

# General Quality and Regulatory Criteria for Establishment and Dissemination of human Pluripotent Stem Cell Lines (hPSCs)

## Key Objectives

- To provide criteria for researchers to use in selection of suppliers of cell lines
- To enable comparison of different suppliers for researchers to resource the best cells for their purposes
- To drive standards for quality of hPSCs provided for research and industry in SEURAT-1

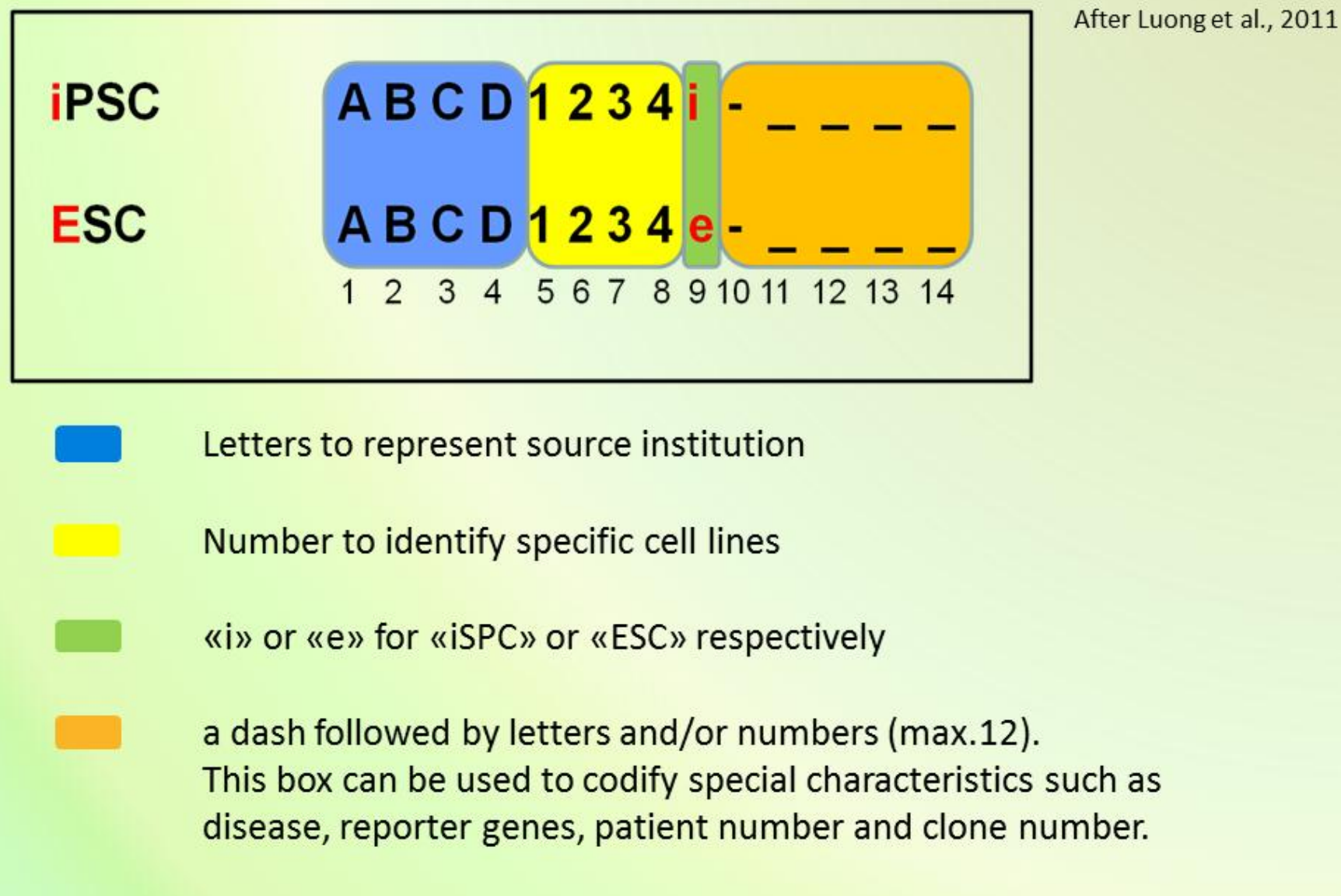
There are several key factors that must be considered to ensure that cell lines used in research provide relevant and valid data: correct identity, absence of microbial contamination and stability of characteristics and function over extended *in vitro* passage.

ToxBank is charged with establishing quality criteria for cell supply which will provide an important quality assessment to help ensure that SEURAT-1 outputs are standardised and valid. Setting these criteria for suppliers of hPSCs has been developed as a new collaboration by ToxBank with SCR&Tox.

It is also important to identify any ethical or commercial issues that could impact on the suitability of the hPSCs for EC funded research and industrial application. We have used key documents representing international consensus in this area for quality control (ISCBI, 2009), cell line nomenclature (Luong et al, 2011) and characteristics for hiPSCs (ESTools).

We are also developing guidelines for evaluation of ethics and commercial issues and to establish core quality control requirements for hiPSCs (Pistollato *et al.*, in press 2012). Procurement criteria will provide a formal standard for the supply of hESCs and hiPSCs within SEURAT-1.

## Proposed nomenclature for cell line names



## Starting Point: A Standard for Reporting Newly Isolated hPSC Lines Cell Line Nomenclature

Naming formats often vary between research groups causing significant confusion for researchers (Luong *et al.*, 2011). hPSC research is already generating large numbers of cell lines. Hence, it will be beneficial to establish a common nomenclature for the naming of hPSCs. Luong *et al.* proposed a naming convention which is designed to be simple, self explanatory and specific and which is not dissimilar to approaches adopted by a number of centres. The proposed convention captures the cell type and origin as shown above.

**Proposal:** any cell lines registered with ToxBank should be allocated a reference based on this naming standard in coordination with the group that derives the line, but using prefixes “h” and “m” before “i/e” to discriminate human and mouse cells. This will provide a common identifier that can provide unequivocal recognition of cell lines throughout the SEURAT-1 programme and beyond.

## Reporting the isolation of hPSCs

It is important that researchers can identify the origin and characteristics of the somatic cells used and the features they exhibit. ESTools ([www.estools.eu](http://www.estools.eu)) established key scientific criteria to demonstrate that a purported iPSC line does in fact have the correct features to justify this description. More recently, Luong *et al.* identified key features that should be reported in first publications of hPSCs. Here we have used these published consensus criteria to establish the features that scientists should expect to see reported for a hPSC intended for use in SEURAT-1.

## Best Practice for procurement, preparation, testing and distribution of hPSCs

Core standards for Good Cell Culture Practice have been established (Coecke *et al.*, 2005) and more recently developed by key hPSC line cell banks and stem cell biologists for the banking and distribution of hESC lines (ISCBI, 2009; [glyn.stacey@nibsc.hpa.org.uk](mailto:glyn.stacey@nibsc.hpa.org.uk)). The criteria established in this guidance cover all aspects of banking and dissemination of hPSCs.

## SCR&Tox and ToxBank Coordination

- SCR&Tox and ToxBank have also collaborated on two aspects relevant to the supply of hPSC:
- An evaluation process for new iPSC lines intended for use to evaluate hPSC lines regarding ethical and commercial issues to ensure that they meet requirements for lines for EC funded research and industrial application.
  - An assessment of the key criteria for scientific quality of hPSCs and the considerations for their quality control (UKSCB and ECVAM) (Pistollato *et al.*, 2012). It is the first stage of the establishment of a guidance on key quality control measures for iPSC lines and the development of differentiation protocols.

## Core Criteria for Selection of hPSC Lines

Criterion	Description
Source of original somatic cells described	Cell type, tissue, passage number, donor age
Derivation method reported	<ul style="list-style-type: none"><li>hESC (e.g. zona pellicula removal, cell isolation and seeding, culture conditions)</li><li>hiPSC- Reprogramming method (e.g. vector system, small molecules, protein, mRNA, or miRNA transduction/transfection)</li></ul>
Characterisation reported*	<ul style="list-style-type: none"><li>Stable ES cell like morphology and growth pattern</li><li>Expanded in culture as established line for &gt; 10 passages</li><li>Human ES cell surface antigen profile: Expression of SSEA3, SSEA4, TRA-1-60/TRA-1-81, L-ALP (TRA-2-54 or TRA-2-49) – quantitated by flow cytometry</li><li>Express key endogenous pluripotency-associated genes: Oct4, Nanog, Sox2, Rex, TDGF</li><li>Primary evidence of pluripotency by qRT-PCR for lineage markers of differentiated cells</li><li>Transgenes down-regulated</li></ul>
Microbial contamination screening reported	<ul style="list-style-type: none"><li>Mycoplasma not detected</li><li>Bacteria and fungi not detected</li><li>(For viruses see ISCBI 2009)</li></ul>

\* ESTools also prescribe “advanced” characterisation of iPSC lines

## Ethics Criteria for Cell Lines Selection

(hiPSCs and hESCs)

In order to establish that all cell lines were obtained from tissue that has been ethically sourced the researchers must be able to provide evidence for the following:

- That fully informed consent was obtained and recorded for the donor tissue
- That consent permits the intended uses of the hPSC lines derived from the donor’s tissue
- That the donor’s identity was anonymised
- A validated copy of the original consent form (with donor details redacted) is available and/or a statement is available from a person authorised by the owner or derivation centre on the ethical provenance of the cell line including a contact that would facilitate confirmation of the original consent without breaking donor anonymity
- There should be a clear statement on any constraints applied by the donor on the use of derivatives from their cells/tissues
- Cell lines are registered within the hESCreg database
- Copies of blank consent form (or an English translation) and any information provided to the donor are available
- Evidence from the donation process that the donor was aware that:
  - derived lines may be exploited commercially but that donors would not receive personal financial benefit
  - the donors’ decision to donate tissue would not influence their personal treatment and there would be no feedback on data from the cell line derived from their tissue
  - derived hPSCs could be used for a wide range of purposes in different laboratories and may be tested for genetic characteristics, microbiological contamination and other features of the cells

## Commercial Criteria for Cell Line Selection

Failure to address key commercial issues relating to the use of any research materials for use in SEURAT-1 projects could invalidate, delay or otherwise compromise their ultimate use to deliver the required outputs from SEURAT-1. It is therefore important that researchers apply suitable vigilance when obtaining hPSCs and other research materials that may be critical at a later stage.

Key criteria for selection of hPSCs should therefore ensure that:

- the owner of the cell line is clearly identifiable (NB numerous cell lines have shared ownership)
- permission has been granted by the owner/s or their agents for the intended use or is the line released for general research without constraint (see also ethics criteria regarding donor constraints).
- intellectual property rights relating to the cell line or any components used to derive the cell line (e.g. DNA constructs) are clear and would not influence their use for commercial application. If there is a potential affect on ultimate use of research materials for commercial purposes this should be discussed with the consortium coordinator and any limitations on the use of the materials agreed.

## Criteria for hPSC Line Characterisation

Analytical Technique	Required Characteristic Reported for Each Cell Line
Identity e.g. DNA profile	Matches parent cell line
Karyotype	Report karyotype from a specified number of metaphase analyses (see Methods and Measurements in ISCBI (2009))
Post-Thaw Recovery	Viable colonies recovered (quantified efficiency of recovery of each bank/lot should be given) NB viable colonies should also be predominantly free of differentiated cells.
Pluripotency	Report data available or traceable to stocks tested for pluripotency*
Growth Characteristics	Report value
Cell antigen expression	High proportion of cells (approx. 70%) positive for each marker*
Cell gene expression	Report data available*
Genetic stability	Report data available*

\* Precise requirements for hESC lines are discussed in ISCBI (2009) and are under development for hESC and hiPSC lines in Scr&Tox.

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We would also like to thank all SEURAT-1 cluster PIs and stem cell researchers who gave up their time and knowledge to assist the authors in preparing the documents.

## Next Steps

- Obtain feedback on the criteria given above from SEURAT-1 partners and where appropriate refine this guidance
- Working with co-authors as part of a coordinated ToxBank/ SCR&Tox effort to develop the detailed scientific quality criteria for hPSCs and facilitate establishment of information on cell lines suitable for SEURAT-1 cluster rough the ToxBank database
- Work with SCR&Tox partners to communicate best practice in procurement and quality control of cell lines used in SEURAT-1