

Q&A ATCC[®] *Excellence in Research* Webinar “STR Profiling for Human Cell Line Authentication”

General Questions

1. Will we be able to download the presentation?
This presentation will be available to watch on-demand on the ATCC website, or [click here](#).
2. Are there other methods to authenticate cell lines besides Short Tandem Repeat (STR) profiling?
In addition to STR profiling, there are a number of ways to resolve the identity and status of a cell line, including growth curve analysis, cytochrome c oxidase 1 (CO1) barcode assay (species identification), karyotyping (chromosome analysis), random amplified polymorphic DNA (RAPD), and single nucleotide polymorphism (SNP) analysis. You can read more about some of these practices here: www.atcc.org/~media/PDFs/Technical%20Bulletins/tb08.ashx
www.ncbi.nlm.nih.gov/pubmed/25877200
3. What is the easiest way to submit a human cell line sample to ATCC for STR DNA Profiling?
ATCC offers a complete Cell Line Authentication Service utilizing STR profiling. You can place your order for the STR profiling service on the ATCC website: www.atcc.org/STR

After you order the service, ATCC will send you the sample collection kits in the mail. Once you receive the kit, spot your cell suspension directly onto the FTA papers and then mail them back to ATCC in the pre-addressed envelope. The collection kits include the barcoded FTA paper, sample preparation instructions, sample submission form, desiccant, and envelope for return mail. Your STR Profile reports will be emailed to you by ATCC within 5 business days of order confirmation and receipt.

4. Considering the extent of cell line contamination present in many labs around the world, what cells were used to develop ATCC’s STR profiles?
ATCC has consistently authenticated our collection of continuous human cell lines through STR profiling for many years. All ATCC human cell lines are included in our database along with cells that have been de-accessioned due to misidentification. The [HeLa \(ATCC[®] CCL-2™\)](#) cell line has been the primary culprit for misidentification and cross-contamination of numerous other cell lines. More information on cell authentication and contamination may be viewed on ATCC’s [Cell Biology Resources](#) webpage and in the November 2014 article “[The Dirty Little Secret of Cancer Research](#)” in *Discover Magazine*, which covers the history and scope of cell line contamination.

5. What is repeat slippage? What is its cause?
Repeat slippage is also known as “stutter.” A stutter is a single repeat unit, smaller than the true peak, which occurs immediately before the true peak. These peaks, which may result in differences between two samples of a cell line at one locus, are typically less than 15% of the true peak. The stutter is caused by errors in DNA polymerase-mediated amplification.
6. What threshold of match probability is utilized for determining identity?
Using the Masters or Alternative Masters algorithms (See: [Capes-Davis A, et al. Int J Cancer 132\(11\): 2510-9, 2013](#)), cell lines with $\geq 80\%$ match are generally considered to be related (i.e., derived from the same patient or donor). Cell lines with a match ranging from 55% to 80% require further profiling for authentication of relatedness.
7. How often should I authenticate my cell lines?
- When you first receive a cell line into the laboratory from a unreliable source
 - After 10 passages
 - After preparing a cell bank
 - When in doubt
8. Is there a recommended maximum passage number for a cell line before it is no longer useful?
The limit to the number of times a given cell line should be passaged must be determined empirically for each cell line based on the nature of the cell line and the type of work being done with the cells. Although many immortalized cell lines have an indefinite life expectancy, we know that the characteristics and properties may sometimes change markedly when animal cells are cultivated for extended periods *in vitro*. ATCC works to maintain the cells at the lowest passage possible using a seed stock system. As a general rule, it is best to passage the cells a limited number of times before returning to a lower passaged frozen stock to ensure that the functionality and characteristics of the cells are maintained. ATCC recommends passaging a culture no more than 8 to 10 times, or 2 months.
9. I understand that STR profiling is only available for human cell lines. Do you have anything for animal cell line authentication?
ATCC is working to validate primer sets for mouse cell line STR profiling. However, the CO1 assay can be used for interspecies identity. The CO1 gene region that is used as the standard barcode for almost all animal groups is a 650 base-pair region in the 5' region of the mitochondrial cytochrome c oxidase 1 gene, and can be used to discriminate between species of the same genus.
10. Is there a list of Journals that require authentication? What exactly do they require?
Well over 30 journals (including the Nature and AACR journals) recommend or require cell line authentication as a prerequisite for publication. Some journals refer to molecular characterization for cell line authentication, while some specifically refer to STR for cell line authentication. We recommend reviewing the Instructions to Authors of each journal for their specific requirement. A partial list is provided in the webinar presentation, which can be viewed

on demand.

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

Submit a sample of your cells to a testing laboratory. In addition to ATCC, other testing laboratories may be able to provide STR profiling, such as:

- Cell Banks
- Paternity testing labs
- Universities
- Core labs

When choosing a testing service, it is important to consider the training and experience of the technicians who perform the service and your access to an STR profile database to compare your cell line profile against for a match.

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

Yes. ATCC offers a complete Cell Line Authentication Service utilizing STR profiling. Simply place your order for the STR profiling service on the ATCC website: www.atcc.org/STR

For any technical inquiries on STR profiling, please contact ATCC Technical Support by Phone: 1.800.638.6597 or 703.365.2700, or by Email: tech@atcc.org

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