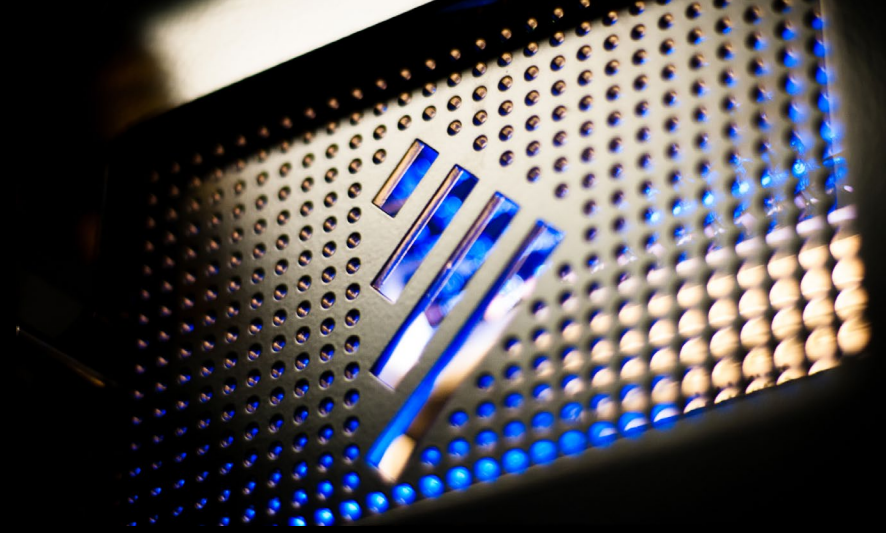
SYNTHEGO

The Genome Engineering Company



Funakoshi

May 27, 2020

Len Goren
VP of Global Sales
len.goren@synthego.com

Kevin Holden, PhD
Head of Science @ Synthego
kevin.holden@synthego.com

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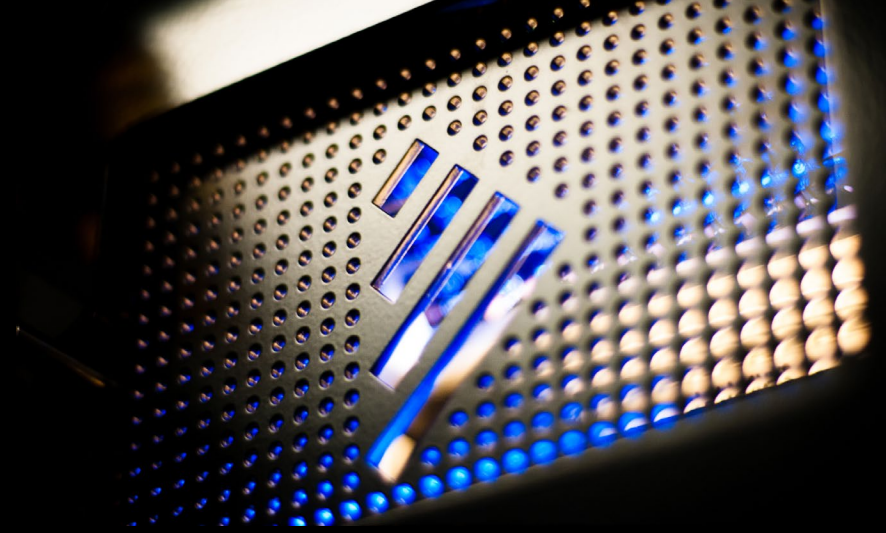
The Genome Engineering Company



How CRISPR technologies are being applied to fight the COVID-19 pandemic

Kevin Holden, PhD
Head of Science @ Synthego
kevin.holden@synthego.com

Agenda

How CRISPR technologies are being applied to fight the COVID-19 pandemic

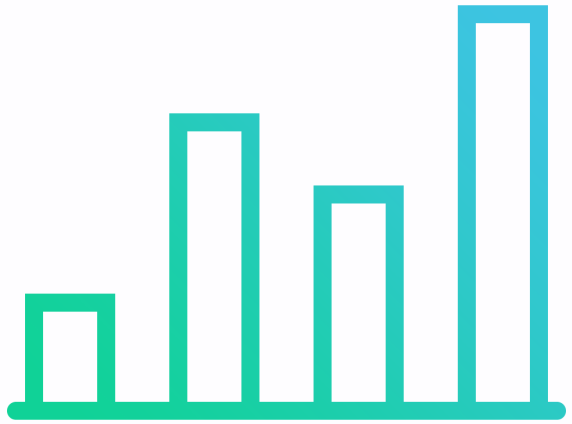
Brief Background

- ❑ A bit on CRISPR
- ❑ A bit on Synthego

How Synthego developed technologies to enable cell engineering at scale

- ❑ Reagent development /optimization
- ❑ Optimizing CRISPR engineering in human cell lines
- ❑ Scaling CRISPR engineering – a relevant application during the COVID-19 pandemic
- ❑ CRISPR engineering in iPSCs (to build better models of disease)

How might you utilize these resources in your own discovery research?

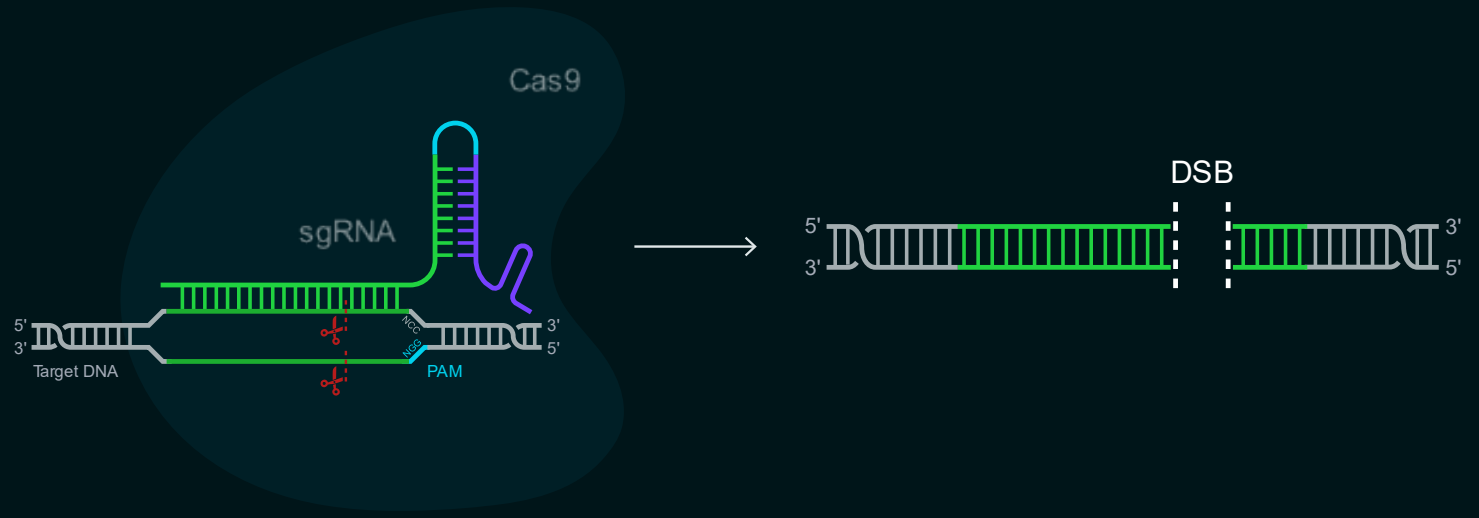


Poll Question

Is your lab currently doing any lab research work during this time?

CRISPR-Cas9: a powerful genome engineering tool

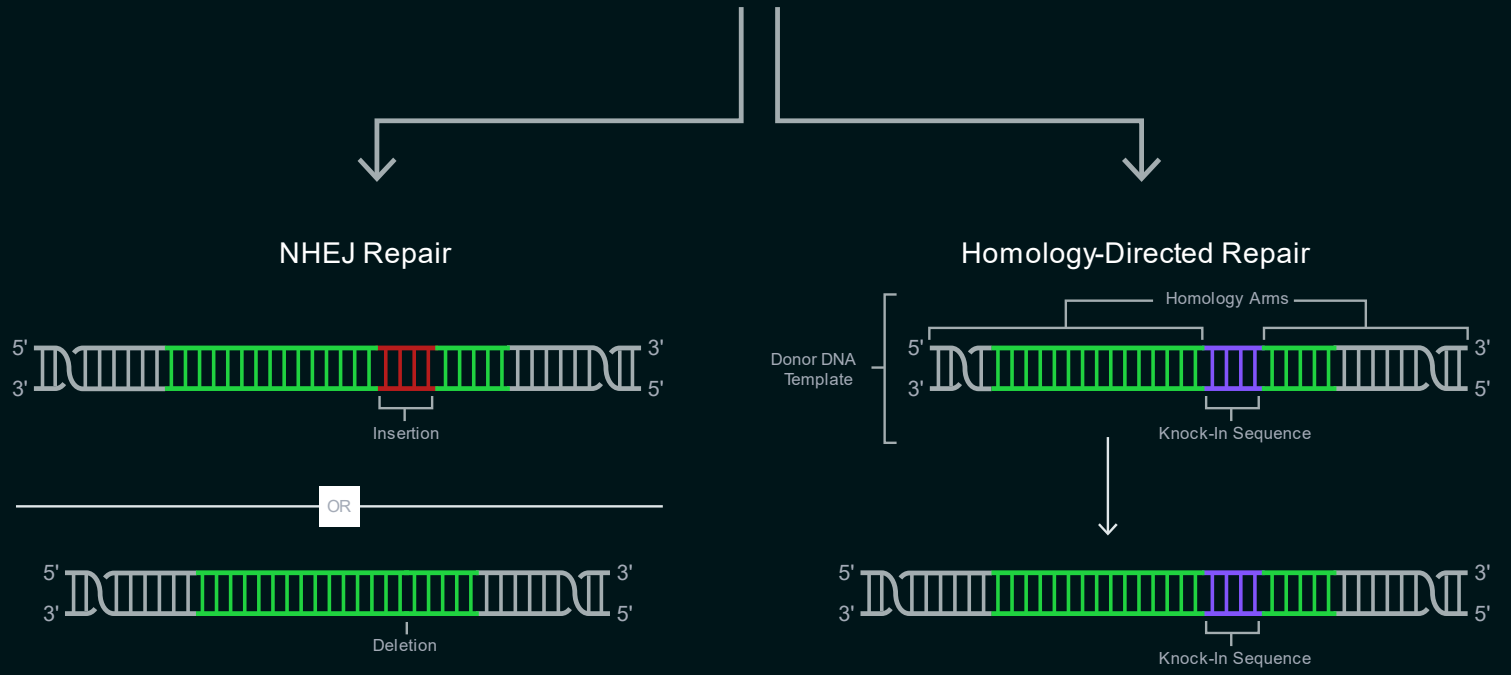
Utilizes a RNA-guided nuclease from bacteria to generate a dsDNA break in a genome



CRISPR is a powerful tool because:

DNA repair is required;

Donor DNA can be introduced to make precise genome edits



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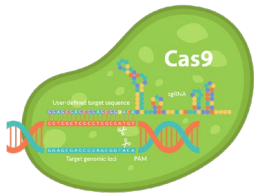
CRISPR applications in disease research & therapeutics

Disease modeling



CRISPR-Cas9 reagents

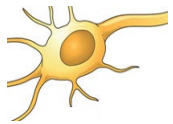
Microinject into
model organism embryo
(Gene KO / KI)

***In vivo* models of disease**

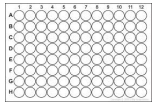
Transfect into cells
(Gene KO / KI)

Primary cell types
(ESCs, iPSCs, T cells etc.)

Immortalized cell lines



***In vitro* models of disease**
(Pathway Analysis,
Immuno-Oncology,
Disease Pathogenesis)



HTP screening
(Drug Discovery)

Immortalize, Differentiate,
Develop Organoid, etc.

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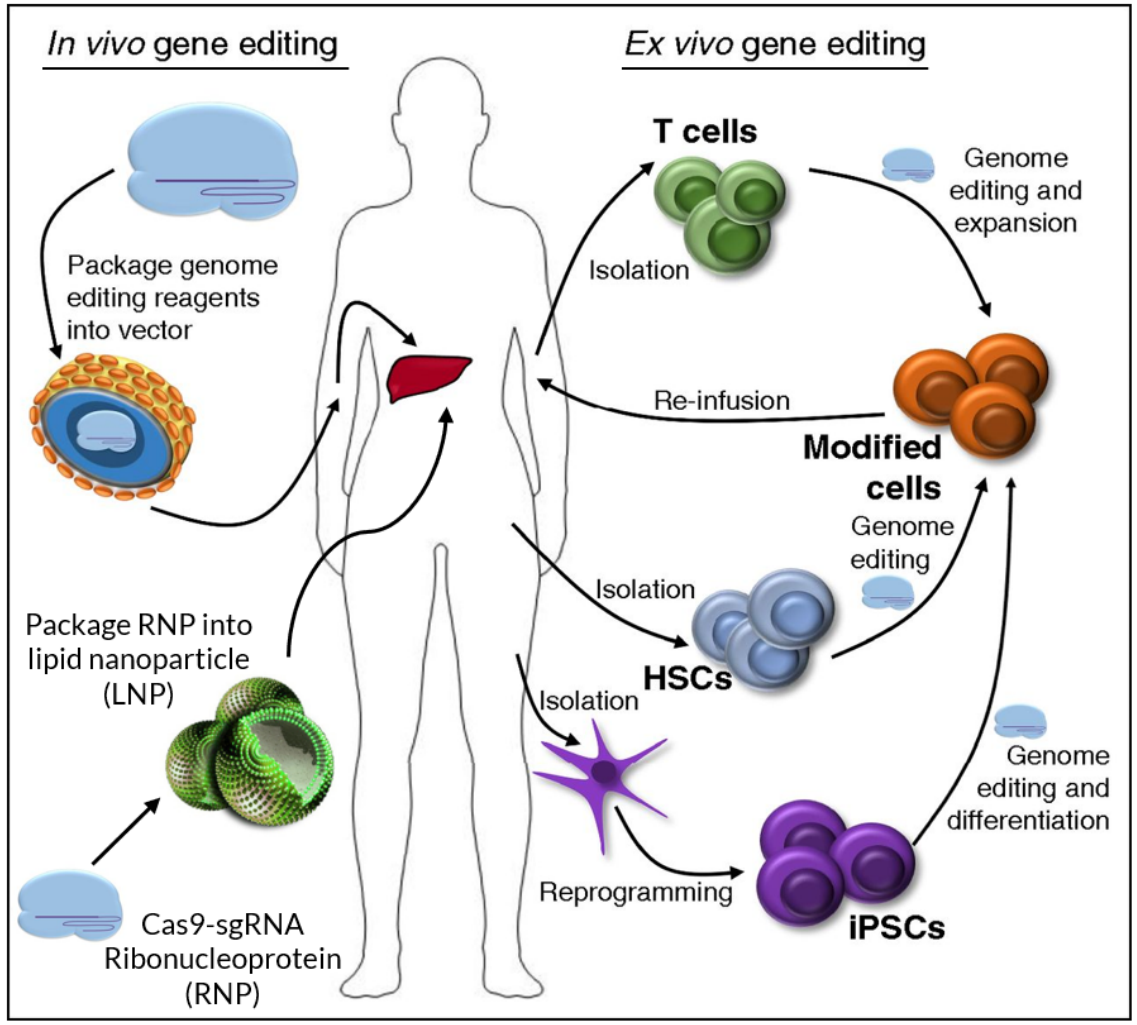


Adapted from: American Society of Hematology, *Blood*, 2016

What does Synthego do?

Provide Access to CRISPR Genome Engineering Tools
In order to build research standardization…
To Enable the Development of Human Therapeutics

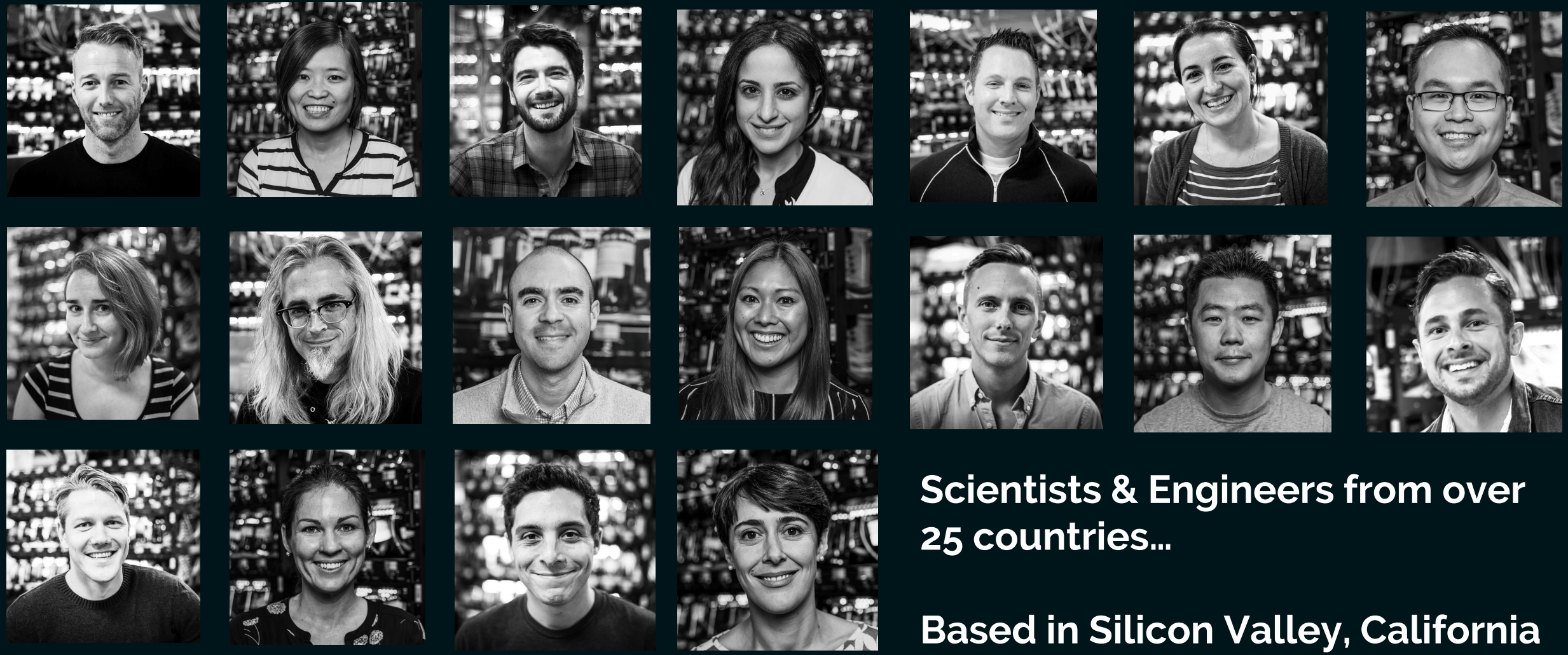
Sell Products & Provide Services for CRISPR Genome Engineering
(Synthetic sgRNA (RUO/GMP), Engineered Cells, Software, etc.

Research Teams: Technology Development
(Biology, Chemistry, Engineering, Bioinformatics, Automation)

Why build standardization into research/discovery platforms?

"The 2016 poll of 1,576 scientists reported that 70% of them had failed to reproduce at least one other scientist's experiment (50% had failed to reproduce one of their own experiments)"

Who are Synthego?

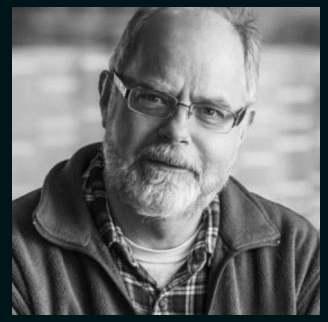


Scientists & Engineers from over 25 countries...

Based in Silicon Valley, California

Who scientifically advises Synthego?

Jennifer Doudna, PhD
Professor
UC Berkeley



Bill Skarnes, PhD
Professor & Director of Cell Engineering
JAX Labs

Matthew Porteus MD, PhD
Professor & Clinician
Stanford University

Sir Andrew Witty
Former CEO
Glaxo Smith Kline

Agenda

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How might you utilize these resources in your own discovery research?

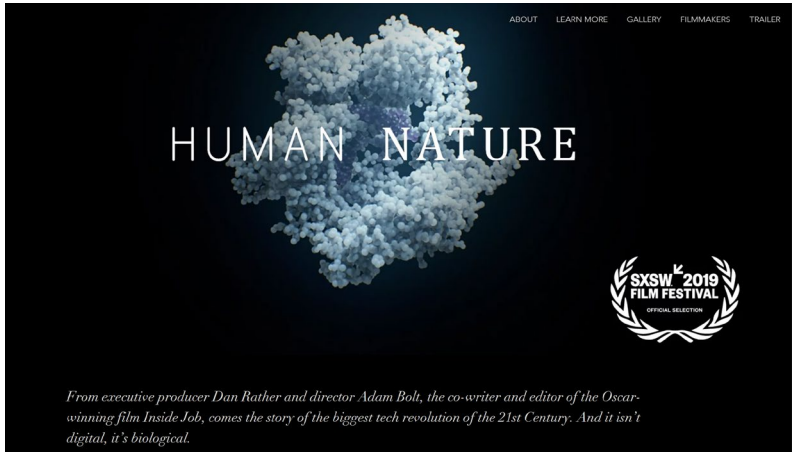
Automated sgRNA Smart Factory

Launched in 2016 as world's first automated HTP sgRNA production factory

Novel synthesis architecture utilizing microfluidics and software management (worldwide patents granted)

Software management = Synthesis accuracy through process control

Synthesized >1 million unique compounds since 2016 (Synthego ships and uses 1,000s of sgRNAs/month)



<u>"Human Nature" Documentary</u>

Theatrical Release March 13, 2020

Available on:
Amazon Prime, iTunes
BBC iPlayer (UK) - FREE

humannaturefilm.com

SYNTHEGO

Ayal Hendel & Rasmus Bak et al. (2015) (Matthew Porteus Lab, Stanford University)

First to demonstrate efficient CRISPR editing in Primary Human Cells – using synthetic RNA

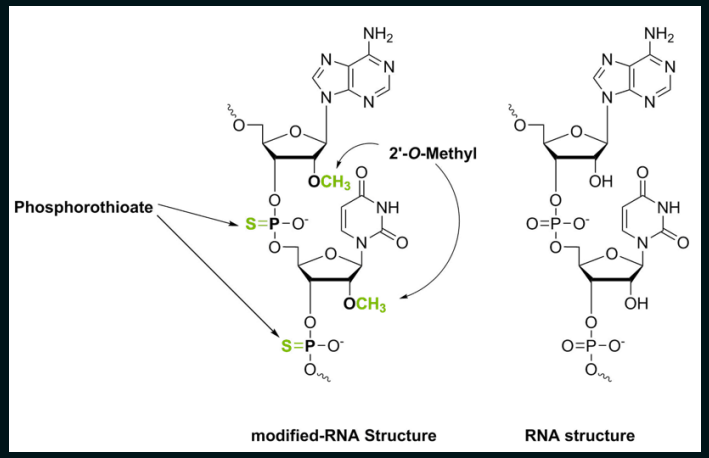
nature biotechnology

LETTERS

29 June, 2015

Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells

Ayal Hendel[1,5], Rasmus O Bak[1,5], Joseph T Clark[1], Andrew B Kennedy[2], Daniel E Ryan[2], Subhadeep Roy[3], Israel Steinfeld[4], Benjamin D Lunstad[3], Robert J Kaiser[2], Alec B Wilkens[1], Rosa Bacchetta[1], Anya Tsalenko[2], Douglas Dellinger[3], Laurakay Bruhn[2] & Matthew H Porteus[1]



Exonuclease resistance

Prevent innate immune cascades caused by plasmids; IVT-derived sgRNAs *(Kim et al, 2018; Weinert et al, 2018)*

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Chemically Modified, Synthetic sgRNA Enables Genome Engineering in Many Primary Cells Types (150+ citations)

ELSEVIER

Methods

Available online 16 June 2019

In Press, Corrected Proof

Improving homology-directed repair efficiency in human stem cells

William C. Skarnes [a], Enrica Pellegrino [b], Justin A. McDonough [a]

nature

c-Jun overexpression in CAR T cells induces exhaustion resistance

Rachel C. Lynn, Evan W. Weber, Elena Sotillo, David Gennert, Peng Xu, Zinaida Good, Hima Anbunathan, John Lattin, Robert Jones, Victor Tieu, Surya Nagaraja, Jeffrey Granja, Charles F. A. de Bourcy, Robbie Majzner, Ansuman T. Satpathy, Stephen R. Quake, Michelle Monje, Howard Y. Chang & Crystal L. Mackall

Science Immunology

B cells engineered to express pathogen-specific antibodies protect against infection

Howell F. Moffett[1], Carson K. Harms[1], Kristin S. Fitzpatrick[1], Marti R. Tooley[1], Jim Boonyaratanakornkit[1] and Justin J. Taylor[1,2,3,*]

nature protocols

Recipes for Researchers

PROTOCOL

CRISPR/Cas9 genome editing in human hematopoietic stem cells

Rasmus O Bak[1,2], Daniel P Dever[1,2] & Matthew H Porteus[1]

nature

CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy

Amira A. Barkal, Rachel E. Brewer, Maxim Markovic, Mark Kowarsky, Sammy A. Barkal, Balyn W. Zaro, Venkatesh Krishnan, Jason Hatakeyama, Oliver Dorigo, Layla J. Barkal & Irving L. Weissman

bioRχiv

THE PREPRINT SERVER FOR BIOLOGY

Efficient CRISPR/Cas9 gene ablation in uncultured naïve mouse T cells for *in vivo* studies

Simone Nüssing, Imran G. House, Conor J. Kearney, Stephin J. Vervoort, Paul A. Beavis, Jane Oliaro, Ricky W. Johnstone, Joseph A. Trapani, Ian A. Parish

doi: https://doi.org/10.1101/730812

nature communications

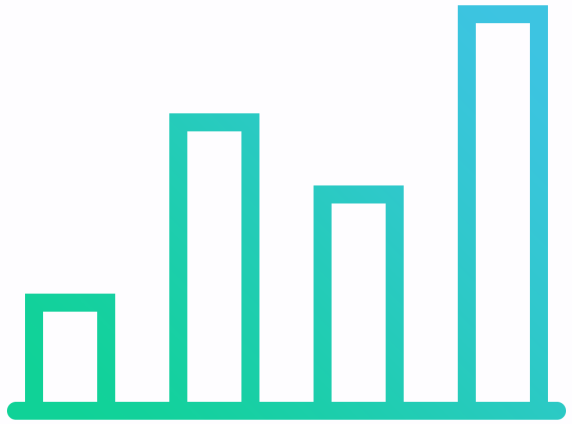
Highly efficient multiplex human T cell engineering without double-strand breaks using Cas9 base editors

Beau R. Webber, Cara-lin Lonetree, Mitchell G. Kluesner, Matthew J. Johnson, Emily J. Pomeroy, Miechaleen D. Diers, Walker S. Lahr, Garrett M. Draper, Nicholas J. Slipek, Branden A. Smeester, Klaus N. Lovendahl, Amber N. McElroy, Wendy R. Gordon, Mark J. Osborn & Branden S. Moriarity

January, 2020

Synthego Announces sgRNA GMP Manufacturing for CRISPR–based Therapeutics & Diagnostics

SYNTHEGO

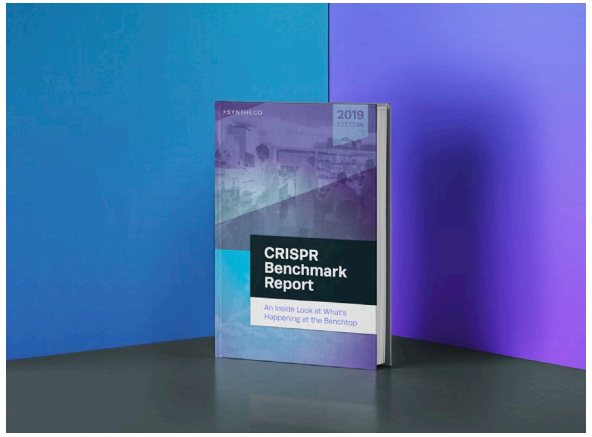


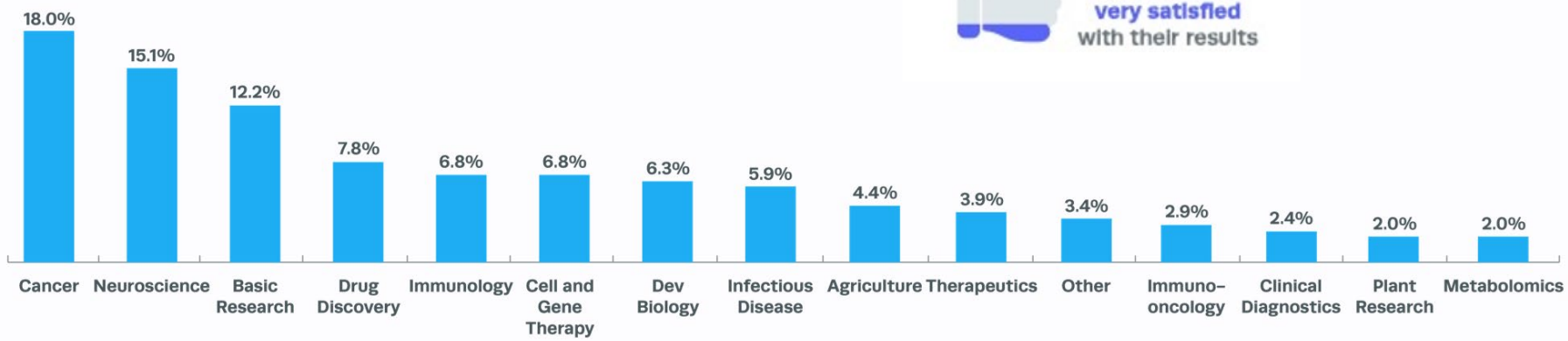
Poll Question

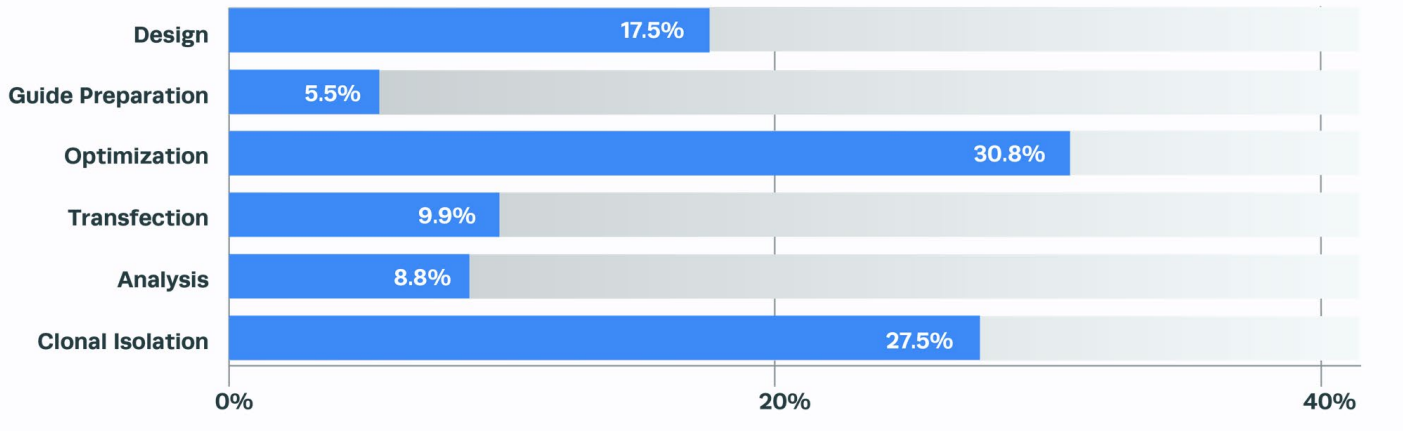
Are you or people in your lab already using CRISPR?

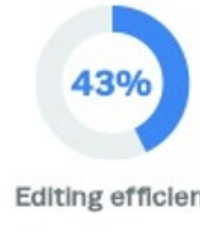
We conducted a blinded CRISPR survey of 200+ scientists…

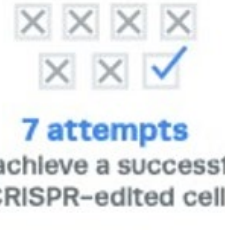
https://www.synthego.com/crispr-benchmark





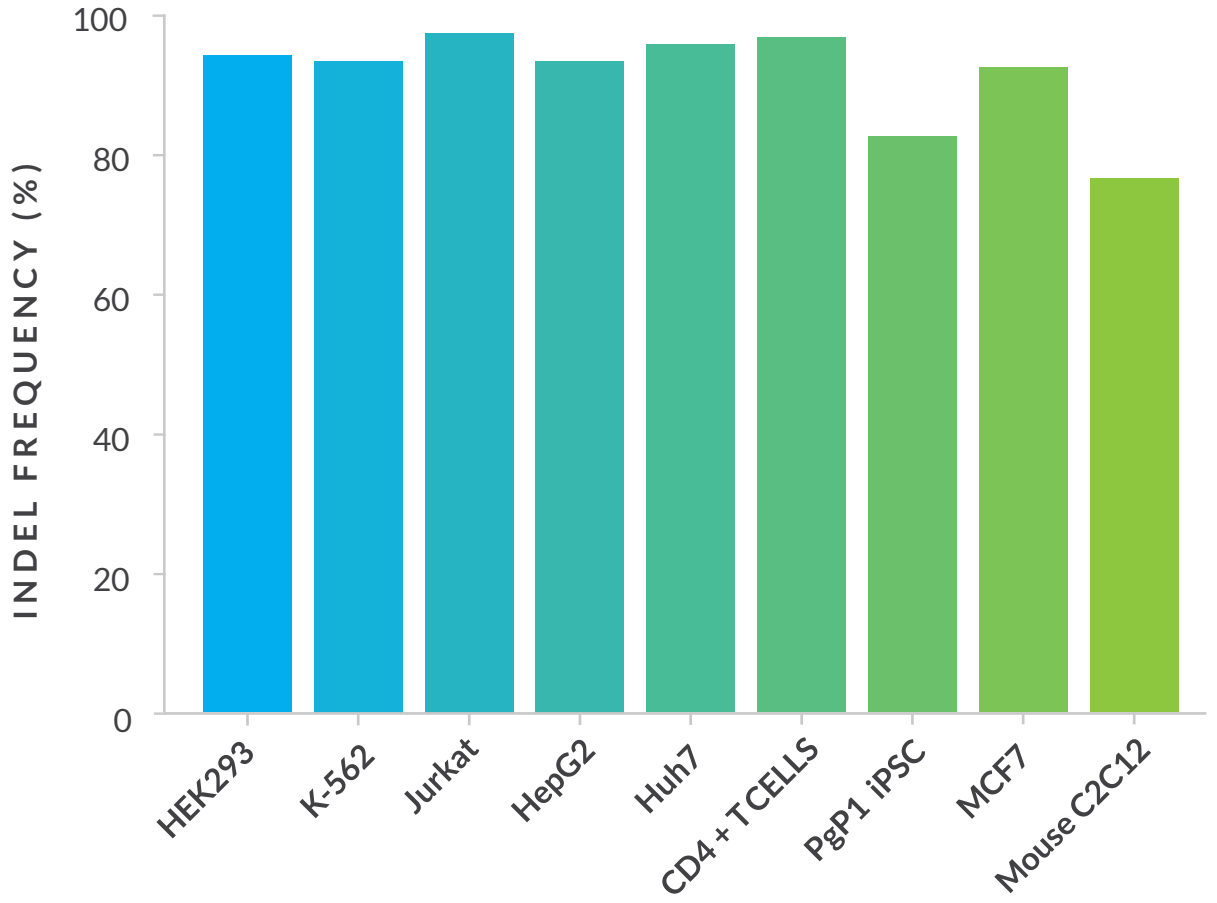






SYNTHEGO

RNPs (modified synthetic sgRNAs) efficiently edit many cell types



sgRNA targeting *RelA*

% Indels seen in Sanger Sequencing of PCR

How we do CRISPR @ Synthego

<u>Ribonucleoprotein (RNP) Complexes</u>

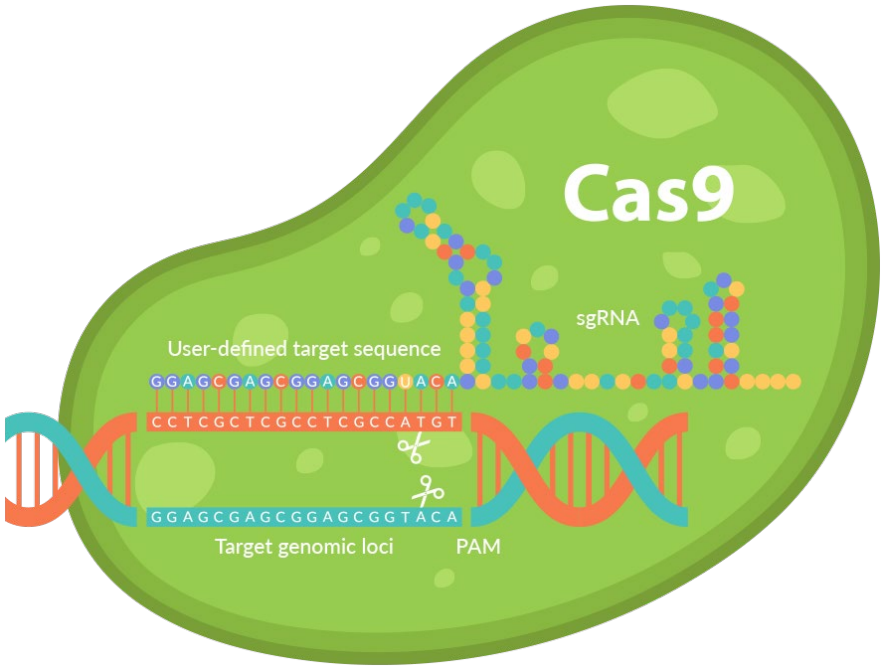
Purified Cas9 nuclease

+

Chemically synthesized (end) modified 100nt sgRNA

Nucleofected into cells

(16 / 96 / 384)



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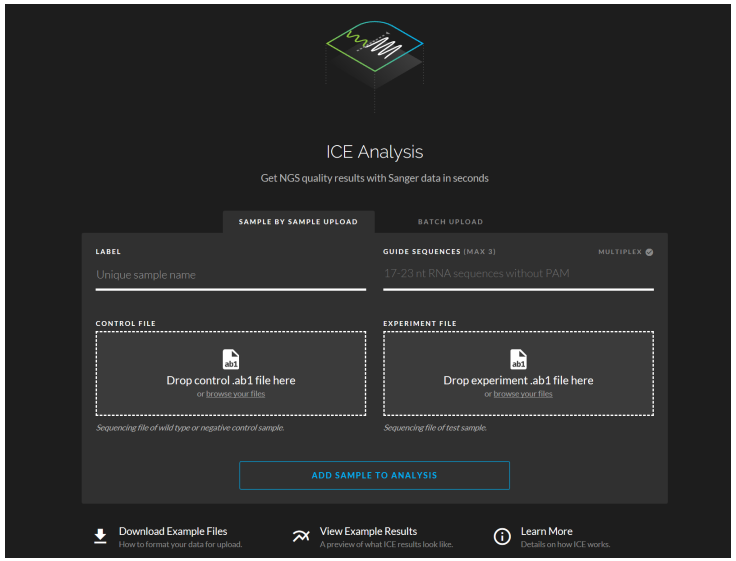
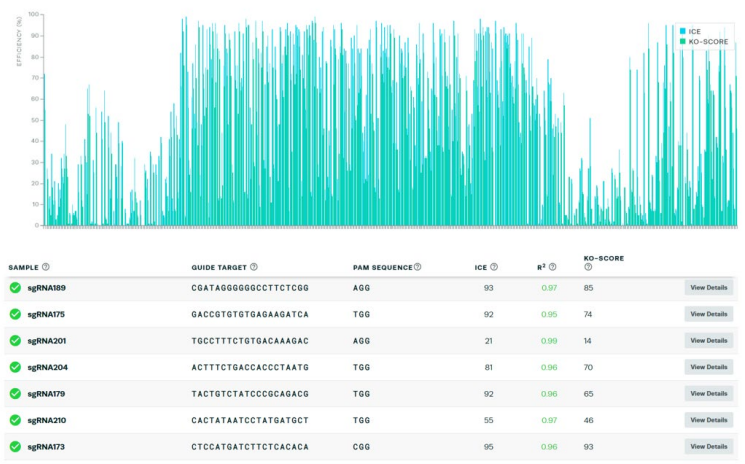
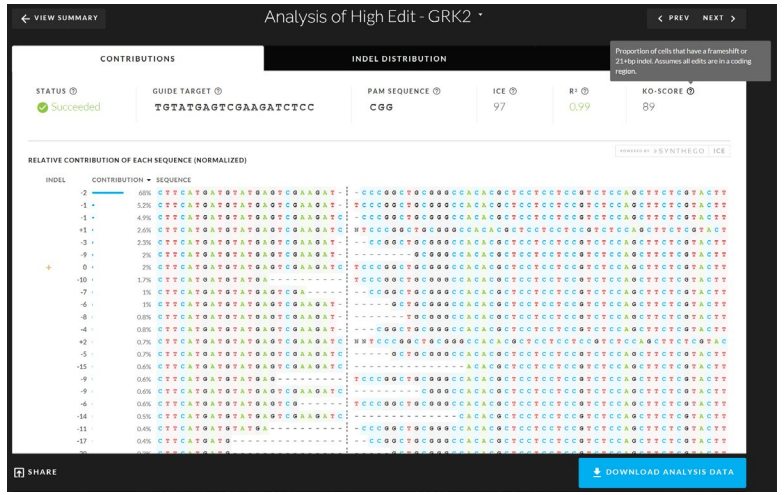
Inference of CRISPR Edits (ICE) from Sanger Sequencing:

HTP editing analysis







- Indel frequency
- Knockout % score
- Knock-in % score
- FREE to use

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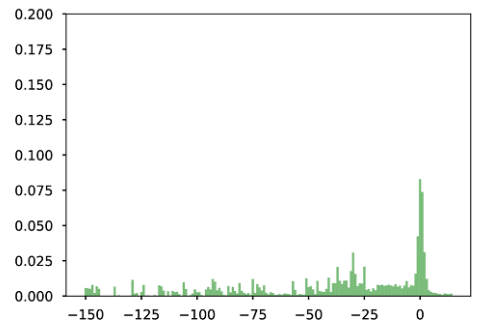
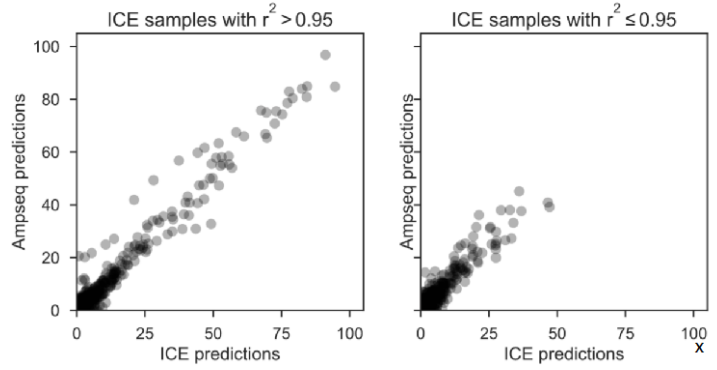
(in peer review)

SUPPORT FOR LARGE DELETIONS



COMPARED AGAINST NGS



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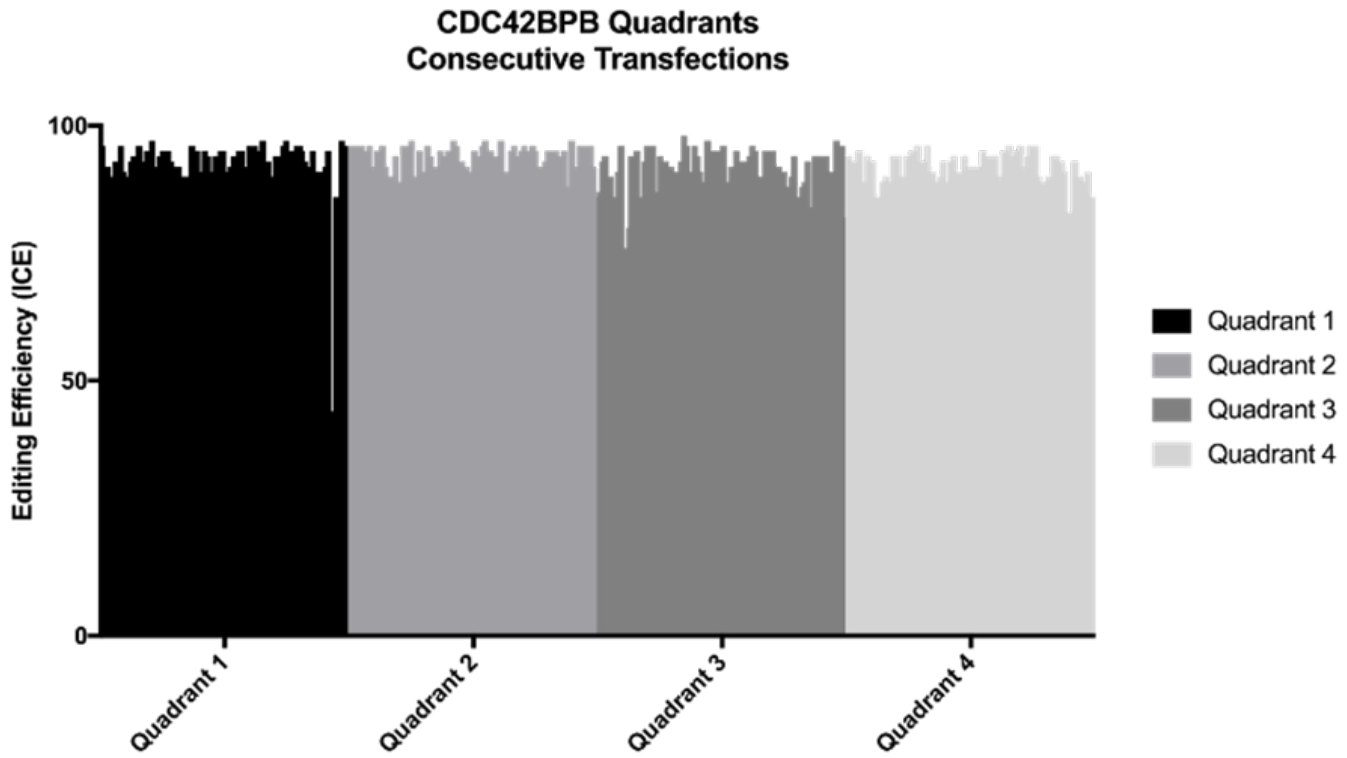
Highly consistent reagent synthesis + optimized transfection = reproducible CRISPR experiments…

…ideal for automation, CRISPR engineering @ scale

Methods

384 well of same positive control sgRNA (CDC42BPB gene)
RNP
HEK293 cells
384 Nucleofection (+4 days)
Indel formation (NHEJ)
Sequencing/ICE analysis



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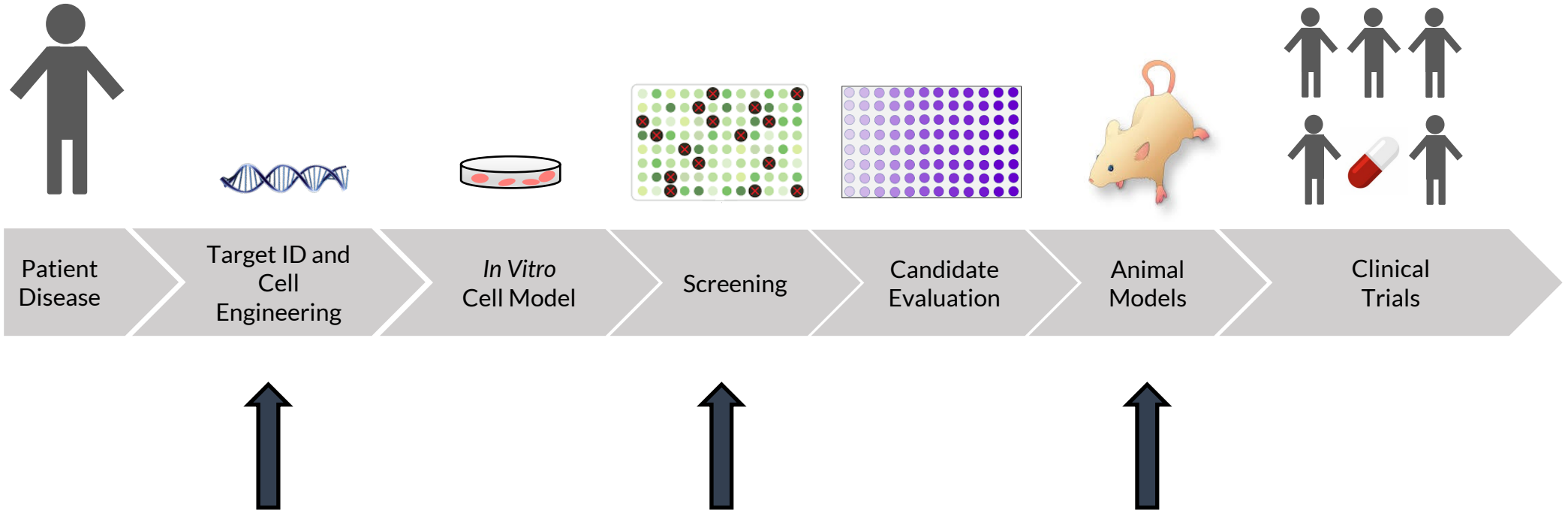
Where can CRISPR be used in discovery pipelines?



Common CRISPR applications for discovery research

Gene Knockouts (KO)

Discovery of Protein Function / Pathway Analysis

Antibody & Assay Validation

Small Molecule Screening / Assay

Gene Knockins (KI)

Disease Modelling (SNV)

Endogenous Protein Tagging

Enabling Standardized CRISPR-Engineering of Cells … at scale

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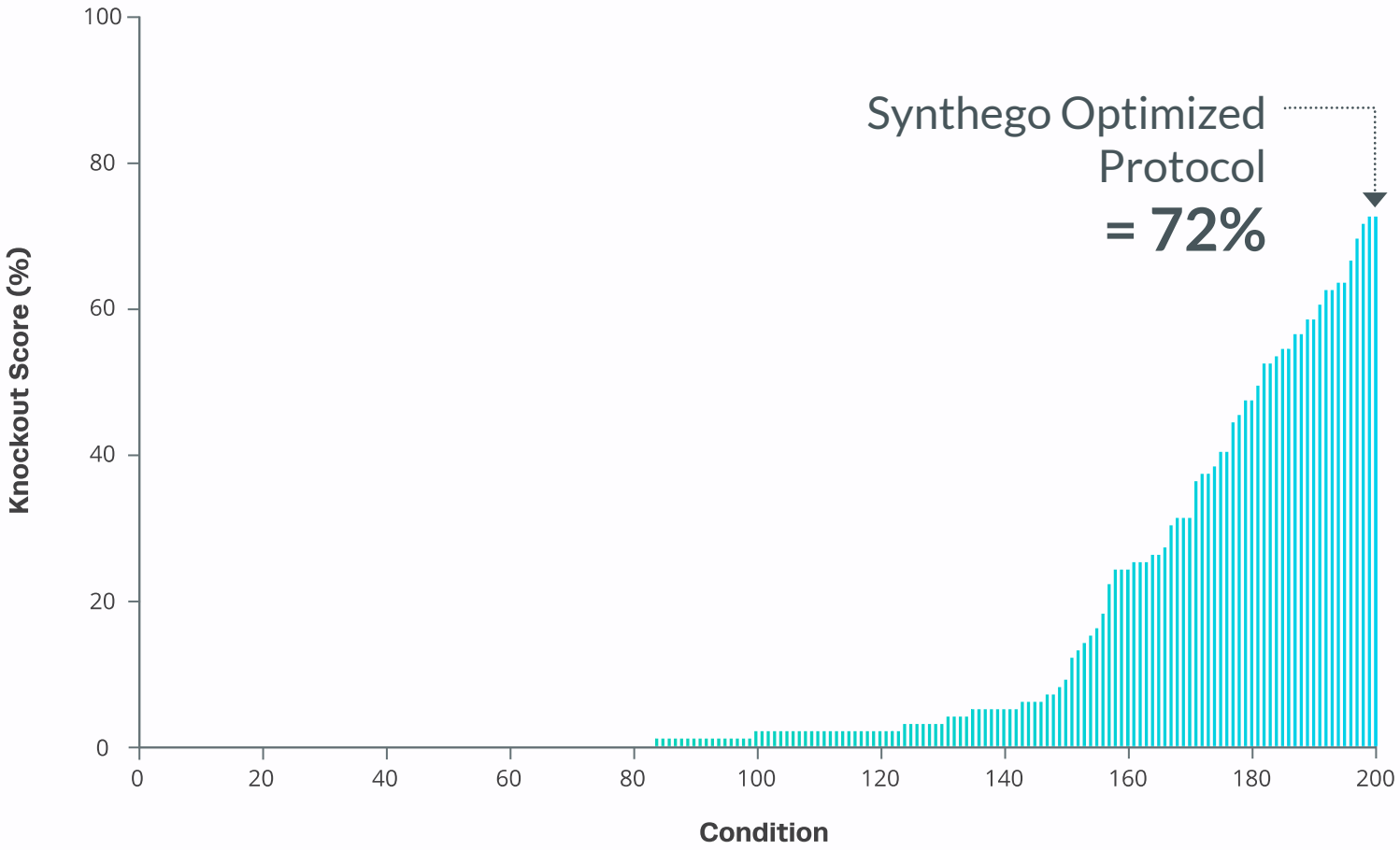
Optimization of RNP transfection/editing (e.g., THP-1)

192 Conditions to optimize:

- **Nucleofection code/pulse (64)**
- **Nucleofector buffer (3)**



Methods

Positive control sgRNA (RelA gene)
RNP (192 conditions)
THP-1 cells
384 Nucleofection (+3 days)
Indel formation (NHEJ)
Sequencing/ICE analysis

***THP-1* Cell line**

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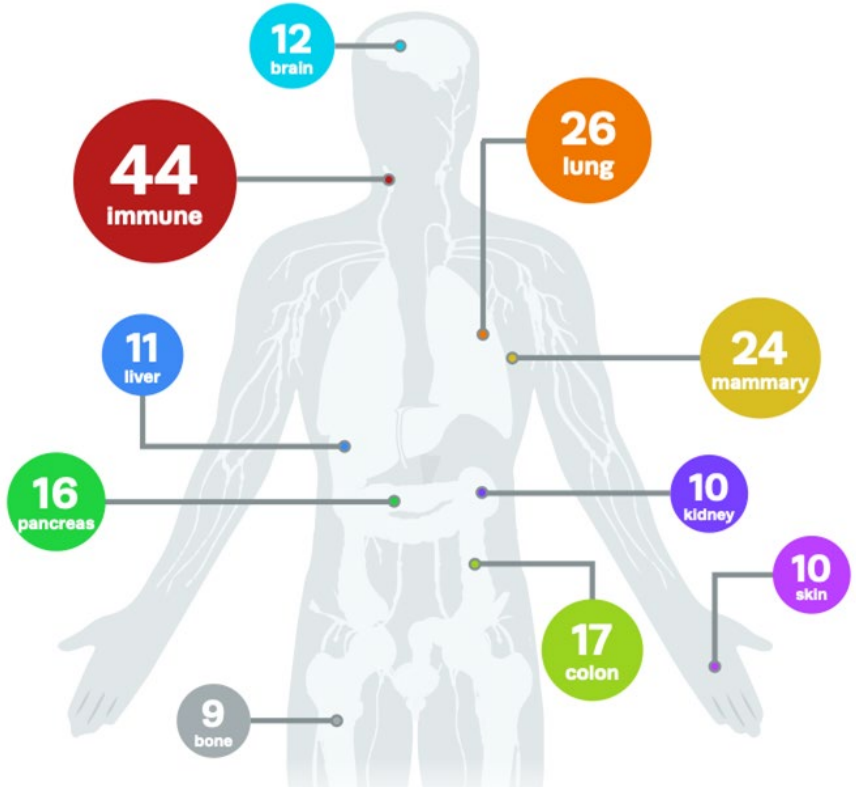
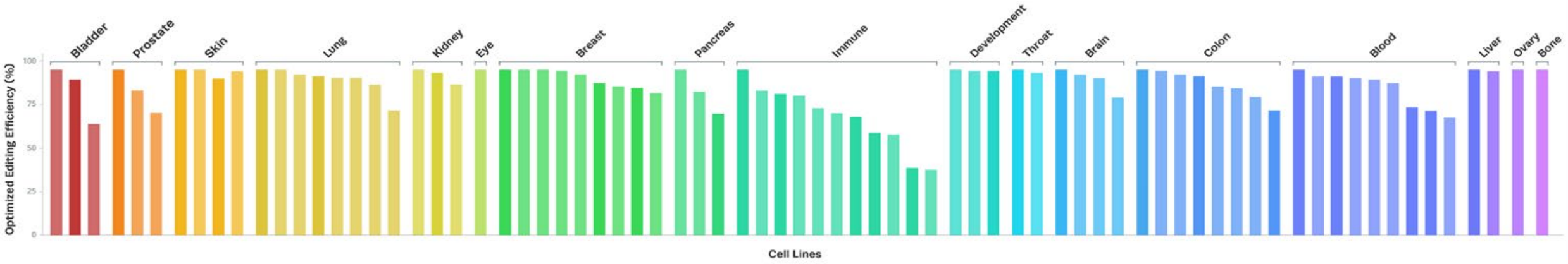
Library of optimized transfection conditions

350+ optimized cell lines

~700 cell lines in ATCC





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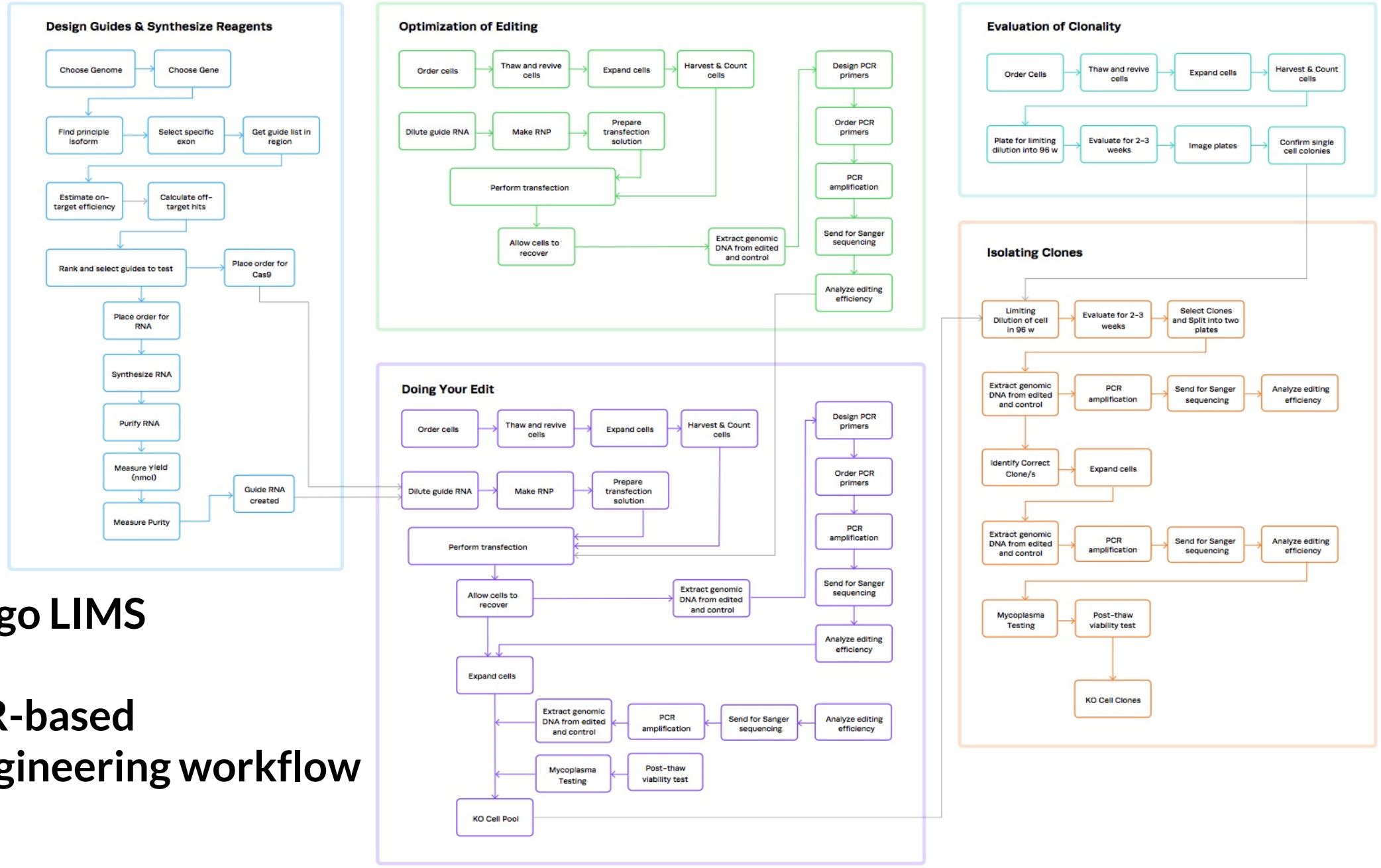

Synthego LIMS

CRISPR-based Cell Engineering workflow

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Design & Make sgRNA

Optimize Cell Transfection

Single Cell Printing for Clonal Isolation

CRISPR Editing & Analysis

Clonal Expansion & QC

Synthego LIMS

CRISPR-based Cell Engineering workflow

Automated Cell Culture

Integrated systems for:

- Single cell printing post transfection
- Continual imaging and clonal tracking
- Cell feeding/splitting
- Incubation nursery

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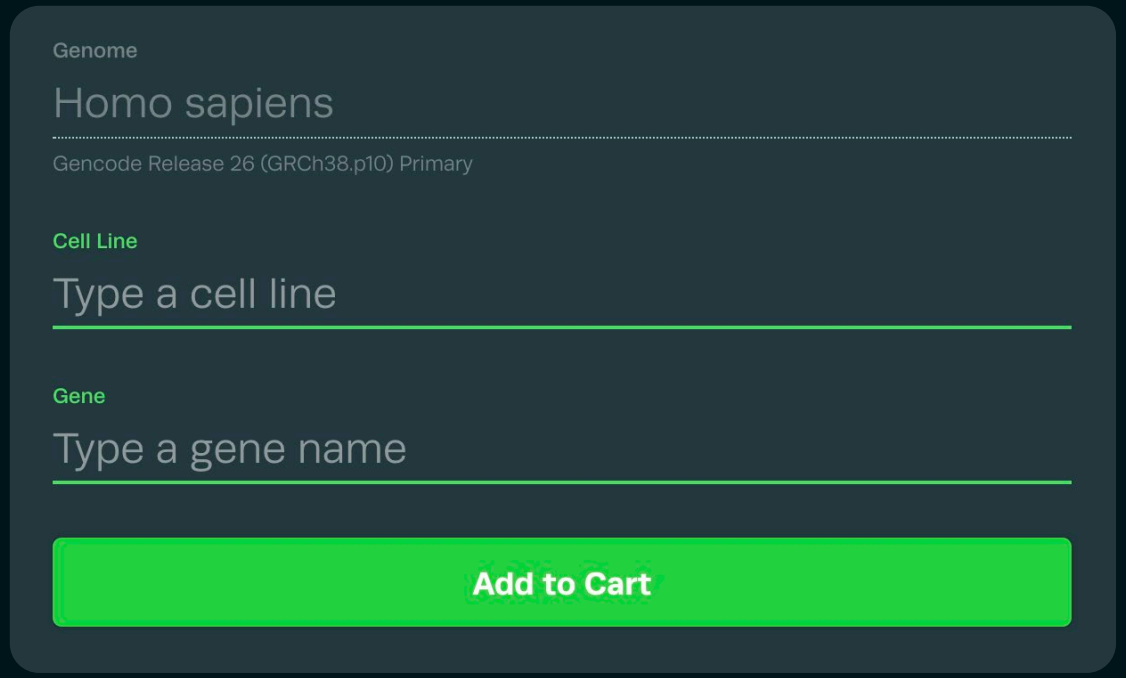
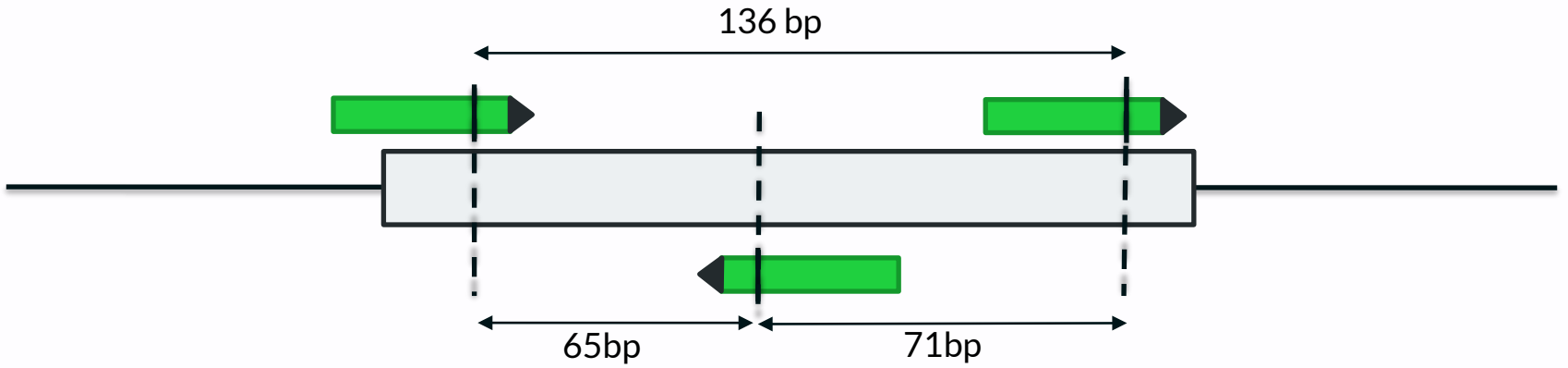
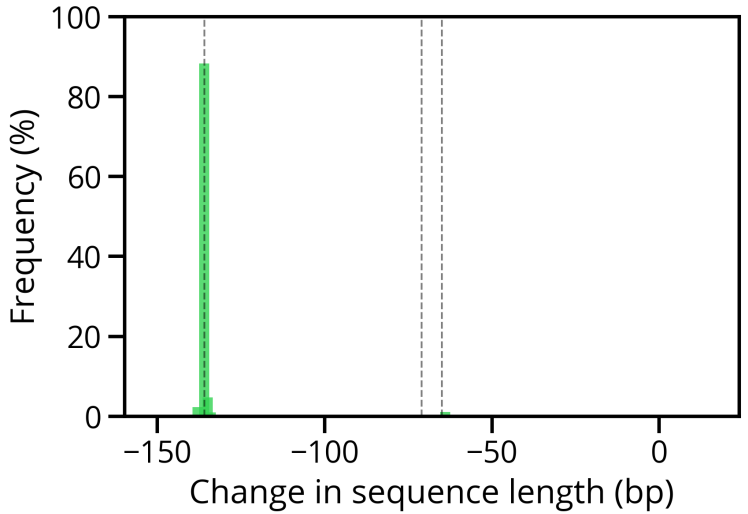
Goal: Build this Gene KO interface

Solution: Generate a foolproof way to engineer cells and optimize transfection of any cell



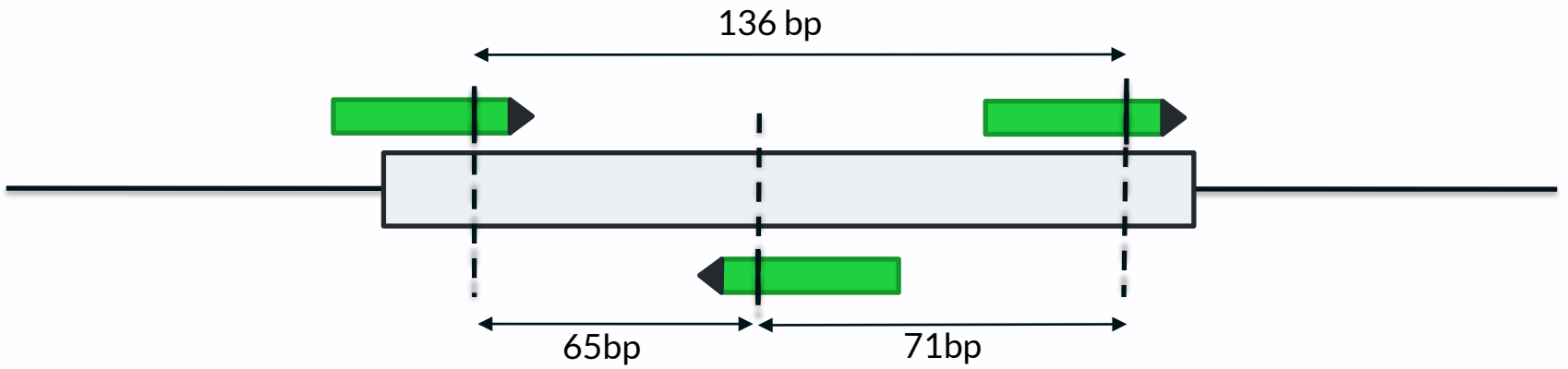
Multi-guide Gene KO Design (early exon)





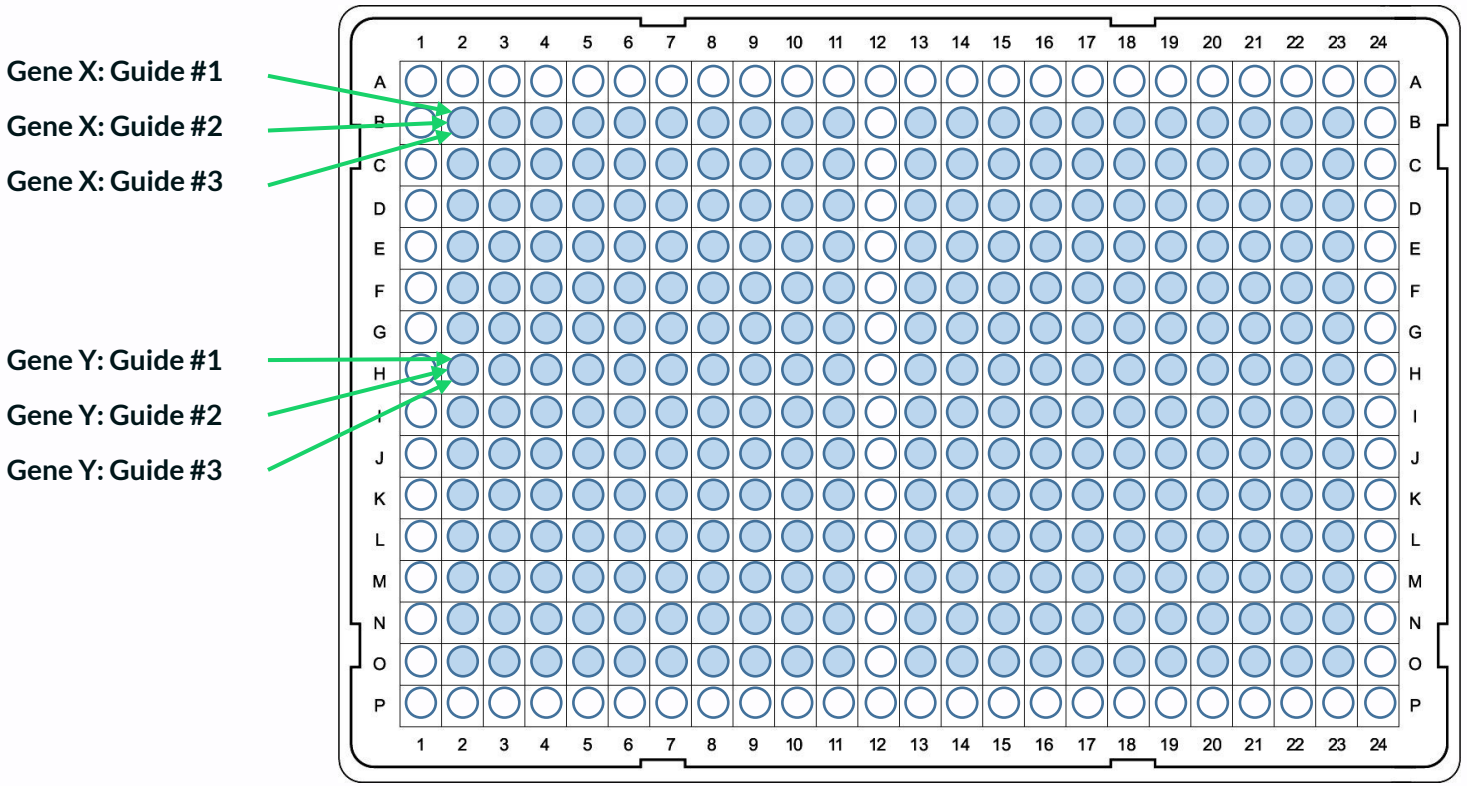
Editing outcomes are stereotyped and predictable

Multi-guide design algorithm for Whole Human Genome



Guide spacing and orientation are critical to success

Gene KO design and construction for the genome



Benefits:

- Pre-designed so no screening of sgRNAs by Cell Engineering Production Group
- Manufactured at large scale = arrayed set of guide RNAs ready for gene KO the next day

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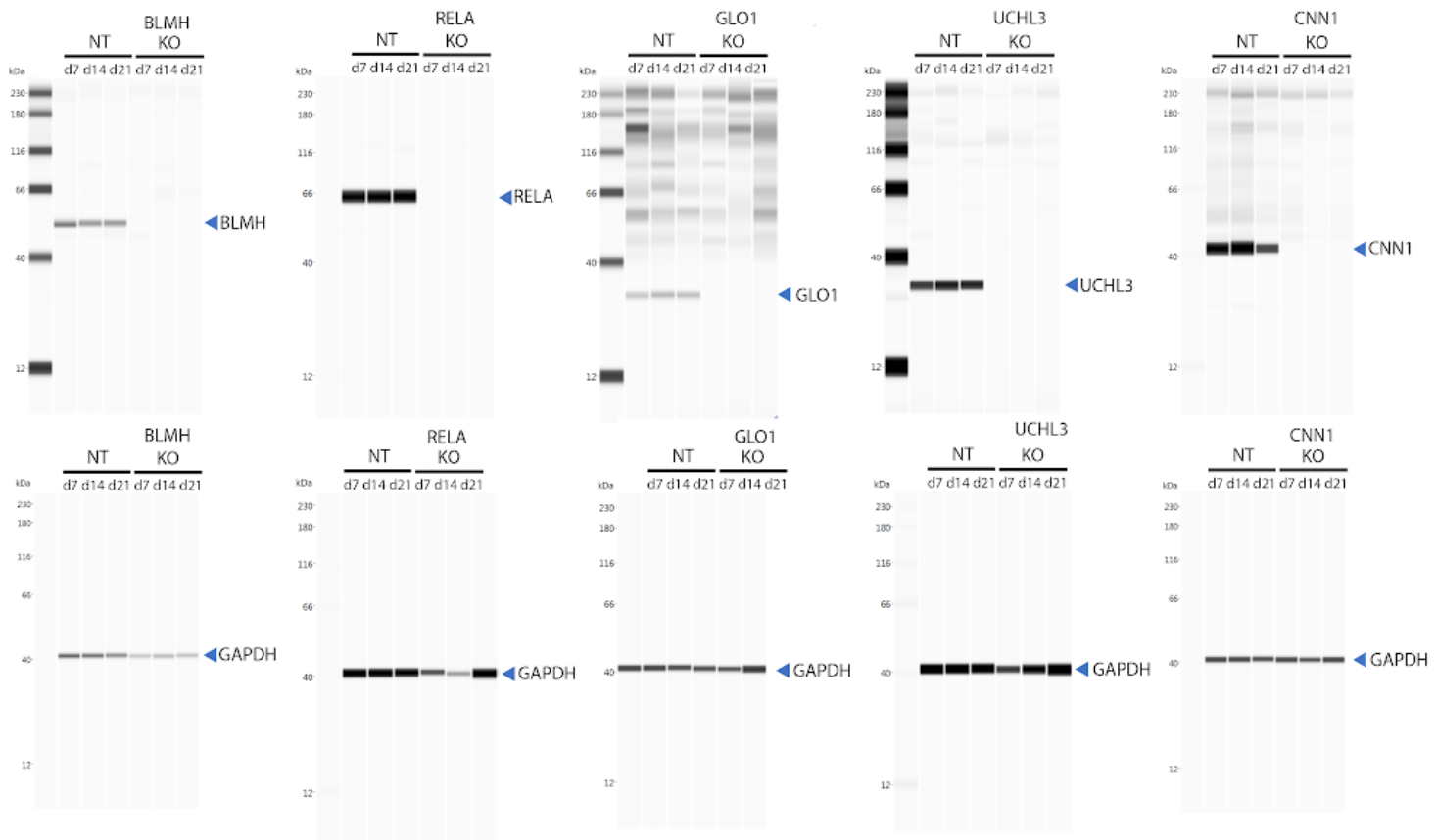
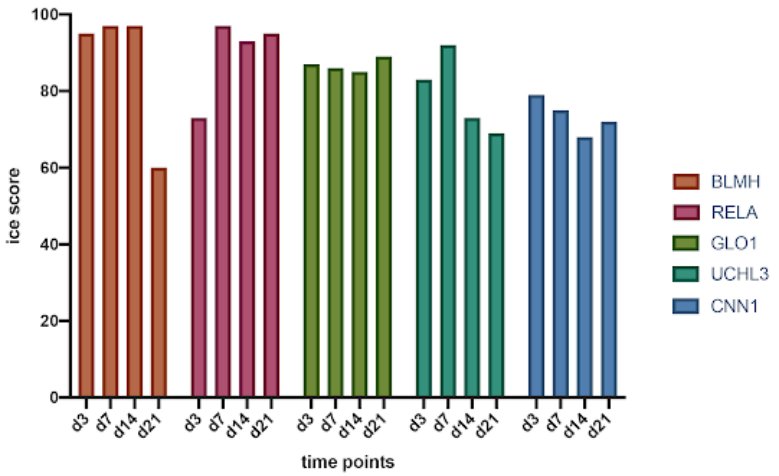
Multi-guide Gene KOs yield corresponding protein loss





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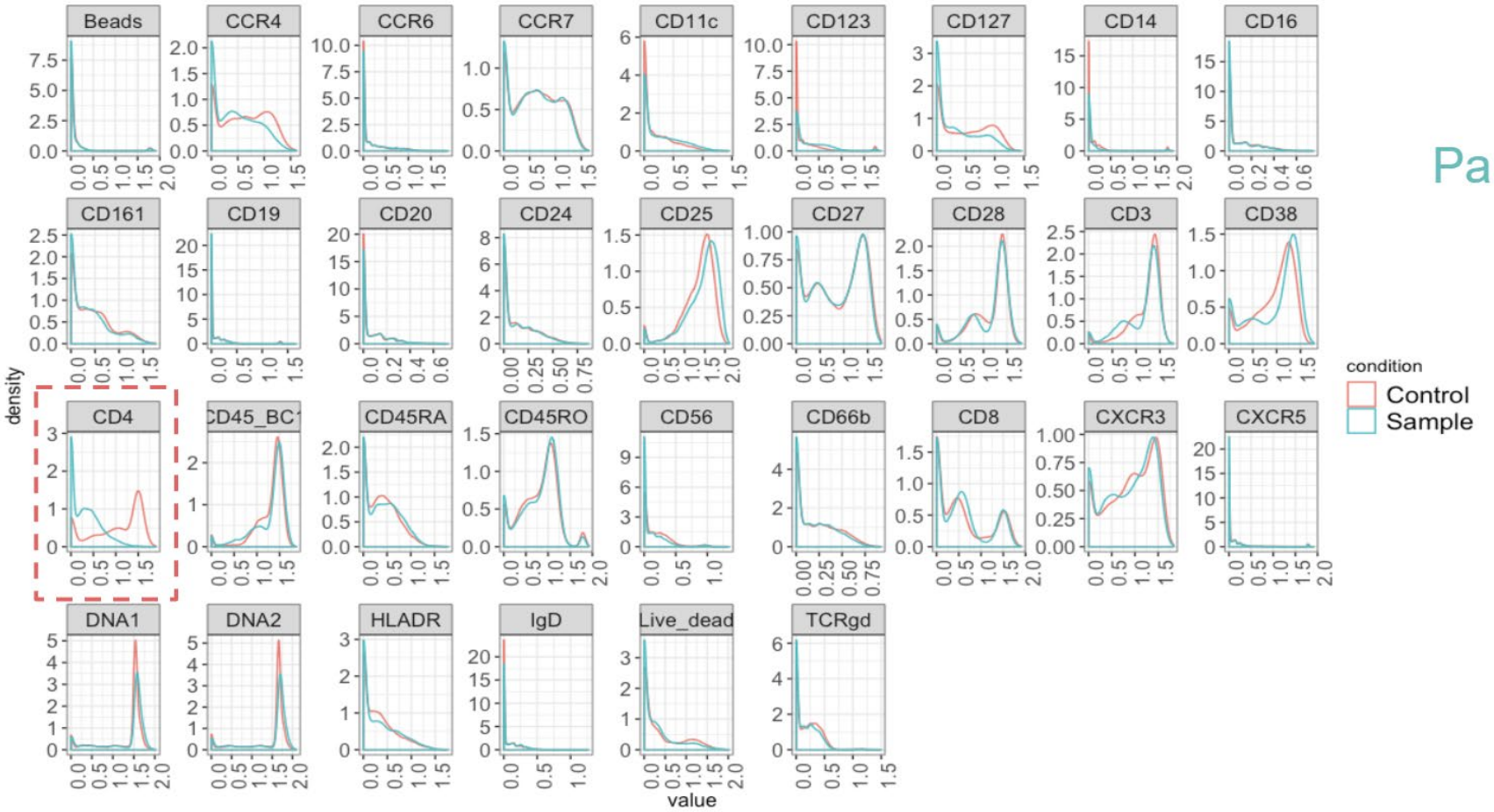
KOs in Clinically Relevant Cell Types



PanT

VS

PanT+**CD4 KO**

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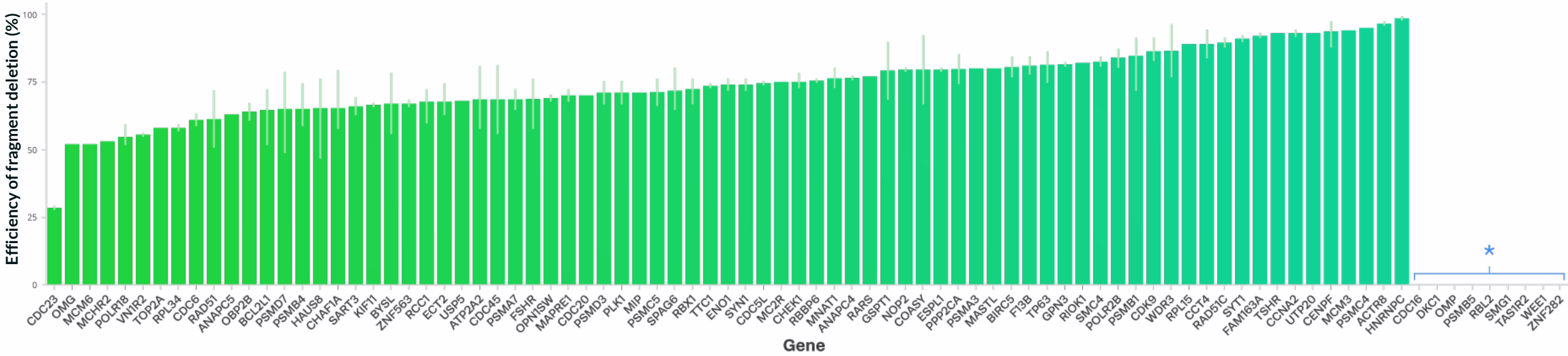
The multi-guide approach yields reliable genotypes



For 77 genes, multi-guide sgRNAs resulted in an average fragment deletion efficiency of 75%

- HEK293 cells, RNP Nucleofection

* No information of editing efficiency due to sequencing failure.

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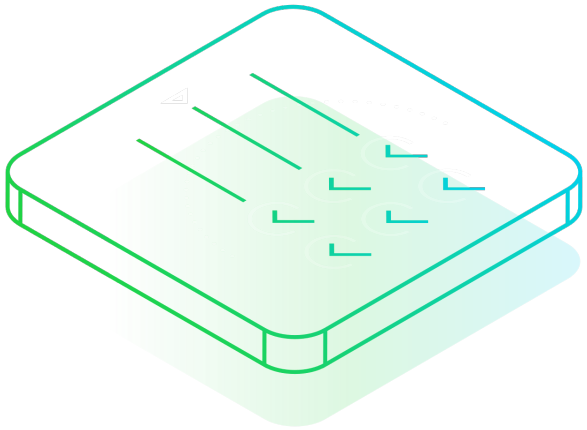
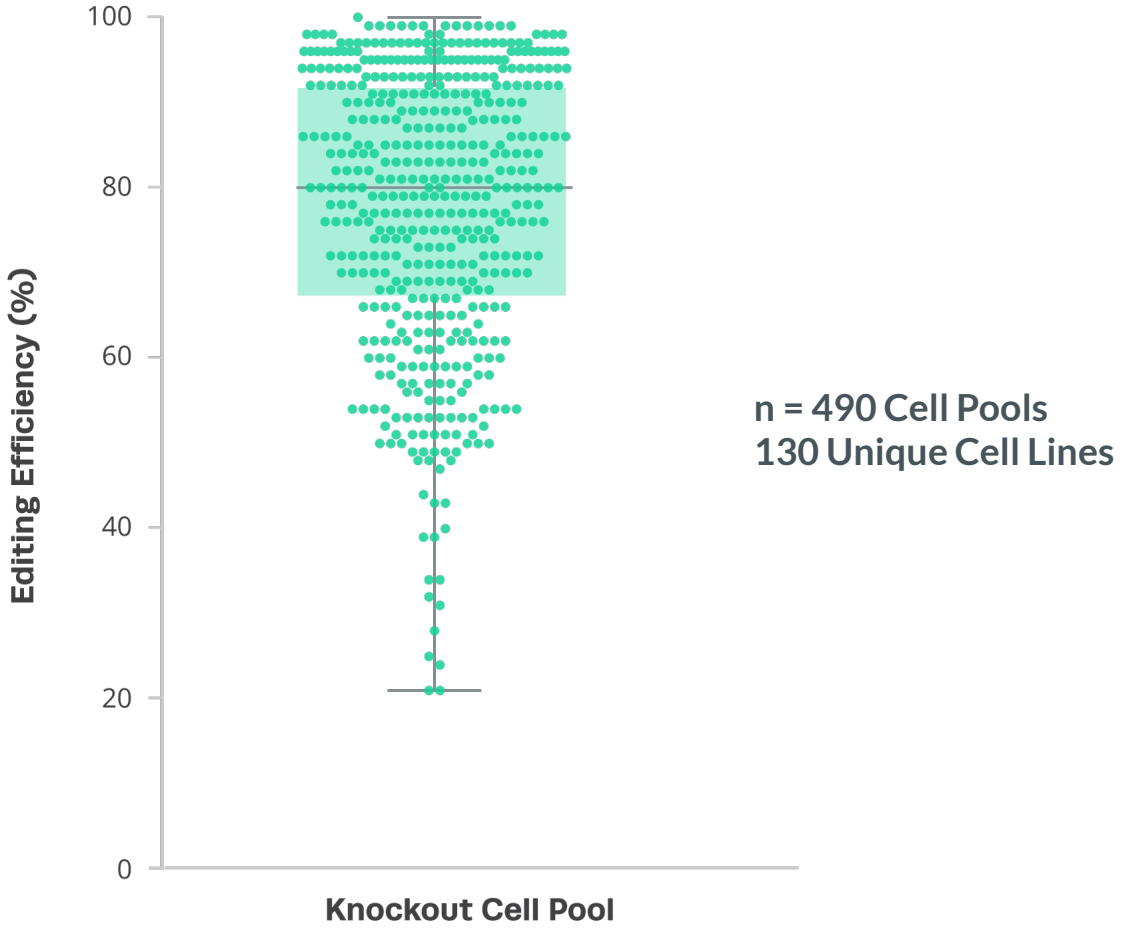
Synthego's CRISPR-engineered cells program

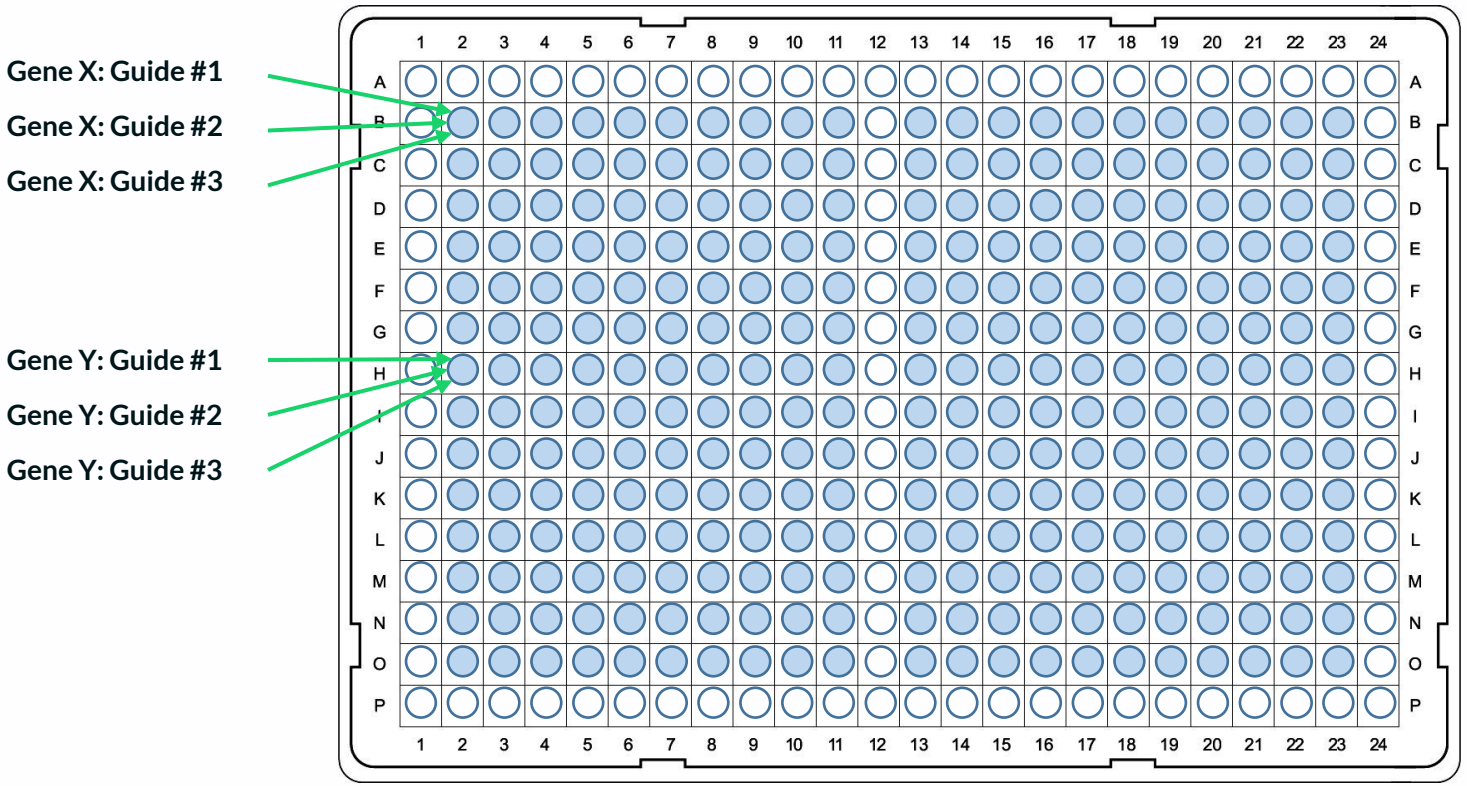


Average of 80% efficiency across ~ 500 KO projects



n = 490 Cell Pools
130 Unique Cell Lines

Gene KO design and construction for the genome



Benefits:

- Pre-designed so no screening of sgRNAs by Cell Engineering Production Group
- Manufactured at large scale = arrayed set of guide RNAs ready for gene KO the next day

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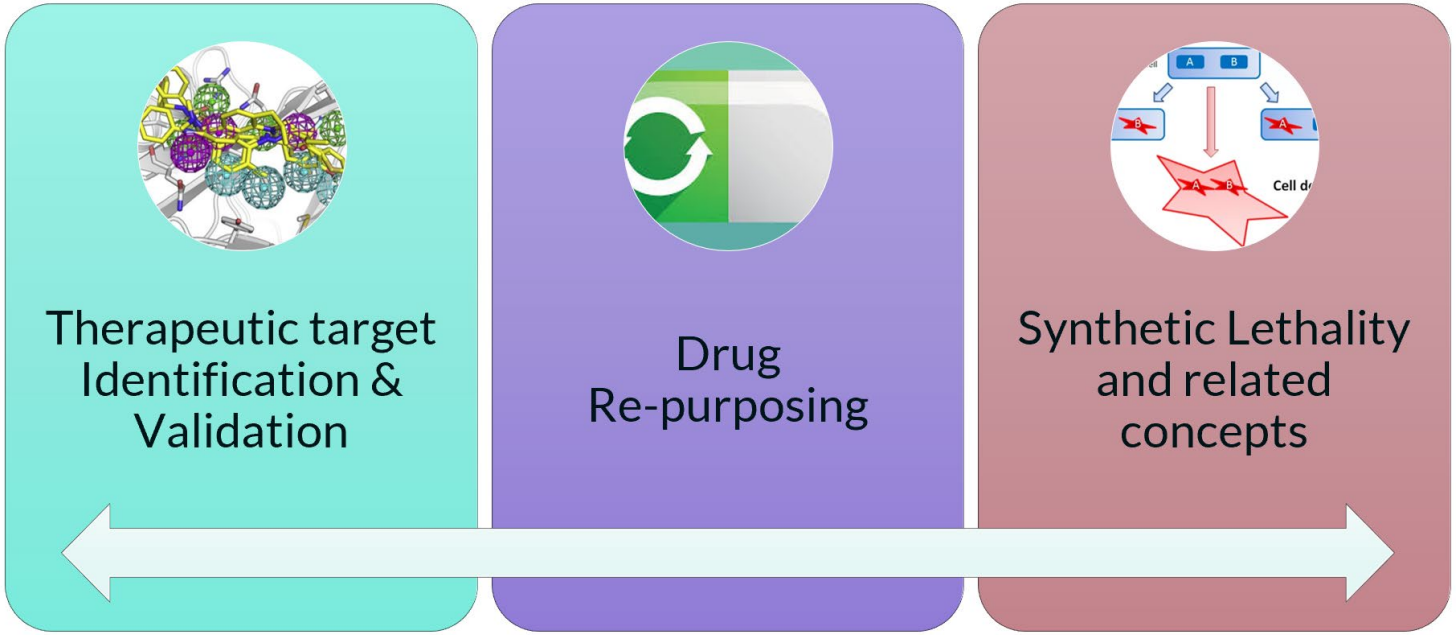
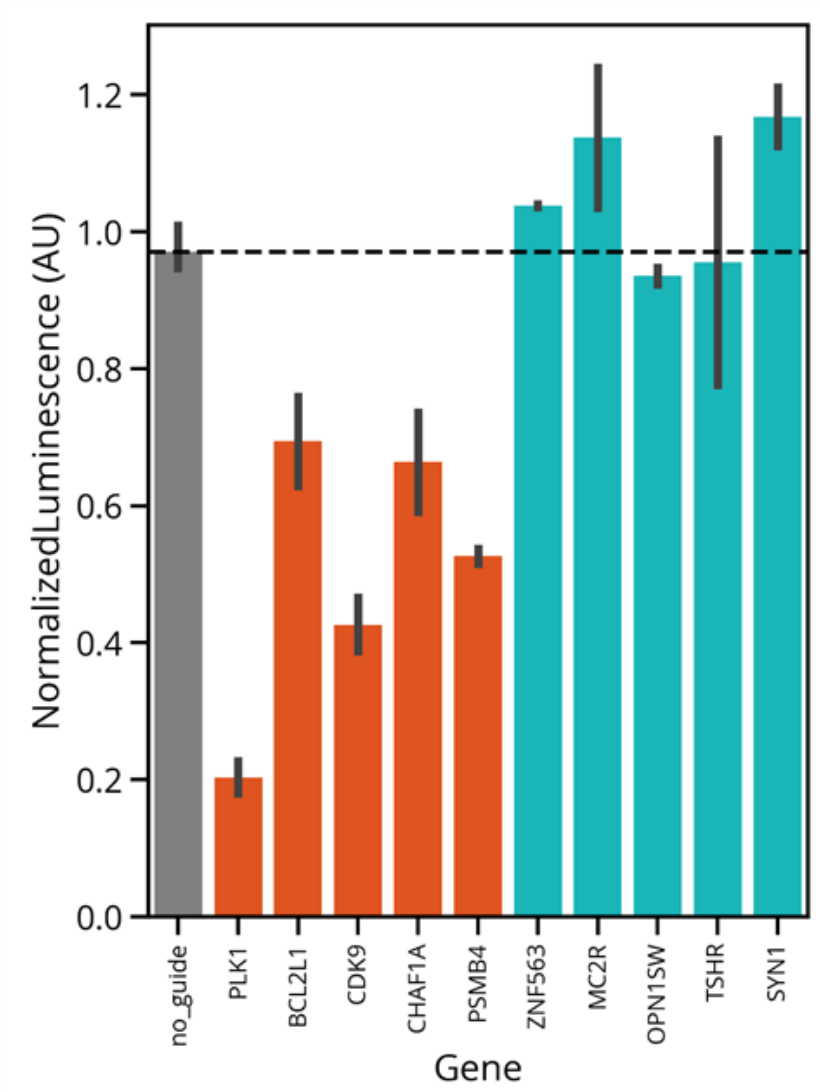
Functional Genomics Screening with gene KO libraries



Gene Knockout Kit v2

Guaranteed Protein Knockout for Any Human-Derived Cell Types Using a Novel Multi-guide Strategy.

Screening with Cas9-expressing cell lines



Methods

Multi-guide (Essential Gene Library Genes)
Mod-sgRNAs
Cas9-expressing U2OS cell line
RNAimax (+5 days)
Viability assay
Cell Titer Glo (ATP) analysis
(normalized to non-essential genes)

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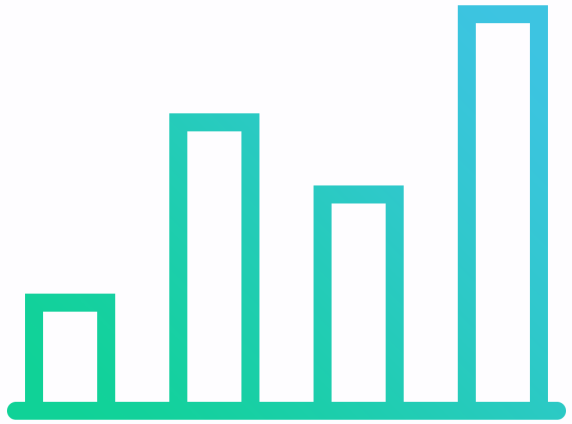


Poll Question

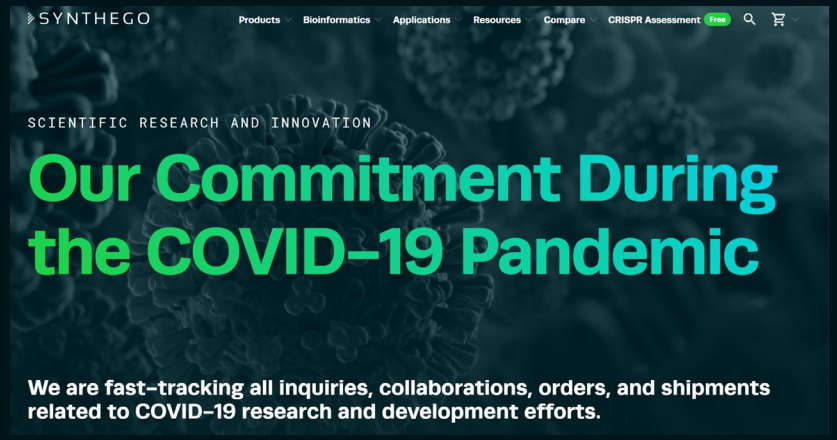
What would be the first thing you do when you return to your lab?

Synthego during the COVID-19 pandemic

Providing COVID-19 resources to our customers and the community…

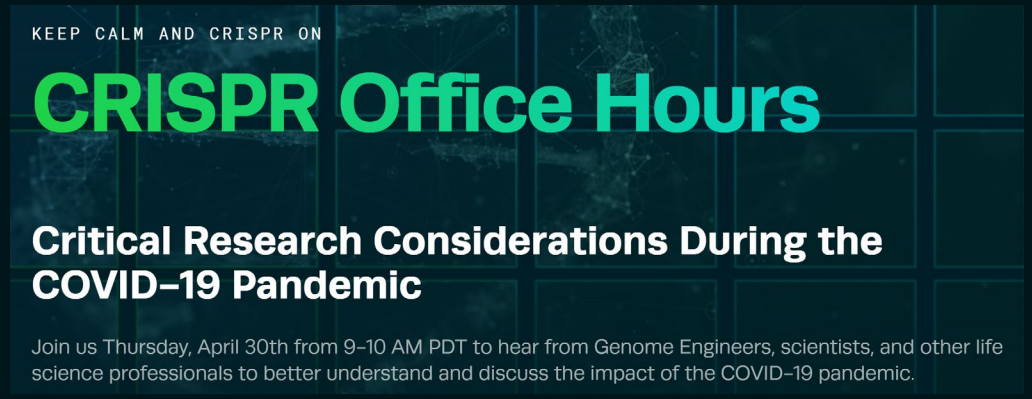
- COVID-19 interactome libraries, COVID-19 diagnostics
- Continuation of RNA/Cell Manufacturing & Production

www.synthego.com/coronavirus



Engaging the scientific community through Virtual Events…

www.synthego.com/office-hours



Synthego during the COVID-19 pandemic

Hosting virtual research mini-symposia…

- Virtual Cancer Conference: CRISPR in Cancer Research
- Poster presentation and scientist Q&A
- Now available on YouTube

www.synthego.com/events/crispr-cancer-webinar-05-20-2020

May 20

Virtual Cancer Conference: CRISPR in Cancer Research

WEBINAR VIRTUAL 9 AM PDT | 12 PM EDT | 6 PM CEST

Deepak Parashar, Ph.D.

MEDICAL COLLEGE OF WISCONSIN, DEPARTMENT OF OBSTETRICS & GYNECOLOGY

Webinar Title: miRNA551b-3p Activates an Oncostatin Signaling Module for the Progression of Triple-Negative Breast Cancer

John Daly, M.Sc.

NATIONAL UNIVERSITY OF IRELAND, GALWAY

Poster Title: Hypersialylation protects Multiple Myeloma cells from NK cell-mediated immunosurveillance and this can be overcome by targeted desialylation or genetic modification of NK cells

Dr. Mark Gurney MB BCh BAO MRCPI

NATIONAL UNIVERSITY OF IRELAND, GALWAY

Poster Title: CD38 Gene Edited Primary Natural Killer (NK) Cells Transiently Expressing an Affinity Optimized CD38 CAR for Enhanced Acute Myeloid Leukaemia (AML) Targeting.

Anna Bakewell

INDUSTRIAL PLACEMENT STUDENT AT LIFEARC

Poster Title: Validating eIF4A1 by CRISPR-Cas9 knockout as a potential target for cancer therapeutics

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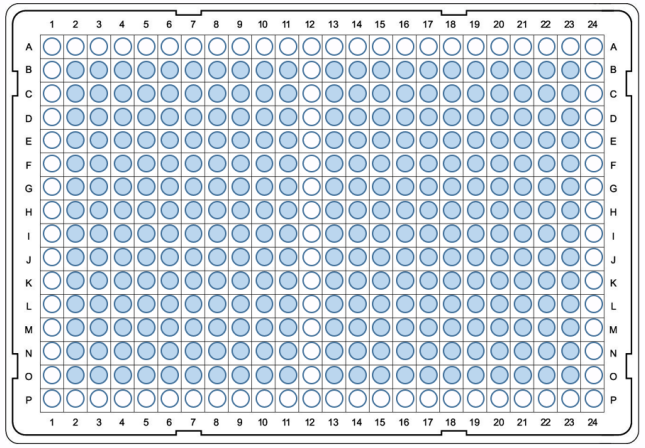
Arrayed Gene KOs = Arrayed, screen ready, gene KO cells



Pre-manufactured or rapidly manufactured arrayed gene KO libraries

+

HTP genome engineering capabilities / Cell Optimization

= Industrialized printing of designed cells

- **Plates of cells, not plates of RNA**
- **Directly usable in assays or experiments**

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Application of Arrayed Cells during COVID-19 pandemic

nature

Article | Published: 30 April 2020

A SARS-CoV-2 protein interaction map reveals targets for drug repurposing

David E. Gordon, Gwendolyn M. Jang, [...] Nevan J. Krogan

Nature (2020) | Cite this article

- Team of 120 scientists in US, UK, France, led by Nevan Krogan (UCSF)
- Used affinity mass-spec to ID human proteins interacting with expressed SARS-CoV-2 proteins in human cells to make an “interactome”
- Analysis of interacted targets revealed 332 candidates genes and 66 druggable human proteins or host factors targeted by 69 compounds (FDA-approved or in trials)
- Viral infection screening (BSL3) revealed several have antiviral effects

Application of Arrayed Cells during COVID-19 pandemic

- In order to validate these interactors in infection studies, and also validate potential drug hits, the human genes need to be KO'd
- Synthego rapidly synthesized the interactome library of 332 and 66 druggable
- Began collaboration with Nevan Krogan to provide KO cells rapidly, for free for the follow-on study
- Rapidly optimized transfection conditions for cell lines provided from SARS-CoV-2 infection studies at Mt. Sinai and Pastuer Institute
- Synthego will deliver Arrayed Gene KO plates of live cells directly to BSL3 facilities in US and France in less than 2 weeks

SYNTHEGO

A SARS-CoV-2 protein interaction map reveals targets for drug repurposing

David E. Gordon, Gwendolyn M. Jang, [...] Nevan J. Krogan

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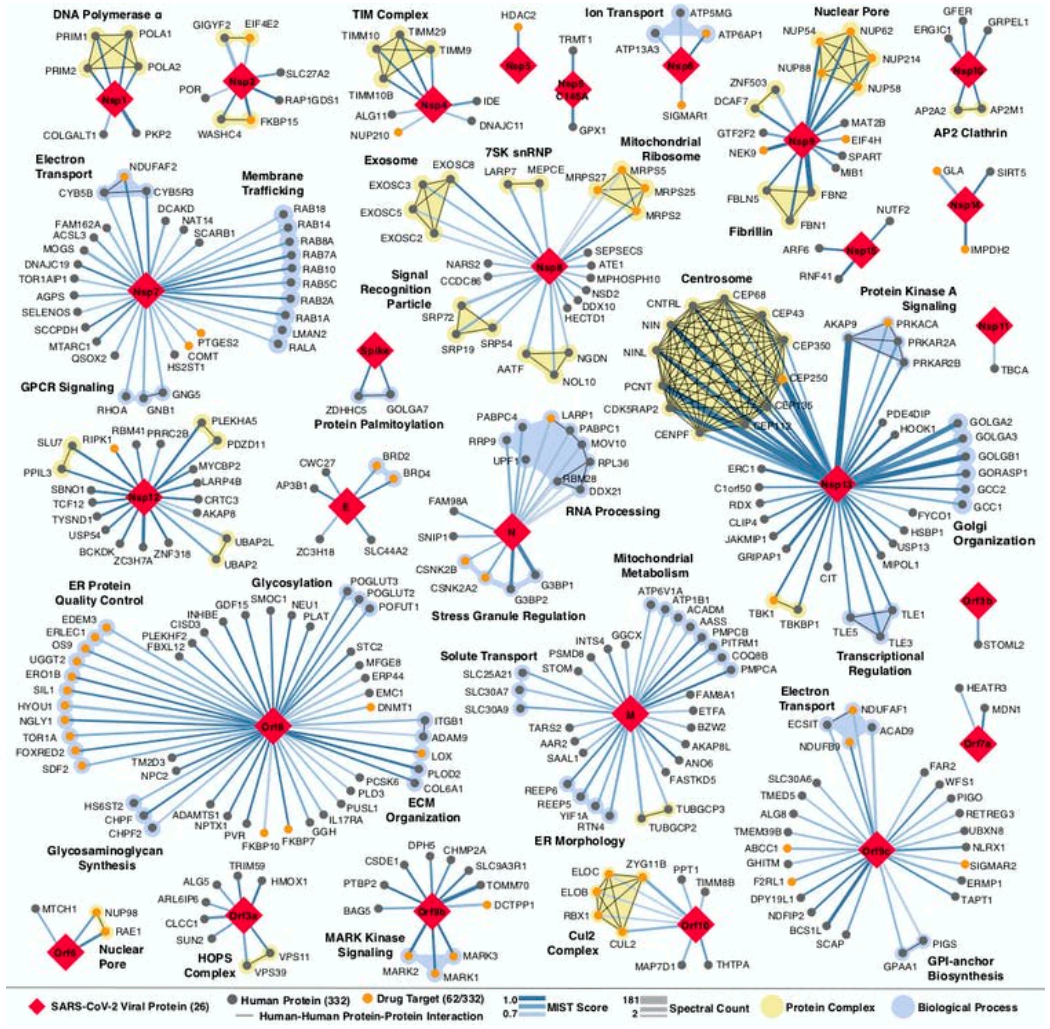
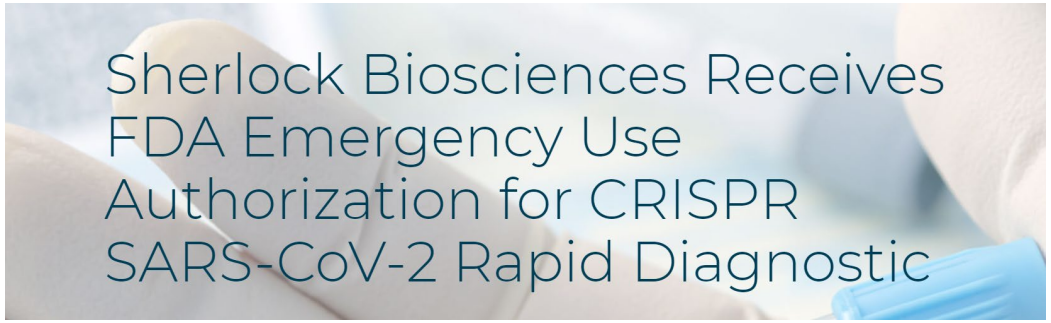


Supplying Critical Reagents for COVID-19 Testing

A protocol for detection of COVID-19 using CRISPR diagnostics

(v.20200311)

Feng Zhang[1,2,3,4,5], Omar O. Abudayyeh[3], Jonathan S. Gootenberg[3]



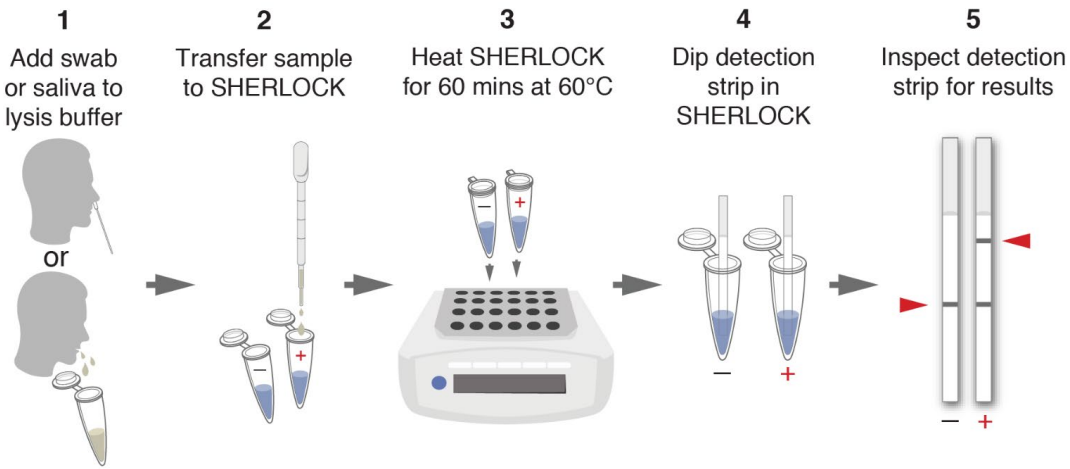
Requires synthetic **Cas13a** gRNA (Synthego)

STOPCOVID

Point-of-care testing for COVID-19 using SHERLOCK diagnostics

(v.20200505)

Julia Joung[1,2,3,4]*, Alim Ladha[1,2,3,4]*, Makoto Saito[1,2,3,4,5], Michael Segel[1,2,3,4,5], Robert Bruneau[10], Mee-li W. Huang[10], Nam-Gyun Kim[10], Xu Yu[6,7,9], Jonathan Li[6,8,9], Bruce D.Walker[1,9], Alexander L. Greninger[10,11], Keith R. Jerome[10,11], Jonathan S. Gootenberg[3,9#], Omar O. Abudayyeh[3,9#], Feng Zhang[1,2,3,4,5,9#]



Requires synthetic **Cas12b** gRNA (Synthego)

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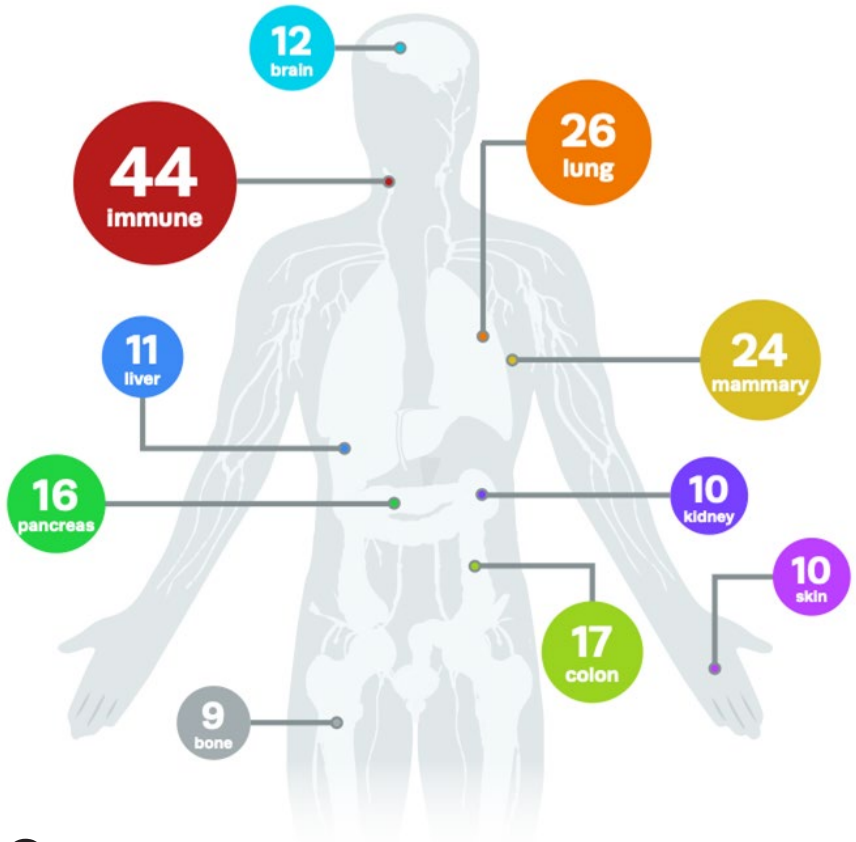
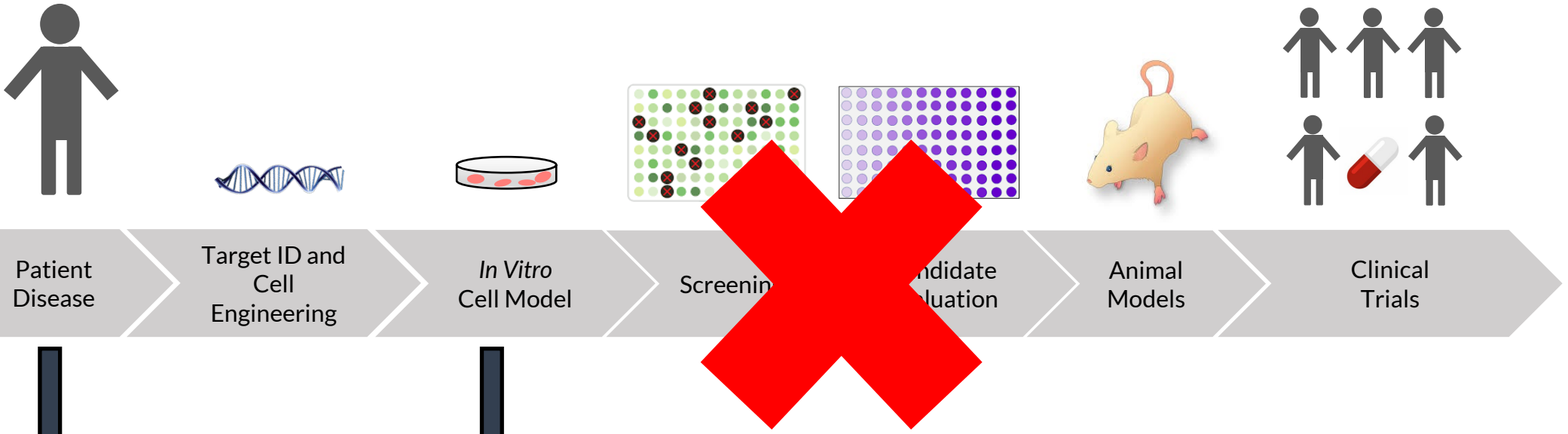
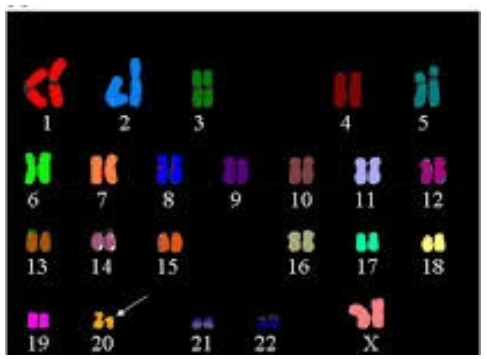
Immortalized cancer cell lines…

…the best proxy for disease modeling?



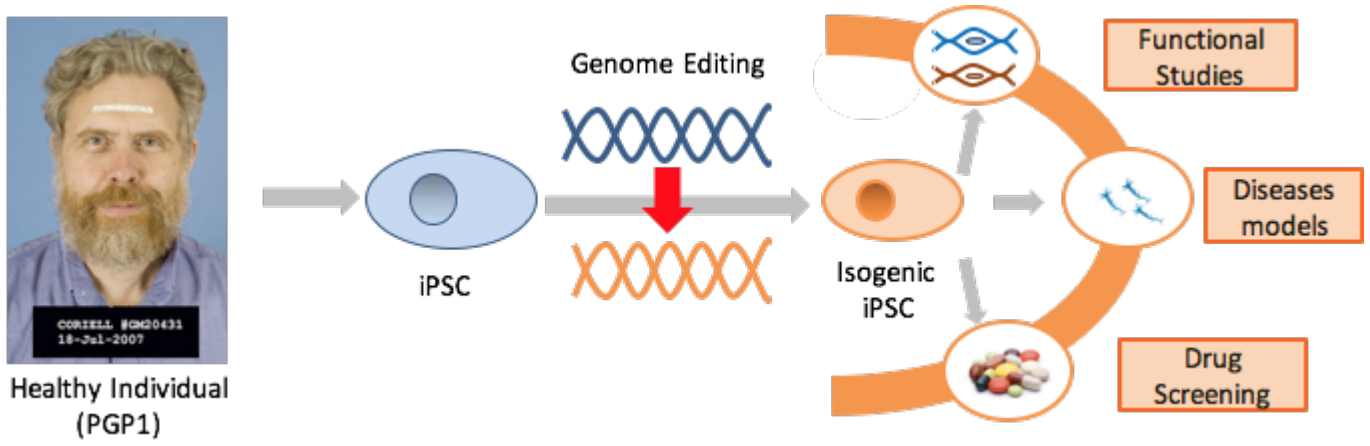
What cell models are really needed?

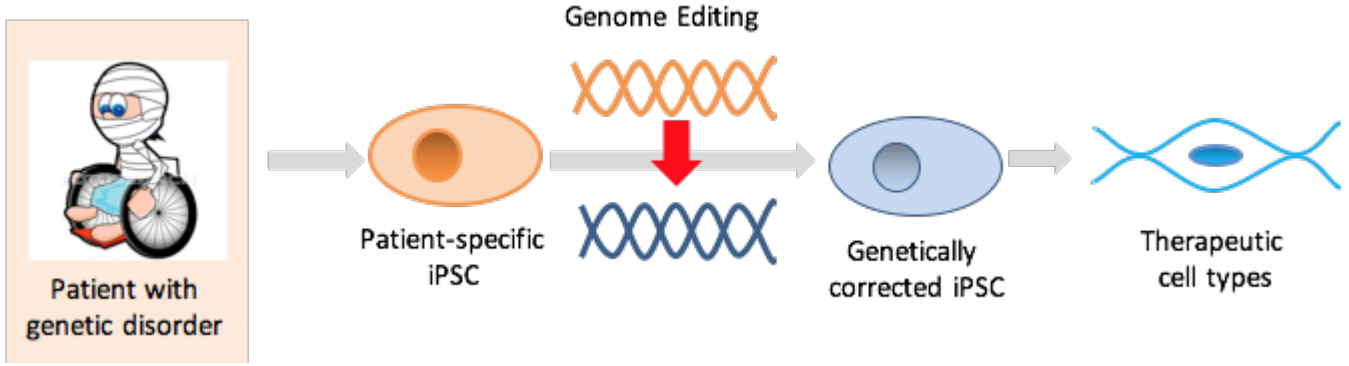




Not really “human”

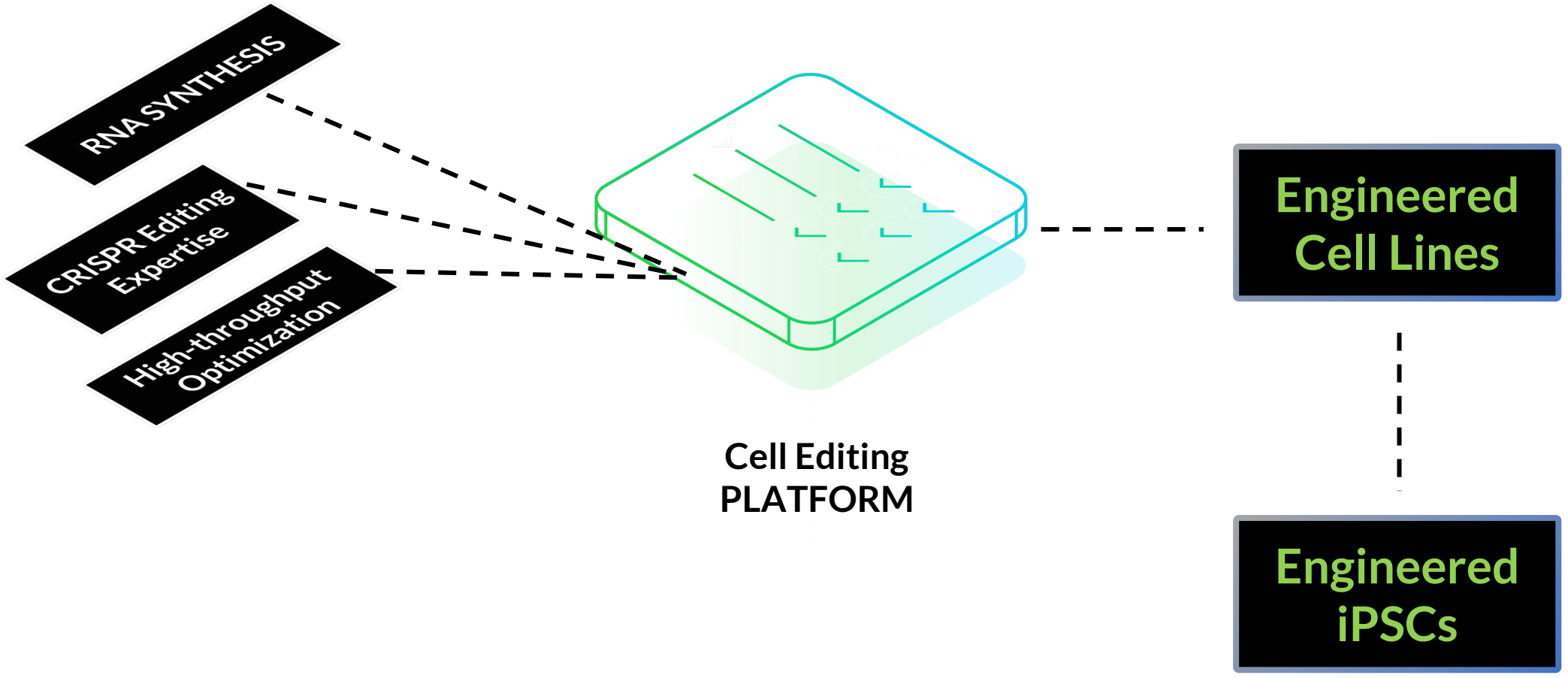
Induced Pluripotent Stem Cells (iPSCs)





More Accurate
Disease Modeling
And
Translational Biology

Automated Cell Editing Platform



iPSC Product Offering – Launched October 2019

Knockouts (KO)

Discovery of Protein Function

Pathway Analysis

Knockins (KI)

Disease Modelling (SNV)

Endogenous Protein Tagging

Control or **patient-derived** iPS cell lines available as **homozygous** or **heterozygous** clonal lines.

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Agenda

How CRISPR technologies are being applied to fight the COVID-19 pandemic

Brief Background

- ✓ A bit on CRISPR
- ✓ A bit on Synthego

How Synthego developed technologies to enable cell engineering at scale

- ✓ Reagent development /optimization
- ✓ Optimizing CRISPR engineering in human cell lines
- ✓ Scaling CRISPR engineering – a relevant application during the COVID-19 pandemic
- ✓ CRISPR engineering in iPSCs (to build better models of disease)

How might you utilize these resources in your own discovery research?

Standardized CRISPR-engineered cells

Knockout Cell Pools

High % gene KO cell pools

- Replaces siRNA
- Utilize directly in assay or isolate clone
- Study gene KO before cellular adaptation

Knockout Cell Clones

100% verified gene KO clones

Advanced Cells

Clonal knock-ins;
complex editing projects;
non-human cell lines

iPSC projects

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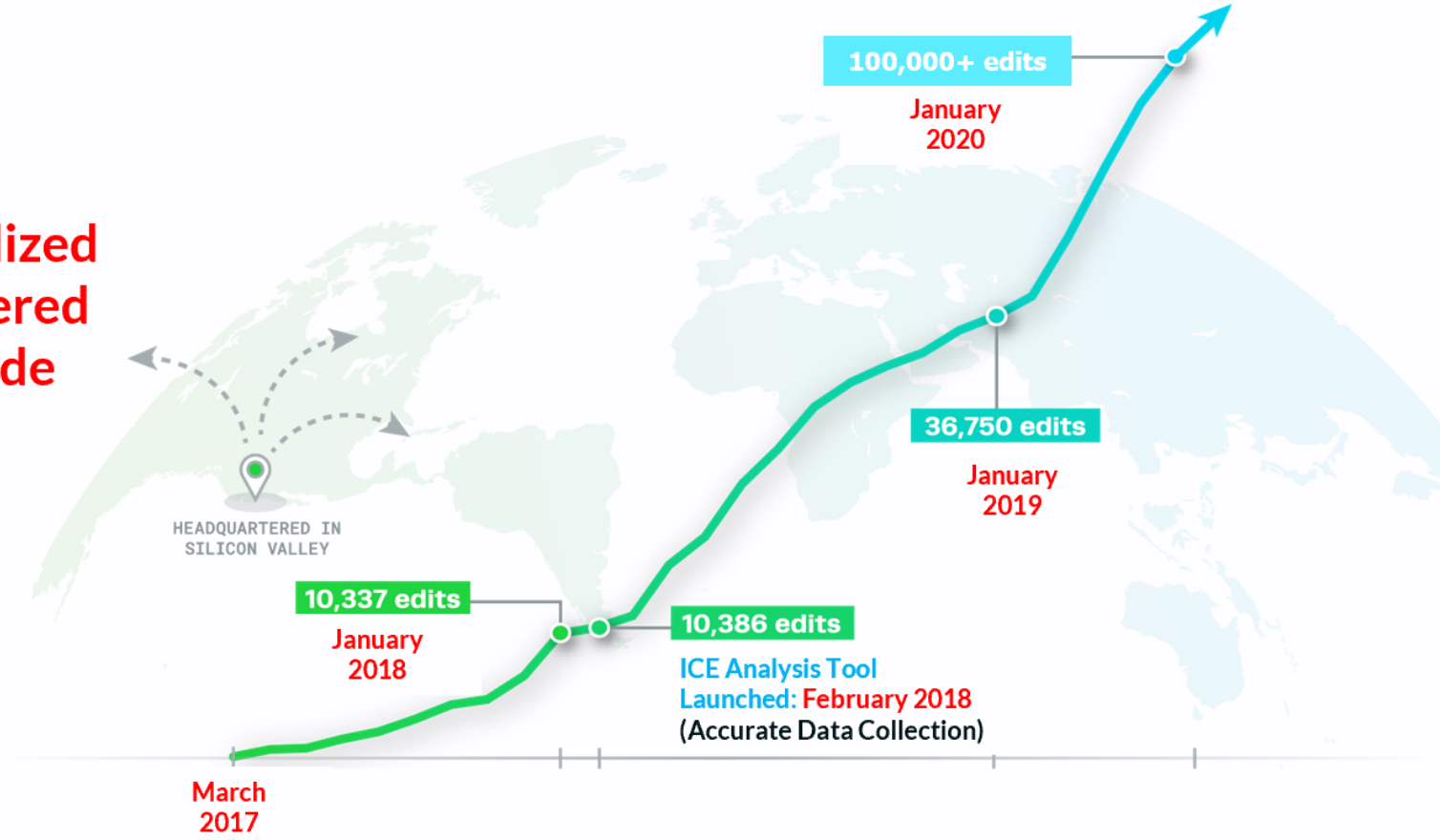
Synthego's goal to bring standardization & expertise

100,000 Sequence-verified CRISPR edits (Mar 2017 – Jan 2020) and counting…



Enabling Standardized CRISPR & Engineered Cells … Worldwide

2,000+ edits per month

200+ citations in primary literature

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Thank You for listening!

Len Goren
VP of Global Sales
len.goren@synthego.com

Please contact Funakoshi to access:

- synthetic sgRNA (R&D or GMP-grade)
- multi-guide gene KO kits and libraries
- CRISPR engineered cell lines (KO/KI)
- CRISPR engineered iPSCs (KO/KI)

Kevin Holden, PhD
Head of Science @ Synthego
kevin.holden@synthego.com

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