# Worksheet: Match Criteria for Human Cell Line Authentication

## Background

Cell lines may undergo genetic drift with passage. This can be minimised through good cell culture practice – for example, by returning to early passage frozen stocks rather than passaging a cell line for prolonged periods. However, even with good cell culture practice, genetic drift is known to occur in some cell lines. This may result in differences when comparing STR profiles from the same donor.

A Standard was recently published on authentication of human cell lines by STR profiling (1). The Standard recommends a set of match criteria, allowing for some degree of genetic drift when interpreting STR profile results from human cell lines. Recommendations in the Standard are supported by more than ten years of publications in this area (2,3).

This worksheet will help you apply the match criteria recommended in the Standard to your sample.

## Step 1: Obtain an STR Profile for your Test Sample

Refer to the Standard for more information on authenticating human cell lines. The quality of the STR profile produced is particularly important for your Test Sample to be interpreted correctly (1). Kits can be purchased by laboratories who wish to develop their own STR profiling. Authentication testing is also performed commercially by various suppliers, including several of the cell repositories.

## Step 2: Identify a Reference Sample for Comparison

To interpret an STR profile result, you must compare to other samples. DNA from the same donor is the best comparison, if available, showing whether the cell line comes from the same donor. If donor DNA is not available, you will need to compare your sample to a range of other cell lines to demonstrate that cross-contamination is unlikely to have occurred. Online interactive databases of STR profiles from human cell lines are currently available for comparison via the [ATCC](http://www.atcc.org/CulturesandProducts/CellBiology/STRProfileDatabase/tabid/174/Default.aspx) and [DSMZ](http://www.dsmz.de/services/services-human-and-animal-cell-lines/online-str-analysis.html) websites. These databases (which require login access) will compare your sample to all available STR profiles (Step 3) and return a list of closest matches.

The “Reference Sample” referred to in this worksheet is defined as a previously authenticated sample, coming from any of these sources.

## Step 3: Compare Test and Reference Samples using the Match Criteria given

### 3a) Enter your STR profile results into Table 3A on the following page

The table normally has space for the eight core STR loci (plus amelogenin for gender determination) recommended as the minimum for authenticating human cell lines (1). If test results have been obtained using a greater number of loci, add additional rows to the table to include all loci tested.

Normally either one or two alleles are amplified at each locus. Two alleles are indicated by two numbers separated by a comma e.g. 7,8. Three or more alleles at some loci are consistent with a mixture, although this finding can occur in the absence of a mixture in a small number of cell lines. Compare to the reference profile before you conclude whether a mixture is present.

### 3b) Perform the calculations listed in Table 3B on the following page

Count the total number of alleles at each locus and add these up to give the TOTAL ALLELES for your Test Sample and Reference Sample. Then add up the number of alleles shared by the Test Sample and the Reference Sample to give the SHARED ALLELES. Finally, use the Match Algorithm to calculate a percent match result for these two samples.

### 3c) Use the questions in Table 3C on the following page to help interpret your result

Comparison of many cell line samples over time has shown that there is a clear gap between related (same donor) and unrelated (different donor) samples for the vast majority of cell lines (1, 2). Related samples generally yield a result in the 80-100 % match range, and unrelated samples in the 0-55 % match range. A small number of samples may give results in the 56-79 % match range. These usually arise from a set of known variable cell lines and may need additional testing to interpret correctly – for example, using an increased number of loci or a different test method.

# Sample Comparison

## Table 3A: STR Profile Results

|  |  |  |
| --- | --- | --- |
|  | ***Test Sample*** | ***Reference Sample*** |
| *Cell line designation**e.g. name and catalog number* |  |  |
| *Date sample collected**(if known)* |  |  |
| *Passage or Population Doubling**(if known)* |  |  |
| *STR Profile Result**(enter name of kit if used)* |  |  |
| **D5S818** |  |  |
| **D13S317** |  |  |
| **D7S820** |  |  |
| **D16S539** |  |  |
| **vWA** |  |  |
| **TH01** |  |  |
| **Amelogenin** |  |  |
| **TPOX** |  |  |
| **CSF1PO** |  |  |

## Table 3B: Percent Match Calculations

|  |  |
| --- | --- |
| TOTAL ALLELES in the Test Sample |  |
| TOTAL ALLELES in the Reference Sample |  |
| SHARED ALLELES, Test and Reference Samples |  |
| Percent Match for Test and Reference Samples |  |

Percent Match can be calculated using an appropriate Match Algorithm (2,3). For this Worksheet:

Match Algorithm = SHARED ALLELES x 2

TOTAL ALLELES in the Test Sample + TOTAL ALLELES in the Reference Sample

Percent Match = Match Algorithm result x 100

## Table 3C: Interpretation of Results

|  |  |
| --- | --- |
| *Are there multiple peaks that would be consistent with a mixture?* | YES/NO |
| *Is the percent match result in the range 0-55 %?*This result is consistent with the two samples being unrelated (different donors) | YES/NO |
| *Is the percent match result in the range 56-79 %?*This result is indeterminant and may need further testing | YES/NO |
| *Is the percent match result in the range 80-100 %?*This result is consistent with the two samples being related (same donor) | YES/NO |

## Notes

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|  |

## References

1. ANSI/ATCC ASN-0002-2011. Authentication of Human Cell Lines: Standardization of STR Profiling. [ANSI eStandards Store](http://webstore.ansi.org/RecordDetail.aspx?sku=ANSI%2fATCC+ASN-0002-2011), 2012.
2. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, Toji LH, Ohno T, Tanabe H, Arlett CF, Kelland LR, Harrison M, et al. Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc Natl Acad Sci U S A* 2001;**98**:8012-7. PMID: 11416159.
3. Tanabe H, Takada Y, Minegishi D, Kurematsu M, Msui T, Mizusawa H. Cell line individualization by STR multiplex system in the cell bank found cross-contamination between ECV304 and EJ-1/T24. *Tiss Cult Res Commun* 1999;**18**:329-38.

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ANSI/ATCC ASN-0002-2011. Authentication of Human Cell Lines: Standardization of STR Profiling. [ANSI eStandards Store](http://webstore.ansi.org/RecordDetail.aspx?sku=ANSI%2fATCC+ASN-0002-2011), 2012.