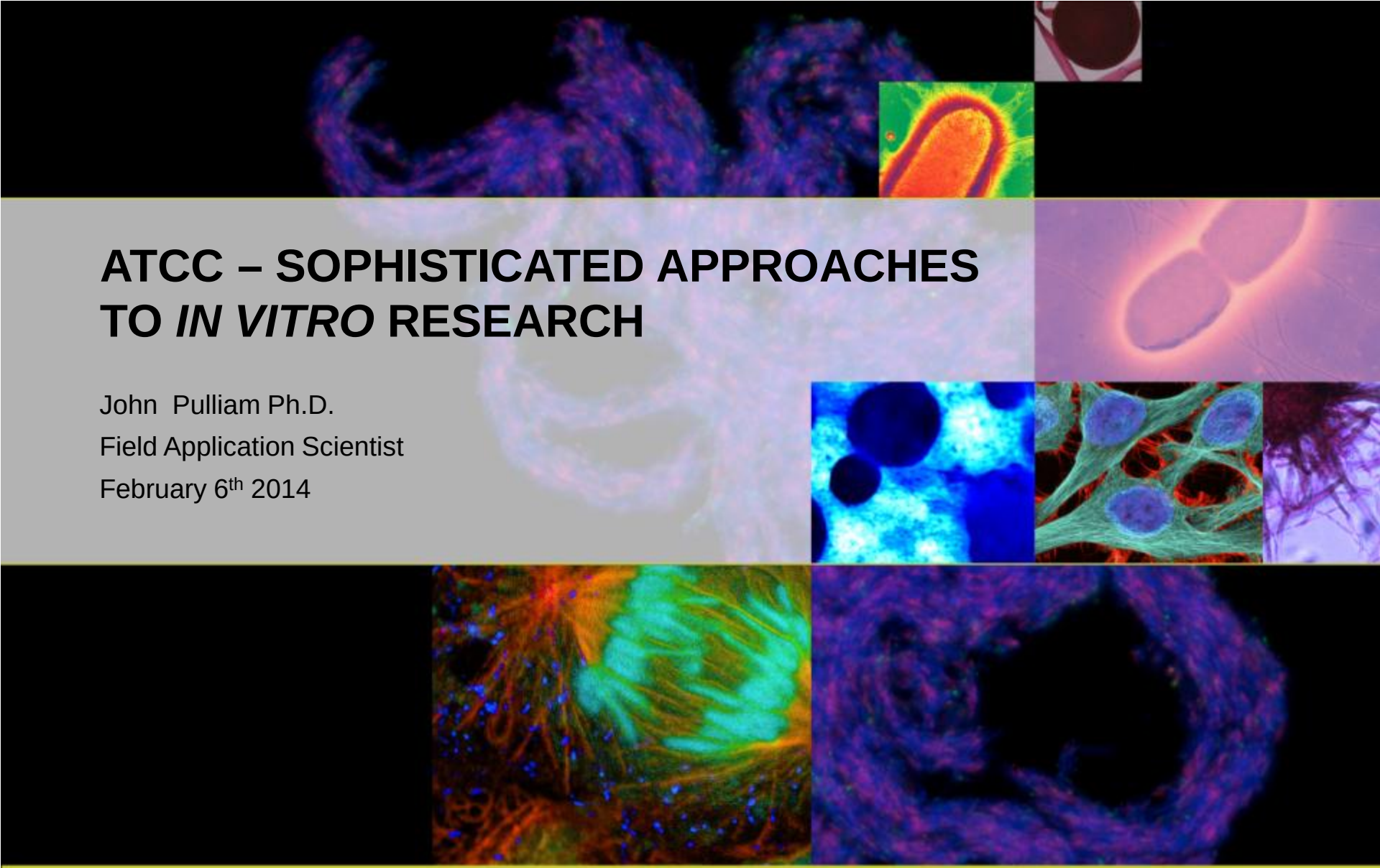

ATCC – SOPHISTICATED APPROACHES TO *IN VITRO* RESEARCH

John Pulliam Ph.D.
Field Application Scientist
February 6th 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
  - Microorganisms
  - Cell lines
  - Derivatives
  - Bioproducts

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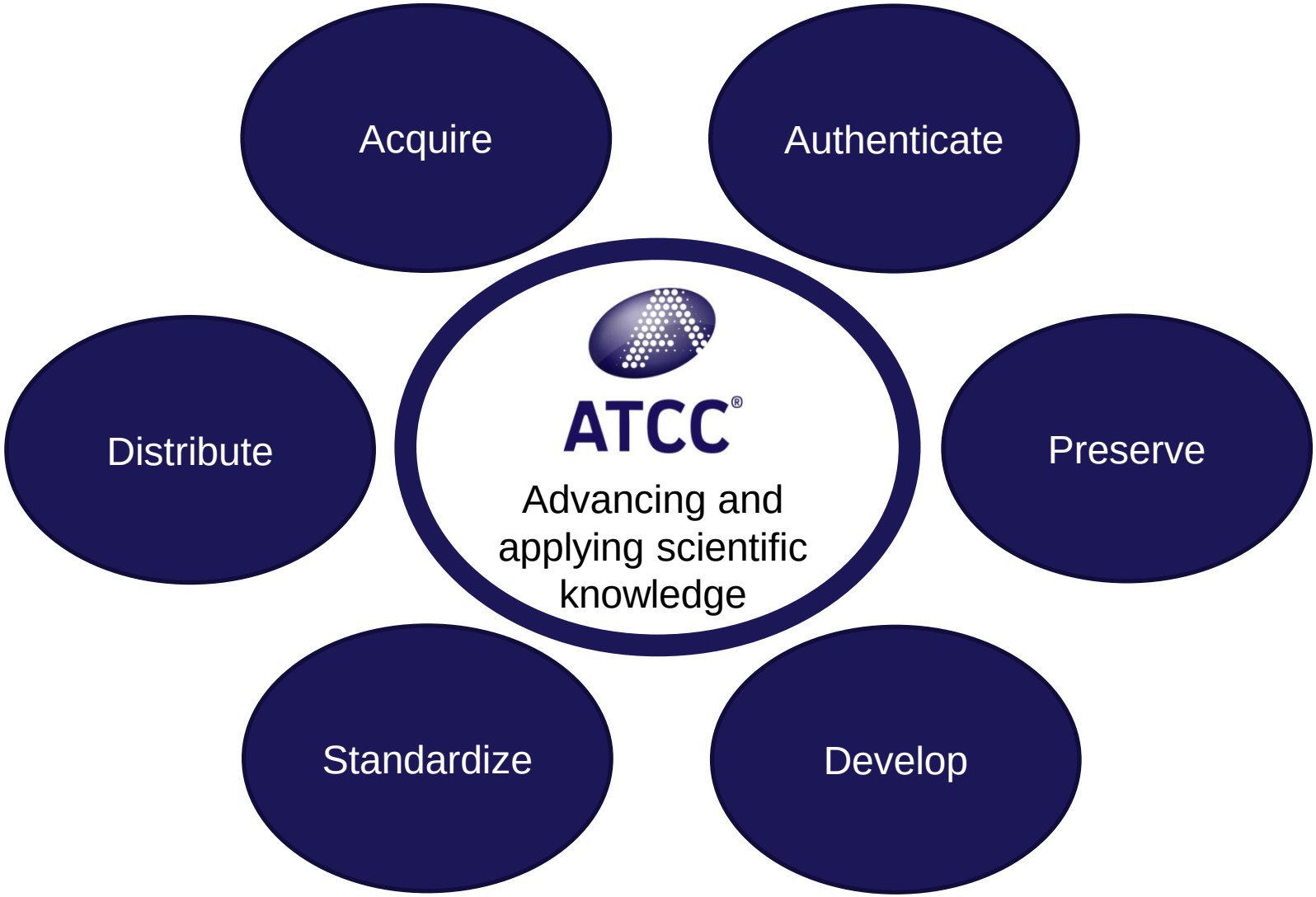
ATCC


Acquire
Authenticate
Distribute
ATCC®
Advancing and applying scientific knowledge
Preserve
Standardize
Develop

ATCC: Keystone of biological research

Cell Biology Systems

Supporting the vast majority of cellular models used in basic research, drug discovery, and biotherapeutic development

Life Science Standards

Developing and maintaining biological standards that protect public interest and provide quality reference material, education, accreditation, and certification services

Microbiology

Providing authenticated strains for use in bioproduction, bioenergy, quality control, and basic research

Government Contracts

Acquiring and managing contracts awarded from both government agencies and private foundations

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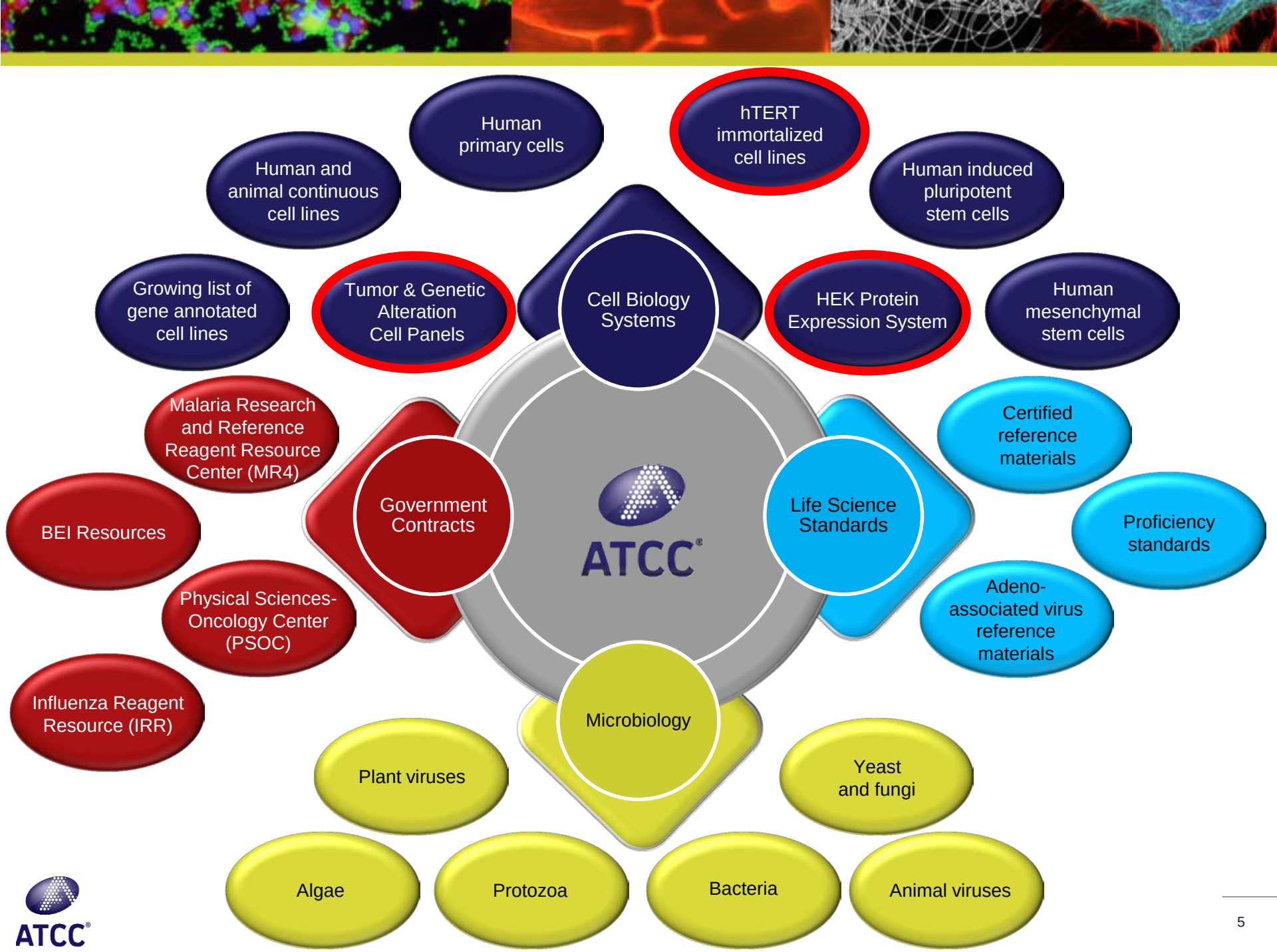
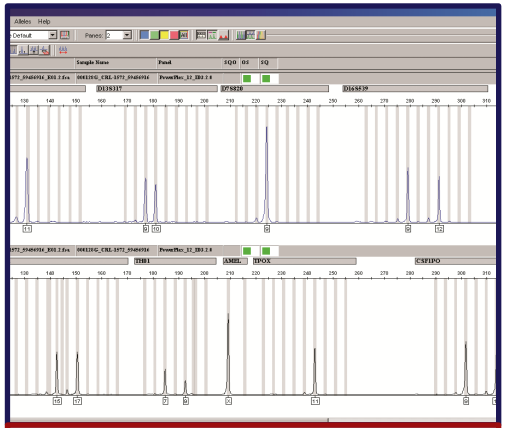

Human primary cells
hTERT immortalized cell lines
Human and animal continuous cell lines
Human induced pluripotent stem cells
Growing list of gene annotated cell lines
Tumor & Genetic Alteration Cell Panels
Cell Biology Systems
HEK Protein Expression System
Human mesenchymal stem cells
Malaria Research and Reference Reagent Resource Center (MR4)
Certified reference materials
BEI Resources
Government Contracts
Life Science Standards
Proficiency standards
Physical Sciences-Oncology Center (PSOC)
Adeno-associated virus reference materials
Influenza Reagent Resource (IRR)
ATCC
Microbiology
Plant viruses
Yeast and fungi
Algae
Protozoa
Bacteria
Animal viruses

Standardized methods and verified materials to support credible research results

Authentication



Short tandem repeat (STR) testing/analysis

Species verification

-Cytochrome C Oxidase (COI)

Morphology

Characterization

Viability testing

-MTT Kit (ATCC® 1010K™)

-XTT Kit (ATCC® 1011K™)

Analysis of cell growth

-Kinetics

-Optimized media

Sterility Testing

BacT3D/ALERT

Virus testing

-HIV, HepB, CMV, HPV, EBV

Mycoplasma testing service (ATCC® 119-X)

-Direct culture

-Hoechst staining

Universal Mycoplasma Detection Kit (ATCC® 30-1012K™)

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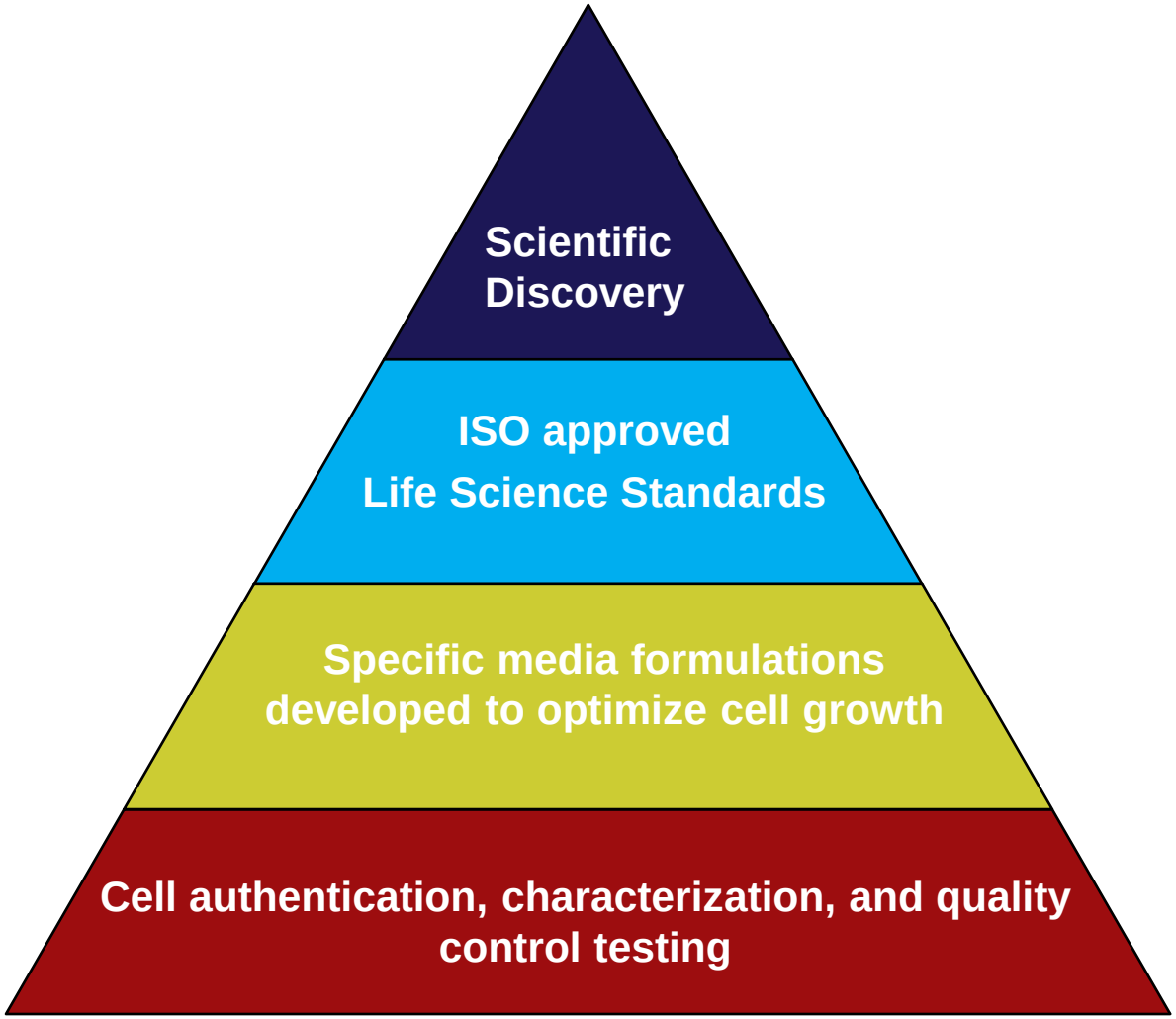
Continuing to expand opportunities for *in vitro* discovery



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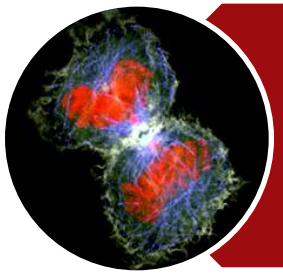
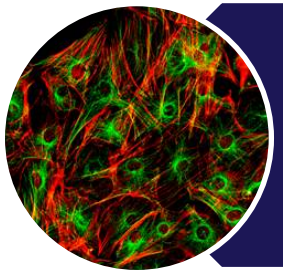
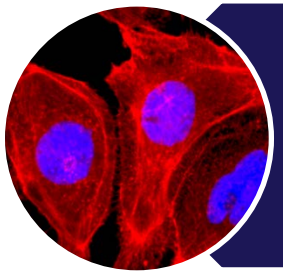
Outline



Tumor and Genetic Alteration Cell Panels



hTERT Immortalized Cell Lines



HEK*Plus* Protein Expression System

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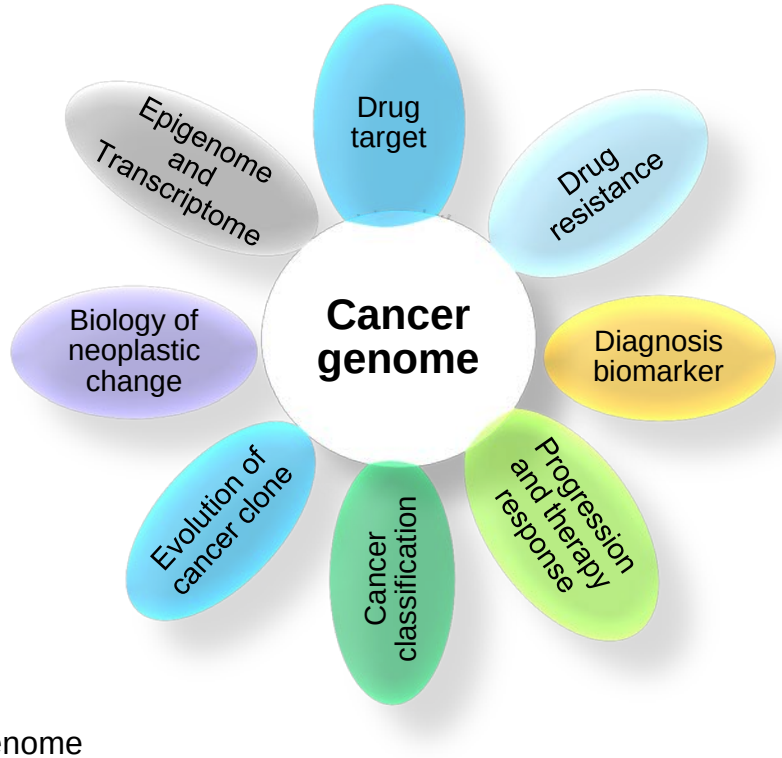
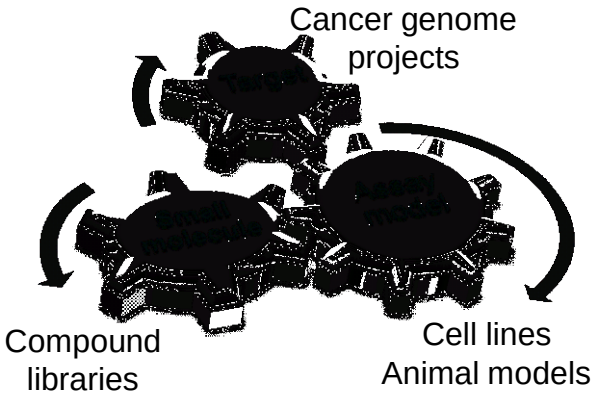
Photo credit: P. Andrews, K Mackenzie

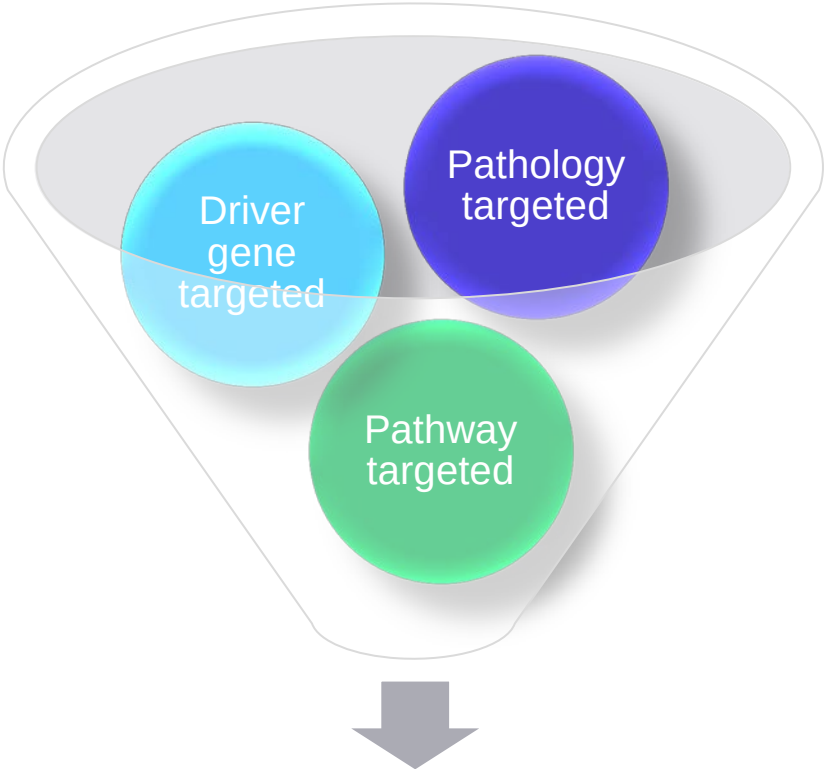
The changing landscape of diagnostics

- Next-generation sequencing has spawned new drug development paradigms
- Large scale sequencing programs
  - The Cancer Genome Atlas
  - International Cancer Genome Consortium (ICGC)
  - Catalogue of Somatic Mutations in Cancer (COSMIC)
  - Cancer Cell Line Encyclopedia





Tumor Cell Panels: investigating the complexity of genetic alterations in disease



Unique tools
Time savings
Convenience

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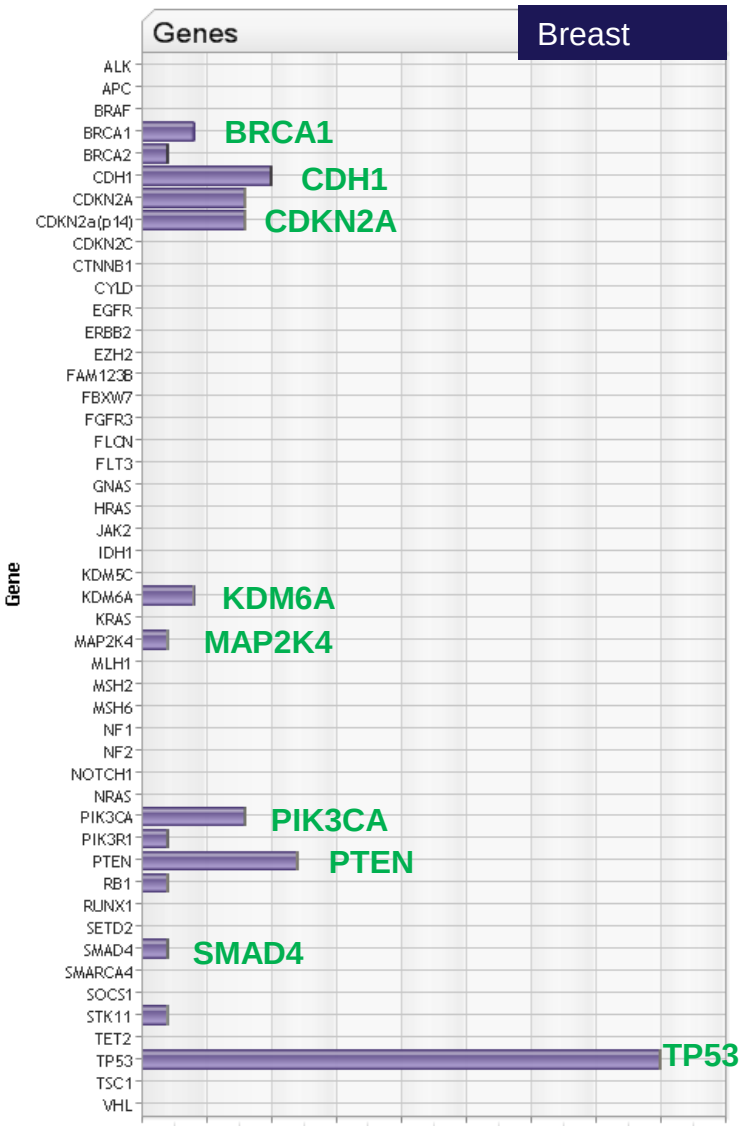
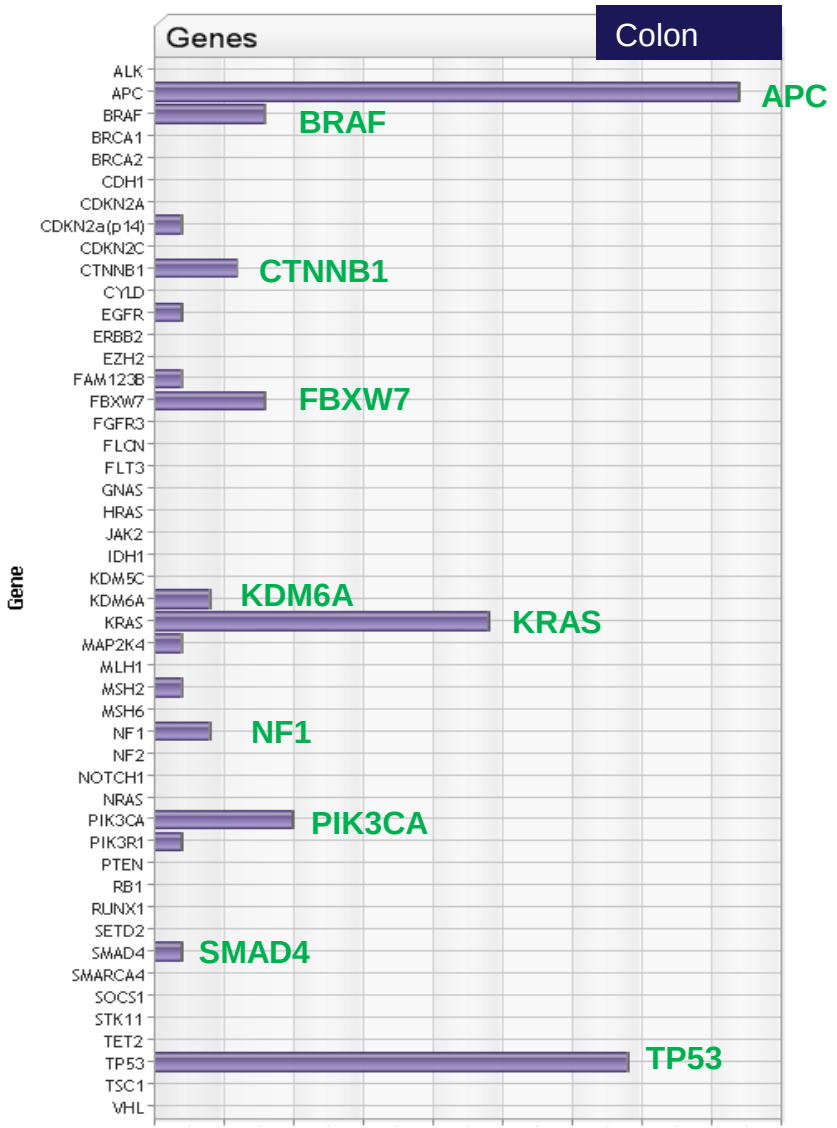
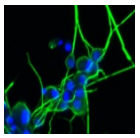
Organization of panels by tissue and mutation



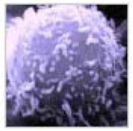


ATCC Tumor Cell Panels

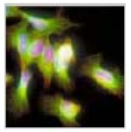


Brain cancer cell panel

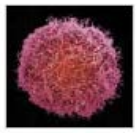
Bone cancer cell panel



Lymphoma cell panel

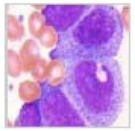


Soft-Tissue Sarcoma cell panel

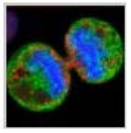


Head/Neck cancer cell panel

Lung cancer cell panel

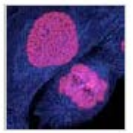


Leukemia cell panel



Melanoma cancer cell panel

Pancreatic cancer cell panel

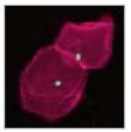


Breast cancer cell panel

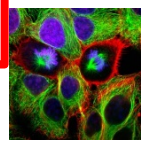
Liver cancer cell panel

Stomach cancer cell panel

Colon cancer cell panel



Bladder cancer cell panel



Gynecologic cancer cell panel

Annotated with genetic alterations

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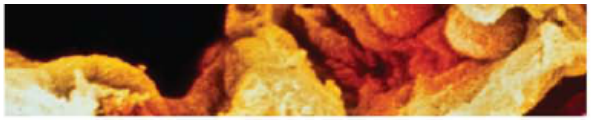
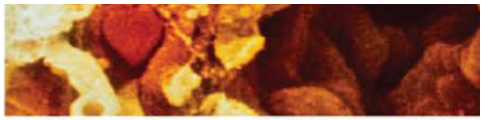
ATCC Tumor Cell Panels

THE ESSENTIALS OF
LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™





ATCC™

COLON CANCER PANELS 1 AND 2

Colon Cancer Panel 1, KRAS (ATCC® No. TCP-1006™) is comprised of eight colon cancer cell lines. Seven of the eight cell lines carry a KRAS mutation as well as other mutations with varying degrees of genetic complexity.

Colon Cancer Panel 2, BRAF (ATCC® No. TCP-1007™) is comprised of eight colon cancer cell lines. Six of the eight cell lines carry a BRAF mutation in addition to mutations in other genes. The table below provides more information for the cell lines included in each panel.

Colon Cancer Panel 1, KRAS (ATCC® No. TCP-1006™)

ATCC® N	Cell line	Tissue	Histology	Source	Mutation	Zygosity	Gene sequence	Protein sequence
CRL-5972™	SNU-C1	Colon	Adenocarcinoma	metastasis, peritoneum	TP53	homozygous	c.497C>A	p.S166*
HTB-39™	SK-CO-1	Colon	Adenocarcinoma	metastasis, ascites	APC	heterozygous	c.3266delT	p.F1089fs*37
					APC	heterozygous	c.4328delC	p.P1443fs*30
					KRAS	homozygous	c.35G>T	p.G12V
CCL-233™	SW1116	Colon	Adenocarcinoma	primary	APC	heterozygous	c.4287_4296delAACCATGCCA	p.Q1429fs*41
					APC	heterozygous	c.35G>C	p.G12A
					KRAS			
					TP53		c.476C>A	p.A159D
CCL-237™	SW948	Colon			APC		c.3340C>T	p.R1114*
					APC	heterozygous	c.4285C>T	p.Q1429*
					KRAS		c.182A>T	p.Q61L
					PIK3CA		c.1624G>A	p.E542K
CCL-248™	T84	Colon	Carcinoma		APC		c.4464delA	p.L1488fs*19
					KRAS	heterozygous	c.38G>A	p.G13D
						heterozygous	c.1624G>A	p.E542K
						homozygous	c.376-1G>T	p.?
CCL-255™	LS123	Rectum	Adenocarcinoma	primary		heterozygous	c.1873C>T	p.Q625*
						heterozygous	c.4348C>T	p.R1450*
						heterozygous	c.34G>A	p.G12S
					SMAD4	homozygous	c.988G>T	p.E330*
					TP53	heterozygous	c.524G>A	p.R175H

Colon | Adeno-carcinoma | Rectum | Metastasis lung | Primary | APC KRAS | APC FAM123B FBXW7 KRAS TP53 | Homo-zygous | Hetero-zygous | c.35G>C | p.G12A

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ATCC Breast Cancer Cell Panels

If you are interested in	Supportive materials
Using large number of cell lines to identify other rare or novel mutations/targets	• Breast Cancer Cell Panel (ATCC® 30-4500K™) • 45 breast cancer cell lines
Basic or translational research focused on triple negative breast cancer	• Triple-Negative Breast Cancer Cell Panels (ATCC® TCP-1001™, TCP-1002™, TCP-1003™)
Patient therapeutic treatment history or biomarker expression	• Breast Cancer Biomarkers Cell Line Panel 1 (ATCC® TCP-1004™)
Breast cancer metastasis, *in vitro* mouse models of breast cancer, or the EGFR-MEK signaling pathways	• Breast Cancer Mouse Model Cell Panel (ATCC® TCP-1005™)
P53 hotspot mutations, or characterization and validation data	• Breast Cancer p53 Hotspot Mutation Cell Panel (ATCC® TCP-2010™)

ATCC®

ATCC Genetic Alteration Cell Panels

ATCC cell lines

Cancer genome

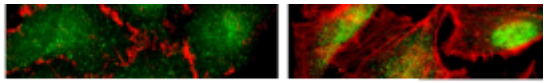
- Systematic analysis
- Gene sequencing validation
- Functional characterization
- Integrative design

- Driver gene focused
- Signaling pathway focused
- Integrative design

PANELS BY MOLECULAR SIGNATURE

Each panel in the molecular signature collection is composed of cell lines that have been sequenced and validated for mutations in specific genes, such as p53. These panels harness the combined forces of genomic data and highly reliable, authenticated ATCC tumor cell lines to provide solid experimental platforms for cancer research and drug discovery.

p53 hotspot mutation panels



NON-SMALL CELL LUNG CANCER P53 HOTSPOT MUTATION CELL PANEL

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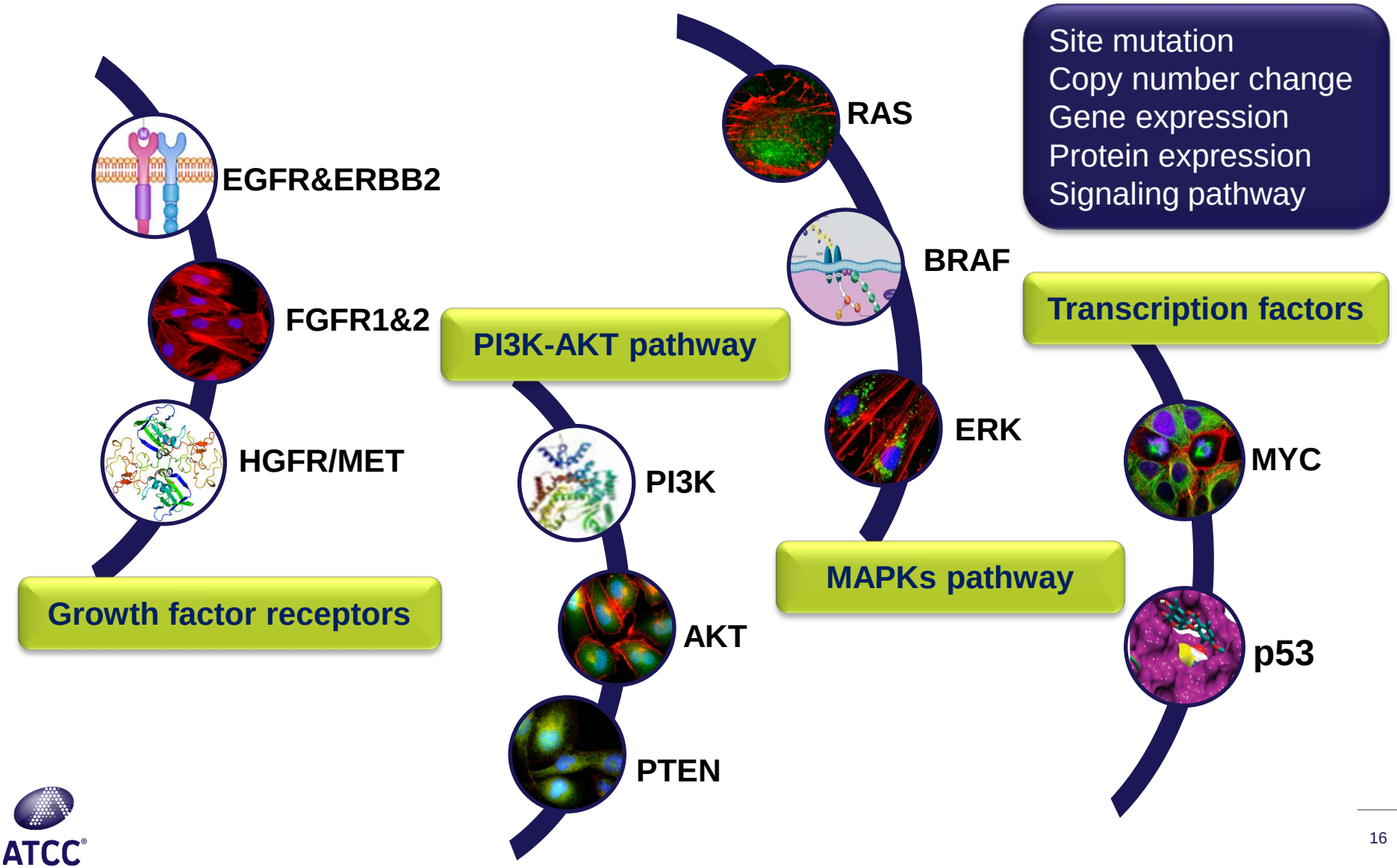
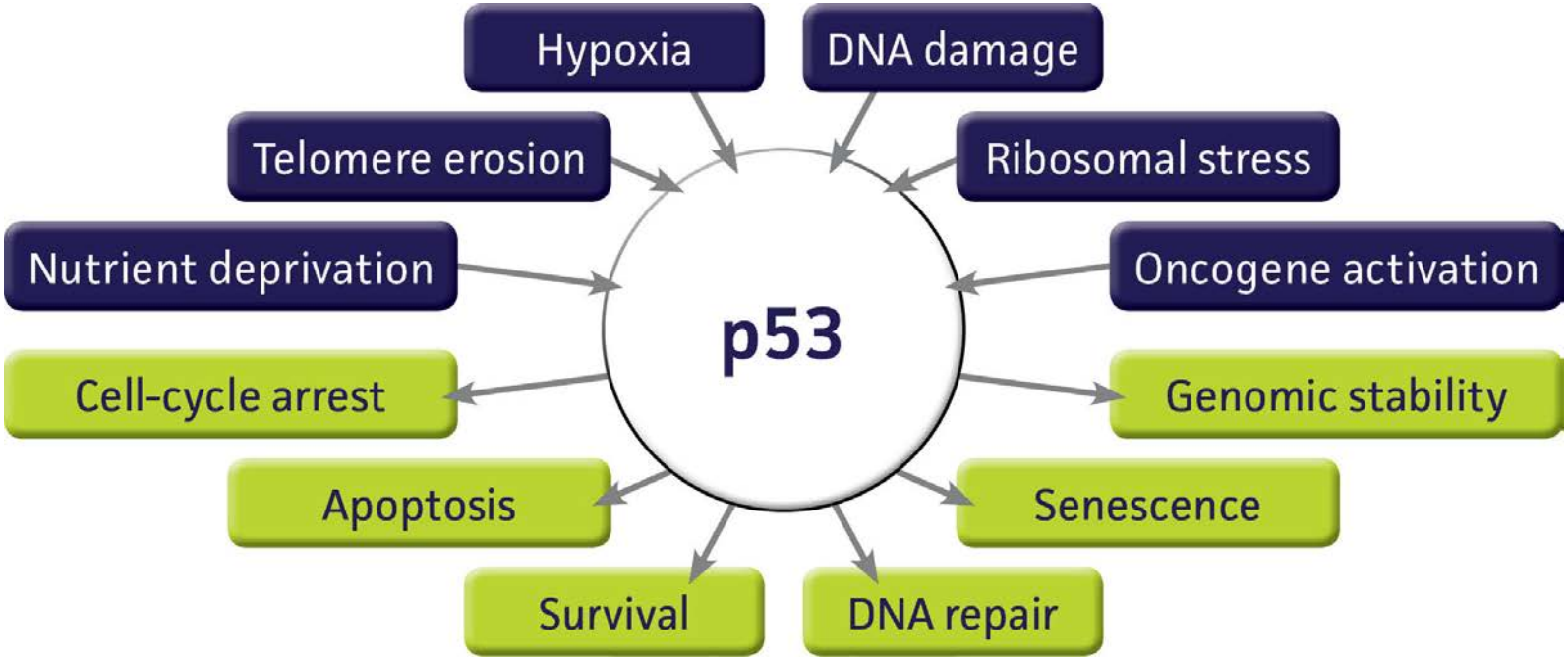
ATCC Genetic Alteration Cell Panels



p53 background

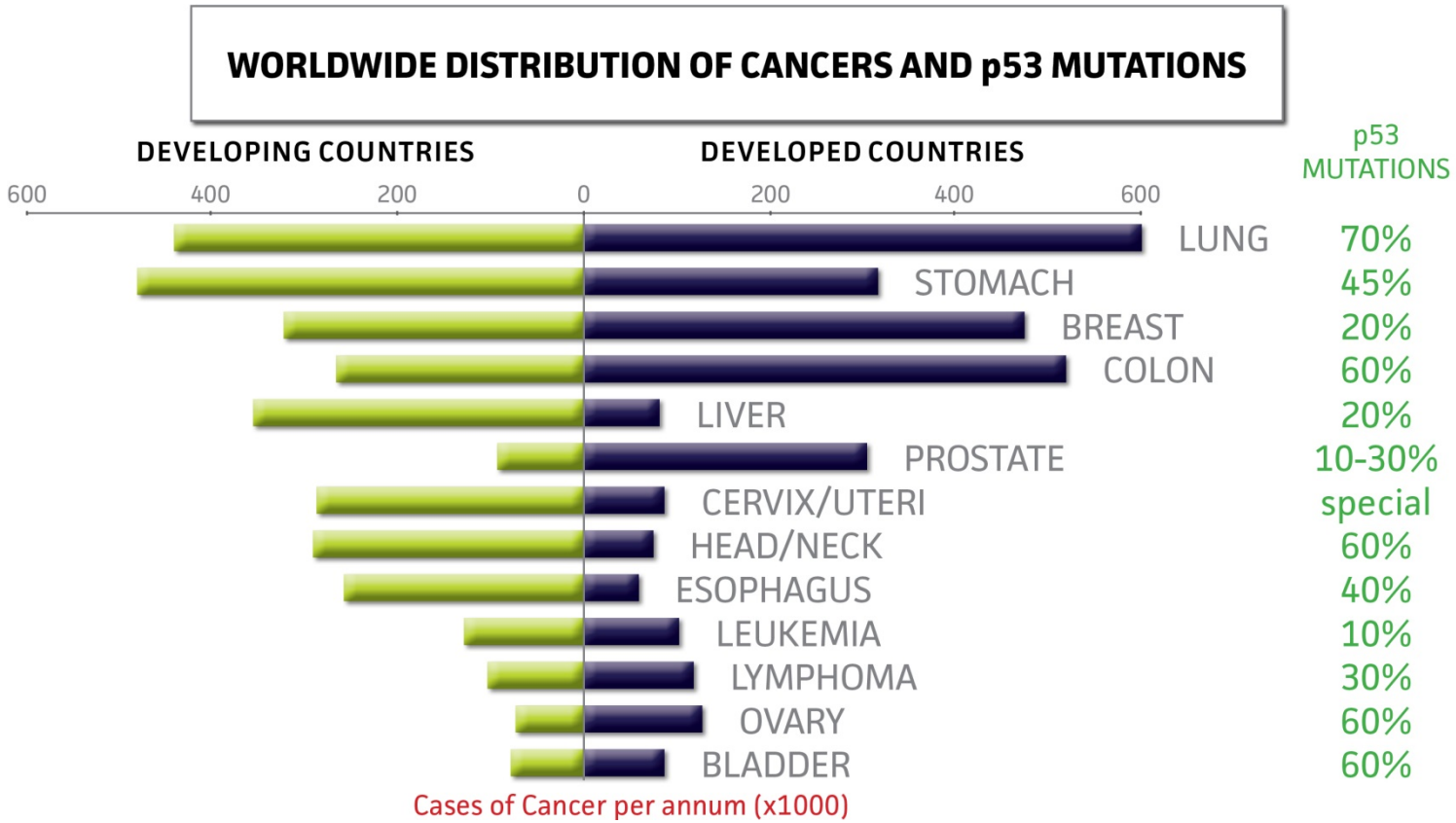
- Tumor suppressor protein encoded by the TP53 gene
- p53 controls the cellular response to stress signals through the induction of cell-cycle arrest, apoptosis, and senescence



Karen H. Vousden and David P. Lane, *Nature Reviews*, 2007

p53 mutations in cancer

- 50% of human tumors contain mutations or deletions in the TP53 gene
- A p53 mutation is vitally important in tumor progression



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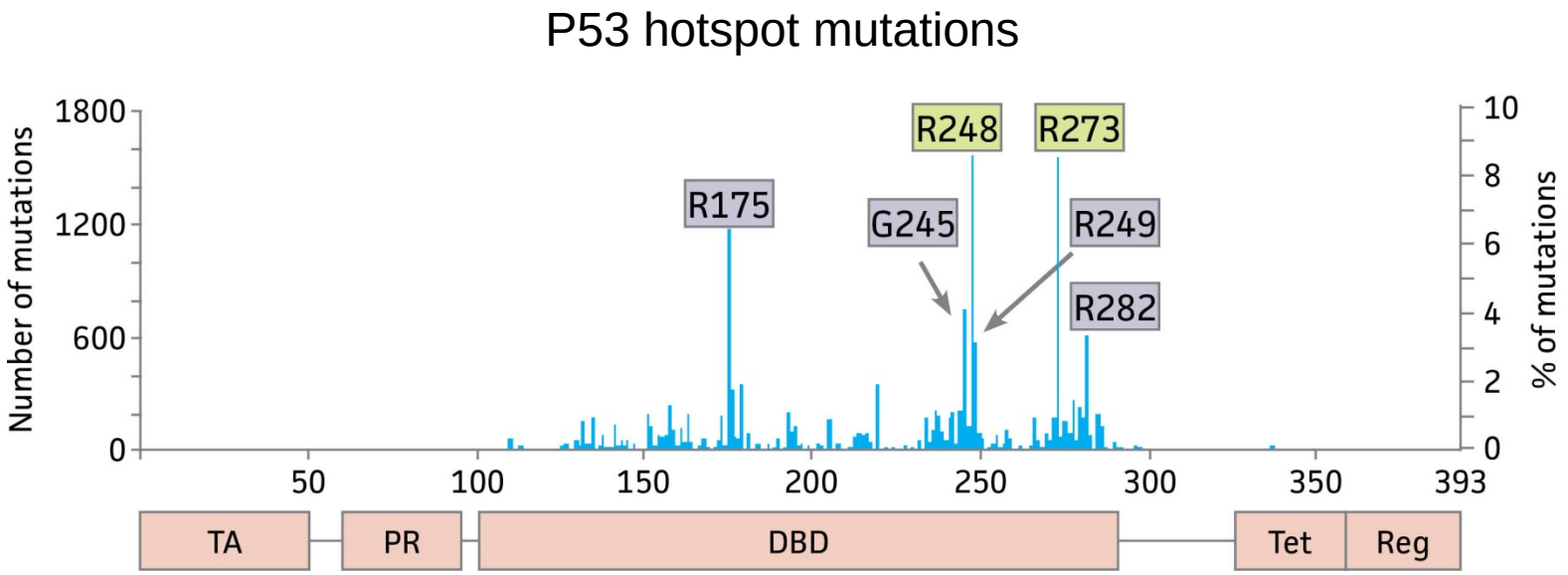
The TP53 website http://p53.free.fr/index.html

p53 hotspot mutations

- Hotspot mutations in the TP53 gene most frequently occur in the DNA binding domain



Christopher J. Brown *et. al.*, *Trends in Pharmacological Sciences*, 2011

The design of a p53 hotspot mutation panel

NSCLC Lung cancer p53 hotspot mutation cell line panel						
ATCC No.	Designation	Tissue	Disease	TP53 status	Gene sequence	protein sequence
CRL-9609	BEAS-2B	lung	normal tissue,SV-40 immortalized	WT	-	-
CCL-185	A549	lung	non-small cell lung carcinoma (NSCLC)	WT	-	-
CRL-5803	NCI-H1299	lung	non-small cell lung carcinoma (NSCLC)	NULL	c.(del)	-
HTB-178	NCI-H596	lung	non-small cell lung carcinoma (NSCLC)	MUT	c.733G>T	p.G245C
CRL-5893	Calu-1	lung	non-small cell lung carcinoma (NSCLC)	MUT	c.742C>T	p.R248W
CRL-5908	NCI-H1975	lung	non-small cell lung carcinoma (NSCLC)	MUT	c.818G>A	p.R273H

Disease	TP53	Gene	protein
normal tissue	WT	-	-
NSCLC	WT	-	-
NSCLC	NULL	c.(del)	-
NSCLC	MUT	c.733G>T	p.G245C
NSCLC	MUT	c.742C>T	p.R248W
NSCLC	MUT	c.818G>A	p.R273H

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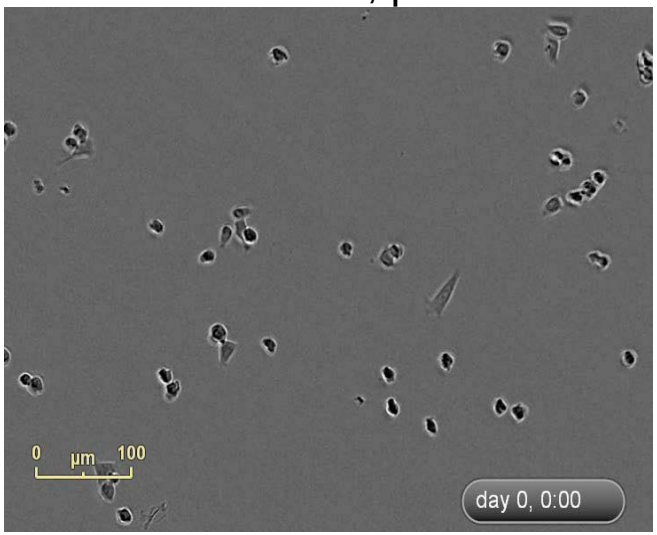
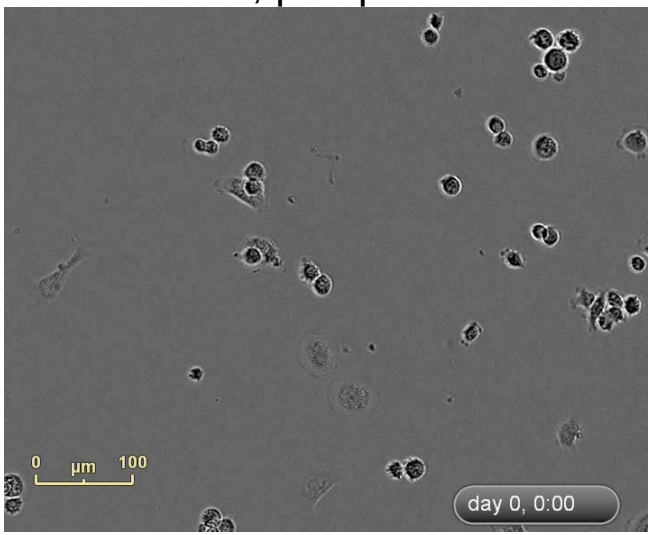
Shorter doubling time in cells with mutant p53

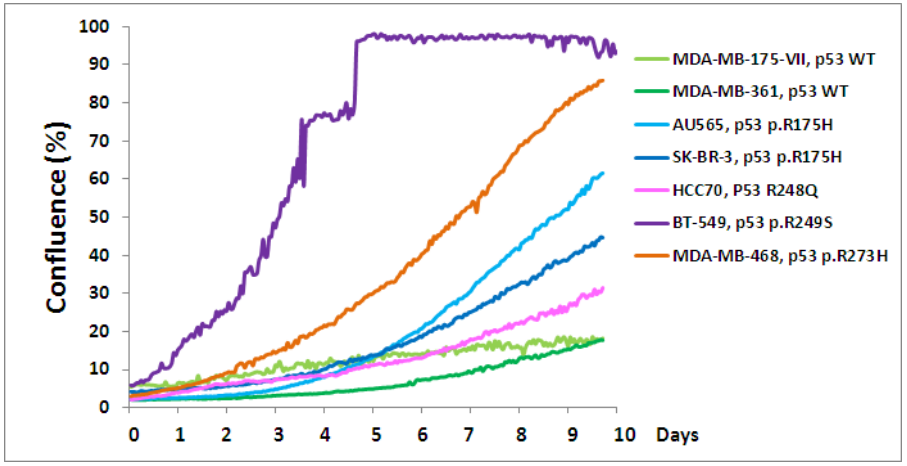
MDA-MB-361, p53 WT



BT549, p53 p.R249S

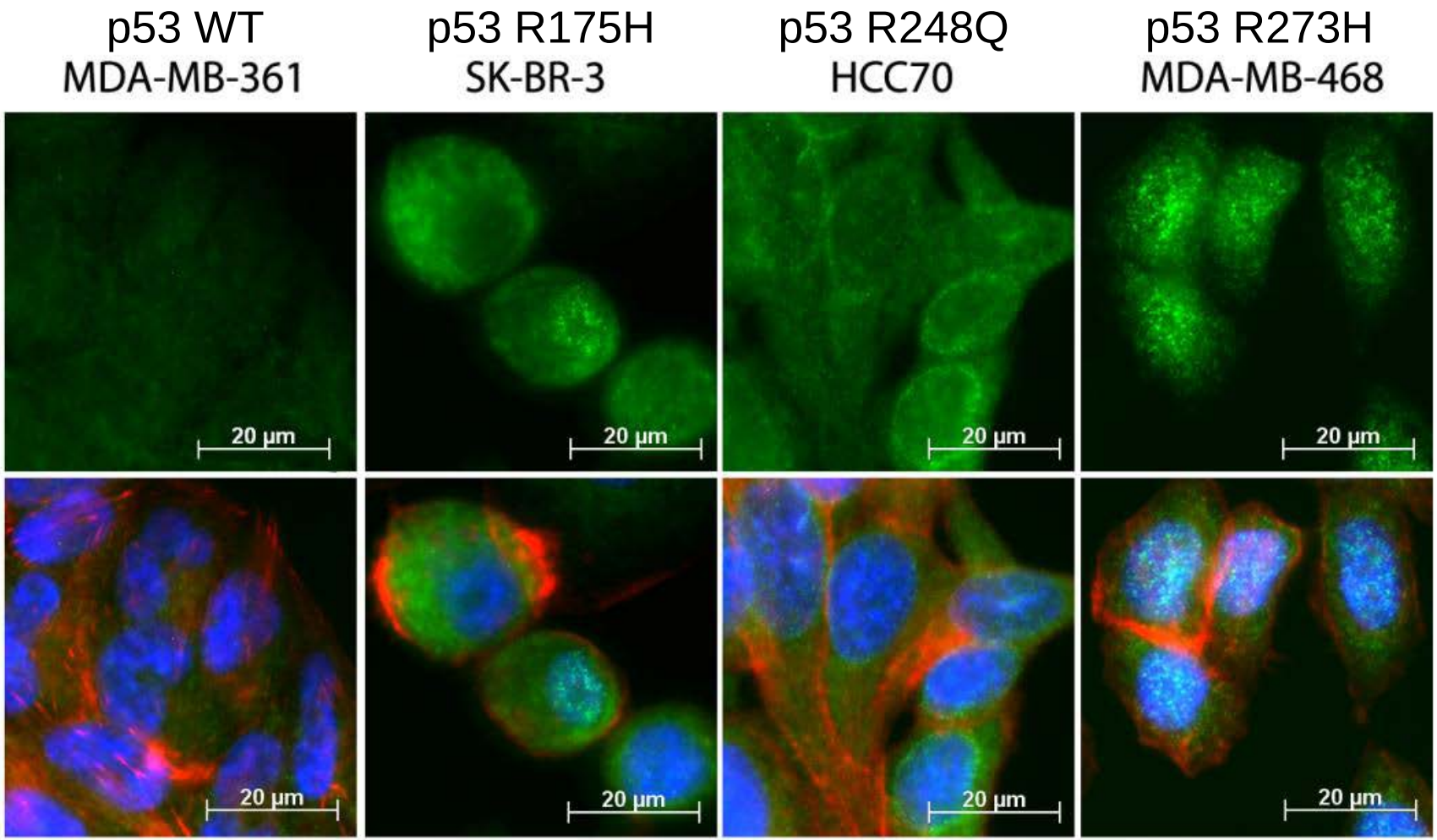


Wild type vs.
mutant kinetics



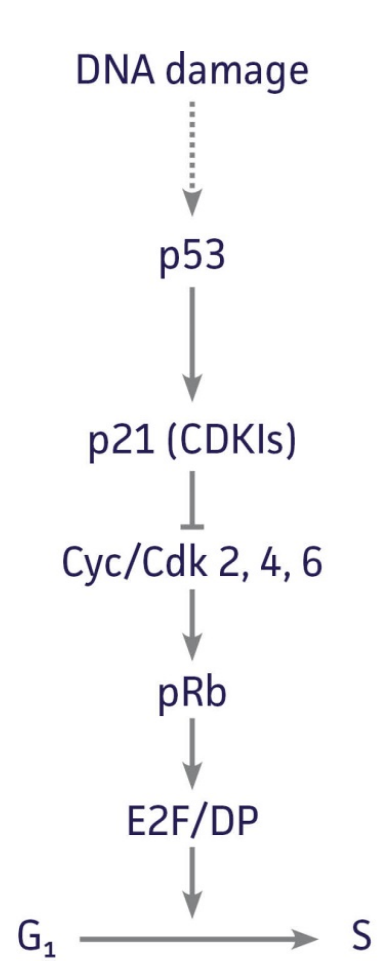
ATCC® TCP-2010™ Breast Cancer p53 Hotspot Mutation Panel

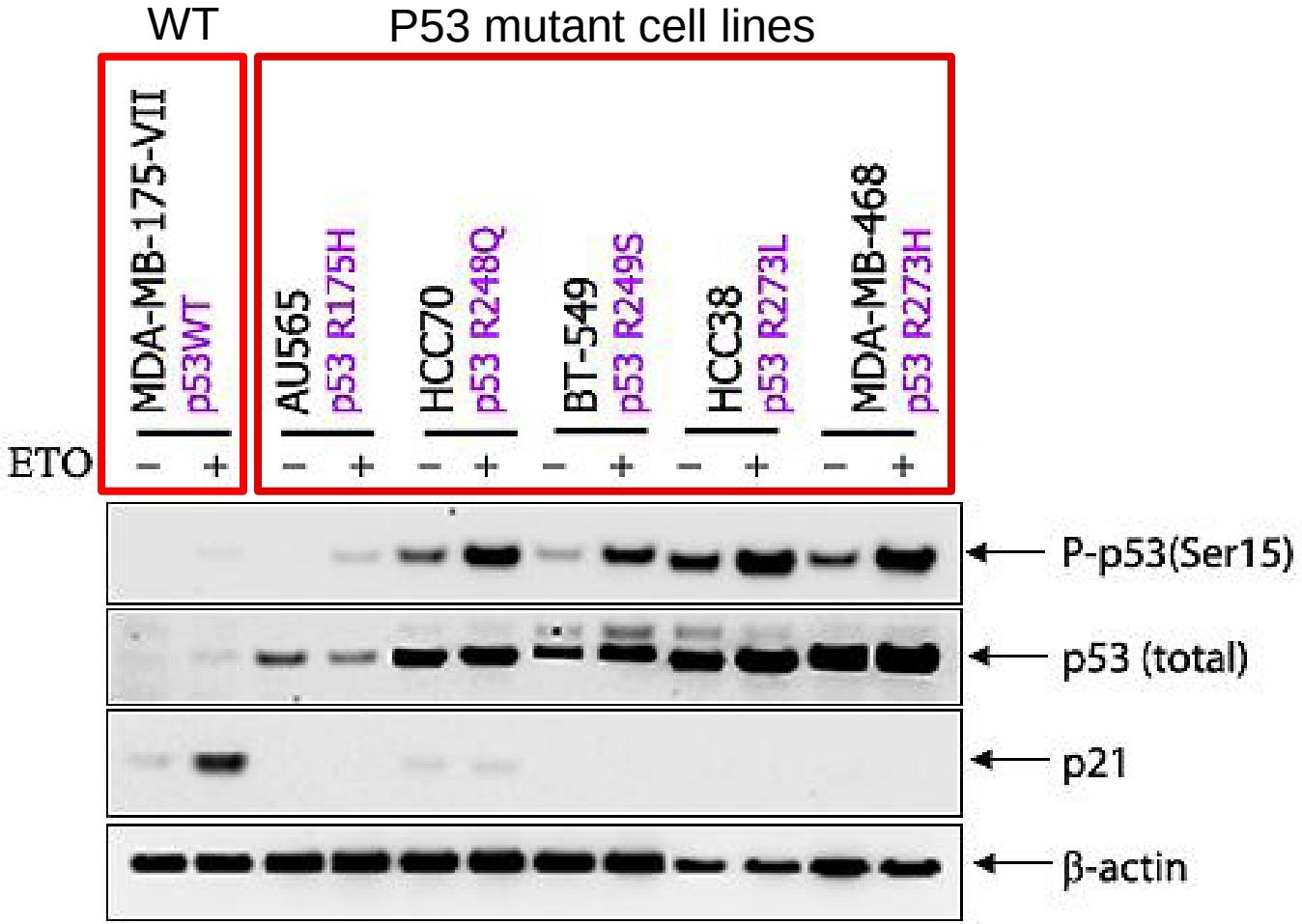
p53 protein expression in cells with mutated p53



(IF staining: P53, F-actin, Hoechst 33342)

p21 expression in cells with mutant p53





ATCC® TCP-2010™ Breast Cancer p53 Hotspot Mutation Panel

Other available Genetic Alteration Panels

Panels

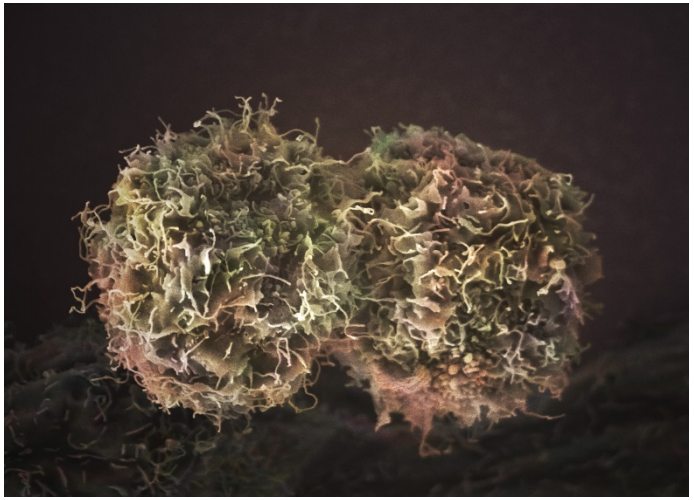
1. EGFR (ATCC® TCP-1027™)
2. PI3K (ATCC® TCP-1028™)
3. AKT (ATCC® TCP-1029™)
4. PTEN (ATCC® TCP-1030™)
5. RAS (ATCC® TCP-1031™)
6. BRAF (ATCC® TCP-1032™)
7. ERK (ATCC® TCP-1033™)
8. FGFR (ATCC® TCP-1034™)
9. MYC (ATCC® TCP-1035™)
10. MET (ATCC® TCP-1036™)

New!

ATCC®

Unique applications for Tumor and Genetic Alteration Cell Panels

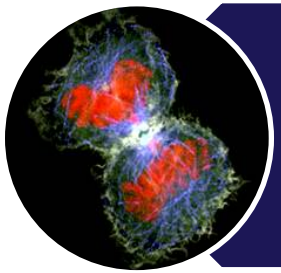
- Validation and characterization of potential cancer driver genes
- Functional and molecular profiling of cancer cell lines which are subtype-specific
- Analyzing top genetic alterations across tumor types and their roles in drug sensitivity and resistance
- Testing small molecules or biologics for cancer drug development



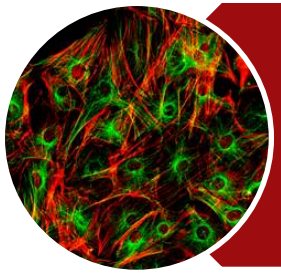
Human skin cancer cell dividing

ATCC®

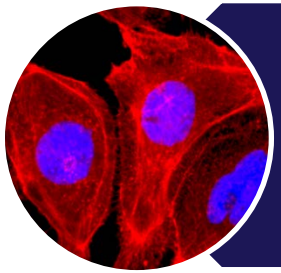
Outline



Tumor and Genetic Alteration Cell Panels



hTERT Immortalized Cell Lines



HEK*Plus* Protein Expression System

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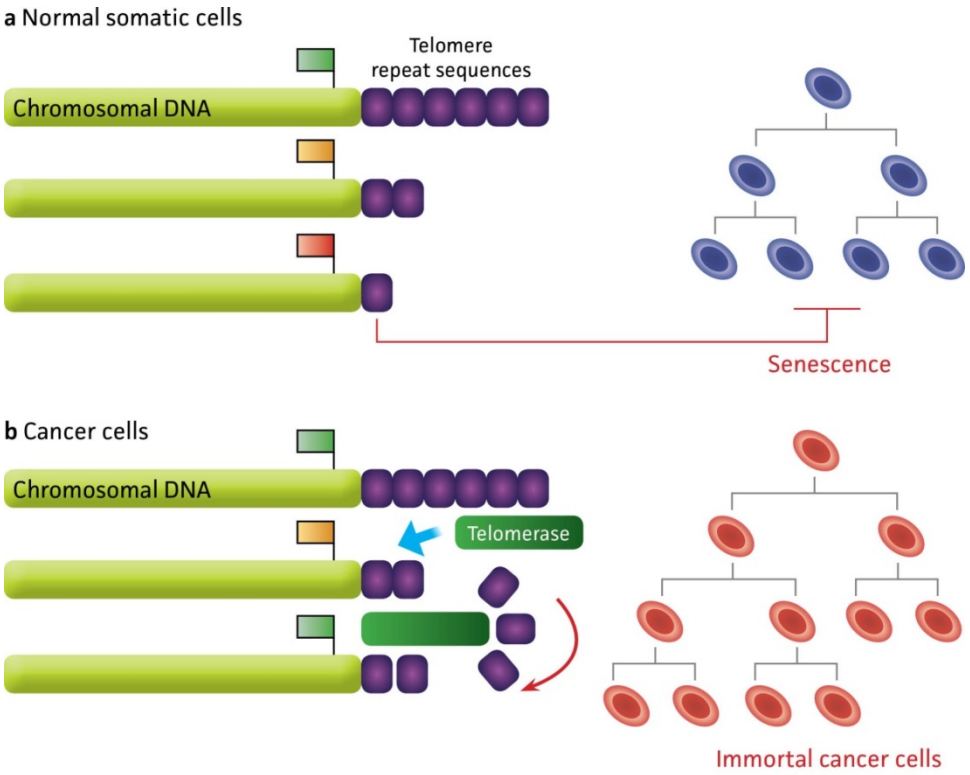
Photo credit: P. Andrews, K Mackenzie

Bypass replicative senescence using telomerase



Regulation of telomere length in normal and cancer cells by telomerase

Note: Viral (Large T antigen, HPV-16 E6/E7) and non-viral (Cdk-4 and Bmi-1) onco-protein vectors may also be used to support the hTERT immortalization vector

hTERT-immortalized cells are unique tools

	Primary Cells	hTERT-Immortalized	Onco, Viral-Transformed	Cancer Cell Lines
Mimic *in vivo* Tissue Phenotype	++++	+++	++	+
Genotypic Stability	Diploid	Diploid / Near-Diploid	Near-Diploid / Aneuploid	Aneuploid
Proliferative Capacity	+	+++	+++	+++
Supply	+	+++	+++	+++
Inter-Experimental Reproducibility	Low	Good	Good	Good
Cost	High	Medium	Low	Low
Ease of Use	+	++	++	+++

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hTERT-immortalized cell lines from ATCC

Tissue	Cell Type	ATCC® No.	Designations	Comments
Breast	Mammary Epithelial	CRL-4010™	hTERT-HME1	Normal
Bone	Bone Cartilage Fibroblast	CRL-2846™, CRL-2847™	CHON-001, CHON-002	Normal
Esophagus	Barrett's Esophageal Epithelial	CRL-4027™, CRL-4028™, CRL-4029™, CRL-4030™	CP-A, CP-B, CP-C, CP-D	Pre-malignant sample
Eye	Retinal Pigment Epithelial	CRL-4000™	hTERT-RPE1	Normal
Kidney	Angiomyolipoma	CRL-4004™	UMB1949	Angiomyolipoma
		CRL-4008™	SV7tert PDGF tumor-1	Autocrine transformation and epigenetic changes
	Proximal Tubule Epithelial	CRL-4031™	RPTEC/TERT1	Normal
Lung	Bronchial Epithelial	CRL-4011™	NuLi-1	Normal
		CRL-4013™, CRL-4015™, CRL-4016™, CRL-4017™	CuFi-1, CuFi-4, CuFi-5, CuFi-6	Cystic Fibrosis
Pancreas	Pancreatic Duct Epithelial	CRL-4023™	hTERT-HPNE	Normal
		CRL-4036™, CRL-4037™, CRL-4038™, CRL-4039™	hTERT-HPNE E6/E7, E6/E7/st, E6/E7/K-RasG12D, E6/E7/K-RasG12D/st	Stepwise oncogenic manipulation
Skin	Dermal Microvascular Endothelial	CRL-4025™	TIME	Normal
	Foreskin Fibroblast	CRL-4001™	BJ-5ta	Normal Neonatal
Uterus	Endometrium Fibroblast	CRL-4003™	T HESCs	Normal

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TIME-GFP cell line

- hTERT-immortalized microvascular endothelial (TIME)-GFP cell
- Derived by transfecting TIME (ATCC® CRL-4025™) cells with linearized pWE2-EmGFP plasmid
- Stably expresses the GFP under a CMV promoter
- Diploid cell line of male origin with a chromosome number of 46 (the line shows some karyotypic instability at later passages)
- Positive for CD31(endothelial cell marker)
- Positive for the uptake of Low Density Lipoprotein (LDL)
- Capable of 15 population doublings after recovery from cryopreservation

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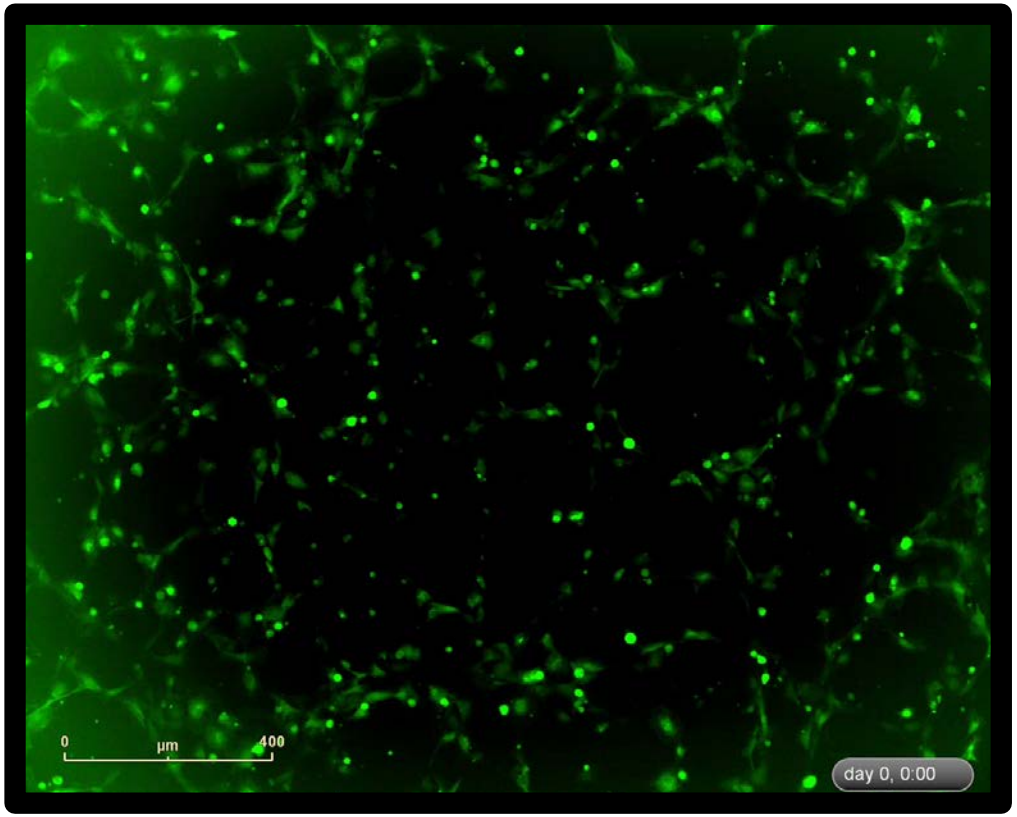
TIME-GFP *in vitro* tubule formation assay

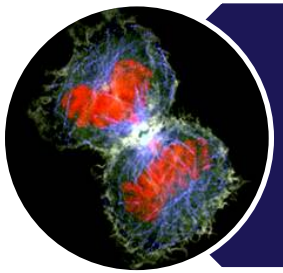


- Grown with:

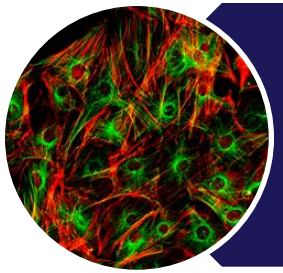
  Vascular Cell Basal Medium (ATCC® PCS-100-030) supplement with Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040) or Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041)

- Blasticidin and G418 added to support cell modification

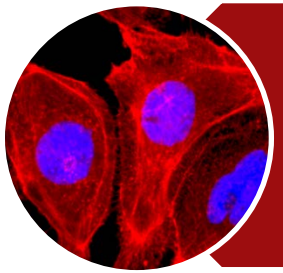
Outline



Tumor Cell and Genetic Alteration Panels



hTERT Immortalized Cell Lines



HEK*Plus* Protein Expression System

ATCC®

Photo credit: P. Andrews, K Mackenzie

HEK*Plus* Protein Expression System (ATCC® ACS-4800K)

A complete mammalian protein expression system using serum-free suspension cell cultures of HEK293 cells

Components	ATCC® No.
HEK*Plus* SF Suspension Cells	ACS-4500™
HEK*Plus* SFM Medium	ACS-4002
HEK*Plus* Boost Solution	ACS-4003
GeneX*Plus* Transfection Reagent	ACS-4004
L-Alanyl-Glutamine, 200 mM	30-2115

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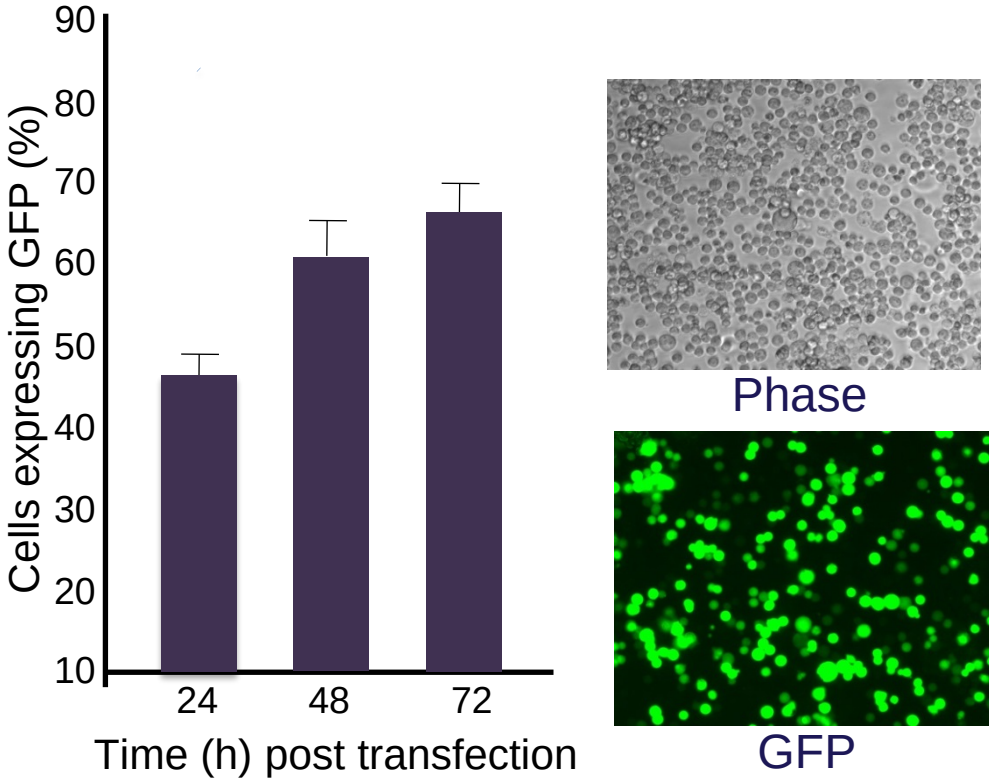
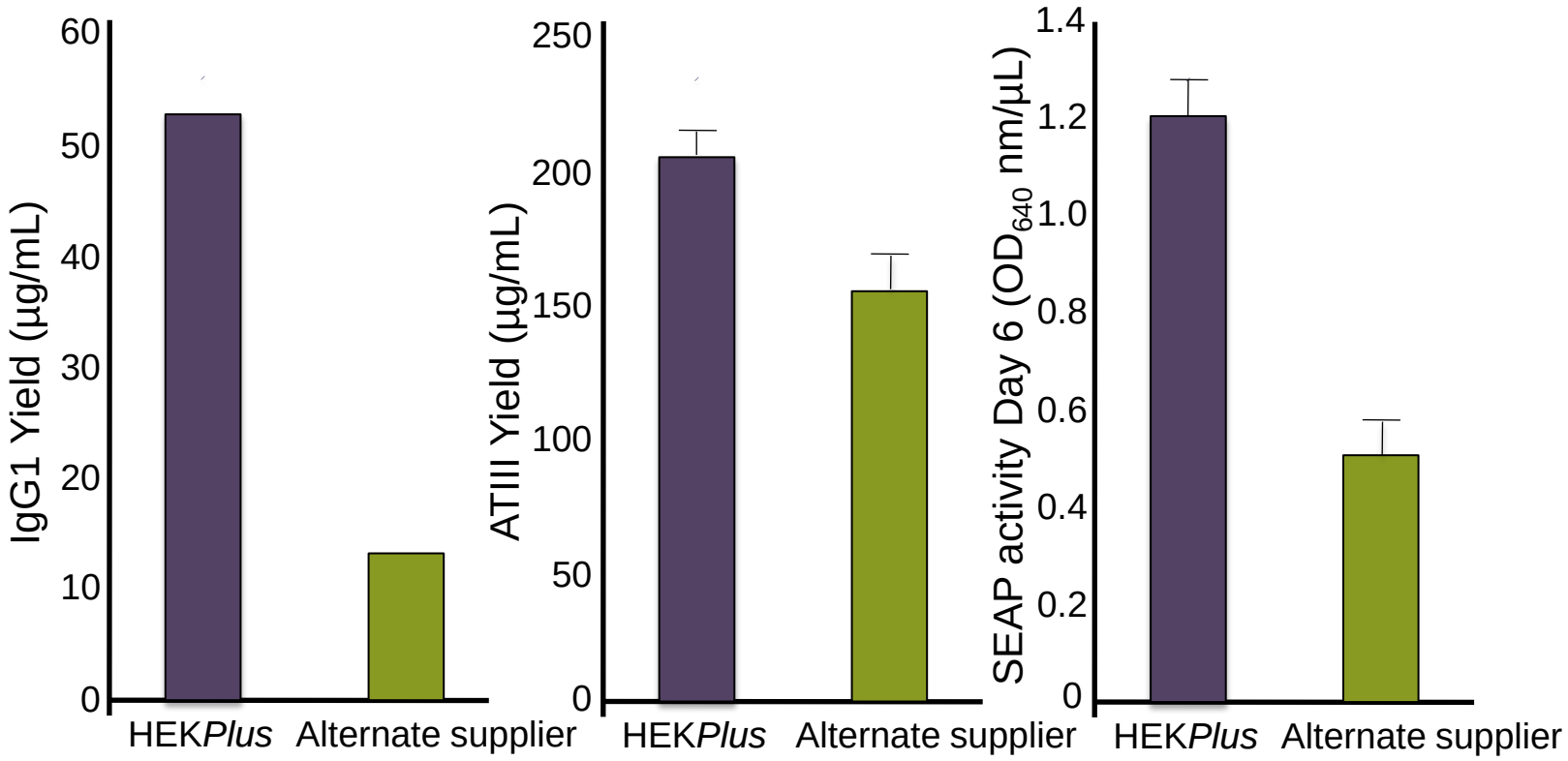
HEK*Plus* system: High transfection efficiency



The HEK*Plus* Expression System consistently achieves **high transfection efficiency**, with 65% to 70% of cells expressing the construct 48 to 72 hours after transfection

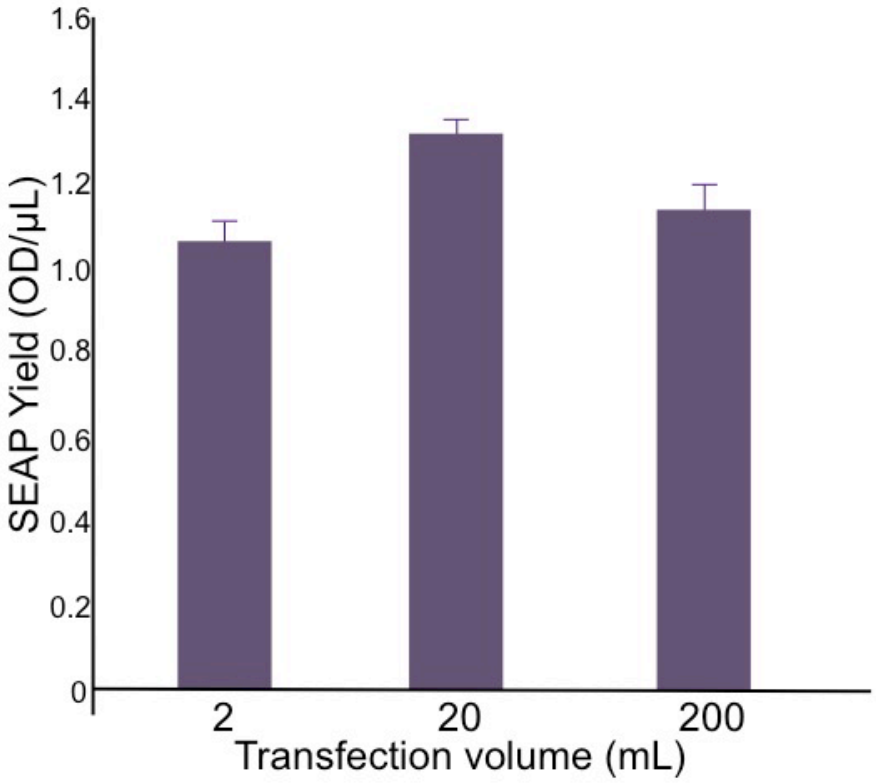
HEK*Plus* system versus control



The HEK*Plus* Expression System results in **protein yields higher than the expression systems of an alternate supplier**.

SEAP was assayed using a phosphatase reaction, which suggests that the expressed protein is **functional**.

Scalability of the HEK*Plus* system



The kit components are offered as a **cost-effective complete system** or **individually** to meet the needs of the investigator.

The kit is **scalable**. It is tested to ensure a comparable yield of SEAP when either 2 mL or 200 mL of cells (1 x 10^6 cells/mL) are transfected.

Summary

- Tumor and Genetic Alteration Cell Panels utilize cancer genomics to support drug discovery and the *in vitro* investigation of clinically relevant cancer pathology.
- hTERT immortalized cells exhibit *in vitro* tissue phenotypes while demonstrating a high proliferative capacity, supporting various long-term studies of biochemical and physical aspects of cell growth.
- HEK*Plus* transfection system offers an easy-to-use system to express your protein of interest, and may be ideal for the expression and detection of functional human proteins.
- Collectively, these tools can support *in vitro* cancer research, drug discovery, and therapeutic target development.

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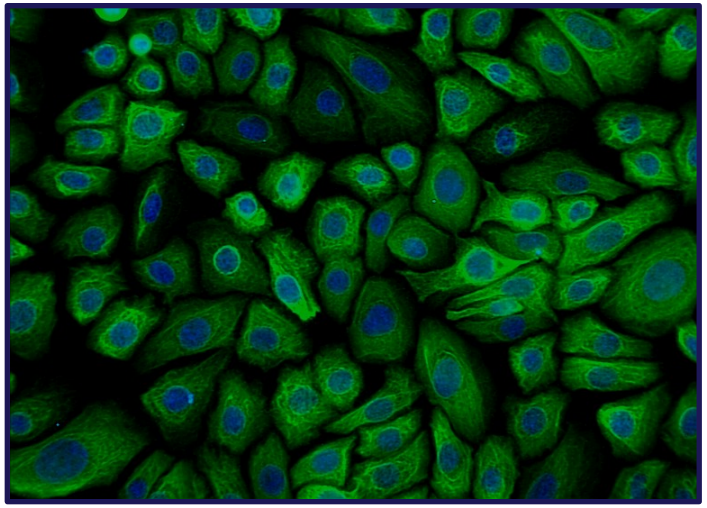
Thank you!

Register for the next installment in the ATCC "*Excellence in Research*" webinar series on February 27, 2014 at www.atcc.org/webinars.

Dr. Yvonne Reid will discuss the current technologies used to authenticate and characterize animal cell lines.



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

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