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ATCC® connection™

Misidentification of the Human Breast Ductal Carcinoma Cell Line, MDA-MB-435s (ATCC® HTB-129™): Historical Perspective

Yvonne A. Reid, PhD, Cell Biology Program, ATCC

MDA-MB-435s (ATCC® HTB-129™) is a spindle variant of the parental cell line MDA-MB-435 isolated from the pleural effusion of a 31-year-old female with metastatic ductal adenocarcinoma of the breast (deposited at ATCC in 1980 by EG & G Mason Research Institute).

Recent studies[1] have generated concern about the authenticity of the parental cell line MDA-MB-435 and its variant, MDA-MB-435s. In 2000, Ross and his colleagues[1] from Stanford University were among the first investigators to demonstrate that MDA-MB-435 was melanocyte in origin. Using cDNA microarray to explore the expression profile of approximately 8,000 unique genes among the NCI-60 cell panel, they showed, unexpectedly, that the parental cell line, MDA-MB-435, clustered with melanoma cells and expressed a large number of melanoma-associated genes. However, the data suggest that the gene expression profile between MDA-MB-435 and its derivative MDA-MB-435s (ATCC) may be similar.

In 2002, Ellison and his colleagues[2] established what they described as "further evidence to support the melanocytic origin of MDA-MB-435." Through literature search and *in silico* cDNA library subtractions, the investigators identified several genes that were differentially expressed in breast epithelium and melanomas. Studying several breast cell lines using quantitative reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry of tumors generated in severe combined immunodeficiency (SCID) mice, the derivative cell lines, MDA-MB-435s and MDA-MB-435-HGF, were shown to be devoid of breast genes (pS2, prolactin-inducible proteins and mammoglobulin) expression. The MDA-MB-435-HGF cell line expressed the four common melanocyte genes (retinoid X receptor, ACP5, tyrosine-related protein, and tyrosine D) at high levels; the MDA-MB-435s derivative expressed only three of the four melanocyte genes, at varying degrees, and lacked the expression of tyrosine D gene. The immunohistochemical studies showed that the derivative cell lines, MDA-MB-435s and MDA-MB-435-HGF, were positive for S100, a neuroendocrine marker, as well as for melan-A, a melanocyte differentiation marker. In addition, the typical markers for epithelium or mammary tumors (cytokeratin, epithelial membrane antigen [EMA], progesterone receptor [PR] and estrogen receptor [ER])

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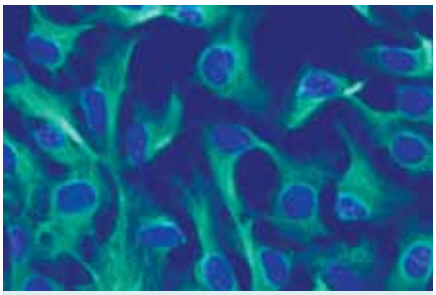


In This Issue

Development of a Consensus Standard for the Authentication of Human Cell Lines: Standardization of STR Profiling

The misidentification and cross-contamination of mammalian cell cultures used in biomedical research continues to be a widespread problem despite 45 years of published data on the subject. The causes of cross-contamination and appropriate preventative measures have also been published and have led to the application of robust methods for the authentication of cell lines. Inexpensive methods are now available to overcome this problem. Yet, the problem continues. Researchers continue to publish data derived from misidentified and cross-contaminated cell lines. Estimates of the incidence of research papers flawed by the use of misidentified and cross-contaminated cell cultures approximate 15-25%. This has resulted in millions of dollars of wasted funds and volumes of misleading research data. The situation called for a strategy that would deliver a remedial message of authentication to cell culture researchers and ensure compliance with the message.

John RW Masters, PhD
Workgroup Chair
University College London

Yvonne A. Reid, PhD
Workgroup Co-Chair
ATCC

A proposal was submitted by Professor John RW Masters, PhD, University College London, and Dr. Roland M. Nardone, PhD, Professor Emeritus, Catholic University of America, to the ATCC® Standard Development Organization (ATCC SDO) that called for a consensus standard to be developed that addressed the authentication of human cell lines. The intent of the standard is to delineate a standardized, relatively inexpensive and universally applicable method for authenticating new and established human cell lines and their criteria for use. The new standard is now in the draft stage and the overall objective is to provide researchers, vendors, and other stakeholders with a standardized methodology (protocols and procedures for STR analysis) for authenticating human cell lines.

The standard will begin with a historical review of the misidentification of human cell lines, the extent of the misidentification and the impact it has on biomedical science. A review on human cell line authentication methodologies and the selection of STR profiling as the recommended technology for the standard will be included. Within the context of this standard, authentication of human cell lines using STR profiling will result in the following: verification of human origin; evaluation of profile consistency between provisionally related cell isolates; comparison to a profile database; and detection of "contaminating human DNA" (intra-species cross contamination). However, authentication using STR-profiling may not imply freedom from inter-species (non-human) cell contamination, since the procedure described in this standard will not lead to direct detection of non-human DNA contamination. The capabilities and limitations of STR profiling will also be addressed. The workgroup is currently meeting and establishing the format and structure of a database of STR profiles of human cell lines, which has been determined to be necessary to support the standard.

The workgroup is chaired by John R W Masters, PhD of the University College London, and co-chaired by Yvonne A. Reid, PhD of ATCC. They have been meeting monthly since early 2009. The standard is expected to be completed in early 2010.

The approach of developing consensus standards is new to the area of cell line authentication. A distinguishing feature of a consensus standard is that procedures and protocols are not the product of a single researcher or laboratory but instead represent the collective work of members of the workgroup. The development of consensus standards offers a new tool to the cell biology community that will foster reproducibility and comparability of cell lines used in different laboratories. It is hoped that the use of the standard will ultimately lead to a marked decrease in the misidentification and cross-contamination of mammalian cell cultures used in biomedical research.

The ATCC SDO was formed to ensure the development of nationally and internationally accepted standards for biomaterials that meet International Standards Organization (ISO) guidelines for standards development, and that are recognized by the American National Standards Institute (ANSI). The mission of the SDO is to develop best practices (standards) for use in the life science industry and to promote their use globally, using a consensus-driven process that balances the viewpoints of industry, government, regulatory bodies and academia.

For Many Cell Lines, Timing is Everything

Temperature, $C0_2$ concentration and media composition are obvious, well-established contributors to the health and quality of a cell line. **Did you know that *when* you culture your cell line also can have a direct impact on its integrity?**

To ensure optimal viability, genetic stability and consistent phenotype, it is critical that cell lines are maintained in the exponential phase of growth. They need to be subcultured on a regular basis *before* they enter the stationary growth phase, i.e., before the cells become 100% confluent or before a suspension reaches the maximum recommended cell density.

Disregarding this simple subculture guideline can have a dramatic impact on a cell line, as is the case with the widely used NIH/3T3 cell line (ATCC® CRL-1658™). This cell line has an easily identifiable phenotypic trait—contact inhibition—a phenotype marked by the inhibition of over-lapping three-dimensional growth of cells in a confluent monolayer (Figure 1).

When the culture is allowed to become confluent, the cells will simply stop growing. Conversely, if the culture is allowed to approach confluence *before* subculturing, variants that are not contact inhibited will be selected. Within a few passages, the entire culture will lose its contact inhibition marked by foci of growth at high density (Figure 2).

Simply put, if your NIH/3T3 cell line has lost this contact inhibited phenotype, it is no longer representative of the original culture and other characteristics (such as surface markers, cell signaling, etc.) may be different than the original population. This factor can significantly skew experimental data and undermine reproducibility. ATCC strongly recommends subculturing this cell line between 60%–70% confluence.

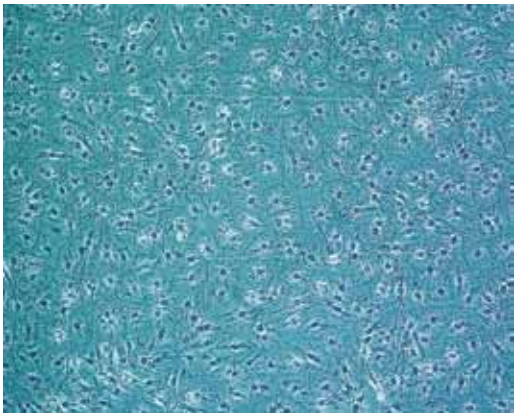


Figure 1. NIH/3T3 cells subcultured correctly retain the property of contact inhibition and are contact inhibited at 100% confluence.

Figure 2. NIH/3T3 cells subcultured incorrectly will lose the property of contact inhibition. Cells no longer grow to confluence and form foci.

Tissue-Culture Adapted

Edward Cedrone[†], Erin Eckard-Amar[‡], Kirsten Jacob[†], Raquel Manteiga[‡], Yvonne Pyla[†],

Introduction

The MDCK cell line is a well-studied model for many applications, including drug absorption, propagation of influenza viruses and epsilon toxin studies.[1,2] When MDCK cells were grown on plastic culture dishes, they displayed 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 74 to 81 and 84 to 90 and a heterogeneous morphology consistent with both fibroblast-like and epithelial-like shapes. Immunocytochemistry staining with pan-cytokeratin antibodies established the epithelial origin of the cells.

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

We explored the diverse morphology by cloning the MDCK cells and selected a single population of cells manifesting high susceptibility 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 medium containing Eagle's Minimal Essential Medium with 10% fetal bovine serum (EMEM [ATCC® 30-2003] + 10% FBS [ATCC®30-2020]). Cells were cloned at a seeding density of 0.5 cells/well in a 96-well plate by limiting dilution. Wells with single colonies were expanded and cryopreserved for later studies. Of the 52 clones identified, several clones with different fibroblast- and epithelial-like morphologies were selected. Subsequently, five clones (ATCC® CRL-2936™, MDCK clone 4, MDCK clone 7, MDCK clone 33, MDCK clone 42) were identified for their ability to support the growth of different influenza isolates. These MDCK clones and the MDCK parental cells were further authenticated to confirm their species of origin by cytochrome oxidase subunit I (COI) analysis and by G-banded karyotyping. Further characterization was performed to determine the: optimal culture conditions (growth curves and doubling time); phenotypic traits (expression of tight junctions, receptors for human and avian influenza), and; tumorigenicity ability in immunocompromised mice (Table 1).

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*

Table 1. Authentication and Characterization of MDCK and MDCK Clones

Table 2. Viruses Used to Evaluate MDCK and Derivatives for Susceptibility to Influenza Virus Infection

ATCC® No.	Virus	Strain	Original Host	Growth Host
VR-95™	Influenza A	A/PR/8/34	Human	Embryonated eggs
VR-1469™	Influenza A	A/PR/8/34	Human	MDCK (CCL-34™)
VR-99™	Influenza A	A/Swine/1976/31	Swine	Embryonated eggs
N/A	Influenza A	A/California/04/2009	Human	MDCK

Phenotypic Characterization of MDCK and MDCK Clones

MDCK and MDCK Clones Express the Receptors for Human and Avian Influenza Virus

A major determinant of influenza virus infection is the presence of receptors on MDCK susceptible cells. Influenza virus binds to cell receptors via sialic acid (SA)–linked glycoproteins. Avian viruses preferentially bind to sialic acid α2,3-galactose (SAα2,3-Gal)–linked receptors, whereas human strains bind to sialic acid α2,6-galactose (SAα2,6-Gal)– linked receptors.[4] The detection of α2,3-linked and α2,6-linked SA by lectin immunocytochemistry (data not shown) and flow cytometry (Figure 1) was determined by the binding of *Sambucus nigra* agglutinin (SNA) for SAα2,6-Gal and *Maackia amurensis* agglutinin (MAA) for SAα2,3-Gal in the MDCK cells.

Immunocytochemistry and Flow Cytometry

Cells were harvested, washed, fixed with methanol, blocked with FBS and then incubated with either 5.0 µg/mL of MAA-conjugated FITC or 2.0 µg/mL SNA-FITC. To confirm binding, 200 mM lactose was used to inhibit the binding of sialic acids to the receptors. For the flow cytometry assays, the cells were treated with 20 µg/mL of SNA and 10 µg/mL of MAA. The sialic receptor sites were blocked with 200 mM lactose.

Isolation and Propagation of Influenza Viruses

Because isolation and propagation of viruses is a major application for the parental MDCK line, susceptibility to infection with influenza viruses was pursued as an initial screen for clone characterization.

Influenza Virus Strains

Quwana Spencer[†], Sujata Suri[†], Wendy Ullmer[†], Peggy Fahnestock[‡], PhD and Yvonne Reid[†], PhD.
[†] ATCC [‡] Formerly with ATCC

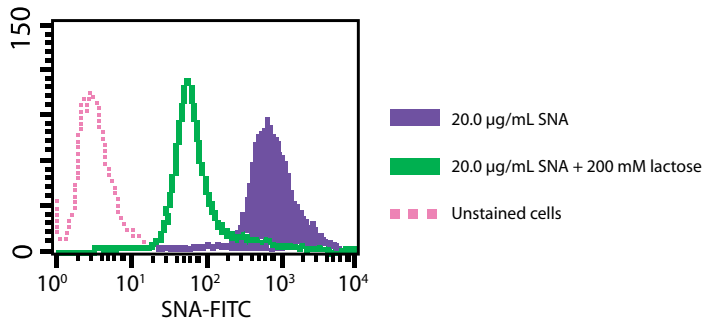
A series of influenza viruses was assembled from the ATCC Animal Virology collection, including different types, subtypes, species of origin, and growth substrates, including cell culture versus embryonated eggs (Table 2). For all experiments, cells were cultured for infection in EMEM with 10% FBS at 37°C in a humidified, 5% CO_2 atmosphere. Infections were completed by virus inoculation and incubation of infected cells in serum-free EMEM with 1 µg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (~250 units/mg), at 35°C in a humidified, 5% CO_2 atmosphere. These conditions are not optimal for all influenza strains, but were adopted as a standardized "middle ground" to allow identification of relative differences.

First, ATCC CCL-34 cells and its derivatives were screened for susceptibility to infection by H1N1 influenza A viruses of swine or human origin, using a simple assay of virus spread. Cells planted in 24-well trays 1 day prior to use were infected with ~750 $TCID_{50}$ per well. After 24 hours of incubation, all cultures were fixed with 1:1 methanol: acetone, stained with antibody to H1N1 influenza A, followed by an FITC-conjugated anti-immunoglobulin secondary antibody and examined using a fluorescence microscope. Representative photomicrographs were taken at 20X magnification.

Cells positive for influenza antigen were observed in all inoculated cultures but not in negative controls (not shown). However, infection patterns varied by virus and by cell line (not shown). Although propogated in cell culture, A/California/04/09, a very recent isolate of the novel H1N1 pandemic influenza A virus, produced only isolated antigen-positive cells in all cell lines. Most other viruses produced foci of infected cells, comet-like aggregates or extended fields. Infected foci in MDCK cells appeared ragged by comparison with foci in most clonal cell lines. Clone 7 appeared to host cell culture-adapted (ATCC® VR-1469™, A/California/04/09), but not egg-grown virus (ATCC® VR-95™, ATCC® VR-99™). Clones ATCC CRL-2936, 4 and 33 showed robust infection by all three well-established viruses, regardless of propagation host. These subjective observations were used to pick the series of six cell lines—the ATCC CCL-34 parent and five clonal derivatives—for further study.

The six cell lines were inoculated with a broader series of viruses, listed in Table 3, to evaluate plaquing efficiency. Included were influenza viruses A and B, multiple influenza A HA/NA types, viruses from multiple species, and preparations grown in eggs or cell culture-adapted. A stock of each virus was titrated by plaque assay in MDCK cells prior to use[5], and inoculated onto three wells of a six-well plate at a dilution expected to produce 35 plaques per well. After adsorption, a semi-solid overlay was added and the plates were incubated for 2 days, then fixed and stained for influenza antigen to visualize plaques. Plaques per well were counted and the average number for each virus in each cell line was expressed as a percentage relative to plaque numbers observed for the same virus in ATCC CCL-34 cells (Figure 2).

Figure 1. MDCK clone 4 expresses the (SAα2,6-Gal) receptors (*purple*) for human influenza virus A. Cells pretreated with lactose blocked SAα2,6-Gal binding to the cells, relatively no staining (*green*); unstained cells (*broken pink bars*). SNA-FITC: 10 µg/mL.



ATCC® VR-297™, the equine H7N7 influenza A virus, did not produce cytopathic effects in any cell line, so it was not used for further analysis or experiments. Plaquing efficiencies for all other viruses followed a fairly consistent pattern. Cell culture-adapted influenza A viruses, ATCC® VR-822™, and influenza B strains gave the highest number of plaques in all cell types, with results for Clones 7 and 33 approaching or exceeding MDCK plaque numbers for those viruses.

Finally, cultures of each of the six cell types were infected with each viral isolate at a multiplicity of infection (MOI) of 0.01, and then incubated under standard conditions in serum-free MEM containing

Table 3. Viruses Used in Further Screening of ATCC® CCL-34™ and Derivatives for Susceptibility to Infection

ATCC® No.	Virus	Strain	Original host	Host in Which Grown
VR-1520™	Influenza A (H1N1)	A/WS/33	Human	CCL-34™
VR-1469™	Influenza A (H1N1)	A/PR/8/34	Human	CCL-34™
VR-99™	Influenza A (H1N1)	A/Swine/1976/31	Pig	Embryonated eggs
VR-822™	Influenza A (H3N2)	A/Victoria/3/75	Human	Embryonated eggs
VR-297™	Influenza A (H7N7)	A/Equine1/Prague/1/56	Horse	Embryonated eggs
VR-1535™	Influenza B	B/Lee/40	Human	CCL-34™
VR-295™	Influenza B	B/Taiwan/2/62	Human	Embryonated eggs
BEI No.				
NR-9695	Influenza A (H3N2)	A/Brisbane/10/2007 X A/PR/8/34	Human; reassorted strain	Embryonated eggs

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Primary cell cultures more closely mimic the physiological state of cells *in vivo* and generate more relevant data representing living systems. The use of primary BTEC and SAEC has been implied in research pertaining to microbial infection and pathogenesis, including: influenza; airway inflammation and wound healing; cancer; asthma; pulmonary fibrosis; chronic obstructive pulmonary disease, such as chronic bronchitis and emphysema; and toxicology testing of pharmaceuticals.

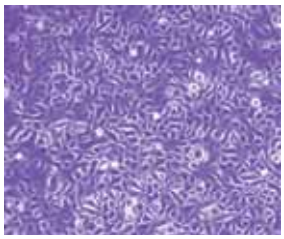

Figure 1. Normal primary bronchial/tracheal epithelial cells.

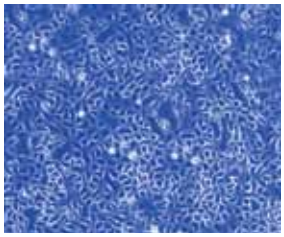

Figure 2. Normal primary small airway epithelial cells.

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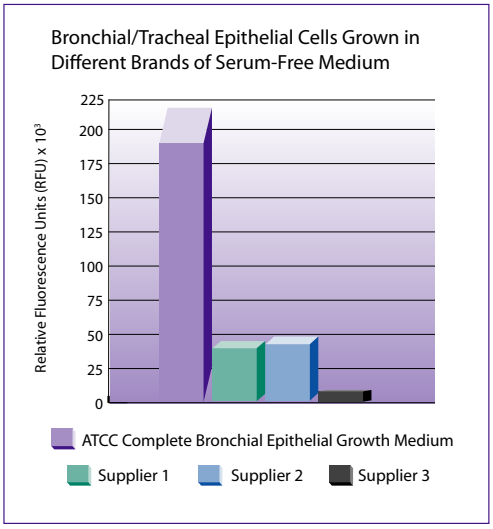
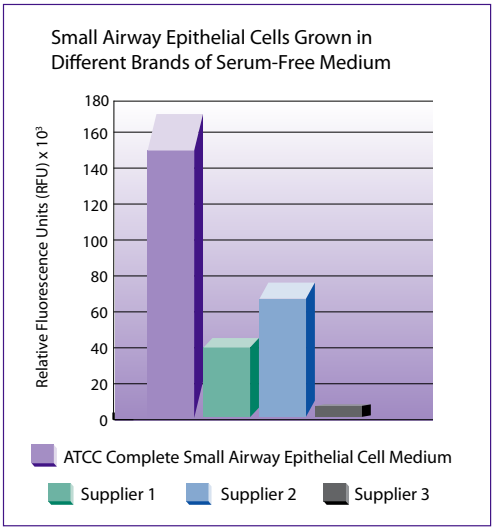
Designed to provide optimal support for cells derived from normal pulmonary tissues, Airway Epithelial Cell Basal Medium is a sterile, phenol red-free, liquid tissue culture medium intended for use as one component in a complete ATCC Primary Cell Solutions system. Used in combination with the Bronchial Epithelial Cell Growth Kit or the Small Airway Epithelial Cell Growth Kit, each complete ATCC Primary Cell Solutions airway epithelial cell system selectively sustains the proliferation and plating efficiency of cells derived from normal human bronchial/tracheal tissues or bronchioles.

Growth of ATCC Primary Cell Solutions Primary Airway Epithelial Cells in Different Brands of Cell-Specific, Serum-Free Media





ATCC Primary Cell Solutions airway epithelial cells were taken from liquid nitrogen and cultures were initiated. The cells were cultured for 3 to 4 days. The cells were then seeded in triplicate into a 24-well plate at 250 cells/cm² (bronchial/tracheal epithelial cells) or 400 cells/cm² (small airway epithelial cells) and grown for 7 days in different brands of serum-free media. Cell proliferation was measured by adding alamarBlue® to each well, incubating for 2 hours, and then measuring fluorescence using a Wallac VICTOR2™ MultiLabel Counter. The medium was not changed during the incubation period; the assay is a measure of a media's capacity to support log-phase growth over time. The higher the relative fluorescence unit (RFU) value, the higher the rate of cell proliferation.

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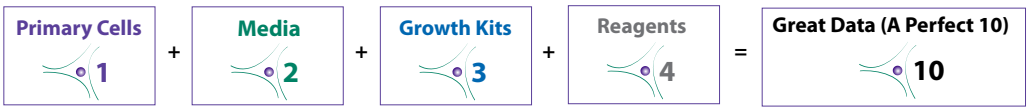

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2	Airway Epithelial Cell Basal Medium – **New!**	485 mL	PCS-300-030
3	Bronchial Epithelial Cell Growth Kit – **New!**	1 kit	PCS-300-040
3	Small Airway Epithelial Cell Growth Kit – **New!**	1 kit	PCS-301-040

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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

	Product Name	Components	Catalog No.
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

	Product Name	Components	Catalog No.
1	Primary Epidermal Keratinocytes; Normal, Human, Neonatal Foreskin	≥ 5 x 10^5 viable cells	PCS-200-010
1	Primary Epidermal Keratinocytes; Normal, Human, Adult — **New!**	≥ 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

	Product Name	Components	Catalog No.
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

	Product Name	Components	Catalog No.
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 — **New!**	≥ 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

	Product Name	Components	Catalog No.
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

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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

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What's New: Cell Lines, Bacteria, Protists, Fungi and Viruses

New Cell Lines

Multiple Myeloma Cell Lines, MM.1S and MM.1R
Depositors: NL Krett and ST Rosen, University of Chicago

Multiple myeloma is an incurable clonal B-cell malignancy of unknown etiology. It is characterized by the accumulation of plasma (antibody-producing) cells in the bone marrow. It accounts for 1% of all cancers and 14% of hematologic malignancies. One of the most commonly used drugs for the treatment of multiple myeloma is the synthetic glucocorticoid (GC) dexamethasone. Most patients respond to this drug but many become refractory to the treatment with steroids.

Two cell lines, MM.1S (ATCC® CRL-2974™) and MM.1R (ATCC® CRL-2975™), derived from the heterogeneous population of the parental cell line, MM.1, were established from the peripheral blood of a 45-year-old African American female patient with an IgA myeloma.[1] The MM.1S cells express high levels of glucocorticoid receptors (GR) and elevated levels of GR mRNAs. The MM.1S cells (dexamethasone-sensitive) when treated with dexamethasone will not proliferate and will undergo cytolysis. Dexamethasone-induced cytolysis is blocked by RU 486, a glucocorticoid antagonist, indicating the specificity of this response for the glucocorticoid receptor.[2] However, the resistant variant, MM.1R, lacks the responsiveness to dexamethasone-induced cytolysis. The phenotypes of both cell lines seem to represent patients in the later stages of the disease. Both cell lines express the same surface markers (lambda light chain, CD38, CD52, CD59) but differ in their sensitivity to GCs.[3]

These cell lines are important in studying the etiology of multiple myeloma, the effects of chemotherapeutic agents, and the development of clinical resistance and also help to elucidate the mechanisms of action of GCs.[1]

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ATCC® No.	Name	Description
CRL-2974™	MM.1S	*Homo sapiens* (human)
CRL-2975™	MM.1R	*Homo sapiens* (human)

Pancreatic Alpha Cell Line, Alpha TC1 clone 6
Depositor: EH Leiter, Jackson Laboratory

The pancreatic alpha cell line, Alpha TC1 clone 6, is a useful model in diabetes research for studying many aspects of islet biology, including glucagon biosynthesis and cytokine sensitivity. It was cloned from the alpha TC1 cell line, which was derived from a pancreatic adenoma created in transgenic mice expressing the SV40 large T antigen oncogene, under the control of the rat preproglucagon promoter. In contrast to the parental cell line, which produces both glucagon and insulin, alpha TC1 clone 6 cells are terminally differentiated and produce only glucagon. Alpha TC1 clone 6 cells exhibit a more differentiated phenotype than alpha TC1 clone 9 (ATCC® CRL-2350™) and express higher levels of glucagon.[1]

Reference

1. Hamaguchi K, Leiter EH. Comparison of cytokine effects on mouse pancreatic alpha-cell and beta-cell lines. Viability, secretory function, and MHC antigen expression. Diabetes 39:415-425, 1990.

ATCC® No.	Name	Description
CRL-2934™	alpha TC1 clone 6	*Mus musculus* (mouse)

Microvascular Endothelium Cell Line, TIME
Depositor: M. McMahon, University of California-San Francisco

The telomerase-immortalized human microvascular endothelium cell line, TIME, represents an effective cell model for studying endothelial cell biology including signal transduction and angiogenesis. TIME cells express a panel of characteristic endothelial cell surface marker proteins including CD31/PECAM-1 and integrin alpha v beta 3. The cells also express the low-density lipoprotein (LDL) receptor and are capable of acetylated LDL uptake. When plated on BD Matrigel™, TIME cells undergo tubule formation exhibiting capillary-like structures. TIME was derived from a primary culture of neonatal foreskin microvascular endothelial cells (HMVEC) of the dermis. The primary HMVECs were immortalized by infection with the retrovirus WZLblast3:hTERT and cultured in complete growth medium containing blasticidin. The immortalized cells do not undergo growth arrest in culture due to the exogenous hTERT expression.

Reference

1. Lagunoff M, et al. De novo infection and serial transmission of Kaposi's sarcoma-associated herpes virus in cultured endothelial cells. J. Virol. 76(5):2440-2448, 2002.

ATCC® No.	Name	Description
CRL-4025™	TIME	*Homo sapiens* (human)

New Bacteria

The Bacteriology collection at ATCC has recently acquired several new *Acinetobacter baumannii* isolates, including two genome sequenced strains and 12 recent multidrug-resistant (MDR) isolates. *A. baumannii* is recognized as an emerging problem in hospital-acquired infections. Recent isolates are increasingly resistant to antibiotics, leaving few therapeutic options. Check our website for detailed antibiotic resistance profiles.

ATCC® No.	Description	Source	Notes
BAA-1709™	*A. baumannii* SDF	Human body louse	Antibiotic-sensitive, sequenced genome
BAA-1710™	*A. baumannii* AYE MDR	Human blood	Sequenced genome
BAA-1789™	*A. baumannii*	Tracheal aspirate	2008 MDR isolate from Maryland, USA
BAA-1790™	*A. baumannii*	Sputum	2008 MDR isolate from Washington DC, USA
BAA-1791™	*A. baumannii*	Sputum	2008 MDR isolate from Maryland, USA
BAA-1792™	*A. baumannii*	Sputum	2008 MDR isolate from Washington DC, USA
BAA-1793™	*A. baumannii*	Sputum	2008 MDR isolate from Washington DC, USA
BAA-1794™	*A. baumannii*	Sputum	2008 MDR isolate from Maryland, USA
BAA-1795™	*A. baumannii*	Nasotracheal aspirate	2008 MDR isolate from Maryland, USA
BAA-1796™	*A. baumannii*	Sputum	2008 MDR isolate from Maryland, USA
BAA-1797™	*A. baumannii*	Blood	2008 MDR isolate from Washington DC, USA
BAA-1798™	*A. baumannii*	Sputum	2008 MDR isolate from Maryland, USA
BAA-1799™	*A. baumannii*	Sputum	2009 MDR isolate from Maryland, USA
BAA-1800™	*A. baumannii*	Trachea	2008 MDR isolate from Washington DC, USA

We have recently added 12 strains of *Neisseria gonorrhoeae* to our collection. These strains were nominated for genome sequencing by a consortium of researchers seeking to correlate genetic difference between strains with different gonococcal disease states. The strains range from antibiotic-sensitive strains isolated in the 1960s to drug-resistant strains from the 1990s. Please visit the *N. gonorrhoeae* group website hosted by the Broad Institute for more information (http://www.broadinstitute.org/annotation/genome/neisseria_gonorrhoeae/Info.html).

ATCC® No.	Description	Notes
BAA-1833™	*Neisseria gonorrhoeae* MS11	Isolated from an uncomplicated gonorrhea infection in the 1960s
BAA-1837™	*Neisseria gonorrhoeae* F62	Serum-sensitive strain isolated in 1960
BAA-1838™	*Neisseria gonorrhoeae* FA19	Sequenced strain from an uncomplicated infection
BAA-1839™	*Neisseria gonorrhoeae* 1291	Low-passage isolate from 1972
BAA-1840™	*Neisseria gonorrhoeae* PID1	Isolated in the 1970s from a woman with severe pelvic inflammatory disease
BAA-1841™	*Neisseria gonorrhoeae* PID18	Isolated in the 1970s from a woman with severe pelvic inflammatory disease
BAA-1842™	*Neisseria gonorrhoeae* PID24-1	Isolated in the 1970s from a woman with severe pelvic inflammatory disease
BAA-1843™	*Neisseria gonorrhoeae* PID332	Isolated in the 1970s from a woman with moderate pelvic inflammatory disease
BAA-1844™	*Neisseria gonorrhoeae* DGI18	Isolated from the blood of a patient with disseminated gonococcal infection
BAA-1845™	*Neisseria gonorrhoeae* DGI2	Isolated from the blood of a patient with disseminated gonococcal infection
BAA-1846™	*Neisseria gonorrhoeae* SK-92-679	Isolated in 1992 from the blood of a patient with disseminated gonococcal infection. Beta lactamase positive and tetracycline resistant.
BAA-1847™	*Neisseria gonorrhoeae* SK-93-1035	AHU strain isolated in 1993 from the blood of a patient with disseminated gonococcal infection

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

New Protists

Seven ichthyosporean strains are now available. Ichthyosporeans are a small group of protists, mostly parasites of fish and other animals. They belong to a taxonomic supergroup that includes animals and fungi, making them of interest to researchers studying their origins. There are only a few species of culturable ichthyosporeans. Of note, *Creolimax fragrantissima* has been isolated regularly and repeatedly from nature and then grown easily through cycles of asexual reproduction. This makes it a potential model organism for further research into marine ichthyosporeans. The strains listed below grow axenically, providing an ideal source of high quality, bacteria-free DNA.

ATCC® No.	Description
PRA-278™	Ichthyosporea g. sp. BM11
PRA-279™	Ichthyosporea g. sp. bPW
PRA-280™	Ichthyosporea g. sp. MB14
PRA-282™	Ichthyosporea g. sp. Le7
PRA-283™	*Sphaeroforma* sp. RU1
PRA-284™	*Creolimax fragrantissima* CH2
PRA-297™	*Sphaeroforma arctica*

New Genomic DNA

ATCC® No.	Description	Source strain
50843D	*Neospora caninum*	Nc-1

continues on page 17

Cell Signaling Expression Vectors and Micro-RNA Interference

AfCS Cell Signaling Expression Vectors

ATCC is the exclusive distributor of over 3,000 molecular vectors constructed by the Alliance for Cell Signaling (AfCS) for murine signal transduction research. The AfCS has concentrated its cloning efforts on key molecules involved in cell signaling and is providing the research community with entry and expression vectors for more than 260 wild-type kinase family members. Expression vectors that express constitutively active kinase family members (i.e., lysine to arginine substitution) are also available for studies characterizing unregulated intracellular kinase activities. Other key molecules associated with cell signaling that are represented in the AfCS vector collection include phospholipases, heterotrimeric G proteins, G-protein coupled receptors, small G proteins, and guanine nucleotide exchange factors.

AfCS has cloned cell signaling genes into the Gateway® entry vector permitting fast and easy transfer to a multitude of compatible expression vectors. The cell signaling vector insertions have been validated by restriction enzyme digestion. For many of the kinase entry vectors, two distinct forms are available for experiments: a T-form which includes the stop codon allowing for N-terminal tagging, and an N-form which lacks the stop codon allowing for C-terminal tagging. The majority of cell signaling vectors available from ATCC are fluorescently tagged at either the N-terminal or C-terminal with cyan fluorescent protein (CFP) or yellow fluorescent protein (YFP) (Figure 1). The fluorescent-tagged vectors are especially useful in cell imaging applications for determining intracellular protein–protein interactions and co-localizations. They can be used with tag antibodies to immunoprecipitate protein complexes during cell signaling experiments. A comprehensive set of 64 vectors that can serve as fluorescent localization markers for various intracellular organelles is available (ATCC® MBA-91).

The AfCS and ATCC websites can be effectively used to browse the AfCS vector collection and search for vectors encoding specific molecular targets. More detailed information on the vectors can be obtained from the AfCS website at **www.signaling-gateway.org/data/plasmid/info.html**, in the referenced publication[1], or by contacting the ATCC Technical Service team. Find specific vectors quickly by using the search function on the AfCS website. By using specific parameters (such as entering the common gene name for a search), the available vector forms of the cell signaling molecule in the AfCS

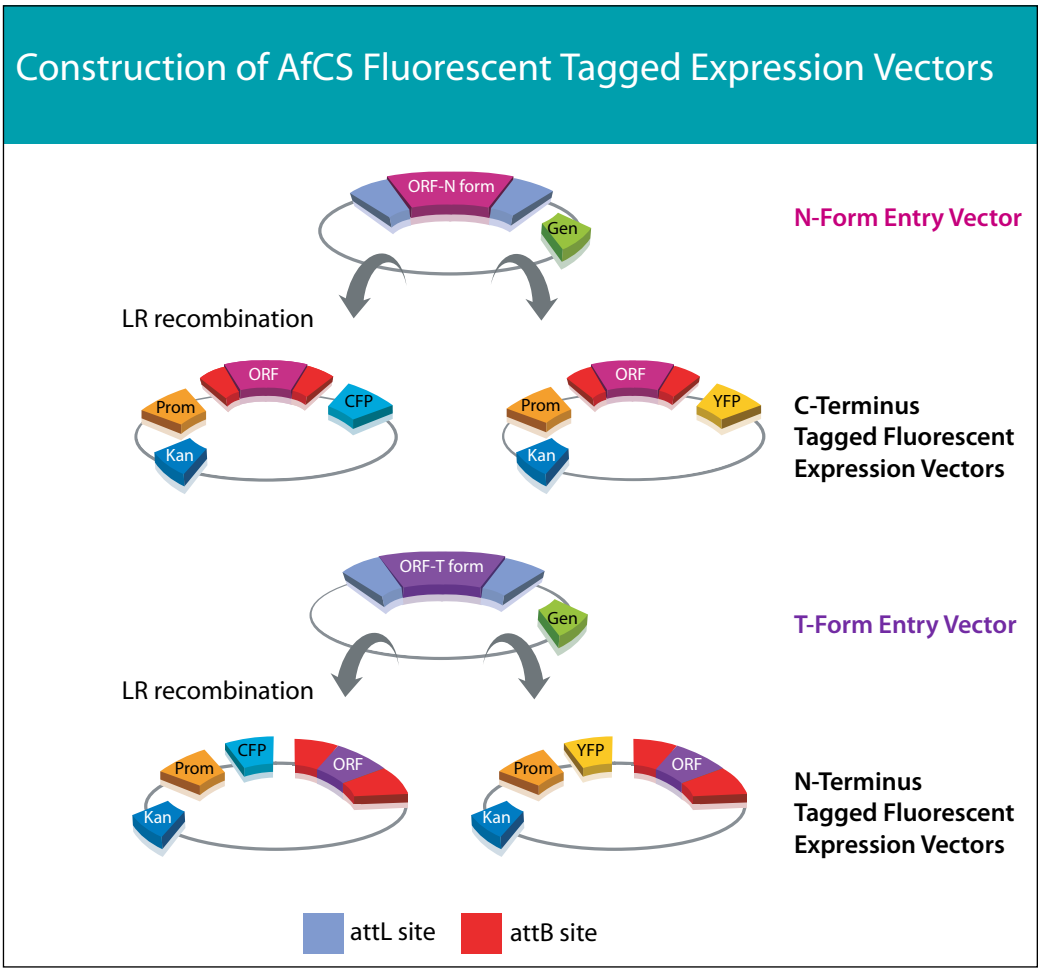


Figure 1. Construction of AfCS Fluorescent Tagged Expression Vectors. Two forms of entry vectors for cell signaling molecules were constructed which differed in the presence (T-form) or absence (N-form) of a stop codon. The T-form entry vectors were used to clone the cell signaling molecules into expression vectors which were CFP- or YFP-tagged at the N-terminus. The N-form entry vectors were used to clone the cell signaling molecules into expression vectors which were CFP- or YFP-tagged at the C-terminus.

Vectors from the Alliance for Cell Signaling (AfCS)

collection are displayed, along with the respective ATCC catalog numbers and direct hyperlinks to the ATCC website for easy ordering. Similarly, different vector forms can be found on the ATCC website (**www.atcc.org**) by using the site search function and the respective gene name.

Reference

1. Zavzavadjian JR, et al. The Alliance for Cell Signaling Plasmid Collection. Mol. Cell. Prot. 6.3:413-424, 2006.

AfCS Micro-RNA Interference Vectors

AfCS has developed a large flexible vector platform that allows multi-gene RNA interference. A single micro-RNA short hairpin RNA (miR-shRNA) transcript can be used to deplete and knock down multiple molecular targets (≥ 3 targets). This multi-gene interference capability is useful for inhibiting a key activity or phenotype in a mammalian cell that expresses either multiple proteins or isoforms with redundant functions. The shRNA entry vector is constructed by directional

continues on page 12

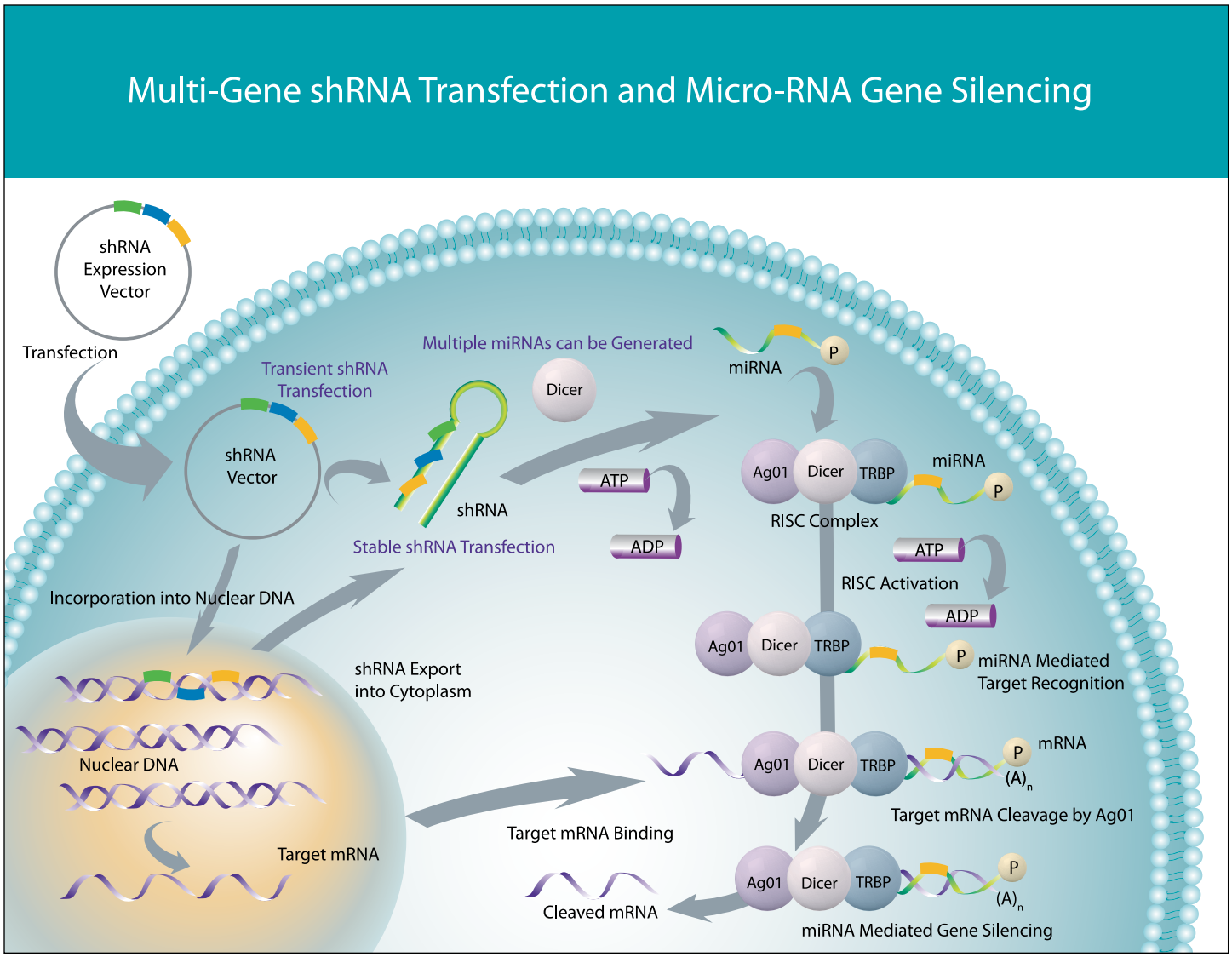


Figure 2. Multi-gene shRNA Transfection and miRNA Gene Silencing. The transfection of a cell is shown using the miR-shRNA vector system which can result in either a transient or permanent transfection model. The shRNA molecule is cleaved into a micro-RNA (miRNA) molecule by Dicer and then is bound by the RNA-induced silencing complex (RISC), ultimately resulting in specific gene silencing.

Cell Signaling, continued from page 11

sub-cloning of the cell signaling genes. Five different promoters (CMV, EF1, β-Actin, Ubi-c, MSCV LTR) are available and can be chosen for the entry vector. The AfCS vector platform can be effectively used with cell types that are difficult to transfect, such as primary cells. For this task, the miR-shRNA constructs can be easily transferred into a comprehensive range of viral vectors (e.g., lentiviral). The efficacy of miR-shRNA transfections and resulting RNA interference can be validated by co-expression with an AfCS fluorescent-tagged expression vector (available from ATCC) for the respective molecular target. The 52 multi-gene interference miR-shRNA vectors developed by AfCS are available individually from ATCC, or obtain the total vector collection in one 96-well microplate (ATCC® MBA-330).

AfCS Lentivirus Vector Platform

AfCS has developed a unique lentiviral vector platform, pSILK, which can be used for tightly regulated expression of miR-shRNAs. The pSILK (single lentivector for inducible knock down) vector works well with intractable cell lines and requires only a single viral infection for effective expression. The expression of miR-shRNAs using the pSILK vector is regulated by tetracycline. Transfected cells show rapid RNAi kinetics after exposure to doxycycline (DOX; a tetracycline antibiotic). Substantial target knockdown (>90%) is often observed after two days of DOX treatment. The RNA interference effect usually lasts longer than a week and can be readily reversed after DOX removal. The parent lentivector is available from ATCC with co-expression of either of three antibiotic selection genes or several fluorescent tags, such as YFP or green fluorescent protein. There are pSILK vectors available for many G protein isoforms coupled with either fluorescent or luminescent-based tags. The 34 vectors in this unique expression system developed by the AfCS are available individually from ATCC, or obtain the total vector collection in one 96-well microplate (ATCC® MBA-268).

The principle of RNA interference elicited by the multi-gene miR-shRNA vector system is shown in Figure 2. Briefly, the multi-gene miR-shRNA vector is transfected into cells where it can be used for either transient or permanent transfections. For a stable, permanent transfection, the miR-shRNA has to enter the cell nucleus and be integrated into chromosomal DNA, which often requires the usage of viral-based vectors. The double-stranded shRNA molecules are specifically cleaved by Dicer into single-stranded, 22-nucleotide long micro-RNA molecules (i.e., miRNA or miR). Separate, distinct miRNAs can be generated from a single multi-gene shRNA transcript. Dicer is a member of the RNAase III family of nucleases that cleaves double-stranded RNAs. Dicer has been shown to be recruited to the cellular RISC by TRBP (the human immunodeficiency virus transactivating response RNA-binding protein). After binding of the micro-RNA to the RISC, the complex undergoes activation leading to recognition and binding of the specific mRNA encoding the target molecule. At this point, Argonaute (Ago1), a key member of the RISC, plays a central role in the cleavage of the target mRNA and the resultant gene silencing. The multi-gene interference capability of the miR-shRNA vector platform makes it especially useful for blocking complex cellular mechanisms.

References

1. Zhu X, et al. A versatile approach to multiple gene RNA interference using microRNA-based short hairpin RNAs. BMC Molecular Biology 8.98 2007.
2. Shin K-J, et al. A single lentiviral vector platform for microRNA-based conditional RNA interference and coordinated transgene expression. PNAS 103(37) 13579-13764, 2006.

ASCB Annual Meeting

Join ATCC in San Diego, CA December 5-9 at the American Society of Cell Biology Annual Meeting. Stop by our **booth #1325** to discuss your assay planning needs, the latest additions to our Cell Biology collection, and how we can support your cell biology research.

Also, plan on attending the **Cell Line Workshop from 2:00 – 3:30 PM on Sunday, December 6, Room 28C:**

Development of a Consensus Standard for the Authentication of Human Cell Lines: Standardization of STR Profiling

- John RW Masters (Kings College London) will present a historical review of the misidentification of human cell lines and the impact it has on biomedical science.
- Yvonne Reid (ATCC) will review human cell line authentication methodologies and the selection of STR profiling as the recommended technology for the standard. The capabilities and limitations of STR profiling will also be addressed.
- Margaret Kline (National Institute of Standards and Technology) will discuss the STR profiling matching criteria used and the establishment and importance of a public cell line database for use by the research and development community to validate cell lines of interest.

Misidentification, continued from page 1

were negative or ambiguous. The investigators concluded that the results provided further evidence defining the origin of the derivative cell lines, MDA-MB-435s and MDA-MB-435-HGF, to melanocyte and not to breast.

In 2004, Sellappan and his colleagues[3] from M.D. Anderson Cancer Center showed interest in the contradictory published information. The two previous reports[1, 2] concluded that the MDA-MB-435 and its derivatives were melanocyte in origin; however, another report showed that MDA-MB-435 cells could produce milk lipid droplets when induced to differentiate.[4] Therefore, the M.D. Anderson Cancer Center embarked to confirm the previous findings. The investigators acquired breast and melanoma cell lines from commercial and private laboratories. Using Western blot analysis, they showed that the parental cell line, MDA-MB-435, expressed beta casein (breast epithelial cell–specific protein), alpha lactoalbumin, EMA (marker of mature phenotype), keratin 19 and pan keratin (epithelial-specific markers). There was no detectable level of keratin 8 level similar to other highly aggressive breast cancer cell lines. Western blot analysis and immunocytochemistry also confirmed that MDA-MB-435 expressed both of the melanocyte-specific markers, melan-A and tyrosinase. The xenograph stained positive for cytokeratin and EMA but only a small region stained positive for the HMB5 (melanocyte-specific marker) antigen. Most cells expressed tyrosinase at a lower level than the melanoma xenographs. In addition, both heregulin beta 1 and vitamin E were capable of inducing beta casein production in MDA-MB-435 cells.

Sellappan and his colleagues[3] maintain that the MDA-MB-435 cells originate from breast cancer. The plausible explanation for the presence of the melanocyte markers (melan-A and tyrosinase) is due to the "lack of adherence of the breast cell line MDA-MB-435 to establish strict adherence to the breast cell lineage." Dedifferentiation may account for the loss of differentiation phenotype characteristic of advanced tumors. This phenomenon has been previously described by Paine and colleagues.[5]

In 2007, Rae and his colleagues[6] showed that MDB-MB-435 was misidentified as the melanoma cell line, M14. In a series of experiments, including gene expression profile, single nucleotide polymorphisms (SNP) analysis, cytogenetic analysis (karyotyping), and DNA profiling, they demonstrated that the melanoma phenotype and genotype were due to misidentification with M14 cell line and not, as was previously described, because of "infidelity" due to dedifferentiation of the tumor.

These results, again, confirm the power of DNA profiling technology for human cell line identification; however, they also remind us that not only is there a need for standard protocols based on best practices, but also a need for a centralized database that is accessible to the scientific community.

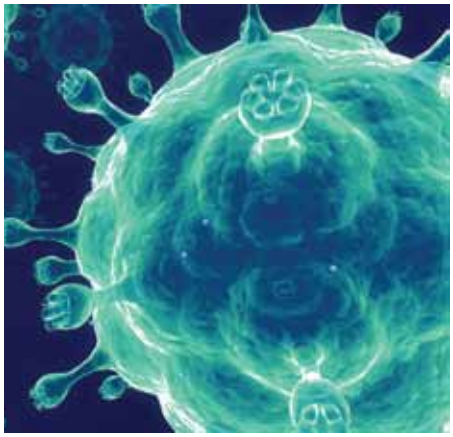
ATCC has distributed MDA-MB-435s, the spindle variant of MDA-MB-435, for more than 28 years. The DNA profiling analysis performed on MDA-MB-435s showed a unique DNA profile, but ATCC did not have the M14 melanoma cell line and would not have detected the misidentified cell line. Furthermore, Rae et al.[6] note that the misidentification of the MDA-MB-435 line occurred sometime prior to 1982, before MDA-MB-435s was deposited at ATCC.

How does ATCC handle misidentified cell lines? Typically, the authentication process at ATCC identifies misidentified cell lines before seed stocks are ever made—these cell lines never get distributed to the scientific community. However, there are rare occurrences, such as the case of MDA-MB-435s, whereby the misidentification is not found until years after the initial deposit. Although ATCC remains vigilant in combing the literature for other potential misidentified cell lines,[7] when a cell line is discovered to be misidentified after distribution to the scientific community, ATCC places the cell line "on hold" while a thorough investigation of the history of the cell line is performed. Once confirmed that the cell line is misidentified, a notification is placed on the ATCC web page, designated for misidentified cell lines (www.atcc.org/MisidentifiedCellLines/tabid/683/Default.aspx). A permit is subsequently placed on the item to halt its sale until the requesting researcher signs a disclaimer acknowledging notification of the questionable nature of the cell line.

References

1. Ross DT, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nat. Genet. 24(3):227-235, 2000.
2. Ellison G, et al. Further evidence to support the melanocytic origin of MDA-MB-435. J. Clin. Path. Mol. Pathol. 55:294-299, 2002.
3. Sellappan S, et al. Lineage infidelity of MDA-MB-435 cells: expression of melanocyte proteins in a breast cancer cell line. Cancer Res. 64:3479-3485, 2004.
4. You H, et al. RRR-alpha-tocopheryl succinate induces MDA-MB-435 and MCF-7 human breast cancer cells to undergo differentiation. Cell Growth Differ. 12:471-480, 2001.
5. Paine ML, et al. Intranuclear post-transcriptional down-regulation responsible for loss of a keratin differentiation marker in tumor progression. Anticancer Res. 15:2145-2154, 1995.
6. Rae MJ, et al. MDA-MB-435 is derived from M14 melanoma cells - a loss for breast cancer but a boon for melanoma research. Breast. Cancer Res. Treat. 104:13-19, 2007.
7. Hughes P, et al. The cost of using unauthorized over-passaged cell lines: how much more data do we need? Biotechniques 43(5): 575-586, 2007.

Genomic Viral DNA and RNA



Viral nucleic acids are now available from ATCC, including viral genomic material, in the form of DNA or RNA from infected cells or allantoic fluid. The package size is 100 µL per vial, dilutable 10-fold or more for amplification. Viral nucleic acids from ATCC can save you the time and expense of isolating DNA or RNA yourself. Applications include positive controls for PCR/RT-PCR, method development and other molecular virology applications. Viral nucleic acids from ATCC have been isolated under aseptic conditions to prevent cross-contamination. Batches are evaluated for integrity, purity and quality by several methods, including viral inactivation, suitability for amplification by PCR and determination of the total amount of the appropriate nucleic acid by PicoGreen® or RiboGreen® measurement.

DNA from Viruses and Chlamydiae

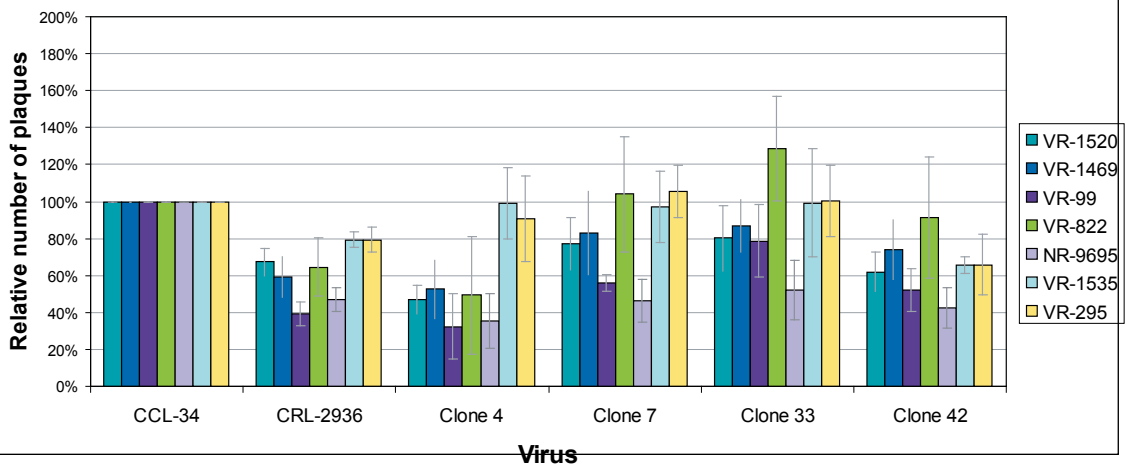
ATCC® No.	Source Organism	Designation	Significance
VR-1D	Human adenovirus 1	Adenoid 71	Species C
VR-846D	Human adenovirus 2	Adenoid 6	Species C
VR-847D	Human adenovirus 3	GB	Species B
VR-1572D	Human adenovirus 4	RI-67	Species E
VR-5D	Human adenovirus 5	Adenoid 75	Species C
VR-6D	Human adenovirus 6	Tonsil 99	Species C
VR-7D	Human adenovirus 7	Gomen	Species B
VR-1604D	Human adenovirus 8	TRIM	Species D
VR-12D	Human adenovirus 11	Slobitski	Species B
VR-863D	Human adenovirus 12	Huie	Species A
VR-15D	Human adenovirus 14	de Wit	Species B
VR-1109D	Human adenovirus 31	1315	Species A
VR-718D	Human adenovirus 35	Holden	Species B
VR-929D	Human adenovirus 37	GW (76-19026)	Species D
VR-931D	Human adenovirus 40	Dugan	Species F
VR-930D	Human adenovirus 41	Tak	Species F
VR-1603D	Human adenovirus 51	BOM	Species D
VR-539D	Human herpesvirus 1	MacIntyre	HSV-1
VR-1493D	Human herpesvirus 1	KOS	HSV-1
VR-540D	Human herpesvirus 2	MS	HSV-2
VR-734D	Human herpesvirus 2	G	HSV-2
VR-1367D	Human herpesvirus 3	Ellen	VZV
VR-538D	Human herpesvirus 5	AD-169	Viral genome has been sequenced
VR-977D	Human herpesvirus 5	Towne	HCMV
VR-878D	*Chlamydia trachomatis*	UW-57/Cx	
VR-886D	*Chlamydia trachomatis*	UW-36/Cx	Type J

RNA from Viruses

ATCC® No.	Source Organism	Designation	Significance
VR-26D	Human respiratory syncitial virus	Long	subsp. A
VR-955D	Human respiratory syncitial virus	9320	subsp. B
VR-1540D	Human respiratory syncitial virus	A2	subsp. A
VR-1580D	Human respiratory syncitial virus	18537	subsp. B

Influenza, continued from page 5

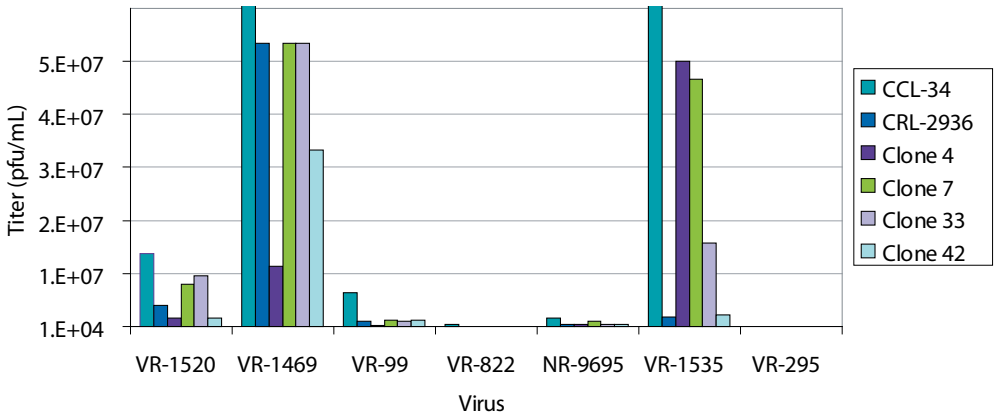
Figure 2. Relative plaquing efficiency of a wide range of influenza viruses in ATCC® CCL-34™ and its derivatives.



1 µg/mL TPCK-treated trypsin. At 3 days post-infection, all inoculated cultures showed extensive degeneration. Culture fluids were harvested, clarified by centrifugation and frozen until titered in ATCC CCL-34, the cell line observed to be most sensitive to infection. Harvest titers – surrogates for virus yield – are shown in Figure 3.

The only viruses that gave appreciable cell-free titers in any cell line were the cell culture-adapted strains of influenza A (ATCC® VR-1520™, ATCC VR-1469) and influenza B (ATCC® VR-1535™). Given variation in plaquing efficiencies no greater than about threefold, the differences of three logs or more in harvest titers between cell culture–adapted and egg grown viruses were unexpected. MDCK gave highest yields in all cases, with titers greater than $5X10^7$ pfu/mL achieved after infection with ATCC VR-1469 and ATCC VR-1535. Clones 7 and 33 were among the highest producers in all cases.

Figure 3. Virus yields from infection of different cell lines (all titrations completed in MDCK – ATCC® CCL-34™)



These results verify heterogeneity with respect to virus susceptibility within the ATCC CCL-34 population. Although not a clonal population, the parental cell line (ATCC CCL-34) supported influenza virus propagation the best, and of the clones, Clones 7 and 33 gave roughly equivalent plaquing efficiency and growth of cell culture-adapted viruses. Interestingly, these three top virus producers are also the three cell lines with the shortest doubling times among those tested (MDCK – 14 hours; Clone 7 – 15.5 hours; Clone 33 – 17 hours). This correlation between cell growth rate and virus production has not been previously described.

Influenza viruses are a heterogeneous group for which a universal optimal cell culture system will be hard to find. As a population, the MDCK cell line works well for many influenza types. Further experience with its clonal derivatives will show whether any single cell within it can provide the same broad-spectrum efficacy.

References

1. Petit L, Gilbert M, Gillet D, Laurent-Winter C, Boquet P, Popoff MR. Clostridium perfringens epsilon-toxin acts on MDCK cells by forming a large membrane complex. Bacteriol. 179(20):6480-6487, 1997.
2. Szymczakiewicz-Multanowska A, Groth N, Bugarini R, Lattanzi M, Casula D, Hilbert A, Tsai T, Podda A. 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. Nicholls JM, Bourne AJ, Chen H, Guan Y, Malik Peiris JS. 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.
5. Matrosovich M, Matrosovich T, Garten G, Klenk H-D. New low-viscosity overlay medium for viral plaque assays. Virology J. 3:63, 2006.

ATCC Hosts Cell Culture Symposium at AAPS

During the American Association of Pharmaceutical Scientists (AAPS) annual meeting in November, ATCC hosted a symposium that covered two very relevant topics in the field of cell culture: automated methods for the scale-up of mammalian cell lines and the importance of comprehensive testing of cell lines in research. The scope of a new ANSI-accredited international consensus standard for the authentication of human cell lines was also unveiled.

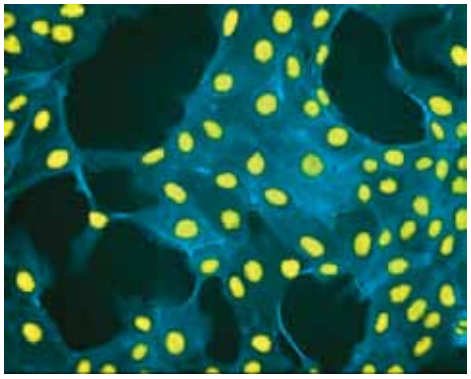
Abstracts of the presentations are presented below. To receive a PDF copy of the full presentations, please go to **www.atcc.org/aaps_preso.**

Automated Methods for Production Scale-up of Mammalian Cell Lines
Emily Osborn, Supervisor, Cell Biology Bioproduction

The SelecT™, an automated cell culture system created by The Automation Partnership (TAP), is being used to expand and maintain a large variety of cell lines at ATCC. This system, used in conjunction with an automated cryovial filler/capper/labeler system, Filamatic®, has revolutionized cell culture at ATCC. The cells produced and preserved with these automated processes are used in the industrial, commercial, medical and research fields. To date, ATCC has greater than 200 cell lines that can be propagated on two SelecT systems, with cell-line specific protocols written for each individual item produced. Some of these cell lines include: HeLa, human cervix epithelial (ATCC® CCL-2™), HCT 116, human colon epithelial (ATCC® CCL-24™), SK-N-SH, and human brain epithelial (ATCC® HTB-11™). These cell lines include suspension, adherent and mixed lines. The closed automation process of the scale-up of human and animal cell lines offers: increased consistency, reduction of technician-introduced contamination, cost reductions and increased capacity. At this time, the only step involving cell-human contact occurs when the ampoule is opened.

The Importance of Comprehensive Testing of Cell Lines in Research
Yvonne Reid, PhD, Collections/Research Scientist, Cell Biology

Propagation of animal cell lines is widely used by scientists in many diverse disciplines. Cell line authentication and characterization are crucial activities and yet are the most under-appreciated tasks performed by research scientists. The validity of conclusions drawn by researchers demands that consistent and unequivocal verification of cell line identity and function is precise. Yet over the years, many cell lines have been shown to be misidentified or cross-contaminated as advances in new technologies have led to more accurate authentication and characterization of cell lines. The financial loss due to these errors is estimated in the millions of dollars. Accurate cell line authentication requires a comprehensive strategy which employs multiple complementary tests including: testing for microbial contamination, testing for cellular identity and pedigree, confirming tissue-associated markers and identifying a unique characterization profile. An overview of the current technologies used to authenticate animal cell lines will be presented and the systematic accessioning process employed at ATCC will be reviewed. Finally, the stakeholders and scope of a new ANSI-accredited international consensus standard for the authentication of human cell lines will be discussed.



What's New *continued from page 9*

New Fungi

Among the 70 new acquisitions completed so far in 2009 in the Mycology collection, 27 are strains of genome sequencing projects. Many of these strains are medically important isolates or fungi having properties related to biomass conversion to biofuel. Another 13 strains are chosen for their unique positions in fungal evolution and phylogeny, as reflected in the NSF-funded AFTOL (Assembling the Fungal Tree of Life) project. We also accessioned approximately 20 type strains. A summary table is presented below.

ATCC® No.	Species	Notes
MYA-4568™	*Candida olivae* Y-1	Type strain
MYA-4577™	*Phytophthora aquatilis* 38J5	Type strain; plant pathogen
MYA-4578™	*Phytophthora aquimorbida* 40A6	Type strain; plant pathogen
MYA-4592™	*Candida blackwellae* AS 2.3639	Type strain; Insect associated yeast
MYA-4593™	*Candida jiufengensis* AS 2.3688	Type strain; Insect associated yeast
MYA-4594™	*Candida oxycetoniae* AS 2.3656	Type strain; Insect associated yeast
MYA-4595™	*Candida pseudojiufengensis* AS 2.3693	Type strain; Insect associated yeast
MYA-4574™	*Stagonospora nodorum* SN15	Genome sequencing strain; plant pathogen
MYA-4575™	*Verticillium dahliae* Ls17	Genome sequencing strain; plant pathogen
MYA-4576™	*Verticillium albo-atrum* Ms102	Genome sequencing strain; plant pathogen
MYA-4579™	*Rhizoctonia solani* Rhs 1AP	Genome sequencing strain; plant pathogen
MYA-4617™	*Magnaporthe grisea*	Genome sequencing strain; plant pathogen
MYA-4620™	*Fusarium graminearum*	Genome sequencing strain; plant pathogen
MYA-4622™	*Nectria haematococca*	Genome sequencing strain; plant pathogen
MYA-4623™	*Fusarium oxysporum* f. sp. *lycopersici*	Genome sequencing strain; plant pathogen
MYA-4604™	*Microsporum gypseum*	Genome sequencing strain; pathogenic to human and/or mammal (Dermatophyte)
MYA-4605™	*Microsporum canis*	Genome sequencing strain; pathogenic to human and/or mammal (Dermatophyte)
MYA-4606™	*Trichophyton tonsurans*	Genome sequencing strain; pathogenic to human and/or mammal (Dermatophyte)
MYA-4607™	*Trichophyton rubrum*	Genome sequencing strain; pathogenic to human and/or mammal (Dermatophyte)
MYA-4608™	*Trichophyton tonsurans*	Genome sequencing strain; pathogenic to human and/or mammal (Dermatophyte)
MYA-4609™	*Aspergillus fumigatus*	Genome sequencing strain; pathogenic to human and/or mammal (Aspergillosis)
MYA-4611™	*Malassezia restricta*	Genome sequencing strain; pathogenic to human and/or mammal (opportunistic Dermatophyte)
MYA-4612™	*Malassezia globosa*	Genome sequencing strain; pathogenic to human and/or mammal (opportunistic Dermatophyte)
MYA-4621™	*Rhizopus oryzae*	Genome sequencing strain; pathogenic to human and/or mammal (Mucormycosis)
MYA-4626™	*Agaricus bisporus* var. *bisporus*	Genome sequencing strain; edible mushroom
MYA-4627™	*Agaricus bisporus* var. *burnettii*	Genome sequencing strain; edible mushroom
MYA-4596™	*Dekkera bruxellensis* Y879	Genome sequencing strain; stress tolerant, bioethanol production candidate
MYA-4618™	*Coprinopsis cinerea*	Genome sequencing strain; mushroom used as model organism
MYA-4614™	*Neurospora crassa*	Genome sequencing strain; used as a model organism in molecular genetics
MYA-4615™	*Neurospora tetrasperma*	Genome sequencing strain; used as a model organism in molecular genetics
MYA-4616™	*Neurospora discreta*	Genome sequencing strain; used as a model organism in molecular genetics
MYA-4619™	*Neurospora crassa*	Genome sequencing strain; used as a model organism in molecular genetics
MYA-4624™	*Podospora anserina*	Genome sequencing strain; used as a model organism in molecular genetics
MYA-4625™	*Podospora anserina*	Genome sequencing strain; used as a model organism in molecular genetics
MYA-4610™	*Malassezia obtusa*	Pathogenic to human and/or mammal (opportunistic Dermatophyte)
MYA-4613™	*Malassezia slooffiae*	Pathogenic to human and/or mammal (opportunistic Dermatophyte)
MYA-4633™	*Insolibasidium deformans* DJM 183	Reference strain for AFTOL project; plant pathogen
MYA-4638™	*Sporisorium scitamineum* MCA 3649	Reference strain for AFTOL project; plant pathogen
MYA-4640™	*Testicularia cyperi* MCA 3645	Reference strain for AFTOL project; plant pathogen
MYA-4628™	*Agaricostilbum hyphaenes* RJB 75 6661-A9	Reference strain for AFTOL project
MYA-4629™	*Agaricostilbum pulcherrimum* F220	Reference strain for AFTOL project
MYA-4630™	*Dioszegia changbaiensis* MCA 3694	Reference strain for AFTOL project
MYA-4631™	*Heterogastridium pycnidioideum* RJB 75-845L	Reference strain for AFTOL project
MYA-4632™	*Helicogloea variabilis* KW 1540 [T-73]	Reference strain for AFTOL project
MYA-4634™	*Sporobolomyces beijingensis* MCA 3740	Reference strain for AFTOL project
MYA-4635™	*Septobasidium burtii* ECS 7 [MIN 878588]	Reference strain for AFTOL project
MYA-4636™	*Septobasidium canescens* ECS 542 [#10]	Reference strain for AFTOL project
MYA-4637™	*Sporobolomyces koalae* WRP 19	Reference strain for AFTOL project
MYA-4639™	*Stilbum vulgare RJB 75-9295D*	Reference strain for AFTOL project

New Viruses

New tissue-culture adapted products add flexibility to ATCC's human- and swine-derived influenza collection.

ATCC® No.		Description
TC-adapted Version	Chicken Embryo Version	
VR-1679™	VR-544™	Influenza A Virus Hong Kong/8/68
VR-1680™	VR-547™	Influenza A Virus Aichi/2/68
VR-1681™	VR-897™	Influenza A Virus New Jersey/8/76
VR-1682™	VR-99™	Influenza A Virus Swine/1976/31
VR-1683™	VR-333™	Influenza A Virus Swine/Iowa/15/30

Attend a Cell Culture Webinar

You're invited to a series of 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. The following online seminar will be presented on the Corning website:

Surface Treatments for Fastidious Cells, presented by John So, MS, Field Applications Scientist, Corning Life Sciences

December 15, 12:00 noon – 1:00 p.m. EST
December 17, 9:00 a.m. – 10:00 a.m. EST

Many variables can influence the cell culture environment and need to be addressed for proper optimization. In this seminar, some of these variables will be discussed, with a focus on cell culture surfaces. The majority of attachment-dependent mammalian cell culture are performed on polystyrene. Therefore, a general overview of different surface treatments will lead into a discussion of why cells attach to some surfaces better than others and how this can influence cellular functionality. Lastly, tips and suggestions will be provided for working with fastidious cells.

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.

Web Tip

Want to quickly find the information that interests you on the ATCC website?

Try the **'Site Search'** function located at the bottom-right of all ATCC website pages. Just enter the keyword or phrase of interest and click **'Go.'** The resulting page will be a list of ATCC website pages and documents that contain your words (highlighted), with brief descriptions and links to the full content.

Note: This search does not include data that are contained in our online catalog. To search the online catalog, use the **'Search Catalog'** function, located at the top-right of all ATCC web pages.

ATCC Homepage

Tech Q

From time to time, ATCC Technical Service 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, addresses the general subject of getting answers to your technical questions.

Q: How can I get quick answers to my technical questions?

Use our new FAQ tool at **www.atcc.org**!

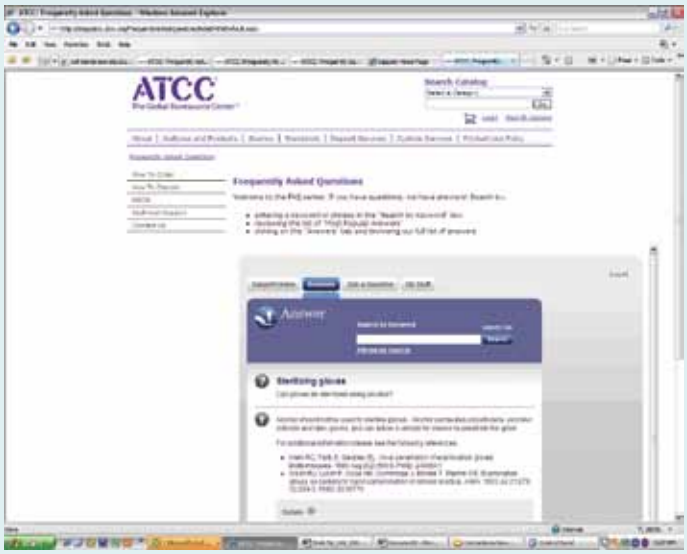
ATCC is pleased to announce the launch of a brand new self-learning knowledge base, designed to provide faster answers for the questions you ask most. We cordially invite you to visit our new FAQ center by going to **www.atcc.org** and then clicking on the 'FAQ' link at the bottom of the page. Search this easy-to-use application for everything you want to know about ATCC cultures and products, including:

- ✓ Animal and microbial cell culture techniques
- ✓ Culture medium formulations
- ✓ Cryopreservation and storage
- ✓ Cell culture terminology
- ✓ And much more…

The FAQ center includes a practical interface allowing users to create their own personal profiles. If you don't find the answer you need, just log in to your profile and e-mail your questions to ATCC Technical Services. We'll respond directly to you and if the answer has the potential to help others, it will also be posted to the FAQ knowledge base.

Once you've had a chance to check out our FAQ center, please let us know what you think! Contact ATCC Technical Services through your FAQ log-in, or send an e-mail to **Tech@atcc.org**. We look forward to hearing from you!

FAQ Center Homepage



Example Answer Page

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Meetings and Conferences

ATCC will be attending the following key events. Stop by and talk to an ATCC representative.

AACR — American Association for Cancer Research
April 17–21, Washington, DC

ASM — American Society for Microbiology General Meeting
May 23–27, San Diego, CA