Integrating ATCC Authenticated Cell Lines Into Your CRISPR Gene Editing Workflows

Utsav Sharma, *Product Manager*
ATCC

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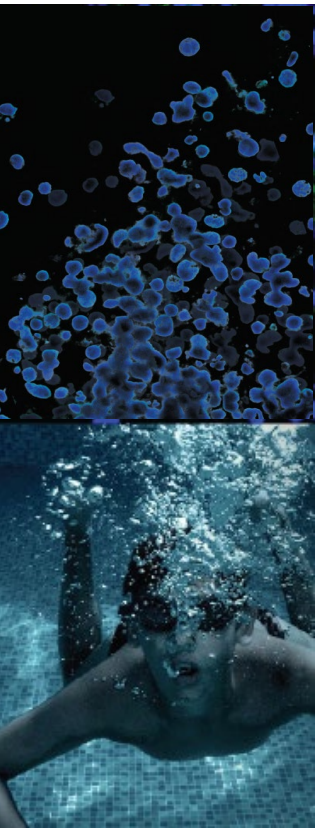
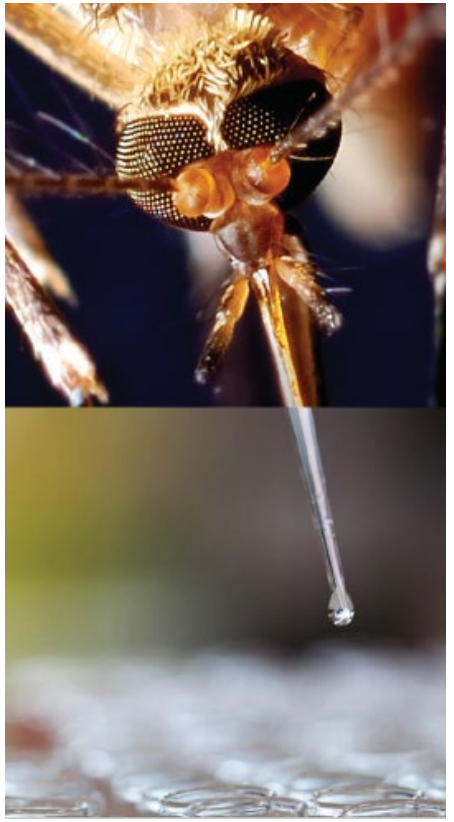
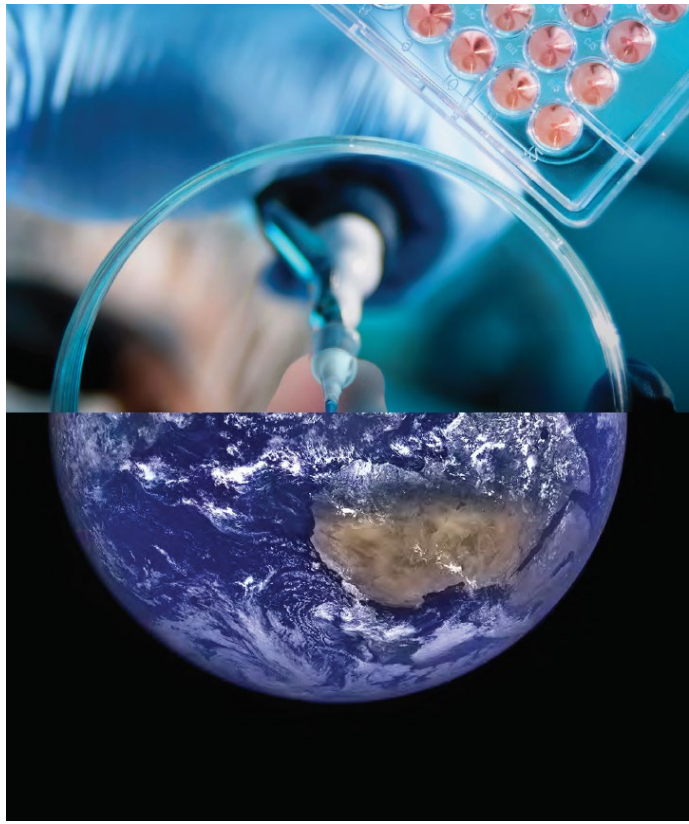
Daniel Orozco, *Senior Research Associate*
EditCo Bio

Credible Leads to Incredible™







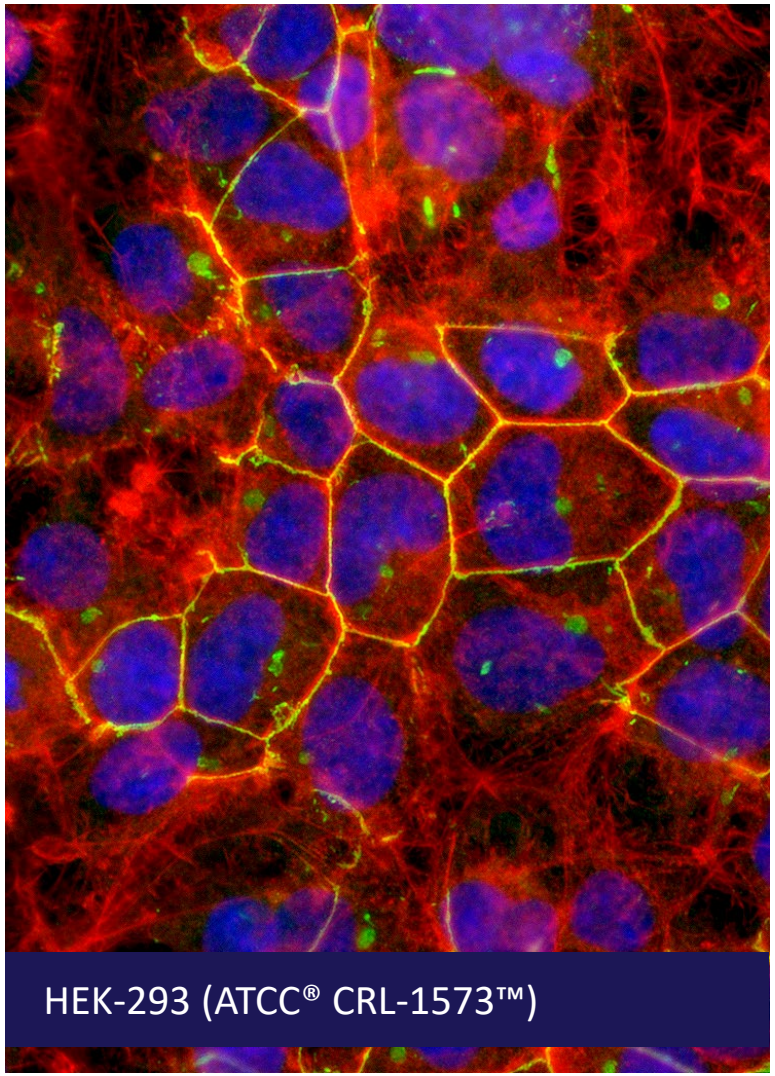
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring advanced models, differentiated stem cells, gene editing
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees

ATCC®

Agenda

- Dr. Fang Tian, Director, Biological Content, ATCC
- Daniel Orozco, Senior Research Associate, EditCo Bio
- Q&A



HEK-293 (ATCC® CRL-1573™)

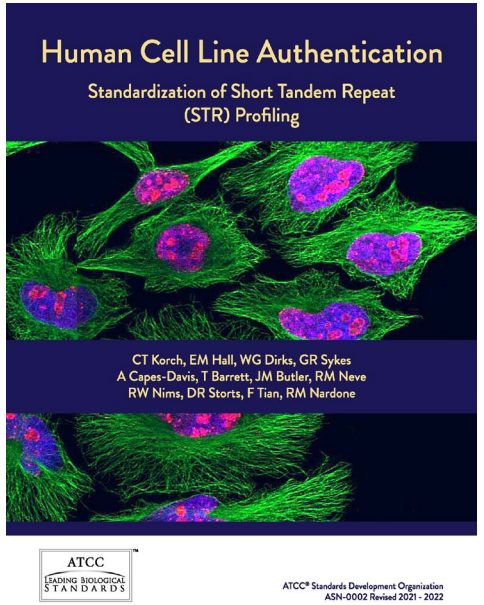
Products for Cell Authentication at ATCC

- STR Services
  - ATCC® 135-XV™ Verified STR Profiling Service (Human)
  - ATCC® 135-XV-10™ 10 STR Profiling Service (Human)
  - ATCC® 135-XV-20™ 20 STR Profiling Service (Human)
  - ATCC® 137-XV™ Mouse STR Profiling Service

Spot → Dry → Mail → Results

- Mycoplasma Testing
  - ATCC® 136-XV™ Mycoplasma Testing Service: PCR-based
  - ATCC® 30-1012K™ Universal Mycoplasma Detection Kit
    - Detect 60 most common mycoplasmas

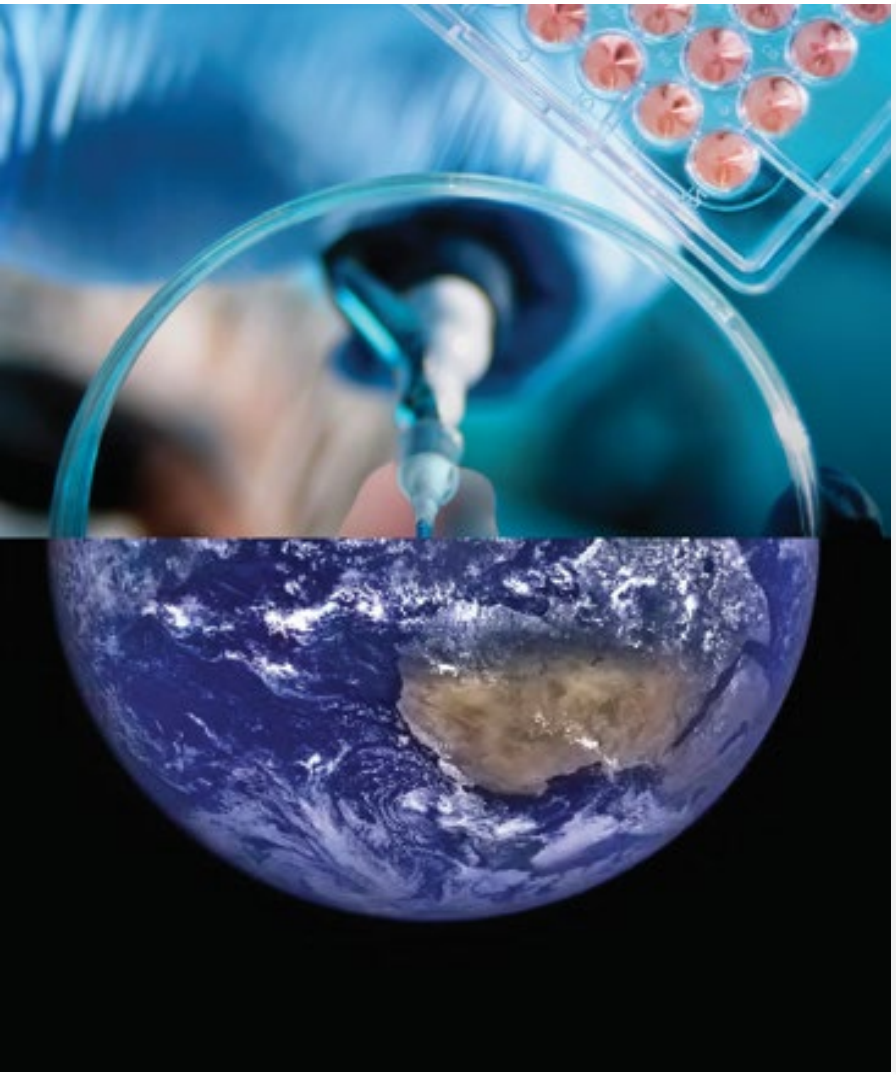
Collect/pellet cells → Cell lysis → Touchdown PCR → Run gel/stain



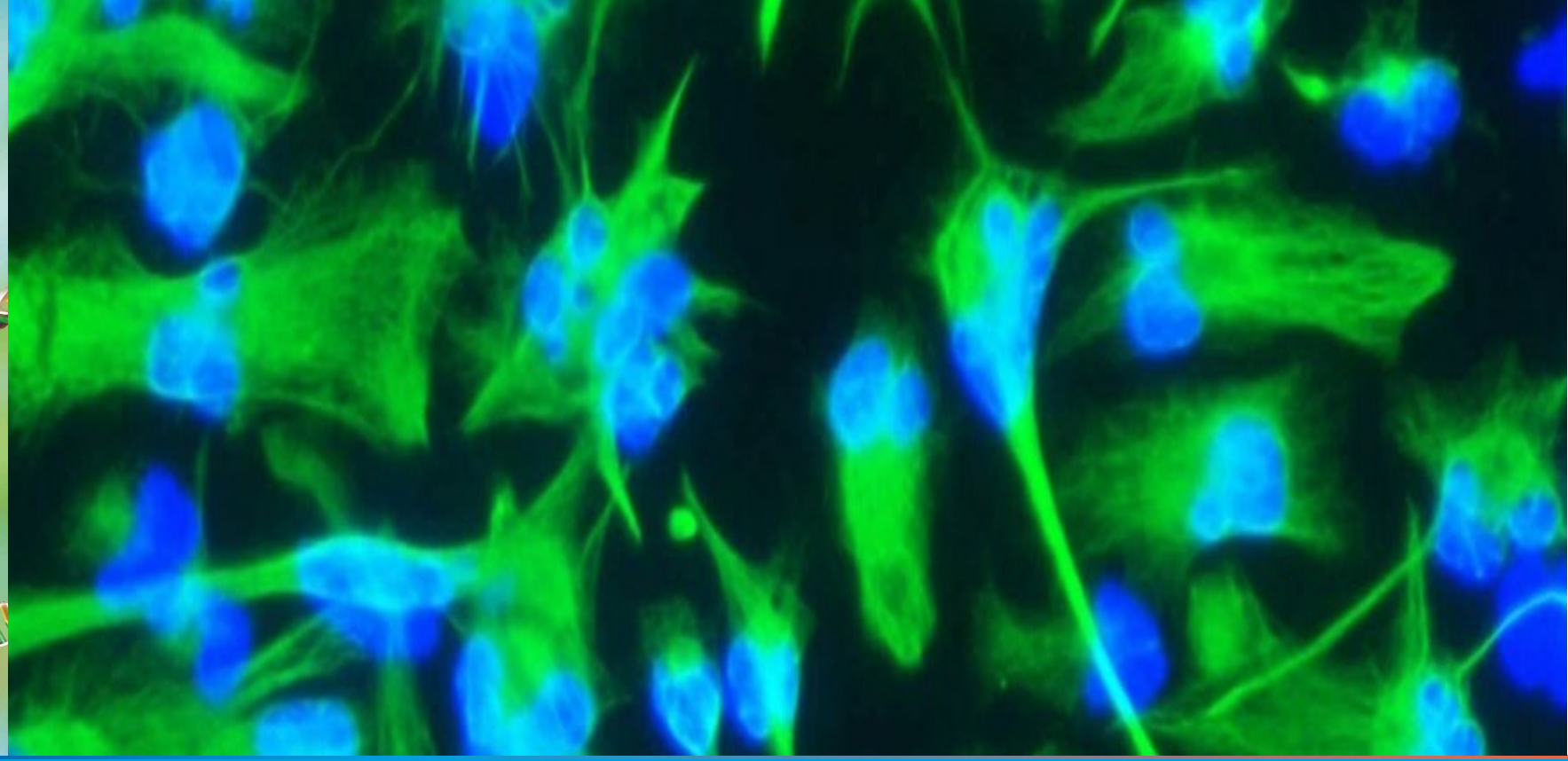
ATCC® 137-ANSI-STR™

ATCC®

Outline

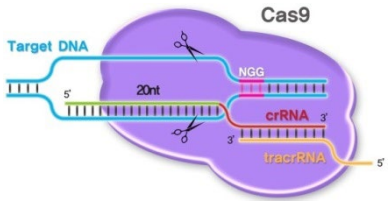


- Points to consider when choosing cells for CRISPR gene editing
- Cell authentication and quality control
- Bioinformatic data associated with ATCC cell lines



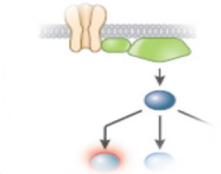
Points to consider when choosing cells for CRISPR gene editing

Power of CRISPR gene editing



CRISPR platform

- Gene knock out
- Gene insertion
- Gene modification



Functional Screening

Generate precision cell models

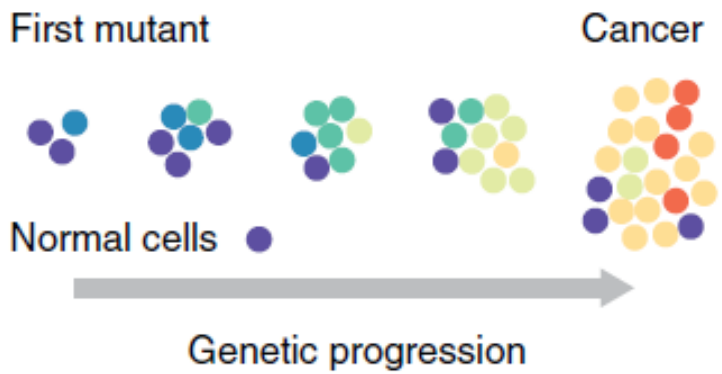
Generate animal models of human disease

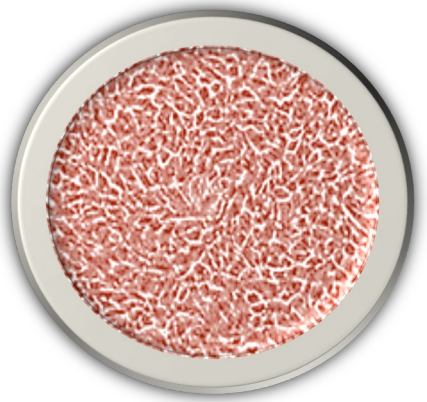
Generate therapeutics

Cells are complex and not all cells are equal

- Cells are complex living organism
- Challenges associated with cells when perform CRISPR gene editing
  - Commonly used and well characterized cell lines are often cancer cell lines
    - tumor heterogeneity
    - genetic abnormality
  - Primary cells
    - limited life span
    - donor-to-donor variability
  - Stem cells – ethical challenge, relative low editing efficiency
- Cell authenticity, cell passage, cryopreservation, culture condition and maintenance have a significant impact on the outcome of gene editing

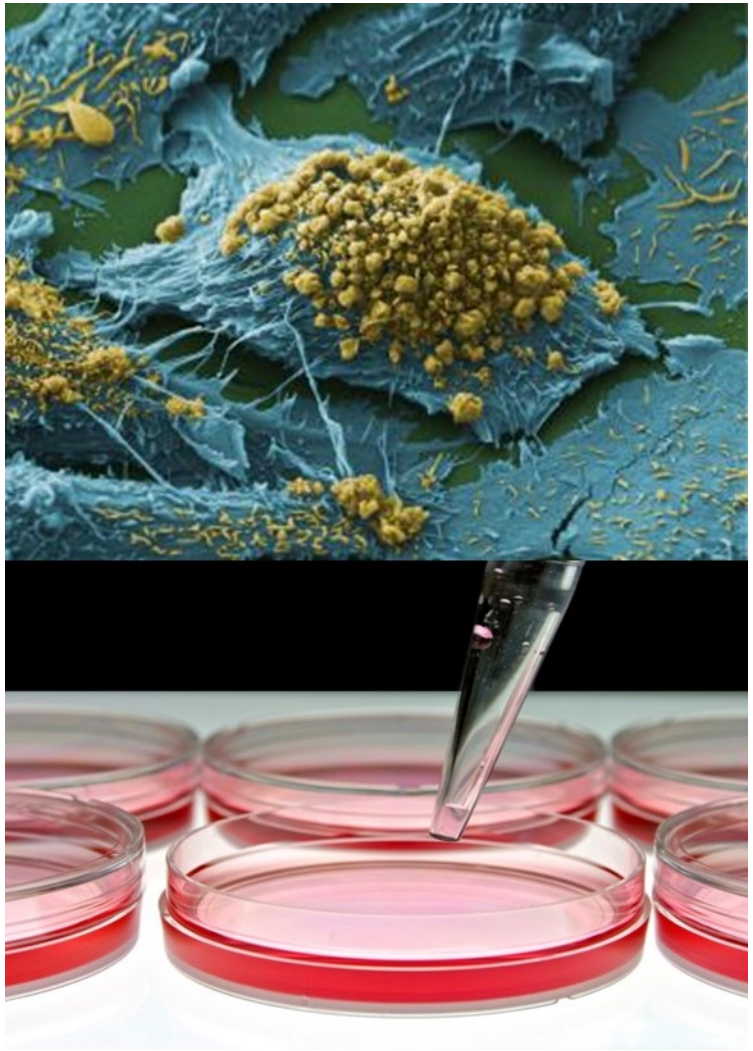
Population dynamics in cancer cells

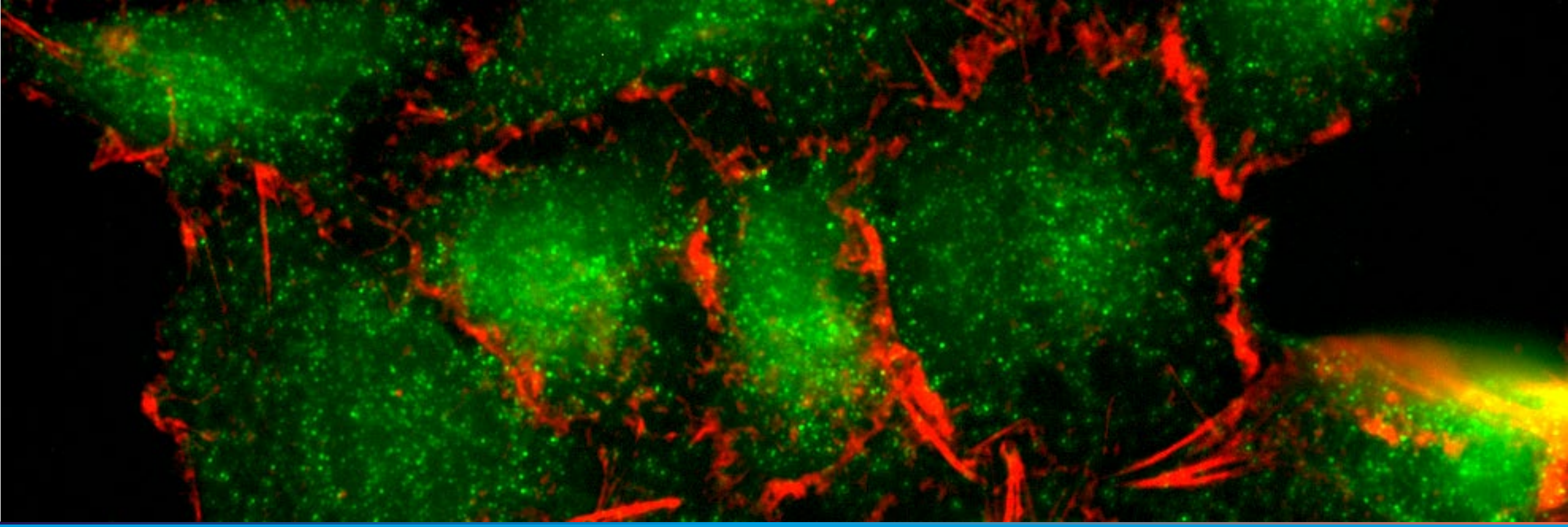




Things to consider when choosing cells for editing

- Cell authenticity
- Cell purity and sterility
- Traceability
- Tissue of origin
- Morphology and growth properties
- Passage number and PDL
- Genetic variants or modifications
- Molecular characteristics
- Unique bio-function
- Cell clonality
- Cell transfectability
- Cell life span

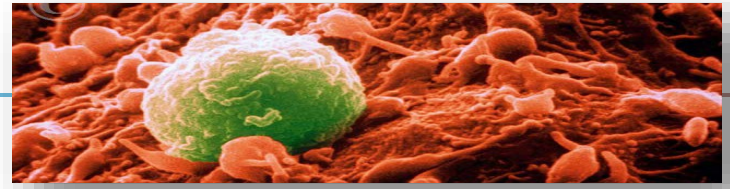




Best practices in cell culture ensures high quality

ATCC®

Cell procurement at ATCC



Record background of cell line

- Cell line name
- Tissue of origin
- Species, strain
- Morphology and growth property
- Passage number and PDL
- Genetic modification information
- molecular characteristics
- Unique biofunctions
- Originator/Institution/laboratory
- Date of origin
- Publication

Record culture information of cell line

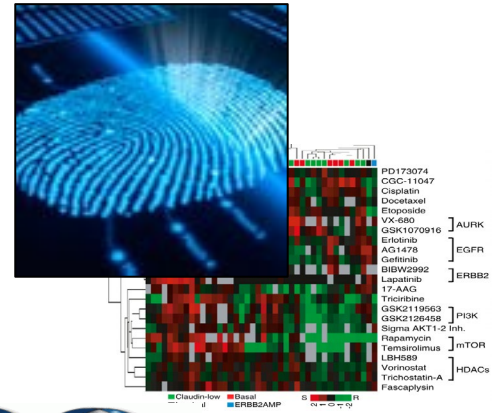
- Complete growth medium
- Serum (to include source)
- Procedure for thawing cells
- Procedure for subculture
- Cryopreservation medium & procedure
- Doubling time
- Expected pre-freeze, post-freeze viability

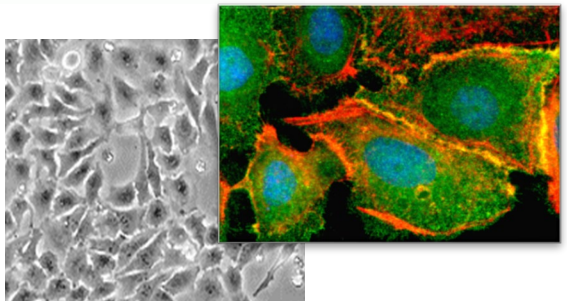
Testing original biomaterials

- Microbial contamination check
- Cell line identity verification

Cell line Authentication and Characterization

Authentication of a cell line is the sum of the process by which a line's identity is verified (demonstrating that it is derived from the correct species and donor), and shown to be free of contamination from other cell lines and microbes.





To obtain reliable and reproducible data:

- Confirm cell identity
- Confirm cell purity and sterility
- Characterize molecular signature and genetic stability
- Characterize bio-function

Use of cross-contaminated or misidentified cell lines is a widespread issue, which leads to irreproducible results, and a significant negative financial impact.

Identify inter-species contamination

ASN-0003

Designation: ASN-0003

Species-Level Identification of Animal Cells through Mitochondrial Cytochrome *c* Oxidase Subunit 1 (CO1) DNA Barcodes

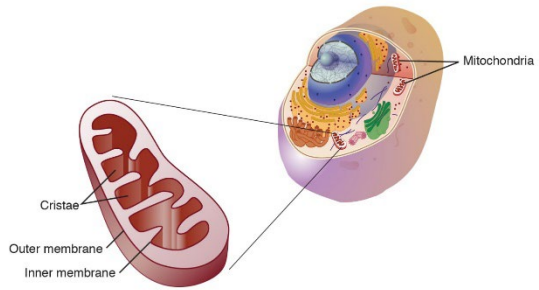
– published –

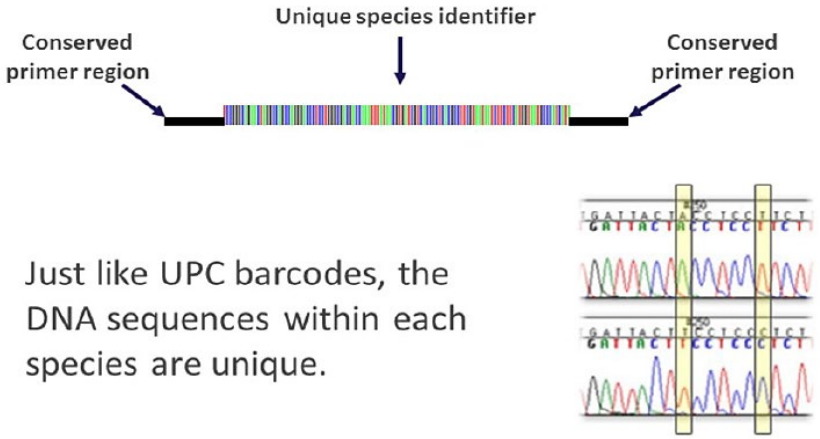
ASN-0004

Designation: ASN-0004

Species-Level Identification and Cross-Contamination Screening in Animal Cells by Multiplex PCR

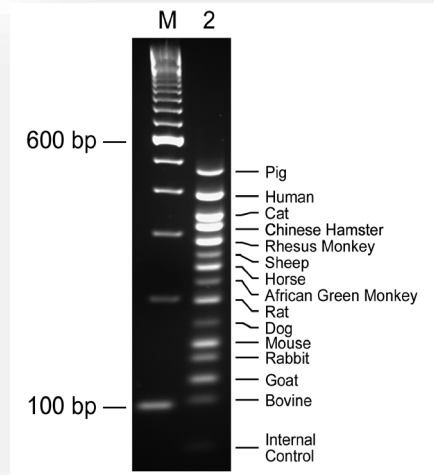
– in progress –





Just like UPC barcodes, the DNA sequences within each species are unique.

Cytochrome c Oxidase I (COI) Assay

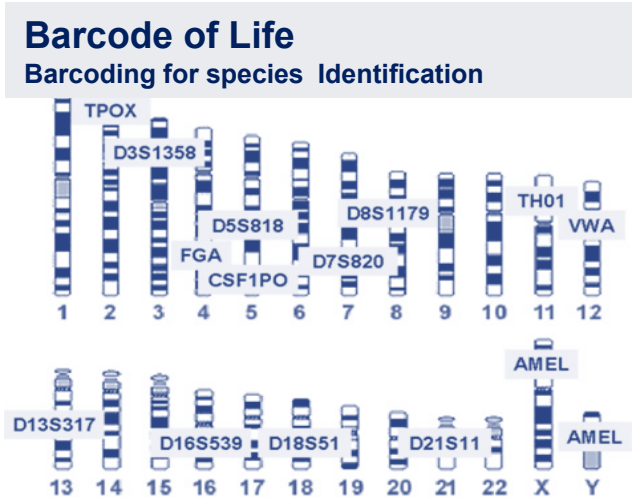


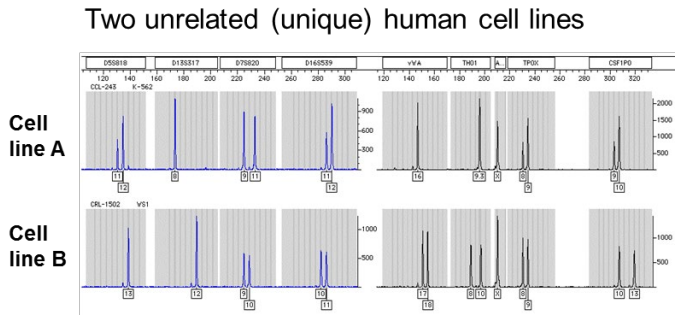
Amplification of the targeted sequence in the mitochondrial DNA for cytochrome *c* oxidase subunit 1

Intra species - Confirm human cell line identity

Gold standard: STR analysis (DNA profiling)

Intra-species identification and authentication of human cell lines

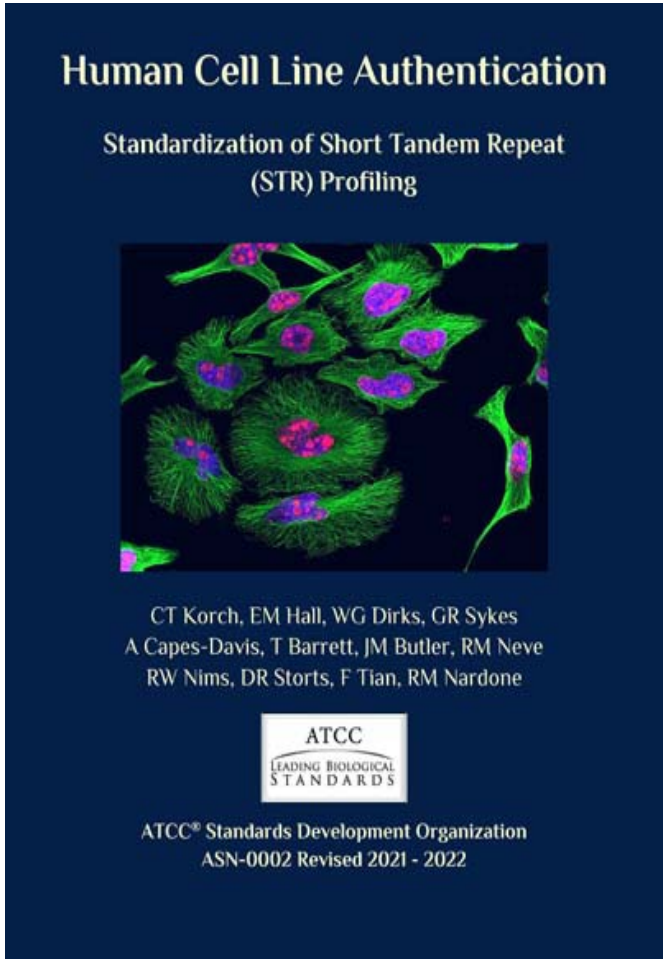




- Target sequence consists of microsatellite DNA contains short tandem repeats
- Highly sensitive, robust and accurate
- Results are highly reproducible
- STR test can determine
  - Cell line identity when compared to a reference
  - Cell line cross contamination
- STR test cannot distinguish
  - Cell lines created from the same individual
  - Cell lines created from identical twins
- STR analysis isn't always easy
  - Cell lines can have tri-allelic pattern and chromosome instabilities

ANS standards – human cell line authentication

ASN-0001
ASN-0002 -2022



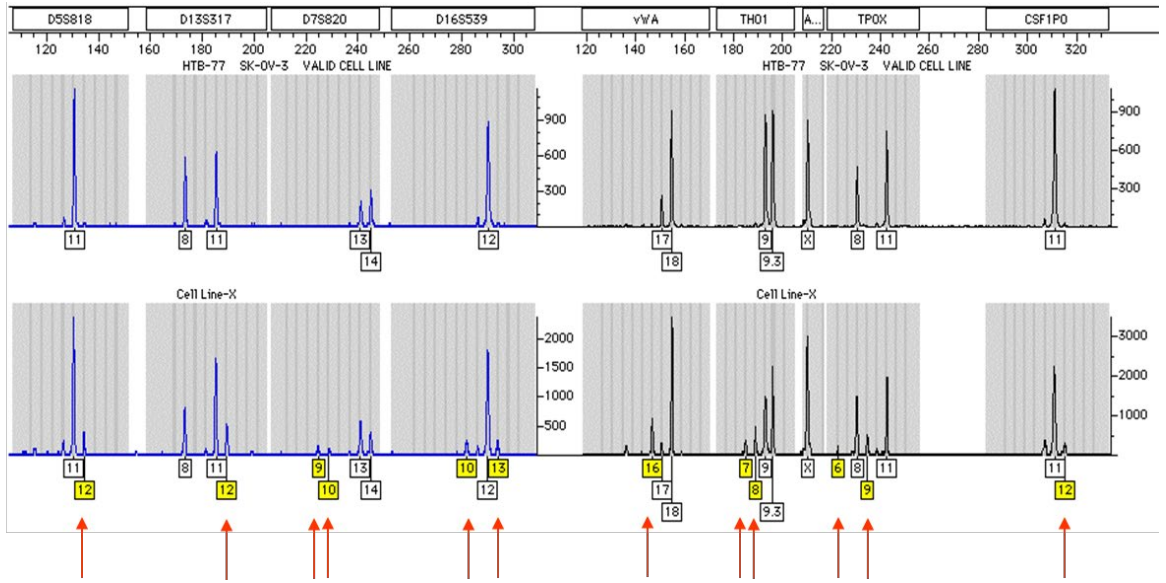
Clonal derivative has identical DNA profile to parental cell line

	D5S818	D13S317	D7S820	D16S539	vWA	TH01	TPOX	CSF1PO	Amel.
BG01 Parent	10, 12	11, 12	10, 11	9, 11	16, 17	7, 9.3	8	10	X, Y
BG01V Clone	10, 12	11, 12	10, 11	9, 11	16, 17	7, 9.3	8	10	X, Y

STR assay detects cross-contamination

SK-OV-3
Ovary

SK-OV-3 +
cell line X



Mouse cell line STR profiling

National Institute of Standards and Technology
U.S. Department of Commerce

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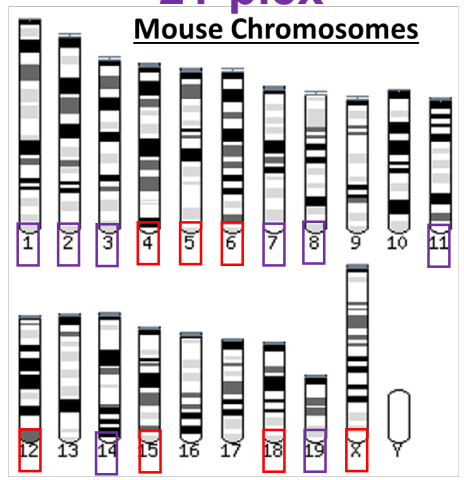
THE ESSENTIALS OF LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™

- A total of 19 mouse STR markers published for mouse cell line authentication
- Two human STR markers included in multiplex assay for contamination control
- New 21 mouseplex showed improved intra-strain resolution
- New 21 mouseplex also increases chromosomal coverage of STR Markers

21-plex



Red boxes depict mouse chromosome represented in the original multiplex carried over to the new assay. Purple boxes indicated the new STR markers added.

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Test for microbial and viral contamination

Bacteria and Fungi

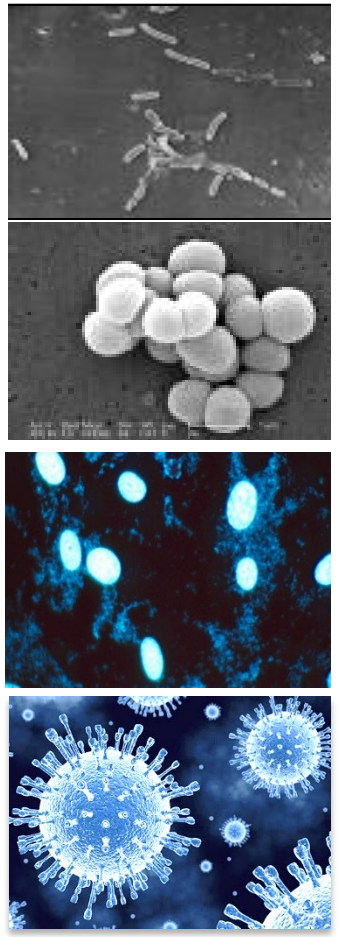
- Microbiological culture (aerobic, anaerobic)
  - Manual test- Streak plate
  - Automated system
- PCR

Mycoplasma

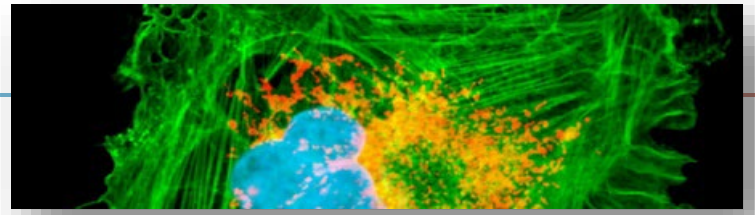
- Direct agar culture
- Indirect Hoechst stain
- PCR
- Sequencing

Viruses

- Cytopathic effect (CPE)
- Indirect immunofluorescent antibody (IFA)
- Enzyme immunoassay (EIA)
- PCR and/or Sequencing

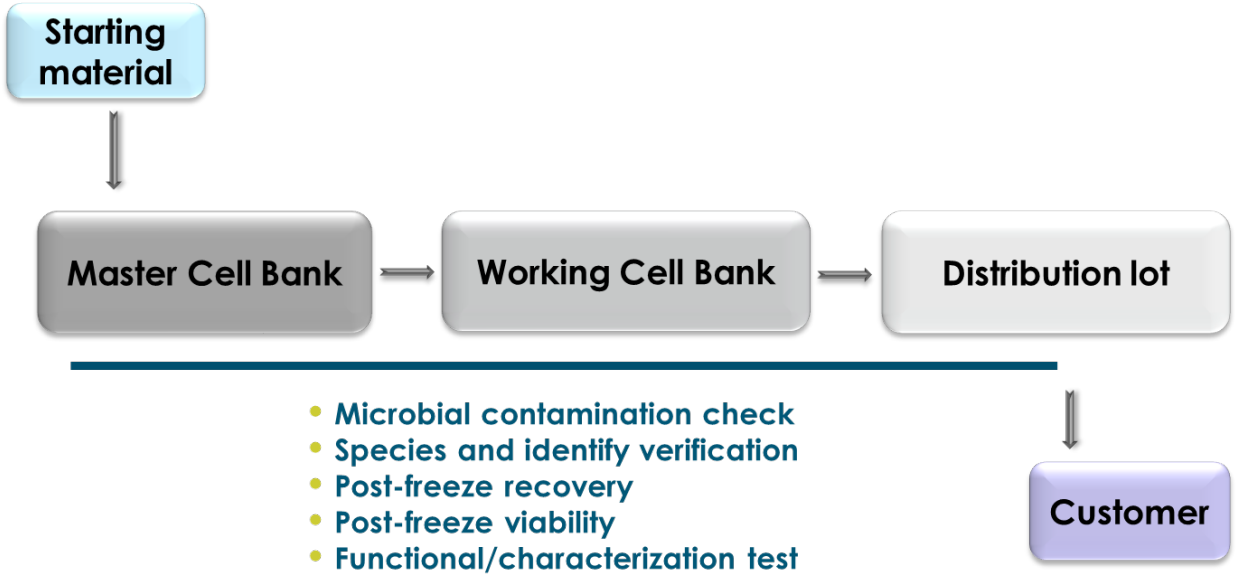


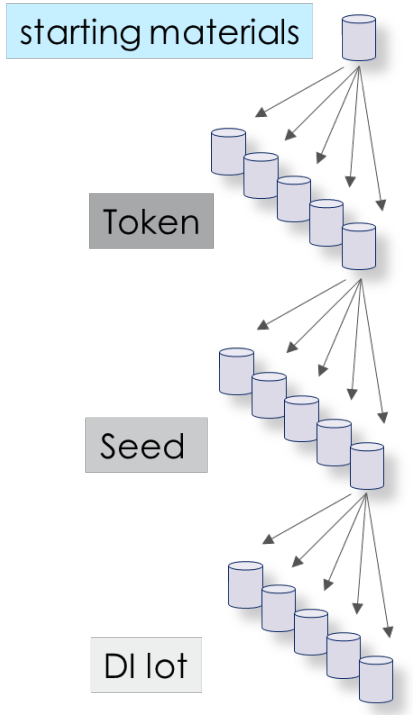
ATCC cell banking

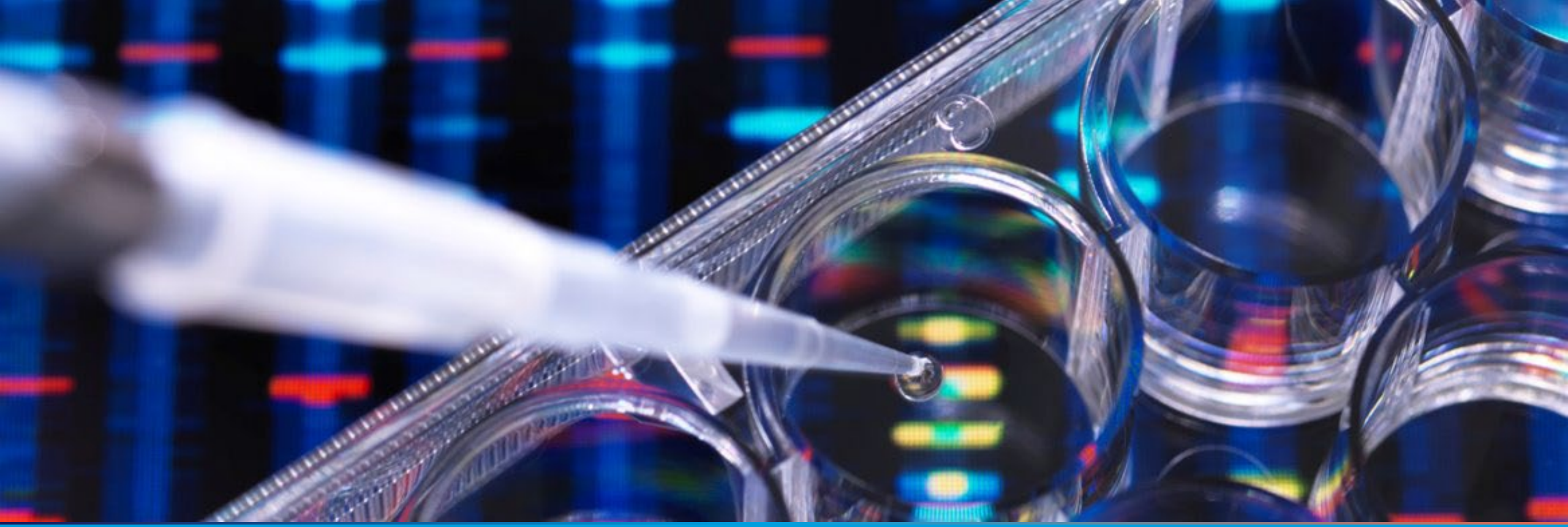


Cell banking systems

- Preserve valuable originally developed source biomaterials
- Reduces passage number
- All frozen at same stage
- Prevents lot-to-lot variation
- Reproducible and consistent production







Bioinformatic data associated with ATCC cell lines

ATCC®

Authenticated genomics data at ATCC

ATCC is focused on data provenance and closing the reproducibility gap

OmicSoft
Ingenuity (IPA)
HGMD

QIAGEN

ATCC Genome Portal
ATCC Cell Line Land

ATCC®

Authenticated Data

- Standardized laboratory methods
- Quality Assurance (ISO)
- Traceable to materials in a biorepository
- ***Maximum data provenance***
- ***Maximum reproducibility***

Expert Curated Data

- Standardized metadata
- Standardized biofx methods
- Improved reproducibility
- FAIR data model
- ***Less risk, more results***

Focused Public Data

- Improved metadata
- Moderate risk
- Often access-controlled
- Limited scope

Uncontrolled Public Data

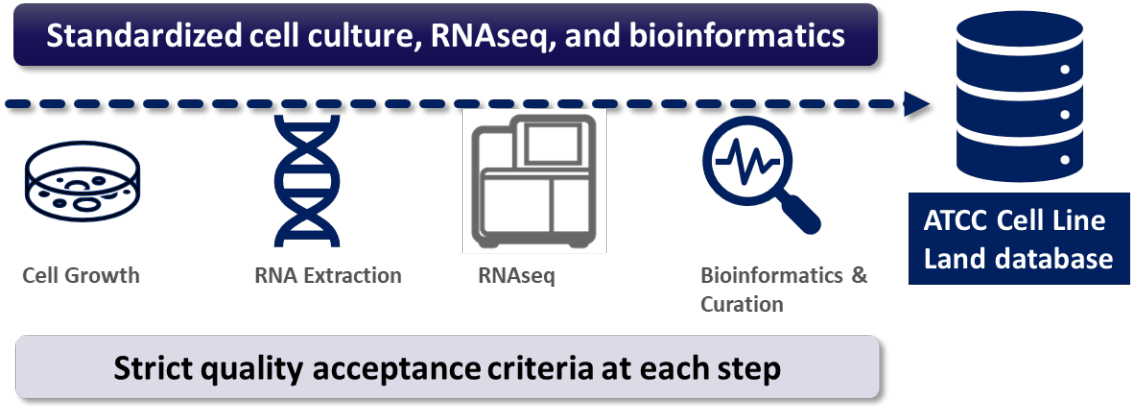
- Unknown quality
- Missing or non-standard metadata
- Risky to use

ATCC®

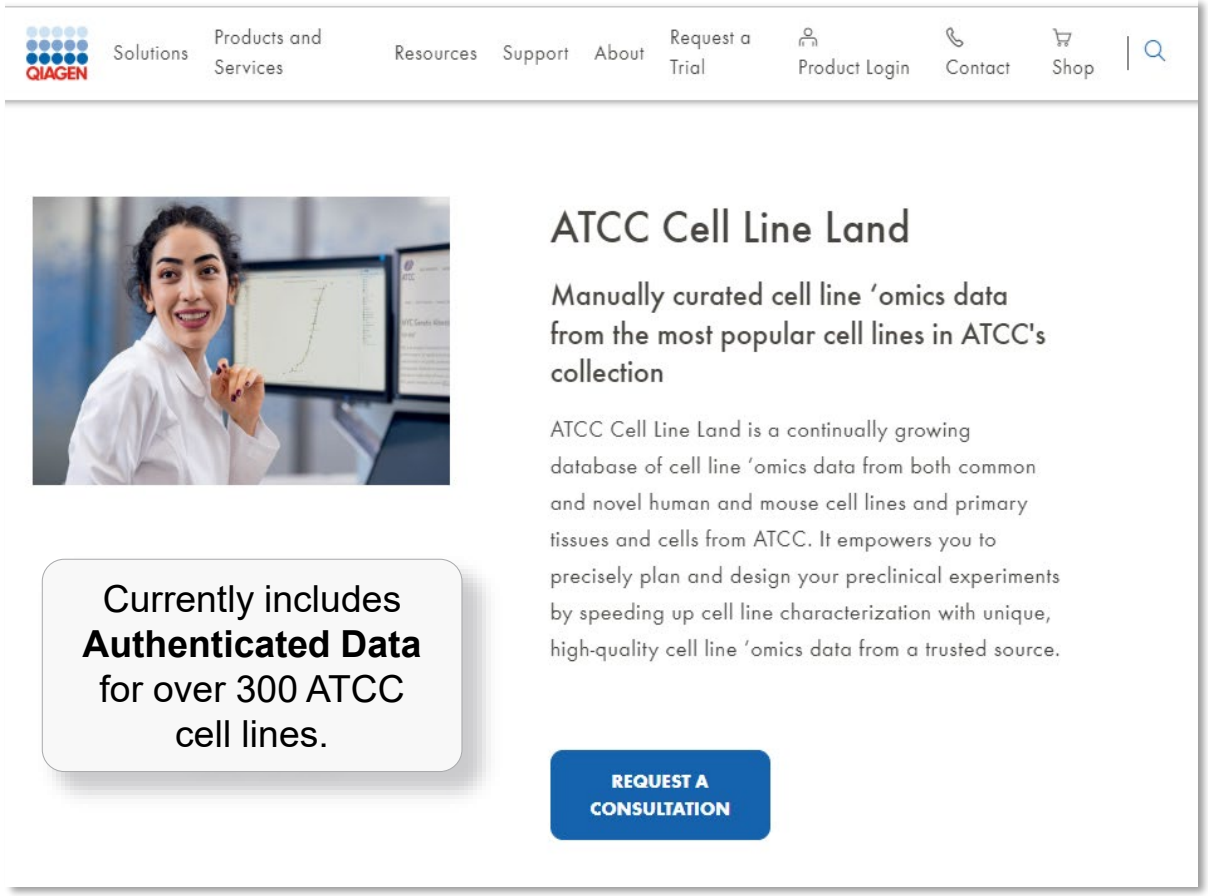
ATCC OmicSoft Cell Line Land

A partnership with QIAGEN Digital Insights

Comparative Transcriptomics Projects



- Repository of ***authenticated 'omics data traceable to physical materials***
- Data production, curation, and analysis uniformly standardized
- Enables the highest level of **scientific reproducibility**.
- End-to-end **Data Provenance**



Currently includes **Authenticated Data** for over 300 ATCC cell lines.

https://digitalinsights.qiagen.com/atcc-cell-line-land/

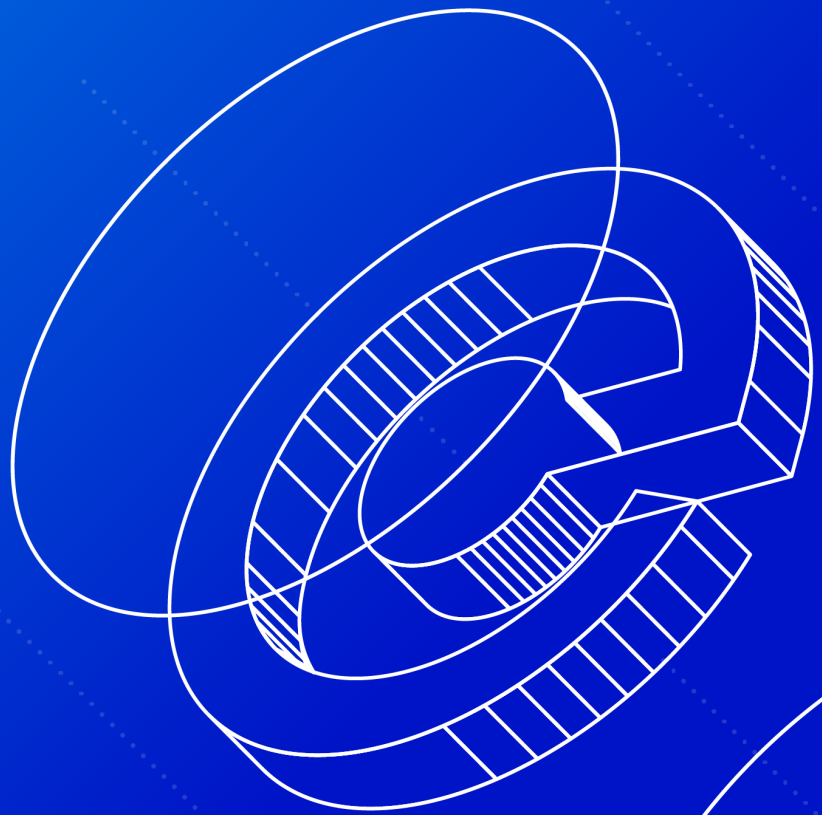
Thank you

ENGINEERED CELLS

Integrating ATCC Authenticated Cell Lines into your CRISPR Editing Workflows

TODAY'S SPEAKERS

Daniel Orozco



WEBINAR OVERVIEW

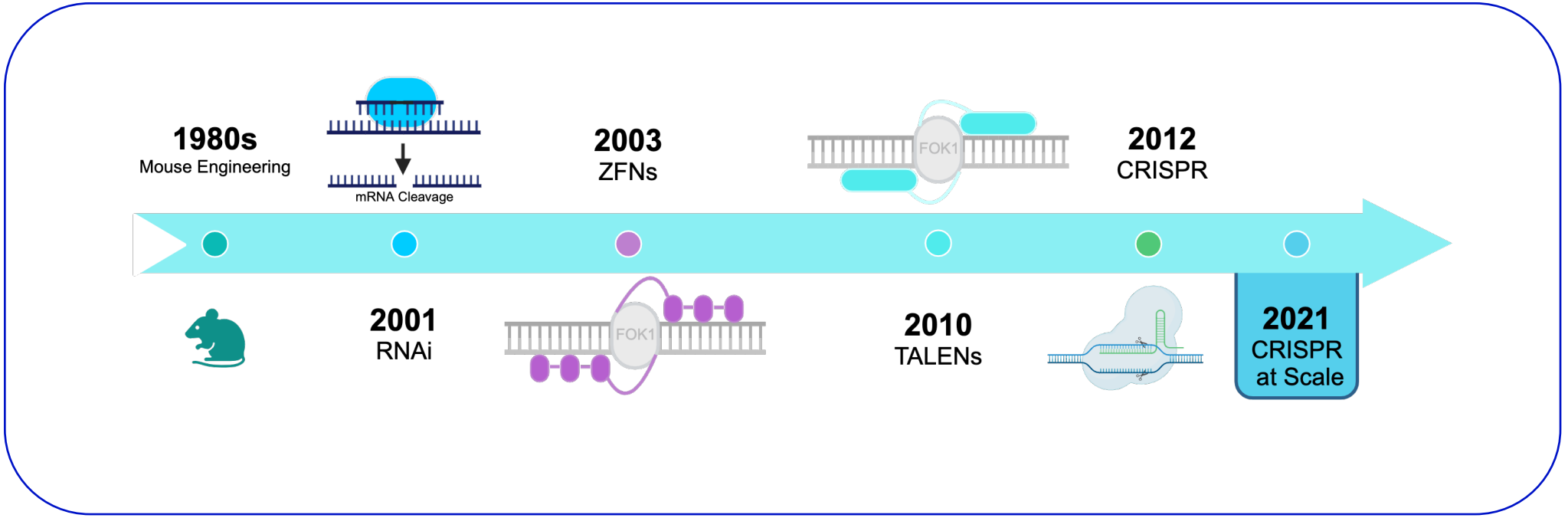
What We Will Cover

- ATCC - Cell Lines, banking and quality
- What EditCo Bio has to offer
- CRISPR
- Cell offerings
  - Immortalized
  - iPSC
  - Primary CD4+ T Cells

EditCo

Cells as reagents

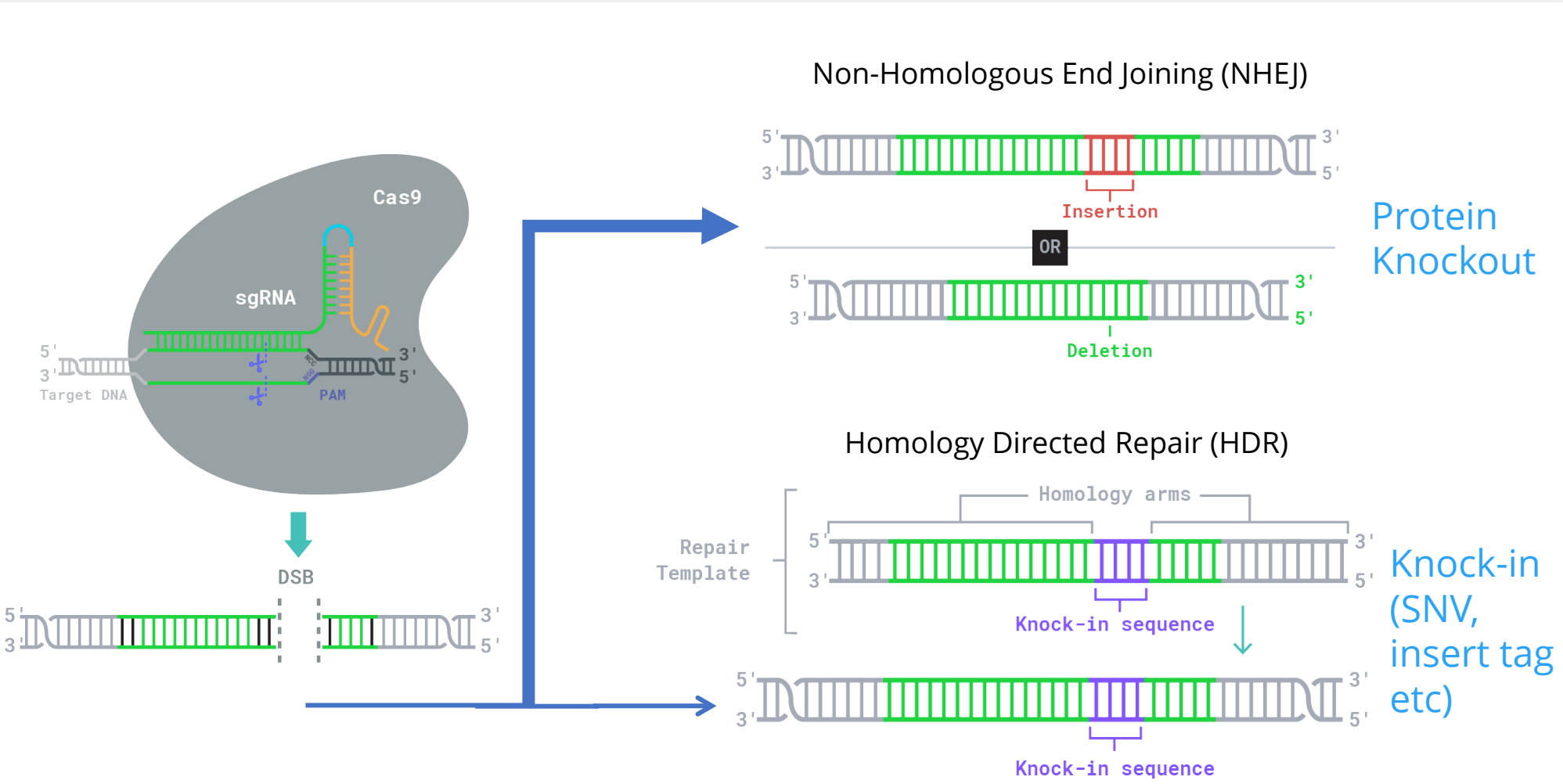
Gene-Editing Evolution



Why CRISPR

- Well studied
- Enzymes have high activity and well understood specificity
- Understood well enough for reproducibility & engineering

How does CRISPR work?



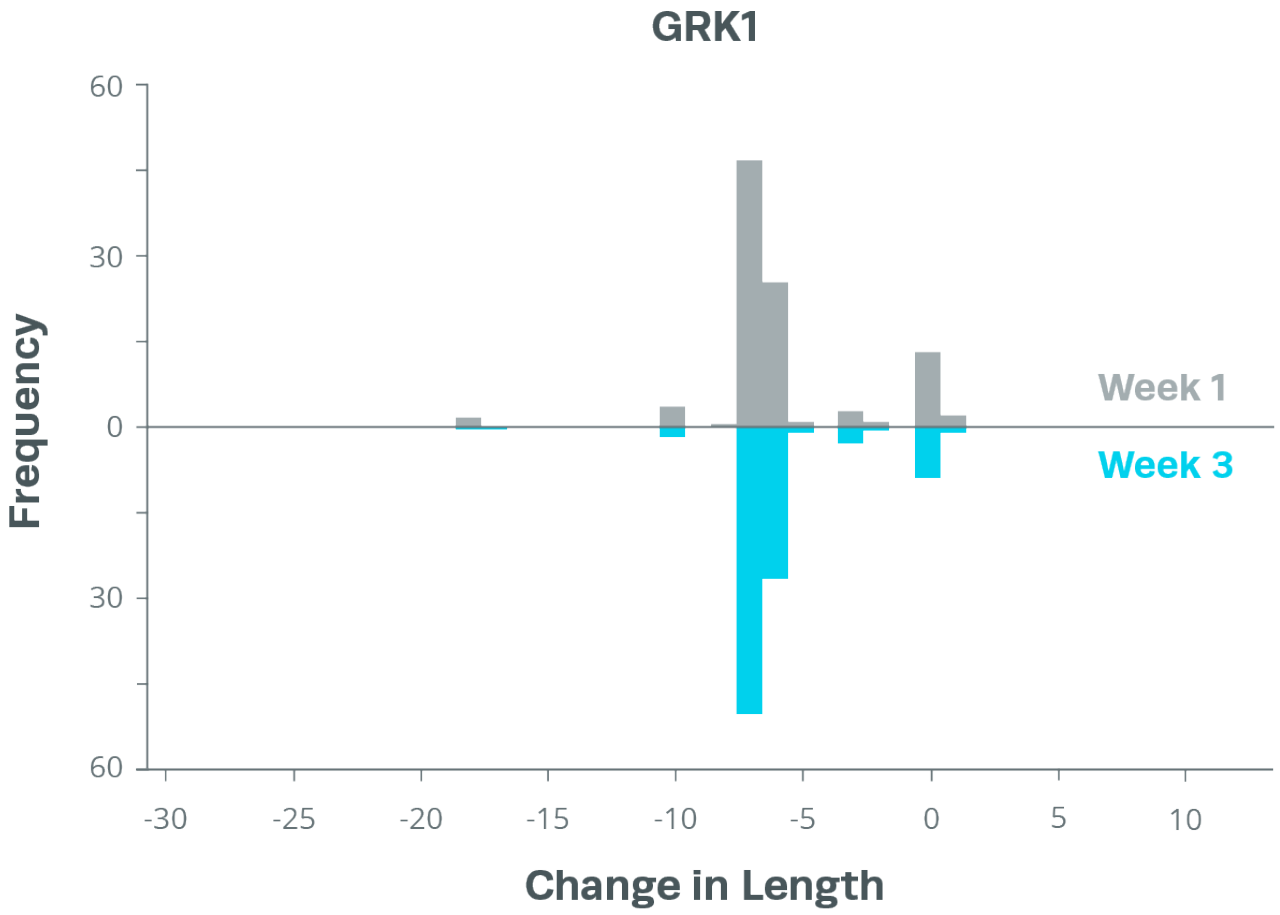
Overcoming the challenges of Experimental Reproducibility

Challenge

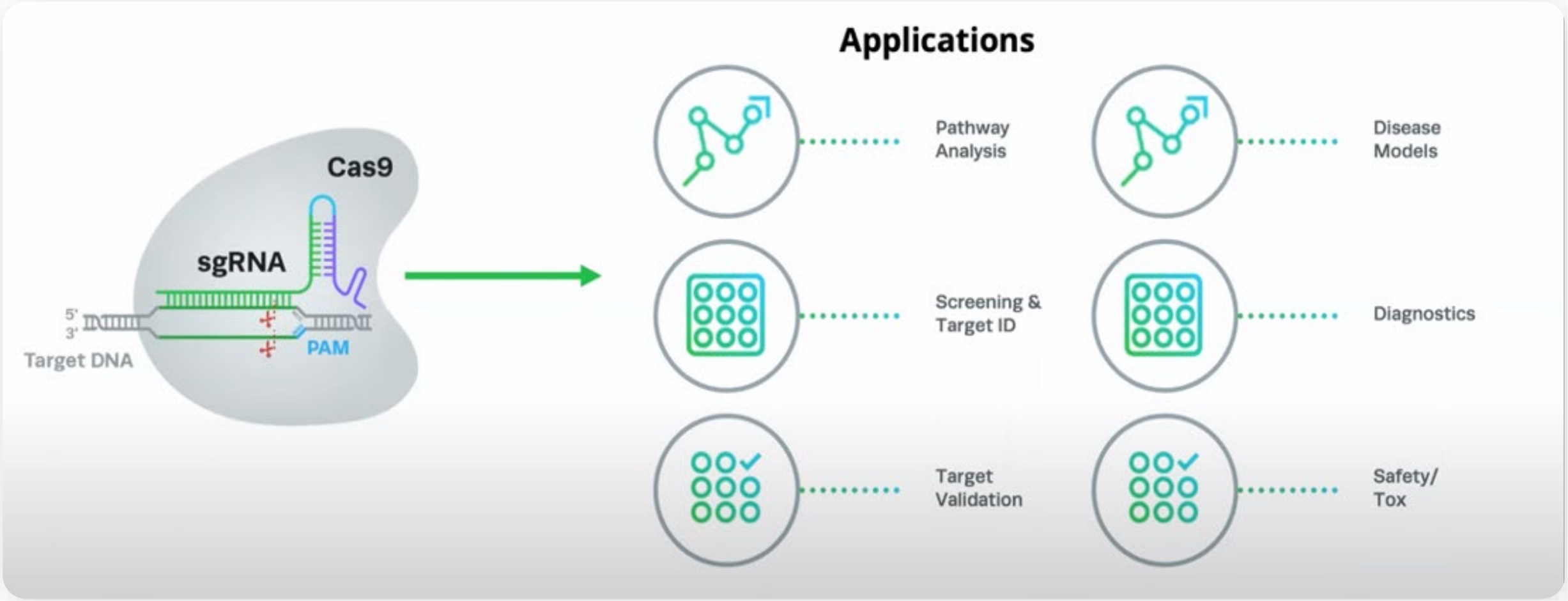
CRISPR Editing Success varies widely among scientists, many not analyzing efficiency prior to functional assay. These factors contribute to reproducibility issues that are troubling scientists.

Solution

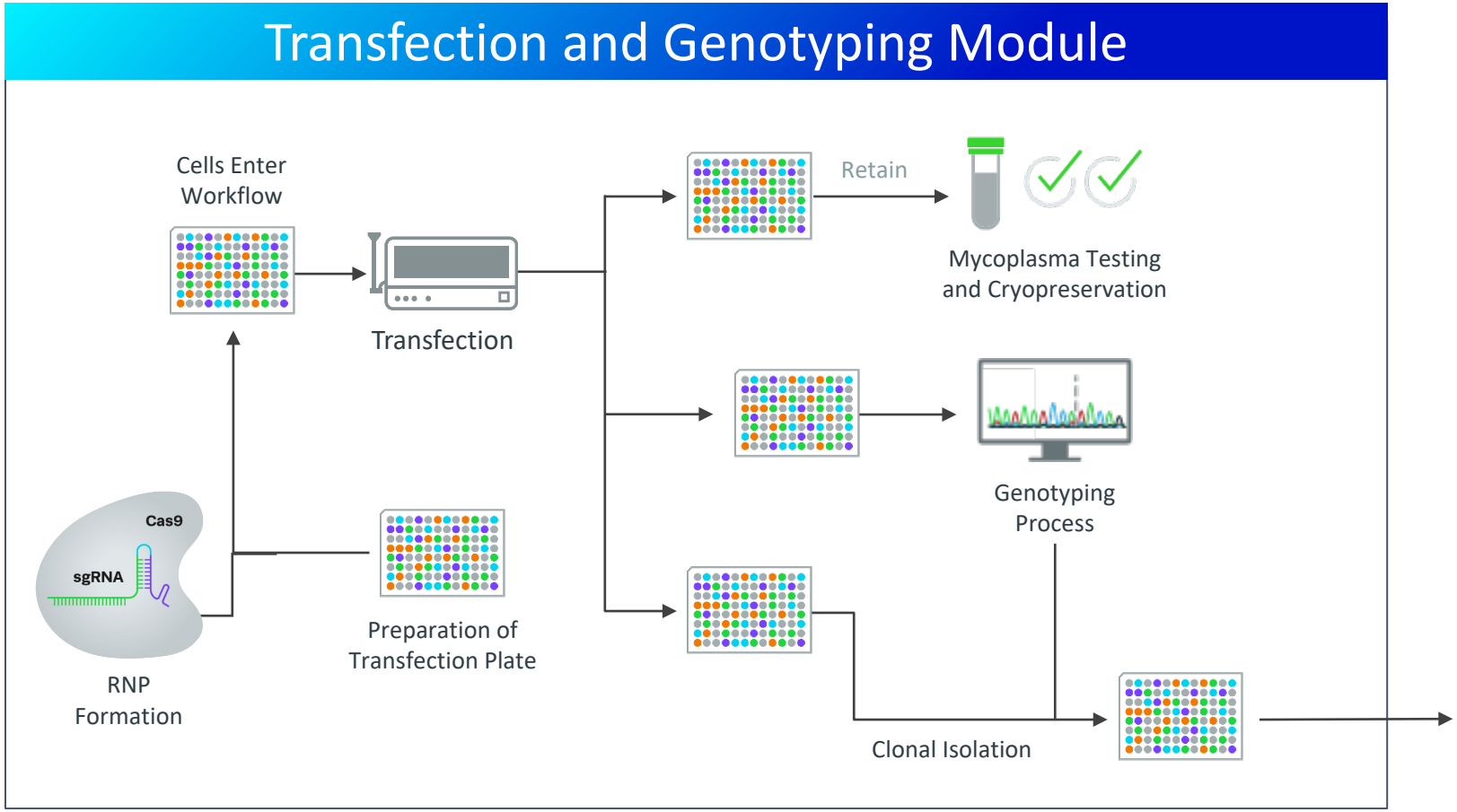
Optimized process results in highly reproduceable edits. Regardless of if we make the same edit today or next month, we will get the same result so you can confidently move your research forward.



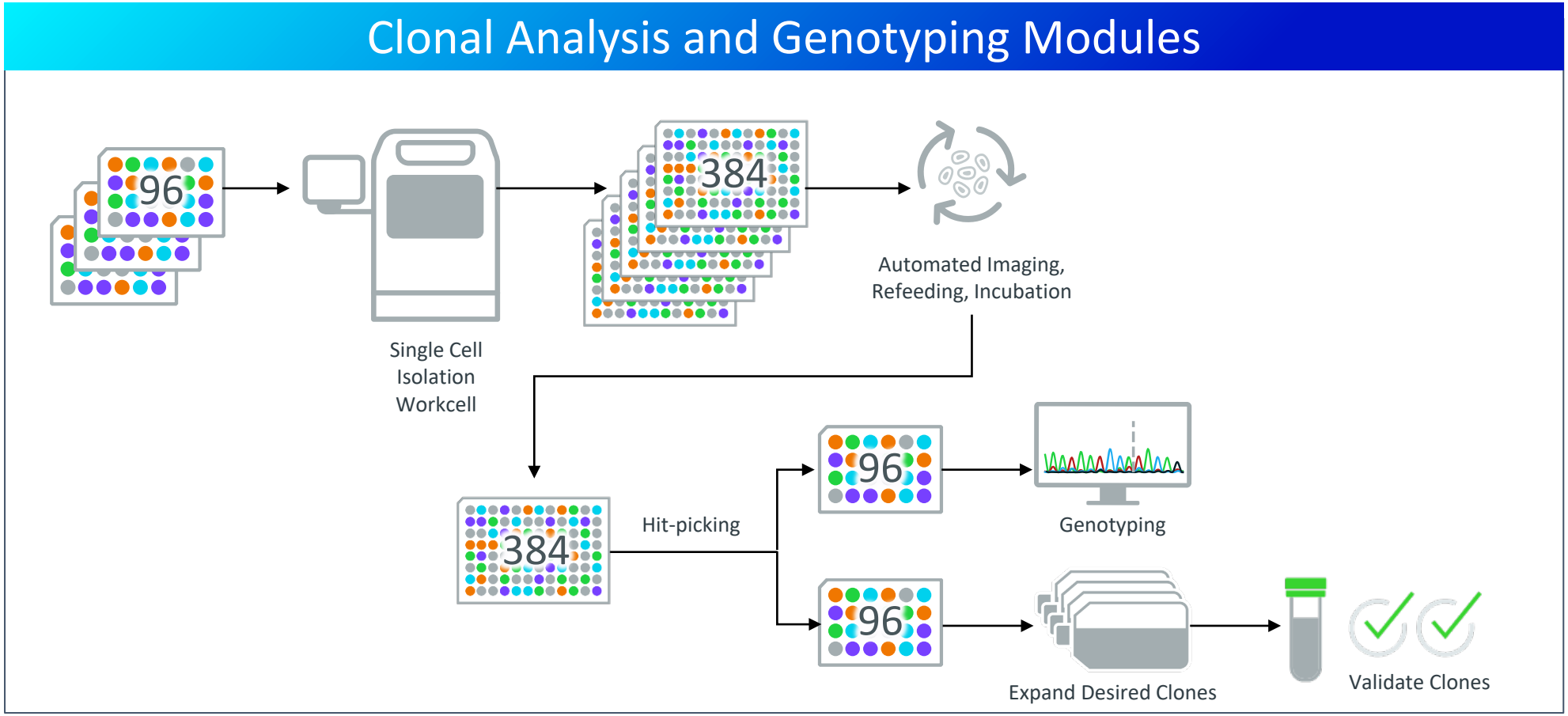
Programmable Genome “Re-Writing”



Cell Engineering Process



Cell Engineering Process



ENGINEERED CELLS

Express Cell Pools

Leverage verified CRISPR gene knockouts with industry leading delivery time.

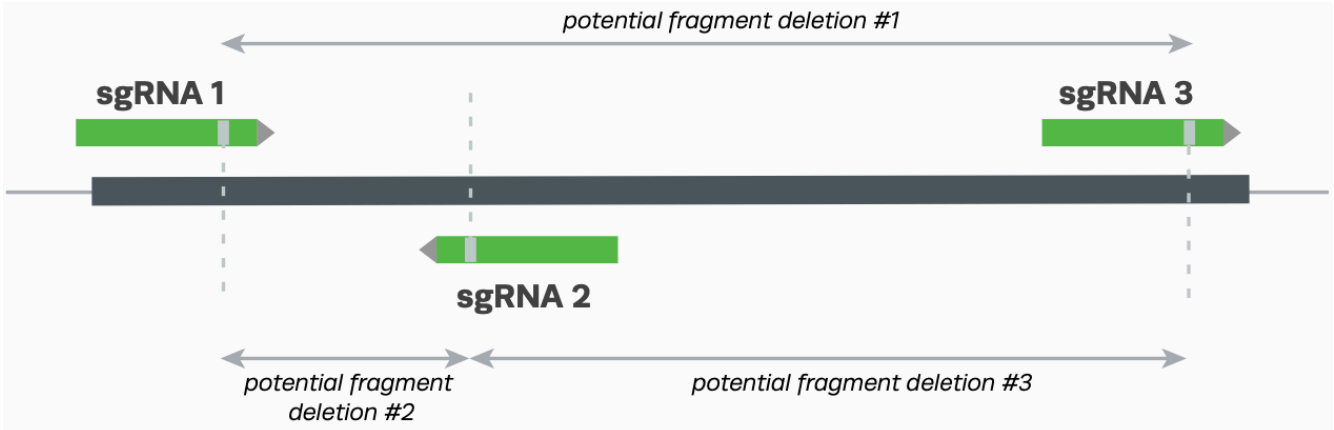
EditCo

Multi-guide Design for Better Knockout

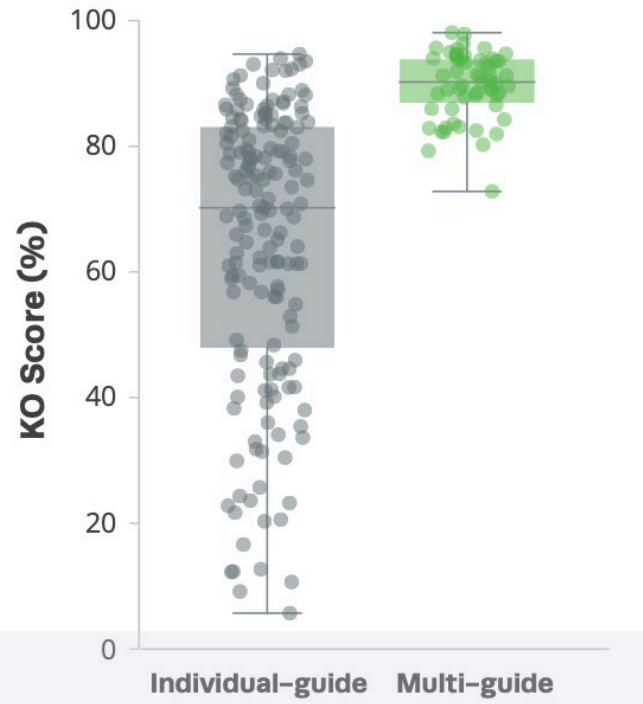
- **Guided Repair:** up to 3 sgRNAs concurrently cut an early exon of the target gene, causing fragment deletions
- **Greater Knockout Confidence:** multi-guide sgRNA achieves more consistent knockout efficiencies than 1 sgRNA alone.

Novel Design Strategy

Proprietary guide design algorithm powers multi-guide design

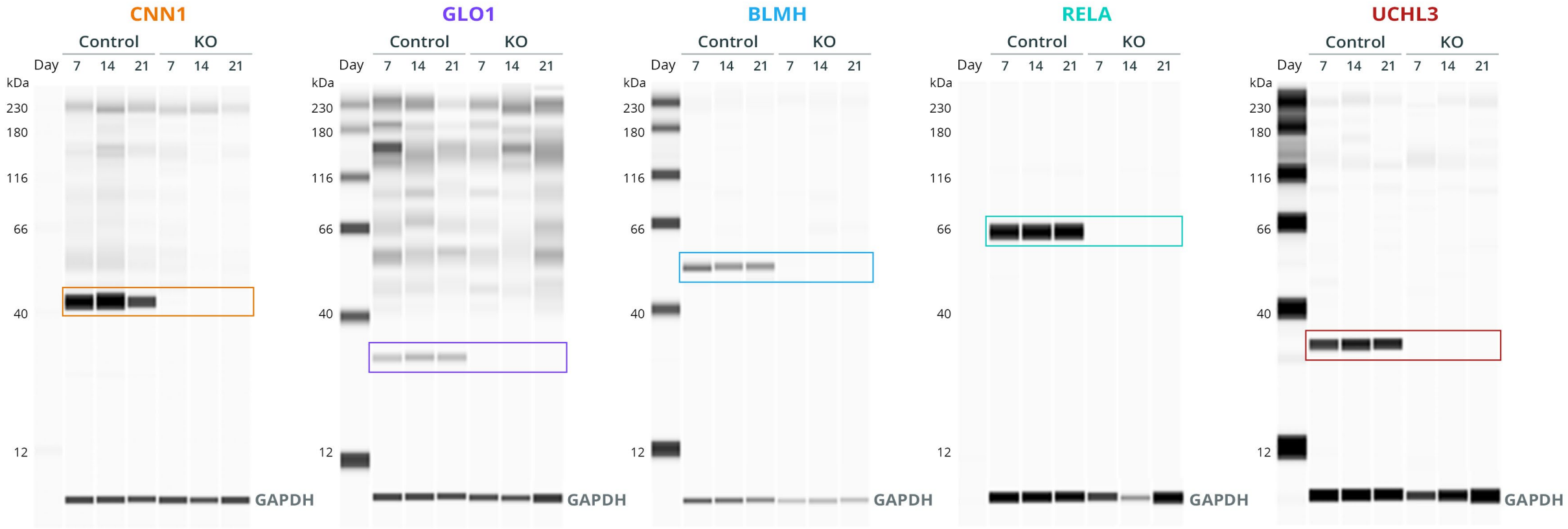


*Multi-guide sgRNA is patent-pending

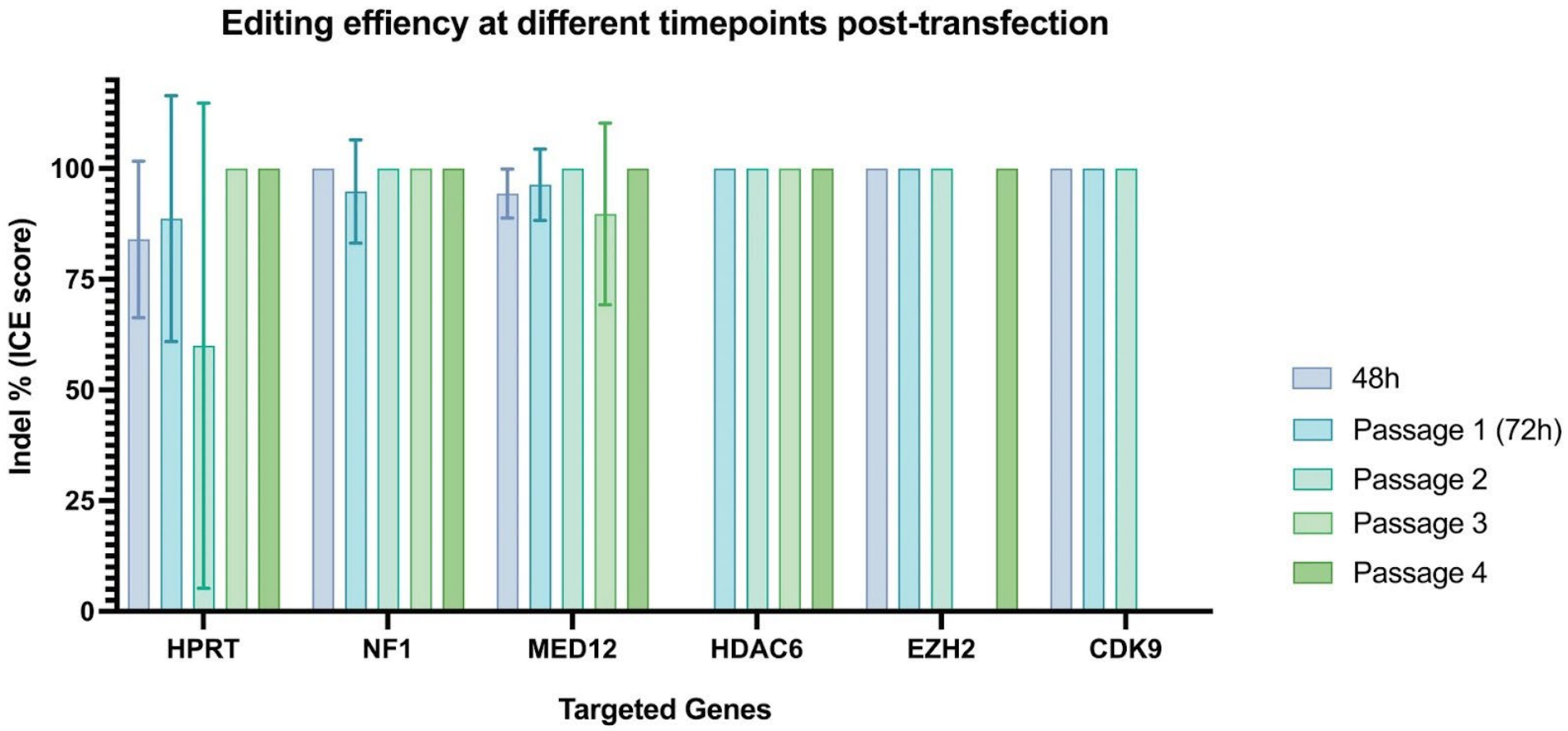


Individual-guide	Multi-guide
69.6%	89.9%

Multi-Guide sgRNA Results in Sustained Protein Depletion in U2OS (ATCC®HTB-96™)



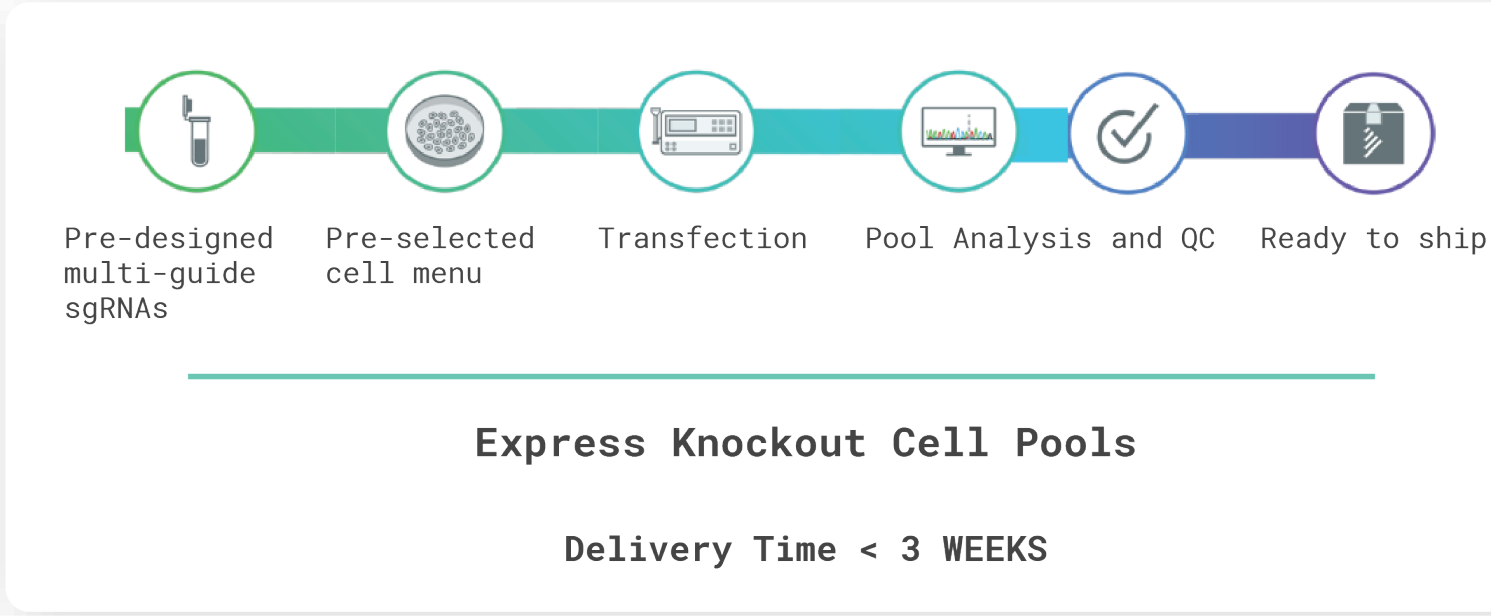
Multi-Guide sgRNA KO pools are stable over multiple passages in A549(ATCC®CCL-185™)



Express Cell Pools

Immortalized Cells

- Knockout Cell Pools
- Speed: delivered in 2 weeks or less!
- Multi-guide technology or custom defined sgRNA
- Selected EditCo Bio-supplied immortalized cell lines:
    - Human



Express Cell Pools Deliverables

Immortalized Cells

- Express Knockout Cell Pools
  - 2 tubes (1.1 mL Micronic)
  - > 300,000 cells/tube
- Wild-type cell pools (Cas9 only mock-transfected)
  - 2 tubes (1.1 mL Micronic)
  - > 300,000 cells/tube
- Sequences of synthetic sgRNAs used
  - Up to 3 sgRNA
- Primer sequences: for PCR and Sanger sequencing
- ICE Analylsis: Sanger sequencing analysis report
- Comprehensive QC report
  - Mycoplasma test (Positive/Negative)
  - Passage number
  - Editing efficiency

EditCo

Handling Procedures For Cells

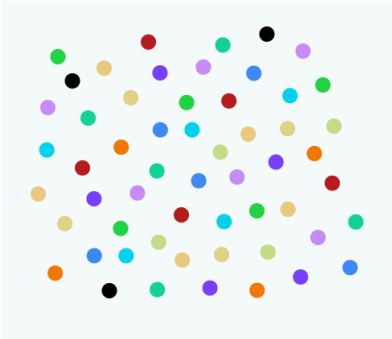
For information on thee culturing conditions and how to store, thaw, and evaluate your Engineered Cells, please refer to our quick start guide at https://hubs.ly/Q01DqKgm0

Edit Information

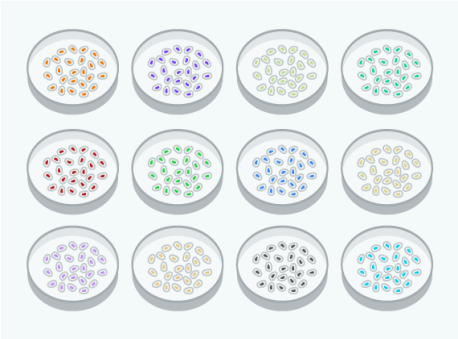
Gene Name	Stat1
Transcript ID	ENSMUST00000186574.6
Guide RNA Sequence	GUGGUUCGAGCUUCAGCAGC GUGGUUCGAGCUUCAGCAGC CUGGAAAAGCAAGACUGGUA
Guide RNA Cut Location	Chr1: 52,122,618 Chr1: 52,122,655 Chr1: 52,122,721
Exon Targeted	3
PCR Primers	FOR Primer (5'-3'): TGCTTTTCAGAAACCAACAGGA REV Primer (5'-3'): AAGAGTCAGCAGGGGTCTGA
Sequencing Primer	Forward
GC Enhancer Used	No

Significance of Cell-Based Disease Models

Already Known Genetic Variants associated With a Disease



Disease Modeling of Variants with Editco Bio's Engineered Cells



Establish Genotype/Phenotype Relationship to Predict Disease Driving Variants

Patient Stratification for Treatment Response Based on Genetic Variants

- CRISPR-edited cells can be excellent tools to study genetic disorders
- They recapitulate the disease biology and help establish the genotype-phenotype correlation
- Cell-based disease models can inform patient stratification in clinical trials

ENGINEERED CELLS

iPS Cell Offerings

CRISPR-edited iPS Cells. Guaranteed.

EditCo

iPS Cell Edit Offerings

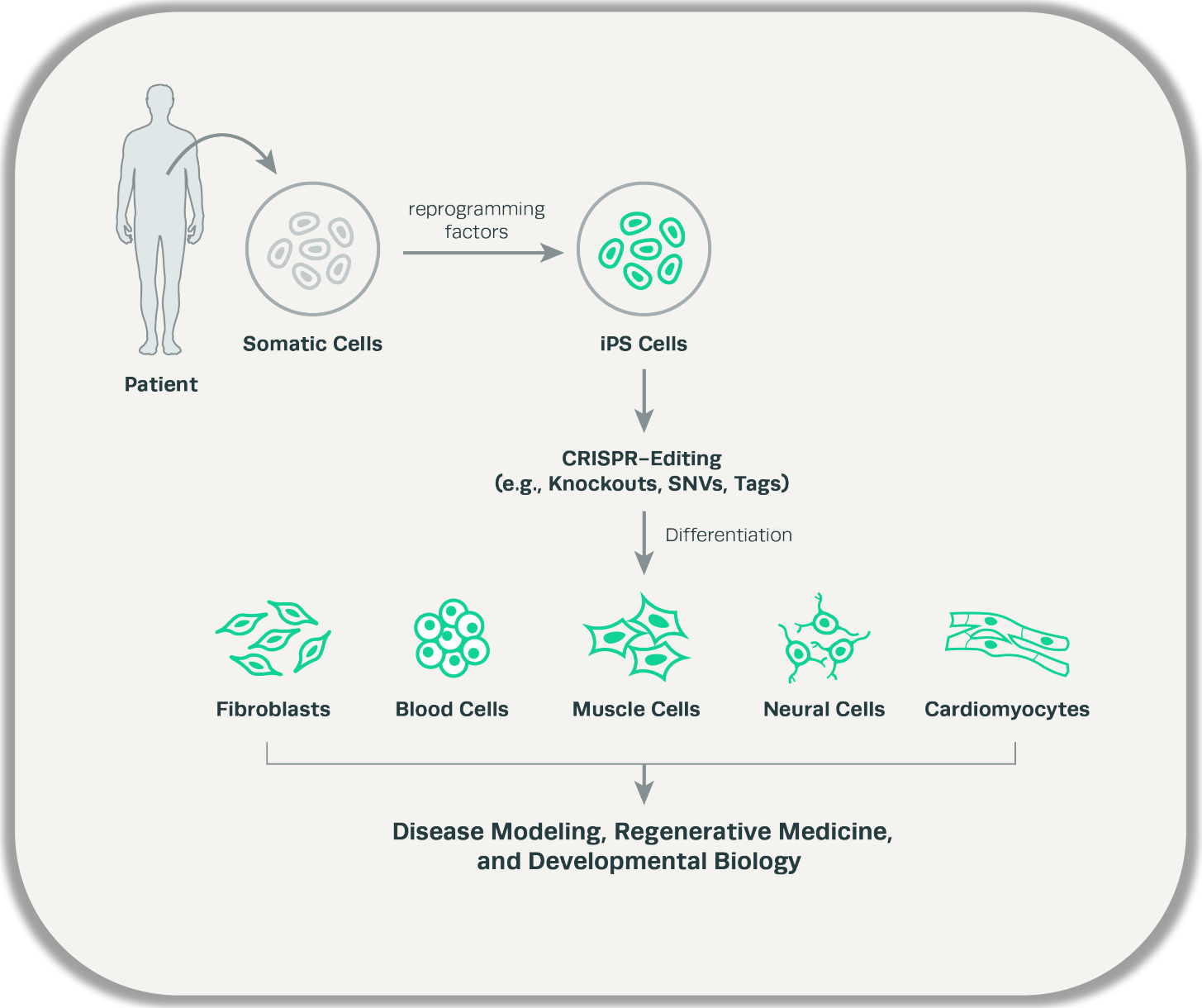
Knockouts, single nucleotide variants, and tag insertions in control or patient-derived iPS cell lines– available in homozygous or heterozygous clone or pool formats.

Knockouts

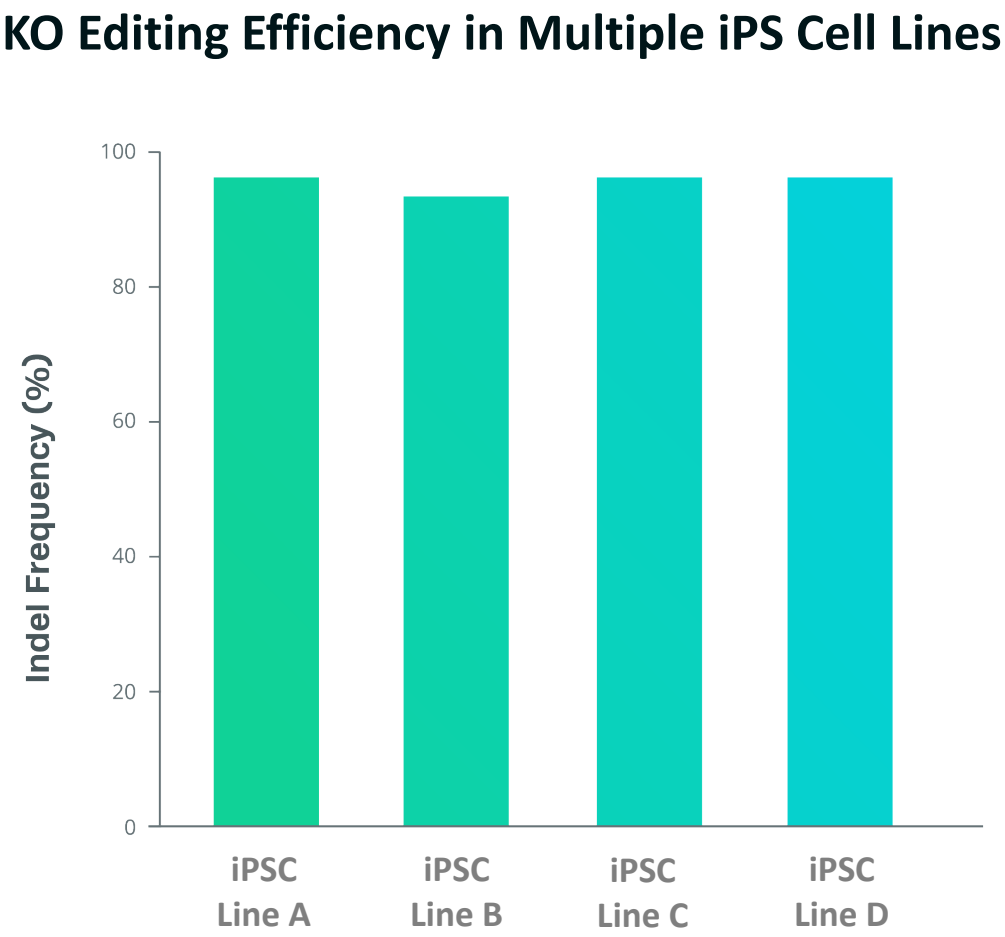
Single Nucleotide Variants

Tags

Versatility of iPS Cell Models

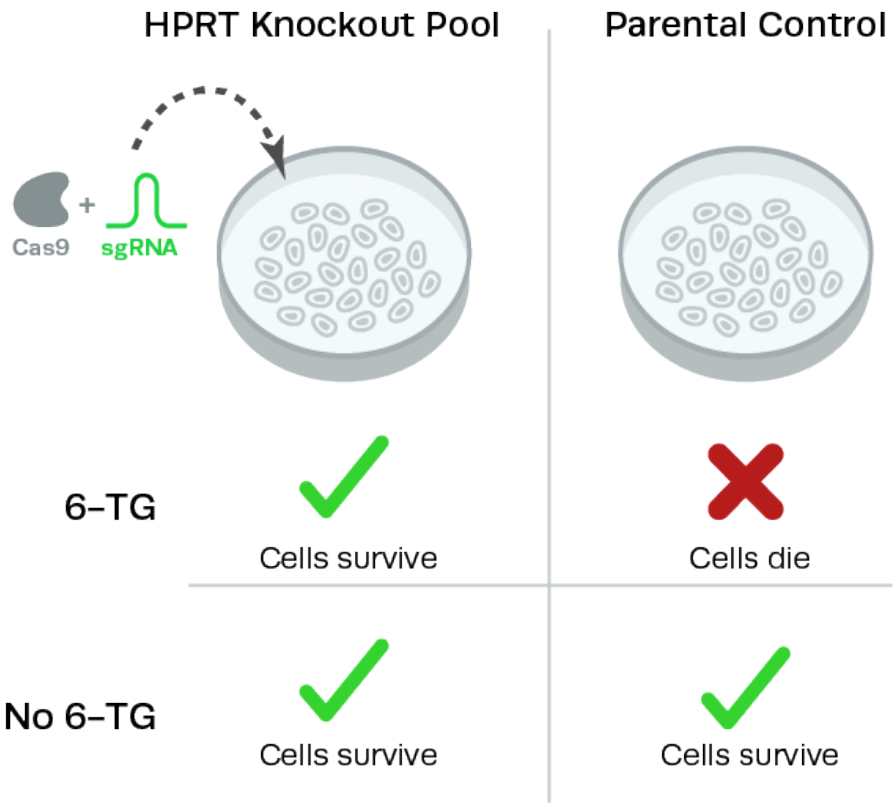


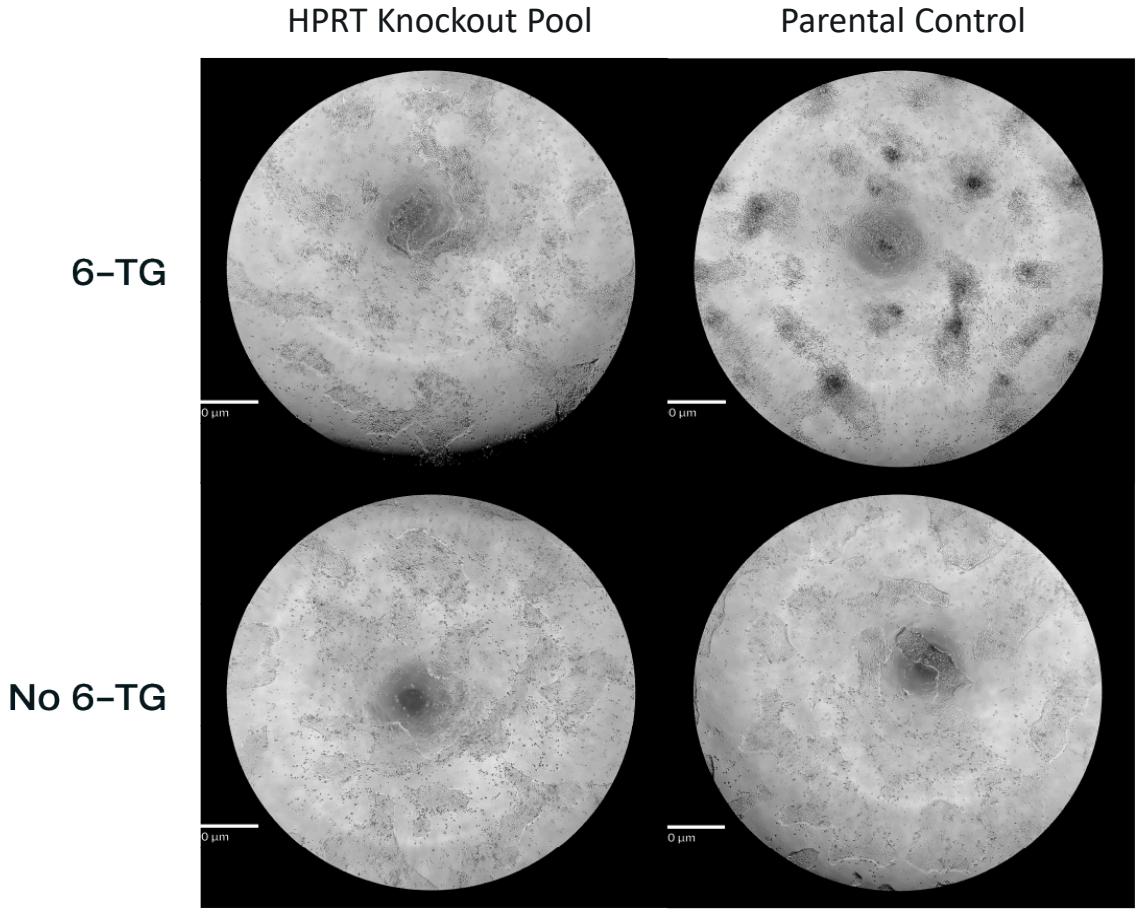
Over 90% Editing Efficiency in iPS Cell Lines



Confirming Functional Knockout in iPS Cell Pools

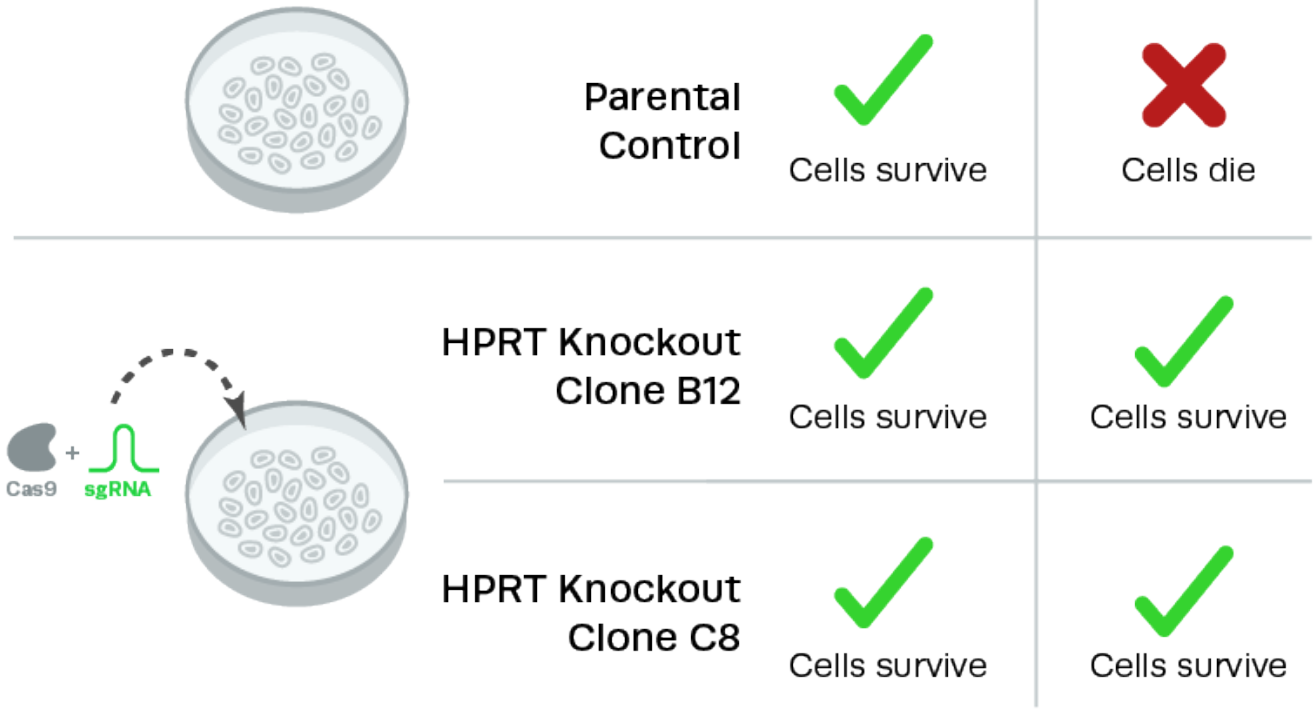
Knockout of HPRT protects cells from 6-TG mediated cell death

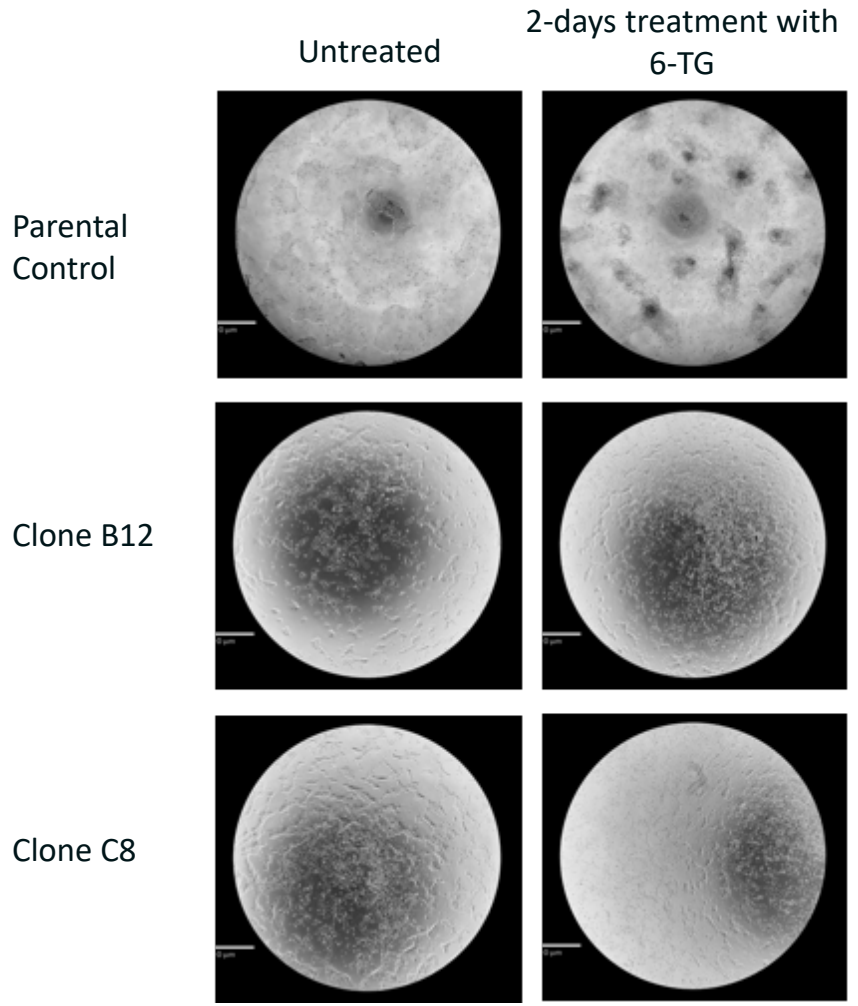




Functional KO in Clonal iPS Cell Lines

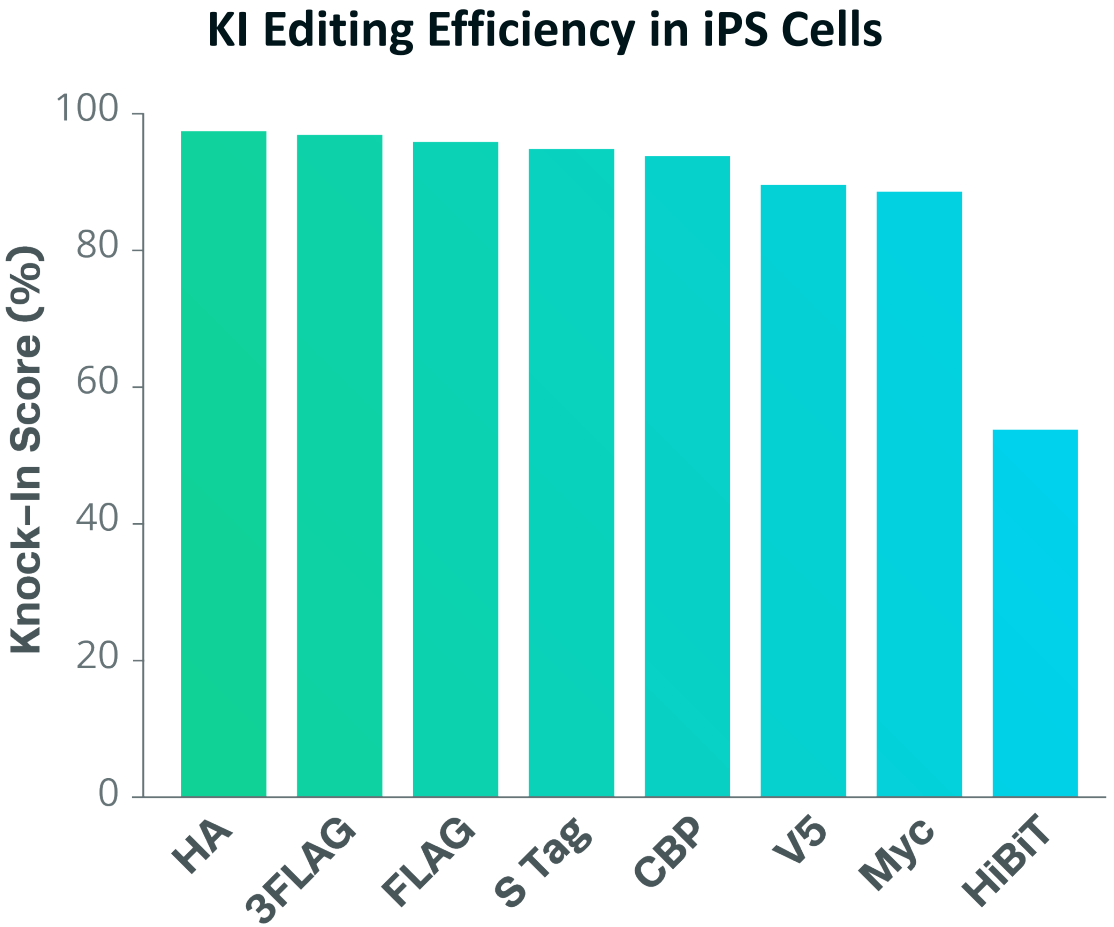
Multiple clones confirm the expected phenotype

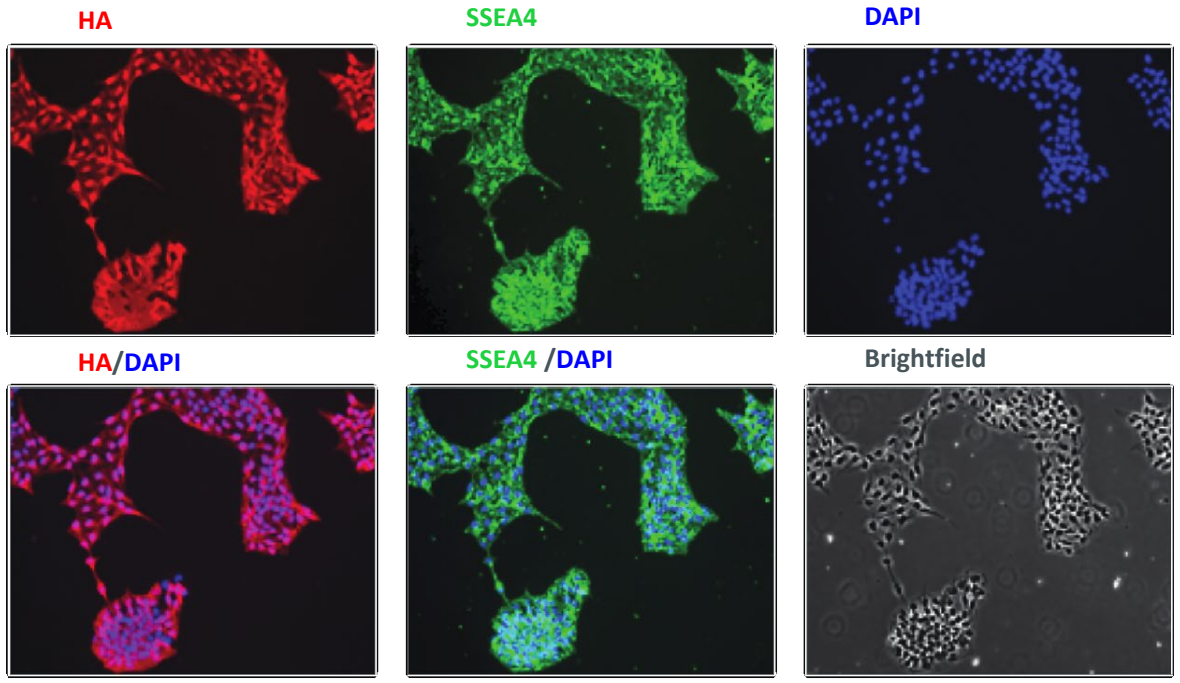




Over 90% Knock-in Efficiency Achieved of Small Tags (<100 bp)

EditCo Bio's process results in incredibly high editing efficiency





iPSC line A

ENGINEERED CELLS

Knockout Primary Immune CD4+ T-Cell Pools

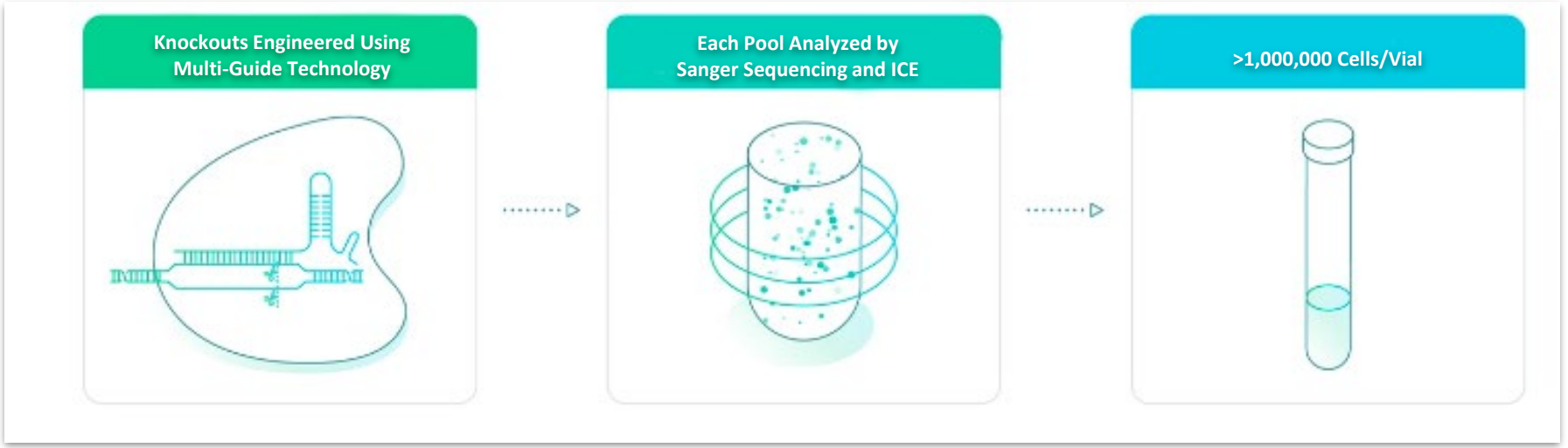
Leverage verified CRISPR gene knockouts in primary human cells with industry leading delivery time.

EditCo

KO Cell Pools

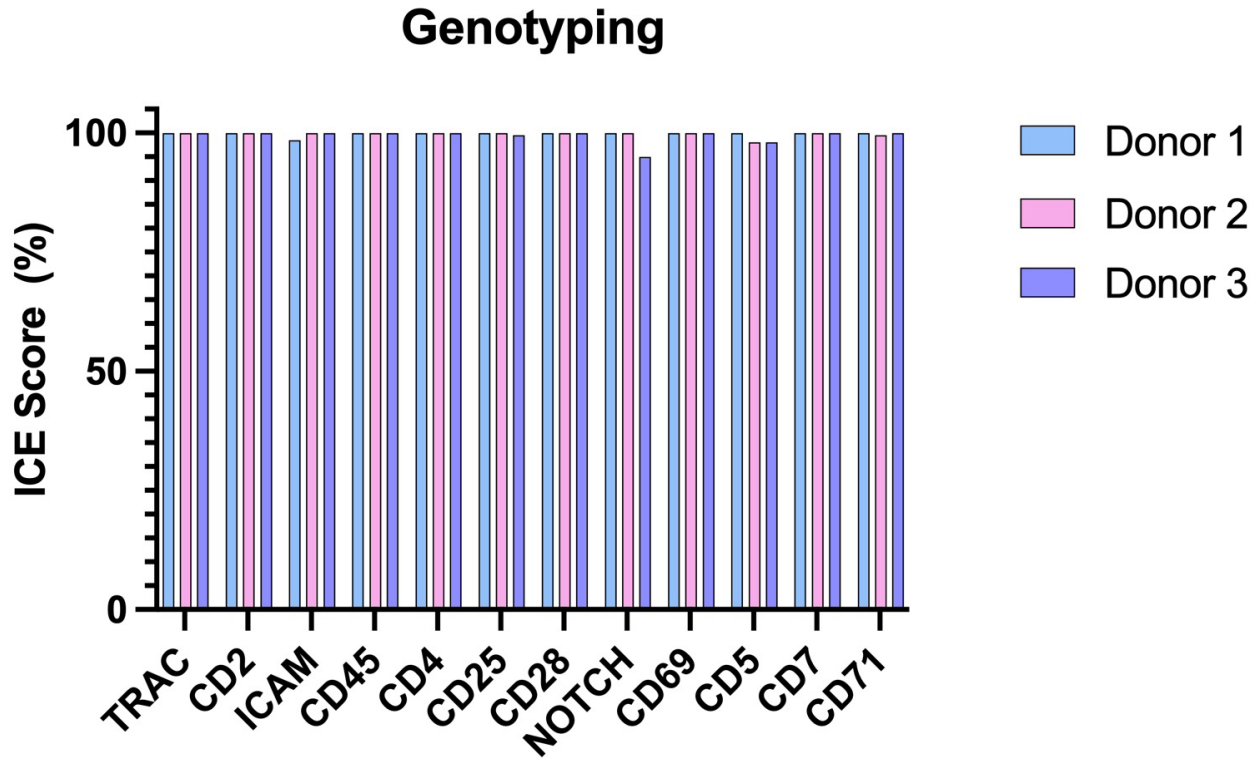
Primary Immune Cells

- Knockout cell pools in CD4+ T-cells
- Multi-guide KO technology
- >80% Editing Efficiency Guarantee
- >80% Pre-freeze viability
- >1M cells/tube



KO Cell Pools

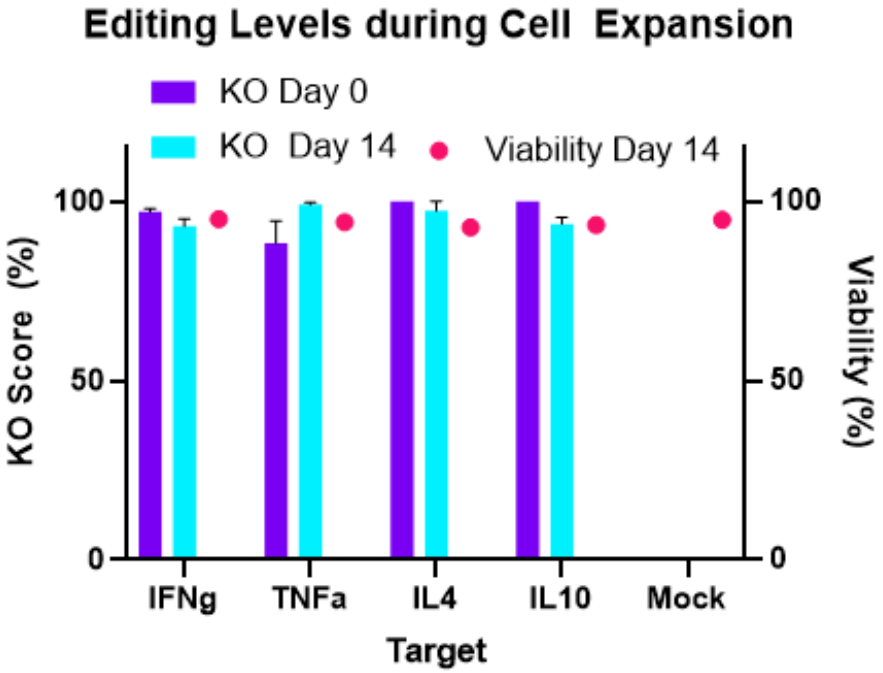
Primary Immune Cells

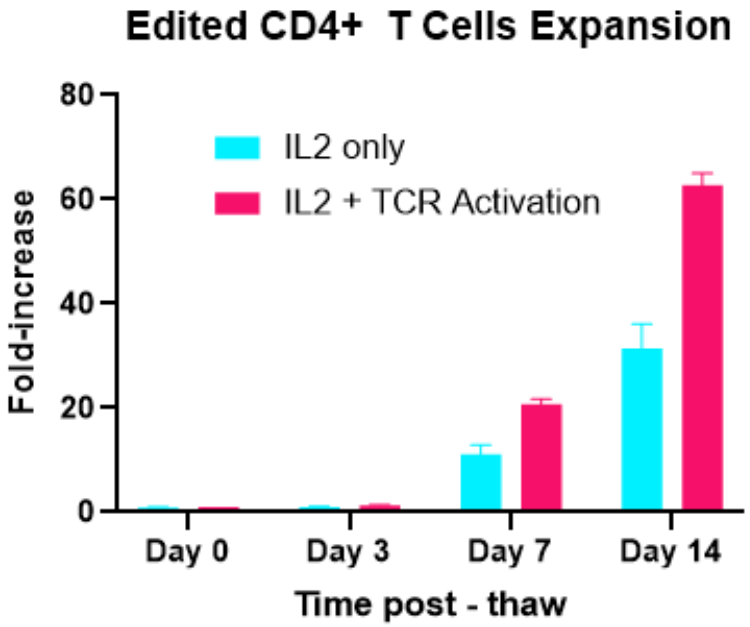


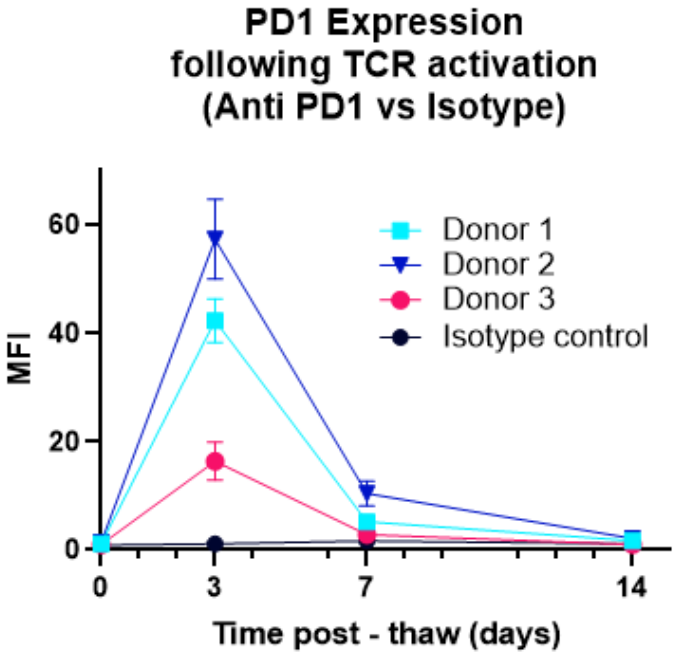
- High editing efficiency across multiple loci and donors

KO Cell Pools

Primary Immune Cells





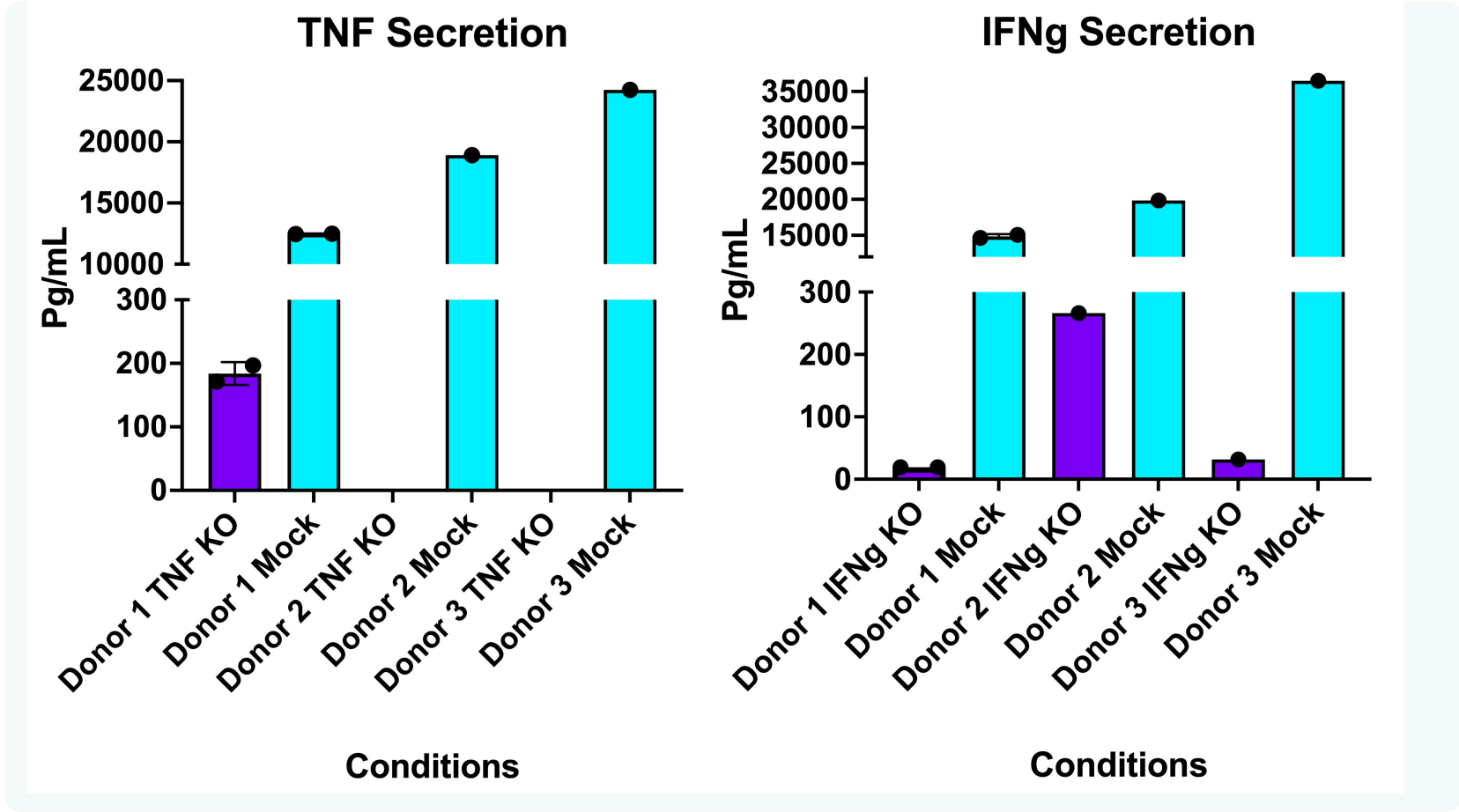


- Editing stability after thaw
- Consistent expansion after thaw
- Low PD1 expression after expansion

KO Cell Pools

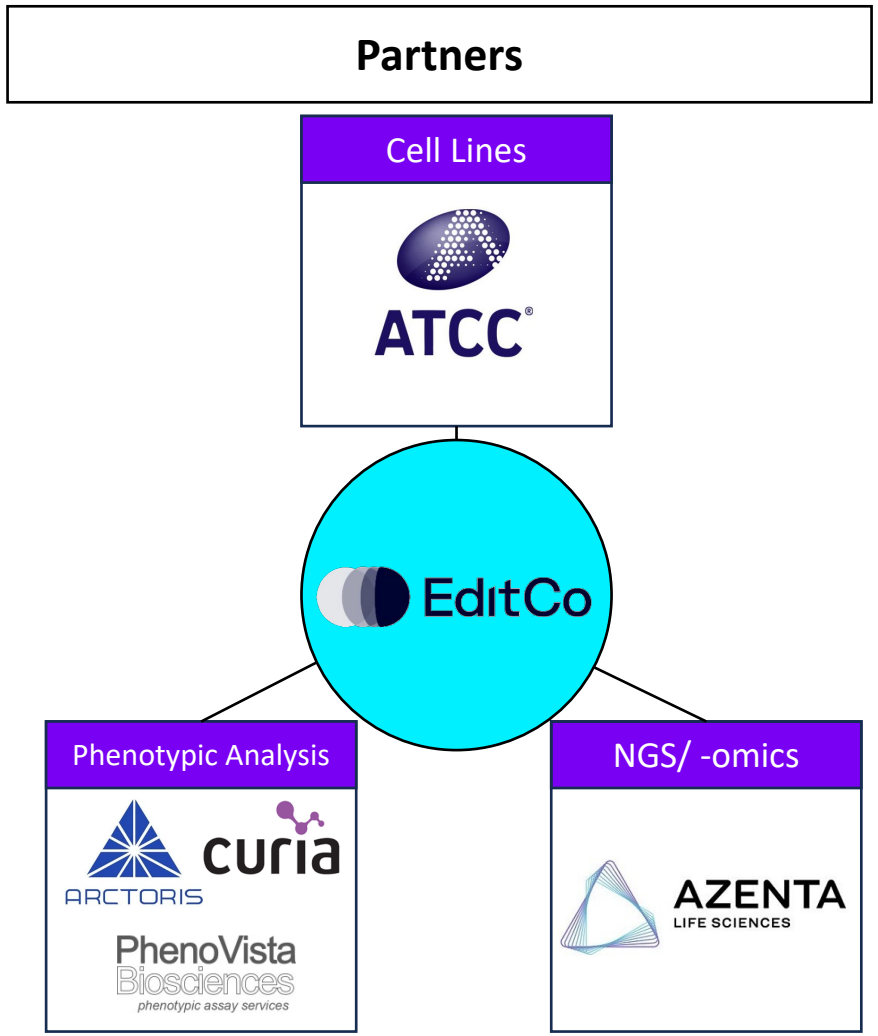
Primary Immune Cells

- Cytokine release assay
- TNFα and IFNg Knock out



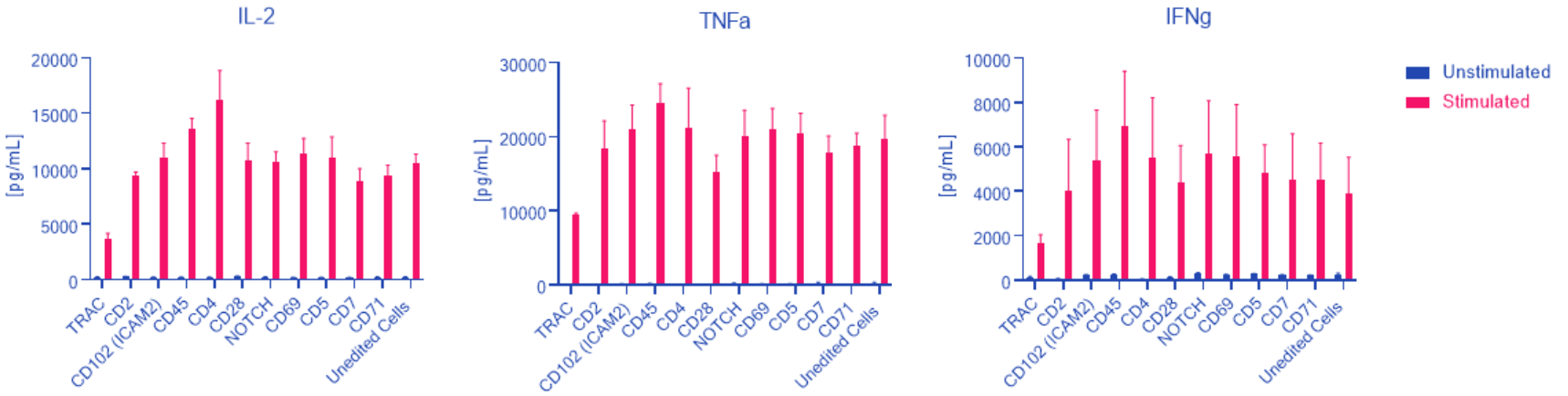
The CRISPR Discovery Partner Ecosystem

- An integrated solution from CRISPR edit to data.
- Access to downstream services who work with us seamlessly.
- Partnerships allow us to offer capabilities outside of our core business.

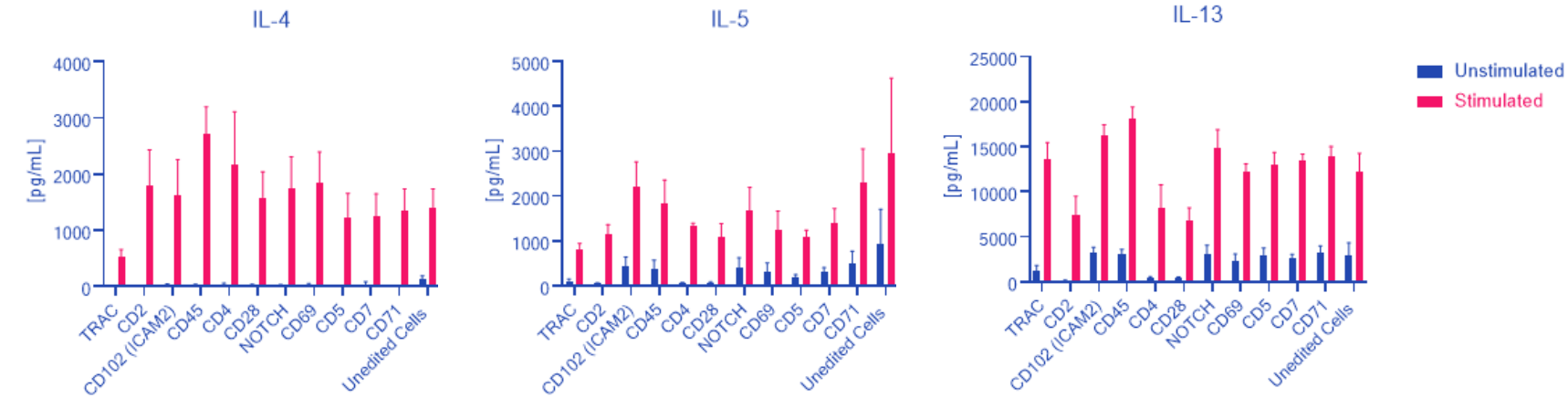


Arctoris partnership for Cytokine release

Th1 cytokines



Th2 cytokines



TALK OVERVIEW

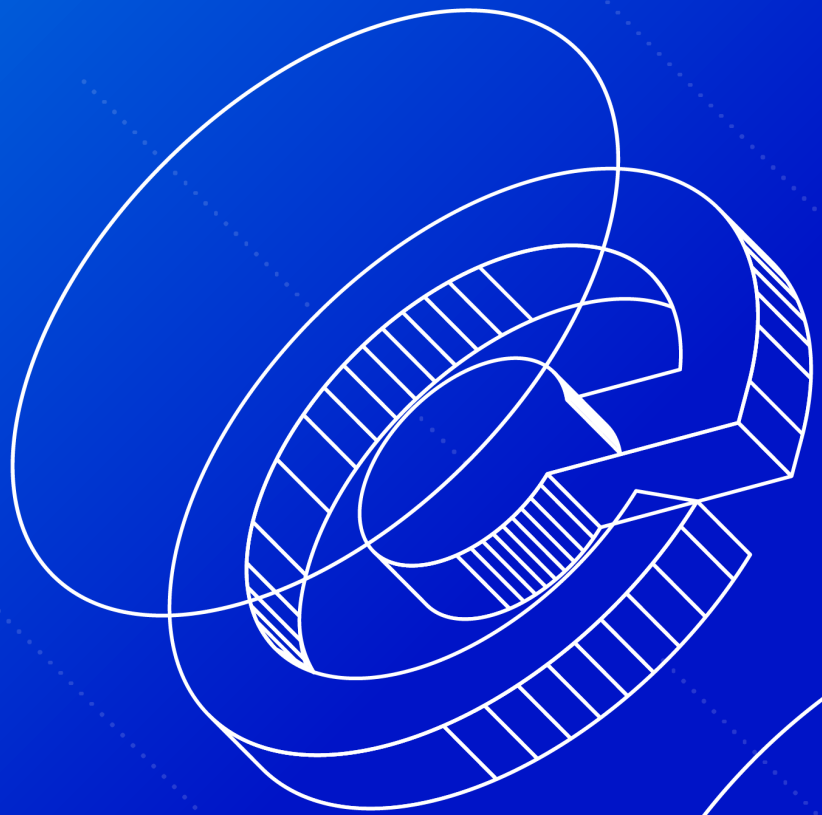
What We Covered

- Express Cell Pools: edited cells in just a few weeks
- iPSC
- CRISPR Discovery Partner Ecosystem
- CRISPR: At an industrial scale

ENGINEERED CELLS

Questions?

Thank you!



New Products:

CAR-T Target Reporter-Labeled Tumor Cells

- Access CAR-T potency and efficacy
- High endogenous expression of CAR-T target antigens
- Available for CD19, CD20, and HER2

Checkpoint Luciferase Reporter Cells

- Enables screening of checkpoint inhibitor molecules
- Wide range of targets such as PD-L1/2, CD-155, B7-H3, PD-1, and others
- Luciferase will be expressed under the control of GAS, NFAT, or NfkB

Human Cancer Models Initiative (HCMI)

- 2-D and 3-D patient-derived models available
- Diverse genetic backgrounds of the same cancer types
- Culturing protocols and organoid growth kits

Assay Ready (Coming soon!)

- Cell lines
- Multi well spheroid assay plates