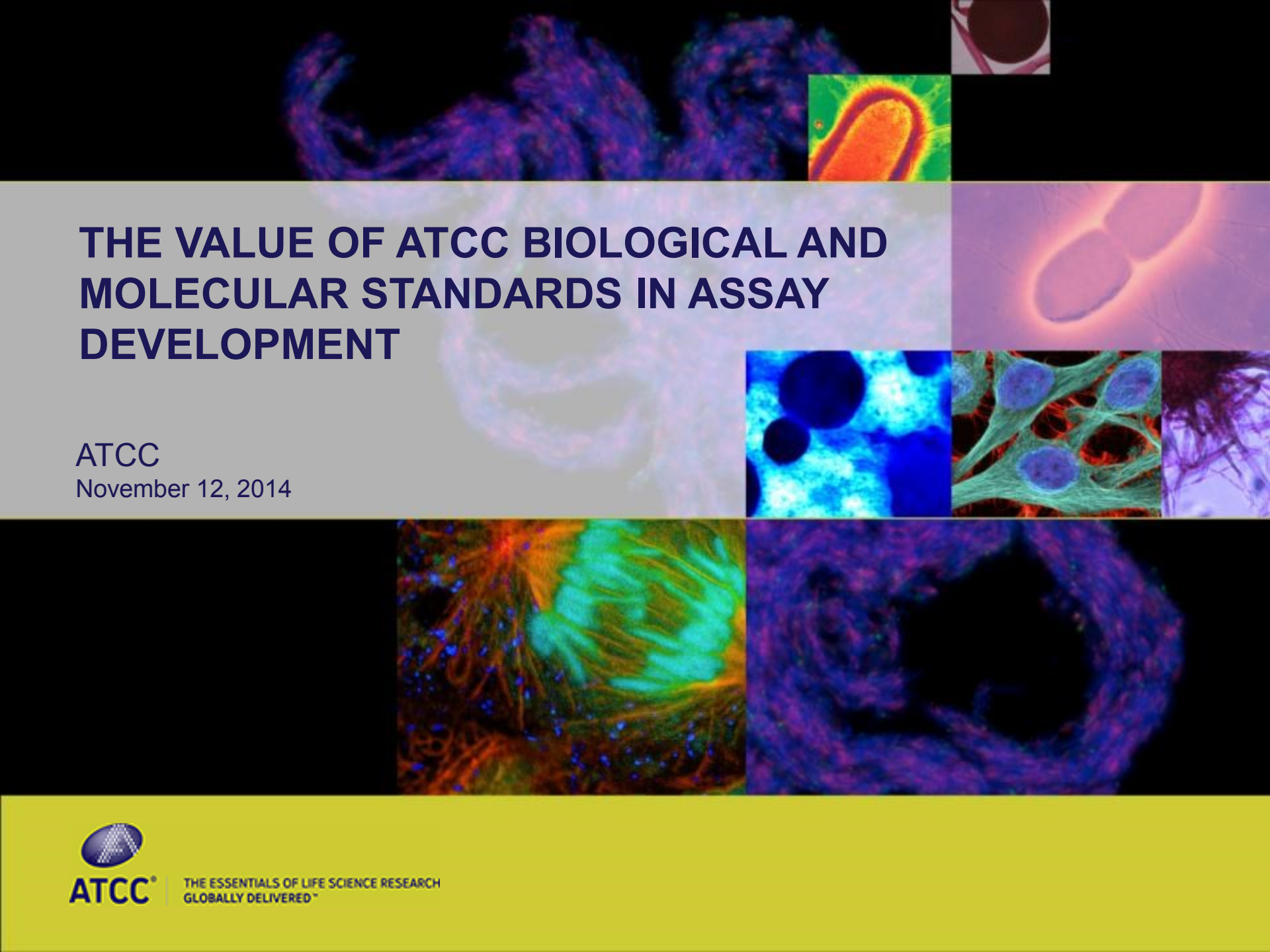

THE VALUE OF ATCC BIOLOGICAL AND MOLECULAR STANDARDS IN ASSAY DEVELOPMENT

ATCC

November 12, 2014

ATCC®
THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

Agenda

ATCC Biological Controls for the Detection and Analysis of Cancer

- **Time:** 2:00 PM – 2:20 PM
- **Speaker:** Dr. Fang Tian, *Lead Scientist*, ATCC Cell Systems

ATCC Standards for Infectious Disease Assay Development

- **Time:** 2:20 PM – 2:40 PM
- **Speaker:** Tracy Vandenbroek, *Product Line Business Manager,* ATCC Microbiology Systems

Real World Application of ATCC Quantitated Synthetic Nucleic Acid Standards

- **Time:** 2:45 PM – 3:30 PM
- **Speaker:** Dr. Benjamin Pinsky, *Assistant Professor of Pathology and Medicine (Infectious Diseases)*, Standford University Medical Center

ATCC®

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 products and innovative solutions
- Broad range of biomaterials
  - Continuous cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, fungi, yeasts, protists, and viruses
  - Microbial and tumor cell panels
  - Genomic and synthetic nucleic acids
  - Certified reference materials
  - Media, sera, and reagents

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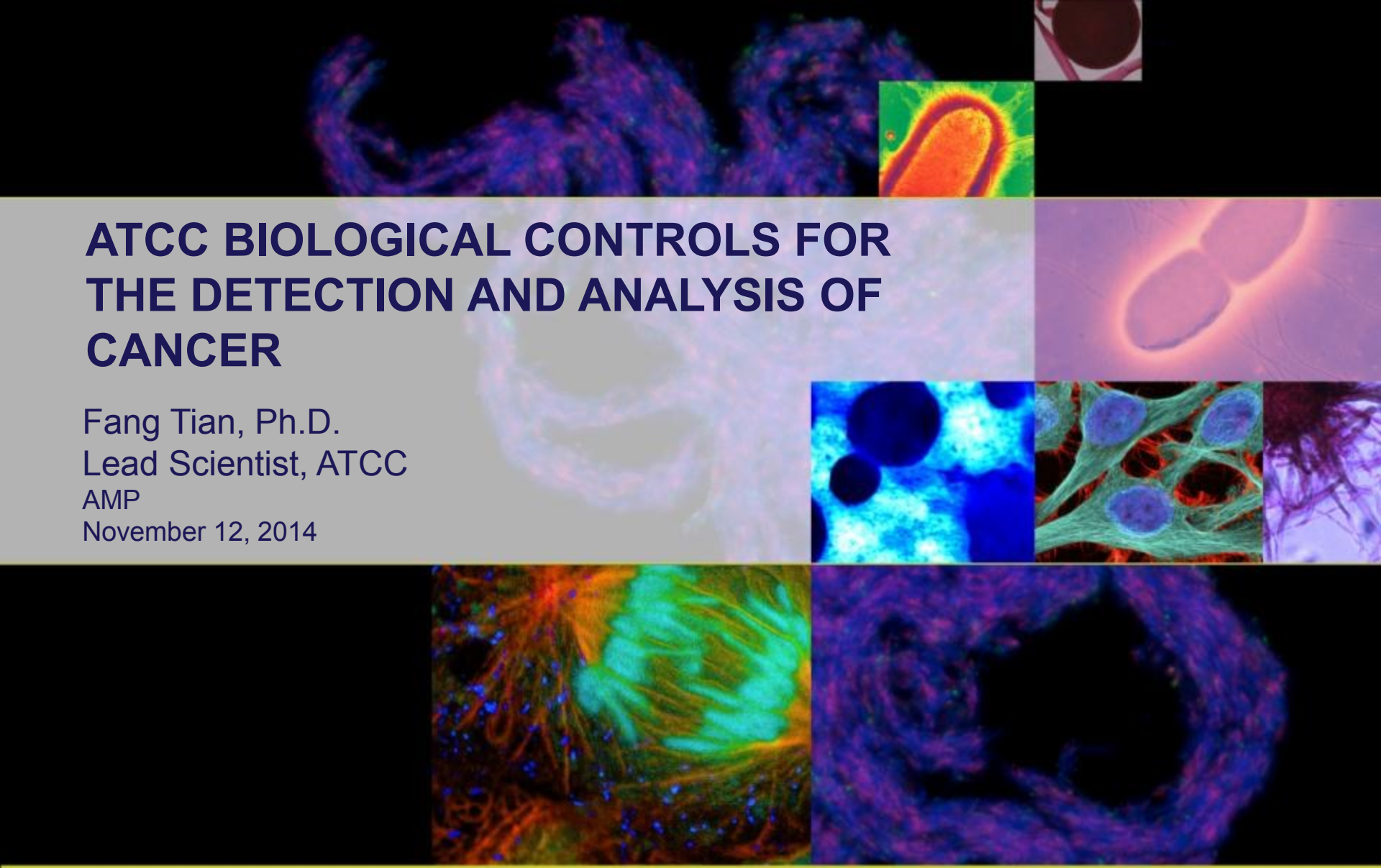

ATCC BIOLOGICAL CONTROLS FOR THE DETECTION AND ANALYSIS OF CANCER

Fang Tian, Ph.D.
Lead Scientist, ATCC

AMP

November 12, 2014

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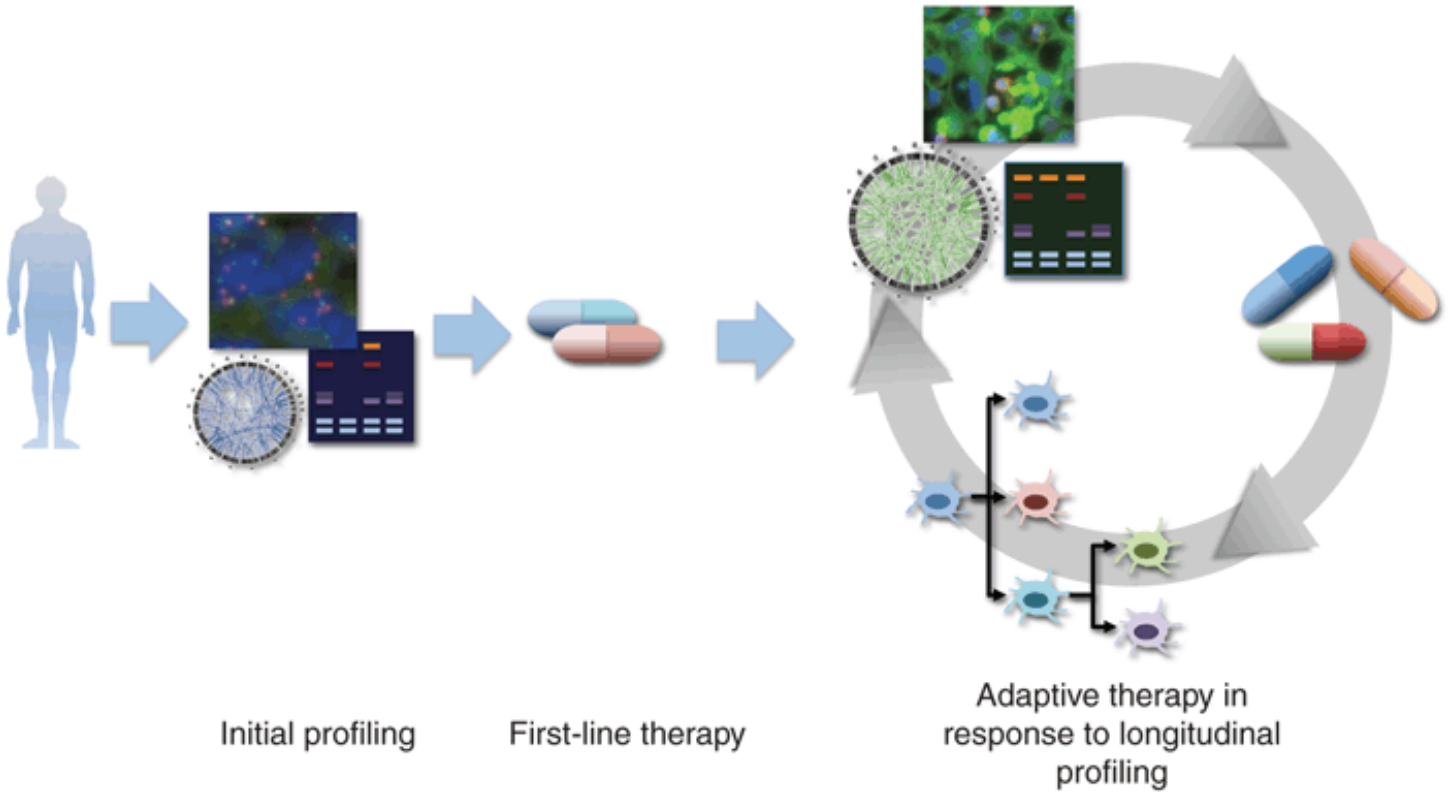
The changing landscape of diagnostics

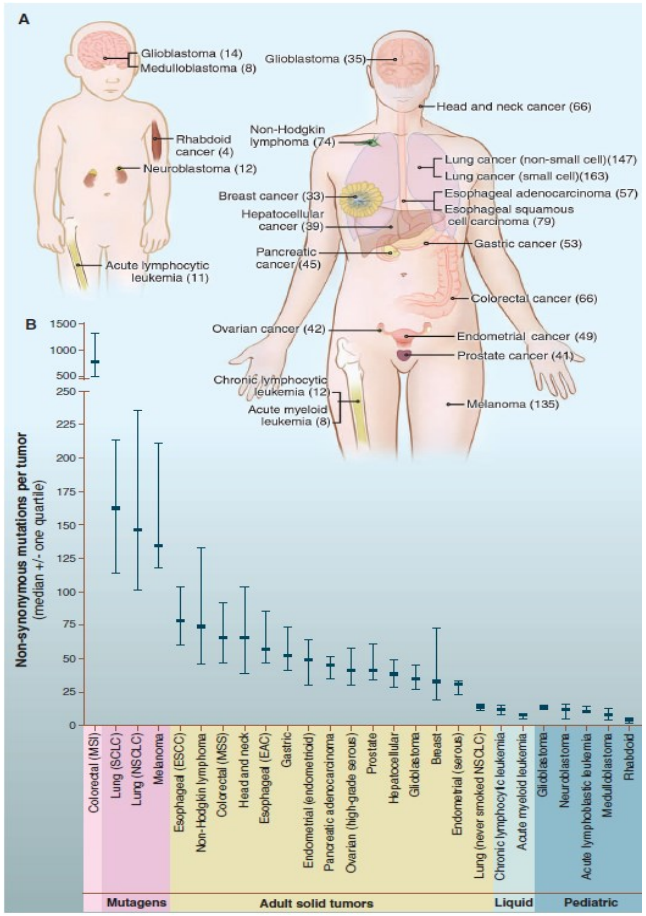
Molecular diagnostics and personalized precision medicine



Gonzalez de Castro D, *et al*., *Clinical pharmacology & Therapeutics* 93(3), 2013

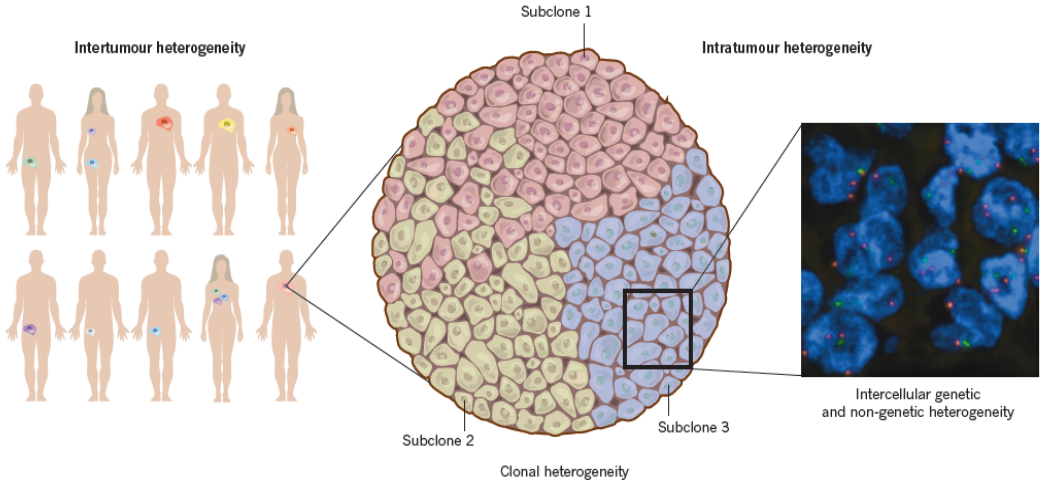
Somatic mutations and tumor heterogeneity

The prevalence of somatic mutations across pediatric and adult tumors



Vogelstein B, *et al. Science* 339, 2013

Intertumor heterogeneity and intratumor heterogeneity



Nature doi:10.1038/nature12625, 2013

Reference material needed to develop molecular-based assays

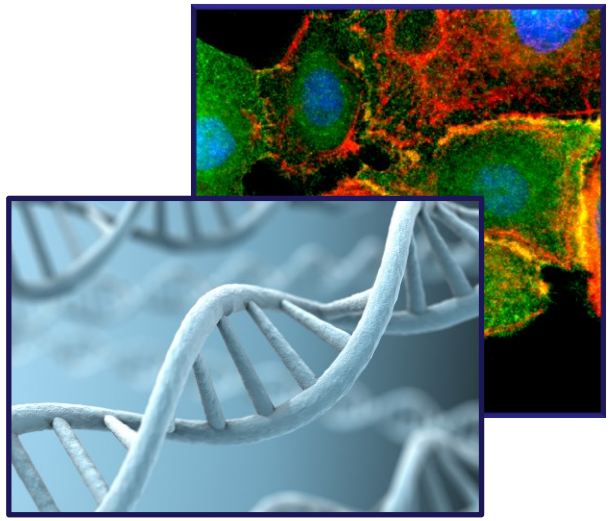
Using reliable biomaterials as controls

Types of materials to choose:

Reference Material	Benefit	Disadvantage
Synthetic oligonucleotides	Easy to design and synthesize	Do not resemble the complexity of the whole genome
Cell lines and cell line genomic DNA	Mimics the complexity of the whole genome	Rare mutations or biomarkers are difficult to obtain
Patient biopsy samples	Representative	Not a sustainable source

Other things to consider:

- Fully authenticated
- Avoid contamination or misidentification
- Characterized genetic alterations
- Stable molecular profiles
- Reproducible results



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ATCC Certified Reference Material (CRM)

- Stable with respect to one or more specified property
- Possess a stated level of confidence for Traceability and Values of Uncertainty, where applicable

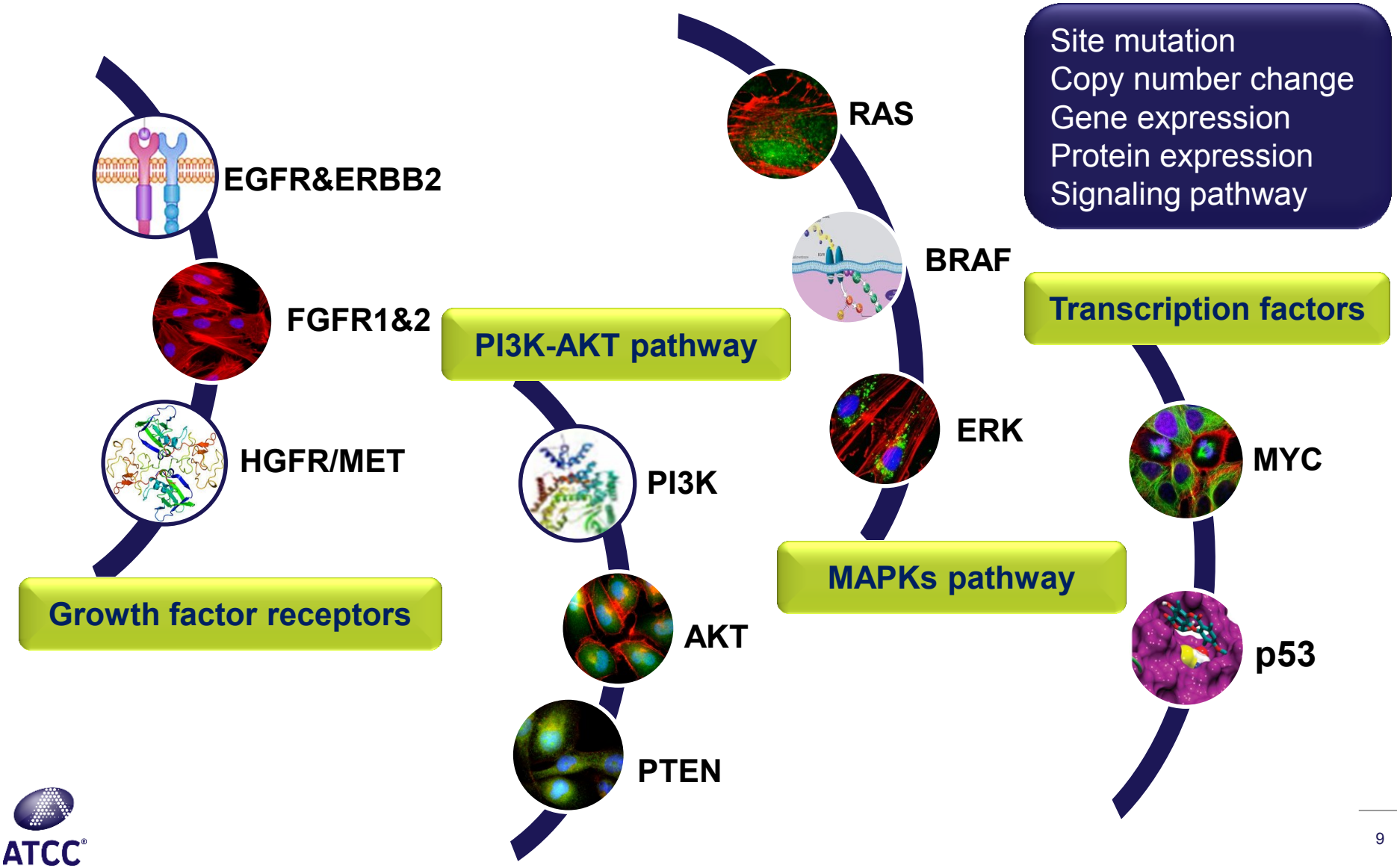
Example: KRAS mutation CRM cell lines and DNAs

ATCC® No.	Cell line name	Amino acid change	DNA change
CRM-TIB-161™	HuT 78	**Wild Type**	WT
CRM-CCL-119™	CCRF-CEM	**p.G12D**	c.35G>A
CRM-CCL-185™	A549	**p.G12S**	c.34G>A
CRM-CRL-1420 ™	MIA PaCa-2	**p.G12C**	c.34G>T
CRM-HTB-174™	NCI-H441	**p.G12V**	c.35G>A
CRM-CRL-3211™	PSN1	**p.G12R**	c.34G>C
CRM-CCL-155™	RPMI 8226	**p.G12A**	c.35G>C
CRM-HTB-26™	MDA-MB-231	**p.G13D**	c.38G>A

KRAS mutation analysis is currently used as a predictive marker of EGFR inhibitor therapeutic response

ATCC®

Molecular Signature Cell Panels



Point mutation validation

Example: RAS Genetic Alteration Panel (ATCC® TCP-1031™)

ATCC® No.	Cell line name	Gene	AA Change	DNA Change	Zygosity	Coverage at Mutation Loci	% Zygosity
CRL-2177™	SW 1271	NRAS	p.Q61R	c.182A>G	Homozygous	26732	G = 99.8%
CRL-2273™	CHP-212	NRAS	p.Q61K	c.181C>A	Heterozygous	49859	C = 50.7, A = 49.1
CRL-7585™	Hs 852.T	NRAS	p.G12V	c.35G>T	Heterozygous	66411	G = 38.0, T = 61.8
CRL-9068™	NCI-H929	NRAS	p.G13D	c.38G>A	Heterozygous	21896	A = 53.9, G = 45.9
TIB-202™	THP-1	NRAS	p.G12D	c.35G>A	Heterozygous	60288	A = 70.1, G = 29.9
CRL-2547™	Panc 10.05	KRAS	p.G12D	c.35G>A	Heterozygous	42708	G = 52.7, A = 47.3
CRL-2549™	Panc 03.27	KRAS	p.G12V	c.35G>T	Heterozygous	58913	G = 47.0, T = 52.9
HTB-174™	NCI-H441	KRAS	p.G12V	c.35G>T	Heterozygous	87521	G = 52.8, T = 47.1
CL-187™	LS 180	KRAS	p.G12D	c.35G>A	Heterozygous	91234	G = 51.3, A = 48.6
CCL-225™	HCT-15	KRAS	p.G13D	c.38G>A	Heterozygous	49764	G = 52.1, A = 47.8

ATCC®

Gene copy number change validation

Example: EGFR Genetic Alteration Panel (ATCC® TCP-1027™)

ATCC® No.	Cell line name	Gene	EGFR copy number variation	Measured CNV of EGFR	ERBB2 copy number variation	Measured CNV of ERBB2	Tumor source
CRL-2868™	HCC827	EGFR	Amplification	63.01	–	–	Lung
HTB-132™	MDA-MB-468	EGFR	Amplification	25.02	–	–	Breast
HTB-19™	BT-20	EGFR	Amplification	15.73	–	–	Breast
HTB-178™	NCI-H596	EGFR	Amplification	0.06	–	–	Lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	Lung
CRL-5928™	NCI-H2170	ERBB2	–	–	Amplification	128.89	Lung
HTB-20™	BT-474	ERBB2	–	–	Amplification	29.70	Breast
HTB-27™	MDA-MB-361	ERBB2	–	–	Amplification	16.85	Breast

EGFR and HER2 are currently used as predictive markers of kinase inhibitor response in NSCLC and breast cancer therapy

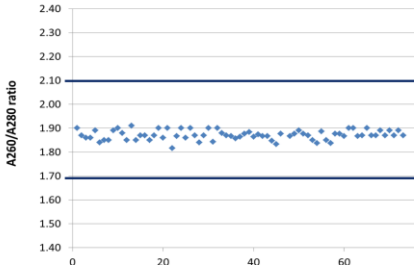
ATCC®

Do you have high quality DNA for your tests?

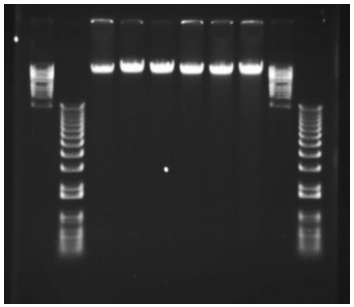
- Quantity
- Integrity
- Purity
- Identity
- Functionality

Validated DNA quantification

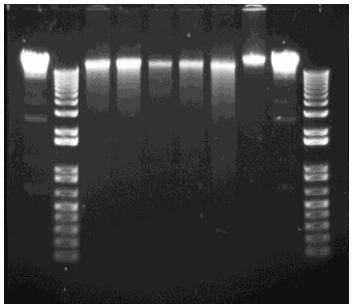
$A_{260}/_{280}$



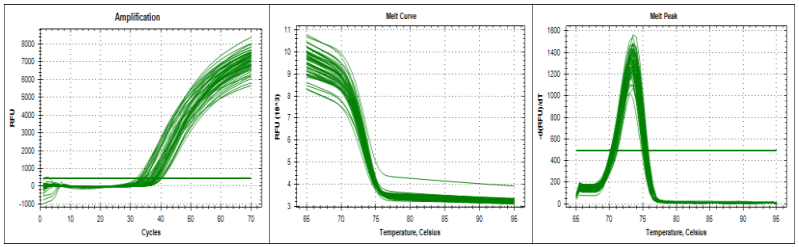
Electrophoresis
–uncut DNA



–DNA digestion



DNA tested in PCR-based assay

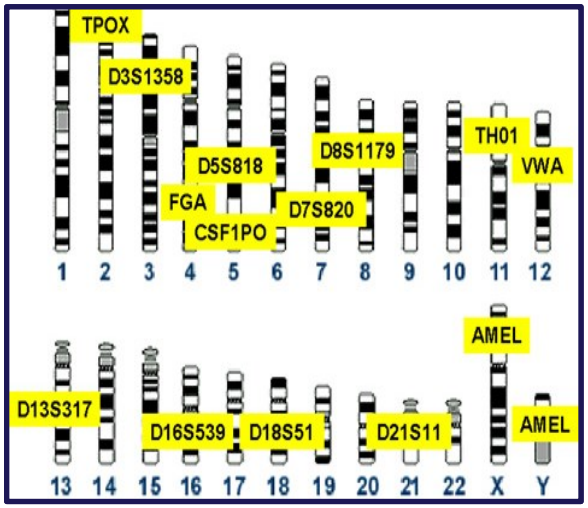


ATCC®

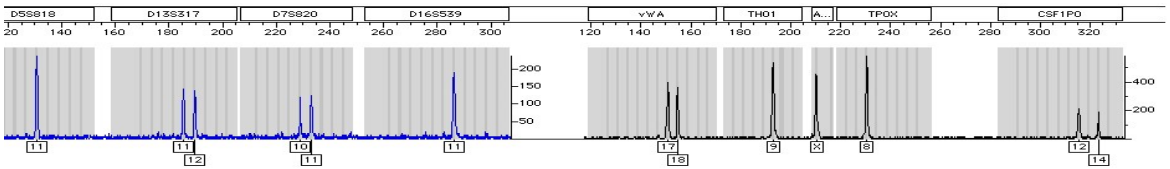
Ensure DNA identity & avoid contamination

- STR analysis (DNA profiling)
- Intraspecies identification and authentication of human cell lines
- Target sequence consists of microsatellite DNA containing short tandem repeats
- STR test can determine:
  - DNA identity when compared to a reference
  - Cross-contamination

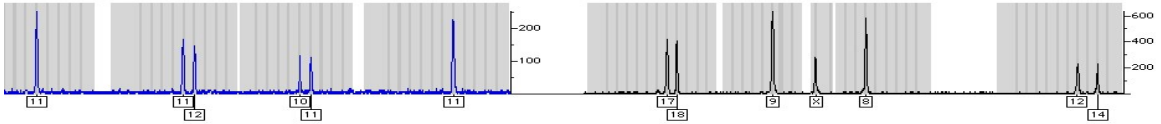
Barcode of Life
Barcoding for species Identification



Parental cell line



Parental cell line derived genomic DNA



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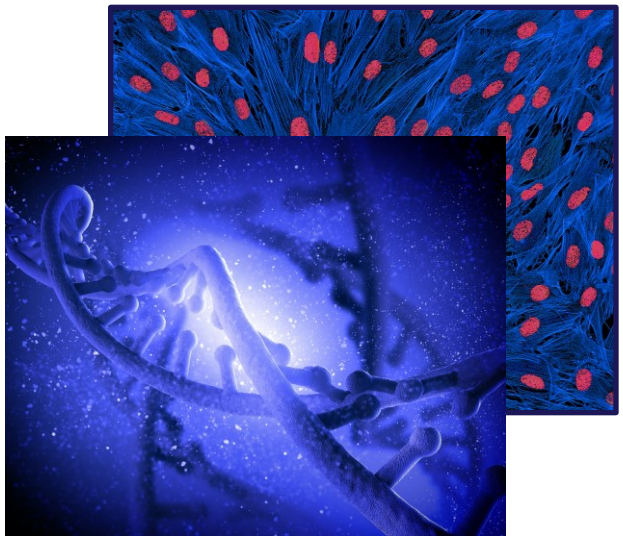
Tumor/normal cell line and DNA pair

COLO829 (ATCC® CRL-1974™)/COLO829BL (ATCC® CRL-1980™)

- First comprehensive catalog of somatic mutations from an individual cancer
- Pleasance *et al*, Nature 2010, 463:191-196
- COLO829 – Malignant melanoma
- COLO829BL – Human lymphoblast
- SNVs, InDels, CNVs, SVs
  - Confirmed by PCR & sequencing
- HiSeq 90x WGS at Illumina & TGEN
- Complete Genomics WGS
- Multiple dilution series analyzed



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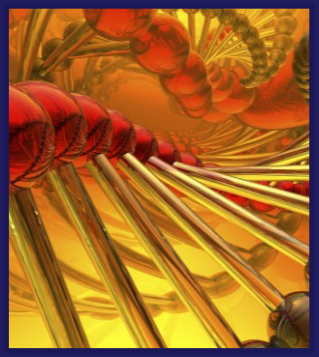
Summary

- Next generation sequencing is revolutionizing medical research, and molecular diagnostics is facilitating the development of personalized medicine
- Challenges still remain, including the areas of technical, computational, data interpretation, standards, and Certified Reference Materials
- Authenticated cell lines and cell derivatives are useful tools in molecular-based assay development
- ATCC purified genomic DNA preparations are isolated from human cell lines and contain relevant oncological biomarkers



ATCC Poster Presentation

Cell Line Genomic DNAs for the Molecular Diagnosis of Cancer
Poster #G55
Abstract #4502
November 15, 2014 from 9:45 AM – 10:45 AM

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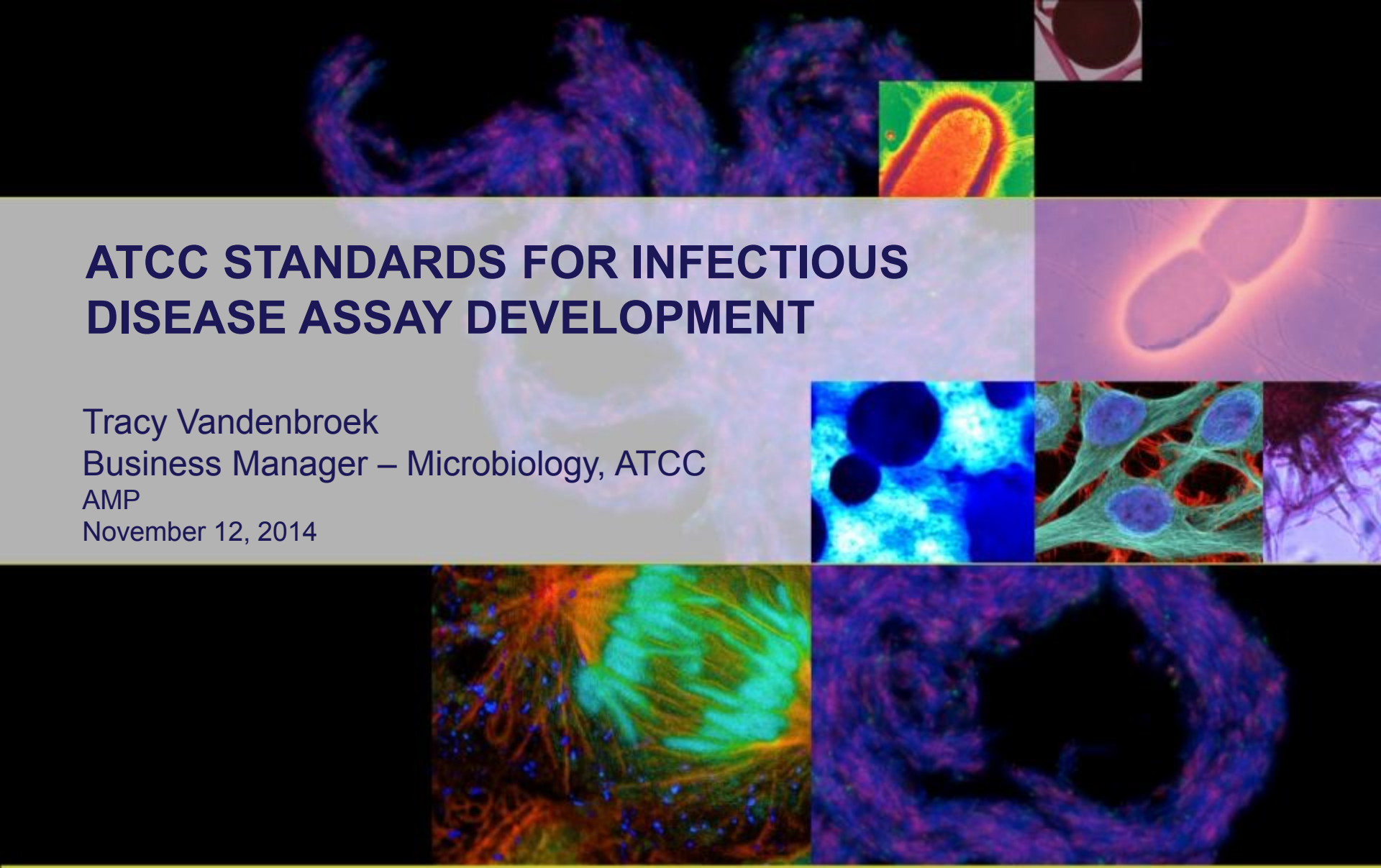

ATCC STANDARDS FOR INFECTIOUS DISEASE ASSAY DEVELOPMENT

Tracy Vandenbroek
Business Manager – Microbiology, ATCC
AMP
November 12, 2014

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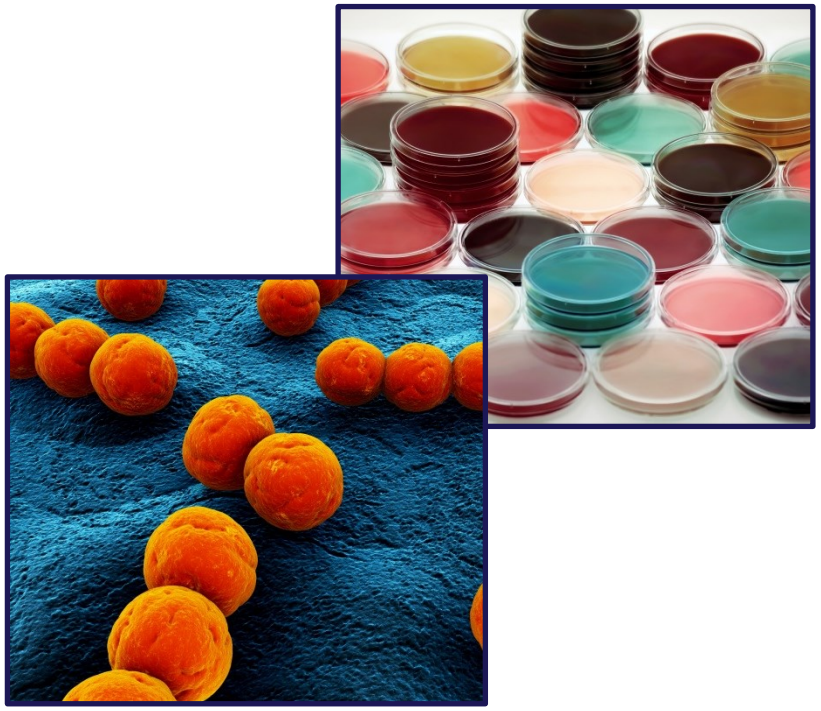
The microbial collection

ATCC® Genuine Cultures

- 18,000 bacteria
- 3,000 animal viruses
- 55,000 yeast and fungi
- 2,000 protozoa
- 1,000 plant viruses



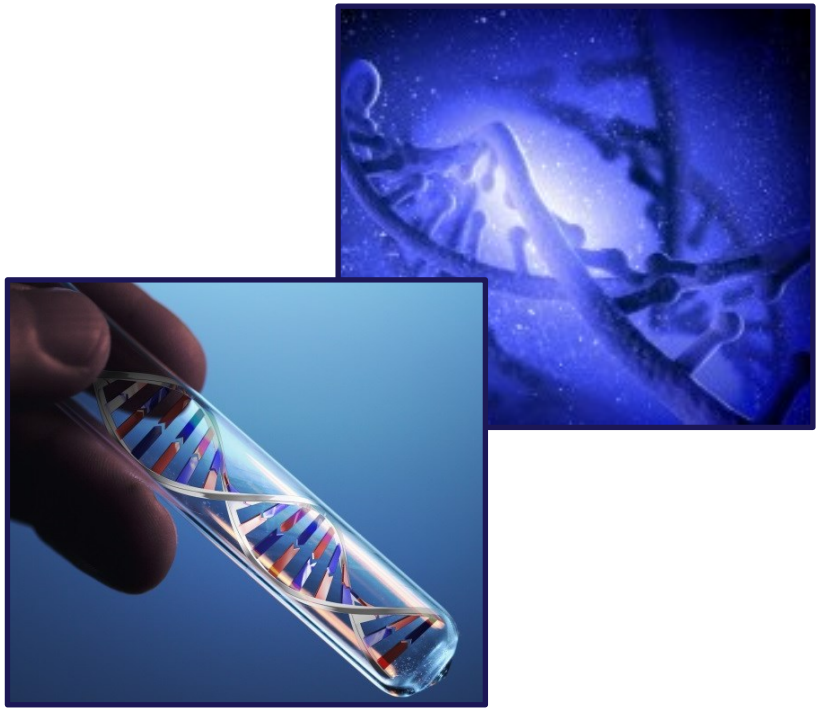
ATCC Genuine Cultures® are authenticated using a polyphasic approach that includes genotypic, phenotypic, and functional analyses.

ATCC®

The nucleic acid collection

ATCC® Genuine Nucleics

- Nearly 1,000 genomic DNA & RNA preparations from the collection
- A growing list of synthetic nucleics
- A custom shop that will purify nucleic acids from most ATCC Genuine Cultures®



ATCC® Genuine Nucleics are authenticated and characterized to ensure integrity, purity, concentration, functional activity, and species identity.

Infectious disease assay development

Optimizing experimental conditions can be challenging during the design phase of assay development, including sourcing organisms or nucleic acids with known traits or relevant genes.

The ATCC portfolio includes:

- Cultures grouped by agent or source of isolation
- Cultures tested for clinically-relevant phenotypic characteristics
- Fully-sequenced strains
- Genomic nucleic acids from close to 1,000 infectious disease strains
- Synthetic nucleic acids containing clinically-relevant gene sequences

ATCC®

Infectious disease assay development

Establishing ideal inclusivity/exclusivity parameters is an essential part of assay validation, particularly for the development of diagnostic and epidemiological assays whose results can affect individual and public health.

ATCC collections are also grouped by:

- Serotype
- Toxin production
- Drug-resistance
- Clinical relevance

ATCC®

Infectious disease assay development

The performance of culture- and molecular-based assays is often evaluated through the examination of sensitivity, specificity, and limit of detection (LOD). Accurate determination of LOD relies on the use of authenticated microbial or molecular controls with known concentrations.

ATCC offers the following products and services:

- Quantified microorganisms
- Genomic nucleic acids with known concentration
- Synthetic nucleic acids with calculated genome copy number
- Extraction and quantification services

ATCC®

Infectious disease assay development

During assay development, or when using a pre-qualified assay or sequencing tool, it is important to select appropriate external controls to evaluate and verify the performance of each process. This testing is imperative in tracking drift and run-to-run variation within a procedure.

To aid in assay validation, ATCC offers an expansive array of authenticated cultures and nucleic acid preparations for use as external controls in:

- Nucleic acid extraction
- Process verification
- Amplification
- Proficiency testing

ATCC®

ISO certification & accreditation

- ISO 9001:2008 certification for quality management system
- ISO 13485:2003 certification for the design, development, production, testing, and distribution of medical devices
  - Applies to synthetic nucleics
- ISO Guide 34:2009 accreditation for production
  - Applies to Certified Reference Materials (CRMs)
- ISO/IEC 17025:2005 accreditation for testing
  - Applies to all ATCC cultures, derivatives, and bioproducts tested in our laboratories

ATCC is the first and only Biological Resource Center to hold all 4 accreditations/certifications

ATCC®

Certified Reference Material (CRM)

Applied in a variety of ways, including:

- Establishing sensitivity, linearity, and specificity during assay validation or implementation
- Challenging assay performance
- Validating or comparing test methods
- Testing and calibration in ISO 17025 labs that stipulate the use of reference materials
- Benchmarking critical assay performance for regulatory submissions and production lot release

ATCC®

Certified Reference Material (CRM)

Quantitative *Mycoplasma* DNA CRMs

- Released early 2014
- Ten common *Mycoplasma* strains found in cell contamination
- Can be used to validate newer molecular-based *Mycoplasma* detection assays that claim to accurately detect lower concentrations of genomic material

ATCC® No.	Species	Specification Range
qCRM-15531D	*Mycoplasma pneumoniae*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-17981D	*Mycoplasma hyorhinis*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-19610D	*Mycoplasma gallisepticum*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-19989D	*Mycoplasma fermentans*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-23064D	*Mycoplasma salivarium*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-23206D	*Acholeplasma laidlawii*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-23714D	*Mycoplasma orale*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-23838D	*Mycoplasma arginini*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-25204D	*Mycoplasma synoviae*	$1x10^6$ – $1x10^7$ genome copies/µL
qCRM-27545D	*Mycoplasma hominis*	$1x10^6$ – $1x10^7$ genome copies/µL

ATCC®

ATCC® Genuine Nucleics

The largest and most diverse array of genomic and synthetic materials for use in molecular-based assays, quality control, and assay development

Genomic

- Isolated under aseptic conditions to prevent cross-contamination
- Fully authenticated and characterized
- Only available from ATCC

Synthetic

- Designed and developed to include key target regions from organisms that are:
  - Difficult-to-culture
  - Unculturable
  - High-containment (BSL3)

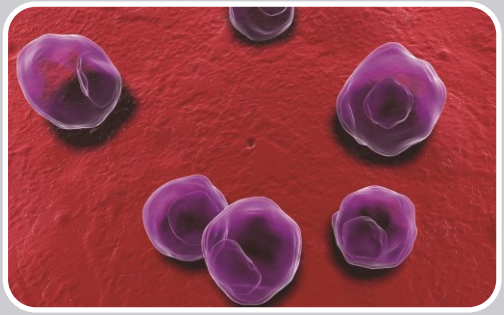
Quality control analyses ensure product identity, stability, quantity, and functionality

ATCC®

Quantitative genomic DNA

- Gene copies are quantitated by Droplet Digital™ PCR (Bio-Rad®)
- Allows for the easy generation of a standard curve for qPCR

New to the collection this year



Vector-borne Disease Research

- *Babesia microti*
  - Tick-borne piroplasm
- *Plasmodium falciparum*
  - Mosquito-borne protozoan parasite known to cause malaria

Waterborne Disease Research

- *Naegleria fowleri*
  - *a.k.a.* “Brain-eating amoeba”

Sexually Transmitted Infection Research

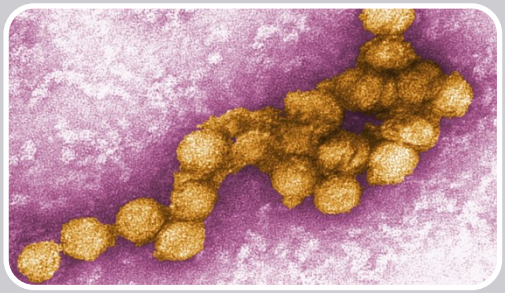
- *Chlamydia trachomatis* LGV serovars I, II, III
  - Nearly 5 million new cases each year in the United States

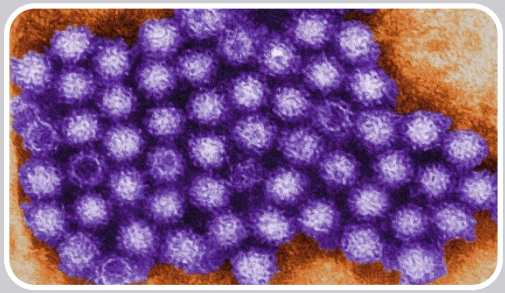
ATCC®

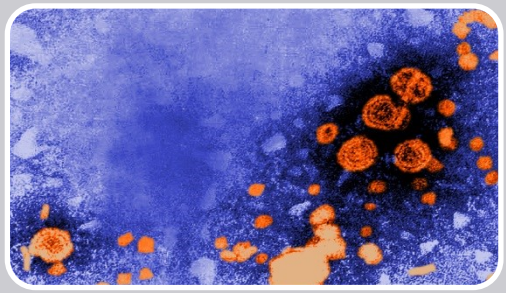
Synthetic molecular standards

- Designed to target regions encompassing common primer sequences
- Stabilized with RNAstable® (Biomātrica®)
- Quantified by Droplet Digital™ PCR (Bio-Rad®)

The current collection







Vector-borne Disease Research

- West Nile virus
- Dengue virus serotypes 1-4

Gastroenteritis Research

- Norovirus GI & GII

Sexually Transmitted Infection Research

- *Mycoplasma genitalium*
- *Treponema pallidum*
- Hepatitis B
- Hepatitis C

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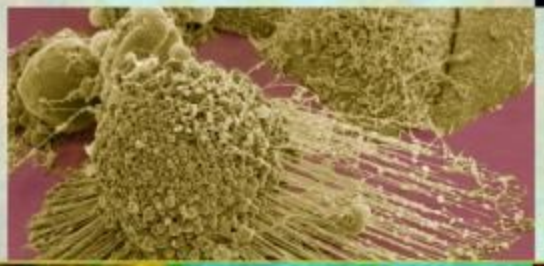
THANK YOU



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