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Competing interests statement
The authors declare no competing financial interests.

DATABASES
OMIM: http://www.ncbi.nlm.nih.gov/omim
keratitis–ichthyosis–deafness
UniProtKB: http://www.uniprot.org
caveolin 1 | Cx26 | Cx32 | Cx37 | Cx43 | Cx45 | N-cadherin | NOV | TSG101

FURTHER INFORMATION
Christian C. Naus' homepage:
http://www.cellphys.ubc.ca/faculty_pages/naus.html
Dale W. Laird's homepage:
http://www.uwo.ca/anatomy/laird/index.htm

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MODELS OF CANCER SERIES — SCIENCE AND SOCIETY

Cell line misidentification: the beginning of the end

American Type Culture Collection Standards Development Organization Workgroup ASN-0002

Abstract | Cell lines are used extensively in research and drug development as models of normal and cancer tissues. However, a substantial proportion of cell lines is mislabelled or replaced by cells derived from a different individual, tissue or species. The scientific community has failed to tackle this problem and consequently thousands of misleading and potentially erroneous papers have been published using cell lines that are incorrectly identified. Recent efforts to develop a standard for the authentication of human cell lines using short tandem repeat profiling is an important step to eradicate this problem.

Cell lines are used extensively in biomedical research as *in vitro* models. The validity of the data obtained often depends on the identity of the cell line, particularly when it is being used as a surrogate for the tissue of origin. Surprisingly, the frequency of cell line misidentification is high, and consequently the ascribed origin of a cell line is often incorrect. This problem has been known for over 50 years and has been described as the most compelling quality-control issue confronting the scientific community[1]. Based on analyses of cell lines submitted to international cell banks, the incidence of misidentification in 1977 was 16%[2] and in 1999 was 18%[3]. Until recently, the authenticity of cell lines used in biomedical research has received little attention. This Science and Society article has been written by the members of the American Type Culture Collection (ATCC) Standards Development Organization (SDO) Workgroup ASN-0002

(BOX 1), a working group currently developing a standard for human cell line authentication. The ATCC SDO was formed in 2007 to develop best practices (standards) for use in the life sciences and to promote their use globally, using a consensus-driven process that balances the viewpoints of industry, government, regulatory agencies and academia. We expect that the draft standard (BOX 2) will be available for public review and comment in 2010 and subsequently the final draft will be approved by the American National Standards Institute (ANSI).

Here we describe the causes and scientific effects of cell line misidentification, its history and the efforts taken to solve the problem. The various methods currently available for authenticating cell lines are discussed and a recommendation is made for the use of short tandem repeat (STR) profiling for authenticating human cell lines. Perhaps of the greatest importance, a universal database of human cell line STR profiles is under construction.

Discovery of cell line misidentification

Misidentification of human and animal cell cultures is a long-standing problem, and awareness of this problem dates back to the 1950s (TIMELINE). Karyotyping and immunological approaches were first used for cell line authentication[4–6]. Extensive species misidentification was reported, leading to the establishment of a bank of authenticated cell lines at the ATCC in 1962.

Misidentification within species could not be detected in 1962, but in 1966 Stanley Gartler (FIG. 1a) introduced the concept of biochemical polymorphisms to distinguish human cell lines on the basis of their isozyme expression. At the Second Decennial Review Conference on Cell, Tissue and Organ Culture in 1966, Gartler reported that 18 human cell lines supposedly of independent origins were all HeLa cells[7], the first human cancer cell line to be established in culture[8]. The examples included cells claimed to be derived from normal intestinal epithelium (Int-407), normal amnion (WISH), normal liver (Chang liver), laryngeal cancer (Hep-2) and oral cancer (KB). The HeLa cell line was derived from a glandular cervical cancer in a female patient named Henrietta Lacks and, because of its celebrated status, was distributed internationally and passed from laboratory to laboratory. Then, as today, many scientists were oblivious to the possibility of cross-contamination. HeLa cells are particularly robust and fast-growing and consequently can rapidly overgrow other cells.

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Denial and complacency

There was resistance and some hostility to Gartler's findings — even among scientists "who should have known better", according to Gartler — but one scientist, Walter Nelson-Rees (FIG. 1b), took particular note of Gartler's talk. Nelson-Rees ran a cell bank at Berkeley under contract for the National Cancer Institute. With his colleagues he developed karyotyping methods for authenticating cell lines and in a series of papers he showed there was extensive cross-contamination among the supposedly unique cultures sent to the bank (for example, see REF. 9). Nelson-Rees's work showed widespread cross-contamination by HeLa cells and for some years all cell lines were under suspicion of being HeLa cells until proven otherwise. He developed methods for cell identification and raised awareness of the problem in the scientific literature and through correspondence with individual scientists affected by the problem. Nelson-Rees's last contribution to the subject was published in 2009, soon after his death[10].

When Nelson-Rees first published his findings, some scientists ignored or denied the evidence and continued to publish papers containing false information[11]. As a consequence, Nelson-Rees felt that he had no option but to highlight the papers (and consequently the individuals) using cross-contaminated cell lines. At that time (and possibly today), Nelson-Rees's behaviour was regarded as unscientific and he was attacked by many colleagues. He was branded a self-appointed vigilante and his contract terminated by the National Institutes of Health (NIH) in 1981. After this, cell line misidentification went largely unchecked and the problem escalated. For the next 10–20 years, cell banks distributed many cell lines under their false names.

Estimating how much misleading and erroneous research is attributable to cross-contamination or misidentification of cell lines has been difficult. The use of misidentified cultures increased about 10-fold in the PubMed database (see Further information for a link) between 1969 and 2004, and the papers that used cultured cells increased only 2–2.5-fold during the same time period[12,13]. By 2004, HeLa was just the tip of the iceberg, and many other cell lines masqueraded under various guises in laboratories worldwide.

A survey that profiled active cell culture workers found that of 483 respondents, 32% used HeLa cells, 9% unwittingly were using HeLa contaminants, only 33% of the investigators tested their cell lines for authenticity and 35% obtained their cell lines from other laboratories rather than from a major repository[12].

Although complacency and, in some cases, denial have been the primary responses to cell line misidentification over the past five decades, a few individuals have devoted a great deal of personal effort into remediation of the problem. Among the largely independent efforts were letters to editors from concerned individuals requesting that readers be alerted about the problem, and that authors be required to provide evidence that the cell lines used in their studies were neither cross-contaminated nor misidentified. These efforts were largely ignored in the period after Nelson-Rees's contract was terminated, despite the development of DNA-fingerprinting techniques, which brought new and more reproducible methods that once again revealed the extent of cell line misidentification in the early 1990s[14].

Roland Nardone (FIG. 1c) started the second crusade in 2004. He gained the support of Joseph B. Perrone, who was then Vice President for Standards at ATCC and provided ideas and the matching outrage needed to fuel the crusade. Together with other concerned scientists, Nardone developed a comprehensive and coordinated initiative that simultaneously sought to raise awareness of the nature and magnitude of the problem and canvassed the involvement of individuals and organizations concerned or affected by the problem[1,15]. Such organizations included the NIH, the Howard Hughes Medical Institute, heads of funding organizations and their attorney generals, leaders of professional societies and editors of science journals.

Copies of a white paper, 'Eradication of cross-contaminated cell lines: a call for action' (subsequently published by Nardone in 2007 (REF. 15)) were distributed to thousands of scientists. The white paper presented what seemed to be a straightforward solution: funding agencies would require cell line authentication as a condition for the receipt of funds and journals would have a similar requirement for manuscripts submitted for publication. This approach was met initially with indifference. Nevertheless, over a period of 4 years, several substantial milestones were reached[1]. An open letter[16] to Michael O. Leavitt, Secretary of Health and Human Resources, led the NIH to re-examine the situation. On November 28 2007, the NIH published an addition to its guidelines for research in the form of a notice regarding authentication of cultured cell lines calling for diligence and more careful peer review[17].

Two factors have driven this progress. One is heightened awareness. The other is the outrage of scientists angered by the failure of funding agencies and journals to address the problem and allowing it to fester and amplify for 50 years. Many scientists now accept the need for a standardized method of human cell line authentication to satisfy the new requirements. ASN-0002 will be the first step towards a universally adopted standard.

Examples and impact

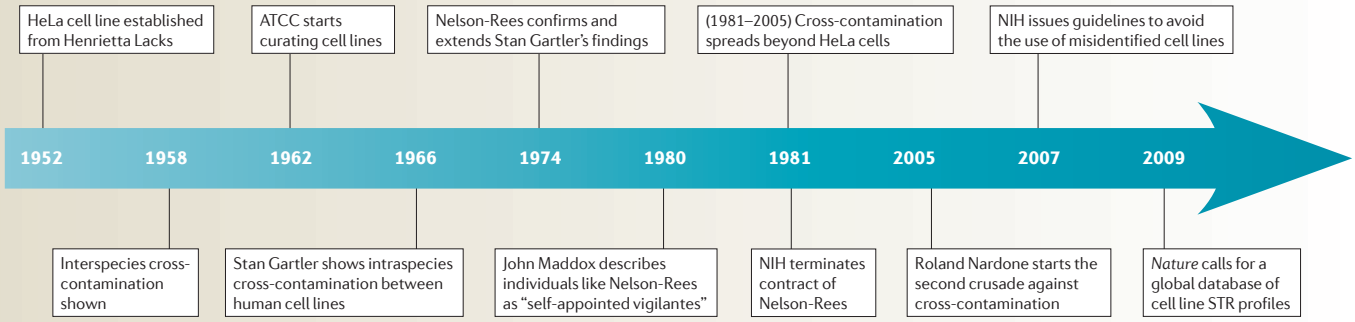
Cross-contamination and misidentification have a long history with many examples, but it is difficult to judge which have been the most substantial and costly.

The classic case already described is contamination by HeLa cells, of which there are several examples (see REFS 7,9 for example). It is astonishing that many of these cell lines

Box 2 | ATCC SDO standards development process

- American Type Culture Collection (ATCC) Standard Development Office (SDO) Consensus Standards Partnership (CSP) members recommend a new standard.
- Recommendation forwarded to ATCC SDO steering committee for review and vote.
- Project Initiation Notification System (PINS) published in *American National Standards Institute (ANSI) Standards Action* for 30-day public comment period, concurrent with CSP (ATCC SDO members) review.
- Recommendation for workgroup chair(s) sent to ATCC SDO steering committee for vote.
- Workgroup established; (ASN-0002), which includes stakeholders from academia, industry and government, and proceeds to draft the standard (see BOX 1 for members of the workgroup).
- ASN-0002 workgroup forwards draft standard to steering committee for internal review. Workgroup edits draft standard and forwards to ANSI and CSP (ATCC SDO membership) for concurrent 45-day public review.
- ASN-0002 workgroup responds to all comments and resolves any differences. If there are no substantial changes to the standard, the standard is submitted to the ANSI board of standards review for final action and publication as an ANSI-approved standard.

Timeline | **Key milestones in the effort to address cell line misidentification**



ATCC, American Type Culture Collection; NIH, National Institutes of Health; STR, short tandem repeat

have continued to be used under their false descriptions in respected journals for over 40 years after they were first shown to be HeLa cells (BOX 3).

T24 is another fast-growing cell line that has contaminated many supposedly distinct bladder cancer cell lines (BOX 3). ECV304 was originally claimed to be a spontaneously transformed human normal endothelial cell line, but later shown to be T24 bladder cancer cells[18]. Surprisingly, the demonstration that ECV304 cells are not endothelial cells had little effect on its use as a model for endothelial cells in publications (FIG. 2).

The putative human prostate cancer cell lines TSU-Pr1 and JCA-1 are also derived from T24 bladder cancer cells[19]. These findings were published in *Cancer Research*, but that did not prevent TSU-Pr1 cells being used as a prostate cancer cell model in a later paper in *Cancer Research* (BOX 3).

DNA-fingerprinting analysis revealed that the NCI/ADR-RES cell line was actually an ovarian tumour cell line, OVCAR-8, rather than a breast cancer cell line. Around 300 papers have been published using the incorrect identification of the NCI/ADR-RES cell line[20]. NCI/ADR-RES is included in the NCI60 panel of cell lines, which has been subject to STR profiling (discussed below)[21].

A paper describing misidentification of oesophageal cell lines stated "Experimental results based on these contaminated cell lines have led to ongoing clinical trials recruiting EAC [oesophageal adenocarcinoma] patients, to more than 100 scientific publications, and to at least three National Institutes of Health cancer research grants and 11 US patents"(REF. 22).

The consequences of widespread misidentification and cross-contamination of cell lines are immeasurable. In addition to the waste of millions of dollars of public money, time and intellectual resources, there is the loss of confidence in published work, and the integrity of science suffers.

Over 50 years of suppression. Why?

Three constituencies share responsibility for cell line misidentification — individual scientists, scientific journals and funding agencies. For most of the past 50 years it is only individual scientists who have addressed the issue. Nevertheless, it is hard to escape the conclusion that many scientists have knowingly used misidentified cell lines in publications (for example, the evidence in FIG. 2). Furthermore, authors are often reluctant to publish corrections to the literature based on cell line misidentification.

John Maddox, the editor of *Nature* in 1980, wrote an editorial about a high-profile case of cross-contamination entitled 'Responsibility for trust in research' (REF. 23). With an almost complete lack of insight into the problem he suggested that "there is no reason to suppose that the few cases [of cross-contamination] that have come to light are in any sense the tip of the iceberg". In the same editorial, scientists like Nelson-Rees were vilified, as the article made the point that it would be tragic if these civilized habits (that is, truth in research) "were to be corrupted by the activities of self-appointed vigilantes". The history of cell line cross-contamination indicates that truth and trust are not as universal among the scientific community as many scientists wish to believe.

The responses of editors of scientific journals to the problem continue to be illuminating. There have been hundreds of papers in scientific journals describing examples of misidentification and, until recently, no remedial action has been taken to eradicate the problem by journals or funding agencies. The editor of an influential tissue culture journal was asked to consider introducing authentication as a requirement for publication and replied that it would be financial suicide. Editors of other journals also refused to consider such quality-control measures on the basis that introducing such a hurdle to publication would substantially reduce the number of authors willing to submit manuscripts to their journal.

Over the past 2 years attitudes have begun to change, with journals, such as *In Vitro Cellular and Developmental Biology*, *International Journal of Cancer*,

Figure 1 | **Pioneers of awareness of cell line misidentification. a** | Stanley Gartler **b** | Walter Nelson-Rees **c** | Roland Nardone

Cell Biochemistry and Biophysics and the American Association for Cancer Research (AACR) journals, demanding that all cell lines are authenticated before publication. *Nature* has indicated that first the funding organizations have to demand authentication and provide the necessary funds. Once they do, *Nature* will require cell line identification prior to publication[24]. In the meantime, the funding organizations continue to ignore the problem.

The constituency with the most power to maintain standards in science is the funding agencies. Surprisingly, these have been resistant to addressing or even acknowledging the problem of cell line misidentification. For example, the NIH advisory note issued in 2007 ignores the fact that individual scientists and reviewers have failed to overcome this problem. As an editorial in *Nature* pointed out, the advisory note merely enforces the status quo[24].

Attempts to address the problem by individual scientists have met with unhelpful responses from funding bodies, which have tended to deny or belittle the problem. A recent public statement by a senior scientist from Cancer Research UK made light of cell line misidentification, saying that "this issue raises its head every few years". Funding bodies seem to be threatened by the issue and are resistant to engaging with scientists who try to address the problem and often attempt to disparage and discredit those who try to find a solution.

Any of the major funding organizations that support biomedical research in the United States or United Kingdom could have eradicated cell line misidentification during the past 10 years for less than the cost of the average project grant by funding the measures outlined in this Science and Society article. Yet, these funding agencies have repeatedly ignored and in some cases suppressed debate, and continued to provide grants for research using false cell lines. There could be wider implications concerning the role of funding agencies in the control of scientific misrepresentation and fraud.

Zero tolerance of cell line misidentification is needed from both journals and funding agencies. There are signs that Nardone's crusade is gaining influence and the standard for human cell line identification will be tangible evidence of Nardone's legacy.

Causes of cell line misidentification

Most cell lines are established in academic environments in which tissue culture is often regarded as a technique requiring little skill and essential facilities, such as flow cabinets and incubators, are used without restriction. In these circumstances, it is not surprising that attempts to establish new cell lines often lead to cross-contamination. Among 550 leukaemia and lymphoma cell lines submitted to the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ; German Collection of Microorganisms and Cell Cultures; please see Further information for a link) cell bank, 59/395 (15%) submitted by originators and 23/155 (15%) submitted by secondary sources were false[25]. Presumably most of the cell lines submitted by the secondary sources had also been cross-contaminated or misidentified by the originators.

There are many causes of cell culture misidentification and every laboratory is at risk. Perhaps the most straightforward cause is mislabelling of a cell culture vessel during routine manipulation. Factors contributing to this error include operator workload, lack of attention, or distractions during manipulation of cell lines.

Cross-contamination of a culture and subsequent overgrowth by the contaminating cell type is another frequent cause of cell line misidentification. The chances of this occurring are increased by the use of shared reagents, repeated use of the same pipette during re-feeding operations and manipulation of multiple cultures at the same time without adequate isolation of one cell type from another. When cross-contamination happens, one cell type may rapidly outgrow the other, leading to a pure culture of the contaminating cells in four or five passages[26].

Intentional co-cultivation during propagation of human stem or primary cells using a feeder layer derived from another species

Box 3 | Examples of the use of cell lines under false descriptions

The examples discussed below were picked at random from PubMed searches. The impact of the false descriptions ranges from minor to invalidation of the conclusions. The individual authors have been failed by peer review. The papers indicate that the editors and some of the reviewers of these journals (and by inference most scientific journals) are unaware of the extent of cell line misidentification, and indicate a general lack of awareness throughout the scientific community.

HeLa cervical cancer cells

- Int-407 (described as "non-transformed intestinal epithelial cells") in *Br. J. Cancer* **101**, 1596 (2009), *EMBO J.* **22**, 5003 (2003) and *J. Biol. Chem.* **280**, 13538 (2005)
- WISH (described as "non-transformed amniotic epithelial cells") in *Mol. Pharmacol.* **69**, 796 (2006), *Endocrinology* **147**, 2490 (2006) and *J. Biol. Chem.* **278**, 31731 (2003)
- Chang liver (described as "normal liver cells") in *Oncogene* **28**, 3526 (2009), *Proteomics* **14**, 2885 (2008) and *J. Biol. Chem.* **279**, 28106 (2000)
- HEp-2 (described as "laryngeal cancer") in *Investig. New Drugs* **26**, 111–118 (2008), *Carcinogenesis* **29**, 1519 (2008) and *J. Biol. Chem.* **283**, 36272 (2008)
- KB (described as "oral cancer") in *Biochem. Pharmacol.* **73**,1901–1909 (2007), *Clin. Cancer Res.* **14**, 8161(2008) and *J. Biol. Chem.* **280**, 23829 (2005)
- HeLa, Int-407 and HEp-2 cells were used as three distinct cell lines in the same study in *Cancer Res.* **69**, 632 (2009)

The scientists that use these cell lines sometimes use them under their false descriptions in many publications. For example, one group has used Int-407 as a model of normal intestinal cells since 1988 and during the past 10 years has published in the *Biochemical Journal* (2 papers), *Biochemical Society Transactions*, *British Journal of Cancer*, *Cancer Research* (2 papers), *Carcinogenesis*, *Experimental Cell Research* (3 papers), *Gastroenterology* (2 papers), *Journal of Biological Chemistry* (3 papers), *Journal of Cell Physiology*, *Journal of Cell Science* (3 papers), *Oncogene*, *PLoS One* and several other journals.

T24 bladder cancer cells

In 1999, ECV304 cells (originally described as spontaneously immortalized normal endothelial cells) were shown to be T24 cells[18].

Yet, many papers continue to describe ECV304 cells as endothelial, for example *Nature Immunol.* **6**, 497 (2005) and *Nature Biotechnol.* **25**, 921 (2007). Some studies use ECV304 cells in endothelial research without claiming that they are endothelial cells, but not stating that they are T24 bladder cancer cells, such as *Proc. Natl Acad. Sci. USA* **106**, 6849 (2009). Some studies have used T24 and one or more of its cross-contaminants as distinct bladder cancer cell lines, for example *J. Urol.* **181**, 1372–1380 (2009). Some studies describe ECV304 as bladder cancer cells, but fail to state that they are T24 cells, such as *J. Biol. Chem.* **285**, 555–564 (2010).

In *Cancer Research* in 2001, it was shown that TSU-Pr1 are T24 bladder cancer cells (*Cancer Res.* **61**, 6340–6344 (2001)). In the same journal, less than 3 years later, TSU-Pr1 cells were used as a prostate cancer model (*Cancer Res.* **64**, 1058–1066 (2004)). TSU-Pr1 continue to be used in some studies as a model for prostate cancer, such as *Endocrinology* **147**, 530–542 (2006) and *Cancer Cell* **5**, 67 (2004).

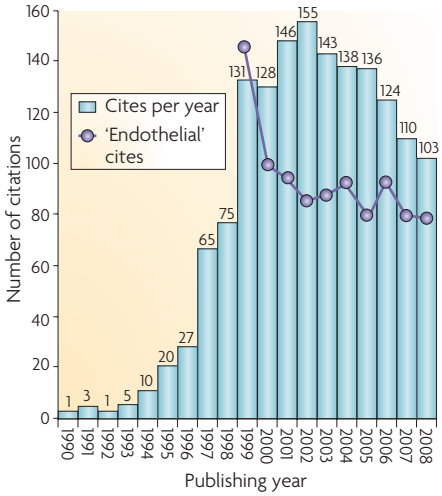


Figure 2 | **Citations of T24 bladder cancer cells referred to as normal endothelial cells.** The demonstration that ECV304 cells are not endothelial cells had little effect on its use as a model for endothelial cells in publications, as shown by the graph. Data generated courtesy of R.A.F. MacLeod, National Institute of Standards and Technology.

(such as mouse 3T3 cells) can result in cross-contamination and overgrowth of the human cell line. Normally, feeder cells are rendered incapable of proliferating, but if the growth arrest procedure is inadequate, the feeder cells can proliferate and displace the human cells. Somatic cell hybridization is unusual but can occur, as found in the human mantle cell lymphoma line NCEB-1, which carries seven mouse chromosomes[27].

Xenografting can also lead to cell line cross-contamination and misidentification[28]. Recovered cell lines from xenografts can be replaced by cells derived from the host animal.

In general, cross-contamination results in the complete and rapid displacement of the less fit cell type. Two cell lines cannot co-exist in the same culture environment for extended periods unless there is a symbiotic relationship, which as far as we know has never been reported. Consequently, cell mixtures are discovered rarely. The only known situation in which a cell population contains a stable mixture of genomes over many passages is following somatic cell hybridization.

Simple, cheap quality-control measures can prevent or at least minimize the consequences of misidentification. Misidentification is rife because of a combination of lack of awareness and the failure to include quality-control measures. The extensive quality-control measures demanded of the biopharmaceutical industry and mandated in the applicable regulatory documents are believed to have contributed to the relatively low frequency of cell line misidentification reported in this industry[29].

Detection of cross-contamination

Many methods have been used to detect cross-contamination, including isoenzyme analysis, karyotyping, human leukocyte antigen (HLA)-typing, immunotyping and DNA fingerprinting. These methods can authenticate a cell line, but with differing levels of ambiguity and powers of discrimination (Supplementary information S1 (table)). However, the data produced by these methods are not sufficiently reproducible between laboratories to allow any of them to be used for a standardized reference database.

Many laboratories have adopted STR profiling to identify human cell lines. STR profiling is the method used by forensic analysts and depends on the simultaneous amplification of multiple stretches of polymorphic DNA in a single tube. STR loci consist of repetitive DNA sequences that have varying numbers of repeats. Each STR locus can be amplified and the amplified products labelled with fluorophores of different colours, making the products easy to distinguish by size and colour (FIG. 3). STR analysis is rapid, inexpensive, amenable to automation and generates reproducible data in a format suitable for a standard reference database. For the quick, unambiguous authentication of cell lines, STR analysis has the greatest value.

STR profiling — potential and limitations

DNA repeat sequences of 3–5 bases have been used routinely for paternity testing, forensic casework, and the identification of victims of mass disaster for more than two decades[30–33]. Consequently, STR profiling was applied to cell line identification[34–36]. There are several advantages to using STRs for the authentication of human cell lines (Supplementary information S2 (box)).

Cancer cell lines contain many genetic alterations, and therefore the criteria used to compare them using STR profiling must be different to those used for normal tissue (Supplementary information S3 (box)). Cancer cells often show loss of heterozygosity (that is, loss of an allele, which cannot be distinguished easily from homozygosity) and can contain multiple copies of alleles owing to DNA duplication. Similarly, during culture, cancer cell lines can lose or more rarely gain a copy of an allele (for examples, see REF. 34). Consequently, sub-lines of the same cell line may not have identical STR profiles. Comparing identical alleles, a threshold of 75% identity has distinguished all known cross-contaminated cell lines in published datasets, and no two cell lines thought to be derived from different individuals showed more than 50% identity[21,34]. Consequently, there is a comfortable cushion of 25% between cell lines that are unique and those that show evidence of cross-contamination. Any cell line found with an identity level between 50 and 75% should be regarded with suspicion.

Major issues in the interpretation of genotypes from human cell lines include heterozygote peak height imbalance (that is the peak height or area of one allele is much larger than the peak height or area of the second allele), multiple alleles at a locus, and allele dropout (no amplification product of the expected size). Cancer cell lines are aneuploid and consequently STR profiles typically show heterozygous peak height imbalances and/or multiple alleles at one or more loci.

The cost of genotyping is a major concern, but trivial in relation to the cost of the work being done with the cell line. The cost of STR profiling includes DNA extraction, polymerase chain reaction (PCR) amplification of STR loci, separation of amplified products by capillary electrophoresis and data analysis. Increasing the number of STR loci, for example, from 6 to 15 would achieve a much higher power of discrimination (Supplementary information S4 (table)).

A major limitation of STR profiling is that it will not detect contaminating cells of another species, although if human cells are overgrown by cells of another species, the DNA will not amplify using human or higher primate-specific STR primers. PCR using species-specific primers can be used to detect contaminating cells from other species. If STR profiles have been established for the other species (currently restricted to a few commercially important species), STR can be carried out to definitively identify the contaminating cells.

For most of the established cell lines, donor tissue is not available and many originators of widely used cell lines are retired or deceased. In these cases, an assumption has to be made, based on the oldest possible cell stocks in repositories. These profiles will need to be labelled as provisional to indicate the absence of authentication back to the original donor tissue.

Until the database described below is available, there are limited resources available for comparing STR profiles. The ATCC and DSMZ cell bank websites and Cell Line Integrated Molecular Authentication

(CLIMA; see Further information for links) database[37] provide some information, and at least two series of STR profiles have been published[21,34]. Currently, one of the most useful resources is the list of misidentified cell lines collected by Amanda Capes-Davis and Ian Freshney (supplementary table in REF. 38), which can also be seen on the European Collection of Cell Cultures (ECACC; see Further information for a link) website. All scientists should check the names of the cell lines they are using against this list.

The interactive database

It is proposed that a database will be established to exploit available STR data to validate the identity of human cell lines. The interactive database will be accessible to everyone, but only the database administrators can make changes or additions. The database will provide DNA profiles and will allow laboratories to compare the STR profiles of their lines, thereby facilitating the validation of experimental data.

Universal criteria are needed for what constitutes a good database. The standard for cell line authentication will establish an interactive database of validated DNA profiles for each unique cell line and will also put in place requirements for carrying out and interpreting the STR assays. The members of the standard committee in conjunction with the National Center for Biotechnology Information (NCBI) will develop the requirements for the database and the database will be maintained by NCBI. The database will initially contain around 500 validated cell lines frequently used by scientists and banked in major cell repositories. The profile of each cell line will be validated before it is submitted to the database.

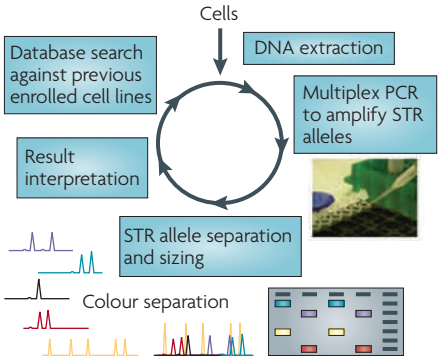


Figure 3 | **Short tandem repeat profiling methodology.** Short tandem repeat (STR) loci consist of repetitive DNA sequences with varying numbers of repeats. Each STR locus can be polymerase chain reaction (PCR) amplified and the amplified products labelled with fluorophores of different colours, making the products easy to distinguish by size and colour. Images courtesy of J. Butler, National Institute of Standards and Technology.

The most effective database to compare cell line STR-profiling data would consist of a common set of markers. However, not all data have been collected for the same STR loci or using the same generation of sequencing instruments. The use of different primer sets for the same STR markers is a common practice for the forensic and human identity community, which in the United States uses a core set of 13 STR markers for data input into the Federal Bureau of Investigation-maintained Combined DNA Index System (CODIS). To maintain the integrity of the data entered into CODIS, laboratories must use CODIS-approved STR-typing kits and instrumentation, and follow strict quality assurance standards[39]. Approved CODIS STR kits have undergone extensive validation studies that include concordance studies designed to elucidate STR-typing differences that may be seen with the use of different primer sets. Similar protocols will be needed for STR profiling of cell lines.

The future

Cell line verification by STR profiling will have a substantial effect on scientific research in terms of increased data credibility and less time, money and effort spent studying misidentified cell lines. Accurate identification of cell lines is crucial during the development of cell-based medical products to avoid the risks of exposing human subjects to misidentified cells. Although such misidentification can largely be avoided by adherence to quality-control measures, such as proper labelling and tracking schemes during manufacture of a cell-based product, the availability of a standardized method for unambiguous cell and tissue identification could contribute to safety assurance when used to confirm that a cell product came from the intended donor and was not inadvertently mixed with cells from other donors. This issue is of great importance to personalized medicine and the application of stem cell-based technologies, including induced pluripotent stem cells.

No single method is available that provides all the information needed to authenticate a human cell line. STR profiling represents the optimal candidate at this time. Consequently, the standard is intended to evolve as new information becomes available. The interactive, searchable database openly available to everyone will largely eradicate the use of misidentified cell lines. Funding bodies and journals are encouraged to adopt a policy of zero tolerance and demand proof that all cell lines are as claimed.

For members of the ATCC Standards Development Organization (SDO) Workgroup ASN-0002 see BOX 1

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Competing interests statement
The authors declare competing financial interests; see Web version for details.

DATABASES

CLIMA database: http://bioinformatics.istge.it/clima
PubMed: http://www.ncbi.nlm.nih.gov/pubmed

FURTHER INFORMATION

ATCC SDO homepage: http://www.atccsdo.org
ATCC cell bank: http://www.atcc.org
DSMZ cell culture collection: http://www.dsmz.de/human_and_animal_cell_lines
ECACC cell culture collection: http://www.hpacultures.org.uk/collections/ecacc.jsp

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (box) | S3 (box) | S4 (table)

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