



Q&A ATCC® Excellence in Research Webinar “Authentication and Characterization of Animal Cell Lines: Towards Best Practices in Cell Culture”

General Questions

1. Will we be able to download the presentation?
This presentation will be available to watch on demand [here](#).
2. Is there a recommended maximum number of passages for all cell lines before the cell characteristics will change?
Increased passage number can have different effects on different cell lines, therefore ATCC cannot give a specific passage limit that is acceptable for all cell lines. ATCC recommends limiting passaging of most continuous cell lines to 10 passages or no longer than 2 months, whichever comes first. It is well documented that cell characteristics can change when cell lines are cultivated for extended periods. Cell lines that have been over-subcultured can experience phenotypic as well as genotypic changes (genetic drift) (Hughes et al., BioTechniques 43:575-586, 2007). It is good cell culture practice to start experiments with fresh, low-passage cells and to use the cells in a predetermined range of passage numbers for best results. If you start to experience sudden and inexplicable variations in your experimental results, it may be that the cell line has been subcultured too often and needs to be replaced. By recording passage number, monitoring your cells with morphology checks, establishing and observing changes unique to the line you work with, you can help to promote the integrity of the cell banks which you have created in your research institution. Refer to [ATCC Technical Bulletin No. 7](#) for more information.
3. Do frozen cells work as well as growing a cell line for a functional assay?
This will depend on what you are specifically looking for. For example, if you are looking at markers that are expressed when cells are at confluence, it will be necessary to examine actively growing cells.
4. I understand that STR profiling is only available for human cell lines. Do we have anything for animal cell line authentication?
STR markers are available in all mammals. They have been selected and optimized in humans by the forensic community for use in PCR reactions. This method is not widespread for animal cell lines as, to our knowledge, there are no STR marker assays for non-human cell lines that are polymorphic and informative enough to distinguish within a species. However, [NIST](#) is currently working on developing STR assays for non-human cell authentication and ATCC is following these developments.

5. If you change the medium you are using for a cell line culture, should the PDT be the same with a similar media?

Any change to the culturing conditions has the potential to change the characteristics of the cell line. Be particularly cautious when working with a new cell line as media formulations vary among suppliers, even for media with similar or identical names. This change in formulation can consequently affect cell growth and population doubling time (PDT) differently. A growth curve/profile can be performed with the cell line of interest for each type of culture medium used, keeping the seeding inoculum constant. To confirm complete adaptation to the new medium, perform functional tests on cells derived from the original and new medium.

6. Is there a reason why ATCC does not test their cell lines for SMRV (Squirrel Monkey Retrovirus)?

It is not possible for ATCC to test every cell line for every virus; we therefore rely on the tests performed by the depositor. ATCC tests all human cell lines in our collection for common human pathogens such as Human Papilloma virus (HPV), Epstein-Barr virus (EBV) also referred to as Human herpes virus 4, Cytomegalovirus (CMV), Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) by qPCR. Testing is performed on each lot when it is prepared, and the test results are reported on the Certificate of Analysis for the specific lot.

7. Is there a list of Journals that require authentication? What exactly do they require?

Many peer-reviewed scientific journals now require some level of cell line authentication, including Nature and all the AACR Journals. A listing of journals and their requirements for cell authentication can be found [here](#). ATCC, in collaboration with Promega, offers the STR-based human cell line authentication service, found [here](#). Also refer to [ATCC Technical Bulletin No. 8](#) for more information on cell line authentication.

8. What are the kinds of markers that you prefer to characterize specific organs like lung, breast, or brain?

STR markers cannot tell you what tissue a specific cell line is from, only that it is from the same individual. ATCC does assay for tissue-specific markers for select cell lines, such as the ATCC human telomerase reverse transcriptase ([hTERT](#)) immortalized cell lines, for example, Mucin1 expression in hTERT-HME1 (ATCC® CRL-4010™) human mammary epithelial cells.

9. What would you suggest to do if a cultured batch of cell lines has lost one of the peaks? Buy a new one, or confirm the identity with a different method, or continue with this batch?

If the tested cell line shows loss of a peak or gain of an additional allele peak, this may be indicative of genetic instability (as in cancerous cell lines) or that the cell line has been contaminated or misidentified. Additional authentication testing should be done on earlier passages to identify where the problem originated, or a new sample from a

repository should be obtained. It is not recommended that research and experiments be continued on a potentially contaminated or misidentified cell line. ATCC uses the Promega PowerPlex® 18D System to confirm the identity of human cell lines by STR (Short Tandem Repeat) analyses. STR profiles reported are compiled, reviewed, and compared between different lots of the same cell line and against all previously ATCC-generated profiles. The ATCC has established baseline DNA fingerprints for human cell line master stocks in the collection. Distribution stocks are derived from the master stocks and the fingerprints are compared to the master stocks and all preceding distribution stocks.

10. Do you get stutter band products from PCRing microsatellites?

Yes, we do occasionally get artifacts or stutter peaks. We have been performing STR profiling for many years, and during that time we have optimized our protocols to reduce the occurrence of stutter peaks. By having good primers, and by optimizing your conditions, you can reduce the occurrence of stutter bands in your laboratory.

11. I work at a small university with few resources. However, I'm able to do cell culture. What is the most basic, cost-effective way for me to identify cell lines when publishing?

There are a number of commercial entities that are performing cell authentication services. ATCC offers custom testing services for mycoplasma detection and human cell line authentication (STR analysis). If you are interested in learning more about these services, please visit our services page [here](#).

12. Could you explain how to use PDL information?

Passage number is generally the number of times the cells have been subcultured into a new vessel. Diploid cell lines have a finite replicative capacity and will begin to slow down and eventually stop dividing after a few population doublings. In contrast, continuous (or immortalized) cell lines have infinite replicative capacity. For diploid cultures, passage number is roughly equal to the number of population doublings (or population doubling level, PDL) since the culture was started. This is not the case for continuous cell lines as they are passaged at higher split ratios. Consequently, the PDL is not determined for continuous cell lines. In most cases, the PDL is an estimate as it does not account for any cells that were lost due to death from necrosis or apoptosis or cells which are nearing senescence and no longer divide.

Calculate the population doubling level with the following formula:

$$PDL = 3.32 (\log X_e - \log X_b) + S$$

X_b is the cell number at the beginning of the incubation time.

X_e is the cell number at the end of the incubation time.

S is the starting PDL.

Calculate the population doubling time, or the time required for a culture to double in number, with the following formula:

$$DT = T \ln 2 / \ln(X_e / X_b)$$

T is the incubation time in any units.

X_b is the cell number at the beginning of the incubation time.

X_e is the cell number at the end of the incubation time.

ATCC tracks the PDL and passage number for many adherent cell lines when the depositor supplies this information at the time of deposit.

13. Do you have any stem cells?

Yes, ATCC offers different types of [stem](#) cells, including human induced pluripotent stem cells, human mesenchymal stem cells, mouse embryonic stem cells, as well as associated stem cell culture reagents and [media](#).

14. Are stem cells authenticated differently than other cell lines?

ATCC uses STR profiling in addition to karyotyping to authenticate stem cells.

15. Do you provide a service of checking cell lines that a lab may want to use?

Yes, ATCC offers cost-effective testing services for mycoplasma detection and human cell line authentication (STR analysis).

With ATCC's Mycoplasma Testing Service, DNA staining and direct culture methods are performed simultaneously for each sample. Together, these methods can detect mycoplasma infection for most of the known species.

ATCC uses the Promega PowerPlex® 18D System to confirm the identity of human cell lines by STR (Short Tandem Repeat) analysis. STR profiles are compiled, reviewed, and compared against all previously ATCC-generated STR profiles in our database. If you are interested in learning more about these services, please visit our services page [here](#).