THE IMPORTANCE OF STANDARDS IN MOLECULAR-BASED ASSAYS

Liz Kerrigan
Director, Standards
May 8, 2014

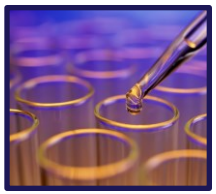
ATCC® | THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- ATCC serves and supports the scientific community with industry-standard products and innovative solutions
- World's leading biological resource center and provider of biological standards
- Broad range of biological materials
  - Cell lines
  - Microorganisms
  - Native & synthetic nucleic acids
  - Reagents

ATCC®

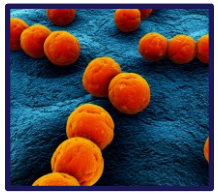
Overview



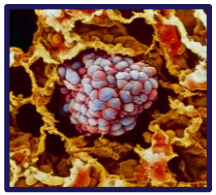
Principles of assay development

Assay design

- Assay sensitivity
- Analytical specificity & microbial interference
- Controls for nucleic acid based assays



Microbial standards & tools for assay development



Challenging assay design with cancer cell lines & nucleic acids

Using Biological Standards in Proficiency Testing programs

Standards for Next-Gen sequencing

ATCC®

Utility for molecular testing

- Improved sensitivity
- Improved specificity
- Time to results
- Ability to multiplex

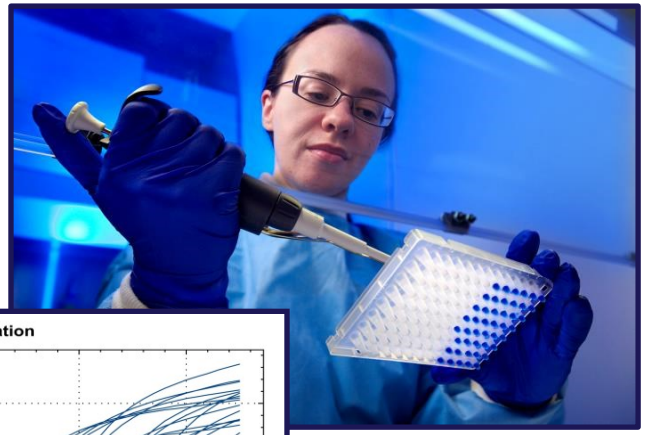
ATCC®

Assay development

- Assay design
  - Design goals
  - Qualitative or quantitative assay
  - Target genes
  - Relevant strains and variants
  - Updates

Analytical sensitivity, specificity & interference

- Analytical sensitivity
  - Limit of Detection (LoD)
  - Reactivity
- Analytical specificity
  - Cross reactivity
- Interference
  - Clinical specimens



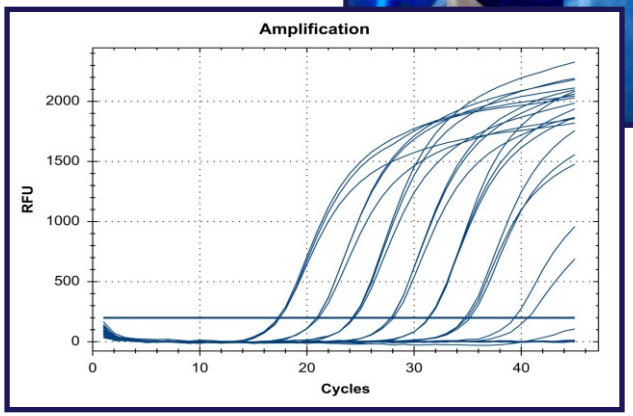


Photo credit: James Gathany, CDC

Controls for nucleic acid based assays

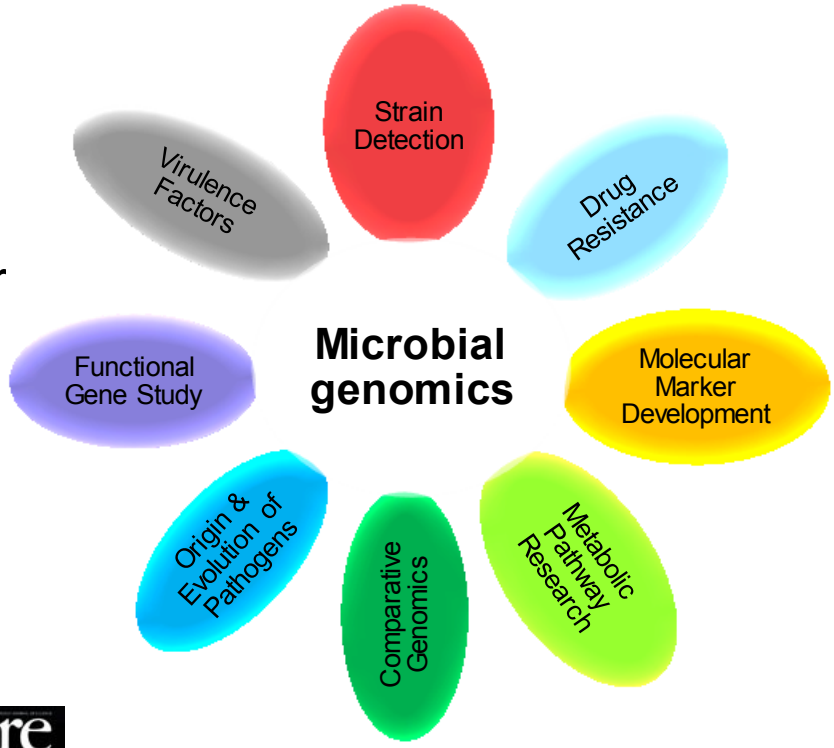
- Used for verification of assay performance in your analytical and clinical studies:
  - **Negative Control:** to rule out contamination
  - **Positive Control:** to verify PCR reagents & instrument are running properly
  - **Internal Control:** to verify that a sample result is not caused by PCR inhibitors
  - **External Control:** to verify that lysis & extraction processes are functioning properly

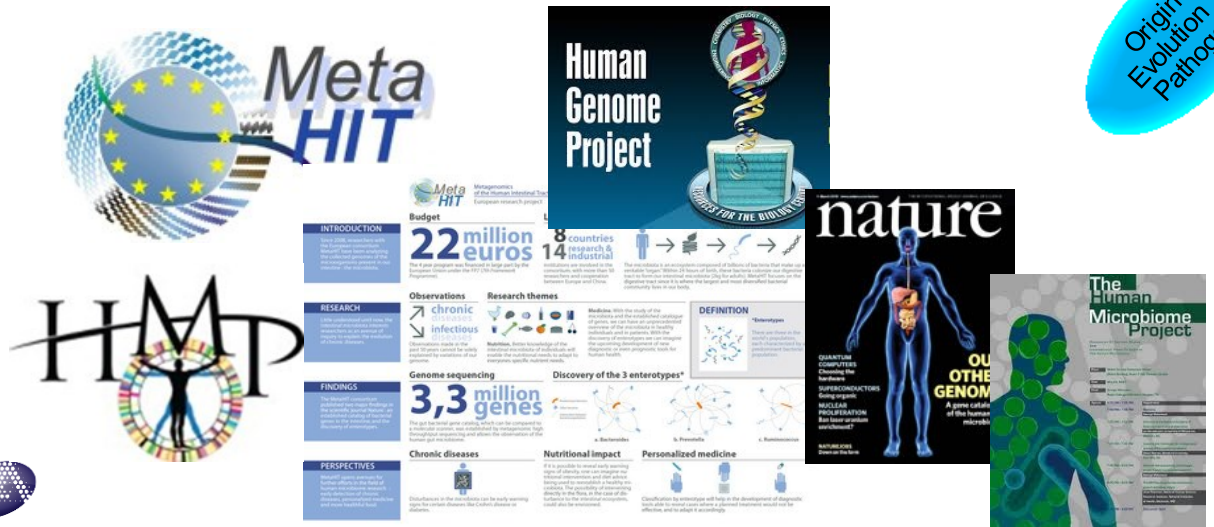
ATCC®

The changing landscape of microbial genomics

Large scale sequencing programs:

- The Human Genome Project
- Human Microbiome Project
- JCVI Genomic Sequencing Center for Infectious Diseases (GSCID)
- MetaHIT Project





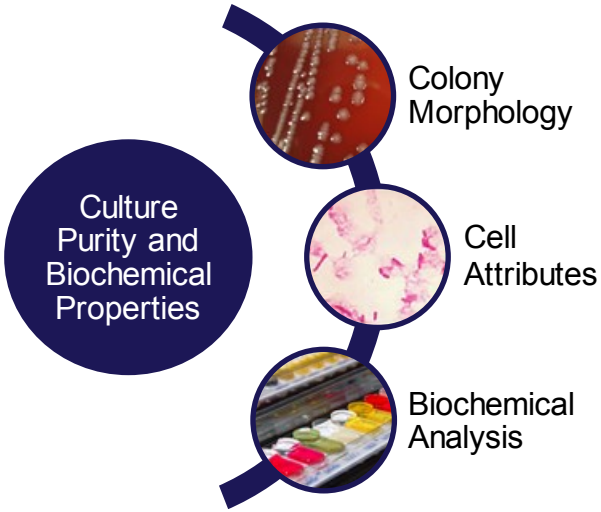
Highly characterized microbial strains for assay development

ATCC utilizes both classical and modern techniques

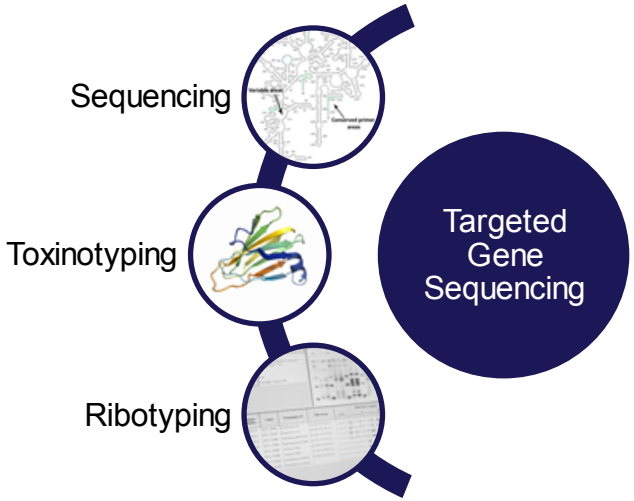
- Phenotypic analysis
- Genotypic analysis
- Functional analysis

No single method of identification is sufficient

Phenotypic testing



Genotypic testing



ATCC®

40 *Clostridium difficile* strains

ATCC® No.	Strain	Toxinotype	Binary Toxin	EIA*	Ribotype	Isolation Source
BAA-1804™	--	0	ND	+	053	Clinical isolate, USA
BAA-1870™	4118	IIIb	Y	+	027	Clinical isolate, USA ME
BAA-1803™	--	IIIc	Y	+	027	Clinical isolate, USA
BAA-1801™	3232	*tcdA*-, *tcdB*-	ND	-	010	Human feces, Belgium
BAA-1875™	5325	V	Y	+	078	Clinical isolate, USA GA
43598™	1470	VIII	ND	+	017	Human feces, Belgium
BAA-1812™	--	XII	ND	+	024	Clinical isolate, USA
BAA-1814™	--	XXII	Y	+	251	Clinical isolate, USA

*Enzyme Immuno Assay (EIA) performed with the Wampole™ C. DIFF QUIK CHEK COMPLETE® kit or the equivalent
ND= Not Detected

Native DNA is available from six different strains

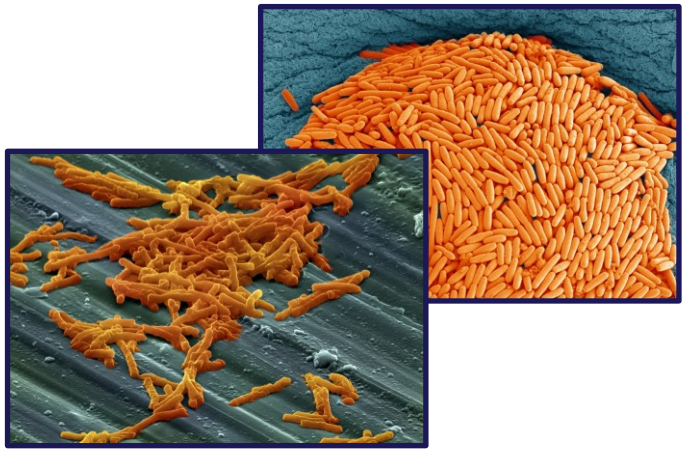


Photo credit: Annie Cavanagh, David Goulding, Wellcome Trust Sanger Institute

Quantitated, frozen, *Clostridium difficile* strains

Tools for Assay Development

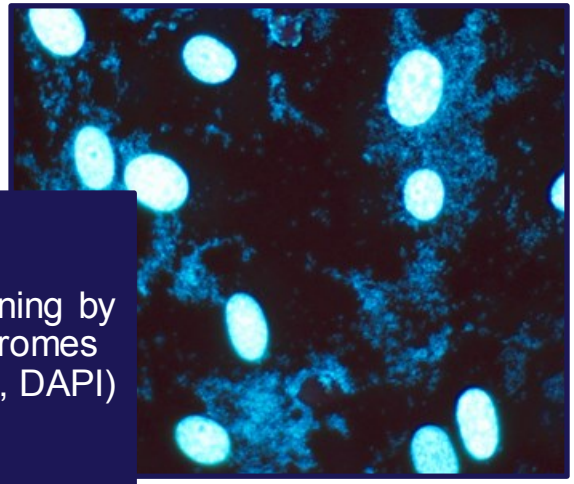
ATCC® No.	Strain	Toxinotype	Ribotype	Titer (CFU/vial)	ddPCR™ (Bacteria/vial)	Isolation source
BAA-1382-FZ™	630	0	012	4.9×10^6	9.6×10^8	Genome sequenced, clinical isolate, Switzerland
17858-FZ™	1253	0	054	1.0×10^8	5.9×10^8	Unknown
9689-FZ™	90556-M6S	0	001	2.2×10^7	3.97×10^8	Type strain
43255-FZ™	VPI 10463	0	087	1.2×10^8	9.2×10^8	Abdominal wound
43593-FZ™	1351	*tcdA*-, *tcdB*-	060	1.5×10^8	5.4×10^8	Human feces, Belgium
43596-FZ™	545	0	012	1.1×10^8	2.62×10^8	Human feces, Belgium
43599-FZ™	2022	0	001	4.5×10^5	7.38×10^8	Human feces, Belgium
43601-FZ™	7322	*tcdA*-, *tcdB*-	031	4.0×10^7	3.86×10^8	Human feces, Belgium
51695-FZ™	BDMS 18 AN	0	001	3.3×10^6	3.98×10^8	
700792-FZ™	14797-2	0	005	4.6×10^7	1.5×10^9	Human feces, Michigan, 1977

*Post preservation material is checked for total bacterial cell count (per vial) using ddPCR™

Detecting mycoplasma

Microbiological culture method

DNA staining by fluorochromes (Hoechst, DAPI)



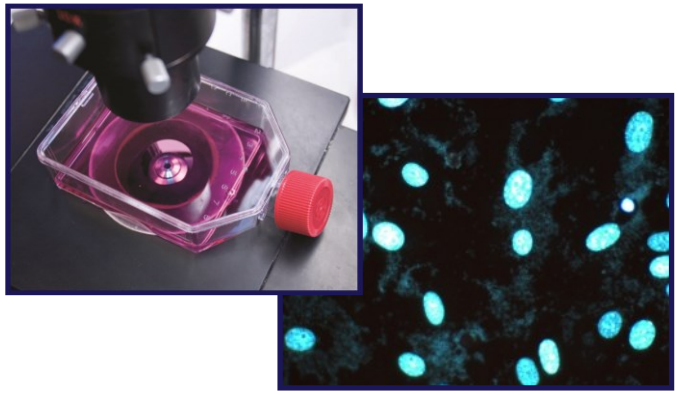
Polymerase chain reaction

ATCC®

Tools for mycoplasma assay development

Titered Mycoplasma Reference Strains Panel (ATCC® MP-7™):

- A panel of 10 titered mycoplasma reference strains with calculated genome copy numbers
- Highly viable and dispersed
- Useful for assay development
- Useful for comparing culture- and molecular-based detection assays*



*Dabrazhynetskaya A, et al. *Biologicals* 41(6): 377-383, 2013. PubMed: 23910092

Titered Mycoplasma Reference Strains Panel (ATCC® MP-7™)

ATCC® No.	Species	Post Preservation Titer	GenBank (Strain Specific)
15531-TTR™	*Mycoplasma pneumoniae*	1.0 x 10^8 cfu/mL	CP002077.1
17981-TTR™	*Mycoplasma hyorhinis*	6.6 x 10^7 cfu/mL	AF258792.1
19610-TTR™	*Mycoplasma gallisepticum*	2.17 x 10^8 cfu/mL	JN935873.1
19989-TTR™	*Mycoplasma fermentans*	1.0 x 10^9 cfu/mL	AP009608.1
23064-TTR™	*Mycoplasma salivarium*	1.67 x 10^9 cfu/mL	AB680625.1
23206-TTR™	*Acholeplasma laidlawii*	7.10 x 10^8 cfu/mL	CP000896.1
23714-TTR™	*Mycoplasma orale*	3.08 x 10^8 cfu/mL	NR_043199.1
23838-TTR™	*Mycoplasma arginini*	3.7 x 10^9 cfu/mL	NR_041743.1
25204-TTR™	*Mycoplasma synoviae*	1.0 x 10^9 cfu/mL	NR_044811.1
27545-TTR™	*Mycoplasma hominis*	1.3 x 10^8 cfu/mL	JN935871.1

Each strain is prepared with a low genome copy (GC) to colony forming unit (CFU) ratio, which is ideal for use in the development and validation of PCR-based methods of detection.

ATCC®

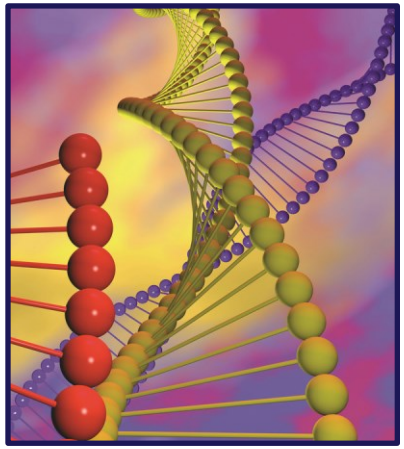
CRM quantitated mycoplasma genomic DNA

Key Features:

- Certified Reference Material
- Quantitated – Genome copy number is based on the quantification of the 16S rRNA gene from nine averaged samples using ddPCR™. ($1.0x10^6$-$1.0x10^7$ genome copies/µL)
- Extracted from the titered mycoplasma reference strains (MP-7™)

Use as a quantitative external control for:

- Inclusivity/exclusivity testing
- Establishing limits of detection (LoD)
- Verification or comparison of test methods
- Other molecular applications



ATCC®

Droplet Digital™ PCR is a trademark of Bio-Rad Laboratories, Inc.

CRM quantitated mycoplasma genomic DNA

Certified Reference Materials

- Produced under an ISO Guide 34 accredited process
- Established chain of custody

ACLASS ACCREDITED
REFERENCE MATERIAL PRODUCER
AR-1384

Extracted from ATCC Genuine Cultures®

- Agarose gel electrophoresis to ensure integrity
- Spectrophotometry to evaluate purity
- QX100 Droplet Digital™ PCR to calculate quantity
- PCR to confirm functional activity
- Sequence analysis of 16S rRNA to confirm species identity

ATCC®

Droplet Digital™ PCR is a trademark of Bio-Rad Laboratories, Inc.

CRM quantitated mycoplasma genomic DNA

ATCC® No.	Species	Genome Size (bp)	16S RNA Copy Number	GenBank
qCRM-15531D	*Mycoplasma pneumoniae*	811088	1	CP002077.1
qCRM-17981D	*Mycoplasma hyorhinis*	806507	1	GCA_000383515.1
qCRM-19610D	*Mycoplasma gallisepticum*	920684	1	GCA_000428645.1
qCRM-19989D	*Mycoplasma fermentans*	1004014	2	AP009608
qCRM-23064D	*Mycoplasma salivarium*	TBD	1	GCA_000485555.1
qCRM-23206D	*Acholeplasma laidlawii*	1496992	2	CP000896.1
qCRM-23714D	*Mycoplasma orale*	710549	1	GCA_000420105.1
qCRM-23838D	*Mycoplasma arginini*	758854	2	GCA_000428625.1
qCRM-25204D	*Mycoplasma synoviae*	736709	2	GCA_000385095.1
qCRM-27545D	*Mycoplasma hominis*	684158	2	GCA_000385075.1

ATCC®

ATCC® Genuine Nucleics

Electrophoresis

Integrity

Spectrophotometry

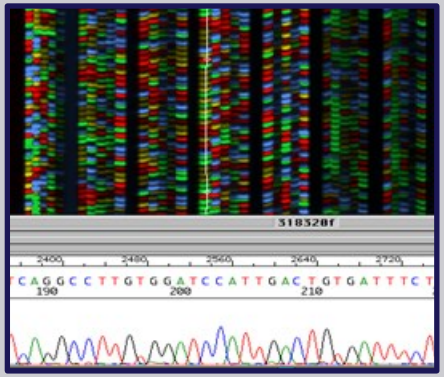
Purity

PicoGreen® and RiboGreen®

Concentration

PCR

Function



Sequencing

Identity

PicoGreen® and RiboGreen® are registered trademarks of Molecular Probes, Inc.

ATCC native nucleic acids

Whole genome preparations extracted from ATCC Genuine Cultures®

- More than 750 catalog items
- Agarose gel electrophoresis to ensure integrity
- Spectrophotometry to evaluate purity
- PicoGreen® or RiboGreen® to calculate concentration
- PCR to confirm functional activity
- Sequence analysis of conserved genomic regions to confirm species identity
- Custom preparations available
- DNA from bacteria, fungi, protists, and viral strains
- RNA from viral strains

ATCC®

ATCC synthetic nucleic acids

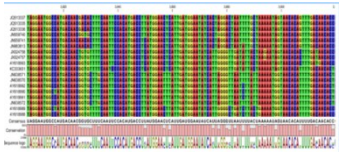
- Synthetically created nucleic acids to serve as a genetic surrogate for microorganisms that are:
  - Difficult to culture
  - Can not be cultured *in vitro*
  - Select agents and/or BSL 3.

ATCC®

Synthetic molecular standards for Dengue virus

DENV Genome

Multiple sequence alignment



Artificial RNA synthesis

Stabilization (RNAstable®)

Sequence Verification
(Next generation sequencing)

Absolute Quantification
(Droplet Digital™PCR)

ATCC Synthetic Molecular Standards	
DENV-1	ATCC® VR-3228SD™
DENV-2	ATCC® VR-3229SD™
DENV-3	ATCC® VR-3230SD™
DENV-4	ATCC® VR-3231SD™

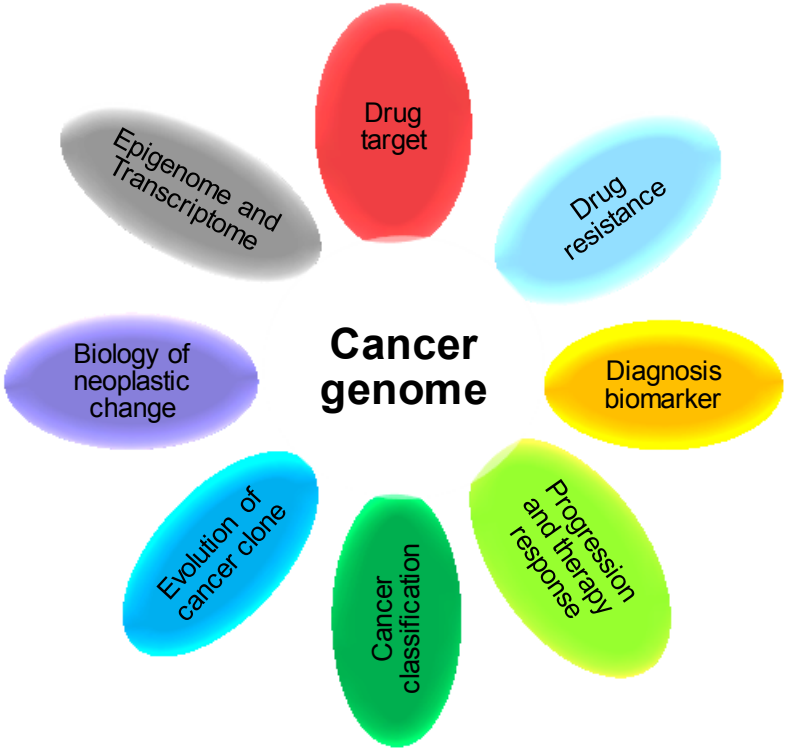
Advantages	Applications
• ATCC fully authenticated & characterized Genuine Nucleics • Consistent and accurate results • BSL-1 ready-to-use control • No shipping restrictions • Quantitative format • Stabilized RNA	• Generation of a standard curve for quantitative RT-PCR (qRT-PCR) • Positive control for RT-PCR assays • Independent standard for validation and verification studies • Monitor assay-to-assay and lot-to-lot variation

ATCC®

The changing landscape of the cancer genome

Large scale sequencing programs:

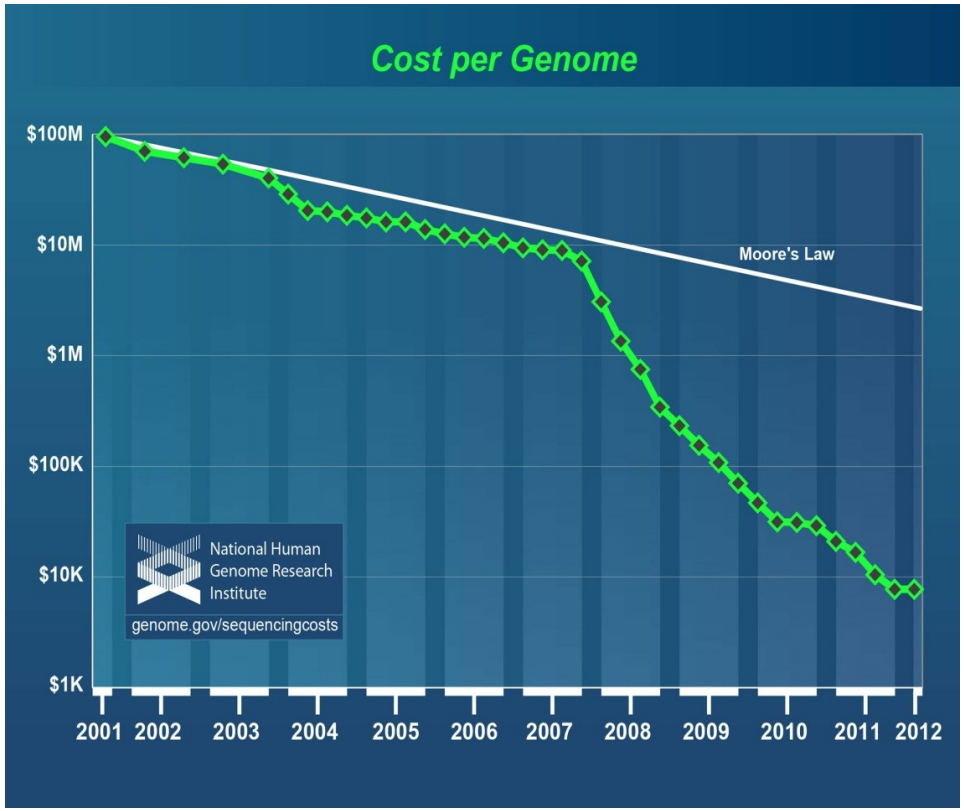
- The Cancer Genome Atlas (TCGA)
- International Cancer Genome Consortium (ICGC)
- Catalogue of Somatic Mutations in Cancer (COSMIC)



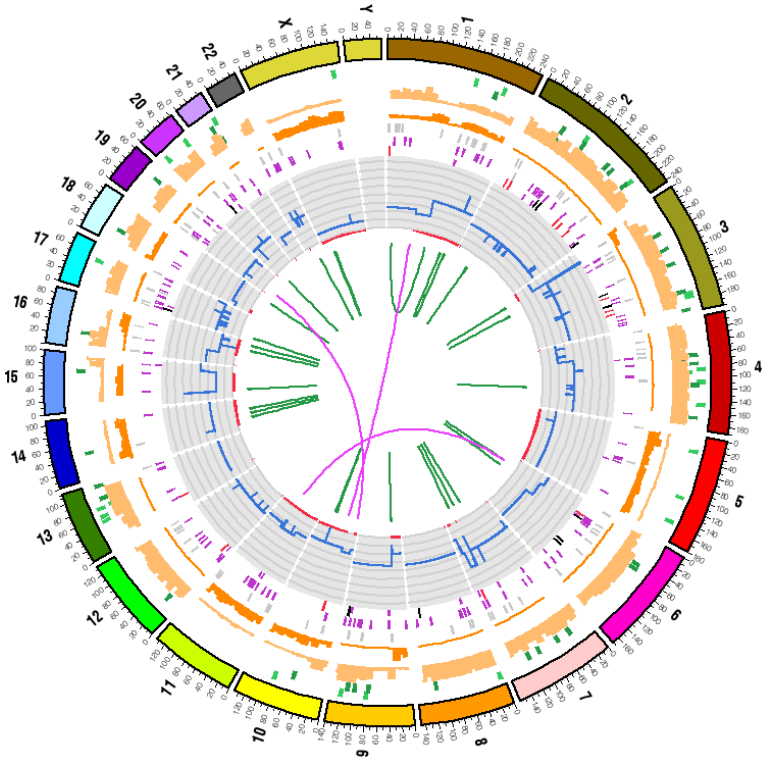
ATCC®

NGS leads to genomic age

Next generation sequencing



Cancer genome



Circos cancer genome display

ATCC®

Challenges in molecular testing

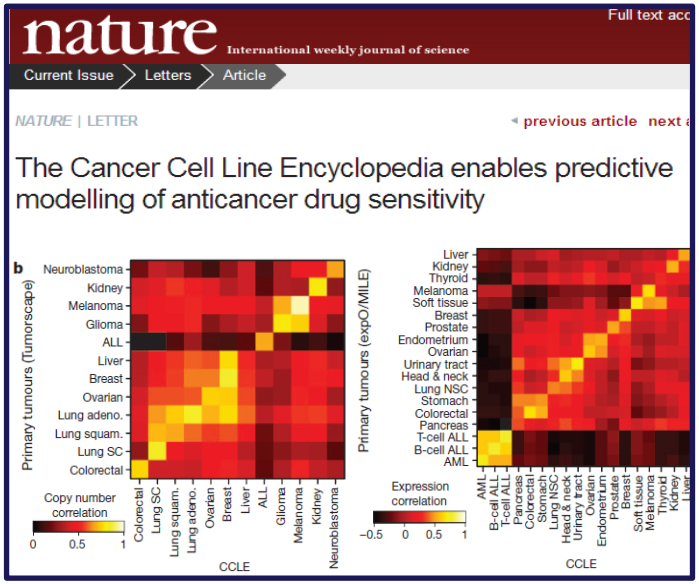
- Omics data storage
- Data analysis
- Quality standards for each step from sample to answer
- Appropriate and reliable controls

Although over 1900 genetic tests are available, the majority of tests still need characterized references or QC materials

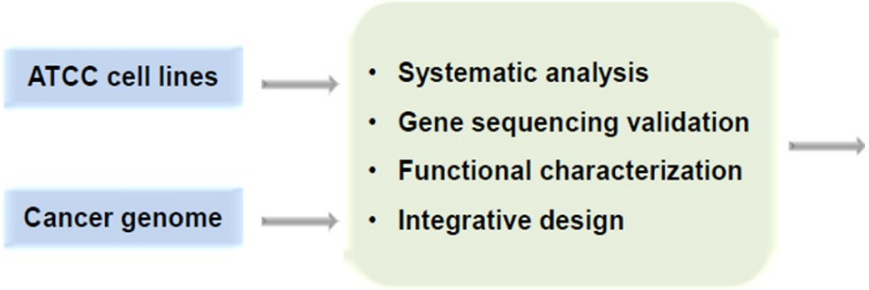
ATCC®

Using authenticated cell lines as controls

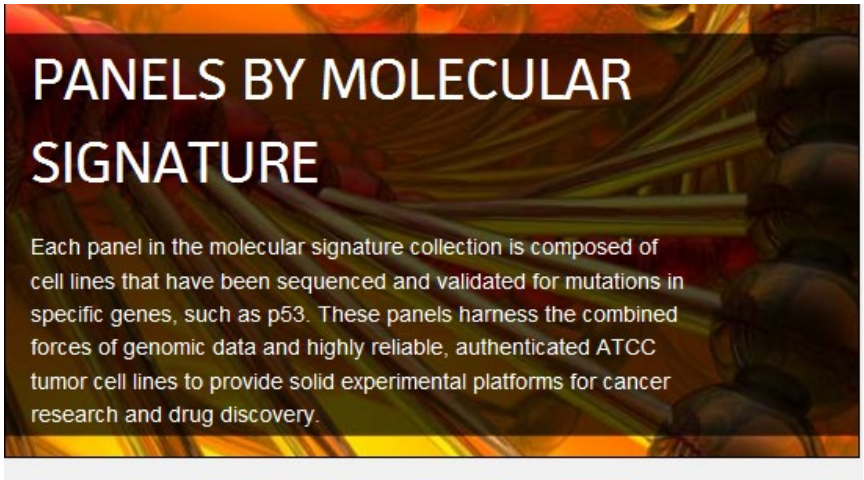
- Fully authenticated
- COI and STR testing to avoid inter-species and intra-species contamination or misidentification
- Characterized tumor genetic alterations
- Stable molecular profiles
- Control FFPE process
- Control IF or IHC staining process



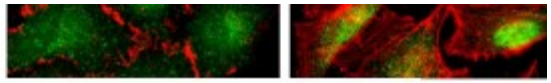
ATCC Molecular Signature Cell Panels



Molecular Signature Cell Panels
- Driver gene focused
- Signaling pathway focused
- Integrative design

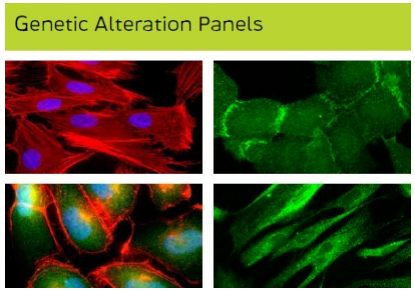


p53 hotspot mutation panels



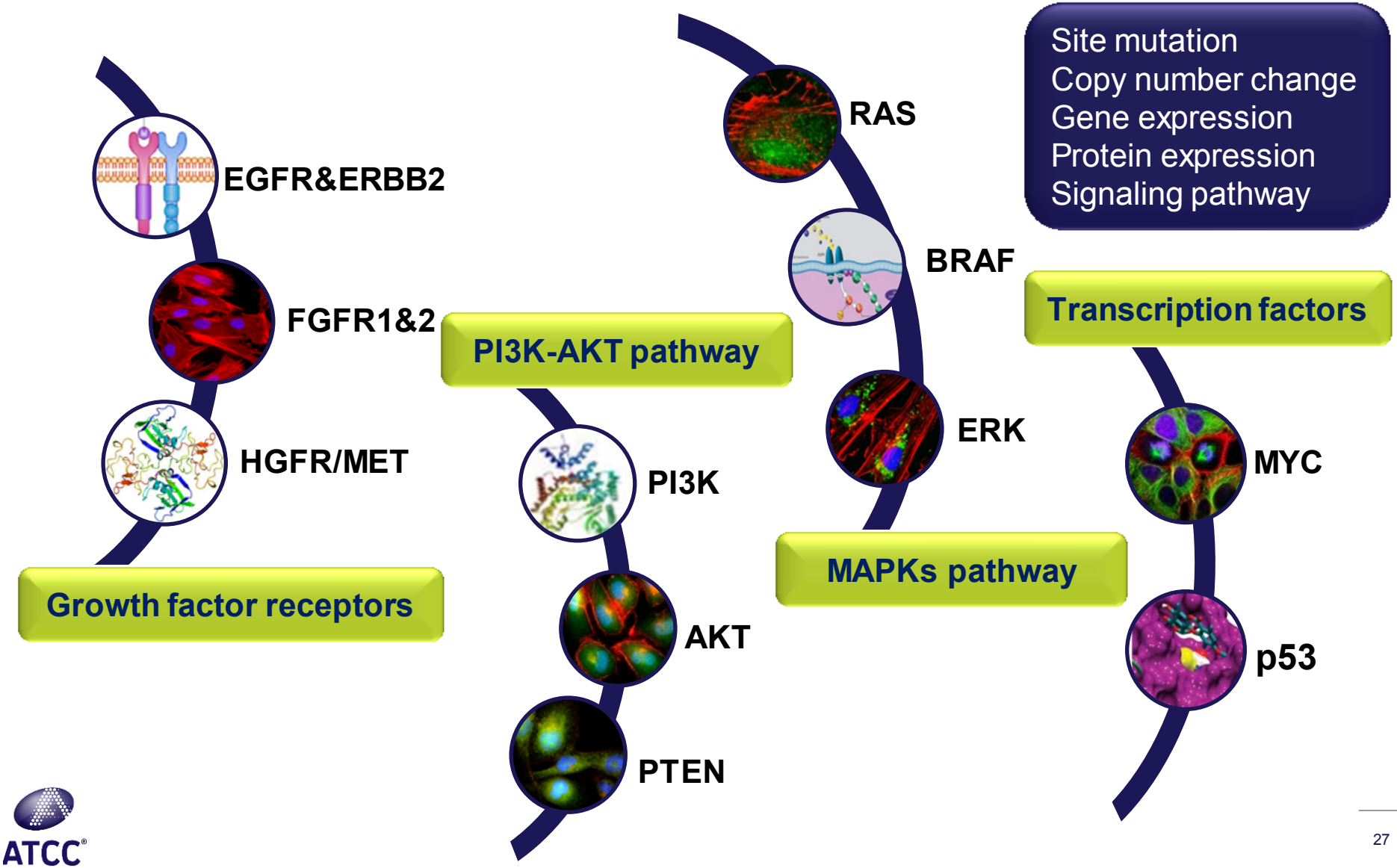
NON-SMALL CELL LUNG CANCER P53 HOTSPOT MUTATION CELL PANEL

Genetic Alteration Cell Panels



ATCC®

Molecular Signature Cell Panels



ATCC®

EGFR cell panel characteristics

EGFR Genetic Alteration Cell Panel (ATCC® TCP-1027™)								
ATCC® number	Cell line name	Gene	cDNA Change	Zygosity	Amino acid Change	EGFR copy number variation	ERBB2 copy number variation	Tumor source
CRL-2868™	HCC827	EGFR	c.2236_2250delGAA TTAAGAGAAGCA	Heterozygous	p.ELREA746del	amplification	–	lung
CRL-2871™	HCC4006	EGFR	c.2236_2244delGAA TTAAGA	Heterozygous	p.ELR746del	–	–	lung
CCL-231™	SW48	EGFR	c.2155G>A	Heterozygous	p.G719S	–	–	colon
CRL-5908™	NCI-H1975	EGFR	c.2369C>T	Heterozygous	p.T790M	–	–	lung
			c.2573T>G	Heterozygous	p.L858R		–	
HTB-132™	MDA-MB-468	EGFR	–	–	–	amplification	–	breast
HTB-19™	BT-20	EGFR	–	–	–	amplification	–	breast
HTB-178™	NCI-H596	EGFR	–	–	–	amplification	–	lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	–	lung
CRL-5928™	NCI-H2170	ERBB2	–	–	–	–	amplification	lung
HTB-20™	BT-474	ERBB2	–	–	–	–	amplification	breast
HTB-27™	MDA-MB-361	ERBB2	–	–	–	–	amplification	breast

ATCC®

ATCC Certified Reference Material (CRM)

Reference materials are:

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

The intended uses of ATCC Certified Reference Materials are:

- To challenge assay performance.
- Validate or compare test methods.
- Establish sensitivity, linearity, and specificity during assay validation or implementation.
- To benchmark critical assay performance during development/validation for regulatory submissions and production lot release.
- For use in testing and calibration in ISO 17025 accredited laboratories.

ACCREDITED
REFERENCE MATERIAL PRODUCER
AR-1384

Certified Reference Materials from ATCC are accompanied by a Certificate of Analysis.

KRAS mutation CRM cell lines and DNAs

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

ATCC® No.	Cell line name	AA change	DNA change
CRM-TIB-161™	HuT 78	WT	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

CRM DNAs are now available

ATCC®

Need for Biological Standards in Proficiency Testing

Key issues in Molecular testing

Rapidly moving field

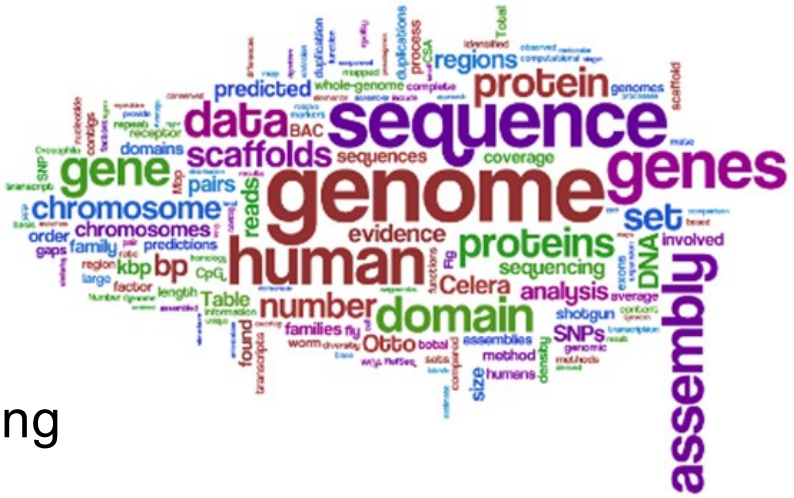
- Test relevant, actionable targets
  - Need high quality, challenging, well characterized PT materials
  - Consistent, traceable
  - Representatives of contemporary strains
  - Genetic variations/mutations
  - New emerging strain variants
- New Technology Developments
  - NGS
  - Maldi-Tof
  - Multiplex assays

ATCC®

Reference material for Next-Gen Sequencing

Characterize platforms & methods:

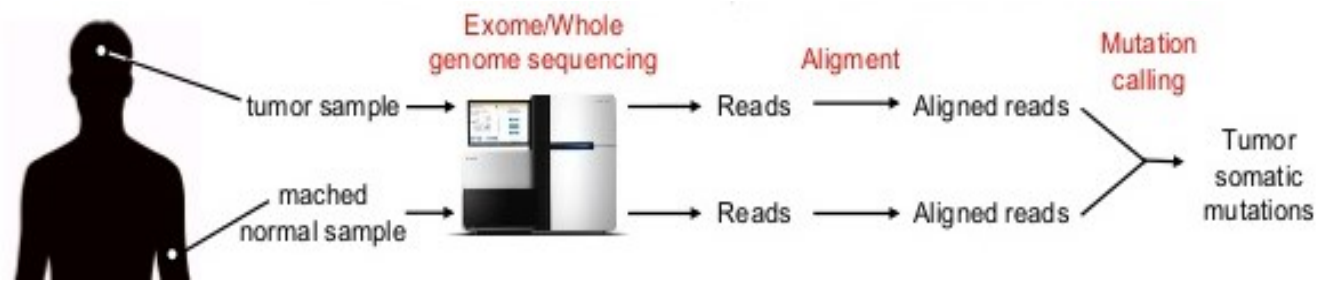
- DNA sequencing
- NGS technologies and platforms
- Research applications
- Clinical diagnostic applications
  - Assay validation
- Facilitate tool development for evaluating performance
  - Somatic calls for:
    - Single nucleotide variants (SNVs)
    - Indels
    - Structural variants
    - Copy number variation
    - RNA fusion detection



Advantages of tumor/normal pairs

ATCC® No.	Name	Cancer Type	Tissue Source	ATCC® No.	Name	Tissue Source
CRL-1974™	COLO 829	Melanoma, malignant	Skin	CRL-1980™	COLO 829BL	B lymphoblast

- First comprehensive catalog of somatic mutations from an individual cancer
- Both cell lines sequenced in 2009
- Somatic calls published in 2010*



*Pleasance *et al. Nature* 463: 191-196, 2010

Tumor/normal cell line and DNA pair

COLO 829 / COLO 829BL

- Whole genome sequencing of COLO 829 and COLO 829BL at a depth of 90x is being generated by Illumina and TGen to build a set of consensus calls
- HiSeq 90x WGS at Illumina & TGen
- SNVs, Indels, Copy NVs, SVs
  - Confirmed by PCR & sequencing
- Complete genomic WGS
- Multiple dilution series analyzed

ATCC®

Conclusions

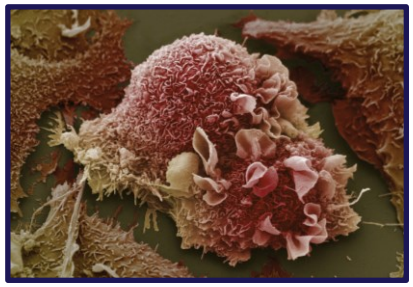
Why are Standards needed?

- To establish sensitivity, linearity, and specificity during assay validation or implementation
- To benchmark critical assay performance during development/ validation for regulatory submissions and production lot release
- Move from growth based methods to molecular methods, establish method equivalence
- Evaluation of assay variability & assay comparison
- Validate or compare test methods
- For use in inter-laboratory studies and comparisons
- For use in testing and calibration in ISO 17025 accredited laboratories
- Production of laboratory reference materials
- Development of standard protocols
- Method maintenance
- External quality assessment

ATCC®

Thank you!

Register for more webinars in the ATCC "*Excellence in Research*" webinar series at www.atcc.org/webinars.



June 5, 2014
10:00 am or 3:00 pm (ET)
Dr. Doug Storts and Dr. Yvonne Reid will discuss the recent advances in STR profiling technologies and how the Standard STR protocol is transforming scientific practices.

Thank you for joining today!
Please send additional questions to tech@atcc.org

ATCC®