

Quality Control and Qualification of hiPSC Banks for Clinical Translation

Abstract

Human induced pluripotent stem cell (hiPSC) banks provide cells for the development and manufacture of cell therapies. Although guidance on their quality control is available, testing practices and criteria for bank release still vary between institutions. This review discusses current approaches to quality control and standardization of hiPSC banks intended for clinical use, with a focus on HLA-homozygous banks. It considers aspects of testing and reporting that can be harmonised and others where the characteristics of the line or its intended use require case by case interpretation. Particular attention is given to the limitations of available assays and the evidence needed to define acceptance criteria. More consistent testing and reporting would help laboratories compare banked lines and assess their suitability for specific products. This is especially relevant to haplobanks, where the same line may be used to manufacture therapies for many patients.

1. Introduction

Over the past two decades since the development of induced pluripotent stem cell (iPSC) technology (1,2), the field has progressed from proof of concept to clinical translation. Induced PSCs can be generated from somatic cells and subsequently differentiated into diverse cell types. As source material for advanced therapy medicinal products (ATMPs) products in clinical trials target a growing list of indications, including age-related macular degeneration, Parkinson's disease, chronic heart failure, and gene-modified allogeneic immune cell therapies (3,4). Nevertheless, the clinical translation of hiPSC-derived products remains uneven across regions, centres, and therapeutic areas.

A significant barrier to furthering the development and application of hiPSCs is the absence of globally harmonised and implemented standards for hiPSC biobanking and quality control, which differ considerably between the EU, USA, Japan, Korea, China, and other countries (5,6). Even for fundamental assessments such as genomic stability (7) and confirmation of the undifferentiated state (8,9) standardised quality control testing and acceptance criteria are not universally applied. International consensus guidelines have been prepared by the International Stem Cell Banking Initiative (ISCBi) (10), the European Bank for induced Pluripotent Stem Cells (EBiSC) (11,12), and, more recently, the International Society for Stem Cell Research (ISSCR) (13). A 2018 consensus paper proposed international guidelines for quality control of iPSC lines intended for clinical use (14). Current guidance generally agrees on the attributes for bank assessment and the available methods. However, it seldom defines acceptance criteria or clarifies the placement of tests within the biobank workflow or specify which processes may continue while results are pending (3,4,15). This paper addresses the gap between an established list of assays and a practical release decision.

Ensuring that new iPSC-based products comply with manufacturing, regulatory, and clinical requirements necessitates comprehensive phenotypic characterisation and quality control of banked lines, as well as evaluation of their microbiological, genetic, and epigenetic status. Culture-derived genetic abnormalities, especially those involving cancer-associated genes and pathways, may compromise genomic integrity and increase the tumorigenic potential of the final product (7,16). Adequate release testing, data traceability, and risk management are also critical for products that incorporate extensive genetic engineering (17). The development of biobanking information data

infrastructure that provides an integrated and auditable link between sample provenance, quality control results, and distribution history is increasingly seen as essential for clinically oriented iPSC biobanking (12,18).

A compelling rationale for standardisation are HLA-matched allogeneic iPSCs. HLA-homozygous haplobanks can, in principle, provide immunological coverage for substantial portions of a national population using a limited number of lines prepared for clinical use. For example, the original HLA-homozygous haplobank of 27 iPSC lines derived from 7 donors at the CiRA Foundation is expected to enable immunological matching for 40% of the Japanese population (19). Similar haplobank initiatives are underway or planned in Spain, South Korea, Finland, France, and other countries (20). The scale of haplobanks also amplifies associated risks. Because haplobanked lines are intended for use in large numbers of HLA-matched patients, an undetected release failure affects every recipient of that line rather than a single patient. Achieving comparability and reliability across HLA-matched products requires more extensive testing than for an autologous product of analogous technical risk.

This review has been developed within the framework of the HAPLO-iPS COST Action (CA21151), a European network focused on the generation, characterisation, and clinical translation of HLA-homozygous iPSC lines. The paper reviews quality control testing of hiPSC banks intended for clinical translation. Key topics include identity testing, phenotypic and purity assessment, genomic stability, microbiological safety, and epigenetic status. The review also addresses specific considerations for HLA-typed and HLA-homozygous haplobanked lines.

2. Quality Control Testing of hiPSC Banks

Quality control of hiPSC banks assesses the identity, biological characteristics and safety of cells intended for the manufacture of cell therapies. Bank qualification combines information on the origin and derivation of the line with analytical data obtained during cell expansion and banking. Testing at this stage forms part of a broader QC strategy extending from donor material and reprogramming through gene editing, where applicable, to intermediate and final products (Figure 1) (4). Several assessments recur across this sequence, although the material tested and the purpose of testing change. Authenticity testing, for example, establishes continuity between the donor, the bank and subsequent cell preparations, whereas repeated genomic testing can reveal abnormalities that emerge or become enriched during culture. Tests for residual reprogramming or editing components address the particular methods used to generate the line. Bank qualification thus provides a reference for subsequent testing, while further assessments address changes associated with continued culture and the requirements of the differentiated product. Comparisons of QC practices between banks need to account for these differences, including the sampling stage, analytical sensitivity and criteria used to interpret the results. Standardisation of these aspects would support more consistent decisions about bank release and facilitate the assessment of banked lines for their intended applications.

HAPLO-iPS

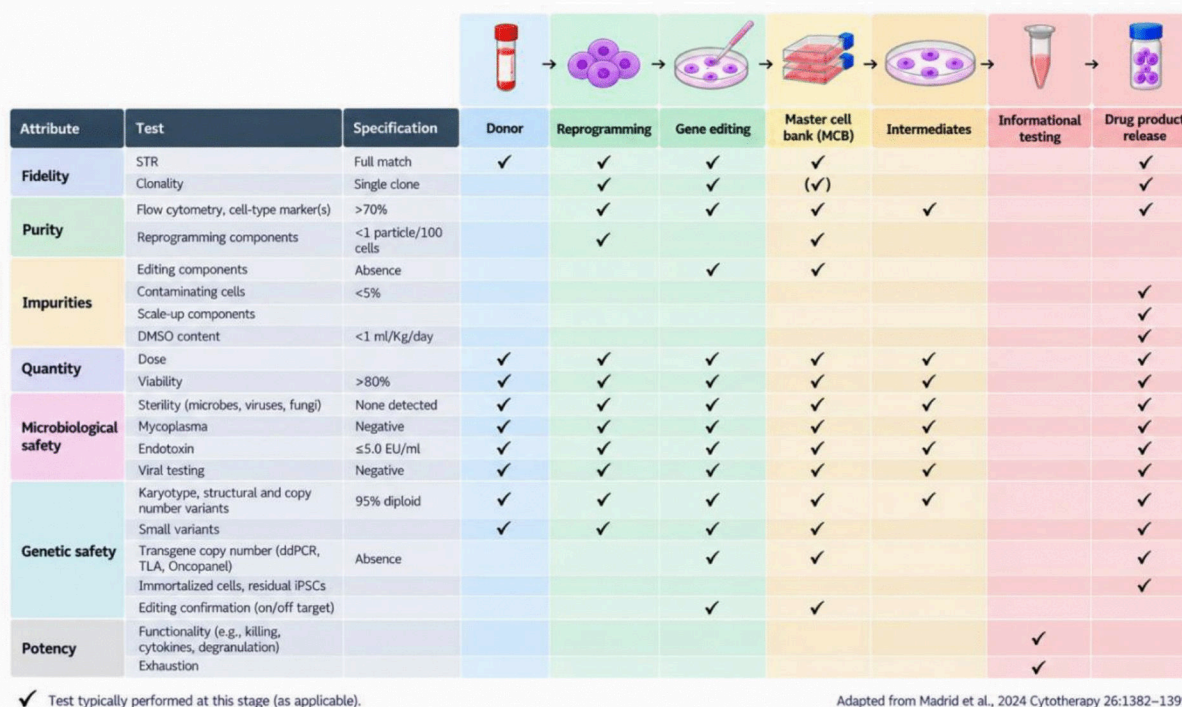


Figure 1. Quality control testing across the iPSC product cycle. Overview of quality control testing at key stages of iPSC-derived cell therapy manufacturing, from donor material and reprogramming through gene editing and MCB establishment to intermediate and final drug products. The scheme summarises representative tests for identity, purity, process-related impurities, quantity, microbiological and genetic safety, and potency, together with indicative specifications and stages at which testing may be performed. Testing strategies and acceptance criteria should be defined for each product using a risk-based approach.

2.1 Identity Testing

Confirming cell line authenticity is an important part of quality control in hiPSC banking. It helps ensure that the cells match the intended donor material and enables the detection of sample misidentification or cross-contamination. Short tandem repeat (STR) profiling is the primary method used to confirm that banked material originates from the designated donor and has not been replaced by another cell line, while human leukocyte antigen (HLA) typing may provide complementary evidence of donor identity. STR profiling requires determination of allele identities at polymorphic STR loci. The ANSI/ATCC ASN-0002-2022 standard recommends eight core microsatellite STR loci for human cell line authentication: TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, and D5S818, together with amelogenin (21,22). Since commercial STR kits analyse the same loci, profiles can be compared between laboratories. As ASN-0002 uses a percentage match value for profile comparison, release specifications should include the required threshold, loci analysed, and procedures for handling partial matches. The ASN-0002 eight-locus set is the minimum requirement for research cell line authentication; however, banking applications may require a larger panel, particularly when siblings are represented in the same collection, since eight loci may not distinguish them. Haplobanks derived from umbilical cord blood collections and populations with high consanguinity or increased HLA homozygosity may face the same issue. Expanding the panel to twenty or more loci enhances discriminatory power. Because the probability of a match depends on allele frequencies within the reference population, banks serving defined populations should report results using population-

specific allele frequencies. Standardised reporting of donor genetic data, especially of ethnicity associated genetic features, is an ongoing focus for international banking consortia (23).

Some STR results may require more careful interpretation. A clean profile excludes substitution and gross cross-contamination, but low-level contamination may remain undetected. A second genotype is usually identified only when it contributes more than a few per cent of the mixture, as weaker additional alleles may be difficult to distinguish from stutter peaks and background. Partial mismatches do not necessarily indicate cell line misidentification. They may result from allele dropout due to degraded or inhibited template, polymorphisms at primer-binding sites, somatic mosaicism, or acquired copy-number changes or loss of heterozygosity during cell culture (22). Most of the eight core loci lie outside chromosomal regions recurrently altered in cultured pluripotent stem cells. One exception is vWA on 12p, where recurrent copy-number gain can alter peak ratios while both alleles remain detectable (7,24). If unequal peak heights are observed at vWA, copy-number data from the same passage should be checked for 12p gain before the STR result is considered abnormal. To provide a reference for these comparisons, a donor reference profile should be established from the initial material prior to reprogramming and subsequently utilised for comparison at the banking stage. Lines obtained from external institutions should also be compared with the accompanying STR profile upon receipt (11,25). STR profiling should be performed on the donor starting material and repeated on each bank lot, with concordance to the donor profile as the acceptance criterion, rather than only once at line establishment.

For haplobanked lines, HLA typing is conducted to define the line's haplotype and assess potential population matches. Two-field typing specifies alleles at the protein level (e.g., HLA-A*02:01). The third and fourth fields, which denote synonymous and non-coding variants, are not used for matching purposes. Allele nomenclature is standardised by the WHO Nomenclature Committee for Factors of the HLA System and curated by the IPD-IMGT/HLA Database, which currently lists over 35,000 alleles (26). Typing of at least HLA-A, -B, and -DRB1 is required to define the HLA haplotype, although most current protocols also include HLA-C and -DQB1. The choice of loci is determined by clinical relevance, as class II matching requirements differ between clinical indications (27). The resolution used for bank qualification should be consistent with that applied in clinical matching. Increasing the number of loci included in donor matching reduces the proportion of the population that each donor can cover. In a survey of 5,421 umbilical cord blood units genotyped for HLA-A, -B, -C, -DRB1, and -DQB1 by next-generation sequencing (NGS), thirteen homozygous donors defined at HLA-A, -B, and -DRB1 loci covered 37.0% of the population, while eleven homozygous donors defined at all five loci covered 33.0%. Expanding this panel to 100 haplotypes increased coverage to 72.7% and 67.9% at three and five loci, respectively. It was estimated that 600 five-locus haplotypes would cover over 90% of the Chinese population (28). Among donor populations from eighteen countries represented in the Global Alliance for iPSC Therapies, the ten most common homozygous haplotypes accounted for 19.5% to 63.8% of the respective populations, with a mean coverage of 33.3%. The 180 most frequent haplotypes represented 54.6% to 81.7% of each population, averaging 68.4% coverage (29). The specific haplotype carried by a line determines the number of patients it can potentially serve, making the typing result a release-critical attribute. Coverage is inherently region-specific. Principal component analysis of published HLA data demonstrates that haplotype frequencies in Asian populations differ from those in European populations, although some overlap exists (28). Coverage estimates should only be transferred between populations when supported by underlying data. Similarly to STR analysis,

allele dropout can affect HLA typing and may lead to a heterozygous line being incorrectly classified as homozygous. Apparent HLA homozygosity should be confirmed using an alternative primer set and copy-number analysis (29).

STR profiling and HLA typing provide complementary information. STR profiling is used to confirm donor authenticity, whereas HLA typing defines the haplotype relevant to matching. Since individuals within a population may share the same haplotype, a matching HLA profile cannot substitute for STR-based authentication of a haplobanked line. Additional authentication methods include single-nucleotide polymorphism (SNP) arrays, which deliver genome-wide genotyping, donor identification, and copy-number variation (CNV) analysis in a single assay. Karyotyping may contribute to identity only where a distinctive constitutional abnormality is present (30,31). hiPSC lines derived from T cells retain the clonally rearranged T cell receptor (TCR) locus of the parent lymphocytes. Its clonal specificity and ease of PCR-based detection make the TCR locus a valuable secondary confirmatory identity marker alongside STR profiling. However, because the rearranged locus does not correspond to the donor germline sequence, this information should be documented in the line records to prevent future confusion (32).

STR and HLA profiles, as genetic information linked to a donor, are typically retained under conditions analogous to those governing other identifiers used to associate a cell line with a donor, in accordance with current data protection legislation. When hiPSCs and associated information are transferred to another institution, the recipient assumes responsibility for the handling of these data. International banking consortia are actively developing governance frameworks and standards for the collection and sharing of donor genetic data in hPSC biobanks (11,23). Authenticity testing can detect sample misidentification only if the reference profile is correctly attributed; it cannot reveal an incorrect donor linkage shared by both the banked material and its reference. Such errors must instead be controlled through an auditable chain of authentication within the quality management system.

2.2 Phenotypic and Purity Assessment

Characterisation of a hiPSC bank for phenotype and purity requires evaluation of three attributes: maintenance of the undifferentiated state, differentiation potential, and absence of differentiated subpopulations. The undifferentiated state is characterised by the expression of cell surface markers TRA-1-60, TRA-1-81, SSEA-3, and SSEA-4, as well as specific transcription factors (e.g., NANOG, OCT4, and SOX2) (14,33). Flow cytometry, a quantitative method for measuring surface markers, is a widely employed and validated technique for assessing these markers in consensus protocols for hiPSCs. At least two of these markers should be quantitatively tested, with a threshold of 80% positive cells at the time of release. An international interlaboratory study involving over twenty laboratories demonstrated the best concordance for OCT3/4, TRA-1-60, and SSEA5 (9). Although expression of these markers indicates an undifferentiated pluripotent state, it does not imply differentiation competency or exclude undesirable cell states. For example, embryonal carcinoma cells can express classical pluripotency markers (34). Results must also be interpreted in the context of the reprogramming methodology. If transgenes encoding OCT4, SOX2, or KLF4 were introduced exogenously, the presence of these markers may reflect transgene expression rather than activation of the endogenous pluripotency network. A marker panel must therefore include at least one protein not encoded by the reprogramming vector. The interpretation should also account for residual or reactivated

reprogramming transgenes, as hiPSC lines with reactivated transgenes may retain a pluripotent phenotype but exhibit severely impaired differentiation (34).

Flow cytometry provides an estimate of the percentage of marker-positive cells, which can inform purity specifications. Variability in sample processing, staining, acquisition, and analysis methods can lead to interlaboratory differences, as has been observed (9). Immunocytochemistry can support characterisation; however, the selection of imaging fields, parameters, and thresholding precludes the establishment of quantitative criteria. Transcript-based assays such as quantitative PCR (qPCR) are complementary and measure average expression in the sampled population, but they cannot directly determine the fraction of marker-positive cells or exclude minor differentiated subpopulations.

Differentiation potential should be evaluated independently from the undifferentiated phenotype. Trilineage differentiation assays demonstrate that a cell line can generate derivatives of all three embryonic germ layers. However, these assays do not definitively assess the ability to produce all cell types within each germ layer, nor do they predict the success of specific differentiation protocols. When a bank is intended for a defined product, a product-specific differentiation assay may be necessary to determine the suitability of a given line for that application. The teratoma assay is a widely used method for evaluating pluripotency *in vivo*; however, it is limited by poor standardisation and the requirement for animal experimentation (35). The principle of replacement, reduction, and refinement (the 3Rs) advocates for the use of validated *in vitro* assays when sufficient evidence of pluripotency and differentiation potential is available. If a teratoma assay is performed, detailed reporting of experimental conditions and histological criteria, including those for malignancy, is required. The hPSC ScoreCard assay can evaluate pluripotency and lineage-specific differentiation potential (36,37). Recently, markers and epigenetic profiles of the pluripotent state and the three germ layers have been developed and compiled into classifiers of hiPSC state and differentiation potential, such as hiPSCore or EpiPluriScore (8,38). These *in vitro* approaches provide overlapping but not identical information and are not interchangeable measures of pluripotency or differentiation potential.

Purity addresses the presence of differentiated cells within the banked population. Even when a high proportion of cells are positive for pluripotency markers, some differentiated cells may still be present. Lineage-specific markers can provide additional information regarding culture heterogeneity; examples include CER1, EOMES, and GATA6 for endoderm, APLNR, HAND1, and HOXB7 for mesoderm, and HES5, PAMR1, and PAX6 for ectoderm (8). Such testing may supplement the assessment of undifferentiated hPSC morphology, but it is not commonly included in the standard release criteria for hiPSC biobanks.

Automated image analysis provides a fast, non-destructive method for monitoring morphology and heterogeneity during culture expansion. Image-based techniques can distinguish between undifferentiated and differentiated colonies, as well as other morphological states, without depleting material from existing banked stocks (39,40). However, as these assessments are based on morphology rather than marker expression, they should not replace any marker-based characterisation during monitoring. The control of analytical variability is essential for quantitative phenotyping. Flow cytometric data acquisition can be influenced by sample preparation, staining, acquisition parameters, compensation, gating strategy, and controls used to define positive populations. Fixed samples should be analysed promptly, as epitope and scatter characteristics may change during storage. Analytical

methods should define these parameters, and any out-of-specification results need to be investigated for both analytical and biological causes.

2.3 Genomic Stability Assessment

A cell bank provides the material and records genomic data at defined stages. It documents the methods used and the scope of testing, while the recipient assesses variant significance in the context of the intended cell type, target tissue and clinical indication. The culture of hiPSCs may lead to genetic changes, including variants that confer a selective growth advantage and increase in frequency with passage. A meta-analysis of more than 100 studies revealed 738 recurrent genetic abnormalities in human pluripotent stem cells. These were defined as overlapping abnormalities reported in at least five independent studies (41). Detection of a genomic alteration does not by itself establish that it is biologically significant or unacceptable. Each method can detect only certain types of genetic changes. A “normal result” provides information only about the types of abnormality detectable by the method used. This section describes the available laboratory methods and the factors relevant to interpreting detected variants. Their scope, main applications and limitations are summarised in Table 1.

Table 1. Comparison of methods used for genomic stability testing in hiPSC banks, including their applications, limitations and sensitivity to subclonal variants.

Method	Detected	Not detected	Sensitivity to a subclone	Position in current practice
Banded karyotype	Aneuploidy; balanced and unbalanced translocations; inversions; deletions and duplications above ~10 Mb	Copy-number change below resolution; copy-neutral loss of heterozygosity; sequence variants	Each metaphase scored separately; depends on the number counted	Release assay in published manufacturing programmes
Array CGH	Gains and losses of genomic material, of the order of 1 kb	Balanced translocations and inversions; copy-neutral loss of heterozygosity; sequence variants	Limited below 30% of cells	Reference or informational assay
SNP array	Gains and losses; copy-neutral loss of heterozygosity; uniparental disomy	Balanced rearrangements; variants not represented on the array	Broadly as for array CGH	Reference or informational assay; also yields a genome-wide genotype
Whole-exome sequencing	Sequence variants within captured coding regions	Non-coding variants; structural change outside capture	Depends on read depth	Used for cancer-associated variant screening
RNA sequencing (RNA-seq)	SNVs and indels in expressed genes	Variants in transcriptionally silent regions or alleles	Depends on sequencing depth, transcript abundance and allele-specific expression	Supplementary characterisation alongside DNA-based genomic testing
Short-read whole-genome sequencing	Sequence variants, indels, copy-number change, many structural rearrangements	Internal structure of complex and repetitive regions	Depends on read depth	At least 50× recommended in United States draft guidance for allogeneic expanded cells
Long-read sequencing	Structure and breakpoints of	Determined by coverage	Depends on read depth	No specific regulatory position; applied to

	complex and tandem rearrangements			characterise defined rearrangements
Targeted ddPCR panel	Predefined recurrent abnormalities; applicable to DNA from culture supernatant	Any locus outside the panel	Detects subclones well below the sensitivity of genome-wide methods	No formal regulatory position; suited to monitoring between scheduled assessments

Banded karyotyping can detect abnormalities larger than approximately 10 Mb. It is a relatively low-resolution test, but can detect balanced translocations and inversions in which there is no gain or loss of genomic material. Evaluation of individual metaphases also allows the detection of mosaic abnormalities, although sensitivity depends strongly on the number of metaphases analysed (42). Trisomy of chromosomes 12, 17 and X, as well as amplification of specific loci, have been reported as recurrent chromosomal and subchromosomal changes in pluripotent stem cell cultures (43).

Array comparative genomic hybridisation (aCGH) and SNP arrays detect gains and losses of genomic material at a resolution that depends on probe density, platform design and analytical criteria. Some platforms used for cell line quality control can detect changes of approximately 1 kb. Copy-neutral loss of heterozygosity and uniparental disomy can be detected by SNP arrays. In these cases, allelic composition is altered, but copy number remains unchanged. Array technology does not detect balanced rearrangements, which involve no net gain or loss of genetic material. Arrays also have limited sensitivity for detecting mosaicism, particularly if the proportion of abnormal cells is below 30% of the analysed cell population. Results are interpreted using quality criteria, mainly the log R ratio and B-allele frequency. A workflow published for use in a repository environment reported chromosomal abnormalities in nine of 32 lines screened, including gain of 20q11.21 (44). Ntai et al. reported two examples illustrating the different detection ranges of karyotyping and aCGH. In an iPSC line derived from a patient with primary progressive multiple sclerosis, karyotyping showed no abnormality, whereas array CGH detected copy-number variants ranging from 20 to 700 kb. In a separate control fibroblast line, karyotyping identified a chromosome 4 rearrangement (46,XX[40]/46,XX,-4,+mar[10]) in 20% of metaphases. The rearrangement was not detected by array CGH since it involved no detectable gain or loss of genomic material and was present at a low level of mosaicism (42). These findings illustrate that the two approaches are complementary, and that neither is superior to the other.

Sequencing allows analysis at the level of single nucleotides and identifies types of variants not fully addressed by karyotyping or microarrays. Whole-genome sequencing (WGS) can detect single-nucleotide variants, insertions and deletions, copy-number changes and structural rearrangements in one test. Sensitivity for detecting a subclone depends on sequencing depth, data quality and the analytical pipeline. Whole-exome sequencing offers less expensive coverage of protein-coding regions; however, it does not capture non-coding variants or structural changes outside the regions covered. Long-read nanopore sequencing was used to identify the internal organisation of the 20q11.21 amplicon. The amplicon copies were arranged head-to-tail, with microhomology sequences flanking or overlapping the proximal and distal breakpoints (24). In 2024, the US FDA published draft guidance on allogeneic cells expanded for use in cell-based products. It recommends whole-genome sequencing at a depth of at least 50× and the reporting of all cancer-associated mutations, with particular attention to TP53 (45).

Genome-wide approaches may not identify low-frequency subclones, which can be detected using targeted assays. However, this capability is limited to loci included in the assay design. For example, the meta-analysis by Assou et al. describes a droplet digital PCR (ddPCR) panel designed to detect recurrent abnormalities. The panel detects over 90% of the reported abnormalities using DNA isolated from culture supernatant. This enables repeated monitoring without harvesting cells between scheduled genome-wide assessments (41). Genomic testing can be performed at various stages of the banking process: donor sample, clone selection, master cell bank, working cell bank and distributed vial. The frequency of a selected variant may change during this process. Each result should include the sampling stage and passage number. Without the passage number, findings from the same line at different time points cannot be compared reliably. When donor material is available, its cytogenomic profile should be used as a baseline. This helps distinguish variants present in the donor from those acquired during reprogramming, clone selection or expansion. Results from the master and working cell banks can then be compared with the donor profile and with each other. Results from the same assay may vary between laboratories. qPCR-based genomic integrity testing showed consistent results among laboratories participating in international Quality Assessment Rounds (9). Karyotyping was not included in that comparison, and comparable data on its reproducibility across service providers are unavailable. Since karyotype results depend on assay performance and the analyst's judgement, the testing laboratory, banding resolution and number of metaphases examined should be recorded.

Acquired sequence variants in pluripotent stem cell cultures are not distributed randomly across the genome. Exome sequencing of 140 human embryonic stem cell lines, including 26 prepared for potential clinical use, identified six TP53 mutations in five unrelated lines. All were dominant-negative and corresponded to mutations frequently found in human cancers. Their variant allele fractions increased with passage number, suggesting selection rather than random drift. Analysis of published RNA-sequencing data from another 117 lines identified nine TP53 mutations, all affecting coding sequences within the DNA-binding domain. In three lines, the variant allele fraction exceeded 50%, consistent with loss of heterozygosity at the locus (46). A survey of more than 2,200 transcriptomes from 146 independent lines and their differentiated derivatives found at least one cancer-related mutation in 22% of samples. Of these, 64% carried a TP53 mutation. The TP53 variants conferred a selective advantage, altered target-gene expression and affected differentiation (47). These effects may alter cell function and increase the number of residual undifferentiated cells. Recurrently acquired variants have also been reported in BCOR and PPM1D. BCOR encodes a component of the non-canonical PRC1.1 complex and regulates pluripotency and developmental differentiation. BCOR mutations were found in approximately 25% of blood-derived hiPSC lines. In a large analysis of coding mutations, BCOR was the only gene with statistically significant positive selection (48). BCOR dysfunction has also been linked to changes in gene expression and differentiation capacity (48–50). PPM1D regulates the DNA-damage response. Truncating mutations in this gene can weaken p53-mediated responses and increase resistance to genotoxic stress. This may give PPM1D-mutant clones a selective advantage under DNA-damaging conditions (51). TP53, BCOR and PPM1D are relevant examples for iPSC quality control, but they should not be considered a complete testing panel. Selection during culture is not limited to sequence variants. Copy-number changes may also give cells a growth advantage. Gain of 20q11.21 is the most frequently reported subchromosomal abnormality in cultured pluripotent stem cells. Its smallest recurrent region includes BCL2L1 and TPX2 (7), genes that may contribute to the selection of cells carrying the gain. Increased TPX2 expression has also been linked to mitotic defects, chromosome misalignment and polyploidy (52).

Cell banks should specify in their release documentation which methods were applied. The variant allele fraction used for reporting a finding should be separate from the applicable acceptance threshold. This allows low-frequency findings to be reported without preventing release of the cell bank. Increasing sequencing depth may help identify low-frequency subclones. A clone with a selective advantage may expand during long-term culture and become detectable, but evidence of selection requires comparison of its frequency across multiple passages (23). The MCB should be tested using G-banded karyotyping in combination with validated whole-genome sequencing. Together, these methods can detect balanced chromosomal abnormalities, copy-number changes, single-nucleotide variants and many structural variants. Sequencing costs are decreasing and analytical pipelines are improving, so that WGS may become more commonly used for primary screening and clone selection. However, WGS will not replace SNP arrays, optical genome mapping or targeted ddPCR until it is validated to detect the relevant variant classes and levels of mosaicism. RNA sequencing can provide additional information on the effect of transcription on developmental programmes, cellular state and oncogenic pathways (50,53). However, RNA sequencing should be regarded as supplementary characterisation, not a substitute for genomic testing, as passage number, confluence, the fraction of spontaneously differentiating cells, and the choice of normalisation can influence the expression profile. TP53 variants affecting the DNA-binding domain warrant separate acceptance criteria because they recur in pluripotent stem cell cultures, increase in frequency under standard culture conditions and affect differentiation. For variants in other cancer-associated genes, the cell bank should document the allele fraction and the passage at which the variant was detected. These findings should be assessed by the recipient in the context of the intended product and its clinical use.

The genomic record supplied with each distributed lot should include the methods used and the stage of the banking process at which each was applied; the types of change detectable by each method; all detected variants, with their allele fractions and passage numbers; and the types of change not analysed. Information on unanalysed changes is necessary to distinguish a negative result from the absence of testing. Registration extends the availability of this record beyond the direct exchange between the cell bank and the recipient. The human pluripotent stem cell registry (hPSCreg) includes structured fields for genetic features, genetic stability, karyotype, passage number, donor information and reprogramming data (54). A pipeline developed with the European Bank for induced Pluripotent Stem Cells permits data from line originators and quality-control testing performed by cell banks to be imported, standardised and stored under a common nomenclature (18). Data deposited in the registry may also support retrospective analysis of associations between culture conditions and particular variants. Genomic findings identified after distribution should be reported to the cell bank because they may be relevant to the remaining vials from the same lot or to other lots derived from the same clone.

The detection of a cancer-associated variant in a cell line does not necessarily indicate a risk of carcinogenesis in recipients. Interpretation of such findings depends partly on reference databases, which are updated over time and may classify the same variant differently between releases. Cancer-associated variants are also found in tissues from healthy individuals, indicating that additional events are required for malignant transformation (55). More than 1,200 patients have received pluripotent stem cell-derived products in clinical trials (56). One case of tumour formation has been reported following administration outside a standardised clinical trial. An immature teratoma developed two

months after intramuscular injection of autologous iPSC-derived beta cells, metastasised to the axillary lymph nodes and did not respond to chemotherapy used for immature teratomas (57). Genomic findings have also affected clinical development without being linked to an adverse event. A phase I trial for age-related macular degeneration in Japan was discontinued after the detection of variants that were absent from the original cells used for reprogramming. In a first-in-human study of allogeneic iPSC-derived corneal endothelial cells, an in-frame deletion of exon 22 of EP300 was identified, but no adverse event was attributed to it (55). The significance of an individual variant must be assessed in relation to the cell type, target tissue, manufacturing process and intended clinical use. Assessment of tumourigenic potential is expected before the clinical use of products derived from human pluripotent stem cells, although a stand-alone in vivo study is not always required. When such a study is performed, the animal model, route of administration, cell dose and observation period should be justified, and assay sensitivity should be established. Consensus guidelines provide a framework for evaluating recurrent variants in pluripotent stem cells (58), although methods for consistent assessment across cell banks are still being developed. At a 2024 workshop organised by the United Kingdom Regenerative Medicine Platform, proposed measures included standardised registries recording variants and the conditions under which they arose, methods for classifying and predicting variants of concern, multiplexed assays for assessing their effects, and improved control of culture conditions to reduce genomic abnormalities (55). Until standardised methods are available, data on detected variants and the associated culture conditions should be retained and made available to recipients to support future interpretation.

2.4 Microbiological Safety Testing

Microbiological safety testing for hiPSC banks comprises a series of steps throughout the cell-banking process, with test results serving as a decision point for subsequent phases. The primary established tests include sterility, mycoplasma, endotoxin, and screening for adventitious viral agents (59). Regulatory standards for these tests are defined (60,61). ICH Q5D outlines the two-tier cell-banking model and the required characterisation of cell substrates, while ICH Q5A(R2) details a risk-based approach to viral safety evaluation for biotechnology products derived from human cell lines. Neither guideline specifically addresses hiPSC banks. A major challenge is the application of these regulations to starting materials intended for clinical use when the final product, manufacturing process, and clinical application remain undetermined. The distribution of tests within the workflow and the activities permitted while awaiting results determine whether a contamination event is contained or spreads throughout the facility. The International Stem Cell Initiative evaluated 59 human embryonic stem cell lines from 17 laboratories and found no significant contamination with mycoplasma, bacteria, or cytopathic viruses (62). This outcome indicates that low contamination rates are achievable under properly controlled conditions. However, these findings cannot be extrapolated to materials obtained from external sources, for which the receiving institution holds responsibility for microbiological evaluation prior to banking.

The microbiological risks associated with hiPSC banking differ from those of conventional continuous cell lines because hiPSC banking involves prolonged culture and multiple manual steps between collection of donor material and establishment of the MCB. Some of these steps may involve open-vessel procedures such as colony picking. Raw materials used for reprogramming, culture, and expansion are another potential source of microbial contamination and should be accounted for in the

contamination risk assessment. Cell banks provide starting material for subsequent expansion and differentiation. Contamination that remains undetected at the point of bank release can be transferred to downstream cultures and manufacturing processes. This is especially relevant for HLA-haplotype banked lines, which may be used to generate products for large numbers of recipients.

The timing and frequency of sterility testing should depend on the risks associated with each stage of cell banking. Compendial methods described in Ph. Eur. 2.6.1 and USP <71> remain the reference standards, but their 14-day incubation period may be impractical for cell-based products requiring rapid release (63,64). Automated blood-culture systems such as BacT/ALERT have been validated as faster alternatives that meet compendial sensitivity criteria for clinical cell therapy products (65). Alternative methods should be validated using representative samples and the reference microorganisms specified by the relevant pharmacopoeia. Validation should include anaerobic organisms, which may be more difficult to detect. In routine testing, sensitivity can also depend on inoculum volume and the time between sampling and inoculation, both of which can affect the recovery of reference anaerobes (65). For in-house testing, it is important to distinguish product-release testing from clinical diagnostic microbiology, as they are subject to different regulatory requirements. Accreditation for diagnostic testing alone does not demonstrate that a laboratory is qualified to perform GMP release testing of a cell product (66). Sterility testing examines only the sample tested and therefore cannot establish the sterility of the entire bank. Effective sterility control also requires environmental monitoring, aseptic process simulation, and control of the filling process.

Slow-growing and nutritionally demanding microorganisms produce little or no visible change in cell cultures and may be difficult to recover by standard sterility testing. This is particularly problematic when antibiotics are routinely added to the culture medium. Instead of eliminating contamination, antibiotics may suppress susceptible microorganisms, which can reappear once antibiotic treatment is discontinued. Cultures intended for banking should be maintained without antibiotics for a defined period before sterility testing, with this period documented in the release records. At least two antibiotic-free passages may be necessary, as a single medium change may not allow sufficient recovery of previously suppressed microorganisms. The required period should, however, be defined based on the culture process and the associated contamination risk. Media prepared in-house also require appropriate microbiological control. Filtration through a 0.22 µm filter removes most bacteria and fungi but does not reliably remove mycoplasmas and cannot generally replace microbiological control of raw materials and media preparation.

Mycoplasmas are capable of surviving multiple passages and transferring between cell lines within the facility. They do not cause turbidity or other visible signs of contamination. Additionally, mycoplasmas exhibit resistance to beta-lactam antibiotics, which are often used in cell culture laboratories. Although mycoplasmas may be present at concentrations exceeding 10⁷ organisms per millilitre without altering morphology, they can significantly impact cell proliferation, metabolism, and differentiation. As a result, data generated during cell line characterisation, such as marker expression, growth kinetics, and differentiation, may be compromised, even if this is not apparent from the information provided to recipients. While compendial culture and indicator-cell methods remain valid, these approaches are problematic due to extended incubation periods. Nucleic acid amplification tests may be accepted if they are properly validated and meet the sensitivity requirements of the pharmacopoeia, including the ability to detect 10 CFU/mL where applicable. Validated in-house real-time PCR assays have

demonstrated the capability to detect all species required by the European Pharmacopoeia, provided that an appropriate sample quantity is analysed (67). However, the analytical performance of molecular assays can vary significantly. For example, a study comparing five commercial tests found that some did not achieve the 10 CFU/mL sensitivity required to serve as potential replacements for culture-based testing according to European and Japanese pharmacopoeial methods, and results may differ when testing various mycoplasma species on the same platform (68). Both the master cell bank (MCB) and working cell bank (WCB) should be tested independently for mycoplasma contamination, since contamination may be introduced during the culture and handling steps between the two banking stages.

Endotoxin testing provides information that is not obtained from sterility testing. Endotoxin may remain detectable after Gram-negative bacteria are no longer viable and can also be introduced during cell culture, formulation, or filling. A bank may therefore be negative in a sterility test but positive for endotoxin. For a hiPSC bank, the endotoxin result mainly provides information on the manufacturing process and possible contamination during processing. It does not directly establish the clinical safety of the cells. Similarly, a negative result does not demonstrate compliance with the endotoxin limit for the final cell-based product. Under Ph. Eur. 5.1.10 and USP <85>, the endotoxin limit for a medicinal product is calculated from the route of administration and the maximum clinical dose. These parameters are generally not defined for a hiPSC bank because the bank is a starting material rather than a product administered to a patient. The endotoxin specification for the bank must therefore be defined separately, taking into account the manufacturing process, possible carry-over or removal of endotoxin during subsequent processing, and the expected use of the cells. The measured endotoxin level should be recorded in the bank documentation and made available to developers of downstream products. The analytical method also needs to account for the characteristics of the material being tested. The kinetic chromogenic Limulus amoebocyte lysate (LAL) assay is more sensitive than the gel-clot method and has shown a higher proportion of valid spike recoveries in cellular products (69). This is relevant because cell suspensions and supernatants containing cryoprotectants can interfere with the assay. Validation for each sample type should include inhibition and enhancement testing and determination of the dilution needed to obtain valid recovery. Recombinant Factor C reagents provide an animal-free alternative to LAL lysate, but their performance also needs to be established for the sample type being tested (70).

Donor screening and qualification of the starting material identify viral agents that may be present before reprogramming. The derived hiPSC lines are tested for adventitious agents that may be introduced during reprogramming, culture, or handling. Legislation in the relevant jurisdiction requires testing for HIV-1/2, HBV, HCV, and HTLV-I/II where applicable, together with additional agents selected on the basis of donor history and country of origin. Potential sources of viral contamination vary with the derivation method, culture conditions, and raw materials used. Information on the origin of the cells and materials is needed to decide which tests are appropriate. ICH Q5A(R2) describes testing of cell substrates using assays in permissive indicator cells, tests for viruses specific to the species of origin, and assessment for retroviruses. The revised guideline also recognises NGS as a method that can complement or, in some cases, replace conventional assays. If a viral vector is used for reprogramming, testing for residual vector should be performed separately from adventitious virus testing. The SARS-CoV-2 pandemic provided an example of how testing may need to be modified when a new viral agent emerges. Cell lines used in biomanufacturing, including CHO, HEK-293, HT-1080, and Vero cells, were

tested for susceptibility to SARS-CoV-2, and routine adventitious virus assays using culture supernatants were evaluated for their ability to detect the virus (71). Pluripotent stem cell lines were not included in the study. Nevertheless, the appearance of a previously unrecognised virus may require additional testing beyond the panel originally established for a cell bank.

Microbiological results need to be interpreted in relation to the material tested and the time of sampling. For a cryopreserved hiPSC bank, samples can be taken from the cell suspension before aliquoting, from culture medium during the final expansion, or from a vial thawed after completion of the bank. A sample collected before freezing reflects the culture up to that point but does not account for contamination that may occur during filling. Culture supernatant from actively growing cells maintained without antibiotics is suitable for mycoplasma testing because organisms released into the medium can be detected without suppression by antimicrobial agents. A vial taken from the completed bank allows the cryopreserved material itself to be tested, although this requires use of a banked vial and additional handling (72). Pre-freeze material can be tested for sterility and mycoplasma, while vials from the completed bank can be used for post-freeze qualification together with viability and identity testing, as proposed in the minimum dataset for iPSC lines intended for clinical use (14). When only a limited number of vials are available, destructive testing has to be taken into account when the bank is established. Release records should state what material was tested and at which stage the sample was collected (11). Cryopreservation also affects the timing of microbiological testing. Unlike fresh cell therapy products with a short shelf life, an MCB or WCB can remain in quarantine under cryogenic storage until microbiological tests are completed (72). Rapid methods may be useful in facilities processing large numbers of banks, but rapid release is not usually dictated by the nature of the cryopreserved material itself. The choice of method can be based on the actual needs of the banking process. Quarantine needs to cover the period between bank establishment and completion of the required testing. Material with pending microbiological results should be kept separate from qualified cultures. SOPs should define the tests required before release, the conditions for entering and leaving quarantine, and any work that can be performed while results are pending (11). This separation limits the possibility of an undetected contaminant being transferred to other cultures. Material received from another institution presents an additional issue. Microbiological records and certificates supplied with the line need to be reviewed, but the receiving facility must decide which results from the originating institution it will accept and which tests it will repeat before introducing the cells into shared culture or banking areas. Experience with collections receiving lines from multiple depositors has shown the need for common acceptance criteria and defined rules for repeat testing (11,25). If the origin of the material or its testing history cannot be adequately documented, the line should remain in quarantine until its microbiological status has been established by the receiving facility.

Procedures for handling microbiological failures should be defined before a contamination event occurs (73). When a positive result is confirmed, the affected material is placed in quarantine and the source and extent of the contamination are investigated. This includes WCBs, retained samples, and other material derived from the same line. Cultures handled in the same area during the relevant period may also need to be examined if cross-contamination is suspected (11). If material from the affected bank has already been distributed, the investigation also extends to the recipients. The decision on whether an affected bank can be retained or has to be rejected depends on the microorganism identified, confidence in the analytical result, evidence for the source of contamination, and the intended use of the cells. These factors can be evaluated according to the quality risk management principles described

in ICH Q9 (74). A confirmed mycoplasma-positive bank intended for clinical translation would generally not be suitable for release (14). A sterility-test failure linked to a documented problem with sampling, handling, or analysis may require further investigation before the bank is rejected. Repeat testing can form part of such an investigation when scientifically justified, but should not be used simply to replace an initial failing result with a passing one. The quality system and distribution agreements should also define how recipients are notified when contamination is detected after material has been distributed (11). The procedure needs to state which findings require notification, what information is provided, and what action recipients are expected to take. For hiPSC banks, this may involve several institutions because a single line can be distributed and extensively expanded before contamination is detected.

Microbiological testing performed when a bank is established documents the status of the material at that time (61). An MCB, however, may remain in cryogenic storage for many years before individual vials are used or distributed. Whether microbiological testing needs to be repeated during this period depends on the conditions under which the bank has been stored and any changes that could affect the original test results. Microbial growth is unlikely in an intact vial kept continuously under validated cryogenic conditions (72), and storage time alone does not provide a reason for periodic microbiological testing. Repeat testing may be needed if container integrity is compromised, storage conditions change, or new information raises questions about the adequacy of the original testing. Container integrity and temperature excursions are particularly relevant for material stored under cryogenic conditions (72,75). For banks kept for long periods, the conditions that require microbiological requalification should be specified in the bank documentation. This may involve testing retained vials at defined intervals or before distribution after prolonged storage. Alternatively, the original microbiological results may remain sufficient when storage conditions and container integrity have been maintained and no additional risk has been identified. The frequency and scope of repeat testing should be defined and justified in the bank documentation. Microbiological requalification is not the same as stability testing. Stability studies examine whether defined quality attributes remain within specification during long-term cryogenic storage and may include post-thaw viability, recovery, identity, genetic integrity, pluripotency, and other relevant characteristics (14,76). Microbiological requalification determines whether additional testing is needed to confirm the microbiological status of the stored bank. The same vials and time points can be used for both purposes where appropriate, but the tests and their acceptance criteria need to be defined separately.

Microbiological safety of a hiPSC bank cannot be determined by a single release test. Results must be interpreted in relation to the material tested, the sampling point, and the analytical method, together with the conditions under which the bank was derived, stored, and released. For banks intended for clinical translation, this information should remain traceable throughout subsequent distribution and use.

2.5 Epigenetic Status

Unlike identity, viability, genomic stability, and microbiological safety, which are established release criteria, characterisation of epigenetic status is not currently required for release, and no universally accepted principles have been established for hiPSC lines deposited in banks. Epigenetic abnormalities can occur in hiPSC lines and vary both between lines and between clones derived from the same donor, with the somatic cell type of origin contributing to that variation. Methylation profiles, imprinting status

and X-chromosome inactivation can all be measured by established methods, but epigenetic testing is not required for the release of pluripotent cell lines, and is not included in the current consensus minimum characterisation for clinical use of hiPSCs (14).

The significance of epigenetic changes in hiPSCs depends on when and how they arise during reprogramming and culture. Somatic epigenetic memory is strongest at early passages and diminishes with continued culture (77). Erosion of X-chromosome inactivation (XCI) accumulates with passage number (78,79). Imprinting defects are set during reprogramming and differ between clones from the same donor, showing no dependence on passage number (80). Memory therefore depends on when the line was banked, XCI erosion on how long the cells have been cultured beforehand, and imprinting on which clone was chosen.

Reprogramming does not eliminate all somatic DNA methylation patterns. Early-passage hiPSC lines derived from hepatocytes, fibroblasts, and melanocytes, representing all three germ layers, can retain epigenetic memory of their somatic cell origin. This is evidenced by the persistence of somatic gene expression and incomplete resetting of promoter DNA methylation. Such retention has been observed in hiPSC lines generated by various laboratories using different methodologies (81). This epigenetic memory can also influence differentiation outcomes. For example, hiPSC lines derived from beta cells maintain open chromatin and altered DNA methylation marks at beta cell genes, resulting in a preference for insulin-producing cell fate compared to either ESCs or isogenic hiPSC lines not derived from beta cells (82). hiPSC lines banked at later passages may exhibit reduced residual somatic gene expression; however, prolonged culture could compromise other epigenetic features, making this approach less desirable.

Genomic imprinting can be disrupted during reprogramming, and loss of imprinting is associated with developmental disorders and with certain cancers. Comparison of human nuclear-transfer embryonic stem cell lines with isogenic induced pluripotent stem cell lines derived from the same somatic cultures identified aberrant DNA methylation or altered allelic expression at imprinted genes in both (80). The defects were clone- and gene-specific, affecting different loci in different clones, which indicates that they arise from the reprogramming process itself. The culture state also influences imprinting. Primed hiPSC lines are globally hypermethylated but can show loss of methylation at imprinted loci. Lines maintained in the naïve state are globally hypomethylated and can display loss of imprinting at numerous loci (83). Imprinting status has to be assessed on each clone under consideration, since a result obtained on one clone describes only that clone.

XCI is a defining epigenetic feature of female hiPSCs. In early culture, such hiPSC lines generally retain the XCI state of the original somatic cell. XCI can become compromised during extended culture, as evidenced by loss of XIST expression, loss of X-chromosome-associated H3K27me3 foci, and loss of transcriptional silencing of X-linked genes. Because most genes subject to X inactivation are derepressed, the entire line is affected, and the erosion persists through differentiation and through further reprogramming (78). Erosion progresses in stages. Allele-specific expression analysis of 165 female hiPSC lines found that biallelic expression increases gradually and begins after only a modest reduction in XIST, and that the affected genes are preferentially those which escape X inactivation in human tissues; the lines fell into three groups at different stages of erosion (79). Since complete loss

of XIST is a late event, a line in which XIST can still be detected may already have significant biallelic expression.

Various assays provide different types of information regarding epigenetic status. Genome-wide methylation arrays are commonly used to assess DNA methylation across the genome, with results interpreted relative to the chosen reference dataset. Currently, there is no standardised criterion for selecting hiPSCs for inclusion in a bank on that basis. Other assays address narrower questions. The Epi-Pluri-Score is a binary classifier that distinguishes pluripotent from non-pluripotent cells based on a DNA methylation signature at three CpG sites, and can be performed using pyrosequencing. It has been validated on 264 pluripotent and 1,951 non-pluripotent samples, demonstrating 99.9% specificity and 98.9% sensitivity (84). Another technique, EpiTyping, utilises RNA sequencing to detect biallelic expression of imprinted genes and to map X inactivation, full activation, and erosion of X inactivation in female cell lines (85). The analysis can also be conducted using existing RNA-sequencing data generated by biobanks for other applications.

Because only a limited number of epigenetic profiling studies have been performed on clinically relevant hiPSC lines, and most existing studies have focused on research-grade lines or mechanistic questions, it remains challenging to establish a meaningful range for banked lines. The passage number at which residual somatic expression becomes acceptable is also unclear. Until these parameters are defined, epigenetic data should be considered characterisation data, accompanied by the passage number corresponding to each measurement. Maintaining records of both raw data and summary interpretations enables recipients to evaluate the line against any criteria that may be established in the future.

3. Conclusions

The main limitation of current hiPSC bank QC is the gap between detecting an abnormality and determining whether it is acceptable for the intended use. Increasing analytical sensitivity can reveal additional low-frequency variants, but does not by itself establish their biological significance. Conversely, a normal result cannot exclude changes outside an assay's detection range, and a pluripotent phenotype does not establish differentiation competence. Release decisions therefore depend on explicit acceptance criteria and an understanding of what each assay can and cannot resolve. Bank qualification also has limits as evidence for the safety of a downstream product. Genomic findings must be interpreted in relation to the differentiated cell type, manufacturing process and clinical application, while microbiological results apply to the material and sampling stage examined. For HLA-homozygous haplobanks, a single line may avoid the need for immune suppression for many recipients, extending the consequences of an undetected failure. Traceable records, quarantine procedures and mechanisms for communicating findings after distribution are therefore integral to the testing strategy. Harmonisation should consequently address how results support release decisions, rather than stop at agreement on an assay list. Priorities are comparable analytical performance, defined reporting and acceptance thresholds, and records linking findings to passage number and banking stage. Epigenetic profiling illustrates why characterisation and release testing must remain distinct: measurable differences cannot yet be translated into widely applicable release criteria. Preserving these data allows future reinterpretation without assigning them a release function that the available evidence does not support.

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Appendix 1. Annotated Literature on Quality Control and Biobanking of hiPSCs

1. Scope and Objectives of hiPSC Biobanking for Clinical Translation

1. Ntai, Aikaterini, Simona Baronchelli, Alberto La Spada, et al. "A Review of Research-Grade Human Induced Pluripotent Stem Cells Qualification and Biobanking Processes." *Biopreservation and Biobanking* 15(4): 384–392 (2017).

DOI: <https://doi.org/10.1089/bio.2016.0097>

Qualification and biobanking of research-grade hiPSCs are reviewed in relation to pluripotency, genetic integrity, identity and microbiological safety. The authors examine the variability associated with different reprogramming and culture methods and stress the need for uniform biobanking and documentation procedures to support reproducibility and clinical translation.

2. Martins, Fernando, Maria H. L. Ribeiro. "Quality and Regulatory Requirements for the Manufacture of Master Cell Banks of Clinical Grade iPSCs: The EU and USA Perspectives." *Stem Cell Reviews and Reports* 21(3): 645–679 (2025).

DOI: <https://doi.org/10.1007/s12015-024-10838-9>

Martins and Ribeiro examine the quality and regulatory requirements for producing hiPSC master cell banks for clinical translation from European (EMA) and US (FDA) perspectives. They define minimum requirements for hiPSCs intended for first-in-human clinical trials and review approaches to characterising banks for identity, purity, adventitious agents and stability. They also argue that existing biotechnological guidelines need to be expanded to address cell banks used in cell therapy manufacturing more adequately.

3. Andrews, P. W., D. Baker, N. Benvenisty, et al. "Points to Consider in the Development of Seed Stocks of Pluripotent Stem Cells for Clinical Applications: International Stem Cell Banking Initiative (ISCB)." *Regenerative Medicine* 10(2 Suppl): 1–44 (2015).

DOI: <https://doi.org/10.2217/rme.14.93>

Although somewhat dated, the ISCB guidance provides an extensive account of the establishment and operation of hPSC biobanks for clinical and translational applications. Governance, donor consent, quality control, traceability and long-term storage are discussed, with a distinction between banking for research use and banking intended to support clinical translation.

4. Escribá, Rubén, Meral Beksac, Annelise Bennaceur-Griscelli, et al. "Current Landscape of iPSC Haplobanks." *Stem Cell Reviews and Reports* 20(8): 2155–2164 (2024).

DOI: <https://doi.org/10.1007/s12015-024-10783-7>

The global development of hiPSC haplobanks is examined in relation to the prospects for scalable, immunologically compatible cell therapies. The authors compare national and international initiatives, including differences in donor selection, HLA coverage strategies, quality requirements and legal frameworks.

5. Madrid, Marinna, Uma Lakshmipathy, Xiaokui Zhang, et al. "Considerations for the development of iPSC-derived cell therapies: a review of key challenges by the JSRM-ISCT iPSC Committee." *Cytotherapy* 26(11): 1382–1399 (2024).

DOI: <https://doi.org/10.1016/j.jcyt.2024.05.022>

Madrid and colleagues examine regulatory and quality considerations for hiPSC-derived cell therapy products, including differences in product definition, manufacturing methods and regulatory requirements across countries. Harmonised standards are presented as important for safe, scalable clinical translation.

6. Hirai, Takamasa, Satoshi Yasuda, Akihiro Umezawa, Yoji Sato. "Country-specific regulation and international standardization of cell-based therapeutic products derived from pluripotent stem cells." *Stem Cell Reports* 18(8): 1573–1591 (2023).
DOI: <https://doi.org/10.1016/j.stemcr.2023.05.003>
National regulations for therapies derived from pluripotent stem cells differ across regions. Hirai and colleagues review these disparities and the difficulties they create for international regulatory harmonisation.
7. Petricciani, John, Takao Hayakawa, Glyn Stacey, Jean-Hugues Trouvin, Ivana Knezevic. "Scientific considerations for the regulatory evaluation of cell therapy products." *Biologicals* 50: 20–26 (2017).
DOI: <https://doi.org/10.1016/j.biologicals.2017.08.011>
Petricciani and colleagues examine the scientific and regulatory evaluation of cell therapy products, identifying differences in regulatory views across countries and regions and the need for a global effort to establish common principles. Their core consensus package of requirements for evaluating human cell therapy products is relevant to defining the scope of biobanking guidelines for clinical translation.
8. Barry, Jacqueline, Elsa Abranches, Ricardo P. Baptista, et al. "Charting the Translational Pathway: ISSCR Best Practices for the Development of PSC-Derived Therapies." *Stem Cell Reports* 21(2) (2026).
DOI: <https://doi.org/10.1016/j.stemcr.2025.102784>
The ISSCR Best Practices for the Development of Pluripotent Stem Cell-Derived Therapies is a comprehensive, interactive resource for academia and industry. It covers critical stages of the translational pipeline and key decisions on the path to a licensed therapy. Its global account of the regulatory landscape includes resources for critical processes and links to jurisdiction-specific regulatory information.
9. European Medicines Agency, Committee for Advanced Therapies (CAT). "Reflection paper on stem cell-based medicinal products." EMA/CAT/571134/2009. European Medicines Agency, 2011.
URL: <https://www.ema.europa.eu/en/reflection-paper-stem-cell-based-medicinal-products-scientific-guideline>
The EMA reflection paper covers the development, manufacturing and quality control of stem-cell-based medicinal products. It complements the general EMA guideline on human cell-based medicinal products (EMA/CHMP/410869/2006).
10. U.S. Food and Drug Administration. "21 CFR Part 1271: Human cells, tissues, and cellular and tissue-based products (HCT/Ps)." Code of Federal Regulations, Title 21.
URL: <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-L/part-1271>
This US federal regulation governs human cells, tissues, and cellular and tissue-based products (HCT/Ps), including donor eligibility, current good tissue practices, registration and reporting requirements. It provides a reference for the US regulatory framework applicable to hPSC biobanking for clinical translation.

2. Biobanking Workflow for Human Pluripotent Stem Cells

11. Orzechowski, Marcin, Maximilian Schochow, Michael Kühl, Florian Steger. "Donor Information in Research and Drug Evaluation with Induced Pluripotent Stem Cells (iPSCs)." *Stem Cell Research & Therapy* 11(1): 126 (2020).
DOI: <https://doi.org/10.1186/s13287-020-01644-4>
Ethical and legal questions surrounding donor information in hiPSC research and pharmaceutical development include informed consent, the selection of clinical and demographic data to collect, and the retention of appropriate documentation. Orzechowski and colleagues also consider the data governance needed to support future translational use.

12. Abberton, Keren M., Tricia L. McDonald, Mary Diviney, et al. "Identification and Re-consent of Existing Cord Blood Donors for Creation of Induced Pluripotent Stem Cell Lines for Potential Clinical Applications." *Stem Cells Translational Medicine* 11(10): 1052–1060 (2022).

DOI: <https://doi.org/10.1093/stcltm/szac060>

Re-consenting existing cord blood donors for the creation of hiPSC lines intended for clinical translation raises ethical and practical issues. Abberton and colleagues discuss re-contacting donors, preparing consent forms and providing donor clinical information linked to new hiPSC lines for translational research.

13. Yoshida, Shinsuke, Tomoaki M. Kato, Yoshiko Sato, et al. "A clinical-grade HLA haplobank of human induced pluripotent stem cells matching approximately 40% of the Japanese population." *Med* 4(1): 51–66.e10 (2023).

DOI: <https://doi.org/10.1016/j.medj.2022.10.003>

Yoshida and colleagues report the production of an HLA-homozygous hiPSC haplobank for clinical translation. Donor selection is based on HLA genotyping, and the lines are generated under GMP-compliant conditions for allogeneic use.

14. Steeg, Rachel, Sabine C. Mueller, Nancy Mah, Bjørn Holst, Alfredo Cabrera-Socorro, Glyn N. Stacey, Paul A. De Sousa, Aidan Courtney, Heiko Zimmermann. "EBiSC Best Practice: How to Ensure Optimal Generation, Qualification, and Distribution of iPSC Lines." *Stem Cell Reports* 16(8): 1853–1867 (2021).

DOI: <https://doi.org/10.1016/j.stemcr.2021.07.009>

The workflows and governance of the European Bank for induced Pluripotent Stem Cells (EBiSC) encompass donor consent, reprogramming, cell line generation, documentation and shipping across contributing centres. Steeg and colleagues discuss the practical challenges of harmonising these activities and operating at scale.

15. Alvarez-Palomo, Belén, Joaquim Vives, Ricardo P. Casaroli-Marano, et al. "Adapting Cord Blood Collection and Banking Standard Operating Procedures for HLA-Homozygous Induced Pluripotent Stem Cells Production and Banking for Clinical Application." *Journal of Clinical Medicine* 8(4): 476 (2019).

DOI: <https://doi.org/10.3390/jcm8040476>

Alvarez-Palomo and colleagues adapt cord blood processing and banking SOPs to the generation and banking of HLA-homozygous hiPSCs for clinical applications. Practical guidance covers the choice of a suitable cord blood unit, reprogramming to hiPSCs under defined conditions, documentation and quality control.

16. Li, Yan, Teng Ma. "Bioprocessing of Cryopreservation for Large-Scale Banking of Human Pluripotent Stem Cells." *BioResearch Open Access* 1(5): 205–214 (2012).

DOI: <https://doi.org/10.1089/biores.2012.0224>

Cryopreservation procedures for stem cells and other biological agents include freezing and thawing protocols designed to maximise viability and function. Li and Ma discuss these procedures alongside criteria for post-thaw cell recovery and the prediction of long-term storage performance.

17. Shibamiya, Aya, Elisabeth Schulze, Dana Krauß, et al. "Cell Banking of hiPSCs: A Practical Guide to Cryopreservation and Quality Control in Basic Research." *Current Protocols in Stem Cell Biology* 55(1): e127 (2020).

DOI: <https://doi.org/10.1002/cpsc.127>

Shibamiya and colleagues provide practical protocols for freezing, thawing and handling hiPSC banks in basic research laboratories. The guide covers cryopreservation and quality control, including viability assessments and basic QC checkpoints at different stages of cell banking.

18. Francis, Natalie, Joy Aho, Inbar Friedrich Ben-Nun, et al. "Scaling up Pluripotent Stem Cell-Based Therapies - Considerations, Current Challenges and Emerging Technologies: Perspectives from the ISCT Emerging Regenerative Medicine Working Group." *Cytotherapy* 27(9): 1031–1042 (2025).

DOI: <https://doi.org/10.1016/j.jcyt.2025.04.058>

The ISCT Emerging Regenerative Medicine Working Group examines the challenges of scaling iPSC-derived therapies for manufacturing. Topics include manufacturing processes appropriate to each development stage, when to introduce automation, the choice of expansion and differentiation technologies, and the integration of manufacturing and analytical automation throughout the process. Quality-by-design approaches and design-of-experiment methods are discussed as ways to improve product characterisation. The paper also provides an in-depth review of expansion and differentiation technologies, their compatibility with GMP requirements and process analytical technologies.

3. Quality Management, Data Management and Traceability

Quality management framework and SOPs

19. O'Shea, Orla, Rachel Steeg, Charlotte Chapman, Peter Mackintosh, Glyn N. Stacey. "Development and implementation of large-scale quality control for the European Bank for induced Pluripotent Stem Cells." *Stem Cell Research* 45: 101773 (2020).

DOI: <https://doi.org/10.1016/j.scr.2020.101773>

O'Shea and colleagues describe the QC system established for EBISC's large-scale hiPSC bank and the quality management system (QMS) adapted to support it. Full traceability, stringent release testing and continuous process improvement ensure the safety and quality of the distributed hiPSC lines.

20. Magri, Mara. "Writing a Quality Management Plan." In: *Quality Management and Accreditation in Hematopoietic Stem Cell Transplantation and Cellular Therapy: The JACIE Guide*. Aljurf, M., Snowden, J. A., Hayden, P., Orchard, K. H., McGrath, E. (eds.). Springer, 2021.

URL: <https://www.ncbi.nlm.nih.gov/books/NBK584266/>

Book chapter

Magri sets out the core elements of a QMS for cell and tissue establishments: document control, SOPs, deviation management, CAPA, change control and personnel training. The chapter provides a structured framework applicable to quality systems in hiPSC biobanking.

21. European Directorate for the Quality of Medicines & Healthcare (EDQM). "Guide to the Quality and Safety of Tissues and Cells for Human Application." 4th edition. Council of Europe, 2019.

URL: <https://www.aebt.org/wp-content/uploads/2020/03/Guide-to-the-quality-and-safety-of-tissues-and-cells-CoE-4th-ed.pdf>

Regulatory guidance document, Council of Europe.

The EDQM guide defines mandatory QMS elements for cell and tissue establishments, including controlled documentation, validated SOPs, deviation handling, CAPA implementation and staff qualification.

22. Aljurf, Mahmoud, John A. Snowden, Patrick Hayden, Kim H. Orchard, Eoin McGrath (eds.). "Quality Management and Accreditation in Hematopoietic Stem Cell Transplantation and Cellular Therapy: The JACIE Guide." Springer, 2021.

DOI: <https://doi.org/10.1007/978-3-030-64492-5>

Book

Practical requirements for implementing quality management systems in cell and gene therapy are set out in this book. The chapters detail structures for document control, SOP management, deviation handling, CAPA, change control and personnel training in regulated manufacturing and banking settings.

- 23.** Hägg, Alice, Rachel Wood, Ayako L. Mochizuki, et al. “Toward Standardized iPSC Testing: Insights from a Multi-Year International Quality Assessment Round.” *Stem Cell Reports* 21(4) (2026).

DOI: <https://doi.org/10.1016/j.stemcr.2026.102857>

Two international Quality Assessment Rounds (QARs), involving 18 sites in 11 countries in 2019 and 23 sites in 12 countries in 2023, evaluated flow cytometry assessment of the undifferentiated state and qPCR testing of genomic integrity in iPSC lines. Genomic integrity testing showed high inter-laboratory consistency. Flow cytometry initially showed substantial variability, which decreased after standardised workflows were introduced in the 2023 round. OCT3/4, TRA-1-60 and SSEA5 were identified as consistently reproducible markers across sites. The study also describes a model for QC benchmarking across sites that is relevant to interoperable iPSC banking.

- 24.** Ludwig, Tenneille E., Peter W. Andrews, Ivana Barbaric, et al. “ISSCR standards for the use of human stem cells in basic research.” *Stem Cell Reports* 18(9): 1744–1752 (2023).

DOI: <https://doi.org/10.1016/j.stemcr.2023.08.003>

The ISSCR standards for laboratory research with human pluripotent and tissue stem cells define reporting criteria and best practices intended to improve reproducibility and rigour. They complement the ISSCR Best Practices for the Development of PSC-Derived Therapies (Barry et al., 2026), which addresses clinical translation rather than basic research.

- 25.** International Organization for Standardization. “ISO 24603:2022 Biotechnology — Biobanking — Requirements for human and mouse pluripotent stem cells.” Geneva: ISO, 2022.

URL: <https://www.iso.org/standard/78708.html>

ISO 24603:2022 defines requirements for biobanking human and mouse pluripotent stem cells, covering quality management, donor consent, cell line characterisation, documentation and long-term storage. It is the first international standard specific to PSC biobanking.

Data management, sample identification and traceability

- 26.** Knoppers, Bartha M., Rosario Isasi. “Stem cell banking: between traceability and identifiability.” *Genome Medicine* 2(10): 73 (2010).

DOI: <https://doi.org/10.1186/gm194>

Knoppers and Isasi examine the importance of traceability and identifiability in stem cell banking. They describe how unique coding systems and linked donor and sample data track biological materials throughout the biobanking process, and discuss the challenges of maintaining accurate records that support scientific use and meet ethical and regulatory requirements.

- 27.** Alkhatib, Ramez, Karoline I. Gaede. “Data Management in Biobanking: Strategies, Challenges, and Future Directions.” *BioTech* 13(3): 34 (2024).

DOI: <https://doi.org/10.3390/biotech13030034>

Data governance and metadata standardisation link biobank samples to their associated data. Alkhatib and Gaede explain how these practices support traceability and audit readiness throughout the biobank lifecycle.

- 28.** Mah, Nancy, Andreas Kurtz, Antonie Fuhr, Stefanie Seltsmann, Ying Chen, Nils Bultjer, Johannes Dewender, Ayuen Lual, Rachel Steeg, Sabine C. Mueller. “The Management of Data for the Banking, Qualification, and Distribution of Induced Pluripotent Stem Cells: Lessons Learned from the European Bank for Induced Pluripotent Stem Cells.” *Cells* 12(23): 2756 (2023).

DOI: <https://doi.org/10.3390/cells12232756>

The data infrastructure used by EBISC and hPSCreg supports the registration, banking, qualification and distribution of hiPSC lines. Mah and colleagues describe how structured databases link donor consent data, cell line provenance, manufacturing records, QC results and distribution information to ensure traceability and regulatory transparency.

- 29.** Shekhovtsov, Volodymyr A., Johann Eder. “Metadata Quality for Biobanks.” *Applied Sciences* 12(19): 9578 (2022).

DOI: <https://doi.org/10.3390/app12199578>

Shekhovtsov and Eder define the core attributes of data and metadata quality in biobanks and their role in maintaining data integrity. They explain how metadata quality supports traceability and the reliability of audits in biobanking systems.

4. Quality Control Testing of hiPSC Banks

- 30.** Sullivan, Stephen, Glyn N. Stacey, Chihiro Akazawa, et al. “Quality Control Guidelines for Clinical-Grade Human Induced Pluripotent Stem Cell Lines.” *Regenerative Medicine* 13(7): 859–866 (2018).

DOI: <https://doi.org/10.2217/rme-2018-0095>

Consensus QC standards for hiPSC lines intended for clinical translation focus on quality attributes relevant to safety and comparability. Sullivan and colleagues outline harmonised assays for identity, pluripotency, genomic integrity and microbiological safety to enable consistent evaluation across sites.

- 31.** Baghbaderani, Behnam Ahmadian, Xinghui Tian, Boon Hwa Neo, et al. “cGMP-Manufactured Human Induced Pluripotent Stem Cells Are Available for Pre-clinical and Clinical Applications.” *Stem Cell Reports* 5(4): 647–659 (2015).

DOI: <https://doi.org/10.1016/j.stemcr.2015.08.015>

Baghbaderani and colleagues report a cGMP-compliant process for producing hiPSC master cell banks for pre-clinical and clinical applications. Manufacturing extends from donor tissue through reprogramming with non-integrating techniques to cell banking and release testing for identity, pluripotency, genomic stability and microbiological safety.

- 32.** Dobner, Jochen, Sebastian Diecke, Jean Krutmann, Alessandro Prigione, Andrea Rossi. “Reassessment of Marker Genes in Human Induced Pluripotent Stem Cells for Enhanced Quality Control.” *Nature Communications* 15(1): 8547 (2024).

DOI: <https://doi.org/10.1038/s41467-024-52922-1>

Commonly used pluripotency-associated marker genes are assessed in both hESCs and hiPSCs, distinguishing those with uniform expression from those that vary. Dobner and colleagues suggest revised gene marker sets to improve the distinction between cell states and the evaluation of pluripotency in vitro.

- 33.** Steichen, Clara, Zara Hannoun, Eléonor Luce, Thierry Hauet, Anne Dubart-Kupperschmitt. “Genomic integrity of human induced pluripotent stem cells: Reprogramming, differentiation and applications.” *World Journal of Stem Cells* 11(10): 729–747 (2019).

DOI: <https://doi.org/10.4252/wjsc.v11.i10.729>

Strategies for assessing genomic stability in hiPSCs include evaluating chromosomal integrity and detecting large-scale genetic abnormalities. Steichen and colleagues review the mechanisms that lead to genomic alterations, methods for monitoring these changes and the implications of instability for research and clinical applications of hiPSCs.

- 34.** Park, Ji Won, Su Ji Bae, Jun Ho Yun, Sunhee Kim, Misun Park. “Assessment of Genetic Stability in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes by Using Droplet Digital PCR.” *International Journal of Molecular Sciences* 25(2): 1101 (2024).

DOI: <https://doi.org/10.3390/ijms25021101>

Park and colleagues assess genomic stability in hiPSCs and their cardiac derivatives using droplet digital PCR to quantify targeted mutations. They compare methods for detecting such changes, including karyotyping, array-based methods and sequencing, and discuss the relevance of genomic integrity to safe clinical applications.

- 35.** Ntai, Aikaterini, Simona Baronchelli, Alberto La Spada, et al. “A Review of Research-Grade Human Induced Pluripotent Stem Cells Qualification and Biobanking Processes.” *Biopreservation and Biobanking* 15(4): 384–392 (2017).

DOI: <https://doi.org/10.1089/bio.2016.0097>

Cross-listed from Section 1, this review addresses key steps in establishing hiPSC collections: handling donor material, documenting reprogramming, performing QC testing and maintaining long-term storage.

- 36.** European Medicines Agency (EMA). “Human Cell-Based Medicinal Products — Scientific Guideline.” 21 May 2008.

URL: <https://www.ema.europa.eu/en/human-cell-based-medicinal-products-scientific-guideline>

Regulatory guidance document, EMA.

The EMA guideline covers the development, manufacturing and quality control of cell-based medicinal products, together with their non-clinical and clinical development. Its scope includes somatic cell therapy medicinal products and tissue-engineered products.

- 37.** International Stem Cell Initiative. “Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage.” *Nature Biotechnology* 29(12): 1132–1144 (2011).

DOI: <https://doi.org/10.1038/nbt.2051>

The ISCI screened 125 hESC and 11 iPSC lines from 38 laboratories worldwide for genetic changes during culture. The study found a progressive tendency to acquire changes during prolonged culture, commonly affecting chromosomes 1, 12, 17 and 20. A minimal 20q11.21 amplicon driven by BCL2L1 was identified in over 20% of lines. These findings provide a basis for QC strategies addressing recurrent karyotypic changes in PSC banks.

- 38.** Benvenisty, Nissim, Jonathan S. Draper, Paul J. Gokhale, et al. “A Call to Action for Deciphering Genetic Variants in Human Pluripotent Stem Cells for Cell Therapy.” n.d. Accessed 12 August 2026.
URL: [https://www.cell.com/cell-stem-cell/abstract/S1934-5909\(25\)00093-1](https://www.cell.com/cell-stem-cell/abstract/S1934-5909(25)00093-1)
An international workshop on culture-acquired genetic variants in hPSCs prompted this call to action on the risks such variants pose for cell therapy. The paper summarises concerns about variants linked to cancer and calls for better strategies to stratify variants and for definitive guidelines for hPSC-based therapy.
- 39.** Krupalnik, Vladislav, Shin Kawamata, Joanne Mountford, et al. “Consensus Perspectives on Quality Control and Safety Strategies for the Clinical Translation of iPSC-Derived Cell Therapies.” *Cytotherapy* (2026).
DOI: <https://doi.org/10.1016/j.jcyt.2026.102969>
International consensus perspectives on QC and safety strategies for iPSC-derived cell therapies call for safety testing to be established case by case, according to the therapeutic indication. Krupalnik and colleagues review safety considerations in neural, cardiac and immune-cell applications, including gene-modified allogeneic platforms such as CAR-T and CAR-NK. They support harmonisation of QC frameworks while recognising indication-specific differences in release testing panels.
- 40.** Vales, John P., Ivana Barbaric. “Culture-acquired genetic variation in human pluripotent stem cells: Twenty years on.” *BioEssays* 46(12): e2400062 (2024).
DOI: <https://doi.org/10.1002/bies.202400062>
Vales and Barbaric review 20 years of research on culture-acquired genetic aberrations in hPSCs. They describe the characteristic spectrum of recurrent aberrations identified over this period, the evidence that these changes confer a selective growth advantage, and the implications of culture-acquired variants for basic research and clinical applications.
- 41.** Rossi, Andrea, Selene Lickfett, Soraia Martins, Alessandro Prigione. “A call for consensus guidelines on monitoring the integrity of nuclear and mitochondrial genomes in human pluripotent stem cells.” *Stem Cell Reports* 17(4): 707–710 (2022).
DOI: <https://doi.org/10.1016/j.stemcr.2022.01.019>
Rossi and colleagues call for consensus guidelines on monitoring the integrity of both nuclear and mitochondrial genomes in hPSCs. Mitochondrial genome monitoring is typically overlooked; the authors propose a set of frequently asked questions as the basis for a practical guide for hPSC users.
- 42.** Kong, Sek Won, In-Hee Lee, Young Cha, Bob Carter, Kwang-Soo Kim. “Safeguarding genomic integrity in pluripotent stem-cell therapies.” *Lancet* 407(10538): 1492–1494 (2026).
DOI: [https://doi.org/10.1016/S0140-6736\(26\)00095-4](https://doi.org/10.1016/S0140-6736(26)00095-4)
Kong and colleagues discuss the need to safeguard genomic integrity as more PSC-derived cell therapy products progress towards clinical use. This Lancet commentary outlines the responsibilities of developers, regulators and clinicians.

5. Risk Identification and Mitigation

- 43.** Kim, Jung-Hyun, Andreas Kurtz, Bao-Zhu Yuan, Fanyi Zeng, Geoff Lomax, Jeanne F. Loring, Jeremy Crook, Ji Hyeon Ju, Laura Clarke, Maneesha S. Inamdar, Martin Pera, Meri T. Firpo, Michael Sheldon, Nafees Rahman, Orla O'Shea, Patricia Pranke, Qi Zhou, Rosario Isasi, Ruttachuk Rungsiwiwut, Shin Kawamata, Steve Oh, Tenneille Ludwig, Tohru Masui, Thomas J. Novak, Tsuneo Takahashi, Wataru Fujibuchi, Soo Kyung Koo, Glyn N. Stacey. "Report of the International Stem Cell Banking Initiative Workshop Activity: Current Hurdles and Progress in Seed-Stock Banking of Human Pluripotent Stem Cells." *Stem Cells Translational Medicine* 6(11): 1956–1962 (2017).

DOI: <https://doi.org/10.1002/sctm.17-0144>

The ISCB workshop report identifies key risks in PSC seed-stock banking and examines ways to mitigate them. Standardised banking workflows, defined QC checkpoints and coordinated international practices are proposed to reduce biological and operational risks.

- 44.** Kim, Jung-Hyun, Andreas Kurtz, Ivana Barbaric, Maneesha S. Inamdar, Martin F. Pera, Nissim Benvenisty, Nika Shakiba, Rosario Isasi, Tadaaki Hanatani, Glyn Stacey. "Perspectives from the 2025 ISCB/ISCI joint workshop on genetic stability, clonal monitoring, ethical data governance, and global inclusion in stem cell banking." *Stem Cell Research & Therapy* 17(1): 2 (2026).

DOI: <https://doi.org/10.1186/s13287-025-04797-2>

The report from the 2025 ISCB/ISCI joint workshop examines culture-derived genetic variants in hPSCs, with particular attention to recurrent cancer-associated mutations and clonal selection in culture. It includes recommendations for routine high-coverage whole-genome sequencing and stress-testing of cell banks, and presents DNA barcoding as a feasible way to monitor clonal dynamics during manufacturing. The report advocates replacing race and ethnicity labels with genetically and ethically sound donor metadata. It also calls for FAIR, interoperable registries and persistent identifiers to support global harmonisation and the safe clinical translation of hPSC-based products.

- 45.** Dashnau, Jennifer L., Qiong Xue, Monica Nelson, Eric Law, Lan Cao, Derek Hei. "A Risk-Based Approach for Cell Line Development, Manufacturing and Characterization of Genetically Engineered, Induced Pluripotent Stem Cell-Derived Allogeneic Cell Therapies." *Cytotherapy* 25(1): 1–13 (2023).

DOI: <https://doi.org/10.1016/j.jcyt.2022.08.001>

Dashnau and colleagues propose a risk-based framework for developing and manufacturing allogeneic iPSC-derived cell therapies. They link key biological and process-related risks to targeted quality and process controls intended to mitigate those risks during cell line development and manufacturing.

- 46.** Madrid, Marinna, Uma Lakshmipathy, Xiaokui Zhang, et al. "Considerations for the development of iPSC-derived cell therapies: a review of key challenges by the JSRM-ISCT iPSC Committee." *Cytotherapy* 26(11): 1382–1399 (2024).

DOI: <https://doi.org/10.1016/j.jcyt.2024.05.022>

Cross-listed from Section 1, this white paper examines risks associated with hiPSC-derived cell products, including variability in starting materials, genomic instability and gaps in process control. Mitigation approaches include risk-based manufacturing, the definition of critical quality attributes and testing strategies.

47. European Medicines Agency / European Commission. “Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.” (EU GMP for ATMPs). 24 November 2017.

URL: https://health.ec.europa.eu/document/download/e1786e5d-1587-42af-b8ff-6a810ccb6836_en?filename=pharm731_2ib_atmps_guidelines.pdf

Regulatory guidance document, EMA / EC.

Chapter 2 of the EU Guidelines on GMP for ATMPs (2017) is dedicated to the risk-based approach, with examples of its application to raw materials and testing strategies. Other chapters cover GMP requirements for personnel and training, documentation, cell bank systems, production, validation and batch release.

Appendix 2. Reprogramming for clinical hiPSC banking: considerations and practical example

Human iPSC lines intended for clinical translation can be established using several reprogramming technologies that differ in efficiency, integration risk, residual transgene material, and technical complexity. The method selected impacts the documentation, release testing, and long-term monitoring needed for a clinical-grade cell bank. Integrating methods include transposons and retroviral or lentiviral vectors. Non-integrating methods involve episomal DNA, synthetic mRNA, and RNA viral vectors, of which Sendai virus (SeV) is the most widely used (86). SeV is a single-stranded RNA virus that replicates exclusively in the cytoplasm and does not produce a DNA intermediate. Temperature-sensitive SeV vectors can be eliminated from reprogrammed cells by shifting the culture temperature (87). Although mRNA- and episome-based methods are designed to avoid chromosomal integration, their reprogramming efficiencies, rates of aneuploidy, and technical demands vary considerably (88). The assessment of a reprogramming method for biobanking should address four questions: (i) how the delivery method, such as transfection, transduction, or electroporation, affects the recipient cell; (ii) whether reprogramming material can integrate into the genome; (iii) how long integrated or residual material persists and whether it affects genomic integrity during expansion; and (iv) whether partial reprogramming or an intermediate cell state could affect downstream manufacturing.

The reprogramming batch record should include the lot numbers and certificates of analysis for vectors, cytokines, media components, matrices, and other raw materials with their relevant storage and handling conditions. These records should allow the reprogramming experiment to be reproduced. Because vector titres can vary between lots, the vector dose should be recorded in a standardised form rather than solely as an input volume. Appropriate in-process controls and predefined acceptance criteria should be established for both the reprogramming platform and the source cells. Clearance of the reprogramming factors and vector should be demonstrated using orthogonal methods before the line is banked and confirmed during preparation of the master cell bank (13,76).

The reliability of the bank also depends on the quality of reprogramming. Cells that pass an early morphological assessment may still be incompletely reprogrammed (78,81,82). Similarly, expression of early pluripotency markers may confirm an undifferentiated phenotype without demonstrating that the cells have acquired a fully reprogrammed pluripotent state (89). Incomplete reprogramming may persist during subsequent culture and affect the differentiation properties of the resulting line. Its

detection may therefore require evaluation over extended culture. These characteristics should be assessed before MCB release, so that lines with evidence of incomplete or unstable reprogramming are not introduced into downstream product development.

The following worked example describes reprogramming of T cells with the CytoTune 2.0 Sendai virus kit (Thermo Fisher Scientific). It illustrates how the principles above translate into batch-record entries, in-process controls and predefined acceptance criteria for a clinically oriented line; it is not offered as a recommended protocol. Cells are thawed and activated for seven days with anti-CD3/CD28 beads (2:1 bead:cell ratio) in IL-2-supplemented medium, transduced on day 0, plated onto vitronectin- and iMatrix-511-coated dishes on day 2, and transferred to feeder-free medium (Essential 8 or mTeSR Plus) from day 3. All steps involving untested human material or vector are performed in a quarantine ML2 laboratory (microbiological containment level 2) before release.

Vector dose. Vector titre varies between manufacturing lots, so the dose is not recorded as a fixed volume. The volume of each vector is calculated from the target multiplicity of infection (MOI), the live cell count on the day of transduction and the titre stated on the lot-specific certificate of analysis (volume = $\text{MOI} \times \text{number of cells} \div \text{titre}$), and a certificate is filed with the batch record. The KOS, c-Myc and Klf4 vectors are used at MOIs of 5:5:3 as a starting point, in duplicate, and this can be optimised.

In-process controls. Two controls are run in parallel with each reprogramming: a fluorescent-reporter Sendai vector (CytoTune EmGFP) to show that the cells are transducible under the conditions used, and a medium-only culture to monitor morphology over time. Both have predefined acceptance criteria at days 4-6: approximately 30% GFP-positive cells in the reporter control, and a change in the transduced cells from small and round to larger and elongated. They are feasibility checks rather than release tests, but they allow a failure of transduction to be identified early, before weeks of culture have been committed.

Clone selection. At least ten colonies are picked at two time points (days 16 and 18) and each clone is expanded separately. Clones advance only if they meet the early-passage criteria (more than 80% NANOG-, OCT4- and SOX2-positive cells, no genomic abnormality, mycoplasma-negative).

Vector clearance. Clearance is assessed on a duplicate plate taken at passage by two orthogonal methods: immunocytochemistry with an anti-SeV monoclonal antibody (clone 1A6), which detects residual vector protein at the level of individual cells, and RT-PCR for SeV sequences with snRNP U1A as the reference gene, so that a negative result can be distinguished from a failed RNA extraction or reverse transcription. Footprint-free status is confirmed at later passages, before the definitive bank is laid down, and is an acceptance criterion for banking rather than a post-banking observation.

HAPLO-iPS

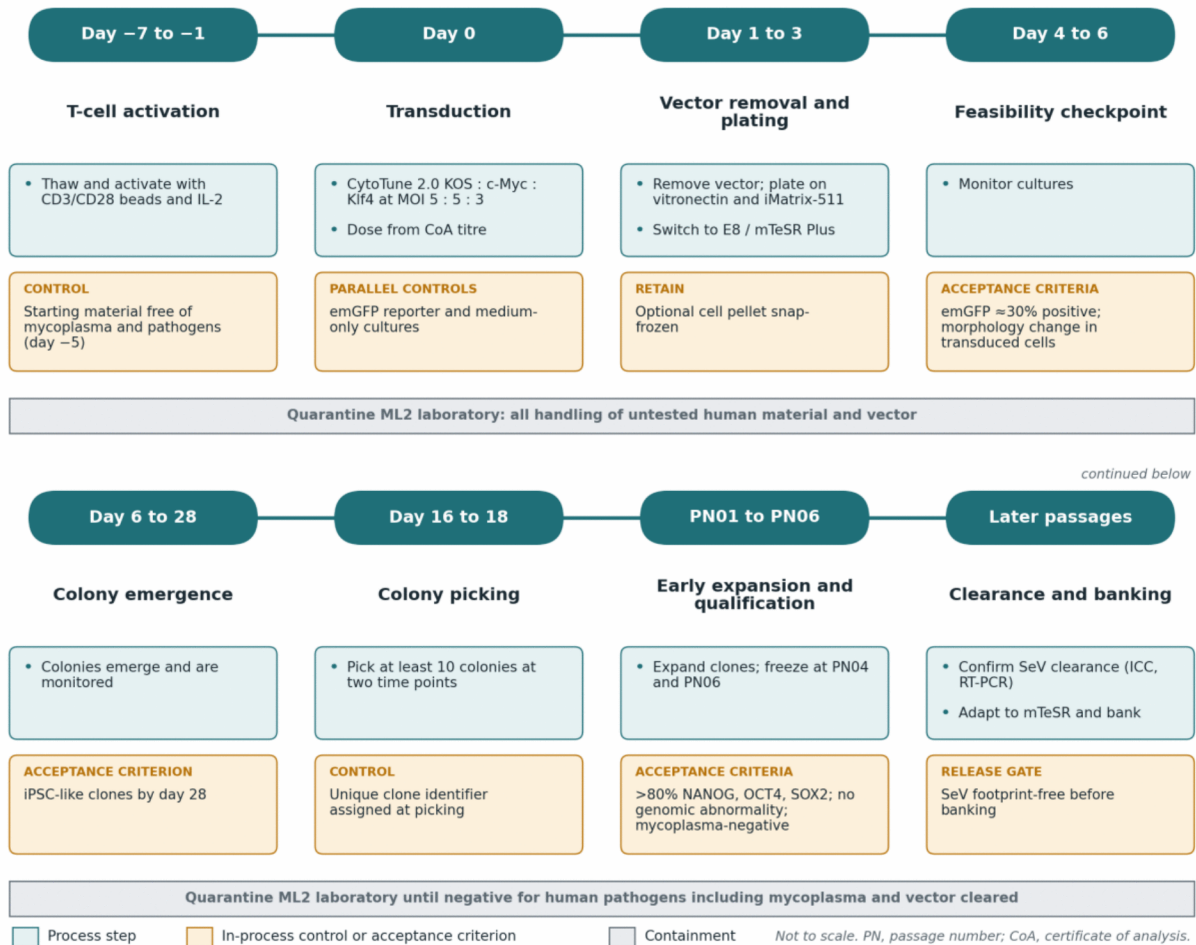


Figure 2. Sendai virus reprogramming of T cells: workflow and control points. Illustrative workflow for generating hiPSC lines from T cells using the CytoTune 2.0 Sendai virus system. The timeline covers T-cell activation, transduction, vector removal and plating, early feasibility checks, colony selection, clonal expansion, and confirmation of vector clearance before banking.