

PRIM-TECH3R

Standards for mouse stem cell use in CIVM development

Deliverable 1.1

Dissemination level: Public

WP1

**Standardised isolation of relevant mouse cells and pilot
mouse biobanking**



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Introduction

In recent years, technological advances in microfluidics, 3D bioprinting and multicellular co-culture systems have enabled the emergence of Complex In Vitro Models (CIVMs), providing complementary alternatives to animal models for studying tissue / organ-level functions. However, their widespread adoption is constrained by high inter-laboratory variability, limited protocol harmonisation and heterogeneous cell sourcing, which compromise robustness, reproducibility and data comparability. A major source of variability lies in the insufficient standardisation of the origin, derivation, characterisation and handling of precursor mouse cells.

Within “Preclinical in vitro Models for the Replacement, Reduction and Refinement of Mouse Models in Preclinical Studies (PRIM-TECH3R)”, WP1 addresses this limitation by establishing rigorous and harmonised standards for the isolation, characterisation, differentiation and biobanking of mouse-derived cells used in CIVMs. In accordance with the Reduction principle of the 3Rs, isolated mouse cells are systematically banked to maximise reuse and minimise additional animal experimentation, making traceability, quality control and controlled downstream use essential to the project’s scientific and ethical goals.

While the International Society for Stem Cell Research (ISSCR) has defined quality standards for human pluripotent stem cells, no equivalent framework exists for mouse cell types despite their central role in preclinical research. Deliverable D1.1 (“Standards for mouse stem cell use in CIVM development”) aims to fill this gap. Given that the PRIM-TECH3R biobank includes embryonic, primary and immortalised murine cell types, D1.1 was expanded to provide comprehensive recommendations for cell characterisation, reporting and use, thereby enhancing consistency, interoperability and reproducibility across laboratories and CIVM applications.

Methodology

The methodological approach was designed to ensure transparency, reproducibility, and comprehensive coverage of recommendations for cell culture and cell line use. The activities were structured in sequential steps, as detailed below.

- 1) **Preliminary Review and Compilation of Existing Guidelines.** ICS initiated the work with a comprehensive review of existing cell culture and cell line recommendations, which included ISSCR guidance for human pluripotent stem cells. To ensure broader applicability and identify gaps, additional guidance was consulted like Good Cell Culture Practice (GCCP 2.0); Organization for Economic Cooperation and Development (OECD) Test Reporting Guidelines, including Guidance Document on Good In Vitro Method Practices (GIVIMP); Guidance for Good In Vitro Reporting Standards (GIVReSt); and the European HYBRIDA project, notably the “Minimal Information About an *Organoid* and its Use” (MIAOU) questionnaire for Pluripotent Stem cells (PSC)-derived organoids. Building on this review and based on critical steps in established isolation



protocols, we developed specific recommendations for mouse embryonic stem cells (mESC). Relevant items were either adopted directly or adapted to meet mESC-specific requirements.

- 2) **Organization and Structuring of Recommendations.** To enhance clarity and usability, all recommendations were organized in an Excel spreadsheet. This table format enables (1) the collection of precise answers through defined questions, (2) rapid verification that all mandatory items are completed, and (3) improved searchability of appropriate cell lines for future users of the PRIM-TECH3R database.
- 3) **Iterative Review.** A first draft of the general recommendations was circulated in October 2025 within a small working group comprising UKRI, UOULU, CIPHE, HMGU, and ICS. Contributors were asked to review, amend, and correct the document. In parallel, a focused working group addressing mESC validation—composed of HMGU, NKI, UOULU, CNR, and ICS—reviewed the validations performed at each centre and finalized a list of common recommendations for mESC validation.
- 4) **Finalization and Consensus.** During the first two weeks of January 2026, the final Excel document was shared with all contributing centres. Each centre evaluated the recommendations, ensuring consensus and applicability across institutions.

Results

Our recommendations are designed to be relevant for all CIVMs in PRIM-TECH3R and, potentially, for all types of mouse cells. Their purpose is not to impose unnecessary validations, but to provide clear guidance for collecting and reporting critical information, thereby supporting reproducibility and experimental success.

The recommendations are compiled in an Excel file with four sheets, each containing multiple lines describing specific aspects of a mouse cell line:

1. **Sheet 1 – Vocabulary:** Defines key terms to ensure consistent understanding among all users.
2. **Sheet 2 – Cell Line Information:** Key information to be completed for each new submission to the PRIM-TECH3R biobank. It is relevant for any mouse cell line, although specific recommendations and required levels (defined in Sheet 1) vary depending on the cell type (embryonic stem cells, primary cultures, or immortalized cells), and some entries may remain blank. This sheet covers:
 - Overview of the cell line for rapid identification
 - Cell line characterization
 - Donor information
 - Isolation/derivation protocols
 - Culture conditions
 - Key parameters to monitor
 - Master and/or daughter cell bank details



D1.1 Standards for mouse stem cell use in CIVM development

Columns are structured as follows: Columns A & B describe the point requiring an answer, Column C clarifies the request with examples or guidance, and the remaining columns indicate the cell type, as reporting recommendations may differ. (A column for recording the response may be added separately.)

3. **Sheet 3 – Genetic Engineering:** Records any genetic modifications performed to introduce or correct mutations or to immortalize a cell line. If no such experiments have been performed, the information specified in this sheet does not have to be filled.
4. **Sheet 4 – Protocols and Usage:** Collects key procedural steps for proper use of the cell line under different scenarios:
 - *In vitro* differentiation to another cell type
 - *In vitro* co-culture
 - *In vivo* injection

This structured format ensures that all critical information is systematically captured, facilitating reproducibility, transparency, and efficient use of the PRIM-TECH3R biobank.

Discussion and next steps

While these recommendations will initially be applied internally within the PRIM-TECH3R consortium, our goal is for any researcher isolating or deriving a new mouse line to be able to use them. The guidelines will thus be published on the PRIM-TECH3R website. Subsequently, the White Paper describing *in vitro* resources of INFRAFRONTIER, BBMRI, and EUBI (deliverable D4.4) will publicize these D1.1 guidelines.

Link to other deliverables and milestones

Deliverable number	Deliverable title	Remarks
D1.2	Collection of standardized protocols for the isolation and differentiation of stem cells and primary cells for CIVMs	The standardised protocols will be written according to D1.1 guidelines, in particular ensuring that all mandatory items are enough described.
D1.3	Biobanking best practice guidelines for mouse cell biobanks	This deliverable will use the recommendations made in D1.1 to collect relevant information on submitted cell lines
D4.4	White Paper on a shared <i>in-vitro</i> resource of INFRAFRONTIER, BBMRI and EUBI	This white paper will make publicity/cite D1.1 guidelines so



		that they could be followed by any scientist.
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