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Biotechnology — Biobanking — Requirements for human and mouse pluripotent stem cells

Biotechnologie — Biobanking — Exigences relatives aux cellules souches pluripotentes humaines et murines

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have been extensively studied in scientific research in order to improve the understanding of developmental biology and diseases, to create organoids for drug screening, and to be applied in cell-based therapies. In just a few years, thousands of PSC lines have been established in laboratories around the world. PSC lines hold unique characteristics and behaviour due to their capability for both self-renewal and differentiation into multiple cell types. However, the stem cell phenotype can be changed by suboptimal cell culture technique, prolonged passage or changing the culture conditions. Clearly the consequences of using adversely affected cells would be wasted time and resources but, even more seriously, the generation of erroneous data in the literature which could both confuse and delay scientific progress in this area. Accordingly, mouse PSCs have been used to establish our fundamental understanding of PSC biology and these discoveries have been translated into human PSC research to drive the development of new human-cell-based *in vitro* assays and potential regenerative medicines. Mouse PSCs and human PSCs have become the most widely studied species in this field and many significant scientific advances have been made by using PSCs from these two species. Of course, PSC lines have been established from other species such as rat, porcine, canine, bovine, primate, etc. and those from primates in particular have provided understanding of the biology of these cells which can be more relevant to human stem cell biology than data from mouse PSCs. However, PSCs from these species are much less used in research laboratories than mouse and human and are therefore not described specifically in this document although much of this document will be relevant to them.

Human PSCs developed in research environments will give the clues to the development of cell therapies, thus ensuring that cell lines used in this dynamic field have been prepared and documented appropriately and have the correct identity and characteristics, which is critical to help ensure reproducibility in PSC-based research. This document aims to meet the current demand for standardized PSC procedures of biobanks and builds on international consensus agreed by PSC resource centres[9]. This document specifies the establishment, maintenance, characterization, storage and distribution requirements for mouse and human PSCs, providing a general guideline for both biobanking and fundamental research of PSCs.

Biotechnology — Biobanking — Requirements for human and mouse pluripotent stem cells

1 Scope

This document specifies requirements for the biobanking of human and mouse pluripotent stem cells (PSCs), including the collection of biological source material and associated data, establishment, expansion, characterization, quality control (QC), maintenance, preservation, storage, thawing, disposal, distribution and transport.

This document is applicable to all organizations performing biobanking with human and mouse PSCs used for research and development.

This document does not apply to cell lines used for *in vivo* application in humans, clinical applications or therapeutic use.

NOTE International, national or regional regulations or requirements, or multiple of them, can also apply to specific topics covered in this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8601-1, *Date and time — Representations for information interchange — Part 1: Basic rules*

ISO 20387:2018, *Biotechnology — Biobanking — General requirements for biobanking*

ISO/TS 20388:2021, *Biotechnology — Biobanking — Requirements for animal biological material*

ISO 21709:2020, *Biotechnology — Biobanking — Process and quality requirements for establishment, maintenance and characterization of mammalian cell lines*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20387:2018 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
biobank
legal entity or part of a legal entity that performs *biobanking* (3.2)

[SOURCE: ISO 20387:2018, 3.5]

3.2
biobanking
process of acquisitioning and storing, together with some or all of the activities related to collection, preparation, preservation, testing, analysing and distributing defined biological material as well as related information and data

[SOURCE: ISO 20387:2018, 3.6]

3.3
cell line master file
complete dossier of all procedures and records used to generate and maintain a cell line

3.4
cell morphology
form and structure of the cell

Note 1 to entry: Morphology can be represented by a single parameter or a combination of two or more parameters.

[SOURCE: ISO 21709:2020, 3.3]

3.5
cell population purity
percentage of a particular cell type in a population, of which has the same specific biological characteristics, such as cell surface markers, genetic polymorphisms and biological activities

[SOURCE: ISO/TS 22859:2022, 3.8]

3.6
cryopreservation
process by which cells are maintained in an ultra-low temperature in an inactive state so that they can be revived later

[SOURCE: ISO 21709:2020/Amd 1:2021, 3.6]

3.7
differentiation
process to bring the cells into a defined cell state or fate

[SOURCE: ISO/TS 22859:2022, 3.11]

3.8
differentiation potential
ability that refers to the concept that stem and progenitor cells can produce daughter cells which are able to further differentiate into other cell types

[SOURCE: ISO/TS 22859:2022, 3.12]

3.9
embryonic stem cell
ESC
pluripotent stem cell (3.21) derived from the inner cell mass of a blastocyst, i.e. an early stage pre-implantation embryo

3.10
ethics review committee
body which is responsible for the evaluation and review of the ethical issues involved in the research

3.11
expansion
cell culturing process by which the cell number increases *in vitro*

3.12
feeder cell
mitotically inactivated cell used to support the growth of *pluripotent stem cells* (3.21)

3.13
genetic integrity
genome of cells that has not been altered

3.14
genetic state
phenotype of genetic profile of individual organism, including but not limited to *karyotype* (3.18), integrity, mutation and knock-in of exogenous sequence

3.15
harvest
process of obtaining cells from a cell culture environment

3.16
identity verification
part of the process of verifying authenticity of a cell line in which cell origin is genetically confirmed

[SOURCE: ISO 21709:2020, 3.10]

3.17
induced pluripotent stem cell
iPSC
pluripotent stem cell (3.21) that is generated from somatic cells through artificial reprogramming by the introduction of genes or proteins, or via chemical or drug treatment

3.18
karyotype
characteristics of the chromosomes of a cell, including its number, type, shape and structure, etc.

3.19
passage
subculture
process of further culturing of cells in a culture vessel to provide higher surface area/volume for the cells to grow

Note 1 to entry: A passage can be performed by harvesting an aliquot from the parent vessel and reseeding it into another vessel.

[SOURCE: ISO/TS 22859:2022, 3.18]

3.20
passage number
number of subculturing that occurred

Note 1 to entry: For this document, P_0 is understood as the starting population of the cells.

[SOURCE: ISO 21709:2020, 3.13, modified — Note 1 to entry added.]

3.21
pluripotent stem cell
PSC
stem cell (3.26) that can differentiate into all cell types of the body and is able to self-renew indefinitely *in vitro*

Note 1 to entry: PSCs include *embryonic stem cells (ESCs)* (3.9) (including fertilization derived ESCs, *somatic cell nuclear-transferred stem cells* (3.25), etc.) and *induced pluripotent stem cell (iPSCs)* (3.17).

Note 2 to entry: ESC-like cells can also be isolated by parthenogenetic division of oocytes or other haploid cell sources, and these cells have many of the characteristics of ESCs. However, certain features of these pluripotent cell types can require specific characterization approaches.

3.22
population doubling time
PDT
doubling time
time taken for cultured cell count to double

Note 1 to entry: The time is measured in hours.

[SOURCE: ISO 21709:2020, 3.8, modified — "population doubling time" and "PDT" added as the preferred term. Note 1 to entry added.]

3.23
self-renewal
ability of *stem cells* (3.26) to divide symmetrically, forming two identical daughter stem cells

Note 1 to entry: Adult stem cells can also divide asymmetrically to form one daughter cell which can proceed irreversibly to a differentiated cell lineage and ultimately lead to specialized functional differentiated cells, while the other daughter cell still retains the characteristics of the parental stem cell.

[SOURCE: ISO/TS 22859:2022, 3.23]

3.24
separation
process of obtaining target cells from biological samples

3.25
somatic cell nuclear-transferred stem cell
embryonic stem cells (3.9) derived from *in vitro* transfer of a donor cell nucleus into an enucleated oocyte

3.26
stem cell
non-specialized cells with the capacity for *self-renewal* (3.23) and *differentiation potential* (3.8), which can differentiate into one or more different types of specialized cells

Note 1 to entry: Based on potency, stem cells can be divided into: *totipotent stem cell* (3.29), *pluripotent stem cell* (3.21), multipotent stem cell, oligopotent stem cells, and unipotent stem cells (see Annex A).

[SOURCE: ISO/TS 22859:2022, 3.24, modified — Note 1 to entry replaced.]

3.27
stem cell marker
protein or gene specifically expressed in *stem cells* (3.26), usually used to isolate and identify stem cells

Note 1 to entry: Stem cell markers vary depending on stem cell type.

3.28
teratoma
tumour containing representative differentiated tissues and cells from the three germ layers

3.29
totipotent stem cell
stem cell (3.26) that can differentiate into an intact new organism including embryonal and extra embryonal cells

3.30
viability
attribute of being alive (e.g., metabolically active, capable of reproducing, have intact cell membrane, or have the capacity to resume these functions)

[SOURCE: ISO 21709:2020, 3.17, modified — "as defined based on the intended use" deleted.]

4 Abbreviated terms

bFGF	basic fibroblast growth factor
EMRO	embryo research oversight
ESC	embryonic stem cell
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HTLV	human T-lymphotropic virus
IFU	instructions for use
iPSC	induced pluripotent stem cell
KLF4	krueppel-like factor 4
KSR	knockout serum replacement
mLIF	mouse leukaemia inhibitory factor
MTA	materials transfer agreement
OCT4	octamer-binding transcription factor 4
OriP	origin of replication
PBMC	peripheral blood mononuclear cell
PSC	pluripotent stem cell
QC	quality control
SOX2	SRY (sex determining region Y)-box 2
SSEA3	stage-specific embryonic antigen 3
SSEA4	stage-specific embryonic antigen 4
SSEA1	stage-specific embryonic antigen 1
STR	short tandem repeat
SV40LT	Simian virus 40 large T

TP treponema pallidum

5 General requirements

5.1 General

The biobank shall follow ISO 20387 and ISO 21709, in addition to this document. ISO/TR 22758 can be used as additional reference for the implementation of ISO 20387. For mouse PSCs, ISO/TS 20388 shall also be followed.

The biobank shall establish criteria and procedures for the isolation, establishment, expansion, storage, thawing and transport of PSCs.

A data analysis procedure shall be established, documented, implemented, regularly reviewed and updated.

The biobank shall use validated and/or verified methods and procedures for activities pertaining to PSCs in accordance with ISO 20387:2018, 7.9.2 and 7.9.3, at all stages of the biological material life cycle (as defined in ISO 20387:2018, 3.29).

According to the characteristics of PSCs, procedures, QC documents for collection, separation, expansion, storage, transportation and testing, and data analysis shall be established, documented, implemented, regularly reviewed and updated.

5.2 Legal and ethical requirements

ISO 20387:2018, 4.1.6, 4.3, 7.2.3.4, 7.3.2.4, A.7 a), and ISO 21709:2020, 4.2, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 4.2, shall also be followed.

The biobank shall collect relevant information on ethical requirements, implement and regularly update them, where relevant.

It is important to recognize that PSC lines are potentially not acceptable for use in research or development or both in some countries, and shipment of cells to collaborating organizations will require consideration of these differences. The biobank shall establish, document and implement policies on the procurement and supply of PSCs.

Experimental plans using or establishing human PSCs should be consulted in a specialized ethics review committee with particular expertise in topics relevant to the type and intended use of the PSC lines in the biobank.

The biobank shall establish a process to verify and document cell line provenance, to be able to provide evidence of ethical and regulatory compliance.

The biobank shall be aware whether reimbursement was made for the donation of human embryos/tissues and whether the human embryo was created for research as this can be illegal in some countries.

For derivation of new pluripotent cell lines from human embryos, the ethical review process shall refer to relevant expert ethical reviews.

EXAMPLE The human embryo research oversight (EMRO) process (ISSCR guidelines 2016, Chapter 2.1)[10].

Ethical requirements relevant for distribution are provided in 18.1.

5.3 Personnel, facilities and equipment

ISO 20387:2018, Clause 6, and ISO 21709:2020, 4.3, 4.4, 4.7, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 4.3, shall also be followed.

