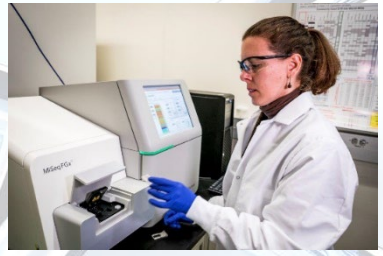
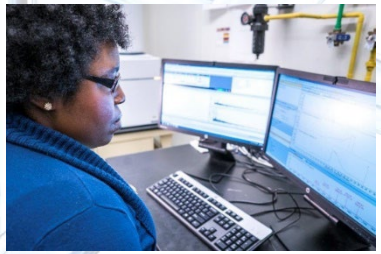




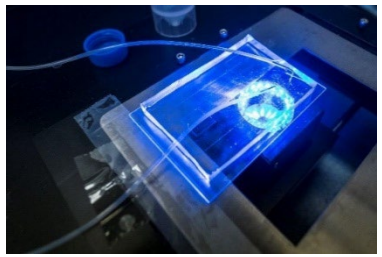
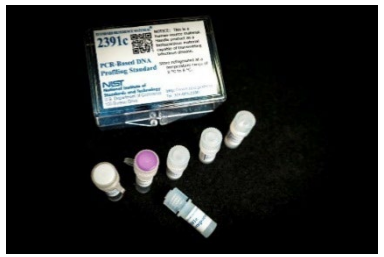

Standards Needed in Mouse Cell Line Authentication

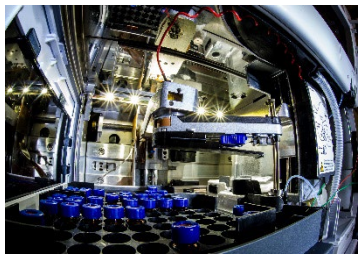
Jamie Almeida

University of Lausanne

January 11, 2024



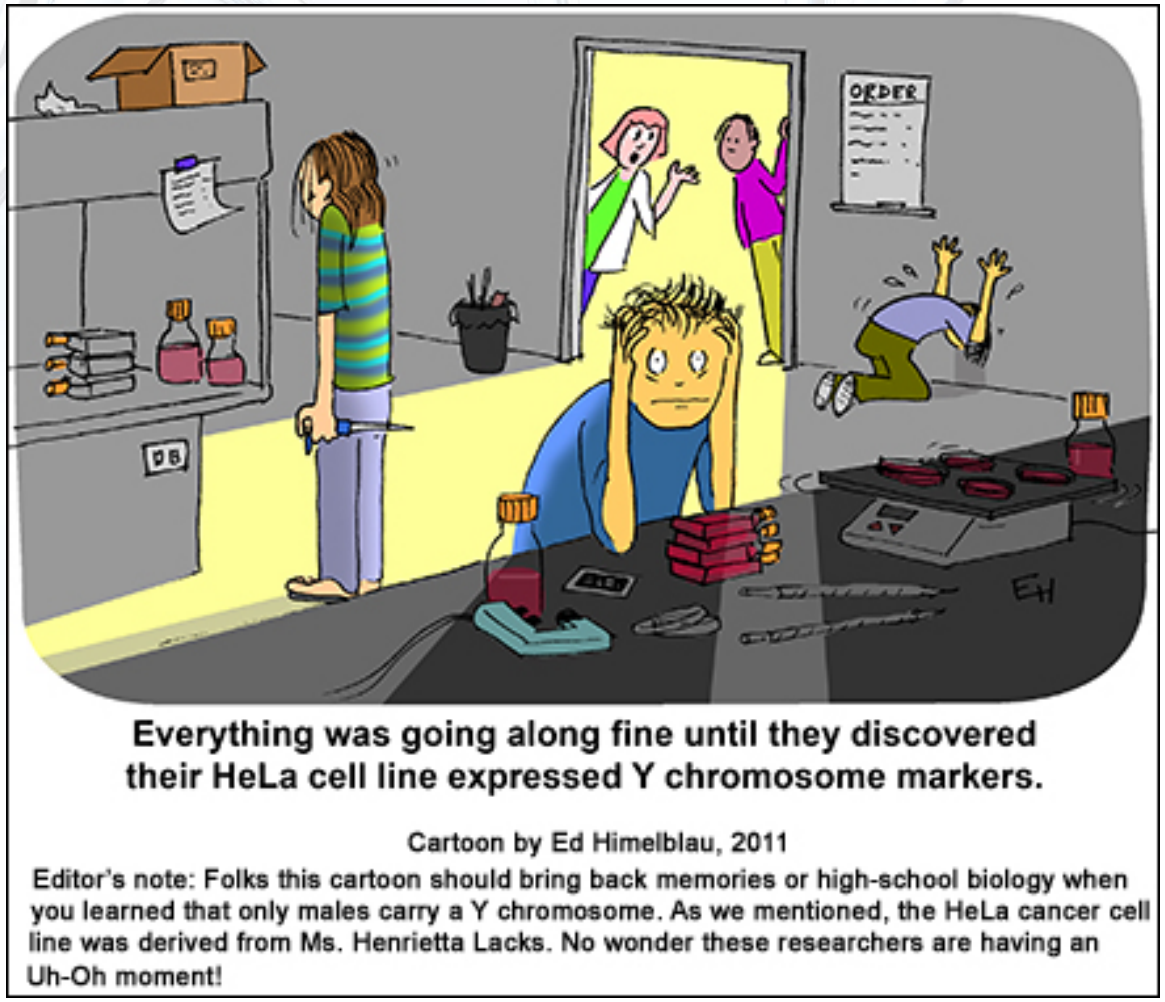


Overview

- Background
  - History of misidentified cell lines and pioneers in the field
  - Introduction to cell line authentication
- Lack of standards in nonhuman cell line authentication
  - Mouse Cell Line Authentication Consortium
- Reference Materials Needed
  - Mouse allelic ladder –what is it?
  - Interlaboratory study results
  - Stability study – in progress
  - Preparation of bulk material
- Needs and gaps

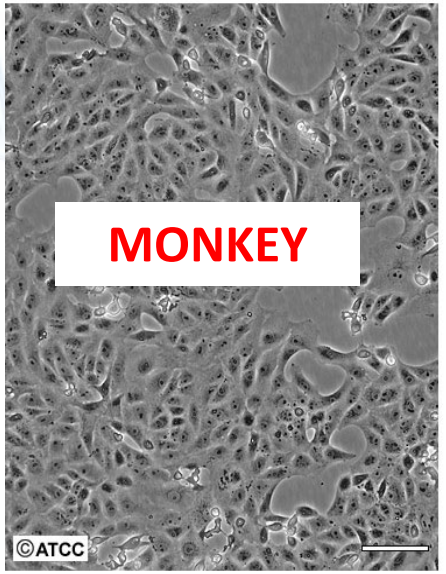
The Problem

Case of mistaken identity



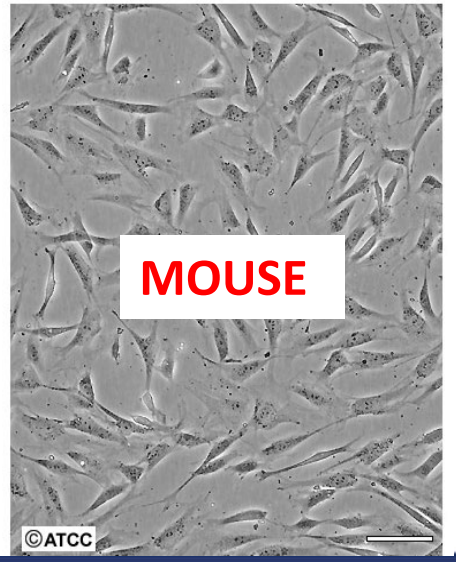
Dunham JH and Guthmiller P. Doing Good Science: Authenticating Cell Line Identity. [Internet] 2012. http:// http://www.promega.com/resources/pubhub/cell-line-authentication-with-strs-2012-update/#ArticleBody-7a4d59f0-8b2d-43c1-9dad-e8d8509275a6

ATCC Number: **CCL-81**
Designation: **Vero**

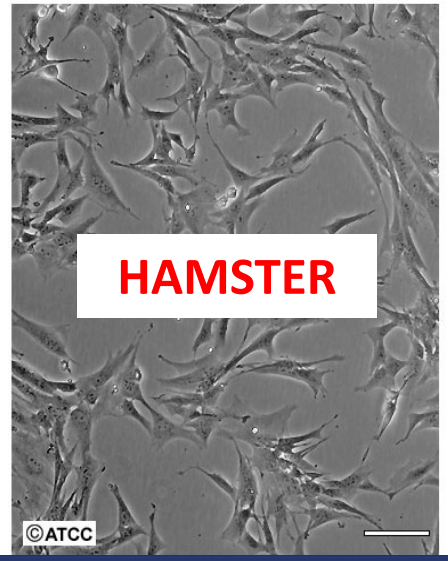


ATCC Number: **CCL-2**
Designation: **HeLa**

ATCC Number: **CRL-1658**
Designation: **NIH/3T3**



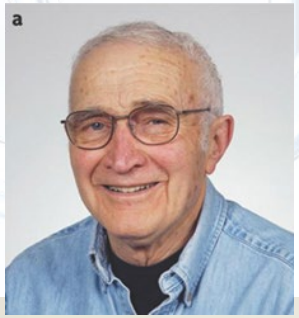
ATCC Number: **CCL-10**
Designation: **BHK-21**



Pioneers in Cell Line Authentication

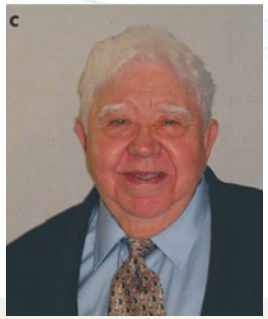
Pioneers of awareness

Stanley Gartler

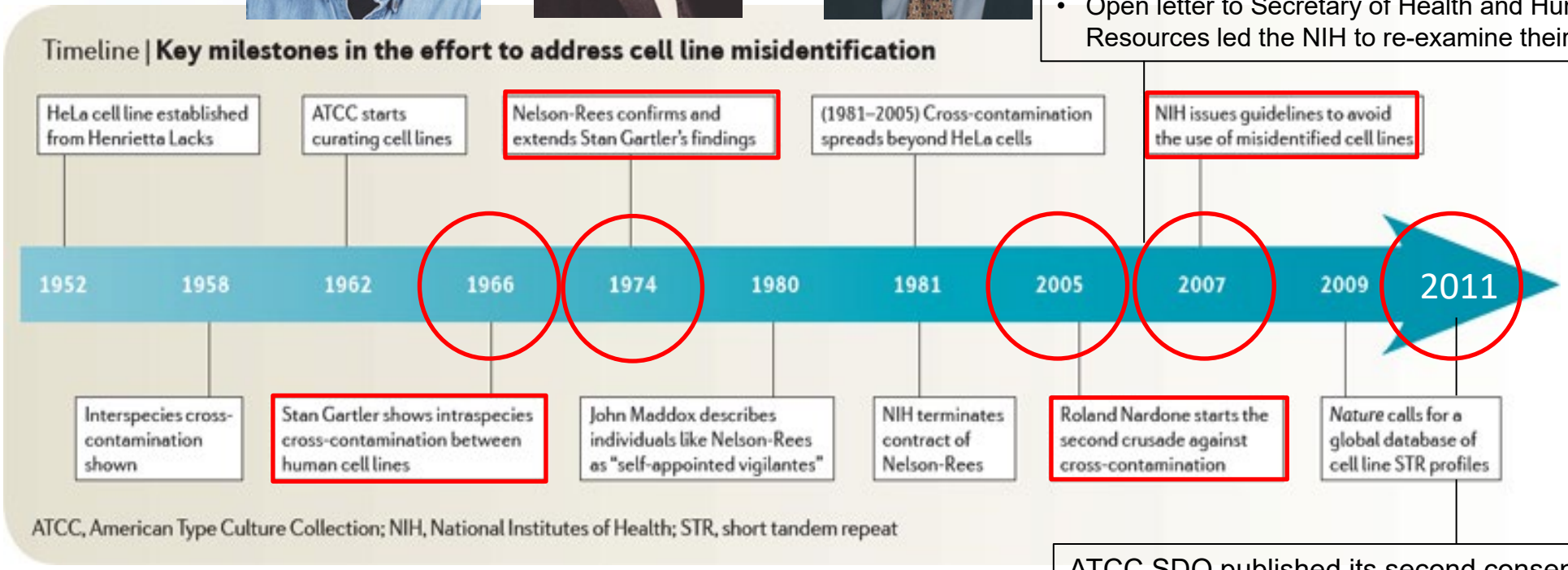


Walter Nelson-Rees

Roland Nardone



- 2007 White paper: Eradication of cross-contaminated cell lines: a call for action
- Open letter to Secretary of Health and Human Resources led the NIH to re-examine their guidelines



***Nat Rev Cancer* 2010, 10(6):441-448.**

ATCC SDO published its second consensus standard, ASN-0002: Authentication of Human Cell Lines: Standardization of STR Profiling.

Moving Forward

- ANSI Standard (ASN-0002): *Authentication of Human Cell Lines: Standardization of STR Profiling* was published in 2012, updated version 2021 **(GOLD STANDARD)**

- Numerous journals and granting agencies are now requiring authentication of cell lines prior to publication and funding

- The FDA has instituted a requirement for the authentication of cell lines used to produce pharmaceuticals
  - 21 CFR 211.160 (b)
  - 21 CFR 610.18 (b)

Important for mouse and CHO cell lines used in the manufacturing of biotherapeutics

- NIH has revised guidelines to applications for funding and provided guidelines for reporting
  - Enhanced Reproducibility through Rigor and Transparency (effective Jan. 25, 2016) Notice Number: NOT-OD-15-103
  - NIH Rigor and Reproducibility: Principles and Guidelines for Reporting Preclinical Research and Endorsement by major journals

The Impact

Science. 2015 Feb; 347(6225): 938-40.

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

The ghosts of HeLa: How cell line misidentification contaminates the scientific literature

Serge P. J. M. Horbach, Willem Halffman

Published: October 12, 2017 • https://doi.org/10.1371/journal.pone.0186281

Article | Authors | Metrics | Comments | Media Coverage

Abstract

While problems with cell line misidentification have been known for decades, an unknown number of published papers remains in circulation reporting on the wrong cells without warning or correction. Here we attempt to make a conservative estimate of this 'contaminated' literature. We found 32,755 articles reporting on research with misidentified cells, in turn cited by an estimated half a million other papers. The contamination of the literature is not decreasing over time and is anything but restricted to countries in the periphery of global science. The decades-old and often contentious attempts to stop misidentification of cell lines have proven to be insufficient. The contamination of the literature calls for a fair and reasonable notification system, warning users and readers to interpret these papers with appropriate care.

A tale of two impostors

Christopher Korch estimated the impact of research on two cell lines, HEp-2 and INT 407. Due to contamination long ago, both are now widely acknowledged to be composed of cancer cells called HeLa.

5789
ARTICLES
in **1182** journals may have used HEp-2 inappropriately, producing an estimated **174,000** citations

1336
ARTICLES
in **271** journals may have used INT 407 inappropriately, producing an estimated **40,000** citations

$713
MILLION
Estimated amount spent on the original articles published on **INT 407** and **HEp-2**

$3.5
BILLION
Estimated amount spent on subsequent work based on those papers

Ongoing Problem

Perspective

SLAS Discovery
2021, Vol. 26(10) 1268–1279

DOI: 10.1177/24725552211051963
journals.sagepub.com/home/jbx
SAGE

The Extensive and Expensive Impacts of HEp-2 [HeLa], Intestine 407 [HeLa], and Other False Cell Lines in Journal Publications

Christopher T. Korch[1] and Amanda Capes-Davis[2]

"As of June 2021, 8497 articles that used HEp-2 [HeLa] inappropriately, published in 2130 journals (Table 1). Within this data set, 3162 (37%) articles described it as a laryngeal or head and neck carcinoma model. The HEp-2 [HeLa] literature is currently growing at about 250 publications annually.

Ongoing Problem

BioTechniques
The International Journal of Life Science Methods

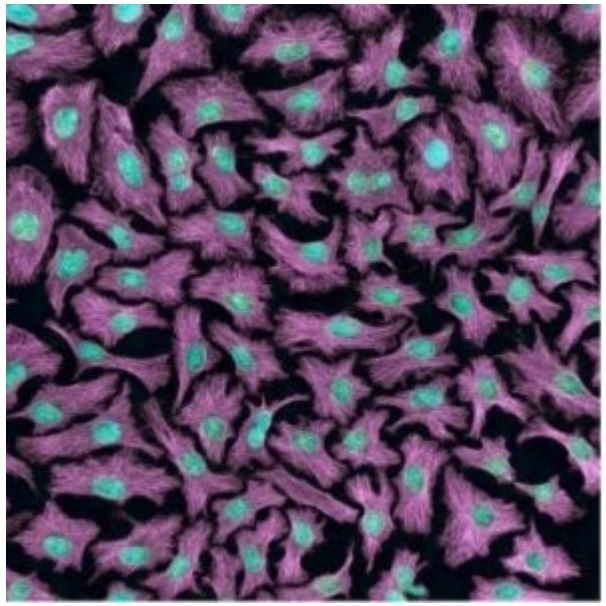
Home Journal Current issue News Multimedia Features Topics LEARN

Quality control: the dark side of cell culture

24 AUG 2018 | WRITTEN BY FRANCESCA LAKE (EDITOR-IN-CHIEF)

CELL AND TISSUE BIOLOGY | NEWS | TECH NEWS

Cell culture is integral to the future of drug discovery, but it suffers from a lack of reproducibility owing to inconsistent quality control.



Meanwhile, various initiatives, such as PRINTEGER and the International Cell Line Authentication Committee, are underway to improve things for future research. Some aim at improving protocols and the laboratory environment while some, such as the Cellosaurus and the Cell Line Data Base, aim to provide a knowledge resource. These resources also aim to help solve the naming issue, whereby the lack of guidance has led to duplication of names, and thus further issues with misidentification.

Accessed 12/21/23
https://www.biotechniques.com/cell-and-tissue-biology/quality-control-the-dark-side-of-cell-culture/

Need for Mouse Cell Line Authentication

- Many funding agencies and editors of journals now recommend identity testing of cell lines → for human cell line testing, this is easy (standards are in place)
- Mouse cell lines (2nd to human cell lines) are the most prevalent models for many types of research
  - Not much is known about the level of misidentification among mouse cell lines
  - SNP testing was used to determine strain level in live mice, but was not being implemented for mouse cell line identity testing
  - DNA barcoding can be used to determine interspecies identity but is not able to distinguish intraspecies samples (within the same species)

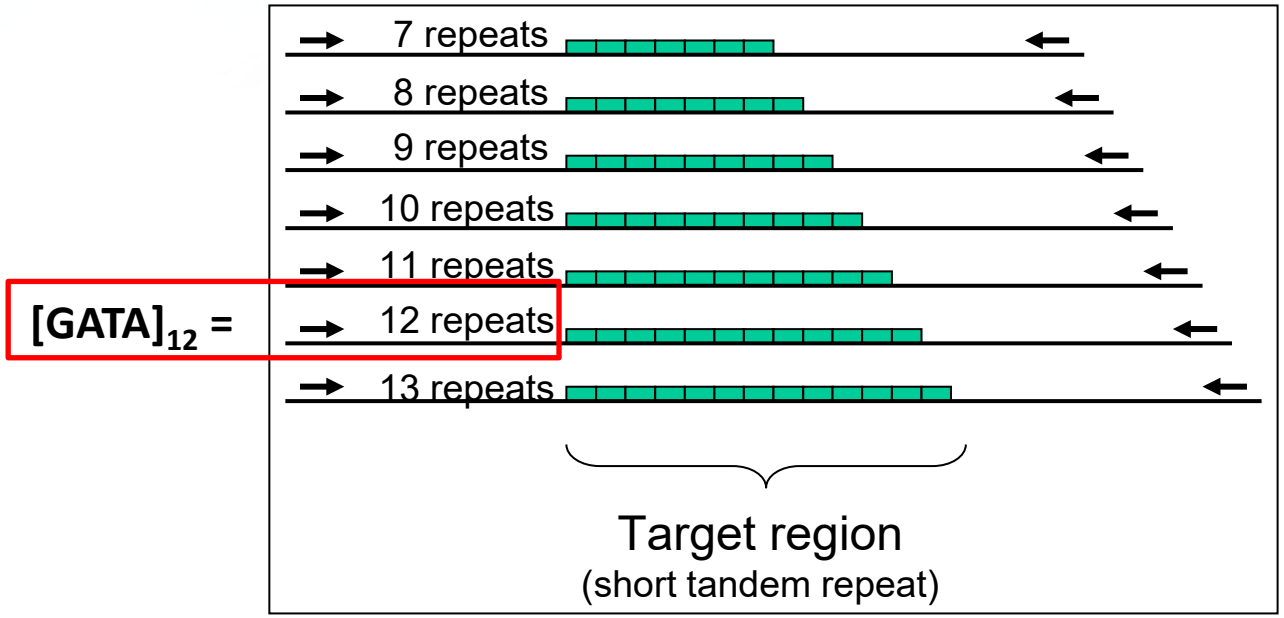
Around 2010, reproducibility was a huge concern in the scientific community. NIST was tasked to help the community in cell line identity measurements for nonhuman cell lines.

Short Tandem Repeat (STR)

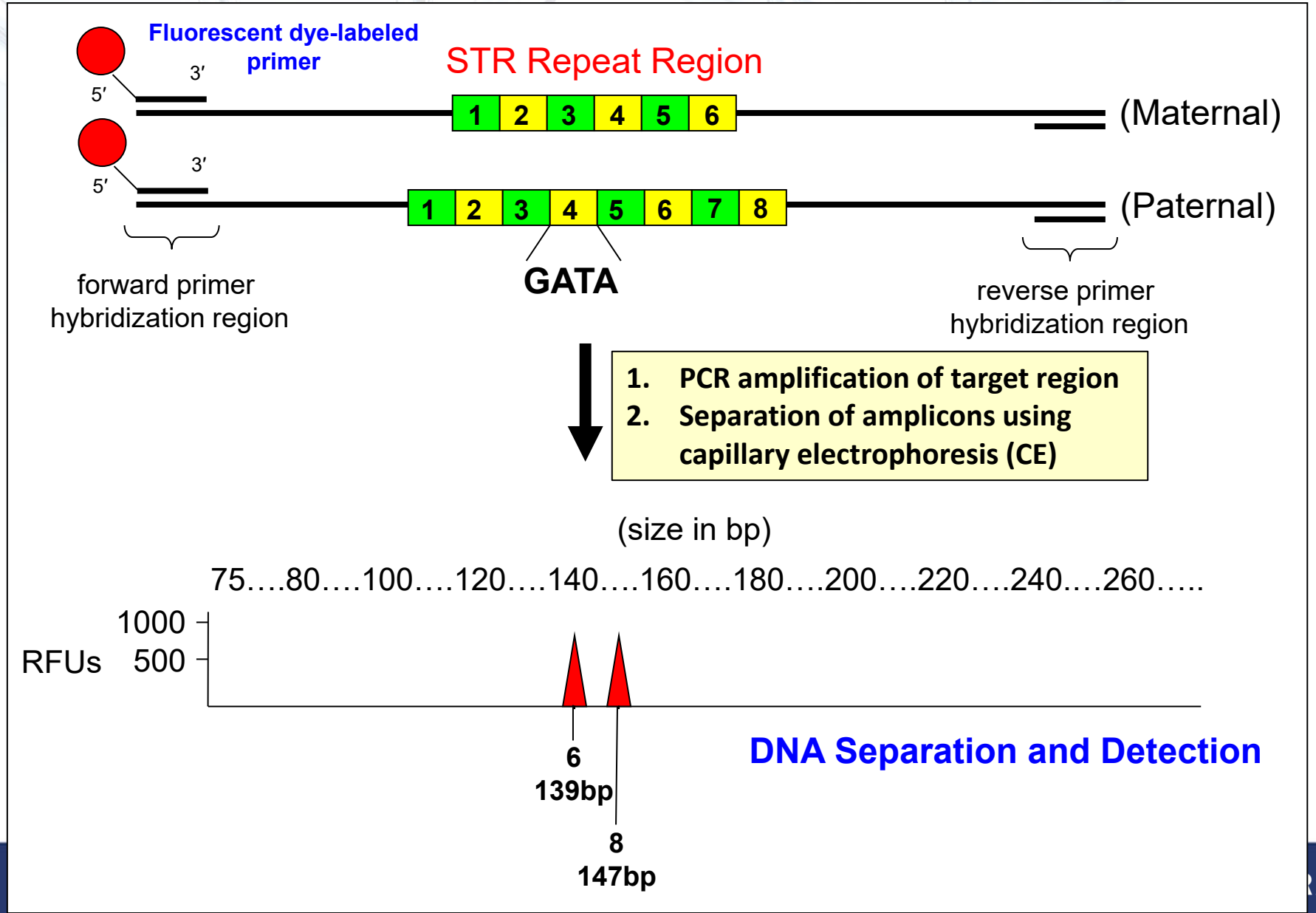
TCCCAAGCTCTTCCTCTTCCCTAGATCAATACAGACAGAAGACAGGTGA
GATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATA
TCATTGAAAGACAAAACAGAGATGGATGATAGATACATGCTTACAGATG
CACAC

- Simple repeats
  - Dinucleotide (CACACA)
  - Trinucleotide (CATCAT)
  - Tetranucleotide (CATGCATG)
  - Pentanucleotide (CATGACATGA)
- Complex repeats
  - (CATG)R(TA)(TAGA)

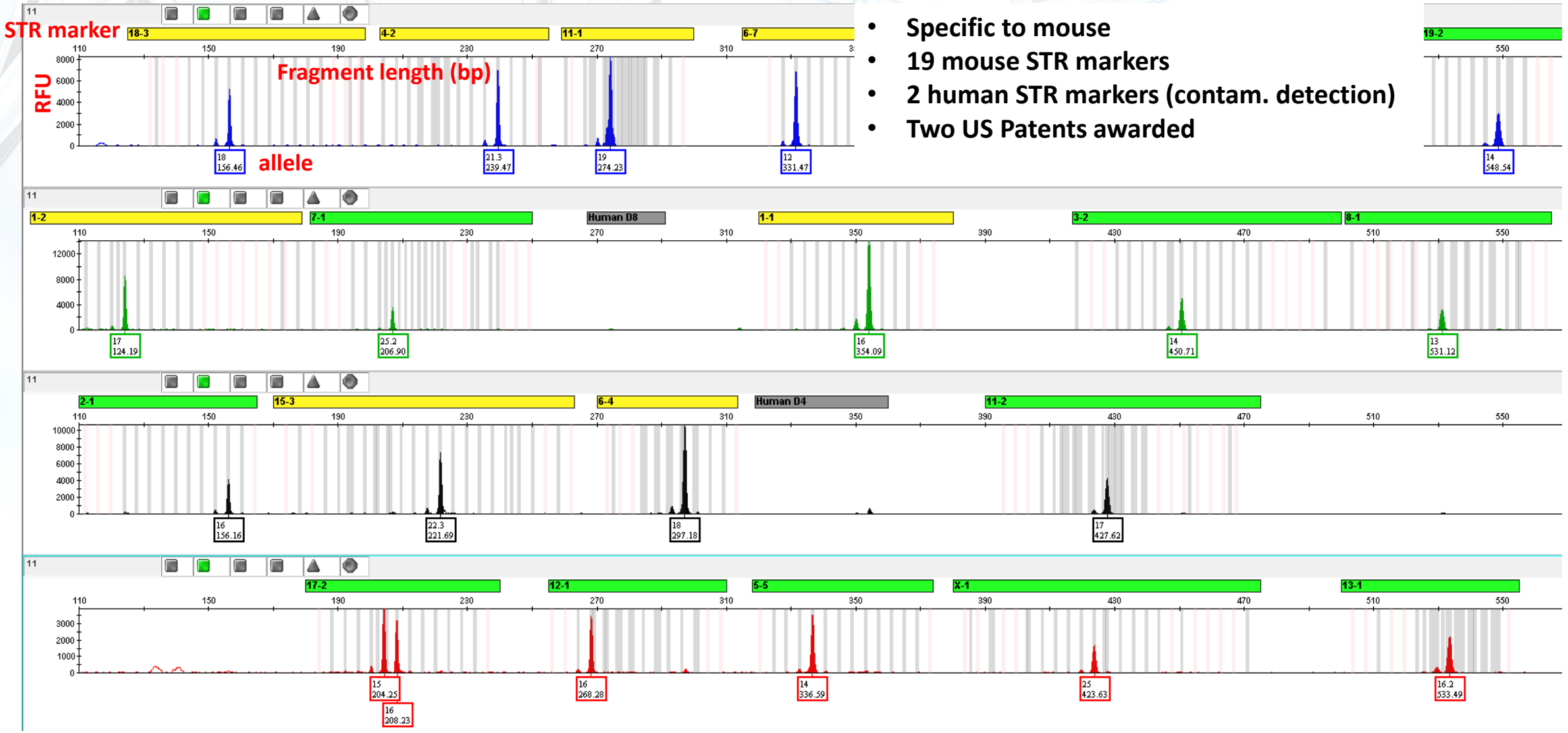
The number of consecutive repeat units can vary between individuals



STR Genotyping



Data Output from CE - Electropherogram



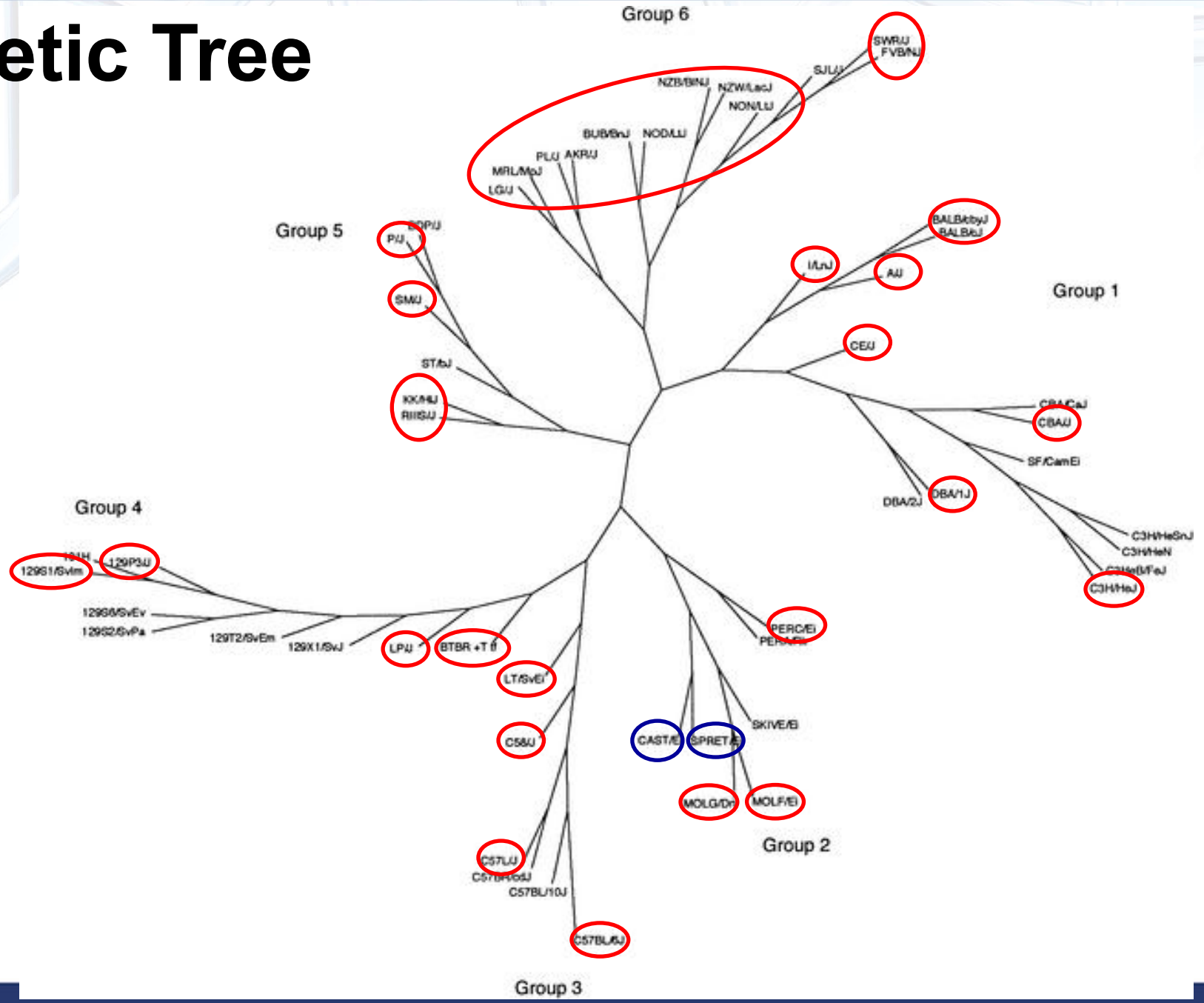
- Specific to mouse
- 19 mouse STR markers
- 2 human STR markers (contam. detection)
- Two US Patents awarded

Mouse Phylogenetic Tree

Will the mouse cell line authentication assay work on ALL mouse strains? → NO

Optimal for the following:

Mus musculus musculus
Mus musculus domesticus
Mus musculus molossinus



Mouse Cell Line Authentication Consortium

National Institute of Standards and Technology
U.S. Department of Commerce

ATCC®

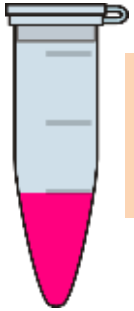
GOALS

- Interlaboratory study to validate 19 mouse STR markers and establish a functional, reliable and standardized STR based method for mouse cell line authentication.
- Provide the scientific community with validated STR profiles for 50 of the most commonly used mouse cell lines by submitting the data to the NCBI BioSample Database.
- Publish consortium results in a peer reviewed journal.

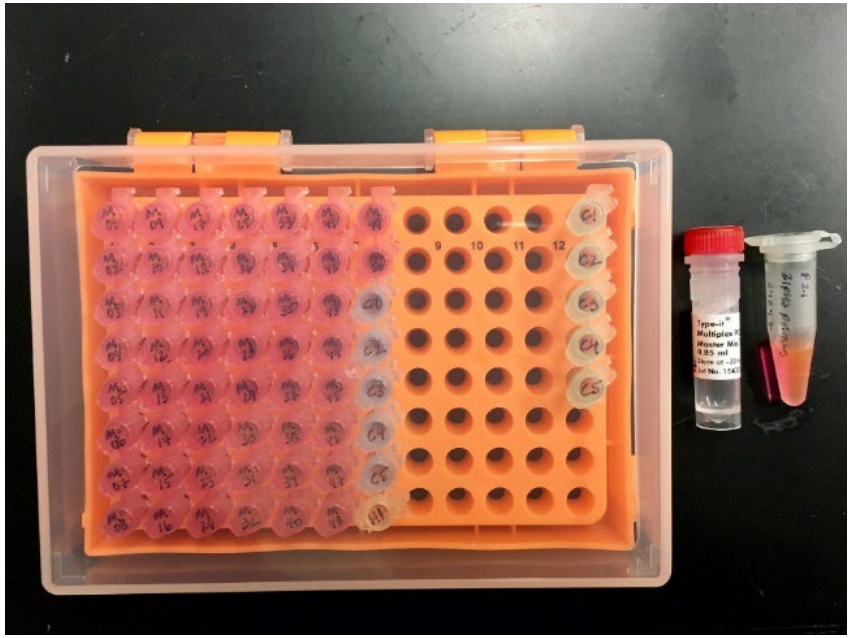
Interlaboratory Study – Method Validation

	1	2	3	4	5	6	7	8	9	10	11	12
A	M01	M09	M17	M25	M33	M41	M49				C1	
B	M02	M10	M18	M26	M34	M42	M50				C2	
C	M03	M11	M19	M27	M35	M43	C1				C3	
D	M04	M12	M20	M28	M36	M44	C2				C4	
E	M05	M13	M21	M29	M37	M45	C3				C5	
F	M06	M14	M22	M30	M38	M46	C4					
G	M07	M15	M23	M31	M39	M47	C5					
H	M08	M16	M24	M32	M40	M48	H1					

Qiagen Type-It Microsatellite PCR Mastermix



Mouse 21plex Primers



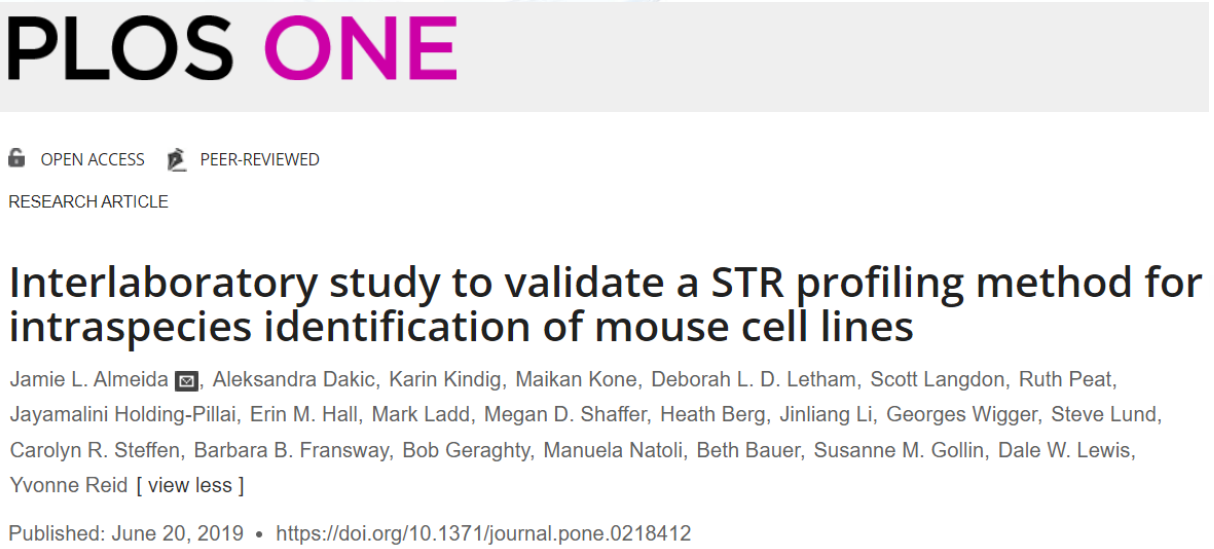
50 Mouse DNA samples (2ng/µL) (M01-M50): red
5 Calibrant DNA samples (2ng/µL)(C1-C5): blue
1 Human DNA positive control (2ng/µL)(H1): yellow

5 Calibrant DNA samples (2ng/µL)(C1-C5): green

Consortium Achievements

- ✅ Completed an interlaboratory study involving 12 consortium members to validate a method to genotype 50 mouse cell lines from ATCC

✅



PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

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

Jamie L. Almeida, Aleksandra Dakic, Karin Kindig, Maikan Kone, Deborah L. D. Letham, Scott Langdon, Ruth Peat, Jayamalini Holding-Pillai, Erin M. Hall, Mark Ladd, Megan D. Shaffer, Heath Berg, Jinliang Li, Georges Wigger, Steve Lund, Carolyn R. Steffen, Barbara B. Fransway, Bob Geraghty, Manuela Natoli, Beth Bauer, Susanne M. Gollin, Dale W. Lewis, Yvonne Reid [view less]

Published: June 20, 2019 • https://doi.org/10.1371/journal.pone.0218412

- ✅ Deposited validated mouse STR profiles on the NCBI BioSample Database
  - STR profiles, electropherograms, and background information for validated mouse cell lines have been submitted and are now searchable in the database.
  - BioProject accession # is PRJNA539973 and the URL to retrieve the cell line data is https://www.ncbi.nlm.nih.gov/biosample/?term=mouse+cell+line+STR +profile%5Battribute+name%5D

NCBI BioSample Database Entry

Search results

Items: 1 to 20 of 42

<< First < Pre

1. RAW 264.7, **mouse cell line STR profile** from ATCC
   Identifiers: BioSample: SAMN11397665; Sample name: RAW 264.7
   Organism: Mus musculus
   strain: BALB/c
   Package: Model organism or animal; version 1.0
   Accession: SAMN11397665 ID: 11397665
   BioProject

2. WEHI-3, **mouse cell line STR profile** from ATCC
   Identifiers: BioSample: SAMN11397664; Sample name: WEHI-3
   Organism: Mus musculus
   strain: BALB/c
   Package: Model organism or animal; version 1.0
   Accession: SAMN11397664 ID: 11397664
   BioProject

3. P815, **mouse cell line STR profile** from ATCC
   Identifiers: BioSample: SAMN11397663; Sample name: P815
   Organism: Mus musculus
   strain: DBA/2
   Package: Model organism or animal; version 1.0
   Accession: SAMN11397663 ID: 11397663
   BioProject

4. 2E8, **mouse cell line STR profile** from ATCC
   Identifiers: BioSample: SAMN11397661; Sample name: 2E8
   Organism: Mus musculus
   strain: BALB/c.xid
   Package: Model organism or animal; version 1.0
   Accession: SAMN11397661 ID: 11397661
   BioProject

RAW 264.7, mouse cell line STR profile from ATCC

Identifiers: BioSample: SAMN11397665; Sample name: RAW 264.7

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	RAW 264.7
culture collection	ATCC: TIB-71
strain	BALB/c
age	adult
sex	male
morphology	monocyte/macrophage
tissue	ascites
repository	American Type Culture Collection (ATCC)
disease	Abelson murine leukemia virus-induced tumor
cell line name alias	RAW264; RAW2647; RAW264.7; RAW-264.7; Raw 264.7; Raw264.7 (Cellosaurus)
date established	1978
about cells	adherent
mouse cell line STR profile	yes
mouse cell line STR profile status	NIST verified

Links: American Type Culture Collection
ATCC TIB-71

BioProject: PRJNA539973 Mus musculus
Retrieve all samples from this project

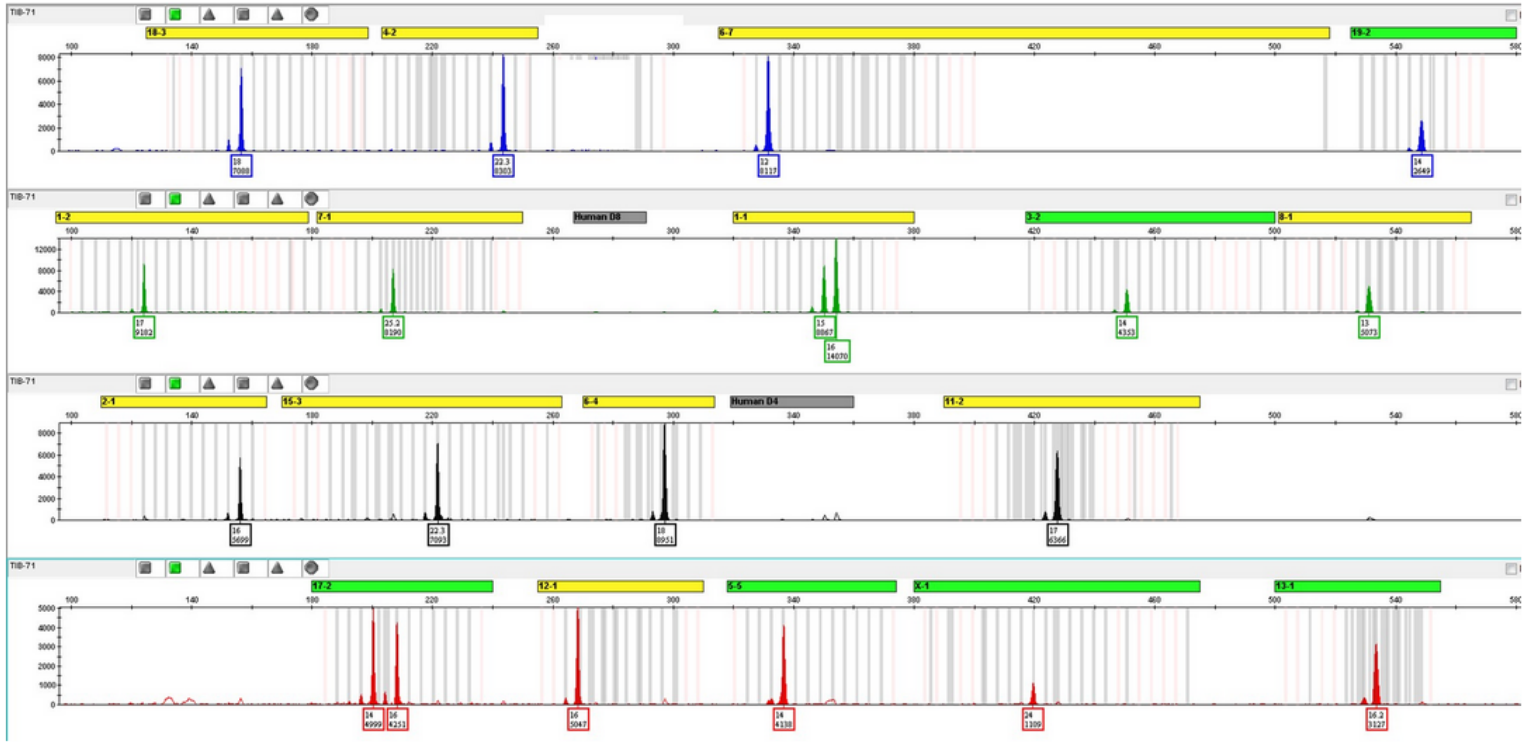
NCBI BioSample Database Entry

STR profile

STR 18-3	18
STR 4-2	22.3
STR 6-7	12
STR 19-2	14
STR 1-2	17
STR 7-1	25.2
STR 1-1	15,16
STR 3-2	14
STR 8-1	13
STR 2-1	16
STR 15-3	22.3
STR 6-4	18
STR 11-2	17
STR 17-2	14,16
STR 12-1	16
STR 5-5	14
STR X-1	24
STR 13-1	16.2

STR analysis

kit	Type-IT Microsatellite PCR kit (Qiagen) using 18 Mouse STR Markers
DNA source	Frozen vial of cells
DNA isolation method	Quick-DNA Miniprep (Zymo Research, Irvine, CA, USA)
method of DNA quantification	Quant-iT Qubit dsDNA HS Assay Kit (Invitrogen) and fluorescence measured using the Qubit 3 Fluorometer (Invitrogen).
amount of DNA used	2 ng DNA



Accession: SAMN11397665 ID: 11397665
BioProject

Cellosaurus Database

Cellosaurus | Home | Browse | Tools | Download | Help | Contact

Search | Clear

Cellosaurus RAW 264.7 (CVCL_0493)

[Text version]

Cell line name	RAW 264.7
Synonyms	RAW264; RAW2647; RAW264.7; RAW-264.7; Raw 264.7; Raw264.7
Accession	CVCL_0493
Resource Identification Initiative	To cite this cell line use: RAW 264.7 (RRID:CVCL_0493)
Comments	Part of: Tumor Immunology Bank (TIB) collection from Salk (transferred to ATCC in 1981). Virology: Not susceptible to infection by SARS coronavirus 2 (SARS-CoV-2) (COVID-19) (PubMed=33389257). Doubling time: 11 hours (Note=From cell counting), 12 hours (Note=From absorbance) (DOI=10.5897/IJBMBR2013.0154); ~30 Transformant: NCBI_TaxID; 11788; Abelson mouse leukemia virus (AbMuLV). Omics: Metabolome analysis. Omics: Phagosome quantitative phosphoproteome analysis. Omics: Phagosome proteome analysis. Omics: SNP array analysis. Miscellaneous: PubMed=23430347 has a different value for STR 6-4 (17) than that of NIST (18) due to a change in the marker Misspelling: RAW 267.4; Note=Occasionally. Misspelling: Raw 267.4; Note=Occasionally. Misspelling: RAW267.4; Note=Occasionally. Misspelling: RAW 274; Note=Occasionally. Misspelling: RAW-274; Note=Occasionally. Misspelling: RAW274; Note=Occasionally. Derived from site: In situ; Ascites; UBERON=UBERON_0007795. Cell type: Macrophage; CL=CL_0000235.
Disease	Mouse leukemia (NCIt: C21604)
Species of origin	Mus musculus (Mouse) (NCBI Taxonomy: 10090) Breed/subspecies: BALB/c.
Hierarchy	Parent: CVCL_4478 (RAW 264) Children:

CVCL_B0YM (Abcam RAW 264.7 Ccl7 KO)	CVCL_B7VG (Abcam RAW 264.7 Ccr2 KO)	CVCL_B0YN (Abcam RAW 264.7 Cd68 KO)
CVCL_B7VK (Abcam RAW 264.7 Cdkn1b KO)	CVCL_B7VH (Abcam RAW 264.7 Cx3cr1 KO)	CVCL_B0YP (Abcam RAW 264.7 Prkaa1 KO)
CVCL_B7VI (Abcam RAW 264.7 Sirpa KO)	CVCL_KB45 (CellSensor NFkB-bla RAW 264.7)	CVCL_DD02 (EA13.5)
CVCL_7189 (eCAS)	CVCL_HF55 (ImKC)	CVCL_B417 (M9A)
CVCL_C8RU (RAW 264.7 Ern1 KO)	CVCL_6517 (RAW 264.7 gammaNO(-))	CVCL_C8RT (RAW 264.7 Gba KO)
CVCL_C8HB (RAW 264.7 Irf3 KO)	CVCL_UL72 (RAW 264.7 LRRK2 KO)	CVCL_C8HC (RAW 264.7 Lrrk2 KO [Montreal])
CVCL_UL71 (RAW 264.7 LRRK2 parental)	CVCL_UL73 (RAW 264.7 LRRK2 T1348N mut)	CVCL_C8RV (RAW 264.7 Ppm1h KO)
CVCL_C8RW (RAW 264.7 Rab10 KO)	CVCL_C8RX (RAW 264.7 Rab29 KO)	CVCL_C8RY (RAW 264.7 Rab8a KO)
CVCL_ZD89 (RAW 264.7 shRNA-RSK2)	CVCL_C8HD (RAW 264.7 Sting1 KO)	CVCL_C8HE (RAW 264.7 Sting1 R330A/R333A)
CVCL_C8HF (RAW 264.7 Xbp1 KO)	CVCL_C8WQ (RAW 264.7-EGFP)	CVCL_6717 (RAW 264.7/LR5)
CVCL_A7ZG (RAW-ASC)	CVCL_X594 (RAW-Blue)	CVCL_X595 (RAW-Blue ISG)
CVCL_F681 (RAW-D)	CVCL_A8CB (RAW-Difluo mLC3)	CVCL_A7ZK (RAW-Dual)
CVCL_C3NB (RAW-E6)	CVCL_C3NC (RAW-EGFP)	CVCL_C8H9 (RAW-Kb)

Sex of cell	Male
Age at sampling	Adult
Category	Cancer cell line
STR profile	**Source(s):** PubMed=23430347; PubMed=31220

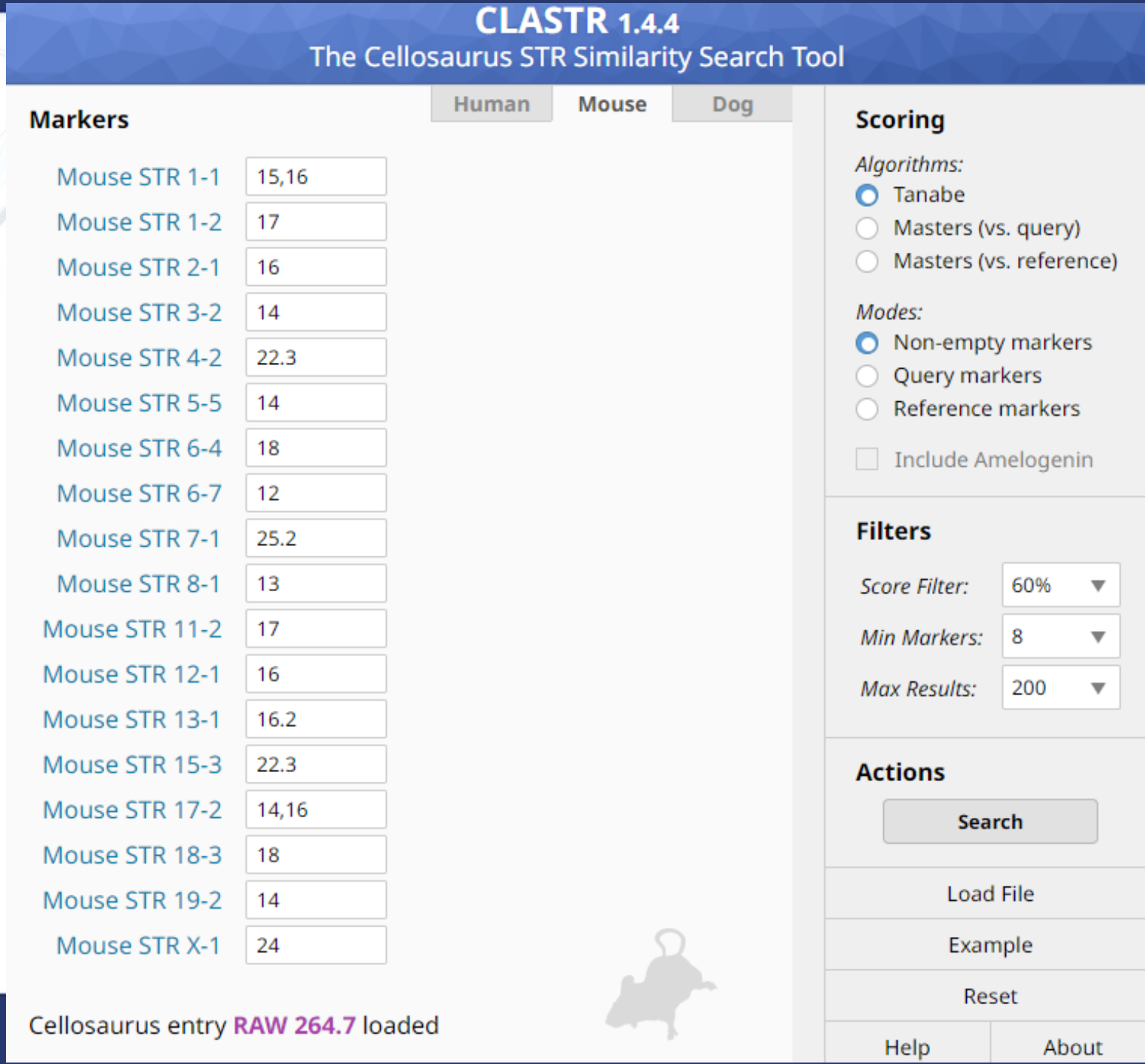
Markers:

Mouse STR 1-1	15,16
Mouse STR 1-2	17
Mouse STR 2-1	16
Mouse STR 3-2	14
Mouse STR 4-2	22.3
Mouse STR 5-5	14
Mouse STR 6-4	18
Mouse STR 6-7	12
Mouse STR 7-1	25.2
Mouse STR 8-1	13
Mouse STR 9-2	15
Mouse STR 11-2	17
Mouse STR 12-1	16
Mouse STR 13-1	16.2
Mouse STR 15-3	22.3
Mouse STR 17-2	14,16
Mouse STR 18-3	18
Mouse STR 19-2	14
Mouse STR X-1	24

Run an STR similarity search on this cell line

Cellosaurus – CLASTR Tool

Search a mouse STR profile against all profiles within Cellosaurus to find similarities.



Next Step: Mouse Allelic Ladder

Reproducibility

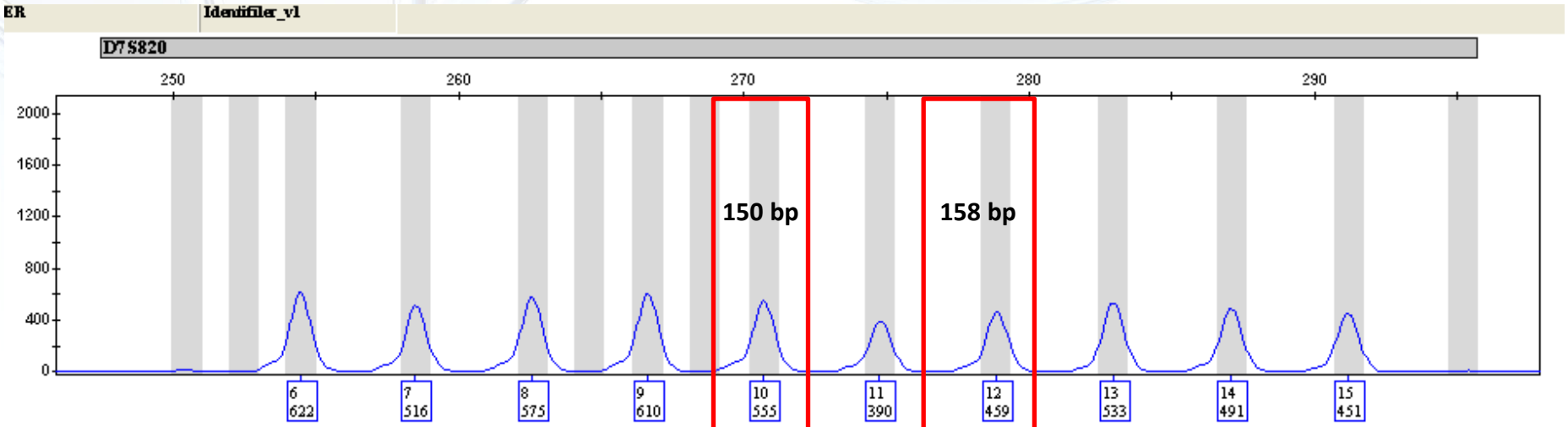
The STR profiles available on the NCBI BioSample Database and Cellosaurus can be obtained using the mouse multiplex PCR assay if proper protocols are followed and a calibrant material is used for allele call determination. This material must be well characterized, sequenced, and stable.

Different CE platforms using various polymers and arrays introduce changes in DNA mobility and cause variations in fragment lengths. How to normalize?

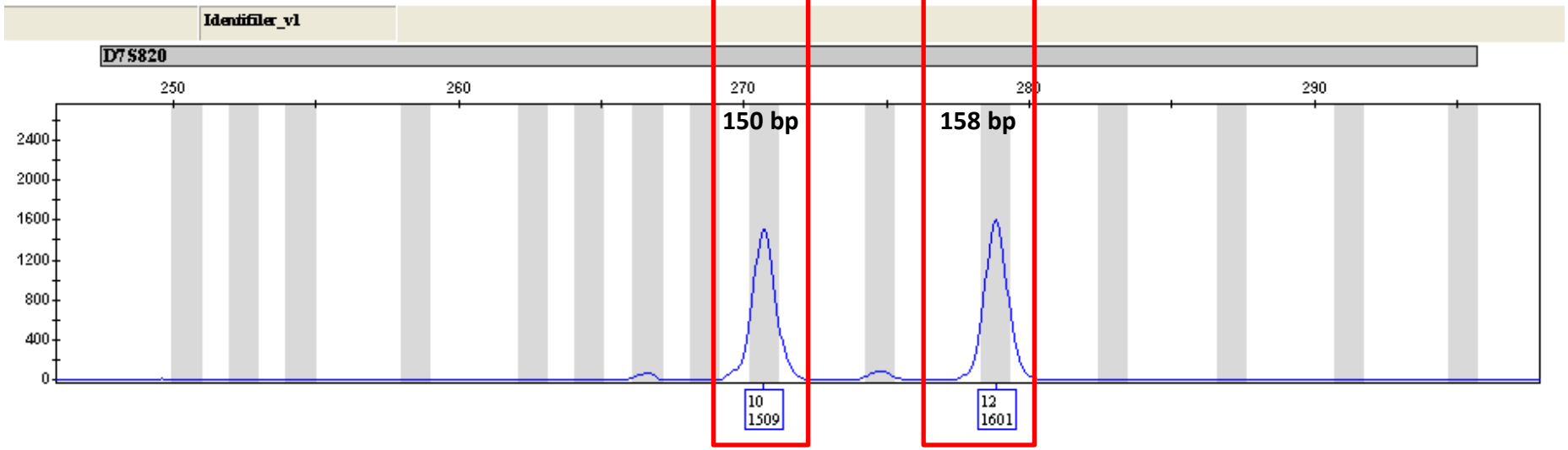
- **Internal size standard**: DNA mixture of known fragment sizes (present in all samples, runs in a different dye channel).
- **Allelic ladder**: DNA mixture of alleles with known number of tandem repeats (GATAGATA) for each STR marker. It correlates fragment length with repeat number (allele). Run as a separate sample during CE.

How Do You Use An Allelic Ladder?

Allelic Ladder



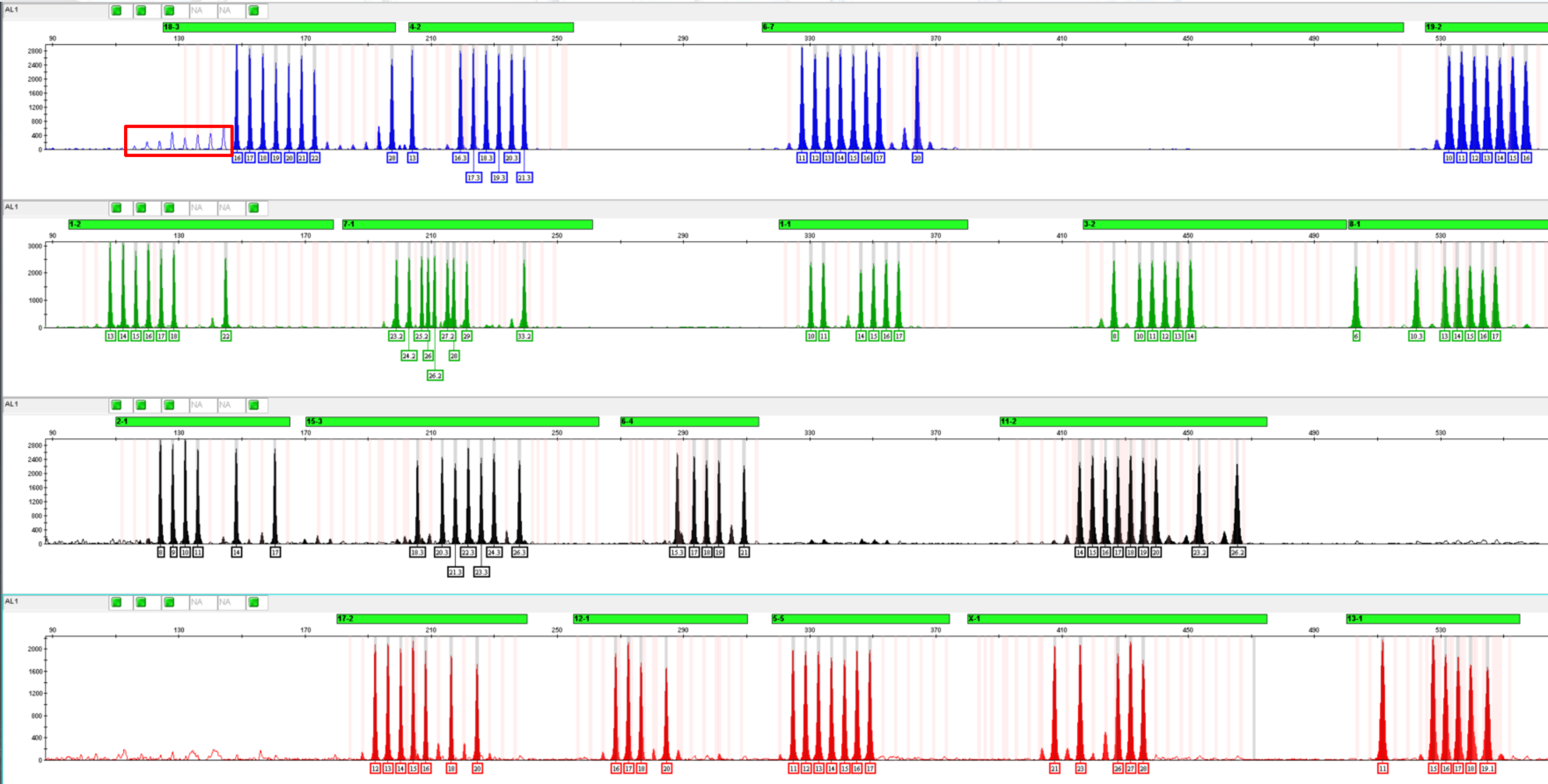
Unknown Sample



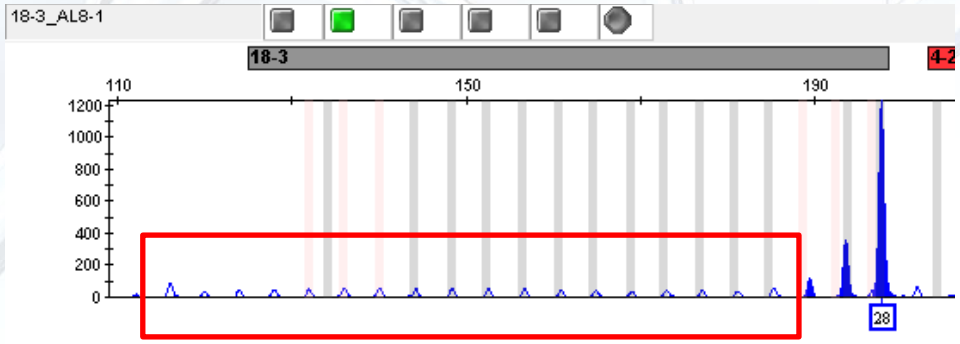
Build the Allelic Ladder - Help from ATCC

- A 2-year postdoc position was funded by ATCC to complete the development of plasmids required for a mouse allelic ladder.
- Known alleles from normal diploid mouse DNA samples from Jackson Laboratories and rare alleles from a several cell lines were amplified, isolated, and cloned into plasmids.
- Hundreds of clones were screened.
- Constructs were sequenced to confirm the correct allele.
- 121 plasmid constructs were completed (each containing a single allele).

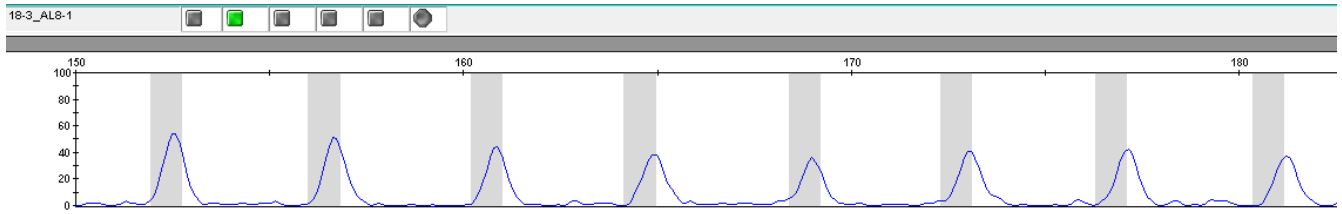
Mouse Allelic Ladder



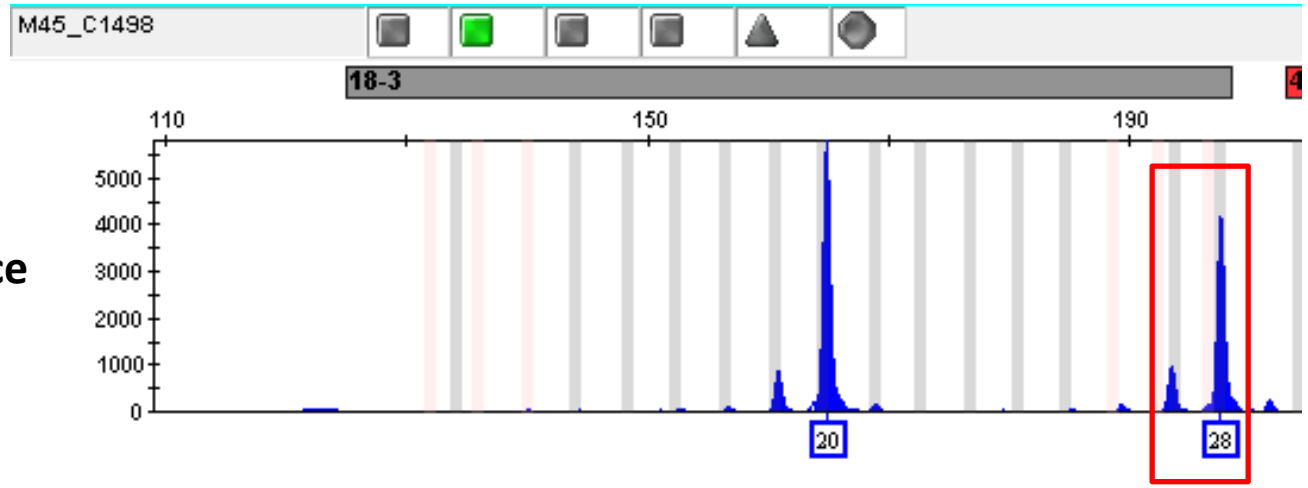
Artifacts in the 18-3 Constructs



Zoom



Original DNA source

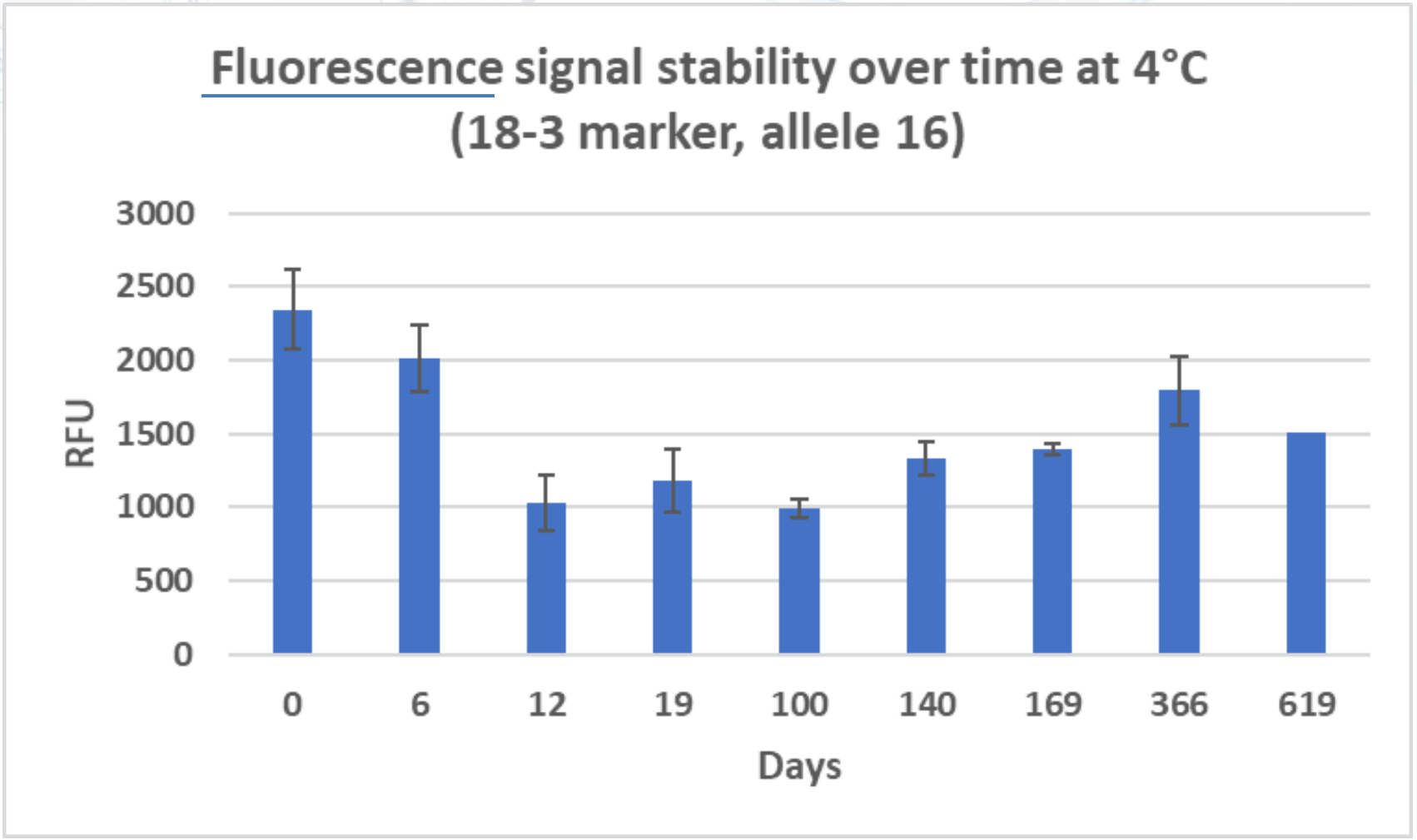


Created all new 18-3 constructs, used all new reagents, and the artifacts remain

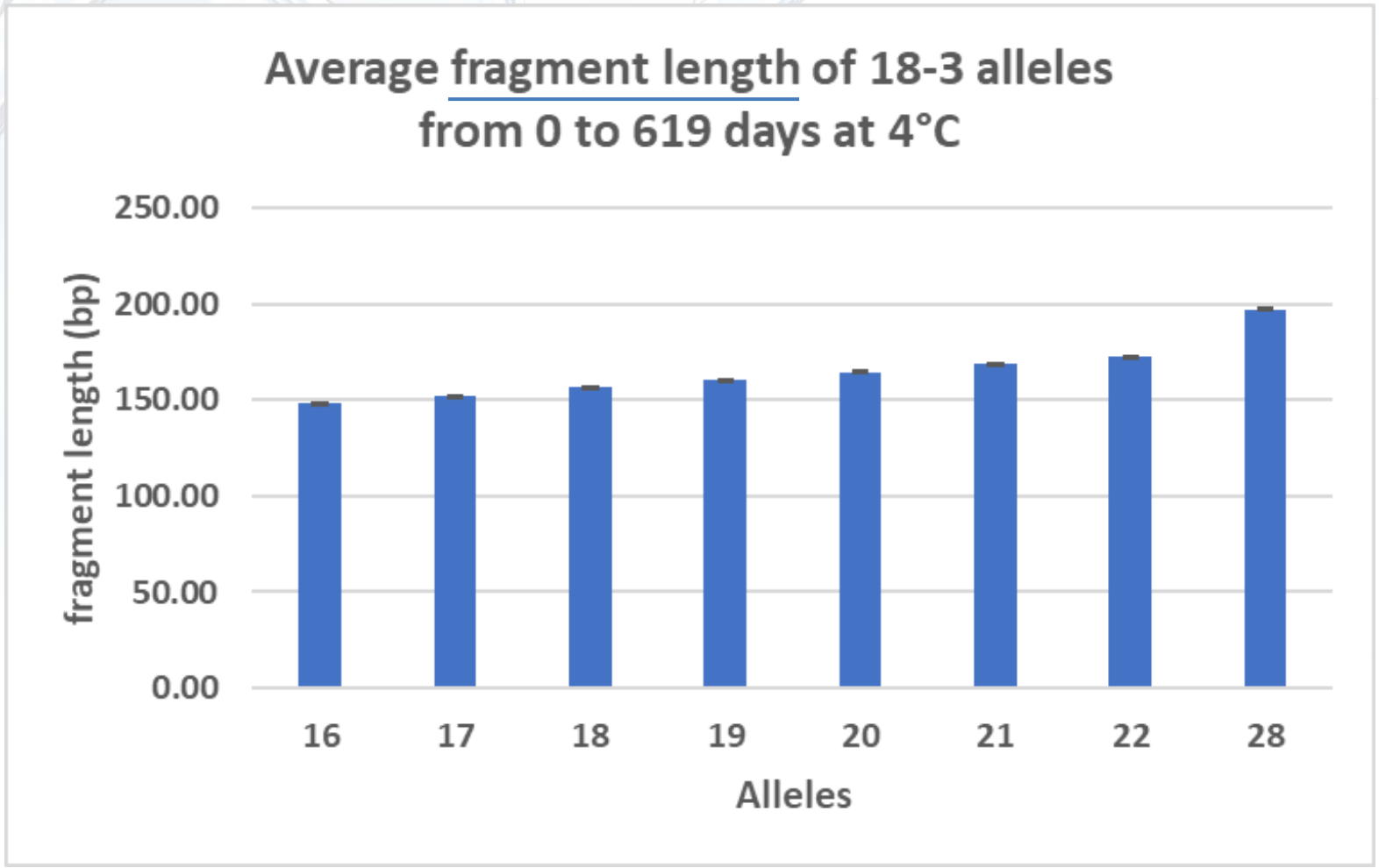
Reference Material Measurements

- Stability
  - Storage temperatures
    - How long is the material stable over time? At room temperature? 4°C? -20°C?
    - Changes in fragment length? Fluorescence?
- Homogeneity
  - Random sampling of vials
- Reference values
  - Allele = Sequence of repeat motif

Preliminary Stability Study at 4°C

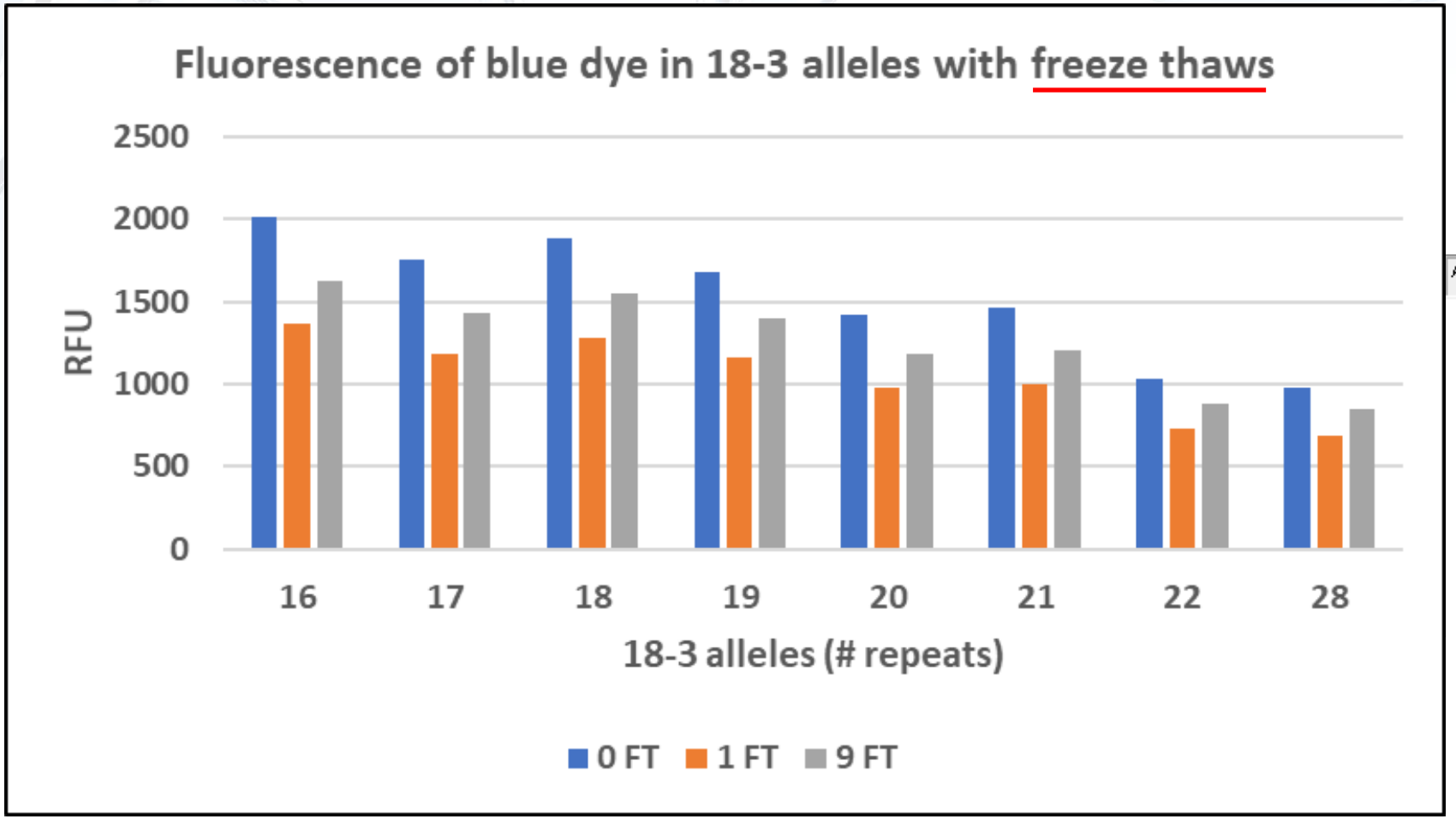


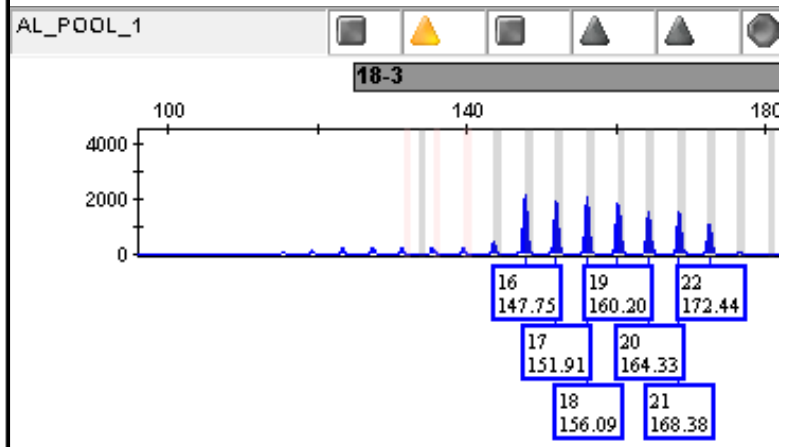
Preliminary Stability Study at 4°C



Allele	AVG bp	STD DEV
16	148.09	0.32
17	152.23	0.32
18	156.38	0.31
19	160.52	0.30
20	164.59	0.31
21	168.66	0.30
22	172.73	0.30
28	197.34	0.27

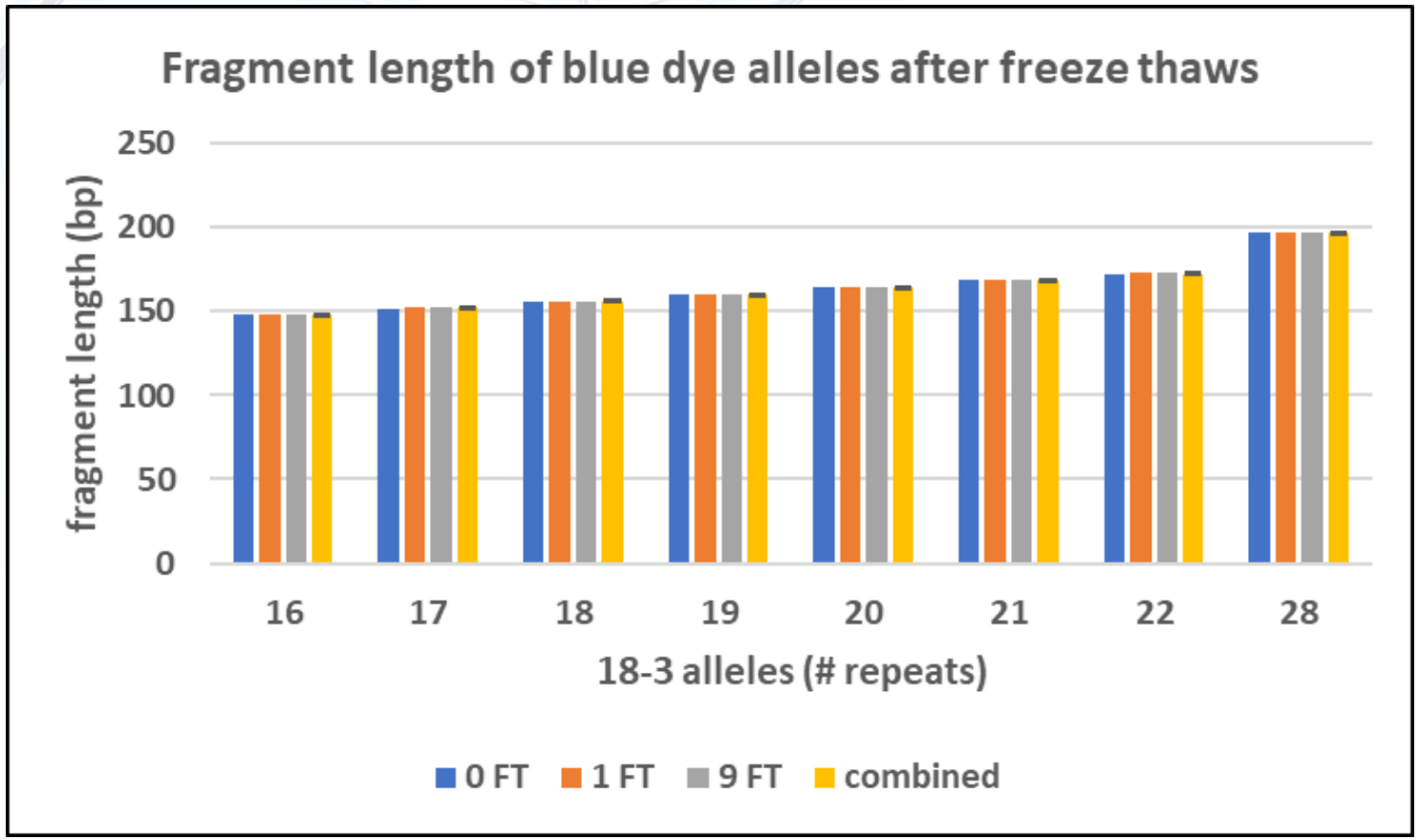
Preliminary Stability Study at -20°C





Preliminary Stability Study at -20°C

Fragment length measurements with freeze thaws, based on CE data



Allele	0 FT	1 FT	9 FT	Combined AVG	AVG STD DEV
16	147.69	147.92	147.95	147.85	0.14
17	151.83	152.06	152.13	152.01	0.16
18	156.00	156.27	156.28	156.18	0.16
19	160.15	160.44	160.39	160.33	0.16
20	164.22	164.50	164.48	164.40	0.16
21	168.30	168.61	168.49	168.47	0.15
22	172.35	172.63	172.61	172.53	0.15
28	197.02	197.23	197.21	197.15	0.12

NIST RGTM 10161 Interlaboratory Study

NIST RGTM 10161 is a Research Grade Test Material and a precursor to NIST Reference Material RM 8399 (Mouse Allelic Ladder)

Aim

Can the RGTM 10161 Mouse Allelic Ladder Test Material be used to make accurate allele calls on any CE platform? Are accurate allele calls obtained in samples that contain alleles that are not represented in the allelic ladder?

Study Details

To determine STR profiles for 10 mouse DNA samples (blinded) using RGTM 10161 for allele call determination. Mouse cell lines were not used so the profiles could not be searched in databases. Mouse samples were selected based on the presence of alleles not represented in the allelic ladder.

Data

Received data back from 6 labs and all allele calls are 100% concordant using allelic ladder. Still waiting on 5 labs to return data.

RGTM 10161 Interlaboratory Study

Constructive feedback from participants:

- Add alleles for the human STR markers to the allelic ladder
  - This would require sequencing the D4 and D8 STR marker regions in several human samples to obtain the alleles needed
  - Clone alleles into plasmids
  - Amplify and purify plasmids
  - Add amplified alleles from plasmids to the current AL, rebalance all of the peak heights within that dye channel

This could be included in an updated version of the AL to be available in the future. We don't want to delay this material any longer.

Needs and Gaps

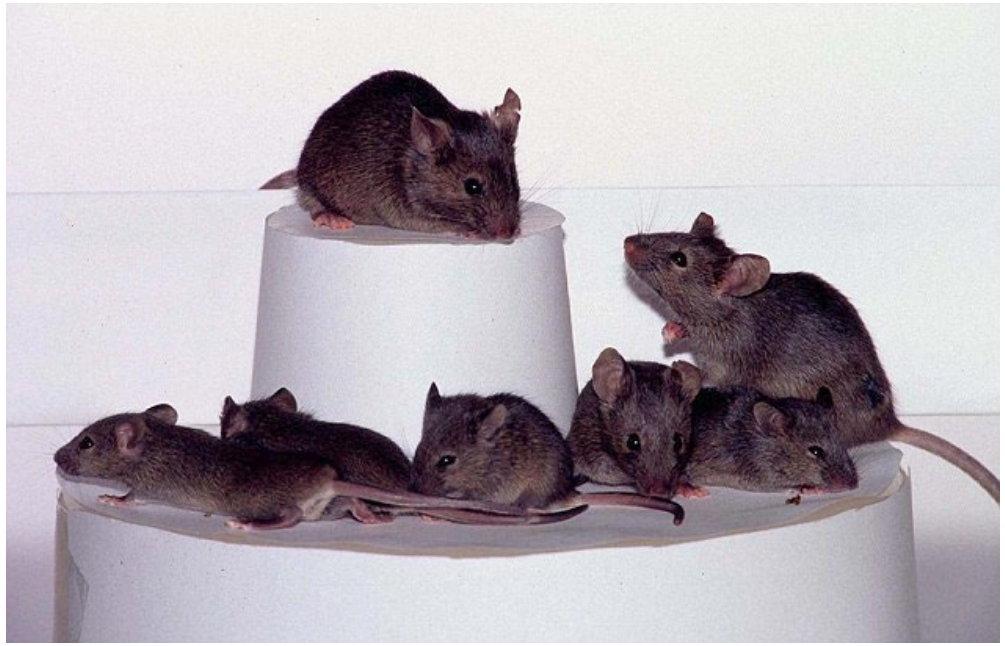
- Poor quality control of genotyping services that were not part of the original Mouse Cell Line Authentication Consortium (European company)
  - Errors found in their data analysis
  - NIST cannot regulate – only have US Patent Rights
  - I've personally reached out to this company to give them needed tools - unresponsive

- Studies are needed to determine relatedness of cell lines from same strain and closely related strains to improve existing matching algorithms for mouse (some labs were using the same stipulations in place for human cell lines)
  - We don't have the bandwidth to do this

- More resolution is needed for these clonal mouse samples
  - Some researchers are trying to use this method to distinguish cell lines created from mice from the same colony. This assay wasn't meant for that kind of resolution.

Limitations

- Cell lines that are derived from the same parent line will most likely have matching STR genotypes
- This assay may not work for all mouse strains
  - The primers were based on the genome for *Mus musculus domesticus (*NCBI build 38.1)
  - We already know that *Mus dunni* does not amplify at every loci
- Loss of heterozygosity due to inbreeding leads to a highly clonal population



Solutions

- Next generation sequencing (NGS)
  - Has the power to distinguish between alleles that are the same length using CE detection but contain different sequence.
  - Intra-STR SNPs cannot be distinguished between using CE methods
  - Whole genome sequencing is becoming less and less expensive, but expertise in bioinformatics is needed.

Can use targeted NGS using a MiSeq to distinguish between samples

Figure 3: NGS Detection of Intra-STR SNPs

TCTATCTGTCTGTCTGTCTATCTATCTATCTATCTATCT D3S1358 16 allele

TCTATCTGTCTGTCTATCTATCTATCTATCTATCTATCT D3S1358 16 allele with SNP

SNP

QUESTIONS

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