Mitochondria-Targeting PCR and CO1 Barcode Sequence Analyses as Alternatives to Isoenzymology

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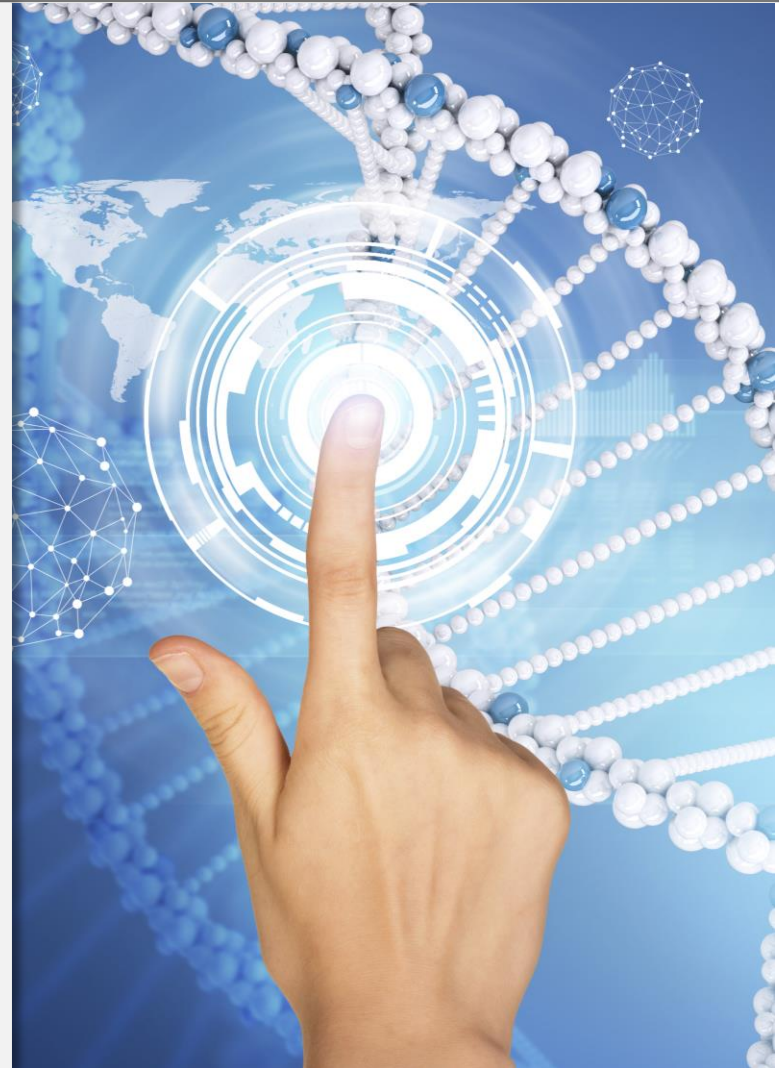December 13, 2016

ATCC

Overview

This presentation will cover:

- American Type Culture Collection (ATCC) overview

- Need for species identification

- Current DNA-based species ID at ATCC:
  - Multiplex PCR-Based Analysis
 - CO1 Barcode Sequencing

- Authentication

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

Scope of This Presentation
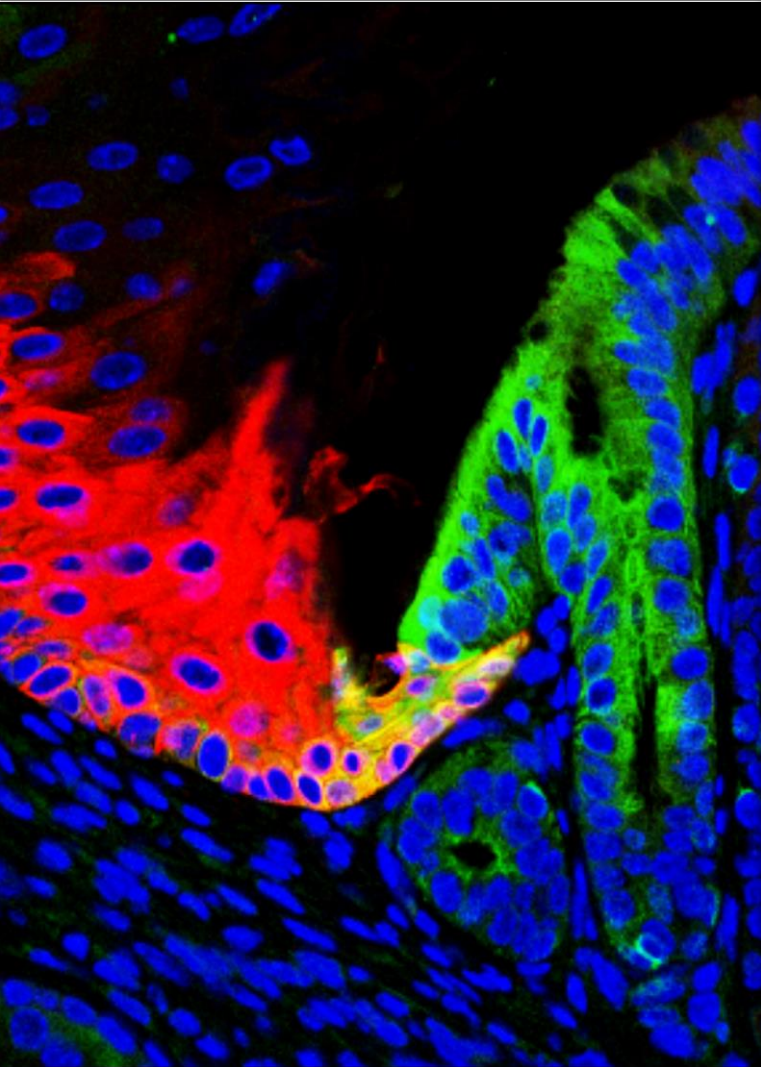
- Animal cell culture

- Interspecies cross-contamination and misidentification

- Other molecular-based detection techniques are not addressed here

Why perform species identification on cells?

Because Bharati Hukku and colleagues (1984) found interspecies problems with 35% of cell lines examined!

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Historical Technique: Karyotyping

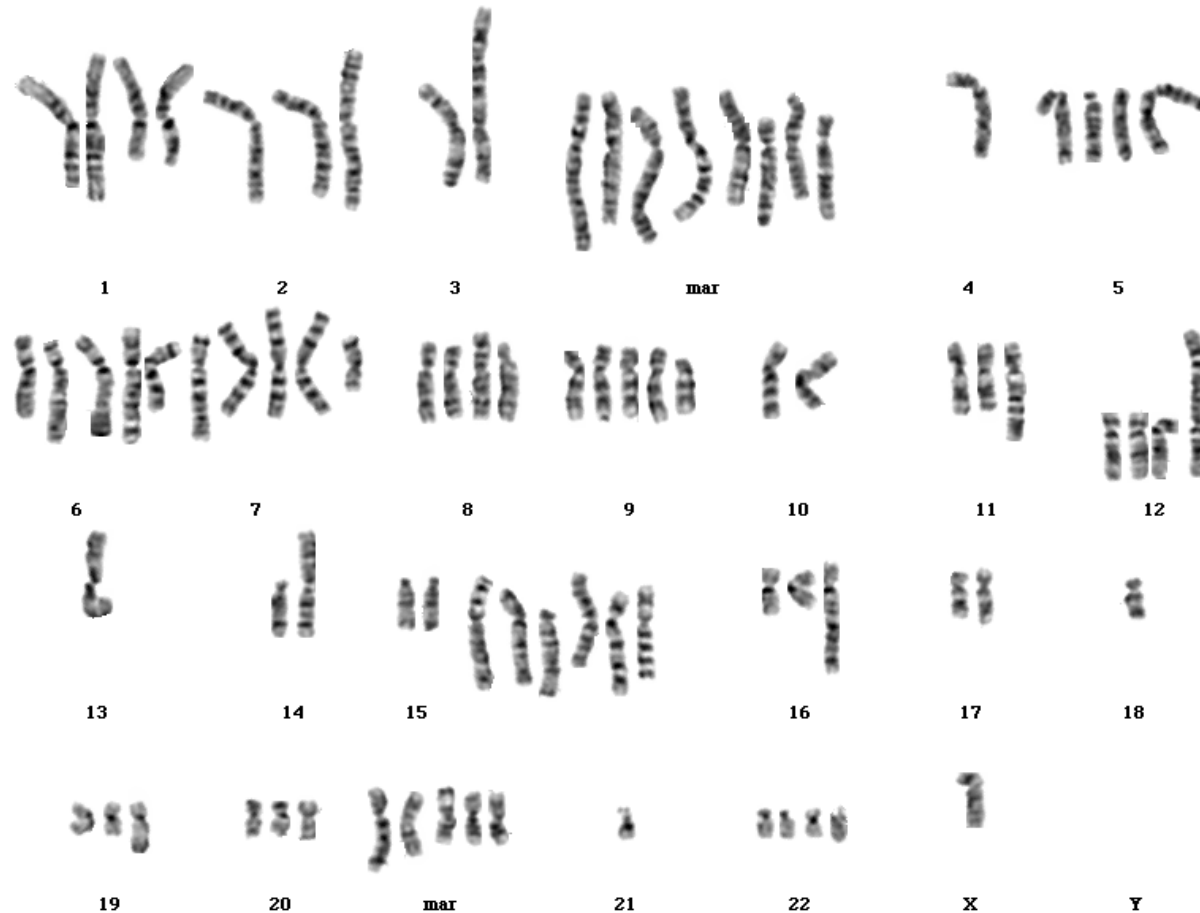

Figure 1. This complex karyogram of human cell line SJCRH30 (ATCC® CRL-2061™) is n=84, a significant departure from normal human n=46.
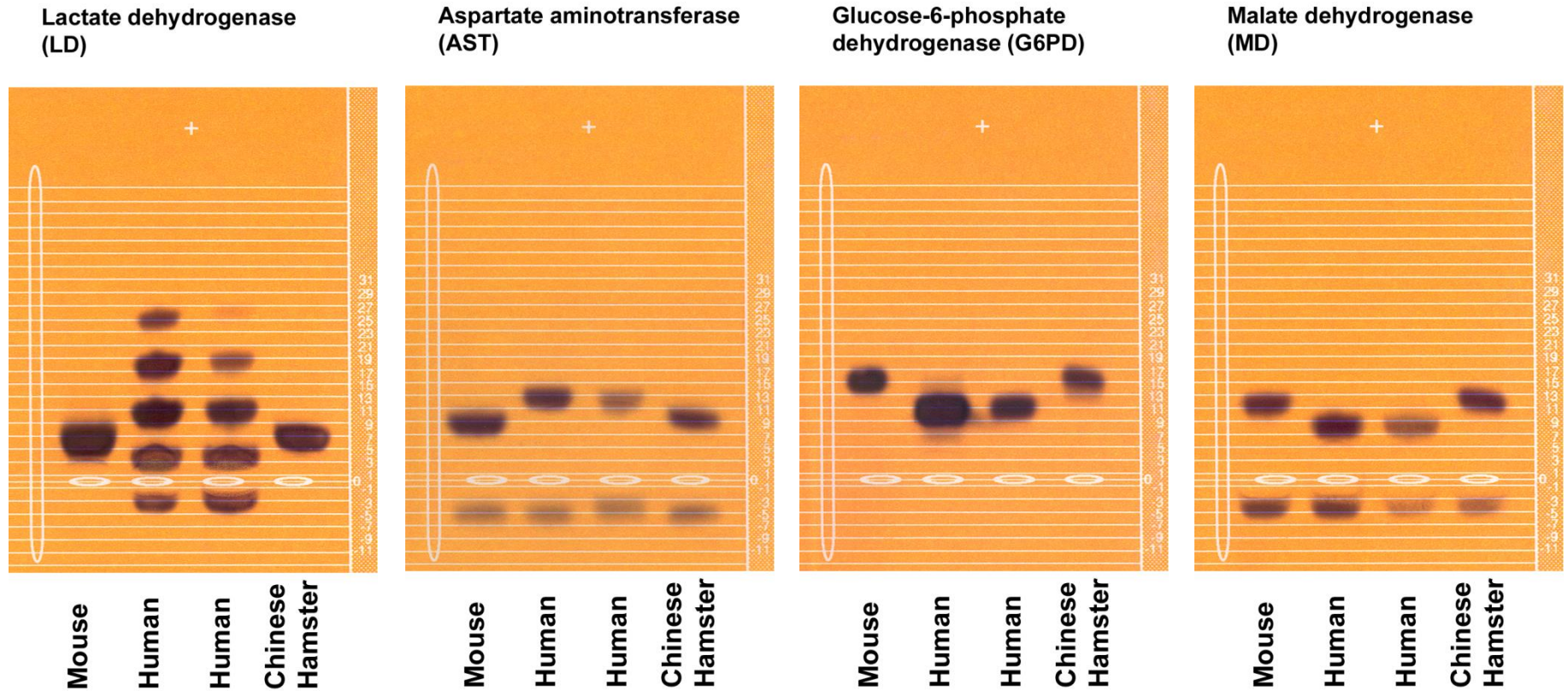
Historical Technique: Isoenzyme Tests

Figure 2. Four of the seven isoenzyme tests are seen here. Resolving related species may be challenging.

The Big Question:

I can't get a hold of isoenzyme reagents anymore!!

What do I do now?

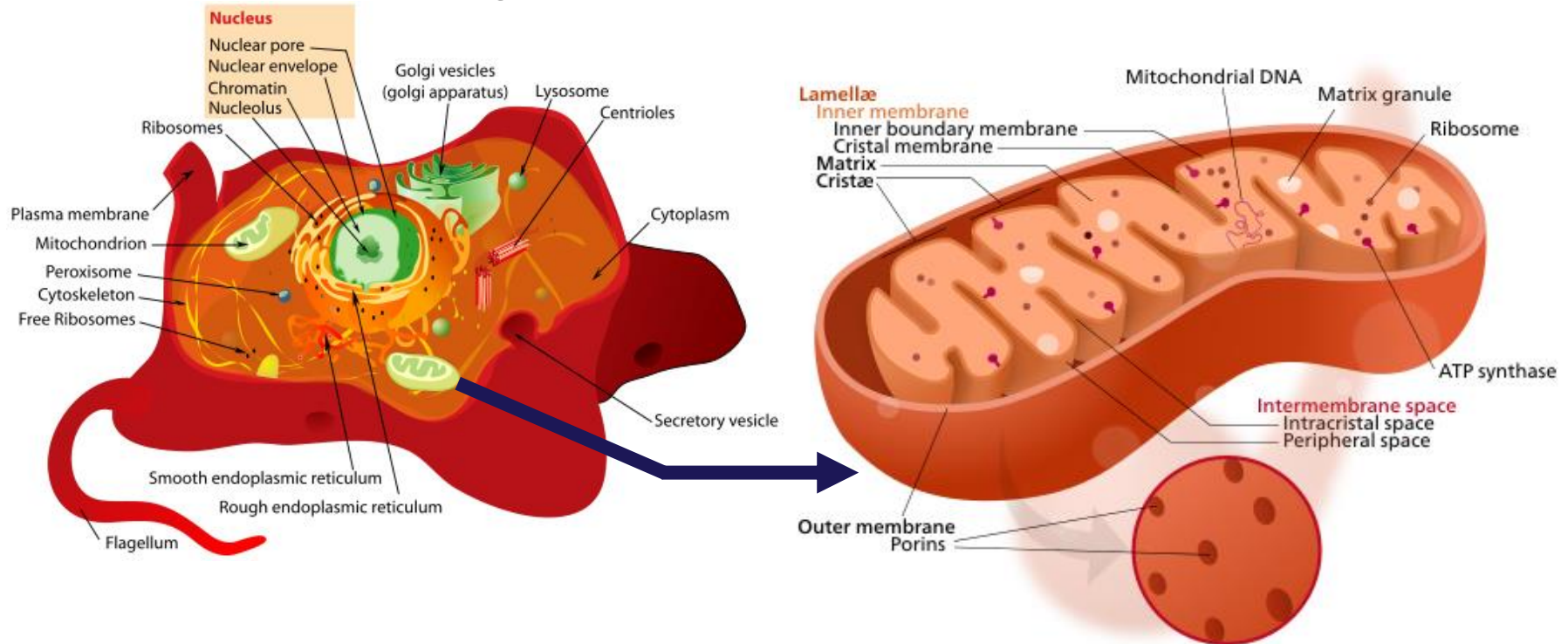# Molecular-Based Species Identification

Designation: ASN-0004

Species-Level Identification and Cross-Contamination Screening in Animal Cells by Multiplex PCR

– in progress –

Molecular-Based Species Identification

Mitochondrial DNA regions are popular in identification

Cytochrome *c* Oxidase I (CO1)

- Parodi et al. (2002): PCR fragment analysis

- Hebert et al. (2003): universal sequencing "barcode" region

Fragment Analysis Assay

Target species and expected size fragments (tentative):

Tier 1: Industry Focus Multiplex

Pig	480 bp
Human	393 bp
Chinese hamster	268 bp
African green monkey	224 bp
Rat	206 bp
Dog	174 bp
Mouse	151 bp
Syrian hamster	124 bp
Bovine	103 bp

Tier 2: Academic Focus Multiplex

Chicken	427 bp
Cat	378 bp
Guinea pig	335 bp
Rhesus monkey	289 bp
Horse	245 bp
Rabbit	138 bp

Both Sets

Internal Control ....70 bp

Fragment Analysis Assay (early generation)
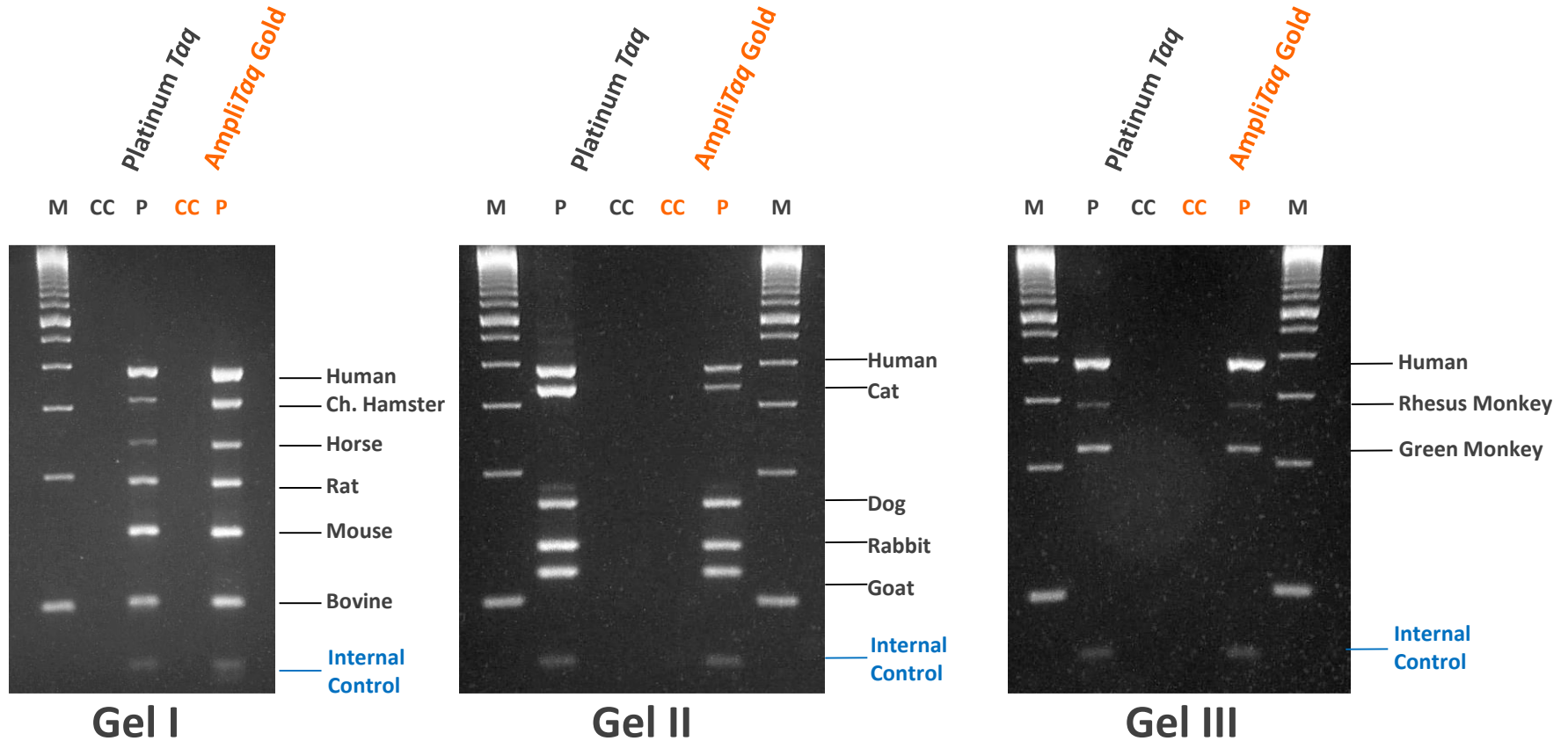

Gel I

Gel II

Gel III

Figure 3. Three gels demonstrate how positive bands appear (P) along with the contamination check (CC). The marker (M) is a 100 bp ladder.
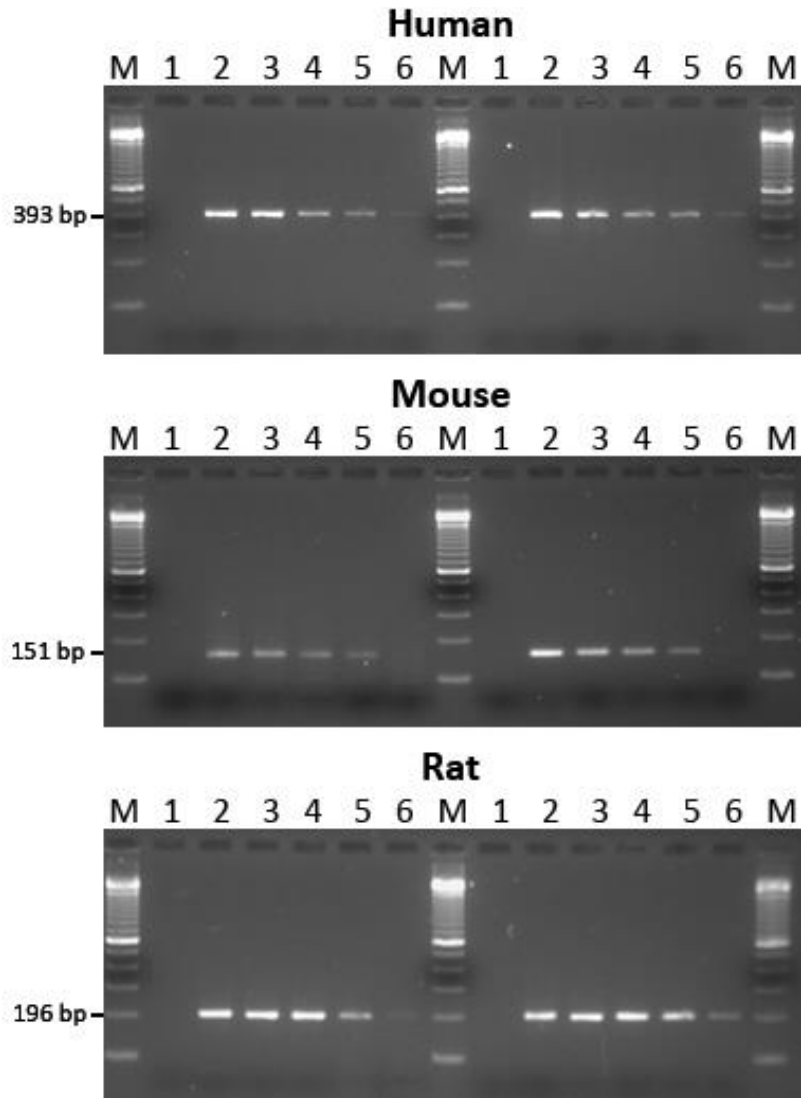
Note: The Chinese hamster and rat bands here are from an earlier primer version; goat was later eliminated.

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Fragment Analysis Assay Sensitivity (early generation)

Figure 4. Gels depicting CO1 Assay's sensitivity. It uses 5 ng of total genomic DNA per 25 µL reaction. The rat band is from an earlier primer set.

KEY
1) Ø control
2) 5.0 ng
3) 1.0 ng
4) 0.5 ng
5) 0.1 ng
6) 0.01 ng
M) 100 bp

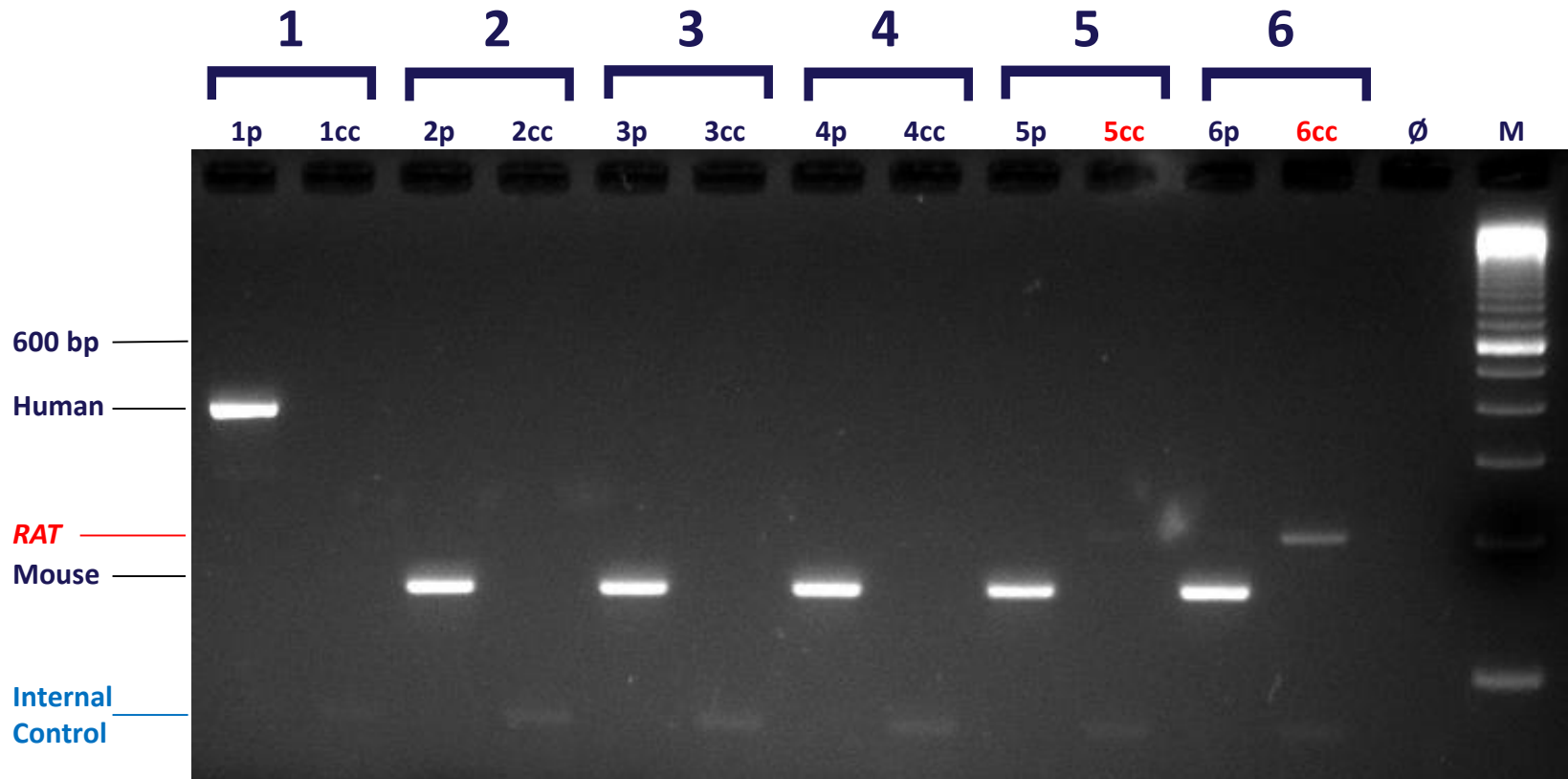# Fragment Analysis Assay (early generation)

Figure 5. In this set, "p" PCRs contain the putative species' primers and the "cc" contamination check has all primers except for the tested species. Set 1 tested for human; Sets 2-6 tested for mouse. Samples 5 and 6 are positive for mouse but also are contaminated with rat cells.

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Molecular-Based Species Identification

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Designation: ASN-0003

Species-Level Identification of Animal Cells through Mitochondrial Cytochrome *c* Oxidase Subunit 1 (CO1) DNA Barcodes
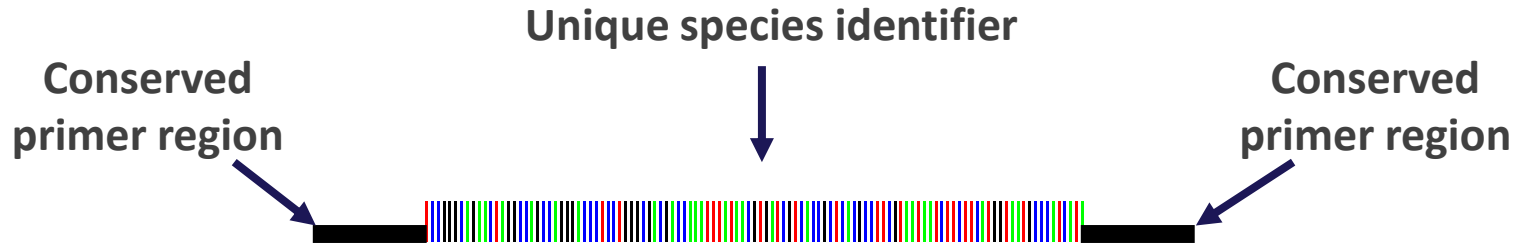
– published –

The Genetic Barcoding Concept

The Universal Product Code (UPC) system developed by the industrial sector employs 10 options at each of 12 positions to uniquely identify over 100 billion individual products.
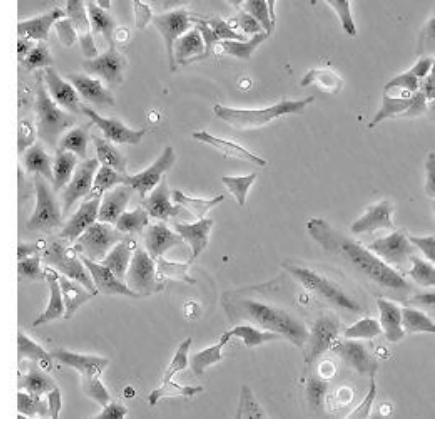
The Genetic Barcoding Concept

Unique species identifier

**Conserved
primer region**

**Conserved
primer region**

Just like UPC barcodes, the
DNA sequences within each
species are unique.
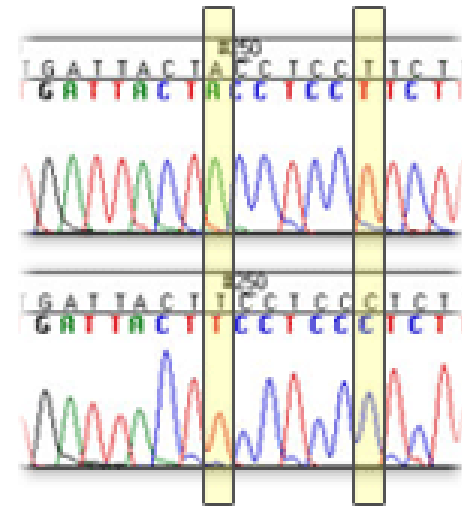
Applied Genetic Barcoding

ATCC Collection	**Primary Molecular Target**
▪ Bacteria	16S: 30S small ribosomal RNA subunit
▪ Fungi and Yeasts	ITS: Internal Transcribed Spacer; D1D2: large-subunit RNA gene
▪ Protists	18S: small subunit nuclear ribosomal RNA
▪ *Animal Cells*	*CO1: cytochrome oxidase I*

Consortium for the Barcode of Life (CBOL)
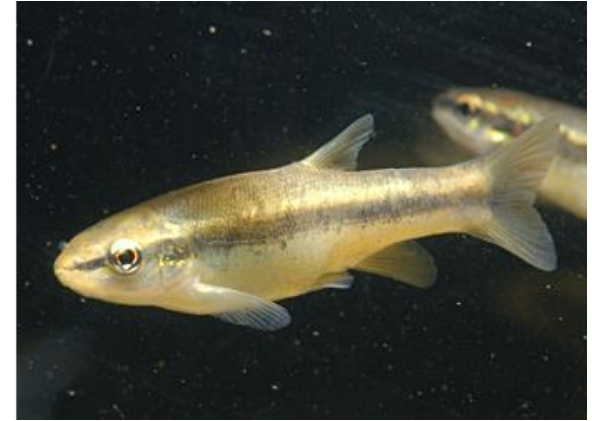
- The CBOL initiative
- Uses CO1 (648 bp)
- Major database efforts: insects, birds, and fish
- Websites:
    - Background: http://www.barcodeoflife.org/
    - Database Queries:
      http://www.boldsystems.org/views/login.php

CBOL Search Query

IgH-2 (ATCC® CCL-108™)

Organism: **iguana** /

GENERAL INFORMATION	CHARACTERISTICS	CULTURE METHOD	HISTORY	DOCUMENTATION

Permits and Restrictions	⚑ View Permits
Organism	iguana
Product Format	frozen
Morphology	epithelial
Culture Properties	adherent
Biosafety Level	1
Age	immature
Gender	male
Storage Conditions	liquid nitrogen vapor phase

PCR and Sequencing

CO1 Barcode for CCL-108

```
>CCL-108_sample
CACCCTATACTTAGTCTTCGGTGCCTGAGCCGGCATAGTCGGAACTGCCC
TCAGCCTGCTAATTCGAGCAGAACTCAGCCAGCCAGGGGCCCTTCTTGGT
GACGACCAAATTTACAACGTCATTGTAACCGCCCATGCCTTTGTTATAAT
TTTCTTCATAGTAATGCCCGTGATAATCGGAGGATTTGGAAACTGATTAG
TTCCCCTAATAATCGGCGCACCAGACATAGCCTTCCCCCGAATAAACAAC
ATAAGCTTCTGACTCCTACCCCCATCCTTTCTACTCCTTTTAGCCTCCTC
TGGCATTGAAGCCGGAGCCGGCACAGGCTGAACTGTCTACCCCCCACTAG
CGGGCAACCTAGCACACGCAGGCGCTTCAGTAGACCTTACAATTTTCTCC
CTCCACCTAGCCGGAATCTCATCCATCCTAGGAGCAATCAACTTTATCAC
AACATCTATCAACATAAAGCCCCCTACAATAACCCAATATCAAACATCCT
TGTTTGTCTGATCCGTACTAATTACAGCCGTACTACTTCTGTTATCCCTA
CCCGTCCTAGCAGCAGGTATTACAATACTACTCACCGACCGCAACTTAAA
TACTTCATTCTTTGACCCCGCAGGAGGGGGAGACCCAATCCTTTACCAAC
ACTTATTT
```
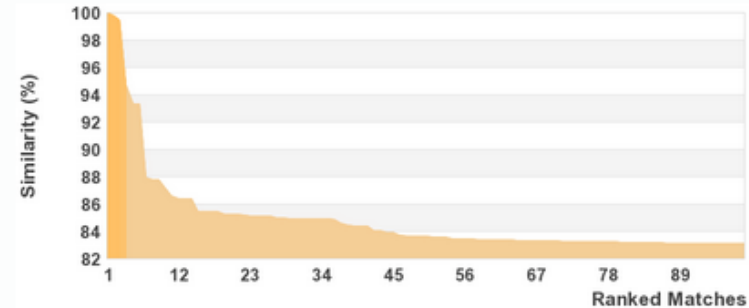
CBOL Search Query

CBOL Search Results

Identification Summary:

Taxonomic Level	Taxon Assignment	Probability of Placement (%)
Phylum	Chordata	100
Class	Reptilia	100
Order	Squamata	100
Family	Iguanidae	100
Genus	Iguana	100
Species	Iguana iguana	100

Similarity Scores of Top 99 Matches:

TOP 20 Matches :

Display option: Top 20

Phylum	Class	Order	Family	Genus	Species	Similarity (%)	Status
Chordata	Reptilia	Squamata	Iguanidae	Iguana	*iguana*	100	Private
Chordata	Reptilia	Squamata	Iguanidae	Iguana	*iguana*	99.82	Private
Chordata	Reptilia	Squamata	Iguanidae	Iguana	*iguana*	99.44	Published
Chordata	Reptilia	Squamata	Iguanidae	Iguana	*iguana*	94.75	Private
Chordata	Reptilia	Squamata	Iguanidae	Iguana	*iguana*	93.36	Early-Release
Chordata	Reptilia	Squamata	Iguanidae	Iguana	*iguana*	93.33	Private
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*ater*	87.96	Published
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*ater*	87.81	Published
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*ater*	87.81	Published
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*varius*	87.19	Early-Release
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*ater*	86.57	Published
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*ater*	86.42	Published
Chordata	Reptilia	Squamata	Iguanidae	Sauromalus	*ater*	86.42	Published
Chordata	Reptilia	Squamata	Iguanidae	Amblyrhynchus	*cristatus*	86.42	Private
Chordata	Reptilia	Squamata	Iguanidae	Amblyrhynchus	*cristatus*	85.45	Published
Chordata	Reptilia	Squamata	Iguanidae	Amblyrhynchus	*cristatus*	85.45	Published

Genetic Barcoding Catches a Misidentification

The ATCC website lists horse…

Horse (ATCC® CRL-6583™)
Organism: *Equus caballus*, horse / Tissue: **unknown** /

GENERAL INFORMATION	CHARACTERISTICS	CULTURE METHOD	HISTORY	DOCUMENTATION

Permits and Restrictions   ⚑ View Permits

Organism	*Equus caballus*, horse
Tissue	unknown
Product Format	flask
Biosafety Level	1

…but DNA Barcode tests show that ATCC® CRL-6583™ is actually Chinese hamster.

NOTE: CRL-6583 is part of the NBL collection, neither produced nor authenticated by ATCC.
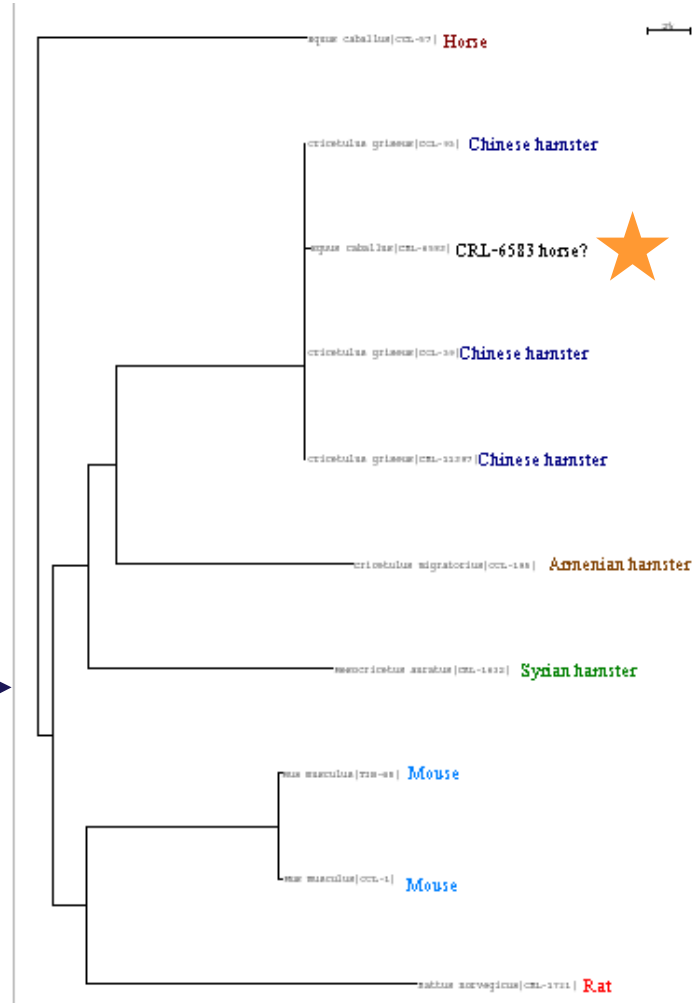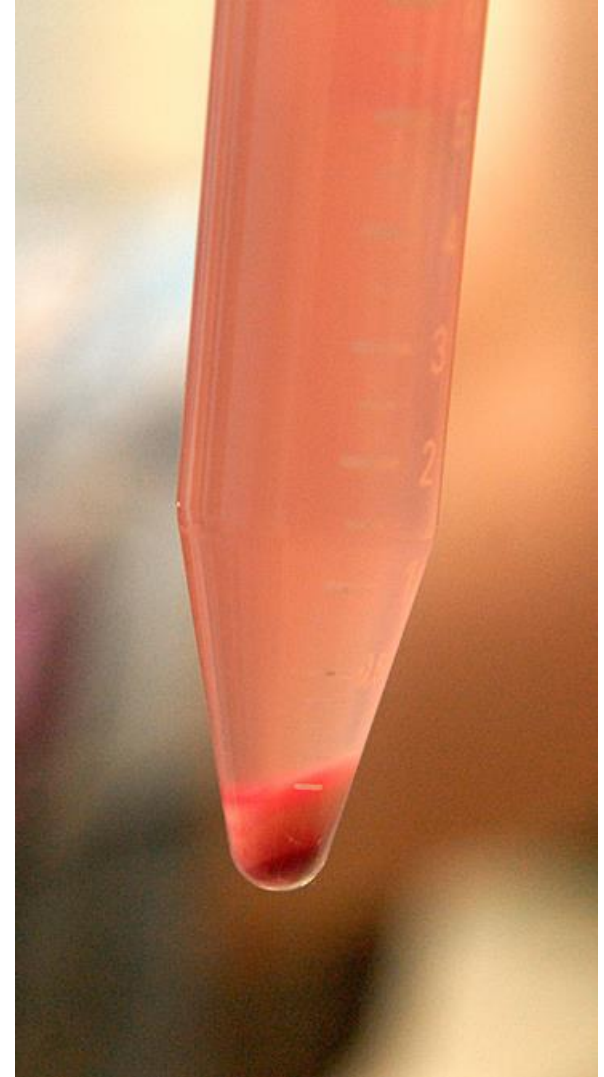
Genetic Barcoding

Genetic Barcoding Cautions

Cell Culture Mixes

and

Low Level Contamination

# *EXTREMELY IMPORTANT:* **AUTHENTICATION**

AUTHENTICATE
NOW!

Use only authenticated cell lines in all of your research.

ATCC

Summary

- ATCC is a biorepository performing cell line authentication

- Genetic methods are preferable species determination approaches

- PCR-based CO1 Assay + DNA Barcoding = complimentary approaches to identify nearly every animal species

- **Use authenticated cell lines**

- Further reading:

  Cooper J, *et al*. 2007. Species identification in cell culture: a two-pronged molecular approach. *In Vitro Cell Dev Biol – Animal* 43(10):344-351.
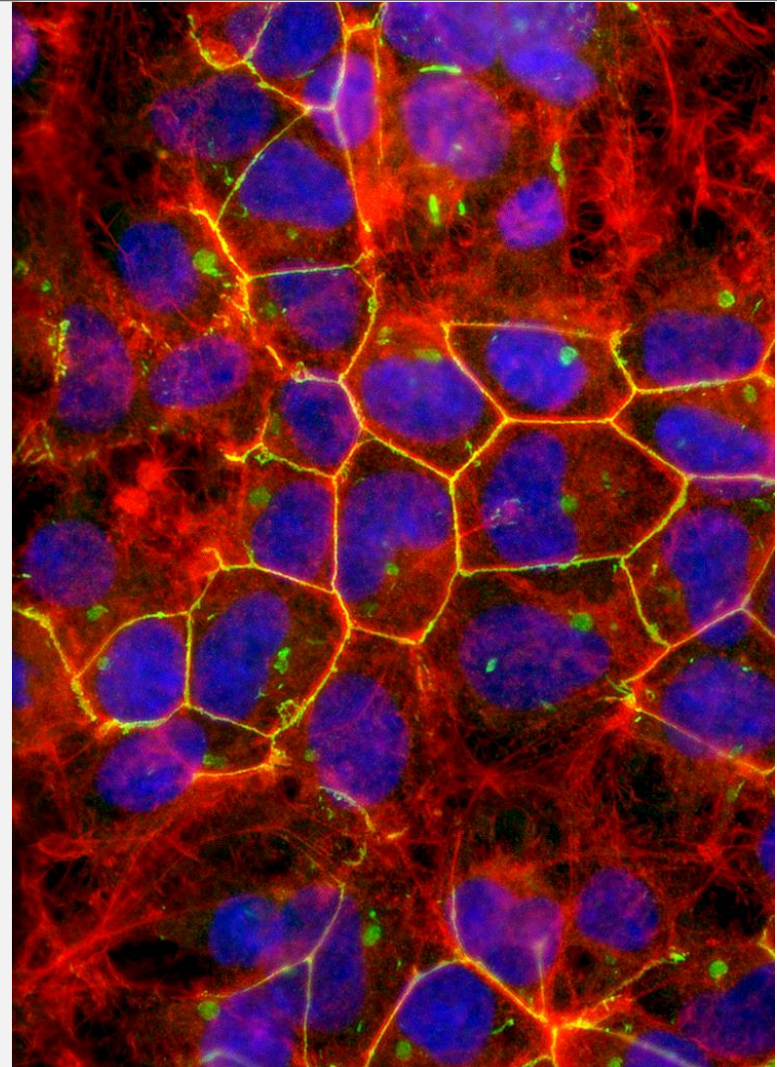
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Thank You