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Beyond the gold standard: why STR profiling should be viewed as the starting point, not the end, of cell line authentication

Junyu Zheng#, Lili Qing#, Haodi Yuan, Xinrui Guo, Shengjie Nie, Linlin Liu

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INTRODUCTION

Recently, Li *et al.* developed STRaM, an innovative bioinformatic framework designed to improve cell product provenance[1]. While traditional capillary electrophoresis-based short tandem repeat (CE-STR) profiling remains the indispensable gold standard for routine identity authentication[2], emerging platforms like STRaM highlight the necessity of transitioning to multi-dimensional molecular screening. However, advanced sequencing-based frameworks should complement, rather than replace, traditional STR approaches.

As Li *et al.* acknowledged, traditional CE-STR struggles when confronting closely related derivative strains (e.g., HEK293 and its derivatives, 293T and 293FT)[1]. Here, a fundamental distinction must be drawn between cell identity authentication - confirming that a cell line originates from the correct donor - and molecular and functional fidelity, which refers to the preservation of genomic stability, cellular states, and expected biological behaviors. While traditional CE-STR primarily verifies identity consistency, it does not capture functional and molecular drift. This limitation manifests in three dimensions: first, as neutral genetic microsatellite loci, traditional STRs merely reflect fragment length, failing to detect targeted mutations or exogenous gene insertions that dictate cellular function; furthermore, they cannot detect macroscopic copy number variations (CNVs)[3]; nor can they reflect transcriptomic remodeling and phenotypic differentiation.

Indeed, even within the same lineage, the biological fates and experimental behaviors of cellular derivatives often vary drastically. For instance, although the foundational strain HEK293 and its derivative 293T present high similarity in traditional STR profiles, 293T stably expresses the SV40 large T antigen, giving it distinct functional properties and genome-wide copy-number differences[4]. Similarly, late-passage SKOV3 cells can spontaneously differentiate into distinct substrains with divergent

NHC Key Laboratory of Drug Addiction Medicine, School of Forensic Medicine, Kunming Medical University, Kunming 650500, Yunnan, China.
#These authors contributed equally to this work.

Correspondence to: Prof. Shengjie Nie, Assoc. Prof. Linlin Liu, NHC Key Laboratory of Drug Addiction Medicine, School of Forensic Medicine, Kunming Medical University, Kunming 650500, Yunnan, China. E-mail: nieshengjie@126.com; liull0818@126.com

transcriptomic features and drug sensitivities, despite maintaining identical STR profiles[5]. Therefore, ensuring both cell-identity authentication and molecular and functional fidelity requires a tiered authentication framework where STR profiling serves as the starting point. If ruling out cross-species contamination and gross mislabeling represents the minimum requirement of experimental quality control, simply holding this line is insufficient in today's era, where gene editing, targeted therapies, and complex in vitro models prevail.

HOW CRYPTIC HOMOLOGOUS EVOLUTION AND CROSS-CONTAMINATION CHALLENGE SINGLE-METHOD QUALITY CONTROL

During prolonged in vitro passaging, tumor cell genomes frequently undergo subtle but functional alterations. Li *et al.* demonstrated that traditional CE-STR fails to capture nucleotide variations within repeats or flanking regions, causing highly homologous strains or trace cross-contaminations to appear identical[1]. Since genetic manipulations like lentiviral transduction do not alter STR repeat lengths, consistent STR profiles can mask underlying genomic and phenotypic divergence.

This represents a systemic challenge. For example, a survey found a 20.5% cell line misidentification rate, showing that single-locus STRs struggle to rule out cryptic cross-contaminations[6]. Thus, relying solely on STR confirms the origin of the cell line but cannot evaluate its genomic stability, ploidy levels, or functional drift over time. To fully characterize cell lines and assess mosaic situations or ploidy changes, classical and molecular cytogenetics must be integrated. Cytogenetic approaches remain among the most practical and established methods for evaluating mosaicism and ploidy alterations at the single-cell level.

MOVING TOWARDS A MULTI-DIMENSIONAL, TIERED AUTHENTICATION FRAMEWORK

To prevent experimental failures and associated financial losses, cell authentication must be matched to specific research needs. While some laboratories avoid authentication due to perceived costs, the financial consequences of cell misidentification - such as retracted papers and wasted consumables - are far more severe[7,8]. To resolve this, we propose a cost-effective tiered authentication framework inspired by the "fit-for-purpose" principles of Almeida *et al.*[9]. Rather than replacing STR, this framework layers complementary technologies hierarchically based on research risks and scenarios:

Tier 1: routine banking and basic identity screening

For routine cell banking and baseline cell identity authentication, CE-STR combined with species-specific PCR remains the most practical and cost-effective approach[6]. It provides a rapid baseline to exclude HeLa contamination and common mix-ups. As demonstrated by Zhang's team, CE-STR successfully validated human cell lines resuscitated after 34 years of cryopreservation[10]. To supplement this, classical cytogenetics (e.g., G-banding karyotyping) should be integrated at this stage to reliably assess ploidy levels and mosaic situations.

Tier 2: genetic modification and structural variation tracking

For gene-edited cell lines, cell derivatives, or long-term passaged cultures, traditional STR is insufficient. At this stage, NGS-based STR (STR-NGS) offers superior resolution by analyzing sequence variations in flanking regions to detect gene edits[1,11]. Nanopore-based pipelines, such as Nanopore Autosomal Short Tandem Repeat Analysis (NASTRA), enable rapid lineage tracking without reference genome constraints[12]. Furthermore, optical genome mapping (OGM-ID) provides high-resolution detection of structural variations (> 500 bp), yielding a 25% detection rate for intra-species contamination compared to 5% for STR[13].

Tier 3: preclinical translation and fidelity validation

In translational studies, high-throughput drug screening, and preclinical model validation, cell identity authentication must be performed alongside assessments of molecular and functional fidelity. This requires integrating multi-omics approaches, including transcriptomics (RNA-seq) and proteomics, with molecular cytogenetics [e.g., multiplex fluorescence in situ hybridization (M-FISH)][14]. Only when molecular phenotypes align with expected biological behaviors can a cell line be verified for high-stakes preclinical applications.

REDEFINING CELL QUALITY CONTROL BASELINES IN THE MULTI-OMICS ERA

Researchers must recognize that no single method can permanently guarantee the genomic and phenotypic stability of cells. The bioinformatic advances of STRaM[1] and the historical cryopreservation insights of Zhang's team[10] highlight a key transitional phase in quality control paradigms: while cryopreservation halts physical cell aging, our definition of "authenticity" must evolve to match modern multi-omics capabilities.

Obtaining cells from reliable sources is merely the first step. Academic journals, industry societies, and funding agencies should re-evaluate existing guidelines for cell line authentication. In the future, for research involving complex cell derivatives and critical translational medicine, merely submitting a basic STR profile should no longer be viewed as sufficient evidence for passing quality control. We recommend establishing comprehensive quality control dossiers that document the genetics, morphology, and passage numbers of cell lines, while promoting and implementing a multi-dimensional, tiered authentication framework to safeguard research. This is not only a protection of precious scientific resources but also an essential step toward ensuring scientific rigor.

DECLARATIONS

Author contributions

Conception and design: Zheng J, Qing L, Nie S, Liu L
Administrative support: Nie S, Liu L
Provision of study materials: Yuan H, Guo X
Manuscript writing: Zheng J, Qing L
All authors read and approved the final manuscript.

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Conflicts of interest

Liu L and Nie S serve as Junior Editorial Board Members of the *Journal of Translational Genetics and Genomics*. They were not involved in any stage of the editorial handling of this manuscript, including reviewer selection, peer-review management, or editorial decision-making. The other authors declare that they have no competing interests.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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