ATCC® Quantitated Nucleic Acids – Empowering Molecular-based Assay Development

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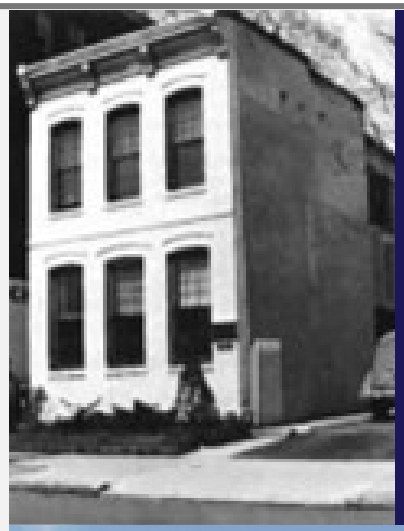
About ATCC

- 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

Agenda

- **Methods of nucleic acid quantitation**
- **Applications of quantitated nucleic acids**
- **Quantified genomic and synthetic microbial nucleic acids**
- Precision medicine and unmet needs in genetic tests
- ATCC human genomic DNAs as control materials
- ATCC quantified human genomic DNAs
  - Gene mutation allelic frequencies
  - Gene copy numbers

Utility for molecular testing

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

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Analytical sensitivity, specificity, & interference

Analytical sensitivity

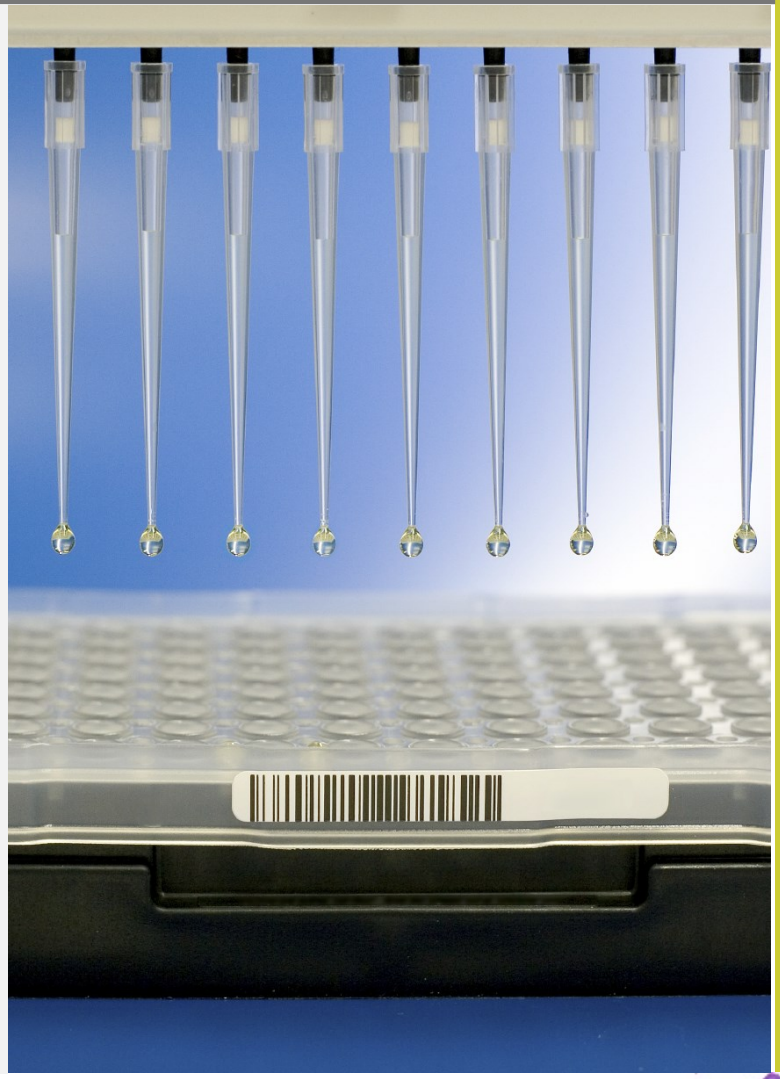
- Limit of detection (LoD)
- Reactivity

Analytical specificity

- Cross reactivity

Interference

- Clinical specimens

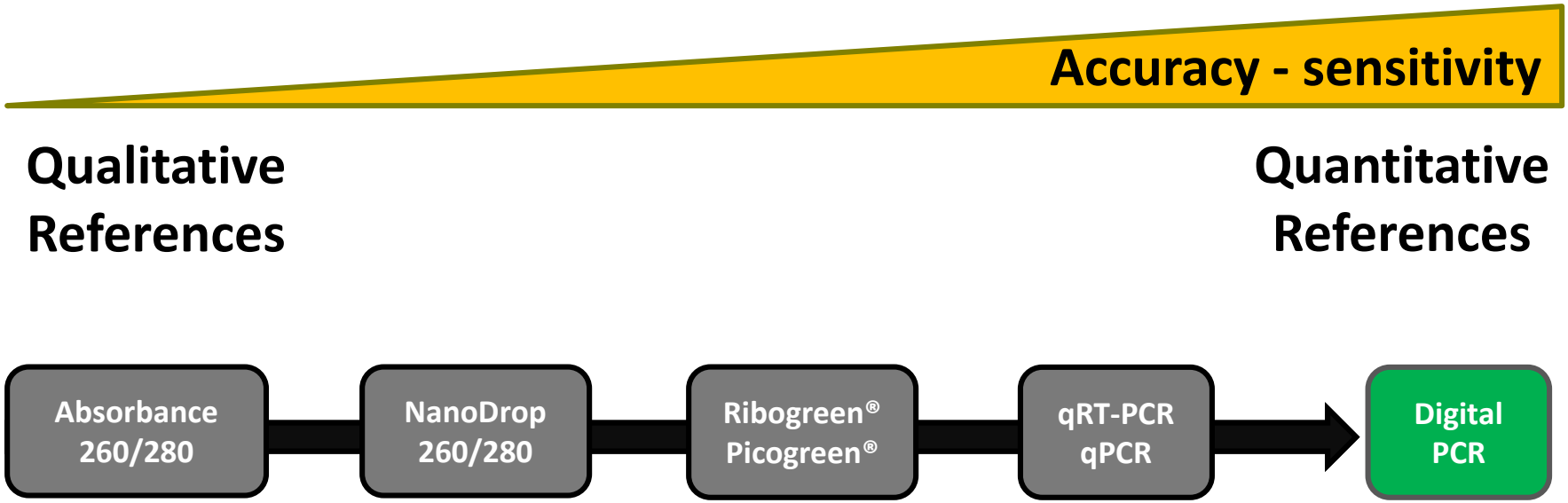


Controls for nucleic acid-based assays

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
- **Inclusivity/Exclusivity**

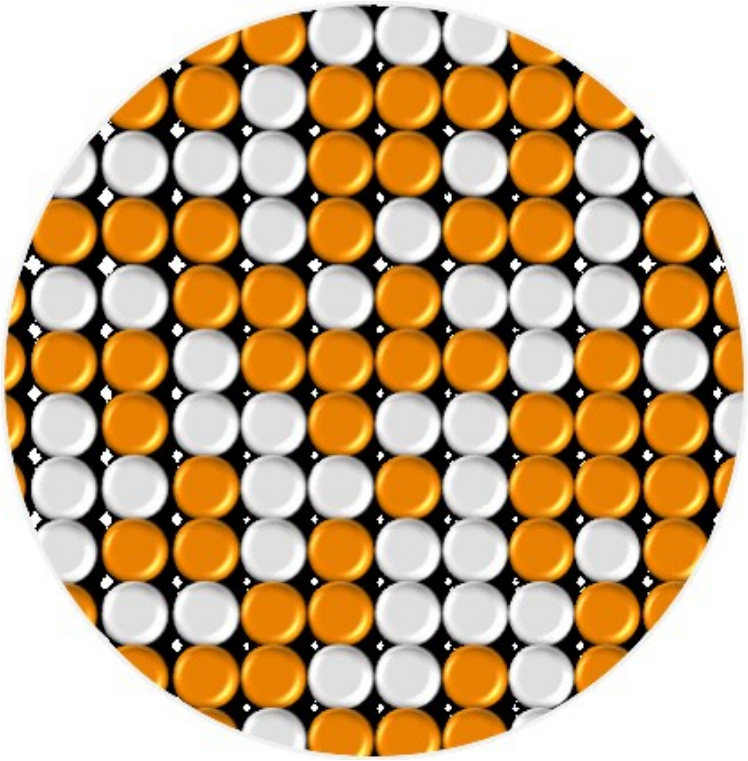
Towards quantification



Advantages of digital PCR

Allows for absolute quantification of nucleic acids

- High precision and accuracy
- Target-specific quantification
- Copy number of individual genes
- Cost effective
- No need to generate cloned standards for a standard curve



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Quantitative nucleic acid standards

Salient features	Applications
• Fully authenticated & characterized • Quantitated by Droplet Digital™ PCR (ddPCR™) • Compatible with several lab-developed and commercially available assays • BSL-1 ready-to-use control	• Generation of a standard curve • Positive control for molecular-based assays • Independent standard for validation and verification studies • Monitoring assay-to-assay and lot-to-lot variation • New assay development • Limit of detection studies

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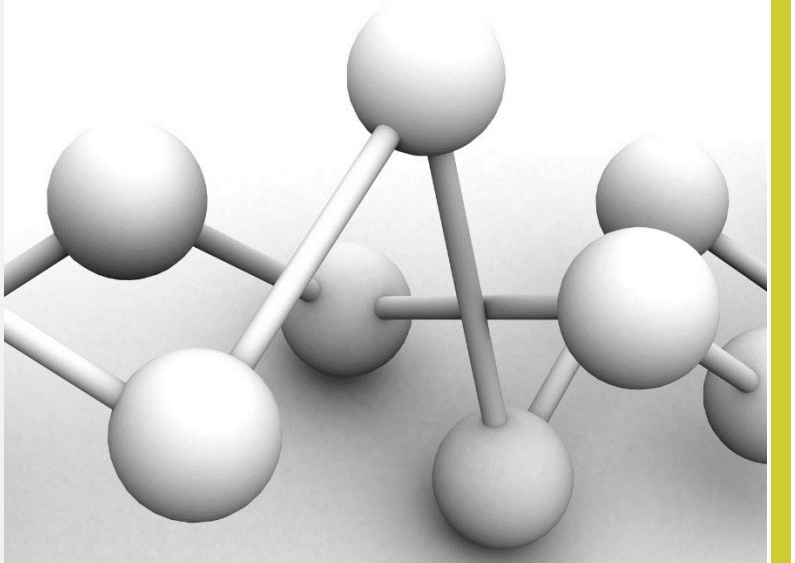
Quantitative nucleic acid standards

The collection encompasses over 60 quantitative preparations

- Synthetic nucleic acids
- Genomic nucleic acids
- Certified reference materials



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ATCC Synthetic Molecular Standards

Can be used as a positive control for:

- Difficult-to-culture or unculturable strains
- Strains requiring BSL-3 containment
- Strains on the commerce control list

Advantages of synthetic nucleic acids:

- Eliminate the need to culture microorganisms
- Can be used in a BSL-1 facility
- No shipping restrictions
- Manufactured under ISO 13485:2003
- Quantified using ddPCR™
- Useful for monitoring assay-to-assay or lot-to-lot variation

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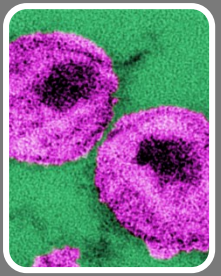
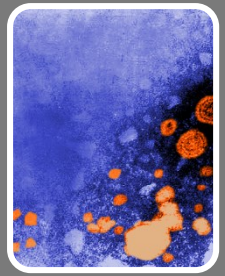
ATCC Synthetic Molecular Standards



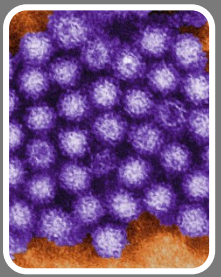
Blood-borne Disease

- BK virus
- Hepatitis B & C virus
- Epstein-Barr virus
- Human immunodeficiency virus



Sexually Transmitted Infections

- Hepatitis B & C virus
- Human immunodeficiency virus
- Human papillomavirus 16 & 18
- *Mycoplasma genitalium*
- *Treponema pallidum*

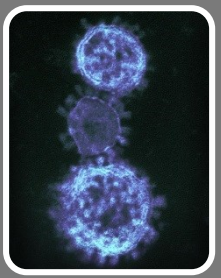


Enteric Disease

- Astrovirus
- *Cyclospora cayetanensis*
- Norovirus GI & GII
- Sapovirus

Vector-borne Disease

- Chikungunya virus
- Dengue virus types 1-4
- Eastern equine encephalitis virus
- St. Louis encephalitis virus
- West Nile virus
- Zika virus



Respiratory Disease

- Human Bocavirus
- MERS-CoV
- Human metapneumovirus

Available online at
www.atcc.org/synthetics

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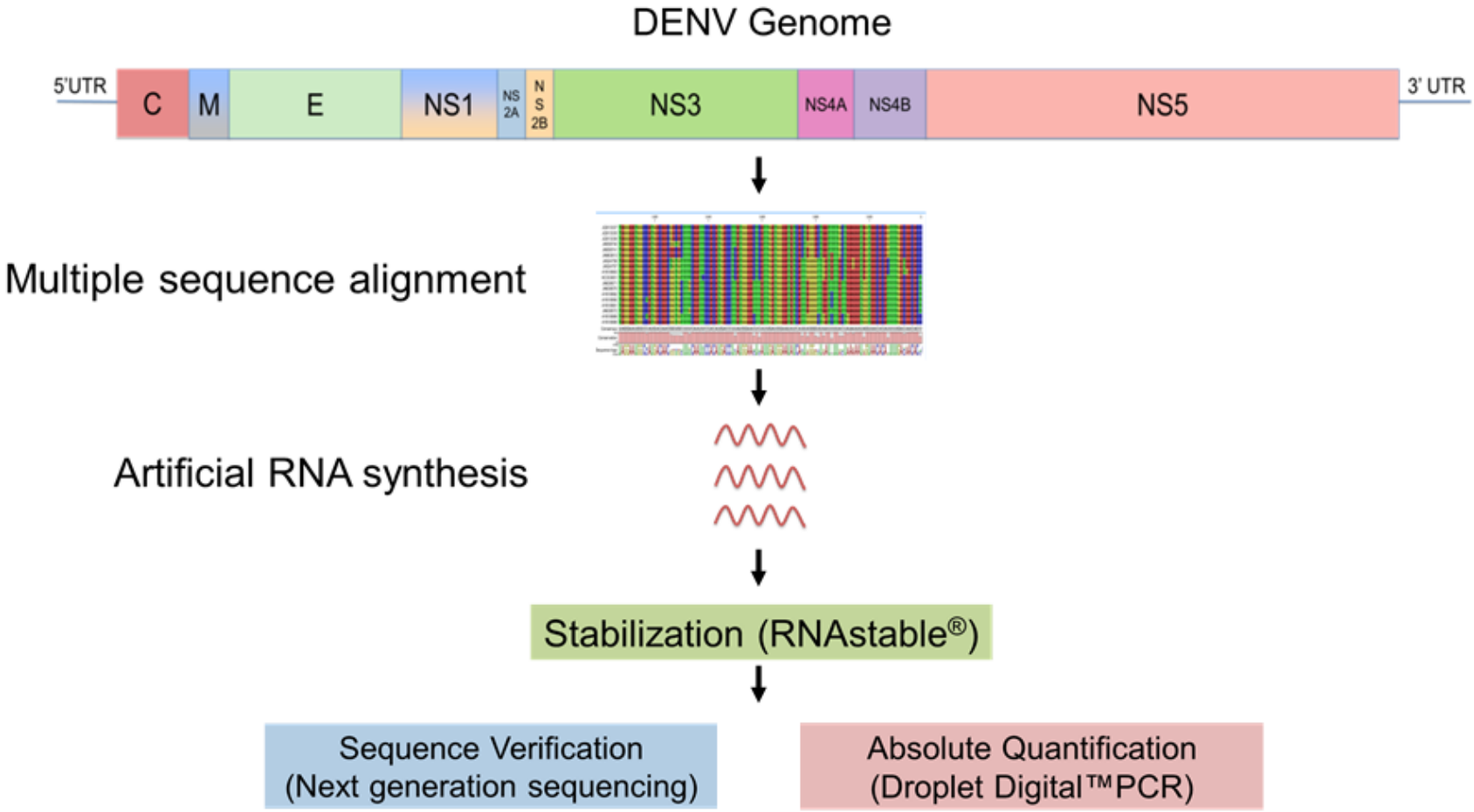
Synthetic Dengue virus RNA



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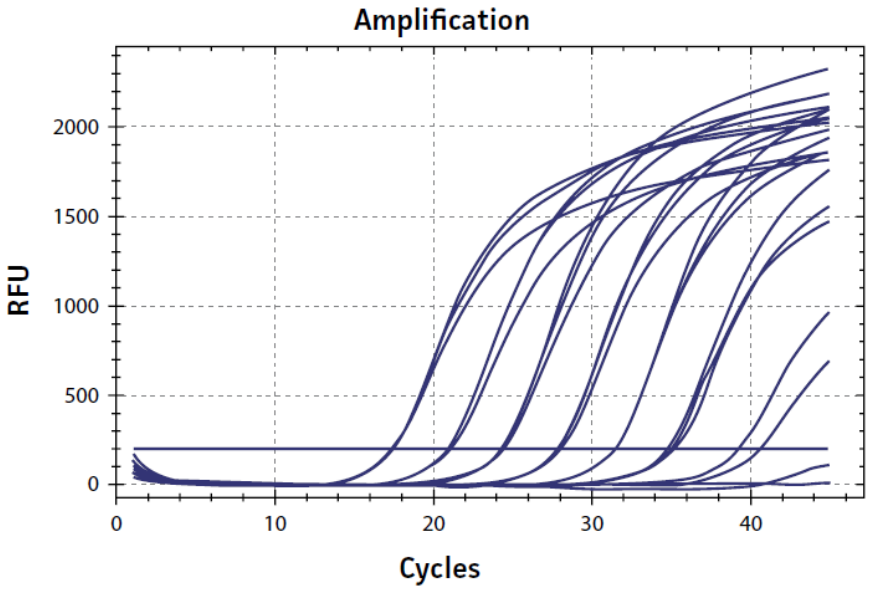
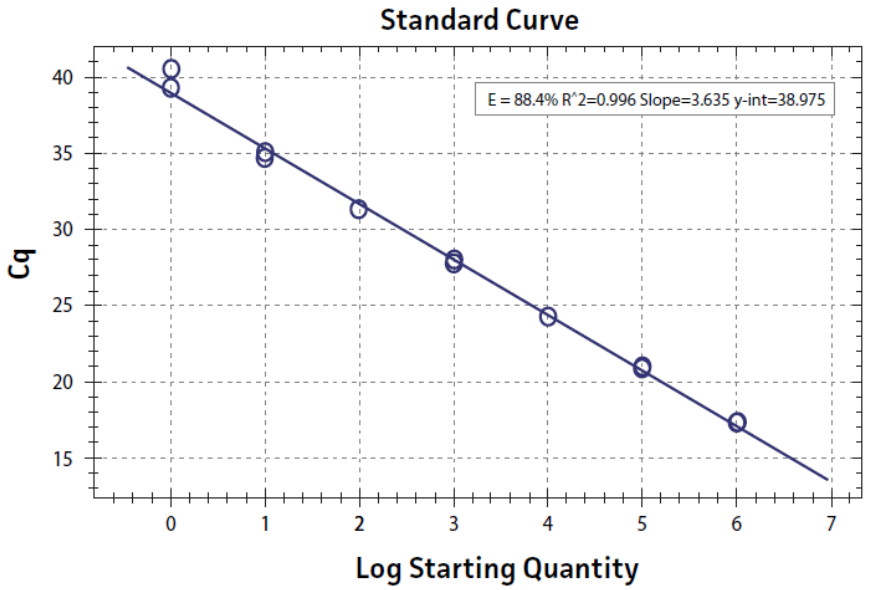
Ashraf S, et al. Development and use of synthetic molecular standards for Dengue virus serotypes 1-4 [Application note], 2014.

Synthetic Dengue virus RNA

Generation of standard curves using the DENV-4 Molecular Standard





Primer and Probe		DENV-1	DENV-2	DENV-3	DENV-4
CDC Assay	Slope	-3.244	-3.277	-3.315	-3.642
	R^2	0.990	0.996	0.987	0.996
Waggoner Assay	Slope	-3.536	-3.535	-3.705	-3.775
	R^2	0.991	0.997	0.989	0.996

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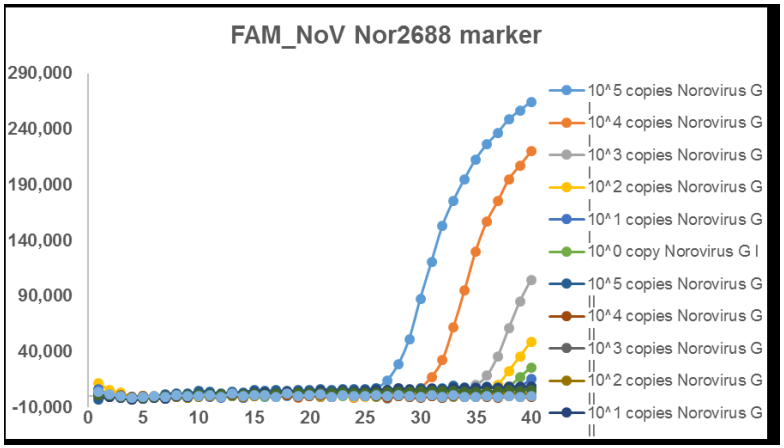
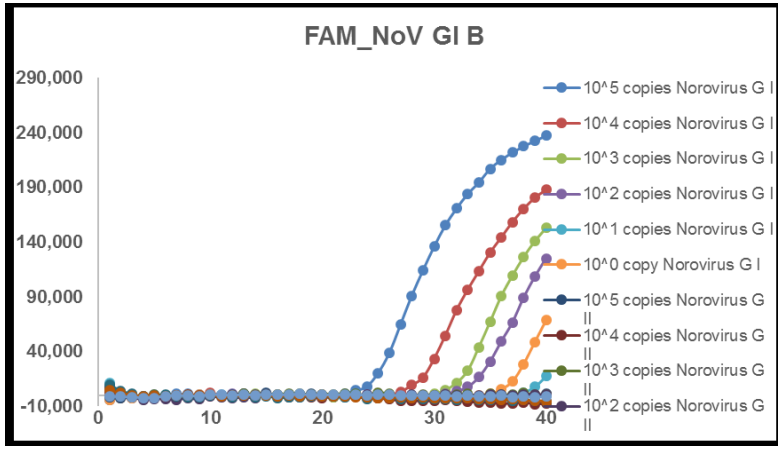
Ashraf S, et al. Development and use of synthetic molecular standards for Dengue virus serotypes 1-4 [Application note], 2014.

New assay development with Norovirus GI and GII





Primer	Probe Target	Limit of Detection	
		NoV GI Template	NoV GII Template
Set 1	FAM_NoV GI Nor2688	500 copies	-
	Cy5_NoV GII Nor21932	-	500 copies
Set 2	VIC_NoV GI A	5 copies	-
	FAM_NoV GI B	5 copies	-
	ROX_NoV GII C	-	5000 copies

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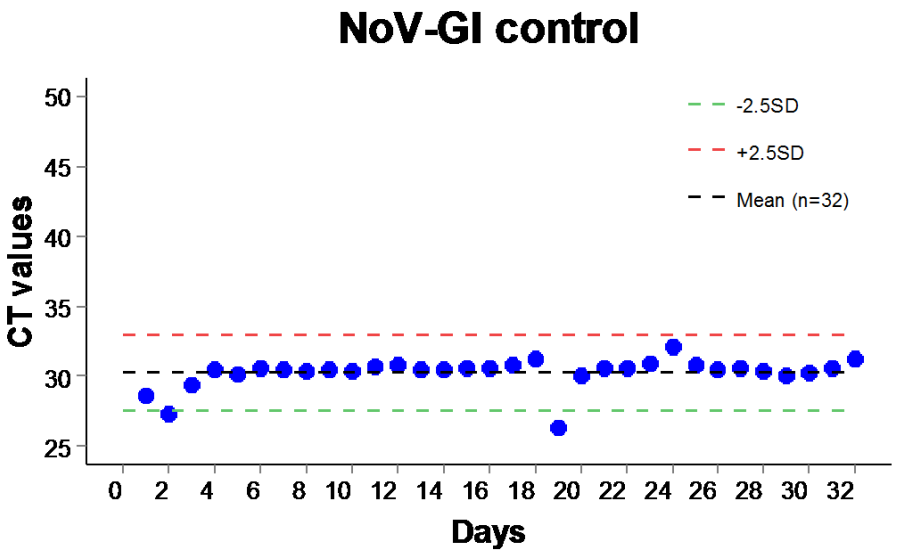
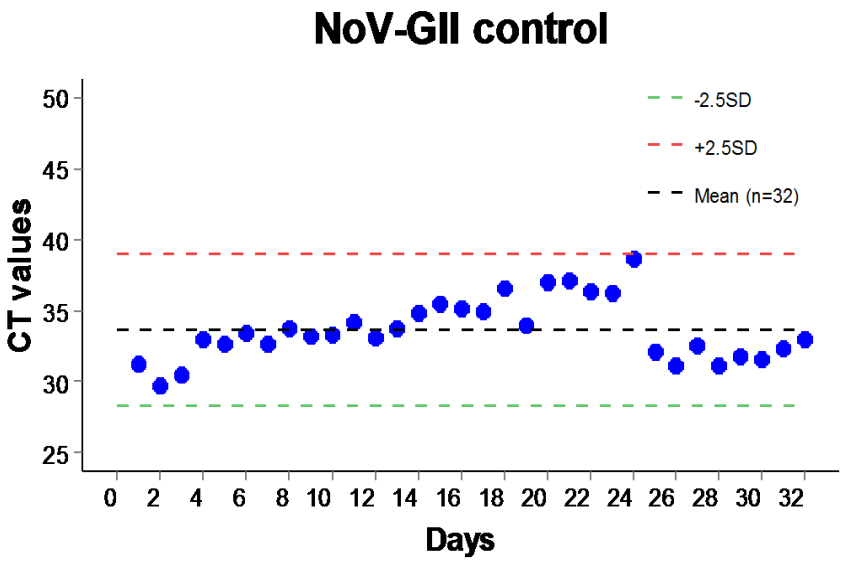
Ashraf et al, Utility of ATCC Synthetic Molecular Standards for Norovirus GI and GII for New Assay Development and as an Independent Control [Poster], AMP, November 2015

Independent assay validation



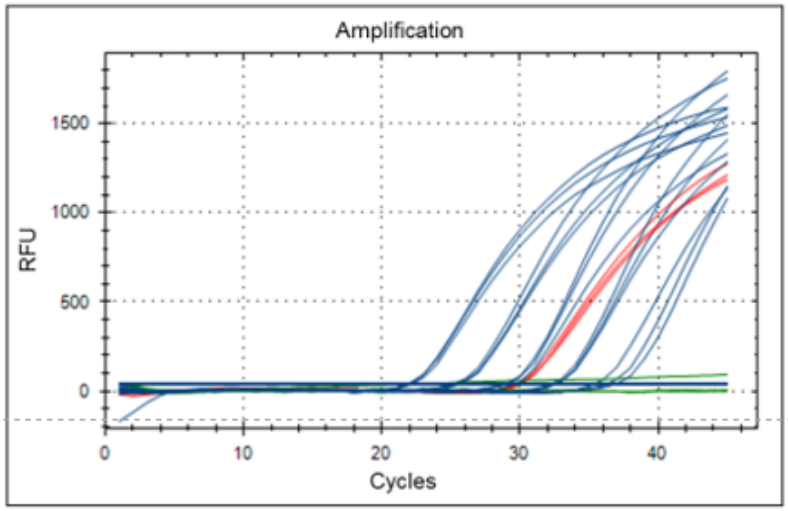


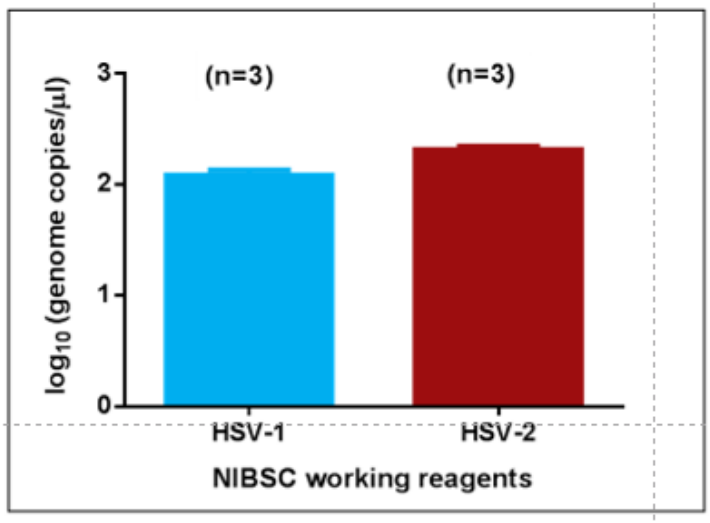
ATCC Synthetic Molecular Standards	Average CT	Standard Deviation (SD)	Co-efficient of Variation (CV)	2.5 SD Range
NoV GI	30.27	1.09	3.6%	27-34
NoV GII	33.69	2.15	6.4%	29-39

Ashraf et al, Utility of ATCC Synthetic Molecular Standards for Norovirus GI and GII for New Assay Development and as an Independent Control [Poster], AMP, November 2015

Utility of quantitated molecular standards as controls

Quantification of NIBSC working reagents for HSV-1 and HSV-2 using ATCC quantitative molecular standards





National Institute of Biological Standards and Control (NIBSC) working reagents for HSV-1 and HSV-2

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Ashraf et al, External Validation of ATCC Molecular Standards for Herpes Simplex Virus (HSV-1 and HSV-2) [Poster], CVS, May 2016

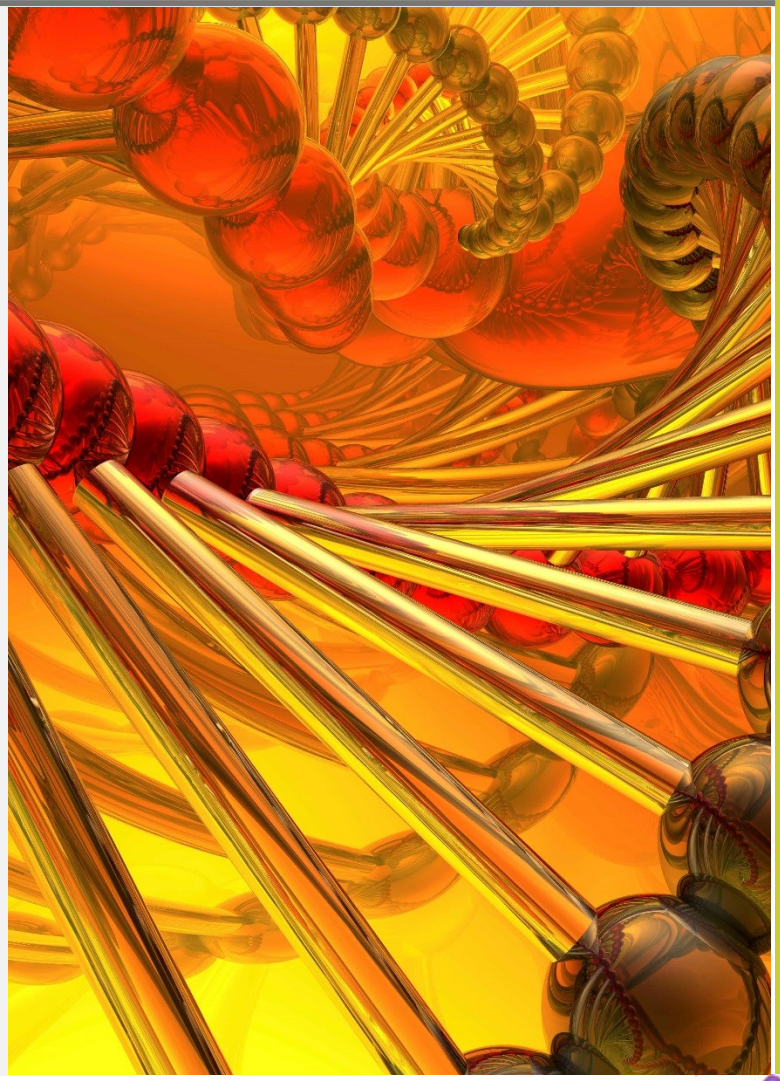
CRM Quantitated Mycoplasma Genomic DNA

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 titered mycoplasma reference materials

Use as a quantitative external control for:

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



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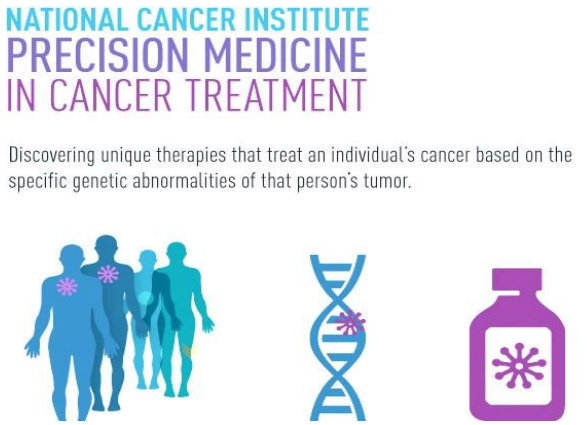
- **Precision medicine and unmet needs in genetic tests**
- **ATCC human genomic DNAs as control materials**
- **ATCC quantified human genomic DNAs**
  - **Gene mutation allelic frequencies**
  - **Gene copy numbers**

Precision medicine

Precision Medicine Initiative®

- Prevention and treatment strategies that take individual variability into account
  - Large-scale biologic databases
  - Molecular approach
    - Near-term focus on cancers
    - Longer term aim for a wide range of disease

The website of the White House (www.whitehouse.gov)

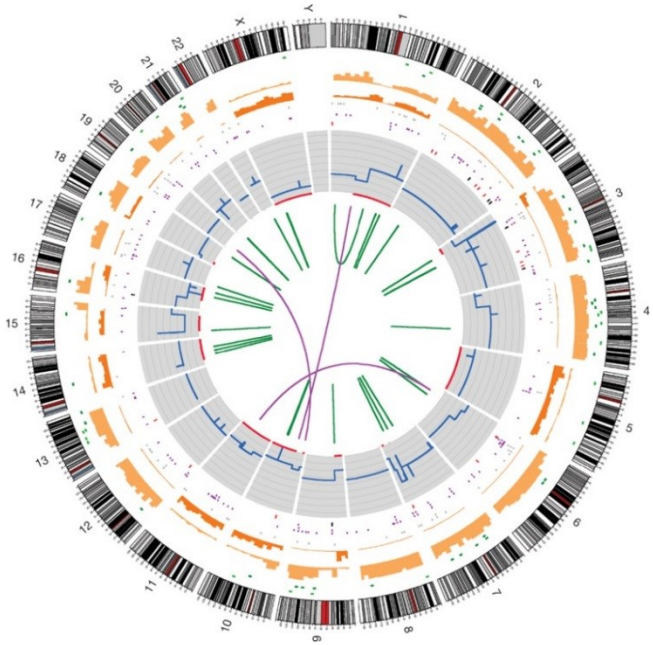


The website of the National Cancer Institute (www.cancer.gov)

Genetics of cancer

Genetic changes and cancer

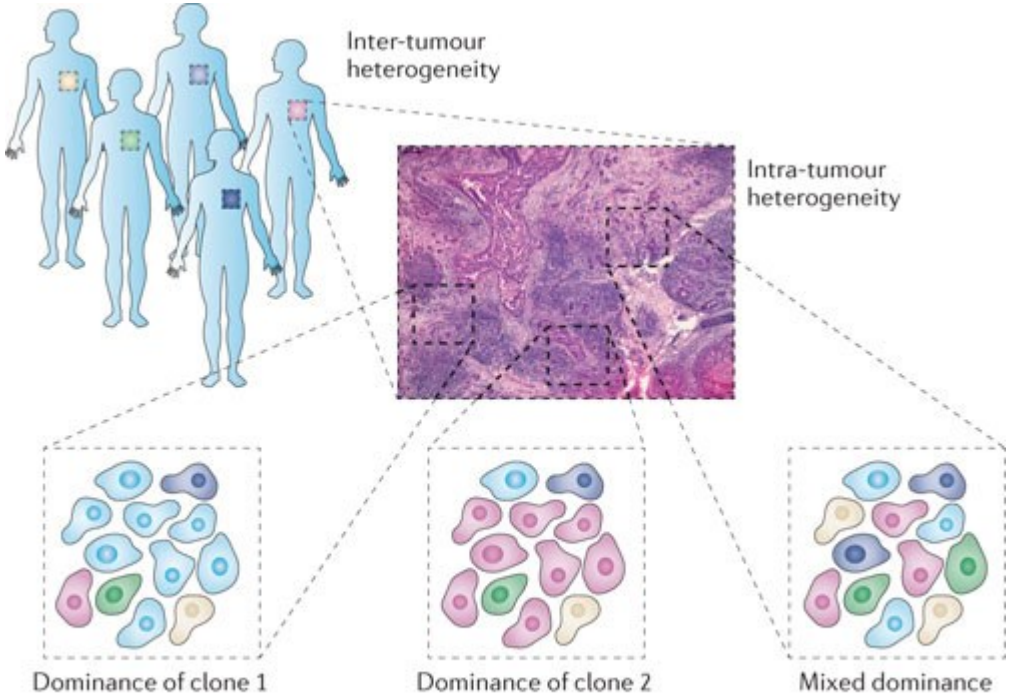
The catalogue of somatic mutations from a human cancer genome



Nature 463(7278): 191-196, 2010.

Heterogeneity of cancer

Every cancer is unique



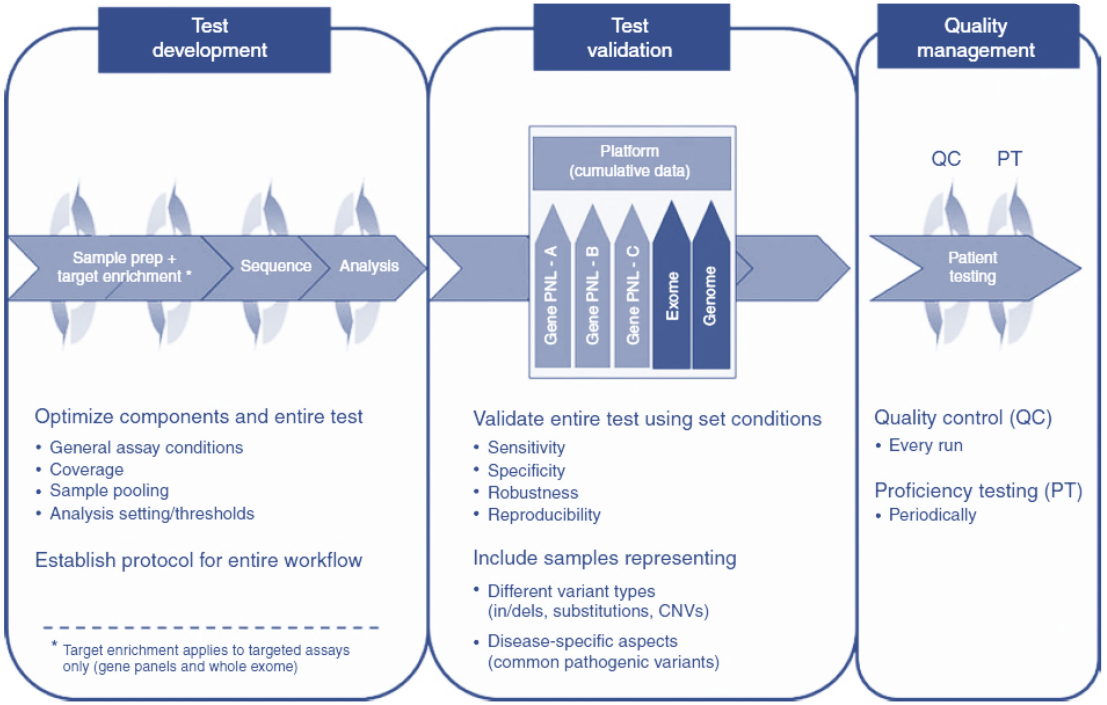
Nature Reviews Cancer 12: 323-334, 2012.

Nature Reviews | Cancer

Molecular oncology diagnostics is one of the most rapidly growing areas

ACMG guidelines for NGS

ACMG clinical laboratory standards for next-generation sequencing



Genet Med 15(9): 733-747, 2013.

Using reference materials

- Test validation
- Quality control
- Proficiency testing

A need for established, fully characterized, globally accepted reference materials

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Unmet need - Reference materials

What is a reference material?

A material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of a measuring system, the assessment of a measurement procedure, or for assigning values to materials (ISO 15195:2003)

A variety of reference materials, including:

- Certified reference materials
- Standard reference materials
- Calibrators
- Characterized genomic nucleic acids

Reference material properties

- Qualitative
- Quantitative

A need for established, fully characterized, globally accepted reference materials

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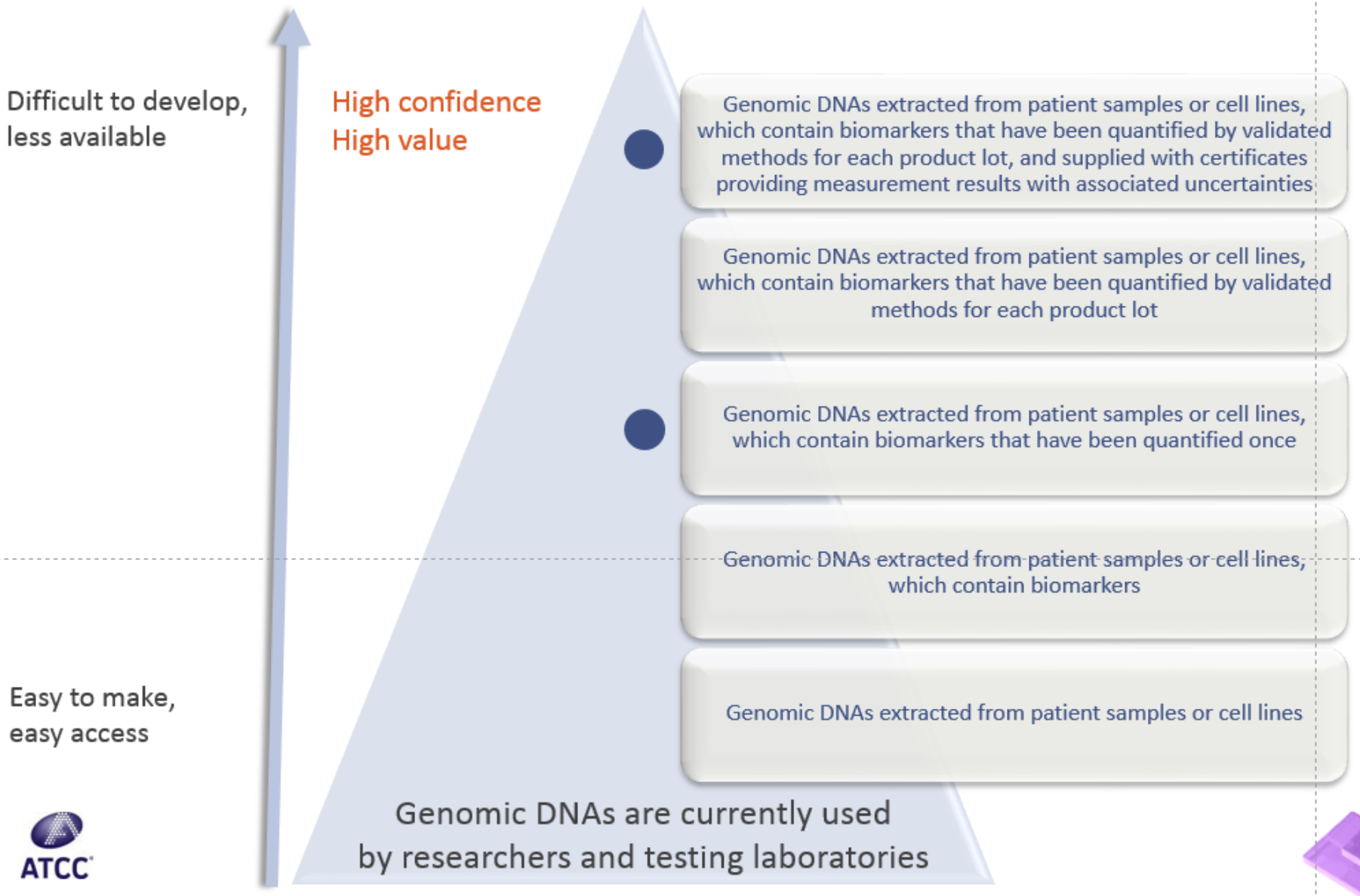
Example of various genomic DNA materials



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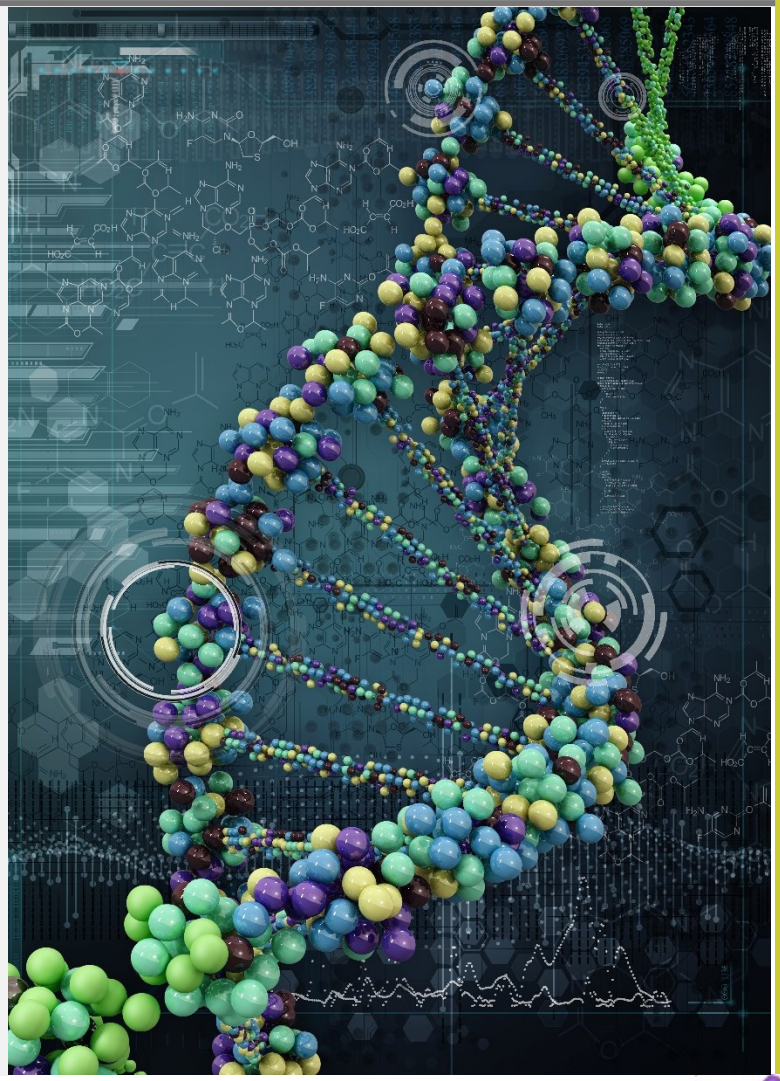
ATCC human Genomic DNA (gDNA) control materials

Purified human gDNAs with oncology biomarkers

- Fully authenticated
- Short Tandem Repeat (STR) testing used to avoid contamination or misidentification
- Characterized genetic alterations
- Faithfully capture human cancer genome
- Reproducible results



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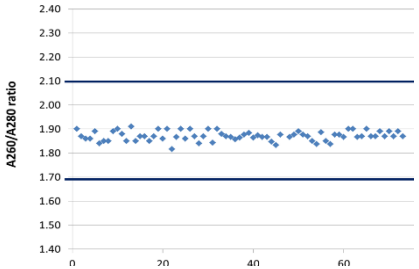
High quality gDNA for molecular tests

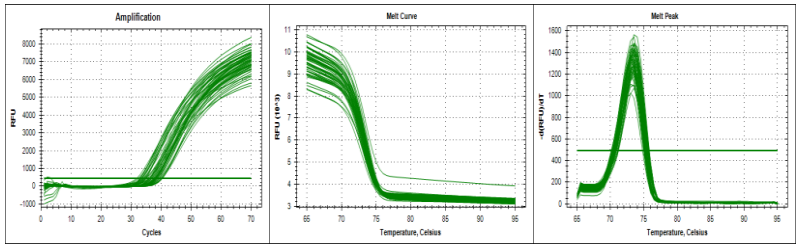
- Quantity
- Integrity
- Purity
- Identity
- Functional testing

PicoGreen® dsDNA Quantitation

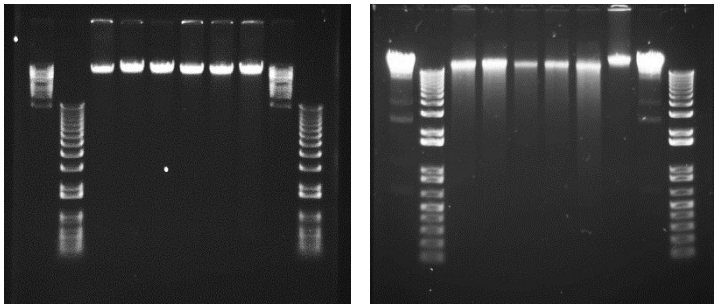
A260/280



DNA tested in PCR based assay



Electrophoresis –uncut DNA –DNA digestion



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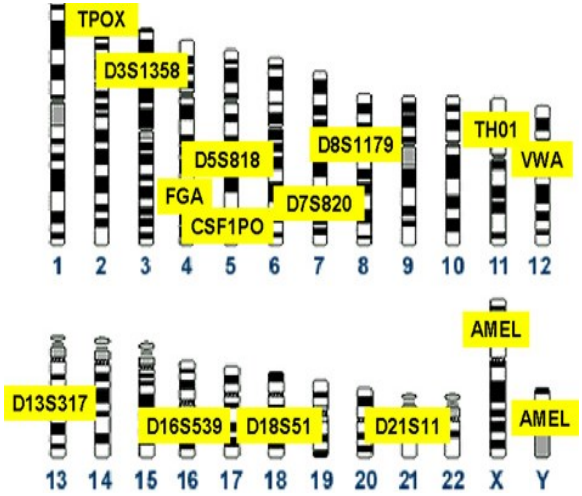
Ensure DNA identity & avoid contamination

STR analysis (DNA profiling)

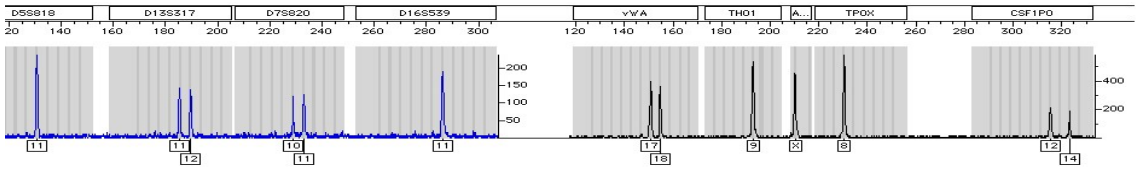
Intraspecies identification and authentication of human cell lines

- Target sequence consists of microsatellite DNA contains short tandem repeats
- STR test can determine:
  - DNA identity when compared to a reference
  - Cross contamination

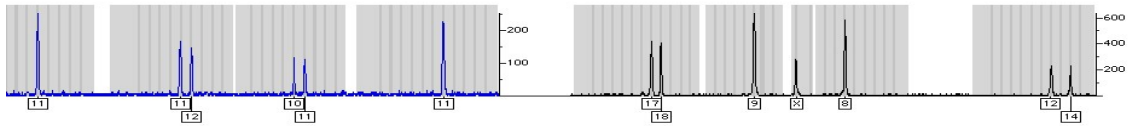
Barcode of Life
Barcoding for species Identification



Parental cell line



Parental cell line derived genomic DNA



Characterize cancer lines genomic DNAs

Cell Line Genomic DNAs for Molecular Diagnosis of Cancer

*Michael Jackson, John Foulke, Luping Chen, Anupreet Bal, Lysa-Anne Volpe, Ana Fernandes, Karin Kindig, Afshin Sohrabi, Fang Tian**

American Type Culture Collection, Manassas, VA 20110

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GLOBALLY DELIVERED™

Abstract

Introduction: Large-scale cancer genome programs have generated rich data of genetic abnormalities observed in thousands of clinical patient tumors, which provides a major opportunity for molecular diagnosis of cancer. However, the lack of control materials for molecular tests has been a challenge. Because of the reproducible nature of the cell lines, genomic DNAs of the fully characterized and authenticated cell lines provide a solution.

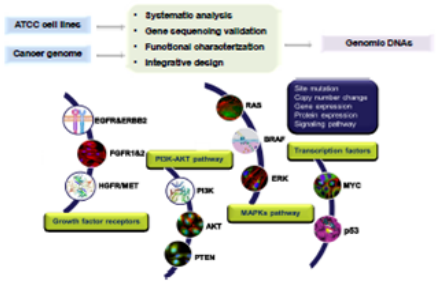
Methods: genomic DNAs were extracted from over 70 common used human cancer cell lines derived from breast, lung, colon, pancreas as well as haematopoietic and lymphoid tissue. Cancer gene mutations were identified by next generation sequencing. Gene copy number changes were analyzed by qBiomarker copy number PCR assay kit. Moreover, the selected cell lines were analyzed by qPCR, western blot and IF staining to verify gene expressions and protein expressions.

Results: Here, we present a large list of over 70 genomic DNAs isolated from authenticated cancer cell lines that contain the desired biomarkers for oncology molecular diagnosis. In addition to driver mutations such as BRAF V600, KRAS G12, PI3K E545 and EGFR T790, the gene copy number amplifications of AKT, FGFR, MET, ERBB2 and deletion of PTEN are presented in the cell lines that have been used to extract the genomic DNAs. In addition, we show systematic molecular characterization and clustering of those human tumor cell lines, which represent the most common human cancer types found in the clinic, such as lung, breast, colon, pancreatic, skin cancer and so on. By next generation sequencing, the genomic DNAs of those tumor cell lines were fully analyzed to capture the driver gene mutations and allelic frequency. Gene DNA copy number variations were determined as well. Moreover, the gene expressions, protein expressions and relevant cell signaling pathway activations of the cell lines have also been profiled. To be paired with mutations, a set of wild type controls that were derived from normal tissues were characterized in parallel.

Conclusions: Overall, the genomic DNAs from authenticated cell lines provide useful control materials to molecular diagnostic labs for genetic testing.

Introduction

ATCC tumor cell panels and related genomic DNAs



Results

Human cancer cell lines contain biomarkers - mutations and copy number variation

ATCC® No.	Cell line name	Gene	AKT1 copy number variation	Measured CNV of AKT1	AKT2 copy number variation	Measured CNV of AKT2	Tumor source
CRL-2321	HCC1143	AKT1	Amplification	6.21	-	-	breast
CRL-1469	PANC-1	AKT2	-	-	Amplification	25.46	pancreas
CRL-1622	KLE	AKT2	-	-	Amplification	12.51	endometrium
HTB-161	NIH:OVCAR-3	AKT2	-	-	Amplification	9.76	ovary
HTB-183	NCI-H661	AKT2	-	-	slight amplification	5.56	lung

ATCC® No.	Cell line name	Gene	FGFR1 copy number variation	Measured CNV of FGFR1	FGFR2 copy number variation	Measured CNV of FGFR2	Tumor source
HTB-23	MDA-MB-134-VI	FGFR1	Amplification	14.22	-	-	breast
CRL-2066	DMS 114	FGFR1	Amplification	7.17	-	-	lung
CCL-235	SW837	FGFR1	slight amplification	3.9	-	-	colon
CCL-246	KG-1	FGFR1	slight amplification	3.87	-	-	leukemia
CRL-5974	SNU-16	FGFR2	-	-	Amplification	451.16	stomach
HTB-103	KATO III	FGFR2	-	-	Amplification	136.62	stomach

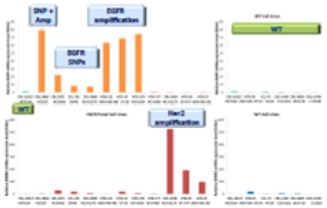
ATCC® No.	Cell line name	Gene	MYC copy number variation	Measured CNV of MYC	Tumor source
CRL-5974	SNU-16	MYC	Amplification	50.48	stomach
CRL-2081	MSTO-211H	MYC	Amplification	36.92	mesothelioma
HTB-175	NCI-H82	MYC	Amplification	35.63	lung
HTB-171	NCI-H446	MYC	Amplification	19.06	lung
CCL-240	HL-60	MYC	slight amplification	9.43	leukemia

ATCC® No.	Cell line name	Gene	MET copy number variation	Measured CNV of MET	Tumor source
CRL-5973	SNU-5	MET	Amplification	71.56	stomach
HTB-135	Hs 746T	MET	Amplification	23.96	stomach
CRL-2351	AU565	MET	slight amplification	1.99	breast

ATCC® No.	Cell line name	gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
CRL-2577	RXD	MAPK3	p.R96R	c.288C>T	3346	C = 36.5, T = 40.5	colon
		PIK3CA	p.H1047R	c.3140A>G	1359	A = 45.5, G = 54.4	
		BRAF	p.V600E	c.1799T>A	257	T = 29.6, A = 69.6	
HTB-9	5637	MAPK1	p.R79K	c.236G>A	67777	G = 56.1, A = 43.8	bladder
HTB-65	MeWo	MAPK3	p.P246S	c.736C>T	9476	C = 41.4, T = 58.6	skin
CRL-9446	CHL-1	MAPK3	p.D28V	c.682A>G	9124	G = 99.8	skin
HTB-2	RT4	MAPK3	p.A109A	c.327G>A	14152	G = 62.6, A = 37.2	bladder

ATCC® No.	Cell line name	gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-31	C-33-A	PTEN	p.R233*	c.697C>T	65522	C = 51.3, T = 48.0 ceh/s	
HTB-111	ANS CA	PTEN	p.R130fs	c.389_389delG	14373	Deletion = 99.3	endometrium
CRL-1718	CCF-STTG1	PTEN	p.L112R	c.335T>G	20249	G = 99.6	brain

Real time PCR analysis of mRNA levels



ATCC® No.	Cell line name	Gene	Amino acid Change	cDNA Change	NGS Coverage	% Zygosity	Tumor source
CCL-231™	SW48	EGFR	p.G719S	c.2155G>A	35993	G = 69.4, A = 30.3	Colon
CRL-5908™	NCI-H1975	EGFR	p.T790M	c.2369C>T	11704	C = 33.0, T = 66.9	Lung
			p.L858R	c.2573T>G	9441	T = 33.7, G = 66.2	

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	126.89	Lung
HTB-20™	BT-474	ERBB2	-	-	Amplification	29.7	Breast
HTB-27™	MDA-MB-361	ERBB2	-	-	Amplification	16.65	Breast

ATCC® No.	Cell line name	gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
CRL-2177	SW 1271	NRAS	p.Q61R	c.182A>G	26732	G = 99.8%	lung
CRL-2273	CHP-212	NRAS	p.Q61K	c.181C>A	49659	C = 50.7, A = 49.1	brain
CRL-7585	Hs 852.T	NRAS	p.G12V	c.35G>T	66411	G = 38.0, T = 61.8	skin
CRL-9068	NCI-H929	NRAS	p.Q13D	c.38G>A	21596	A = 53.9, G = 45.9	myeloma
TIB-202	THP-1	NRAS	p.G12D	c.35G>A	60268	A = 70.1, G = 29.9	leukemia
CRL-2547	Panc 10.05	KRAS	p.G12D	c.35G>A	42708	G = 52.7, A = 47.3	pancreas
CRL-2549	Panc 03.27	KRAS	p.G12V	c.35G>T	56913	G = 47.0, T = 52.9	pancreas
HTB-174	NCI-H441	KRAS	p.G12V	c.35G>T	87521	G = 52.8, T = 47.1	lung
CL-187	LS 180	KRAS	p.G12D	c.35G>A	91254	G = 51.3, A = 48.6	colon
CCL-225	HCT-15	KRAS	p.G13D	c.38G>A	49764	G = 52.1, A = 47.8	colon

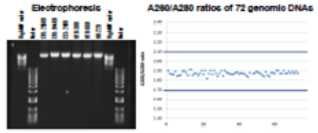
ATCC® No.	Cell line name	gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-19	BT-20	PIK3CA	p.H1047R	c.3140A>G	7062	A = 64.3, G = 35.6	breast
HTB-131	MDA-MB-453	PIK3CA	p.H1047R	c.3140A>G	10415	A = 35.6, G = 64.2	breast
HTB-112	HEC-1-A	PIK3CA	p.G106R	c.3145G>C	5961	G = 38.8, C = 61.0	endometrium
HTB-178	NCI-H596	PIK3CA	p.E545K	c.1633G>A	2869	G = 68.5, A = 31.4	lung
CRL-1739	AGS	PIK3CA	p.E545A	c.1634A>C	9277	A = 23.6, C = 76.3	stomach
CCL-237	SW948	PIK3CA	p.E542K	c.1624G>A	13713	G = 52.7, A = 47.2	colon
HTB-121	BT-483	PIK3CA	p.E542K	c.1634A>C	11779	A = 49.8, C = 50.0	breast
HTB-27	MDA-MB-361	PIK3CA	p.E545K	c.1633G>A	4681	G = 79.7, A = 20.1	breast
			p.K567E	c.1700A>G	916	A = 64.2, G = 35.8	

ATCC® No.	Cell line name	gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-66	RPMI-7951	BRAF	p.V600E	c.1799T>A	1599	T = 52.5, A = 37.1	skin
CCL-238	SW1417	BRAF	p.V600E	c.1799T>A	3697	T = 58.6, A = 41.2	colon
CRL-7896	A101 D	BRAF	p.V600E	c.1799T>A	5643	T = 43.8, A = 55.8	skin
CCL-224	COLO 201	BRAF	p.V600E	c.1799T>A	4122	T = 22.0, A = 77.8	colon
HTB-67	WM-266-4	BRAF	p.V600D	c.1798_1800TG >AT	6776	T = 37.7, A = 62.1; G = 37.4, T = 62.5	skin

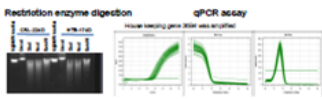
ATCC® No.	Cell line name	gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-62	P3HR-1	MYC	p.Y27S	c.80A>C	50463	A = 9.6, C = 89.9	Burkitt's lymphoma
			p.E54D	c.162G>A	66405	G = 10.8, A = 82.6	
			p.P72S	c.214C>T	65462	C = 17.8, T = 81.2	
			p.Q113H	c.339G>C	66396	G = 9.6, C = 89.2	
CRL-1646	CA46	MYC	p.V29I	c.86G>A	22792	G = 12.1, A = 86.5	Burkitt's lymphoma
			p.P72S	c.214C>T	30466	C = 13.8, T = 85.3	
			p.P75H	c.224C>A	29467	C = 17.2, A = 82.3	
			p.L193V	c.577C>G	21112	C = 54.8, G = 44.7	
			p.G321H	c.962G>C	30065	G = 50.1, C = 49.5	
CRL-1647	ST486	MYC	p.Q61L	c.182A>T	50033	A = 8.1, T = 87.8	Burkitt's lymphoma
			p.P72T	c.214C>A	49363	C = 21.5, A = 77.7	
			p.T110P	c.329A>C	50765	A = 7.2, C = 92.2	
			p.A196V	c.587C>T	31630	C = 5.7, T = 93.0	

Genomic DNAs produced by a validated automation system

Genomic DNA purity



Genomic DNA functional tests

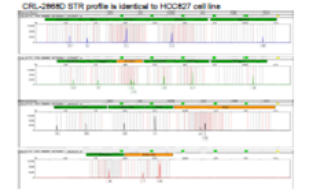


Next generation sequencing -Illumina

Genomic DNAs extracted from different passages of cells were compared

Cell line	DNA samples	gene	AA Change	DNA Change	NGS Coverage	% Zygosity
RPMI-7951 (p5)	gDNA e1	BRAF	p.V600E	c.1799T>A	9244	T = 61.1, A = 38.8
RPMI-7951 (p6)	gDNA e2	BRAF	p.V600E	c.1799T>A	8787	T = 61.8, A = 38.1
RPMI-7951 (p9)	gDNA e3	BRAF	p.V600E	c.1799T>A	9644	T = 59.7, A = 40.2
Panc 10.05 (p5)	gDNA e4	KRAS	p.G12D	c.35G>A	56929	A = 53.5, G = 46.3
Panc 10.05 (p6)	gDNA e5	KRAS	p.G12D	c.35G>A	49775	A = 52.8, G = 47.0
Panc 10.05 (p9)	gDNA e6	KRAS	p.G12D	c.35G>A	49951	A = 52.7, G = 47.2

STR verify genomic DNA identity



Summary

Over 70 genomic DNAs isolated from authenticated human cancer lines contain the desired biomarkers for oncology molecular diagnostics, which are useful tools and are helping accelerate nucleic acid-based detection for cancer.

Point mutation validation

Example: RAS genetic alteration

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

Point mutation validation

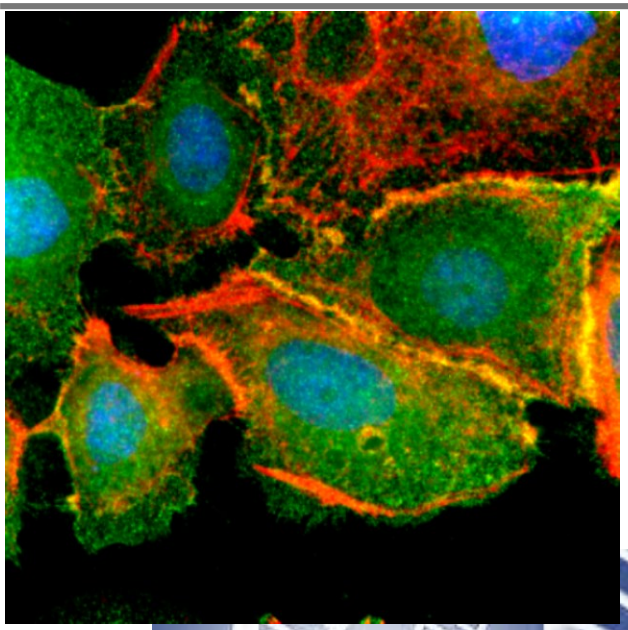
Example: EGFR genetic alteration

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 non-small cell lung cancer (NSCLC) and breast cancer therapy

ATCC

ATCC quantitated human gDNA



- Purified from authenticated ATCC cell lines
- Contain oncology biomarkers
- Quantified gene mutation allelic frequencies, gene copy numbers
- Quantified by validated methods for each product lot
- Supplied with certificates providing measurement results with associated uncertainties for each product lot

ATCC

Quantification of genetic variations in gDNAs

Coming soon: Quantitative gDNAs

ATCC® No.	Gene	Mutation
HTB-131DQ™	PIK3CA	H1074R
CRL-5908DQ™	EGFR	T790M; L858R
CRL-2868DQ™	EGFR	ELREA746del
CCL-231DQ™	EGFR	G719S
CRL-7898DQ™	BRAF	V600E
CCL-225DQ™	KRAS	G13D
CL-187DQ™	KRAS	G12D
CRL-2177DQ™	NRAS	Q61R
CCL-227DQ™	KRAS TP53	G12V R273H
HTB-30DQ™	TP53	R175H
CRL-2158DQ™	TP53	R245S
CRL-1648DQ™	TP53	R248Q
HTB-122DQ™	TP53	R249S
HTB-111DQ™	pTEN	R130fs

Use NGS to quantify mutations

ATCC

Next-generation sequencing

Workflow overview

Genomic DNA extraction and quantification

Genomic DNA fragmentation

Adapter ligation and PCR enrichment

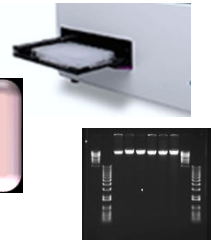
Clean-up/purification and QC

Cluster generation on flow cell

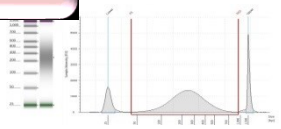
Sequencing reaction

Data analysis

High quality purified genomic DNA



Bioanalyser to ensure good quality library prep



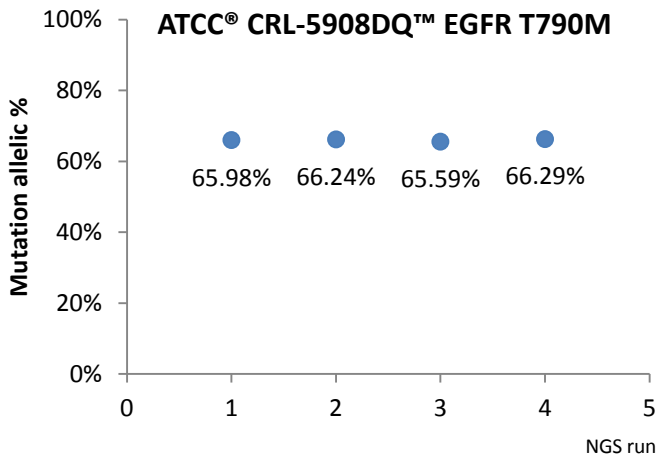
Deep sequencing to ensure the accuracy

ATCC

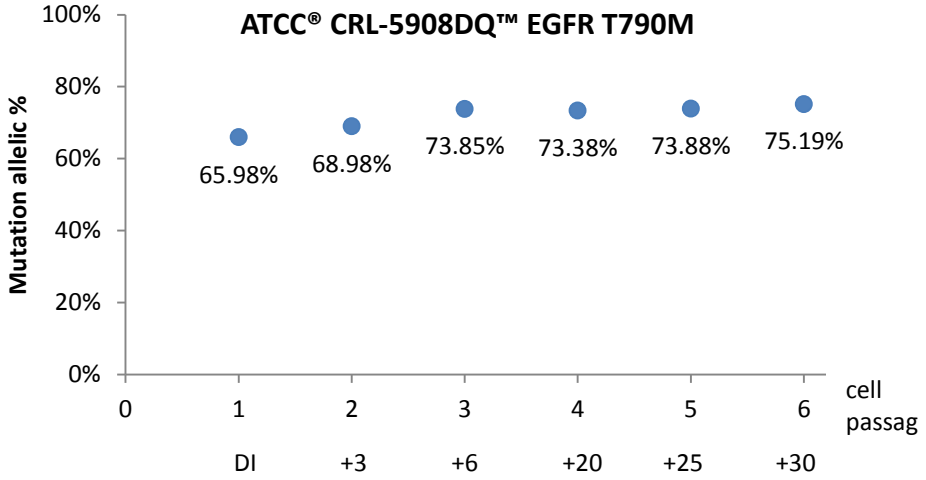
EGFR mutation quantification

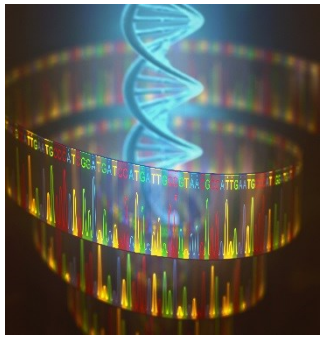
Example of how cell passage number can affect mutation allelic frequency in some lines

Reproducibility in independent runs



Mutation allelic frequency shifting

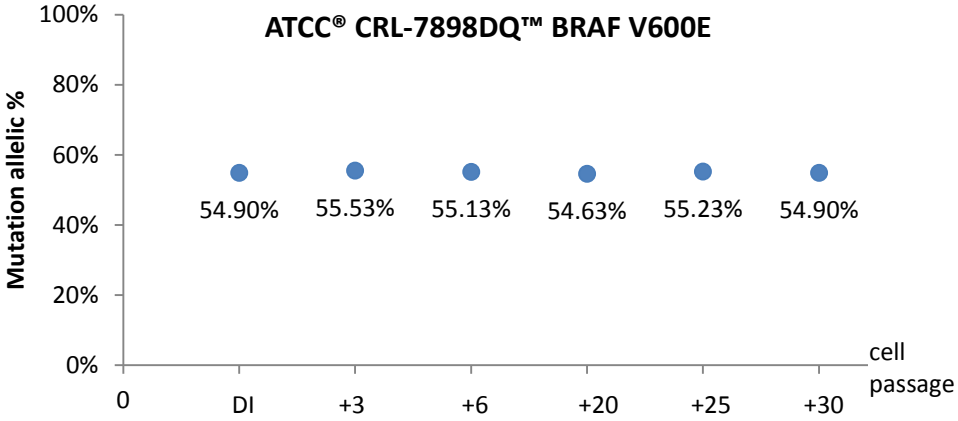


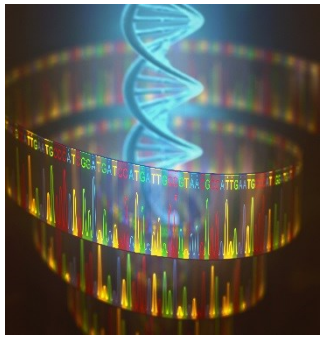


NGS
coverage >50,000X

NGS is performed on each lot to ensure biomarker quantification - your trusted control materials

BRAF mutation quantification





NGS
coverage >50,000X

The impact of passage number is cell line dependent, and some lines are relatively stable

Gene copy number quantification

Gene amplifications have emerged as therapeutic targets and diagnostic biomarkers

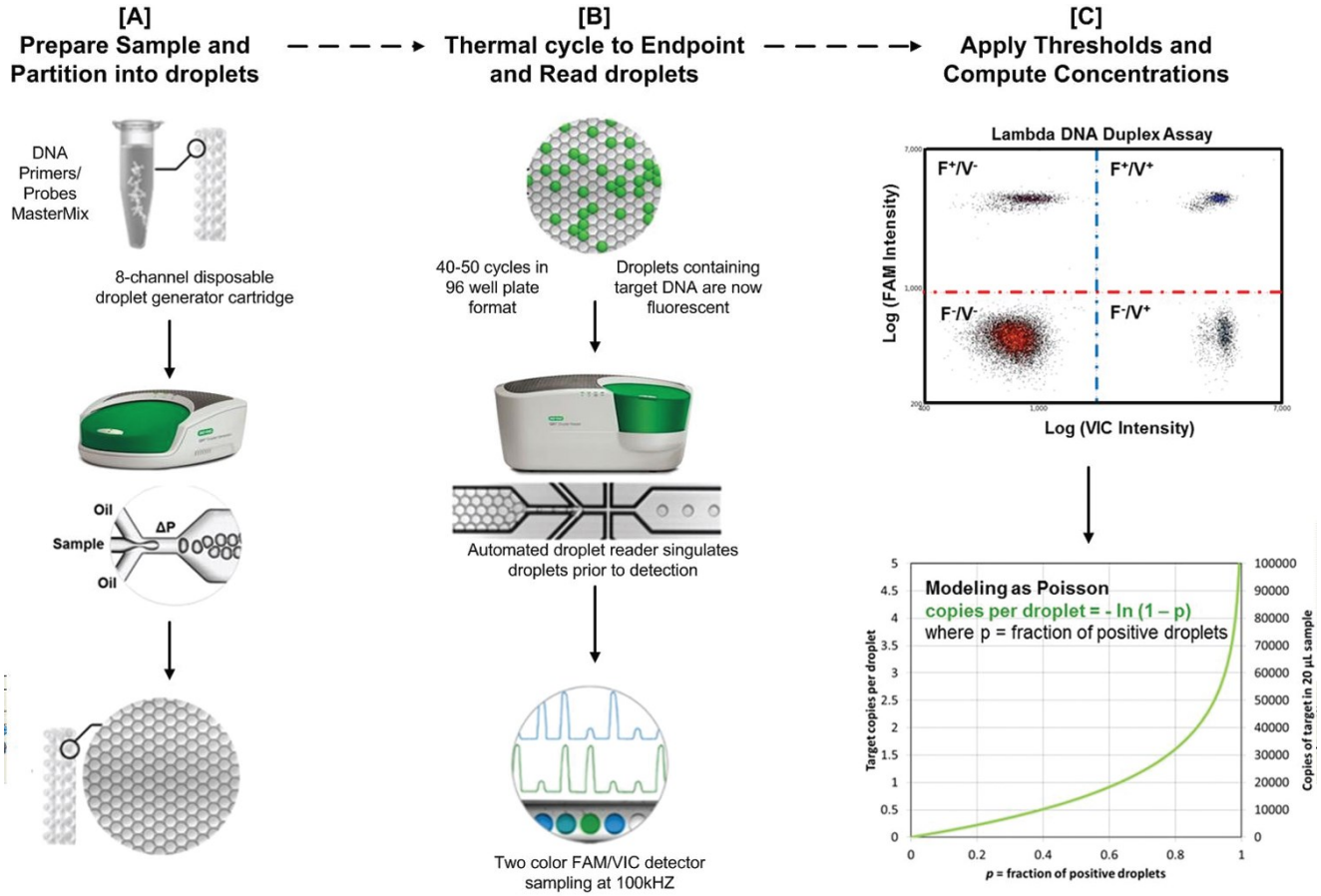
ATCC® No.	Gene	Gene copy number variation	Parental cell line
CRL-2868DQ™	EGFR	amplification	HCC827
CRL-5928DQ™	ERBB2	amplification	NCI-H2170
CRL-5973DQ™	MET	amplification	SNU-5
CRL-5974DQ™	MYC	amplification	SNU-16

Use QX200 ddPCR™ (Bio-Rad) to perform quantification

- Absolute copy number of target gene
- Absolute copy number of housekeeping gene EIF2C1
- Relative copy number/ copy number variation

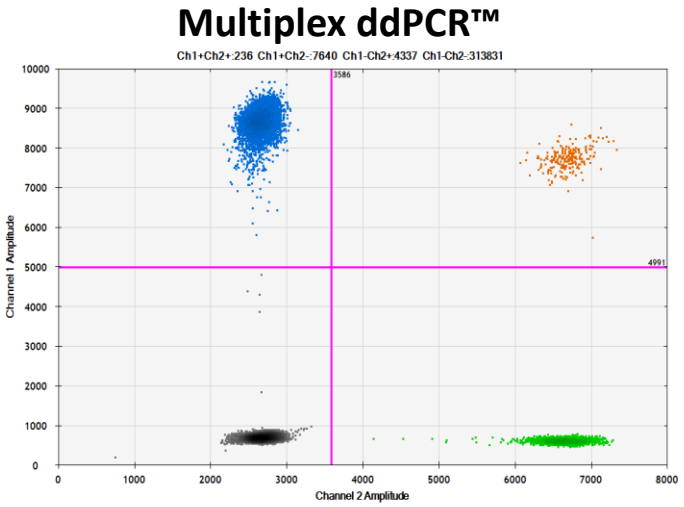
ATCC

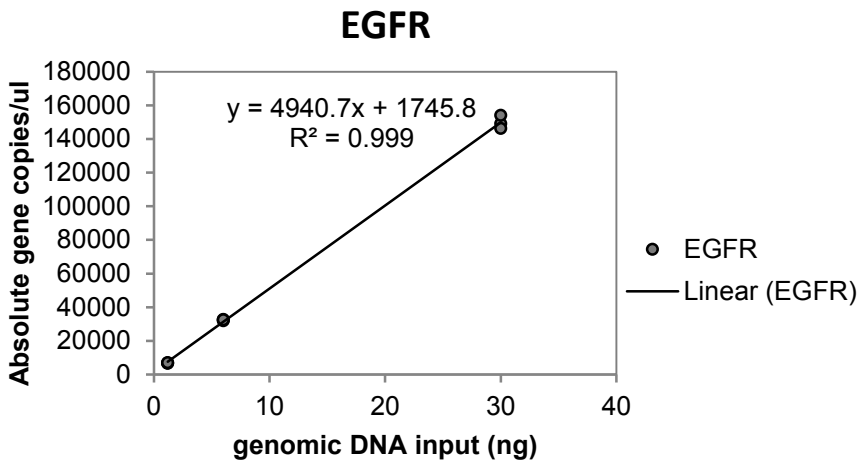
Overview of ddPCR™ workflow

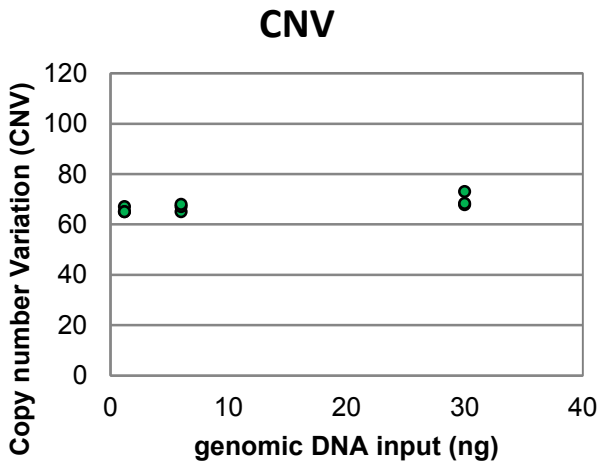


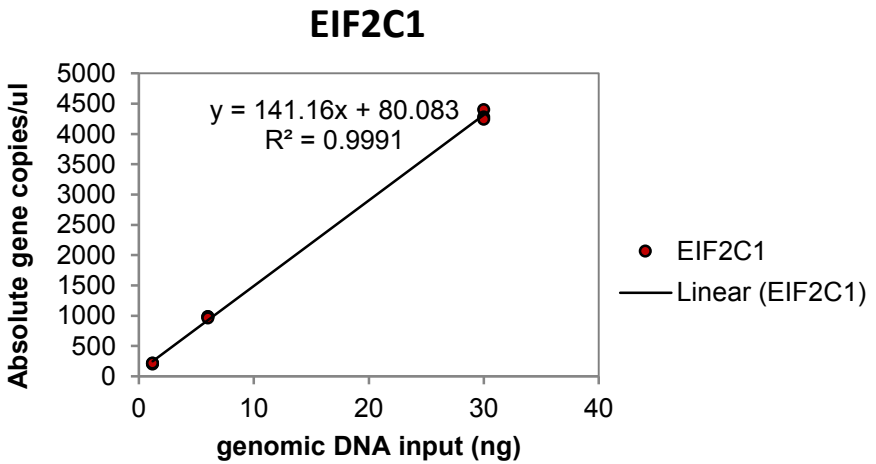
Anal Chem 84(2): 1003−1011, 2012.

Quantify EGFR amplification









ddPCR™ is performed on each lot to ensure gene copy number quantification - your trusted control materials

ATCC

Batch specific test results for each production lot

Example: ATCC® CRL-2868DQ™ quantitated human gDNA

- **Lot:** 63788713
- **Gene:** EGFR
- **Mutation:** ELREA746del
- **Mutation percentage:** 100%
- **EGFR absolute gene copy number:** 8217.83 copies/ng
- **EGFR relative gene copy number:** 65.83 copies

CoA report result – NGS (Coverage > 10,000X)

NGS result uncertainty is equal or smaller than ±5%. The reported uncertainty represents uncertainty expressed at approximately the 99% confidence level using a coverage factor of k=3.

CoA report result – ddPCR™ (Average of nine data points)

ddPCR™ uncertainty is equal or smaller than ± 25%. The reported uncertainty represents uncertainty expressed at approximately the 99% confidence level using a coverage factor of k=3.

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Conclusion

- Next-generation sequencing and other molecular tests have been used in clinical diagnostics, which is facilitating advances in disease prediction and therapeutic decision making
- Globally accepted, well-established reference materials are needed to ensure the reliability and reproducibility of diagnostic test results
- Quantitative gDNAs with known mutation allelic frequency and gene copy number provide a reliable and sustainable alternative to variable patient tissue derived controls in oncology molecular diagnostic assays

ATCC

Acknowledgements

- Brian Chase
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- Lysa-Anne Volpe
- Melisa Wilson

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Disclaimers

ATCC nucleic acids are for research use only

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- **September 29, 2016**
  12:00 PM ET
  Keeping Cells Happy – Topics in Cell Health Maintenance and Viability
  Steve Budd, M.S., *Product Line Business Specialist,* ATCC

- **November 3, 2016**
  12:00 PM ET
  The Biology of Anaerobic Bacteria and Predominant Propagation Practices
  Allison Faust, *Senior Biologist,* ATCC
  Nancy Krueger, *Senior Biologist,* ATCC