

Advice to Scientists Incorporating Authentication into Everyday Culture Practice

1) Planning a project or grant application

- a) Identify a validated source of cell lines you wish to use, e.g., a cell bank that will provide evidence to guarantee authenticity and absence of microbial contamination including mycoplasma.
- b) If such a source is not available:
 - i) Do a PubMed and Google search using the name of the cell line.
 - ii) Identify the originator of the cell line – only if cells are not available from the originator is it necessary to approach a secondary user of the cells.
 - iii) Check that cell line is not on ICLAC list of misidentified cell lines:
http://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac/Cross_Contaminations_v7_1.pdf
 - iv) Confirm that source laboratory has some evidence of authenticity.
 - v) Confirm that cell line designation is unique and cannot be confused with a different cell line.

2) Initiating work on a new project

- a) Obtain cell lines from a validated source if possible (as in 1a above).
- b) Expand and freeze your own working stocks (as in 4 below).
- c) Take additional precautions while preparing stocks if a validated source is not available:
 - i) Handle under quarantine.
 - ii) Freeze token stock (2-3 vials to guard against future loss).
 - iii) Confirm freedom from mycoplasma and other microorganisms.
 - iv) Confirm identity by performing authentication testing (as in 5 below).
 - v) If clean and authentic, release from quarantine and freeze additional stocks (as in 4 below).

3) Initiating new cell lines

- a) Retain sample of patient's blood and/or biopsy in liquid nitrogen for DNA extraction and STR profiling.
- b) When cell line is subcultured:
 - i) Freeze token stock (2-3 vials to guard against future loss).
 - ii) Check medium supernatant for mycoplasma contamination.
- c) When cell line is established as a finite or continuous cell line (and before distributing as the originator of the new cell line):
 - i) Confirm identity with reference to donor DNA from blood or biopsy (as in 5 below).
 - ii) Confirm freedom from mycoplasma and other microorganisms.
 - iii) If clean and authentic, freeze additional stocks (as in 4 below).
 - iv) Keep records for all stored vials including total number of passages, date, and test results.
 - v) Deposit cell line at an international cell bank or distribute to others with appropriate data on validation.

4) Freezing down stocks

Laboratories needing a small number of vials, and who receive those vials 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.

Cell banks or laboratories needing a larger number of vials will prepare stocks at several passages:

- A small stock (12-20 vials), also known as “seed stock” or a “master bank”, is prepared first and used only to replenish stocks later on.
- A single vial from that stock is expanded to give a large number of vials (up to 100 vials), also known as “distribution stock” or a “working bank”. Vials from that stock are suitable to release to members of that laboratory for experimental work. Cell banks have permission from the originator to use those stocks for wider distribution.
- A lab member receiving such a vial will prepare a working stock (~20 or more vials) before commencing experimental work using that cell line. Lab members should return to those stocks periodically to avoid overpassaging. Working stocks should not be distributed to other users,

even within the same laboratory. Other users should obtain cells from the validated distribution stock.

When freezing down stocks for future use, always:

- Keep records for all stored vials including total number of passages, date, test results, any unique distinguishing growth behavior, and any known genetic features (e.g., mutations).
- Confirm identity of stocks by authentication testing.
- Store frozen stocks across at least 2 independent storage systems, to reduce the risk of losing all stocks in case of equipment malfunction.

5) Authentication testing

Authentication testing aims to compare the DNA profile for that cell line to other samples from the same donor to confirm the cell line is not cross-contaminated or otherwise misidentified. If donor tissue or cell lines are not available, the comparison is made with reference to online databases containing DNA profiles from a large number of widely used cell lines.

For human cell lines, it is recommended that cell lines are tested using short tandem repeat (STR) profiling in accordance with the standard ANSI/ATCC ASN-0002-2011 Authentication of Human Cell Lines: Standardization of STR Profiling. Recommendations from the standard should be followed, including the use of at least eight core STR loci and application of match criteria (80% match threshold) to allow for a small amount of genetic drift in some cell lines.

For non-human cell lines, best practice will vary with the species being tested. As a minimum, it is recommended that non-human cell lines are tested for species-specificity and absence of human and rodent cell lines. Appropriate test methods include karyotyping, isoenzyme analysis, and mitochondrial DNA typing (DNA barcoding). Test methods, including STR profiling, are available commercially at a modest cost from various suppliers, including some of the cell repositories.

6) More Information

For additional information on good cell culture practice or testing for contamination, see:

- Freshney RI. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications* (6th edition). Wiley-Blackwell 2010.
- Cree IA (editor). *Cancer Cell Culture: Methods and Protocols* (2nd edition). *Methods Mol Biol* 2011; 731. Humana Press.

For more on contaminated cell lines or authentication requirements, and procedures, see:

- ICLAC web page
http://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac/
- List of cross-contaminated or misidentified cell lines
http://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac/Cross_Contaminations_v7_1.pdf
- ANSI/ATCC ASN-0002-2011. Authentication of human cell lines: Standardization of STR profiling. The Standard can be purchased at the ANSI eStandard store:
<http://webstore.ansi.org/RecordDetail.aspx?sku=ANSI%2fATCC+ASN-0002-2011>
- Guide to human cell line authentication (Authentication SOP)
See ICLAC web page