Standards for Cell Line Authentication and Beyond

Jamie L. Almeida, Kenneth D. Cole, Anne L. Plant*

Biosystems and Biomaterials Division, The National Institute of Standards and Technology, Gaithersburg, Maryland, United States of America

* anne.plant@nist.gov

Abstract

Different genomic technologies have been applied to cell line authentication, but only one method (short tandem repeat [STR] profiling) has been the subject of a comprehensive and definitive standard (ASN-0002). Here we discuss the power of this document and why standards such as this are so critical for establishing the consensus technical criteria and practices that can enable progress in the fields of research that use cell lines. We also examine other methods that could be used for authentication and discuss how a combination of methods could be used in a holistic fashion to assess various critical aspects of the quality of cell lines.

A Comprehensive Approach to Assessing the Quality of Human and Nonhuman Cell Lines

Cell line authentication is still poorly reported, despite the prevalence of misidentification, the calls for community action, and the estimates of wasted research dollars [1–6]. Studies that are carried out with misidentified cell lines add misinformation to the literature, are likely not to be reproducible, and can spur additional studies that are also of questionable value [7]. The general reluctance by research labs to perform and report results that establish the identity and purity of their cell lines is one of the contributing factors to irreproducibility of biomedical research results [8,9]. Cell line authentication is an example of the kind of data that add confidence to the results of a scientific study. The lack of reporting of cell line authentication data reflects a broader failure to appreciate the need for more complete reporting of experimental details that qualify data and provide confidence in research results [10]. Without sufficient control data that provide assurance that the study results are based on reasonable assumptions, the value of the data is questionable. To establish a high level of confidence that published data are reliable and can be confidently built on by others, data that support assumptions about reagents, instrument performance, assay validity, software algorithm accuracy, and cell lines are critical [10]. One might argue that these control data are as important as the study data themselves.

The critical nature of the issue of cell line authentication has led to requirements being put in place by funding agencies and publishers for authentication and purity of cell lines. The
National Institutes of Health (NIH) has revised guidelines to applications for funding [11] and provided guidelines for reporting that many journals (which are listed in the announcement) have endorsed [12]. Since 2013, the Nature publishing group has required authors to report the status of the authentication of cell lines used. A web site (http://www.scoop.it/t/cell-line-contamination/p/4040895974/2015/04/08/which-journals-ask-for-cell-line-authentication) lists journals with authentication policies and links to those policies. The Prostate Cancer Foundation has had an initiative requiring cell line authentication and contamination testing for grantees since 2013, and since then a campaign to encourage cell line authentication has been initiated by the Global Biological Standards Institute [13] with cosignatories that include a number of nonprofit research funding organizations. The University of Texas MD Anderson Cancer Center has an institutional policy requiring cell line testing. Workshops on the topic include the NIH Workshop on Reproducibility in Cell Culture Studies [14].

Why are more labs not authenticating their human cell lines? Possibly, many researchers are unaware of the existence of a documentary standard that codifies specific guidance and laboratory protocols and the availability of authentication services that can make the process relatively easy. The American National Standards Institute (ANSI) and the American Type Culture Collection (ATCC) provide a documentary standard (ASN-0002) for human cell lines based on the use of short tandem repeats (STR) entitled \textit{Authentication of Human Cell Lines: Standardization of STR Profiling}. This is a very thorough and helpful document that explains why, and precisely how, to authenticate human cell lines [15], and provides a definitive compilation of methods and results from recognized experts.

Unlike the situation for human cell lines, authentication of nonhuman cell lines is less clear and is burdened by the immature state of development of kits, testing services, databases, and accepted standards for STR or other DNA markers.

In addition to authentication, there are other issues that should be considered for assessing the quality of an experimental cell line. By quality, we mean those characteristics that allow assessment of the appropriateness of the cell line for its purpose. As we consider how to develop and encourage the use of methods and standards for qualifying cell lines, we should keep in mind that the use of cell lines in biomedical research is only one application that requires quality assessment. The need for quality assessment of cell lines that are used for clinical products in the form of cell and/or gene therapies and for production of pharmaceutical products will inform and be informed by the research community’s needs for rapid and reliable quality control data. These methods and standards may also find application to identification and quality assurance of biopsied tissues and food products. Similarly, cell lines modified in the context of synthetic biology also require technologies for qualification.

Here we suggest a comprehensive view of cell line assessment in order to lay out a larger landscape that might be considered as the community makes decisions about technologies and standards in this arena.

\textbf{Comprehensive Assessment of the Quality of Cell Lines}

There are a number of methods available that provide relevant data on the quality of cell lines, and some of the more commonly used methods appear in Table 1. Karyotyping is most often used for detection of gross abnormalities in chromosomes, but chromosome number was the basis of early reports of cell line misidentification [16]. Karyotyping is a specialized technique requiring high technical expertise, time, and significant expense. DNA sequence-based methods such as STR profiling [17,18], single nucleotide polymorphisms (SNP) profiling [19–21], and the use of species-specific primers [22–24] allow detection of mutations, genetic drift, and the presence of adventitious agents [25]. Verification of the species of a cell line can be
performed by probing the sequence associated with species-specific cytochrome c oxidase subunit 1 (CO1), commonly referred to as DNA barcoding [26,27], or by employing PCR assays with other species-specific primers [23].

Sequence-specific profiles of SNP and STR provide the power to discriminate between individuals of the same species. Interspecies contamination can also be detected with these methods if appropriate primers or probes are included in the test. Whole genome sequencing (WGS) is a powerful technique that, in principle, can provide information about species identity as well as the presence of mutations and contaminating species, including adventitious agents. At the current time, the technical and bioinformatic resources required make WGS expensive; however, as the cost continues to decrease for sequencing and bioinformatic approaches become more routine, this approach will become more practical for routine characterization of cell lines. Phenotypic characteristics such as morphology or gene expression profiles can also be indicative but can be strongly influenced by the culture environment, so strict experimental controls would have to be in place to conclude misidentification. But if one wants to reproduce an experiment, quantitative phenotypic and gene expression profiles can support a claim that cell culture environment is highly similar. Depending on circumstances, it is possible that all of the methods listed in Table 1 would be appropriate for thoroughly assessing a particular cell line. No one method will provide all of the qualifying information that might be desired. As the biomedical community considers how to approach comprehensive cell line qualification, all the methods listed are likely to play a role. Some methods, such as STR profiling and DNA barcoding, are better developed than others at this time, and standards based on them can be developed and put into practice more quickly for qualifying cell lines than can other methods.

The development of community-vetted documentary standards for these methods can play an important role in their acceptance and ease of use. The ANSI/ATCC document on STR profiling for cell line authentication that was developed by an international working group of experts led by ATCC and contributed to by NIST and others is an excellent example of how to

| Quality assurance metric                      | CO1 Barcode | Karyotype | STR | SNP | Species-specific primers* | WGS** |
|----------------------------------------------|-------------|-----------|-----|-----|----------------------------|-------|
| Species identification                       | X           |           | X   | X   | X                          | X     |
| Identity of donor individual                 | X           |           | X   | X   | X                          | X     |
| Large chromosomal structural changes         | X           |           |     |     | X                          | X     |
| Spontaneous mutations/genetic drift          | X           |           | X   | X   | X                          | X     |
| Contamination with adventitious agents*      | X           |           |     |     | X                          | X     |
| Interspecies contamination                   | X           | X         | X   | X   | X                          | X     |
| Intraspecies contamination                   | X           |           | X   | X   | X                          | X     |

* [22]

** Whole genome sequencing (WGS) is capable of addressing all of these metrics provided there is sufficient bioinformatics support for the interrogation of genomic sequence databases.

% Chromosome number was the basis of early reports of cell line misidentification [16].

@ These methods will only be able to detect mutations in the regions of DNA that are covered by the probes or amplicons in the specific assay.

* Other tests (including enzymatic) that are specific for mycoplasma are readily available and are strongly recommended to determine this frequent contamination.

# If probes or primers specific for other species are included in the assays.

** Changes due to culture conditions and independent of any genomic changes would have to be carefully controlled for. Quantitative methods for assessing would need to be accompanied by benchmarks that allow comparison of data between laboratories.

doi:10.1371/journal.pbio.1002476.t001
develop comprehensive standards. While most if not all of the supporting information for the standard is provided in traditional peer-reviewed publications such as [28], official standards produced under the auspices of ANSI and ANSI-accredited standard development organizations (SDOs) such as ATCC are developed under a formal framework that ensures that appropriate principles and procedures are followed. The formality of the process adds to the credibility of the product and provides confidence to the community that the recommendations are the result of fully expert consideration and consensus. ANSI standards require the participation of all major stakeholders in their development, and comments from the public are generally encouraged. The formality of the process enhances the level of confidence the community has in the resulting document. Because of the adherence to a formal prescribed process, the inclusivity of opinions, the level of knowledge from the contributing experts and the cited literature, and the completeness of the document in terms of rationale, methodologies, and caveats, the ASN-0002 document is a dependable and definitive resource for guiding the community in implementing these methods in a manner in which the results and interpretations from different laboratories can be compared and trusted. The copyright for the standard document is held by ATCC, and the document is readily available for a fee of $240; the fee helps to offset the administrative costs incurred during development and distribution. The standard document resulted from an effort by an international community of 25 experts, who are listed in S1 Text.

**Human Cell Line Authentication: STR Profiling**

For the development of ASN-0002, STR profiling was chosen as the preferred method for human cell line authentication because of the extensive work that has been done in human identification for forensics purposes. Forensic identification is almost exclusively based on STR analysis for the following reasons: (1) discriminatory power, (2) established testing infrastructure, (3) cost, (4) effectiveness for degraded DNA samples, (5) comparability of STR profiling data from various platforms, and (6) ability to detect human DNA mixtures [29]. As a result of work in forensics, STR markers that provide good discriminatory value are available for human cell line authentication, and services for paternity and forensics testing are readily adaptable to human cell line authentication.

The ASN-0002 documentary standard is summarized in an electronic book that is available online [30] at [http://www.ncbi.nlm.nih.gov/books/NBK144066/](http://www.ncbi.nlm.nih.gov/books/NBK144066/). STR profiling was selected as the basis of a standard for human cell line authentication because of its ability to discriminate human cell lines with a relatively small number of allelic markers; comparable data can be achieved across different laboratories, making it feasible to establish a database against which data can be compared; STR profiling kits are available so tests can be run in individual laboratories; it is a relatively rapid and low-cost test; and it doesn’t require as high a level of technical expertise as some other methods.

The standard consists of over 100 pages that include detailed protocols for DNA extraction, STR profiling, data analysis, quality control of the data, interpretation of results, and implementation of a public searchable database. Different methods are compared, all necessary algorithms are provided, and guidance on how to evaluate and interpret data and troubleshoot anomalies is described. How to assess the quality of the data and the interpretation are addressed rigorously, including the use of an allelic ladder (a DNA mixture containing the different alleles permitting calibration), how to assess assay artifacts such as stutter and allelic dropout, appropriate controls, accurate quantification of DNA, etc. A carefully considered basis for assigning a "match" or "mismatch" is based on a matching metric of ≥80% [31]. The careful analysis of what STR typing will provide and what its limitation are vis a vis cell line
authentication and the detailed protocols provided make this document a vital resource for the practitioner even beyond its usefulness as a standard. The document is a source of concepts and a thorough reference for good laboratory practice. It is a document that should be familiar to every laboratory performing work with cell lines or providing services. This document also provides a basis for additional standards and refinements that take into account new data and knowledge as they become available. For example, a question regarding differences in the algorithms for determination of matches has recently appeared [32]. The existence of an expert group that is responsible for the formal document provides an excellent forum for discussions and clarifications as needed.

As a result of this standard, the STR profiles of many human cell lines have been collected by the Biological Resource Centers (BRCs), namely the ATCC, the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), the Japanese Collection of Research Bioresources (JCRB), and RIKEN, and these data are available in public databases (http://www.atcc.org/STR%20Database.aspx; http://www.jhsf.or.jp/bank/Category-Index.html; https://www.dsmz.de/services/services-human-and-animal-cell-lines/online-str-analysis.html). The DSMZ database allows profile searching against cell lines from all of these databases. The existence of STR profile databases is critical for providing a benchmark profile against which a user can compare their cell line by searching by name of the cell line or by STR profile. The National Center for Biotechnology Information (NCBI) also hosts the BioSample database (http://www.ncbi.nlm.nih.gov/biosample/), which contains the STR profiles of the most frequently used cell lines from the BRCs (listed above) as well as the International Cell Line Authentication Committee (ICLAC) database (http://iclac.org/databases/cross-contaminations/), which list human cell lines that are misidentified based on their STR profiles.

At this time, there is no definitive determination regarding how often authentication and contamination testing should be performed, although a number of publications have addressed this issue [1]. As more testing is done, collection and consolidation of supporting data may provide evidence on which recommendations or standards could be based.

Alternative Methods for Human and Nonhuman Cell Line Authentication

While STR profiling is the only method that is part of a formal consensus standard for distinguishing cell lines from the same species, this standard pertains only to human cell lines. **Table 2** compares the state of development of the common methods used to establish the identity of cell lines for human and non-human species.

Standards for using SNP markers for authentication of human cell lines have been proposed [20,21] and some data have been collected (http://www.ncbi.nlm.nih.gov/snp). Standard methods for CO1 DNA barcoding for distinguishing animal cell lines at the level of species have been developed by the Consortium for the Barcode of Life [35]. The ATCC/ANSI standard (ASN-0003) for DNA barcoding entitled *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit I (CO1) DNA Barcodes* is now available [36]. The contributors to this standard are listed in **S2 Text**. DNA barcoding is extremely useful in determining the species of a cell line by targeting the CO1 gene and searching the databases (BOLD, http://www.barcodinglife.org, and NCBI, http://www.ncbi.nlm.nih.gov/genbank/barcode) for matches. Species-specific primer assays [22,23] provide a quick and inexpensive way for a lab to confirm the species identity of a sample by running a multiplex PCR assay and separating the amplicons by fragment length. The only species that will be detected are those that are represented in the assay. Not all animal species are included in the assays. A consensus standard for the use of species-specific multiplex PCR is being organized by ANSI/ATCC.
Species-specific primers have the advantages that they use low-cost instrumentation and the analysis is straightforward, but they do not allow cell line identity testing or detection of intraspecies contamination.

**Authentication of Nonhuman Cell Lines**

When considering authentication of nonhuman cell lines, the extensive body of technical knowledge on STR profiling, which is captured in the ANSI standard and by other organizations (e.g., The International Society for Forensic Genetics), and the proven record of practicality and fit-for-purpose of STR profiling, make it an obvious choice for consideration as a standard method for nonhuman cell lines.

A number of issues that accompanied the development of the ANSI/ATCC standard for human cell lines will have to be considered for nonhuman cell lines. These include the following: identifying reliable polymorphic STR markers that provide intraspecies discrimination; having many laboratories collect STR data on the same cell lines, establishing and accumulating consensus data for informative STR markers that produce reliable results to consistently characterize a cell line; determining the metrics by which the raw data will be deemed to be of sufficient quality; and determining the best methods for assessing concordance in STR marker data and what level of concordance is sufficient to call a “match.” These are tasks that will need to be undertaken in a community effort. STR databases for nonhuman species need to be developed using this process.

Having community-vetted documentary standards is a critical factor that is helpful for facilitating implementation of cell line authentication. Table 2 summarizes the progress so far on the tools that are beginning to appear for authentication of human and most commonly used nonhuman cell lines and indicates the existing gaps. For mouse cell lines, SNP [19] and STR [33] markers have been identified, and STR markers have been identified for African green monkey [34]; markers are under development for Chinese hamster ovary (CHO) and rat cell lines. However, comprehensive testing of existing markers for their discriminatory power and

---

**Table 2. Current status of SNP, STR, and DNA barcode technologies as standard methods for assessing the identity of cell lines from different species.**

| Species                  | Assays      | Consensus Standard Method | Commercially Available Kit | Commercial Service | Comparative Data |
|--------------------------|-------------|---------------------------|---------------------------|--------------------|------------------|
| Human                    | STR         | ASN-0002                  | Yes                       | Yes                | ATCC, DSMZ, JCRB, NCBI** |
| Mouse                    | SNP         | No                        | No                        | Yes                | [21], [32], NCBI  |
| African green monkey     | STR*        | No                        | No                        | No                 | None             |
| Chinese hamster ovary    | STR*        | No                        | No                        | No                 | None             |
| Rat                      | STR*        | No                        | No                        | No                 | None             |
| Species-level identification | CO1 DNA barcode | ASN-0003                  | Yes                       | Yes                | Barcode of Life Data System, NCBI** |
| Species-specific primers | No          | No                        | Yes                       | None               | None needed      |

These methods are currently the most developed for this application. There are extensive data on human cell lines, but while there are some kits and services for some nonhuman species, there is little available data for nonhuman species, except for DNA barcoding, which only distinguishes cell lines on the basis of species, not individuals.

* STR markers have been identified [33,34]. Markers for rat and Chinese hamster ovary cells are still under development by NIST.

** These sources contain a significant amount of data from multiple sources. See text for URLs.

*doi:10.1371/journal.pbio.1002476.t002*
databases for different cell lines against which researchers can compare their cell lines need to be developed. As authentication becomes common practice in more laboratories, it will likely be made easier, because more companies will provide the needed services. Only a small number of companies currently provide STR profiling services for mouse cell lines. To spur the development and availability of services, a consortium of organizations and companies is being planned to create an STR marker kit and compile data on STR profiles of mouse cell lines to develop a public database.

Conclusions

Measurements can provide confidence in the use of cell lines. Community action around how to apply measurements reliably and the development of comparative data are key to achieving a comprehensive toolkit for assuring the quality of cell lines. The existence of the ANSI/ATCC standard (ASN-0002) for STR profiling of human cell lines and ASN-0003 for species barcoding are critical first steps and provide excellent examples that can be built on. Similar activities should be undertaken for the other methods discussed here.

Despite the existence of the very thorough and highly vetted documentary standard, human cell line authentication is still not commonly performed. In part, this may be due to the lack of awareness of this document and the need for increased education and training programs on cell line authentication. The ASN-0002 document could provide an excellent basis for educational materials to be used for training in procedures for achieving robust qualifying data. It is also likely that greater availability of contract testing services and clearer funding and publishing priorities will be required to stimulate the application of the capabilities and standards that are already in place for human cell line identification and to spur the development of similar standards for the important nonhuman cell lines. Consensus standards that are produced in a careful, open, and official process are an integral part of the success of this endeavor. Standards help to assure that data are sharable and can be the basis of decision-making and compliance. The collection of high-quality data from a large range of cell lines and different labs is an initial necessary step. What will follow are consensus standards and access to the data, which will facilitate labs and companies to provide the testing services and encourage the widespread adoption of this essential part of quality assurance of any process that utilizes cell lines.

Supporting Information

S1 Text. Participants in the Working Group for ASN-0002, Authentication of Human Cell Lines: Standardization of STR Profiling.

S2 Text. Participants in the Working Group for ASN-0003, Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes.

Acknowledgments

Any mention of commercial products is for information only; it does not imply recommendation or endorsement by NIST.

References

1. Hughes P, Marshall D, Reid Y, Parkes H, Gelber C. The costs of using unauthenticated, over-passaged cell lines: how much more data do we need? BioTechniques. 2007; 43(5):575, 7–8, 81–2 passim. Epub 2007/12/13. PMID: 18072586.
2. Stacey GN. Cell contamination leads to inaccurate data: we must take action now. Nature. 2000; 403 (6768):356. Epub 2000/02/10. doi: 10.1038/3500394 PMID: 10667765.

3. Masters JR. False cell lines: The problem and a solution. Cytotechnology. 2002; 39(2):69–74. Epub 2000/11/13. doi: 10.1023/a:1022908930937 PMID: 19003294; PubMed Central PMCID: PMC3463980.

4. Nelson-Rees WA, Flandermeyer RR. HeLa cultures defined. Science (New York, NY). 1976; 191 (4222):96–8. Epub 1976/01/09. PMID: 1246601.

5. Freedman L. Irreproducibility: A $28B/Year Problem with some Tangible Solutions. GEN. 2015; June 30.

6. Freedman LP, Gibson MC, Ethier SP, Soule HR, Neve RM, Reid YA. Reproducibility: changing the policies and culture of cell line authentication. Nature methods. 2015; 12(6):493–7. Epub 2015/05/29. doi: 10.1038/nmeth.3403 PMID: 26020501.

7. Neimark J. Line of attack. Science (New York, NY). 2015; 347:938–40.

8. Dirks WG, MacLeod RAF. Nakamura Y, Kohara A, Reid Y, Milch H, et al. Cell line cross-contamination initiative: an interactive reference database of STR profiles covering common cancer cell lines. Int J Cancer. 2010; 126:302–4.

9. Freedman LP, Gibson M, C, Wisman R, Ethier SP, Soule HR, Reid YA, et al. The culture of cell culture practices and authentication—Results from a 2015 Survey. Biotechniques. 2015; 59:189–90. doi: 10.2144/000114344 PMID: 26458546

10. Plant AL, Locascio LE, May WE, Gallagher PD. Improved reproducibility by assuring confidence in measurements in biomedical research. Nature methods. 2014; 11:895–8. doi: 10.1038/nmeth.3076 PMID: 25166868

11. Enhanced Reproducibility through Rigor and Transparency (effective Jan. 25, 2016) Notice Number: NOT-OD-15-103 (published June 9, 2015). http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html

12. NIH Rigor and Reproducibility: Principles and Guidelines for Reporting Preclinical Research and Endorsement by major journals. http://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research.

13. Global Biological Standards Institute, Authenticate. http://www.gbsi.org/work/authenticate/.

14. National Institutes of Health, Rigor and Reproducibility, Meetings and Workshops. http://www.nih.gov/research-training/rigor-reproducibility/meetings-workshops.

15. ANSI/ATCC ASN-0002-2011, Authentication of Human Cell Lines: Standardization of STR Profiling. http://webstore.ansi.org/RecordDetail.aspx?sku=ANSI%2fATCC+ASN-0002-2011.

16. Nelson-Rees WA, Daniels DW, Flandermeyer RR. Cross-contamination of cells in culture. Science (New York, NY), 1981; 212:446–52.

17. Dirks WG, Drexler HG. Authentication of cancer cell lines by DNA fingerprinting. Methods Mol Med 2004; 88:43–55. PMID: 14634217

18. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, et al. Short tandem repeat profiling provides an international reference standard for human cell lines. Proc Natl Acad Sci USA. 2001; 98:8012–7. PMID: 11416159

19. Didion JP, Buus RJ, Naghashfar Z, Threadgill DW, Morse HC 3rd, de Villena FP. SNP array profiling of mouse cell lines identifies their strains of origin and reveals cross-contamination and widespread aneuploidy. BMC genomics. 2014; 15:847. Epub 2014/10/04. doi: 10.1186/1471-2164-15-847 PMID: 25277546; PubMed Central PMCID: PMC4198738.

20. Castro F, Dirks WG, Fahnhrich S, Hotz-Wagenblatt A, Pawlita M, Schmitt M. High-throughput SNP-based authentication of human cell lines. International journal of cancer Journal international du cancer. 2013; 132(2):308–14. Epub 2012/06/16. doi: 10.1002/ijc.27675 PMID: 22700458; PubMed Central PMCID: PMC3492511.

21. Liang-Chu MM, Yu M, Haverty PM, Koeman J, Ziegler J, Lee M, et al. Human biosample authentication using the high-throughput, cost-effective SNPtrace(TM) system. PLoS ONE. 2015; 10(2):e0116218. Epub 2015/02/26. doi: 10.1371/journal.pone.0116218 PMID: 25714623; PubMed Central PMCID: PMC4340925.

22. Parodi B, Aresu O, Bini D, Lorenzini R, Schena F, Visconti P, et al. Species identification and confirmation of human and animal cell lines: a PCR-based method. BioTechniques. 2002; 32:438–40.

23. Cooper JK, Sykes G, King S, Cottrill K, Ivanova NV, Hanner R, et al. Species identification in cell culture: a two-pronged molecular approach. In vitro cellular & developmental biology Animal. 2007; 43(10):344–51. Epub 2007/10/16. doi: 10.1007/s11626-007-9060-2 PMID: 17934781.
24. Liu M, Liu H, Tang X, Vafai A. Rapid identification and authentication of closely related animal cell culture by polymerase chain reaction. In Vitro Cell Dev Biol Anim 2008; 44:224–7. doi:10.1007/s11626-008-9121-1 PMID: 18553210

25. Pruckler JM, Pruckler JM, Ades EW. Detection by polymerase chain reaction of all common Mycoplasma in a cell culture facility. Pathobiology. 1995; 63:9–11. PMID: 7546276

26. Herbert PDN, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA barcodes. Proc R Soc Lond B. 2003a; 270:313–21.

27. Herbert PDN, Ratnasingham S, deWaard J, R. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc Lond B 270 Suppl 1 2003b;270 Suppl. 1:S96–S9.

28. Alston-Roberts C BR, Bauer SR, Butler J, Capes-Davis A, Dirks WG, Elmore E, Furtado M, Kerrigan L, Kline MC, Kohara A, Los GV, MacLeod RA, Masters JR, Nardone M, Nardone RM, Nims RW, Price PJ, Reid YA, Shewale J, Steuer AF, Storts DR, Sykes G, Taraporewala Z, Thomson J. Cell line misidentification: the beginning of the end. Nature reviews Cancer. 2010; 10(6):441–8. Epub 2010/05/08. PMID: 20448633. doi:10.1038/nrc2852

29. Butler J. Fundamentals of Forensic DNA Typing. San Diego: Academic Press; 2010.

30. Reid Y, Storts D, Riss T, Minor L. Authentication of Human Cell Lines by STR DNA Profiling Analysis. Bethesda (MD): NCBI.

31. Capes-Davis A, Reid YA, Kline MC, Storts DR, Strauss E, Dirks WG, et al. Match criteria for human cell line authentication: Where do we draw the line? Int J Cancer 2013; 132:2510–9. doi:10.1002/ijc.27931 PMID: 23136038

32. Yu M, Selvaraj SK, Liang-Chu M, Aghajani S, Busse M, Yuan J, et al. A resource for cell line authentication, annotation and quality control. Nature. 2015; 520:307–11. doi:10.1038/nature14397 PMID: 25877200

33. Almeida JL, Hill CR, Cole KD. Mouse cell line authentication. Cytotechnology. 2014; 66(1):133–47. Epub 2013/02/23. doi: 10.1007/s10616-013-9545-7 PMID: 23430347; PubMed Central PMCID: PMCPMC3886540.

34. Almeida JL, Hill CR, Cole KD. Authentication of African green monkey cell lines using human short tandem repeat markers. BMC biotechnology. 2011; 11:102. Epub 2011/11/09. doi: 10.1186/1472-6750-11-102 PMID: 22059503; PubMed Central PMCID: PMCPMC3221628.

35. Barcode of Life, Identifying Species with DNA Barcoding. http://www.barcodeoflife.org/.

36. Cooper JK, Almeida JL, Bauer BA, Burnett EC, Cole KD, Cooper JW, et al. ANSI/ASN-0003-2015, Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes. 2015; http://webstore.ansi.org/RecordDetail.aspx?sku=ANSI%2fATCC+ASN-0003-2015.