Guide to Mouse Cell Line Authentication

It is good practice to authenticate cell lines when preparing master and working cell banks, prior to starting a new research project that may result in a publication, and to qualify reagents when submitting a proposal to a funding agency. Best practices are compiled in the Baust et al. [1] publication where it describes the method using short tandem repeat (STR) profiling to authenticate human cell lines and the updated version of the ATCC/ANSI standard ASN-0002-2021 by Korch et al. [2]; whereas nonhuman cell lines use DNA barcoding to determine species identity (detailed in ASN-0003-2015) [3]. Building on the same principles as the human genotyping assay, a mouse STR genotyping method was developed [4] and validated by a consortium [5]. This method will be described briefly here, and more detailed information can be found in The Assay Guidance Manual (AGM) written by Almeida and Korch (2023) [6], published by the National Center for Biotechnology Information.

SUMMARY

1. Send cells or DNA to an experienced commercial provider of STR genotyping service for mouse cell lines using the validated methods described by the Mouse Cell Line Authentication Consortium [5].
2. Compare STR profile to donor and all other known cell line STR profiles including other possible spellings of the same cell line.
   - ➢ Recommend using Cellosaurus as a search tool (https://www.cellosaurus.org)
3. Check the cell line name against the ICLAC Register of Misidentified Cell Lines (https://iclac.org/databases/cross-contaminations/).
4. Refer to ICLAC resource document “Advice to Scientists: Making Cell Line Authentication Part of Everyday Culture Practice” (https://iclac.org/resources/advice-scientists/).
5. If in doubt, ask ICLAC.
6. If the cell line is false, discontinue use, and contact ICLAC so information regarding that cell line can be updated and looked into further.

More detailed information and caveats are included below.

The right start of cell line authentication

As a rule, cell lines should be obtained from reliable and confidential sources such as public cell banks. If cell lines are available in a running laboratory, their identity should be checked before starting a new project. Always check the name of the cell line for existing problems before you start experiments and before you test it. This can be done using Cellosaurus, which provides a broad range of cell line information [7-8], and the ICLAC Register of Misidentified Cell Lines, which focuses specifically on false cell lines [9].

The latest versions of Cellosaurus and the ICLAC Register are available at:

https://www.cellosaurus.org

http://iclac.org/databases/cross-contaminations/

This step will alert you to possible problems with the cell line and may save you considerable time and effort. However, it should be noted that if a cell line is reported to be cross-contaminated, it does not necessarily mean that all samples are affected. There may be multiple stocks of cell lines with the same name from many sources, only some of which may be cross-contaminated. New cell lines that do not enter a laboratory through public cell banks should always be validated using STR genotyping.

A. Prepare starting material for STR profiling

Starting material should include:

1. **DNA from the donor of the cell line if established within your laboratory**
   Snap freeze a few milligrams of tissue in liquid nitrogen (e.g., part of the biopsy, tail snip, ear punch) from the donor. DNA from this sample can be used later to provide evidence (STR profiling) that the cell line is derived from the donor material.
2. **DNA from the cell line**
   Where possible, it is advisable to purchase a cell line from a reputable supplier that provides characterization and an STR profile. This STR profile can then be used for comparison when testing your own stock.

At minimum, testing should be performed at the beginning and end of experimental work. Test each batch of frozen vials (cell bank) that you prepare for that cell line.

Some scientists prefer to check the STR profile of the cells before they are banked, others after they are banked. Either way, it is essential that all batches of cells are STR profiled at the time they are frozen down and before they are used or distributed. Confidence is then assured for the length of time the sample is available that the cells have the determined STR profile. Any other method of maintaining the cell stock (e.g., continuous culture or multiple freeze/thaw cycles) is poor tissue culture practice.

For more information on cryopreservation of cell line stocks, see Baust et al. and Stacey et al. [1,10].

B. Perform STR profiling

STR profiling can be performed in-house or outsourced. STR profiling should only be done in-house if it is routinely used, and experienced personnel adhere to the criteria set out in the validated method for mouse cell line authentication [5]. Commercial service providers can be found online (e.g., enter “mouse cell line authentication” or “mouse STR genotyping service” into the search engine). It is essential that the supplier chosen provides a rapid and reliable service with appropriate accreditation and quality assurance. The supplier should provide an STR profile and quality control data. Some suppliers ask for DNA, while others accept either cells or DNA. Suppliers may express a preference for samples spotted onto FTA paper, allowing shipping and storage at room temperature. A single T25 tissue culture flask will provide more than enough cells or DNA to obtain an STR profile.

C. What to do with the result when it arrives: STR profile comparison

An STR profile is obtained by PCR amplification of a set of STR loci. The profile consists of a series of numbers corresponding to the number of repeats seen at each allele for that locus. Eighteen mouse STR loci are required for mouse cell line authentication and two human STR markers to detect both human and African green monkey contamination if present.

The next step is to compare the STR profile to an appropriate reference sample – for example, DNA from the donor of that cell line. A useful tool for mouse STR profile comparison is CLASTR on the Cellosaurus website (https://www.cellosaurus.org/str-search/). Cellosaurus sources data from the NCBI BioSample Database which contains the validated mouse STR profiles from the Mouse Cell Line Authentication Consortium
(https://www.ncbi.nlm.nih.gov/biosample/?term=mouse+cell+line+STR+profile%5Battribute+name%5D)
. Precise matching algorithms have not been established for mouse cell lines as they are highly inbred and share many alleles. It is important to note that rules for human cell line authentication matching should not be used for mouse cell lines.

Points to be aware of when comparing cell line samples:

1. To be completely certain that a cell line is authentic, you need to compare it to another sample from the same donor. For many cell lines, another donor sample is not accessible. In those cases, comparison to a database with STR profiles from many different cell lines will give you a high degree of confidence that your sample is not misidentified.

2. STR profiling does not allow you to distinguish between cell lines arising from the same donor. All cell lines from that donor will have the same, or highly similar, STR profiles.
3. A small amount of STR profile variation may be seen between cultures derived from the same donor. This can be caused by genetic drift with passage, particularly in cell lines with microsatellite instability. Variation may also relate to laboratory differences in test methods or interpretation.

To use the CLASTR profile database:

1. Enter your STR profile as set out in the search form. STR profiles include results for different loci (e.g., STR 1-1, STR 2-1, etc). For each locus on the search form, find the corresponding locus in your sample and enter its results, separating multiple alleles for the same locus with a comma.
2. Search the database to find the closest matches to your sample. Samples are compared using a match algorithm (Tanabe for example); search results will list the closest matches first.
3. To interpret your results, it helps to look at the percent match (score). Samples from the same donor generally yield a result in the 90-100 % match range for mouse, while samples from different donors generally lie in the 0-80 % (keep in mind that some mouse strains are very closely related based their phylogenetic tree placements [11] and we are still learning how their STR profiles are related).
4. It also helps to know the provenance of your cell line. Was it derived from another cell line, or are there other cell lines known to be established from the same donor? If so, these cell lines would legitimately have the same STR profile. A misidentified cell line is one where the STR profile fails to correspond to the expected donor, or where the STR profile unexpectedly corresponds to an unrelated cell line.

D. What to do with misidentified cell lines

If your testing shows that a cell line is misidentified, stocks of it should be discarded and any colleagues who have other stocks of that cell line should be informed. If you discover a novel cross-contamination, you may choose to publish your finding. If the cell line is used widely and you believe that no authentic stocks exist, publication is an important step to alert others in the scientific community. Please also contact ICLAC at info@iclac.org so the cell line can be added to the Register of Misidentified Cell Lines. If you wish to add a new misidentified cell line or edit entries within the database, we will ask for more information to help us review your findings and decide on the best course of action.

For additional resources relating to contaminated cell lines and authentication testing, see:

- ICLAC web page http://iclac.org/
- Register of Misidentified Cell Lines http://iclac.org/databases/cross-contaminations/
- Advice to Scientists: Making Cell Line Authentication Part of Everyday Culture Practice https://iclac.org/resources/advice-scientists/

References

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