

Human Cell Line Authentication FAQ's

What is cell line authentication?

Cell line authentication uses short tandem repeat (STR) profiles to establish a genetic identity of a cell line. Once this identity is established, cell lines should be tested periodically to make sure that misidentification or contamination has not occurred.

Why should cell lines be authenticated?

Contaminated or misidentified cell lines can result in invalid and misleading data. The NIH recently issued a notice discussing the need for cell line authentication and a number of Journals are now requiring authentication of cell lines prior to publication.

How are cell lines authenticated?

Our facility uses the Promega GenePrint® 10 System. This system allows for the co-amplification and three-color detection of nine human STR loci and Amelogenin for gender identification. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92×10^9 .

CELL ID™ System STR Locus	Chromosomal Location	Repeat Sequence 5' → 3'
D21S11	21q11-21q21	TCTA Complex (16)
TH01	11p15.5	AATG (16)
TPOX	2p23-2pter	AATG
vWA	12p12-pter	TCTA Complex (16)
Amelogenin	Xp22.2-22.3 and Y	NA
CSF1PO	5q33.3-34	AGAT
D16S539	16q24-qter	GATA
D7S820	7q11.21-22	GATA
D13S317	13q22-q31	TATC
D5S818	5q23.3-32	AGAT

How often should cell lines be authenticated?

Cell lines should be tested:

When a new cell line is established or acquired.

Before cells are frozen, or once every two months while the culture is actively growing.

At the start of a new series of experiments, and prior to publication.

When the performance of the cell line is not consistent or results are unexpected.

If more than one cell line is used in a Lab. All lines should be tested initially to rule out contamination.

How do I place an order?

Orders are placed online using iLab as with other DNA Analysis Facility services.

What do I need to provide the DNA facility?

Samples should be submitted in individual tubes with between 0.5×10^6 and 1×10^6 cells. The cells should be pelleted with the media removed and frozen. The samples will remain frozen at the facility until the gDNA extraction is done. Please write the number of cells contained in the 1.5 ml tube.

What happens next?

The facility will extract gDNA . DNA quality and quantity will be checked on the NanoDrop. A dilution will be made to 2ng/uL and verified on the NanoDrop. Samples will then be amplified using the Promega GenePrint® 10 System. These fluorescently labeled PCR products will be run on the ABI 3130 Avant Genetic Analyzer. The data will be analyzed using GeneMapper™ v5.0 software. A gene table will be exported from GeneMapper™ including allele calls, size in bp, and peak height. A Cell Line Authentication Report will be generated including the allele calls only.

What format can I expect to see my results?

You will receive three types of files. The data files generated on the 3130 Avant Genetic Analyzer are in a .fsa format. These files can be viewed in GeneMapper™ (the DNA facility has a copy) or Peakscanner (freeware from ABI). The gene table exported from ABI is an excel file. The Cell Line Authentication Report generated by the facility is a word document.

How can I compare my results from the facility with a known reference?

The following are a number of STR databases available to search commercially available cell lines:

- ☉ Cell Line Integrated Molecular Authentication (CLIMA)

<http://bioinformatics.istge.it/clima/>

- ☉ American Type Culture Collection (ATCC)

<https://www.atcc.org/CulturesandProducts/CellBiology/STRProfileDatabase/tabid/174/Default.aspx>

- ☉ Japanese Collection of Research Bioresource (JCRB)

http://cellbank.nibio.go.jp/cellbank_e.html

- ☉ German Collection of Microorganisms and Cell Cultures (DSMZ)

http://www.dsmz.de/human_and_animal_cell_lines/main.php?contentleft_id=101

What do I do if my cell line is not found in a database?

If your cell line is not found in a database, you can use the results to establish a genetic profile for your cell line for future comparisons.

What to include on grant or paper submissions:

If found in database:

Cell lines were validated at the Vermont Cancer Center DNA Analysis Facility by STR DNA fingerprinting using the Promega GenePrint® 10 System according to manufacturer's instructions (Promega #B9510). The STR profiles were compared to known ATCC fingerprints (ATCC.org), and to the Cell Line Integrated Molecular Authentication database (CLIMA)

database version 0.1.200808 (<http://bioinformatics.istge.it/clima/>) (Nucleic Acids Research 37:D925-D932 PMID: PMC2686526). The STR profiles matched known DNA fingerprints.

If not found in database:

Cell lines were validated at the Vermont Cancer Center DNA Analysis Facility by STR DNA fingerprinting using the Promega CELL ID™ System according to manufacturer's instructions (Promega #B9510). The profiles did not match known DNA fingerprints in the Cell Line Integrated Molecular Authentication database (CLIMA) database version 0.1.200808 (<http://bioinformatics.istge.it/clima/>) (Nucleic Acids Research 37:D925-D932 PMID: PMC2686526), but will serve as a reference for future work with these cell lines.