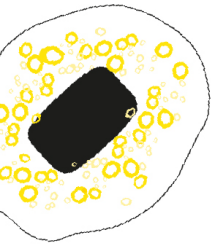
COMMISSIE
COGEM
GENETISCHE
MODIFICATIE

ONDERZOEKSRAPPORT
2026-02

IMPACT OF MISIDENTIFIED CELL LINES IN DUTCH INSTITUTIONS (IFCELL-NL)

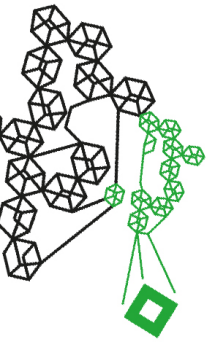
ONAFHANKELIJKE WETENSCHAPPELIJKE ADVISERING EN SIGNALERING VAN DE RIJKSOVERHEID

UNIVERSITY OF TWENTE.



Report - final version

Impact of Misidentified Cell Lines in Dutch Institutions (IFCELL-NL)



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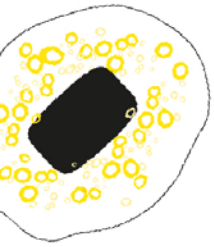

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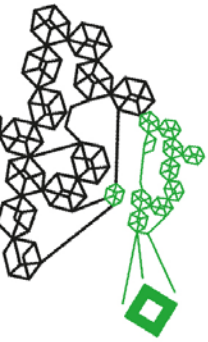
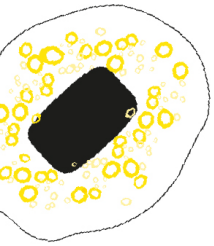


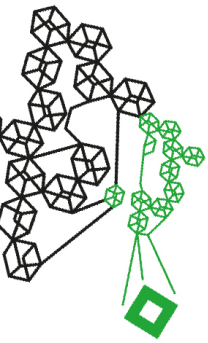


Foreword

Human and animal cell lines are widely used in biomedical research. They are often used as models for specific cells, tissues, and organs, frequently to understand the causes and consequences of diseases. This requires that the materials used originate from the source assumed and are therefore as representative as possible of the cells that the researcher wishes to study. This may seem trivial, but in practice it proves to be more difficult: the literature shows that cells used in research are sometimes mixed up and are much less representative of the cells, tissues, or organs that are the subject of study.

Sometimes the incorrectly described origin of the cells is decades old and the result of an old procedure for culturing slow-growing cells in used medium from rapidly dividing cells. If that used medium was not made cell-free, there was a high chance that the rapidly dividing cells would take over the cell culture. More recent analyses demonstrated that a significant proportion of the old cell lines have properties identical to HeLa cells. Even today, mix-ups still occur from time to time. Many journals therefore require publications to clearly state that the cell lines used are authentic. The aim is to increase the usability and reproducibility of the research. In addition, to assess environmental risks associated with experiments with GMO's, researchers are required to authenticate the cells used in the experiments.

Researchers at the University of Twente, commissioned by COGEM, have conducted research into the extent of the problem of misidentification of cells in the Netherlands and into the procedures used to mitigate it. The aim of the research was to provide insight into the use of cell line authentication methods in biomedical research in the Netherlands. The researchers approached the research questions in different ways. On the one hand, the researchers themselves had 32 cell lines used in the lab authenticated. In addition, the researchers conducted a survey among Biological Safety Officers (BSOs) to investigate the practices and procedures in their institutions. Finally, the researchers conducted a literature review of the techniques available for authentication, including the advantages and limitations of the methods.

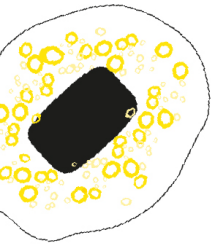


The results of the project are presented in this report and led to several recommendations.

I am convinced that this report will contribute to our awareness on this issue and that it can provide background and guidance for researchers to have their cell lines authenticated on a regular basis. Ultimately, this will increase the usefulness and applicability of our research.

Prof. Rob C. Hoeben

Chair of the supervisory committee



Voorwoord

Cellijnen van mens en dier worden veel gebruikt in biomedisch onderzoek. Ze worden daarbij veelal gebruikt als model voor specifieke cellen, weefsels en organen, vaak om de oorzaken en gevolgen van ziekten te doorgronden. Dat vraagt dat de gebruikte materialen ook daadwerkelijk de herkomst hebben die verondersteld wordt en daarmee maximaal representatief zijn voor de cellen die de onderzoeker wil bestuderen. Dat lijkt triviaal, maar de praktijk is weerbarstiger: uit de literatuur blijkt dat cellen die in het onderzoek gebruikt zijn soms verwisseld zijn en veel minder representatief zijn voor de cellen, weefsels of organen die het onderwerp van studie zijn.

Soms is de foutief-beschreven herkomst van de cellen al decennia oud, en het gevolg van een oude procedure om moeizaam groeiende cellen te kweken in gebruikt medium van snel-delende cellen. Als dat gebruikte medium niet absoluut cel-vrij gemaakt was, bestond er een grote kans dat de snel-delende cellen de celcultuur overnamen. Een aanzienlijk deel van oude cellijnen blijkt bij testen identieke eigenschappen te hebben als HeLa-cellen. Ook tegenwoordig vinden er nog af en toe verwisselingen plaats. Veel tijdschriften vragen dan ook in publicaties duidelijk te maken dat de gebruikte cellijnen authentiek zijn. Hiermee wordt gestreefd de bruikbaarheid en de reproduceerbaarheid van het onderzoek te vergroten. Daarnaast is het voor het beoordelen van de milieurisico's van werkzaamheden met ggo's noodzakelijk dat onderzoekers de cellijnen waarmee gewerkt wordt verifiëren.

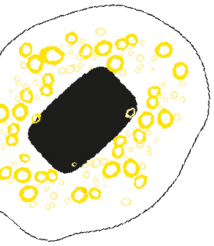
Onderzoekers aan de Universiteit Twente hebben, in opdracht van de COGEM, onderzoek gedaan naar de grootte van dit probleem in Nederland en naar de procedures die worden gebruikt om deze problematiek te beheersen. Het onderzoek had als doel inzicht te geven in het gebruik van cellijn authenticatiemethoden in biomedisch onderzoek in Nederland. De onderzoekers hebben de onderzoeksvraag op verschillende manieren benaderd. Ten eerste hebben ze zelf 32 cellijnen, die in het lab gebruikt werden, laten authentiseren Daarnaast hebben de onderzoekers middels een enquête onder Biologisch Veiligheidsfunctionarissen (BVF's) onderzocht wat de gebruiken en procedures zijn in hun instituten. Tenslotte hebben de onderzoekers een literatuuronderzoek uitgevoerd naar voor authenticatie beschikbare technieken, inclusief de voor- en nadelen van de verschillende methoden.

De resultaten van de drie benaderingen staan beschreven in dit rapport en hebben geleid tot een aantal aanbevelingen.

Ik ben ervan overtuigd dat dit rapport bijdraagt aan ons bewustzijn over deze problematiek en dat het achtergrondinformatie en leidraden bevat voor onderzoekers voor het regelmatig authentiseren van de cellijnen die in het onderzoek worden gebruikt. Dit zal uiteindelijk de bruikbaarheid van de resultaten van ons onderzoek kunnen doen toenemen.

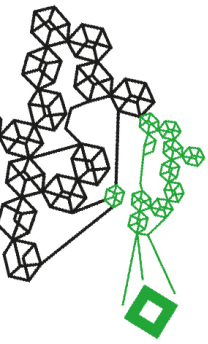
Prof. Rob C. Hoeben

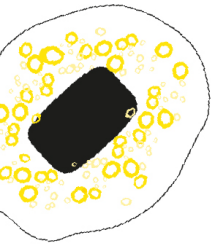
Voorzitter van de begeleidingscommissie



Nederlandse samenvatting

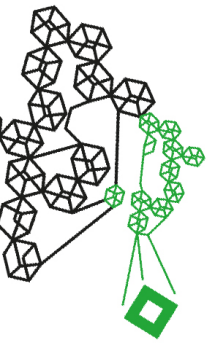
Het doel van dit project was om inzicht te verkrijgen in de prevalentie, impact en perceptie van verkeerd geïdentificeerde cellijnen binnen Nederlandse onderzoeksinstellingen, evenals in de huidige praktijk rondom celidentificatie aangezien foutieve celidentificaties mogelijk kunnen leiden tot wetenschappelijke, gezondheids- of milieurisico's. Hiervoor werd een gecombineerde aanpak toegepast bestaande uit een literatuurstudie, een kleinschalig intern experimenteel inventariserend onderzoek naar de frequentie van foutieve celidentificatie, en een enquête onder biologische veiligheidsfunctionarissen. De resultaten laten zien dat er brede consensus bestaat over het belang van celidentificatie voor de reproduceerbaarheid, geloofwaardigheid en efficiëntie van wetenschappelijk onderzoek. Desondanks blijkt de routinematige toepassing van celidentificatie niet vanzelfsprekend: ongeveer de helft van de deelnemende organisaties voert structureel geen identiteitstesten uit. Genoemde belemmeringen zijn onder andere kosten, tijdsdruk, gebrek aan expertise, en onduidelijkheid over beschikbare protocollen en wettelijke verwachtingen. Het experimentele onderzoek bevestigde reeds bekende beperkingen van standaard Short Tandem Repeat (STR)-profilering, waarbij contaminaties onder circa 25% niet betrouwbaar worden gedetecteerd, gebaseerd op een STR-snapshot analyse van 32 routinematig gebruikte cellijnen. Dit benadrukt dat celidentificatie niet uitsluitend als een eenmalige controle moet worden gezien, maar bij voorkeur worden geïntegreerd als onderdeel van een doorlopend kwaliteitsborgingsproces, met name bij langdurige culturen. Hoewel de Nederlandse wet- en regelgeving geen specifieke authenticatiemethoden voorschrijft, vereisen onder andere de Regeling Genetisch Gemodificeerde Organismen (GGO) en de Arbeidsomstandighedenwet wel een adequate risico-inventarisatie en -beheersing bij het werken met biologische agentia. Een dergelijke risicoanalyse veronderstelt voldoende zekerheid over de identiteit van gebruikte cellijnen. Uit de enquête blijkt dat dit wettelijke aspect niet altijd wordt herkend als stimulans voor celidentificatie. Samenvattend toont dit project aan dat er een duidelijke kloof bestaat tussen het brede bewustzijn van het wetenschappelijke belang van celidentificatie en de daadwerkelijke implementatie ervan. Duurzame verbetering vraagt om ondersteunende maatregelen op organisatieniveau, waaronder duidelijke richtlijnen, gestandaardiseerde protocollen, toegang tot externe testfaciliteiten, gerichte training en een verhoogde bewustwording bij zowel onderzoekers als management.

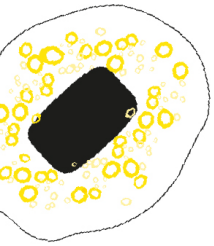




English Summary

The primary objective of this project was to assess the prevalence, impact, and perception of misidentified cell lines within Dutch research institutions and to evaluate current practices related to cell line authentication as erroneous cell identifications may result in scientific, health, or environmental risks. This was achieved through a combined approach consisting of a literature review, a small-scale internal experimental assessment of cell line authentication, and a survey among Biological Safety Officers representing a range of organizations. The findings demonstrate overall strong awareness among respondents of the scientific consequences of using misidentified cell lines, particularly with respect to research reproducibility, credibility, and the efficient use of research funding and resources. Nevertheless, routine implementation of cell line authentication procedures remains inconsistent, with approximately half of the surveyed organizations not performing identity verification. Perceived barriers include costs, workload, limited in-house expertise, and unfamiliarity with available protocols and regulatory expectations. Short Tandem Repeat (STR) profiling is a commonly used technique to validate cell line identity. Experimental evaluation of 32 in-use cell lines confirmed known limitations of STR-profiling, showing that contaminations below approximately 25% remained undetected. This underscores the importance of viewing authentication not as a one-time confirmation, but as part of an ongoing quality control strategy, particularly for long-term cultures. Although Dutch legislation does not explicitly prescribe specific authentication methods, it does require adequate risk assessment and control when working with biological agents. These obligations implicitly rely on sufficient certainty regarding the identity of cell lines in use. Survey results suggest that this regulatory dimension is not always recognized as a driver for implementing authentication procedures. Overall, the project highlights a clear gap between awareness of the scientific value of cell line authentication and its practical implementation. The results indicate that successful and sustainable adoption will require coordinated organizational support, including clear institutional guidelines, standardized protocols, access to external authentication services, dedicated training, and increased awareness at both researcher and management levels.





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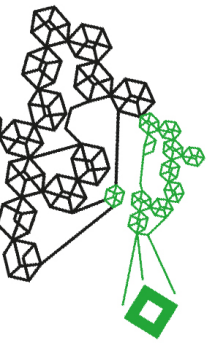
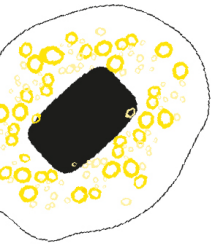
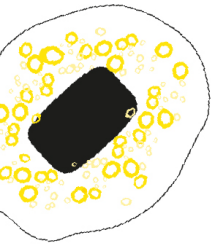




1. List of abbreviations

AACR	= American Association for Cancer Research
ALP	= Alkaline phosphatase
ANSI	= American National Standards Institute
ATCC	= American Type Culture Collection
BVF	= Biologische Veiligheidsfunctionaris
CFI	= Category of physical containment
CLASTR	= Cell Line Authentication using STR
CNA	= Copy Number Alteration
CNV	= Copy Number Variation
COGEM	= Commission on Genetic Modification
DSMZ	= German Collection of Microorganisms and Cell Cultures
ECACC	= European Collection of Authenticated Cell cultures
ESCs	= Embryonic stem cells
GDPR	= General Data Protection Regulation
GGO/GMO	= genetisch gemodificeerde organismen/genetically modified organisms
HPV18	= Human papillomavirus 18
ICLAC	= International Cell Line Authentication Committee
indels	= Insertions/deletions
iPSCs	= Induced pluripotent stem cells
JCRB	= Japanese Collection of Research Bioresources
LC-MS	= Liquid chromatography mass spectrometry
LDH	= Lactate dehydrogenase
LOH	= Loss of Heterozygosity
ML	= Machine learning
MSI	= Microsatellite instability
NIZO	= NIZO food research
NMR	= Nuclear magnetic resonance
OGM	= Optical Genome Mapping
(q)PCR	= (quantitative) polymerase chain reaction
RFLP	= Restriction fragment length polymorphism
SAAZ-UNie	= Samenwerkende arbo- en milieudiensten van Academische Ziekenhuizen en Universiteiten
SNP	= Single nucleotide polymorphism
SNV	= Single-nucleotide variants
STR	= Short tandem repeat
SV	= Structural variant
UT	= University of Twente
VNTRs	= Variable number tandem repeats
WGS	= Whole Genome Sequencing



2. Aim and objectives and approach

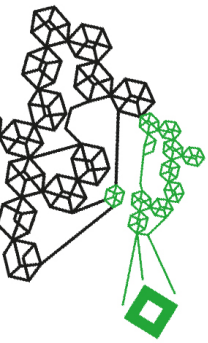
2.1 Aim and objectives

The aim of the work described in this report is to create insight into and to determine the impact of the use of misidentified cell lines in Dutch institutions. Based on these findings, recommendations and guidance will be developed to promote the implementation of cell line identification policies in the Netherlands.

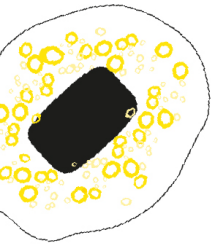
The objectives contributing to this overarching aim are defined as follows:

1. To conduct a literature review describing the extent and impact of cell misidentification on research data acquired using those cells, and to inventory established and newly described methodologies that are used for cell authentication purposes.
2. To conduct an inventory on the awareness and use of cell identification procedures in Dutch institutions through questionnaires distributed among biological safety officers employed at Dutch universities, research organizations and industry.
3. To perform a small-scale experimental verification within the University of Twente (UT), and UT collaborators to evaluate whether local findings are consistent with trends reported in the literature.
4. To determine the overall impact of cell line misidentification on research in the Netherlands based on experimental data, questionnaire results and literature data.
5. To develop evidence-based recommendations to address the most commonly reported challenges in cell authentication, drawing on data from questionnaires, the literature, and small-scale experimental analyses, and to provide advisory input to the Commission on Genetic Modification (COGEM) on the dissemination of these recommendations and the project's findings.

2.2 Approach



To provide a substantiated advice to the COGEM, the prevalence, causes, consequences, and (available) preventive measures related to cell line misidentification were assessed. By analyzing existing methods and discussing recent innovations based on a literature search, we offer an overview of the current state-of-the-art of cell line identification technologies. Additionally, previously reported case studies were identified that underscore the impact of misidentification, and literature was reviewed to assess the reported prevalence of cell line misidentification, its consequences, and preventive measures. We generated internal data using STR, as one of the main suggested methods for (human) cell line identification, to provide us with more insight regarding practical issues and to highlight limitations of authentication and critically address the definition of authentication. To establish on what scale cell line misidentification occurs within Dutch organizations and identify potential causes of resilience toward testing, a custom-designed questionnaire was distributed with (biological) safety officers from Dutch laboratories that are affiliated with safety and cell line identification. These questionnaires were actively featured and shared during the annual BVF platform and SAAZ-Unie symposia in 2025. The collective data generated through all these routes were combined and critically assessed to develop advisory input to the COGEM.



3. Literature study

3.1 Introduction

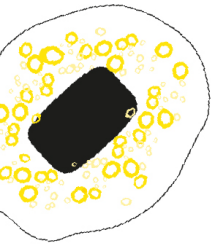
Cell line misidentification is a widespread problem in biomedical sciences that threatens the reliability of research results, leading to erroneous conclusions and wasted research funds, and can, at times, introduce safety hazards or pose environmental risks. Misidentification can be the result of mislabelling, cross-contamination, or poor research hygiene. Academic journals are disseminating increasing awareness of the vital importance of cell line authentication. For example, an extending number of journals explicitly require or strongly enforce cell line authentication for manuscripts reporting research with cell lines during peer review. These requirements are implemented to improve reproducibility and integrity of experimental results. Many of them are defined in journals' Instructions for authors, editorial policies, or peer-review checklists. For example, journals of the American Association for Cancer Research (AACR), which include Cancer Discovery and Cancer Research, typically require authentication of all cell lines used in submitted manuscripts. Also, Endocrine Society Journals, such as Endocrinology and Molecular Endocrinology, require that all cell lines used or described in submitted studies are authenticated. Nature journals generally require strong authentication documentation for cell line materials included in research.

One of the seminal papers describing the impact of misidentification was published in 2017 and identified nearly 33,000 articles that reported data based on misidentified cell lines (Horbach et al., 2017). This, and other studies show that a significant percentage of cell lines used in research do not match their original descriptions, endangering scientific validity. The International Cell Line Authentication Committee (ICLAC) initiated a registry of misidentified cell lines (Capes-Davis et al., 2010), largely recognizing the major impact of misidentification for the validity of research data. Well-documented incidents, such as the contamination of many cell lines with the highly proliferative HeLa cell, illustrate the scale of the problem and the need for adequate identification methods. Various articles describe methods such as STR profiling and DNA barcoding as essential for verifying cell line identity before, during, and after experiments. In this literature study we will describe the applications of cell lines and reported methodologies to authenticate their identities, discuss sources of misidentification and outline a few case studies.

3.2 Cell lines and their applications

3.2.1 Background and relevance

Cell lines are *in vitro* cultured populations of cells derived from specific tissues that can continue to proliferate under laboratory conditions (de Bardet et al., 2023; Voloshin et al., 2023). This characteristic distinguishes them from primary cells, which have a limited lifespan and are directly isolated from tissues. Cell lines may become immortalized, which occurs either spontaneously or via chemical, viral, or genetic manipulation (de Bardet et al., 2023; Chalak et al., 2024; Shay and Wright, 2000). This process of immortalization allows them to bypass normal cellular senescence processes and maintain virtually indefinite division potential (Voloshin et al., 2023). To maintain these characteristics, cell lines require a controlled environment, including nutrients, temperature, humidity, and gas composition, to support their ongoing growth and function (de Bardet et al., 2023; Voloshin et al., 2023). Their recognized wide-spread application potential triggered them to form an essential part of biomedical research globally. As such, they are widely used as model systems in biological and biomedical research for studying cellular processes, disease mechanisms, and drug responses (Voloshin et al., 2023; Wei et al., 2021; Narayana, 2023; Jack et al., 2014). Cell lines do not only provide insight into important cellular mechanisms through research, they also contribute to the development of therapies for (genetic) diseases (Brandt et al.,



2016; Littlewood et al., 1995; Gossen et al., 1995; Sauer and Henderson, 1988; Muir et al., 2020). One of the most employed and best-known cell lines is the HeLa cell, derived from a cancer patient in 1951 (Gey et al., 1952), which has contributed to many medical breakthroughs (Masters, 2002), but has also been responsible for large-scale contaminations (Lucey et al., 2009). This cell line naturally acquired mutations in genes regulating cell death and division, such as activating telomerase or disabling apoptosis leading to its immortalization (Ivanković et al., 2007; Horner et al., 2004; Nishimura et al., 2006). Besides HeLa, other cell cultures are employed to study various biomedical processes. Cells may be genetically modified, engineered to express or knock out specific genes, hence requiring careful authentication and quality control to ensure that both the modification and the cell line identity are as intended.

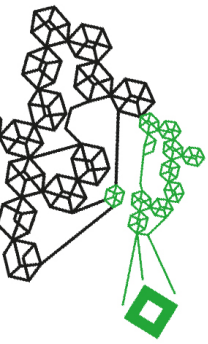
Below, we will discuss the most frequently used types of cell cultures for research, as well as their applications in research and industry.

3.2.2 Types of cell cultures

Cell types used in the laboratory for research purposes can be classified as follows:

3.2.2.1 Primary cells

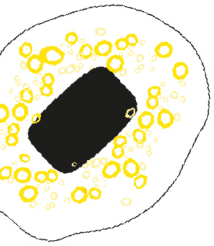
Primary cells are non-immortalized cells obtained directly from tissues of a human or animal donor and maintained in culture for a limited number of passages. They exhibit a finite replicative lifespan and restricted proliferative capacity, eventually undergoing senescence, which limits scalability and long-term experimentation (Alcock et al., 2024; Chan et al., 2022). Because primary cells retain many *in vivo*-like physiological, molecular, and phenotypic characteristics, they provide high biological and translational relevance compared with immortalized cell lines, but they are more difficult to isolate, more sensitive to culture conditions, and display donor-to-donor and batch variability challenging the comparison of interlaboratory research findings (Pan et al., 2009). Commonly used examples include primary neurons, hepatocytes, and intestinal epithelial cells, which are frequently employed for short-term experiments, functional assays, toxicity testing, and studies of tissue-specific physiology (Segeritz and Vallier, 2017; Richter et al., 2021; Cristofalo et al., 1998).



Although primary cells originate from defined donor material, they remain susceptible to mislabelling, cross-contamination, and mix-ups during isolation, expansion, and distribution, particularly when multiple donors or cell types are processed in parallel. Authentication is therefore recommended to confirm donor origin, verify species and human identity, and to detect overgrowth by faster-growing contaminant cell lines (including established tumour lines) during early passages. Establishing a genetic reference profile (for example, STR or single nucleotide polymorphism (SNP) profile) from the initial donor sample or early-passage stock enables later confirmation that experimental primary cultures correspond to the intended donor and have not been replaced or mixed with unrelated cells (Richter et al., 2021). STR authentication of human primary cells uses the same core STR-profiling workflow as for established lines, but results are validated against donor material or early-passage reference stocks.

3.2.2.2 Immortalized cell lines

Immortalized cell lines are derived from primary cells that have acquired the ability to proliferate indefinitely (de Bardet et al., 2023; Chalak et al., 2024; Shay and Wright, 2000). These cells are easier to culture, highly reproducible, and scalable as opposed to primary cells. As a result of their immortalization, these cells may diverge significantly from native physiology. Examples of frequently used immortalized cell lines are cervical adenocarcinoma HeLa, human embryonic kidney HEK293, colorectal adenocarcinoma Caco-2 and neuroblastoma-derived SH-SY5Y.

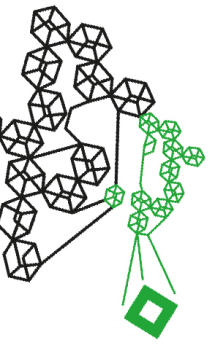


Immortalized cell lines are widely employed in biotechnology, drug development, and basic research. In the Netherlands, GMO regulations require that risks associated with genetically modified cell lines or cell lines in combination with GMOs are assessed, including verification of cell line authenticity and routine quality control measures. Although the GMO regulations do not state how verification should be performed, examples for verification of cells in culture could include contamination checks, for example by means of STR profiling. Tumour-derived cell lines, often immortalized or transformed, such as HeLa, MCF-7 (breast cancer) and A549 (lung adenocarcinoma), are frequently used in cancer research but are particularly involved in misidentification or contamination. The main reason is that they are extremely widely used, highly robust, and fast-growing, and were historically exchanged long before the development of routine authentication procedures, so they readily overgrow or get confused with other cultures. This has been explicitly discussed in the misidentification literature, especially for HeLa, and, to a lesser extent, for MCF-7 and A549 (Huang et al., 2017; Kniss et al., 2014). Moreover, in the past, newly established tumour cell cultures were initiated using HeLa-conditioned medium, which was rendered 'cell-free' by centrifugation. However, early centrifugation protocols were not always sufficient to remove all viable HeLa cells. As a result, inadvertent HeLa contamination frequently occurred, allowing these highly proliferative cells to overgrow slower-growing primary tumour cultures (Gartler, 1967, Nelson-Rees et al. 1981, Masters, 2022).
A large STR-based survey investigating 278 cell line samples from 28 institutes, found that 46% (128 out of 278) of these samples involved cross-contamination/misidentification. Nearly half of all these contaminated cell lines (≈47%) contained HeLa (Huang et al., 2017).

3.2.2.3 Stem cells

Stem cells are cells with self-renewal and differentiation capacity. They can be derived from embryonic, foetal, or adult origin. While embryonic stem cells are derived from blastocysts and, hence, pluripotent, adult stem cells are multipotent and therefore tissue-specific (*e.g.*, neural stem cells). Induced pluripotent stem cells (iPSCs) are reprogrammed somatic cells with ESC-like properties. Stem cell lines can differentiate into multiple lineages and cell types following a wide range of published protocols.

3.2.2.4 Hybrid cultures



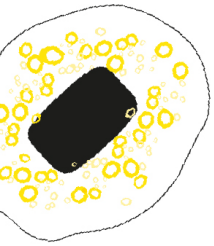
A mixture of the aforementioned categories of cell lines includes the intentionally generated hybrid cell lines, which are the result of fusion of two distinct cell types from any of the above-mentioned categories, and are often used to combine specific desirable properties, for example in hybridoma lines for monoclonal antibody production (*e.g.* Winzeler and Wang, 2013).

3.2.2.5 Alternative types of classification

Alternative types of classification of cell types are described, including 2D versus 3D cultures, adherent versus non-adherent, classification according to regulatory and ethical status, tissue lineage, species, and genetic status. For example, a recent review compares cell types to biological species and contrasts morphology, lineage, and transcriptomic similarity as alternative classification bases (Doyle, 2022). For neurons specifically, morphological, electrophysiological, connectivity and molecular/transcriptomic classification schemes in retina and cortex and propose a staged, multi-modal taxonomy were described and reviewed (Zeng and Sanes, 2017).

3.2.3 Applications of cell cultures in research and industry and cell culture banks

For biomedical research, cultured cells are commonly used to study disease mechanisms, drug development, and gene therapy. Besides, cell cultures play a crucial role in the production of biological drugs and vaccines but also to study virus replication, production of vector particles. For example, CHO cells are used for the generation of monoclonal antibodies (Yang et al., 2022), and Vero cells are used as platform to produce polio vaccines (Barret et al., 2009; 2017). Cell cultures,

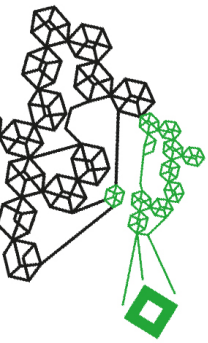


both in 2D as well as 3D format, are also being used to test the toxicity of substances (Nguyen et al., 2024; Wang et al., 2021). Several cell culture banks and cell repositories have been established and operate internationally to accommodate the expanded use and application of cell cultures (Weiskirchen et al., 2024; Wrigley et al., 2014). These organizations play a critical role in advancing biomedical research by providing standardized, reproducible biological materials. They differ in scope (human, animal, microbial), regulatory status, and intended use (research versus clinical). The most relevant and widely used cell banks include the American Type Culture Collection (ATCC), European Collection of Authenticated Cell cultures (ECACC), German Collection of Microorganisms and Cell Cultures (DSMZ), Japanese Collection of Research Bioresources (JCRB Cell Bank), and the RIKEN BioResource Research Center. Next to these cell banks, various specialized and thematic cell banks exist, including the Coriell Institute for Medical Research with a strong focus on genetic diseases and population studies, the European Bank for induced Pluripotent Stem Cells, supplying the community with clinical-grade and research-grade human iPSC lines, and the UK Stem Cell Bank.

3.3 Molecular authentication methods.

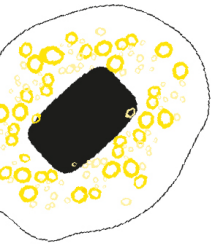
Accurate verification of cell line identity is crucial in biomedical research to maintain experimental quality, reproducibility, and overall reliability of scientific findings. The identity of all cell types employed in such biomedical research are typically established through authentication methods. An overview of all cell line identification techniques discussed in this report can be found in **Table 1**.

3.3.1 Morphological identification



Morphological identification assesses cells by their shape, size, and growth pattern under the microscope, based on the idea that changes in morphology can signal genetic drift or cellular senescence (Hayflick and Moorhead, 1961; Driscoll et al., 2019). Several comprehensive reviews have been published describing morphology-based cell identification methods (Chen et al., 2012; Bourn et al., 2025; Zinchenko et al., 2023). However, its reliability currently remains limited, because cell lines from different tissues may look very similar, and culture conditions such as medium composition, natural variability of serum used, and passage number can markedly alter morphology, increasing the risk of misidentification (Tong et al., 2022). To address these limitations, newer approaches combine image analysis with machine learning and standardized automated or high-throughput imaging. For example, an automated workflow has been reported that allows for the authentication of cell lines from the AstraZeneca Cell Bank, using deep learning on brightfield images acquired with high-throughput IncuCyte microscopy. This trained network (CLCNet) learned image-level features linked to specific cell line labels and then predicted the identity of test images, achieving 99.8% accuracy on a training set, a coefficient of determination of 0.927 for incubation time prediction, and 97.7% precision with 95.8% recall for new cell lines (Tong et al., 2022). Other deep neural network architectures, such as InceptionResNet-V2 (Mzurikwao, et al., 2020) and MobileNet (Mzurikwao et al., 2020), have likewise been explored for extracting image-based features to classify cell lines suggesting that advanced image analysis can substantially enhance morphology-based identification.
Despite these promising advances, most machine learning-driven morphology studies have been conducted on relatively small data sets and still require validation on broader, independent cohorts before they can be adopted as stand-alone authentication tools. At present, morphology-based and machine learning (ML)-enhanced identification methods are best positioned as complementary tools alongside established assays such as STR profiling, where they can provide additional, orthogonal evidence to support cell line identity.

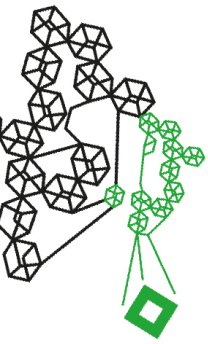
3.3.2 Biochemical identification



3.3.2.1 Enzyme activity assays

Biochemical identification using enzyme activity is one of the earliest reported methodologies, dating back to the 1970s, used to distinguish interspecies cell type cross-contamination (Halton et al., 1983; O'Brien et al., 1980; Peterson et al., 1984; Peterson et al., 1979; Stacey et al., 1997). The principle of this assay is based on the differences in the electrophoretic banding patterns of specific intracellular enzymes from different species. The distinguishing power of such assays is further based on the principle that certain cell lines produce specific enzymes and enzyme levels that can be detected. For example, mixed cell cultures of rat and mouse origin were correctly identified based on isoenzymatic assays (Steube et al., 1995). Using this assay, it was estimated that contaminating cells must be present at a level of at least 25% to be detected by isoenzymatic analysis (Steube et al., 1995). A specific example is the use of the enzyme alkaline phosphatase (ALP), as a histochemical marker for ESCs or iPSCs as these elicit high ALP-activity (Štefková et al., 2015; Plotnikov et al., 2017). The commercially available Authentikit System (Innovative Chemistry) enables the identification and discrimination of a limited range of cell types routinely used in laboratories by exploiting differences in enzyme activity profiles, specifically nucleoside phosphorylase, glucose-6-phosphate dehydrogenase, malate dehydrogenase, and lactate dehydrogenase. Using this system, contamination of MRC-5 human cells with AA8 Chinese hamster (CHO-K1) or mouse L929 cells was detectable with each of the enzymes analysed (Nims et al., 1998). Conversely, contamination of L929 or CHO-K1 cells with MRC-5 cells was also detectable using all enzymes included in the kit (Nims et al., 1998). Contamination of CHO-K1 cells with L929 cells was detectable only through lactate dehydrogenase analysis, whereas detection of CHO-K1 contamination in L929 cells required the additional analysis of peptidase B. Using this approach, interspecies cross-contamination could be detected when the contaminating cells constituted at least 10% of the total cell population (Halton et al., 1983; Nims et al., 1998).

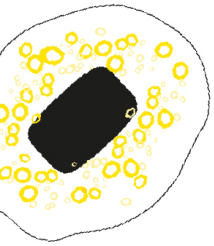
3.3.2.2 Metabolite profiling



Metabolite profiling involves metabolomics techniques using nuclear magnetic resonance (NMR) or mass spectrometry to capture dynamic snapshots of intracellular/extracellular metabolites, for example amino acids, lipids, and sugars. A few reports evaluated the use of metabolomic profiling to identify cell types. For example, Alden et al. (2020) profiled six CHO cell lines, derived from two different hosts and each cell line producing a different monoclonal antibody, using an untargeted metabolomics approach by using liquid chromatography mass spectrometry (LC-MS) on spent media. This approach allowed for the identification of a specific set of metabolites accumulating in the stationary phase, highlighting lineage-specific signatures. Silva et al. (2017) defined volatile metabolomic signatures of breast cancer cells, by collecting volatile organic compounds from the headspace of breast cancer cells, followed by analysis using gas chromatography combined with mass spectrometry (GC-MS), effectively distinguishing breast cancer cells from healthy human mammary epithelial cells. Lichtarge et al. (2025) applied a newly developed data analysis algorithm, MetaboLINK, to NMR data from hESC-derived neural lineages, revealing cell-specific pathways across neural developmental stages and neural lineages.

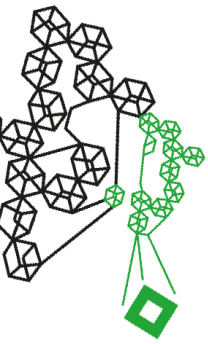
While species-specific metabolic signatures exist (*e.g.*, phospholipid profiles distinguishing human versus rodent cells), they overlap significantly with culture conditions, limiting reliability for contamination detection. Therefore, metabolite profiling is primarily leveraged for process and culture optimization rather than direct authentication, as the metabolite spectrum usually reflects physiological state or culture conditions rather than genetic identity (Evie et al., 2017). In this way, such profiles do potentially support quality control and detection of significant phenotypic drift.

3.3.3 Short tandem repeat (STR) profiling

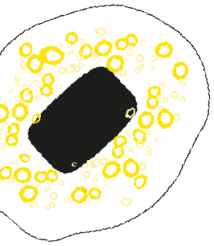


STR profiling is the most used method for identifying and authenticating human cell lines (Dirks and Drexler, 2013, Chen et al. 2023, ICLAC, 2023 Guide to Human Cell Line Authentication). It relies on the fact that each cell line has a unique DNA 'fingerprint' based on short, repetitive DNA sequences, and it is recommended as the gold standard by the ATCC (Almeida et al., 2016). In practice, STR profiling examines a defined set of variable DNA regions (microsatellite loci) and compares the resulting profile with reference data stored in curated databases such as Cellosaurus, ATCC, or DSMZ (Dirks and Drexler, 2013).
The method uses polymerase chain reaction (PCR) to amplify these DNA regions, after which the resulting pattern is computationally compared with reference profiles using dedicated software. While STR profiling is considered to be highly accurate and reproducible, it requires specialized laboratory infrastructure and data analysis tools. Interpretation of the results also requires specific expertise (Azari et al., 2007). Software tools such as Cell Line Authentication using STR (CLASTR) generate similarity scores that indicate how closely the tested cell line matches known reference profiles (Robin et al., 2020).
In general, a similarity score above 80% compared with the expected reference cell line (based on at least 13 loci) is considered strong evidence that the tested cell line is correctly identified (Capes-Davis et al., 2013). Scores below 80% raise concerns about possible contamination or misidentification. In some cases, a second reference cell line may also show a high similarity score (>80%). If this second hit corresponds to a different cell line than expected, the result is considered inconclusive and may indicate a mixed culture or incorrect cell line identity, as outlined in ICLAC guidelines.
Despite its strengths, STR profiling has limitations (Bian et al., 2017). Genetic changes such as microsatellite instability and loss of heterozygosity, particularly common in cancer-derived cell lines, can complicate data interpretation (Almeida et al., 2016). In addition, STR profiling is not yet widely standardized for non-human (animal) cell lines, largely because validated STR marker panels and reference databases are still limited for these species.

3.3.4 Non-STR-related PCR for cell line authentication



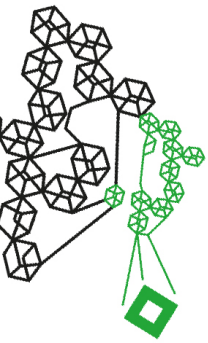
PCR is a versatile tool for assessing the genetic identity of cell lines. PCR is employed in STR profiling, but it can also be used outside the STR context for broader authentication needs (Vicente et al., 2022). PCR enables targeted amplification of specific genomic regions of interest, such as transgenes, knockout alleles, integration sites, or other genetically engineered modifications, using custom primers designed against known sequences (Saiki et al., 1985). This approach verifies the presence, absence, copy number, or integrity of engineered constructs (*e.g.*, CRISPR edits, lentiviral inserts, reporters), providing a rapid method to monitor the genetic composition of cell lines. For example, endpoint or quantitative (q)PCR can confirm homozygous/heterozygous knockouts, quantify plasmid copy number in stable transfectants, or detect off-target integrations, complementing STR by linking identity to functional modifications. Therefore, this method is primarily useful to assess specific genetic modifications introduced by recombinant DNA techniques alongside the STR profile of a cell line, ensuring that observed phenotypes align with the intended engineering in an authenticated cellular background. In genome-edited lines, PCR-based genotyping (*e.g.*, T7E1 mismatch cleavage, allele-specific PCR) rapidly screens clones before full authentication, while multiplex PCR panels can simultaneously check for multiple edits and contamination markers.
Standards like those from ATCC recommend PCR for construct verification after STR authentication, particularly in biopharma, where transgene stability directly impacts product safety and efficacy. PCR offers high specificity, low DNA input (ng scale), and fast turnaround (hours), making it suitable for iterative quality control during cloning or selection. However, it requires prior sequence knowledge (unlike unbiased Whole Genome Sequencing (WGS) or Optical Genome Mapping (OGM)), risks primer mismatches in drifted lines, and cannot independently confirm cell line identity without STR/SNP integration, positioning it as a targeted adjunct rather than standalone authentication method.



3.3.5 DNA fingerprinting

DNA fingerprinting is a molecular approach used to verify the identity of cell lines by detecting polymorphic DNA regions across the genome. Historically, techniques such as restriction fragment length polymorphism (RFLP) analysis were employed, but modern implementations often use PCR-based amplification of variable number tandem repeats (VNTRs) or other highly polymorphic loci (Almeida et al., 2016; Chen et al., 2025). DNA fingerprinting generates a multi-locus profile by amplifying or digesting hypervariable genomic regions, producing a characteristic banding pattern or allele set that serves as a unique identifier for a cell line. Unlike STR profiling, which targets 8-21 specific loci, DNA fingerprinting surveys dozens to hundreds of polymorphic sites simultaneously, offering broader genomic coverage that can capture insertion/deletions, VNTRs beyond standard forensic panels, and even transgene integrations in genetically modified lines (Almeida et al., 2016). It is particularly useful when STR panels are unavailable or insufficient, for example, for non-human cell lines (*e.g.*, rodent, insect) or in cases requiring interrogation of additional genomic regions, such as verifying construct stability in engineered cells. Studies like those from Almeida et al. (2016) highlight its role in resolving ambiguities in hybrid or contaminated lines where STRs show partial matches, as the multi-locus pattern provides higher discriminatory power.

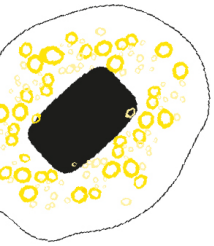
Compared with STR profiling, DNA fingerprinting can provide broader genome coverage, which may facilitate detection of genetic modification elements or inserted constructs, enabling more precise monitoring of engineered cell lines. This method can differentiate between cell lines, even if they are morphologically similar or share STR alleles due to Loss of Heterozygosity (LOH) or drift, making it valuable for authentication of complex models like iPSCs or tumour lines (Almeida et al., 2016; Chen et al., 2025). For instance, multi-locus VNTR analysis has been used to confirm identity in non-human mammalian lines and detect cross-contamination events missed by species-specific STRs (Almeida et al., 2016). However, this approach generally requires higher quantities of DNA (often 100-500 ng vs. 1-10 ng for STR), more complex gel-based or sequencing analysis, and lacks the extensive reference databases of STR systems, which limits its routine use relative to standardized STR profiling and typically leads to higher costs and longer turnaround times. Standards bodies like ATCC and ICLAC thus recommend it as a complementary tool for challenging cases rather than a primary screen.



3.3.6 Whole genome sequencing (WGS)

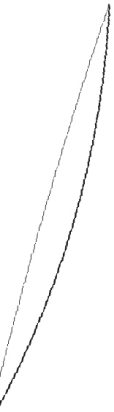
WGS provides a comprehensive framework for cell line authentication and identification by determining the complete genetic composition of a cell line, including the detection of genome-wide single-nucleotide variants (SNVs), insertions/deletions (indels), structural variants (SVs), and copy number alterations (CNAs). Compared to STR, WGS offers substantially higher resolution that can reveal subtle genomic drift, contamination events, or complex karyotypic changes undetectable by STR analysis (Almeida et al., 2016; Ben-David et al., 2018; Lung et al., 2021). For example, in genome-edited lines, WGS was used to confirm parental identity alongside edit integrity (on/off-targets, integrations) (Lung et al., 2021; Almeida et al., 2016).

The principle of WGS is based on the generation of base-pair resolution data across the entire ~3 Gb diploid human genome, enabling extraction of virtually unlimited STR alleles, millions of SNPs, mitochondrial haplotypes, and genome-wide SV/Copy Number Variation (CNV) profiles for comparison against reference genomes from banks like ATCC/DSMZ or Cellosaurus (Almeida et al., 2016). One of the main limitations of WGS is its time-consuming character. Lung et al. (2021) developed a high-throughput WGS methodology suitable for authentication, using k-mer analysis for species identity, SNP/STR calling for intra-species matching, and resolving 509 ICLAC misidentified lines with >99% accuracy, outperforming barcoding for hybrids and contaminants like HeLa. For verification, reads are mapped to reference cell line genomes or *de novo* assembled to

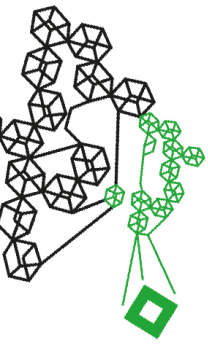


assess SNP concordance (>99.9%), STR sharing, and SV/CNV patterns. Tools like CLASTR facilitate database comparisons to flag misidentification (*e.g.*, HeLa rearrangements in non-cervical lines).
WGS can provide discriminative, sequence-based identity and copy-number information that complements STR profiling, and it may be especially useful in aneuploid or genetically unstable cell lines where STR profiles can be difficult to interpret due to LOH, copy-number changes, or drift. Almeida et al. (2016) emphasize the value of WGS to facilitate the identification of inter-species contamination (*e.g.*, rodent in human), genetic purity, and adventitious agents like viruses. Bioinformatics pipelines for WGS are becoming increasingly standardized (*e.g.*, through services such as GenomeScan). This standardization is helping WGS gain wider adoption in biopharmaceutical producer cell lines and academic research, where it complements STR profiling as a confirmatory method endorsed by ICLAC and ATCC (Almeida et al., 2016).The approach currently remains limited for routine analysis as it is time-consuming (days to weeks vs. hours for STR), with high costs, data complexity (terabytes per sample), and need for bioinformatic expertise (Mora-Castilla et al., 2016). Relevant standards organizations thus position WGS for complex cases like novel lines, contamination investigations, or validating engineered cells rather than everyday screening (Lung et al., 2021; Almeida et al., 2016).

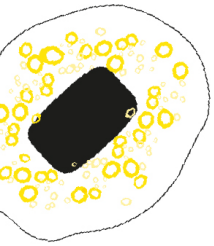
3.3.7 Optical genome mapping (OGM)



OGM can be used as a high‐resolution, genome‐wide method for human cell line authentication by exploiting each line's unique pattern of large structural variants as a "fingerprint" (Chialastri et al., 2025). OGM labels ultra-high-molecular-weight DNA (typically >150 kb) at specific sequence motifs, linearizes molecules in nanochannels, and records the ordered pattern of fluorescent labels along each molecule. *De novo* assembly of these molecules generates a genome‐wide SV map showing insertions, deletions, inversions, translocations, and copy-number changes, that is highly characteristic for a given cell line (Chan et al., 2018; Savara et al., 2021). Chialastri et al. (2025) developed OGM-ID, an OGM-based approach that uses genome-wide large insertions and deletions (>500 bp) as features to uniquely identify and authenticate human cell lines. In this framework, the set and configuration of SVs function as a line‐specific barcode, analogous to how STR profiles are used, but at megabase scale (Chialastri et al., 2025). In OGM-ID, the SV pattern of a test sample is compared to a reference OGM profile of the same line or donor to confirm identity and detect deviations. The method can distinguish between unrelated lines, identify inter-species and intra-species contamination, and authenticate genome-edited clones derived from a common donor, even when SNP/STR markers are identical. OGM-ID was demonstrated to correctly match a reference iPSC line (GM24385) to its donor and discriminated it from parental and family-member lines and authenticate clones after CRISPR editing and single-cell cloning. Because OGM robustly captures large insertions, deletions, and transgene integrations, the same dataset can simultaneously report karyotype-like information (balanced and unbalanced rearrangements) and support identity checks, which is especially valuable for quality control of engineered cell products. Villarejo et al. (2024) used OGM to reveal copy-number changes and complex rearrangements in CRISPR-edited hiPSCs that were not evident from morphology, underscoring its use as a genome‐integrity and identity tool in advanced cell models (Villarejo et al., 2024).



Compared with classical karyotyping, OGM offers higher resolution (down to a few hundred base pairs) and is not strongly affected by culture conditions used to obtain metaphases, while still detecting most clinically relevant structural rearrangements. Unlike STR profiling, it does not rely on a small panel of short tandem repeats but instead assesses thousands of SVs across the genome, which can be particularly informative for highly engineered or aneuploid lines where large-scale changes carry line-specific signatures (Chan et al., 2018; Villarejo et al., 2024; Chialastri et al., 2025). However, current OGM implementations have limited sensitivity for very small variants and some classes of complex rearrangements and require high-quality high-molecular-weight DNA plus specialized instrumentation, so they complement rather than replace established STR-based authentication. In practice, OGM is emerging as an orthogonal, whole-



genome authentication and integrity assay: it can confirm identity, detect contamination, and simultaneously evaluate large structural changes that may impact the reliability and safety of human cell lines in research and cell therapy workflows (Pei et al., 2025; Finlay et al., 2025; Villarejo et al., 2024; Chialastri et al., 2025).

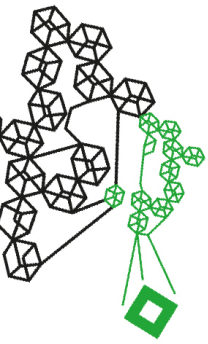
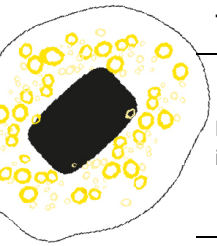
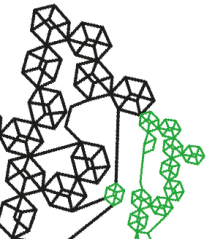
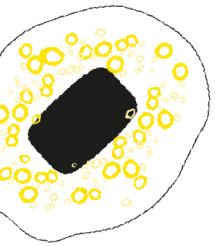


Table 1. Overview of cell line identification techniques.

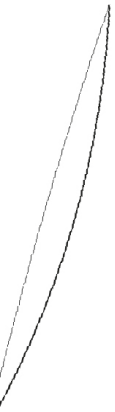


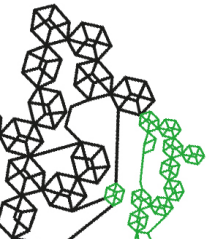
Technique	Principle / Method	Purpose / Application	Advantages	Limitations / Disadvantages	Examples / Notes
Morphological identification	Analysis of cell shape, size, and growth patterns via microscopy; can be automated using machine learning and deep learning	Complementary identification of cell lines; detection of morphological changes due to senescence or genetic drift	Simple, inexpensive, high-throughput capable; recent ML methods improve accuracy	Low discriminative power for morphologically similar lines; influenced by culture medium and passage number	Deep learning networks such as CLCNet, InceptionResNet V2; accuracy up to 99.8% on training sets
Biochemical tests	Detection of enzyme activity, isoenzymes, or metabolite profiles	Identification of interspecies contamination; characterization of cells	Fast, relatively inexpensive; detects ≥10–25% contamination	Limited to certain cell types; sensitive to culture conditions; low differentiation between similar lines	Authentikit System (enzymes: ALP, G6PD, lactate dehydrogenase (LDH), etc.); esterase profiling; NMR/metabolomics for quality control
PCR (non-STR)	Targeted amplification of specific genes or transgene loci	Verification of presence/absence of genetic modifications, plasmid copy number, CRISPR edits	High specificity, low DNA input, fast	Requires known sequences; cannot independently confirm identity	qPCR, multiplex PCR, mismatch analysis
STR profiling	Amplification of polymorphic microsatellite loci and comparison with reference databases	Standard for human cell line authentication	Accurate, reproducible, standardized via ATCC/CLASTR	Limited for animal cell lines; susceptible to microsatellite instability or LOH	>13 loci, ≥80% match required; ATCC, DSMZ, Cellosaurus references
DNA fingerprinting / VNTR	Multi-locus PCR or RFLP of polymorphic DNA regions	Identification of non-STR cell lines; detection of genetic modifications; interspecies contamination	Broad coverage, high discrimination; useful for hybrid lines	Requires more DNA (100–500 ng), more expensive and time-consuming than STR, limited databases	Multi-locus VNTR analysis; detection of transgene integrations
Whole Genome Sequencing (WGS)	Sequencing the entire genome; detection of SNPs, indels, CNVs, SVs	Comprehensive authentication; detection of contamination and genetic drift	Maximum resolution; detects subtle changes; detects all variants	Very expensive, time-consuming, large data output, requires bioinformatic expertise	K-mer analysis for species ID; SNP/STR concordance; ≥99% accuracy for ICLAC-misidentified lines

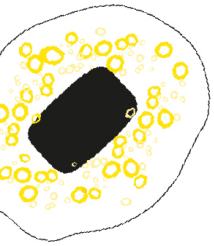




Technique	Principle / Method	Purpose / Application	Advantages	Limitations / Disadvantages	Examples / Notes
Optical Genome Mapping (OGM)	Detection of large structural variants (>500 bp) via fluorescent labelling and nanochannel mapping	Authentication of human cell lines; contamination detection; karyotyping	High resolution; independent of small STR panels; detects CRISPR edits	Limited for small variants; requires high-molecular-weight DNA and specialized equipment	







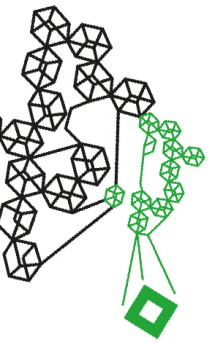
3.3.8 Data analysis in cell line authentication data

Data analysis is a critical component of cell line authentication, as it determines whether a cultured cell population corresponds to its claimed origin. For STR profiling, analysis typically involves comparing the allelic patterns at standardized loci to reference profiles, often using algorithmic matching and defined thresholds for confirming identity. Databases, such as the ICLAC database or profiles provided by major cell banks (*e.g.*, ATCC, DSMZ), are commonly used to validate results. Many reference cell lines STR patterns are available In Cellosaurus (CLASTR). The number of references within the database or misidentified hits impact the opportunities to eventually authenticate cell lines based on reference databases.

DNA fingerprinting or VNTR-based methods similarly rely on pattern recognition across multiple polymorphic loci, which can be compared against historical or published reference data. With the advent of genome-wide approaches, such as Optical Genome Mapping (OGM), analysis involves more complex bioinformatic pipelines to detect structural variants, copy number changes, and chromosomal rearrangements, which can then be interpreted in the context of reference genomes or known cell line profiles. Across all methods, challenges in data analysis include distinguishing genuine genetic drift or subline variation from misidentification, dealing with mixed or contaminated samples, and integrating results from multiple assays to generate a robust conclusion. Effective authentication thus requires not only accurate data generation but also standardized computational workflows, appropriate thresholds for calling matches, and careful interpretation within the biological context of the cell line. Importantly, the definition of identity with respect to cell lines is a crucial factor for data interpretation.

3.4 Misidentification

An important factor to consider when discussing misidentification are the definitions of ‘’identity’’ and ‘’authenticity’’. **Figure 1** shows an overview of the different definitions and sources of misidentification and cross-contamination. For this report, we consider the definition of identity with respect to cell lines as: “*the verified correspondence between a cell culture and its claimed origin, established by a reproducible molecular profile*” (paraphrased from the ICLAC Terms of Reference and ATCC/ANSI ASN-0002 guidelines). The ICLAC states that ‘’*A cell line is considered to be misidentified if its DNA profile (genotype) is no longer consistent with the donor from whom it was first established.*’’ (ICLAC.org, terms of reference). Moreover, they acknowledge that ‘’*Researchers who are unaware they are working with a false cell line may use it without realizing it comes from a different cell type, tissue, or even species, and is likely unfit for their intended application. To determine whether a cell line is authentic or misidentified, the genotype (e.g., STR profile) should be compared to the original donor’s profile rather than relying on its observed characteristics (phenotype).*’’ (ICLAC.org, terms of reference).



Their best practice recommendations include genotyping by STR profiling as a key verification step for cell lines. Long-term cultivation of cell lines *in vitro* frequently results in genomic alterations such as aneuploidy, chromosomal rearrangements, loss of heterozygosity, or even chromosomal loss (*e.g.* loss of Y chromosome, LOY) (Yu et al. 2015). The accumulation of such genomic alterations during prolonged culture complicates the concept of cell line identity as it might result in a ‘’shift of identity’’ due to changes in molecular profiling attributed to genomic alterations. These phenomena might contribute to the suggestion that authentication may not represent a fixed verification, in contrast to current perspectives on this, but rather a dynamic reference point, comparable to a baseline measurement, against which subsequent genomic evolution should be evaluated.

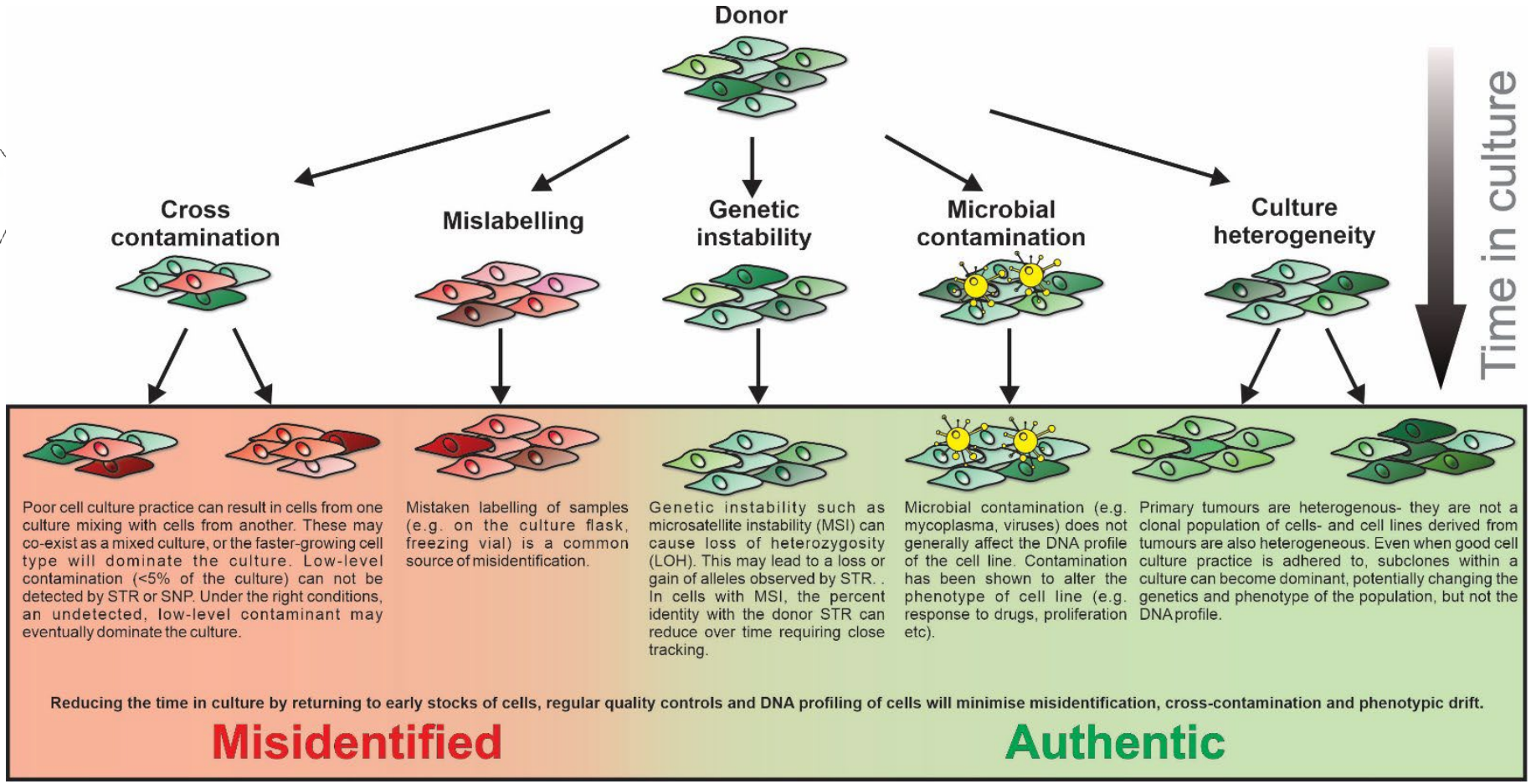
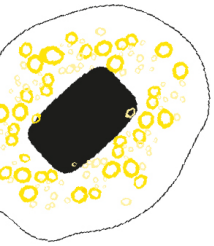
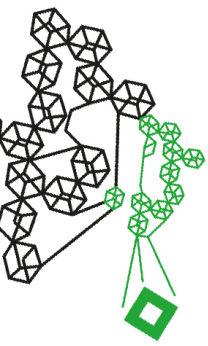


Figure 1 Schematic overview of experimental situations in laboratory experiments regarding misidentified and authentic cell lines. *Source: https://iclac.org/education/training-seminar/. Note: very extensive genetic instability may ultimately result in misidentification.*



Studies have shown that misidentification of cell lines is a long-lasting problem for biomedical research (A.T.C.C.S.D.O.W. ASN-0002 (2010) via ICLAC) and can occur due to human errors, cross-contamination, and inadequate documentation. Interestingly, this also relates to cell lines that have been used for many years and have been included in many publications (see Stacey, 2000, and Masters, 2010 for reviews regarding this topic). Misidentification can reduce the value of certain research results (Masters, 2010) which leads to financial and ethical consequences regarding the opportunities to develop further knowledge based on these data. Examples of financial and ethical consequences are: unreliable results, wasted research funds, and retractions of publications. This has consequences for both the pharmaceutical industry and academia.
Misidentification of cell lines has been the subject of several published studies in leading peer-reviewed journals. Researchers from the Radboud UMC determined that, even though this is probably an underestimate, that at least 32755 research articles have been published that include misidentified cell lines and gave rise to almost half a million citations and of which 92% were cited at least once (Horbach & Halffman, 2017).

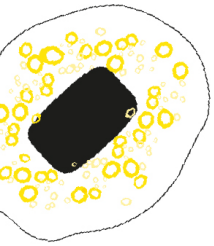
Horbach and Halffman distinguish primary articles from secondary articles in which primary articles are original articles in which the cell line was firstly introduced and characterized whereas a secondary article depicts information from primary articles solely. It is estimated that there are approximately 4.5 to 5 million research articles containing cell lines, with the percentage of "contaminated primary literature" being slightly below 0.8% of the total number of articles, and that the percentage of "contaminated secondary literature" is around 10%, according to the authors. However, this most likely includes false positives regarding contaminated secondary literature (Horbach and Halffman, 2017). Based on multiple studies, it is estimated that, in general, around 5% of cell lines are misidentified, but that the estimate of the percentage of misidentified cells can vary depending on factors such as the origin of the tissue and the country of origin (Souren et al., 2022). The emergence of technology, particularly STR analysis, has played a significant role in facilitating the identification of cell lines. Although the literature describes a worrying problem with far-reaching implications for research fields such as oncology, molecular biology, and biochemistry, surprisingly, there appears to be a certain degree of reluctance within research institutions, as the vast majority of researchers within research institutions both ten years ago and still today indicate that they do not test for cell line authenticity (Freedman et al. 2015, Buehring et al. 2004, Vaught and Korch 2021 and "Announcement: time to tackle cells' mistaken identity. 2015. Nature 520:264."; Weiskirchen, 2025; Weiskirchen et al., 2025; Chen et al., 2025). In October 2013, COGEM issued an advisory opinion on the verification of cell lines and identification, focusing specifically on which cell line studies are eligible for verification (CGM/131002-01). This opinion included, among other things, that cell lines containing retroviruses or retroviral sequences are eligible for verification. The COGEM recommendation could be expanded and updated to include other aspects if practice shows that other types of studies, agents, or analytical methods more often involve a higher category of physical containment (CFI) and an increase in environmental and/or health risks than initially assumed.



3.5 Case studies and examples

As previously mentioned, a study conducted by Horbach and Halffman (2017) identified 32,755 publications reporting research involving misidentified cell lines, which in turn were cited by an estimated half a million other papers. In addition, 103 publications were identified in the PubMed database using the search terms *“cell line” AND misidentification (*Horbach and Halffman, 2017). Specific examples within this selection include:

1. Yang et al. (2024) performed authentication testing based on STR loci on MGC-803 cells, a cell line originally reported as derived from gastric carcinoma. The study demonstrated that MGC-803 cells contain genetic material from HeLa, including human papillomavirus 18 (HPV18) sequences, likely resulting from cell-cell fusion. Further genetic analyses revealed that MGC-803 originated from donors with distinct genetic ancestries, one African (HeLa) and one Asian. Transcriptomic analyses showed strong similarity between MGC-803, HeLa, and

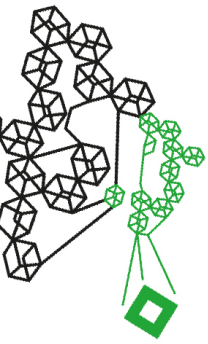


another nasopharyngeal-HeLa hybrid line, CNE-2. Based on these findings, the authors concluded that MGC-803 represents a hybrid cell line derived from HeLa and another Asian-origin cell line.

2. Capes-Davis et al. (2010) used STR profiling, karyotyping, and HPV testing to show that the widely used "breast adenocarcinoma" line Hs 588T is a HeLa contaminant, lacking breast-specific markers and carrying HeLa's characteristic HPV18 integration; the study urged its discontinuation from repositories.
3. Nelson-Rees and Flandermeyer (1976) reported enzyme profiling with various other biochemical read-outs to show that the supposed human embryonic intestinal line Int 407 is a hybrid contaminated with HeLa cells. Cellosaurus entry CVCL_1907 confirms Int 407 as ICLAC-listed HeLa hybrid via STR (60-70% HeLa alleles) and HPV18+ (https://www.cellosaurus.org/CVCL_1907).
4. Recent RNA-seq reanalysis by Shankar et al. (2025) detected viral contamination in ~10% of public MCF7 datasets through unmapped reads and machine learning classifiers, identifying HBV, SARS-CoV-2, and others. However, not all detected viral signals necessarily reflect unintended contamination, as some experiments may have intentionally introduced viral infections as part of the experimental design (Shankar et al., 2025). In addition, SARS-CoV-2 signals detected in RNA-seq data are known to be particularly susceptible to sequencing- and library preparation–related artefacts and therefore do not necessarily indicate genuine cellular infection (Yan et al., 2021). Biomarker validation across 1,000+ samples showed gene expression shifts consistent with active infection.

3.5.1 Cell lines frequently associated with misidentification or contamination

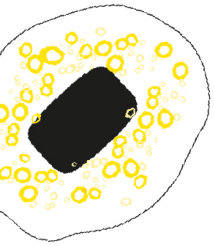
Cell lines that are most often implicated in misidentification or contamination are typically cell lines that are widely distributed, frequently shared between laboratories, or of unclear origin. Such issues often arise from cross-contamination or incorrect labelling during cell culture handling. The International Cell Line Authentication Committee (ICLAC) maintains a comprehensive database on cell line misidentification, which is accessible on the website https://iclac.org/databases/cross-contaminations/. The most recent version, version 13, was released on April 26, 2024. To date, the register lists 545 cell lines that are established as misidentified with no known authentic stock (ICLAC; https://iclac.org/databases/cross-contaminations/). Although the ICLAC register is widely used as a curated repository of misidentified or cross-contaminated human cell lines, occasional inaccuracies or omissions cannot be entirely excluded. Therefore, results should be corroborated with additional authentication resources, and users are encouraged to interpret results in the context of complementary authentication data.



The cell line most associated with misidentification, reported in 145 cases, is HeLa, one of the earliest and most extensively used human cell lines. HeLa cells are known for their robust and rapid growth, enabling them to easily overgrow other cultures in the event of contamination.
Examples from the literature of cell lines that were contaminated with HeLa cells include:

- 2564 (MAC-21) human lung carcinoma cells (Nelson-Rees et al., 1981)
- 5-8F (SUNE1) human nasopharyngeal carcinoma cells (Ye et al., 2015)
- ACC2 human salivary gland adenoid cystic carcinoma cells (Phuchareon et al., 2009; Zhao et al., 2011)
- H-494 human prostate carcinoma cells (Williams, 1980)
- HEK human embryonic kidney cells (Nelson-Rees et al., 1981b)

In 21 reported cases, the intended cell line was contaminated with T-24, a human bladder carcinoma cell line frequently confused with other urothelial lines. Reported examples of T-24 contamination include the ECV-304 human endothelial cell line (Dirks et al., 1999; MacLeod et al., 1999), the GHE human astrocytoma cell line (MacLeod et al., 1999), and several human bladder tumorigenic urothelial cell lines such as HCT-29Tmv, Hu456, and Hu549 (Christensen et al., 1993), as well as the JCA-1 human prostate carcinoma line (Van Bokhoven et al., 2001). Other cell lines

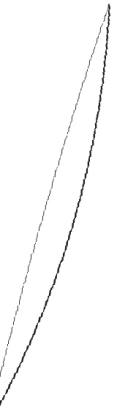


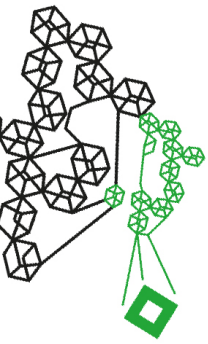
frequently associated with misidentification or genetic variability include cell lines are shown in **Table 2**.

Table 2. Commonly misidentified or cross-contaminated cell lines and associated challenges.

Cell line	Typical reason for challenges	Common challenges (incl. factors complicating what is considered misidentified)
CHO (Chinese Hamster Ovary)	Widely used in biopharmaceutical production and research, leading to multiple sublines	Genetic drift and ambiguous subline classification
MCF-7 (Human Breast Cancer)	Extensively used in breast cancer research	Cross-contamination and subline variability (
A549 (Human Lung Adenocarcinoma)	Common in toxicology and cancer research	Cross-contamination and mislabelling
U87MG (Human Glioblastoma)	Known for variability due to passage history and sublines	Misidentification and genetic divergence
L929 (Mouse Fibroblast)	Frequently used in cytotoxicity assays and as a feeder layer	Cross-contamination with other murine lines
HEK293 (Human Embryonic Kidney)	Extensive use in molecular biology and subcloning	Genetic drift and subline misclassification
PC-3 (Human Prostate Cancer)	Widely used in prostate cancer research	Cross-contamination and misidentification in mixed cultures
J774A.1 (Mouse Macrophage-like)	Commonly used in immunological studies	Mislabelling and contamination during extended use

Footnote: Data adapted from multiple sources on cell line misidentification and contamination (ICLAC database, Nelson-Rees et al., 1981; Ye et al., 2015; Christensen et al., 1993; Van Bokhoven et al., 2001; others).





3.6 Standardization and guidelines

Various organizations and commercial parties published guidelines for cell line authentication.

3.6.1 ICLAC

Minimal requirements for cell line authentication and quality control under ICLAC are built around routine STR genotyping plus basic provenance and reporting standards (https://iclac.org/resources/human-cell-line-authentication/). ICLAC's Guide to Human Cell Line Authentication specifies that human cell lines must be authenticated by STR profiling against a suitable reference, using at least 13 nuclear STR loci plus Amelogenin for sex determination, and applying an ≥80% allele-match threshold as defined in the American National Standards Institute (ANSI)/ATCC standard. The STR profile from the test sample should be compared against reference profiles from major cell banks and databases such as Cellosaurus, and against any donor or historical reference available, to confirm identity or reveal misidentification/cross-contamination. The guide recommends authenticating cell lines when they are newly acquired or

established, before starting a new project, when creating or using master/working banks, after major manipulations (*e.g.*, selection, genetic modification), and whenever unexpected results raise suspicion. For lines already in use, authentication at the beginning and end of a project is suggested, with additional checks if cultures are maintained long term or shared between labs. Minimal quality control also includes verifying that a line is not listed in the ICLAC Register of Misidentified Cell Lines before use and avoiding use of known false lines unless there is a specific, well-justified reason that is clearly disclosed. Good practice requires sourcing cells from reputable banks, maintaining clear records of cell line name, origin, passage history, STR profiles, mycoplasma status, and any known issues, and following ICLAC's checklists and naming guidelines when reporting cell lines in manuscripts or grant applications.

Several major biological resource centres complement ICLAC by issuing practical guidelines, test recommendations, and standards that aim to reduce misidentification, contamination, and reporting errors.

3.6.2 ATCC (American Type Culture Collection)

ATCC has also played a role in formalizing cell line authentication standards, co-leading development of the ANSI/ATCC ASN-0002 documentary standard for STR-based authentication of human cell lines. ATCC technical documents recommend a minimal panel of STR loci (aligned with ASN-0002), routine authentication at key points (on receipt, before banking, after major manipulations, and before publication), and use of reference profiles from their catalogue to confirm identity and detect cross-contamination. Although ATCC defines a comprehensive quality control framework that includes mycoplasma testing, sterility checks, morphology monitoring, and documentation of passage number and provenance, it also recognizes that some cell lines deposited many years ago were originally misidentified or cross-contaminated and has reclassified these where appropriate. Therefore, even reference collections should still be used alongside routine authentication and contamination screening. This underscores the importance of independent verification.

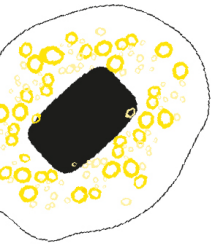
3.6.3 German Collection of Microorganisms and Cell Cultures (DSMZ)

The German Collection of Microorganisms and Cell Cultures (DSMZ), similarly to ATCC, authenticates its human cell lines, participating in the same STR-profiling framework and contributing profiles to shared databases referenced in standards discussions. DSMZ and other major banks (e.g., JCRB, ECACC) apply routine identity testing, contamination screening, and standardized nomenclature, and share reference STR profiles that underpin cross--lab comparability and error detection (Almeida et al., 2016).

3.6.4 Comparison of guidelines

In conclusion, ATCC, DSMZ, and partner organizations seem to not only implement these practices internally but also advocate for community adoption of ASN-0002, development of complementary SNP/species-barcoding standards, and alignment with journal and funder requirements for authentication and quality control. Together with ICLAC, these organizations define a convergent set of expectations: use of validated STR panels for human lines, regular identity and contamination checks, checking against misidentified-line registries, and transparent reporting of cell line origin and testing in publications.

ATCC, DSMZ, and ICLAC show strong convergence on STR loci for human cell line authentication, with no substantive differences in "mandatory" sets, all reference the ANSI/ATCC ASN-0002 standard and endorse using at least 8 core loci (often expanded to 13+ for robustness). The foundational 8 core loci (D5S818, D13S317, D7S820, D16S539, vWA, TH01, TPOX, CSF1PO) plus Amelogenin (for sex) form the minimal shared panel across all three, derived from forensic standards and adapted for cell lines via ATCC-led consensus. ATCC's ASN-0002 (updated 2022)

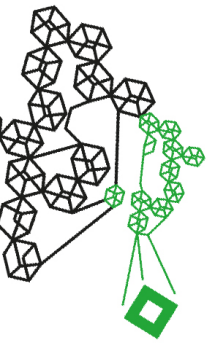


explicitly recommends a minimum of 13 autosomal STR loci to enhance discrimination, especially for lines with LOH or MSI, and both DSMZ and ICLAC align with or cite this threshold for reliable matching (≥80% allele concordance). Specific panel differences and practice differences between the three organizations are outlined in **Table 3** below.

Table 3. Specific panel and practice differences between ATCC, DSMZ and ICLAC to define identification criteria of cell lines.

Organization	Minimal loci specified	Preferred/extended panel	Key notes
ATCC	8 core + Amelogenin; recommends ≥13 for authentication	Full ANSI ASN-0002 panel (13+ loci); uses Identifiler/PowerPlex kits internally	Leads standard development; provides searchable database with merged data from DSMZ/JCRB/RIKEN for cross-comparison
DSMZ	8 core + Amelogenin; compatible with ATCC	≥13 loci in practice; contributes to consensus databases	Harmonizes kits/loci with ATCC for merged profiles; emphasizes additional loci for ambiguous matches
ICLAC	≥13 STR loci + Amelogenin explicitly required	Directs to ATCC/DSMZ/Cellosaurus databases using core+extended loci	No independent panel, relies on bank standards; stresses ≥13 for >80% match criterion to account for cultured-sample variation

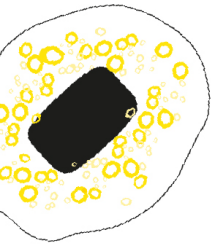
Overall, differences are minor and implementation-focused: ATCC/DSMZ generate and publish profiles using compatible commercial kits (*e.g.*, ABI Identifiler, PowerPlex) that amplify 15-24 loci, allowing flexible use of the full dataset while meeting the 8/13 minimum. ICLAC prescribes the ≥13 threshold in guidance without specifying kits, directing users to bank databases where profiles use overlapping loci. All support database merging (ATCC+DSMZ+JCRB+RIKEN) to resolve discrepancies from LOH/MSI or kit variations, ensuring interoperability without rigid "mandatory" divergence.



3.7 Safety related to misidentification of cell lines

GMOs can also be associated with misidentifications. Dutch organizations should comply to the national law and regulations regarding GMOs meaning that identity determination of those materials is required. The European Union (EU) has developed regulations and directives regarding GMOs. For example, contained use of GMOs in research falls under the Directive 2009/41/EC on the contained use of genetically modified micro-organisms (GMMs). There are other specific Directives and Regulations for more specific situations, such as the deliberate release of GMOs, for instance when GMO plants are used in field trials or clinical trials related to gene therapy or GMO-vaccines. In the Netherlands, the law and regulations that are applicable to GMOs (Besluit GGO 2013 and Regeling GGO 2013 under ''Wet milieubeheer'') is derived from the Directive from the European Union. The Regeling GGO describes specific actions that are needed to allow appropriate and safe contained use of GMOs within Dutch organizations.

In the Regeling GGO, it is described that Dutch organizations that work with GMOs should include and execute specific procedures regarding quality control to establish whether a GMO batch is not contaminated. Failure to adequately perform these quality control procedures may result in non-compliance with GMO regulations and can have broader regulatory, operational, and biosafety consequences for the organization, as well as potential risks with the environmental introduction of GMOs. For example, mouse cell lines contain endogenous retroviral sequences. Failure to correctly identify such cell lines prior to the use of retroviral vectors may allow recombination events to occur, leading to the formation of replication-competent retroviruses. Such viruses may pose a

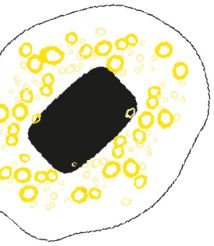


potential environmental risk due to persistence and dissemination, as well as a health risk to humans or animals resulting from unintended exposure or infection.
A comparable example is the use of COS-1 and COS-7 cell lines, which express SV40 large T antigen. Failure to distinguish these cell lines from non-SV40-expressing “COS” derivatives, for example due to mislabelling or inadequate cell line identification, may result in the unintended co-presence of SV40 large T antigen and SV40 origin-containing sequences, enabling episomal replication or reconstitution of infectious SV40 particles. This represents a potential biosafety concern arising from cell line misidentification, potentially leading to an environmental risk through accidental release or spread of replication-competent SV40 viruses.
Moreover, if a cell line is misidentified and is originally a cell line that should be contained in a level-II facility, but is thought to be a level-I cell line, researchers may be exposed to for instance viral particles as level-I conditions are not sufficient in terms of safety measures.

3.8 Remaining considerations regarding cell line authentication

How genetically engineered cell lines are used in research and how their misidentification or contamination can distort the interpretation of genetic modifications is a matter of debate. Genetic modification of a cell line implies the deliberate alteration of its genome (*e.g.*, by means of CRISPR knock-out, overexpression, or reporter insertion) while (ideally) maintaining correct identity and provenance. When a genetically modified line is misidentified or contaminated, the observed phenotype (*e.g.*, drug response, gene expression, growth behaviour) cannot be reliably attributed to the intended modification, because the underlying cell identity is wrong. For example, if a CRISPR-edited “lung cancer line” is a HeLa derivative, any “lung-specific” conclusions are invalid, and the modification may be acting in a cervical cancer background instead (Weiskirchen, 2025; Huang et al., 2017).
Fast-growing lines like HeLa, HEp-2, or T24 are common contaminants that can overgrow slower genetically modified lines during routine culture, especially if authentication is not performed regularly. Once contamination occurs, the resulting culture is a mixture or pure contaminant, so any phenotypic effects of the genetic modification may be masked or confounded by the contaminant’s biology. Many widely used “standard” lines have been shown by STR profiling to be HeLa or other contaminants. If a researcher uses such a misidentified line as the parental strain for CRISPR, lentiviral transduction, or other modifications, all derived GMO lines inherit the wrong identity, and any conclusions about tissue-specific or disease-specific mechanisms become suspect. Misidentified lines that are then genetically modified and deposited in repositories or shared between labs can propagate errors: a “GMO line X in tissue Y” may actually be a modified HeLa or another contaminant, misleading future users about the true cellular context of the modification.
Genetic modification itself does not usually prevent STR-based authentication, but it can make cell line authentication more challenging in several practical ways, mainly by introducing genetic heterogeneity, altering the reference profile, and complicating the interpretation of “identity”. CRISPR/Cas9, lentiviral transduction, or other editing methods often generate a polyclonal population, and subsequent cloning yields multiple subclones that may differ in copy number, integration site, and off-target mutations. Each clone can acquire distinct structural variants (deletions, duplications, translocations) or mosaic CNAs during editing and expansion, so that different clones from the same parental line show divergent karyotypes and SNP/CNV profiles, even if their STR profile remains unchanged. This means that two “isogenic” edited clones may behave very differently in functional assays, and simply matching STRs to the parental line does not guarantee that the clones are functionally equivalent or that observed phenotypes are solely due to the intended edit (Chen et al., 2025; Panda et al., 2023).

Although STR loci are usually located in non-coding regions, extensive genetic engineering (*e.g.*, large deletions, chromosomal rearrangements, or whole‑chromosome loss/gain) can occasionally affect STR loci, particularly when edits occur nearby or in genomically unstable regions. Consequently, the STR profile of the engineered line may differ from the parental line due to deliberate or unintended structural alterations rather than stochastic genetic drift. This complicates



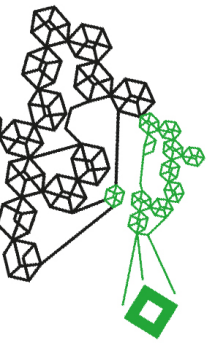
the distinction between true misidentification and genuine alterations arising from cell line modification (Masters et al., 2001; Chen et al. 2025). Moreover, long‐term passaging of edited lines can lead to additional STR instability (slippage, loss of heterozygosity), further blurring the match to the original reference and requiring updated in‐house STR profiles for each engineered line (Chen et al., 2025).

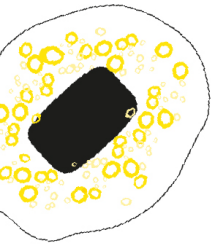
For a wild-type line, the reference is usually the profile from a major cell bank (e.g., ATCC, DSMZ) or the original depositor. For a genetically modified line, there is no universal reference: the “correct” profile depends on whether one compares to the parental line, an early passage of the edited line, or a specific clone (Panda et al., 2023). If the parental line itself is not well authenticated, or if multiple labs use different clones or passage numbers, the same “GMO line name” can correspond to several genetically distinct populations, undermining reproducibility even when STRs appear to match locally.

Genetic modification often involves selection (*e.g.*, antibiotic resistance, fluorescence, growth advantage), which can favour the outgrowth of contaminating lines that happen to carry the same selection marker or have a fitness advantage. For example, a HeLa contaminant that acquires the same resistance cassette as the intended line can be mistaken for a valid edited clone, especially if authentication is done only once at low passage and not repeated after selection.

These challenges in authentication of GMOs may be mitigated using an approach including:

- Authentication of the parental line before any genetic modification, using STR/SNP profiling and species testing.
- Generation and banking of multiple clones, and performing STR/SNP profiling on each clone to document clonal heterogeneity.
- Establishing an in-house reference STR profile for each engineered line at an early passage and re-testing regularly, especially after selection and long-term culture.
- Combination of STR with orthogonal methods (e.g., SNP arrays, WGS, karyotyping) when working with heavily edited or unstable lines, particularly for critical experiments or biobanking.





4. Experimental STR-profiling

To experimentally validate the findings of the literature study, commonly used cell cultures at the UT and NIZO food research were collected and submitted for authentication by STR profiling.

4.1 Experimental set-up

4.1.1 Cell culture and sample preparation

Thirty-two different human cell lines were cultured at UT, NIZO food research or at Saxion under defined conditions in the appropriate growth medium for each cell type until sufficient biomass was obtained. The Eurofins Sample Submission Guide was followed to collect and submit sample. For this, prior to harvesting, culture medium was removed, and cells were washed twice with phosphate-buffered saline (PBS) to remove residual serum and media components. Cells were then collected by centrifugation to form a visible pellet. Pellets were rinsed with either PBS or Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) to remove remaining extracellular proteins and media constituents before submission. Cell preparation criteria specified by Eurofins were >10 000 cells per pellet with a clearly visible pellet in a 2 mL reaction tube (no Falcon tubes) for STR analysis. Cell pellets containing ≥10 000 cells, following Eurofins guidelines, were shipped at room temperature.
Note: Even though the Eurofins Sample Submission Guide does not specify exact centrifugation forces (g) or durations for pellet formation, typical practice for suspension cultures involves pelleting at ~300–500 × *g* for 5–10 min at room temperature; for adherent cells, trypsinization was followed by centrifugation under similar conditions prior to washing. This ensures a compact pellet suitable for shipment and downstream DNA extraction.

As an internal control and to establish sensitivity to contamination of human cell lines, we generated we generated mixtures of two common in-use human cell lines, C20A4 and SW1353, of 100—0%, 98-2%, 95-5%, 75-25%, 50-50%, 25-75%, and 0-100%. Cell masses were submitted for authentication as described above.

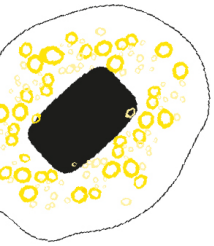
4.1.2 Shipment

Collected samples were submitted to Eurofins Genomics (APG Department, Anzinger Str. 7a, 85560 Ebersberg, Germany) with prepaid barcodes assigned via the Eurofins online order system. During transfer, samples were secured in protective containers to prevent leakage.

4.1.3 STR analysis and data handling

The service selected employs an Applied Biosystems™ AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit to genotype 16 short tandem repeat (STR) loci (including Amelogenin for sex determination) according to ANSI/ATCC ASN-0002 standards. Upon receipt, Eurofins extracted DNA from the submitted pellets and performed STR amplification using the Identifiler Plus system. The STR profiles were analysed with Capillary Electrophoresis. The output shared with us as recipient included allele calls at each locus and quality metrics for heterozygosity, peak height ratios, and allelic balance, allowing detection of misidentification or cross-contamination.

To analyse the dataset, STR profiles obtained with Capillary Electrophoreses were compared with published reference profiles using the Cellosaurus database, applying the International Cell Line Authentication Committee (ICLAC) criteria for identity and contamination assessment. According to these ICLAC criteria, a match of ≥80% with the expected donor indicates an authenticated cell line, a match between 56–79% suggests a related or partially matching cell line, and a match of



<56% is considered no match, indicating misidentification or potential cross-contamination. In addition to this, ≥13 loci should be analysed, with similarity to the query profile and potential misidentification or cross-contamination should be excluded, which is defined by another cell line showing >80% similarity.

4.2 STR-analysis results

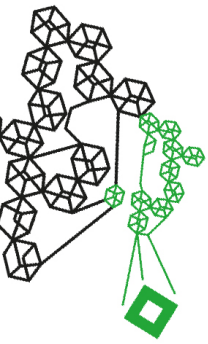
4.2.1 Overview of Snapshot analysis

A total of 32 selected and actively maintained human cell lines were submitted for authentication, from UT and NIZO food research. For the cell lines analysed in this study, the STR profiles of all the expected parent/original cell lines were found to be deposited in the Cellosaurus database, except for one cell line (PDL-hTERT or derivatives). ICLAC STR match criteria for data interpretation that is used for this section:

Criterion 1: The STR profile shows >80% similarity to a reference profile.

Criterion 2: The comparison includes more than 13 loci.

Criterion 3: No other reference/hit in the database meets both Criterion 1 and Criterion 2.



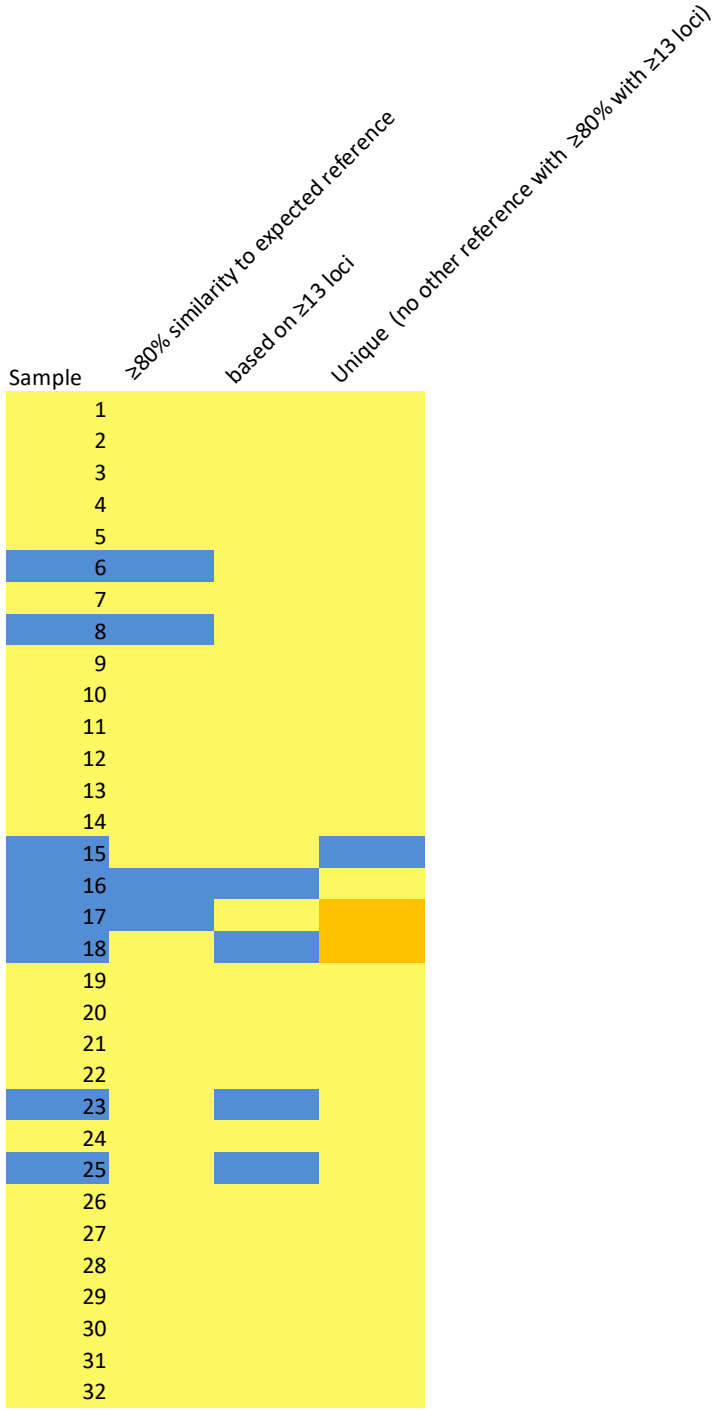


Figure 2 Heatmap of STR-data analysis including the three ICLAC criteria on the x-axis and the anonymized cell lines on the y-axis. According to ICLAC guidance, STR-based authentication of human cell lines is assessed using three key criteria: (1) the test STR-profile must match the reference profile with ≥ 80% similarity; (2) the comparison must include a sufficient number of loci (typically ≥ 13 for human cell lines); and (3) no other cell line in the database should show ≥ 80% similarity over the same set of loci, to ensure the match is unique and the cell line is correctly identified. (ICLAC Guide 2023). Yellow indicates compliance with the criterion, orange indicates it is uncertain or indecisive whereas blue indicates non-compliance.

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A total of 24 out of the 32 submitted cell lines (75%) showed >80% similarity to the query profile based on at least 13 loci, indicating full or near-full concordance with the reference cell line. These cell lines included common cell lines C20A54 batches, THP-1, Hela, CH-2879, Hek293 derivatives, SW1353 batches and derivatives, PC3, WTC-11 derivatives, HUVEC derivatives, MCF7, MG63, MDA-MB-231, PDL hTERT, SCP-1, MSOD-B, SHSY5, Saos-2, T24, HT-29, and Caco-2 from UT or NIZO food research. **Figure 2** shows an overview of the extent by which authenticated cell lines comply with the three ICLAC criteria. Eight cell lines (25%) did not meet the criteria for authentication, either because they showed <80% similarity to an expected reference profile, less than 13 loci were detected, or another cell line type with >80% similarity. In case criterium 2, less than 13 loci detected, is applicable, this could be due to technical errors such as suboptimal PCR test conditions, inappropriate DNA quality or quantity, or contamination with non-human cell lines. This suggests that repeating the analysis with better DNA quality and quantity might lead to a more complete and reliable STR-profile or that these lines are either misidentified, cross-contaminated, or genetically divergent due to genetic drift.

4.2.2 Cell lines failing authentication criteria

Four out of 32 cell lines showed <80% similarity to the expected reference profile (**Figure 2** blue boxes in column 1), falling below the authentication cut-off. One of these cell lines, had a first hit with <80% similarity to an unexpected reference cell line. However, the expected reference STR profile of this cell line was not available in the CLASTR database. Moreover, reliable readouts could only be determined for 8 loci for this sample. CLASTR results that show max. 8 loci reads for human cell lines indicate potential technical errors such as suboptimal PCR test conditions, inappropriate DNA quality or quantity, or potential contamination with non-human cell lines. While authenticity cannot be confirmed, there is a possibility of cross-contamination. One of these cell lines was expected to be HEK293. For this cell line, <80% similarity when compared to the expected reference may have been the result of its known high genetic instability (Malm et al., 2020), as most CLASTR hits corresponded to known HEK293 derivatives. This indicates partial similarity, but the overall match did not meet the >80% threshold for authenticity.

Three cell lines, despite demonstrating >80% similarity to the expected reference profile, identified less than the required 13 loci suggesting that the similarity levels are based on an unreliable number of loci. This can be due to technical errors such as suboptimal PCR test conditions, inappropriate DNA quality or quantity, or contamination with non-human cell lines. Therefore, for these cell lines, authentication results were regarded inconclusive and we would recommend to repeat the assay for such sample with improved quality and quantity of the DNA (Figure 1, red column 2). Interestingly, two cell line cultures derived from the same stock but thawed at different time points showed 90% and 78% similarity to the query profile, indicating that either the stock might contain contaminations or that the cell line might be contaminated during cultivation of the cells. Moreover, cell lines that contained a genetically modified element within the genome did not present anomalies in similarity percentage to the query profile when compared to the unmodified parental cell line. Interestingly, the LNCAP cell line, a prostate cancer cell line, that had been in culture for several passages appears to have lost the Y chromosome, a phenomenon common for this cell line (Bose et al. 2025).

Upon authentication of our internal control samples, matching with ICLAC STR criteria for the reference cell type is failing upon intended contamination with another human cell line from a level of 25% upwards (**Figure 3**). Interestingly, % similarity to the reference in databases in all mixtures with >25% contaminations were below 80%, also for the ‘’contaminant’’ reference profile whereas the appropriate number of loci could be included (>13). In other words, the mixed samples used in

this analysis never had a hit above 80% similarity to a reference in the Cellosaurus database (max. ±77%).

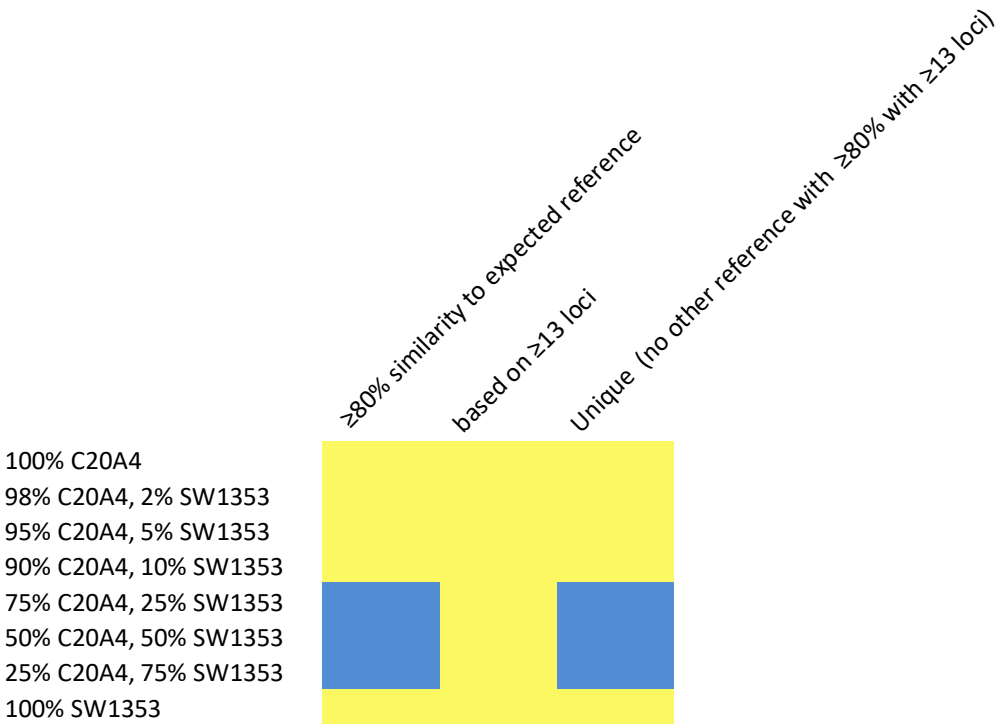
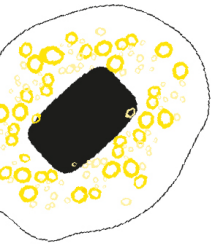


Figure 2 **Heatmap of STR-data analysis from mixtures of C20A4 and SW1353 cell line and the ICLAC criteria were scored to expected reference.** According to ICLAC guidance, STR-based authentication of human cell lines is assessed using three key criteria: (1) the test STR-profile must match the reference profile with ≥ 80% similarity; (2) the comparison must include a sufficient number of loci (typically ≥ 13 for human cell lines); and (3) no other cell line in the database should show ≥ 80% similarity over the same set of loci, to ensure the match is unique and the cell line is correctly identified. (ICLAC Guide 2023). Yellow indicates compliance with the criterion, whereas blue indicates non-compliance. The 100% SW1353 sample matches the three key criteria for the SW1353 reference profile.

4.3 Concluding remarks experimental STR analysis

In conclusion, the performed profiling analysis indicates that most cell lines are authentic (>80% similarity to the query profile based on 13 loci and no other second hit). However, a small subset shows low or ambiguous similarity, suggesting potential misidentification, cross-contamination, or genetic divergence. Among this small subset were also cell lines with low passage numbers and none of the cell lines were in the Register of Misidentified Cell Lines or known to be frequently misidentified. Overall, according to the snapshot analysis, 8 cell lines require additional analysis to confirm authenticity as it is currently questioned or inconclusive. This could include another round of STR-profiling as technical issues at the third party could not be excluded in most cases. Moreover, morphological analysis and specific biochemical tests, if available, could be considered. When authenticity is critical to the research, WGS can be considered, provided that a suitable reference genome is available for comparison. Appropriate subsequent analyses highly depend on the available information for each cell line and is out of scope for this study. Importantly, this snapshot underscores the importance of regular STR profiling to ensure the validity of experimental results and the integrity of cell line-based research.



5. Survey

A questionnaire was designed in order to determine whether cell line identity testing occurs within Dutch organizations, to evaluate awareness of the relevance of regular cell line authentication, to identify any factors playing a role in resilience against testing, and whether misidentifications have been observed among those organizations and subsequent measures taken upon identification of a cell line that does not comply with authentication guidelines.

5.1 Survey methodology

A questionnaire-based survey was designed at the University of Twente and implemented through the Crowdtech online survey platform (Crowdtech BV, Amsterdam, the Netherlands) to investigate cell line identification within Dutch organizations. The survey consisted of multiple-choice and open-ended items addressing cell culture practices, authentication frequency, and statements. Invitations were distributed electronically via the Biologische Veiligheidsfunctionarissen (BVF)-network, the BVF-symposium, and the SAAZ-Unie symposium. Informed consent was obtained digitally prior to participation. All responses were collected anonymously in compliance with General Data Protection Regulation (GDPR) standards and internal ethical review.

5.1.1 Ethical approval and data management

This project was reviewed by the Ethics Committee of the University of Twente, which granted a positive opinion for its conduct. The anonymized raw data obtained from the questionnaire are managed by the University of Twente. The survey tool, Crowdtech, was used for creating and distributing the questionnaire and is provided by a Dutch company. All raw data are stored and secured on servers located in the Netherlands. In the disclaimer it was mentioned that this report containing the anonymized aggregated survey results will be published on the website of COGEM (Commission on Genetic Modification, the commissioning body). The questionnaire did not include any questions requesting disclosure of participants' names or the names of their research group, department, or institution, and these identifiers were also not requested to enter in open-text fields.

Participants were informed that, if they had questions about their rights as research participants, or wished to obtain additional information, ask questions, or discuss any concerns about this study with someone other than the researchers, they could contact the Ethics Committee of the University of Twente. Participants were also provided with the option to contact the researchers directly. The questions and responses are depicted in Appendix I.

5.2 Results of survey/questionnaire

A small number of preliminary screening questions were included to identify and differentiate organisations that currently perform cell line testing. Based on the responses to these initial questions, the questionnaire was subsequently branched accordingly. For each question, the number of respondents is indicated in the figures, including responses from participants who did not complete the entire questionnaire.

5.2.1 Approximately half of the Dutch organizations perform cell line authentication with a slight bias toward commercial organisations and university medical centres.

A total of 77 respondents initiated the questionnaire. Of these respondents, 34% (n=26) is currently affiliated with an academic organization or university, 25% (n=19) are working for a university

medical hospital (UMC), 21% (n=16) works for a commercial company, 7% (n=5) at a contract research organization, 4% (n=3) at a government agency, and 9% (n=7) responded 'other' (**Figure 4**). Out of 70 respondents, 64 work with human cell lines, 62 with animal cell lines and 10 with plant cells. Among the 71 respondents, 83% (n = 59) reported working with cell lines at their respective institutions that have been in use for more than 10 years, whereas 13% (n = 9) indicated working with more recently introduced cell lines, and 4% (n = 3) reported being unsure.

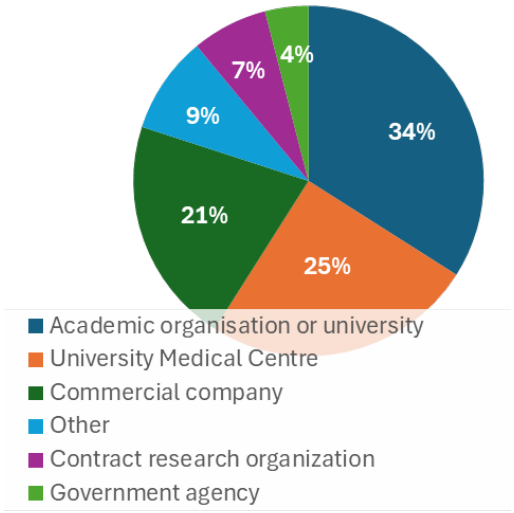


***Figure 3* Affiliation of total (n=77) respondents.** The majority of respondents works at an academic organization or hospital, university medical centre or commercial company.

Of 67 respondents, 51% (n = 34) indicated that cell line identity testing is performed within their research group or organisation, while 49% (n = 33) reported that such testing is not conducted. Responses indicating the absence of cell line identity testing were evenly distributed across organisational types and backgrounds. Consequently, respondents were nearly equally divided at this stage for the subsequent questions.

The large proportion (38%) of the respondents indicated that annually approximately 1 to 50 cell lines are subjected to authentication evaluation with 15% mentioning that 50 to 100 cell lines are tested during this time span, and 8% of the respondents perform over 100 identifications annually. The respondents further indicated that less than 1% of all tested cell lines eventually turn out to be misidentified.

5.2.2 STR analysis is the main assay used for cell line authentication purposes.

In total, 59% (n=20) out of 34 respondents that confirmed cell line identity tests within their group or organization indicated that STR is among the methods of cell line identity testing. Other frequently employed methods used for cell line authentication purposes includes DNA sequencing (n=14), PCR for specific regions (n=12) and functional analysis (n=13), and comparison of obtained data against known databases**Figure** **5**).

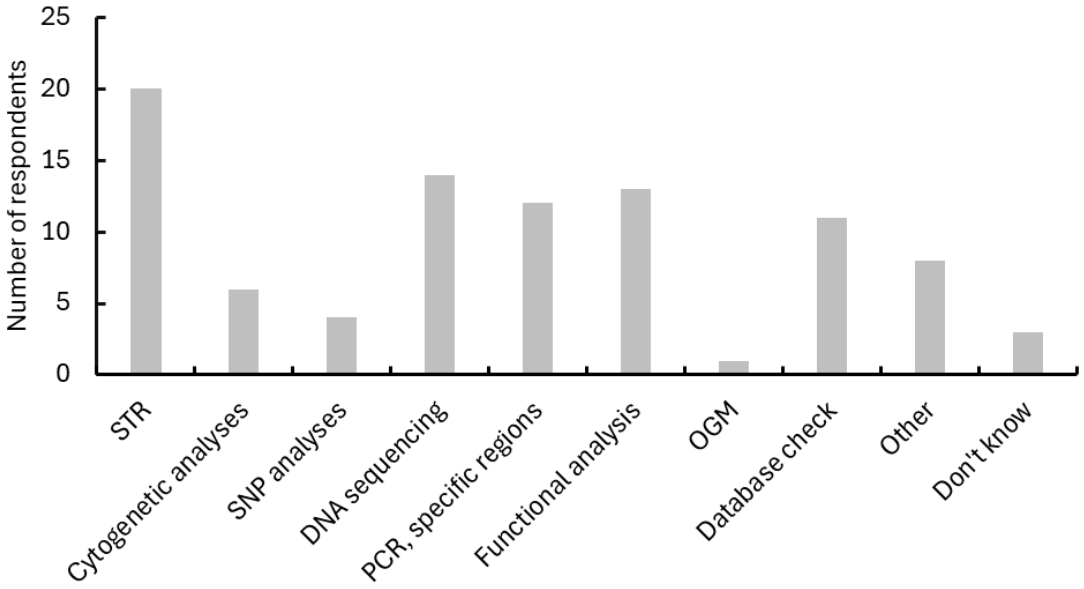


Figure 4 Reported use of different types of cell line authentication procedures. *STR = DNA profiling, short-tandem repeat; Cytogenetic analyses = e.g. karyotyping, FISH or SKY), SNP = DNA profiling by single nucleotide polymorphism analysis, DNA sequencing = by means of a PCR fragment or other, PCR for specific regions = e.g. by means of a fragment in a cell line obtained by means of recombinant DNA technology, functional analysis = e.g. by detection of fluorescence, identification of specific morphological features, OGM = optical genome mapping, Database check = by ICLAC or Cellosaurus etc. Other included response such as Whole genome sequencing, detection of a marker gene or antigen, optical, flow cytometry-based or dependent on the research line. One respondent commented that for animal studies an IDEXX test is required.*

Analysis of the responses indicated that many organizations reporting the use of cell authenticity testing employ multiple authentication methods. Of the 20 respondents who indicated the use of STR profiling for cell line authentication, 15 reported that additional identification methods are applied within their group or organization **Figure 6**). Notably, one of the 20 respondents who reported using STR profiling as an internal method for cell line identification in a previous question indicated that human cell lines are not used within their group or organization. This discrepancy may reflect a reporting error or, alternatively, the use of a validated STR-based authentication method for animal cell lines.

Of 30 respondents, 63% (n=19) indicated to use external services for authentication, while 30% (n=9) mentioned using available in-house services (7% don't know).

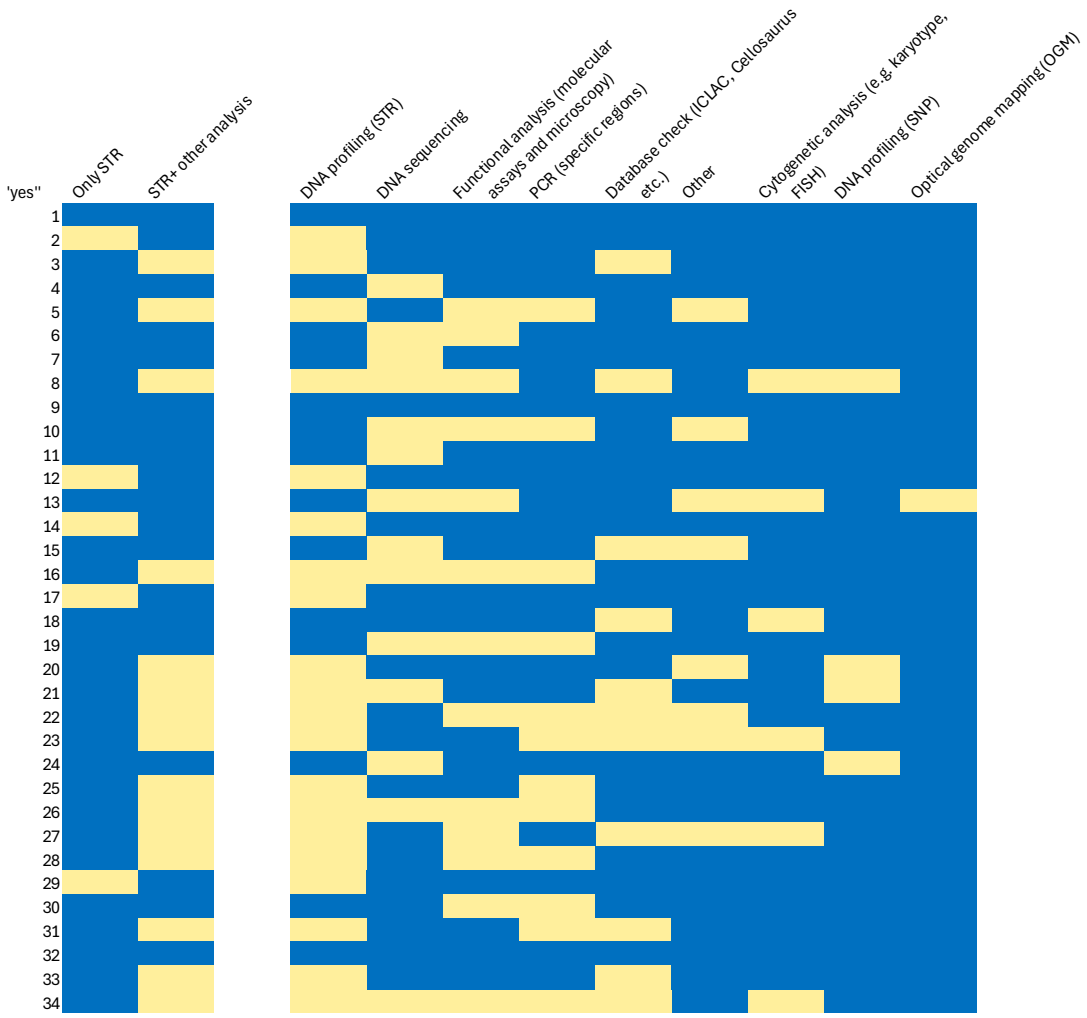


Figure 5 **Parallel use of different authentication analysis types** reported by respondents that confirm cell line identification performance within group or organization (''yes'') and that perform STR solely or in combination with other techniques. Blue depicts that the use of the analysis type (whether or not in combination with STR) occurs within the organization whereas yellow depicts that the use of the analysis type (whether or not in combination with STR) does not occur within the organization.

5.2.3 Frequency of authenticity testing among the different organizations is highly diverse.

The reported frequency of cell line authentication testing varied considerably among respondents (n = 31) who indicated that they perform such testing (**Figure 7**). While many respondents reported conducting routine, periodic testing (*e.g.* monthly), the response option “different” was selected by 7 of the 31 respondents (22%). Explanations provided for this choice included variability in testing frequency between research groups, authentication performed at the time of cell freezing, or the use of a combination of different testing intervals. In addition, several respondents indicated that authentication testing is performed upon receipt of cell lines from collaborators. Some respondents noted that they apply multiple of the listed testing frequencies; however, the questionnaire did not allow the selection of more than one response option.

Further analysis revealed that the five respondents who reported to exclusively rely on STR profiling as their sole authentication method indicated that this analysis is performed only under

specific circumstances, such as upon suspicion of misidentification, at the time of cell stock freezing, or when required by a journal or collaborative partner. In contrast, respondents who reported using STR profiling in combination with additional cell line identification methods described a broader range of testing frequencies, including authentication at the start and end of a project or on a periodic basis.

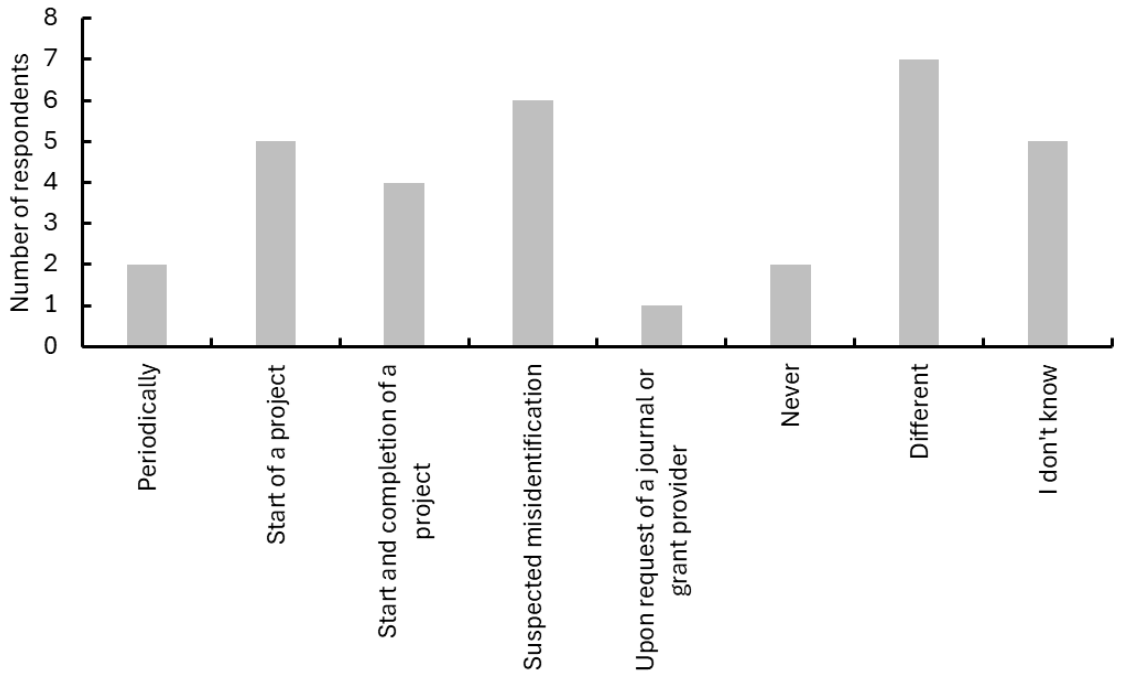


Figure 6 **Frequency of cell authentication** (n=31 total).

5.2.4 Measures taken upon misidentification.

Thirty-eight percent of respondents (11 of 27) who had previously indicated to perform cell line authentication, indicated that misidentification exclusively involved human cell lines. Cell lines mentioned as reported misidentified within the various organizations included tumor macrophage KG-1, lymphoblast blood-derived MV4-11, colon adenocarcinomacSw480 and cervical adenocarcinoma HeLa cells. Misidentification of non-human animal lines or plant lines was not reported in our survey. In contrast, 10 of the 27 respondents (34%) reported that they had not encountered misidentified cell lines. The remaining respondents (n=8, 28%) indicated that the type of misidentified cell line was unknown. Subsequent analysis showed that respondents reporting misidentification primarily of human cell lines were affiliated with academia (n=5), university medical centres (n=4), companies (n=1), or other organizations (n=1). Respondents who reported not having encountered misidentified cell lines were affiliated with companies (n=5), academia (n=2), university medical centres (n=1), contract research organizations (n=1), or other organizations (n=1).

According to subsequent analysis, most cell lines were obtained either from commercial cell banks, such as ATCC or DSMZ, or through collaborative partners. One respondent indicated that cell lines were generated in-house from primary tissue. A few respondents (n=3) answered that the origin of the cell lines used was unknown. Table 4 outlines the responses outlining the origin of the cell lines in use by the respondent organizations.

List describing origin of cell lines in use by respondent organizations.

- Through a collaborating organization
- Originally purchased from commercial cell line providers, culture collections, and biobanks including ATCC/DSMZ
- Obtained directly from patients or animals, or sometimes generated in-house
- Unknown
- iPSC lines sometimes generated from skin, sometimes purchased from companies, or obtained from other research groups
- Developed in-house for antibody production

The majority of respondents (68%, 17/25) reported that previously misidentified cell lines did not lead to reduced safety, such as changes in containment or facility requirements. For a subset of these respondents, no safety issues were observed despite uncertainty regarding the identity of the misidentified cell line at the time of completing the questionnaire. Twenty-four percent (n=6) of the respondents did not know whether the misidentification of cell lines led to safety risks. Only one respondent (4%) indicated that misidentification had resulted in safety concerns; however, no additional details were provided in the follow-up question. One respondent chose not to answer this question.

We next surveyed any measures taken by the participating respondent organizations upon detection of a misidentified cell line (**Figure 8**). In general, organizational authorities were notified, including lab or research managers (n=16), the Biological Safety Officer (n=12) or the line manager (n=8) or any other relevant operational department or local responsible party (**Figure 8**). In 12 out of 26 cases, cell lines were terminated. While reasons for retaining misidentified cell lines were not specifically addressed in this survey, laboratories may opt to do so for various reasons, for instance; in case cell lines are not actively cultivated (and obtained long time ago) and only tested after receiving or sending the materials. First, if the misidentified cell line retains the relevant biological characteristics necessary for the ongoing research, its experimental utility may remain valid despite the discrepancy in identity. Second, switching to a correctly identified alternative may involve substantial logistical challenges, including the need to repeat experiments, re-validate protocols, or acquire new reagents, which can significantly delay project timelines. Third, historical data generated with the misidentified line may still hold scientific value, allowing for continuity in longitudinal studies or comparative analyses. Finally, risk assessments may indicate that the continued use of the misidentified line does not compromise laboratory safety or regulatory

compliance, further supporting its ongoing use under controlled conditions. A last reason may include the inadverted mislabelling of cell lines.

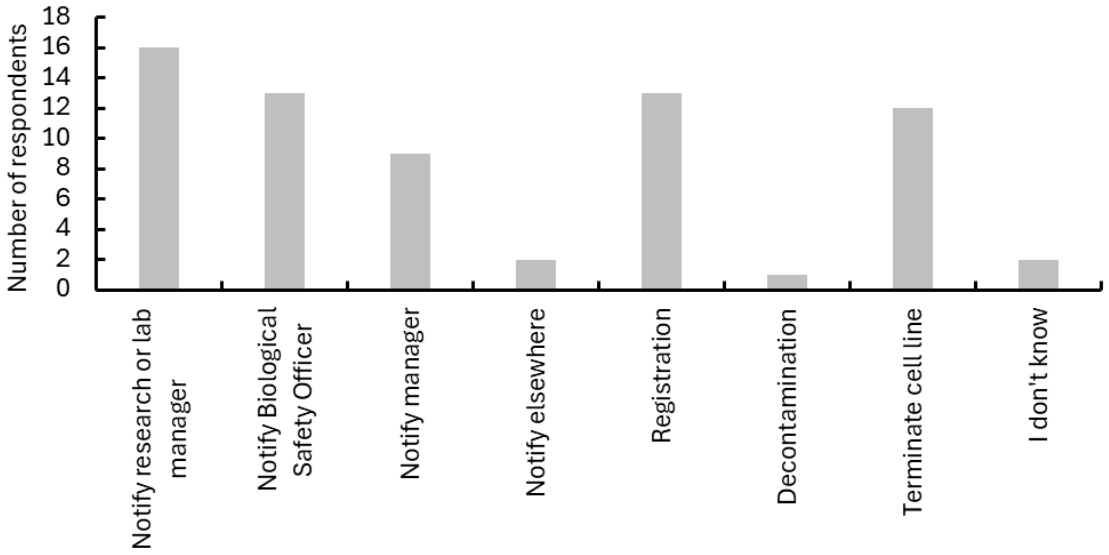


Figure 7 Measures taken upon detection of a misidentified cell line *(total n=26). Responses to 'Notify elsewhere' included the local Quality Assurance department or the Director Operations.*

5.2.5 Identification of resistance against implementation of testing for cell line authentication.

We next aimed to explore the potential reasons underlying the reluctance of the subset of respondents who reported not performing any authentication testing to implement cell line authentication assays. Among respondents who indicated that no cell line identifications are currently conducted within their group or organization, a large proportion (45%, n=15) reported uncertainty as to whether their group or organization is considering implementing cell line identification (**Figure 9**). Slightly more than one-third of respondents (36%, n=12) indicated that such implementation is being considered, while a minority reported that cell line identification is not being considered at their facility.

As shown in **Figure 10**, most respondents expressed confidence in the accuracy of cell line identity upon receipt. However, other factors were also cited as contributing to the decision not to perform cell line identification. These include the expense of the analysis (11%), time restrictions (11%), lack of internal technical expertise to support the analysis (13%), unavailability of protocols or SOPs for this purpose (14%), researchers that do not see the value of such analysis (11%), and a high level of trust on the delivering party (16%).

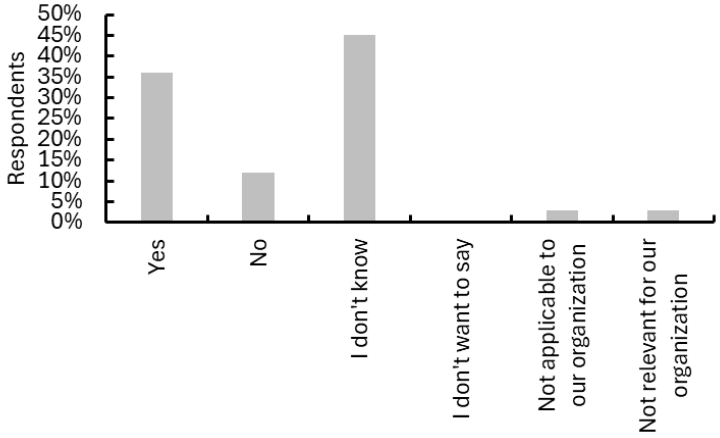


Figure 8 **Gauging future consideration to implement cell authentication assays.** Total n=33.

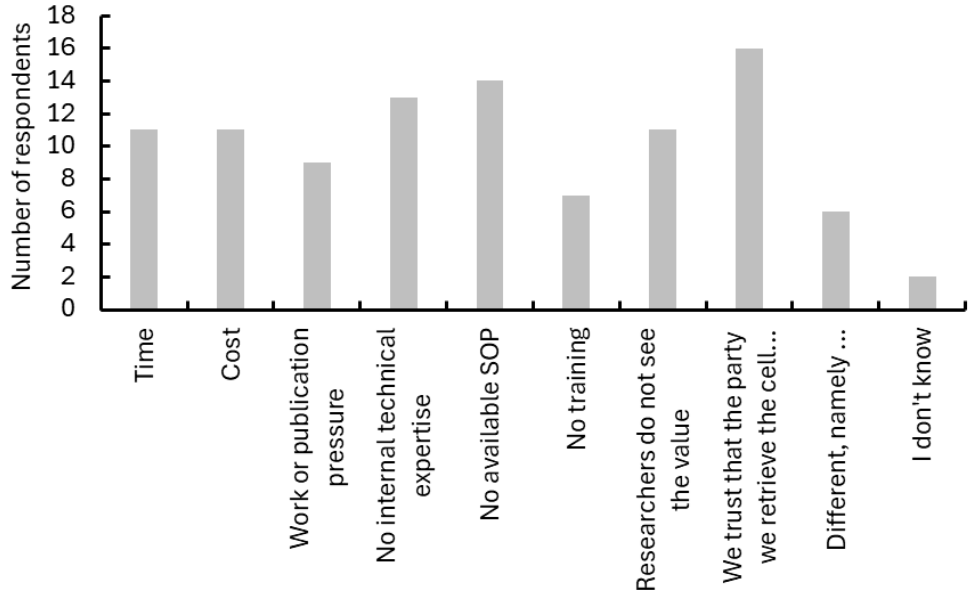


Figure 9 **Factors contributing to resilience against implementation of cell line authentication assays** (n=33). Selection of multiple responses was allowed. Responses to 'Different, namely …' included that the cells in culture served for training purposes for students without primary research activities, that cell lines were submitted externally for authentication, unawareness of involved researchers, the culture of only one cell line at a time, and the use of a functional read-out as main essential validation framework.

When respondents were asked about potential factors that could facilitate the implementation of cell culture authentication analyses, several key themes emerged. Many highlighted the importance of raising awareness of the significance of authentication and providing training for personnel, including researchers and research leaders. Building knowledge and clear protocols, including standardized workflows within and across research groups, was frequently mentioned, as was the need for easily accessible testing services or kits with built-in positive and negative controls. Financial support, such as dedicated budgets for authentication, was also considered critical, along with practical measures that reduce the time and effort required to perform testing. Other suggestions included establishing mandatory requirements to provide evidence of cell line

identity, offering informative sessions, central coordination, and purchasing cell lines from reliable sources rather than informal channels. Overall, respondents emphasized that a combination of education, accessible resources, clear protocols, and institutional support would be necessary to encourage consistent implementation of cell line authentication.

Respondents emphasized that trust in upstream suppliers plays a significant role in current practices, with several indicating that they assume appropriate controls have been performed when cell lines are obtained from commercial providers, typically accompanied by certification. At the same time, respondents expressed a clear need for greater clarity regarding acceptable authentication methods, particularly in relation to regulatory requirements, noting that guidance outlining which approaches are sufficient to meet applicable legislation would be valuable. Practical considerations were also frequently raised, including the preference for outsourcing authentication to reliable external providers, provided that services are affordable and deliver results in a timely manner.

Several responses highlighted that implementation is often deprioritized due to competing operational demands, such as laboratory relocations, or because authentication is perceived as offering limited immediate benefit in the absence of explicit requirements from journals or regulatory bodies. Accordingly, external drivers, such as mandates from journals, legislation, or funding agencies, were widely viewed as key incentives for adoption. Respondents further noted that supporting materials, such as evidence-based factsheets from trusted platforms, standardized SOPs, and opportunities for knowledge exchange, could substantially facilitate implementation.

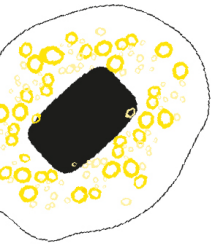
Importantly, respondents underscored that responsibility for cell line authentication should not rest solely with individual researchers. Instead, effective implementation was described as requiring coordinated efforts among management, biosafety offices, and end users, as researchers often lack the time, expertise, or resources to address this independently. Experiences with contamination incidents were cited as illustrative of broader risks associated with insufficient controls. Finally, respondents highlighted the importance of education and training, particularly for students and early-career researchers, to ensure that cell line identification and routine quality control practices, such as mycoplasma testing, are embedded as standard components of good cell culture practice.

5.2.6 Cell line authentication values

We next sought to assess the values and beliefs behind cell line authentication by means of collecting responses to a set of statements. We hypothesized that these values and beliefs may provide critical information for successful implementation of new policies, following an evaluation of the status of current policies within organizations. These survey statements to assess values and beliefs were presented to the entire population of respondents, regardless of their current use of cell line authentication procedures within their respective organizations. A total of 46 (61%) respondents filled in the statements.

5.2.6.1 The value of authentication to improve reproducibility and credibility of research based on cell lines is largely underscored.

A large majority of 91% (n=42) respondents agreed with the statement that reproducibility and credibility of research can be negatively affected by the use of cell lines with uncertain identity. Only 4% of the respondents (n=2) disagreed with this statement. Some respondents may disagree with the statement because their practical experience suggests that cell line misidentification does not always materially affect experimental outcomes. In certain research contexts, particularly exploratory or proof-of-concept studies, the biological behavior of the cell line may remain sufficiently consistent to support the intended conclusions, even if the exact identity is uncertain. Respondents may therefore perceive reproducibility as being driven more by experimental design,



assay robustness, and technical execution than by formal cell line authentication. Additionally, respondents who rely on well-established suppliers or long-standing in-house cell lines may have a high level of confidence in cell line identity and may not have encountered reproducibility issues attributable to misidentification. In such cases, authentication may be viewed as a confirmatory rather than a critical determinant of research credibility. Finally, some respondents may interpret reproducibility narrowly, focusing on internal reproducibility within a single laboratory rather than broader inter-laboratory reproducibility, leading them to underestimate the impact of cell lines with uncertain identity on the wider scientific record.

1.1.1 Researchers are aware that the use of cell lines with uncertain identity can lead to waste of research funding and resources

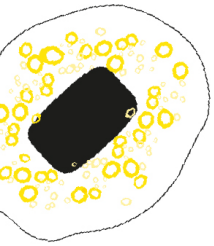
A majority of 86% (n=40) of the respondents agreed with the statement that the use of cell lines with uncertain identity may lead to waste of research sources. Two respondents (4%) expressed their disagreement to this statement. Fundamental reasons for this may be similar as to those outlined in the previous paragraph. A small number of respondents may disagree because they perceive cell lines with uncertain identity as still yielding usable and internally consistent data, particularly when experiments are exploratory or rely on established supplier-provided lines. This finding implies that, despite broad consensus on the risks, a minority of researchers continue to underestimate the potential downstream costs of misidentification, reinforcing the need for clearer guidance and stronger external incentives to promote routine cell line authentication. The responses to the question whether the international reputation of a research organization may sustain damage when using cell lines with uncertain identity cell lines underline these findings, showing that 80% (n=37) respondents agree with this statement.

5.2.6.2 Implementation of cell line authentication may benefit from dedicated supporting actions.

Responses to the statement, "*The amount of work, costs, and/or required expertise associated with cell line authentication do not outweigh the benefits*", were mixed. Nearly half of the respondents (48%, n=22) disagreed or strongly disagreed with this statement, while 30% (n=14) selected a neutral response and 22% (n=10) agreed. Notably, neutral and agreeing responses were more frequently observed among respondents from groups or organizations that do not currently perform cell line identity testing (see Appendix I). Overall, respondents expressed neutral to positive attitudes toward integrating training in cell line identification as a standard element of education or as part of dedicated courses for laboratory technicians and PhD students (see Appendix I).

Responses to the statement "*In general, there are no workable protocols or sufficient scientific literature available that lead to correct cell line identification*" were evenly distributed. Subsequent analysis indicates that 9 agreed with the statement, 9 were neutral, and 6 disagreed among respondents from organizations that do not perform cell line identification. Among respondents from organizations that do perform cell line identification, 6 agreed, 9 were neutral, and 6 disagreed.
Many respondents (87%, n=40) agreed with the statement that their host organizations should generate and enforce clearer guidelines to facilitate cell line authentication processes. At the same time, over half of the respondents (54%, n=25) agree that cell line identification is insufficiently discussed or monitored within their respective organizations. Six respondents (13%) disagree with this statement.

5.2.6.3 Survey respondents indicate insufficient awareness of implications of cell authentication and misidentification within the cell research community.



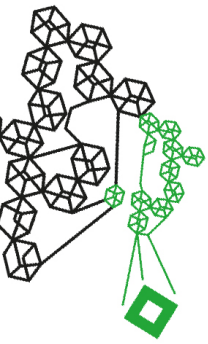
While most respondents indicated sufficient awareness of the benefits of cell authentication and risks of misidentification with themselves, more than half of the respondents felt that other researchers, such as their colleagues, are insufficiently aware of the risks for quality (61%, n=28), and safety and environment (65%, n=30) upon using misidentified cell lines. Respondents also agree that a culture change is required to integrate cell authentication evaluation into good laboratory practice (74%, n=34) and that the implementation of authentication will substantially increase upon raising awareness of the value, risks and purpose of such assays (74%, n=34).

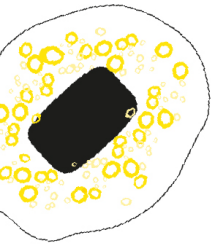
Overall, perceptions of the availability of workable protocols and scientific literature were divided and appeared largely independent of whether respondents came from organizations that currently perform cell line identification. Although a slightly higher number of respondents from non-identifying organizations agreed with the statement, the small sample size limits firm conclusions. This suggests that perceived limitations in protocols and literature are not confined to organizations that do not perform cell line identification. Differences in organizational expertise is, however, an argument raised by about one thirds of respondents from organizations that do not perform cell line identification (12 of 32).

5.2.6.4 Concluding remarks of the survey results

Overall, the survey results indicate strong consensus among respondents regarding the scientific and reputational importance of cell line authentication. The vast majority recognize that the use of cell lines with uncertain identity can negatively affect reproducibility, credibility, and the efficient use of research resources. Despite this broad agreement, perceptions diverge when considering the practical burden of implementation, particularly with respect to costs, workload, and required expertise. These reservations are more pronounced among respondents from organizations that do not currently perform cell line authentication, potentially indicating that lack of experience may reinforce perceived barriers, but these suggestions should be further investigated in follow-up studies.

Importantly, the findings highlight structural and cultural challenges rather than a lack of perceived value. Respondents consistently point to insufficient awareness, limited organizational guidance, and unclear responsibilities as key impediments. The strong support for clearer institutional guidelines, dedicated training, and awareness-raising initiatives underscores that successful implementation of cell line authentication will likely require coordinated organizational action rather than reliance on individual researchers alone. Together, these results suggest that while the scientific rationale for authentication is widely accepted, targeted supporting measures are essential to translate awareness into routine practice.





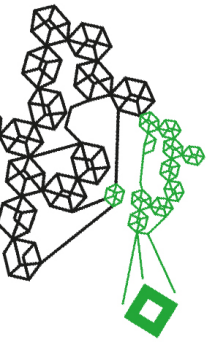
6. Conclusion and discussion

The overarching objective of this project was to assess the prevalence and impact of misidentified cell lines within Dutch research institutions. This objective was addressed through a multi-method approach combining a literature review, a small-scale internal experimental authentication study, and a survey distributed among Biological Safety Officers. Based on the integrated findings, recommendations and guidance could be developed to support the development, implementation, and dissemination of cell line identification policies in the Netherlands. The study indicates that assessing the overall impact of cell line misidentification within Dutch organizations remains challenging, partly because the extent to which such misidentification occurs is still not fully understood.

6.1 Practical and legal challenges in cell line authentication.

Overall, survey respondents underscored that effective cell line authentication requires both practical support and strong institutional commitment. Practical and institutional support includes consistent implementation of cell line authentication procedures based on a coordinated mix of awareness, training, standardized procedures, accessible resources, and institutional support. In a 2014 review, Harput and colleagues emphasized that researchers should authenticate cell lines obtained from collaborators upon receipt and consult available resources when doing so (Harput et al., 2014). In practice, however, most Dutch research organizations lack in-house capabilities for cell line authentication and therefore rely on third-party service providers. Furthermore, material transfer agreements (MTAs) commonly stipulate that providers are not liable for the distribution of unauthentic or contaminated materials. Therefore, MTAs might impose restrictions to users that are in conflict with Dutch law and regulations regarding GMOs. This situation creates a tension between the scientific responsibility to verify cell line identity and the legal and logistical constraints faced by recipient organizations.

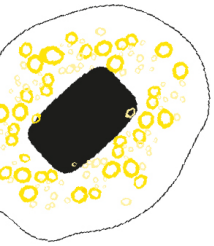
6.2 Detection limits of STR profiling for cell line contamination



STR profiling is widely regarded as the gold standard for human cell line authentication. Application notes from Promega and Applied Biosystems indicate that contaminations of approximately 20% HeLa cells fall within the detection limits of standard STR profiling using capillary electrophoresis. Although coupling STR analysis with next-generation sequencing (NGS) can improve sensitivity, routine authentication workflows typically rely on conventional STR profiling. To evaluate these limitations in practice, we assessed two commonly used cell lines in our laboratory by generating defined mixtures with contamination ratios ranging from 2% to 75%. Using standard STR profiling, deviation from the reference could be reliably detected when the contaminating cell line constituted at least 25% of the culture. These findings are consistent with published application notes and the existing literature, underscoring the inherent sensitivity limitations of standard STR-based authentication.

6.3 Defining cell line authentication

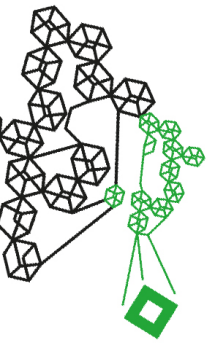
In this report, cell line identity is defined as the verified correspondence between a cell culture and its claimed origin, established through a reproducible molecular profile. Such profiles typically involve STR analysis and may be complemented by morphological assessment or additional molecular assays, in accordance with established guidelines (*e.g.* ANSI/ATCC ASN-0002, ATCC Technical Bulletins, DSMZ Guidelines). However, for many widely used and historically established cell lines, independent verification of the original reference profile is challenging. Reference profiles were often generated after extensive passaging, and molecular authentication

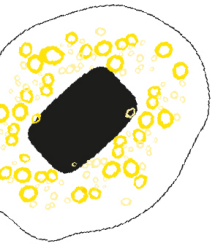


technologies were not routinely available at the time these cell lines were first derived (Geraghty et al., 2014). Consequently, authentication should not be interpreted solely as confirmation of an absolute origin, but rather as the establishment of an initial reference benchmark, or “zero measurement”, against which future genetic changes can be assessed. Viewed in this way, cell line authentication becomes a dynamic component of quality control rather than a one-time endpoint. This perspective is particularly relevant for long-term cultures and genetically unstable cell types, such as tumor-derived cell lines, where genomic drift has been shown to influence phenotypic behavior, experimental outcomes, and drug responses (Ben-David et al., 2018).

6.4 Cell line identification practices in relation to Dutch legislation

A central question in the survey was whether cell line identification procedures are performed within respondents’ groups or organizations. Approximately half of the respondents indicated that such procedures are in place. This finding is notable considering Dutch legislation, including the Regeling GGO, which requires users to ensure that the purity and/or correct identity of microorganisms and nucleic acid preparations used in GMO construction are adequately controlled. Although not all cell lines qualify as GMOs, similar considerations apply to non-GMO cell lines when conducting proper risk assessments. The Working Conditions Act (Arbowet) and Working Conditions Decree (Arbobesluit) mandate the identification, assessment, and control of risks associated with biological agents but do not explicitly prescribe specific authentication methods such as STR profiling or sequencing. Nevertheless, it can be argued that meaningful risk assessment and risk management presuppose a sufficient degree of certainty regarding the identity of the biological agent in use. The survey results suggest that awareness of the regulatory relevance of cell line identity testing may be limited, even though respondents broadly acknowledge its importance for research reproducibility, prevention of resource waste, and scientific integrity. These findings indicate that efforts to promote cell line authentication may benefit from explicitly linking its role in research quality and reproducibility to broader considerations of safety, regulatory compliance, and responsible research conduct.





7. Recommendations

The recommendations formulated in this report are based on the integrated insights obtained from a literature review, a targeted questionnaire, and an internal experimental reference dataset. Together, these sources highlight both technical and organizational factors that influence current practices in cell line authentication and identity control.

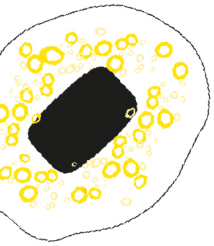
First, it is recommended to further develop the questionnaire into a **structured assessment or self-evaluation tool that can be used by research groups and organizations**. Such a tool could support organizations in systematically assessing their current practices, awareness levels, and potential gaps related to cell line identification. Follow-up actions could focus on providing tailored advice or guidance based on the assessment outcomes, rather than direct intervention or enforcement. In this context, biological safety officers (BSOs) could play a facilitating role by coordinating follow-up activities within a dedicated workgroup, for example under existing platforms such as SAAZUnie or the BVF platform. COGEM could consider supporting or endorsing such an initiative to promote harmonization and knowledge exchange at the national level.

Organizations are advised to **give careful consideration to the choice and interpretation of cell line authentication methods**. While STR profiling remains the internationally accepted standard, its role can be framed in different ways: either as a one-time confirmation of identity or as the establishment of a temporal baseline (“zero measurement”) against which future changes can be monitored. Explicitly defining this perspective within internal policies may help clarify expectations and responsibilities. At the same time, organizations should be encouraged to **remain informed about emerging and innovative authentication approaches**, such as brightfield imaging combined with machine learning or optical genome mapping. Although these techniques are not yet established standards, they may become valuable complementary tools in the future once sufficiently validated and standardized.

To ensure **biosafety** and regulatory compliance, organizations working with (genetically modified) cell lines should require routine cell line authentication and contamination checks before genetic modification, vector use, or assignment of containment level, thereby preventing unintended exposure, recombination events, and environmental release. In addition, careful cell line authentication is essential to mitigate potential environmental and health risks. Misidentification or mislabelling of cell lines, particularly those containing specific endogenous (retro)viral sequences or (retro)viral elements, can lead to the inadvertent generation or dissemination of replication-competent viruses. To reduce these risks, organizations are recommended to routinely authenticate cell lines and to maintain accurate labelling, and implement documentation procedures to ensure traceability, thereby protecting both environmental and human or animal health.

To strengthen ongoing oversight, it is recommended that BSOs **incorporate periodic authentication checks or identity “snapshots”** (for example on an annual basis) into internal monitoring protocols. This approach aligns with existing obligations under GMO legislation and the Working Conditions Act and supports a culture of continuous quality assurance. Importantly, the identification of misidentified or cross-contaminated cell lines should be framed as a positive outcome of quality control rather than as a failure. Promoting awareness, transparency, and corrective action, rather than punitive responses, supports good scientific practice and research integrity.

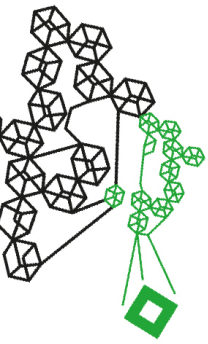
COGEM may further **stimulate the development of pragmatic, organization-level protocols** for cell line identification within the Netherlands. This could take the form of high-level

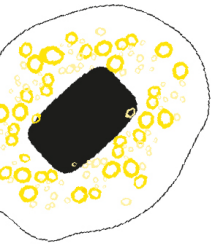


recommendations or guidance documents that outline key considerations and decision points, without providing direct technical implementation support.

In addition, organizations are encouraged to **assess whether there is a need for targeted training** or education of staff in cell line authentication and protocol development. Questionnaire responses indicate that lack of expertise and awareness is a significant barrier, particularly in organizations where authentication is not routinely performed. Guidance can therefore focus on helping organizations identify required expertise and appropriate external resources, while leaving implementation decisions to the organizations themselves.
A **risk-based and proportional approach to cell line authentication** is strongly recommended. Authentication requirements should be tailored to the potential impact and risk profile of the work, for example by integrating considerations into existing internal risk assessments such as the RI&E (Risico-inventarisatie en -evaluatie) or the GMO Risk Assessment (Risicobeoordeling, Regeling GGO).

Finally, organizations should be encouraged to **define clear and explicit triggers for cell line authentication**, such as upon receipt of new material, following genetic modification, after prolonged passaging, prior to distribution, or in response to incidents. Suggested decision logic for such triggers is provided in Appendix II (Decision Tree). In parallel, maintaining formal documentation and traceability of cell line identity within internal governance and quality systems is recommended to support accountability, reproducibility, and regulatory compliance. Moreover, we recommend developing protocols that outline procedures for handling contaminated or mislabelled cell lines.





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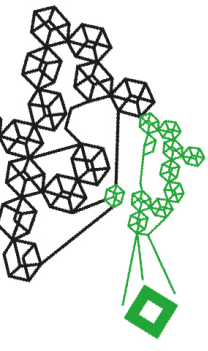

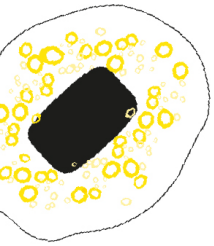

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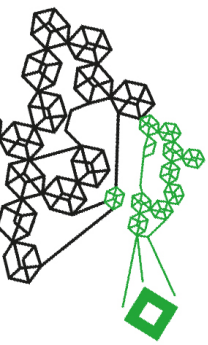

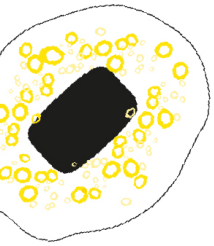

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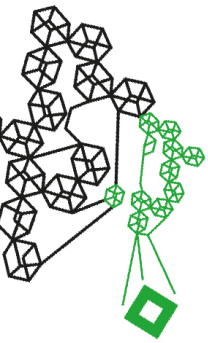

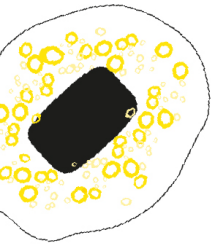

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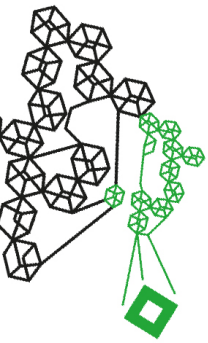

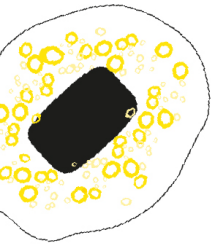

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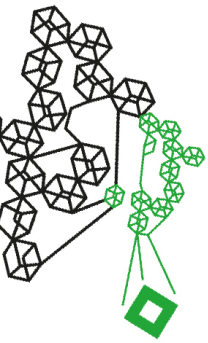

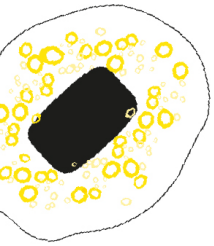

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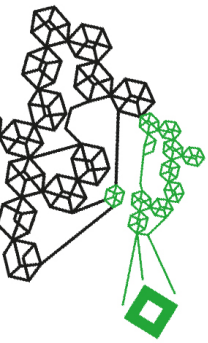

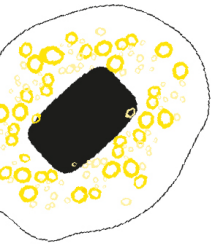

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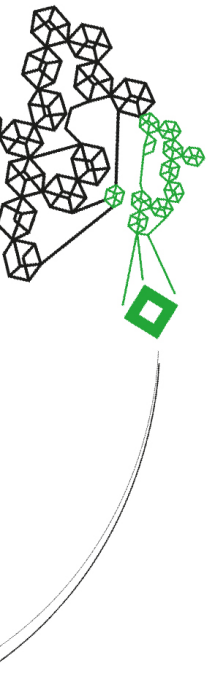


Appendix I Overview of questionnaire results

Resultaten - 23-04-2026	
Onderzoeksnaam	**Inventarisatie van cellijn identificaties**
Selectiedatum	**26-08-2025 - 01-02-2026**
Introductietekst (''vraag 1'')	Het project is gereviewd door de corresponderende ethische commissie van de Universiteit Twente en deze heeft een positief advies gegeven voor dit project. De ruwe data van deze vragenlijst is in beheer van de Universiteit Twente. De tool die gebruikt wordt voor de vragenlijst, Crowdtech, is afkomstig van een Nederlands bedrijf waarbij de ruwe data dus ook opgeslagen en beveiligd is op Nederlandse servers. De geanonimiseerde rapportage waarin de samengevatte resultaten van deze enquête staan, wordt gepubliceerd via de website van de COGEM (Commissie Genetische Modificatie, opdrachtgever). In de vragenlijst zullen wij niet vragen naar namen of naam van groep/afdeling/instelling en wij vragen van u om deze ook niet in open velden te noemen. Als u vragen heeft over uw rechten als deelnemer aan het onderzoek, of als u informatie wilt verkrijgen, vragen wilt stellen of eventuele zorgen over deze studie wilt bespreken met iemand anders dan de uitvoerende onderzoeker(s), neem dan contact op met: NES Ethics Committee ethicscommittee-nes@utwente.nl Ethics Committee Natural Sciences and Engineering Sciences Universiteit Twente 7522NB Enschede, Nederland Indien u in contact wil komen met de uitvoerende onderzoeker(s), neem contact op met: Dr. Joyce Mulder, Biologische veiligheidsfunctionaris (BVF) Universiteit Twente 7522NB Enschede, Nederland

Vraag	**Balk met verhouding aantal respondenten t.o.v. het totaal**	**% respondenten**	**Aantal respondenten**
2. Doe je vrijwillig en anoniem mee?	*Aantal respondenten bij deze vraag: 77*		
Hierbij verklaar ik dat ik vrijwi en anoniem meedoe aan he invullen van deze vragenlijst.		100.0	77
Nee [einde]			0
3. Bij welke soort instelling werkt u?	*Aantal respondenten bij deze vraag: 76*		
Academisch/universiteit		34.21	26
Universitair medisch centrum		25.00	19
Groep		3.95	3
Contract research organisatie (CRO)		6.58	5
Commercieel bedrijf		21.05	16
anders		9.21	7
4. Antwoord u namens een groep binnen de instelling of de gehele instelling?	*Aantal respondenten bij deze vraag: 75*		

Groep		53.33	40
Gehele instelling		46.67	35
5. Wordt er met cellijnen gewerkt die al lang in gebruik zijn (>10 jaar)? *Aantal respondenten bij deze vraag: 71*			
Ja		83.10	59
Nee		12.68	9
Weet ik niet		4.23	3
6. Met welke type cellijnen wordt er gewerkt binnen de instelling of groep? *Aantal respondenten bij deze vraag: 70*			
Humane cellijnen		91.43	64
Dierlijke cellijnen		88.57	62
Plantaardige cellijnen		14.29	10
7. Wordt de identiteit van cellijnen binnen uw instelling getest? *Aantal respondenten bij deze vraag: 67*			
Ja		50.75	34
Nee		49.25	33

8. Welke methoden worden gebruikt voor de identificatie en authenticatie van cellijnen in uw instelling? Selecteer 1 of meerdere: *Aantal respondenten bij deze vraag: 34*			
DNA profiling: short-tandem repeat (STR)		58.82	20
Cytogenetische analyse (bijvoorbeeld karyotypering, FISH, SKY)		17.65	6
DNA profiling: single nucleotide polymorphism (SNP) analyse		11.76	4
DNA sequencing (bijvoorbeeld fragment van PCR of anders)		41.18	14
PCR; specifieke regio's (bijvoorb recombinante DNA technieken verkregen is)		35.29	12
Functionele analyse, bijvoorbeeld; detectie van fluorescentie, het identificeren van morfologische eigenschappen d.m.v. microscopie, andere moleculaire assays die eigenschappen van de cellijn in kaart kan brengen.		38.24	13

Optical genome mapping (OGM)		2.94	1
Database check; ICLAC, cellosaurus etc.		32.35	11
Anders, namelijk: [Open antwoorden] (zie bijlage)		23.53	8
Weet ik niet		8.82	3
9. Welke methode(n) vindt u dat minstens zou(den) moeten worden uitgevoerd voor de identificatie van cellijnen in uw instelling? Selecteer 1 of meerdere: *Aantal respondenten bij deze vraag: 33*			
DNA profiling: short-tandem repeat (STR)		54.55	18
Cytogenetische analyse (bijvoorbeeld karyotypering, FISH, SKY)		15.15	5
DNA profiling: single nucleotide polymorphism (SNP) analyse		18.18	6
DNA sequencing (bijvoorbeeld fragment van PCR of anders)		27.27	9
PCR; specifieke regio's (bijvoorb recombinante DNA technieken verkregen is)		24.24	8
Functionele analyse, bijvoorbeeld; detectie van fluorescentie, het identificeren van morfologische eigenschappen d.m.v. microscopie, andere moleculaire assays die eigenschappen van de cellijn in kaart kan brengen.		18.18	6
Optical genome mapping (OGM)		6.06	2
Database check; ICLAC, cellosaurus etc.		24.24	8
Anders, namelijk: [Open antwoorden] (zie bijlage)		15.15	5
Weet ik niet		9.09	3
10. Indien de vorige vraag niet overeenkomt met wat in de praktijk wordt gedaan: kunt u aangeven waarom? *Aantal respondenten bij deze vraag: 33*			
Ja, namelijk: [Open antwoorden] (zie bijlage)		24.24	8
Nee, namelijk: [Open antwoorde		9.09	3
Voor mij is deze vraag niet van toepassing: de uitkomsten van beide vragen komt overeen		54.55	18
Weet ik niet		12.12	4
11. Kunt u aangeven wat de frequentie is voor cellijn identificatie? Selecteer 1 of meerdere *Aantal respondenten bij deze vraag: 32*			

Periodiek, namelijk; (bijvoorbeeld: wekelijks, maandelijks etc.) [Open antwoorden] (zie bijlage)	6.25	2
Projectmatig: bij aanvang project	15.63	5
Projectmatig: bij aanvang en einde project	12.50	4
Als ik vermoed dat de identiteit niet overeenkomt met de aangenomen of geregistreerde identiteit	18.75	6
Wanneer dit verzocht wordt door een journal, grant etc.	3.13	1
Nooit	6.25	2
Anders, namelijk: [Open antwoorden] (zie bijlage)	21.88	7
Weet ik niet [Open antwoorden] (zie bijlage)	15.63	5

12. Hoe vaak of wanneer zouden cellijnen getest moeten worden op authenticiteit conform intern beleid of interne reinheidsprotocollen? Selecteer 1 of meerdere;

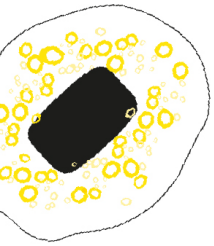
Aantal respondenten bij deze vraag: 31

Periodiek, namelijk; (bijvoorbeeld: wekelijk, maandelijks etc.) [Open antwoorden] (zie bijlage)	6.45	2
Projectmatig: bij aanvang project	9.68	3
Projectmatig: bij aanvang project en einde project	22.58	7
Als ik vermoed dat de identiteit r overeenkomt met de aangenom geregistreerde identiteit	3.23	1
Wanneer dit verzocht wordt door een journal, grant etc.	3.23	1
Nooit	6.45	2
We hebben de frequentie of andere details daarover niet officieel vastgelegd	22.58	7
Anders, namelijk: [Open antwoorden] (zie bijlage)	16.13	5
Weet ik niet	9.68	3

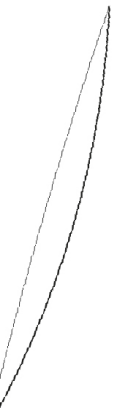
13. Maakt uw instelling gebruik van externe diensten buiten de instelling voor de authenticatie van cellijnen? Selecteer 1;

Aantal respondenten bij deze vraag: 30

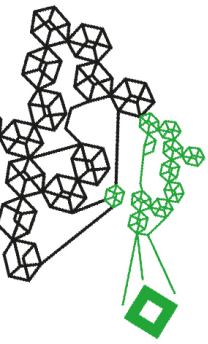
Ja, altijd	33.33	10
Ja, soms	30.00	9
Ja, alleen in specifieke samenwerkingen binnen consortium of project		0



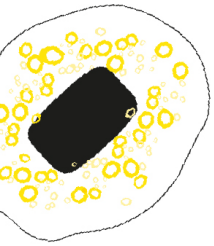
Nee, wij identificeren cellijnen in-house		30.00	9
Weet ik niet		6.67	2
14. Welk type cellijnen worden met name foutief geïdentificeerd? Selecteer 1; *Aantal respondenten bij deze vraag: 27*			
Humane cellijnen, namelijk; (indien geen specifieke namen bekend, dan schrijf in open veld: weet ik niet) [Open antwoorden] (zie bijlage)		40.74	11
Dierlijke cellijnen, namelijk; (indien geen specifieke namen b dan schrijf in open veld: weet ik			0
Plantaardige cellijnen, namelijk; (indien geen specifieke namen bekend, dan schrijf in open veld: weet ik niet) [Open antwoorden] (zie bijlage)			0
Wij zijn geen foutief geïdentificeerde cellijnen tegengekomen		37.04	10
Weet ik niet		29.63	8

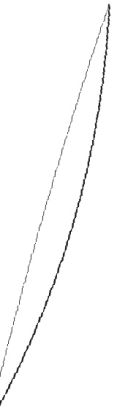


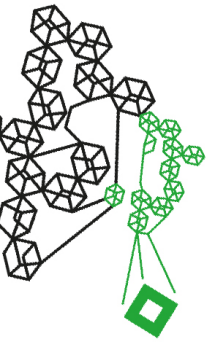
15. Hoe ben je aan deze cellijn gekomen? *Aantal respondenten bij deze vraag: 26*			
Geef in het open veld je antwoord. Let op dat er geen namen van instellingen, personen of andere herleidbare zaken worden genoemd. Indien onbekend, schrijf op weet ik niet of deze kennis heb ik nu niet paraat o.i.d.		100.0	26
16. Wat zijn de stappen die in de praktijk worden ondernomen als een cellijn foutief geïdentificeerd wordt? Selecteer 1 of meerdere; *Aantal respondenten bij deze vraag: 26*			
Melding maken bij onderzoeksleider (OL) en/of labbeheerder		61.54	16
Melding maken bij de BVF		50.00	13
Melding maken bij de leidinggevende		34.62	9
Melding maken elders, namelijk; [Open antwoorden] (zie bijlage)		7.69	2
Administratief registreren		50.00	13
Decontamineren		3.85	1
Termineren		46.15	12

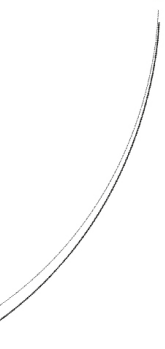


Weet ik niet	7.69	2
17. Hoeveel cellijn identificaties vinden plaats per jaar? Een schatting is ook goed. *Aantal respondenten bij deze vraag: 26*		
0	7.69	2
1-50	38.46	10
50-100	15.38	4
100-200	3.85	1
200-500		0
500-1000	3.85	1
>1000		0
Weet ik niet; ik kan ook geen schatting maken	30.77	8
18. Hoeveel procent van de geïdentificeerde cellijnen zijn foutief geïdentificeerd? *Aantal respondenten bij deze vraag: 26*		
Dit is in procenten: [Open antwoorden] (zie bijlage)	19.23	5
Dit is een schatting in procenten [Open antwoorden] (zie bijlage	7.69	2
Weet ik niet; ik kan ook geen schatting maken	65.38	17
Wil ik niet zeggen	7.69	2
19. Heeft een foutief geïdentificeerde cellijn ooit geleid tot veiligheidsrisico's zoals bijvoorbeeld andere inschaling en Categorie Fysische inperking (CFI)? *Aantal respondenten bij deze vraag: 25*		
Ja	4.00	1
Nee	68.00	17
Weet ik niet	24.00	6
Wil ik niet zeggen	4.00	1
20. Optioneel: indien paraat of van toepassing: heeft u misschien ervaringen met betrekking tot cellijn identificatie en/of foutieve cellijn identificaties die u wilt delen? Bijvoorbeeld een anekdote, specifieke gevolgen of een genomen maatregel waar u trots op bent en die u wilt delen? Let er op dat dit niet herleidbaar en anoniem weergegeven wordt. *Aantal respondenten bij deze vraag: 14*		
[Open antwoorden] (zie bijlage)	100.0	14
21. Overweegt uw instelling om dit te gaan doen? *Aantal respondenten bij deze vraag: 33*		
Ja	36.36	12
Nee	12.12	4
Weet ik niet	45.45	15
Wil ik niet zeggen		0
Het is voor ons niet van toepassing	3.03	1
Wij hechten hier geen belang aan	3.03	1









22. Welke factoren zijn van invloed op het niet testen van de identiteit van cellijnen of waarom weet ik niet geselecteerd is? Selecteer 1 of meerdere *Aantal respondenten bij deze vraag: 33*			
Tijd		33.33	11
Kosten		33.33	11
Werkdruk of publicatiedruk		27.27	9
Wij hebben intern niet de techni		39.39	13
Wij hebben hier (nog) geen SOP voor of deze is in de maak		42.42	14
Gebrek aan training		21.21	7
Onderzoekers zien het nut er niet van in		33.33	11
Wij vertrouwen erop dat de partij die ons de cellijn overhandigt dit heeft gedaan		48.48	16
Anders, namelijk; [Open antwoorden] (zie bijlage)		18.18	6
Weet ik niet		6.06	2
23. Indien geen andere maatregelen ten behoeve van de kwaliteitscontrole ingebouwd zijn (zoals intake incl. afspraken over het materiaal): waarom niet? *Aantal respondenten bij deze vraag: 32*			
Weet ik niet		53.13	17
Wil ik niet zeggen			0
Geen tijd voor gehad		9.38	3
Ik zie het nut niet in van maatregelen ten behoeve van de kwaliteitscontrole voor een cellijnidentificatieproces		3.13	1
Anders, namelijk; [Open antwoorden] (zie bijlage)		34.38	11
24. Wat is er nodig zodat dit binnen uw instelling of groep opgezet kan worden? *Aantal respondenten bij deze vraag: 28*			
Wat er nodig is: [Open antwoorder		67.86	19
Weet ik niet		32.14	9
Wil ik niet zeggen			0
25. Wat wilt u verder over dit onderwerp kwijt? Bijvoorbeeld wat u van dit onderwerp of specifieke procedure vindt, of u ergens ruimte voor verbetering ziet die u zou helpen om protocollen op te zetten, of u van bepaalde diensten of organisaties verwachtingen hebt voordat u de protocollen opzet etc. Indien u niets kwijt wilt, geef dan het antwoord " niets". *Aantal respondenten bij deze vraag: 40*			
[Open antwoorden] (zie bijlage)		100.0	40
26. Dit is het laatste onderdeel waar we uw mening willen horen over een aantal stellingen. De volgende stellingen gaan over uw waardering van cellijn identificatie. *Aantal respondenten bij deze vraag: 52*			

		100.0	52

27. De volgende stellingen gaan over uw waardering van cellijn identificatie

Aantal respondenten bij deze vraag: 46

1. Reproduceerbaarheid en geloofwaardigheid van onderzoek kan negatief beïnvloed worden bij gebruik van niet-geïdentificeerde cellijnen. *Aantal respondenten bij deze vraag: 46*			
Helemaal mee oneens		2.17	1
Mee oneens		2.17	1
Niet eens/niet oneens		4.35	2
Mee eens		50.00	23
Helemaal mee eens		41.30	19
2. Het gebruik van niet-geïdentificeerde cellijnen kan leiden tot verspilling van onderzoeksgeld en middelen *Aantal respondenten bij deze vraag: 46*			
Helemaal mee oneens			0
Mee oneens		4.35	2
Niet eens/niet oneens		8.70	4
Mee eens		43.48	20
Helemaal mee eens		43.48	20
3. Internationale reputatie van een onderzoeksinstelling kan geschaad worden wanneer deze niet-geïdentificeerde cellen gebruiken *Aantal respondenten bij deze vraag: 46*			
Helemaal mee oneens			0
Mee oneens		2.17	1
Niet eens/niet oneens		17.39	8
Mee eens		54.35	25
Helemaal mee eens		26.09	12
4. De hoeveelheid werk en/of kosten, en/of benodigde expertise die gepaard gaan met cellijn authenticatie weegt niet op tegen de voordelen *Aantal respondenten bij deze vraag: 46*			
Helemaal mee oneens		13.04	6
Mee oneens		34.78	16
Niet eens/niet oneens		30.43	14
Mee eens		21.74	10
Helemaal mee eens			0
5. Goede cellijn identificatie draagt bij aan transparantie en integriteit van wetenschappelijk onderzoek. *Aantal respondenten bij deze vraag: 46*			

Helemaal mee oneens			0
Mee oneens		2.17	1
Niet eens/niet oneens		2.17	1
Mee eens		52.17	24
Helemaal mee eens		43.48	20

6. Instellingen zouden duidelijke richtlijnen moeten opstellen en hand-haven voor cellijn identificatie.

Aantal respondenten bij deze vraag: 46

Helemaal mee oneens			0
Mee oneens			0
Niet eens/niet oneens		13.04	6
Mee eens		52.17	24
Helemaal mee eens		34.78	16

7. Binnen mijn groep of instelling wordt cellijn identificatie niet structureel besproken of gemonitord.

Aantal respondenten bij deze vraag: 46

Helemaal mee oneens		8.70	4
Bij opniew		4.35	2
Niet eens/niet oneens		32.61	15
Mee eens		30.43	14
Helemaal mee eens		23.91	11

8. Veel onderzoekers lijken onvoldoende op de hoogte zijn van de risico's voor de kwaliteit van hun onderzoek bij gebruik van foutief geïdentificeerde cellijnen.

Aantal respondenten bij deze vraag: 46

Helemaal mee oneens			0
Mee oneens		13.04	6
Niet eens/niet oneens		26.09	12
Mee eens		47.83	22
Helemaal mee eens		13.04	6

9. Veel onderzoekers lijken onvoldoende op de hoogte zijn van de risico's zowel voor veiligheid en milieu bij gebruik van foutief geïdentificeerde cellijnen

Aantal respondenten bij deze vraag: 46

Helemaal mee oneens			0
Mee oneens		13.04	6
Niet eens/niet oneens		21.74	10
Mee eens		47.83	22
Helemaal mee eens		17.39	8

10. Het lijkt er op dat er een cultuurverandering nodig is om cellijn-identificatie vanzelfsprekend te maken binnen onderzoeksgroepen

	Aantal respondenten bij deze vraag: 46		
Helemaal mee oneens		2.17	1
Mee oneens		4.35	2
Niet eens/niet oneens		19.57	9
Mee eens		54.35	25
Helemaal mee eens		19.57	9

11. Het aantal cellijn identificaties binnen instellingen zal drastisch omhoog gaan wanneer het belang hiervan begrepen wordt in het kader van het verkrijgen reproduceerbare resultaten

	Aantal respondenten bij deze vraag: 46		
Helemaal mee oneens			0
Mee oneens		8.70	4
Niet eens/niet oneens		17.39	8
Mee eens		58.70	27
Helemaal mee eens		15.22	7

12. De mate van cellijn identificatie binnen instellingen zal drastisch omhoog gaan wanneer het belang hiervan begrepen wordt in het kader van veiligheid, gezondheid en milieu voor de medewerkers

	Aantal respondenten bij deze vraag: 46		
Helemaal mee oneens			0
Mee oneens		4.35	2
Niet eens/niet oneens		39.13	18
Mee eens		45.65	21
Helemaal mee eens		10.87	5

13. Training in cellijnidentificatie zou een vast onderdeel moeten zijn binnen de opleiding of een cursus voor laboranten en promovendi

	Aantal respondenten bij deze vraag: 46		
Helemaal mee oneens		2.17	1
Mee oneens		2.17	1
Niet eens/niet oneens		28.26	13
Mee eens		52.17	24
Helemaal mee eens		15.22	7

14. Cellijn identificatie d.m.v. STR-analyse is voldoende om een uitspraak te doen of een cellijn de juiste cellijn is.

	Aantal respondenten bij deze vraag: 46		
Helemaal mee oneens			0
Mee oneens		6.52	3
Niet eens/niet oneens		67.39	31

Mee eens	21.74	10
Helemaal mee eens	4.35	2

15. In het algemeen zijn er geen werkbare protocollen of voldoende beschikbare wetenschappelijke literatuur die leiden tot een juiste vorm van cellijn identificatie

Aantal respondenten bij deze vraag: 46

Helemaal mee oneens	2.17	1
Mee oneens	23.91	11
Niet eens/niet oneens	41.30	19
Mee eens	28.26	13
Helemaal mee eens	4.35	2

Inventarisatie van cellijn identificaties.
Open antwoorden die gegeven zijn door de respondenten bij de corresponderende vragen.

8. Welke methoden worden gebruikt voor de identificatie en authenticatie van cellijnen in uw instelling? Selecteer 1 of meerdere:

Aantal respondenten bij deze vraag: 34

9. Anders, namelijk:					
Meer weet ik niet	voor gebruik in dier studies wordt een IDEXX test geeist. (contaminatie en cellcheck) Op verschillende laboratoria worden lijnen opnieuw aangeschaft als ze ouder dan 10 jaar zijn.	Whole genome sequencing	markergen of antigeen herkenning, westernblot, FACS analyse, HLA typering	Optisch	Afhankelijk van de onderzoekslijn
flowcytometrie	Optisch				

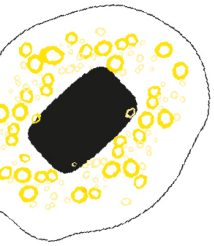
9. Welke methode(n) vindt u dat minstens zou(den) moeten worden uitgevoerd voor de identificatie van cellijnen in uw instelling? Selecteer 1 of meerdere:

Aantal respondenten bij deze vraag: 33

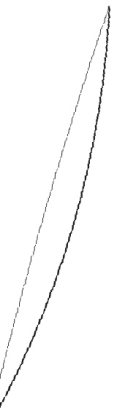
9. Anders, namelijk:					
testen/uitsluiten van aanwezige virale fragmenten	Whole genome sequencing	markergen of antige westernl FACS analyse, H	Optisch	Indien van toepassing	

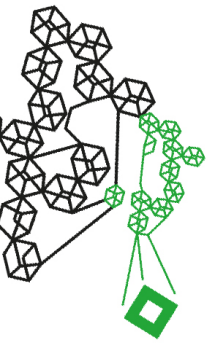
10. Indien de vorige vraag niet overeenkomt met wat in de praktijk wordt gedaan: kunt u aangeven waarom?

Aantal respondenten bij deze vraag: 33



1. Ja, namelijk:					
Het is bij ons aan de individuele onderzoeker om de gebruikte cellijnen te testen	Voor GGO identificatie	Vaak wordt (Antwoord niet afgemaakt)	Beschikbaarheid van methode in-house	We doen meer, afhankelijk van het type of al dan niet gebruikte modificatie	Alleen bij twijfel gaat men meestal testen
Verschil in doel, m'n creeen van deleties/ mutaties en t vasstellen	wij testen de cellijnen niet regelmatig met de genoemde technologieen				
2. Nee, namelijk:					
Wat we incidenteel hebben gedaan vs wat we structureel zouden willen doen	Cellijnen worden vaak aangeleverd met alle identiteits testen al uitgevoerd door de klant				





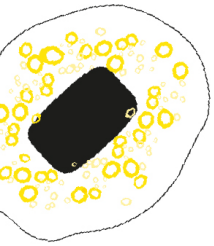
11. Kunt u aangeven wat de frequentie is voor cellijn identificatie? Selecteer 1 of meerdere

Aantal respondenten bij deze vraag: 32

1. Periodiek, namelijk; (bijvoorbeeld: wekelijks, maandelijks etc.)					
Niet vastgesteld	maandelijks				
7. Anders, namelijk:					
Kwaliteits controle	Bij aanvang project en periodiek waar nodig	Bij opnieuw invriezen	Per groep anders. Periodiek tot projectmatig	Periodiek bij invriezen 5 passages na STR en bij een vermoeden dat er iets mis is en als een journal hier om vraagt.	als we cellijnen krijgen vanuit samenwerking en wanneer dit verzocht wordt door een journal/grant; daarnaast schaffen we cellijnen aan van gerenomeerde celbanken (ATCC, DSMZ) die STR geprofileerd zijn. (Ik kon geen meerdere opties aanvinken)
Journal, start, periodiek					
8. Weet ik niet					

Helaas weet ik niet	iedere groep doet dit anders				
12. Hoe vaak of wanneer zouden cellijnen getest moeten worden op authenticiteit conform intern beleid of interne reinheidsprotocollen? Selecteer 1 of meerdere; *Aantal respondenten bij deze vraag: 31*					
1. Periodiek, namelijk; (bijvoorbeeld: wekelijk, maandelijks etc.)					
Jaarlijks	1x per 2 jaar als cellen vaak gekweekt worden elk jaar				
8. Anders, namelijk:					
Kwaliteits controle	Bij aanvang/einde project en bij twijfel over identiteit	Projectmatig icm bij vermoeden	wanneer twijfel is over reinheid (bullet 4), op verzoek van journal (bullet 5),	Zelfde als vorige	
14. Welk type cellijnen worden met name foutief geïdentificeerd? Selecteer 1; *Aantal respondenten bij deze vraag: 27*					
Humane cellijnen, namelijk; (indien geen specifieke namen bekend, dan schrijf in open veld: weet ik niet)=					
Ons onderzoek betreft voornamelijk humaan	Sw480	Weet ik niet	KG1, MV4;11	HeLa	

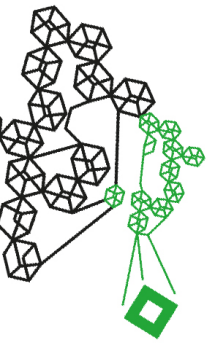
15. Hoe ben je aan deze cellijn gekomen? *Aantal respondenten bij deze vraag: 26*					
1. Geef in het open veld je antwoord. Let op dat er geen namen van instellingen, personen of andere herleidbare zaken worden genoemd. Indien onbekend, schrijf op weet ik niet of deze kennis heb ik nu niet paraat o.i.d.					
Cellijnen worden aangeschaft bij bedrijven die cellijnen commercieel verkopen.	Cultuur collecties	Andere instellingen met MTA's	Collega universiteiten	verschillende herkomst: van officiele cellijn verkopers, gekregen van andere onderzoekers	Divers, het kunnen oude cellijnen zijn, verkregen materiaal of inherent aan het werk zijn dat er iets mis gaat
Atcc	weet ik niet of deze kennis heb ik nu niet paraat	Zijn alle afkomstig van bekende commerciele providers van cellijnen.	Van universitaire instelling	Gekocht via ATCC, Via andere instellingen, direct uit patient en/of dier, worden soms zelf gegenereerd.	Geen idee



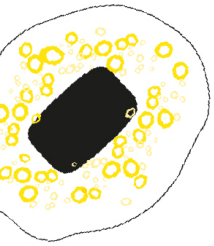
Hg	Inhouse ontwikkeld voor productie van antibodies	commercieel en via samenwerkingen	Via bevriende organisatie	Cellijnen ooit gekocht bij ATCC/DSMZ daarna al vele jaren in kweek	nvt
weet ik niet	als [redacted] verkrijgen wij cellijnen van partners in projecten	weet ik niet	soms genereren iPS lijnen vanuit huid, soms aankopen van bedrijven, soms van andere onderzoeksgroepen		

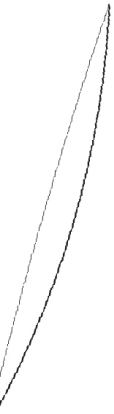
16Wat zijn de stappen die in de praktijk worden ondernomen als een cellijn foutief geïdentificeerd meerder *Aantal respondenten bij deze*					
4. Melding maken elders,					
Quality afdelin	Director				

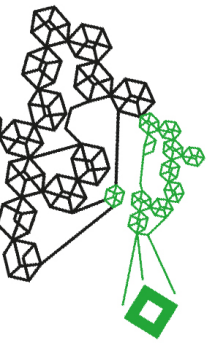
18. Hoeveel procent van de geïdentificeerde cellijnen zijn foutief geïdentificeerd? *Aantal respondenten bij deze vraag: 26*					
1. Dit is in procenten:					
0	100	0	minder dan 1%	0	
2. Dit is een schatting in procenten:					
1	0				



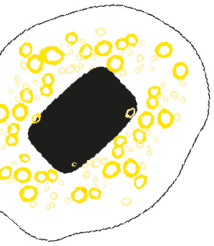
20. Optioneel: indien paraat of van toepassing: heeft u misschien ervaringen met betrekking tot cellijn identificatie en/of foutieve cellijn identificaties die u wilt delen? Bijvoorbeeld een anekdote, specifieke gevolgen of een genomen maatregel waar u trots op bent en die u wilt delen? Let er op dat dit niet herleidbaar en anoniem weergegeven wordt. *Aantal respondenten bij deze vraag: 14*					
1.					
Geen ervaring mee	GFP in "Wildtype"	Geen ervaring	n.v.t.	-	-
n.v.t.	Hh	Door een verkeerde aanname bleek een celline gekweekt op ML-I, gemaakt was met een muisvirus en ingeschaa had moeten worden op ML-II. De cellijn is later met een omlaagschaling verzoe vrijgegeven voor ML-I	Foutief hernoe men van lijnen verkeer de Latijnse naam	Niet van Toepassi ng	nee
Geen	geen ervaring mee				







22. Welke factoren zijn van invloed op het niet testen van de identiteit van cellijnen of waarom weet ik niet geselecteerd is? Selecteer 1 of meerdere *Aantal respondenten bij deze vraag: 33*					
9. Anders, namelijk;					
We hebben maar 1 cellijn in gebruik, wel meer in het stikstof. Maar al meer dan 10 jaar niet gebruikt.	de activiteit van de cellijn wordt met een test bewezen en als gevolg hiervan hoeft de cellijn niet precies te zijn wat het was als het maar volgens de standaard meegaat in de test en binnen de levels die gezet zijn voor de waardes die uit de testen komen.	Waarschijnlijk van alles e	Cellijnen worden opgestuurd ter identificatie	Onderzoekers zijn zich veelal niet bewust van het feit dat cellijnen mogelijk niet zijn wat ze lijken	we leren de studenten kweken; doen er geen echt onderzoek mee
23. Indien geen andere maatregelen ten behoeve van de kwaliteitscontrole ingebouwd zijn (zoals intake incl. afspraken over het materiaal): waarom niet? *Aantal respondenten bij deze vraag: 32*					
5. Anders, namelijk;					
Ik snap de vraag niet	Check bij leverancier	Cellijn gekocht bij [redacted], en maar 1 cellijn in gebruik	wij hebben wel maatregelen voor kwaliteit, steriliteit etc. misschien niet identiteit maar wel of ze nog voldoen aan wat we van de cellijn verwachten	y	Alles komt binnen met MTA, maar door vele passages en verkeerd terugplaatsen/pakken uit vriezer gaat er ws. wel iets fout

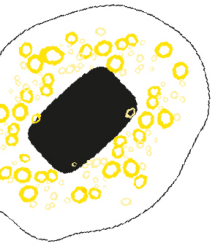


Zijn ingebouwd	Er worden zo veel mogelijk besteld via officiële leveranciers en nieuwe batches besteld	Nooit geimplementeerd	kopen ze		

24. Wat is er nodig zodat dit binnen uw instelling of groep opgezet kan worden?
Aantal respondenten bij deze vraag: 28

1. Wat er nodig is:

Een stok achter de deur. Informatieve sessies met onderzoekers, technieken die gebruikt kunnen worden	Onderwijs van onderzoeksleiders en onderzoekers. verplichting om bewijs te leveren, informatie over gevolgen een risico's	Ik weet niet waar ik dergelijke testen kan laten uitvoeren.	Niet, gebeurt bij leverancier	Bewustwording	De overtuiging van de onderzoekers dat ze wel eens niet kunnen werken waarmee ze denken te werken.
Kant en klare methode, liefst een kitje. Ook voor onze niet veel gebruikte cellijn, BGM cellen	besef van belang, bereidheid tot medewerking, training personeel, beschikbaarheid financiële ondersteuning	Cellen aankopen ipv via via verkrijgen	Expertise, of een duidelijk werkprotocol, zodat dit binnen de verschillende onderzoeksgroepen gestandardiseerd kan worden.	Kennis / protocol voor identificatie	Een makkelijke manier om te testen, in de vorm van een kit os iets dergelijks waarbij, positieve en negative controles zijn ingebouwd.Of een bedrijf waar we het makkelijk naar op kunnen sturen zoals bijvoorbeeld voor de mycoplasma testen die we uitsturen.
Geld, protocollen, makkelijke route om te lat n checken	Voorlichting aan onderzoekersVerplichting bij opzetten onderzoek, incl. begroting voor controle op cellijnen	Bewustwording en minder werkdruk	Budget	Geld en tijd	Meer bewustwording en misschien centrale coordinatie
Kennis opbouwen, tot nu toe vertrouwen op leveranciers van cellijn					



25. Wat wilt u verder over dit onderwerp kwijt? Bijvoorbeeld wat u van dit onderwerp of specifieke procedure vindt, of u ergens ruimte voor verbetering ziet die u zou helpen om protocollen op te zetten, of u van bepaalde diensten of organisaties verwachtingen hebt voordat u de protocollen opzet etc. Indien u niets kwijt wilt, geef dan het antwoord " niets".

Aantal respondenten bij deze vraag: 40

1.					
Niets	Niets	Als we cellen van andere bedrijven krijgen, dan verwacht ik dat zij de juiste controles hebben gedaan. Normaal gesproken zit er een certificaat bij de cellen.	Extern laten uitbesteden, graag niet te duur en snel	Niets	Nee bedankt
Ik verwacht de eis van extern (journals, wetgeving)	Niets	Niets	niets	In verleden een collega gehad die pipetten in medium of buffers terug stopte om pipetten te besparen. Dit heeft enorme contaminatie veroorzaakt onder de cellen	Probleem direct opgelost!
In principe verantwoordelijkheid van de groep	niets	Onze ervaring is dat wanneer blijkt dat de identiteit van een cellijn niet klopt dit vooral gevolgen heeft voor het onderzoek (en dan wordt opgelost), maar niet zozeer voor de inschaling.	-	niets, op dit moment zijn wij er nu niet mee bezig ivm verhuizingen van locaties. Ik denk zolang de eis van journal niet terugkomt bij publicaties zien onderzoekers ook niet het nut er van in omdat het tijd kost en eerst uitgezocht moet worden.	Niets

Het zou fijn zijn als er ergens een overzicht is van welke methodes volstaan om aan dit artikel in de Regeling GGO te kunnen voldoen. Al kan ik me voorstellen dat dit van veel dingen afhankelijk is.	Niets	n.v.t.	Op dit moment gaan we dit nog niet invoeren, maar misschien wel in de toekomst. Mocht er een gerenomeerd bedrijf zijn die de testen aanbied of een kit die makkelijk uit te voeren is.	niets	Hh
niets	niets	niets	Een onderbouwing (factsheet) vanuit BVF platform waarom dit essentieel is zou helpen	Niets	niets

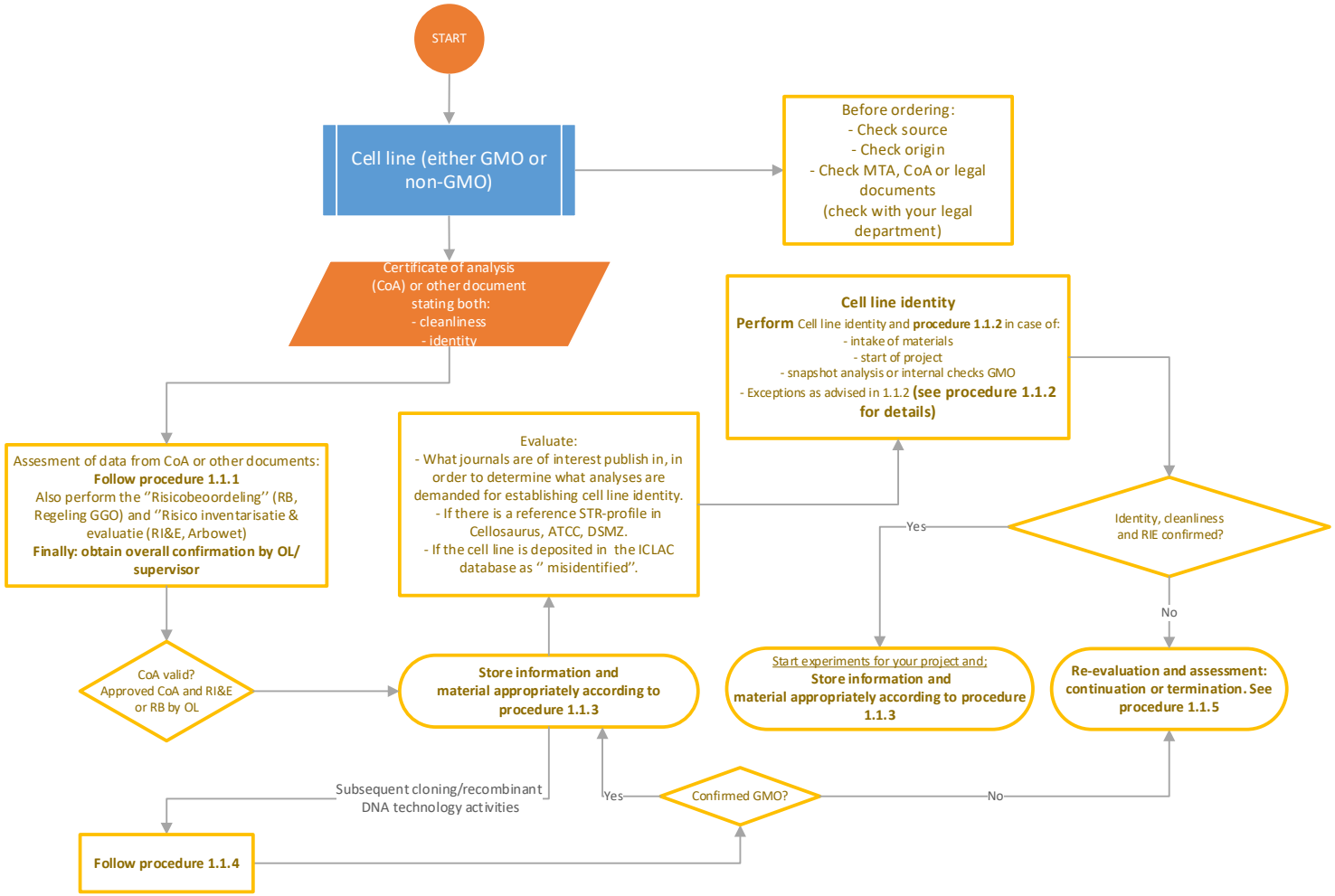
25. Wat wilt u verder over dit onderwerp kwijt? Bijvoorbeeld wat u van dit onderwerp of specifieke procedure vindt, of u ergens ruimte voor verbetering ziet die u zou helpen om protocollen op te zetten, of u van bepaalde diensten of organisaties verwachtingen hebt voordat u de protocollen opzet etc. Indien u niets kwijt wilt, geef dan het antwoord " niets".

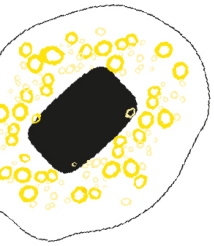
Aantal respondenten bij deze vraag: 40

Dit hele onderwerp is een samenwerking vanuit management, bioveiligheids afdeling en (eind)gebruiker. Hier wordt vaak de (eind)gebruiker aan zijn lot overgelaten om het maar uit te zoeken, terwijl die er geen kennis, tijd of de middelen voor heeft.	Niets	Niets	niets	Maken en implementeren SOPs staat momenteel op de to-do lijst van de BVF. Kennisuitwisseling rondom dit onderwerp zou heel fijn zijn	[redacted] ligt de taak bij [redacted] om bewijs mee te leveren van de cellijnen voor productie processen. [redacted] onder GMP valt, worden dit soort zaken strikt gecheckt.
het is goed als studenten leren hoe je cellijnen identificeert en controleerd (ook voor mycoplasma bv)	niets	Geen	...		

Appendix II For consideration; an example decision tree including example procedures.

Example decision tree for your consideration regarding cell line identification performance in practice for Dutch organizations including example procedures (1.1.1-1.1.5).





Procedure 1.1.1. Tips and tricks for assessment of data in Certificates of analysis (CoA)

Tips and tricks

- Check if your cell line was already assessed for authentication: what method was used, when was this performed, evaluate outcomes (percentage match to reference, what database), passage number check. Red flags: only confirmation of cell line without information on how and when this was performed and evaluated.
- Check if the cell line was assessed for mycoplasma infection and, if so; what test method was used, when was this tested, which species of mycoplasma was tested for. Red flags: if only a DNA staining procedure was performed and no date of experiment performance is included.
- In case the cell line was tested for other bacterial infections; also check method and date of testing.
- Check if your cell line was checked for contamination/presence of viruses such as HIV-1/2, HTLV-I/II, CMV, EBV, HBV, HCV.
- Check in the MTA or CoA if anything regarding identification of Materials is mentioned: if unsure, please contact your legal department.
- Check passage number of the purchased materials versus testing. If no passage numbers are depicted on the CoA, you could check this with the company.
- Check Lot/Batch number and if this matches with the received materials.
- Check trackability; presence of a website link to the Master Cell Bank (MCB) or Working Cell Bank (WCB)
- Is the original depositor mentioned? In case not, please check in references are request information at the company of which you received the materials from.
- If the materials are GMP/clinical grade and that this was confirmed via, for instance, ISO norms, these are signs that the materials were assessed to strict protocols.
- Was the CoA signed by the company of which the materials were purchased?

Certificate of Analysis (CoA): Review Checklist for Cell Lines

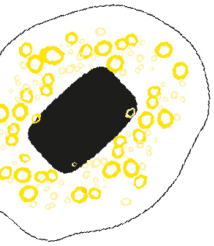
Purpose: Rapid but critical review of a Certificate of Analysis (CoA) when purchasing/receiving cell lines, prior to performing in-house identity and contamination testing.

1. General information

- ☐ Cell line name (exact designation)
- ☐ Alternative names/synonyms listed
- ☐ Supplier / cell bank (ATCC, DSMZ, ECACC, etc.)
- ☐ Lot / batch number
- ☐ Research Use Only / GMP / clinical grade
- ☐ CoA issue date

2. Identity and authentication

- ☐ Identity testing performed? (yes/no)
- ☐ Method used:
    - ☐ STR profiling (human cell lines)
    - ☐ SNP analysis / karyotyping
    - ☐ Isoenzyme analysis
- ☐ Date of identification



- ☐ Passage number at time of identification
- ☐ Reference database specified (ATCC / DSMZ / internal)
- ☐ Match score / conclusion reported
- ☐ STR raw data available (attached / upon request)

Red flags: no method specified, outdated testing, or only “identity confirmed”.

3. Species verification

- ☐ Species confirmed (*e.g. Homo sapiens*, *Mus musculus*)
- ☐ Method specified (species-specific PCR / isoenzyme analysis)
- ☐ Relevant for co-culture or cross-contamination risk addressed

4. Mycoplasma testing (mandatory)

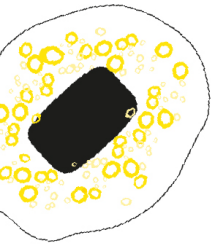
- ☐ Mycoplasma tested? (yes/no)
- ☐ Method:
    - ☐ PCR / qPCR
    - ☐ DNA staining (DAPI / Hoechst)
    - ☐ Culture method
- ☐ Test date
- ☐ Result clearly stated (“not detected”)
- ☐ Mycoplasma species / panel specified
- ☐ Assay sensitivity / detection limit reported

Red flags: staining-only methods, missing test date.

5. Virus / adventitious agent testing

- ☐ Viral testing performed? (yes/no)
- ☐ Test panel specified (list provided)
- ☐ Method:
    - ☐ PCR / qPCR
    - ☐ *In vitro* assay
    - ☐ *In vivo* assay
- ☐ Test date
- ☐ Panel relevance for cell line type assessed

Note: panel selection often depends on human vs animal origin and intended application.



6. Sterility and microbial contamination

- ☐ Bacterial contamination tested e.g. mycoplasma
- ☐ Fungal / yeast contamination tested
- ☐ Method specified (USP <71>, direct inoculation, etc.)
- ☐ Endotoxin tested (if relevant)

7. Passage & production information

- ☐ Passage number at delivery
- ☐ Passage number at testing
- ☐ Master Cell Bank (MCB) / Working Cell Bank (WCB) indicated
- ☐ Traceability ensured

8. Missing information / risk assessment

- ☐ No raw data available
- ☐ Incomplete viral test panel
- ☐ Outdated test results
- ☐ Tests not batch-specific

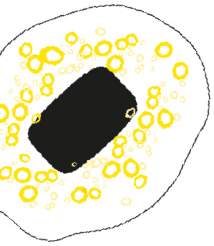
Note: if one or more boxes are checked: **in-house verification strongly recommended**.

9. Internal follow-up upon receipt

- ☐ Mycoplasma test upon receipt
- ☐ STR / identity testing upon receipt
- ☐ Initial aliquot stored as reference
- ☐ Documentation archived (CoA + internal test results)

Overall evaluation:

Reviewed by: _____ **Date:** _____
Assessed by: _____ **Date:** _____



Procedure 1.1.2. Cell line identifications

We recommend performing cell line identification in case of:

- Intake of materials (newly purchased cell lines or cell lines that were received in-kind or any other way)
- Start of project
- During snapshot analysis or internal checks GMO according to GMO law and regulations
- Specific exceptions, such as instable cell lines (assessed by researcher), prior to publication or external data sharing, after thawing long-stored cell lines, after extended passaging, after major genetic manipulations, in case of inconsistent or unexpected results.

The method for cell line identification depends on a few conditions, such as:

- Presence of a reference profile or baseline in the assay (for instance STR-profile)
- Available data on original tissue or cell line
- Available biochemical analyses

We recommend assessing this specifically for your cell line of interest prior to proceeding towards selecting a method for authentication.

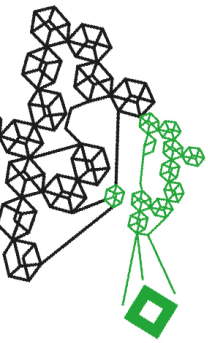
STR analysis

STR is globally recognized as the golden standard for (human) cell line identification and a familiar technique within Dutch organizations for cell line identification (see outcomes of the questionnaire regarding cell line identification in this report). However, STR analysis can only be performed for cell line authentication if a reference STR-profile is available for the cell line of interest.

- Check beforehand whether an STR-profile of the reference material is present.

Generic STR sample prep & submission protocol (lab procedure)

Use this as a one-stop SOP for preparing either cell pellets or purified genomic DNA to send to most commercial STR services. Please use the Sample Submission Guide specifically from the company you are providing the materials to. The information in this SOP is generic and provides an idea on how an STR could be performed and updated to specific company requirements accordingly.
We recommend to also perform a parallel analysis for morphology (bright field microscopy) or biochemical analysis specific for the cell line of interest.

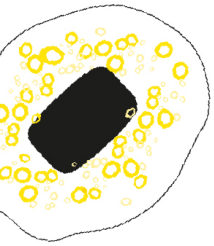


A. Decide sample type to send

- Preferred options (in order): 1) purified genomic DNA (gDNA), 2) cell pellet, 3) FTA card / spotted cells (if vendor accepts). Many vendors will extract DNA for you from pellets but will accept gDNA if you provide it. Please check at the company you are planning to send the materials to for analysis.

B. Prepare a cell pellet (if you will send cells)

1. Grow cells to exponential phase; avoid over-confluence and heavy apoptosis.
2. **Before harvesting, check your cells under a bright field microscope and save pictures. Assess whether the morphology is as expected for this cell line.**
3. Harvest:
    - Adherent cells: trypsinize (or appropriate dissociation), neutralize, transfer to tube.
    - Suspension cells: harvest directly.
4. Wash twice with PBS (or TE buffer if vendor requests) to remove medium and serum (residual proteins can inhibit extraction). Please note that some companies, for instance Eurofins, explicitly ask for medium completely removed and washed with TE or PBS.



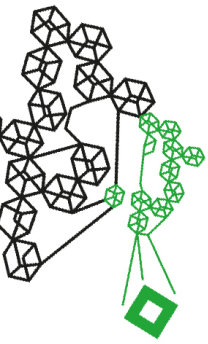
5. Pellet: centrifuge at low speed (*e.g.*, 300-500 × g for mammalian cells) and remove supernatant completely.
6. Target pellet size / cell number: make a visible pellet; vendors differ but typical minimums are:
    - Eurofins: >10,000 cells (visible pellet).
    - Typical core labs: ~5×10^5 cells recommended if you will ship frozen pellets (for safe extraction).
    - ATCC FTA workflow: spot cells at ~1×10^6 cells/mL and apply 40 µL to card (so roughly ~4×10^5 cells per spot) if using FTA.
7. If shipping pellets: resuspend in a *small* volume of PBS/TE (e.g., 50-200 µL) so the pellet stays compact; label tube clearly.

C. Purified genomic DNA (preferred if you can extract in-house)

1. Extract high-quality gDNA using a column or kit known for high molecular weight DNA (Qiagen DNeasy / QIAamp or equivalent) and avoid shearing.
2. Quantify both by fluorometric method (Qubit) and optionally NanoDrop (260/280) for purity. Run on a 0.8% agarose gel if you suspect degradation.
3. Typical vendor requirements (range):
    - DSMZ example: ~20 µL at 10-100 ng/µL (*i.e.*, ~200-2000 ng total).
    - Eurofins: commonly request >100 ng total and volume >10 µL (different docs: check current guide). Eurofins also offers free DNA extraction from pellets if you prefer to send pellets.
    - Many cores accept ≥10 ng/µL, 10-20 µL (so ≥100-200 ng total). If in doubt, aim for ≥200 ng total at 10-50 ng/µL to be safe.

D. QC before shipping

- gDNA: Qubit concentration, 260/280 ~1.8-2.0, no smear on gel.
- Cell pellet: count cells before pelleting and note the count on the submission form; remove medium completely. For FTA cards, follow vendor cell density instructions (ATCC recommends ~1×10^6 cells/mL and 40 µL spot).



E. Packaging & shipping

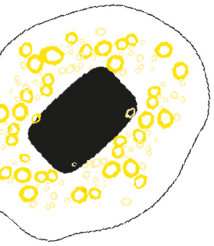
- Follow vendor sample submission form exactly. Many accept ambient-shipped DNA (stabilized) but prefer chilled/frozen pellets on dry ice if sending live/frozen cells. Eurofins accepts pellets shipped ambient or cooled (not necessarily dry ice) if medium removed; DSMZ has rules about GMOs. Always check the current submission guide.

F. Metadata to include

- Cell line name (exact), passage number, lot/batch, culture conditions, date of harvest, contact & PO number, and whether sample is blinded. If you send DNA, include quantification method and values. Vendors often require a signed submission form or barcode.

2) What commercial providers typically do / what platforms they use

- Marker panels / kits: many services use commercial multiplex STR kits such as Promega PowerPlex® 21 / 16 / 18 or similar, 17–21 loci panels are common (ATCC/DSMZ follow ANSI/ATCC standards recommending 8 core loci up to 17 loci). Using more loci increases resolution. Eurofins advertises a 21-locus service (PowerPlex 21).



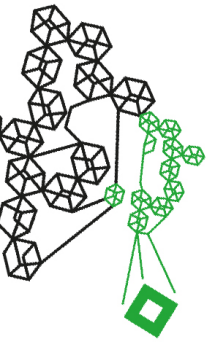
- Typical workflow at vendor: DNA extraction (if required) → PCR multiplex STR amplification → capillary electrophoresis → allele calling and electropherogram QC → STR profile generation → database matching (ATCC/DSMZ/Cellosaurus/CLASTR) → PDF report / certificate.

3) Key differences between vendors (quick summary)

- Sample acceptance: some (Eurofins, many cores) will extract from pellets; others (DSMZ) prefer or require purified gDNA for some services or for GMO samples. Check submission forms.
- Minimum cell numbers / DNA input: variable - Eurofins says visible pellet / >10,000 cells; core labs often want ≥5×10^5 if shipping frozen pellets; DSMZ requests ~20 µL at 10-100 ng/µL DNA for direct DNA submission. ATCC gives FTA card density guidance. Always check vendor doc before prepping.
- Loci panel size: DSMZ/ATCC often use the 17-locus (ANSI/ATCC) standard, many providers extend to 21 loci for higher discrimination (PowerPlex 21). This affects downstream matching stringency, a 21-loci match is more discriminating than 8-loci.
- Reporting style: ATCC/DSMZ provide signed reports with raw allele calls and electropherograms; Eurofins provides PDF certificate + can compare to reference databases. Formats vary; some provide raw data on request.

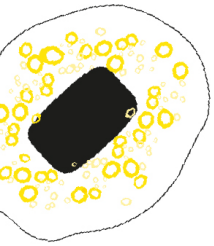
4) Practical values to use in your lab (recommended, conservative)

- If you can provide gDNA: send ≥10 µL at ≥10 ng/µL (aim for 20 µL at 10–50 ng/µL, i.e. 200–1000 ng total). This will satisfy most providers (DSMZ example).
- If you provide cell pellets: aim for ≥5×10^5 cells (safe for in-house extraction), but if following Eurofins minimum, ensure >10,000 cells with a visible pellet; label cell counts on the form. If using FTA cards, follow ATCC instructions (~1×10^6 cells/mL; 40 µL spot).
- Always include an internal negative control (blank tube) and wear gloves and change tips to avoid operator DNA contamination.



5) ICLAC / authoritative interpretation criteria (what to apply to STR matches)
ICLAC's Guide to Human Cell Line Authentication (2023 update) and companion resources set the accepted interpretation rules used by major banks (ATCC, DSMZ). Key points to apply when comparing your STR profile (*e.g.*, CLASTR / Cellosaurus / DSMZ / ATCC matches):

1. *Minimum loci to use: analyse ≥13 STR loci (ICLAC / ANSI guidance). Results using fewer loci have lower confidence.*
2. *Percent match rule (practical):*
    - *If ≥80% match across ≥13 loci, the sample is considered authenticated / consistent with the reference (original donor cell line).*
    - *100-80% generally marked as authentic (may reflect some allelic drift in long-term culture). <80% indicates possible misidentification or cross-contamination and requires follow up. (DSMZ/ATCC use similar color-coded thresholds in their search tools).*
3. Mixed profiles / contamination detection:
    - Presence of more than two alleles at many loci suggests mixed human cell populations (cross-contamination). Quantitative interpretation of mixtures is not straightforward by STR alone, repeat testing from a new aliquot and orthogonal testing are recommended.

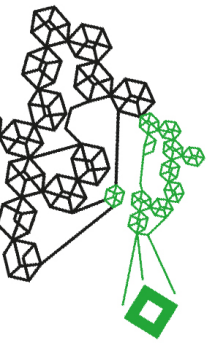


4. Allelic drift & MMR-deficient lines: lines with mismatch repair defects (MMR-) can show STR allele loss/gain over passages, interpret with caution (DSMZ notes potential drift). If you see small differences and know the line is MMR-deficient, still document and consider additional loci or genomic methods.
5. Report raw data: you should retain electropherograms and raw allele calls. If vendor results are surprising, request raw data for re-analysis. ATCC/DSMZ highlight the importance of signed reports and raw data review.

6) How to interpret CLASTR / Cellosaurus output practically (stepwise)

1. Run CLASTR with your allele calls (enter the alleles at each locus). CLASTR will return matches with a % similarity.
2. Check how many loci were used in the match (some database entries have fewer loci recorded). If your test is 17-21 loci but the matched record has only 8 loci, treat % similarity cautiously. Prefer comparisons where both profiles have ≥13 loci.
3. Apply the 80% rule: if CLASTR / Cellosaurus reports ≥80% match across ≥13 loci, mark as consistent/authenticated. If <80%, treat as not authenticated and investigate
4. If you get an intermediate match (*e.g.*, 70-79%):
    - Check electropherograms for stutter, low peak height, allele drop-out, or off-ladder alleles.
    - Re-run STR from an independent aliquot or provide higher quality DNA.
    - Consider orthogonal checks (karyotype, SNP-array, WGS) if critical.
5. If multiple high-scoring hits appear (more than one cell line scoring similarly high), inspect locus-level concordance and known problematic lines (ICLAC/Cellosaurus list of misidentified lines). Some lines are derivatives of others, consider lineage and investigate cell line.

In case the cell line remains inconclusive in terms of decision on authentication after subsequent analysis, please discontinue the cell line and terminate.

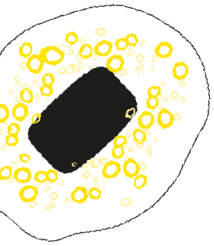


7) Short practical checklist you can paste into your LIMS / SOP

- Did I send cell line exact name, passage number, lot, and culture conditions?
- If sending pellet: was medium removed and pellet washed with PBS/TE? (Eurofins: yes).
- Cell count noted and met vendor minimum (*e.g.*, ≥10k visible or recommended ≥5×10^5 depending on provider).
- If sending DNA: concentration (Qubit) and volume (aim for 20 µL @ 10–50 ng/µL).
- Include submission form and barcode; avoid operator DNA contamination (gloves, new tips).
- On receipt of STR result: request raw electropherograms if not provided; run CLASTR/Cellosaurus; apply ≥80% (≥13 loci) rule for authentication.

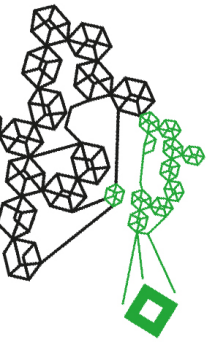
8) Caveats and final recommendations

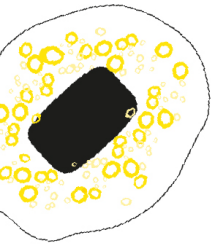
- Always check the vendor's current submission guide before you prepare/shipping samples as vendors update their forms and acceptance criteria.
- For any critical or regulatory work (GMP, publication, IP), prefer purified gDNA and a vendor that returns signed reports + raw data.



References

- Eurofins Genomics - Cell Line Authentication / sample submission guides (STR, pellet / DNA requirements). eurofinsgenomics.eu+1
- DSMZ - STR typing / sample submission form and database (17-loci standard, sample requirements). dsmz.de+1
- ATCC - Human STR testing & sample prep instructions (FTA card workflow, submission requirements). https://www.atcc.org
- ICLAC - Guide to Human Cell Line Authentication (2023) - match criteria and recommendations (≥13 loci, 80% rule). ICLAC
- CLASTR / Cellosaurus - STR similarity search tool and guidance for interpreting matches. ICLAC+1





Procedure 1.1.3 Storage and use of certificates of analysis and cell line identification data (GMO)

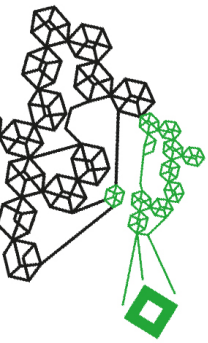
All Certificates of Analysis (CoAs) and cell line identification data (such as STR profiles, origin information and authentication reports) relating to genetically modified organisms (GMOs) are centrally stored on the designated GMO Microsoft Teams site of your organization.
Storage takes place in the assigned document library and is structured by project, cell line and/or experiment number, to ensure traceability and reproducibility. Documents are stored in a non-editable format (preferably PDF) to safeguard data integrity. Access to the Teams site is restricted to authorised personnel and managed in accordance with your organization's information security policy.
The responsible researcher ensures that the CoAs and cell line identification data are:

- complete and up to date;
- available prior to the physical arrival of the material at your organization;
- uploaded in a timely manner to the GMO Teams site following receipt or verification.

This information is incorporated by the researcher into the risk assessment as required under applicable GMO regulations. The researcher informs the Principal Investigator (PI) of the relevant findings and submits the completed or updated risk assessment to the PI for review and approval, in accordance with established procedures.
Where your organization operates a procurement system that supports this process, an optional workflow may be applied whereby:

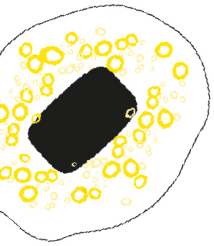
- CoAs and other relevant documentation are reviewed prior to purchase as part of the risk assessment;
- approval of this documentation is linked to the release of the purchase within the procurement system;
- the approved documents are subsequently stored on the GMO Teams site as part of the project documentation.

This approach supports timely risk assessment, compliance with GMO regulations, and controlled intake of GMO-related materials within your organization.

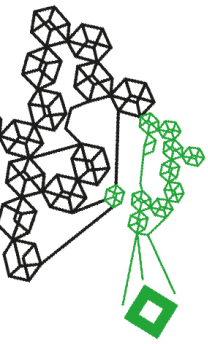


RASCI Overview: Control of Documented Information and Risk Assessment (GMO)

Activity	Researcher	Principal Investigator (PI)	Biosafety Officer (BSO)
Identify and collect relevant documented information (CoAs, cell line identification data)	R	I	S
Verify accuracy, completeness and currency of documented information	R	A	S
Ensure documented information is available prior to operational activities (receipt of material)	R	A	I
Manage and store documented information in accordance with the established structure (GMO Teams site)	R	A	I
Perform the risk assessment in accordance with GMO regulations	R	A	S
Communicate relevant risks and control measures to the PI	R	I	I

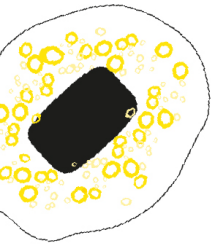


Activity	Researcher	Principal Investigator (PI)	Biosafety Officer (BSO)
Review the risk assessment as part of operational control	C	A	S
Formally approve the risk assessment prior to commencement of work	I	A	C
Ensure that work does not start without an approved risk assessment	R	A	I
Assess impact on containment level, permits and notifications (compliance obligations)	C	A	S
Identify and escalate changes or deviations with potential impact on risks	R	A	I
Advise on additional risk control measures and preventive actions	I	C	R
Verify compliance with legal and other requirements (GMO regulations, permits)	I	C	R
Review documentation prior to procurement (if applicable) as part of operational planning	R	A	S
Link document review to procurement release (optional control measure)	R	A	S
Update the risk assessment following changes (change management)	R	A	S
Ensure traceability and archiving of documented information	R	A	I
Support internal audits, inspections and compliance evaluations	R	A	S



Legend:

- R - Responsible: performs the activity
- A - Accountable: ultimately accountable for effectiveness
- S - Support: actively supports or facilitates
- C - Consulted: provides expert input
- I - Informed: kept informed



Procedure 1.1.4 Example Standard operating procedure (SOP) for subsequent cloning/recombinant DNA technology activities within cell lines

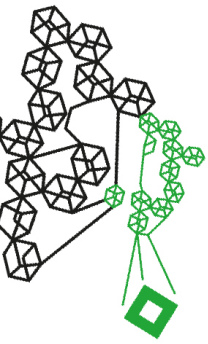
SOP: Identity Verification of Genetically Modified Elements in Cell Lines
SOP ID: [to be assigned]
Version: 1.0
Date: [YYYY-MM-DD]
Author: [Name]
Approved by: [Name/Position]

1. Purpose
This SOP describes the procedure for verifying the identity of a genetically modified (GG) element, such as recombinant DNA or a transgene, in a host cell line. It ensures that the intended genetic modification is present and correctly integrated, and helps detect misidentification or cross-contamination.

2. Scope
Applies to all laboratory personnel working with genetically modified cell lines under institutional and biosafety guidelines. Covers DNA extraction, PCR/qPCR verification, sequencing, and documentation.

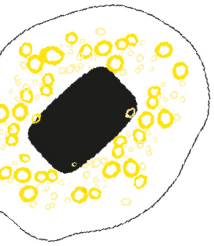
3. Responsibilities
- Laboratory personnel: Execute the procedure accurately, maintain records.
- Principal Investigator / Lab manager: Approve SOP use and review results.
- Quality Assurance (QA)/ biological safety officer: Audit records, ensure compliance with institutional and regulatory standards.

4. Materials and Equipment
Materials:
- Cell culture samples (adherent or suspension)
- DNA extraction kit suitable for eukaryotic cells
- PCR/qPCR reagents
- Primers specific to the GG-element (target-specific and flanking regions)
- Positive control DNA (verified GG-element)
- Negative control DNA (host cell line without GG-element)



Equipment:
- Biological safety cabinet
- Thermocycler or qPCR instrument
- Gel electrophoresis system (optional if using PCR)
- DNA sequencing facility or instrument

5. Procedure
5.1 Sample Preparation
1. Collect cells at appropriate passage number (preferably early to avoid drift).
2. Pellet 1-5 × 10^6 cells and proceed with genomic DNA extraction following manufacturer instructions.



5.2 PCR/qPCR Verification

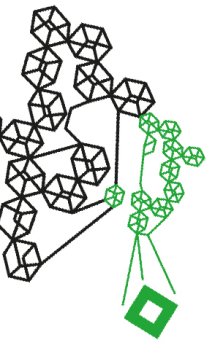
1. Design primers targeting:
    - Specific sequences of the GG-element
    - Flanking genomic regions (to verify integration site, optional)
2. Run PCR or qPCR with:
    - Sample DNA
    - Positive control
    - Negative control
3. Analyse results:
    - Correct amplicon size (PCR) or expected amplification curve (qPCR) indicates presence of GG-element.

5.3 Sequencing (Optional / Recommended)

1. Sequence PCR amplicons to confirm:
    - Correct GG-element sequence
    - Integrity of junction regions if integrated into the genome
2. Compare to reference sequence using alignment software.

5.4 Interpretation

- Confirmed identity: Sequence matches reference, correct amplicon observed.
- Uncertain / Partial match: Minor deviations or ambiguous results—repeat analysis or perform additional verification (e.g., Southern blot).
- Misidentified / Absent: GG-element not detected or sequence mismatched—flag cell line, consider termination or quarantine.



6. Documentation

- Record all experimental data in the lab notebook or electronic LIMS.
- Include: sample ID, passage number, primer sequences, PCR/qPCR results, gel images, sequencing data.
- Record interpretation and any follow-up actions.

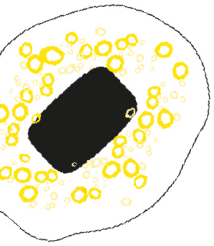
7. Quality Control and Safety

- Include positive and negative controls in every run.
- Follow institutional biosafety level regulations.
- Periodically re-verify GG-element in long-term cell cultures.
- Quarantine and re-test any cell lines with ambiguous or unexpected results.

8. References

- ICLAC: Guidelines on cell line authentication

- Institutional Biosafety Committee regulations
- Manufacturer manuals for PCR, qPCR, DNA extraction kits



Procedure 1.1.5 Re-evaluation and assessment

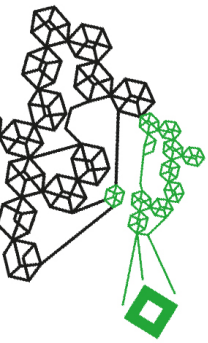
1. Initial Assessment

Upon reaching the “Re-evaluation and Assessment” point in the decision tree, perform:

1. An overall documentation review (source, passage number, cryopreservation records, relevant metadata) including the STR profiling data
2. Phenotypic evaluation (morphology, growth characteristics, marker expression)
3. Reference check against:
    - Known cell line STR profiles
    - ICLAC misidentified cell line database

2. Classification and Actions

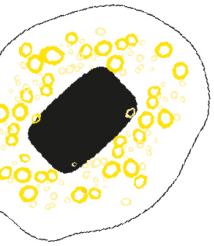
Category	Criteria	Recommended Action
Clearly Misidentified / Terminate	- STR profile mismatches reference on ≥2 loci or matches a different known cell line - Listed in ICLAC as misidentified/cross-contaminated - Irretrievable or inconsistent provenance/documentation - Phenotypic traits incompatible with reported cell line	Discontinue use immediately. Document findings. Seek alternative verified cell line.
Uncertain / Conditional Use	- STR does not meet ICLAC match threshold (<80%) - Morphology or marker expression differs but no clear evidence of misidentification - STR-data based on less than 13 loci - Limited historical data or incomplete metadata	Use only with restrictions: - STR verification at subsequent passages - Limit experiments to internal validation or methodological studies (not for publications) - Label as “identity under review” -Mention a note in your research article on the cell line - Monitor phenotypic characteristics over time
Confirmed / Proceed	- STR profile matches reference - Provenance verified - Morphology and markers consistent with expected phenotype	Resume normal workflow. Document verification.



3. Monitoring and Follow-up

For cell lines under Conditional Use:

1. Determine appropriate defined passage intervals to repeat STR analysis
2. Document any phenotypic changes or inconsistencies.
3. Reassess and reclassify if new evidence indicates misidentification.
4. Decide to terminate or to proceed along with supervisor (PI, Prof.)



4. Documentation

All assessments, STR results, and decisions must be recorded in the cell line registry (procedure 1.1.3), including:

- STR profiles and reference comparison
- Source and passage history
- Rationale for conditional use or termination