# Best Laboratory Practices for Cell Line and Tissue Sample Authentication to Ensure Valid and Reproducible Research

## Supplementary Information and Guidelines

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Below are additional descriptions of different aspects of cell line and tissue authentication and suggestions of how to prepare manuscripts for submission of grant applications and for publication of results in journals.

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## Section 1 - Human Genotyping by the STR / DNA Profiling Method

Human cell lines and tissue samples must be authenticated before they are used (for each new lot of cryopreserved cells, for instance), during their usage, and prior to publication of research results. Two seminal references describe how to perform authentication by STR genotyping of human tissue and cell lines. The ANSI-ATCC ASN-0002 Standard by Korch et al. [22] published in 2021 with minor revisions in 2022 [23] is an update of the 2011 ANSI-ATCC Standard [4]. The update describes in great detail how to perform STR genotyping of human samples by laboratories offering this service and how to interpret STR data appropriately by both the service personnel and the researcher. The Assay Guidance Manual (AGM) written by Almeida and Korch in 2022 [3], published by the National Center for Biotechnology Information, describes how to perform the STR analysis for both human and mouse cell line and tissue samples and shows how researchers can understand and interpret the resulting data.

Human STR data can be obtained by using one of several commercial kits (e.g., ABI Identifiler Plus or GlobalFiler, or Promega Powerplex -16, -18D, 21, Fusion, or Fusion 6C), which test 13 or more STR loci on human autosomal chromosomes and of the allele(s) at the Amelogenin locus on the human gonosomal chromosomes by PCR amplification. Numerous STR kits from different suppliers are listed in the 2022 ANSI-ATCC Standard for authentication of human cell lines [23]. The mention of specific products is only for illustrative purposes and should not be construed as a recommendation of any specific product. At a minimum, the STR alleles at the following 13 loci must be determined: CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, and vWA. Some commercial kits may present additional loci that can be helpful to identify culture samples that have very few (<4) diallelic STR loci.

Historically, allelic data for the amelogenin locus on the X and Y chromosomes has been included in the calculations of the STR genotype match scores. This is not valid, as discussed elsewhere [3, 22], since it is not an STR locus and approximately 40% of male-derived cell lines lack the Y-linked amelogenin locus [24]. Therefore, the amelogenin locus should not be included in calculating the genotype match scores.

Calculations of match scores by three different algorithms are available by using the CLASTR tool and associated data entry template on the Cellosaurus website (https://www.cellosaurus.org/). Explanations for using the different algorithms are explained in the help menu and elsewhere [3, 22]. The resulting STR genotypes should then be used to search different databases to confirm the identity of the culture samples being used. Recommended guidelines and other resources, including links to STR databases, are available at the ICLAC website. The most useful STR genotyping search engine is the CLASTR tool at the Cellosaurus knowledge resource on cell lines website [7]. From the literature, as of September 2022, this database has compiled information about 141,885 cell lines, of which 106,170 are human-derived, 21,928 are from mouse, and 2,637 from rat. The CLASTR search tool contains the STR profiles for 8,159 human cell lines, 78 mouse cell lines, and 36 dog cell lines.

SNP analysis is also becoming an accepted method for genetic cell line identification, with a minimum of 48 SNPs [24]. However, a search tool for identifying cell lines based on SNPs is not currently available.

## Section 2 - Additional Authentication Considerations

### Section 2.1 - Growth Media and Cultivation Conditions

- Media components are variable between suppliers and between batches from the same supplier [33]. Therefore, each batch of culture media and their components should be recorded and validated, if necessary, for suitability before being used so that results are reproducible. The media will not affect the STR genotype, but can alter the phenotype of the cells (for example, changing the expression of specific genes being used to characterize the cell line).
- The level of confluence of cells can affect the expression of many genes in cell lines [28]. Therefore, one should harvest cells at consistent levels of confluence to maintain reproducibility between assays.

### Section 2.2 - Cell Line Heterogeneity and Genetic Drift

Researchers need to be aware that cell line cultures are heterogeneous mixtures of cells with slightly different genotypes and phenotypes that have arisen in the original tumor or during culturing due to genetic drift and selection. This may or may not give rise to variant STR genotypes.

Kleensang et al. described how two aliquots of the same batch of MCF-7 (RRID: CVCL_0031) obtained at the same time from the ATCC could diverge phenotypically within 7 passages even though their STR genotypes at 8 loci were identical [20]. It might seem that establishing cell lines from single cells would yield genotypically pure clones that were derived from single cells. However, these can also evolve at the genetic and physiological levels anew and develop into genetically heterogeneous cultures as shown for MCF-7 and other cell lines by Ben-David et al. [8] and for HeLa cells (RRID:CVCL_0030) by Liu et al. [25].

Genetic instability can be due to microsatellite instability (MSI) caused by defects in the cell's DNA mismatch repair or from defects in the DNA replicative machinery. However, MCF-7 and HeLa cells are MSI-Stable. Researchers should be aware that microsatellite instability can, with culturing and selection, give rise to variant STR profiles, which may complicate the interpretation of the STR data. Assaying for shared SNPs may be used to confirm relatedness of MSI-Unstable cell lines that show variable STR profiles as shown by Korch et al. [21].

### Section 2.3 - Identification of Cell Line Species by PCR Assays

The presence of interspecies contaminants (e.g., non-human cells in human-derived cell lines) can further confuse research endeavors and conclusions. The STR profiling kits used for human-derived samples cannot detect the presence of non-human cells in tissue samples and cultures. It has been shown that interspecies contamination of cell lines (~ 9%) can be as frequent as intra-species contamination [10]. This is especially important when working with cell lines from different species and with xenograft models.

Cooper et al. described a multiplex species-specific PCR-based assay, which tests for the presence of two mitochondrial genes, to rapidly identify the most common cell culture species and quickly detect inter-species contaminations (>2-5%) in a cell line culture/tissue sample [13].

The so-called universal DNA barcoding method of the International Barcode of Life organization based on determining the sequence of the standard barcode for almost all metazoan animal groups (mitochondrial cytochrome c oxidase I gene, "COI") is a highly effective method for identifying many animal groups [13]; however, this method cannot detect contaminations < 20%.

If rodent tissues and cell cultures need to be authenticated, one should consider using STR genotyping for mouse lines [2, 3] or SNP analysis for either mouse or rat [11, 14, 27, 30, 34]. If dog cell lines need to be authenticated, one can use the STR genotyping method described by O'Donoghue et al. [29]. Also, authentication by STR and microsatellite genotyping methods of canine, mouse, and rat cell lines are commercially available, for example, using LabCorp's Cell Line Authentication and Research Services or the CellCheck service at Idexx BioAnalytics. The CLASTR search tool in the Cellosaurus can search for genotype matches in the dog and mouse STR databases [6, 7, 32].

Detection of interspecies contamination using COI, cannot detect interspecies hybrid cells. To partially address this possibility, Huang et al. described a method that screens for both mitochondrial COI regions and some autosomal regions of 10 species [18]. Almeida et al. have included two human STR loci in their mouse-specific STR genotyping assay, which would allow the detection of two human chromosomes among mouse chromosomes in a sample [1]. Another approach to detect mouse-human cell hybrids ("hybridomas") could be to test a DNA sample for chromosomal-linked STR alleles with both a mouse STR kit and a human STR kit.

## Section 3 - Templates for Grant and Manuscript Descriptions of Guidelines and Cell Line Authentication Procedures used in Report

Assuming that the policy guidelines are fully implemented in a researcher's laboratory, below are a few generic examples of short descriptions which should be modified appropriately for the methods section of grant applications and manuscripts. The highlighted underlined spaces are intended for specifying the indicated information.

### Section 3.1 - Established Human Cell Lines, Patient-derived Explants and Xenografts of Human Tissue, and Development of New Human Cell Lines

- *The authenticity of cell lines used in these experiments was checked by verifying that the cell line's name, or variants of the name, was not present in the list of known misidentified cell lines on the International Cell Line Authentication Committee's website or the Cellosaurus cell line database [6].*
- *Prior to their use, all established human cell lines used in these experiments were genotyped by STR analysis using the _____ kit[1] or by the _____ laboratory[2] .*

[1] E.g., ThermoFisher Applied Biosystems AmpFLSTR® Identifiler® PCR Amplification Kit, catalog number 4322288; or Promega Powerplex 16HS System. catalog number DC2101)
[2] Specify the resource or commercial entity

- *The identity of the cell line was confirmed by comparing the resulting STR genotype to reference profiles from the originator or source of the cell line or through searching the consolidated STR data from four cell line repositories (ATCC, DSMZ, JCRB, RIKEN) base available at the DSMZ website [15] and/or the Cellosaurus STR database. The percent match with the reference genotype was ___ % using the ___ algorithm*[3] (*and/or* one of the other algorithms available on CLASTR without including the data for the amelogenin result in the search.

In the case of newly established cell lines or if the reference STR genotype of an established cell line is not available in the literature or an STR database, describe whether:

- Attempts were made to obtain a sample of the original tissue from which the cell line was established; and
- Its STR genotype was determined and compared to the cell line to verify that the cell line was established from the specified tissue/patient and that it was not a contaminant.

If the STR genotype was determined, provide the data for publication in a table format in either the main body or the supplemental information of the report and submit it to Cellosaurus for inclusion in this essential resource.

**NOTE** - Actual electropherogram(s) should be only provided for the editors and reviewers and should not be published to avoid being copied by others.

- All cultures were STR genotyped at the end of each series of experiments and prior to submission of grant applications and manuscripts.
- All cultures were confirmed not to be contaminated with mycoplasma by a PCR assay, e.g., Bulldog- Bio eMyco Plus kit (Catalog # 2523448) or the luciferase luminescence-based Lonza MycoAlert™ PLUS Mycoplasma Detection Kit (Catalog # LT07-118).
- If SNP genotyping was used, modify the above descriptions appropriately. Note that as of September 2022 there is no searchable public database for comparing SNP genotyping data between samples.

**Section 3.2 - Additional Descriptions for Xenografts, 3D cultures, and Stem cells**

- *The STR genotype of the source tissue (e.g., original tumor tissue, lymphocytes from blood) was determined and used as a reference genotype for any xenografts that were propagated through, e.g., mice.*
- *Each xenograft tumor passage was checked by STR genotyping to confirm that there had not been any inadvertent mix-ups of samples or contamination by a cell line being used concurrently; and*

[3] E.g., Tanabe algorithm and/or one of the other algorithms available on CLASTR, without including the data for the amelogenin result in the search

- *The presence of inter-species contaminating cells (e.g., mouse cells in a human xenograft) was checked by the method of Cooper et al. [13].*

  **NOTE** – The Cooper et al. method checks for the presence of mitochondrial DNA and not for the presence of autosomal DNA from other species. Therefore, it cannot detect mouse – human cell hybrids which have a mixture of mouse and human chromosomes. Such hybrids need to be screened for by karyotyping of individual cells as was done by Jacobsen et al. [19] or by multiplex PCR analysis that screens for the presence of one or more SNPs or mouse chromosomal STR alleles on multiple mouse chromosomes. This PCR assay should be considerably more sensitive than karyotyping. Alternatively, as noted above a DNA sample may be tested with multiple STR kits.

## Section 4 - The Cell Line Authentication Policy implemented at the *International Journal of Cancer* (IJC) with Modifications.

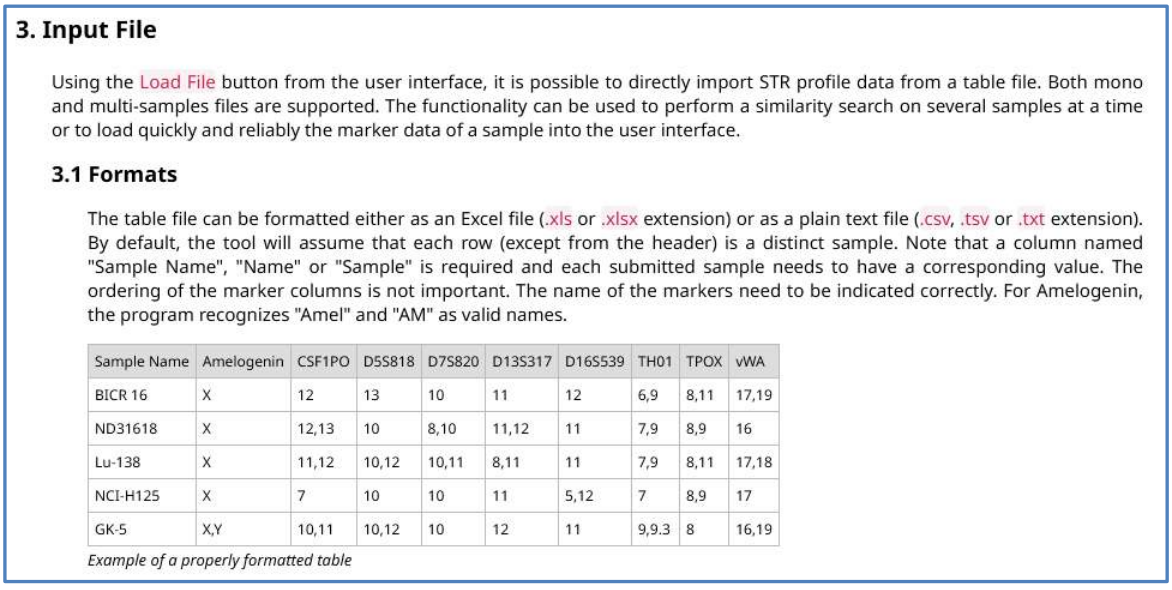
This journal's policy on cell line authentication is among the most straightforward of any such policy. Below, are the seven main stipulations for manuscript submissions to the IJC, as outlined by Souren et al. [35], which are based on the requirements presented on the IJC website, with a few clarifications we recommend:
https://onlinelibrary.wiley.com/page/journal/10970215/homepage/ForAuthors.html#AUCEL

1. *Authors must provide cell line authentication documents that are not older than three years of all continuous human cell lines used in their manuscript.*
   - *Note that IJC requires electropherograms of authentication data and they will check the data against Cellosaurus. Also, Souren et al.[35] suggested that these data be checked by a person qualified to do so.*
   - *Souren et al. [35] note that IJC recommends that the submitted high quality legible electropherograms not be published (i.e., only be included for the reviewers and editors to examine). In the main text or supplementary information, which is to be published, include a table summarizing the STR results. We suggest placing the STR data in an Excel/CSV table using the Cellosaurus / CLASTR format (see CLASTR website) so that it is accessible and easily searchable by the readers.*

     *The reason for not including the electropherograms is that they may be copied by others and claimed as their own as the IJC has reported [35].*
2. *STR profiling is the preferred method for cell line authentication of human, mouse, rat, and dog cell lines. Authors can perform STR profiling in their own laboratory or use the service provided by a laboratory or cell bank with certified quality control. Either way, the cell line authentication documents submitted with a manuscript should include high-quality legible electropherograms.*
3. *For continuous human cell lines obtained within the previous three years from a commercial source that guarantees cell line authenticity through in-house quality control measures (e.g., ATCC, DSMZ), the corresponding purchase orders or invoices are acceptable evidence of cell line authentication.*

- *Note that the ANSI-ATCC Standard for Authentication of Cell Lines by STR Profiling [22, 23] and several cell line authentication policies recommend that profiling be performed more frequently than every three years (e.g., once per year) and when phenotypic changes are noted in the culture.*
- *Note that the IJC has received falsified invoices for some manuscripts [35]. Also, in a recent PubMed literature search for the sources of the false cell line L-02 (a.k.a. HL-7702), some reports claimed it was obtained from the ATCC. However, the ATCC never had this imposter cell line among its offerings (Korch, data not shown, 2022).*

4. *The IJC also requests authentication of human cell lines for which no reference STR profile is available. Prior to submission, the obtained STR profile should be compared to a public database (e.g., Cellosaurus), and should show that the cell line is unique and not cross-contaminated or misidentified.*
5. *The IJC also accepts single nucleotide polymorphism (SNP) based cell line authentication reports from service providers with certified quality control, but only for cell lines for which a SNP-based reference profile is publicly available.*
6. *Authors of studies describing the establishment of new human cell lines are strongly encouraged to include the summarized STR results in the manuscript for future reference, such as inclusion of data in Cellosaurus, preferably in the .csv, .txt, or .xlsx format of the Cellosaurus template for CLASTR searches. See screenshot below from [https://www.cellosaurus.org/str-search/help.html](https://www.cellosaurus.org/str-search/help.html).*



**3. Input File**

Using the Load File button from the user interface, it is possible to directly import STR profile data from a table file. Both mono and multi-samples files are supported. The functionality can be used to perform a similarity search on several samples at a time or to load quickly and reliably the marker data of a sample into the user interface.

**3.1 Formats**

The table file can be formatted either as an Excel file (.xls or .xlsx extension) or as a plain text file (.csv, .tsv or .txt extension). By default, the tool will assume that each row (except from the header) is a distinct sample. Note that a column named "Sample Name", "Name" or "Sample" is required and each submitted sample needs to have a corresponding value. The ordering of the marker columns is not important. The name of the markers need to be indicated correctly. For Amelogenin, the program recognizes "Amel" and "AM" as valid names.

| Sample Name | Amelogenin | CSF1PO | D5S818 | D7S820 | D13S317 | D16S539 | TH01 | TPOX | vWA |
|---|---|---|---|---|---|---|---|---|---|
| BICR 16 | X | 12 | 13 | 10 | 11 | 12 | 6,9 | 8,11 | 17,19 |
| ND31618 | X | 12,13 | 10 | 8,10 | 11,12 | 11 | 7,9 | 8,9 | 16 |
| Lu-138 | X | 11,12 | 10,12 | 10,11 | 8,11 | 11 | 7,9 | 8,11 | 17,18 |
| NCI-H125 | X | 7 | 10 | 10 | 11 | 5,12 | 7 | 8,9 | 17 |
| GK-5 | X,Y | 10,11 | 10,12 | 10 | 12 | 11 | 9,9.3 | 8 | 16,19 |

*Example of a properly formatted table*

7. *The following information must be included in the Materials and Methods section:*
    - *All cell lines used must be listed using the official cell line name and its Research Resource Identifier (RRID) as available in the ExPASy Cellosaurus database*

*(e.g., HeLa (RRID: CVCL_0030). Note that Babic et al. [5] showed that there are fewer problematic cell lines in manuscripts citing the RRIDs.*

- *The source/supplier of all cell lines used must be provided.*
- *A statement confirming that all human cell lines have been authenticated using STR (or SNP) genotyping within the last 3 years.*
- *A statement confirming that all experiments were performed with mycoplasma-free cells.*

## Section 5 - Considerations of Patient Confidentiality when Publishing Human STR Data

When establishing institutional cell line and tissue authentication guidelines as described herein and deciding to publish human STR genotyping data, patient confidentiality may need to be considered. The human STR genotyping assays were developed for the identification of forensic samples, with newer kits detecting the alleles in 25 to 30 STR loci. These alleles are in noncoding chromosomal regions and are of minimal prognostic/diagnostic value. The original STR assays used for cell line identification between 1999 and 2013 [4, 26, 31, 36] were based on kits using nine or fewer STR loci. Between 2013 and 2021 it became evident that data for additional loci were needed to distinguish between cell lines, especially ones that had many monoallelic STR loci. To address this issue, the 2022 ANSI-ATCC standard for authentication of human cell lines recommended that data from a minimum of thirteen STR loci be used for the identification of cell lines [23] .

This expansion of available genetic data increases the potential for identifying a specific patient and familial relatives, which means that patient confidentiality might be breached. To address this concern (as discussed in detail in the 2022 ANSI-ATCC cell line authentication standard [23]) the M.D. Anderson Cancer Center and the Japanese Protection of Personal Information Act require that the alleles at only eight STR loci be published, as opposed to alleles at thirteen STR loci recommended by the 2022 ANSI-ATCC standard. The MD Anderson rule is self-mandated, whereas in Japan it is stipulated by national law. However, as of November 2022, there are already 8,327 human STR genotypes publicly available in Cellosaurus, which were obtained from published and unpublished sources and can include data for up to 31 loci, with most profiles containing data for between 8 and 17 loci.

Under the 2018 General Data Protection Regulation (GDPR), genetic data privacy is regulated in the European Union, but it is not clear to what extent each country can set their own rules and how much genetic information may be revealed in biospecimens. In Australia, there are rules governing the ethical use of human biospecimens, including cell lines. In the USA, patient confidentiality and medical insurability are regulated by the Patient Protection and Affordable Care Act (AFA), Health Insurance Portability and Accountability Act (HIPAA), the Genetic Information Nondiscrimination Act (GINA), and the American Disabilities Act (ADA) [12, 22]. However, to our knowledge, STR genotyping is not specifically addressed in Australia, Europe, or the US.

We are not aware of any journal that limits the number of loci for which STR data can be reported. The International Journal of Cancer (IJC) requests data for a minimum of eight core STR loci as outlined by Souren et al. [35] and described in the IJC instructions to authors. Therefore, the decision of how much human STR genotyping data to publish rests with individual laboratories and research organizations. It is worth considering the recommendation of Souren et al. [35] that only the tabulated STR data be published. The actual electropherograms should not be published, but only be provided to the editor and reviewers for their use. This is because the IJC received several manuscripts in which the authors had copy-and-pasted electropherograms from articles published by other authors.

## Section 6 - Recommended Online Resources

### Section 6 .1 International Cell Line Authentication Committee (ICLAC) Website

The ICLAC website has several recommendations for cell line authentication listed under Resource Documents:

- Cell Line Policy for Research Institutions
- Match Criteria Worksheet for Human Cell Line Authentication.
- Cell Line Checklist for Manuscripts and Grant Applications
- Guide to Human Cell Line Authentication
- Guide to Mouse Cell Line Authentication
- Advice to Scientists; Incorporating Authentication into Everyday Culture Practice
- Naming a Cell Line
- Definitions – Authentication, Cross-contamination, Misidentification
- Register of cross-contaminated or misidentified cell lines
- Links to STR profile databases for Cell Lines at the ATCC, Cellosaurus, the German DSMZ tissue culture facility, and the CLIMA database of STR profile.

### Section 6.2 - Cellosaurus Website Cell Line Knowledge Resource Database with the CLASTR Search Tool for Finding Cell Lines with Matching STR Genotypes

Cellosaurus is a knowledge resource for cell lines. It attempts to describe all cell lines used in biomedical research. Its scope includes:

- Immortalized cell lines
- Naturally immortal cell lines (example: stem cell lines)
- Finite life cell lines when those are distributed and used widely
- Vertebrate cell line with an emphasis on human, mouse and rat cell lines

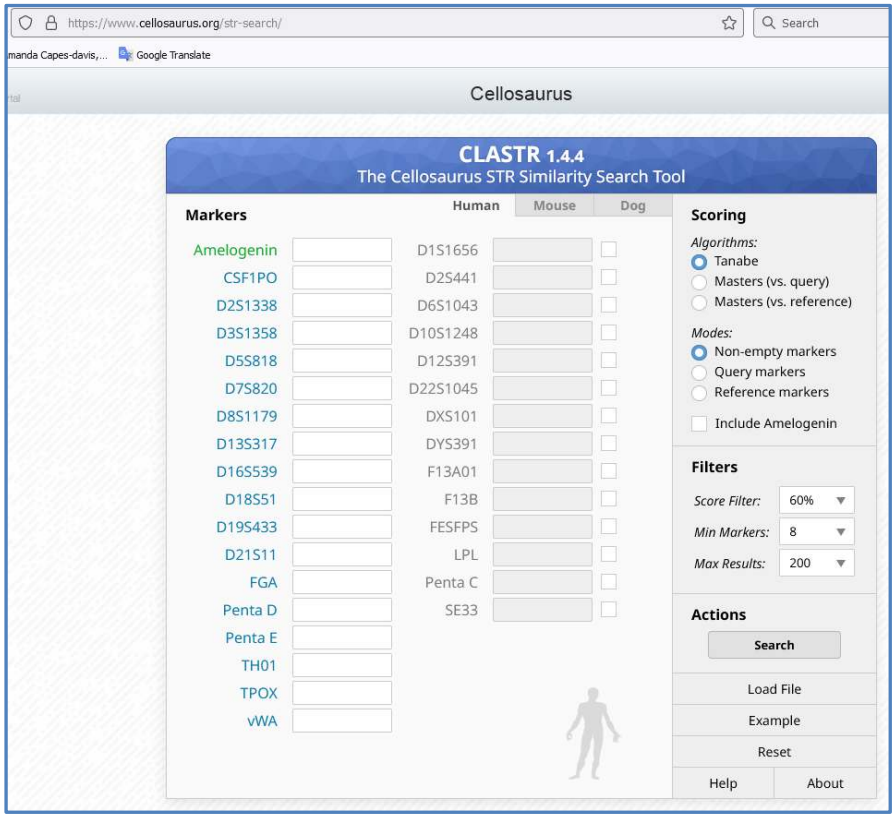
- Invertebrate (insects and ticks) cell lines

Its scope does not include:

- Primary cell lines (with the exception of the finite life cell lines described above)
- Plant cell lines

In addition, it offers the CLASTR - STR similarity search tool (see figure below) which allows the comparison of STR genotypes of human cell lines, mouse cell lines, and dog cell lines. The STR genotypes can be compared using three different algorithms:

- Tanabe algorithm,
- Masters algorithm for comparing reference profiles to query profiles, and
- Alternative Masters for comparing query profiles to reference profiles.




CLASTR STR search tool, available at Cellosaurus (https://www.cellosaurus.org/str-search/)

As of September, 2022, Release 43 contains information about 141,885 cell lines (106,170 of human origin, 21,928 from mouse, and 2,637 from rat). The CLASTR tool can compare one or more query genotype against genotypes for 8,327 human cell lines, 83 mouse cell lines, and 36 dog cell lines.

**Section 6.3 - Published Guidelines for Culturing and Handling of Cell Lines**

- The Guidelines for the use of cell lines in biomedical research by Geraghty et al. [17]
- The 7th edition of *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications* by R. Ian Freshney [16].
- The 8th edition *of Freshney's Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications* by Amanda Capes-Davis and R. Ian Freshney [9]. This replaces the 7th edition cited above.
- The ANSI/ATCC ASN-0002-2022 Authentication of Human Cell Lines: Standardization of STR Profiling of 2022 replaces the standard with a similar name that was published in 2011 [4]. This updated Standard is available online at: https://webstore.ansi.org/standards/atcc/ansiatccasn00022022
- The Assay Guidance Manual (AGM) for Authentication of Human and Mouse Cell Lines by Short Tandem Repeat (STR) DNA Genotype Analysis by Almeida and Korch [3], which is an update of the AGM by Reid et al. [31].

**Section 6.4 - National Institutes of Health (NIH) Notices Pertaining to Cell Lines**

*Notification Regarding Authentication of Cultured Cell Lines, November 28, 2007. NOT-OD-08-017*. In addition, NIH Updated policy (June 2015) *Notification Enhancing Reproducibility through Rigor and Transparency*. and NIH Notices NOT-OD-15-103, NOT-OD-16-011, and NOT-OD-16-012 and the *Principles and Guidelines for Reporting Preclinical Research* .

**Section 6.5 - Other Useful Links including Cell Line Repositories**

- American Type Culture Collection (ATCC) - https://www.atcc.org/
- Biocompare - https://www.biocompare.com/Cell-Line-Authentication/ and https://www.biocompare.com/Reproducibility/Cell-Line-Authentication/
- Biosample - https://www.ncbi.nlm.nih.gov/biosample
- Catalogue of Somatic Mutations in Cancer (COSMIC). Version 97 (29 November 2022) contains curated DNA sequence information on approximately 1,000 cell lines// https://cancer.sanger.ac.uk/cell_lines
- Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) – This organization has a searchable collection of cell line STR genotyping data from DSMZ, ATCC, JCRB, and RIKEN. These data have now been integrated into Cellosaurus. https://www.dsmz.de and https://www.dsmz.de/services/human-and-animal-cell-lines/online-str-analysis.
- The M.D. Anderson's Cell Line Authentication Policy (ACA#1044) requires all researchers to validate their cell lines at least once per year. It is described at the following site: https://www.mdanderson.org/content/dam/mdanderson/documents/core-facilities/Characterized%20Cell%20Line%20Core%20Facility/CCLC_%20Policy_ACA1044.pdf

- JCRB Cell Bank (Japanese Collection of Research Bioresources Cell Bank) of the National Institutes of Biomedical Innovation, Health and Nutrition - https://cellbank.nibiohn.go.jp/english/ is one of the two major repository of cell lines in Japan.
- RIKEN (Rikagaku Kenkyūsho) BioResource Research Center – The cell line repository for a nation-wide group of Japanese institutions that perform research in multiple scientific disciplines, including biomedical research (https://cell.brc.riken.jp/en/). The STR genotypes of its many cell lines are accessible in the databases of STR profiles maintained by the DSMZ and Cellosaurus.

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