Making Cell Line Authentication Part of Everyday Cell Culture Practice

Before starting a research project that uses cell lines:

1) **Select each cell line based on reliable sources of information**
Choose cell lines that are best suited to the research project based on published information about their genotype, phenotype, and origins (including tissue or cell type, disease, donor sex and age, and donor ancestral background). Cellosaurus is a good source for such information [1]: https://www.cellosaurus.org/

2) **Check to see if the cell line is known to be misidentified**
A misidentified cell line no longer corresponds to its original donor due to cross-contamination, mislabeling, or other causes. Cross-contamination initially results in a mixed culture, but a faster growing contaminant will rapidly overgrow and replace the original, authentic cells. ICLAC curates a Register of Misidentified Cell Lines [2]: https://iclac.org/databases/cross-contaminations/

3) **Obtain the cell line from a reputable source**
The cell line should be obtained from a primary source – either a cell repository or the laboratory / institution where it was first established. Cell lines from secondary sources - such as the friendly scientist from the neighboring lab - have a higher risk of being misidentified [3]. Always ask for evidence that the cell line has been tested for authenticity and check that the supplier is reputable. “Pirate” suppliers have no authorization or license to distribute the cell line, and their stocks do not have any Certificate of Analysis/Authentication (CoA) or have poor viability [4].

When testing cell lines for misidentification / contamination:

4) **Test each cell line before starting experiments and before publication**
Testing must be performed when a cell line is established, when it enters a new laboratory, and when a new cell bank is prepared (see point 11). If it is being used for experimental work, the cell line should be tested before and after experimental work (i.e., before submission for publication). Cell lines should also be tested after culturing for long periods of time (months), and after any genetic modification, selection, or changes in phenotype occur.

5) **Perform cell line authentication using a genotype-based method**
Phenotype-based methods are not suitable for cell line authentication because cell behavior and expression patterns vary with passaging and culture conditions. Cell line authentication relies on genotype-based methods that allow discrimination between cell lines from different donors. Suitable genotype-based methods include:

    a) Short tandem repeat (STR) genotyping. A standardized, consensus method has been developed for human cell line authentication [5,6], including a minimal set of STR loci for use when comparing results between laboratories (see point 6). A method has also been developed for mouse cell line authentication using STR profiling [6,7].

    b) Single nucleotide polymorphism (SNP) genotyping. A standardized, consensus method has not been developed for SNP-based cell line authentication. However, this method can be used for in-house testing (e.g., as part of high-throughput sequence analysis) once the cell line has been shown to be authentic using STR profiling [8]. Several sets of SNP loci have been shown to be effective for human cell line authentication [8-9].

6) **Compare authentication results to cell line databases to uncover misidentified cell lines**
Detection of misidentified cell lines relies on comparison of results between laboratories [5,10]. This comparison requires a database of STR profiles from many different cell lines, including the rapidly growing tumor cell lines that are most likely to cause cross-contamination. Suitable cell line databases include the Cellosaurus STR Similarity Search Tool (CLASTR) [11]: https://www.cellosaurus.org/str-search/

7) **Test the species of the cell line using a genotype-based method**
Species testing should be performed if the cell line comes from a species where methods for STR or SNP analysis are not available. A standardized, consensus method has been developed

for species testing using DNA barcoding [12], based on sequencing the mitochondrial gene cytochrome c oxidase subunit 1 (commonly referred to as CO1, COI1, or COX1). A species-specific multiplex PCR assay has also been developed for rapid confirmation of species origin and potential cross-contamination detection based on 14 commonly used species in cell culture [13]. Cytogenetic analysis can also be used for species detection and to reveal disease- or cell line-specific chromosomal anomalies such as rearrangements and duplications.

8) **Test for microbial contamination**
All cell lines should be tested for common microbial contaminants such as mycoplasma. Testing for viruses may also be needed, depending on the cell line and your application. The rationale and methods for mycoplasma or viral testing are discussed elsewhere [14-15].

Whenever working with a cell line:

9) **Freeze tissue or blood samples when establishing a new cell line for future testing**
Store blood or tissue samples from the original donor (e.g., blood samples) at -80°C or in liquid nitrogen. This material can be used to generate reference genotypes for authentication, to investigate sources of microbial contamination, or to perform other characterization testing.

10) **Quarantine each incoming cell line until it has been tested**
Handle the cell line separately until you are confident that it is authentic and free of mycoplasma and other microbial contaminants. Cell lines can be quarantined through physical separation (e.g., using a separate incubator) or, if this is not possible, through separate handling (e.g., by handling after other cell lines at the end of the working day).

11) **Prepare a cell bank for the cell line**
A “cell bank” consists of a set of cryovials that is frozen at early passage, ensuring that the cell line and its unique characteristics are preserved in case of future contamination, genetic drift, or other problems [16]. A tiered approach is typically used when preparing cell banks:

    a) A “master” cell bank or “seed stock” is prepared as soon as sufficient numbers of cells are available. These cryovials are used only to prepare further cell banks.

    b) A “distribution” or “working” cell bank is prepared from a single vial of the master cell bank. These cryovials are distributed to end users or used for other purposes (e.g., to generate antibodies or other biologicals), depending on the application.

    c) Further stocks may be prepared by recipients for their own personal use. Such “user” cell banks should not be distributed to others, even within the same laboratory. Users should always return to the tested stocks from the “distribution” or “working” cell bank.

Note: small laboratories that need a small number of vials, and who receive cell lines from a validated source, may choose to prepare a single working stock (~20 vials). Stocks should be replenished if they reach the last 5 vials from the original stock.

12) **Keep detailed records on the origins and handling of the cell line for future reference**
Good records are essential for cell culture to be reproducible. Records should include:

    a) The name of the cell line in full, as published by the original laboratory / institution. Refer to our separate guidelines for assistance when naming a new cell line [17].

    b) The cell line’s Research Resource Identifier (RRID). This numerical identifier should be reported in the Materials and Methods section along with the cell line name, ensuring that cell line designations are unique and easily searchable [18]. The RRID can be located by searching the cell line in Cellosaurus [1].

    c) The origins of the cell line including the tissue or cell type, disease, the donor’s sex and age, and their ancestral background (e.g., strain for non-human cell lines).

    d) The source of the cell line including the cell repository or laboratory from which it was received and the catalog number (if received from a cell repository).

    e) Cell line handling information including the medium, substrates (e.g., coatings) and environment (e.g., gas percentages) required for culture.

f) Previous testing including the passage number and date on which the test sample was obtained, the test method used, and the test result.

g) Characterization – varies with the application but is likely to include images, growth data, sequencing data (e.g., mutations), and expression of cell type-specific markers.

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