ATCC

APPLICATION NOTE

EXPLORING THE TRANSCRIPTIONAL LANDSCAPE OF HEK CELL LINES VIA ATCC CELL LINE LAND

Ajeet P. Singh, PhD; Amy L. Reese, MS; Rula Khairi, MS; Corina Tabron, MS; Noah Wax, MS; Jade L. Kirkland, MS; Robert Marlow, BS; James Duncan, BS; Steve King, MS; Ana Fernandes, BS; Briana Benton, BS; John Bagnoli, BS; Jonathan L. Jacobs, PhD
ATCC, Manassas, VA 20110
Correspondence: asingh@atcc.org, jjacobs@atcc.org

ABSTRACT

The lack of trusted genomic data from authenticated biological materials remains a persistent challenge, often leading to irreproducibility and inaccurate scientific conclusions when relying on public domain data. To address this, ATCC® is conducting next-generation sequencing (NGS) analysis to define the molecular characteristics of authenticated cell lines within our biorepository and making those data available to the scientific community. As part of this effort, we present here the gene expression analyses between ATCC's authenticated HEK-293 parental cells and 9 derivative cell lines. Our analyses revealed distinct shifts in expression patterns that intimately influence cellular component organization, motility, and adhesion processes. Deviations in gene expression between adherent and suspension derivatives offer insights into cholesterol biosynthesis and key gene modulation. Furthermore, we highlight substantial genetic differences between the parental HEK-293 cell line and a STAT1 and BAX CRISPR-edited double knockout HEK-293 cell line that significantly impacts cell proliferation, apoptosis, and viral production. Our findings underscore the pivotal role of RNA sequencing in unlocking the latent potential of human and mouse cell lines used in drug development, biotherapeutics production, and viral vector production, paving the way for future advancements in biotechnological applications.

INTRODUCTION

It's estimated that over 20% of all experiments use incorrect cell lines, potentially leading to skewed results, incorrect conclusions, and wasted time and resources.[1] This problem stems from a range of issues including cross-contamination, sample misidentification, labeling errors, lab-adaption, and genetic drift from undocumented long-term passaging.[2] In response to this challenge, many scientific journals and cell line repositories have mandated cell line authentication.[3-6] Surprisingly, less than half of all researchers routinely verify the authenticity of their cell lines.[2] Despite various measures taken to rectify cell line misidentification,[3,7,8] the prevalence of irreproducibility remains a major concern in life science research. A 2015 paper reported an estimated spending of USD $56.4 billion on preclinical research with a cumulative irreproducibility rate of about 50%, resulting in approximately USD $28 billion per year spent on non-replicable research. Several factors contribute to this issue, with poor biological reagents and reference materials (including genomic data) identified as significant contributors.[1]

Ensuring the use of authenticated cell lines is crucial for addressing the issue of irreproducibility in research. As a leader in cell line authentication, ATCC employs gold-standard methods to verify the identity of our cell lines. Taking a step further, ATCC provides comprehensive whole-transcriptome data for our human and mouse cell lines through ATCC Cell Line Land. This dataset is invaluable as it offers a complete gene expression profile, eliminating molecular ambiguities. Utilizing RNA-seq methodology, which generates high-throughput

quantitative datasets, allows for the visualization and analysis of transcript expression within cells. Beyond its foundational role, RNA-seq facilitates various analyses including differential gene expression, variant detection, and profiling of all RNA species produced in cells, among others. These capabilities significantly contribute to our understanding of how transcriptome sequencing serves to provide a molecular profile unique to each cell line model. [9,10,11]

In this use-case study, we demonstrate the value and functionality of ATCC Cell Line Land as a resource for transcriptomics data analyses by utilizing its bioinformatic capabilities to analyze RNA-seq data from authenticated HEK-293 parental cells and selected derivative cell lines. Here, we include the comparative expression patterns of genes, identifying key differentiator pathways that offer a unique molecular identity for each specific cell line.

MATERIALS & METHODS

CELL CULTURE

All cell lines mentioned in this study (Table 1) were obtained directly from ATCC's repository. Multiple biological replicates (n=5) of HEK-293 parental and derivative cell lines were cultured adhering to ISO 9001 standards and following ATCC process parameters tailored to each cell line. Cells were harvested during the logarithmic growth phase at 70-80% confluency and were subsequently pelleted by centrifugation at 150-450 x g.

Table 1: HEK-293 and derivatives used in this study

Designation	ATCC® No.	Culture
HEK-293	CRL-1573™	Adherent
293 c18	CRL-10852™	Adherent
293/CHE-Fc	CRL-2368™	Adherent
293T	CRL-3216™	Adherent
293T/17 [HEK 293T/17]	CRL-11268™	Adherent
293TT	CRL-3467™	Adherent
HEK 293 STF	CRL-3249™	Adherent
HEK-293.2sus	CRL-1573.3™	Suspension
HEK293S GnTI	CRL-3022™	Adherent
293.STAT1 BAX KO	CRL-1573-VHG™	Adherent

RNA EXTRACTION AND QUALITY CONTROL (QC)

RNA isolation was performed using the QIAcube® automated system with the RNeasy® Mini QIAcube® Kit (QIAGEN®). Frozen samples were thawed and prepared for RNA extraction according to ATCC's work instructions. Extracted samples were tested for RNA purity and RNA quality using the Thermofisher® Nanodrop™ (A260/A280 1.8 ≥ x ≤ 2.2) and Agilent® Tapestation™ (RNA integrity number (RIN) ≥ 6.5), respectively.

RNA-SEQ LIBRARY PREPARATION AND SEQUENCING

Automated RNA-seq NGS library preparation was performed on the Eppendorf® epMotion® 5075 Liquid Handler using the Illumina® Stranded mRNA Prep, Ligation kit. Prepared NGS libraries were assessed by quantitative analysis using the Invitrogen™ Qubit™ dsDNA High Sensitivity Assay Kit and qualitative analysis using the Agilent® 4200 TapeStation™ and D5000 ScreenTape® System. Libraries were loaded on an Illumina® P3 200-cycle Reagent kit and sequenced on the NextSeq® 2000 platform.

RNA-SEQ DATA QUALITY CONTROL (QC)

Raw sequencing reads were corrected for broken read pairs as needed with bbmap's repair.sh (<5% of samples) and trimmed with fastp to remove any lingering adapter sequences or extraneous poly-g tails. Trimmed reads were then mapped to the RefSeq human genome reference GRCh38.p14 with the STAR mapping software utilizing its options for 2-pass mapping for sensitive novel junction discovery. The output logs from STAR were used to ensure ≥ 18M input reads and ≥ 70% uniquely mapped reads via a custom R script.

Following the sequencing quality control process, the potentially repaired read FASTQ files were sent to QIAGEN® Digital Insights for gene expression analysis and then made available through the ATCC Cell Line Land database.

BIOINFORMATICS ANALYSIS

Gene expression analysis was performed using ATCC Cell Line Land, a database accessible within the QIAGEN® OmicSoft bioinformatics portal that contains curated and authenticated human and mouse transcriptomes and exomes from ATCC cell lines. Molecular pathways and gene network analysis of the differentially expressed genes were conducted using QIAGEN® Ingenuity Pathway Analysis (IPA)®.

RESULTS

As a part of our ongoing efforts to characterize the molecular identities of ATCC's human and mouse cell line collection, whole-transcriptome sequencing has been completed on more than 60 human kidney cell lines and over 10 mouse kidney cell lines encompassing a diverse array of disease conditions. In this report, we demonstrate the application of ATCC Cell Line Land, a bioinformatics portal of ATCC's cell lines curated, authenticated data to visualize the baseline abundance of gene expression across human embryonic kidney (HEK) cell lines including the parental HEK-293 cell line and selected clonally derived daughter cell lines. This analysis enables us to understand genetic drift among the HEK cell lines and the inherent molecular identity that shapes cellular behavior and biological functions.

TRANSCRIPTIONAL DISCREPANCIES BETWEEN HEK CELL LINE VARIANTS

The easy growth and high transfectability of HEK cells have spurred the development of newer variants with enhanced biological features; these variants can grow either in suspension or adherently. One adherent cell line of interest is 293 c18 (ATCC® CRL-10852™), which was derived from parental HEK-293 (ATCC® CRL-1573™) cells through the introduction of the Epstein-Barr Virus Nuclear Antigen 1 (EBNA1) gene. 293 c18 has gained attention in research for its extensive use as an in vitro model, enabling a wide range of cellular, molecular, and biochemical investigations.[12,13] The heatmap in Figure 1 highlights the top 50 differentially expressed genes (DEGs) in 293 c18 cells as compared to parental HEK-293 cells, demonstrating significant variation likely linked to cellular transformation and viral replication. Moreover, genetic modifications and bioprocess enhancements have influenced global gene expression patterns across other derivative cell lines (Figure 1).[14] Functional validation confirmed differential enrichment of the indicated candidate genes in the specified suspension (HEK293.2sus) and various adherent (HEK-293, parental and its derivatives) cell lines (Figure 2), underscoring the impact of culture conditions and molecular modifications on gene expression.

Notably, the HEK293.2sus suspension cell line demonstrated significant increases in the expression of ANGPT2, KLF18, CPHXL, and FRG2B when compared to adherent HEK-293 parental and derivative cell lines (Figure 1, 2). ANGPT2, a growth factor closely related to Angiopoietin 1, plays a crucial role in angiogenesis, influencing endothelial cell survival, proliferation, migration, adhesion, cell spreading, and more. It accomplishes this by binding to and activating the TEK/TIE2 receptor and downstream kinases MAPK1/ERK2 and MAPK3/ERK1.[15]

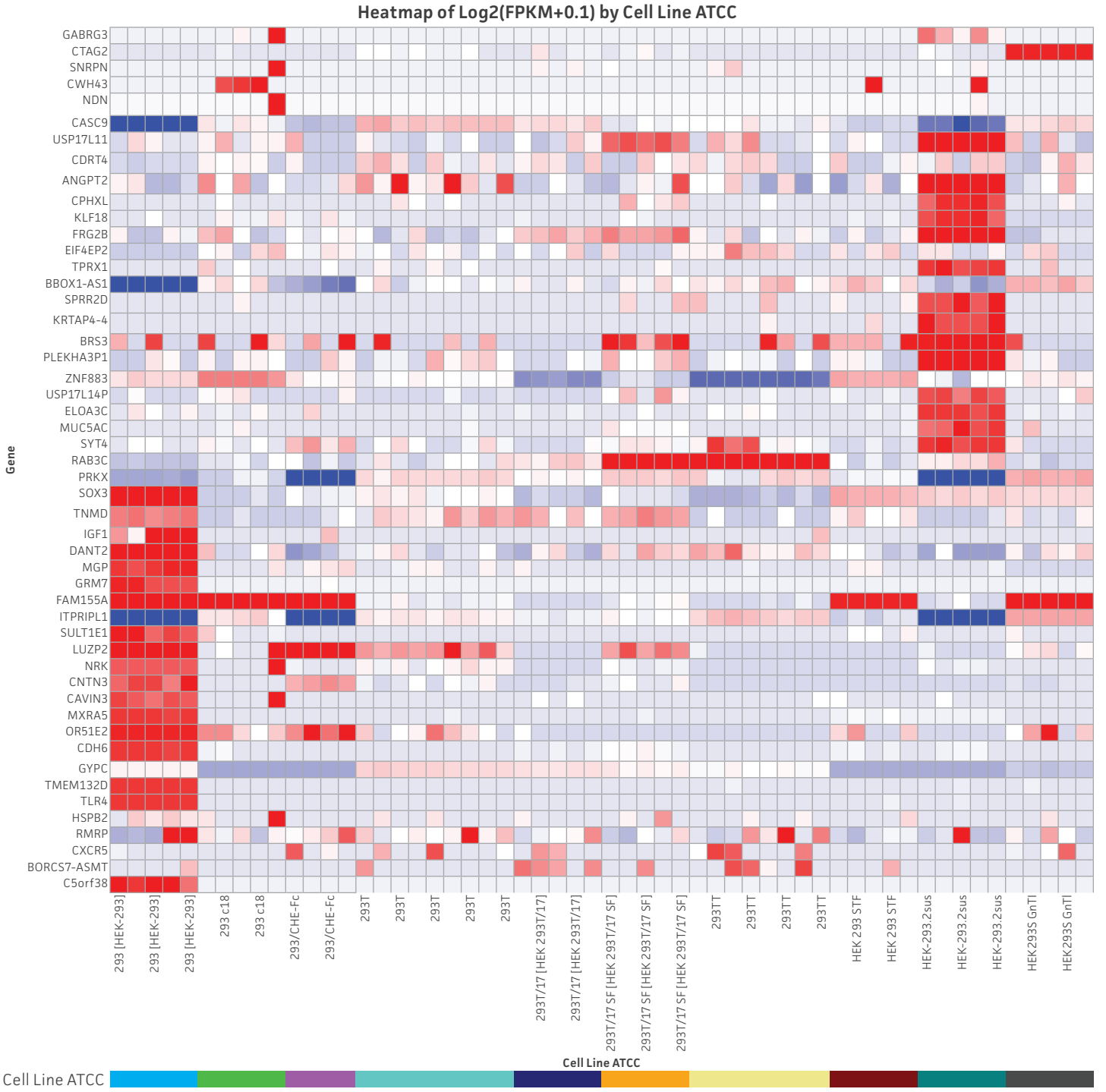

Figure 1: Pattern of genes differentially expressed in HEK-293 cell lines. The heatmap illustrates the expression pattern of the top 50 genes (y-axis) that are differentially expressed in the parental HEK-293 cells and selected derivative HEK cell lines (x-axis), with multiple biological replicates as indicated by the multicolored bar beneath the heatmap. The color code of the heatmap is as follows: red = induced expression, blue = reduced expression.

Similarly, CPHXL and KLF18 were significantly induced in the suspension cell line compared to adherent cell lines (Figure 1, 2). These genes are involved in the regulation of transcription by RNA polymerase II. Furthermore, FRG2B, which controls genes promoting cell survival and proliferation, was also induced in the suspension cells relative to the adherent cells. The activation of these genes indicates that suspension cells have evolved to possess a superior capacity for cell viability and proliferation, making them particularly well-suited for mass production and propagation as compared to adherent cells.

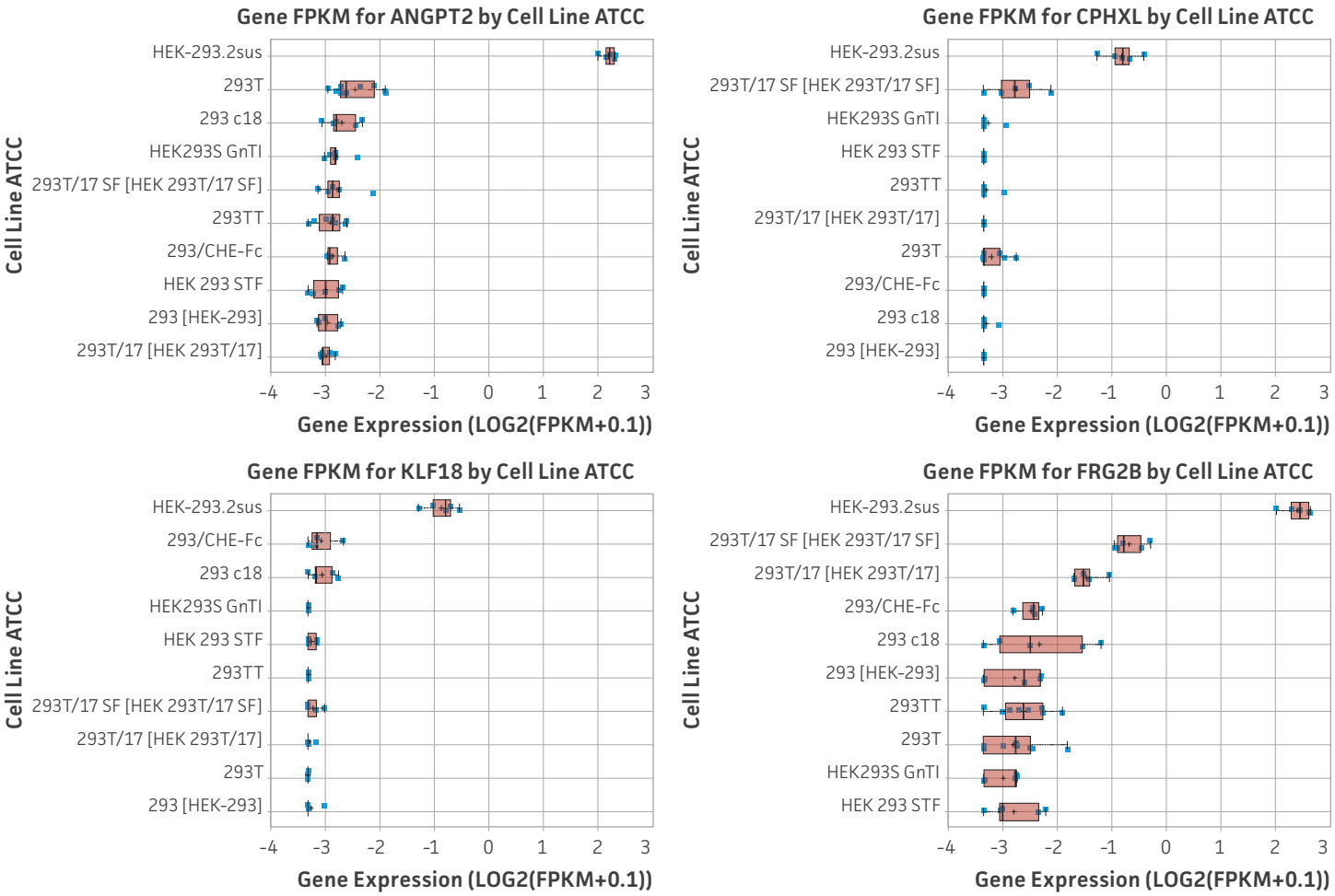

Figure 2: Basal levels of endogenously expressed genes in specified HEK cell lines. The box plots display the fragments per kilobase million (FPKM) normalized read counts of ANGPT2 (top left), CPHXL (top right), KLF18 (bottom left), FRG29 (bottom right) in the cell lines displayed along the y-axis of the plots.

Subsequently, we conducted QIAGEN® Ingenuity Pathway Analysis® (IPA) on the genes that displayed differential expression in the derivative HEK293.2sus (suspension) versus parental HEK-293 (adherent) cell lines. IPA provided insight into the central pathways and upstream regulators likely responsible for orchestrating the observed changes in gene expression within the DEG datasets.

Remarkably, the HEK-293 parental cell line showed activation of the Pathogen-Induced Cytokine Storm Signaling Pathway, which emerged as one of the top dysregulated pathways (Figure 3A). This pathway possesses the intriguing feature of negative interaction with innate immune molecular networks, which are involved in limiting immunity and resisting susceptibility to pathogenic infections (Figure 3B).

Delving deeper, we explored the upstream genes that were likely activating or repressing the target genes in the suspension cell line. Notably, IPA unveiled the induction of TNF, which, in turn, activated the genes NFKB1, RELA, STAT1, and IFNG in addition to others that were upregulated among the DEGs (Figure 3C). TNF is a proinflammatory gene known for its role in inducing chronic inflammation, which can result in cellular transformation and potentially contribute to the development of cancer. However, it's important to note that TNF also plays a context-dependent role in immunogenic cell death. For instance, many genes, including TNF, indirectly trigger the activation of IFNG, which subsequently directly activates downstream genes such as STAT1 and NFKB1 thus influencing complex immune and cellular processes (Figure 3C). To gain insights into the causal network associated with dysregulated gene expression patterns, we turned our attention to the networks identified through IPA. Notably, glucocorticoid emerged as one of the leading candidates on the list of IPA-produced causal networks strongly associated with the altered gene expression patterns in HEK293.2sus cells (Figure 3D).

A

Top Canonical Pathways

Name	p-value	Overlap	
Pathogen Induced Cytokine Storm Signaling Pathway	2.02E-08	14.3 %	53/371
Granulocyte Adhesion and Diapedesis	2.68E-08	18.0 %	34/189
Agranulocyte Adhesion and Diapedesis	1.22E-07	16.7 %	35/210
Phagosome Formation	1.28E-07	11.5 %	80/695
S100 Family Signaling Pathway	6.49E-07	10.9 %	84/771

1 2 3 4 5 6 7 8 9 >

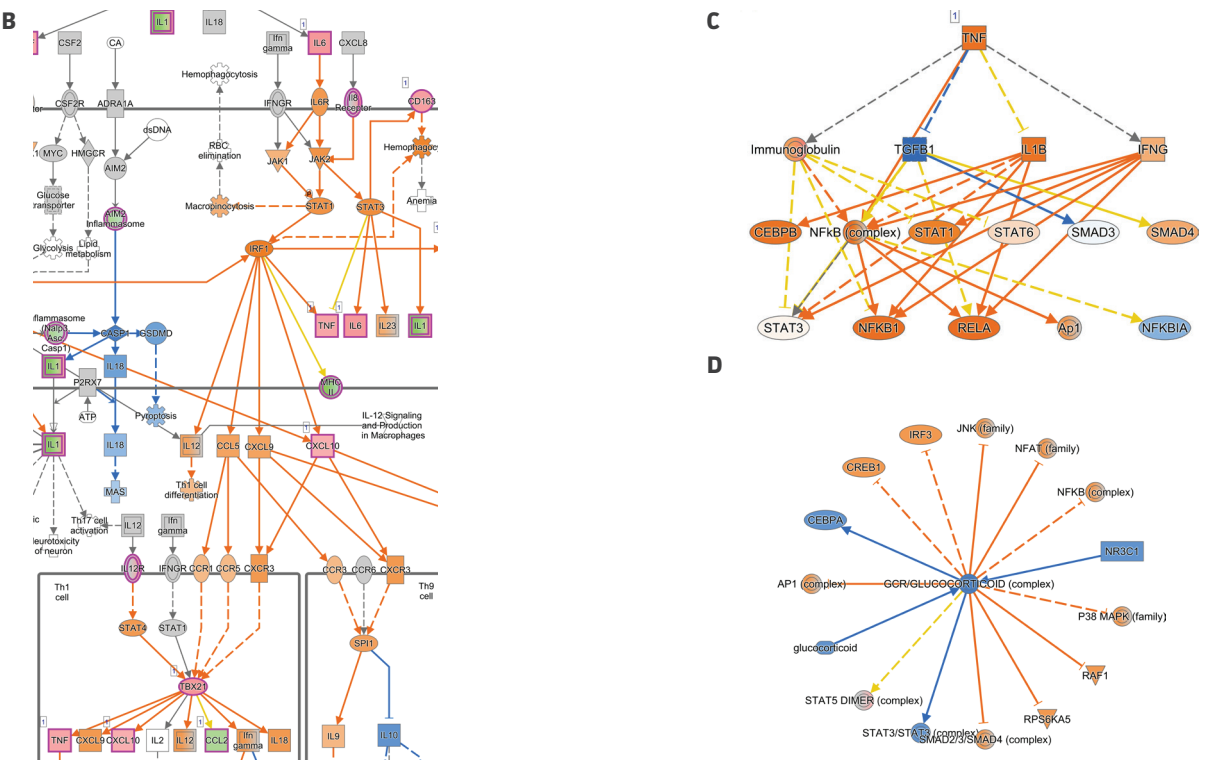

Figure 3: IPA of genes differentially expressed in the parental HEK-293 cells. The IPA results highlight some of the key dysregulated pathways, upstream regulators, and causal networks that exert control over the expression of genes seen in our processed datasets. (A) The Pathogen-Induced Cytokine Storm Signaling (PICSS) Pathway ranked at the top of the list of dysregulated pathways. (B) Within PICSS pathway, several molecules were found to be activated. (C) The gene network analysis shows activation of TNF as an upstream regulator controlling downstream genes in the dataset related to the innate immune system, which prevents viral infections. (D) Causal network analysis reveals the repression of Glucocorticoid/GCR, leading to subsequent changes in direct and indirect target genes. Color legends applied to all figure panels: orange = activated, blue = inactivated, purple = induced, green = repressed, grey/white color = activity not reported.

The activation of immune-related genes within the HEK cell lines offers intriguing possibilities for the development of new derivative cells optimized for high-titer viral production. In combination, our bioinformatics analysis unveiled intricate gene regulatory networks involved in various molecular and cellular functions associated with known diseases and disorders. These findings shed light on the molecules that should be considered when selecting HEK cell lines to produce exogenous proteins or viruses for pharmaceutical and biomedical research purposes.

293.STAT1 BAX KNOCK-OUT VS. PARENTAL HEK-293 CELL LINES

When the STAT1 and BAX genes are activated, they play vital roles in various biological processes like innate immune response, cell survival, and apoptosis.[16,17] In a previous internal study at ATCC, we utilized CRISPR/Cas9 gene-editing technology to knock out these genes in the HEK-293 parental cell line, resulting in the development of the 293.STAT1 BAX KO (ATCC® CRL-1573-VHG™) cell line. A viral transfection assay was conducted previously to assess the effects of the BAX and STAT1 deletion on viral production. Notably, the results indicated a higher viral infection rate with greater dilution in the 293.STAT1 BAX KO cells as compared to the wild-type HEK-293 parental cells. The finding of an increased rate of viral infection was subsequently confirmed by imaging and Droplet Digital™ PCR (ddPCR™; Bio-Rad

Laboratories), showing an increased number of GFP+ cells and copy numbers, respectively. These results highlight the positive impact of the loss of STAT1 and BAX on viral production.

We conducted whole-transcriptome profiling to elucidate the transcriptional changes mediated by the loss of BAX and STAT1 in HEK-293 cells. Subsequently, we used ATCC Cell Line Land to facilitate the analysis of genes differentially expressed in 293.STAT1 BAX KO cells as compared to the parental HEK-293 cells. The results of our analysis revealed that the STAT1 BAX double knockout led to the differential expression of 4,316 genes (Figure 4A). Notably, we identified a substantial depletion (green) of STAT1 and BAX mRNA levels in the KO cells as compared to that of the parental cells (Figure 4B). Specifically, STAT1 and BAX mRNA levels decreased by more than 5-fold and 2.5-fold, respectively, in the 293.STAT1 BAX KO cells compared to that of wild type (WT) parental HEK-293 cells (Figure 4C, 4D).

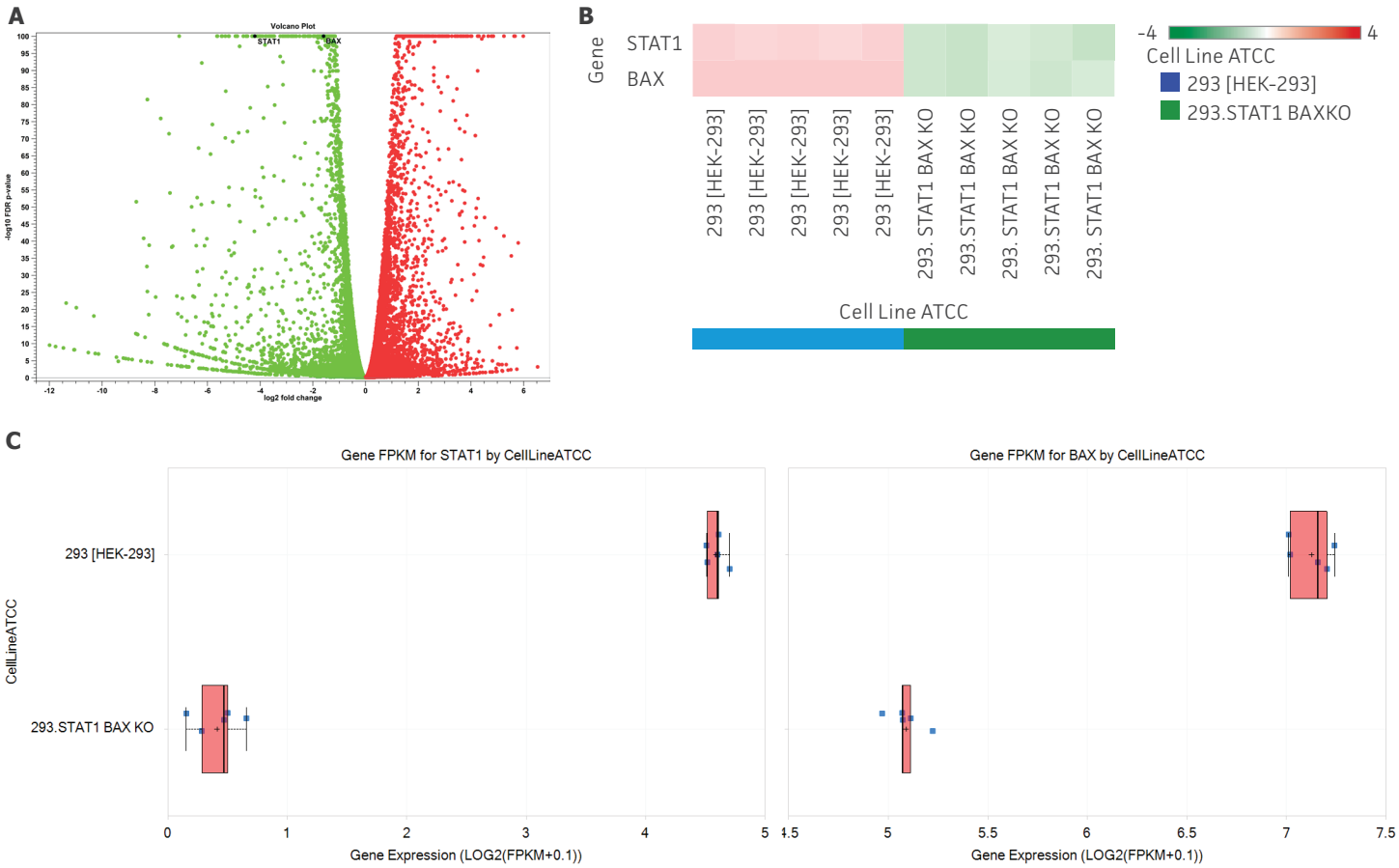

Figure 4: Visualization of DEGs in the 293.STAT1 BAX KO cells as compared to parental HEK-293 cells. (A) The volcano plot illustrates genes that are differentially regulated in 293.STAT1 BAX KO cells as compared to parental HEK-293 cells. (B) The heatmap demonstrates the expression patterns of STAT1 and BAX in 293.STAT1 BAX KO and parental HEK-293 cells. (C, D) The box plots present the quantitative levels of (C) STAT1 and (D) BAX in each cell line.

Further examination of the RNA-seq data revealed that the list of differentially expressed genes were evenly distributed between upregulated and downregulated genes, and that these genes were involved in various biological processes. Collectively, these results provide strong validation that the STAT1 signaling pathway controls genes with diverse activities (Figure 4A). STAT1 is a component of the transcriptional activator interferon-stimulated gene factor-3, consisting of STAT1, STAT2, IRF9, STAT3, STAT4, STAT5a, STAT5b, STAT6, and IRF9, which are linked with JAK family genes.[16] Initially, we investigated the expression patterns and relative abundance of JAK1, JAK2, TYK2, and JAK3. While JAK1, JAK2, and TYK2 appeared decreased and JAK3 remained unchanged in the KO cells (Figure 5), a quantitative assessment revealed minimal or negligible changes in their relative mRNA levels (data not shown). Similarly, we observed differential expression in genes such as STAT3, STAT4, STAT5A, STAT5B, and STAT6 (Figure 5); however, a quantitative comparison revealed that these differences translated into only minor fold change alterations in their relative expression levels (data not shown). Thus, the inactivation of the STAT1 gene did not significantly affect the expression of STAT/JAK family members.

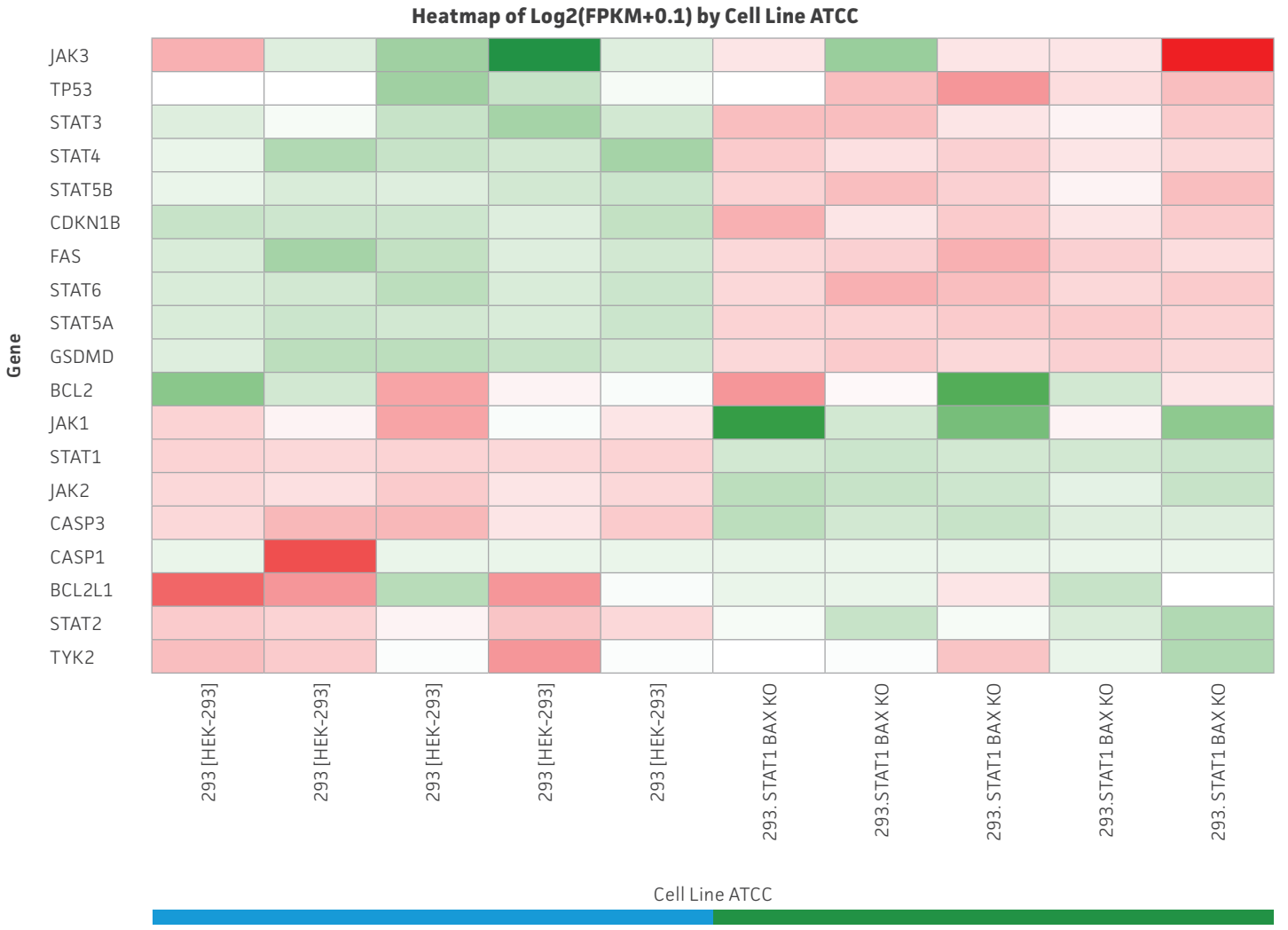

Figure 5: Visualization of specified genes and down regulated pathways in the 293.STAT1 BAX KO cells as compared to HEK-293 cells. The heatmap illustrates the expression patterns of candidate genes that regulate immune response, cell survival, and cell death in both 293.STAT1 BAX KO cells as compared to the HEK-293 parental cells. The color code of the heatmap is as follows: red = induced expression, green = reduced expression.

Moving forward, we examined genes responsible for controlling cell survival and cell death, noting distinctive expression patterns. Yet, their relative mRNA levels remained largely unchanged or showed only slight variations. Next, we processed the differential gene expression data using IPA and discovered key pathways. Interestingly, we found that the knockout of STAT1 and BAX resulted in the inactivation of pathways that had been activated in HEK-293 and other derivative cell lines (data not shown). IPA suggests that STAT1 is an upstream regulator of the Pathogen-Induced Cytokine Storm Signaling Pathway, which controls innate immune signaling and the response to pathogenic invasion. Altogether, RNA-seq analysis revealed that the inactivation of STAT1 and BAX attenuated the expression of genes and pathways that negatively control viral replication and cell proliferation.

CONCLUSION

In this study, we've highlighted the significant advantages offered by the newly developed ATCC Cell Line Land, which houses RNA sequencing data from ATCC's authenticated human and mouse cell lines. This resource allows for the rapid analysis of global gene expression patterns and provides a quantitative assessment of gene choices across the ATCC cell lines designed for various applications.[2,18,19]

We demonstrate the value of our transcriptomics data by utilizing ATCC Cell Line Land to explore the molecular characteristics of highly valued cell lines. Our initial undertaking involved comprehensive RNA sequencing of all kidney tissue cell lines housed in the ATCC biorepository, encompassing 25 derivatives of the HEK-293 cell line. HEK-293 cells are widely utilized in research and development due to their exceptional traits, such as a high transactivation rate, rapid growth, and the ability to thrive in a serum-free, suspension culture environment.[23,24] Most notably, their human origin makes them ideal for producing biologics intended for human use.

We compared gene expression patterns within the HEK-293 cell line and its various subtypes, shedding light on the baseline expression levels of genes, patterns of gene expression, and the relative abundance of key factors/pathways. Additionally, we produced a

quantitative assessment of the choice of genes and signaling pathways related to controlling specific biological attributes (cell proliferation, viability, death, etc.), which can be used as a reference standard for ensuring quality and enhanced experimental reproducibility.[20,25,26,14,27] This provided insight into the intrinsic activities of genes in controlling cellular behavior, aiding in the understanding of positive and negative correlations among gene sets. This comprehensive information guides the selection of the most suitable cell line for applications of functional studies.

Overall, we aimed to showcase ATCC Cell Line Land as a potent tool for hypothesis-free discoveries and as a reference data provider for authenticated cell lines. It aids in understanding disease mechanisms as well as predicting drug responses and resistance, drug toxicity and efficacy, pharmacokinetics, and more. Moreover, it includes complete data provenance, the availability of this resource empowers researchers to make informed decisions when selecting cell lines for their projects. This decision-making process is grounded in a comprehensive understanding of both global and candidate gene expressions.[20,21,22] Unlike publicly available transcriptomics data from various laboratories worldwide, ATCC Cell Line Land's authenticated transcriptomic data are highly reliable and traceable to the biological materials stored in the repository. Moreover, they come with complete data provenance and the entire cell culture and sequencing data production process adheres to ISO 9001 guidelines, instilling a high level of confidence and scientific rigor in research. Considering these advantages, researchers can confidently leverage these authenticated transcriptomics data to inform their cell line selection process.

REFERENCES

1. Freedman LP, Cockburn IM, Simcoe TS. The Economics of Reproducibility in Preclinical Research. PLoS Biol 13(6): e1002165, 2015. PubMed: 26057340.
2. Capes-Davis A. et al. Cell Lines as Biological Models: Practical Steps for More Reliable Research. Chem Res Toxicol 32(9): 1733-1736, 2019. PubMed: 31203605.
3. Almeida JL, Cole KD, Plant AL. Standards for Cell Line Authentication and Beyond. PLoS Biol 14(6): e1002476, 2016. PubMed: 27300367
4. McNutt, M. Journals unite for reproducibility. Science 346(6210): 679, 2014. PubMed: 25383411.
5. Brito JJ, et al. Recommendations to enhance rigor and reproducibility in biomedical research. Gigascience 9(6):giaa056, 2020. PubMed: 32479592.
6. Raphael MP, Sheehan PE, Vora GJ. A controlled trial for reproducibility. Nature 579: 190-192, 2020.
7. Fan H, Chu JY. A brief review of short tandem repeat mutation. Genomics Proteomics Bioinformatics 5(1): 7-14, 2007. PubMed: 17572359.
8. Almeida JL, Korch CT. Authentication of Human and Mouse Cell Lines by Short Tandem repeat (STR) DNA Genotype Analysis. In: Assay Guidance Manual (eds S. Markossian et al., 2004). Published January 17, 2023.
9. Costa V, et al. Uncovering the complexity of transcriptomes with RNA-Seq. J Biomed Biotechnol 2010: 853916, 2010. PubMed: 20625424.
10. Gondane A, Itkonen HM. Revealing the History and Mystery of RNA-Seq. Curr Issues Mol Biol 45(3): 1860-1874, 2023. PubMed: 36975490.
11. Hong M, et al. RNA sequencing: new technologies and applications in cancer research. J Hematol Oncol 13(1): 166, 2020. PubMed: 33276803.
12. Graboski AL, et al. Mechanism-based inhibition of gut microbial tryptophanases reduces serum indoxyl sulfate. Cell Chem Biol 30(11): 1402-1413 e1407, 2023. PubMed: 37633277. PubMed: 37971880.
13. Clark AM, et al. Disruption of CFAP418 interaction with lipids causes widespread abnormal membrane-associated cellular processes in retinal degenerations. JCI Insight 9(1): e162621, 2024.
14. Abaandou L, Quan D, Shiloach J. Affecting HEK293 Cell Growth and Production Performance by Modifying the Expression of Specific Genes. Cells 10(7): 1667, 2021. PubMed: 34359846.
15. Huang H, et al. Targeting the ANGPT-TIE2 pathway in *mali*gnancy. Nat Rev Cancer 10(8): 575-585, 2010. PubMed: 20651738.
16. Hu X, et al. The JAK/STAT signaling pathway: from bench to clinic. Signal Transduct Target Ther 6(1): 402, 2021. PubMed: 34824210.
17. Pawlowski J, Kraft AS. Bax-induced apoptotic cell death. Proc Natl Acad Sci U S A 97(2): 529-531, 2000. PubMed: 10639111.
18. Brodaczewska KK, et al. Choosing the right cell line for renal cell cancer research. Mol Cancer 15(1): 83, 2016. PubMed: 27993170.
19. Terry L, et al. Treating Cells as Reagents to Design Reproducible Assays. SLAS Discovery 26(10): 1256-1267, 2021. PubMed: 34530643.
20. Arzumanian V, Pyatnitskiy M, Poverennaya E. Comparative Transcriptomic Analysis of Three Common Liver Cell Lines. Int J Mol Sci 24(10): 8791, 2023. PubMed: 37240140.
21. Navarro FCP, et al. Genomics and data science: an application within an umbrella. Genome Biol 20(1): 109, 2019. PubMed: 31142351.
22. Han Y, et al. Advanced Applications of RNA Sequencing and Challenges. Bioinform Biol Insights 9(Suppl 1): 29-46, 2015. PubMed: 26609224.
23. Tan E, et al. HEK293 Cell Line as a Platform to Produce Recombinant Proteins and Viral Vectors. Front Bioeng Biotechnol 9: 796991, 2021. PubMed: 34966729.
24. Malm M, et al. Evolution from adherent to suspension: systems biology of HEK293 cell line development. Sci Rep 10(1): 18996, 2020. PubMed: 33149219.

25. Wang J, et al. Comparative Transcriptomics and Proteomics of Cancer Cell Lines Cultivated by Physiological and Commercial Media. Biomolecules 12(11): 1575, 2022. PubMed: 36358924.
26. Yuan J, et al. The Scattered Twelve Tribes of HEK293. Biomedical and Pharmacology Journal 11: 621-623, 2018.
27. Lin YC, et al. Genome dynamics of the human embryonic kidney 293 lineage in response to cell biology manipulations. Nat Commun 5:, 4767, 2014. PubMed: 25182477.

10801 University Boulevard
Manassas, Virginia 20110-2209

703.365.2700 703.365.2701 sales@atcc.org www.atcc.org

AP-072024-v01