Solving Identity Crisis in Animal Cells: Best Practices with DNA Barcodes & STR Analysis

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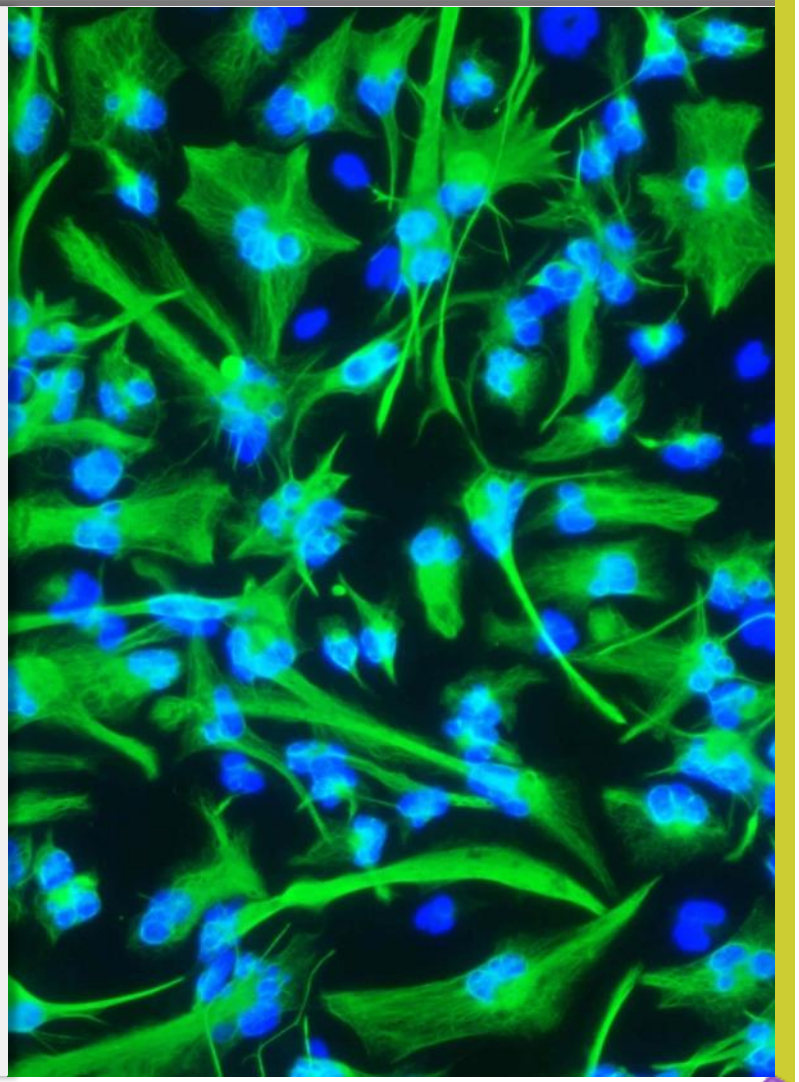
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees

Established partner to global researchers and scientists

Outline

- Define cell line authentication
- History of misidentified cell lines
- Confirm species
  - STR analysis for human cell line identification
  - Case studies
- Steps for reducing cellular and microbial contamination
- New regulations requiring cell line authentication



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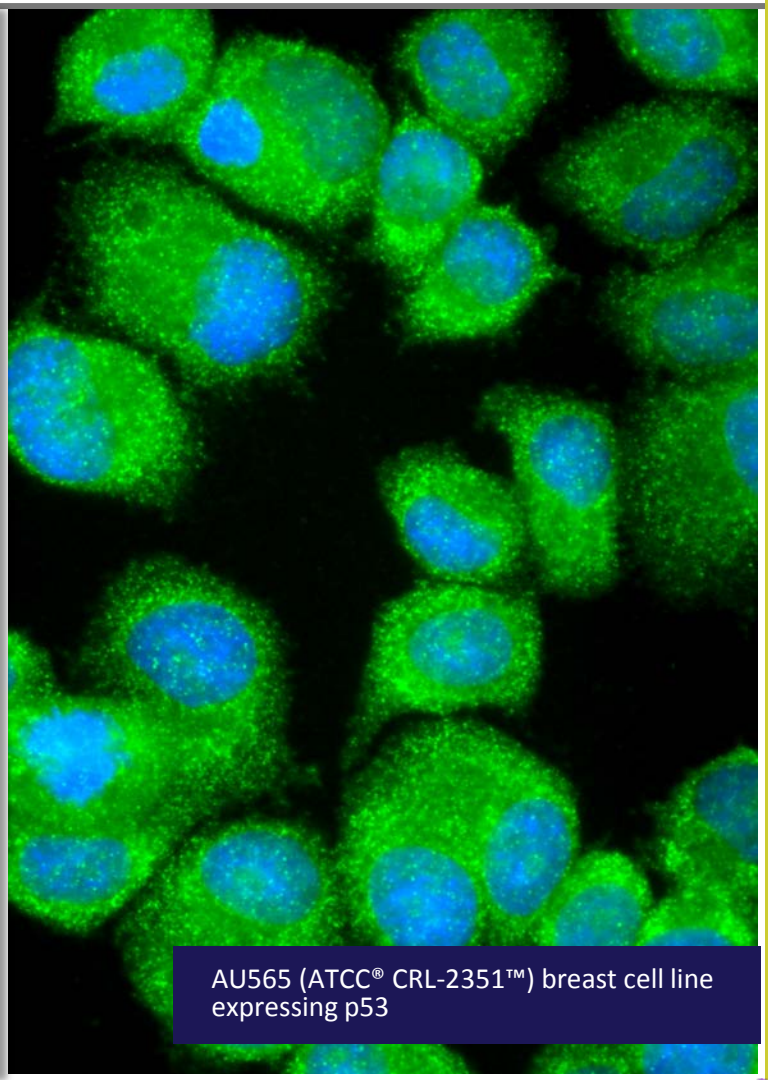
What is cell line authentication?

Cell line authentication – comprehensive approach:

- **Intraspecies identification by STR analysis**
- **Interspecies identification by CO1 assay**
- Tests for adventitious agents: mycoplasma, bacteria, fungi, viruses
- Confirm functional/unique characteristics

Note:

- Use cells at low population doubling levels or passage number to ensure that the cells maintain their unique characteristics when compared to the tissue of origin
- Optimize growth conditions by performing a growth curve



AU565 (ATCC® CRL-2351™) breast cell line expressing p53

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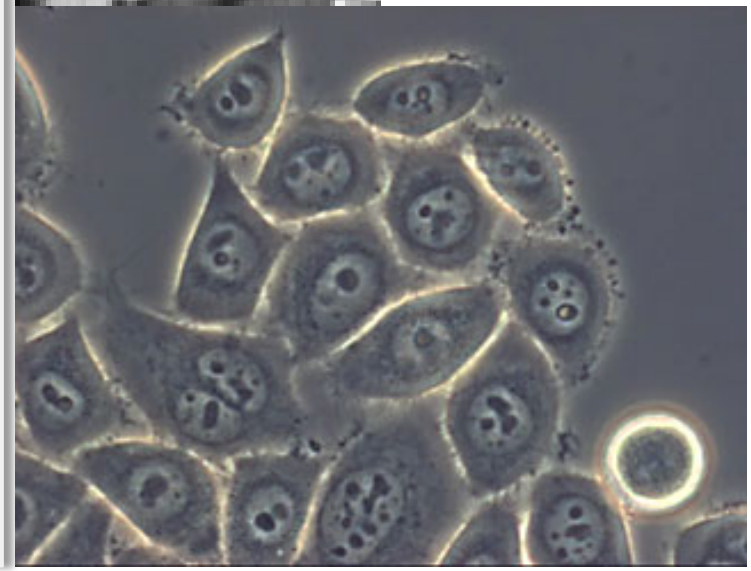
1960s: Poor culture conditions lead to contamination

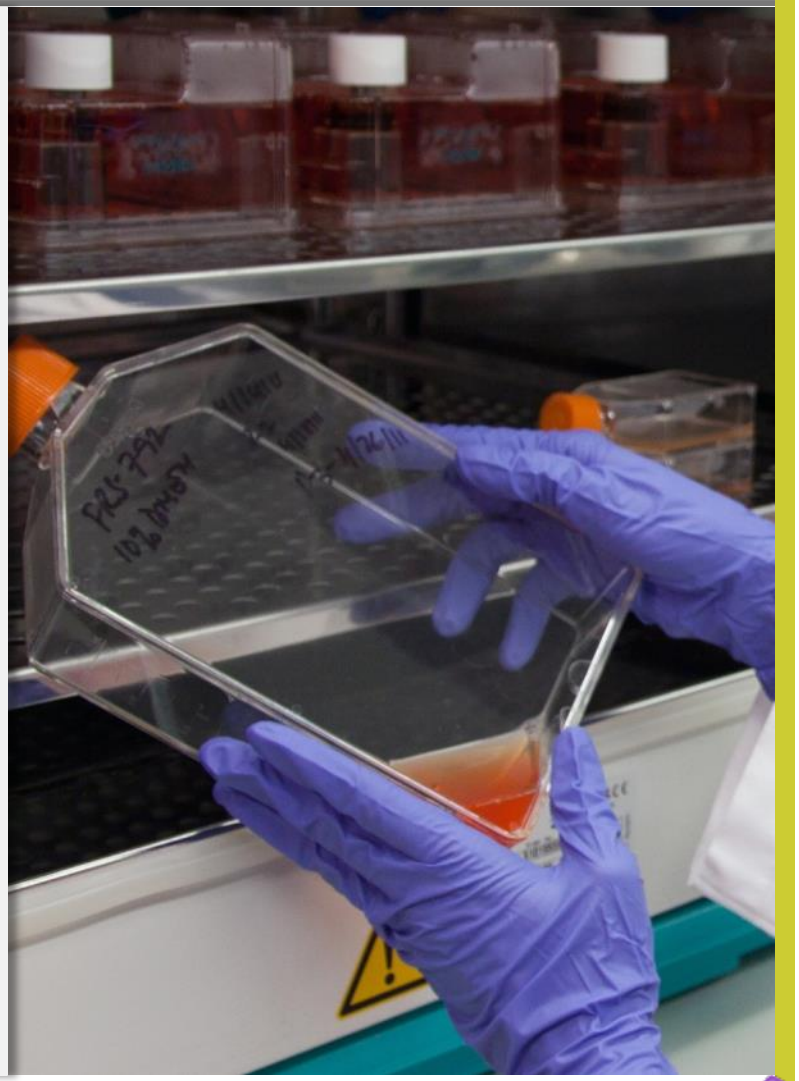
- Poor tissue culture environment
- No disposable, plastic culture dishes
- No commercial media
- No commercial sera
- Cells grown on benchtop
- No laminar flow hoods
- Cryopreservation of cell lines was not routine

1952 HeLa (ATCC® CCL-2™) - first human cancer cell line was derived from a glandular cervical cancer



Better tissue culture conditions - contamination persists…

- Recent data suggest that cross-contaminations are still a major ongoing problem with modern cell cultures
- Estimated that 1/3 of cell lines used are misidentified



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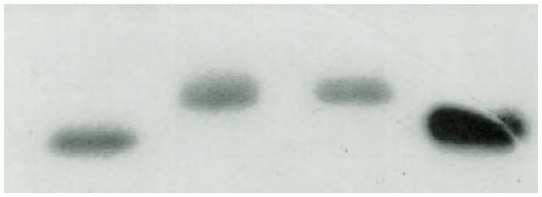
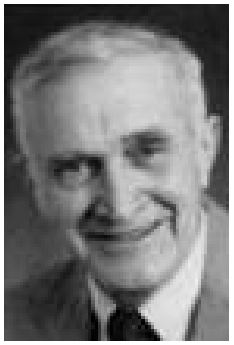
1962: Gartler describes HeLa contamination of cell lines

2nd Biennial Review Conference on Cell Tissue and Organ Culture, Bedford, Pa, 1962



Isoenzyme analysis
Glucose-6-phosphate dehydrogenase (G6PD)



Dr. Stanley Gartler

Name	Description	ATCC® No.	Origin	G6PD variant
HeLa	Cervical adenocarcinoma, human	CCL-2™	African	Type A (fast)
KB	Oral epidermoid carcinoma, human	CCL-17™	Caucasian	Type A (fast)
HEp-2	Larynx epidermoid carcinoma, human	CCL-23™	Caucasian	Type A (fast)
Chang liver	Liver, human	CCL-13™	Caucasian	Type A (fast)
Int-407	Embryonic intestine, human	CCL-6™	Caucasian	Type A (fast)

Conclusion: 90% (18/20) human cell lines are 'HeLa'

Gartler SM, NCI Monogr. 26:176, 1967; Gartler, SM, Nature 217:750, 1968.

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1980-2003: Interspecies and intraspecies misidentification of cell lines

Cellular cross-contamination					
Year	**No.**	**%**	**Type of contamination**	**Technology**	**Reference**
1981	446	9.2%	Inter- and intraspecies	Karyotyping	Nelson-Rees WA, *et al.* Science 212,446, 1981.
1984	275	35%	Interspecies	Karyotyping	Hukku B, *et al.* Eukaryotic cell culture. Plenum Press, 1984.
1999	252	18%	Intraspecies	STR profiling	Drexler HG, *et al.* Leukemia 13:1999.
2003	550	15%	Intraspecies	STR profiling	Drexler HG, *et al.* Leukemia 17:2003.

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2002-2013: Misidentification of cell lines persists…

Year	Title of article	Reference
2004	LCC15-MB cells are MDA-MB-435: A review of misidentified **breast and prostate** cell lines.	Clin Exp Metastasis 21(6):535, 2004.
2007	MDA-MB-435: The questionable use of a melanoma cell line as a model for **human breast** cancer is ongoing.	Cancer Biology & Therapy 6:9, 1355, 2007.
2008	Deoxyribonucleic acid profiling analysis of 40 human **thyroid cancer** cell lines reveals cross-contamination resulting in cell line redundancy and misidentification.	J Clin Endocrinol Metab 93(11):4331, 2008.
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	Misidentification of putative medullary **thyroid cancer** cell lines RO-H85-1 and RO-D81-1	J Clin Endocrinol Metab 98(3):954, 2013.
2013	Beware of imposters: MA-1, a novel MALT lymphoma cell line is misidentified and corresponds to Pfeiffer, a diffuse large B-cell lymphoma cell line	Genes, Chromosomes and Cancer 52 (10):986, 2013.

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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.

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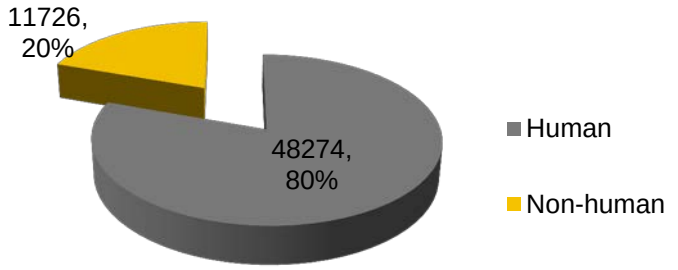
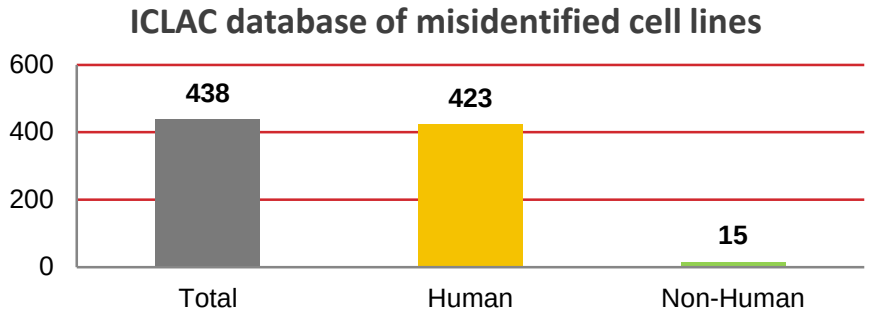
The use of misidentification of cell lines is widespread!

- The majority (80%) of cell lines used in pre-clinical cancer research are human in origin
- About 20% are non-human
- Need for cell line authentication for both human and non-human cell lines



Cellosaurus – Ontology of over 60,000 cell lines; about 80% are human cell lines; and 20 % are non-human cell lines (http://web.expasy.org/cellosaurus/)



Over 96% of misidentified cell lines in the ICLAC database are human in origin (*ICLAC data base of misidentified cell lines: (http://iclac.org/databases/cross-contaminations/)*

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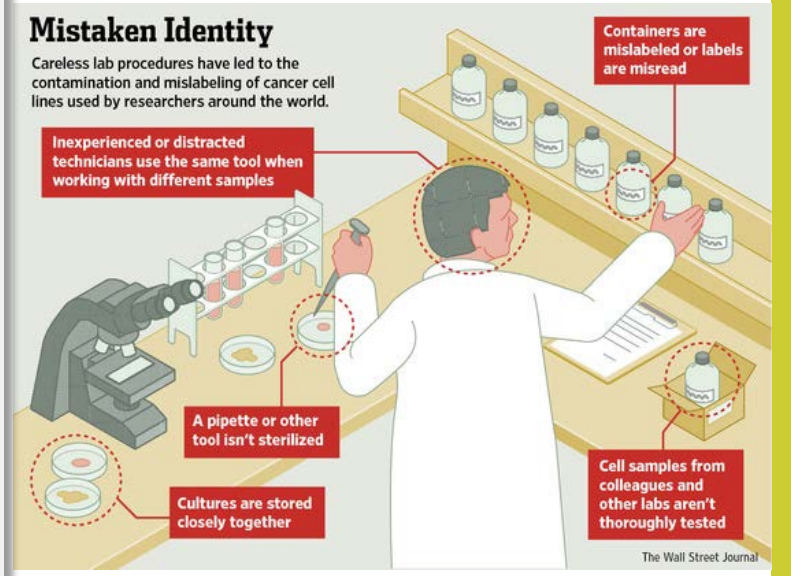
Common sources of misidentification

Common sources of cellular contamination

- Getting cell lines from a colleague down the hall
- Continuous culturing of working cell banks
- Use of feeder cells
- Mislabeling of culture flasks
- Working with multiple cell lines, concurrently
- Using one reservoir of growth medium for multiple cell lines

Are your cells REALLY what you think they are?

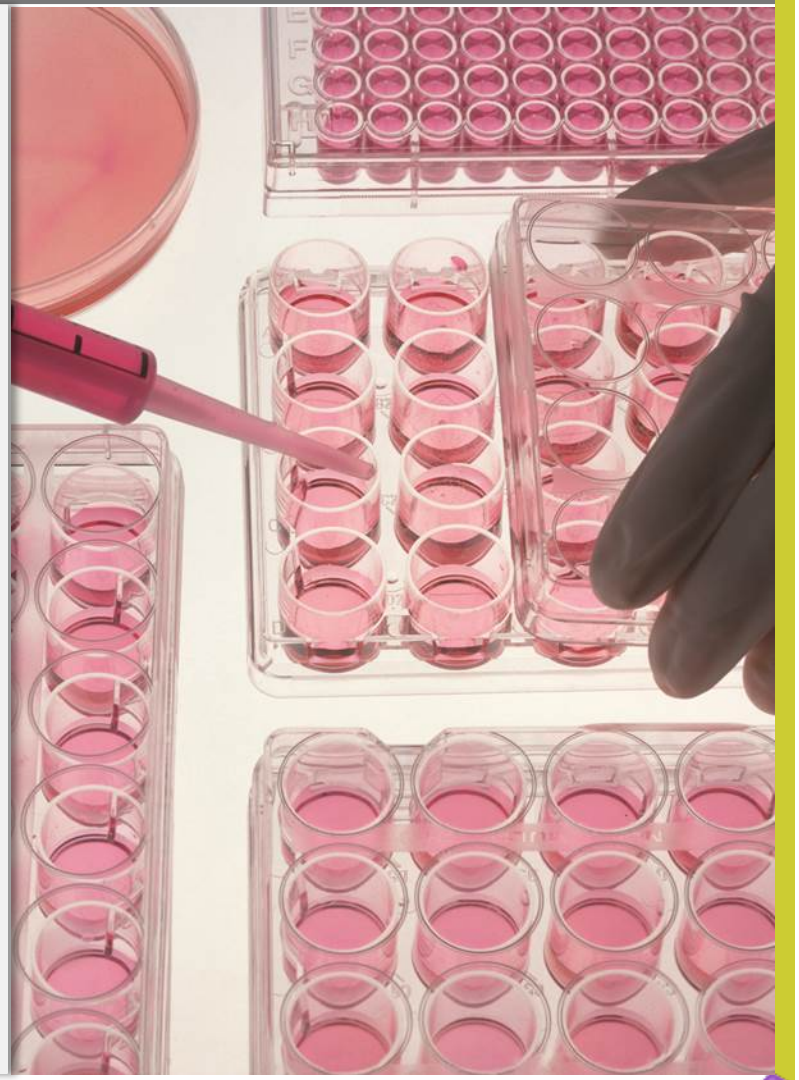


Consequences of using misidentified cell lines

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



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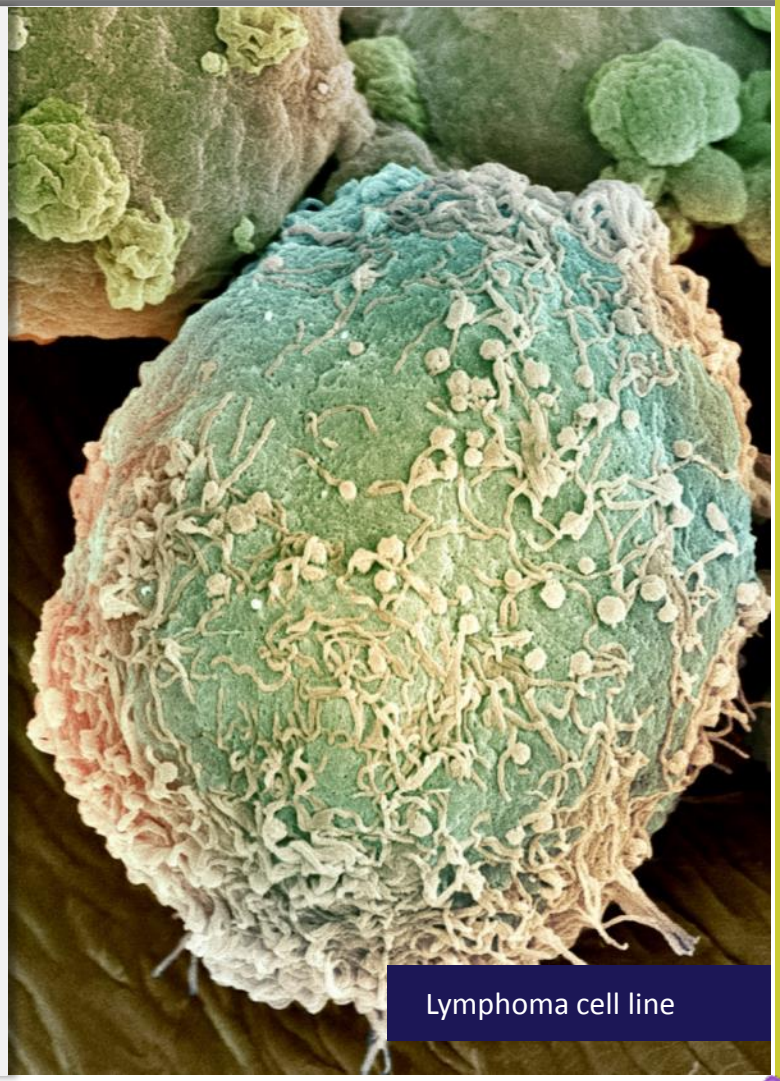
Determining cell line identity – an important first step for cell line authentication

Molecular Technology

- **Intraspecies identification (within species) of human cell lines**
  - **STR profiling: variation in the number of tandem repetitive sequences**
  - SNP analysis: variation in single nucleotide – polymorphism
- **Interspecies identification (between species)**
  - **CO1 analysis: amplification of mitochondrial cytochrome C oxidase 1 gene**
  - Karyotyping: differences in metaphase chromosome numbers for each species

Note: **Isoenzyme analysis**: post-translational modification of enzymes (**no longer available**)



Lymphoma cell line

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Short Tandem Repeat (STR) analysis for intraspecies identification of human cell line

DNA location	Degree of repetition	Number of loci	Repeat unit length
Satellite DNA (centromere)	10^3 to 10^7	1 to 2	2 to several thousand bp
Minisatellite DNA (telomere)	2 to several hundred	Many thousands	9 to 100 bp
Microsatellite DNA (STRs); randomly scattered	**5 to about a hundred**	**10^4 to 10^5**	**1 to 6 bp**

STR profiling - a method for human cell line authentication!

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STR analysis for human cell line identity

- Target sequence consists of microsatellite DNA, human-specific
- Typically use 1-2 ng DNA
- 1 to 2 fragments
- Discrete alleles allow digital record of data
- Banding pattern is reproducible
- PCR amplifiable, high throughput
- Small size range allows multiplexing
- Small product size compatible with partially degraded DNA
- Markers are highly informative
- Rapid processing is attainable

Outline of STR profiling procedure

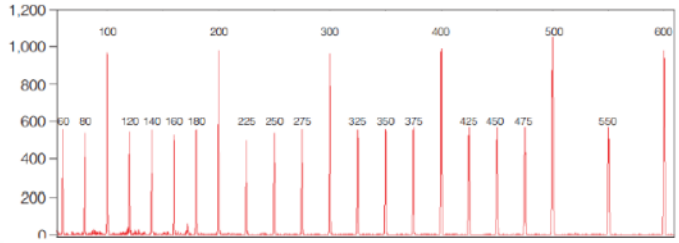
DNA sample
DNA on filter paper

Multiplex PCR → Capillary Electrophoresis (CE) → Data Analysis

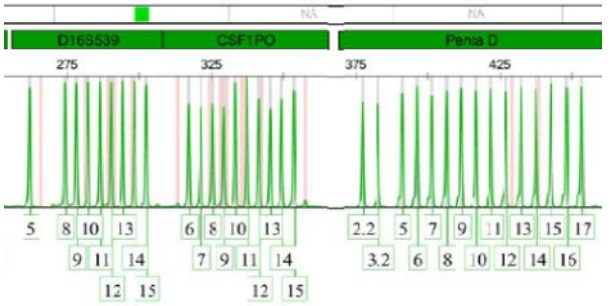
- Amplification of STR loci
- Simultaneous fluorescent labeling

- 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



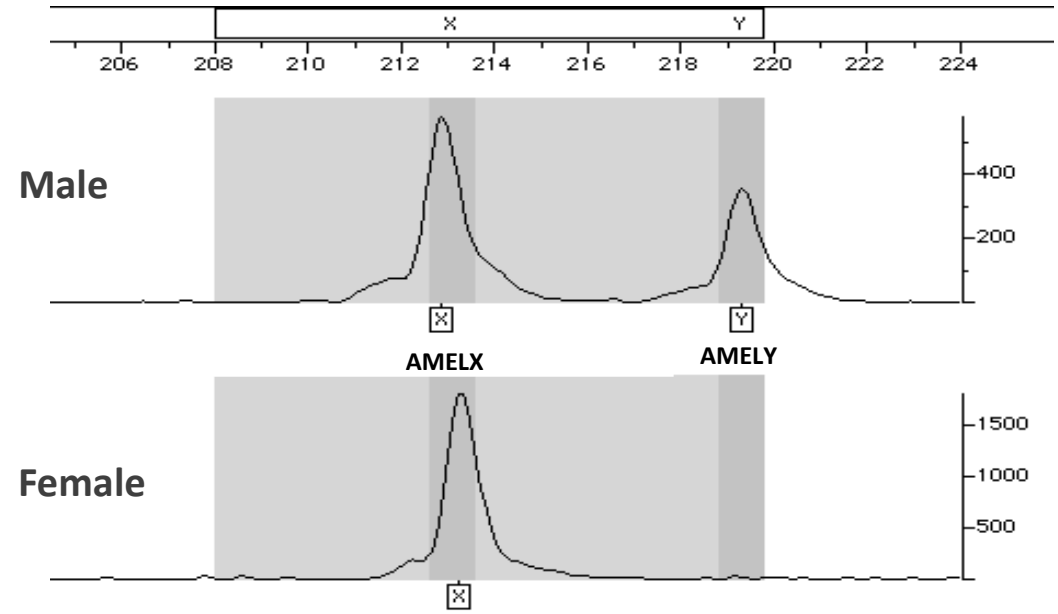
Internal Lane Standards

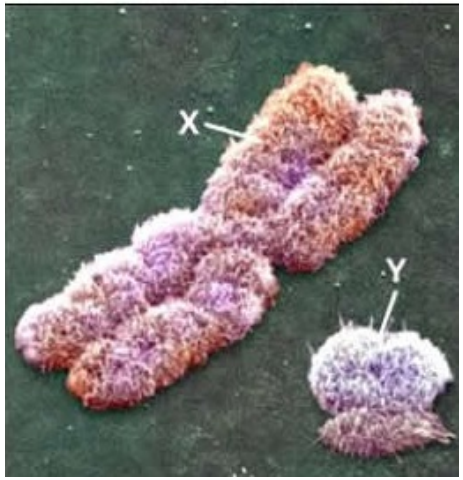


Allelic ladder (3 loci)

Gender is important for cell line identification

Amelogenin gene: AMELX (female), AMELY (male)





AMELX gene contains a 6 bp deletion in the intron 1

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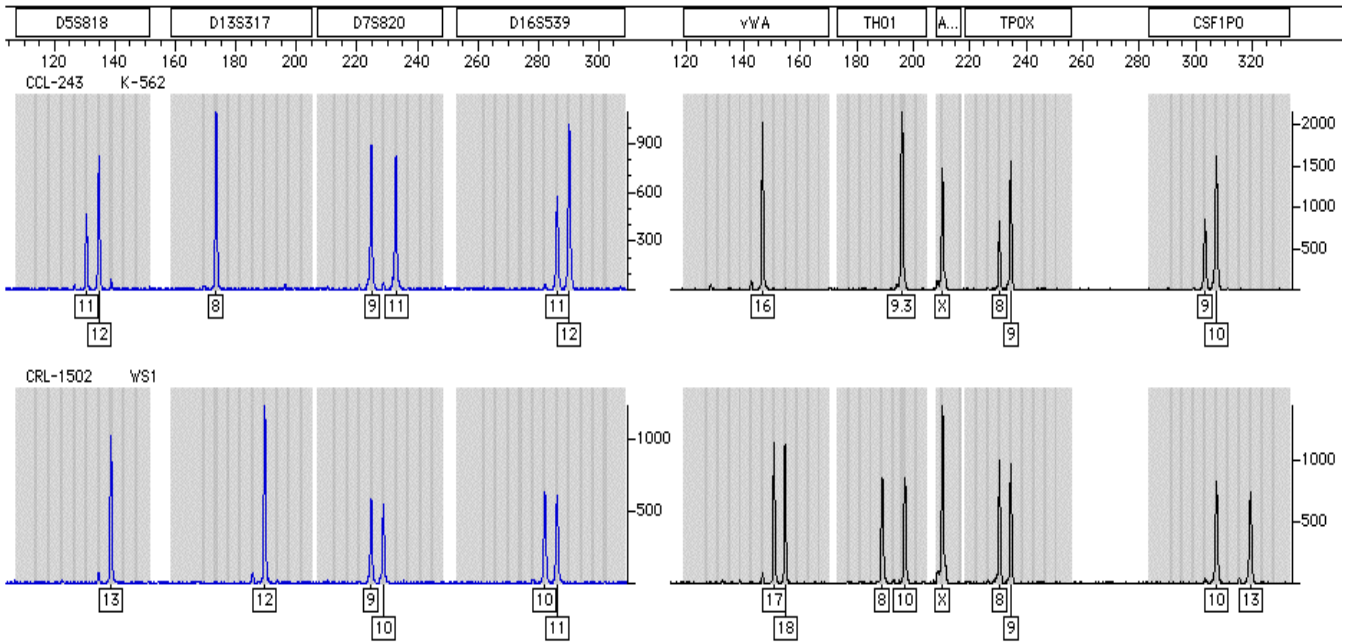
Unrelated human cell lines: STR analysis

K562

WS1



	D5S818	D13S317	D7S820	D16S539	vWA	THO1	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

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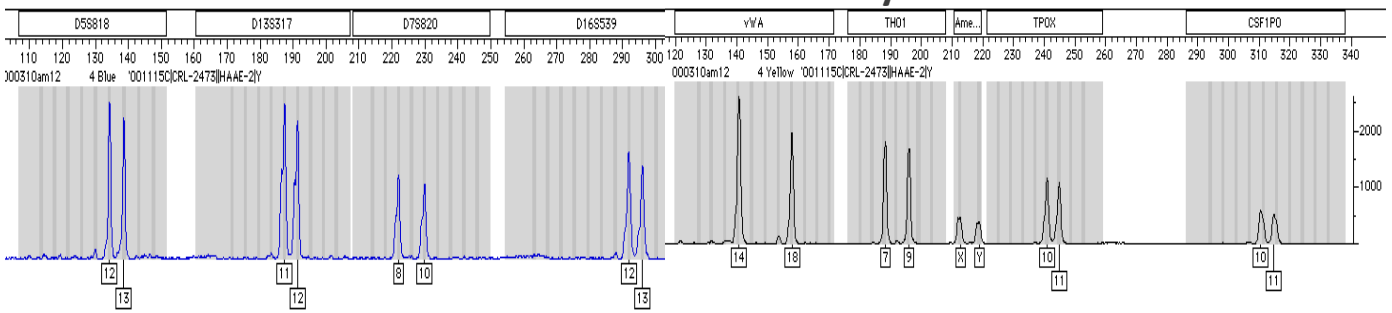
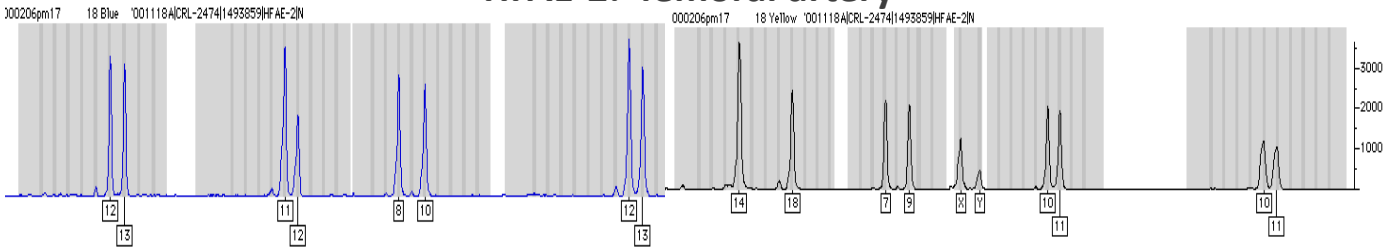
Related human cell line identification: STR analysis

HAAE-2: aortic artery



HFAE-2: femoral artery



	D5S818	D13S317	D7S820	D16S539	vWA	THO1	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

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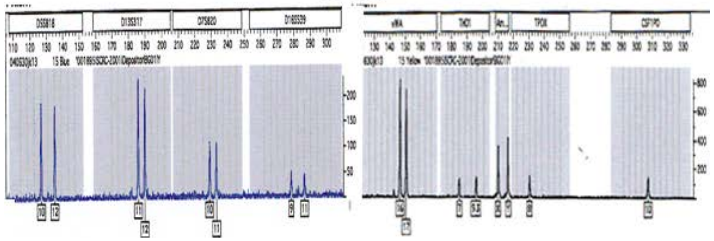
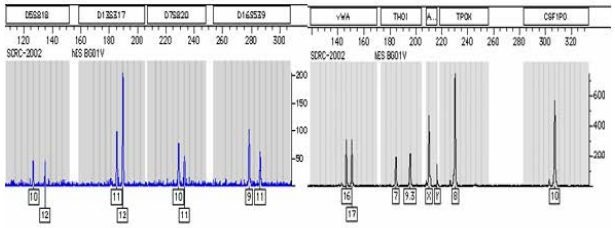
STR DNA profile links clone to parent

Clonal derivative has identical DNA profile to parental cell line

BG01 HuES (parent)

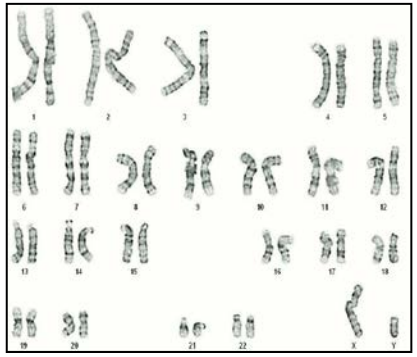


BGO1V HuES (clone)



	D5S818	D13S317	D7S820	D16S539	vWA	TH01	TPOX	CSF1PO	Amel.
BG01	10, 12	11, 12	10, 11	9, 11	16, 17	7, 9.3	8	10	X, Y
BG01V	10, 12	11, 12	10, 11	9, 11	16, 17	7, 9.3	8	10	X, Y

Karyotype (g-band)



Diploid; normal chromosomes

Aneuploid; +1 copy chrs. 12, 17, +1 copy chr. X

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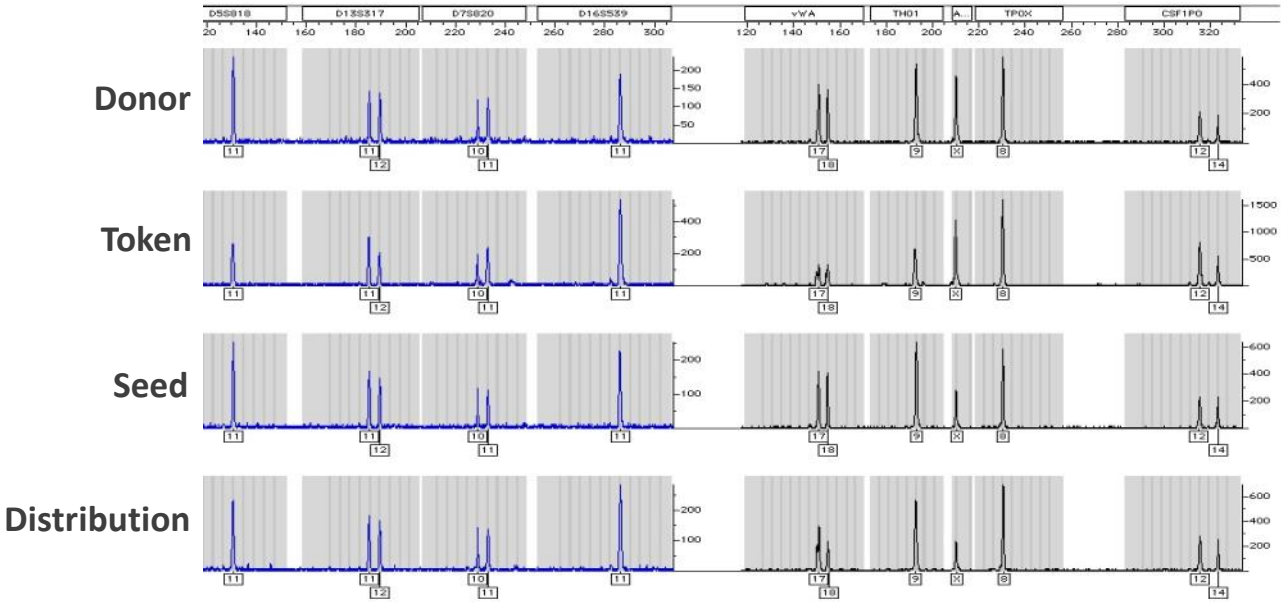
STR analysis for monitoring cell banks

Monitor…

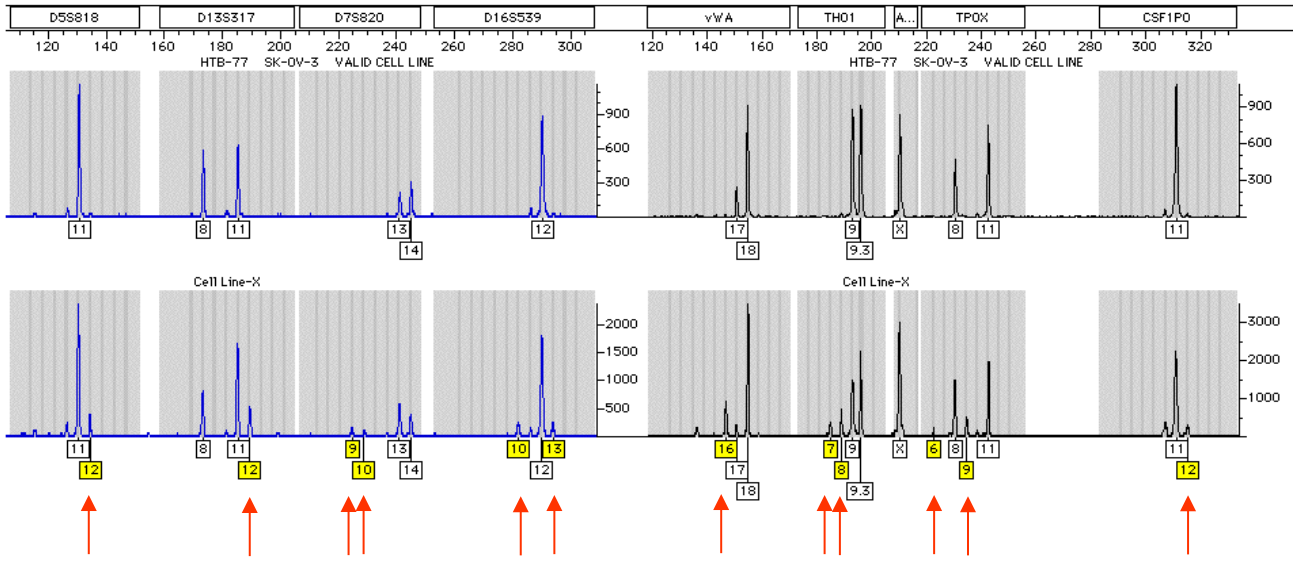
- Genomic stability
- Cellular contamination
- Misidentification



Case study 1: Cellular cross-contamination

SK-OV-3
Ovary

SK-OV-3 +
cell line X

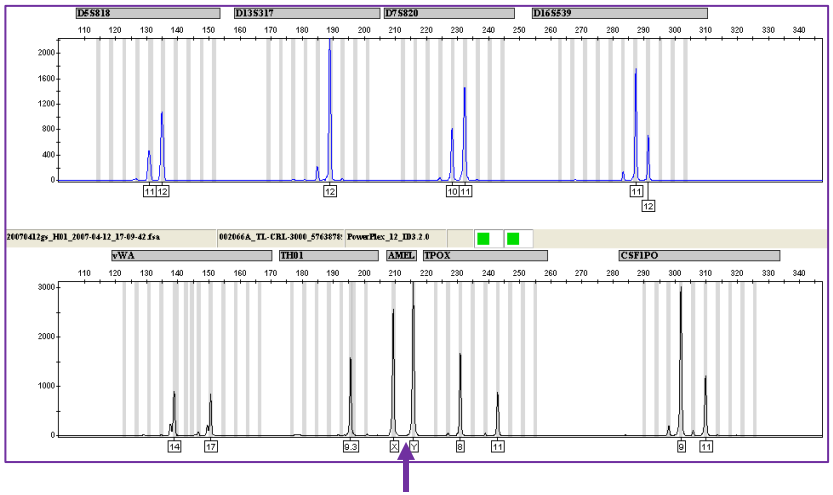


	D5S818	D13S317	D7S820	D16S539	vWA	THO1	Amel.	TPOX	CSF1PO
SK-OV-3	11	8,11	13,14	12	17,18	9,9.3	X	8,11	11
SK-OV-3 + cell line X	11,12	8,11,12	9,10,13,14	10,12,13	16,17,18	7,8,9,9.3	X	6,8,9,11	11,12

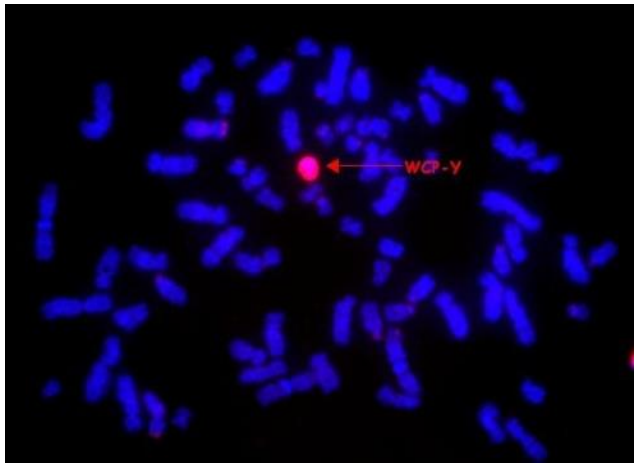
Case study 2: Gender misidentification

Human cell line purported to be of female origin

STR analysis



Y paint



Y material found by STR profiling and confirmed by Y chromosome paint

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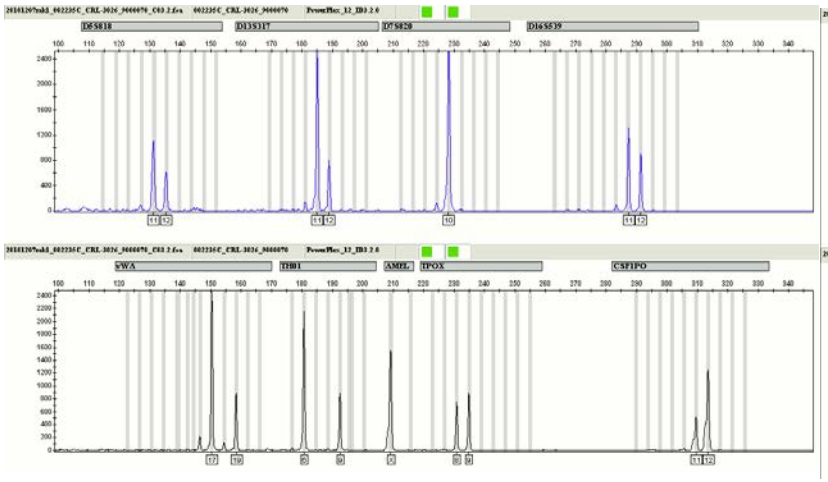
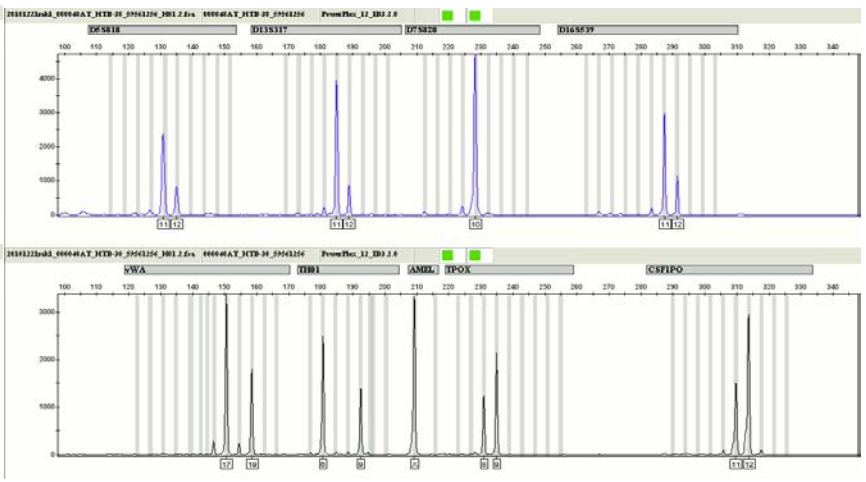
Case study 3: Misidentified cell line

Cell line X



HT-29 (ATCC®HTB-38™)



	D5S818	D13S317	D7S820	D16S539	CSF1PO	vWA	TH01	Amel.	TPOX
Cell line X	11.12	11,12	10	11,12	11,12	17,19	6,9	X	8,9
HT-29	11.12	11,12	10	11,12	11,12	17,19	6,9	X	8,9

HT-29: Human colon adenocarcinoma cell line

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*
- **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.

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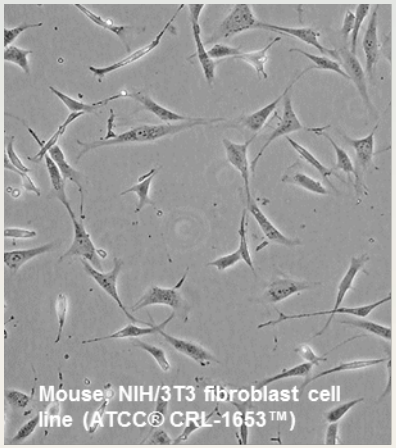
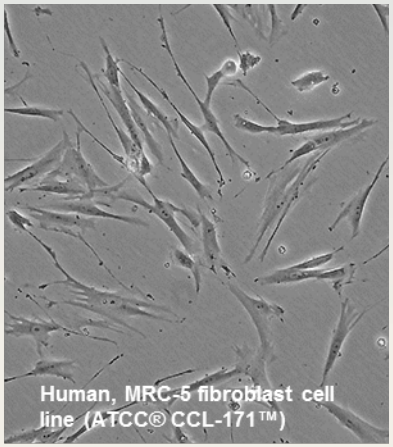
Interspecies Identification of Animal cell lines by CO1 barcode

Jason Cooper, M.S.
Adjunct Professor
Community College of Beaver County

Identifying species in animal cell culture

- Although animals have a variety of shapes and sizes, the cell lines derived from these animals do not
- Looking at cells under a microscope will not provide species identification
- Under the microscope, the pig and cow look very similar to each other
- Yet, cell lines are essential model systems for research.
- How can we have confidence in the identity of our models systems?





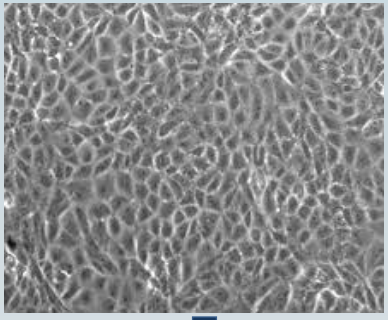
The voucher specimen

- A 'voucher specimen' is any specimen
  - Usually, but not always, a cadaver
  - Serves as a basis of study and is retained as a reference
- ‘Specimen' means the whole animal or a part thereof
  - A voucher should be in an accessible collection
  - Even if it is not, it remains a voucher

S. spilosoma, S. tridecemlineatus, and A. leucurus. **Source***:* Center for Disease Control Public Health Image Library ID#14313

Making the connection: cell line with voucher

- DNA provides the bridge to connect a cell culture model in a laboratory with a voucher specimen sitting in a museum
- **To use DNA, it is essential to:**
  - Choose the correct gene target
  - Use optimized primers
  - Use the correct analysis parameters



Cell line

DNA

Voucher specimen

Source: Daderot, https://en.wikipedia.org/wiki/Zoological_specimen#/media/File:Oslo_Zoological_Museum_-_IMG_9062.jpg

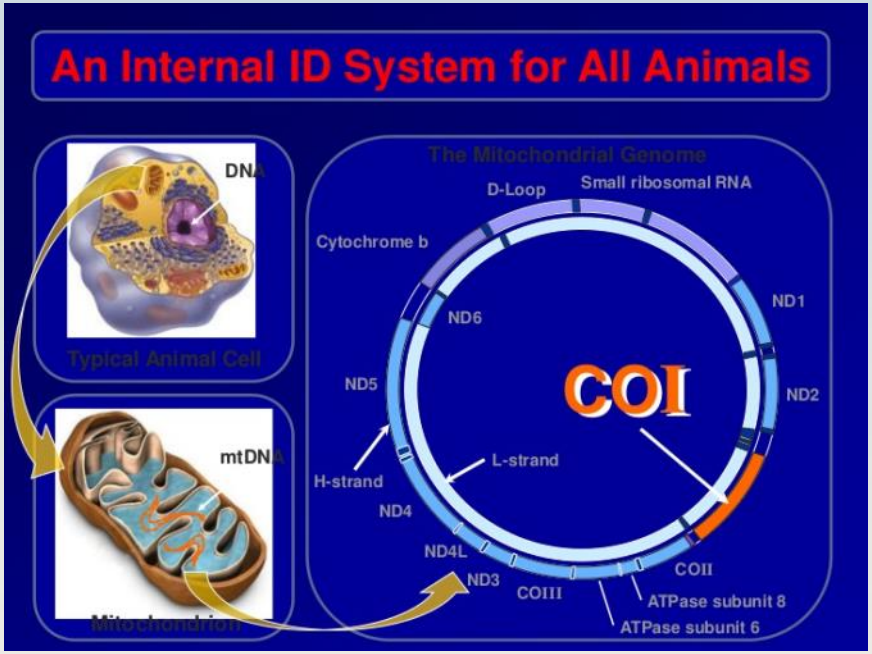
The DNA Barcode

- Species specific patterns of A, C, G, and T's can be used for identification
- Individuals within a species may vary slightly in sequence
- Yet, the demarcation between one species an another can usually be identified
- In this way, DNA serves as a barcode for species identification
- **The key is to choose the right gene target to serve as a barcode!**

DNA Barcode examples

Cytochrome C oxidase I as a DNA Barcode

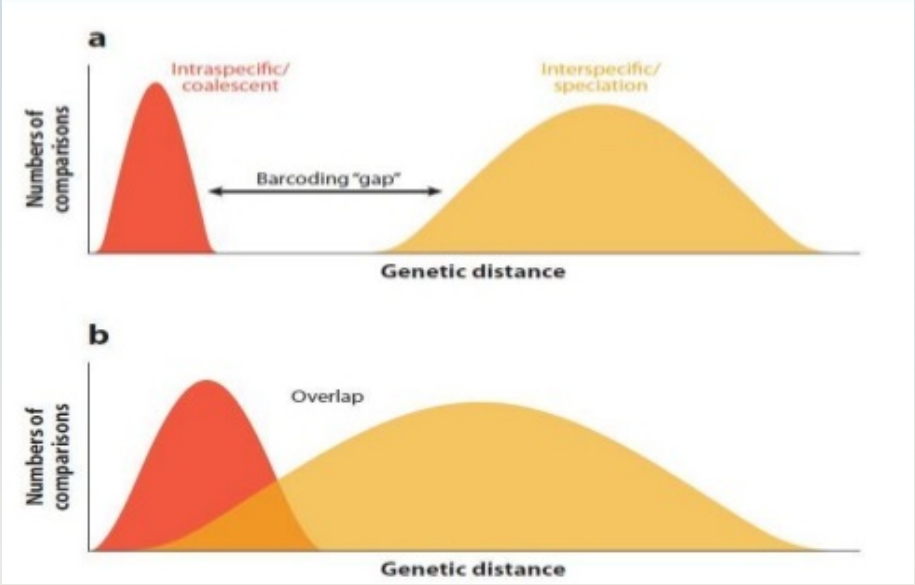
- Cytochrome C oxidase, subunit I (CO1)
  - Mitochondrial gene
  - Part of the electron transport chain universal to all animal species
- Present in large copy number compared with nuclear
  - Amplification of mitochondrial genes is much more robust
  - Inheritance of mitochondrial genes is maternal
- Thus, animals typically have only one variant of each mitochondrial gene



Source: http://www.barcodeoflife.org/psa/files/Schindel.pdf

Sequence diversity in CO1

- Mitochondrial genes show more divergence than many other nuclear genes
- CO1 shows divergence between one species and another
- Yet, conspecific individuals diverge very slightly
- The gap between the sequence cluster of conspecific individuals and individuals of other species is the barcoding gap
- This gap is critical to using DNA as a barcode and it is a special feature of the CO1 gene



Primers for CO1 amplification

Sequence of Barcoding PCR Primers

Primer Name	Primer Sequence (M13 tail is bolded and underlined)
VF1d_t1	**TGTAAAACGACGGCCAGT**TCTCAACCAACCACAARGAYATYGG
VR1d_t1	**CAGGAAACAGCTATGAC**TAGACTTCTGGGTGGCCRAARAAYCA
LepF1_t1	**TGTAAAACGACGGCCAGT**ATTCAACCAATCATAAAGATATTGG
LepR1_t1	**CAGGAAACAGCTATGAC**TAAACTTCTGGATGTCCAAAAAATCA

Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

- Primer cocktails designed to amplify a 648bp 'barcode' region from CO1 have been designed
- The primer cocktail picks up many taxa, but the reach is not completely universal
- A wide range of more targeted primer pairs have been designed, tested, and published
- We recommend trying the most universal cocktail first and moving to more targeted combinations if necessary

Controls for the Barcode assay

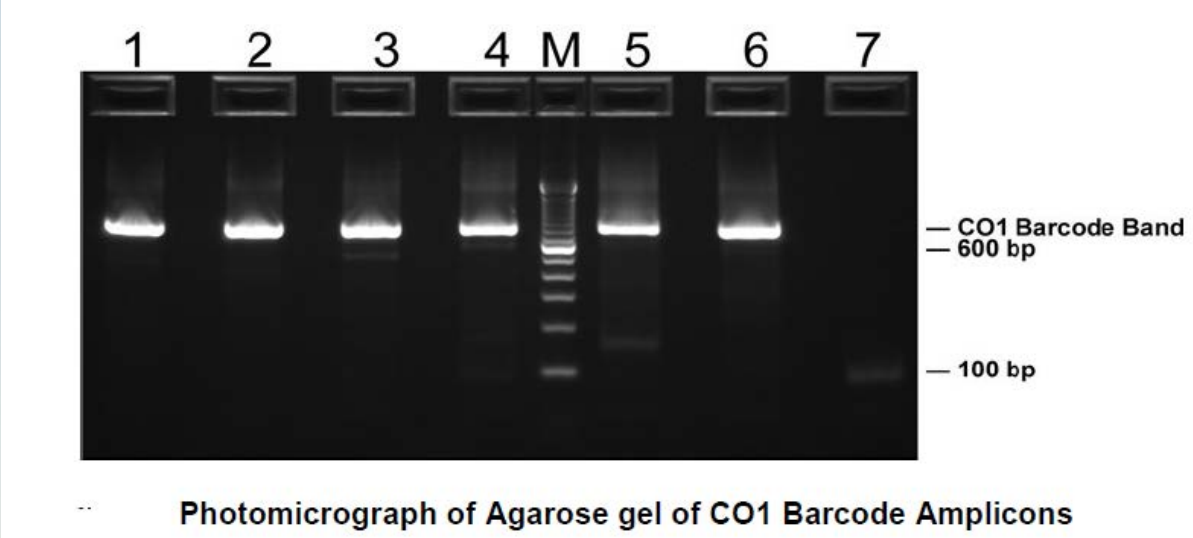
- ATCC houses cell lines from a wide variety of species
- Since the CO1 barcode is used for identification at ATCC, many of these cell lines have been tested
- These cell lines can then serve as positive controls for the barcode assay

PCR positive control DNAs from seven species

Species Group	Authenticated Source	Species
Amphibian	A6 (ATCC® CCL-102™)	*Xenopus laevis* (South African clawed frog)
Avian	QNR/D (ATCC® CRL-2532™)	*Coturnix coturnix japonica* (Japanese quail)
Fish	SJD.1 (ATCC® CRL-2296™)	*Danio rerio* (zebrafish)
Insect	Sf9 (ATCC® CRL-1711™)	*Spodoptera frugiperda* (fall army worm)
Mammalian	SIRC [*Statens Seruminstitut* Rabbit Cornea] (ATCC® CCL-60™)	*Oryctolagus cuniculus* (rabbit)
Reptilian	Gekko lung-1 (ATCC® CCL-111™)	*Gekko gecko* (Tokay gecko)
Example producing faint secondary band	CHO-K1 (ATCC® CCL-61™)	*Cricetulus griseus* (Chinese hamster) **Note:** This is an example of a DNA source that can produce both the expected PCR product along with a secondary product using these CO1 PCR primers.

Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

Amplicon from Barcode region



Photomicrograph of Agarose gel of CO1 Barcode Amplicons

Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

- Lanes 1 through 4: Experimental CO1 barcode samples. Note, some experimental samples in lanes 3 and 4 have secondary bands that should not interfere with sequence analysis.
- Lane 5: Positive control from CHO-K1 cells (Chinese Hamster Ovary), which also produces a known secondary band at ~120 bp.
- Lane 6: Positive control DNA from A6 cells *Xenopus laevis* (South African clawed frog).
- Lane 7: Water no template negative control, shows only the presence of the universal PCR primers. Lane M 100 bp Ladder (Invitrogen) agarose gel: 2% gel is an E-Gel .

Quality Sequencing Data

- Raw sequence data will often appear mixed
- Sequencing analyzers run algorithms that clean up the sequence data
- After the sequence data is cleaned up, clearly distinguishable peaks should be evident

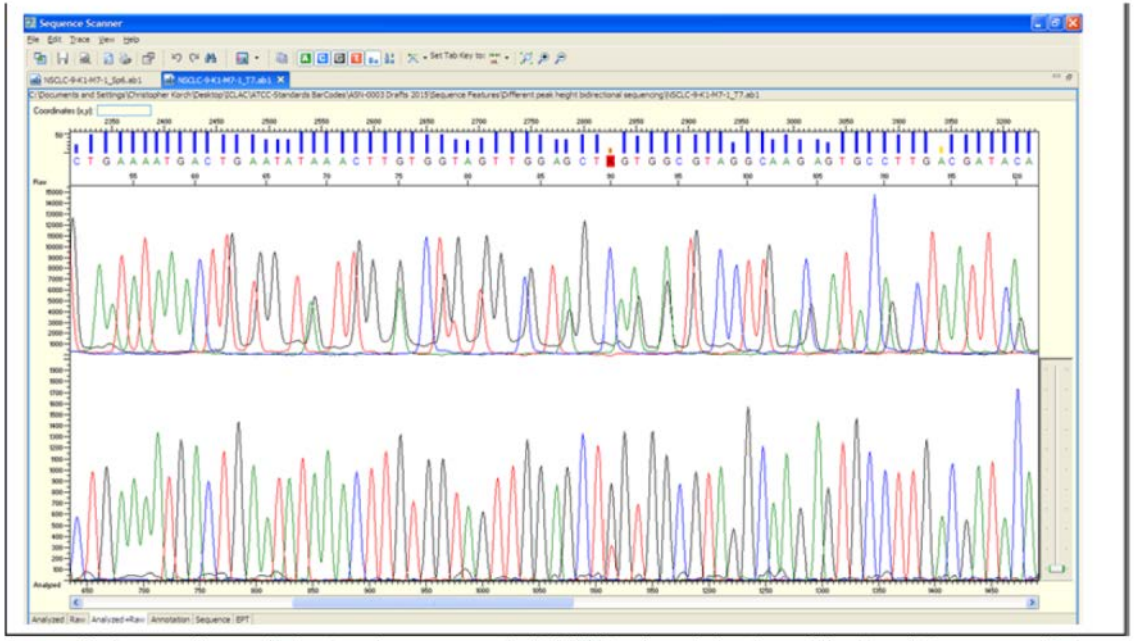
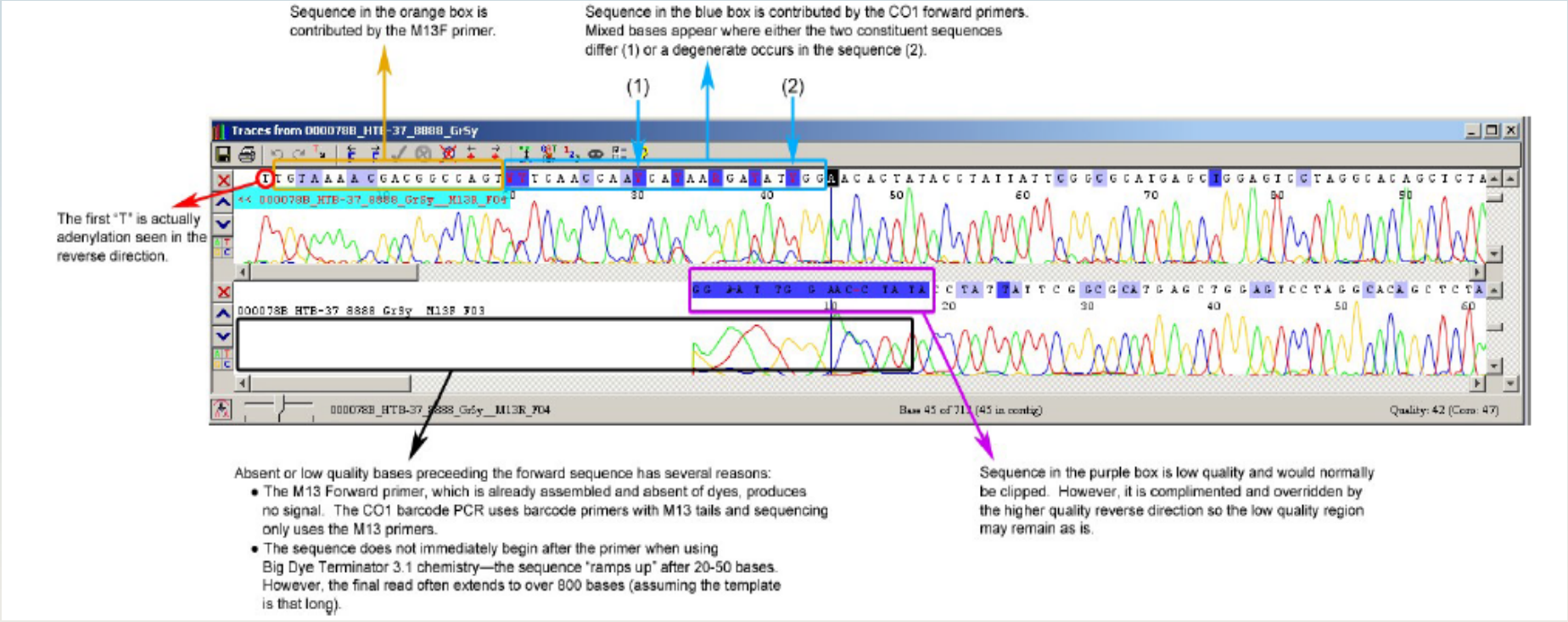


Figure 4: Comparison of electropherograms in RAW (top) and Analyzed (bottom) Sequence Data

Note that peak appears to co-migrate in the RAW data; this is because mobility corrections have not been done.

Building a Consensus Sequence



Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

Quality Data for Analysis

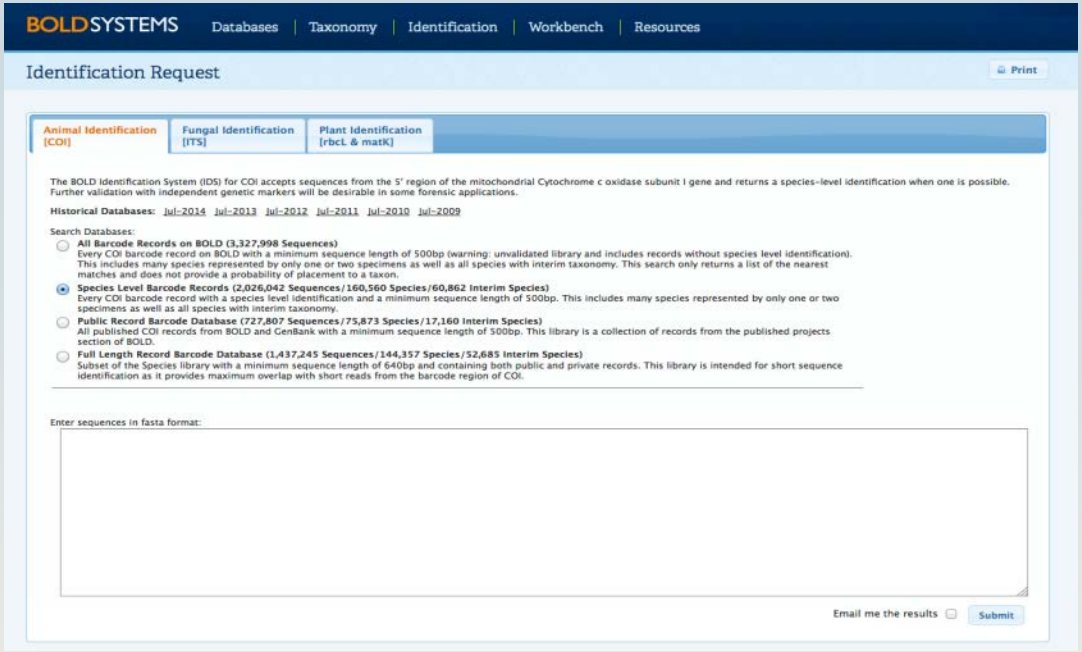
Quality Score Values and Associated Probabilities of Errors in Base Calling

Quality Score	Error Probability
10	1 in 10
20	1 in 100
30	1 in 1,000
40	1 in 10,000
50	1 in 100,000

- Quality scores asses probability of error at every position in the consensus sequence
- Generally Quality Scores above 20 are considered high enough for further analysis
- A barcode sequence should have 500 or more quality base calls (>QS20) to continue the analysis

BOLD ID Engine

- BOLD ID Engine www.boldsystems.org/index.php/IDS_OpenIdEngine
- The default options open this page to the “Animal Identification [CO1]” section, with the “Species Level Barcode Records” selected as the Database
- These options are appropriate for most purposes

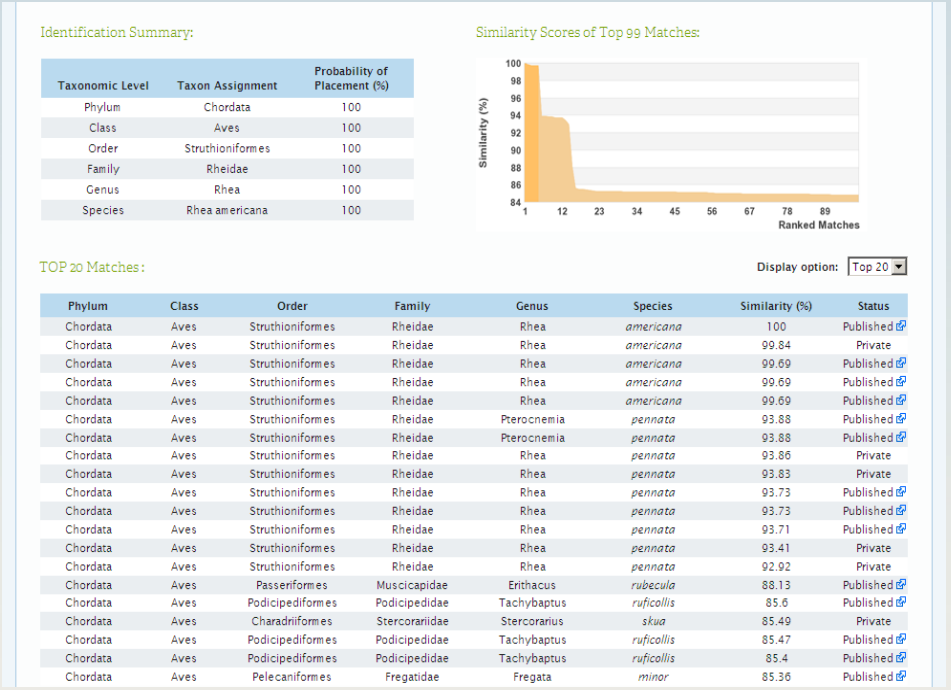


Screen capture: Animal Identification (CO1) section

Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

Identification

- This sequence closely matches (99.69%-100% identity) to five *Rhea americana* barcodes
- The next closest match is to *Rhea pennata* (92.92% to 93.88% identity)



Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Aves	100
Order	Struthioniformes	100
Family	Rheidae	100
Genus	Rhea	100
Species	Rhea americana	100

Similarity Scores of Top 99 Matches:

TOP 20 Matches:

Display option: Top 20

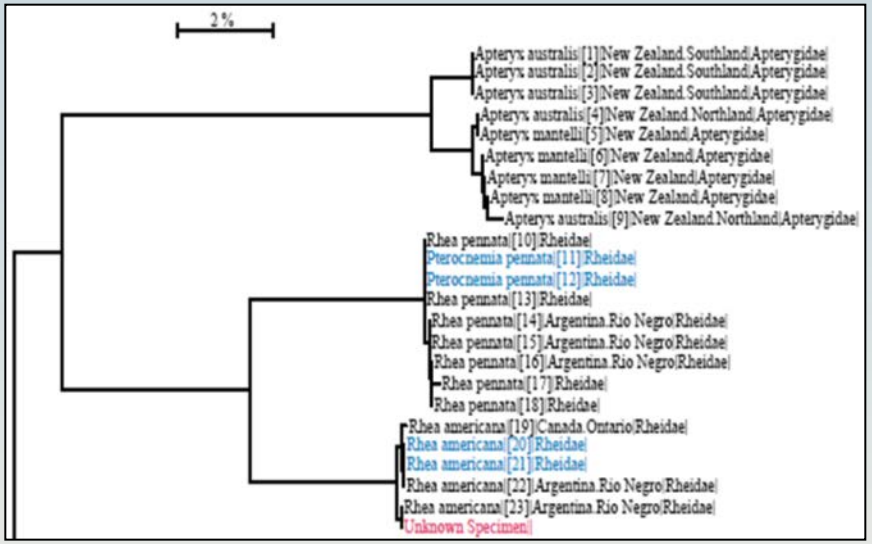
Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*americana*	100	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*americana*	99.84	Private
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*americana*	99.69	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*americana*	99.69	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*americana*	99.69	Published
Chordata	Aves	Struthioniformes	Rheidae	Pterocnemia	*pennata*	93.88	Published
Chordata	Aves	Struthioniformes	Rheidae	Pterocnemia	*pennata*	93.88	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	93.86	Private
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	93.83	Private
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	93.73	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	93.73	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	93.71	Published
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	93.41	Private
Chordata	Aves	Struthioniformes	Rheidae	Rhea	*pennata*	92.92	Private
Chordata	Aves	Passeriformes	Muscicapidae	Erithacus	*rubecula*	88.13	Published
Chordata	Aves	Podicipediformes	Podicipedidae	Tachybaptus	*ruficollis*	85.6	Published
Chordata	Aves	Charadriiformes	Stercorariidae	Stercorarius	*skua*	85.49	Private
Chordata	Aves	Podicipediformes	Podicipedidae	Tachybaptus	*ruficollis*	85.47	Published
Chordata	Aves	Podicipediformes	Podicipedidae	Tachybaptus	*ruficollis*	85.4	Published
Chordata	Aves	Pelecaniformes	Fregatidae	Fregata	*minor*	85.36	Published

Screen capture: Input from BOLD on Species Identification

Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

Tree-based Identification

- Links on the page take you to:
  - The Species Page
  - BIN Page
  - Tree Based Identification
- The tree is often helpful in visualizing the matches
  - Excerpt shown here with the query sample listed as “Unknown Specimen”

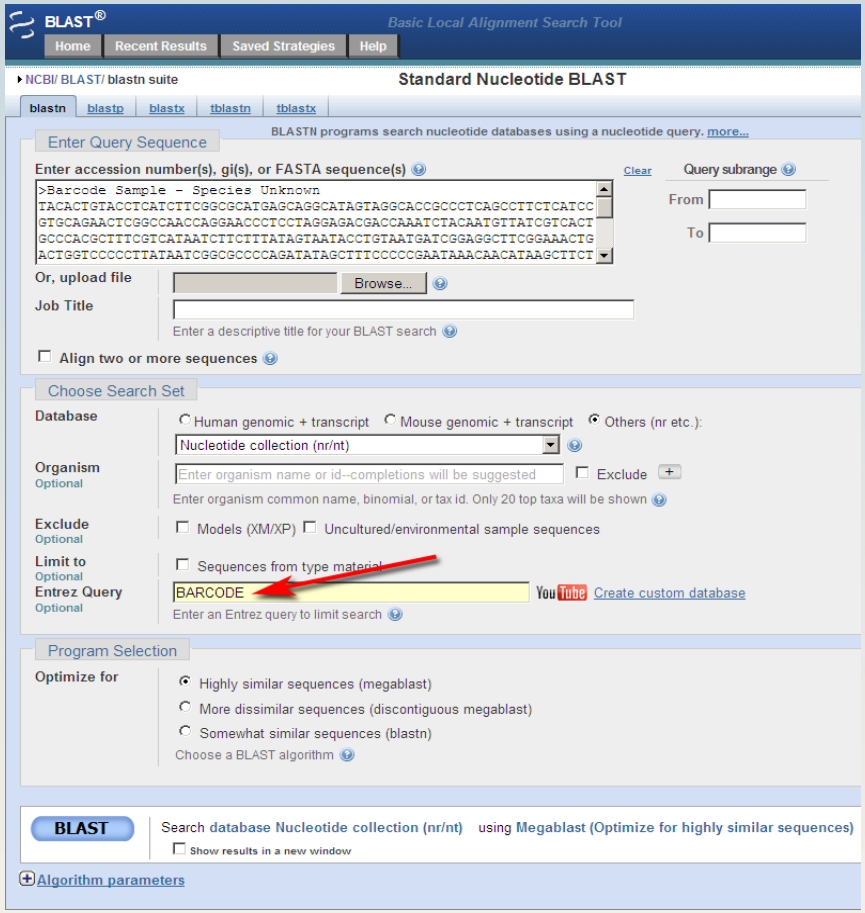


Species Tree

Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

NCBI BLAST

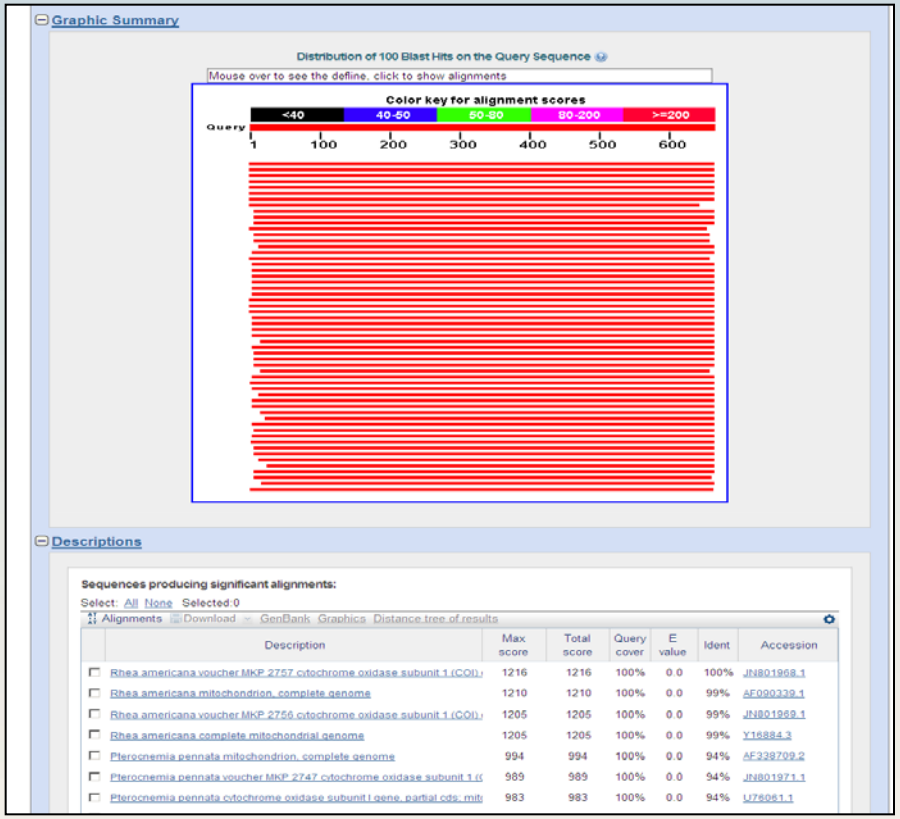
- If a close match is not achieved through a BOLD analysis, try searching the NCBI/GenBank using BLAST (Basic Local Alignment Sequence Tool)
- To use BLAST, go to http://blast.ncbi.nlm.nih.gov/Blast.cgi
- To run a BLAST analysis against only voucher specimens, enter “BARCODE” in the Entrez Query field, designated by the red arrow in the illustration



Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

BLAST Results

- Results are reported as shown
  - The Highest MaxScore on top
  - The percentage match is under "Ident," not MaxScore
- Clicking on the titles under "Description" shows how the sample and reference sequences align
- Clicking on the accession number opens a window with more information about the reference sequence, such as authors and journal publications



Source: *Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1) DNA Barcodes*. ANSI/ASN-0003-2015

New ANSI Standard Document

- Designation: ASN-0003
- Species-Level Identification of Animal Cells through Mitochondrial Cytochrome c Oxidase Subunit 1 (CO1)
- Identifying a specimen's place on the tree of life through DNA barcodes

Source:Williamguion98 [CC BY-SA 4.0 (http://creativecommons.org/licenses/by-sa/4.0)], via Wikimedia Commons

Take the time to identify…

- EPC (ATCC® CRL-2872™) - CO1 testing at ATCC revealed that EPC, originally deposited as a carp cell line (*Cyprinus carpio*), was in fact a fathead minnow cell line (*Pimephales promelas*)
- Since time of deposit, isoenzymology testing has correctly and consistently identified EPC as a fish cell line

However:

- Isoenzymology does not allow for speciation between genus:
  - Information regarding the species of fish was previously provided by the depositor
  - These observations were confirmed via CO1 testing of the original stock

Is it a carp?

Source: By Dezidor [GFDL (http://www.gnu.org/copyleft/fdl.html), CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0/)

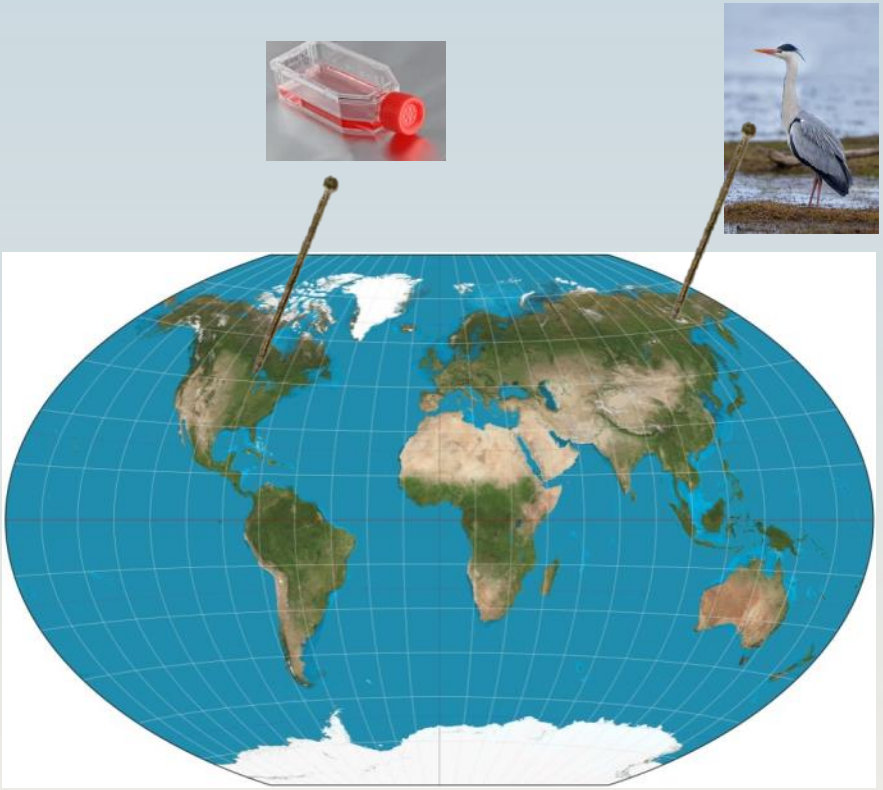
OR

Is it a minnow?

Source: Aimee Roberson, USFWS [Public domain], via Wikimedia Commons

Summary

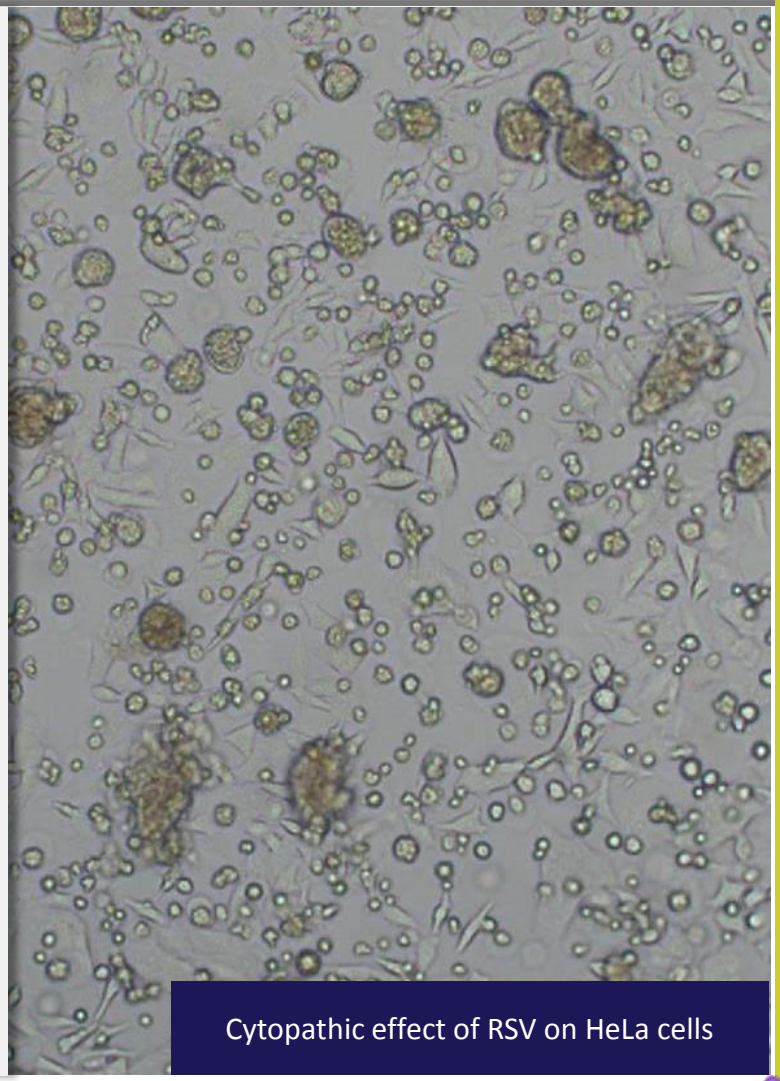
- The mitochondrial gene CO1 can be used as a DNA barcode
- The sequence from a segment of this gene can be analyzed easily with the BOLD or NCBI databases
- Species of animal cell lines can be identified at a high level of confidence
- In this way, a cell line or a cell culture model in a laboratory anywhere in the world can be connected with a voucher specimen sitting in a museum



Source: Andreas Trepte [CC BY-SA 2.5 (http://creativecommons.org/licenses/by-sa/2.5)], via Wikimedia Commons

Outline

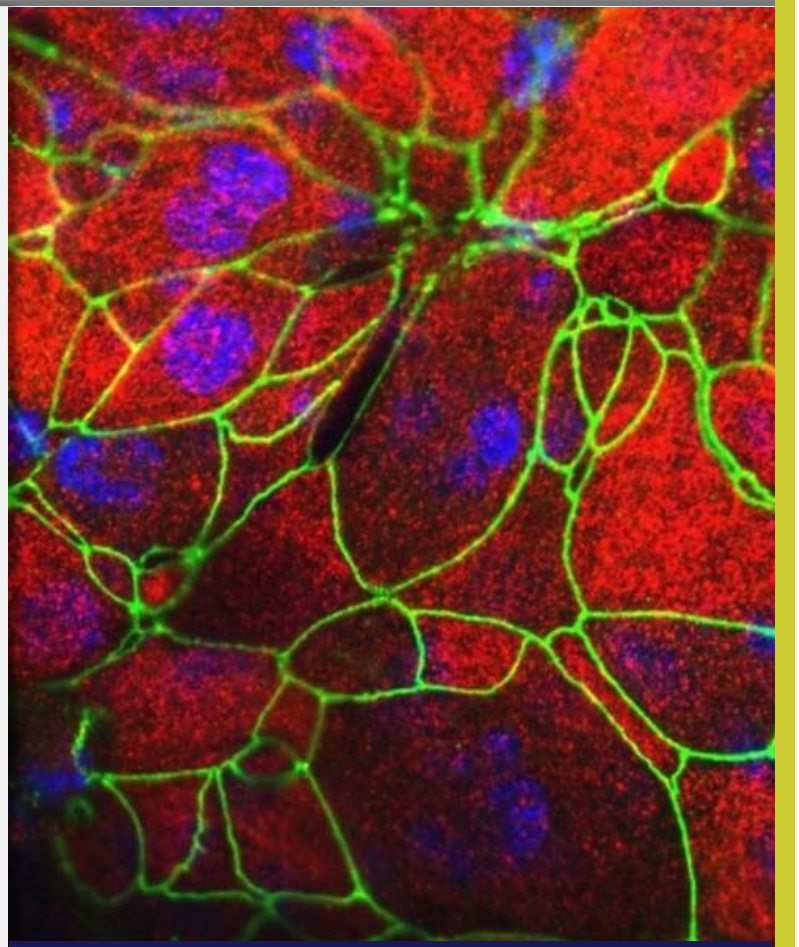
- Steps for reducing cellular and microbial contamination
- New regulations requiring cell line authentication



Cytopathic effect of RSV on HeLa cells

Steps for reducing cellular and microbial contamination

- Good documentation
- Highly trained technicians
- Good aseptic techniques
- Use one reservoir of medium per cell line
- Aliquot stock solutions/reagents

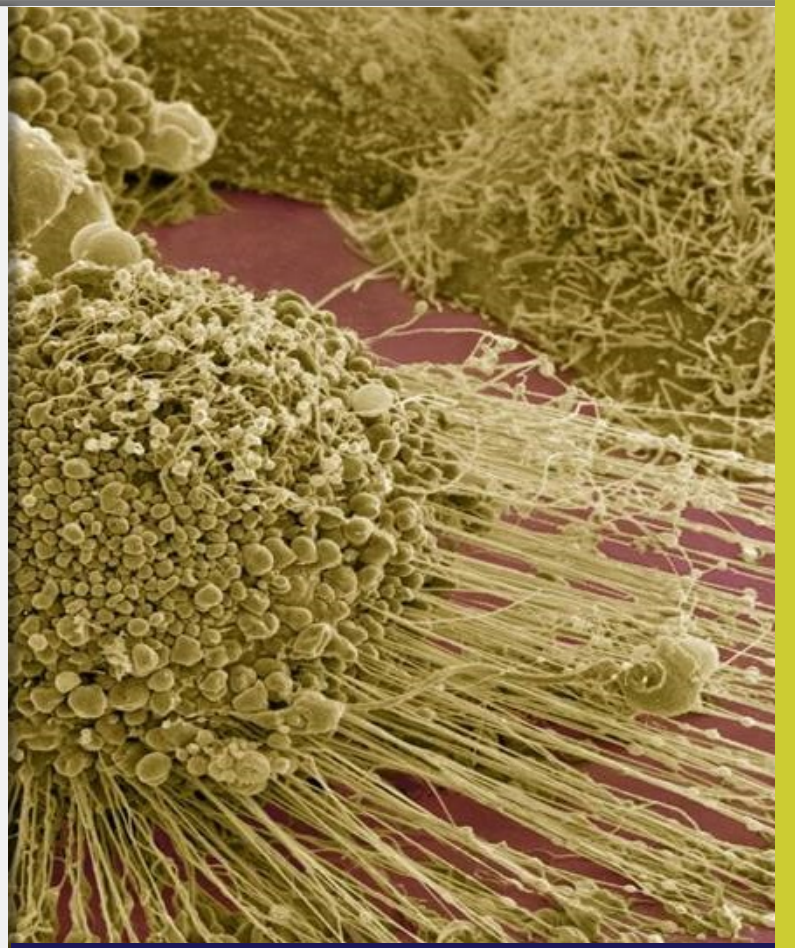


NCI-H441 (ATCC®HTB-174™) human papillary adenocarcinoma differentiated under air-liquid interface conditions

ATCC

Steps for reducing cellular and microbial contamination

- Label flasks with name of cell line, passage number, date of transfer (use barcoded flasks when available)
- Work with one cell line at a time in a biological safety cabinet
- Clean the biological safety cabinet between each cell line
- Allow a minimum of 15 minutes between each cell line

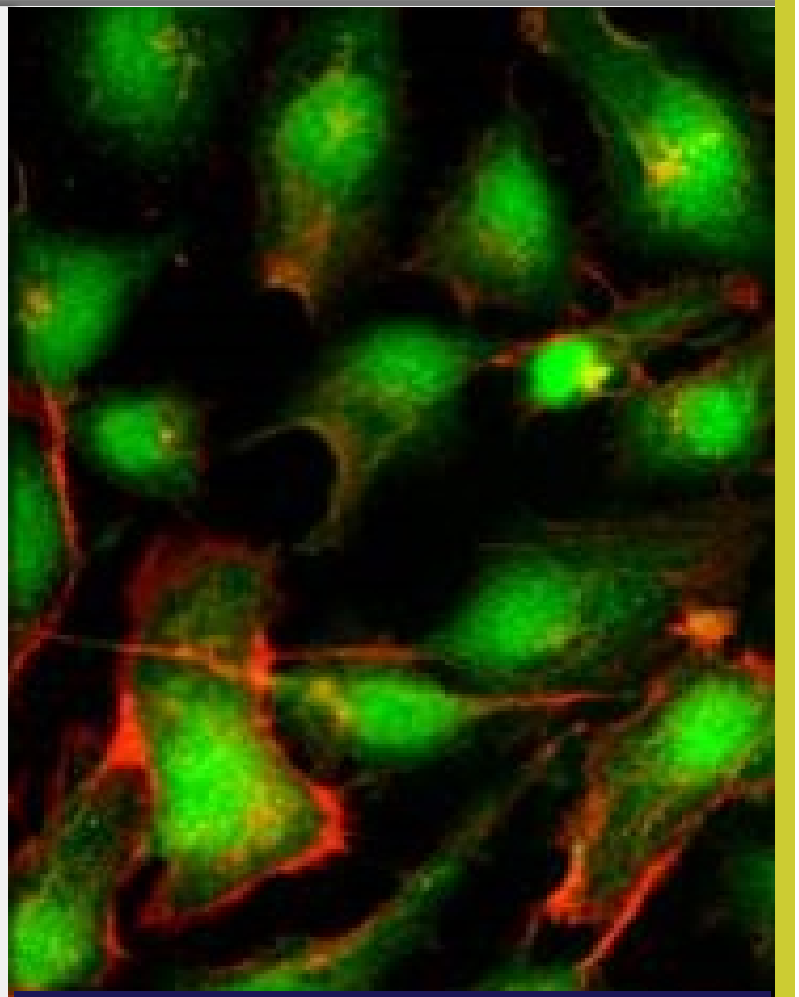


HeLa (ATCC® CCL-2™) cervical carcinoma. Scanning EM of cultured HeLa cell undergoing apoptosis.

ATCC

Steps for reducing cellular microbial contamination

- Quarantine “dirty” cell lines from “clean” cell lines
- Manageable work load (reduce accidents)
- Clean laboratory (reduce bioburden)
- Legible handwriting (printed labels)

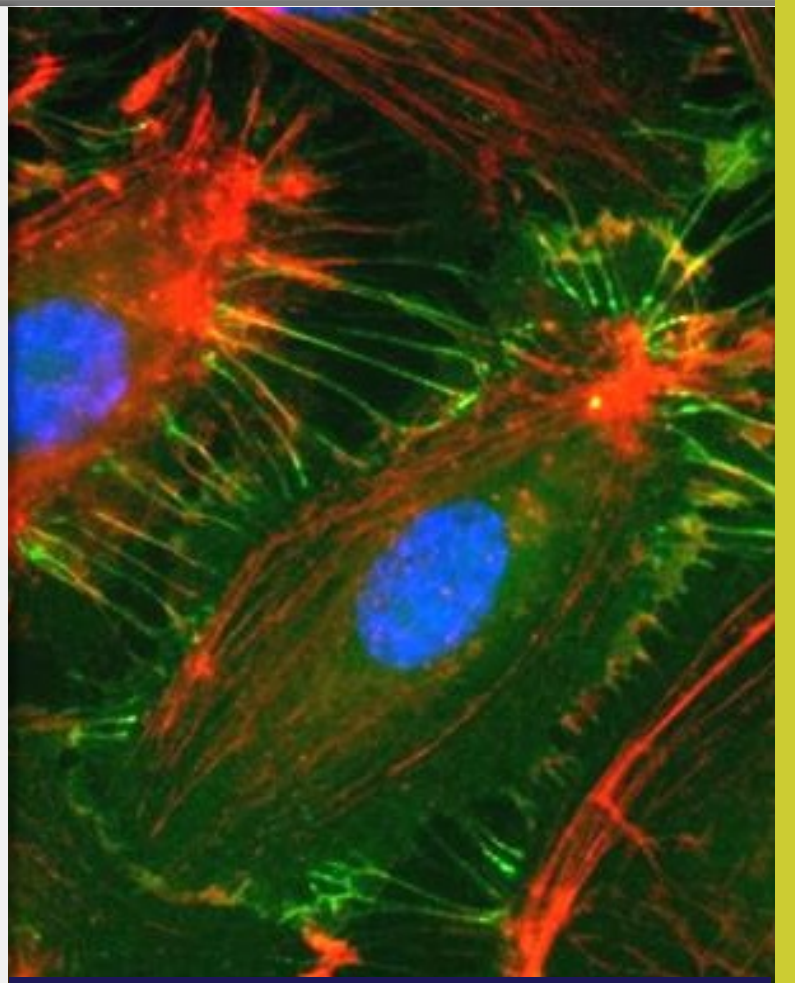


Small Cell Lung Cancer (SCLC) cells expressing p53

ATCC

Steps for reducing cellular and microbial contamination

- Monitor for cell line identity routinely
- Create a “good” working environment
- Review and approve laboratory notebooks
- Obtain each cell line from a reputable source



Human Vein Endothelial Cells (HUVEC, ATCC® CRL-1730™) expressing CD34

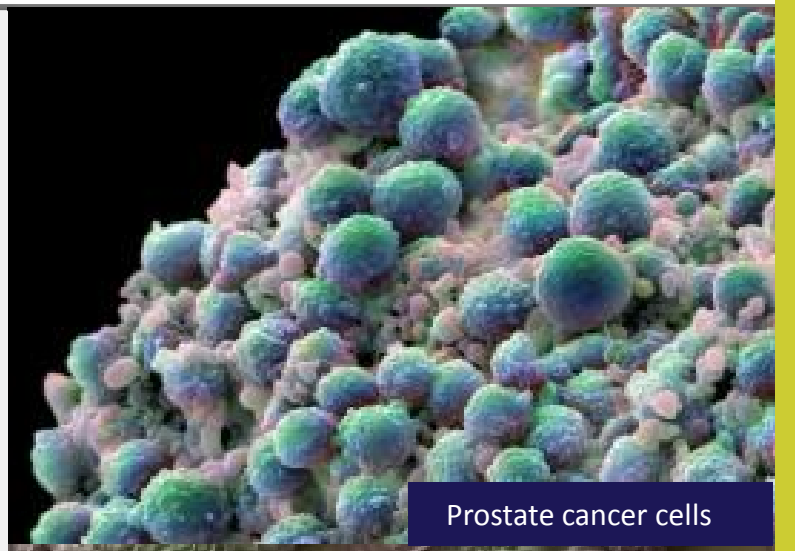
ATCC

Cell line identity to improve reproducibility in cancer research

NIH – Updated Application Instructions to Enhance Rigor and Reproducibility (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
  - Do not limit authentication to **cell lines**; include specialty chemicals, antibodies, and biologics

https://www.nih.gov/research-training/rigor-reproducibility/updated-application-instructions-enhance-rigor-reproducibility



Prostate cancer cells

Lung cancer cells

ATCC

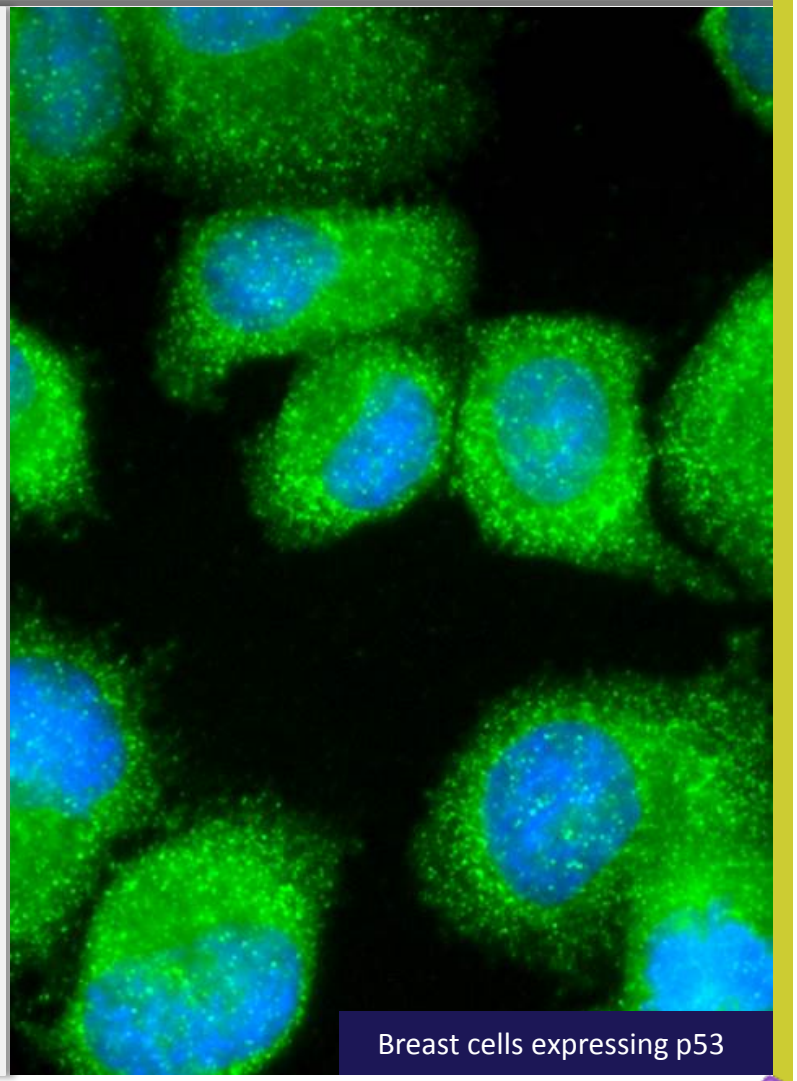
Cell line identity to improve reproducibility in cancer research

NIH Principles and Guidelines for Reporting Preclinical Research

5-points guidelines – No. 5: Consider establishing best practices guidelines for:

- Cell lines: to report source, authentication, and Mycoplasma contamination status
- Over 130 signatories of journals, scientific associations, and societies

https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research



Breast cells expressing p53

ATCC

Publications requiring/recommending cell line authentication

BioTechniques
Cancer Discovery (AACR)
Cancer Epidemiology, Biomarkers and Prevention (AACR)
Cancer Immunology Research (AACR)
Cancer Prevention Research (AACR)
Cancer Research (AACR)
Carcinogenesis
Cell Biochemistry and Biophysics
Cell Biology International
Clinical Cancer Research (AACR)
Clinical Orthopaedics and Related Research
Endocrine Reviews
Endocrine-Related Cancer
Endocrinology
In Vitro Cellular & Developmental Biology – Animal
International Journal of Cancer
Journal of Clinical Endocrinology & Metabolism
Journal of Endocrinology
Journal of Molecular Biology
Journal of Molecular Endocrinology
Journal of the National Cancer Institute
Molecular Cancer Research (AACR)
Molecular Cancer Therapeutics (AACR)
Molecular Endocrinology
Molecular Vision
Nature
Nature Cell Biology
Nature Methods
Neuro-Oncology
PLoS ONE

The number of publications requiring/recommending cell line authentication as re-requisite for publication is growing… (partial listing)

ATCC

When to perform STR profiling and CO1 assays

- When you first receive a cell line into the laboratory from a unreliable source
- After 10 passages
- After preparing a cell bank
- When in doubt

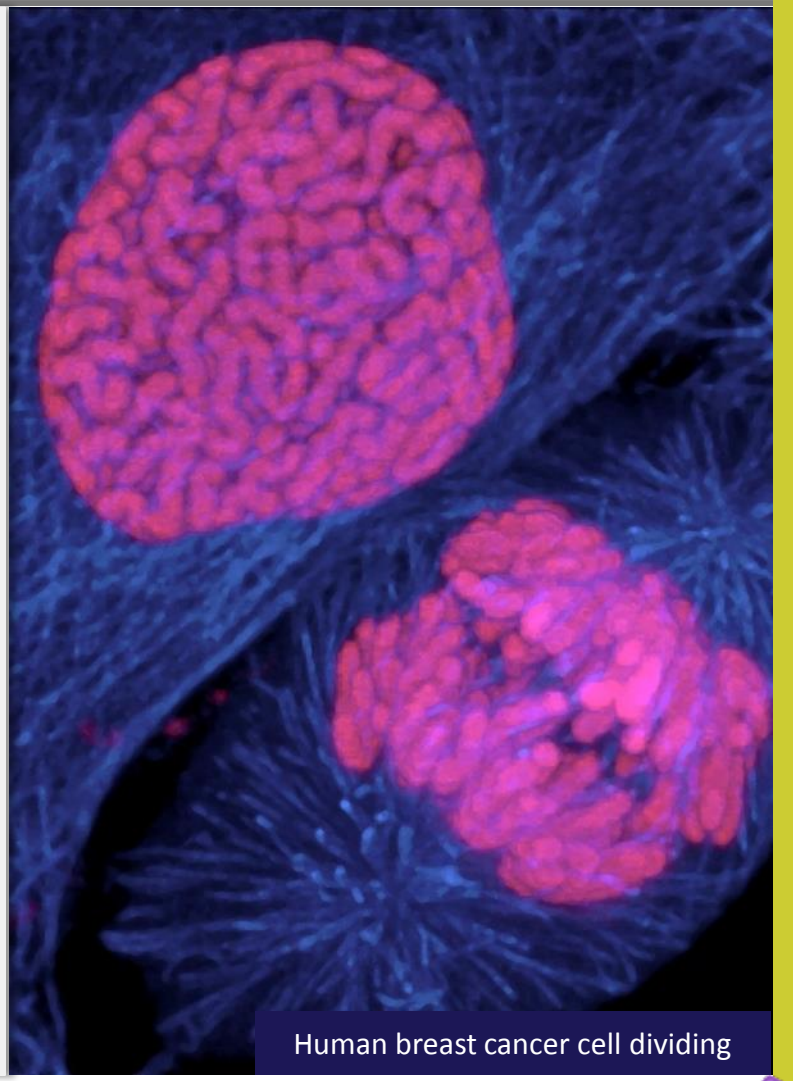
Testing services for STR profiling and CO1 Assays

Testing Services

- Cell Banks
- Paternity testing labs
- Universities
- Core labs

Important consideration when choosing a Testing Service

- Highly trained, experienced technicians
- Access to database of STR profiles



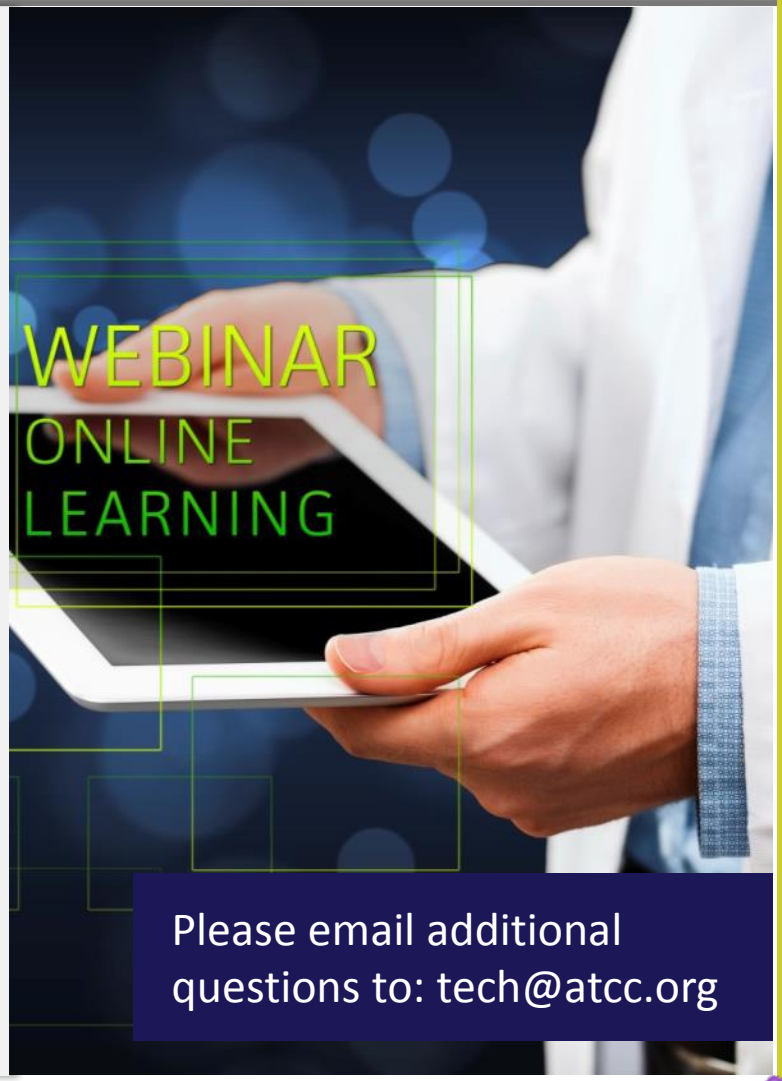
Human breast cancer cell dividing

Thank you for joining today!

Register for more ATCC "*Excellence in Research*" webinars, or watch recorded webinars, at www.atcc.org/webinars.

- **April 21, 2016**
  10:00 AM, 3:00 PM EST
  Steven Budd, M.S., M.B.A., *Product Line Business Specialist*, ATCC
  Best Practices in Cryopreservation

- **April 28, 2016**
  10:00 AM, 3:00 PM EST
  Frank Simione, M.S., *Director, Standards, Standards Resource Organization*, ATCC
  The ATCC Story: A Ninety Year Celebration



Please email additional questions to: tech@atcc.org