linked to the UK Steering Committee deposit reference number to ensure traceability. E.g. **C-17-001**. It is based on the "grade of cells" (**C**linical, **R**esearch, **P**atent) and year (e.g. 2017) and number in year of deposit (e.g. 001). A unique Single European Code, Donation Identification Sequence is also allocated to each cell line as required in the EU.

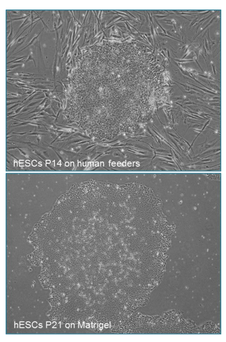
Cell storage in quarantine liquid nitrogen LN_2 vessel

Following receipt and accessioning, EUTCD grade cell lines are stored in a separate LN2 storage vessel until production commences.

Production

Culture and Cryopreservation of EUTCD grade Human Embryonic Stem Cells (hESCs)

- The pre-master cell bank, master cell bank and distribution cell bank are produced as a continuous process but may comprise discrete production runs
- Culture protocols are either taken from the depositor's protocols (UKSCB Cell Line Information Form) or standard UKSCB cell culture protocols are implemented
- EUTCD grade hESCs are:
  - ⇒ Grown in serum-free, serum replacement, or xeno-free media with a formal risk assessment process applied to all critical regents
  - ⇒ Co-cultured on inactivated human feeders or synthetic matrices prior to banking
  - ⇒ Passaged mechanically or using dissociation reagents (e.g. TrypLe^{TM})
  - ⇒ Banked using a controlled rate cooling method in cryogenic vials, as opposed to vitrification in straws
  - ⇒ Placed into a dedicated "in-process" LN_2 storage vessel until QC testing is complete
  - ⇒ The majority of frozen vials are stored at the UKSCB but a number of vials are also stored in a HTA compliant off site storage facility under a disaster recovery plan

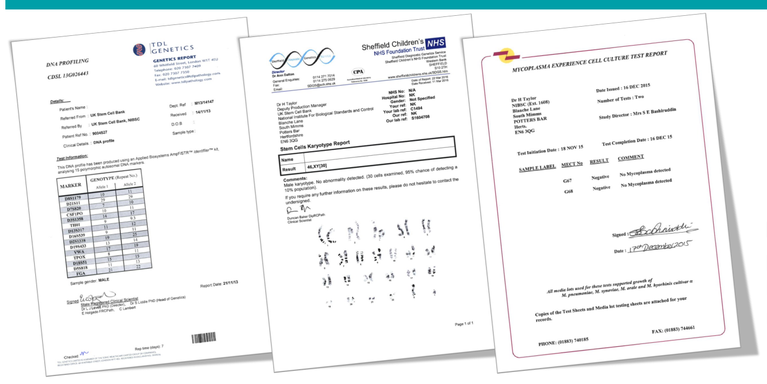


Quality Control Testing

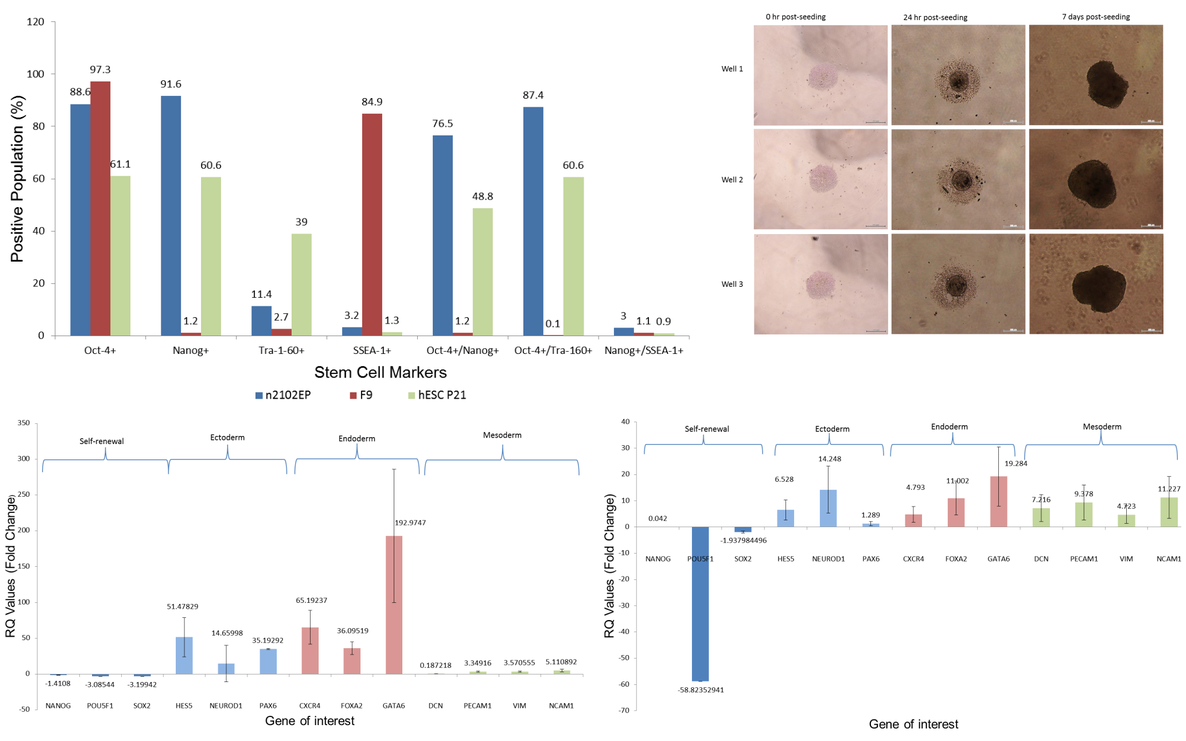
- During production of an EUTCD grade cell line, production records are completed and signed by the operator and reviewed by the Production Manager and Quality Assurance (QA) and consist of:
  - ⇒ Thawing, subculture and cryopreservation records
  - ⇒ Details of reagents and consumables used, including batch numbers and expiry dates
  - ⇒ Record of lab equipment used
- Testing is based on Good Cell Culture Practice (under revision), EU Tissue and Cells Directive (2004) and The ISCBI Consensus Guidance Document (2015)
- Release QC tests are reviewed for consistency between each cell bank and with the Depositor's material

Mandatory Release QC test	Method
Viability	Trypan Blue Exclusion assay and NC3000
Sterility and in-process finger dabs and settle plates	Sterility: Cell supernatant incubated in Tryptone soya broth, Fluid thioglycollate medium and Sabaroid's liquid medium for 14 days; Finger dabs and settle plates are taken at the thawing and cryopreservation stages
Mycoplasma	EU Pharmacopeial broth/culture test and Mycoplasma/ Ureaplasma PCR
Viral PCR	DNA extract tested against a panel of human viruses: hCMV, HTLV1, HIV1, HIV2, HepB, HepC, EBV
DNA profiling	Applied Biosystems AmpF/STR Identifier kit
Informational test	**Method**
G-band karyotyping	Giemsa staining of metaphase chromosomes
"Stemness" markers	Flow cytometric analysis using the following panel of antibodies: Tra-1-60, Oct4, Nanog, and SSEA-1.
Differentiation	Embryoid body Q-PCR analysis of 13 self-renewal/ differentiation genes

Quality Control Testing



Example mandatory release test reports, from left: STR profile; G-banding karyology result; mycoplasma culture report.



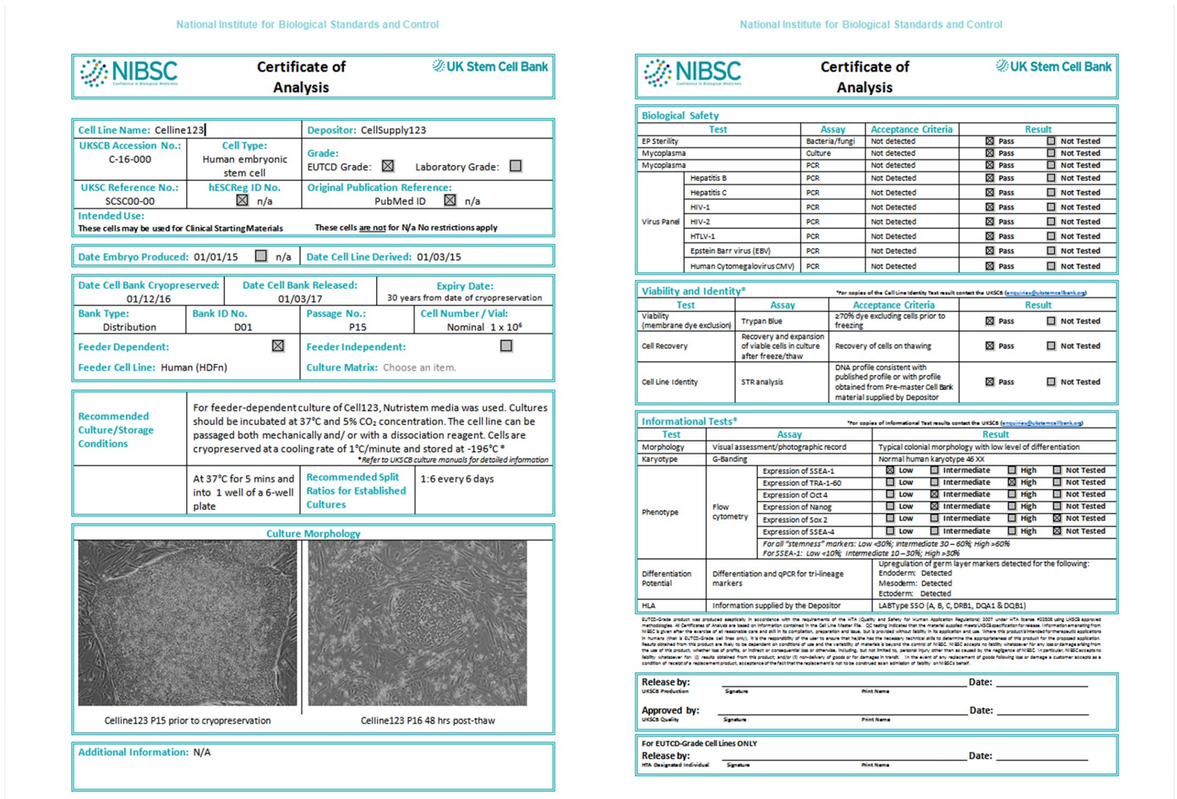
Example informational test results, clockwise from top left: flow cytometry analysis to check for "stemness" markers; photomicrographs of embryoid bodies formed from hESCs to assess differentiation capacity; Q-PCR analysis of embryoid bodies to check for differentiation into the three germ layers (endoderm, ectoderm and mesoderm).

Cell Bank Release Procedure

- **The Cell Line Master File** is firstly completed and reviewed:
  - The Cell Line Master File contains all information related to the cell line including traceability, production and QC documentation.
  - It is reviewed by UKSCB production, NIBSC Quality department and the Bank's designated individual prior to release of the cell line for distribution.
  - Ultimately it will feed into the Cell Line History File which will attribute to future Product Dossiers for regulatory review of stem cell derived cellular therapies
- Cell banks which "pass" QC release tests can then be moved to the "Release" LN_2 storage vessel
- The **UKSCB on-line catalogue** is updated to show that the cell line is now available: along with sex determination and any HLA data

http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/eutcd_grade_.aspx

- Buyers will receive a vial of cells along with a **Certificate of Analysis** (see example below) and UKSCB protocols to assist with the thawing of the EUTCD-grade human embryonic stem cells.



Acknowledgements

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