

## Preparation of EUTCD-Grade Human Embryonic Cell Lines for use as Starting Materials for the Development of Clinical Therapies

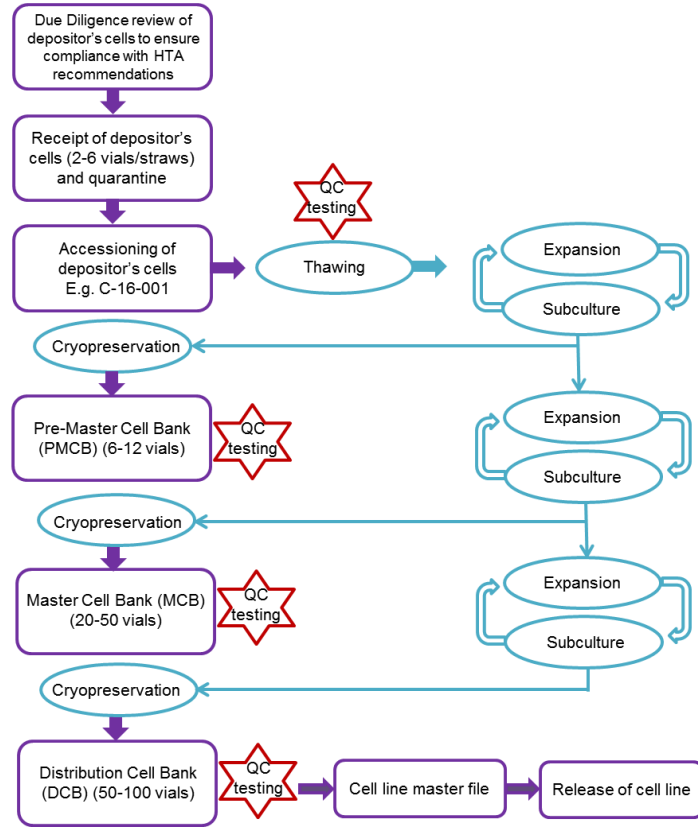
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### Overview of the Cell Banking Process

The UK Stem Cell Bank (UKSCB) is a key component of the UK regenerative medicine infrastructure charged with procuring, processing (banking and testing) and distributing seed stocks of human embryonic stem cell (hESC) lines for research and human application.

One of the key objectives of the UKSCB is to bank and release a panel of carefully selected stem cell lines that meet the EU Tissue and Cell Directives (EUTCD) as set out in Human Tissue Authority (HTA) regulations which set a benchmark for the standards that must be met when carrying out any activity involving tissues and cells for human application.



### Due Diligence, Receipt and Accessioning

#### Due Diligence Process

All cells are subject to ethical scrutiny by a national Steering Committee, following which the UKSCB performs a due diligence protocol which establishes for each individual cell line, whether it could meet the requirements of the EUTCD and is thereby provide a starting material for clinical trials.

#### 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.

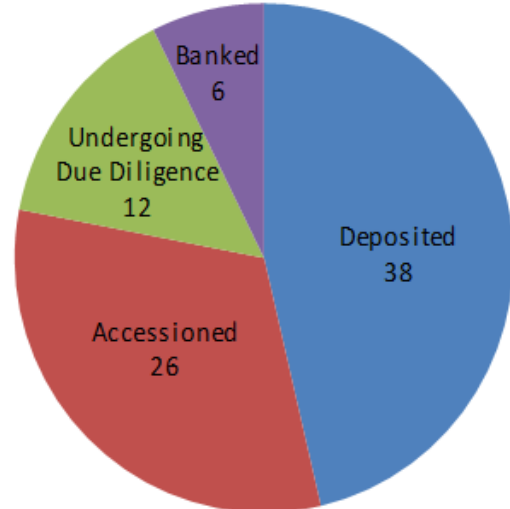
#### Allocation of Accession Number

The cell line is given an accession number which 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. A unique Single European Code, Donation Identification Sequence is also allocated to each cell line as required in the EU.

#### Cell Storage

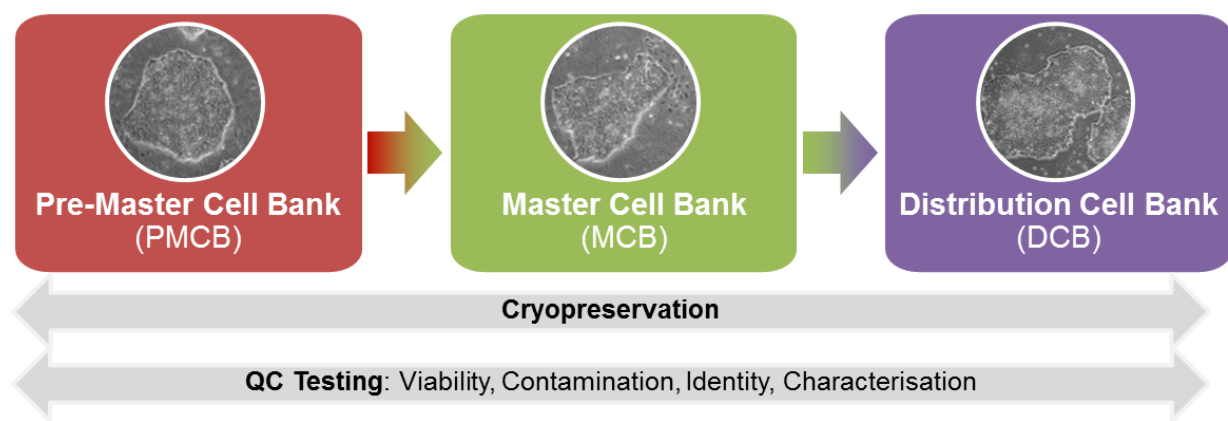
Following receipt and accessioning, EUTCD-grade cell lines are stored in a quarantine vessel prior to processing.

#### EUTCD-Grade Cell Lines at the UKSCB



### Production

#### Cell Culture and Storage



- EUTCD-grade cell lines may be banked in one continuous process but may also comprise discrete production runs.
- A cell line is grown under conditions using standard operating procedures (SOPs) taken from the depositor's protocols (UKSCB Cell Line Information Form) or from validated in-house UKSCB SOPs.
- During production, records are completed and signed by the operator and reviewed by the Production Manager and Quality Assurance (QA).
- All EUTCD-grade cell lines are:
  - ⇒ Grown in serum-free, serum replacement, or xeno-free media with a formal risk assessment process applied to all critical reagents.
  - ⇒ Co-cultured on inactivated human feeders (iHDF) or on synthetic matrices prior to banking.
  - ⇒ Passaged mechanically or by using dissociation reagents.
  - ⇒ Banked using a controlled rate cooling method in cryogenic vials.
  - ⇒ Placed into a dedicated "in-process" liquid nitrogen (LN<sub>2</sub>) storage vessel until Quality Control (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

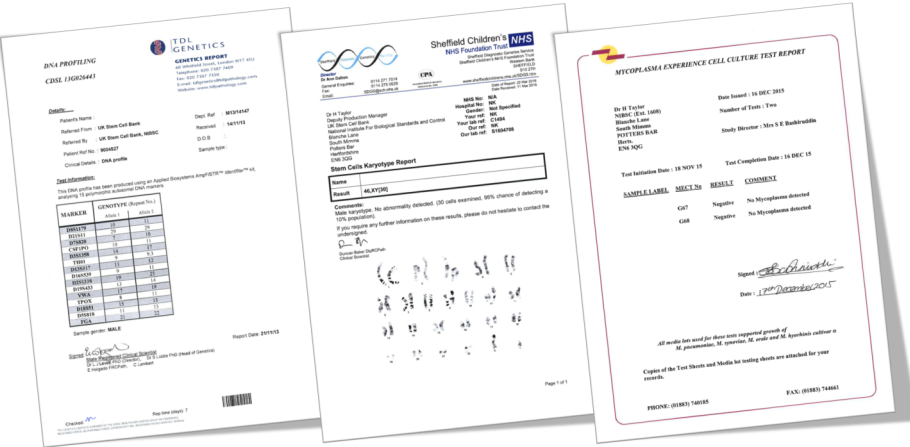
QC tests are based on Good Cell Culture Practice (under revision), EU Tissue and Cells Directive (2004) and The ISCB Consensus Guidance Document (2015).

The UKSCB employ mandatory and information QC tests (Table 1) for the release of a cell line ensuring that there is consistency between each cell bank and with the Depositor's material.

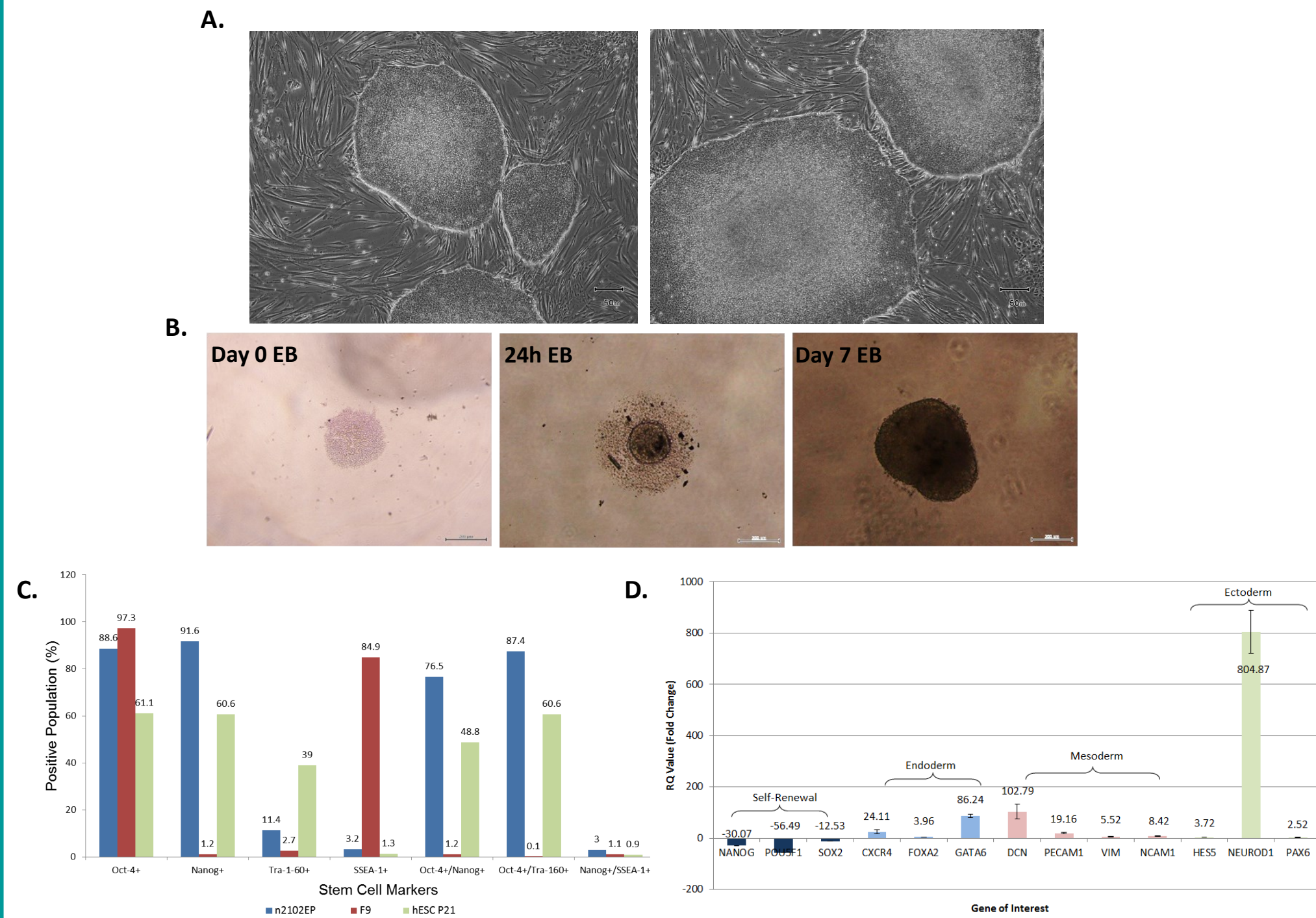
Mandatory Release QC test	Method
Viability	Trypan Blue Exclusion assay and NC3000
Sterility and in-process finger dabs and settle plates	Sterility: Cell supernatant incubated in Tryptone soya broth, Fluid thioglycollate medium and Sabaroid's liquid medium for 14 days; Finger dabs and settle plates are 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

- All cell lines are QC tested at the following time points:
  - ⇒ Upon thaw
  - ⇒ In-process/during expansion
  - ⇒ Prior to banking
  - ⇒ After cryopreservation/post-recovery



Example mandatory release test reports (L-R): STR profile; G-banding karyology result; mycoplasma culture report.



**Example informational test results:** Photomicrographs of typical cell morphology of hESCs cultured on iHDFs (A). Photomicrographs of embryoid bodies formed from hESCs to assess differentiation capacity (B). qPCR analysis of embryoid bodies to assess differentiation potential into the three germ layers (endoderm, mesoderm and ectoderm (C). Flow cytometry analysis to check for "stemness" markers (D).

### Cell Bank Release Procedure

- The Cell Line Master File (CLMF)** is firstly completed and reviewed:
  - ⇒ The CLMF 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<sub>2</sub> storage vessel.
- The **UKSCB on-line catalogue** is updated to show that the cell line is "available" along with sex determination and any HLA data.
- Distribution** of EUTCD-grade human embryonic stem cells will be accompanied by a **Certificate of Analysis** (see example below) and UKSCB protocols to assist with cell recovery.

National Institute for Biological Standards and Control

**NIBSC Certificate of Analysis** UK Stem Cell Bank

Cell Line Name: CellLine123 Depositor: CellSupply123

UKSCB Accession No.: C46-000 Cell Type: Human embryonic stem cell Grade: EUTCD Grade: Laboratory Grade: Original Publication Reference: n/a

UKSCB Reference No.: KSC00-00 HESF ID No.: n/a

Intended Use: These cells are to be used for clinical starting materials. These cells are not for use for any other purpose.

Date Embryo Produced: 01/01/15 n/a Date Cell Line Derived: 01/03/15

Date Cell Bank Cryopreserved: 01/12/16 Date Cell Bank Released: 01/03/17 Expiry Date: 30 years from date of cryopreservation

Bank Type: Distribution Bank ID No.: D01 Passage No.: P15 Cell Number / Vial: Nominal 1 x 10<sup>6</sup>

Feeder Dependent: Feeder Independent: Culture Matrix: Choose an item

Recommended Culture/Storage Conditions: For feeder-dependent culture of CellLine123, Nutrient media was used. Cultures should be incubated at 37°C and 5% CO<sub>2</sub> concentration. The cell line can be passaged both mechanically and/or with a dissociation reagent. Cells are cryopreserved at a cooling rate of 1°C/minute and stored at -130°C. n/a

At 37°C for 5 mins and into 1 well of a 6-well plate. Recommended Split Ratios for Established Cultures: 1:6 every 6 days

Culture Morphology: CellLine123 P15 prior to cryopreservation CellLine123 P16 48 hrs post-thaw

Additional Information: N/A

National Institute for Biological Standards and Control

**NIBSC Certificate of Analysis** UK Stem Cell Bank

Biological Safety: Test Assay Acceptance Criteria Result

ES Sterility: Not detected Pass Not Tested

Mycoplasma: PCR Not detected Pass Not Tested

hepatitis B: PCR Not detected Pass Not Tested

HIV-1: PCR Not detected Pass Not Tested

HIV-2: PCR Not detected Pass Not Tested

HTLV-1: PCR Not detected Pass Not Tested

Epstein Barr virus (EBV): PCR Not detected Pass Not Tested

Human Cytomegalovirus (HCMV): PCR Not detected Pass Not Tested

Viability and Identity: Test Assay Acceptance Criteria Result

Viability (after 48h): 270% Pass Not Tested

Cell Recovery: Recovery of cells on thawing: Pass Not Tested

Cell Line Identity: STR analysis: Pass Not Tested

Informational Tests: Test Assay Result

Genotype: Visual assessment/photographic record: Typical human karyotype: 46, XY

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Expression of SSEA-348: Low Intermediate High Not Tested

Expression of SSEA-349: Low Intermediate High Not Tested

Expression of SSEA-350: Low Intermediate High Not Tested

Expression of SSEA-351: Low Intermediate High Not Tested

Expression of SSEA-352: Low Intermediate High Not Tested

Expression of SSEA-353: Low Intermediate High Not Tested

Expression of SSEA-354: Low Intermediate High Not Tested

Expression of SSEA-355: Low Intermediate High Not Tested

Expression of SSEA-356: Low Intermediate High Not Tested

Expression of SSEA-357: Low Intermediate High Not Tested

Expression of SSEA-358: Low Intermediate High Not Tested

Expression of SSEA-359: Low Intermediate High Not Tested

Expression of SSEA-360: Low Intermediate High Not Tested

Expression of SSEA-361: Low Intermediate High Not Tested

Expression of SSEA-362: Low Intermediate High Not Tested

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Expression of SSEA-401: Low Intermediate High Not Tested

Expression of SSEA-402: Low Intermediate High Not Tested

Expression of SSEA-403: Low Intermediate High Not Tested

Expression of SSEA-404: Low Intermediate High Not Tested

Expression of SSEA-405: Low Intermediate High Not Tested

Expression of SSEA-406: Low Intermediate High Not Tested

Expression of SSEA-407: Low Intermediate High Not Tested