Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2026**; 31(10): 55855
https://doi.org/10.31083/FBL55855

Short Communication

Genomic Characterization and Species Identification of Commercial Human Primary Cell Lines Through Multi-Modal Authentication

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Academic Editor: Graham Pawelec
Submitted: 24 April 2026 Revised: 28 August 2026 Accepted: 7 September 2026 Published: 14 October 2026

Abstract

Background: Accurate determination of cell identity is crucial for the reproducibility and validity of biomedical research. The species identity of two commercially obtained primary cell preparations labeled as human neuronal cells (hNC) and human gallbladder cells (GBC) was investigated. **Methods:** Cell identity was determined using a multimodal authentication strategy comprising morphological examination, independent short-tandem repeat (STR) profiling, and RNA sequencing (RNA-seq). To further characterize the hNC preparation, RNA-seq reads were mapped to the *Cricetulus griseus* reference genome (GCF_003668045.3_CriGri-PICRH-1.0), followed by analysis of hamster-associated non-coding RNAs and canonical neuronal markers. **Results:** Independent STR profiling failed to yield valid human STR profiles for either preparation. RNA-seq revealed that approximately 91–92% of reads from the hNC preparation mapped to *Cricetulus griseus*, whereas the GBC preparation exhibited genomic signals from multiple species. Transcriptomic reanalysis of the hNC preparation showed abundant expression of the hamster-associated long non-coding RNA *Adapt15* (*gadd7*) and detectable expression of *Mir6092*. In contrast, canonical neuronal markers, including *Map2*, *Rbfox3*, *Snap25*, *Syp*, *Nefl*, *Nefm*, and *Nefh*, showed very low or undetectable expression. **Conclusions:** These findings identified substantial discrepancies between the labeled identities and the independently determined molecular characteristics of commercially obtained primary cell preparations. The findings highlight the limitations of relying solely on supplier-provided authentication documentation and underscore the importance of independent, orthogonal authentication using complementary genomic and transcriptomic approaches to ensure experimental reproducibility and data integrity.

Keywords: cell line authentication; primary cell culture; microsatellite repeats; whole genome sequencing; RNA-seq; cricetulus

1. Introduction

Cell line misidentification and cross-species contamination remain pervasive issues in biomedical research, significantly affecting the reproducibility of scientific findings [1]. Short-tandem repeat (STR) profiling has long been established as the gold standard for human cell line authentication [2,3]. However, conventional authentication methods may be insufficient when species identity is ambiguous or when genomic signals from multiple species are detected. To address these challenges, the scientific community increasingly relies on genome-wide sequencing and read-mapping approaches to enable precise genomic characterization and accurate species identification. These methods facilitate comprehensive genome analysis and help resolve ambiguities in species identity [4,5,6]. An early study of 246 cell cultures reported a 14% contamination rate with cells from other species, while nearly 30% were misclassified [7]. A subsequent comprehensive authentication study of 482 human tumor cell lines uncovered multiple instances in which cell lines labeled as human were, in fact, of mouse, Chinese hamster, bovine, or non-human primate origin [8]. These concerns have led to recommendations that authentication should include not only established cell lines but also primary cell cultures and other human cell-based preparations [9]. However, most published systematic studies have predominantly focused on established or tumor-derived cell lines, whereas commercially supplied primary cell preparations have received comparatively limited independent evaluation at the species level.

Commercially available primary cells are widely used as physiologically relevant models because they are believed to retain tissue-specific characteristics better than immortalized cell lines. In practice, researchers frequently rely on supplier-provided documentation, morphology, viability, and expression of selected markers to confirm cell identity. However, these criteria do not directly establish the species of origin. Marker-based assays can be potentially misleading when primers or antibodies recognize conserved genes across species [10,11]. Therefore, independent authentication is crucial for commercial primary cell preparations before use in downstream biological or translational studies [12].

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To address this issue, as part of routine quality control, our laboratories detected unexpected cross-species genomic signals in two commercially obtained cell preparations labeled as human primary cells from a supplier in Torrance, California. To investigate these findings, we implemented a multimodal authentication workflow that combined morphology, STR profiling, direct analysis of material from the original vial, and genome-wide sequencing or read-mapping analyses. Our results showed that the human-labeled neuronal cell preparations lacked detectable human STR alleles and predominantly aligned with the Chinese hamster genome. In contrast, the human-labeled GBC preparation failed human STR authentication and showed no predominant human mapping in sequencing-based analysis. These findings highlight the need for independent cell authentication and careful interpretation of supplier-provided identity information in biomedical research.

2. Materials and Methods

2.1 Cell Line and Cell Culture

Primary human neuronal cells (Celprogen, Cat #36057-01, Lot #1314006, 1314021, Torrance, CA, USA) were cultured according to the supplier's protocol. Briefly, the growth medium (Celprogen, M36057-01) was pre-warmed at 37 °C for 30 minutes. Cells were thawed in a 37 °C water bath for 2 minutes and transferred into a 15 mL tube containing 9 mL of growth medium. After centrifugation at 100 ×g for 7 minutes, the supernatant was discarded, and the cells were resuspended in 8 mL of growth medium before plating in a 10 cm dish.

The human normal gallbladder primary cell line (Celprogen, Cat #36126-53N, Lot #2208112) was cultured in the supplier-recommended medium supplemented with 10% fetal bovine serum (FBS; Gibco™, Grand Island, NY, USA) and 1% penicillin–streptomycin (Gibco™, Grand Island, NY, USA).

OCUG-1 and G415 were obtained from the Health Science Research Resources Bank (Osaka, Japan). NOZ, TGBC2TKB (2TKB) and TGBC24TKB (24TKB) were purchased from the RIKEN BioResource Center (Ibaraki, Japan). OCUG-1 cells were maintained in DMEM (Gibco™), G-415 cells in RPMI (Gibco™), and NOZ, 2TKB, 24TKB cells in Williams' E Medium (Gibco™). All media were supplemented with 10% FBS and 1% penicillin–streptomycin.

2.2 RNA Sequencing

The purified RNA was used to prepare the sequencing library with the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations. Briefly, mRNA was purified from total RNA (1 µg) using oligo(dT)-coupled magnetic beads, and the mRNA was fragmented at elevated temperatures. First-strand cDNA was synthesized using reverse transcriptase and random primers. After double-stranded cDNA synthesis and adenylation of the 3' ends of DNA fragments, the adaptors were ligated. The products were enriched by PCR and purified with the AMPure XP system (Beckman Coulter, Beverly, MA, USA). The libraries were qualified using the Qsep400 System (Bioptic Inc., Taiwan) and quantified with the Qubit 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA). Subsequently, the qualified libraries were sequenced on an Illumina NovaSeq platform with 150 bp paired-end reads generated by Genomics, BioSci & Tech Co., New Taipei City, Taiwan.

For species-mapping analysis, the RNA-seq reads were subjected to quality-control processing before alignment to reference genomes. Read quality was assessed using FastQC v0.11.9 and summarized using MultiQC v1.11, with trimming performed using Trimmomatic v0.39 and fastp v0.23.2. For the GBC samples, cleaned FASTQ reads were analyzed using FastQ Screen against multiple reference genomes to assess the species-associated composition of the sequencing libraries. Reads were categorized according to their mapping patterns, including Human, Mouse, Rat, Multiple Genomes, and No hits. "Multiple Genomes" denotes reads mapping to more than one reference genome included in the screening panel, whereas "No hits" denotes reads that did not map to any of the reference genomes included in the analysis. For the hNC samples, RNA-seq reads were compared with human, mouse, and Chinese hamster reference genomes to evaluate species-associated alignment patterns.

2.3 Human Cell Line Authentication

Genomic DNA was extracted using the EZ1&2 DNA Tissue Kit (QIAGEN, Cat # 953034, QIAGEN GmbH, Hilden, Germany), following the manufacturer's instructions to obtain high-purity DNA. To authenticate the cell line, short tandem repeat (STR) profiling was performed with the AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit (Thermo Fisher, Cat# 4427368, Applied Biosystems, Foster City, CA, USA). The AmpFLSTR Identifiler Plus PCR Amplification Kit detects 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) together with the amelogenin (AMEL) sex marker. PCR products, along with positive and negative controls, were separated and analyzed by capillary electrophoresis on the ABI 3730xL Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed with GeneMapper v4.0 software (Applied Biosystems, Foster City, CA, USA) and compared to the Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) human STR profile database, which contains data from 2455 cell lines curated by ATCC, DSMZ, JCRB, and RIKEN. Cell lines with ≥80% match were considered related, and those with 55–80% match were flagged for further profiling according to ANSI Standard guidelines. This comprehensive workflow

ensures accurate cell line authentication and minimizes the risk of misidentification.

2.4 RNA-seq Re-Alignment and Transcriptomic Analysis

To further characterize the species and cellular identity of the hNC preparation, RNA-seq data obtained from three technical replicates of the hNC preparation were re-analyzed using the Chinese hamster reference genome. Raw sequencing reads were realigned by the sequencing service provider to the Cricetulus griseus reference genome GCF_003668045.3_CriGri-PICRH-1.0 (NCBI Taxonomy ID: 10029) using HISAT2. Gene annotation was based on the corresponding Ensembl gene annotation, and gene expression levels were quantified as transcripts per million (TPM) using the analysis pipeline supplied by the sequencing service provider.

2.5 Data Analysis

Data were presented as mean ± standard deviation (SD) (Fig. 1c) and analyzed by GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Morphological Discrepancies and Species-Mapping Analysis of Neuronal Cells

Our investigation was initiated after observing an atypical morphology in human neuronal primary cells purchased from Celprogen (Cat #36057-01, Lot #1314006). This particular cell line has previously been utilized in the literature to investigate therapeutic options for glioblastoma [13]. At 48 hours post-seeding, the cells exhibited an elongated, spindle-shaped morphology uncharacteristic of typical neuronal cells (Fig. 1a). To address this anomaly, we performed standard STR profiling on both a cryopreserved aliquot (P1hNC, Lot #1314006) and the original cell vial (OrihNC, Lot #1314021). Electropherogram analysis using GeneMapper v4.0 revealed no detectable human STR alleles in either sample (Fig. 1b, **Supplementary Fig. 1a,b**). To ascertain the actual species origin, we employed RNA-seq-based species mapping, which revealed that 91.46–92.22% of reads aligned to the Chinese hamster reference genome, whereas only 3.26–3.60% and 7.31–7.44% aligned to the human and mouse reference genomes, respectively (Fig. 1c). Following the predominant Chinese hamster-associated alignment pattern, the hNC RNA-seq reads were re-aligned to the *Cricetulus griseus* reference genome (GCF_003668045.3_CriGri-PICRH-1.0) for species-directed transcriptomic analysis. To further characterize the transcriptomic identity of the hNC preparation, we examined the expression of selected hamster-associated non-coding RNAs and canonical neuronal markers. *Adapt15* (*gadd7*), a long non-coding RNA originally identified in Chinese hamster cells [14], was abundantly and consistently expressed across three technical replicates, with a mean expression level of approximately 3232 TPM (Fig. 1d). Additionally, *Mir6092*, corresponding to the *Cricetulus griseus*-annotated miRNA cgr-miR-6092, was consistently detected across three technical replicates, with a mean expression level of approximately 28.6 TPM (Fig. 1d). These findings supported a Chinese hamster-associated molecular identity.

In contrast, canonical neuronal marker genes exhibited minimal or undetectable expression in the hNC samples (Fig. 1e). Specifically, *Map2* and *Syp* were only faintly detected, with mean expression levels of approximately 0.35 and 0.41 TPM, respectively. In contrast, markers such as *Rbfox3*, *Snap25*, *Nefl*, *Nefm*, and *Nefh* were not detectable. These transcriptomic findings are consistent with the hamster-associated molecular features identified in the hNC preparation and further suggest that the cells do not express the gene-expression profile characteristic of mature neuronal cells.

3.2 Species-Mapping Analysis and FastQ Screen Mapping of Gallbladder Cells

An independent collaborating laboratory identified similar unexpected genomic profiles in a human normal GBC (Celprogen, Cat #36126-53N, Lot #2208112) that was previously used to evaluate anticancer drug activity [15] (Fig. 2a). STR profiling of the GBC line across a standard human panel yielded flat baselines with no detectable alleles (D5S818, D13S317, D7S820, D16S539, vWA, TH01, AMEL, TPOX, and CSF1PO), confirming the absence of amplifiable human DNA (Fig. 2b, **Supplementary Fig. 2**). RNA-seq-based FastQ Screen analysis provided independent evidence of an atypical species-mapping profile in the GBC preparation. Across the three GBC samples, 48.720–49.930% of reads were classified as “No hits”, indicating that they did not map to any of the reference genomes included in the screening panel. In addition, 35.543–36.411% of reads were classified as “Multiple Genomes”, indicating mapping to more than one reference genome in the screening panel. Only 2.300–2.327% of reads were assigned to the human category, whereas 6.200–6.471% and 5.925–6.115% were assigned to the mouse and rat categories, respectively (Fig. 2c).

To verify that the absence of detectable human STR alleles in the analyzed commercial cell preparations was not attributable to technical shortcomings of the assay, human gallbladder carcinoma cell lines were included as positive controls for STR profiling. Both NOZ and G415 produced clear human STR allele profiles across the tested loci and matched their corresponding reference cell line profiles with 100% similarity in public STR databases (**Supplementary Figs. 3a,4a**). Representative GeneMapper electropherograms showed distinct STR peaks in these positive control samples (**Supplementary Figs. 3b,4b**). These results confirmed the technical validity of the STR profiling workflow and its capability to reliably detect human STR alleles under the experimental conditions employed in this study.

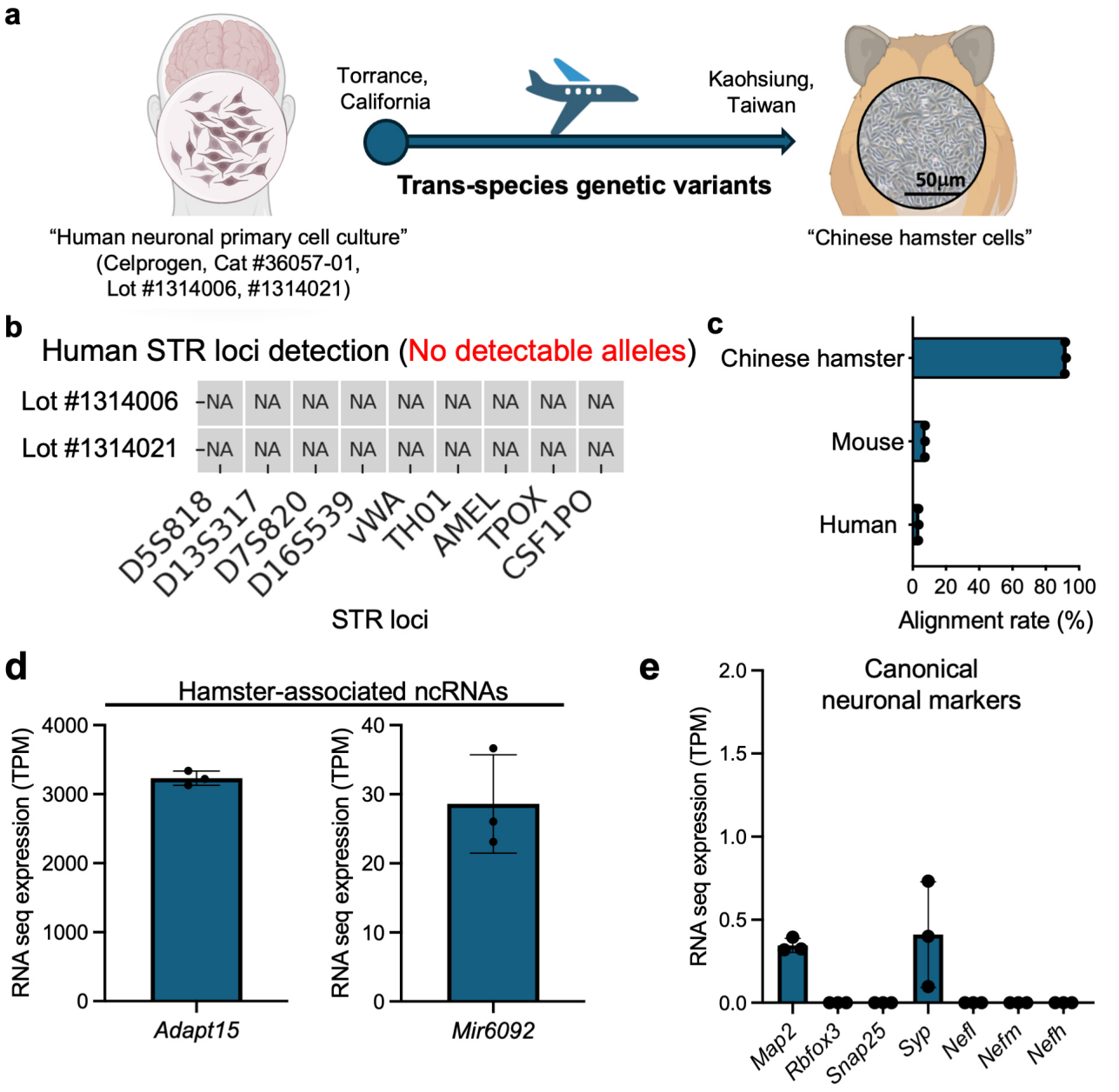

Fig. 1. Characterization of human neuronal cells. (a) Schematic overview of the commercially obtained hNC preparation, labeled as human neuronal primary cells by the supplier, and the Chinese hamster-associated identity identified by subsequent molecular analyses. (b) STR profiling detected no human alleles across nine loci. (c) RNA sequencing revealed >90% alignment to the Chinese hamster genome, with negligible mapping to the human or mouse genomes. (d) Expression of hamster-associated non-coding RNAs *Adapt15* (*gadd7*) and *Mir6092* in hNC samples. (e) Expression of canonical neuronal markers *Map2*, *Rbfox3*, *Snap25*, *Syp*, *Nefl*, *Nefm*, and *Nefh*. Expression levels are shown as TPM. Error bars represent mean ± SD from three technical replicates. STR, short-tandem repeat; TPM, transcripts per million. Created in BioRender. Hsu, W. (2026) https://BioRender.com/1mslgar.

4. Discussion

This study revealed that two widely used commercial cell lines in biomedical research did not originate from human sources. The neuronal cells labeled as human cells showed no detectable human STR alleles and predominantly aligned with the Chinese hamster genomic signature in genome-wide mapping analysis. Similarly, the gallbladder cells labeled as human lacked confirmation of human identity across multiple assays. Collectively, these findings suggest that the commercial cell lines examined could not be independently authenticated as of human origin.

These findings emphasize that supplier-provided product information should not be treated as a substitute for

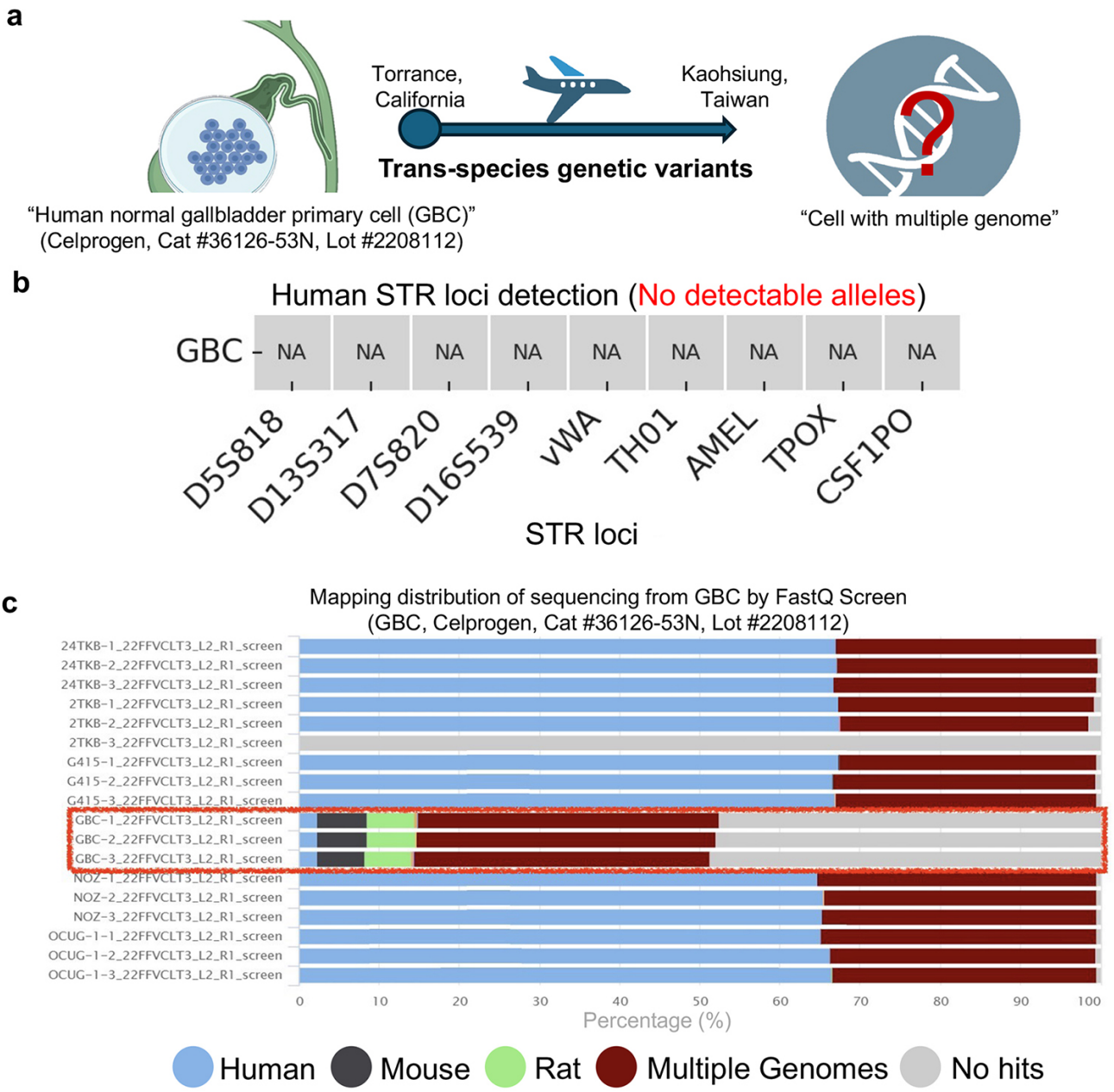

Fig. 2. Characterization of gallbladder cells. (a) Schematic of human normal gallbladder primary cell culture (GBC, Celprogen, Cat #36126-53N, Lot #2208112) showing trans-species transformation. (b) STR profiling detected no human alleles. (c) FastQ Screen mapping of the GBC samples. Across the three GBC samples, 48.720–49.930% of reads were classified as "No hits", whereas 35.543–36.411% were classified as "Multiple Genomes". The "Multiple Genomes" category denotes reads mapping to more than one reference genome included in the FastQ Screen analysis and does not specifically indicate mapping exclusively to multiple non-human genomes. Only 2.300–2.327% of reads were assigned to the human category. The red box highlights the three GBC samples analyzed in this study, distinguishing them from the other samples shown in the FastQ Screen output. Created in BioRender. Hsu, W. (2026) https://BioRender.com/xeknxdg.

independent cell authentication. While data such as catalog identity, morphological characteristics, and viability measurements can indicate the product description or whether cells remain viable under the recommended handling and culture conditions, these descriptions do not directly verify species origin or genomic identity. In the present study, cell preparations consisting of human primary cells failed to be independently authenticated by STR profiling and sequencing-based mapping analyses. Our findings support the recommendation that researchers should independently authenticate cell preparations rather than relying solely on supplier documentation. Incorporating orthogonal valida-

tion steps, including STR profiling and genome-wide read mapping, may help prevent the propagation of inaccurate results caused by unrecognized species-identity discrepancies. These findings underscore the need for more rigorous authentication protocols when using commercial primary cells in biomedical research.

To further investigate the consistency of the observed identity discrepancy across independent commercial lots, we performed STR profiling on cells obtained directly from the original cryopreserved vial of a separate hNC lot (Lot #1314021) immediately after thawing (**Supplementary Fig. 1b**). No detectable human STR alleles were observed in this original vial sample, consistent with the absence of detectable human STR alleles in the independently examined hNC preparation from Lot #1314006 (Fig. 1b, **Supplementary Fig. 1a,b**). Thus, the absence of detectable human STR markers was observed in two independent commercial hNC lots. Identifiable human genetic markers were not detected at the earliest post-thaw stage examined, indicating that the absence of detectable human identity could not be attributed to extended culture or passaging within our laboratory. Nonetheless, this analysis does not elucidate the precise timing or source of the discrepancy. Consequently, no attribution can be made to any specific stage of cell preparation, cryopreservation, transportation, or handling. Our conclusions are confined to the observation that the cell preparations tested could not be independently authenticated as of human origin.

Following these authentication findings, we notified the supplier and provided information regarding the discrepancy observed in our analyses. In response, the supplier provided an STR analysis report for the same neuronal cell catalog and lot examined in this study, stating that its results supported a human origin. According to the supplier-provided documentation, cells from this lot were cultured under the recommended conditions for five days before genomic DNA extraction; two laboratories performed STR analyses; and an additional cell pellet was submitted to ATCC for STR analysis. Therefore, the results provided by the supplier were inconsistent with those obtained in our independent analyses, which showed no detectable human STR alleles and the predominant alignment of sequencing reads to the Chinese hamster genome. Since the two analyses were performed independently on separately processed samples, the data available did not permit us to resolve this discrepancy or determine the specific stage at which it arose. Accordingly, these findings should not be interpreted as assigning responsibility to any specific stage of cell preparation, cryopreservation, transportation, or subsequent laboratory procedures.

For researchers who have previously used these or similar commercial cell preparations, it is recommended that any remaining original vials, archived stocks, early-passage aliquots, or matched lots should be independently authenticated using complementary species-identification methods whenever feasible. The implications of an authentication discrepancy should be evaluated in light of its relation to the specific experimental question. Studies whose conclusions depend directly on human species identity, human-specific molecular targets, or the presumed tissue origin of the preparation may require caution and re-evaluation. However, the present findings do not establish that all results generated using these products are necessarily invalid. When authentication concerns materially affect the interpretation or reproducibility of published findings, transparent disclosure to collaborators, institutions, readers, and, when appropriate, the relevant journal should be considered.

5. Limitation

A limitation of this study is the inability to precisely identify the source and timing of the observed identity discrepancy. While STR profiling of cells collected directly from the original cryopreserved vial suggests that the absence of detectable human identity was already present at the earliest post-thaw stage examined, it does not distinguish among possible causes that may have occurred during cell preparation, cryopreservation, transportation, or other handling steps prior to analysis. In addition, this study examined specific commercial lots rather than multiple batches from various suppliers. Consequently, no attribution is made to a particular stage or source. The conclusions are therefore confined to the independent authentication results obtained from the tested cell preparations.

6. Conclusion

This study demonstrates that commercial cell preparations labeled as human primary cells may not reliably undergo independent authentication via STR profiling and sequencing-based species mapping. These results highlight the importance of supplementary validation beyond supplier-provided information to confirm species origin or genomic identity. Prior to utilization in downstream biological experiments—particularly those impacting disease modeling, pharmacological testing, or translational research—it is essential to perform independent validation. Incorporating routine STR profiling and genome-wide read mapping can mitigate risks associated with unrecognized discrepancies in cell identity, thereby enhancing the reproducibility and reliability of biomedical research outcomes.

Availability of Data and Materials

The processed data supporting the findings of this study are available within the article and its supplementary materials. The raw RNA-seq datasets used for species-mapping analyses are not publicly available but are available from the corresponding authors upon reasonable request.

Author Contributions

CWL: Methodology, Investigation, Data curation, Visualization, Writing – original draft. WYW: Methodology, Investigation. WLH: Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing. MLK: Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The cell preparations investigated in this study were commercially obtained from Celprogen, Inc. (Torrance, CA, USA) as human primary neuronal and gallbladder cells. According to the supplier's published Biomedical Ethical Standards, human tissues used for cell isolation are obtained under the guidance of Institutional Review Board (IRB) protocols and with signed informed consent from donors. The supplier also lists the Declaration of Helsinki among the relevant ethical standards. No human participants were directly recruited, and no human tissue specimens were collected by the authors for this study.

Acknowledgment

Figures and graphic schematics were created with BioRender.com.

Funding

This work was supported by the National Center for Geriatrics and Welfare Research at the National Health Research Institutes (Grant No. 14A1-CGGP11-051) and the Kaohsiung Veterans General Hospital (Grant No. KSVGH111-074).

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used Gemini (Google) for editorial assistance to improve the clarity and flow of the text. All scientific content was verified by the authors, who take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL55855.

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