

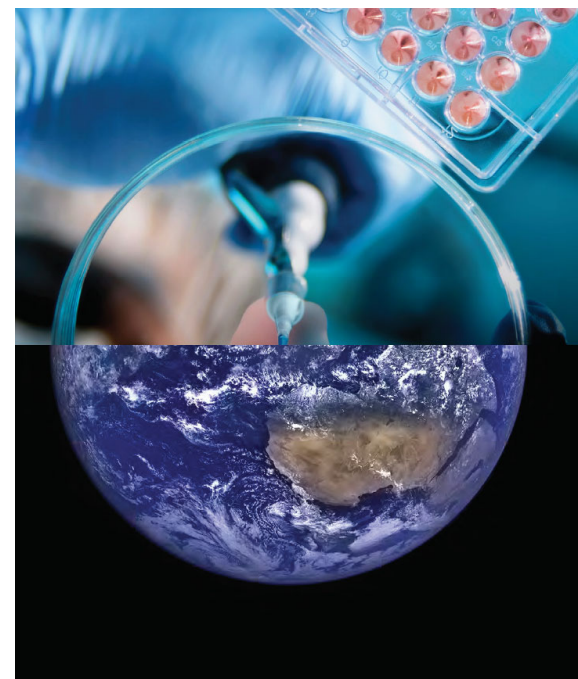
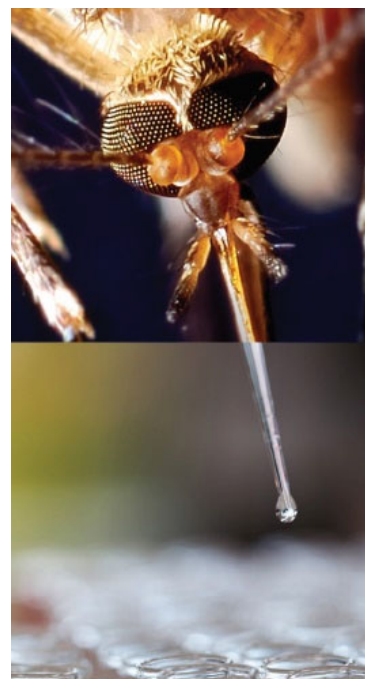
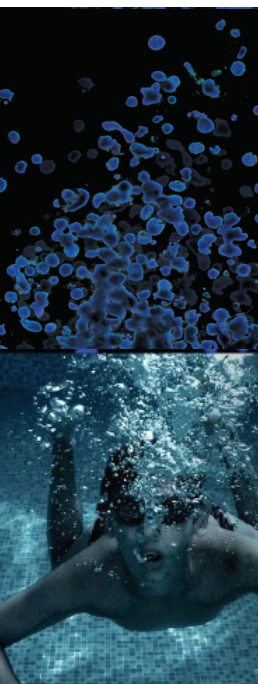


Mouse STR Profiling: A new addition to ATCC's authentication portfolio

Balsam Shawky, M.S.
Senior Biologist, ATCC

Brian Shapiro, Ph.D.
Scientific Content Specialist, ATCC

Credible Leads to Incredible™



About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbes – the “gold standard”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- World leader in cell line and microbe authentication
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

Agenda: Cell line authentication for human and mouse cells

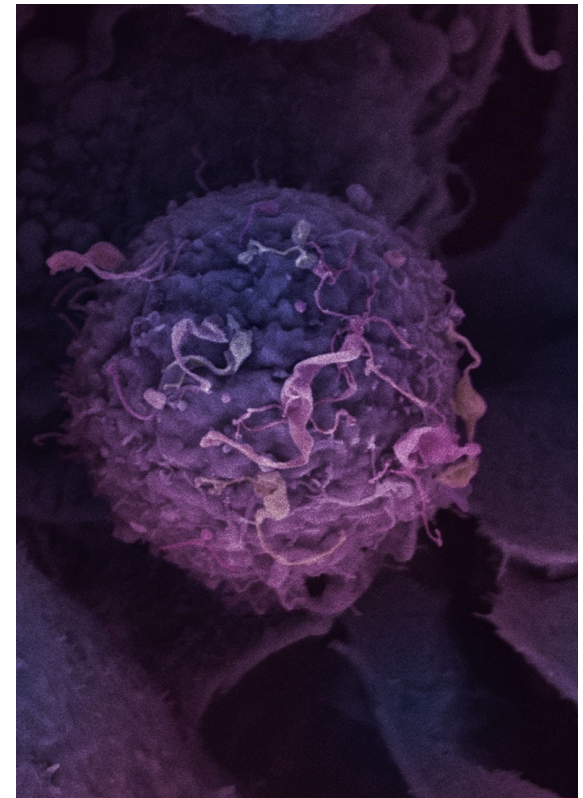
Introduction: Misidentification of cell lines

Cell line authentication

- Short tandem repeat (STR) profiling technique
- Mouse STR profiling

When and why should you authenticate

Summary



Consequences of using misidentified cell lines

- Loss of cell line
- Loss of time and money
- Misinformation in the public domain
- Discordant or irreproducible results
- Publication retraction
- Tarnished reputation

“If we’re not using what we think we’re using, we’re not testing our hypotheses. We’re just gumming up the literature. I’m not sure what we’re doing, but that’s not science.”

Jeffrey Boatright, Emory University, The Big Clean Up, The Scientist Magazine®, September 1, 2015



Misidentification of cell lines in the literature: 1994-2017

| Year | Title of article | Reference |
|------|--|---|
| 1994 | Re-examination of the McCoy cell line for confirmation of its mouse origin | Clin Diagn Virol 2(2):95-103, 1994. |
| 2009 | Genetic profiling reveals cross-contamination and misidentification of 6 adenoid cystic carcinoma cell lines: ACC2, ACC3, ACCM, ACCNS, ACCS and CAC2. | PLoS one 4(6):e6040, 2009. |
| 2010 | Verification and unmasking of widely used human esophageal adenocarcinoma cell lines. | JNCI 102(4):271, 2010. |
| 2013 | A forensic path to RGC-5 cell line identification: lessons learned. | Invest Ophthalmol Vis Sci 54(8):5712-9, 2013. |
| 2014 | SNP Array profiling of mouse cell lines identifies their strains of origin and reveals cross-contamination and widespread aneuploidy | BMC Genomics 15:847, 2014. |
| 2017 | Incorrect strain information for mouse cell lines: sequential influence of misidentification on sublines. | In Vitro Cell Dev Biol Anim 53(3)225-230, 2017. |
| 2018 | A comprehensive analysis of e-CAS cell line reveals they are mouse macrophages | Sci Rep 8(1):8237, 2018 |

Impact of misidentified cell lines on applied research

| Misidentification of frequently used esophageal adenocarcinoma cell lines | | | |
|---|-------------------------------------|---|---|
| Cell Line | Purported | STR confirmed (ATCC STR Profile database) | |
| SEG-1 | Esophageal adenocarcinoma cell line | H460 (ATCC® HTB-177™) | Lung carcinoma (large cell lung cancer) |
| BIC-1 | Esophageal adenocarcinoma cell line | SW620 (ATCC® CCL-227™) | Colorectal adenocarcinoma |
| SK-GT-5 | Esophageal adenocarcinoma cell line | SK-GT-2 | Gastric fundus carcinoma |

Experimental results based on contaminated cell lines...

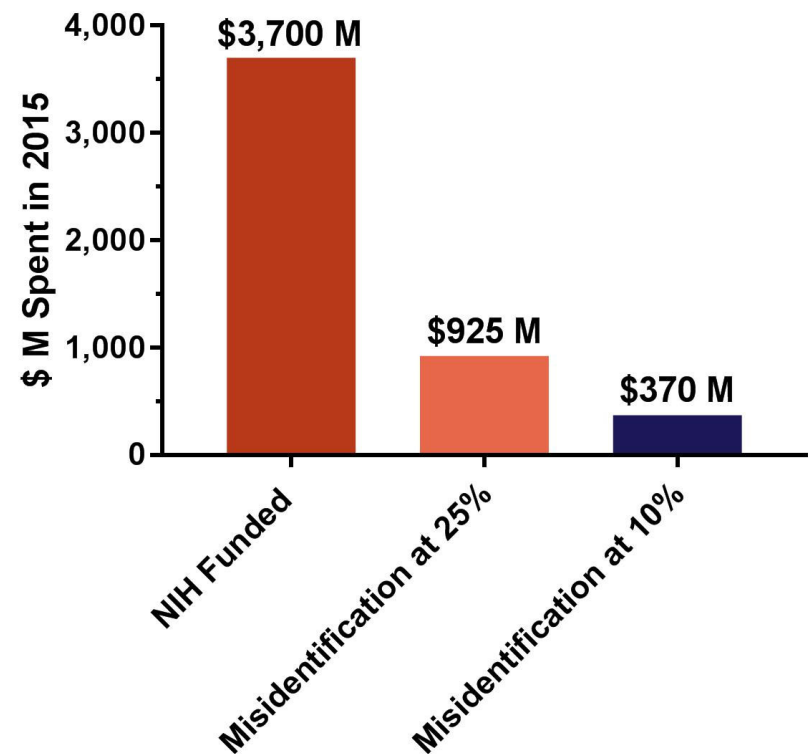
- Clinical trial recruiting EAC patients
- 100 scientific publications
- At least 3 NIH cancer research grants
- 11 US patents

Boonstra, J.J., et al. JNCI.102(4):271, 2010

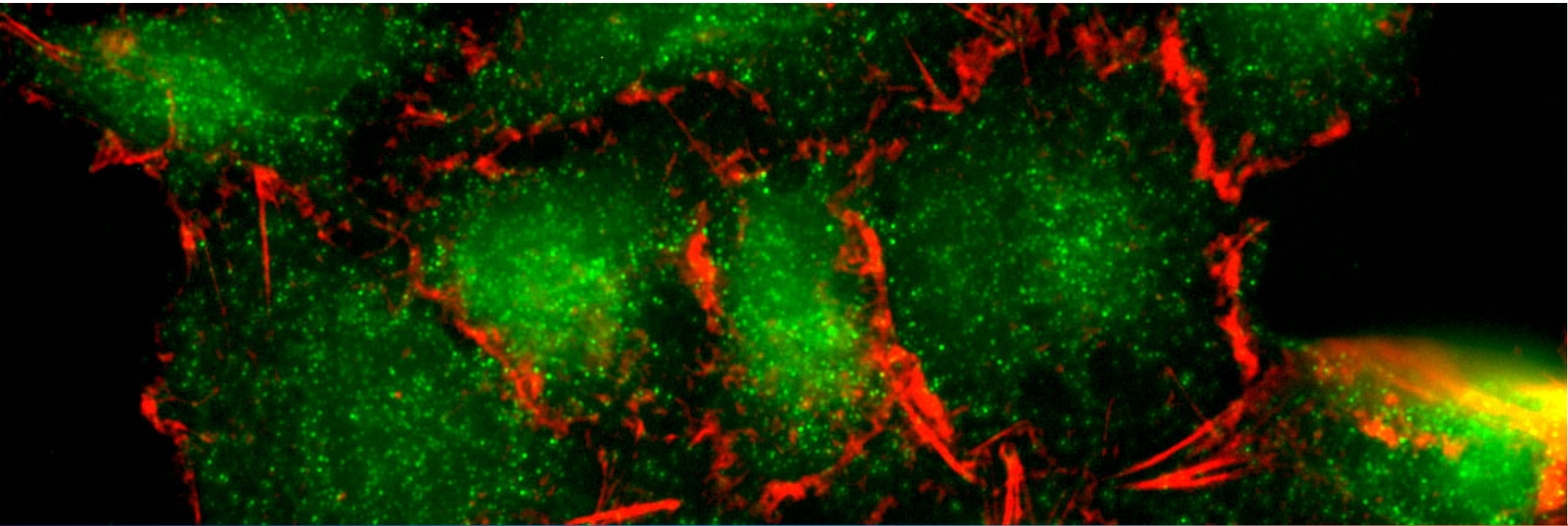
Economic impact of misidentified cell lines

NIH Reporter for projects using “cell line” or “cell culture” – US \$3.7B

- If 25% of research is conducted using misidentified cell lines then the loss could be \$925 M
- If this number could be lowered to 10% then the cost is be reduced to \$370 M



Freeman LP, *The Economics of Reproducibility in Preclinical Research*; PLoS Biology June 9, 2015



Short Tandem Repeat (STR) Profiling

A549 non-small cell lung carcinoma cell line expressing p53

STR – A standard for the authentication of human cell lines

- **ASN-0002 - Authentication of Human Cell Lines: Standardization of STR Profiling**
 - The standard describes a **consistent, inexpensive, and universally applicable method** for authenticating new and established cell lines and their criteria for use
 - *Chair: John R.W. Masters, University College of London*
 - *Co-Chair: Yvonne A. Reid, ATCC (Retired)*
 - **Final action by ANSI: January 25, 2012**
 - **Published date: February 2, 2012**



Barallon, R. et al. *In Vitro Cell Dev Biol Anim* 46: 727, 2010.

STR analysis for cell line identity: characteristics

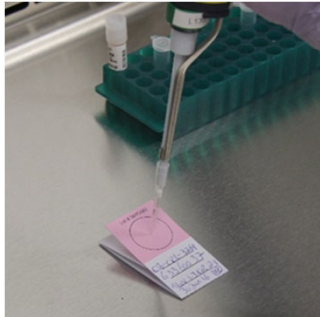
- Target sequence consists of microsatellite DNA (short repeats, 2 – 6 bp, 5 – 50 times)
- Typically use 1-2 ng DNA
- Discrete alleles allow digital record of data
- Markers distributed throughout the genome
- Highly variable within populations; highly informative; high discriminating power
- High observed heterozygosity >70% (more alleles = higher power of discrimination)
- Robust and reproducible results
- Low stutter characteristics
 - 2 bp has high stutter
 - 4 bp has low stutter



Properties of STRs for DNA profiling (Human Loci)

| Locus name | Chromosome location | Repeat motif | No. repeating units |
|---|---------------------------------------|------------------|---------------------|
| D16S539 | 16q24-qtr | GATA | 5-15 |
| D7S820 | 7q11.21-22 | GATA | 6-15 |
| D13S317 | 13q22-q31 | TATC | 5-15 |
| D5S818 | 5p21-q31 | AGAT | 7-16 |
| CSF1PO | 5q33.3-34 | TAGA | 6-16 |
| TPOX | 2p23-pter | GAAT | 6-13 |
| vWA | 12p23-pter | [TCTA] [TCTG] | 10-24 |
| TH01 | 11p15.5 | TCAT | 3-14 |
| Amelogenin | Gender determination (not STR marker) | | |
| Power of discrimination 1 : 1.2 x 10⁸ | | | |
| Retrospective studies on 500 human cell lines – minimum of 8 STR markers required to uniquely identify a human cell line | | | |

Outline of STR profiling procedure



Cells/DNA on
Whatman FTA paper

Multiplex PCR

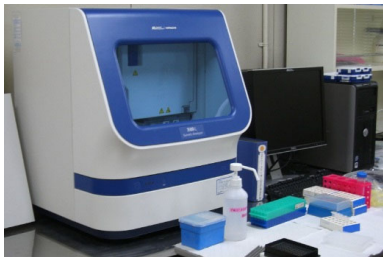
- Amplification of STR loci
- Simultaneous fluorescent labeling

Capillary Electrophoresis (CE)

- Addition of Internal Lane Standard
- CE to separate fragments
- Fluorescent detection
- Run allelic ladder in parallel

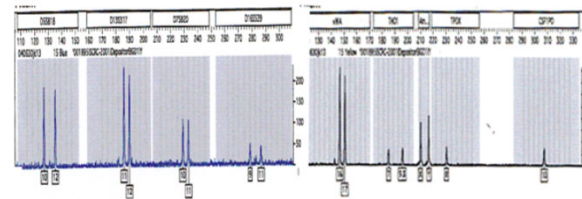
Data Analysis

- Calculate size based on Internal Lane Standard
- Compare fragment sizes to allelic ladders to determine STR alleles
- Compare to databases



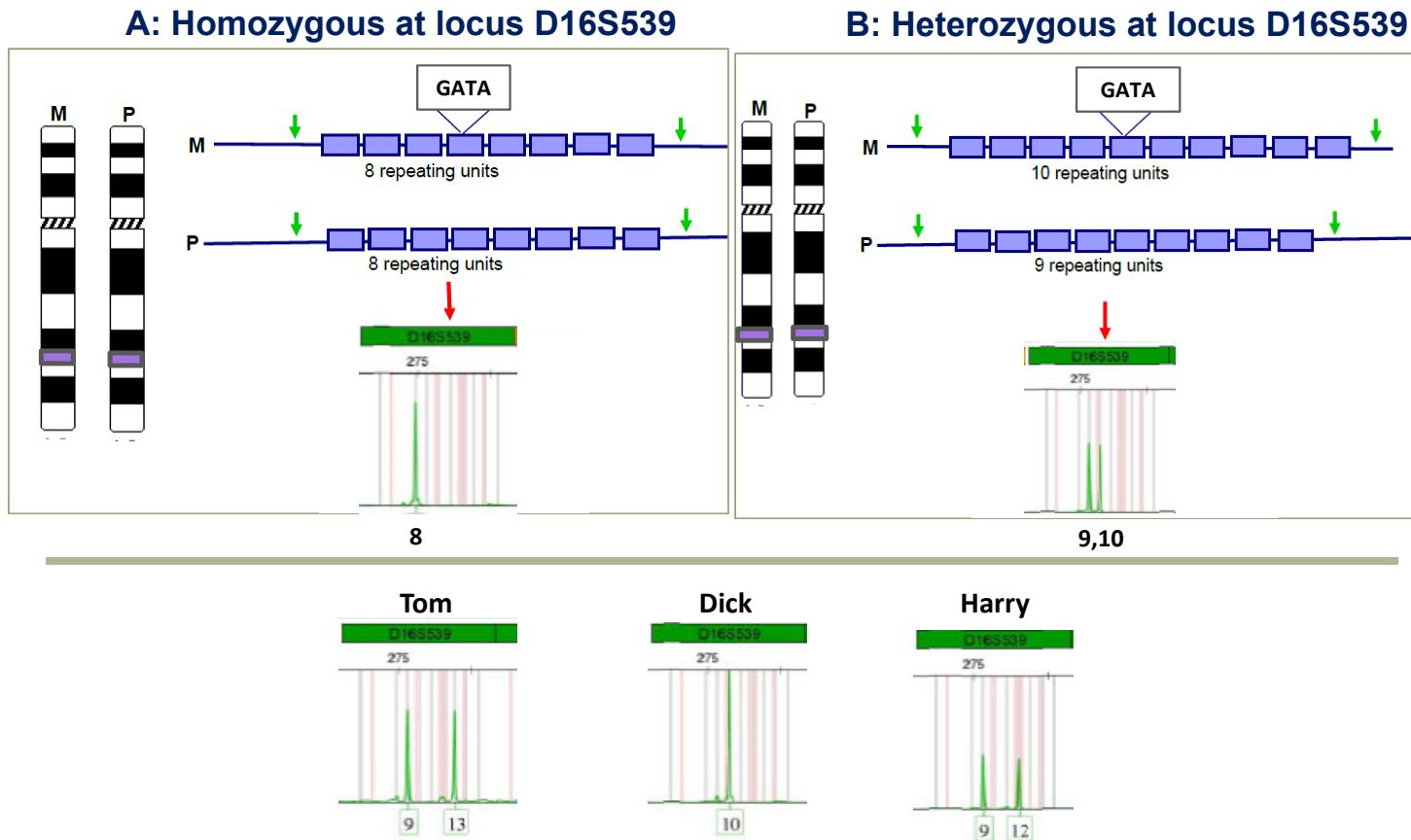
Requirements:

- Gene sequencer
- Thermocycler
- Primer sets
- STR database of cell lines
- Experienced technicians



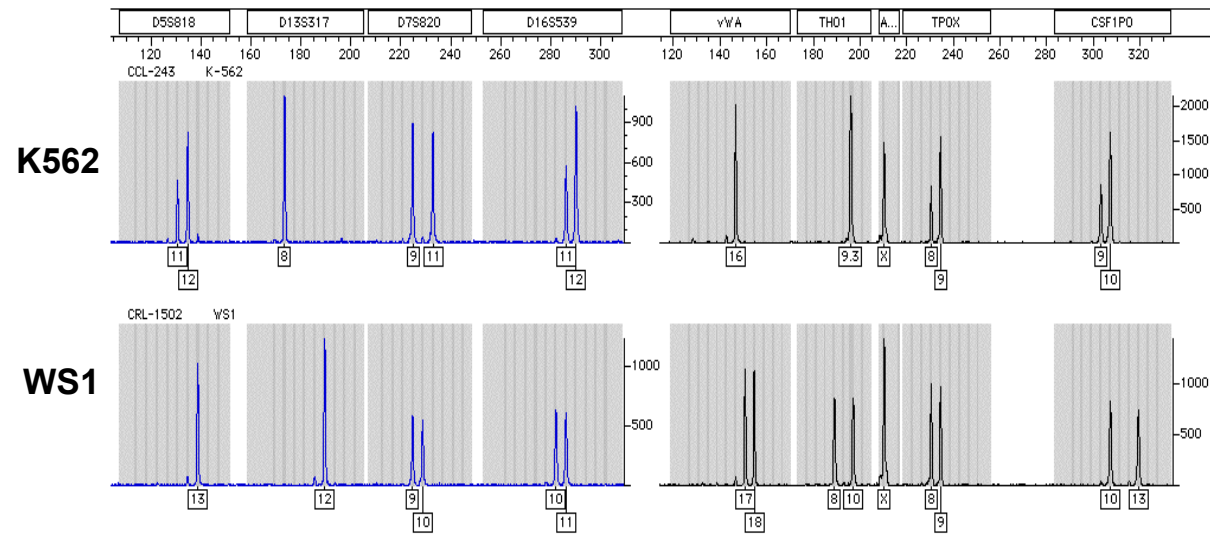
Sample Electropherogram

STR DNA polymorphism



Unique STR DNA profile for each cell line derived from unrelated individuals

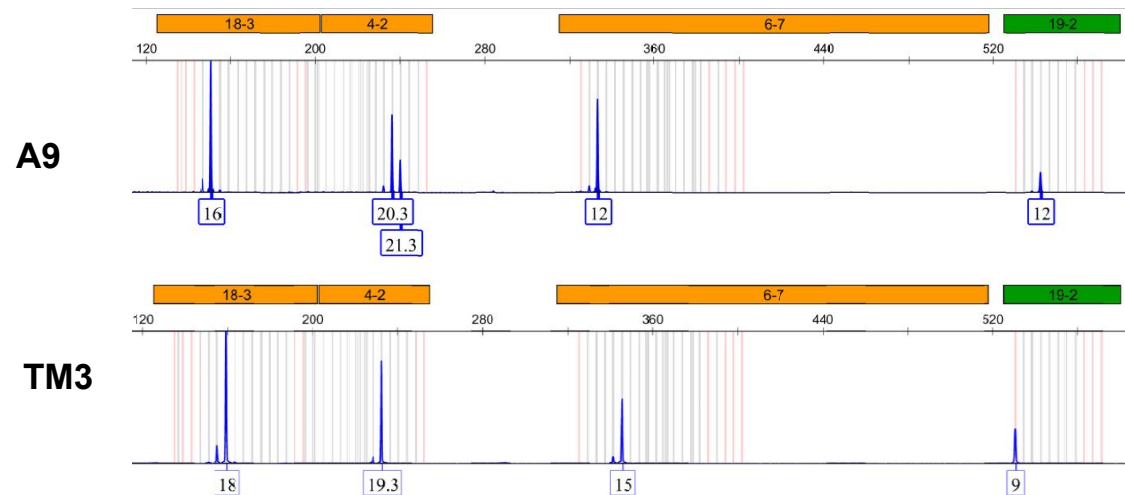
Unrelated human cell lines: STR analysis



| | D5S818 | D13S317 | D7S820 | D16S539 | vWA | TH01 | Amel. | TPOX | CSF1PO |
|-------------|--------|---------|--------|---------|--------|-------|-------|------|--------|
| K562 | 11, 12 | 8 | 9, 11 | 11, 12 | 16 | 9.3 | X | 8, 9 | 9, 10 |
| WS1 | 13 | 12 | 9, 10 | 10, 11 | 17, 18 | 8, 10 | X | 8, 9 | 10, 13 |

2 unrelated cell lines, separate individuals, unique STR DNA profiles

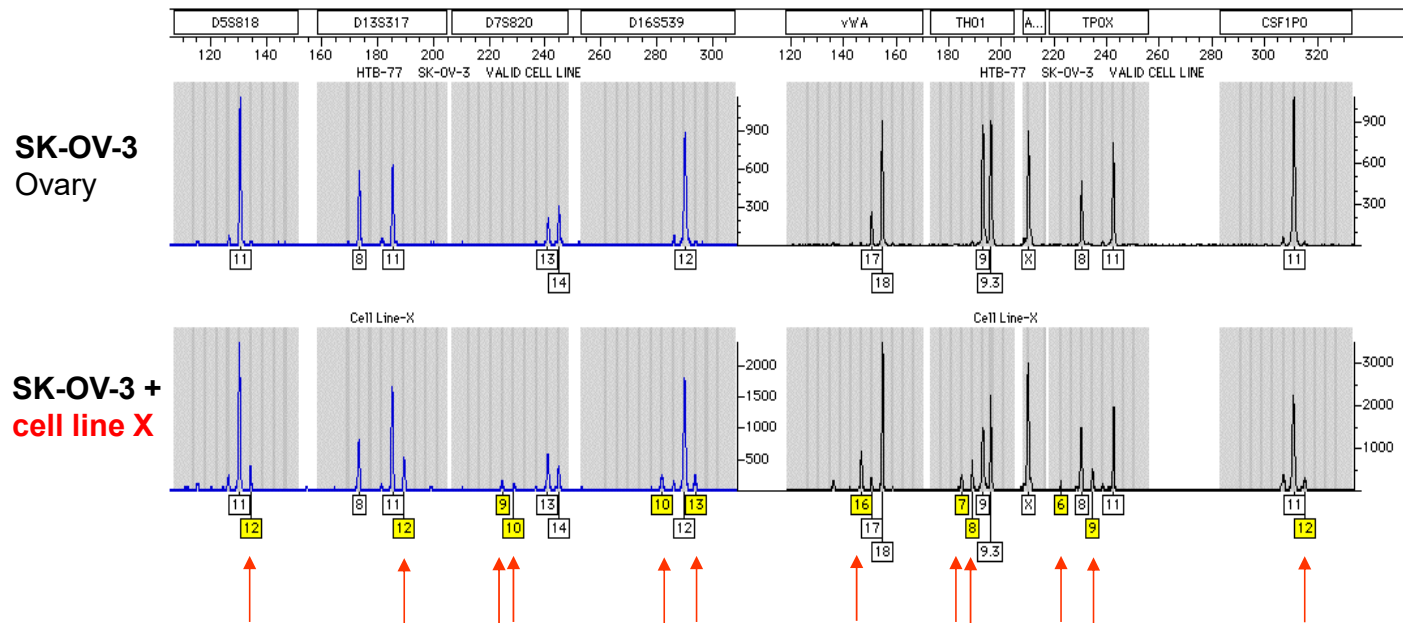
Unrelated mouse cell lines: STR analysis



| | 18-3 | 4-2 | 6-7 | 19-2 |
|-----|------|------------|-----|------|
| A9 | 16 | 20.3, 21.3 | 12 | 12 |
| TM3 | 18 | 19.3 | 15 | 9 |

2 unrelated cell lines, separate individuals, unique STR DNA profiles

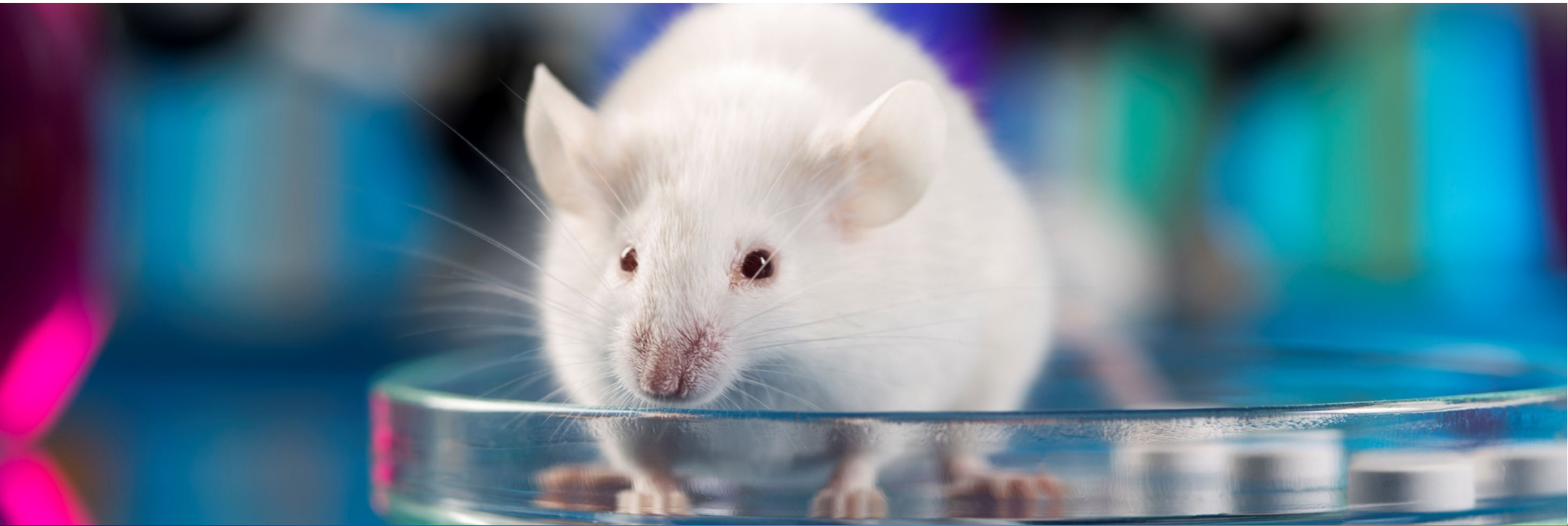
Cellular cross-contamination



ATCC cell line authentication services

| | ATCC® No. | Designation | Where to order |
|-------------------|-----------|--|--|
| Human | 135-XV™ | FTA Sample Collection Kit for Human Cell Authentication Service | www.atcc.org/humanSTR |
| Mycoplasma | 136-XV™ | FTA Sample Collection Kit for PCR-based Mycoplasma Detection Service | www.atcc.org/mycotesting |
| Mouse | 137-XV™ | FTA Sample Collection Kit for Mouse Cell Authentication Service | www.atcc.org/mouseSTR |





Mouse STR Profiling

Mouse STR Consortium

October 2016 12 labs joined the Mouse Cell Line Authentication Consortium

Goal: Validate an STR multiplex PCR assay to distinguish Mouse cell lines

NIST distributed testing kits containing:

- DNA from 50 ATCC mouse cell lines (blinded)
- Primer Mix
- PCR Master Mix
- Calibrants (allelic ladder)
- Control DNA
- Protocols

RESEARCH ARTICLE

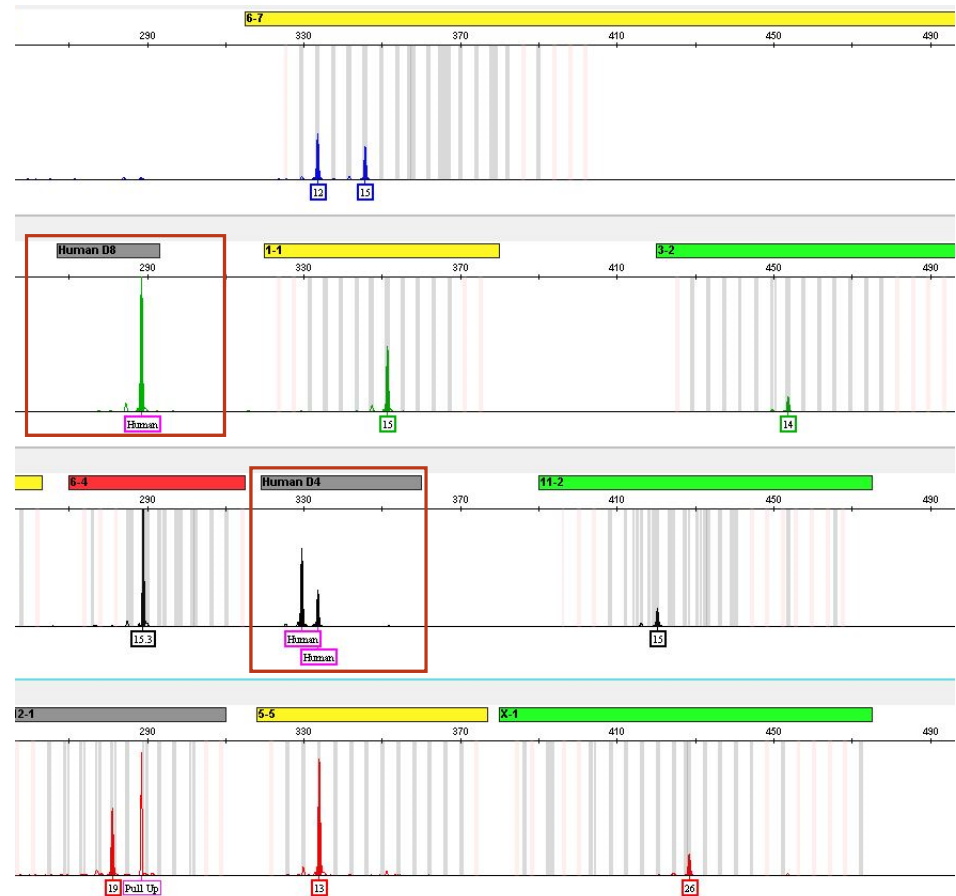
Interlaboratory study to validate a STR profiling method for intraspecies identification of mouse cell lines

Jamie L. Almeida^{1*}, Aleksandra Dakic², Karin Kindig², Maikan Kone², Deborah L. D. Letham³, Scott Langdon⁴, Ruth Peat⁵, Jayamalini Holding-Pillai⁵, Erin M. Hall⁶, Mark Ladd⁶, Megan D. Shaffer⁶, Heath Berg⁷, Jinliang Li⁸, Georges Wigger⁹, Steve Lund¹⁰, Carolyn R. Steffen¹¹, Barbara B. Fransway¹², Bob Geraghty¹³, Manuela Natoli¹³, Beth Bauer¹⁴, Susanne M. Gollin¹⁵, Dale W. Lewis¹⁵, Yvonne Reid¹

¹ National Institute of Standards and Technology, Biosystems and Biomaterials Division, Gaithersburg, Maryland, United States of America, ² ATCC, Standards Resource Center, Manassas, Virginia, United States of America, ³ Charles River Laboratories, Malvern, Pennsylvania, United States of America, ⁴ Duke University, Durham, North Carolina, United States of America, ⁵ The Francis Crick Institute, Cell Services, London, United Kingdom, ⁶ Genetica Cell Line Testing –a LabCorp brand, Burlington, North Carolina, United States of America, ⁷ IDEXX BioAnalytics, Columbia, Missouri, United States of America, ⁸ Laragen Inc., Culver City, California, United States of America, ⁹ Microsynth AG, Balgach, Switzerland, ¹⁰ National Institute of Standards and Technology, Statistical Engineering Division, Gaithersburg, Maryland, United States of America, ¹¹ National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, Maryland, United States of America, ¹² University of Arizona, Genetics Core Lab, University of Arizona Genetics Core, Tucson, Arizona, United States of America, ¹³ University of Cambridge, Cancer Research UK, Cambridge Institute, Cambridge, United Kingdom, ¹⁴ University of Maryland, College Park, Maryland, ¹⁵ University of Pittsburgh, Department of Human Genetics, Pittsburgh, Pennsylvania, United States of America

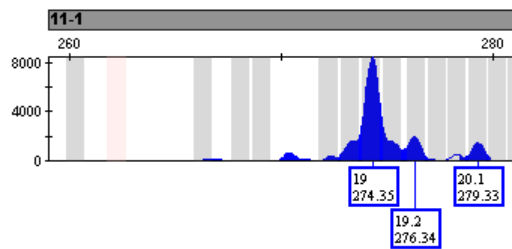
Mouse STR Consortium: the assay

- Primers designed to *Mus musculus musculus* (NCBI build 38.1)
- 18 Mouse STR loci & 2 Human loci for contamination detection
- New STR assay builds on NIST's 9 marker assay published in 2014
 - *Mouse cell line authentication; J.Almeida Cytotechnology* 2014 Jan; 66(1): 133 - 147



Mouse STR Consortium: the results

- 1) Unique STR profiles obtained for each mouse cell line tested
- 2) 42 Validated Mouse STR profiles uploaded to NCBI Biosample ($\geq 98\%$ concordance)
- 3) STR Locus 11-1 removed due to abnormal peak morphology



NCBI Resources How To

BioSample

Advanced

Full

WEHI-3, mouse cell line STR profile from ATCC

Identifiers BioSample: SAMN11397664; Sample name: WEHI-3

Organism [Mus musculus \(house mouse\)](#)
cellular organisms; Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Dipnotetrapodomorpha; Tetrapoda; Amniota; Mammalia; Theria; Eutheria; Boreoeutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Package [Model organism or animal: version 1.0](#)

Attributes

| | |
|------------------------------------|---|
| cell line | WEHI-3 |
| culture collection | ATCC: TIB-68 |
| strain | BALB/c |
| age | unknown |
| sex | unknown |
| morphology | lymphocyte-like |
| tissue | peripheral blood |
| repository | American Type Culture Collection (ATCC) |
| disease | Leukemia |
| cell line name alias | WEHI 3; WEHI3; Wehi-3 (Cellosaurus) |
| date established | unknown |
| about cells | suspension, some adherent |
| mouse cell line STR profile | yes |
| mouse cell line STR profile status | NIST verified |

Links [American Type Culture Collection](#)
[ATCC: TIB-68](#)

BioProject [PRJNA539973 Mus musculus](#)
Retrieve all samples from this project

Submission [NIST, Jamie Almeida; 2019-04-11](#)

STR profile

| | |
|----------|----------------|
| STR 18-3 | 18 |
| STR 4-2 | 21.3 |
| STR 6-7 | 12,13,14 |
| STR 19-2 | 12,13 |
| STR 1-2 | 17 |
| STR 7-1 | 25.2,26.2,27.2 |
| STR 1-1 | 15,16,18 |
| STR 3-2 | 14,15 |
| STR 8-1 | 13,14 |
| STR 2-1 | 16 |
| STR 15-3 | 22.3,23.3,25.5 |
| STR 6-4 | 18,19,20,21 |
| STR 11-2 | 18,19 |
| STR 17-2 | 16 |
| STR 12-1 | 16,17 |
| STR 5-5 | 14 |
| STR X-1 | 26,27 |
| STR 13-1 | 15.2,16.2 |

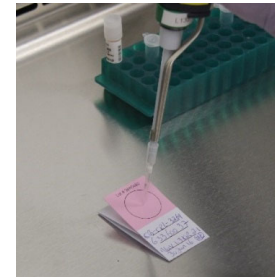


Mouse STR at ATCC

This technology has been licensed to ATCC for commercialization – available NOW!

Methodology

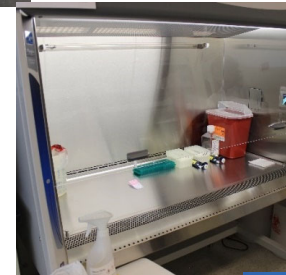
- Samples spotted and shipped on Whatman FTA™ Cards
- Profiles compared to ATCC Mouse STR Database
- Results emailed with three to five business days



Data interpretation

- According to NIST granted US Patent (9,556,482) and 2019 consortium publication
- Database matching follows the Tanabe matching algorithm

$$\% \text{ match} = \frac{2 (\text{number of alleles matching})}{(\# \text{ of query alleles} + \# \text{ of reference alleles})}$$



Mouse STR at ATCC

1 Online Order www.atcc.org/mousestr

The screenshot shows the ATCC website's Mouse STR service page. At the top, there is a navigation bar with links for Products, Services, Standards, Resources, Support, and About. A search bar is located on the right side of the page. The main content area features a large image of a white mouse with the text "MOUSE SHORT TANDEM REPEAT PROFILING" overlaid. Below the image, there is a paragraph of text explaining the service and its benefits. Three buttons are provided: "Order Mouse STR Profiling Service", "Download Sample Submission Form", and "Download Sample STR Profile Report". A section titled "Have confidence in the identity of your cells, and get the data you need in 3 Easy Steps!" follows, with three numbered steps: 1. Place your order for the service, 2. Spot cells onto supplied Sample Collection Card, and 3. Mail dried card to ATCC in pre-addressed envelope. A list of related resources is on the right, including Culture Guides, FAQs, Webinars, Videos, Testimonials, Related Pages, Articles, Infographics, Brochures, and Technical Bulletins. A separate box for "MYCOPLASMA TESTING SERVICE" is also visible.

ATCC | **Credible leads to Incredible™**

Login | Create a Profile | Quick Order | 🛒 (0 items) | USA ▼ | 1.855.912.2611

Search by Keyword or Catalog 🔍

Products Services Standards Resources Support About

MOUSE SHORT TANDEM REPEAT PROFILING

Misidentified and contaminated cell lines undermine your experimental results and discredit preclinical studies. Until now there has been no credible method of authenticating mouse cell lines. To address this need, ATCC has teamed up with an [independent consortium](#) of NIST and 10 independent laboratories to develop a mouse STR profiling service. This service aids in the detection of misidentified, cross-contaminated, or genetically drifted cells, which invalidate research results. ATCC mouse STR analysis meets all requirements for NIH funding, journal publication, and quality control for FDA submissions. Order STR profiling and trust your experimental results.

[Order Mouse STR Profiling Service](#) [Download Sample Submission Form](#) [Download Sample STR Profile Report](#)

Have confidence in the identity of your cells, and get the data you need in 3 Easy Steps!

- 1** Place your order for the service
- 2** Spot cells onto supplied Sample Collection Card
- 3** Mail dried card to ATCC in pre-addressed envelope

1. [Place your order](#).

2. Spot cells onto the Sample Collection Card that you received from the mailer packet in the mail.

3. Mail the dried card back to us in the pre-addressed envelope. (View [Sample Instructions](#))

Results are emailed to you in a PDF format. ([Sample Generic Report](#)).

CELL AUTHENTICATION RESOURCES

- Culture Guides
- FAQs
- Webinars
- Videos
- Testimonials
- Related Pages
- Articles
- Infographics
- Brochures
- Technical Bulletins

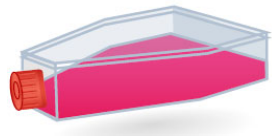
MYCOPLASMA TESTING SERVICE

From the leaders of cell authentication
[Learn More ▶](#)

Mouse STR at ATCC

2 Spot & Ship

Mix & Spot cells or DNA



Cells

1×10^6 cells/ml

OR



DNA

20 ng/ μ l

Sample Submission Form

Thank you for placing an order for the Mouse STR Testing Service. Please read this form in its entirety and follow all steps accurately.



Peace of mind in 3 easy steps

- 1 The Mouse STR Testing Results will be emailed by ATCC to the email address provided below. Customer Information (All fields are required. Please print information to ensure it is legible)

Name _____

Institution/Company _____

Address _____

City _____ State _____ Zip code _____ Country _____

Email

(Please print clearly; authentication results will be sent to the email address above)

- 2 Prepare your sample according to the Sample Preparation Instructions found on the back of this form. Enter the cell line information and sign the hazard statement below (REQUIRED).

Mouse Cell Line Information

Please verify that the barcode number on your sample card matches the barcode number on this form for each sample.

Mouse Cell Line Name/Designation _____ Catalog/Item # (If any) _____

Hazard Statement

ATCC does not accept cultures infected with HIV or Biosafety Level 3 or 4 agents for STR testing. To the best of my knowledge the cell line being submitted is free of hazardous materials, agents, and carcinogens.

Print Name _____

Signature _____ Date _____

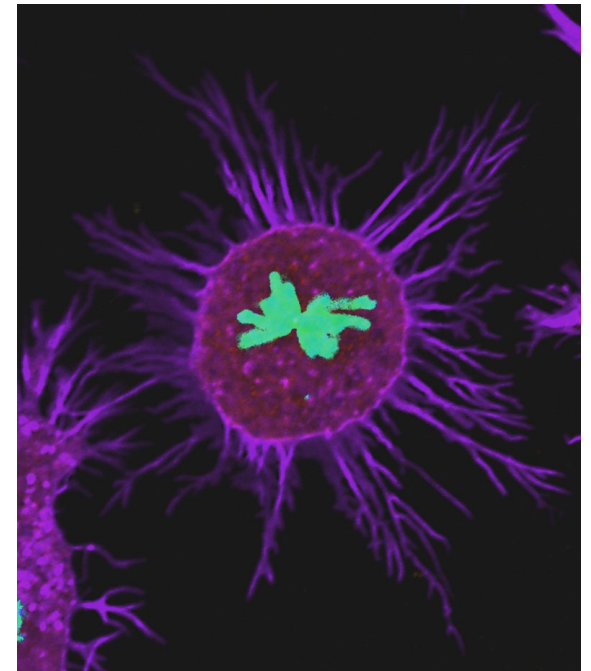
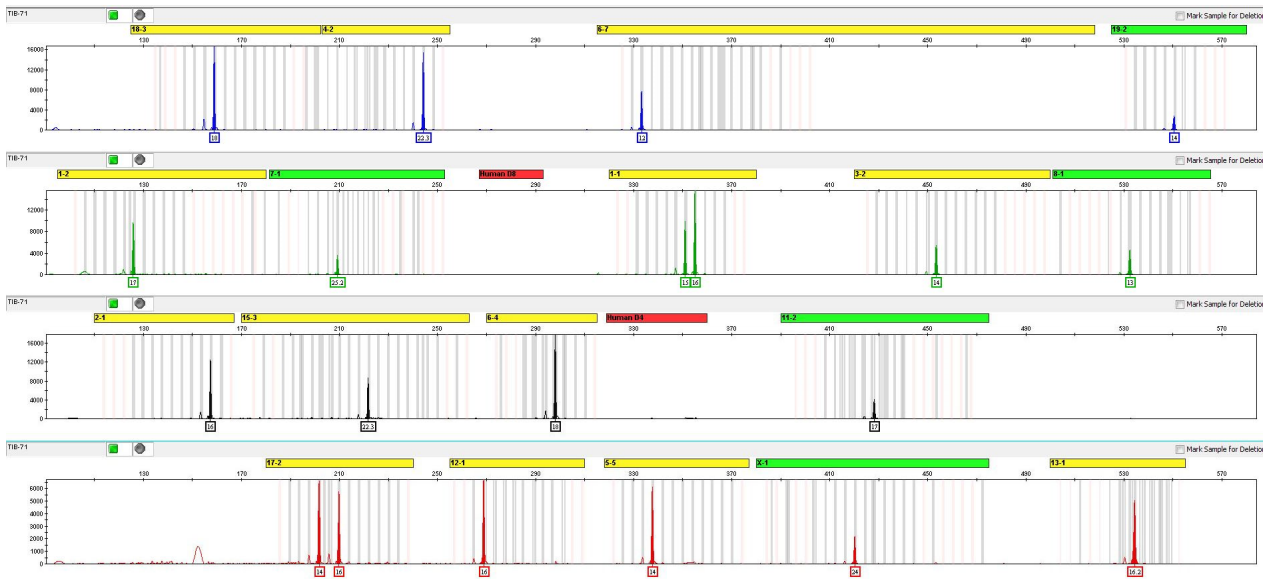
- 3 Mail your sample and this completed form in the pre-addressed Return Envelope included within the kit.



Mouse STR at ATCC

3 In-Lab Processing & Data Analysis

RAW 264.7 (ATCC® TIB-71™)



RAW 264.7 cells
Image courtesy of Donna Stolz, University of Pittsburgh

Mouse STR at ATCC

4 Mouse STR Report

Report e-mailed within 3 to 5 business days

 **Cell Line Authentication Service**
Mouse STR Profile Report

FTA Barcode: MU SA 0001
ATCC Sales Order: S00004536

| Test Results for Submitted Sample | | | | ATCC Reference Database Profile | | | |
|--|--------------------------|----|--|--|----|--|--|
| Locus | Query Profile: RAW 264.7 | | | Database Profile: TIB-71 RAW 264.7; Mouse (Mus musculus) | | | |
| 18-3 | 18 | | | 18 | | | |
| 4-2 | 22.3 | | | 22.3 | | | |
| 6-7 | 12 | | | 12 | | | |
| 19-2 | 14 | | | 14 | | | |
| 1-2 | 17 | | | 17 | | | |
| 7-1 | 25.2 | | | 25.2 | | | |
| 1-1 | 15 | 16 | | 15 | 16 | | |
| 3-2 | 14 | | | 14 | | | |
| 8-1 | 13 | | | 13 | | | |
| 2-1 | 16 | | | 16 | | | |
| 15-3 | 22.3 | | | 22.3 | | | |
| 6-4 | 18 | | | 18 | | | |
| 11-2 | 17 | | | 17 | | | |
| 17-2 | 14 | 16 | | 14 | 16 | | |
| 12-1 | 16 | | | 16 | | | |
| 5-5 | 14 | | | 14 | | | |
| X-1 | 24 | | | 24 | | | |
| 13-1 | 16.2 | | | 16.2 | | | |
| Number of shared alleles between query sample and database profile: | | | | 20 | | | |
| Total number of alleles in the query sample profile: | | | | 20 | | | |
| Total number of alleles in the database profile: | | | | 20 | | | |
| Percent match between the submitted sample and the database profile: | | | | 100 | | | |

Explanation of Test Results:
Cell lines with 80% match are considered to be related, i.e., derived from a common ancestry. Cell lines with a percent match between a 55 - 80% require further investigation for authentication of relatedness.

The submitted sample profile is an exact match for the following ATCC cell line(s) in the ATCC mouse STR database: **TIB-71**

The submitted sample profile is mouse, however a matching reference profile has not previously been established in the ATCC mouse STR database.

The submitted profile is similar to the following ATCC cell line(s):

An STR profile could not be generated from the submitted sample.

Human and/or African Green Monkey Species Detection

Human and/or African green monkey has been detected in the submitted sample profile (see attached electropherogram at Human D8 & D4 loci).

Additional Comments:
The submitted sample profile, RAW 264.7, is an exact match to the ATCC cell line, TIB-71.

FTA Barcode: MU SA 0001

ATCC Sales Order: S00004536

| Test Results for Submitted Sample | | | | ATCC Reference Database Profile | | | |
|--|--------------------------|----|--|--|----|--|--|
| Locus | Query Profile: RAW 264.7 | | | Database Profile: TIB-71 RAW 264.7; Mouse (Mus musculus) | | | |
| 18-3 | 18 | | | 18 | | | |
| 4-2 | 22.3 | | | 22.3 | | | |
| 6-7 | 12 | | | 12 | | | |
| 19-2 | 14 | | | 14 | | | |
| 1-2 | 17 | | | 17 | | | |
| 7-1 | 25.2 | | | 25.2 | | | |
| 1-1 | 15 | 16 | | 15 | 16 | | |
| 3-2 | 14 | | | 14 | | | |
| 8-1 | 13 | | | 13 | | | |
| 2-1 | 16 | | | 16 | | | |
| 15-3 | 22.3 | | | 22.3 | | | |
| 6-4 | 18 | | | 18 | | | |
| 11-2 | 17 | | | 17 | | | |
| 17-2 | 14 | 16 | | 14 | 16 | | |
| 12-1 | 16 | | | 16 | | | |
| 5-5 | 14 | | | 14 | | | |
| X-1 | 24 | | | 24 | | | |
| 13-1 | 16.2 | | | 16.2 | | | |
| Number of shared alleles between query sample and database profile: | | | | 20 | | | |
| Total number of alleles in the query sample profile: | | | | 20 | | | |
| Total number of alleles in the database profile: | | | | 20 | | | |
| Percent match between the submitted sample and the database profile: | | | | 100 | | | |

Mouse STR at ATCC

4 Mouse STR Report

Report e-mailed within 3 to 5 business days

ATCC Cell Line Authentication Service
Mouse STR Profile Report

FTA Barcode: MU SA 0001
ATCC Sales Order: SO0004536

| Test Results for Submitted Sample | | | | ATCC Reference Database Profile | | | |
|--|--------------------------|----|--|---|----|--|--|
| Locus | Query Profile: RAW 264.7 | | | Database Profile: TIB-71 RAW 264.7; Mouse (<i>Mus musculus</i>) | | | |
| 18-3 | 18 | | | 18 | | | |
| 4-2 | 22.3 | | | 22.3 | | | |
| 6-7 | 12 | | | 12 | | | |
| 19-2 | 14 | | | 14 | | | |
| 1-2 | 17 | | | 17 | | | |
| 7-1 | 25.2 | | | 25.2 | | | |
| 1-1 | 15 | 16 | | 15 | 16 | | |
| 3-2 | 14 | | | 14 | | | |
| 8-1 | 13 | | | 13 | | | |
| 2-1 | 16 | | | 16 | | | |
| 15-3 | 22.3 | | | 22.3 | | | |
| 6-4 | 18 | | | 18 | | | |
| 11-2 | 17 | | | 17 | | | |
| 17-2 | 14 | 16 | | 14 | 16 | | |
| 12-1 | 16 | | | 16 | | | |
| 5-5 | 14 | | | 14 | | | |
| X-1 | 24 | | | 24 | | | |
| 13-1 | 16.2 | | | 16.2 | | | |
| Number of shared alleles between query sample and database profile: | | | | 20 | | | |
| Total number of alleles in the query sample profile: | | | | 20 | | | |
| Total number of alleles in the database profile: | | | | 20 | | | |
| Percent match between the submitted sample and the database profile: | | | | 100 | | | |

Explanation of Test Results
Cell lines with 80% match are considered to be related, i.e., derived from a common ancestry. Cell lines with a percent match between a 55 - 80% require further investigation for authentication of relatedness.

- The submitted sample profile is an exact match for the following ATCC cell line(s) in the ATCC mouse STR database: **TIB-71**
 - The submitted sample profile is mouse, however a matching reference profile has not previously been established in the ATCC mouse STR database.
 - The submitted profile is similar to the following ATCC cell line(s):
 - An STR profile could not be generated from the submitted sample.
- Human and/or African Green Monkey Species Detection**
- Human and/or African green monkey has been detected in the submitted sample profile (see attached electropherogram at Human D8 & D4 loci).

Additional Comments:

The submitted sample profile, RAW 264.7, is an exact match to the ATCC cell line, TIB-71.

The submitted sample profile is an exact match for the following ATCC cell line(s) in the ATCC mouse STR database: **TIB-71**

The submitted sample profile is mouse, however a matching reference profile has not previously been established in the ATCC mouse STR database.

The submitted profile is similar to the following ATCC cell line(s):

An STR profile could not be generated from the submitted sample.

Human and/or African Green Monkey Species Detection

Human and/or African green monkey has been detected in the submitted sample profile (see attached electropherogram at Human D8 & D4 loci).

Mouse STR at ATCC

3 Mouse STR Report

Addendum: Comparative Output from ATCC Mouse STR Database



Cell Line Authentication Service Mouse STR Profile Report

Addendum: Comparative Output from the ATCC Mouse STR Profile Database

FTA Barcode: MUSA0001
ATCC Sales Order: SO0004536

| % Match | 100 | 100 |
|----------------|-----------|-----------|
| ATCC® Cat. No. | | TIB-71 |
| Designation | RAW 264.7 | RAW 264.7 |
| 18-3 | 18 | 18 |
| 4-2 | 22,3 | 22,3 |
| 6-7 | 12 | 12 |
| 19-2 | 14 | 14 |
| 1-2 | 17 | 17 |
| 7-1 | 25,2 | 25,2 |
| 1-1 | 15,16 | 15,16 |
| 3-2 | 14 | 14 |
| 8-1 | 13 | 13 |
| 2-1 | 16 | 16 |
| 15-3 | 22,3 | 22,3 |
| 6-4 | 18 | 18 |
| 11-2 | 17 | 17 |
| 17-2 | 14,16 | 14,16 |
| 12-1 | 16 | 16 |
| 5-5 | 14 | 14 |
| X-1 | 24 | 24 |
| 13-1 | 16,2 | 16,2 |

Definitions of terms used in this report:

Artifact:
A non-allelic product of the amplification process, an anomaly of the detection process, or a by-product of primer synthesis.

Pull-up:
A term used to describe when signal from one dye color channel produces artificial peaks in another, usually adjacent, color.

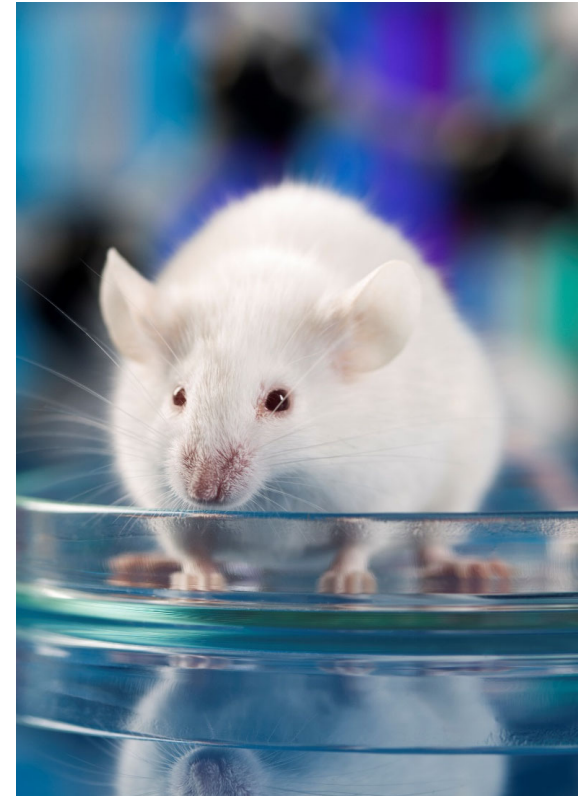
Spike:
An extraneous peak resulting from dust, dried polymer, an air bubble, or an electrical surge.

Dye blob:
Free dye not coupled to primer that can be injected into the capillary.



Mouse STR at ATCC – key points

- ATCC worked with NIST to pioneer STR profiling for mouse cell lines (*Available **NOW***)
- ATCC authentication services are simple and inexpensive, after placing your order:
 - Spot
 - Dry
 - Mail
 - Receive your results in three to five days
- **Report includes:**
 - Submitted & Matched Allele Calls
 - Contamination check
 - Comparative output for database comparison
 - PDF of the submitted sample profile



Why authenticate? Journals now require cell authentication

| Publisher and/or Journal Title | Required | Encouraged |
|---|-----------------|-------------------|
| American Association for Cancer Research journals (8) | | X |
| BioMed Central journals (200+) | | X |
| Endocrine Society journals (5) | X | |
| <i>Nature</i> journals (approximately 150) | X | |
| Society for Endocrinology journals (3) | X | |
| <i>BioTechniques</i> | | X |
| <i>Carcinogenesis</i> | X | |
| <i>Cell Biochemistry and Biophysics</i> | X | |
| <i>Cell Biology International</i> | X | |
| <i>International Journal of Cancer</i> | X | |
| <i>Investigative Ophthalmology & Visual Science</i> | | X |
| <i>In Vitro Cellular & Developmental Biology—Animal</i> | X | |
| <i>Journal of Molecular Biology</i> | | X |
| <i>Journal of the National Cancer Institute</i> | X | |
| <i>Molecular Vision</i> | X | |
| <i>Neuro-Oncology</i> | | X |
| PLOS ONE | | X |

Fusenig NE, The need for a worldwide consensus for cell line authentication: Experience implementing a mandatory requirement at the International Journal of Cancer. 2017. <https://doi.org/10.1371/journal.pbio.2001438>

Why authenticate? Agencies require cell authentication

NIH revised guidelines to applications for funding - Enhancing Reproducibility through Rigor and Transparency (effective Jan. 25, 2016)

- Authentication of key biological and/or chemical resources
 - NIH expects that key biological and/or chemical resources will be regularly authenticated to ensure their identity and validity for use in the proposed studies

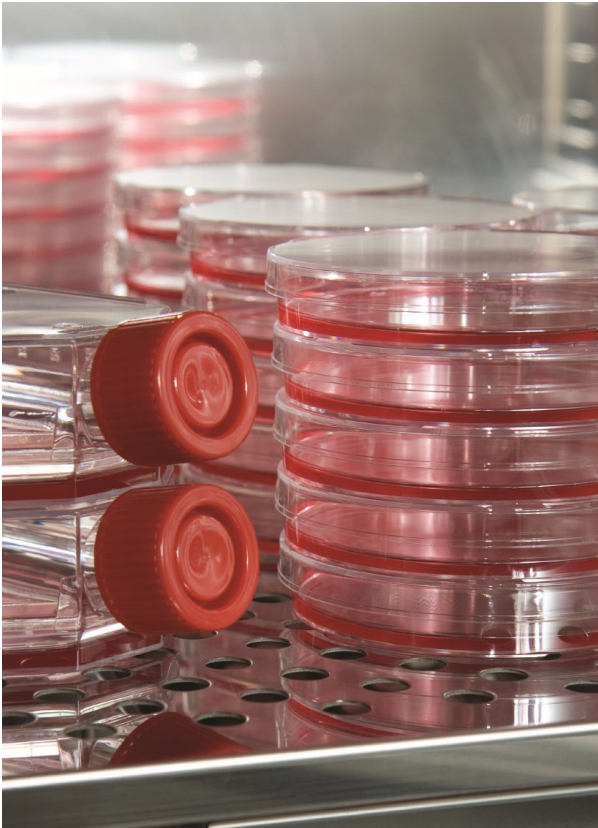
Notice Number: NOT-OD-15-103 (published June 9, 2015): <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html>

FDA Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials

- Provides manufactures of viral vaccines recommendations for the characterization of cell substrates and other biological materials for human use

Docket No: FDA-2006-D-0223; February 2010

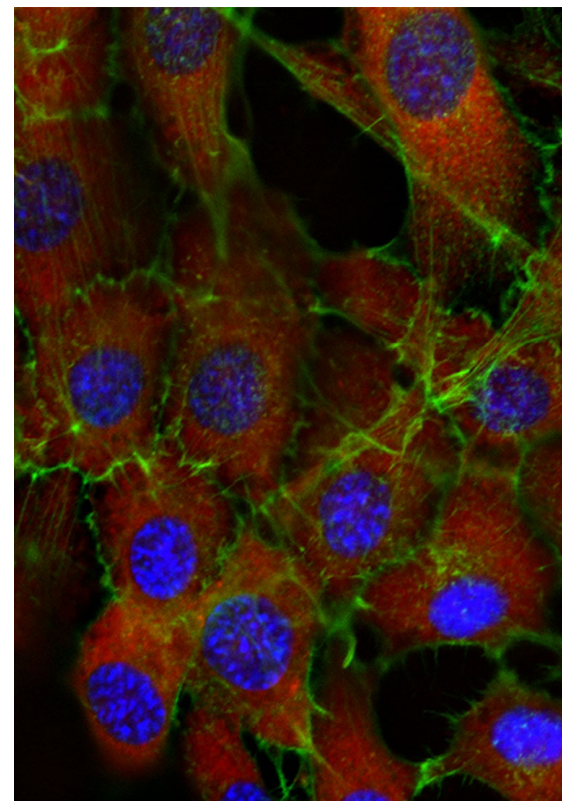
When to authenticate?



- At the beginning and conclusion of a study
 - Before submission of grant applications
 - Before manuscript submissions
- After preparing a cell bank
- At regular intervals throughout study
- When in doubt
 - Novel phenotypic behavior observed

Summary

- Cell line misidentification and contamination bears a high cost
- STR technology offers a powerful means to identify cells
- ATCC has helped pioneer STR profiling for mouse and human cells
- Journals and funding agencies require evidence of authentication
- There is no wrong time to authenticate your cell lines
- www.atcc.org/mouseSTR for more information



NIH 3T3 cells
Image courtesy of Nicole Carrejo, Rice University

Cultivating collaboration to support global health

Go to www.atcc.org/mouseSTR for the ATCC Mouse STR Profiling Service

Upcoming webinars:

- **Simplifying assay development with molecular standards: Remove culturing from the equation**
September 26, 12:00 ET

www.atcc.org/webinars

