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ATCC CONNECTION™

In This Issue

What is in your Vial? The Requirement for Polyphasic Microbial Identification and Strain Characterization of *Escherichia coli (E. coli)* ATCC® 8739™

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Molecular Epidemiology Inc.

In the pharmaceutical, personal care, and food industries, quality control testing is used to monitor and address potential microbial contamination of products, processes and environments. Microbial strains with confirmed identity, viability and purity—produced by meticulous laboratory procedures that minimize subculturing—are important components of quality control testing programs. The testing procedures and the associated use of reference QC organisms are often conducted under United States, European, and Japanese compendia guidelines.

For the pharmaceutical industry, recent regulatory guidelines issued to address current good manufacturing practice (cGMP)[1] have stressed a need for genetic-based techniques for the identification of microorganisms. These techniques have been proposed because of the advantages they can bring to understanding and investigating potential physical and temporal sources of microbial contamination recovered in the course of pharmaceutical manufacturing. In response to industry demands for rapid microbiological testing, instrumentation and associated databases have been developed to allow for the standardized testing of both phenotypic and genotypic traits across a wide array of microorganisms.

A polyphasic approach to identification and strain characterization provides a more definitive confirmation and avoids the pitfalls of misidentification resulting from the limitations of various commercial phenotypic and genotypic microbial ID systems and their associated databases. In the current study, Molecular Epidemiology Inc. (MEI) examined a polyphasic identification approach which combined genetic-based microbial ID (16S rRNA sequencing) with a broad spectrum of phenotypic and biochemical analysis to accurately identify a very common microorganism used in QC compendial testing, *Escherichia coli* ATCC® 8739™. This species also forms the platform for both industrial fermentation bioprocesses in the pharmaceutical industry and can represent an environmental contaminant in various production processes (e.g. food) with a potential for pathogenicity. The current study was further complemented by DNA fingerprinting using pulsed field gel electrophoresis (PFGE) with three restriction

continues on page 4



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CRM-11437™	*Clostridium sporogenes*	L.S. McClung 2006
CRM-8739™	*Escherichia coli*	Crooks
CRM-11229™	*Escherichia coli*	AMC 198
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New Viral Gene Vector Reference Material, Plasmid and Cell Line Now Available

ATCC® VR-1616™ Recombinant Adeno-Associated Virus, Type 2, Reference Material (rAAV2-RSS); ATCC® MBA-331™ pTR-UF-11 Plasmid in *E. coli*; and ATCC® CRL-2972™ HeLa RC32 Cell Line

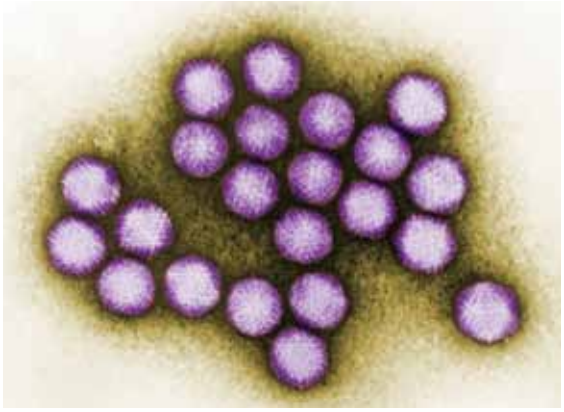
Richard O. Snyder, PhD, Department of Molecular Genetics and Microbiology, University of Florida
Keith L. Carson, *BioProcessing Journal*

Characterization work has been completed for a new viral gene vector reference material. Using helper virus-free transient transfection, an adeno-associated virus type 2 with a green fluorescent protein (AAV2-GFP) viral vector was derived from the vector plasmid, pTR-UF-11. ATCC® VR-1616™ is intended for use in calibrating internal reference materials and assays for AAV viral gene products so that data from different vectors will be more comparable.

The reference material, the plasmid, and the cell line are now available; ATCC® VR-1616™, MBA-331™, and CRL-2972™ respectively. Each 0.5 mL vial of the reference material contains 3.28 x 10^{10} vg/mL. The HeLa RC32 cell line is a stable packaging cell line expressing the rep and cap genes for recombinant adeno-associated virus type 2 (rAAV-2) assembly. The adenovirus type 5 helper virus (ATCC® VR-1516™), used to conduct AAV2 RSM infectious titering, is also available.

With an NIH grant as well as donations from industry and numerous academic organizations, recombinant adeno-associated virus, type 2, reference material (rAAV2-RSS) was produced and purified by a group led by Richard Snyder, PhD, at the Vector Core of the University of Florida's Powell Gene Therapy Center. Characterization was performed by 16 laboratories worldwide.

rAAV vectors are known to be efficient vehicles for gene transfer in animal models. Primarily, this feature results from the long-term gene expression with little or no associated toxicity that is observed after it is administered to a variety of tissues. Clinical trials demonstrate a very good overall safety profile but problems persist due to the lack of any systematic method for normalizing doses administered to animals and humans. To date, most of the published work involves AAV serotype 2 vectors but vector systems based on other AAV serotypes continue to be developed.



Adenovirus image courtesy of Dr. G. William Gary, Jr., CDC

Administered doses are usually based on titer but the defective nature of AAV makes its infectious units difficult to measure. Titering methods based on vector genomes quantified with hybridization, real-time PCR, or spectrophotometry are more reliable but give no information as to the infectivity of the vector. Determining infectious titer is critical, however; the ratio of infectious virions to vector genome-containing virions is needed to determine the dose, potency, and potential effectiveness of the vector preparation.

To date, our knowledge of potential long-term toxicities has been based on the study of insertional mutagenesis and germ-line transmission. It has been recognized that adequate toxicology studies would require animal quantities and other resources that are beyond those available in academic centers and small enterprises involved in the treatment of rare genetic diseases. In May of 1999 at a joint FDA/NIH workshop, members of the rAAV gene therapy community from academia, industry, and the federal government discussed the value of sharing a body of data to address vector-related safety issues. It was generally accepted that sharing and comparing preclinical and clinical data developed with different vector-transgene combinations would be quite valuable for determining vector dose, strength, and potency in terms of equivalent titer units. As a result, it was agreed that a highly characterized rAAV reference material would be needed to facilitate these comparisons and allow researchers to normalize their titer values.

Ordering Information

ATCC® No.	Description
VR-1616™	Recombinant adeno-associated virus 2 reference standard stock (rAAV2-RSS)
MBA-331™	pTR-UF-11 plasmid in *E. coli*
CRL-2972™	HeLa RC32 cell line
VR-1516™	Adenovirus type 5 reference material

Strain Characterization, continued from page 1

enzymes to provide a definitive characterization and differentiation of *E. coli* ATCC 8739 relative to several phylogenetically related organisms associated with the family *Enterobacteriaceae*.

The presented results illustrate the key benefits of polyphasic ID and the limitations of genetic and/or phenotypic (rapid) microbial ID methods when each of these is used individually. Incorrect, incomplete or inadequate identification and characterization of strains of *E. coli* cannot exclude potential pathogenic forms such as serotypes of O157, Enterohemorrhagic strains (EHEC) as well as *E .coli* subtypes ETEC, EPEC, EIEC.

The following organisms: *E. coli* ATCC® 8739™, *Shigella sonnei* ATCC® 25931™, *Klebsiella pneumoniae* ATCC® 10031™, and *Klebsiella oxytoca* ATCC® 43863™ were obtained by MEI from ATCC as lyophilized cultures, reconstituted and subcultured as recommended for isolated colonies. The study was further supplemented with ATCC strains *E. coli* ATCC® 25922™ and *E. coli* O157:H7 ATCC® 35150™ from MEI's QC collection, as well as two additional *E. coli* strains from laboratory and environmental sources. Representative cultures of each microorganism were subjected to 16S rRNA sequencing, colonial, morphological, biochemical (Vitek® GNI, bioMérieux), and PFGE analysis (using *Xba*I, *Bln*I, *Spe*I).

Figure 1 shows representative results of 16S rRNA sequencing of *E. coli* (Figure 1a) versus results from a polyphasic analysis (Figure 1b) of the same isolate. Total genomic DNA was isolated using standard methods. A fragment of the 16S SSU rRNA gene of approximately 500bp was amplified using universal primers in PCR and purified amplicons were subjected to cycle sequencing reaction. Purified labeled DNA fragments (product of cycle sequencing reaction) were run on an automated MegaBACE™ 1000 (GE Healthcare) and the resulting sequence was analyzed by comparing (BLAST followed by ClustalW) to a validated reference sequence library (MEI) containing both type and non-type strains. The resulting genetic distance values are represented as percent differences between the query and subject sequences. The data demonstrate that with genetic ID alone (Figure 1a), identification is possible only to the 'family' level due to insufficient genetic separation. Figure 1b shows that with a polyphasic approach species level identification to *E. coli* is possible (combining phenotypic data). The 16S sequencing data (MEI's comparison of published sequences) further indicate that certain species of *Shigella* (e.g., *S. sonnei*, *S. flexneri*, *S. boydii)* and *Escherichia* (*E. coli, E. fergusonii)* are practically indistinguishable from each other (Table 1) and raise the question of over reliance on solely genetic-based ID methods (16S rRNA) with such critical microorganisms.

Table 1. Genetic Similarity Table. Based on 16S rRNA gene sequence data (portion of gene analyzed).

	Klebsiella oxytoca	*Klebsiella pneumoniae*	*Enterobacter cloacae*	*Escherichia coli*	*Shigella dysenteriae*	*Shigella sonnei*
Klebsiella oxytoca		98	99	99	96	96
Klebsiella pneumoniae	98		97	98	95	95
Enterobacter cloacae	99	97		99	96	96
Escherichia coli	99	98	99		100	100
Shigella dysenteriae	96	95	96	100		99
Shigella sonnei	96	95	96	100	99	

Table 2 presents the phenotypic characteristics and biochemical reactions presented by the microorganisms in this study. The Vitek GNI bio-number generates a unique representation of the combination of biochemical reactions. It is a simplistic but uniform way of comparing certain phenotypic characters and is representative of a rapid phenotypic microbial method.

1a.

Genetic ID
Comparisons to genetically similar microorganisms

Genetic Distance	Genus	Species
0.0000	*Shigella*	*sonnei*
0.0000	*Shigella*	*flexneri*
0.0000	*Escherichia*	*coli*
0.0000	*Shigella*	*boydii*
0.0000	*Escherichia*	sp.
0.0007	*Enterobacter*	*hormaechei*
0.0019	*Shigella*	*dysenteriae*
0.0022	*Enterobacter*	sp.
0.0031	*Cronobacter*	*sakazakii*
0.0081	*Citrobacter*	*freundii*
0.0090	*Enterobacter*	*dissolvens*
0.0100	*Klebsiella*	*oxytoca*

Microbial ID Conclusion	Family: *Enterobacteriaceae*

1b.

Test	Test Results
Basic ID	Bacterium
Gram Stain Reaction	Negative
Microscopic	Rods
Oxidase	Negative
Catalase	Positive
Indologenesis	Positive
Acid from Xylose	Positive
Acid from Sucrose	Negative
Acid from Lactose	Positive
Acid from Sorbitol	Positive
Lysine decarboxylase	Positive
Malonate Utilization	Negative
Acid from Adonitol	Negative
Citrate Utilization	Negative
Acid from Raffinose	Negative

Genetic ID
Comparisons to genetically similar microorganisms

Genetic Distance	Genus	Species
0.0000	*Shigella*	*sonnei*
0.0000	*Shigella*	*flexneri*
0.0000	*Escherichia*	*coli*
0.0000	*Shigella*	*boydii*
0.0000	*Escherichia*	sp.
0.0007	*Enterobacter*	*hormaechei*
0.0019	*Shigella*	*dysenteriae*

Microbial ID Conclusion	*Escherichia coli*

Figure 1. Genetic and Polyphasic Identification of ATCC® 8739™ *E. coli* A: Genetic identification. B: Polyphasic identification.

The first three *E. coli* strains (ATCC 8739) are indistinguishable from each other based on the Vitek bio-number (and by three restriction enzyme PFGE analysis; Figure 2a). They are also indistinguishable from the environmental isolate listed at the bottom of Table 2, although this strain presents what is described subjectively as a "weak" reaction on

Table 2. Phenotypic Data.

Sample Description		Cultural and Phenotypic Data			
Sample Name	**Sample Source**	**Colony Morphology**	**MaC (Lactose)**	**Indol**	**Vitek GNI Number**
Escherichia coli ATCC® 8739™	ATCC	Flat	+	+	GNI 6204724631
Escherichia coli ATCC 8739	MEI Frozen Stock	Flat	+	+	GNI 6204724631
Escherichia coli ATCC 8739	Customer QC Strain	Flat	+	+	GNI 6204724631
Klebsiella pneumoniae ATCC® 10031™	ATCC	Raised (inhibited)	+	-	GNI 6634773631
Klebsiella oxytoca ATCC® 43863™	ATCC	Raised	+	+	GNI 6674773631
Shigella sonnei ATCC® 25931™	ATCC	Flat	-	-	GNI 6003006632
Escherichia coli ATCC® 25922™	MEI Frozen Stock from ATCC	Raised β-Hemolytic	+	+	GNI 6004720633
Escherichia coli O157:H7 ATCC® 35150™	MEI Frozen Stock from ATCC	Raised	+	+	GNI 6004754633
Escherichia coli O157:H7	MEI Lab Isolate	Raised	+	+	GNI 6004754633
Escherichia coli	Environmental Isolate	Flat	Weak	+	GNI 6004724631

MacConkey agar (it is however clearly different based on PFGE analysis; Figure 2b). The other ATCC strain of *E. coli* (ATCC 25922) is markedly different in hemolytic character as well as some biochemical reactions as evidenced by the Vitek bio-number as well as by PFGE patterns. The two strains of *E. coli* O157:H7 are indistinguishable from each other (by comparison of their bio-number). However, they are clearly differentiated from the other *E. coli* isolates based on colonial morphology and specific biochemical reactions (sorbitol and sucrose) as well as by additional PFGE analysis (data not shown).

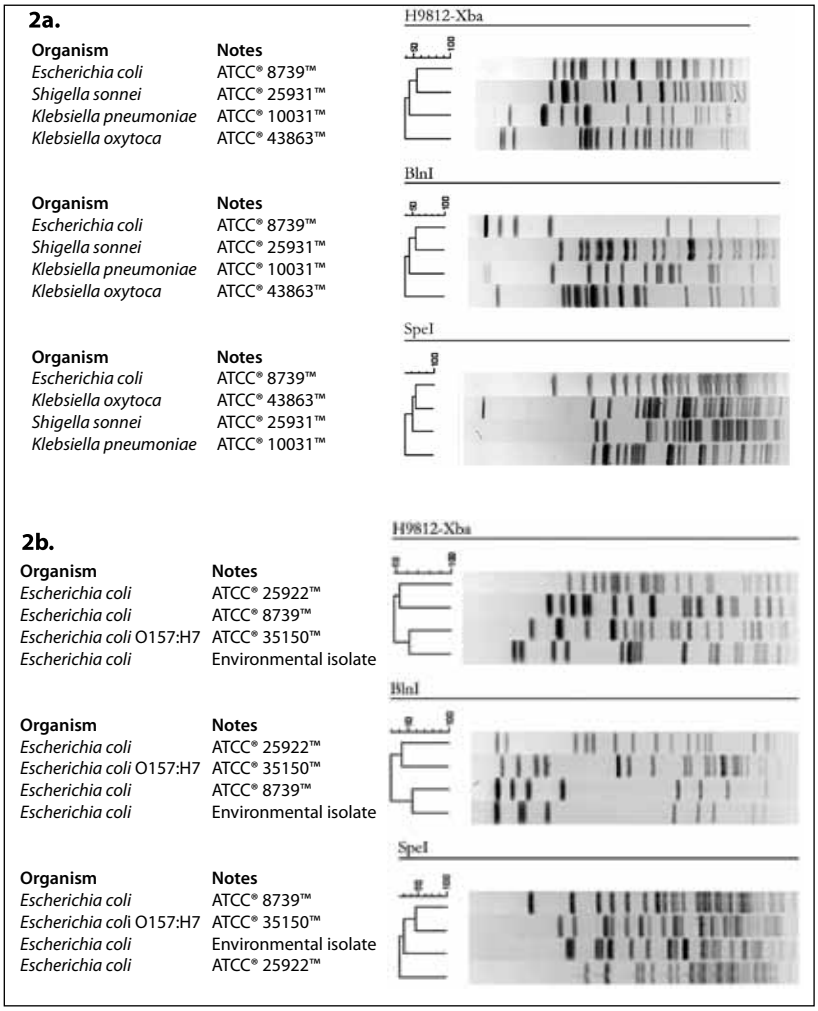


Figure 2. Strain Differentiation by PFGE. A: Comparison with three enzymes for all four ATCC cultures. B: Stark pattern variation between ATCC® 8739™, other ATCC *E. coli* and environmental *E. coli*.

Klebsiella pneumoniae (ATCC 10031) and *K. oxytoca* (ATCC 43863) were easily differentiated from each other and from the *E. coli* (ATCC 8739 and 25922) and *S. sonnei* (ATCC 25931) strains by colonial morphologies as well as the by Vitek and supplemental biochemical reactions (indologenesis). The *S. sonnei* strain was easily differentiated from the O157:H7 strains of *E. coli* as well as the other isolates by the combination of colonial morphologies, Vitek bio-numbers, and supplemental tests.

It is evident that any attempt to identify *E. coli* exclusively by DNA sequencing of the 16S rRNA target would result in an extremely misleading conclusion of not only the genus *(Escherichia, Salmonella, Shigella* or *Enterobacter)* but raises the alarm regarding potential pathogenic strains of *E. coli*. In addition, phenotypic data alone appear insufficient to discriminate between environmental and QC strains. The present study, therefore, demonstrates the critical need for a polyphasic microbial identification approach coupled with DNA fingerprinting for the confident identification and differentiation of *E. coli* ATCC 8739 relative to three phylogenetically related microorganisms as well as potential environmental strains presenting as the same species. This is a critical step in establishing the authenticity and fidelity of commercially acquired QC microorganisms for their subsequent usage in compendial microbiological testing. The benefits of such well-characterized and traceable products are evident when specific contamination isolates are recovered and compared to the established QC strain.

Reference

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New *Clostridium difficile* strains represent the most common North American pulsed-field (NAP) types

Clostridium difficile is an anaerobic, spore-forming bacillus that produces two enterotoxins, toxin A and toxin B. *C. difficile* is the most frequent cause of nosocomial diarrhea worldwide. *C. difficile* infection (CDI) is not a new illness but it is becoming more frequent and difficult to treat. The rate of *C. difficile* infection among hospital patients doubled from 2001 to 2005, according to an April 2008 report from the Centers for Disease Control and Prevention (CDC). *C. difficile* can also cause diarrhea among patients in community settings.

Six *C. difficile* isolates representing the most common North American pulsed-field types (NAP) are now available from ATCC. The strains were characterized by the CDC using pulsed-field gel electrophoresis (PFGE), PCR evaluation of *tcdC*, PCR ribotyping and toxinotyping. Strains were typed by Sma1 PFGE and analyzed with BioNumerics® v5.01 (Applied Maths) as described in Kilgore.[1] Toxinotyping was based on restriction fragment length polymorphisms in the A3 and B1 fragments of the pathogenicity locus as described by Rupnik.[2] Restriction endonuclease analysis (REA) was conducted by Dr. Dale Gerding's laboratory (Hines VA Hospital, Hines, IL).

TOX 0 strains have been reported to be a common cause of healthcare associated CDI. TOX V strains are a frequent cause of CDI in food-producing animals in Europe and the United States.[3] North American PFGE type 1 (NAP-1), toxinotype III, PCR ribotype 027, restriction endonuclease analysis type BI, is often referred to as the epidemic strain.[3] It has been implicated in numerous outbreaks across North America and has been associated with increased severity of disease. Based on data from the CDC, the virulent strain NAP1/027 has been reported in most states throughout the U.S. and in several countries in Europe.

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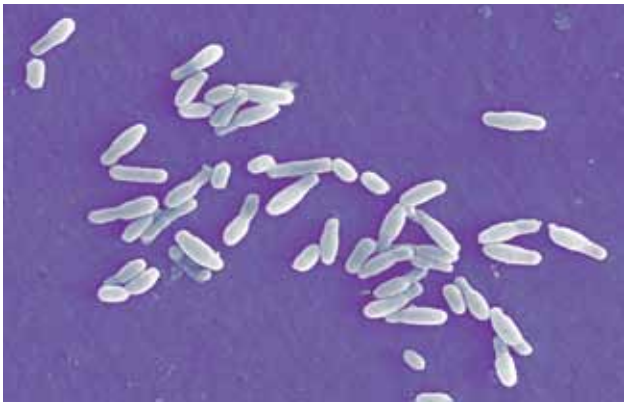

Image of Clostridium difficile courtesy of Janice Carr, CDC

Ordering Information

Clostridium difficile strains representing the most common North American pulsed-field (NAP) types.

ATCC® No.	Description	PFGE Type	REA Type	Toxinotype	PCR Ribotype	Binary Toxin Result	Presence of Genes for Toxins A&B	*tcdC* Deletion	State
BAA-1870™	*Clostridium difficile*	NAP1	BI 8	III	027	positive	positive	18 bp	Maine
BAA-1871™	*Clostridium difficile*	NAP2	J 16	0	001	negative	positive	0 bp	NJ
BAA-1872™	*Clostridium difficile*	NAP4	Y Group	0	154	negative	positive	0 bp	Maine
BAA-1873™	*Clostridium difficile*	NAP5	K3	0	Unknown type	negative	positive	0 bp	NY
BAA-1874™	*Clostridium difficile*	NAP6	G 1	0	002	negative	positive	0 bp	Oregon
BAA-1875™	*Clostridium difficile*	NAP7	BK 16	V		positive	positive	39 bp	GA

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Available to Researchers within the 50 United States*

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Background

The Gram-positive, spore-forming, rod-shaped, ubiquitous *Clostridium perfringens* (*C. perfringens*) is an important cause of human and livestock disease. There are five subtypes of *C. perfringens (A-E),* and the bacteria's virulence is derived from its prolific generation of more than 13 protein toxins. Of these, PFO, CPE, and CPB all play a substantial role in the pathogenesis of *C. perfringens*-mediated disease.

Perfringolysin O (PFO), ATCC® BTX-100

Perfringolysin O is a toxin produced by *C. perfringens*. PFO from ATCC is a recombinant protein expressed in and isolated from *E. coli* and contains a N-terminal histidine tag. It is supplied as a frozen solution of 25 µg of PFO suspended in 50 µL of 50 mM HEPES (pH 8.0), 10% glycerol, 150 mM NaCl and 5 mM DTT. The preparation is qualified for hemolytic assays and Western blots.

Beta Toxin (CPB), ATCC® BTX-110

Beta Toxin is produced by type B and type C strains of *C. perfringens.* CPB from ATCC is a native protein purified from culture supernatants of *C. perfringens* type C, strain CN3685 and is qualified for Western blots and cytotoxicity assays. ATCC Beta Toxin is provided as a solution of 20 µg of beta toxin suspended in 50 µL of 300 mM Tris-HCl (pH 7.5) and 100 mM NaCl.

Enterotoxin (CPE), ATCC® BTX-120

C. perfringens enterotoxin is a native protein isolated from culture supernatants of *C. perfringens*, Type A strain B. Hobbs 3653/50. Each vial of ATCC CPE contains approximately 10 µg of lyophilized CPE ready for reconstitution. The preparation is qualified for Western blots and cytotoxicity assays.

For more information, please visit **www.atcc.org** and click on Cultures and Products/Microbiology/Toxins from the dropdown menus.

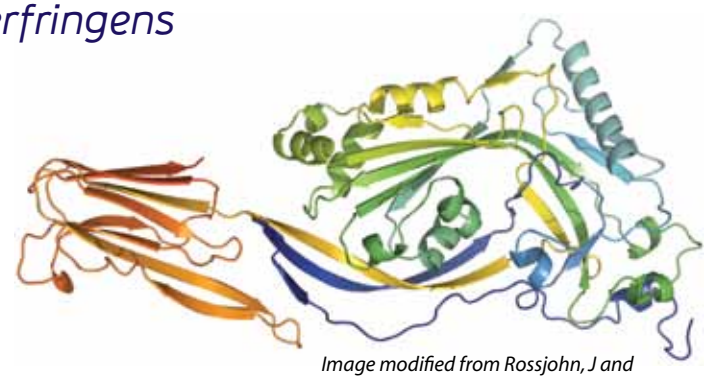

Image modified from Rossjohn, J and Parker, MW. Perfringolysin O. PBD ID : 1PFO

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BTX-110	Beta Toxin (CPB) native protein isolated from *C. perfringens*	20 µg
BTX-120	Enterotoxin (CPE) native protein isolated from *C. perfringens*	10 µg

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HTB-37™	Caco-2, human colon epithelial cell line	vial
CCL-240™	HL-60, human periferal blood myeoblastic cell line	vial
30-2003	Eagle's Minimum Essential Media (EMEM)	500 mL
30-2020	Fetal Bovine Serum	500 mL
30-2021	Fetal Bovine Serum	100 mL



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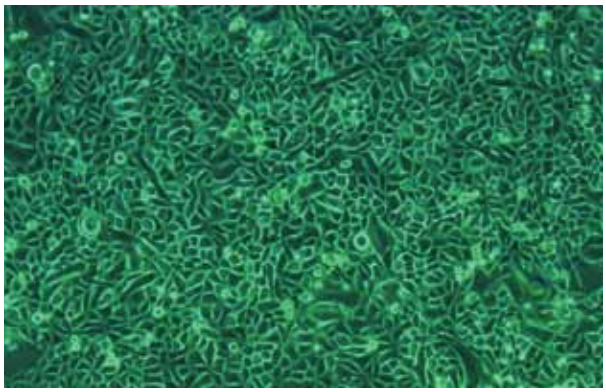
Designed to provide optimal support for cells derived from normal prostate tissues, Prostate Epithelial Cell Basal Medium is a sterile, phenol red-free, liquid tissue culture medium intended for use as one component in a complete ATCC Primary Cell Solutions system. Used in combination with the Prostate Epithelial Cell Growth Kit, the complete medium sustains the proliferation and plating efficiency of cells derived from normal human prostate under serum-free conditions.

Normal primary prostate epithelial cells.

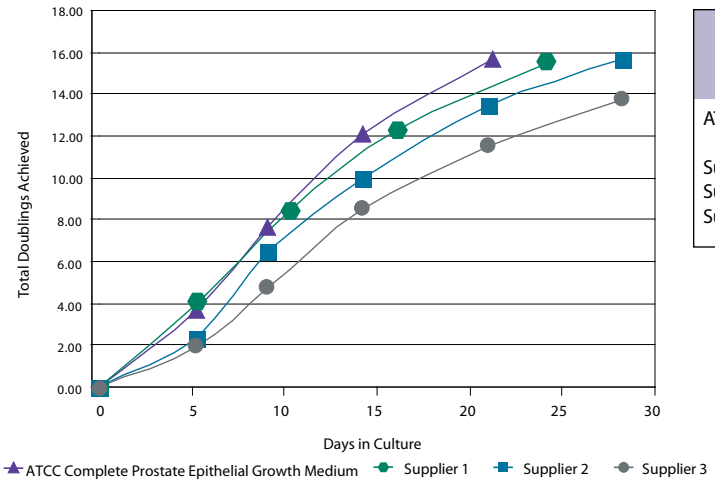


For more information, please visit **www.atcc.org** and click on Cultures and Products/Tissue Biology/Primary Cells/Prostate Epithelial Cell Solutions from the drop down menu.

Supporting prostate cancer research

Prostate cancer is the second most common cancer in American men. According to the American Cancer Society, about one man in six will be diagnosed with prostate cancer during his lifetime. ATCC LNCaP, PC-3, DU 145, and VCaP cell lines have been cited in thousands of publications describing the mechanisms of prostate cancer. To support this valuable research, ATCC will donate $35 to the Prostate Cancer Foundation (**www.prostatecancerfoundation.org**) for every vial of primary prostate epithelial cells sold.

Growth Rate Comparison*: Prostate Epithelial Cells Cultured in Different Brands of Serum-Free Medium

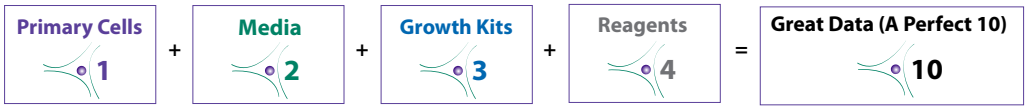


Medium	Number of Doublings	Days in Culture	Average Doubling Time (hrs)
ATCC Complete Prostate Epithelial Growth Medium	15.7	21	32.9
Supplier 1	15.5	24	38.8
Supplier 2	15.6	28	47.7
Supplier 3	13.7	28	52.5

*This experiment was conducted while various lots of ATCC® Primary Cell Solutions™ prostate epithelial cells were undergoing QC testing. When the QC-specification for population doublings was achieved (≥15) the experiment was concluded; cells grown in Supplier 3 medium did not achieve 15 population doublings.

Ordering Information

To achieve the best possible results, we suggest that you order a complete system for each cell type:



PUTTING ALL THE PIECES TOGETHER ADDS UP TO YOUR SUCCESS.

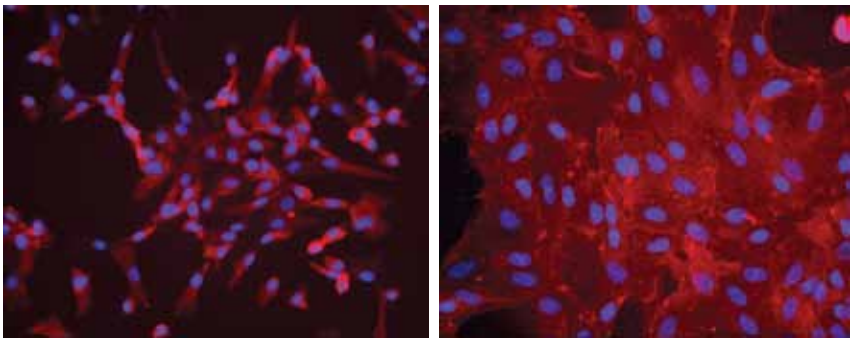
	Product Name	Components	Catalog No.
PROSTATE EPITHELIAL CELLS-NEW!			
1	Primary Prostate Epithelial Cells; Normal, Human – **New!**	≥ 5 x 10^5 viable cells	PCS-440-010
2	Prostate Epithelial Cell Basal Medium – **New!**	485 mL	PCS-440-030
3	Prostate Epithelial Cell Growth Kit – **New!**	1 kit	PCS-440-040
AIRWAY EPITHELIAL CELLS			
1	Primary Bronchial/Tracheal Epithelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-300-010
1	Primary Small Airway Epithelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-301-010
2	Airway Epithelial Cell Basal Medium	485 mL	PCS-300-030
3	Bronchial Epithelial Cell Growth Kit	1 kit	PCS-300-040
3	Small Airway Epithelial Cell Growth Kit	1 kit	PCS-301-040
ENDOTHELIAL CELLS			
1	Primary Umbilical Vein Endothelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-100-010
1	Primary Aortic Endothelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-100-011
2	Vascular Cell Basal Medium	475 mL	PCS-100-030
3	Endothelial Cell Growth Kit–BBE	1 kit	PCS-100-040
3	Endothelial Cell Growth Kit–VEGF	1 kit	PCS-100-041
SMOOTH MUSCLE CELLS			
1	Primary Aortic Smooth Muscle Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-100-012
2	Vascular Cell Basal Medium	475 mL	PCS-100-030
3	Vascular Smooth Muscle Cell Growth Kit	1 kit	PCS-100-042
KERATINOCYTES			
1	Primary Epidermal Keratinocytes; Normal, Human, Neonatal Foreskin	≥ 5 x 10^5 viable cells	PCS-200-010
1	Primary Epidermal Keratinocytes; Normal, Human, Adult	≥ 5 x 10^5 viable cells	PCS-200-011
2	Dermal Cell Basal Medium	485 mL	PCS-200-030
3	Keratinocyte Growth Kit	1 kit	PCS-200-040
MELANOCYTES			
1	Primary Epidermal Melanocytes; Normal, Human, Neonatal	≥ 5 x 10^5 viable cells	PCS-200-012
2	Dermal Cell Basal Medium	485 mL	PCS-200-030
3	Melanocyte Growth Kit	1 kit	PCS-200-041
FIBROBLASTS			
1	Dermal Fibroblasts; Normal, Human, Neonatal	≥ 5 x 10^5 viable cells	PCS-201-010
1	Dermal Fibroblasts; Normal, Human, Neonatal, Mitomycin C treated	≥ 3 x 10^6 viable cells	PCS-201-011
1	Dermal Fibroblasts; Normal, Human, Adult	≥ 5 x 10^5 viable cells	PCS-201-012
2	Fibroblast Basal Medium	480 mL	PCS-201-030
3	Fibroblast Growth Kit–Serum-free	1 kit	PCS-201-040
3	Fibroblast Growth Kit–Low serum	1 kit	PCS-201-041
RENAL EPITHELIAL CELLS			
1	Primary Renal Proximal Tubule Epithelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-400-010
1	Primary Renal Cortical Epithelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-400-011
1	Primary Renal Mixed Epithelial Cells; Normal, Human	≥ 5 x 10^5 viable cells	PCS-400-012
2	Renal Epithelial Cell Basal Medium	485 mL	PCS-400-030
3	Renal Epithelial Cell Growth Kit	1 kit	PCS-400-040
REAGENTS			
4	Phenol Red	1 mL	PCS-999-001
4	Penicillin-Streptomycin-Amphotericin B Solution	1 mL	PCS-999-002
4	Trypsin-EDTA for Primary Cells	100 mL	PCS-999-003
4	Trypsin Neutralizing Solution	100 mL	PCS-999-004
4	Gentamicin-Amphotericin B Solution	1 mL	PCS-999-025
4	0.1% Gelatin Solution	100 mL	PCS-999-027
4	Dulbecco's Phosphate Buffered Saline (D-PBS)	500 mL	ATCC® 30-2200

Isolation and Characterization of MDCK Clones used for

Yvonne A. Reid, PhD, Yvonne Pyla and Edward Cedrone, Cell Biology Program, ATCC

The MDCK cells have been used by industrial and academic scientists in a wide variety of cell-based assays. Key applications include their use as substrate for the growth of influenza viruses and as models for epsilon toxin studies.[1,2] However, when MDCK cells are grown on polystyrene culture flasks they display both genotypic and phenotypic heterogeneity.[3] We have confirmed these results by showing that the cytogenetic analysis of the MDCK parent cell line has an average bimodal karyotype ranging from 76 to 81 and 84 to 90 (Figure 1a) and a heterogeneous morphology consistent with both fibroblast-like and epithelial-like shapes (Figure 1b, 1c). Immunocytochemistry staining with pan-cytokeratin antibodies established the cells to be epithelial in origin (Figures 2a, 2b).

Figure 2. Staining of two MDCK clones ATCC® CRL-2935™ (2a) and CRL-2936™ (2b) with antibody to pan Cytokeratin (CK1, 4, 5, 6, 8, 10, 13, 18, 19). Cells were harvested, washed and fixed with methanol and blocked with 5% goat serum. Unstained and isotype antibody were used as controls.



2a. **2b.**

The MDCK cell line was derived from the distal collecting tubule of a kidney of an adult female dog by S.H. Madin and N.B. Darby, Naval Biological Laboratory, University of California. The MDCK cell line was submitted to ATCC in 1964 at passage 49 and was assigned catalog number ATCC® CCL-34™.

We explored the diverse morphology of the MDCK cells by cloning a single population of cells that were highly susceptible to influenza virus infection, as well as populations that were very sensitive to epsilon toxin.

Parental cell line MDCK (ATCC CCL-34) was propagated in complete growth containing EMEM + 10% FBS. Cells were cloned at 0.5 cells/well in a 96-well plate by limited dilution. Wells with single colonies were expanded and cryopreserved for later studies. Of the 52 clones identified, several clones with different epithelial-like morphologies were selected and subsequently six clones (MDCK clone 4, MDCK clone 7, MDCK clone 33, MDCK clone 42, ATCC® CRL-2936™ and ATCC® CRL-2935™) were identified as substrates for the expansion of various influenza isolates.[4] These MDCK clones and the MDCK parental cells were further authenticated to confirm their species of origin by cytochrome oxidase subunit I (COI) analysis[5] and by G-banded karyotyping. Further characterization to determine the optimal culture conditions (growth curves and doubling time); phenotypic traits (expression of tight junctions, receptors for human and avian influenza[4]); and their tumorigenicity ability in immuno-compromised mice is summarized in Table 1.

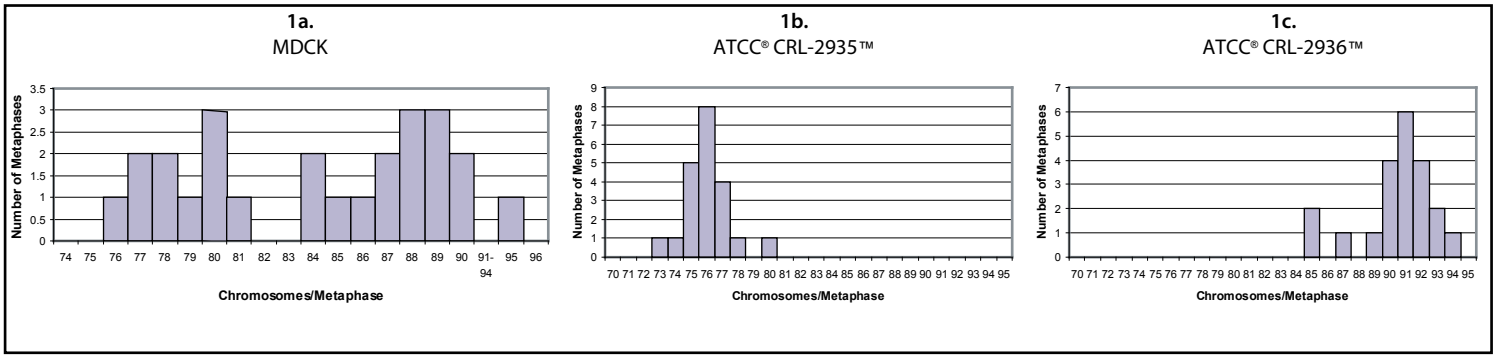


Figure 1. Bimodal distribution of Giemsa banding karyotype of MDCK parental MDCK cell line (mixed morphology) and two clones ATCC CRL-2935 (fibroblast-like) and ATCC CRL-2936 (epithelial-like). Bimodal number: MDCK = 76–81 and 84-90; CRL-2935 = 73–80; CRL-2936 = 85–94.

Influenza Virus Production and Epsilon Toxin Studies

Table 1. Authentication and Characterization of MDCK and MDCK Clones.
*Tumor regressed in most animals after 3 days.

Assays	MDCK Parental CCL-34	CRL-2936 MDCK.2	MDCK Clone 4	MDCK Clone 7	MDCK Clone 33	MDCK Clone 42
Tight Junction ZO-1	Positive	Positive	Positive	Positive	Positive	Positive
Tight Junction E-Cadherin	Positive	Positive	Positive	Positive	Positive	Positive
Growth Curve (PDT)	14.0 hrs	22.0 hrs	20.0 hrs	15.5 hrs	17.0 hrs	22.0 hrs
Sialic Receptor Human	Positive	Positive	Positive	Positive	Positive	Positive
Sialic Receptor Avian	Positive	Positive	Positive	Positive	Positive	Positive
Speciation (COI; barcode)	*Canis familiaris*	*Canis familiaris*	*Canis familiaris*	*Canis familiaris*	*Canis familiaris*	*Canis familiaris*
Tumorgenicity* (1×10^7 cells/ inoculum in each of 10 nu nu mice)	Highly tumorigenic	Weakly tumorigenic	Weakly tumorigenic	Weakly tumorigenic	Weakly tumorigenic	Weakly tumorigenic

In an independent cloning procedure, clone ATCC CRL-2936 (epithelial-like) was identified for its sensitivity (CD_{50} = 0.03 nM) and ATCC clone CRL-2935 (fibroblast-like) for its insensitivity (CD_{50} = 70.2 nM) to epsilon toxin.

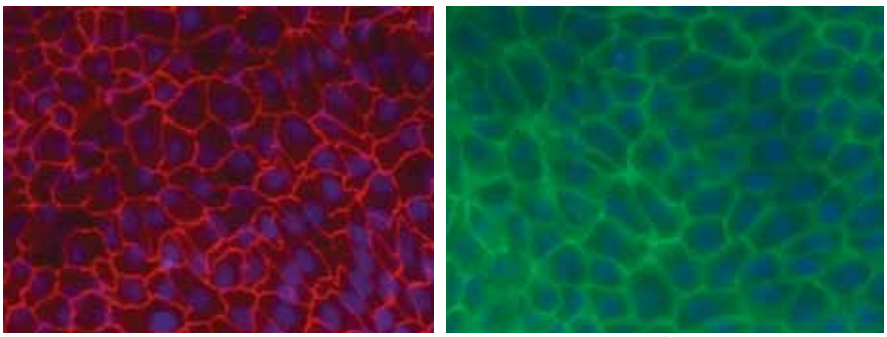
MDCK and MDCK clones are epithelium in origin

ZO1 and E-Cadherin are classical epithelial markers that play an essential role in the preservation of the epithelial tight junction complex and as such are important molecules in maintaining epithelial integrity. Epithelial integrity can be damaged in some diseases, so it was important to verify their presence. The MDCK cells and clones were tested by flow cytometry and immunocytochemistry (Figure 3) to express these markers. All MDCK and clones stained positive for the epithelial markers ZO1, E-Cadherin and for pan-cytokeratin (CK1, 4, 5, 6, 8, 10, 13, 18, 19).

Clones ATCC CRL-2935 and CRL-2936 are now available to the scientific community. For more information, please search for these catalog numbers at **www.atcc.org**.

References

1. Petit L, et al. *Clostridium perfringens* epsilon-toxin acts on MDCK cells by forming a large membrane complex. Bacteriol. 179(20):6480–6487, 1997.
2. Szymczakiewicz-Multanowska A, et al. Safety and Immunogenicity of a Novel Influenza Subunit Vaccine Produced in Mammalian Cell Culture. J. Infectious Diseases. 200:841–848, 2009.
3. Arthur JM. The MDCK cell line is made up of populations of cells with diverse resistive and transport properties. Tissue Cell. 32(5):446–450, 2000.
4. Cedrone E, et al. Tissue-Culture Adapted Influenza Virus Strains. ATCC Connection 29(2):4, 2009.
5. Cooper JK, et al. Speciation identification in cell culture: a two-pronged molecular approach. In Vitro Cell Dev Biol Anim. 43(10):344, 2007.
6. Nicholls, JM, et al. Sialic acid receptor detection in the human respiratory tract: evidence for widespread distribution of potential binding sites for human and avian influenza viruses. Respiratory Research 8:73, 2007.



3a. **3b.**

Figure 3. MDCK clone 7 stained with antibody to Z01 (3a) and E-Cadhedrin (3b). Cells were seeded on culture slides at 1×10^4 cells/cm^2 and incubated for 2 weeks in 95% air and 5% CO_2 to achieve tight cell contact. Cells were washed and fixed with methanol and stained overnight with either 2.0 µg/mL ZO1 or E-Cadherin antibody. Unstained and isotype antibody were used as controls (not shown).



ATCC® Cell Line Heroes: Gold Standard

Many ATCC cell lines are recognized as "gold standard" research models and have appeared in thousands of peer-reviewed papers. Beginning with this issue, each *Connection* newsletter will feature a set of these gold standard ATCC® Cell Line Heroes.

Myeloid Leukemia Differentiation Cell Set

HL-60, THP-1, U937

A number of human cell lines have been established from patients with acute myeloid leukemia (AML) but three have become the gold standard model systems for leukemia research: HL-60, THP-1, and U937. These three cell lines can be ordered together as a Myeloid Leukemia Differentiation Cell Set. The HL-60, THP-1, and U937 cell lines have been comprehensively characterized and have been cited in thousands of peer-reviewed publications concerning their growth, differentiation, apoptosis, and effector functions.

Each of the cell lines is locked at a specific step of maturation but can be triggered to differentiate to a more mature cell type by specific agents. THP-1 and U937 cells are monoblasts that can be induced to differentiate into macrophage-like cells. HL-60, a promyelocyte, can be induced to differentiate along two separate pathways, becoming either a granulocyte-like or macrophage-like cell, depending on the stimulating agent used. Some agents, such as phorbol 12-myristate 13-acetate (PMA, also known as TPA), can induce monocytic differentiation in all three of the cell lines. Other agents, however, show differential effects on the three cell lines, allowing fine examination of the critical molecular alterations and rogue leukemogenic processes occurring during AML. Inducing differentiation in these cell lines causes detectable losses and nascent formations of numerous biological markers and biochemical activities, such as increased production of reactive oxygen metabolites.

One powerful application of the HL-60, THP-1, and U937 cell lines is their usage for analyzing the effects of multiple potential therapeutic agents on intracellular signaling pathways and secretion of biological response modifiers. It is only by having a better understanding of the biology behind AML that new and better differentiation-based therapies can be developed.

Use these 3 gold standard leukemia model systems in your research and receive superior **ATCC Genuine Cultures®** cell line performance. Order HL-60, THP-1, and U937 together at a discount price using promotional code ML001.

HL-60

ATCC® CCL-240™

The HL-60 cell line was derived from the peripheral blood of a patient with acute promyelocytic leukemia in 1979. The cell line has appeared in more than 9,000 publications. Undifferentiated HL-60 cells stain as promyelocytes, are non-adherent, and constitutively express myeloperoxidase and ASD chloroacetate esterase activities. After treatment with 1% DMSO, the HL-60 cell line changes its morphology from promyelocytic to granulocyte-like cells, begins expression of C3d receptors, and expresses CD11b, Mac-1, and Gr-1 granulocyte differentiation markers. Treatment with TPA induces the cell line to become monocytic in nature with loss of azurophilic granules and the appearance of pseudopodia and a convoluted nucleus. Monocytic differentiation using TPA results in loss of myeloperoxidase activity and the appearance of acid phosphatase activity, along with surface Mo1 (CD11b/CD18) and Mo2 expression.

The most commonly used marker to assess HL-60 differentiation seems to be the appearance of nitroblue tetrazolium (NBT) reduction activity, associated with the formation of reactive oxygen metabolites, which occurs during both the granulocytic and monocytic paths of differentiation.

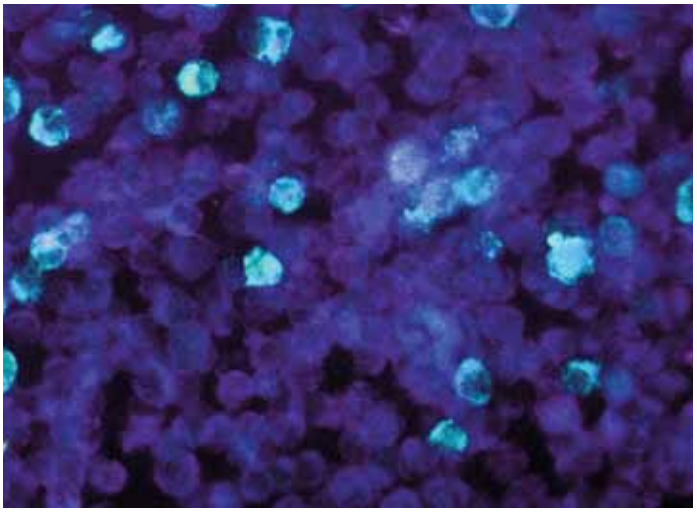
THP-1

ATCC® TIB-202™

The THP-1 cell line was derived from the blood of a boy with acute monocytic leukemia in 1980. The cell line has appeared in

Model Systems for Leukemia Research

Image of leukemia cells courtesy of Dr. Paul M. Feorino, CDC.



more than 4,000 publications. THP-1 has been confirmed as being of monoblast/monocytic origin by: (1) morphology; (2) the presence of alpha-naphthyl butyrate esterase activities that could be inhibited by NaF; (3) lysozyme production; (4) phagocytosis of various particles, and; (5) the ability to restore T-cell responses to Concanavalin A. The cell line has Fc and C3b receptors, but no surface or cytoplasmic immunoglobulins.

Following differentiation to a macrophage-like cell, THP-1 demonstrates cell adherence, cell cycle arrest, NBT reduction activity, and expression of differentiation markers including CD68, CD11b, and matrix metalloproteinase 9 (MMP9). THP-1 has been widely used as a model system to study foam cell formation during atherosclerotic processes.

U937

ATCC® CRL-1593.2™

The U937 cell line was isolated from pleural fluid of a patent with diffuse histiocytic lymphoma in 1976. The cell line has appeared in more than 6,000 publications. U937 has been confirmed as being of monoblast/monocytic origin by: (1) morphology; (2) strong naphthol AS-D acetate esterase activity inhibited by NaF; (3) lysozyme production, and: (4) induction of phagocytosis. The cell line bears few Fc, C3b, and chemotactic peptide receptors.

Following differentiation, the cell line becomes adherent, acquires phagocytic and NBT reduction activities, displays annexin V binding, releases cytokines such as IL-1, and expresses the monocytic differentiation markers CD11b, CD36, and CD14. The U937 cell line is an established model used for HIV research and antibody-dependent cell-mediated cytoxicity (ADCC) effector studies.

Key HL-60, THP-1, U937 Cell Line Reviews

Petrie, K. Differentiation therapy of acute myeloid leukemia: past, present and future. Current Opinion in Hematology 16(2):84-91, 2009.

Ennaciri J., Girard D. Immune system: maturation of myeloid cells. Methods in Molecular Biology 550:195-203, 2009.

Harris P., Ralph P. Human leukemic models of myelomonocytic development: a review of the HL-60 and U937 cell lines. Journal of Leukocyte Biology 37:407-422, 1985.

Koeffler H. Induction of differentiation of human acute myelogenous leukemia cells: therapeutic implications. Blood 62(4):709-721, 1983.

Koeffler H., Golde D. Human myeloid leukemia cell lines: a review. Blood 56(3):344-350, 1980.

See our online catalog at **www.atcc.org** for a full description of each item.

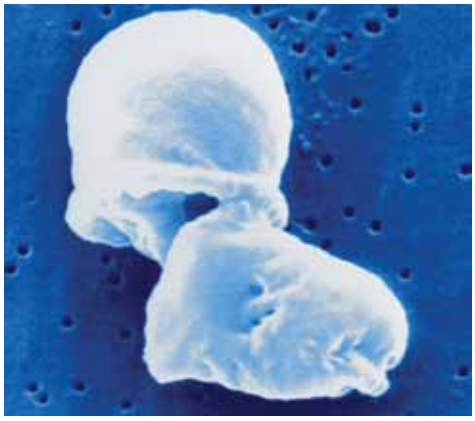


Fully Sequenced Fungal Type Strains Responsible for Dandruff—*Malassezia globosa* and *Malassezia restricta*—are now available

Have you ever had an itchy or flaky scalp, a problem commonly known as dandruff? Scientists at Proctor and Gamble have successfully sequenced the complete genomes of *Malassezia globosa* (ATCC® MYA-4612™) and its close relative, *Malassezia restricta* (ATCC® MYA-4611™). These genomes are the most commonly isolated species from human scalp and are associated with skin disorders, including dandruff. The scientists' goal was to further understand the fungi and how they work and then to gain a better understanding of how to target and rid the scalp of *Malassezia*. Genomic analysis indicates how *Malassezia* adapt to the skin environment and yields important clues to the role they play in human disease. *M. globosa* and *M. restricta* are considered commensal microbes (those causing no harm to their host) but they can be opportunistic pathogens that cause skin disorders such as pityriasis versicolor, psoriasis, atopic dermatitis, seborrheic dermatitis, dandruff, *Malassezia* folliculitis, and tinea versicolor in humans and ear and skin diseases in cats and dogs. Both *M. globosa* and *M. restricta* live on the skin of humans and mammals and are part of the normal cutaneous microflora. They are both present on the dandruff scalp but *M. globosa* is the most likely initiating organism due to its high ability to secrete lipases which are used to aid in its digestion. Both strains require lipids, degrade sebum, free fatty acids from triglycerides, consume specific saturated fatty acids, and leave behind free fatty acids. These free fatty acids can irritate the scalp and lead to dandruff.

ATCC MYA-4612 *Malassezia* globosa, type strain CBS 7966 was isolated from the skin of a patient with Pityriasis versicolor in 1990 in London, United Kingdom. It is the most commonly found strain of *M. globosa* and the sequence has been deposited in the GenBank® database, accession no. AAYY00000000. The *M. globosa* genome is comprised of just nine million base pairs consisting of about 4,285 protein coding genes. It is among the smallest sequenced free-living fungal genome. *M. globosa* lacks the ability to produce fatty acids, making it highly adaptive and niche dependent. It is commonly found on the scalp, back, face, and chest, areas where the highest levels of sebum are produced. *M. globosa* is capable of excreting over 50 different enzymes that help digest and break down materials on the hair and scalp. Studies suggest that it is capable of mating, a key consideration in the organism's ability to change and adapt in the future.

ATCC MYA-4611 *Malassezia restricta* type strain CBS 7877 was isolated in 1990 from the healthy skin of a male in Bristol, United Kingdom and the sequence has been deposited in the GenBank database, accession no. AAXK00000000. There are many similarities in the *M. restricta* and *M. globosa* genomes and only three genes were found in the *M. restricta* genome that were missing in the *M. globosa* genome sequence.



Scanning electron micrograph of Malassezia species courtesy of Janice Carr, CDC

Before both strains were fully sequenced, little was known about the *Malassezia* species at the molecular level. Due to the earlier use of culture-based technologies, scientists incorrectly assumed for years that dandruff and other skin conditions were caused by a different species, *Malassezia furfur*. Genomic analysis offers the promise of defining new treatments for dandruff and seborrheic dermatitis that are targeted at changing the level or activities of *Malassezia* genes. Genomic comparisons provide insights into the mechanisms by which fungi adapt to the mammalian skin environment. The new sequence data was used to assess the phylogenetic position of *Malassezia* and to gain insights into the origin of the genus indicating that they are closely related to plant pathogens. Research studies suggest an ancestral shift in host preference, from plant to animal.

References

Dawson TL, Jr. *Malassezia* globosa and *restricta*: Breakthrough understanding of the etiology and treatment of dandruff and seborrheic dermatitis through whole genome analysis. J. Investig Dermatol *Symp. Proc.* 12**:**15–19, 2007.

Xu J, et al. Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc. Natl. Acad. Sci. USA 104:18730–18735, 2007.

ATCC Names Biological Industries Exclusive Distributor in Israel

On January 4, 2010, ATCC announced a distribution and development partnership with Biological Industries Ltd. to become the exclusive distributor of ATCC products in Israel. The Israel-based organization is now the newest member of the global network of distributors for ATCC.

The agreement, signed on December 1, 2009, will improve access to ATCC products for the Israeli academic, industrial, and quality control testing communities. "It will also streamline logistics for biological materials management and customer services", said Dr. Sabina Glozman, Managing Director, Biological Industries Ltd.

"Israel is a growing center for life sciences intellectual property. Our partnership will provide essential infrastructure for the needs of Israeli academic institutions and commercial biopharma organizations as well as allow us to engage in the joint development of life science reagents of mutual interest," said Dr. Raymond Cypess, CEO & President of ATCC.

"Our relationship with Biological Industries Ltd. is part of our ongoing strategy to form scientific collaborations with companies and research institutions in Israel and around the world," said Cypess.

Please visit **www.bioind.com** for more information on ordering ATCC products through Biological Industries Ltd.

For a complete listing of ATCC authorized distributors, please visit **www.atcc.org** and click on 'Distributors' in the 'Cultures and Products' drop-down menu.

Workshops and Webinars

ATCC Hosts Workshop at ASCB

At the American Society of Cell Biology (ASCB) annual meeting in December 2009, ATCC hosted a workshop, ***Development of a Consensus Standard for the Authentication of Human Cell Lines–Standardization of STR Profiling***. The standard, now in draft stage, will give researchers and other stakeholders a standardized methodology to use to authenticate human cell lines. John R. W. Masters, PhD, Kings College London, presented a historical review of the misidentification of human cell lines and the impact it has on biomedical science. Yvonne Reid, PhD, ATCC, reviewed human cell line authentication methodologies and the selection of STR profiling as the recommended technology for the standard. Margaret Kline, National Institute of Standards and Technology, discussed the STR profiling matching criteria used and the establishment and importance of a cell line database.

The diverse international audience included members of academic and commercial organizations. Copies of the presentations from this workshop can be found at **www.atccsdo.org**.

Webinars

Recent and upcoming webinars include:

What's in your Vial? Best Practices for Maintaining Microbial QC Strains, presented by Liz Kerrigan, Director, Standards and Certification, Microbiology, Biodefense and Emerging Infectious Diseases, ATCC and Jaspreet Sidhu, PhD, Vice President of Business Development and Pharmaceutical Microbiology, Molecular Epidemiology, Inc. Presented on April 28, 2010.

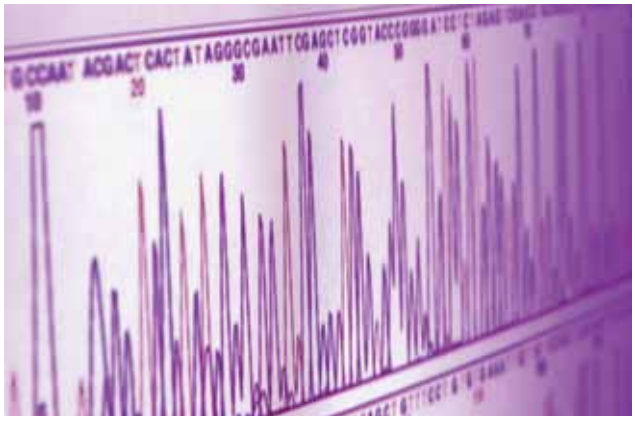
To download this webinar, please visit **www.atcc.org** and click on Science/Learning Center from the drop down menu.

Cell Culture Webinars

Please see the series of cell culture webinars listed on page 19.



What's New: Mycology,



New from Mycology

DNA Sequences Submitted to GenBank®

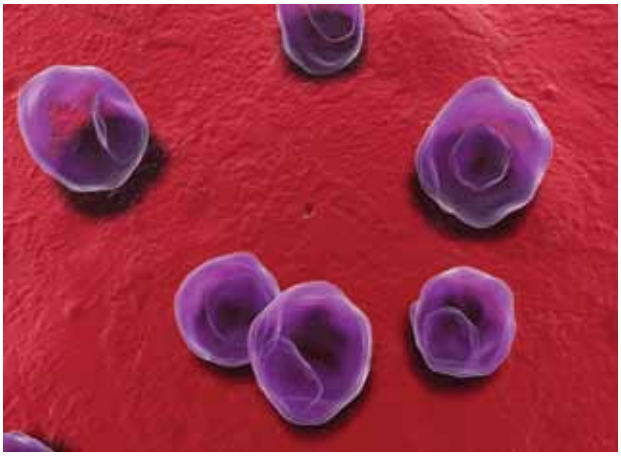
As part of ATCC Mycology Collection's enhanced authentication efforts, we continue to sequence the barcoding region (namely ITS and D1D2) of fungi and yeasts. Recently, ATCC submitted more than 500 such DNA sequences of ATCC Genuine Cultures® fungi to GenBank. To access this information, go to the NCBI's Nucleotide database at **www.ncbi.nlm.nih.gov/nucleotide** and search on 'ATCC authentication'.

New from Virology

Eight new DNA products from *Chlamydia*, Vaccinia and a Koi herpesvirus have been manufactured and released for distribution.

Ordering Information

ATCC® No.	Strain	Comments	Designation
VR-348BD™	*Chlamydia trachomatis*	Serovar E	BOUR
VR-879D™	*Chlamydia trachomatis*	Serovar H	UW-43/Cx
VR-1360D™	*Chlamydia pneumoniae*		CM-1
53592D™	*Chlamydia pneumoniae*		AR-39
VR-885D™	*Chlamydia trachomatis*	Serovar D	UW-3/Cx
VR-1508D™	Vaccinia virus		MVA
VR-1354D™	Vaccinia virus	TC-adapted	WR
VR-1592D™	Koi herpesvirus		F347



New from Protistology

The ATCC Protistology Collection houses nearly 700 parasitic strains. These include causative agents of diseases that affect large populations globally and cause numerous deaths such as diarrheal diseases (i.e., *Entamoeba histolytica*, *Giardia lamblia*), African sleeping sickness (*Trypanosoma brucei*), Chagas' disease (*Trypanosoma cruzi*), and Leishmaniasis (*Leishmania* sp.). We are committed to expanding our collection of pathogenic parasites of medical and veterinary relevance to include type strains, genome strains, and mutant strains used in pathogenesis studies. Our most recent acquisitions are listed below.

Ordering Information

ATCC® No.	Organism	Strain	Source	Comments
PRA-302™	*Babesia duncani*	WA-1	Blood from infected human patient, Washington, USA	Type strain
PRA-309™	*Leishmania major*	Seidman	Cutaneous lesion from infected patient, West Africa	*In vivo* studies
PRA-317™	*Leishmania panamensis*	MHOM/COL/85/2317	Cutaneous lesion from infected patient, Colombia	*In vivo* studies
PRA-318™	*Leishmania panamensis*	MHOM/COL/81/L13	Cutaneous lesion from infected patient, Colombia	*In vivo* studies
PRA-319™	*Toxoplasma gondii*	RHΔ*ku80*Δ*hxg*	Mutant of parent RH strain lacking *ku80* and *hxg* genes	Development of targeted gene knockouts
PRA-320™	*Entamoeba histolytica*	G3	Epimutant of parent HM1:IMSS strain silenced in virulence genes	Avirulent *in vivo*

Virology, Protistology and Cell Biology

New from Cell Biology

Antibiotic Resistant MEF Feeder Cell Lines Support ES Cell Research

The DR4 MEF feeder cell line was developed by ATCC from DR4 mice under license from the Massachusetts Institute of Technology. SNL 76/7 and SNLP 76/7-4 were licensed from and deposited by Dr. Allen Bradley, Baylor College of Medicine.

Mouse embryonic fibroblast (MEF) feeder cell lines are widely used to culture mouse ES cells. They provide both a substrate for ES cell growth and they modulate the microenvironment by secreting many factors needed by ES cells to inhibit differentiation and maintain pluripotency. Propagation of mouse ES cells on feeder cells is particularly important if the ES cells will be used for blastocyst injection and subsequent mouse production.

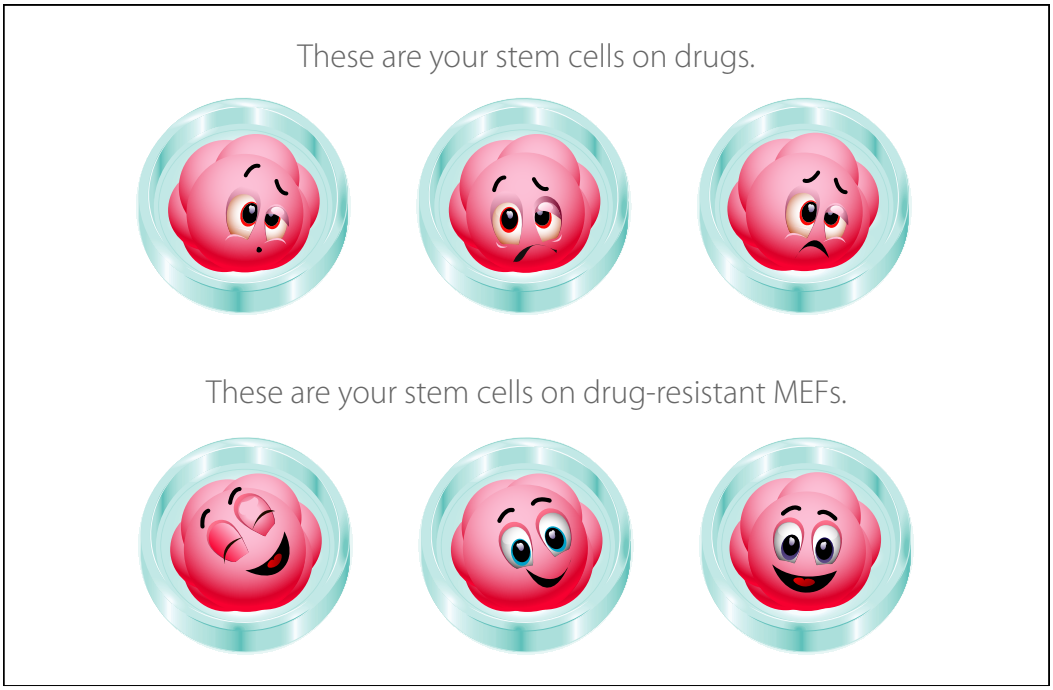
ATCC is one of the world's leading suppliers of ready-to-use MEF feeder cell lines. For nearly 10 years, scientists have valued the consistency derived from ATCC standards of quality and the convenience associated with a source of pre-qualified, ready-to-use MEF feeder cells.

MEF feeder cell lines are useful not only for routine maintenance of ES cells but also in gene targeting experiments. These experiments can involve the sequential cultivation of transfected cells in media supplemented with one or more drugs for the selection of specific drug-resistant clones. The DR-4 strain of mice was developed by researchers in the laboratory of Dr. Rudolf Jaenish to support this type of experimental design[1]. ATCC® SCRC-1045™ was developed by ATCC scientists from DR-4 mice obtained from The Jackson Laboratory. This cell line provides researchers with ability to conduct positive selection experiments using neomycin (G418), puromycin and hygromycin and negative selection using 6-thioguanine (DR-4 cells are *hprt* negative).

ATCC® SCRC-1049™ (SNL 76/7) is a clone derived from the STO cell line that expresses neomycin (G418) resistance and produces murine leukemia inhibitory factor (LIF) at an abundant level.[2] This cell line can be used as a feeder layer to support the growth of mouse ES cells and was used by Takahashi *et al* to develop the first murine induced pluripotent stem (iPS) cell lines.[3]

ATCC® SCRC-1050™ (SNLP 76/7-4) is a puromycin-resistant derivative clone of SNL 76/7 that is resistant not only to neomycin (G418) but also to puromycin.[4] Like ATCC SCRC-1049, ATCC SCRC-1050 produces LIF from a recombinant gene cassette at an abundant level. ATCC SCRC-1050 can be used to support the growth of both ES cells and iPS cells.

continues on page 20





Web Tip: Try our category browse function to find the products you need!

When searching for ATCC products on our website, you can now browse collection catalog categories in three ways:

- By full text search
- By field search
- By numeric/alphabetic index

Each browse choice offers a powerful option for finding ATCC products quickly and easily.

To access this tool, click on the 'Search Options' link located at the top right-hand corner of any page of the ATCC web-site. Once there, select the catalog category of interest from the drop-down list (Figure 1). Then, select one of the browse options.

To use the Full Text Search, simply enter your search text and click on the 'Search' button.

Selecting the Field Search option will load a list of key col-lection-specific fields from which to choose (Figure 2). Select up to six fields, enter your key words/phrases, and click on 'Search'.

Use the Index feature to browse by cell line/organism/prod-uct number or name. Simply select this option then click on '0-9' to search product numbers, or choose a letter (Figure 3).

Regardless of the option you choose, you can view 20, 50, 75 to 100 result items per page. You can also sort the results in ascending or descending order by product number, product description or designation. To sort, click on the result head-ers. Results will be listed in appropriate categories, such as organism type, Reagents and Kits, etc.

Find the right ATCC product today at **www.atcc.org** with quick, easy and flexible search tools!

Figure 1. Select the catalog category to search.

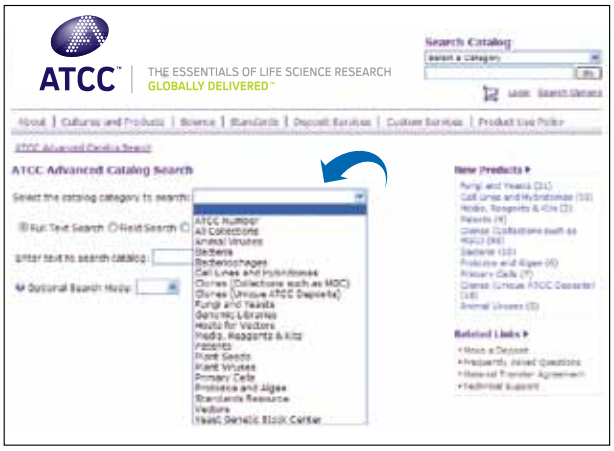


Figure 2. Field Search Option (Cell Lines and Hybridomas example)

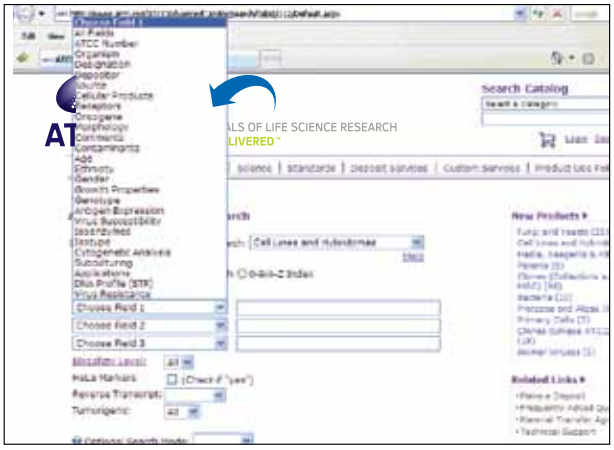


Figure 3. Bacteria Index Search (Bacteria, letter 'A' example)

Attend a Cell Culture Webinar

You are invited to a series of free web-based technical seminars on cell culture. Co-sponsored by Corning, ATCC, and The Society for In Vitro Biology (SIVB), the webinars are designed to provide novel tips, best practices, and proven techniques to help with cell culture research needs.

Impact of Microbial Contamination and Misidentified Cell Cultures on Research, was presented by Yvonne Reid, PhD, Cell Biology Collection Scientist, ATCC on March 9, 2010.

This webinar is now available for download on the Corning website: **http://ems3.intellor.com/index.cgi?c=105&t=13&s=corningweb**

CORNING

Webinar Abstract

Animal cell lines are important *in vitro* systems and tools for scientists in diverse disciplines beyond basic cell biology. Cell line authentication and characterization are crucial in these fields, yet most research scientists underappreciate them. Over the years, numerous cell lines have been shown to be misidentified due, in part, to poor techniques, inadequate authentication protocols, and sharing of unauthenticated cell lines amongst researchers. Technological advances have given rise to improved capabilities. Cell line authentication and characterization now require a comprehensive strategy that employs several complementary technologies for systematic testing for morphology, microbial contaminations, cellular cross-contamination as well as functionality. The validity of conclusions drawn from research data is dependent on consistent and unequivocal verification of cell line identity and function. It is estimated that the financial loss incurred by poorly characterized or misidentified cell lines is in the millions of dollars. An overview of the current technologies used to authenticate and characterize animal cell lines was presented.

The following upcoming online seminars will be presented on the Corning website:

Primary Cell Culture: Tips and Techniques for Getting Started, presented by Jaishree Trikha, PhD, Technical Marketing Manager, Corning Life Sciences

May 11, 12:00 noon – 1:00 p.m. EST
May 13, 9:00 a.m. – 10:00 a.m. EST

Life and Death in Vitro: the Evolution of Techniques for Measuring Cell Growth and Toxicity in Culture, presented by Mark Rothenberg, PhD, Applications Group Manager, Corning Life Sciences

June 8, 12:00 p.m. – 1:00 p.m. EST
June 10, 9:00 a.m. – 10:00 a.m. EST

Optimizing RNAi for Cell Culture, presented by John Shyu, PhD, Field Applications Scientist, Corning Life Sciences

July 13, 12:00 noon – 1:00 p.m. EST
July 15, 9:00 a.m. – 10:00 a.m. EST

Cellular Transfection— More than One Way. A View From Discovery into the Future, presented by Vitaly Klimovich, PhD, Applications Scientist, Corning Life Sciences

September 14, 12:00 noon – 1:00 p.m. EST
September 16, 9:00 a.m. – 10:00 a.m. EST

For more information or to register, please visit **www.corning.com/lifesciences**. Select your region, then click on the 'Online Training' link. Check the website regularly for updates about additional seminars in this series.

What's New, continued from page 17

With the addition of these powerful tools, ATCC can support your increasing sophisticated gene targeting experiments. Select the cell type that best meets your needs:

ATCC® No.	Name	Neomycin resistance	Puromycin resistance	Hygromycin resistance	6-Thioguanine resistance	LIF expression
SCRC-1045™	DR4	●	●	●	●	
SCRC-1049™	SNL 76/7	●				●
SCRC-1050™	SNLP 76/7-4	●	●			●

For more information, please visit **www.atcc.org/MEF5.**

References

1. Tucker KL, et al. A transgenic mouse strain expressing four drug-selectable marker genes. Nucleic Acids Res. 25:3745–3746, 1997.
2. McMahon AP, Bradley A. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. Cell. 62(6):1073–1085, 1990.
3. Takahashi K, et al. Induction of pluripotent stem cells from fibroblast cultures. Nat. Protoc. 2(12):3081–3089, 2007.
4. Cadiñanos J, Bradley A. Generation of an inducible and optimized piggyBac transposon system. Nucleic Acids Res. 35(12):e87, 2007.

Ordering Information

ATCC® No.	Name	Size	Description
SCRC-1045™	DR4	2 x 10^6 cells	MEF feeder cell line; resistant to neomycin, puromycin, hygromycin,and 6-thioguanine
SCRC-1049™	SNL 76/7	3 x 10^6 cells	STO-derived MEF feeder cell line; resistant to neomycin; expresses LIF from a recombinant cassette
SCRC-1050™	SNLP 76/7-4	3 x 10^6 cells	STO-derived MEF feeder cell line; resistant to neomycin and puromycin; expresses LIF from a recombinant cassette

Mouse Embryonic Fibroblast Panel for the Study of Mitochondrial Fusion

Depositor: D. Chan, California Institute of Technology

This panel is comprised of five mouse embryo fibroblast (MEF) cell lines that have been generated with mutations in genes required for mitochondrial fusion: mitofusion Mfn1, mitofusion Mfn2, and the dynamin-related gene, OPA1. Mitochondrial fusion is important not only for maintenance of mitochondrial morphology, but also for cell growth, mitochondrial membrane potential, and respiration. Embryonic fibroblasts lacking Mfn1 or Mfn2 display fragmented mitochondria, a phenotype caused by a severe reduction in mitochondrial fusion. Cells lacking both Mfn1 and Mfn2 have completely fragmented mitochondria and show no detectable mitochondrial fusion activity.

Ordering Information

ATCC® No.	Designation	Organism
CRL-2991™	Wt MEFs	*Mus musculus* (mouse)
CRL-2992™	Mfn1-null MEFs	*Mus musculus* (mouse)
CRL-2993™	Mfn2-null MEFs	*Mus musculus* (mouse)
CRL-2994™	Mfn1/Mfn2-null MEFs	*Mus musculus* (mouse)
CRL-2995™	OPA1-null MEFs	*Mus musculus* (mouse)

Other New Products Featured in this issue of *ATCC Connection*™

Find Just the Right Clone with ATCC

ATCC has the largest, most diverse clone collection on the planet, with over 5.6 million clones. We continue to expand our offering, adding over 60,000 clones just during the last 6 months. Our website search tools make it easy to find the right clone for your research, with options to search by gene name/keyword, GenBank® number, I.M.A.G.E. CloneID, or ATCC catalog number. Our high-quality clones are also economically priced and backed by superior service and support.

The ATCC comprehensive selection of molecular clones includes:

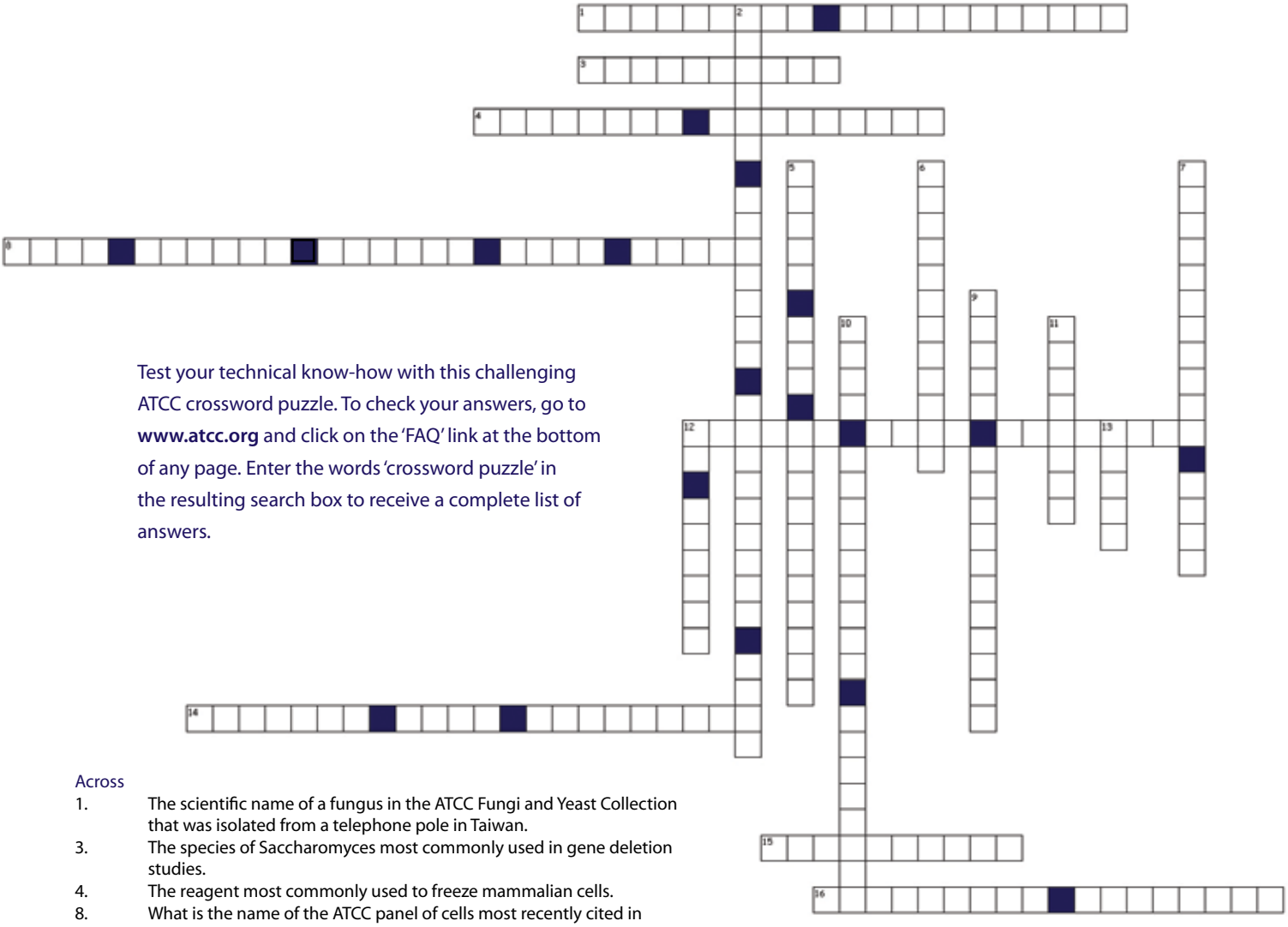
- MGC clones
- I.M.A.G.E. clones
- Clone libraries and sets
- Alliance for Cellular Signaling (AfCS) clones
- Cloned viral genomes
- Apoptosis clones
- Many classic, unique clones

These clones are available in flexible formats: individual clones, BLAST-verified, custom plates (for orders of 30 or more), replicated plates and plate sets.

Find just the right clone with ATCC by visiting **www.atcc.org/clone3.**



Test Your Technical Expertise!



Test your technical know-how with this challenging ATCC crossword puzzle. To check your answers, go to **www.atcc.org** and click on the 'FAQ' link at the bottom of any page. Enter the words 'crossword puzzle' in the resulting search box to receive a complete list of answers.

Across

1. The scientific name of a fungus in the ATCC Fungi and Yeast Collection that was isolated from a telephone pole in Taiwan.
3. The species of Saccharomyces most commonly used in gene deletion studies.
4. The reagent most commonly used to freeze mammalian cells.
8. What is the name of the ATCC panel of cells most recently cited in Breast Cancer Research and Treatment (Jan 24,2009 [Epub] PubMed ID: 19169812)?
12. A tumor of the mantle cells that surround the germinal centers of the lymph nodes (Hint: See ATCC Special Collections).
14. This group of products can take you "one step closer to *in vivo*."
15. An infection of the small intestine caused by the protozoa Giardia lamblia.
16. Which group at ATCC can answer questions about growing a culture from any of the collections?

Down

2. What does "TCID" stand for?
5. The first person to see and describe bacteria (1674); also known as "the father of microscopy."
6. Describes an organism that is capable of surviving and reproducing in extremely cold temperatures.
7. The most commonly used bacteria in molecular cloning.
9. Depositor of ATCC MBA-141, pGRN145 plasmid encoding hTERT (human telomerase reverse transcriptase).
10. An ATCC web-based search tool designed to cross-reference approved ATCC cultures with procedural standards and commercial identification systems.
11. The part of a chromosome representing a tandemly repeated TTAGGG motif.
12. An isopropanol-filled container manufactured by NALGENE® Labware that can be used to mimic a -1 degree C per minute freezing rate.
13. What's the most commonly used acronym for an endothelial cell isolated from a human umbilical cord?

Tech Q

From time to time, ATCC Technical Services receives questions on topical issues with broad applicability across the research spectrum. In this issue of *ATCC Connection*™, Emily Jackson-Machelski, ATCC Technical Service Manager, answers two technical questions.

Q: How does ATCC measure infectivity of its animal virus products?

ATCC uses a simple limiting dilution method to estimate infectivity in its products.

For products grown in cell culture, serial tenfold dilutions of the product are prepared and inoculated onto pre-plated host cells in 1.9-cm wells, 0.2-mL per well, 3–4 wells per dilution. One or more wells are inoculated with 0.2 mL of diluent as a negative control. Inoculated cultures are incubated under appropriate conditions for growth of the organism for the appropriate length of time and then examined for infection. The number of positive and negative wells at each dilution is used to estimate titer, based on commonly accepted statistical methods. Titer is expressed as $10^{x.y}$ $TCID_{50}$ (50% tissue culture infectious dose) per 0.2 mL, where x.y is the inverse of the logarithm of the dilution at which half of the inoculated wells are infected.

For products grown in eggs or animals, a similar procedure is followed. Serial dilutions of the product are made and a specified volume of each dilution is inoculated into 3–4 eggs or animals. The number of infected and uninfected eggs/animals inoculated with each dilution is used to estimate the dilution at which 50% of those inoculated will be successfully infected. Titers are expressed as $CEID_{50}$ or EID_{50} for titrations in eggs (50% chicken egg or egg infectious dose) and ID_{50} (50% infectious dose) or LD_{50} (50% lethal dose) per unit volume for titrations in animals.

Q: Can $TCID_{50}$ / EID_{50} / ID_{50} / LD_{50} titers be converted to PFU ("plaque forming units")?

$TCID_{50}$ assays and plaque assays differ fundamentally in how they measure infectivity. The $TCID_{50}$ titer gives a dilution at which a certain level of function can be expected under a defined set of conditions. A plaque assay measures individual infection events under a defined set of conditions. Because of this difference, and because conditions used for the two assay systems vary, an exact conversion of the results of one assay to results of the other assay is not possible. Assuming that the same cell system is used, that the virus forms plaques on those cells, and that no procedures are added which would inhibit plaque formation, 1 mL of virus stock would be expected to have about half of the number of plaque forming units (PFUs) as $TCID_{50}$. This expectation is based on the probability that each positive well at the endpoint in a $TCID_{50}$ assay may be infected by more than one plaque forming unit and takes into account the 50% difference.

The Poisson distribution has been used to calculate an approximate conversion factor from $TCID_{50}$ to PFU/mL. Using this statistical calculation, it has been determined that the $TCID_{50}$ titer (per mL) can be multiplied by a factor of approximately 0.7 to predict the mean number of PFU/mL. When interpreting this result though, the calculated titer is only valid if the protocol to visualize the plaques for the titer is the same as the conditions employed to calculate the $TCID_{50}$. As a working estimate, one can assume material with a $TCID_{50}$ of $1x10^5$ $TCID_{50}$/mL will produce 0.7×10^5 pfu/mL.

If a precise PFU titer is required (e.g., for performance of an experiment), a preparation must be titered by plaque assay. Where an estimate of infectivity is adequate (e.g., to gauge the amount of virus to use to prepare a new stock), the $TCID_{50}$ measurement can be converted to general "infectious units" per mL by taking the antilog of the log $TCID_{50}$ and dividing by the unit volume used to measure it.

For example, a titer of $10^{7.5}$ $TCID_{50}$ per 0.2 mL is equivalent to a titer of 1.58×10^8 infectious units per mL ([antilog(7.5)] / 0.2 mL = 1.58×10^8/mL).

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ATCC is pleased to announce that we have rebranded our organization! We have realigned and integrated our assets into three divisions: BioServices, BioProducts, and BioStandards. By doing so, we have enhanced our culture of innovation and entrepreneurship in support of product and service development, framed within the context of BioStandards.

Over the last ten years, you have seen us evolve successfully from a large collection, to a repository, to a biological resource center, and now, also to a tools and reagents company. *Sourcing, standardizing and managing biomaterials is our only business and we focus 100% of our resources on it.*

THE ESSENTIALS OF LIFE SCIENCE RESEARCH
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