Abstract

Continuous human cell lines have been used extensively as models for biomedical research. In working with these cell lines, researchers are often unaware of the risk of cross-contamination and other causes of misidentification. To reduce this risk, there is a pressing need to authenticate cell lines, comparing the sample handled in the laboratory to a previously tested sample. The American Type Culture Collection Standards Development Organization Workgroup ASN-0002 has developed a Standard for human cell line authentication, recommending short tandem repeat (STR) profiling for authentication of human cell lines. However, there are known limitations to the technique when applied to cultured samples, including possible genetic drift with passage. In the current study, a dataset of 2279 STR profiles from four cell banks was used to assess the effectiveness of the match criteria recommended within the Standard. Of these 2279 STR profiles, 1157 were grouped into sets of related cell lines – duplicate holdings, legitimately related samples, or misidentified cell lines. Eight core STR loci plus amelogenin were used to unequivocally authenticate 98 % of these related sets. Two simple match algorithms each clearly discriminated between related and unrelated samples, with separation between related samples at ≥80 % match and unrelated samples at <50 % match. A small degree of overlap was noted at 50 % to 79 % match, mostly from cell lines known to display variable STR profiles. These match criteria are recommended as a simple and effective way to interpret results from STR profiling of human cell lines.

Introduction

Research into cancer, and many other life-threatening conditions, relies on the culture of continuous cell lines as effective models for the cell type being studied (1,2). Cell lines cease to be effective models if their behavior changes – for example, due to over-passaging, microbial contamination, or misidentification of the cell line (3,4). Cell line authentication and contaminant testing, and other routine measures to minimize behavioral changes, are an important but often neglected part of good cell culture practice (2).

A cell line is said to be misidentified when its DNA profile is no longer consistent with the individual donor from whom it was first established. Although misidentification has many causes, including accidental substitution of culture samples, it is often caused by cross-contamination – introduction of another cell line into that culture, resulting in overgrowth by the contaminant. Cross-contamination is a common occurrence within the research community. Ongoing work from the German Collection of Microorganisms and Cell Cultures (DSMZ), evaluating deposited leukemia-lymphoma cell lines (n=620), has shown that 31 % of that sample set are contaminated: 18 % with Mycoplasma, 7 % with another cell line, and 6 % with both (5,6). Cross-contamination remains a common occurrence even when cell lines are supplied from the originator's laboratory, suggesting that contamination is frequently an early event – often during establishment of the cell line (5). More than 360 cell lines are known to be cross-contaminated or otherwise misidentified with no known authentic stock, calling into question the validity of any studies involving these cell lines (6).

Misidentified human cell lines, whether due to cross-contamination or other causes, can be detected through authentication testing. Authentication testing aims to compare the test cell line to other samples (either tissue or cultured cells) from the same donor. Correctly authenticated samples may behave differently in culture (i.e., exhibit phenotypic differences), but testing will show that they come from the same donor and are not cross-contaminated by other commonly used cell lines such as HeLa. A variety of methods exist for authentication testing, but short tandem repeat (STR) profiling has been recommended as the most widely used method currently available for human cell lines (7,8).

Human STR profiling relies on a PCR-based assay examining polymorphic tetra- or pentanucleotide repeats (9). The varying number of repeats produces amplified DNA

fragments of different sizes, which are identified and assigned a numerical value after comparison to a set of size standards. The resulting profile is characteristic of that individual and can be entered into a database, where it is readily compared between laboratories so long as appropriate controls and validation are used (7,10). STR profiling was initially shown to be effective for international comparison of cell line samples (7). It was recently recommended by the American Type Culture Collection Standards Development Organization (ATCC SDO) Workgroup ASN-0002 as the best method currently available for human cell line authentication (8,11). The Workgroup has now published a Standard for authentication of human cell lines by STR profiling, approved by the American National Standards Institute (ANSI), aiming to set out sufficient information for laboratories to perform their own testing or to interpret results obtained from testing laboratories (12).

As with all authentication methods, STR profiling has advantages and disadvantages. Advantages include extensive data available through population and forensic studies; standardized kits; and implementation of the technique by cell banks worldwide, resulting in publication of STR profiles for the most commonly used cell lines (9,11). Four of the cell banks have made these STR profiles available through online interactive databases; samples have established provenance, with records of their origin and history kept by the relevant cell bank (13,14). The combined dataset will be used for a global database currently being developed by the ASN-0002 Workgroup and the National Center for Biotechnology Information (NCBI). Researchers will be able to use the NCBI database to compare the STR results for their cell lines to those for other samples of the same cell line and will be able to add STR profiles from their own testing (8,11). Some laboratories have undertaken STR profiling of large cell line panels that would fit well with a combined database approach (15-19). Other groups such as the National Institute of Standards and Technology (NIST) are generating STR profiles specifically for the NCBI database through the Identification of Human Cell Lines Project (20).

The disadvantages of STR profiling include the inability to fully characterize more than a few species using the available kits. Most primer sets used for cell line authentication amplify only human DNA, although primer sets for other species have been developed and both canine and monkey cell lines have been authenticated using a similar approach (21,22). Another disadvantage of STR profiling comes from a tendency for some cell lines to undergo genetic drift with continued passage in culture. STR profiles from malignant tissues are

known to vary, with loss of heterozygosity and a high incidence of microsatellite instability (23,24). In culture, genetic drift may be accentuated by suboptimal culture conditions, for example, if cells are overpassaged or overdiluted when passaged, resulting in selection of variant subclones. Subclone selection through "bottlenecking" can be minimized by the use of low passage cultures, regular feeding and avoidance of overdilution (25).

Most cell lines show only small amounts of genetic drift (7,17,26). Such changes generally relate to loss of heterozygosity rather than changes in alleles (17). However, some cell lines such as CCRF-CEM and Jurkat show considerable variation between subclones, resulting in genetic drift even when good cell culture technique is used (27,28). CCRF-CEM and Jurkat are widely used, and like HeLa, have often been implicated in cases of cross-contamination (29). Thus to interpret authentication test results correctly, one must use standardized match criteria to discriminate between "related" (same donor) and "unrelated" (different donor) samples, with some allowance for genetic drift. Any match criteria must also discriminate effectively between cells derived from different donors; if set incorrectly, samples may be said to come from the same donor when that is not the case.

Match criteria generally incorporate an algorithm to compare two STR profiles (7,16,30-32). One STR profile is defined as the "questioned" profile (the sample being tested), while the other is a "reference" profile, ideally from the same donor. Where another sample from that donor is not available, the questioned STR profile should be compared to other samples from that laboratory and databases online. Comparison to other samples improves the chances of detecting cross-contamination, since most cases of cell line cross-contamination arise from a limited number of commonly used cell lines (5,6). Various match algorithms have been proposed for comparison of DNA fingerprints, some requiring specialized bioinformatics expertise (32,33). Although the more complex algorithms can yield a substantial amount of information on authenticity and instability, early validation of STR profiling has shown that simple match algorithms also work well to discriminate between related and unrelated samples (7,30).

The STR profiles made available by the cell banks generally included eight STR loci plus amelogenin for gender determination (10). Some cell banks and laboratories now use a larger number, typically 16 loci (16,31). To make effective recommendations for the human cell line authentication Standard (12), STR profiles were contributed from four cell banks to give

a combined dataset. The duplicates within the dataset were sorted into sets of "related" (same donor) samples, based on known provenance, and analyzed to determine the minimum number of STR loci when comparing cell line profiles, and the effectiveness of a simple set of match criteria. We also highlighted the commonly used cell lines where match criteria are difficult to apply due to marked genetic drift, and the known misidentified cell lines within the dataset.

Methods

Match Algorithms

Three empirical match algorithms were evaluated for STR profile comparison. The sample undergoing authentication is defined as the "questioned" profile, and a previously authenticated sample used for comparison is defined as the "reference" profile. Homozygous alleles are counted as one allele.

The first match algorithm is referred to as the **Masters algorithm** (7):

Percent match = number shared alleles / total number of alleles in the questioned profile

The second algorithm is a modification of the Masters algorithm:

Percent match = number shared alleles / total number of alleles in the reference profile

The third match algorithm is referred to here as the **Tanabe algorithm** (30) but is more usually known as the Sørensen similarity index or Sørensen-Dice coefficient (34):

Percent match = (number shared alleles x 2) / (total number of alleles in the questioned profile + total number of alleles in the reference profile)

Combined Dataset of STR Profiles

STR profiles for this analysis were contributed by four cell banks: ATCC (n=664), DSMZ (n=465), JCRB (n=577) and RIKEN (n=573), giving a total of 2279 STR profiles. STR profiles were obtained retrospectively, during authentication testing performed by each cell bank as part of its accession process.

Methods used for DNA extraction varied between the cell banks and over time. Extraction methods included spotting onto FTA® cards (Whatman), using FTA Classic or FTA Elute (formerly IsoCode, Schleicher & Schuell), and use of the High Pure PCR Template Preparation Kit (Roche). The PowerPlex® 1.2 System (Promega) was used by all four cell banks for STR analysis. Results were made available for eight core STR loci: D5S818,

D13S317, D7S820, D16S539, vWA, TH01, TPOX and CSF1PO, with the addition of amelogenin as a ninth locus for gender determination. Electropherograms were analyzed and results interpreted by each cell bank, in accordance with their own internal STR method validation and Standard Operating Procedures.

Data were made available anonymously. Donors were deidentified, with the exception of the HeLa cell line, where the donor's identity is in the public domain.

Grouping STR Profiles into Related Cell Line Sets

To identify "related" STR profiles, i.e. those coming from a common donor, entries were tagged by manual sorting of the combined dataset. Entries were sorted by:

1. Cell line designation. All STR profiles associated with the same cell line name, or with the same name followed by a different suffix, were tagged as possibly coming from the same donor. For example, HeLa was tagged along with HeLa 229, HeLa AG, HeLa TG and HeLa S3.
2. STR locus. STR profiles that differed at 0-2 loci were tagged as possibly coming from the same donor.
3. Comparison to a separate database of cross-contaminated or misidentified cell lines (6). Version 6.7 of the database was used for comparison. Cell line designations in common were compared to the published contaminant, and the resulting misidentified cell lines were tagged as coming from the same donor.

The provenance of all tagged entries (14) was assessed, looking for further evidence of relatedness apart from the cell line designation or STR profile. Provenance was determined through the catalogue entry for that cell line and a search of the scientific literature using PubMed. For example, HeLa 229, HeLa AG, HeLa TG and HeLa S3 were all documented as HeLa derivatives on the relevant cell bank websites and through published work (35).

Tagged entries were grouped into related cell line sets. Each set represented all of the STR profiles arising from a single donor. A reference STR profile was selected from each set for further comparison. Normally a reference profile would come from donor tissue, or the parental cell line at the lowest available passage. Because no tissue or passage information

was available for this study, the reference profile was selected arbitrarily from the entries for the parental cell line.

Provenance information was then used to determine relationships between the reference profile and the other STR profiles in that set. STR profiles were grouped into same cell line (duplicate holdings of that cell line across several cell banks); legitimately related cell lines – either derivatives (daughter cell line) or other cell lines established from the same donor (sister cell line) – and known misidentified or cross-contaminated cell lines. In some cases provenance could not be used to establish a relationship between similar STR profiles, and in those cases the entry was labelled as "Relationship Unknown".

Developing a Validated Subset of STR Profiles

To look at the effectiveness of match criteria in distinguishing between unrelated cell lines, a validated subset of STR profiles was used. This subset was initiated as the nucleus for the NCBI database, and consists of cell lines with known identities, whose STR profiles displayed minimal variation when tested by the cell banks contributing to the dataset.

STR profiles were included in this validated subset if two or more cell banks generated identical STR profiles for that cell line. Data were interpreted by the contributing cell bank. The cell lines in the subset were then compared to the database of cross-contaminated or misidentified cell lines (6), using version 6.7 of the database for comparison. Any cell line with evidence for misidentification was excluded before the subset was finalized.

Results

A minimum of eight core STR loci is recommended when comparing STR Profiles

The STR loci used by the cell banks may differ with respect to the specific loci used and the number of loci used, relative to those described in previous studies (7,30). To recommend a minimum number of loci, the ASN-0002 Workgroup examined the number of unique STR profile results within the combined dataset. Relationships between cell line samples were not considered at this stage. For successful authentication, it is important to be able to discriminate between unique STR profiles, just as it is to be able to group related samples together.

Discrimination between unique STR profiles was assessed by progressively reducing the number of loci included in the analysis from nine (eight STR loci plus amelogenin) to one. All possible locus combinations were examined (including elimination of amelogenin), giving a total of 512 combinations across the nine loci evaluated. For each locus combination, the number of unique STR profiles resulting was calculated, expressed as a percentage of the combined dataset (n = 2279). The highest and lowest percentages were recorded for each of the number of loci included in the analysis, giving a range of reductions.

The results are summarized in Figure 1. Nine loci (eight STR loci plus amelogenin) resulted in 77.2 % unique STR profile results within the combined dataset. As might be expected, reduction of the number of loci evaluated resulted in loss of discriminating ability. Some loss of discrimination became evident with removal of one locus; unique STR profile results from eight loci ranged from 76.8 % to 75.5 %. Continued reduction of the number of loci evaluated resulted in a steady loss of discrimination, which was particularly marked when looking at the worst results from all locus combinations.

Because some reduction of discrimination occurred after exclusion of one locus, the recommendation was made that eight core STR loci (plus amelogenin) be used as a minimum when comparing cell line STR profiles. The core loci recommended were those included in this analysis: D5S818, D13S317, D7S820, D16S539, vWA, TH01, TPOX and CSF1PO.

Related cell line samples can be highly variable

To assess the effectiveness of the recommended STR loci for cell line authentication, the combined dataset was examined to identify related STR profiles, i.e. those arising from the same donor. The process is described in the Methods section and summarized in Figure 2.

Relatedness was established for more than half of the combined dataset (n=1157, 50.8 %). A "reference" profile was chosen for each set of related samples (n = 369). Of the remaining STR profiles, approximately one third were duplicate holdings of the same cell line (352/1157, 30.4 %); another third were legitimately related (330/1157, 28.5 %); and almost one in ten samples were known to be misidentified (91/1157, 7.9 %). Misidentification was documented in previous publications or was confirmed during discussion with the relevant cell bank. Misidentified cell lines were clearly labelled as such in the cell bank catalogue or, in other cases, were restricted from distribution to the public.

In a small number of cases (15/1157, 1.3 %), a search of the cell bank catalogues and the literature did not bring up evidence that cell lines were legitimately related. Of these STR profiles, 7/15 were identical to another STR profile in the dataset; all fulfilled the match criteria described in the following section. Because these samples made up only 1 % of the dataset and otherwise fulfilled the match criteria, they were included in data analysis along with the samples for which relatedness could be clearly documented.

The sets of related cell lines were then examined to see how they differed when compared to the reference profile. A total of 369 sets were examined, locus by locus, looking at the maximal locus differences when each profile was compared to the reference profile. The results are shown in Table 1. More than half of the related cell line sets consisted of identical STR profiles (189/369, 51.2 %). Other sets showed more variability. One in four included STR profiles differing at only one locus (93/369, 25.2 %), while one in ten contained STR profiles that differed at three or more loci when compared to the reference profile (37/369, 10.0 %). The latter group included a high proportion of misidentified samples (44/91 misidentified profiles), making this an important group of samples to authenticate correctly.

A simple match algorithm with an 80 % threshold shows relatedness for 98 % of cell lines

A match algorithm is often employed to compare STR profiles from cell line samples, allowing for some degree of genetic drift. We assessed the effectiveness of this approach by applying a commonly used and simple match algorithm, the Masters algorithm (see Methods), to the STR profiles in the related cell line sets. A threshold of 80 % match was used for interpretation of results (7,30).

The results are shown in Table 2. Of all the related cell line sets identified from the combined dataset, 98 % demonstrated percent match between the reference profile and all sample profiles at 80 % or above (362/369, 98.1 %). Four sets included samples displaying percent match in the range 70 % to 79 %, and three had samples displaying <70 % match (total displaying <80 % match in profiles: 7/369, 1.9 %). The seven cell line sets that failed to meet the 80 % match threshold using the Masters algorithm are listed in Supplementary Table 1.

Samples within each set were not equally variable with respect to STR profile match. The seven cell line sets failing to meet the 80 % match threshold with the Masters algorithm included 35 STR profiles; of those profiles, ten fell below the 80 % match threshold. Those ten samples originated from all of the cell banks contributing data (Supplementary Table 1). Six samples came from duplicate holdings of the same cell line, two from legitimately related cell lines, and two from misidentified cell lines (Table 2), suggesting that a more distant relationship between cultures was not the cause of the variability seen in these STR profiles.

Both algorithms tested are effective to show relatedness

Although the Masters algorithm is simple to apply, it can produce different numerical results depending on whether the calculation is based on the number of total alleles in the questioned or reference profiles. Because of this variation, some laboratories prefer to use the Tanabe algorithm (see Methods), which uses the numbers of alleles from both questioned and reference profiles in the calculation. To compare the different approaches, percent match was calculated across all related samples using two additional algorithms. The Masters algorithm was re-applied, but using the total number of alleles in the reference profile rather than in the questioned profile. The last algorithm tested was the Tanabe algorithm. Percent match results for each of the three algorithms across the related cell line sets are compared in Figure 3.

The Masters algorithm resulted in some subtle changes when the calculations were based on the numbers of alleles in the reference profile instead of in the questioned profile – for example, there were fewer results at ≥90 % match (Figure 3). However, almost 98 % of sets gave a percent match of 80 % or above (361/369, 97.8 %). Eight sets failed to meet the 80 % match threshold (8/369, 2.2 %), four at 70 % to 79 % and four at <70 % match. In contrast, the Tanabe algorithm gave a larger number of results at ≥90 % match, but its performance relative to the 80 % threshold was similar (Figure 3). Nearly 99 % of related cell line sets gave a percent match of 80 % or above (364/369, 98.6 %) using the Tanabe algorithm; five sets failed to meet the 80 % match threshold (5/369, 1.4 %), three at 70 % to 79 % and two at <70 % match.

Whichever algorithm was used, close to 98 % of cell line samples were correctly authenticated. However, the profiles for the remaining 2 % of samples were more challenging to interpret correctly. Samples from the related cell line sets that failed to meet

the 80 % match threshold with either algorithm are listed in Supplementary Table 1. Cell lines within these sets have previously been documented as having highly variable STR profiles (7,16,28), and many have known microsatellite instability – for example the leukemia and lymphoma cell lines CCRF-CEM, Jurkat, MOLT-3, MOLT-4, NALM-6 (36) and KCL-22 (37), and the breast carcinoma cell line MT-3 (38).

Results for all known misidentified cell lines in the dataset (n = 91) are listed in Supplementary Table 2. All but two misidentified cell lines gave percent match results at ≥80 % when the contaminant was compared to the reference profile (n = 89), including all HeLa-contaminated samples (n = 32). The two exceptions were RTSG and RMUG-L, both contaminated with SNG-II. Both STR profiles showed more than two allele peaks across at least four loci (Supplementary Table 2), suggesting that these samples were mixtures at the time of testing.

Testing on a subset of unrelated STR profiles shows that an 80 % threshold discriminates effectively between unrelated cell lines

Having examined the effectiveness of an 80 % match threshold when detecting related samples, it was important to assess its ability to discriminate between unrelated samples. To evaluate this, we used a validated subset of the combined dataset containing 223 STR profiles. The combined dataset is likely to contain unrecognised duplicates even after known provenance has been determined, making such a subset necessary for analysis of unrelated samples.

Percent match was calculated for all of the STR profiles in this 223 sample subset, comparing each STR profile to the others in the dataset – a total of 49506 comparisons. Of these comparisons, 282 were found to involve related STR profiles (to same or sister/daughter cell lines) and were excluded from the analysis, leaving 49224 unrelated comparisons. The results are summarized in Figure 4.

All percent match results for unrelated cell lines fell below the 80 % match threshold. Four were found in the 60 % to 69 % match range, and 36 in the 50 % to 59 % range; the remaining comparisons (n = 49184) were each below 50 %. A clear separation can thus be seen between related samples, at ≥80 % match (Figure 3), and unrelated samples at <50 %

match (Figure 4). Note that an overlap can also be seen in the 50 % to 79 % range for a small minority of samples in this dataset.

Discussion

STR profiling is an accepted and reliable method for matching different samples derived from the same human donor (9). However, a tendency towards genetic drift in malignant samples and cultured cells means that clear guidelines are needed for comparison of STR profiles from cell line samples. Researchers using STR profiling for human cell line authentication need to know the minimum number of STR loci that should be used, and how to interpret results to correctly conclude whether samples come from the same donor. Absence of such guidelines mean that laboratories may easily draw incorrect conclusions from authentication data – for example, saying that differences in STR profiles are due to "genomic instability" when cross-contamination has actually occurred. One such example has occurred recently in relation to the T1 neural stem cell line, showing the need for clear recommendations in this area (39,40).

STR profiling has been performed on cell lines over more than ten years and so, inevitably, there are variations in the number of loci and in the specific loci comprising the published profiles. One recent study has questioned the use of only eight STR loci. The authors presented data and performed simulations to show that reliable discrimination between glioma cell lines does not always occur when evaluating eight STR loci plus amelogenin, and that the duplicates present within many databases make interpretation more difficult (31). In the present study, we used the duplicates present within the cell bank holdings and applied known provenance information to generate sets of related cell lines that were then used to analyze these eight STR loci in practice.

Our results are consistent with previous studies showing a clear separation in STR profiles between related and unrelated cell line samples (7,16). When using the Masters algorithm, more than 98 % of related cell line sets (362/369) gave percent match results at 80 % or above; more than 99 % of unrelated comparisons (49184/49224) gave percent match results below 50 %. These results were obtained using eight STR loci plus amelogenin, and involved a large dataset comprising many of the common cancer cell lines, including those most likely to cause cross-contamination (6,13).

Our results also agree with other studies showing that in a small number of cases, determining if two samples are related using STR profiling alone is difficult (28,31). Using the Masters algorithm, 7/369 of related cell line sets gave at least one result at <80 % match; 40/49224 of unrelated comparisons gave results at 50 % to 69 % match. We thus have a documented overlap at 50 % to 79 % match between related and unrelated samples. Although discrimination due to insufficient loci may be responsible, a more likely cause is variation in primer choices, test methods and interpretation of results between the cell banks and over time. STR profiles were obtained retrospectively as part of authentication testing of cell bank holdings over a prolonged period of time (>10 years). A previous study performing STR profiling of 253 cell lines within a single centre did not demonstrate a similar overlap (7), suggesting that laboratory differences are contributory in the current study. A prospective study from a single cell bank or testing organization would help to address this possibility, for example, arising from the NIST Identification of Human Cell Lines Project (20). If such variations are confirmed to contribute to STR profile variability, validation guidelines for all laboratories contributing to the NCBI database of human cell line STR profiles would be essential. Quality criteria for STR profiles have already been developed as part of the human cell line authentication Standard (12).

It should be emphasised that eight core STR loci plus amelogenin are recommended as a minimum number for effective authentication; adding more loci will further increase the discriminatory power of the technique. The discriminatory power of STR analysis depends on the biological nature of each of the loci included, the number of loci, and the population group (12). For the eight core STR loci recommended here, the probability of a random match has been estimated at 2.71×10^{-8} for African American and 1.14×10^{-8} for US Caucasian populations (41). The power of discrimination improves by approximately one order of magnitude for each STR locus added (12); for example, the probability of a random match becomes 5.9×10^{-18} when examining the 15 STR loci of the PowerPlex 16 kit in the US Caucasian population (42). Increasing the number of STR loci used will therefore give greater clarity when comparing cell line samples, but it also increases the risk that the donor may be unambiguously identified if results are compared to other databases online.

Our analysis of a combined dataset containing the cell lines most frequently associated with cross-contamination (6) shows that in most cases, eight core STR loci plus amelogenin

provide sufficient resolving power for effective authentication. A clear separation is seen here between related and unrelated samples even where one or more loci show variation from the reference sample. However, additional loci may be of benefit for a small number of problematic samples. Problematic samples include those related cell line sets with percent match results in the range 50 % to 79 % (n = 7), or those samples at >80 % match with no known relationship (n = 15).

Any percent match result that does occur in the range 50 % to 79 % is best handled by further testing, using additional loci or an alternate test method (31). Six of the sets here that failed to meet the 80 % match threshold included cell lines with known microsatellite instability (36-38). STR loci are known to vary in the presence of microsatellite instability, with the degree of variability depending on the locus being assessed (16,28,43). A greater number of loci may give sufficient data for discrimination, but for cell lines with microsatellite instability, an alternate method such as SNP analysis should also be considered (44).

Previous information on the cell line's provenance should be taken into consideration when performing authentication testing. In some cases, what initially appears to be a case of cross-contamination may prove to be quite otherwise when the history of the cell line is known – for example, showing that a cell line exists under two different names, as with Alexander and PLC/PRF/5 (45). Cell lines from different donors but carrying the same name coincidentally may also cause confusion. Where similarly named cell lines exist, it is advisable for the laboratory that established the later cell line to alter its designation so that each cell line is uniquely identified. Where cell lines are held by a repository, supplying the catalogue number within the Methods section will allow cross-referencing with provenance information collected from the depositor by the cell bank when that cell line was first deposited.

STR profiling will not discriminate between cell lines established from the same donor. Cell lines from different tissues may be distinguishable using phenotypic markers, although it should be noted that phenotypic markers can be affected by the degree of differentiation shown by each cell line and their length of time in culture (1,3). To effectively distinguish between cell lines from the same donor, it may be necessary to use microarray analysis or next-generation sequencing to perform in depth analysis of gene expression patterns, underlying mutations and copy number alterations (46,47).

The ultimate aim of authentication testing is to ensure that cell lines are not misidentified, but rather that they continue to correspond to the individual who first agreed to donate their cells or tissue for research. We have a responsibility to ensure that the donor's gift is used effectively and guarded from misuse. Authentication testing of human cell lines through STR profiling offers an excellent solution, but as with all test methods, results must be interpreted carefully and considered in context. Clear guidelines for authentication testing, documentation of cell line provenance and ongoing validation will help ensure that human cell lines are effective and representative models for biomedical research.

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Figure Legends

Figure 1. Discrimination between STR profiles based on the number of loci used

The combined dataset (n = 2279) comprised results from eight STR loci (D5S818, D13S317, D7S820, D16S539, vWA, TH01, TPOX and CSF1PO), with the addition of amelogenin for gender determination. To assess discrimination, the number of unique STR profiles existing within the combined dataset with these nine loci was calculated and expressed as a percentage of the total number of STR profiles in the combined dataset. The number of loci was then progressively reduced from nine to one and the number of unique STR profiles calculated for each possible locus combination (512 combinations). The "best result" (highest) and "worst result" (lowest) are recorded for each locus number.

Figure 2. Grouping STR profiles into related cell line sets

The combined dataset of STR profiles was sorted into related cell line sets using the process shown. The process is described further in the Methods section.

Figure 3. Comparison of match algorithms

Percent match results obtained when comparing related cell line sets using different algorithms. Samples are compared using the Masters algorithm, using the total number of alleles from the questioned profile in the calculation; the Masters algorithm, using the total number of alleles from the reference profile in the calculation; and the Tanabe algorithm. The lowest percent match result for each set is recorded here.

Figure 4. Comparison of unrelated cell lines

Percent match results obtained when comparing unrelated cell line samples. A subset of 223 validated samples, with all related samples removed, was used. Each sample was compared to all of the others in the subset, resulting in a total of 49224 unrelated results.