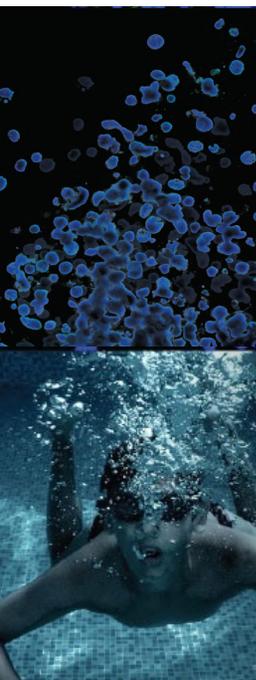




# Restoring Reproducibility in Research: Four Tools to Authenticate Your Cells

Kevin Grady, BS  
*Senior Product Line Business Manger, ATCC*

Credible Leads to Incredible™

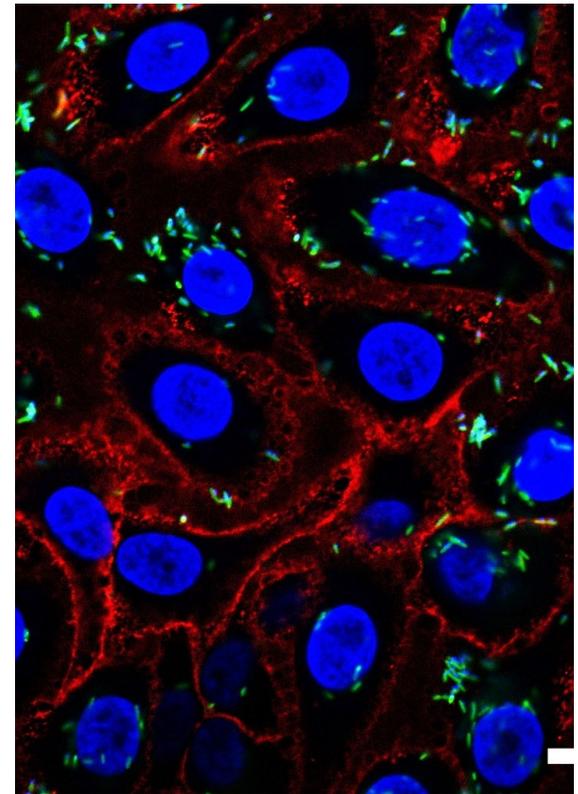


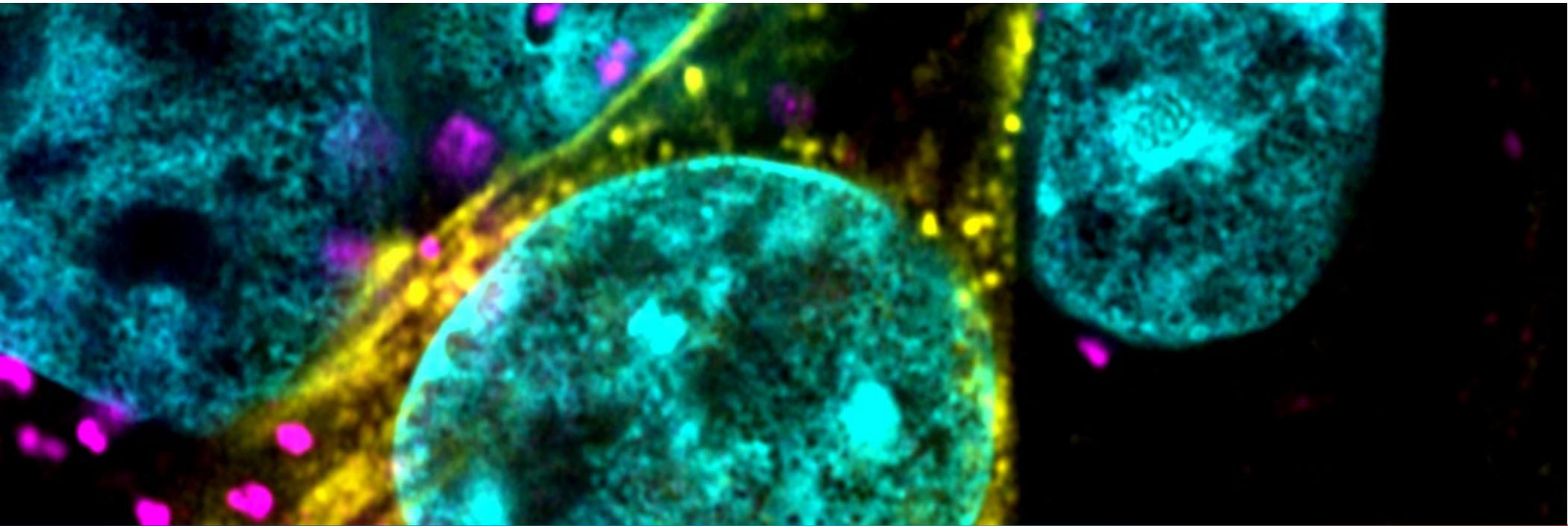
# About ATCC

- 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 cell culture – the “*gold standard*”
- Innovative standards organization featuring multiple products and services around characterizing and authenticating cells and microbes
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

# Agenda

- **Impact of misidentified and contaminated cell lines**
- **STR profiling for cell line identity**
  - Human
  - Mouse
- **Mycoplasma detection methods**
- **CO1 barcoding for species identity**





# Authentication

**Vero cells stained for F-actin and lamp1**

*Image courtesy of Amy Engevik, Vanderbilt University Medical Center*

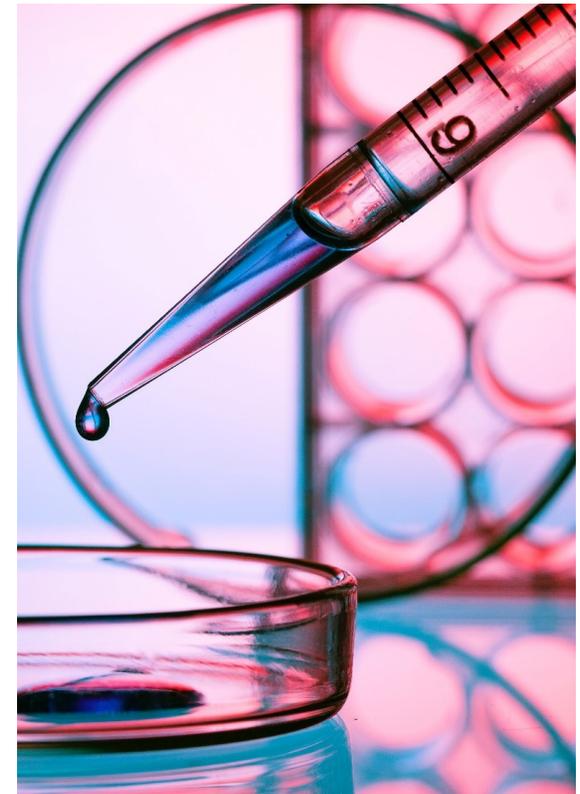
# Cell authentication

## *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*

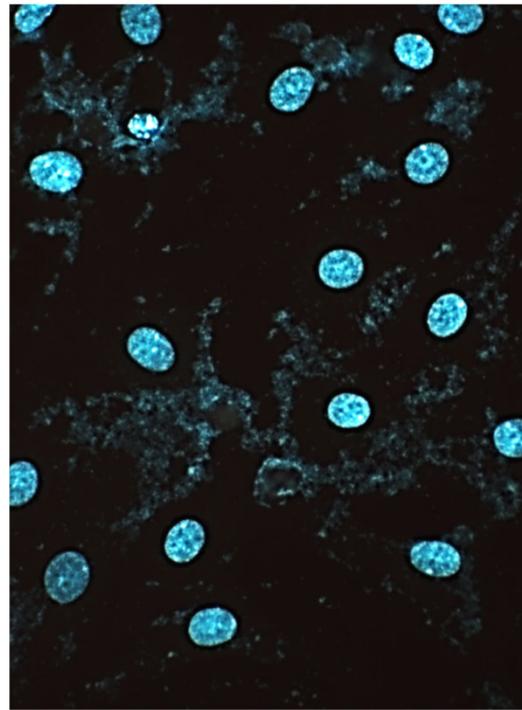


# Cell authentication

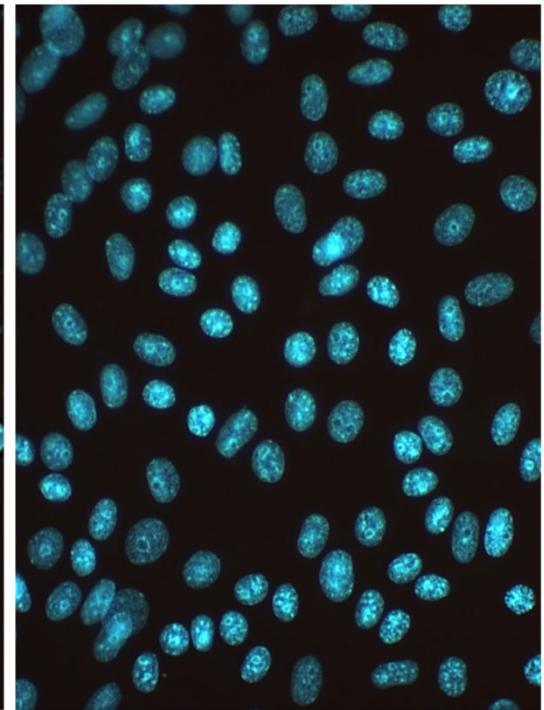
## *Impact of mycoplasma contamination on scientific research*

### Contamination results in a number of deleterious effects

- Chromosomal aberrations
- Disruption of nucleic acid synthesis
- Changes in membrane antigenicity
- Inhibition of cell proliferation and metabolism
- Decreased transfection rates
- Changes in gene expression profiles
- Affects virus production
- Cell death



Vero cells infected with *M. hyorhinis*



Uninfected Vero cells

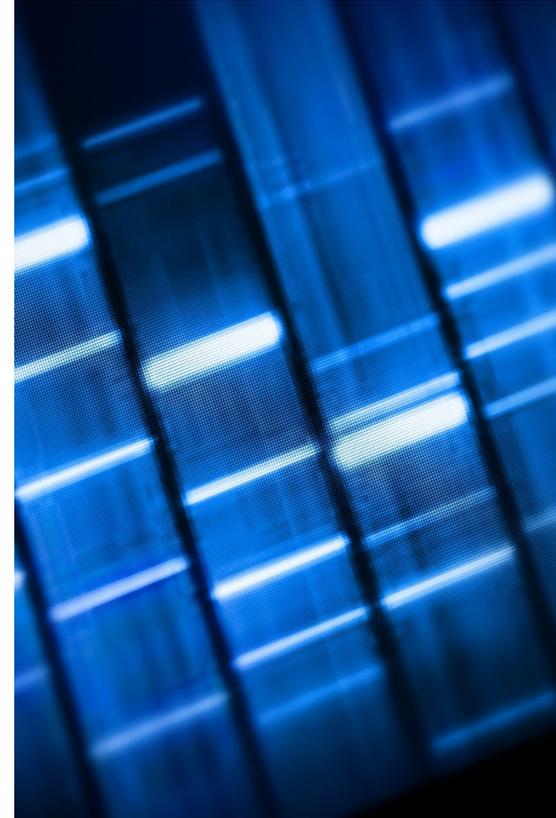
# Cell authentication

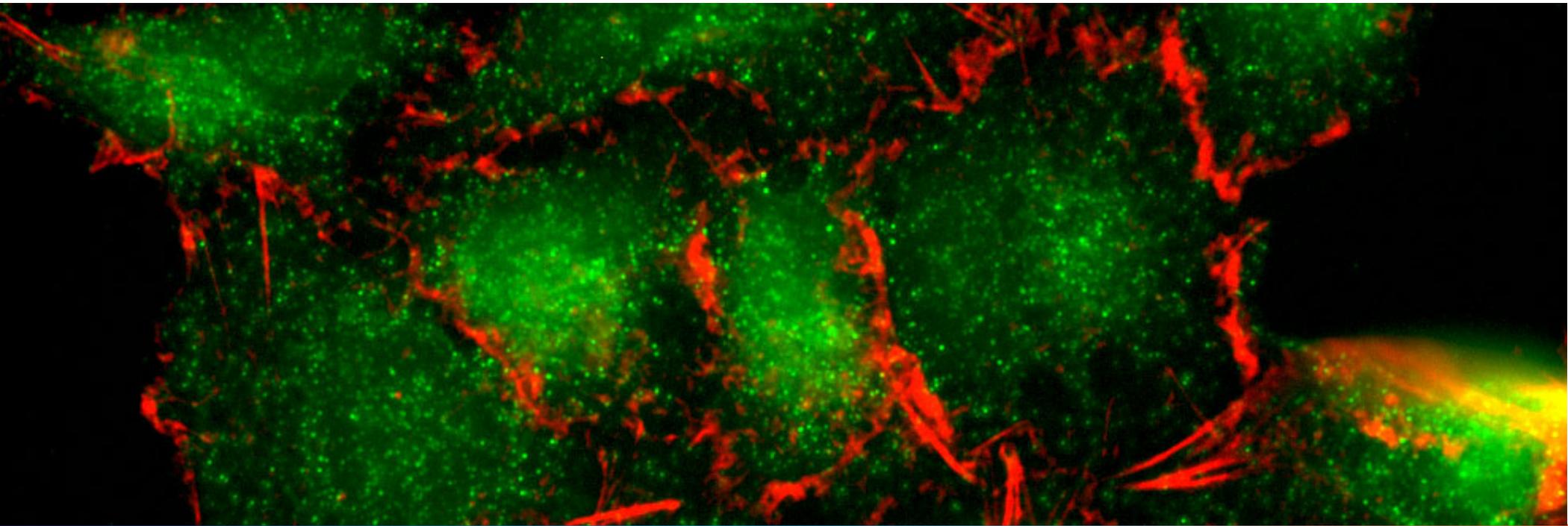
## Available Services

- STR (short-tandem repeat) analysis (human and murine)
- Mycoplasma testing (PCR, direct, and indirect methods)
- CO1 (Cytochrome Oxidase C1) testing
- Sterility testing
- Human pathogenic virus testing
- Phenotyping via ICC, flow cytometry and/or molecular assays

## What we offer

- Standardized methods for cell line authentication
- Comprehensive quality control and analysis by ATCC experts in cell authentication
- Authentication ensures valid and reproducible results
- ISO/IEC 17025 accredited process for quality control





## Short Tandem Repeat (STR) Profiling

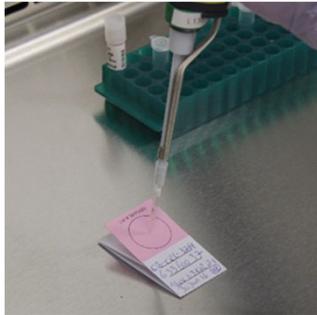
**A549 non-small cell lung carcinoma cell line expressing p53**

# STR analysis for human cell line identity

- Target sequence consists of microsatellite DNA (short repeats, 1 – 6 bp, 5 – 50 times)
- Typically use 1-2 ng DNA
- 1 to 2 fragments
- Discrete alleles allow digital record of data
- Markers distributed throughout the genome
- Highly variable within populations; highly informative



# Outline of STR profiling service procedure



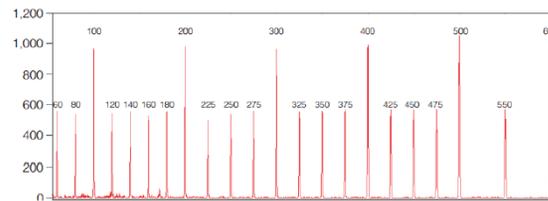
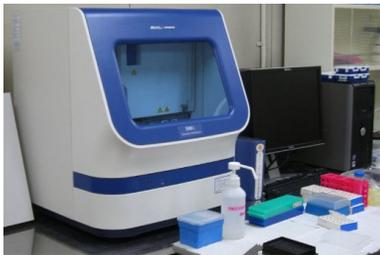
DNA on Whatman FTA paper



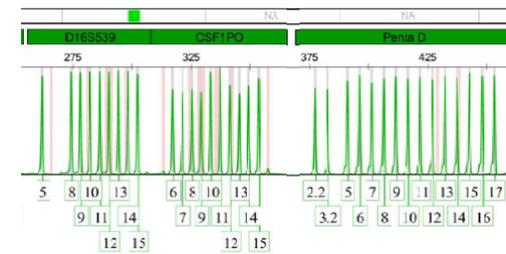
- Amplification of STR loci
- Simultaneous fluorescent labeling

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

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



Internal Lane Standards

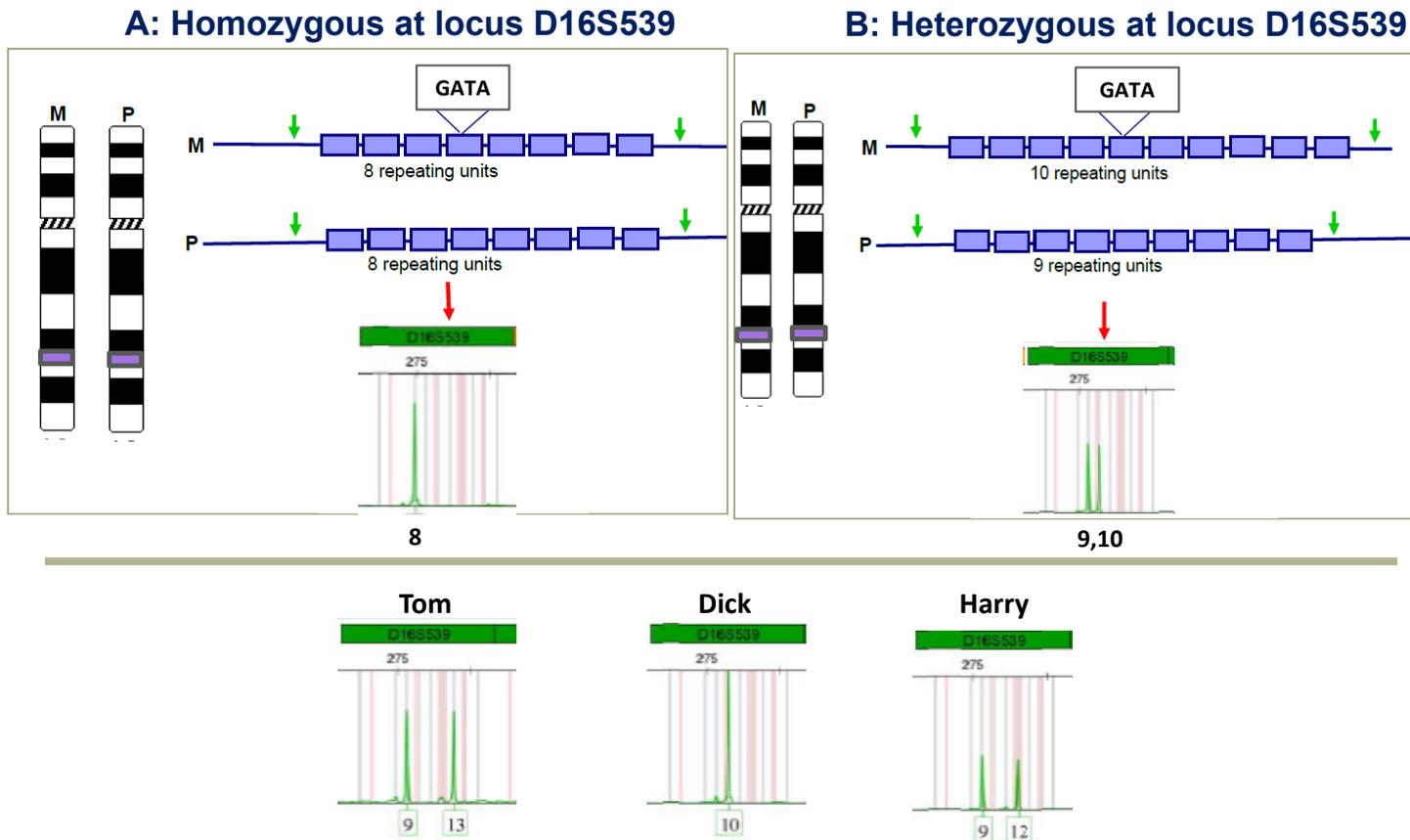


Allelic ladder (3 loci)

## Requirements:

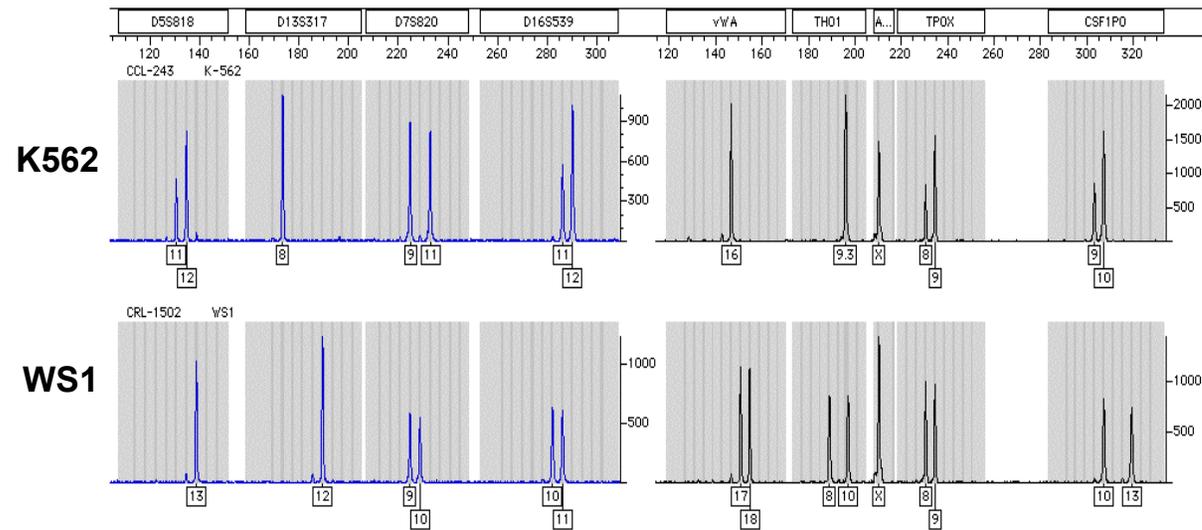
- Gene sequencer
- Thermocycler
- Primer kits from manufacturers
- STR database of human cell lines
- Experienced technicians

# STR DNA polymorphism



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

# Unrelated human cell lines: STR analysis

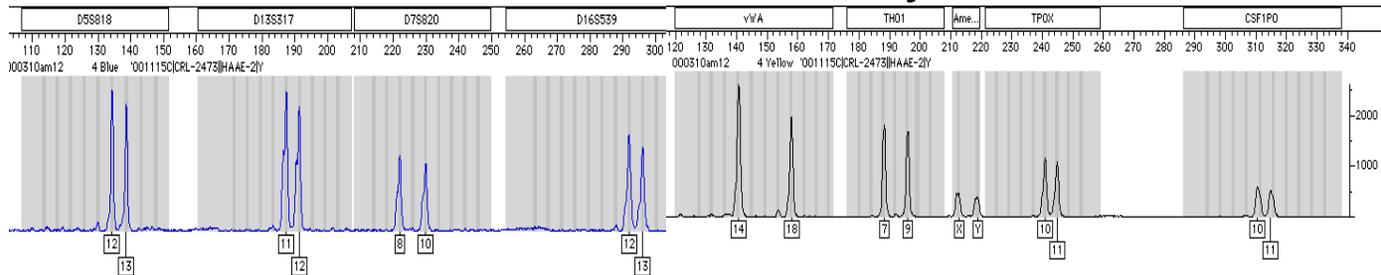


	D5S818	D13S317	D7S820	D16S539	vWA	TH01	Amel.	TPOX	CSF1PO
<b>K562</b>	11, 12	8	9, 11	11, 12	16	9.3	X	8, 9	9, 10
<b>WS1</b>	13	12	9, 10	10, 11	17, 18	8, 10	X	8, 9	10, 13

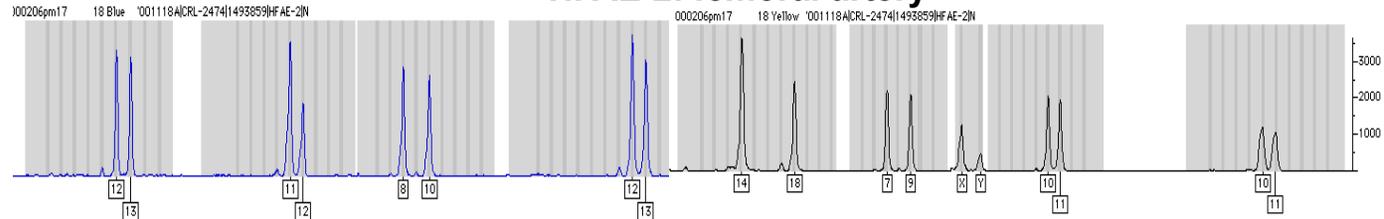
**2 unrelated cell lines, separate individuals, unique STR DNA profiles**

# Related human cell line identification: STR analysis

**HAAE-2: aortic artery**



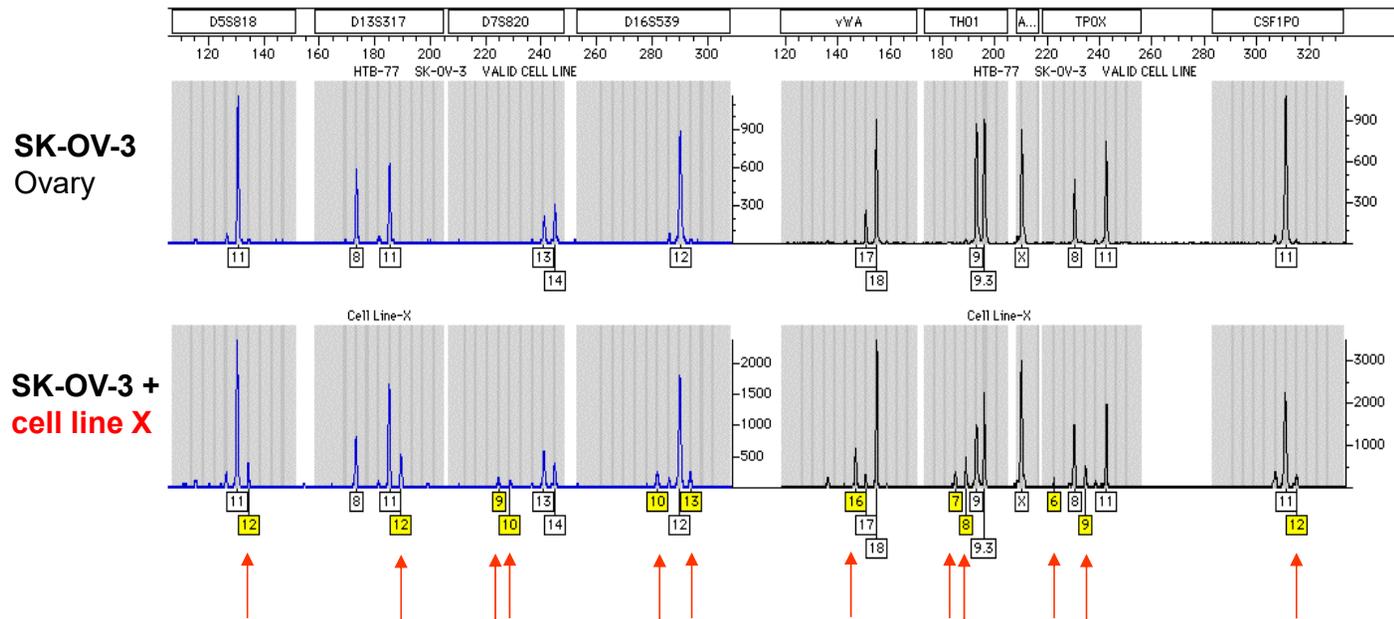
**HFAE-2: femoral artery**



	D5S818	D13S317	D7S820	D16S539	vWA	TH01	Amel.	TPOX	CSF1PO
HAAE-2	12,13	11,12	8,10	12,13	14,18	7,9	X,Y	10,11	10,11
HFAE-2	12,13	11,12	8,10	12,13	14,18	7,9	X,Y	10,11	10,11

**Two related cell lines, same individual, identical DNA STR profile**

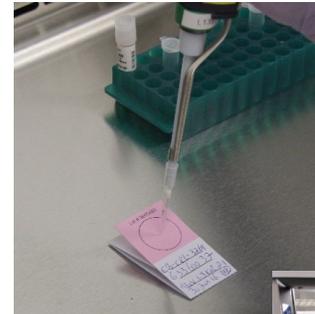
# Cellular cross-contamination

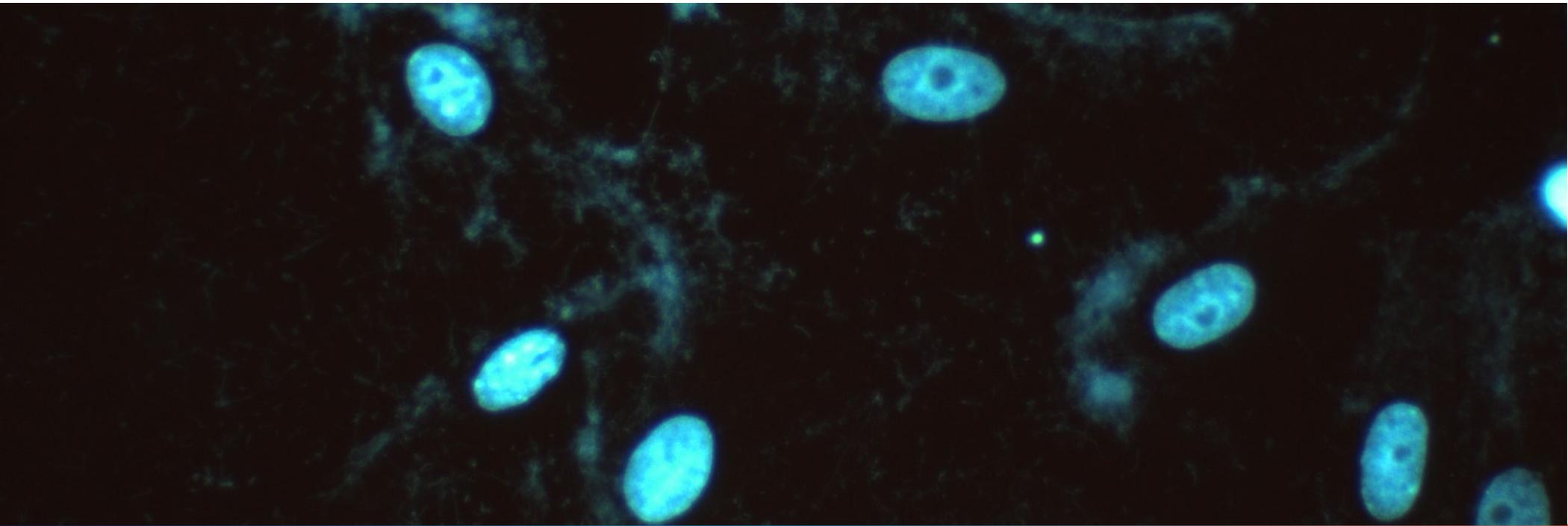


## Key points: STR profiling services

- ATCC works with other standards organizations to identify solid means of authenticating cell identity
- ATCC provides STR profiling for all human cell lines in its collection
- ATCC worked with NIST to pioneer STR profiling for mouse cell lines
- ATCC authentication services are simple and inexpensive, after placing your order:
  - Spot
  - Dry
  - Mail
  - Receive your results in three to five days

**Analysis performed following ISO 9001:2015 and ISO/IEC 17025:2017 quality standards**





## Mycoplasma detection

*M. fermentans* infection of Vero cell line

# How to protect cell cultures from mycoplasma contamination: Routine testing methods

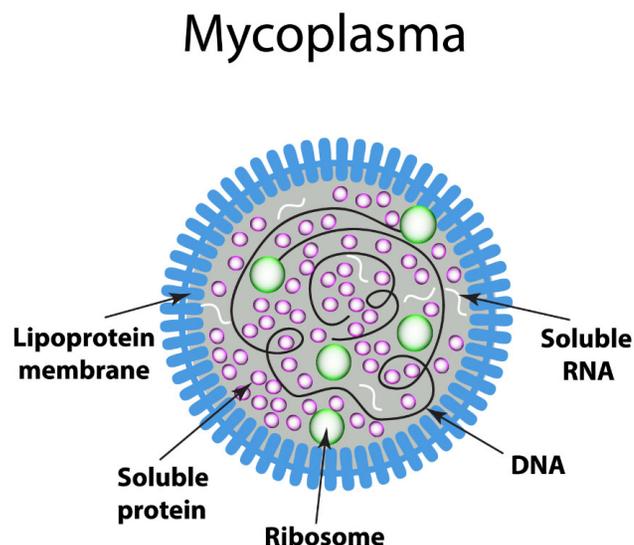
## Why is routine testing is important?

### Mycoplasma contamination is not easily detected

- Does not cause media turbidity
- Does not alter the pH of the media
- Few metabolic byproducts
- Cannot be detected by microscopy

### Common testing methods

- Direct agar culture
- Indirect Hoechst DNA staining
- PCR-based testing



# Direct agar culture

## Advantages

- Considered the “gold standard” for testing
- Easy to perform
- Detects viable cells
- Meets FDA Points to Consider

## Disadvantages

- Time intensive
- Laborious
- Not all mycoplasma are culturable *in vitro*
- May require expert interpretation
- Requires selective media

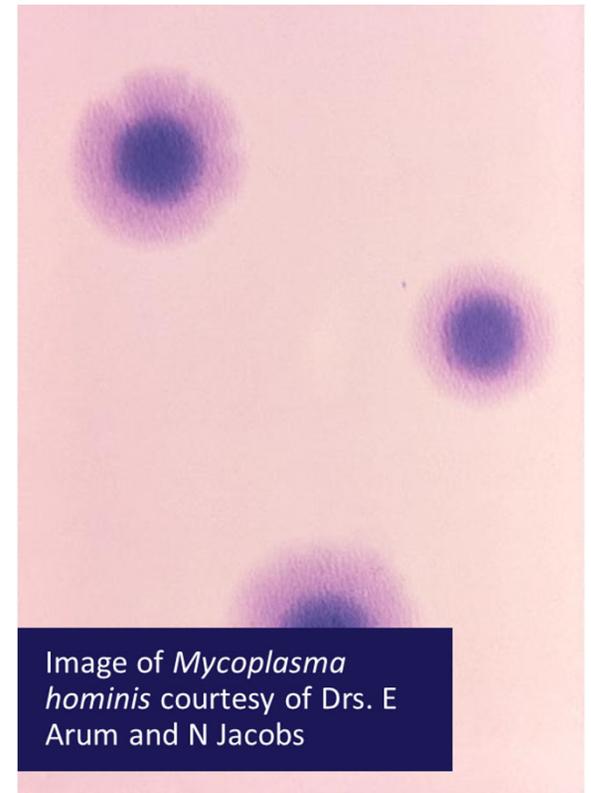


Image of *Mycoplasma hominis* courtesy of Drs. E Arum and N Jacobs

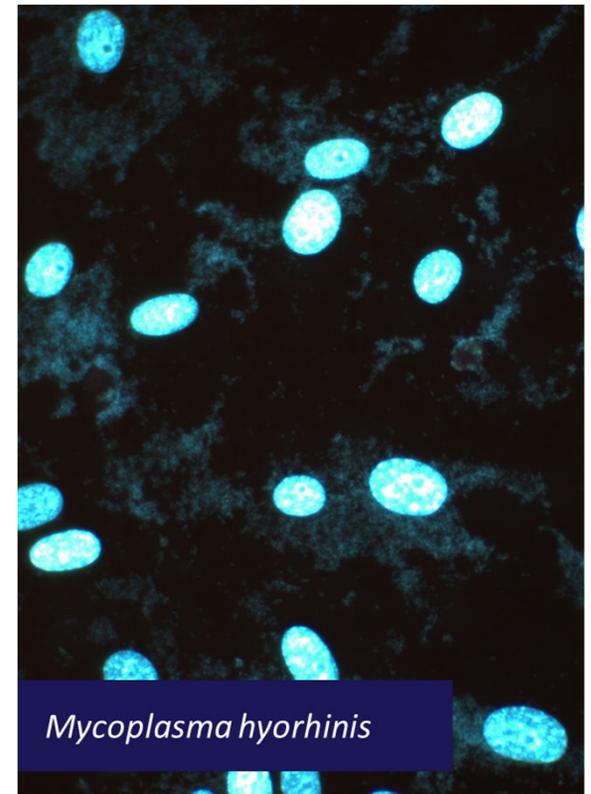
# Indirect Hoechst DNA staining

## Advantages

- Easy to perform
- Rapid analysis
- Cost effective

## Disadvantages

- Interpreting results can be challenging
- Stains all nucleic acids, so you cannot differentiate between:
  - Eukaryotes vs. prokaryotes
  - Mycoplasma vs. other bacteria



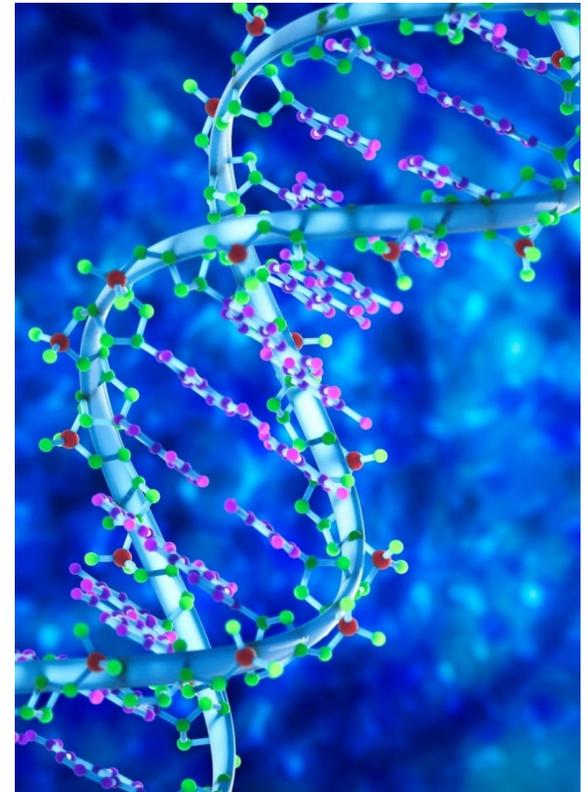
# PCR-based mycoplasma detection

## Advantages

- Easy to perform
- Reproducible
- High sensitivity and specificity
- Efficient
- Cost effective

## Disadvantages

- Cannot distinguish viable and non-viable cells
- Requires primers that are broad enough to amplify different mycoplasma, but specific enough to not amplify other bacterial contaminants
- Requires optimization



# ATCC Mycoplasma Testing Services

## Direct and indirect culture (bundled service)

- Direct culture – Uses both broth and agar
- Indirect culture – Hoechst DNA stain

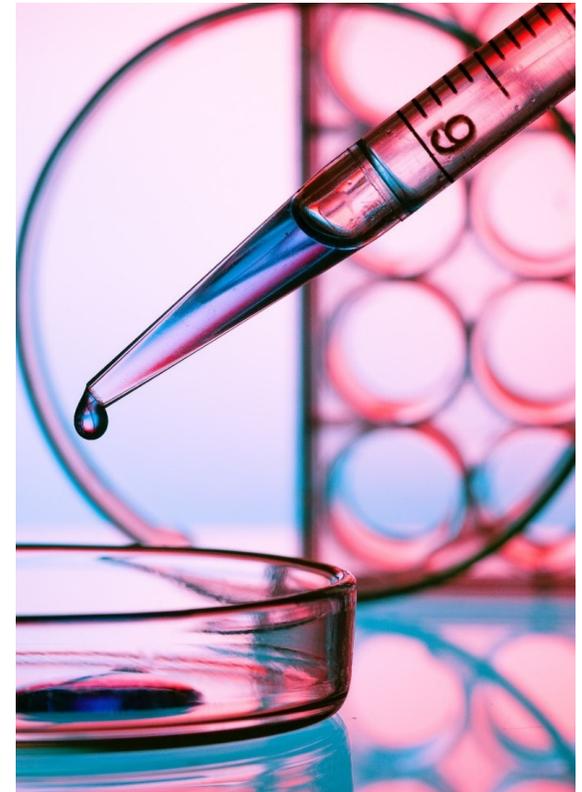
## PCR-based testing service – New!

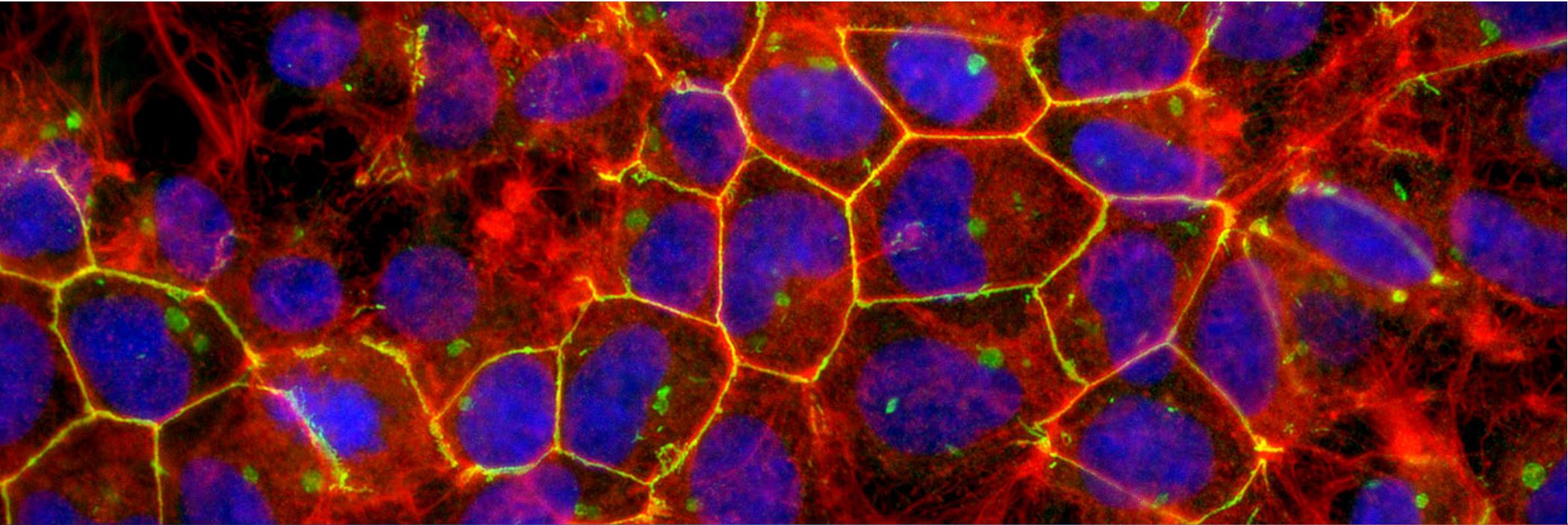
- Sample spotting on FTA paper and mycoplasma detection by using the ATCC Universal Mycoplasma Detection Kit

## ATCC Universal Mycoplasma Detection Kit

- A PCR-based assay that can be purchased and run in the researcher's laboratory

**Analysis performed following ISO 9001:2015 and ISO/IEC 17025:2017 quality standards**

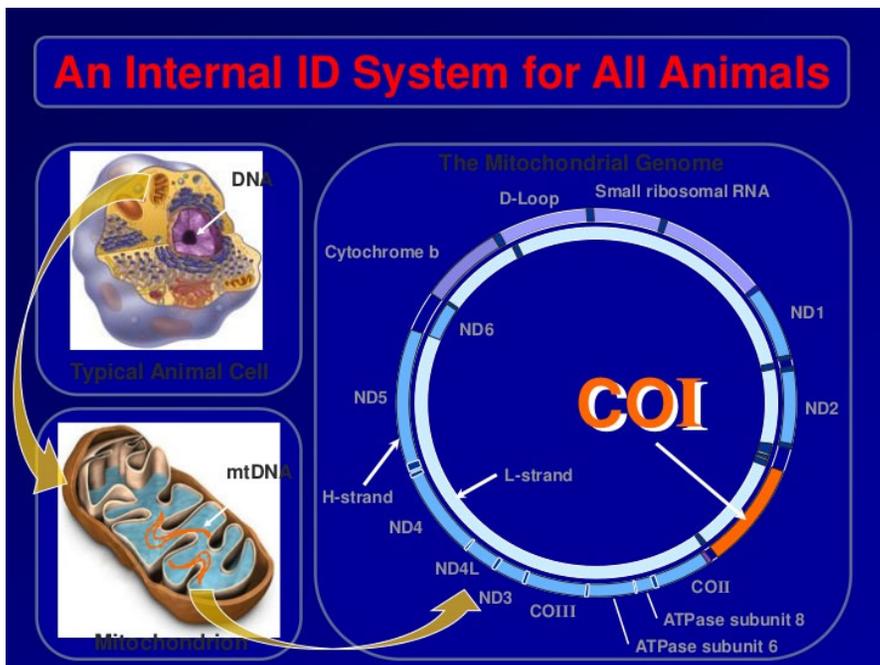




# Cytochrome Oxidase (CO1) Barcoding

HEK293 cells expressing ZO1

# Cytochrome C oxidase I as a DNA Barcode



**COI shows divergence between one species and another**

**Yet conspecific individuals diverge very slightly**

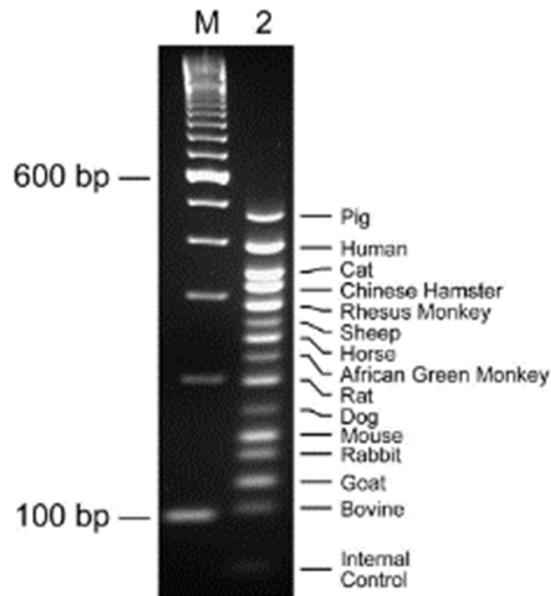
## Cytochrome C oxidase, subunit I (COI)

- Mitochondrial gene
- Part of the electron transport chain universal to all animal species

**Present in large copy number compared with nuclear**

- Amplification of mitochondrial genes is much more robust
- Inheritance of mitochondrial genes is maternal, animals typically have only one variant of each MITO gene
- Mitochondrial genes show more divergence than many other nuclear genes

# CO1 Barcoding at ATCC



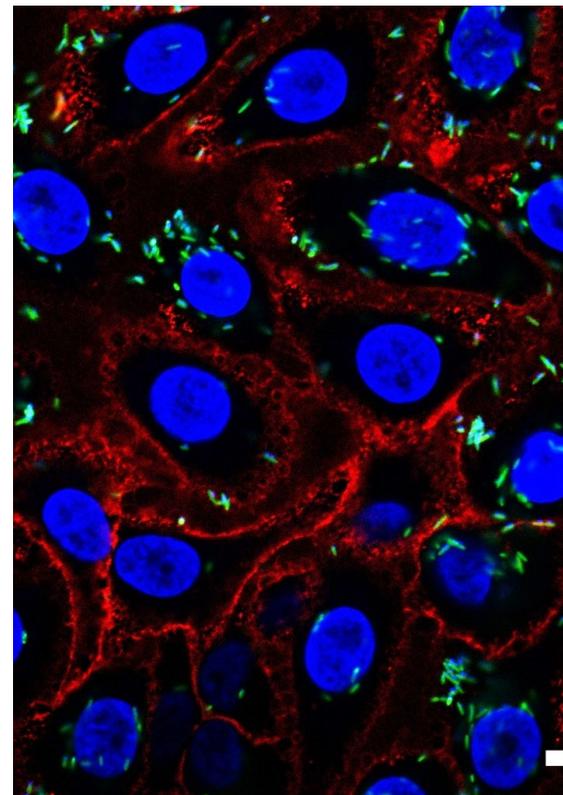
Amplified fragments were detected by ethidium bromide staining on a 4% agarose gel. Lane 1 shows the 100 bp ladder. Lane 2 shows the multiplex performance of oligonucleotide pairs specific for the following 14 species: pig, human, cat, Chinese hamster, Rhesus monkey, sheep, horse, African green monkey, rat, dog, mouse, rabbit, goat, and bovine. The template for the reactions consisted of 0.5 -1.0 ng mixed DNA contributed from all of the species with primers in the master mix.

- Provided for all ATCC cell lines
- Replaces isoenzymology
- Multiplex PCR-based assay
- Capable of distinguishing cell lines of pig, human, cat, Chinese hamster, Rhesus monkey, sheep, horse, African green monkey, rat, dog, mouse, rabbit, goat and cow origin
- When the species of a cell line remains in question a ~650bp 'barcode' region of the COI gene is sequenced for verification

**Analysis performed following ISO 9001:2015 and ISO/IEC 17025:2017 quality standards**

## Conclusions

- **Understand the impact of misidentified and contaminated cell lines and appreciate the best practices to counter this impact**
- **ATCC provides solutions**
  - STR profiling for cell line identity (human and mouse)
  - Mycoplasma detection methods
  - CO1 barcoding for species identity



Learn more: [www.atcc.org/services/cell-authentication](http://www.atcc.org/services/cell-authentication)

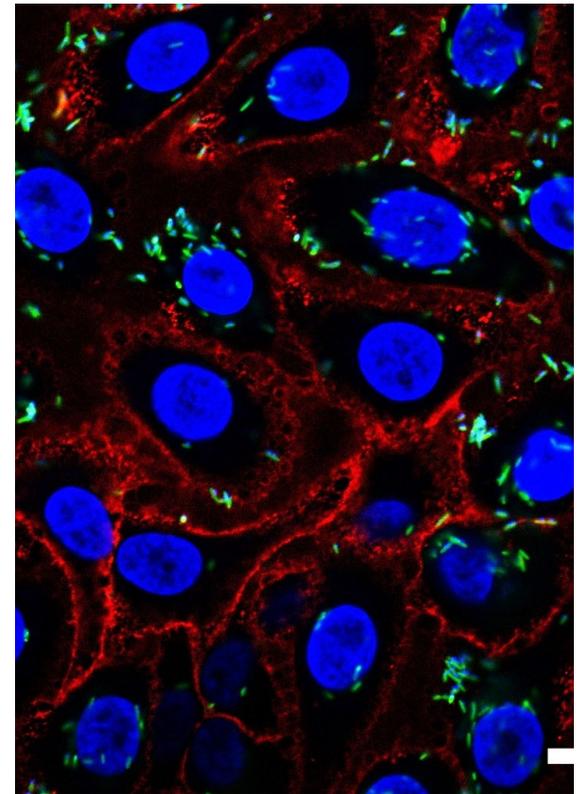
Coming soon!

**Addressing the Need for Accuracy and Traceability in  
Microbial Genomic Data: The ATCC Genome Portal**

Presenter: Jonathan Jacobs, PhD

November 18, 12:00 ET

**ATCC Webinars:** [www.atcc.org/webinars](http://www.atcc.org/webinars)



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