Enabling a Healthier World

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Nucleofector® Technology and Genome Editing

An Introduction to Nucleofection® and CRISPR

Lonza Bioscience | June 2023

Public

Nucleofector® Technology

The principle

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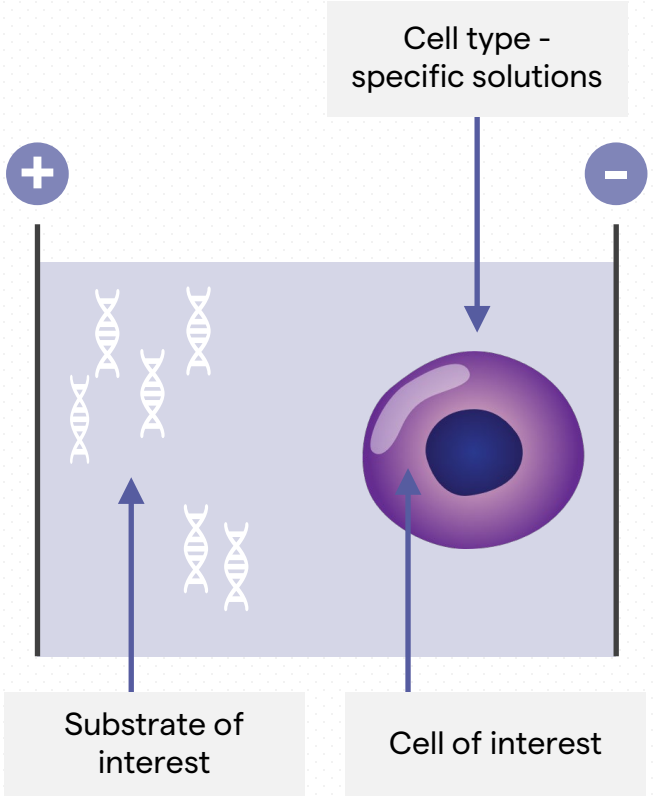
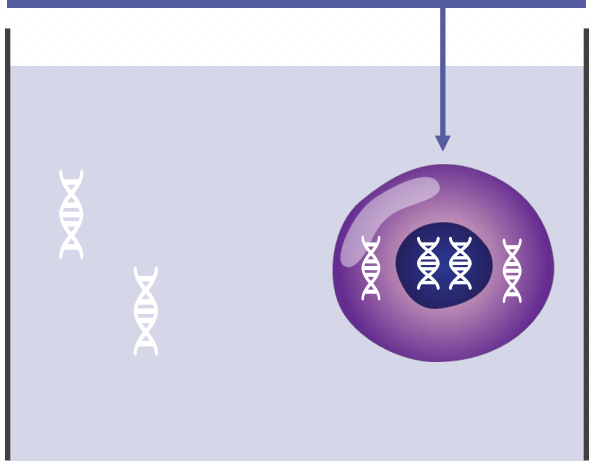


Nuanced electrical pulses

Gene transfer directly into the nucleus



Pores are generated in the cell and nuclear membranes during the Nucleofection® Process, allowing substrates to enter the cell and the nucleus

For more information watch [Nucleofection® Technology Animation - YouTube]

What Is Transfection?

Methods to transfer material (DNA, RNA and proteins) into living cells

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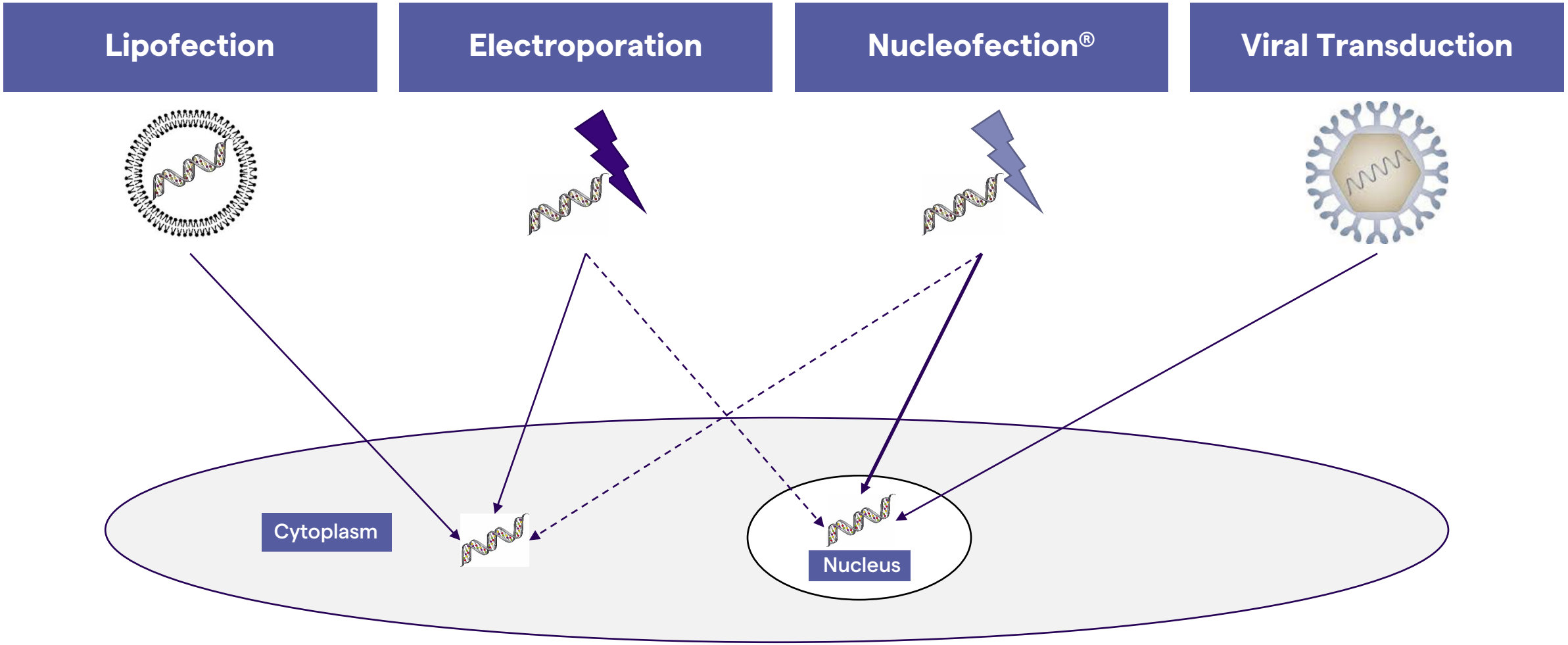

Proven Transport of DNA Into The Nucleus

Methods to transfer material (DNA, RNA and proteins) into living cells

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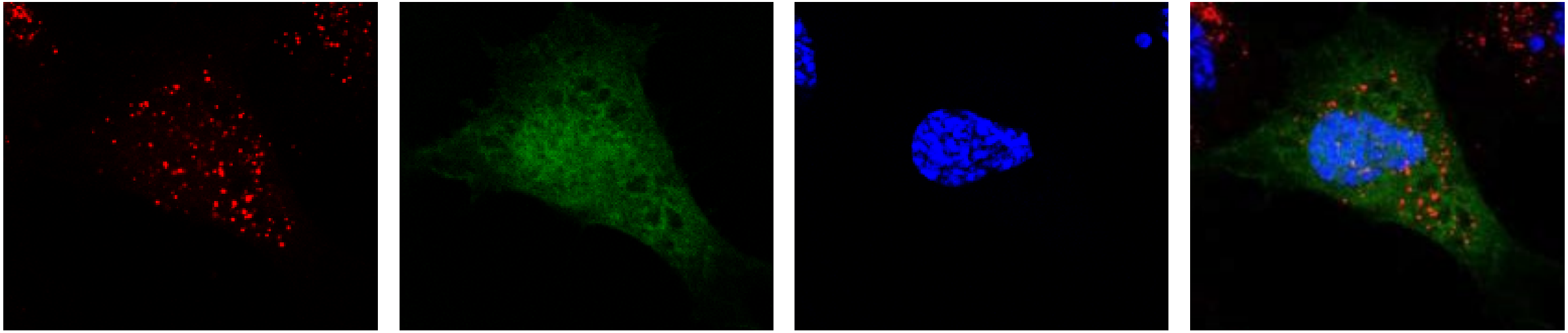
Transport	Expression	Nucleus	
DNA	GFP	DAPI	Merge



- Primary NHDF-neo cells were transfected with labeled plasmid DNA encoding GFP, fixed **after 2h** in 3.5% PFA and analyzed by confocal microscopy.

Transfection – Primary Cells vs. Cell Lines

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Primary Cells:

- Closest to *in vivo* situation (biological relevance)
- Short-term culture
- Usually hard-to-transfect
- Current "trending" cell types: Immune cells (T-cells, NK-cells)

Cell Lines:

- Immortalized cells
- Long-term culture (e.g. HeLa since 1949)
- Usually easier to transfect
- Widely used cell lines: CHO, HEK293, THP-1

More Than 80 Primary Cell Types

Superior transfection efficiencies of difficult to transfect primary cells

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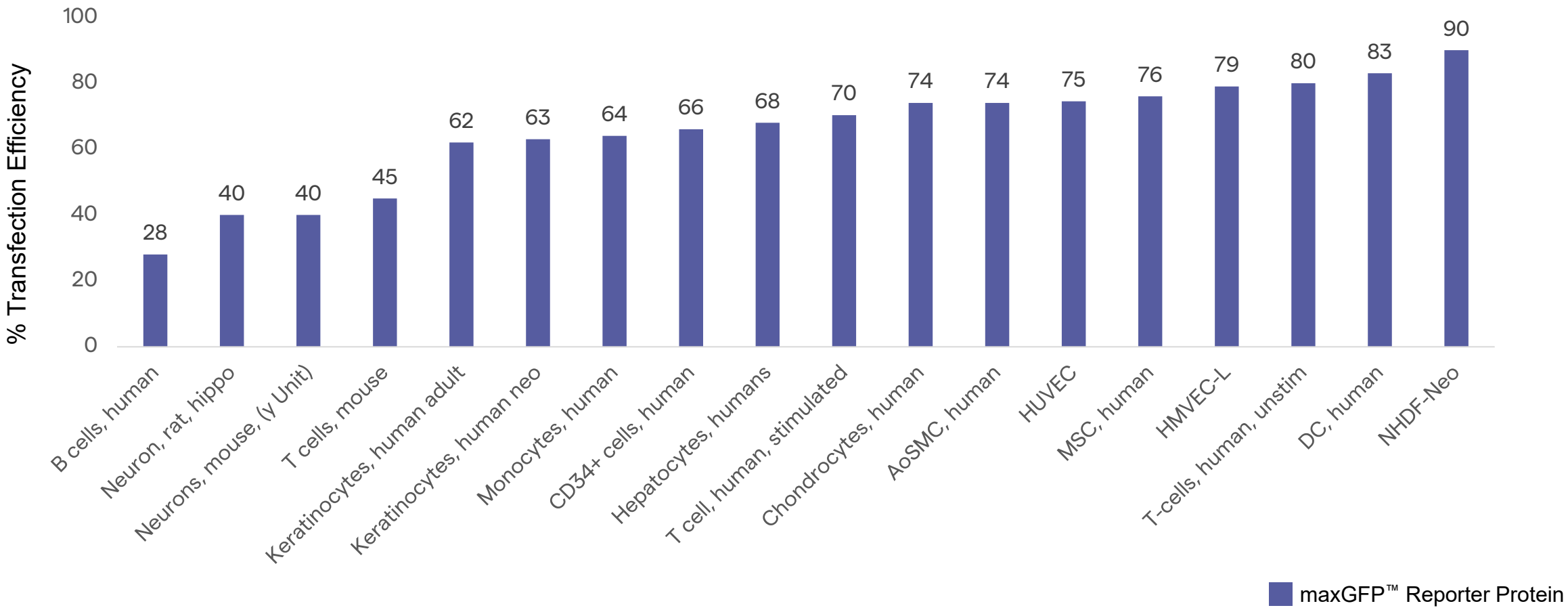

More Than 100 Optimized Protocols for Cell Lines

Analysis time: 24 h post transfection

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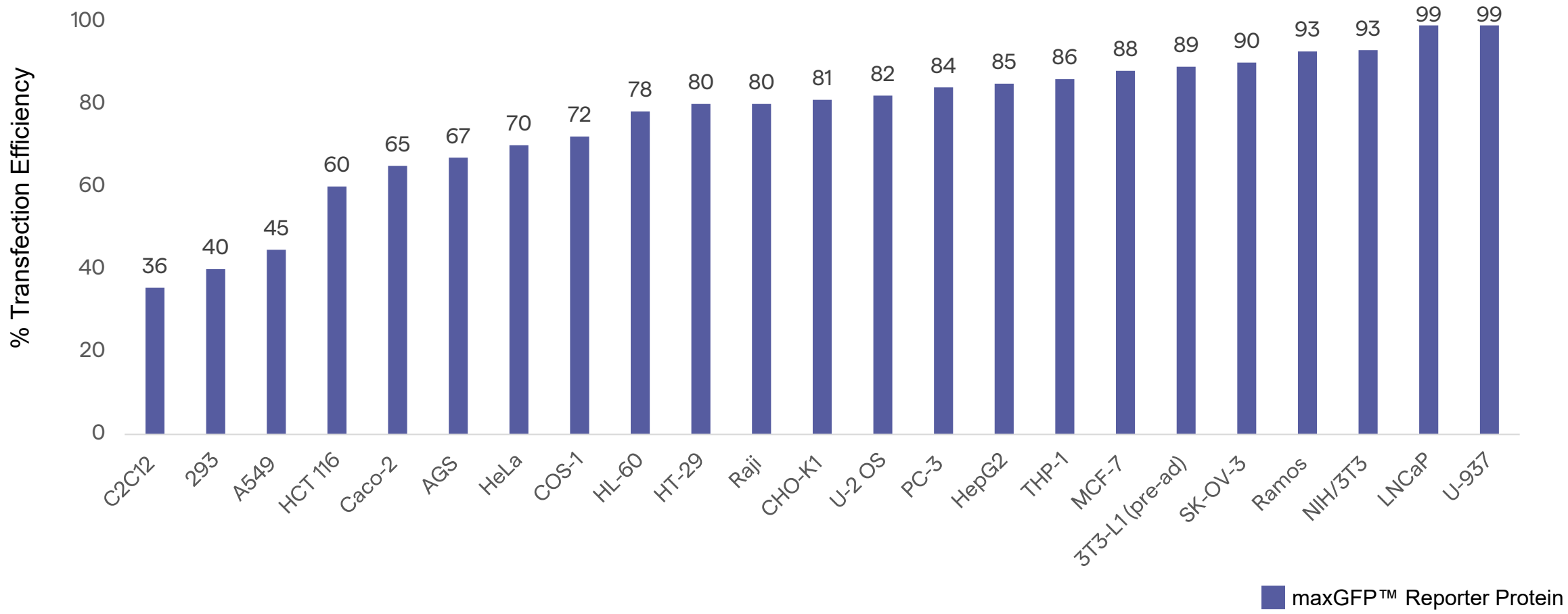

Nucleofector® Technology

Versatility for many applications

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Knock-out generation

Knock-in generation

Screenings

Transfection control

Co-expression of different molecules (e.g. DNA, RNA, protein, RNPs)

Functional genomics

CRISPR

Genome Editing

Co-transfection

iPSC reprogramming

Pathway analysis

Virus production

Target validation

Target identification

Pre-clinical

Generation of stably transfected cell lines

RNAi rescue

Production of recombinant proteins

Drug discovery assays

Signal transduction

Reporter gene assays

Gene expression analysis

Localization studies

Nucleofection® Process in 5 Simple Steps

4D-Nucleofector® X Unit as an example

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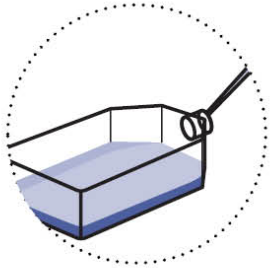
Step 1

Harvest cells of interest.



Step 2

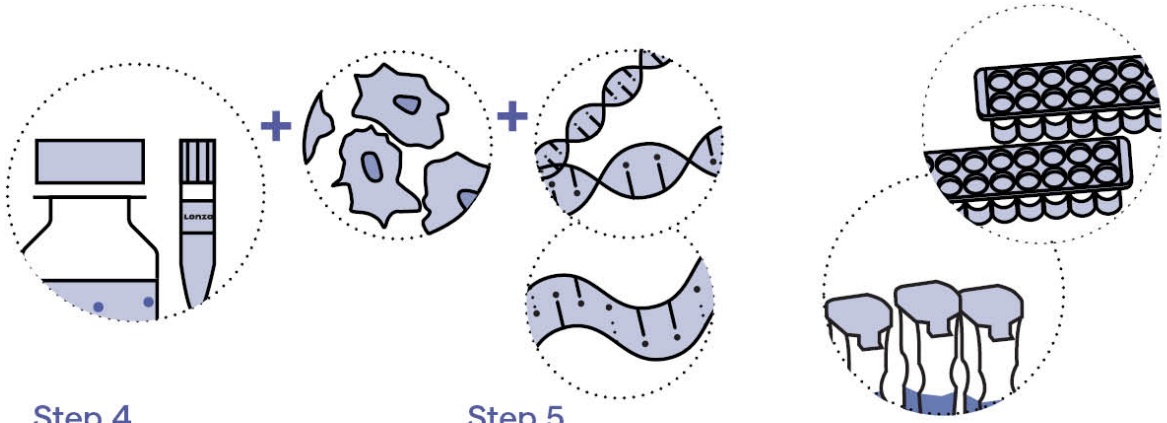
Mix and combine.

Nucleofector® Solution with supplement

Cells

DNA or siRNA

Transfer to a Lonza certified cuvette.

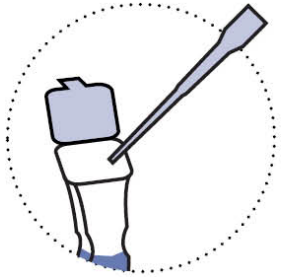


Step 3

Select Nucleofector® Program. Insert cuvette. Press » Start «.

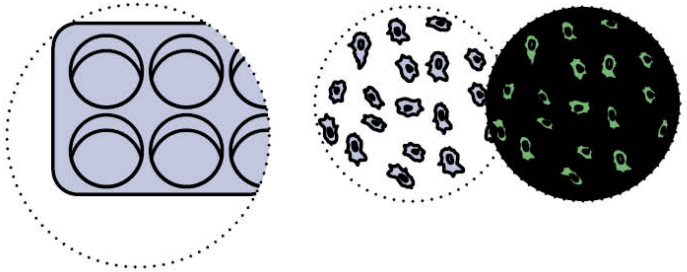
Step 4

Rinse cuvette with culture



Step 5

Transfer to culture dish. Expression can be detected as soon as 3 – 8 hours post Nucleofection® Experiment.



Summary – Nucleofector® Technology

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Principle	Efficient non-viral transport of DNA straight into the nucleus of primary cells and cell lines

Major Benefits

- High transfection **efficiency** and **viability**
- Maintenance of **functionality**
- Allows transfection of **non-dividing cells** such as neurons
- **Nuclear transfer** of cargo enables expression of gene **independent from cell division** and **faster gene expression**
- **Optimized, ready-to-use protocols**
- **Versatility** – can transfect plasmid DNA, DNA oligos, mRNA, peptides, RNPs in either primary cells or cell lines
- **Scalable** to higher throughput or larger volume using the same transfection conditions

Nucleofector® Technology Portfolio

Supporting different throughput and scales

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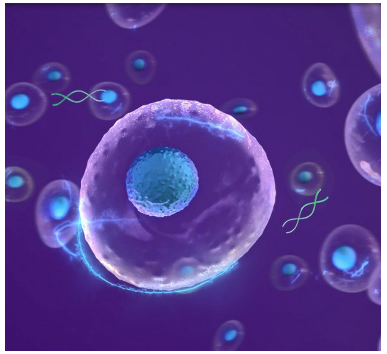
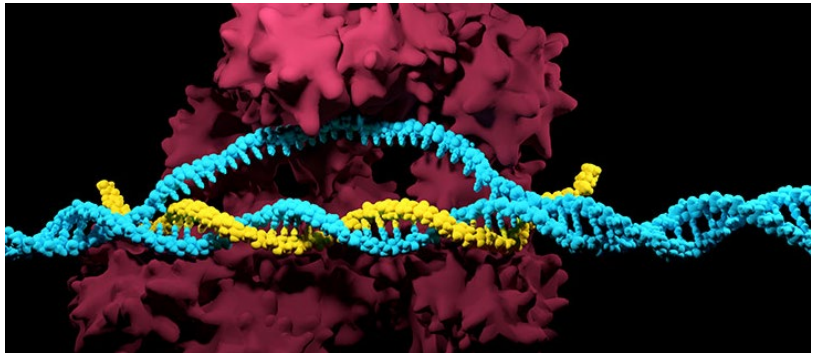
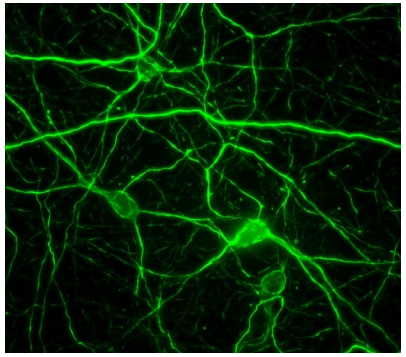
	Small scale, optimization	Large scale	High throughput/ Screening		Small scale
	Suspension		Suspension		Adherence
Platform					
	4D-Nucleofector® X Unit	4D-Nucleofector® LV Unit	4D-Nucleofector® 96-well Unit	HT Nucleofector® System	4D-Nucleofector® Y Unit
Main application (s)					

Nucleofector® Technology Portfolio

Supporting different throughput and scales

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	Small scale, optimization	Large scale	High throughput/ Screening		Small scale
	Suspension		Suspension		Adherence
Platform	**4D-Nucleofector® X Unit**	**4D-Nucleofector® LV Unit**	**4D-Nucleofector® 96-well Unit**	**HT Nucleofector® System**	**4D-Nucleofector® Y Unit**
Throughput	Low - Medium	Very low	High	Very high	Medium
Reaction vessel					
Cell number	20 µL: 10^4 to 10^6 100 µL: 10^5 to 10^7	10^7 to 10^9	10^4 to 10^6	10^4 to 10^6	10^4 to 10^5

Transferability of Transfection Conditions

Example: T cells

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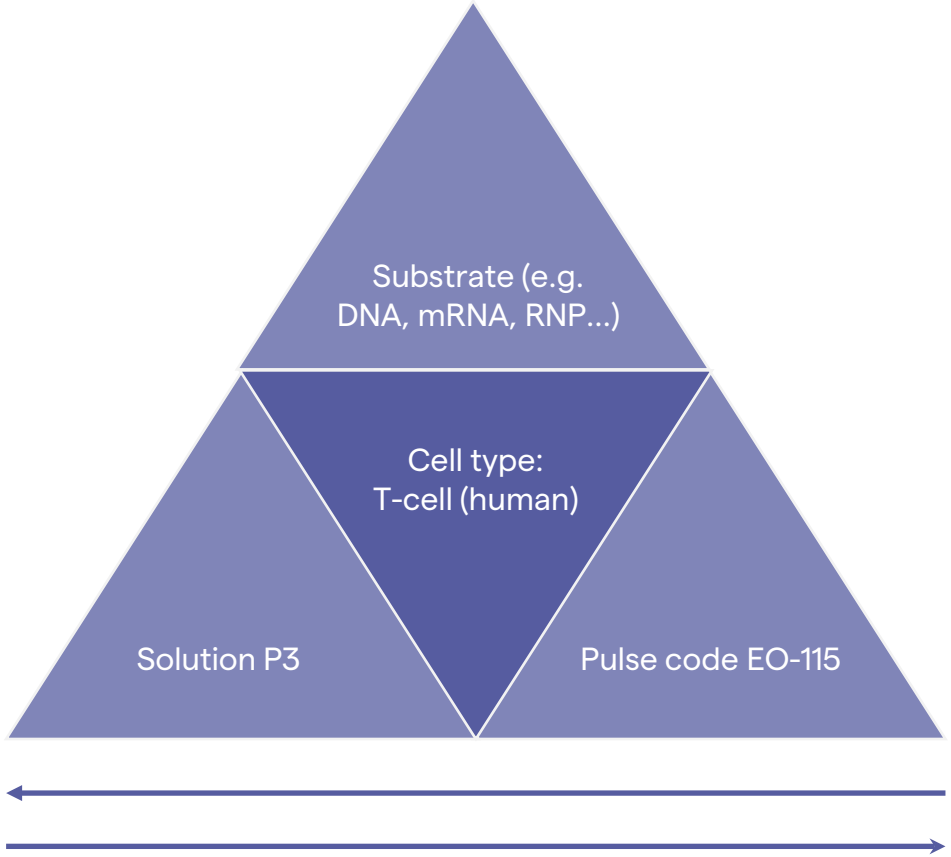
Small scale



Large volume

High throughput screening

Transferability of Transfection Conditions

Comparison of units and vessels

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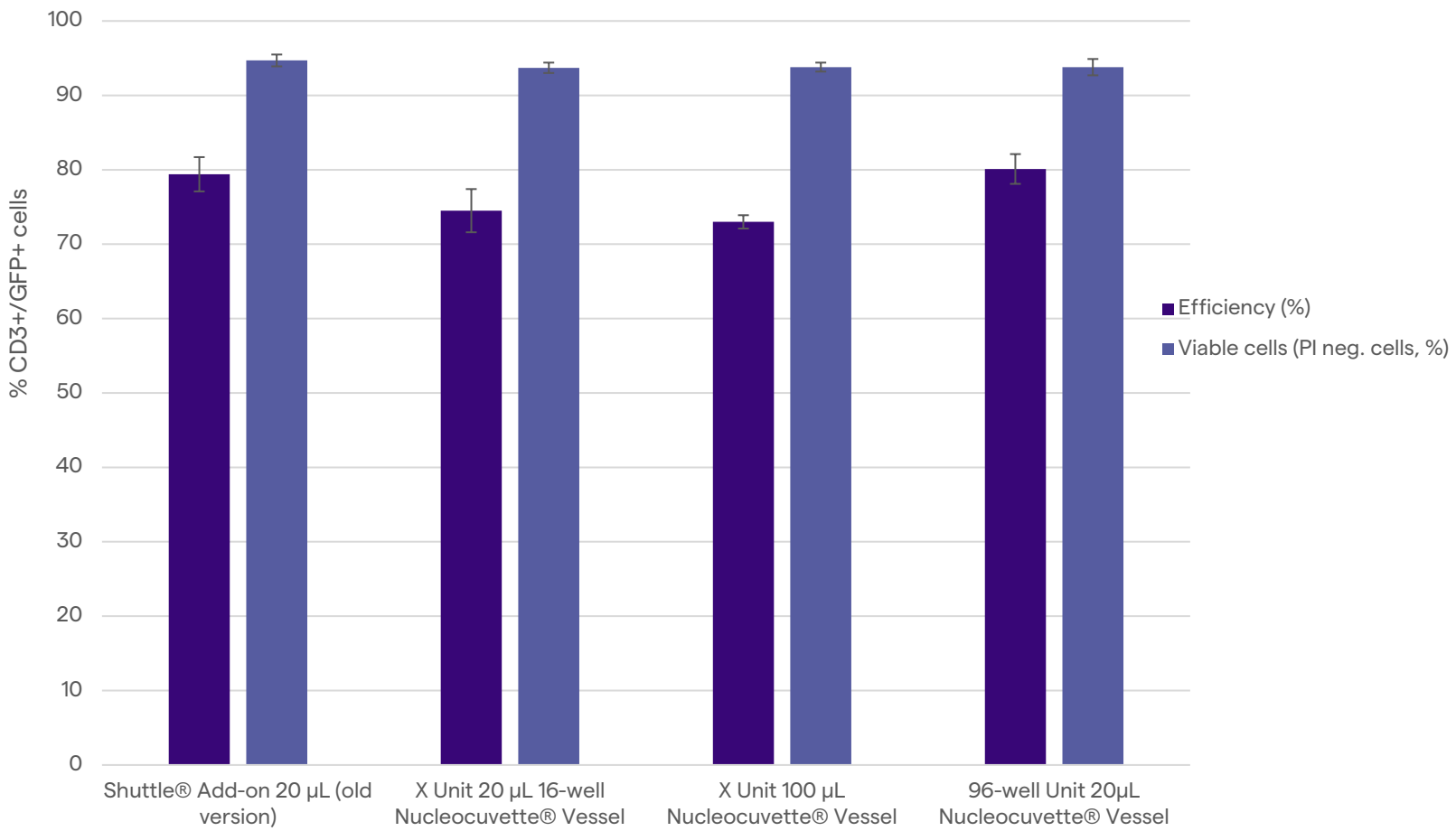

CD3+ T cells were pulsed with pMaxGFP™ in P3 Solution and Pulse FI-115.

The 4D-Nucleofector® X Unit

The Generalist

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Flexible:

- 2 x 10^4 to 2 x 10^7 cells per reaction
- Parallel processing of two 100 µL Nucleocuvette® Samples
- 16-well Nucleocuvette® Strips for smaller cell number with higher throughput

Application:

- Transfection of low and medium cell numbers
- Primary cells and cell lines with various substrates

384-well HT Nucleofector® System

The high-throughput platform

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Throughput and Automation:

- 2×10^4 to 2×10^6 cells per reaction (as in X- or 96-well Unit)
- Parallel processing of up to 384 wells per Nucleofection® Experiment
- Each well can be individually programmed
- Automation/ Integration into LH System possible

Application:

- High throughput screening (CRISPR)
- Genome wide screens to identify
  - New mechanisms involved in diseases
  - Novel drug targets

384-well HT Nucleofector® System

- **Power Supply Unit**
  - Generating high voltage pulses
- **Plate Handler Unit**
  - Carousel with two plate positions
- **HT Software (Laptop included)**
  - Easy set-up of Nucleofection® Experiments
  - Seamless integration into liquid handling systems

Scalable Technology

Condition transfer from research to manufacturing

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X Unit

Research → **Manufacturing**

- $1 \times 10^4 – 1 \times 10^6$ cells
- $1 \times 10^5 – 1 \times 10^7$ cells
- $1 \times 10^7 – 2 \times 10^8$ cells
- $3 \times 10^7 – 1 \times 10^9$ cells

LV Unit

20 µL strips

100 µL cuvettes

1 mL cartridge

LV cartridge

A Short History of Genome Editing

The rise of CRISPR as the genome-editing technology

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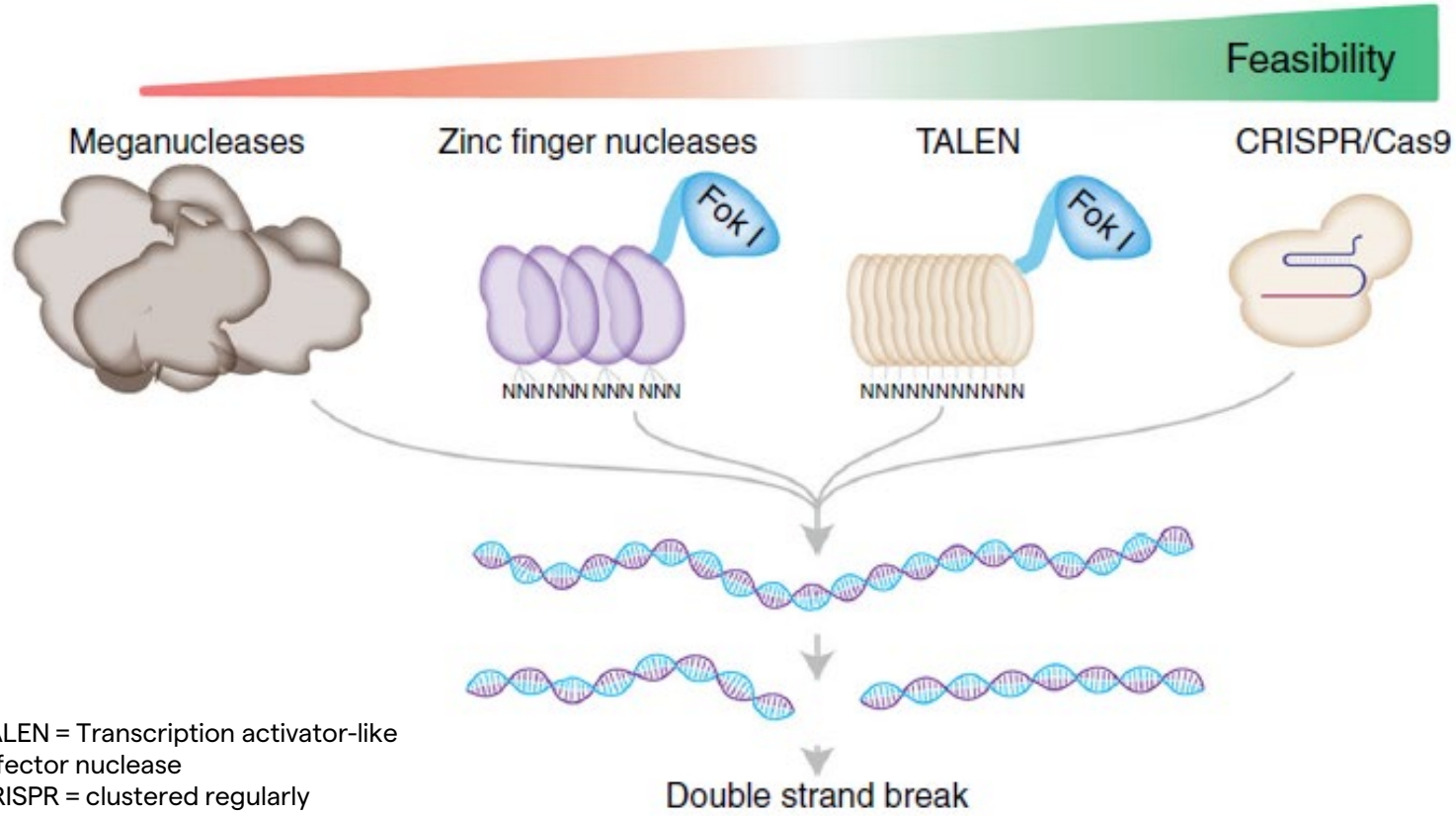

TALEN = Transcription activator-like effector nuclease
CRISPR = clustered regularly interspaced short palindromic repeats

Compared to Mega-nucleases, Zinc finger nucleases and TALENs, CRISPR/Cas 9 is

- Easier to use
- Cheaper

"For the first time ever, researchers had an extremely flexible tool that could be easily guided to target nearly any location in the genome (...)."

Citation and figure adapted from Adli (2018) The CRISPR tool kit for genome editing and beyond | Nature Communications under Creative Commons License (Attribution 4.0 International)

Genome Editing via CRISPR/Cas9

In more detail

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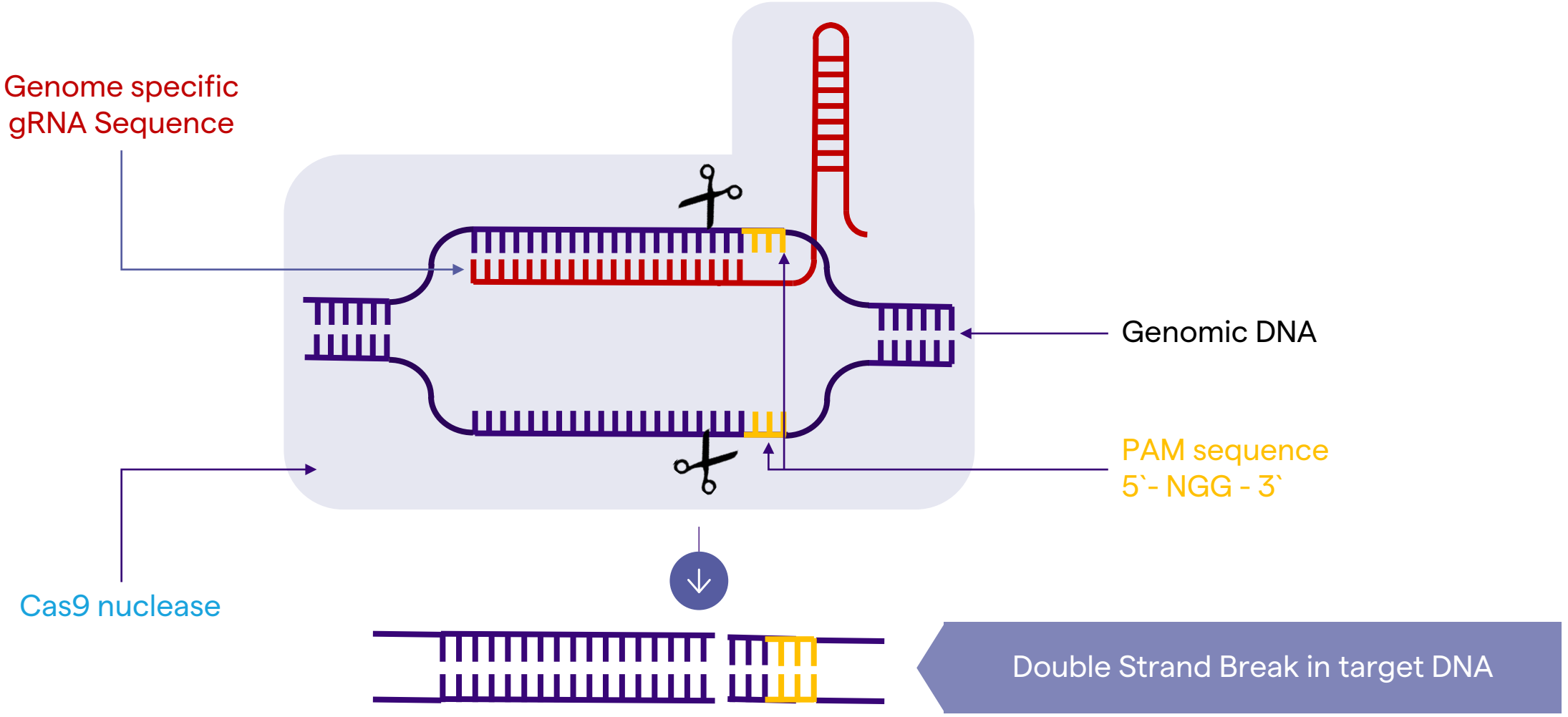

A Short History of Genome Editing

Downstream consequences of double strand break

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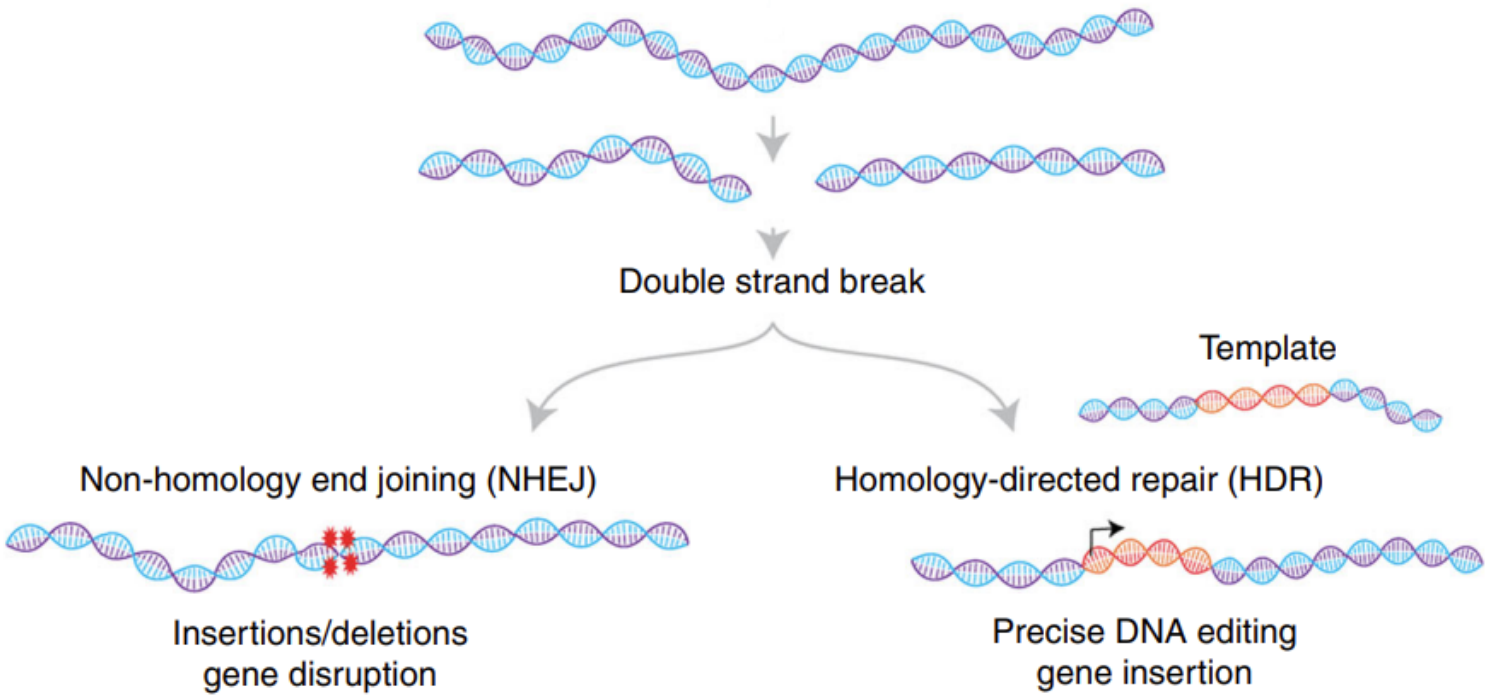

- The downstream consequences of the double-stranded break are dictated by the endogenous DNA repair pathways, not the technology used to create the break.
- Breaks induce DNA repair and increase mutagenesis frequencies by >1000 fold
- **NHEJ for Knock-outs**
- **HDR for DNA repair/insertion/deletion**

Citation and figure adapted from Adli (2018) The CRISPR tool kit for genome editing and beyond | Nature Communications under Creative Commons License (Attribution 4.0 International)

Logistics of CRISPR Cas9 Editing System

Several options and formats

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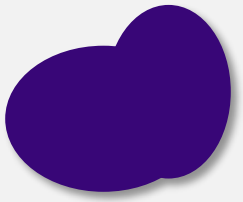
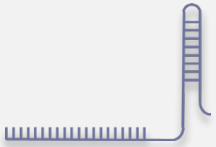
Knock-out



Cas9

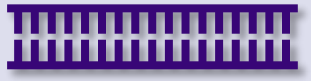
- Plasmid coding for Cas9
- Cas9 mRNA
- Cas9 Protein



Guide RNA

- Plasmid coding for guide
- crRNA + tracrRNA
- sgRNA

Knock-in



Homology Donor DNA

- Plasmid
- Linearized Plasmid
- dsDNA
- ssDNA

Utilizing CRISPR-Cas9 Beyond Genome Editing

Major application areas of CRISPR-Cas-based technologies

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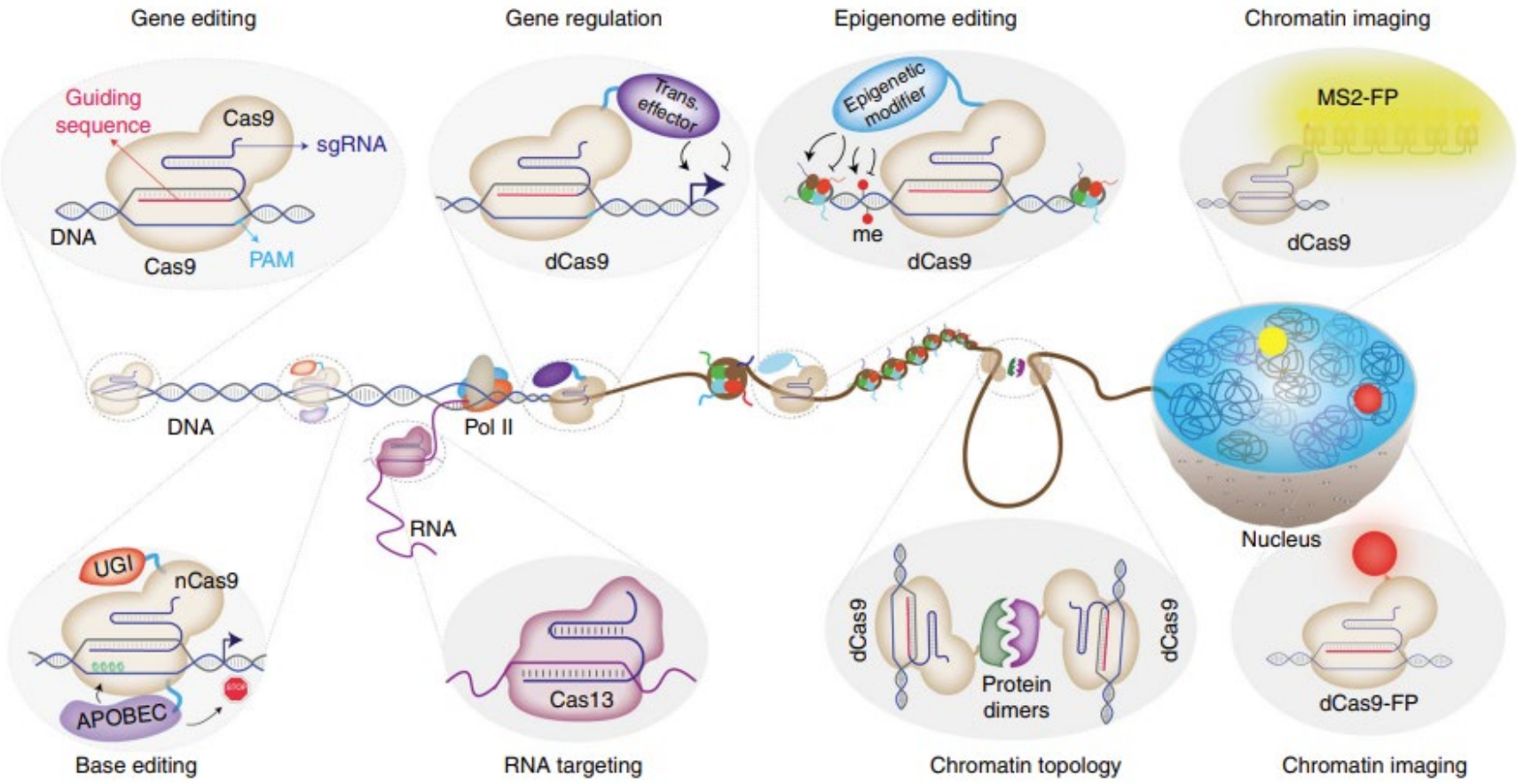

Figure adapted from Adli (2018) The CRISPR tool kit for genome editing and beyond | Nature Communications under Creative Commons License (Attribution 4.0 International)

CRISPR/Cas9 for Genome Editing

Different substrate types

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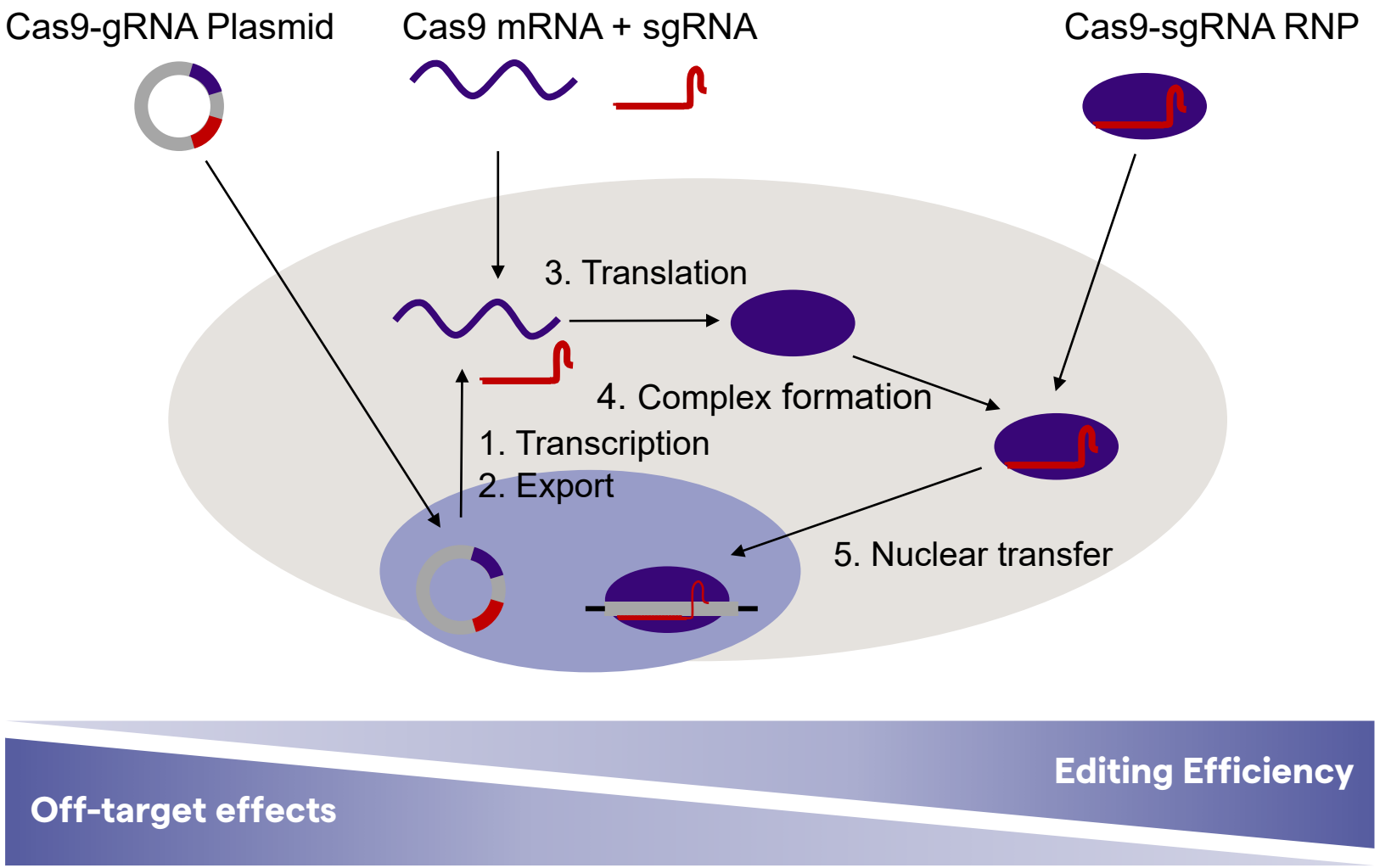

Public

Genome Editing

Setup for co-transfections

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ZFN or TALEN

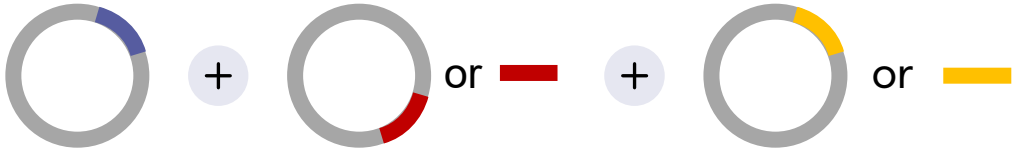
Fok1–ZF or Fok1-TAL fusion plasmid or mRNA

Repair plasmid or ssODN (optional)

CRISPR

Cas9 + gRNA plasmid

Repair plasmid or ssODN (optional)



Cas9 plasmid

gRNA plasmid or PCR cassette

Repair plasmid or ssODN (optional)

RNP (Cas9 protein + gRNA)

Repair plasmid or ssODN (optional)

Note: It is also possible to transfect Sleeping Beauty and PiggyBac® constructs.

Nucleofector® Technology – Enabling Genome Editing

CRISPR – "A tool for rewriting the code of life"

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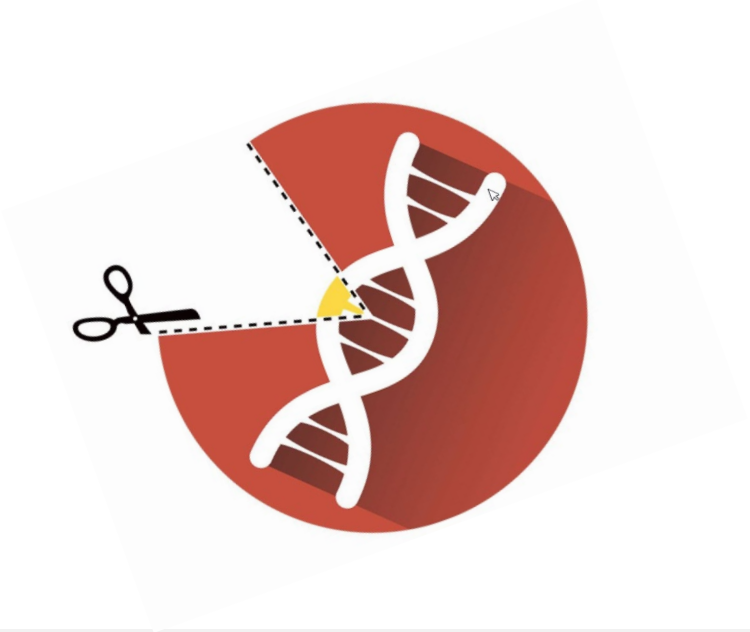
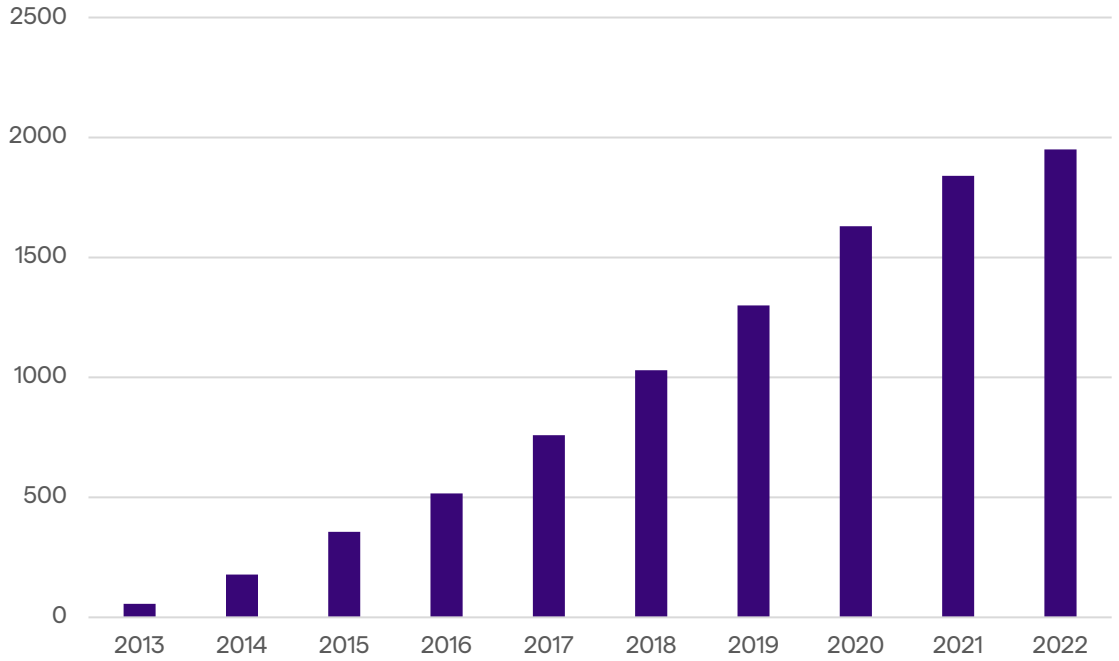


- A "gene scissor" made of an enzyme and RNA that revolutionizes biomedical research
- Nobel Prize in Chemistry 2020 to Emmanuelle Charpentier and Jennifer A. Doudna (citing our technology)

Publications Using Nucleofector® Technology and CRISPR



Google Scholar Search for "Nucleofect* CRISPR", March 2023

Lin-Shiao... J.A. Doudna, Nucleic Acids Research, 2022
Albanese et al., Nature Methods, 2022
Hultquist et al., Nature Protocols, 2019
Shifrut et al., Cell, 2018
Roth et al., Nature, 2018
And many, many more...

How To Start?

Lonza Knowledge Center

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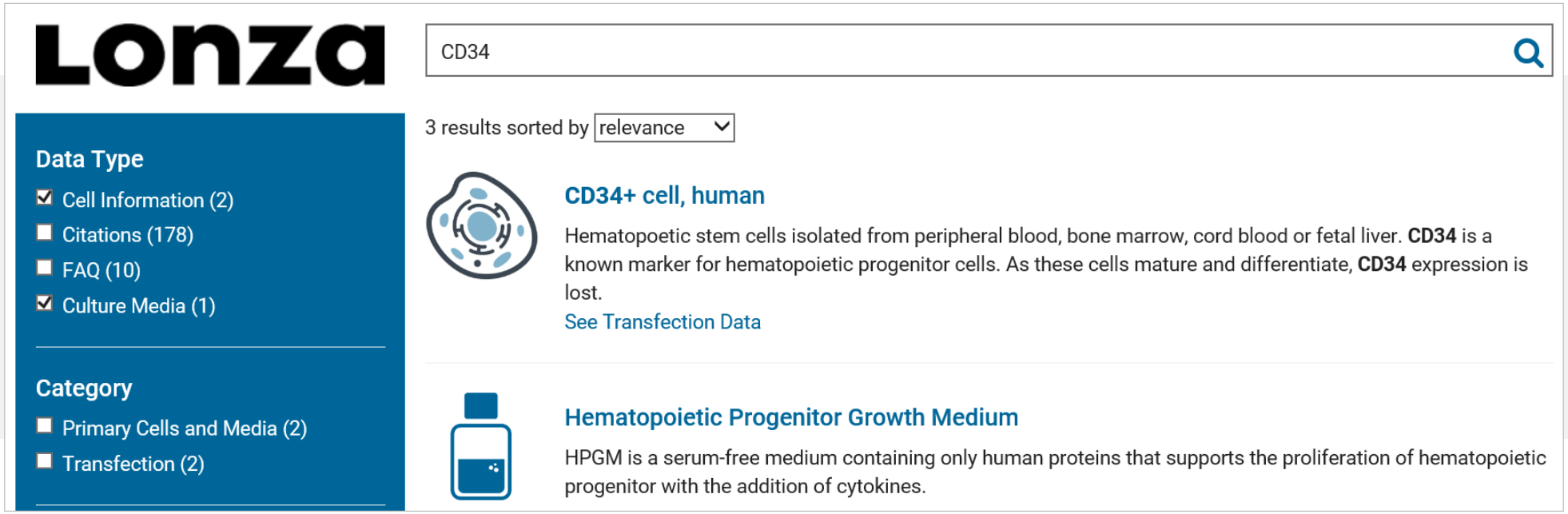
1. More than 650 ready-to-use protocols in Lonza Knowledge Center: knowledge.lonza.com
2. Conditions optimized for plasmid delivery works for other substrates as well
3. Optional fine tuning for RNP delivery



How To Start?

Optimal Nucleofection® Conditions

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Download Optimized Protocol

Transfection Information

Lonza Optimized Protocol

Optimization Guideline

Filter: Any Substrate | Any Platform

The table below shows data for the cell type and Nucleofector™ Platform selected. Those data are either based on Lonza Optimized Protocols or on results shared from customers who performed an optimization based on our guidelines. In case no data are shown for the selected Nucleofector™ Platform, please take a look at our optimization strategy to get further guidance on how to easily determine optimal Nucleofection conditions yourself.

Protocol	Kit	Program	Cells	Efficiency	Viable Cells	Substrate		Format	Platform
	P3	96-EO-100	5e4	78-88%	56-68%	Plasmid (general)	0.4 µg	20 µl	Shuttle
	CD34+, human	U-008	1e6	60-82% ±11		Plasmid (general)	µg	100 µl	I/II/2b
	P3	EO-100	5e4	62-70%	79-91%	Plasmid (general)	0.4 µg	20 µl	4D X-Unit

How To Start?

Optimal Nucleofection® Conditions

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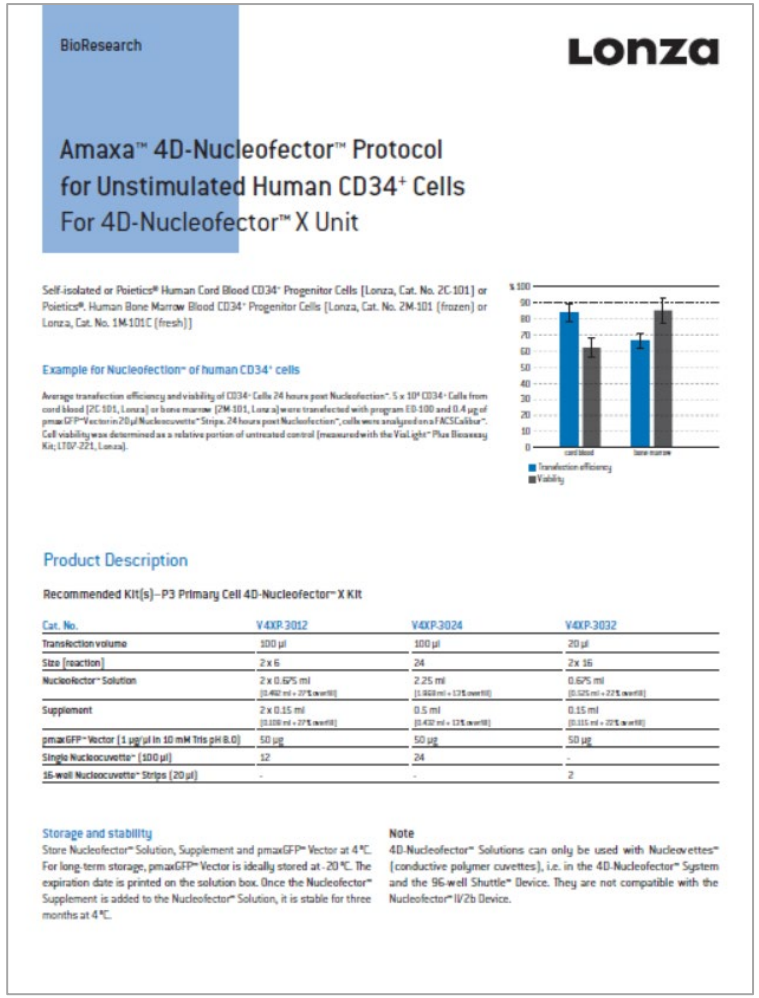
Depending on the cell type,

first verify optimal conditions (given in the ready-to-use protocol) using pmaxGFP™

or determine optimal conditions (e.g. for iPSC clones)



BioResearch

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Amaxa™ 4D-Nucleofector™ Protocol
for Unstimulated Human CD34⁺ Cells
For 4D-Nucleofector™ X Unit

Self-isolated or Poietics® Human Cord Blood CD34⁺ Progenitor Cells [Lonza, Cat. No. 2C-101] or Poietics®, Human Bone Marrow Blood CD34⁺ Progenitor Cells [Lonza, Cat. No. 2M-101 (frozen) or Lonza, Cat. No. 1M-101C (fresh)]

Example for Nucleofection™ of human CD34⁺ cells

Average transfection efficiency and viability of CD34⁺ Cells 24 hours post Nucleofection™. 5 x 10⁶ CD34⁺ Cells from cord blood [2C-101, Lonza] or bone marrow [2M-101, Lonza] were transfected with program ED-100 and 0.4 µg of pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. 24 hours post Nucleofection™, cells were analysed on a FACSCalibur™. Cell viability was determined as a relative portion of untreated control (measured with the ViaLight™ Plus Bioassay Kit; LT07-221, Lonza).

Product Description

Recommended Kit(s)—P3 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-3012	V4XP-3024	V4XP-3032
Transfection volume	100 µl	100 µl	20 µl
Size (reaction)	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.868 ml + 13% overfill)	0.675 ml (0.525 ml + 27% overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 27% overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuvette™ (100 µl)	12	24	-
16-well Nucleocuvette™ Strips (20 µl)	-	-	2

Storage and stability
Store Nucleofector™ Solution, Supplement and pmaxGFP™ Vector at 4 °C. For long-term storage, pmaxGFP™ Vector is ideally stored at -20 °C. The expiration date is printed on the solution box. Once the Nucleofector™ Supplement is added to the Nucleofector™ Solution, it is stable for three months at 4 °C.

Note
4D-Nucleofector™ Solutions can only be used with Nucleovettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. They are not compatible with the Nucleofector™ II/2b Device.

Designing Your Experiment

Plasmid considerations

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Purity

- Use highly purified DNA
- Check A260:A280 ratio, should be ideally 1.8 -2.0, but 1.6 is acceptable
- Use endotoxin removing prep kits

Quality

- Check for nicked or degraded DNA on agarose gel

Backbone

- Promoter strength
- IRES plasmid might give lower efficiency, depending on position of gene of interest relative to IRES sequence

Designing Your Experiment

Plasmid Quality: Nicked DNA may lead to lower efficiency

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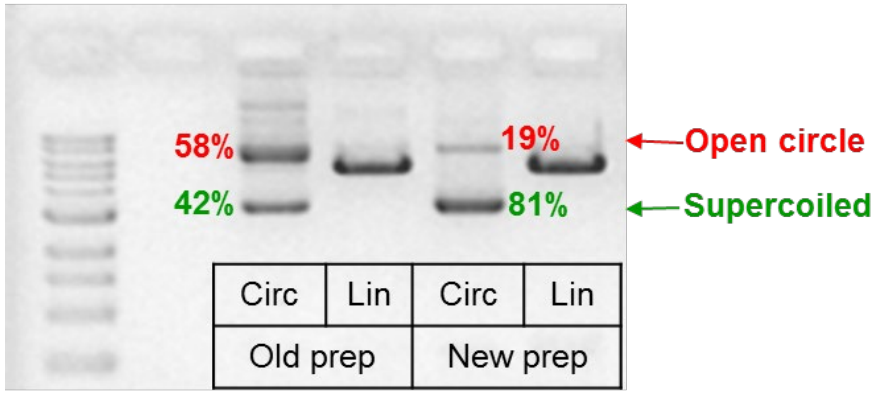

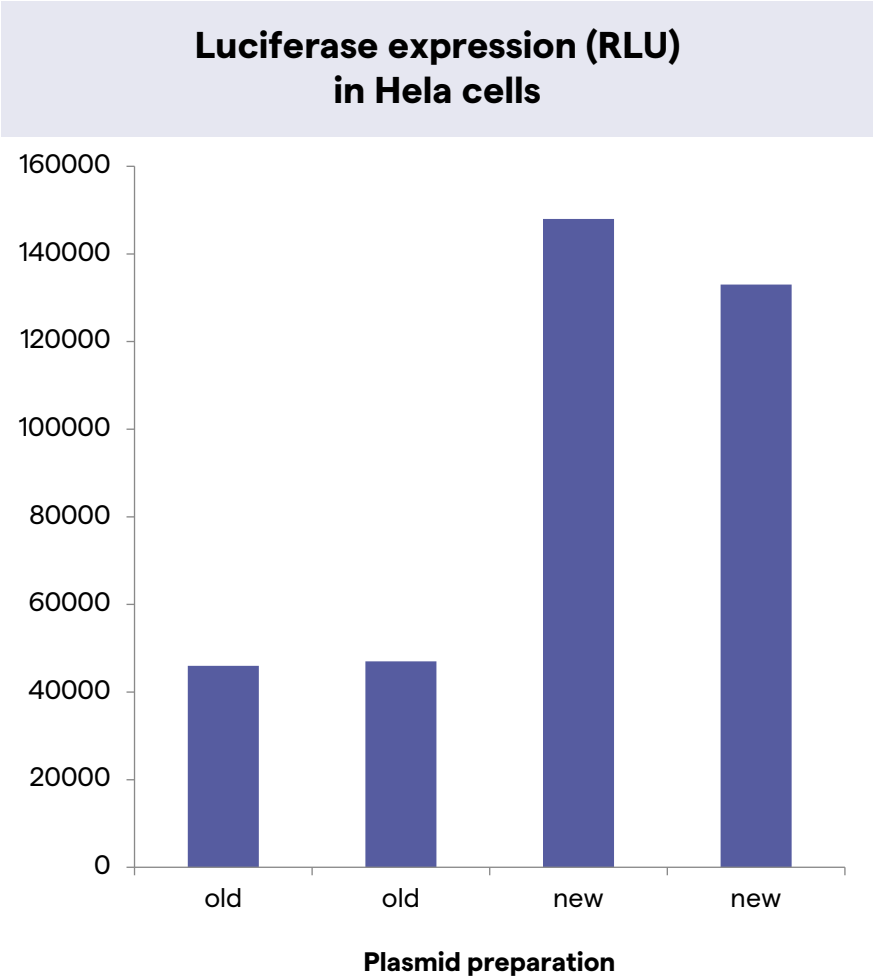

Designing Your Experiment

Plasmid Backbone: Expression level is promoter dependent

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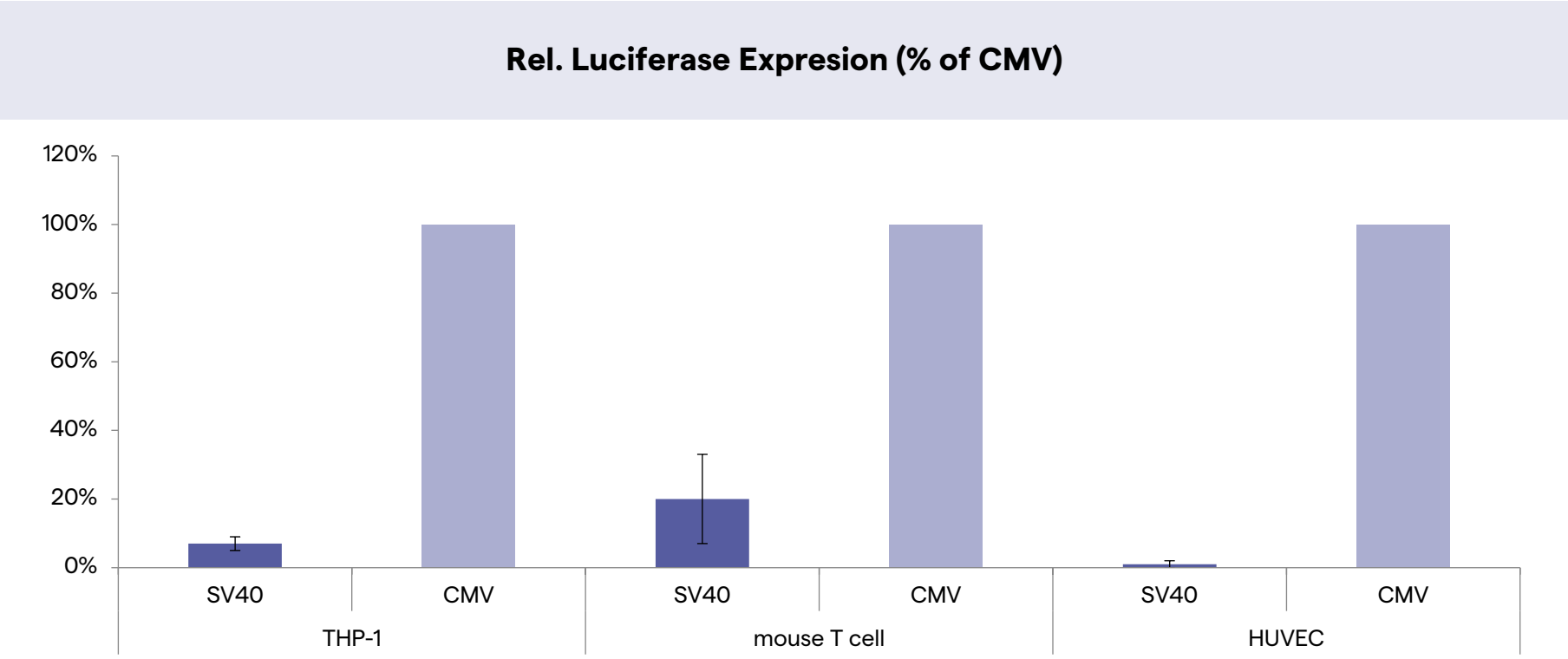
- Luciferase expression using different promoters
  - SV40 promoter: weak Luc expression
  - CMV promoter: strong Luc expression



Designing Your Experiment

Plasmid Backbone: Special sequences can influence expression

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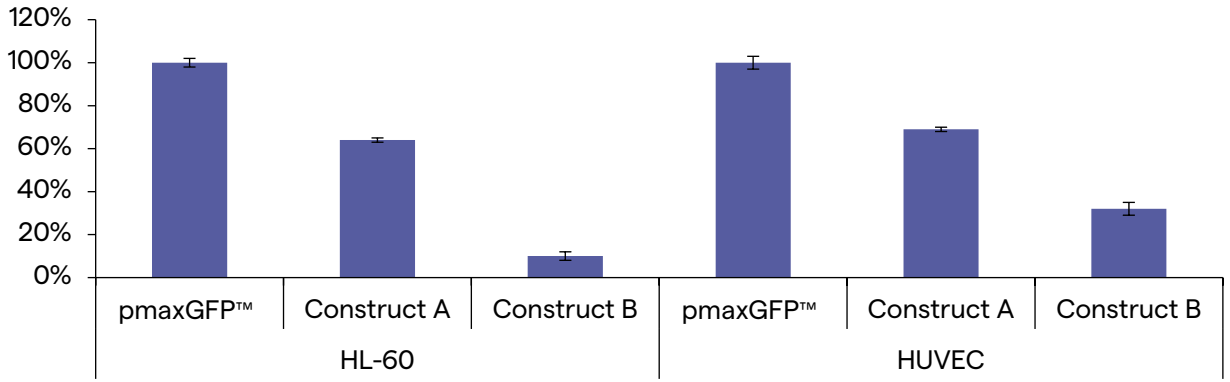
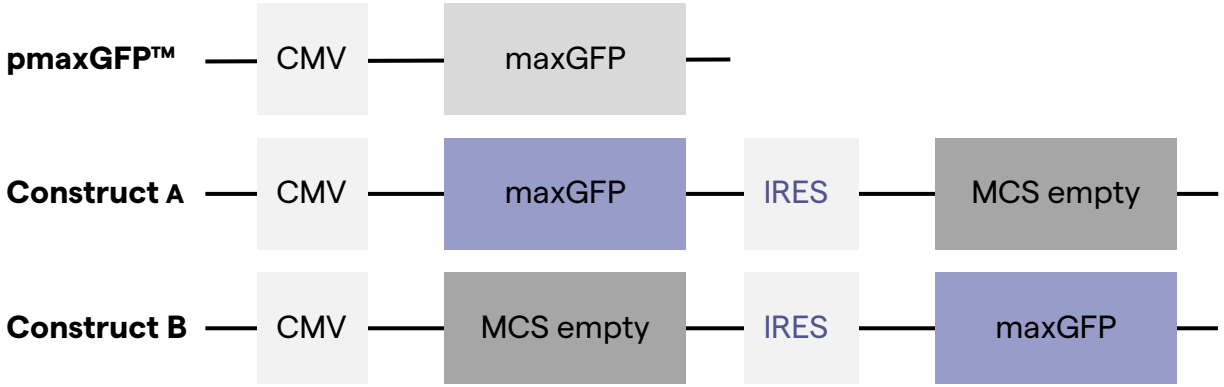
Cell & Gene

Mean fluorescence (% of maxGFP™ Expression)





- Multicistronic plasmids (e.g. IRES or T2A) may result in lower expression levels: IRES plasmids
- Potential reasons for lower expression for protein downstream IRES:
  - Intracellular long mRNA might be degraded
  - Inefficient internal ribosomal entry sites

Designing Your Experiment

Plasmid Backbone: Special sequences can influence expression

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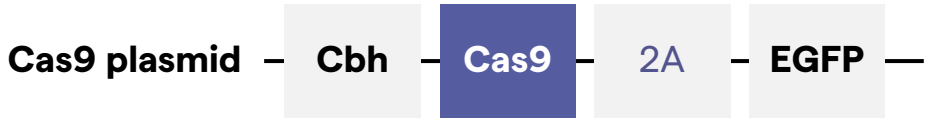
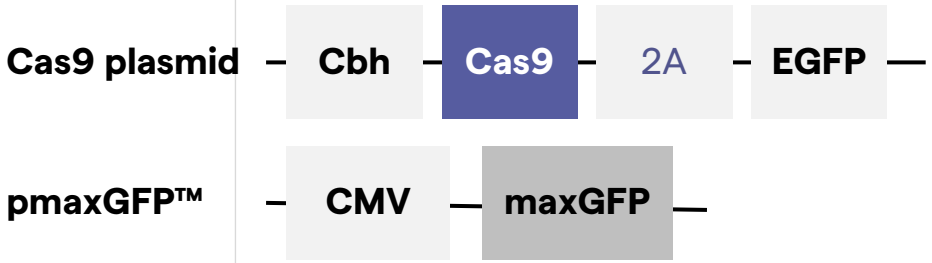
- Example T2A plasmids:



- Potential reasons for lower expression for gene downstream T2A:
  - Intracellular long mRNA might be degraded
  - Long protein (before self cleavage)/ fusion protein
  - Long process to generate functional gene product due to self-cleavage

- Alternative option: Co-transfection of GFP plasmid might help to sort for positive cells
  - Co-transfection works highly efficient in Nucleofection® Experiments
  - Low amounts of pmaxGFP™ co-transfected with Cas9 plasmids are able to mark the positive cells of expressing Cas9



Designing Your Experiment

Nucleofection® of mRNA

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- Follow the same protocol and use the same program that is used for the transfection of DNA with your cells
- The mRNA should be capped and poly-adenylated
- Optimal amount has to be titrated, might be higher than for plasmid: 2 - 20 µg in 100 µL reaction
- When higher amount is required, the volume should not exceed 10% of the total sample volume
- Depending on the cell type (e.g. for DCs) it might be best to add the mRNA directly into the empty cuvettes and then add the cell-solution mix on top. After adding the cell suspension transfect the sample immediately
- For further reading please see our Genome Editing Citation List, e.g.
  - Hendel et al. Nat. Biotechnol. 2015
  - Bak et al. Cell Reports 2017

Designing Your Experiment

Nucleofection® of RNPs

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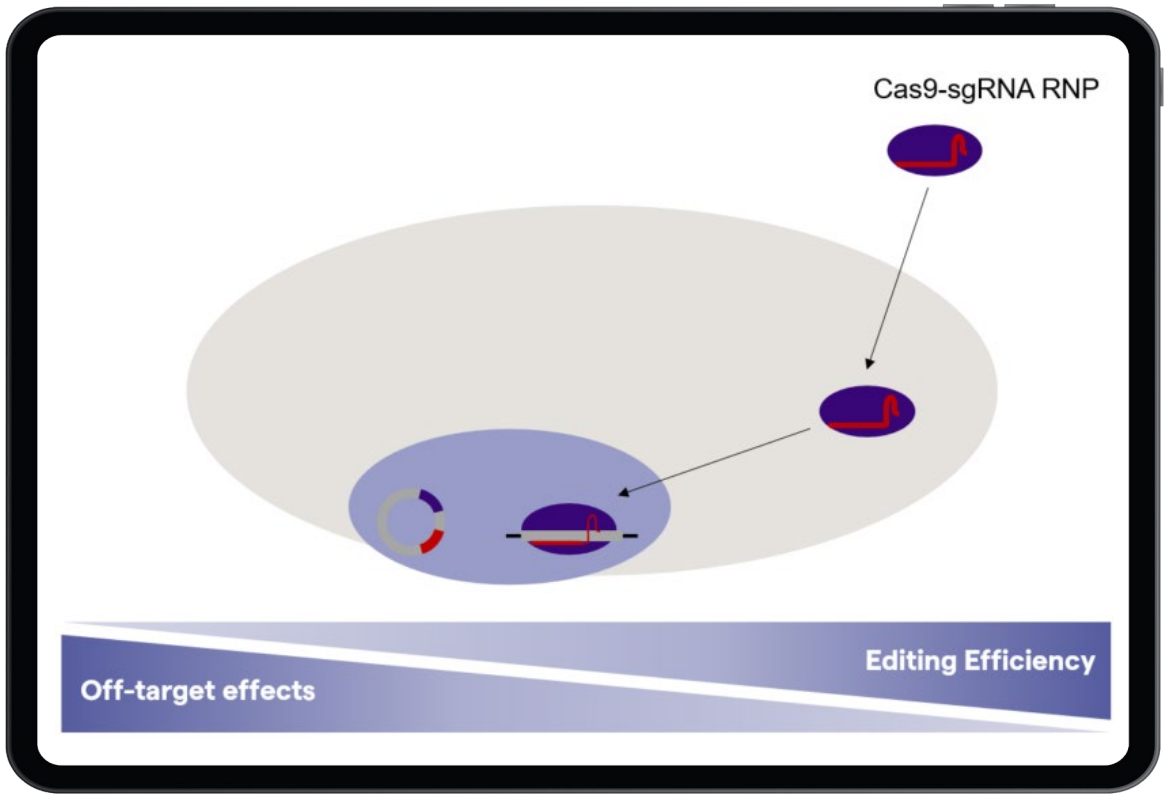
RNP complexes might be the method of choice because of various advantages:

- No cloning of constructs needed
- Less toxic than DNA
- More stable than mRNA
- No transcription/translation process needed: Easier dosage
- Defined action time (short term): Lower risk of off-target effects



Designing Your Experiment

Nucleofection® Conditions for transfection of RNPs

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RNP complexing: Titrate optimal ratio between Cas9 protein and gRNA

- In most publications Cas9:gRNA ratio between 1:1.2 and 1:5 was used

Titrate optimal amount of RNP for transfection

- Suggested starting range for Nucleofection®:

Concentration	20 µL reaction	100 µL reaction
0.08 – 0.8 µg/µL 0.5 – 5 pmol/µL	1.6 – 16 µg 10 – 100 pmol	8 – 80 µg 50 – 500 pmol

Addition of an unspecific ssODN, e.g. Alt-R Cas9 Electroporation Enhancer (IDT)

Avoid too much dilution of Nucleofection® sample by RNP buffer:

- Usually “foreign” buffer should not be more than 10%
- Consider complexing directly in Nucleofector® Solution

Designing Your Experiment

Nucleofection® Conditions for transfection of RNPs

Lonza
Cell & Gene

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Designing Your Experiment

Example: Nucleofection® of RNPs into resting T cells (Knock-in)

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SYNTHEGO | Lonza

PROTOCOL

Case Study

Synthetic sgRNAs Enable Researchers to Study Viral Infection in Resting Human CD4+ T Cells

CRISPR Editing Human Primary Resting CD4+ T Cells with RNPs using Nucleofector® Technology

Developed by researchers at the University of Munich in collaboration with Synthego and Lonza

- Detailed protocol on CRISPR based genome editing in primary resting CD4+ T Cells
- Simultaneous, polyclonal six-gene KO following a single RNP Nucleofection® Process and knock-in experiments
- Protocol can be adapted to other primary cells and cell lines – our Scientific Support Team will help

For further information and detailed protocol see: Case Study and Associated Protocol “Synthetic sgRNAs Enable Researchers to Study Viral Infection in Resting Human CD4+ T Cells” from Synthego and Lonza

Designing Your Experiment

Example: Nucleofection® of RNPs into resting T cells (Knock-in)

Lonza
Cell & Gene

	20 µL Volume	100 µL Volume
Kit	P3 Primary Cell 4D-Nucleofector® X Kit S (V4XP-3032 for 16 x 20 µL reaction) 1.6 – 16 µg 10 – 100 pmol	P3 Primary Cell 4D-Nucleofector® X Kit L (V4XP-3012 for 12 x 100 µL reaction; V4XP-3024 for 24 x 100 µLreaction)
Cell number	1-2 x 10^6	0.5-1 x 10^7
Nucleofector® Solution	20 µl (P3 + supplement)	100 µl (P3 + supplement)
SpCas9	100 pmol	500 pmol
sgRNAs	40 pmol	200 pmol
HDR template	0.8 – 1.12 pmol	4 – 5.6 pmol

For further information and detailed protocol see: Case Study and Associated Protocol “Synthetic sgRNAs Enable Researchers to Study Viral Infection in Resting Human CD4+ T Cells” from Synthego and Lonza

Designing Your Experiment

Example: Nucleofection® of RNPs into resting T cells

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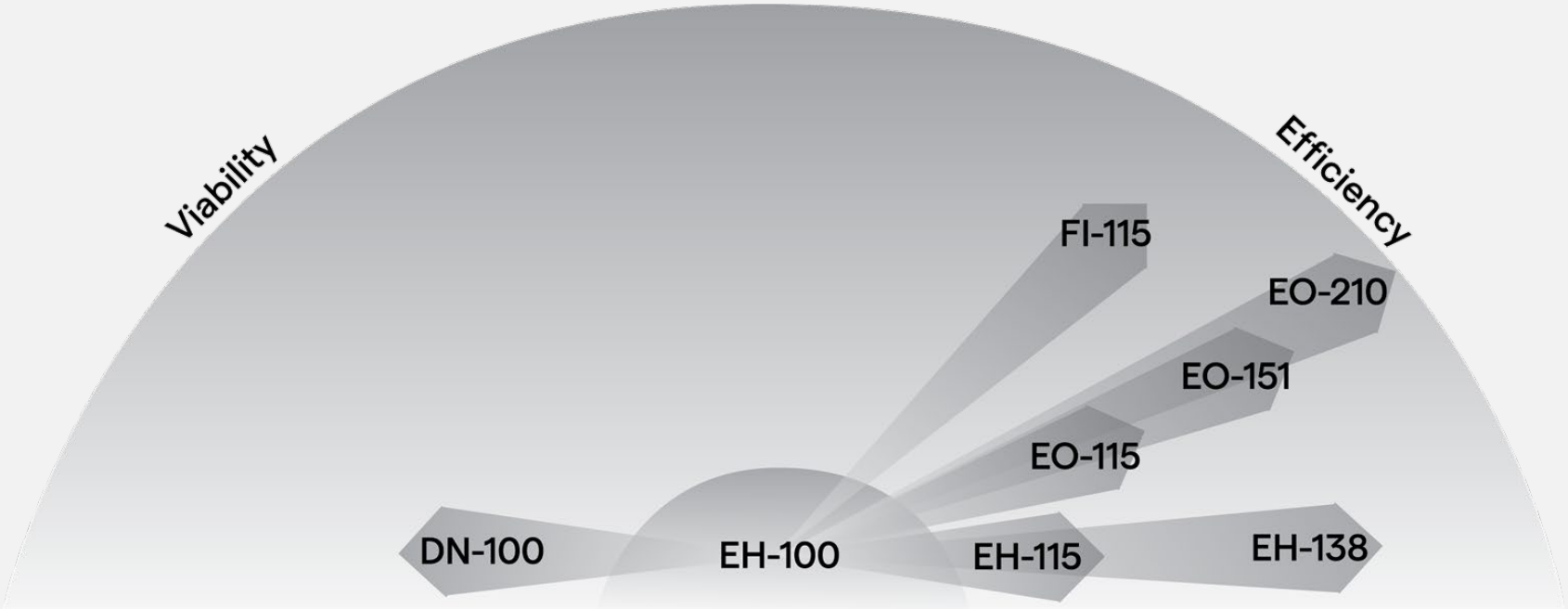
Cell & Gene

Depending on your desired outcome (e.g. editing efficiency, number of viable transfected cells) you might want to test additional pulses. Please test (some of) the following pulse conditions along with a no pulse control:



For further information and detailed protocol see: Case Study and Associated Protocol “Synthetic sgRNAs Enable Researchers to Study Viral Infection in Resting Human CD4+ T Cells” from Synthego and Lonza

Genome Editing Publications Using Nucleofector® Technology

Search for your cell type of interest

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Cell & Gene

Genome Editing Publications Using Nucleofector® Technology

Authors	Citation	Nucleofector® Platform	Cell Types	Substrates
CRISPR				
Afolabi LO et al	Immunology 158(2):63-69 (2019)	4D-Nucleofector® System	Human NK cells	protein (Cas9 RNP)
Agudelo D et al.	Nat Methods 14(6):615-620 (2017)	4D-Nucleofector® System	K562, U2OS, HEK293, CD34	plasmid, protein (Cas9 RNP)
Albanese M et al.	Nat Methods 19(1):81-89 (2021)	4D-Nucleofector® System	human T cell	protein (Cas9 RNP)
Bak RO et al.	Nat Prot 13(2):358-376 (2018)	4D-Nucleofector® System; Nucleofector® II/2b Device	Human T cells, human CD34	protein (Cas9 RNP)
Basar et al.	Blood Adv 4(14):3357-3367 (2020)	4D-Nucleofector® System; 96-well and LV-Unit	human T cells, HEK	protein (Cas9 RNP)
Bexte T et al.	Oncoimmunology 11(1):2081415 (2022)	4D-Nucleofector® System	NK, T cells, MM1.S	n.d.
Borot F et al.	PNAS 116(24):11978-11987 (2019)	4D-Nucleofector® System	Human CD34	protein (Cas9 RNP)
Bothmer A et al.	CRISPR J. 3(3):177-187 (2020)	4D-Nucleofector® System	T cells	protein (Cas9 RNP)
Bothmer A et al.	Nat Commun 8:13905 (2017)	4D-Nucleofector® System	U2OS	plasmid
Bressan R et al.	Development 144(4):635-648 (2017)	4D-Nucleofector® System; Nucleofector® II/2b Device	Human neural stem cell line	plasmid, protein (Cas9 RNP)
Byrne et al.	Curr Prot Stem Cell Biol 35:5A.8: -1-5A.8.22 (2015)	4D-Nucleofector® System	Human iPSC	plasmid
Byrne et al.	Nucleic Acids Res 43(3): e21 (2015)	4D-Nucleofector® System	Human iPSC	plasmid
Chan et al.	Biotechnol J 11(3):399-414 (2016)	4D-Nucleofector® System	CHO suspension	plasmid
Chavez et al.	Nat Meth 12(4): 326–328 (2015)	4D-Nucleofector® System	Human iPSC	plasmid
Choi J et al.	Nature 608(7921):98-107 (2022)	4D-Nucleofector® System	MEF, MES	plasmid
Dalvai et al.	Cell Rep 13(3):621-633 (2015)	4D-Nucleofector® System	K562	plasmid
Dever DP et al.	iScience 15:524-535 (2019)	4D-Nucleofector® System	Human NSCs	plasmid (Cas9, TALEN), mRNA (Cas9)
Dewari PS et al.	Elife 7:e35069 (2018)	4D-Nucleofector® System; 96-well Unit	Human/mouse ESC, mouse NSC line, human glioblastoma	protein (Cas9 RNP)
deWitt et al.	Sci Transl Med 8(360):360ra134 (2016)	4D-Nucleofector® System	CD34, K562	protein (Cas9 RNP)
Doudna JA, Charpentier E	Science 346(6213):1258096 (2014) - Review	n.d.	n.d.	n.d.
Evgin L et al.	Nat Commun 11(1):3187 (2020)	4D-Nucleofector® System	CAR T cells	protein (Cas9 RNP)
Freund EC et al.	J Exp Med 217(7):e20191692 (2020)	4D-Nucleofector® System	Human/mouse Monocytes, Macrophages or DCs	protein (Cas9 RNP)
Fu Y et al.	Nat Biotech 32(3):279–284 (2014)	4D-Nucleofector® System	U2OS	plasmid
Fu Y et al.	Nat Biotech 31(9):822–826 (2013)	4D-Nucleofector® System	U2OS, K562	plasmid
Gaj T et al.	Nucleic Acids Res 45(11):e98 (2017)	4D-Nucleofector® System	HEK293T, U2OS-GFP, C6, human fibroblasts	protein (Cas9 RNP)
Georgiadis C et al.	Leukemia 35(12):3466-3481 (2020)	4D-Nucleofector® System; LV-Unit	human T cell	mRNA
Ghorbal et al.	Nat Biotechnol 32:819–821 (2014)	4D-Nucleofector® System	Plasmodium falciparum	plasmid (linearized)

Citation List available for download: CRISPR Transfection: Efficient Genome Editing | Lonza

Sample Handling – Optimized Protocols

4D-Nucleofector® X Unit

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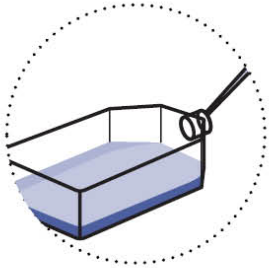
Cell & Gene

Step 1

Harvest cells of interest.



Step 2

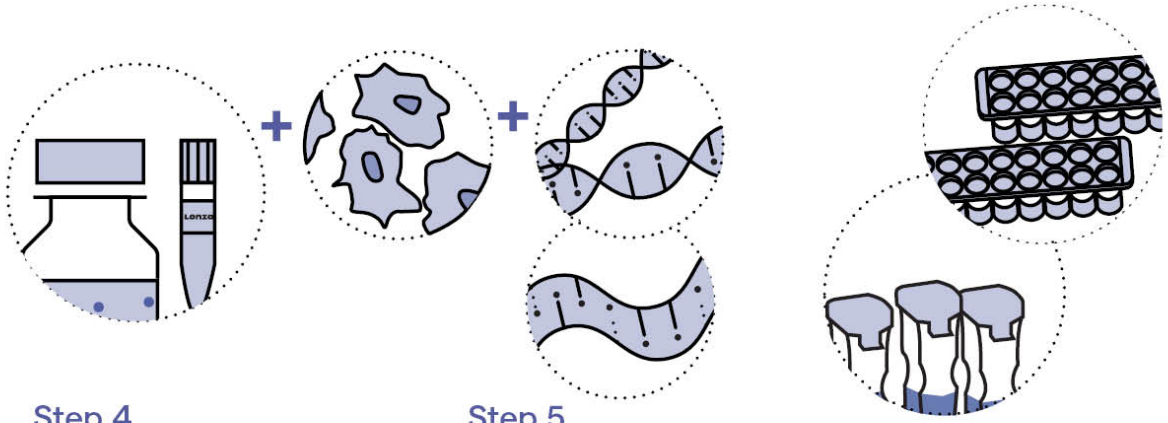
Mix and combine.

Nucleofector® Solution with supplement

Cells

DNA or siRNA

Transfer to a Lonza certified cuvette.

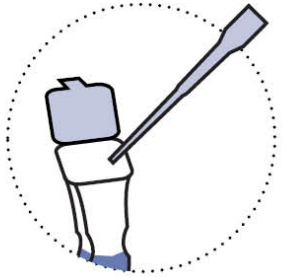


Step 3

Select Nucleofector® Program. Insert cuvette. Press » Start «.

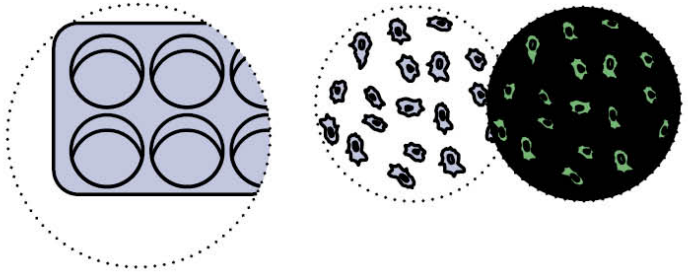
Step 4

Rinse cuvette with culture



Step 5

Transfer to culture dish. Expression can be detected as soon as 3 – 8 hours post Nucleofection® Experiment.



Designing Your Experiment

Cell considerations

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Cell & Gene

Cell condition can influence transfection results

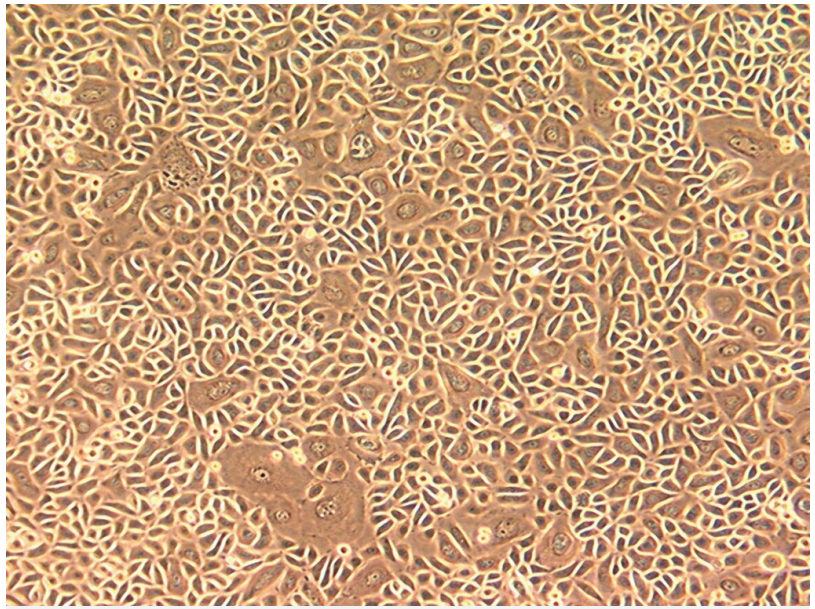
- Cells should be in log growth (confluency or density)
- Use cells at low passage number
- Free of contamination
- Use standardized culture conditions for better reproducibility
- Consider clonal differences with cell lines

Special considerations for primary cells

- Donor differences
- Senescence of cells can affect transfection efficiency *(Seluanov et al., 2004, PNAS 101:7624-7629)*

Ideal conditions

Please review cell type specific Nucleofector® Protocol



Example:
NHBE, Lonza Cat. No. CC-2541

Designing Your Experiment

Step 1: Harvest cells of interest

Lonza
Cell & Gene

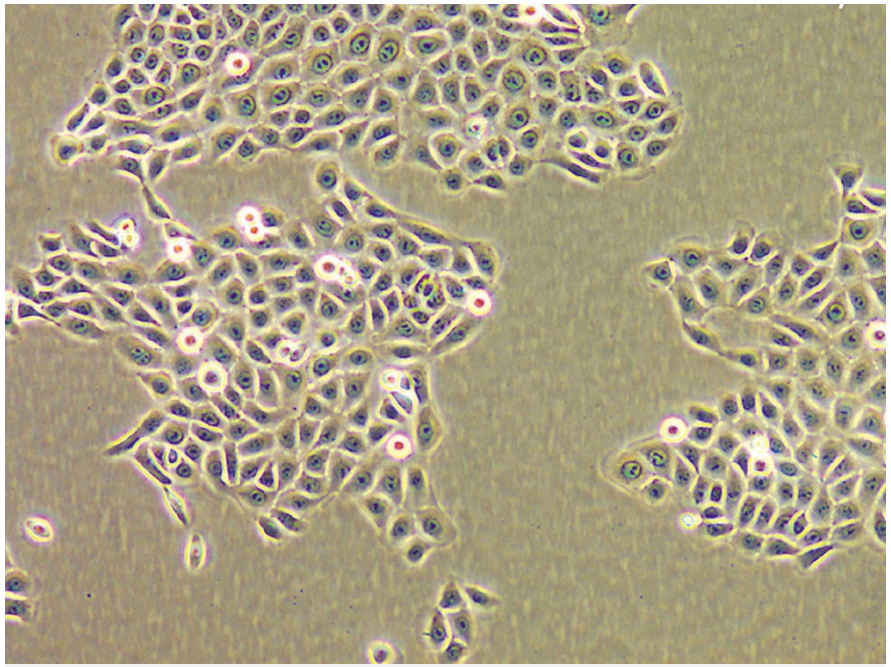
Cell Harvesting

For monolayers, monitor trypsinization, limit exposure to trypsin

Determine cell concentration (cells/ml) of cell suspension every time

If possible, trypsinize at RT

Centrifuge at low force (80-100 x g)



Example:
NHEK-Ad, Lonza Cat. No. 00192627

Tips for a Nucleofection® Experiment

Step 2: Mix and combine

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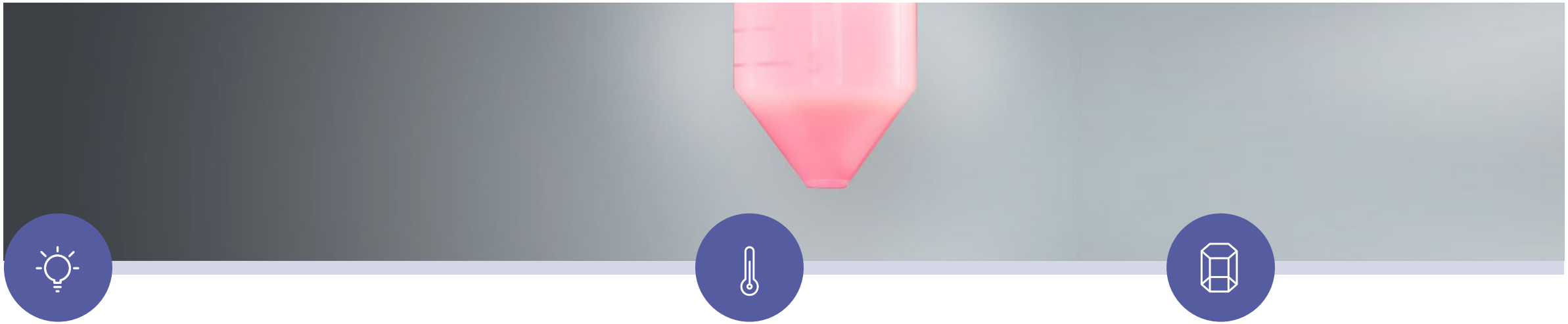


Cell Handling before Nucleofection®

- Drain pellet completely prior to re-suspension in Nucleofector® Solution
- Gentle re-suspension with Nucleofector® Solution
- Cells should be in Nucleofector® Solution for no longer than 15–20 minutes

Nucleofector® Solution at RT (already mixed with Supplement)

Volume of substrate ideally less than 10% of total sample volume

Tips for a Nucleofection® Experiment

Step 2: Mix and combine

Lonza
Cell & Gene

Example: Nucleofection® Experiment for T cells (human, activated)				
	20 µL	**100 µL**	**1 mL**	**20 mL**
Cells	1x10e6	5x10e6	5x10e7	1x10e9
Solution P3	16.4 µL	82 µL	820 µL	16.4 mL
Supplement	3.6 µL	18 µL	180 µL	3.6 mL
pmaxGFP™ (1 µg/ µL)*	0.4 µL	2 µL	20 µL	400 µL

*Volume of substrate ideally less than 10% of total sample volume

Transfer of cell suspension to Nucleocuvette® by avoiding air bubbles

Software / GUI

The home screen

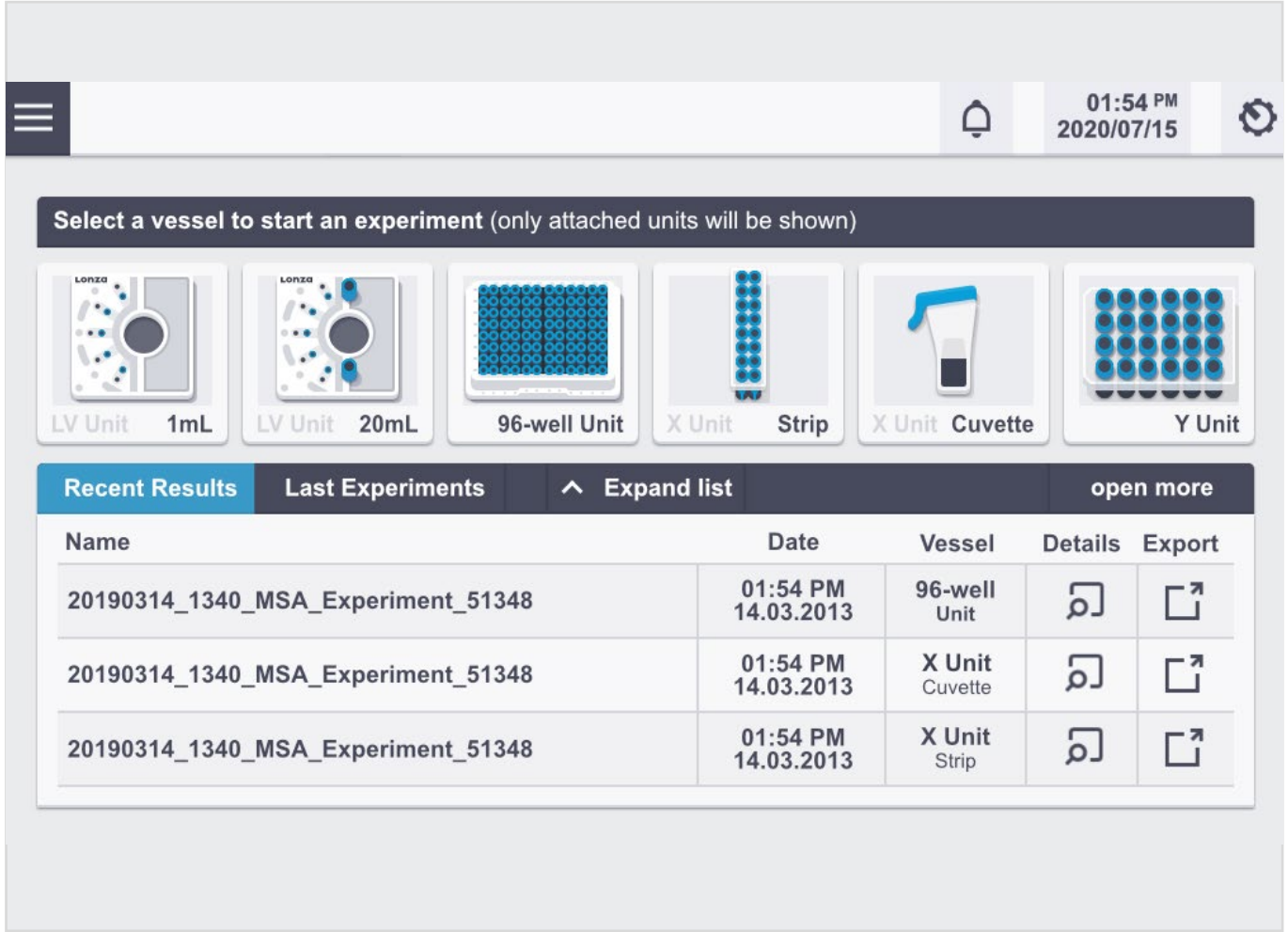
Lonza
Cell & Gene

Up to three functional units can be operated with one Core Unit

Integrated detection of functional modules

Choose vessel type to start

Or go from last results and experiments



Tips for a Nucleofection® Experiment

Step 3: Nucleofection® Process

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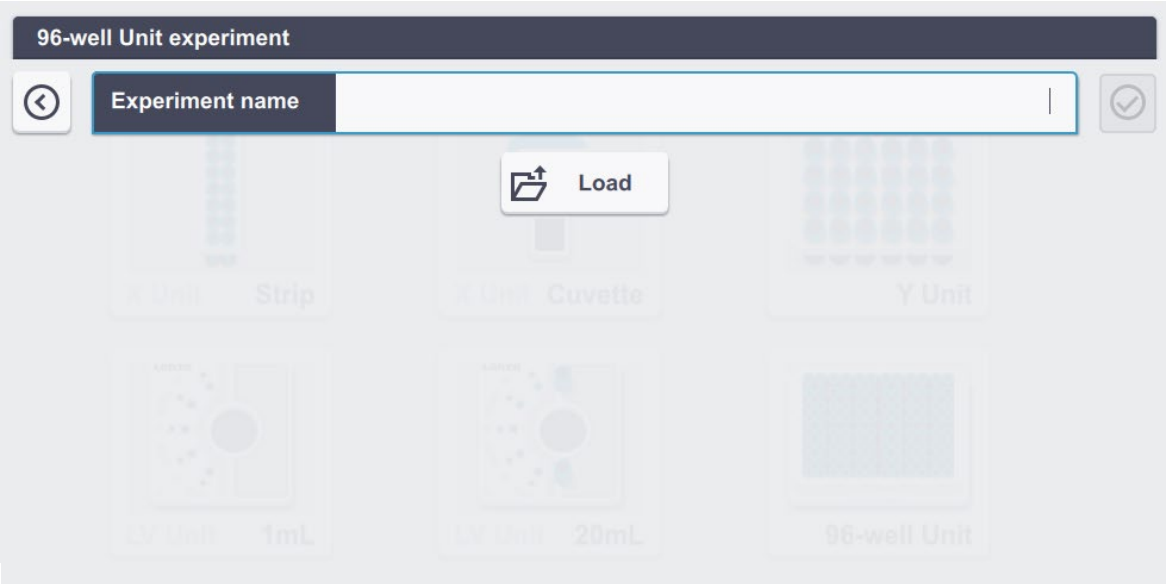
Cell & Gene

Setup of Nucleofector® Program on 4D-Nucleofector® Core Unit or PC Editor

Select Nucleofector® Vessel (e.g. Strip or Cuvette)



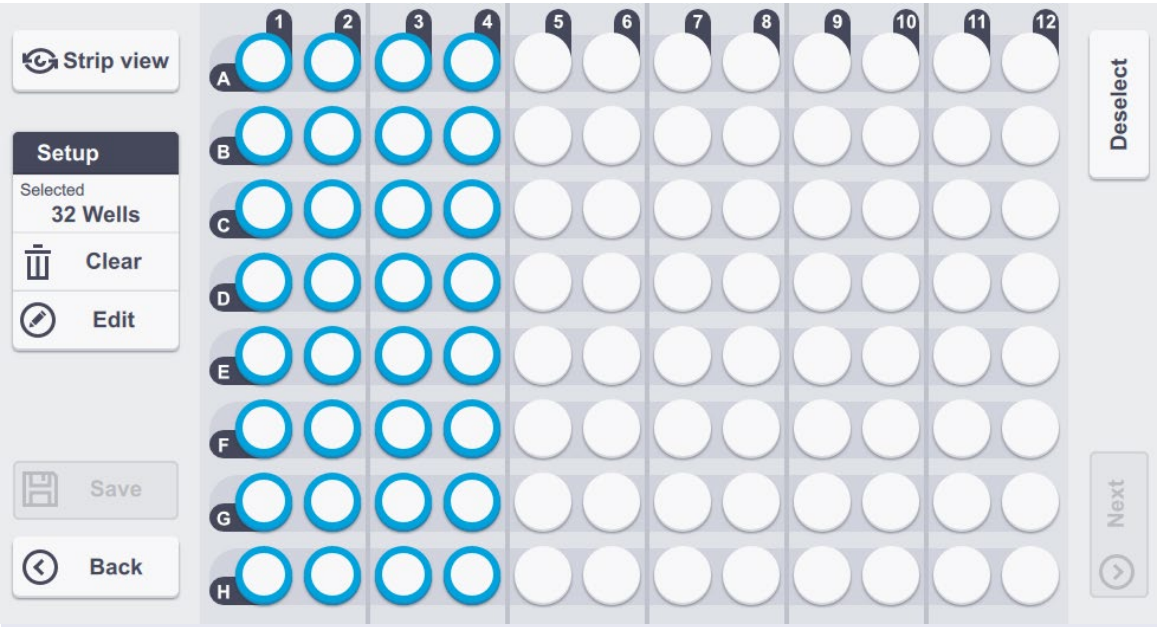
Load Nucleofector® Experiment or enter Experiment name

Tips for a Nucleofection® Experiment

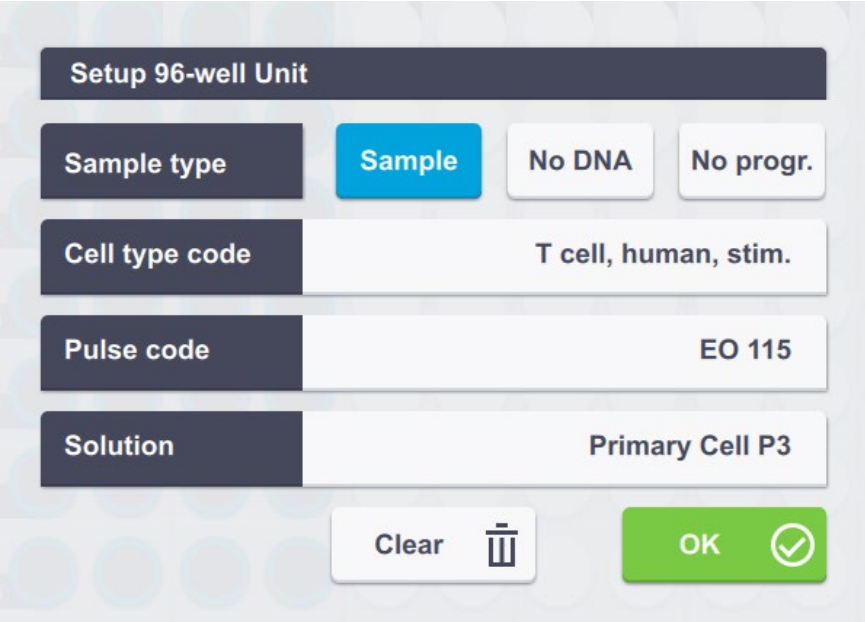
Step 3: Nucleofection® Process

Lonza
Cell & Gene

Setup of Nucleofector® Program on 4D-Nucleofector® Core Unit or PC Editor



Select wells to be used



Edit wells by selecting sample type (sample or control option) and adding cell type code (or pulse code and solution)

Tips for a Nucleofection® Experiment

Step 4: Cell handling post Nucleofection®

Lonza
Cell & Gene

1. Add warm and equilibrated medium to Nucleocuvette® Vessel before removal of transfected cells (volume recommendation in table below)

Format	Single Nucleocuvette® Vessel	16-well Nucleocuvette® Strip	96-well Nucleocuvette® Plate	384-well Nucleocuvette® Plate
Transfection volume	100 µL	20 µL	20 µL	20 µL
Culture medium to be added to the sample post Nucleofection®	500 µL	80 µL	80 µL	40 µL (60 µL max. well volume)

2. Use pipette provided to remove transfected cells (for 100 µL Single Nucleocuvette® Vessel)
3. Avoid foam formation/air bubbles
4. Incubation of sample after addition of media for 10 min at RT may increase viability (recovery step)
5. Always handle cells with care

Tips for a Nucleofection® Experiment

Step 5: Transfer of cells to culture dish

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Cell & Gene

Plate transfected cells in pre-warmed media

Plate transfected cells at appropriate/ recommended density

Use fresh growth medium with correct supplementation for respective cell type

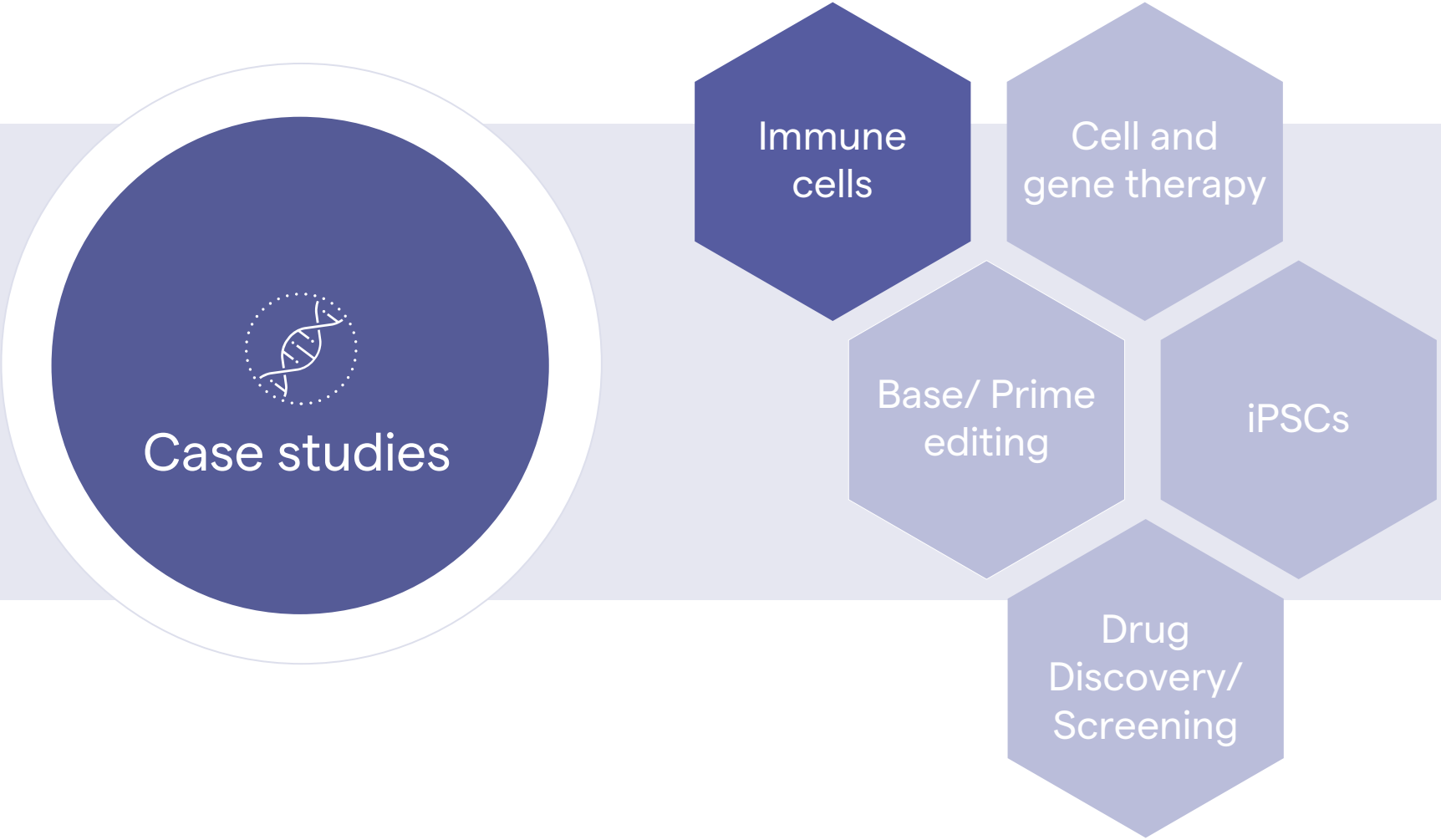
Wait at least 24 hours before adding selective agent to culture medium

Expression can be detected as soon as 3–8 hours post Nucleofection® Experiment

Genome Editing

Case studies

Lonza
Cell & Gene

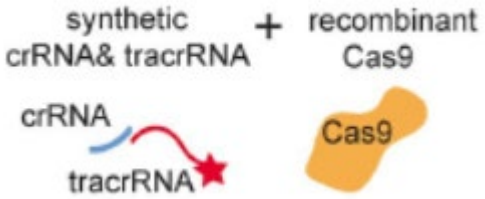


Case Study: Immune Cells

Tips and tricks for RNP Nucleofection®

Lonza
Cell & Gene

Case Study:
Seki and Rutz, J Exp Med, 2018



Duplex of
- Fluorescently labelled Alt-tracrRNA-Atto550 (IDT)
- Alt-crRNA (IDT)

Complexed with Cas9 protein (IDT)

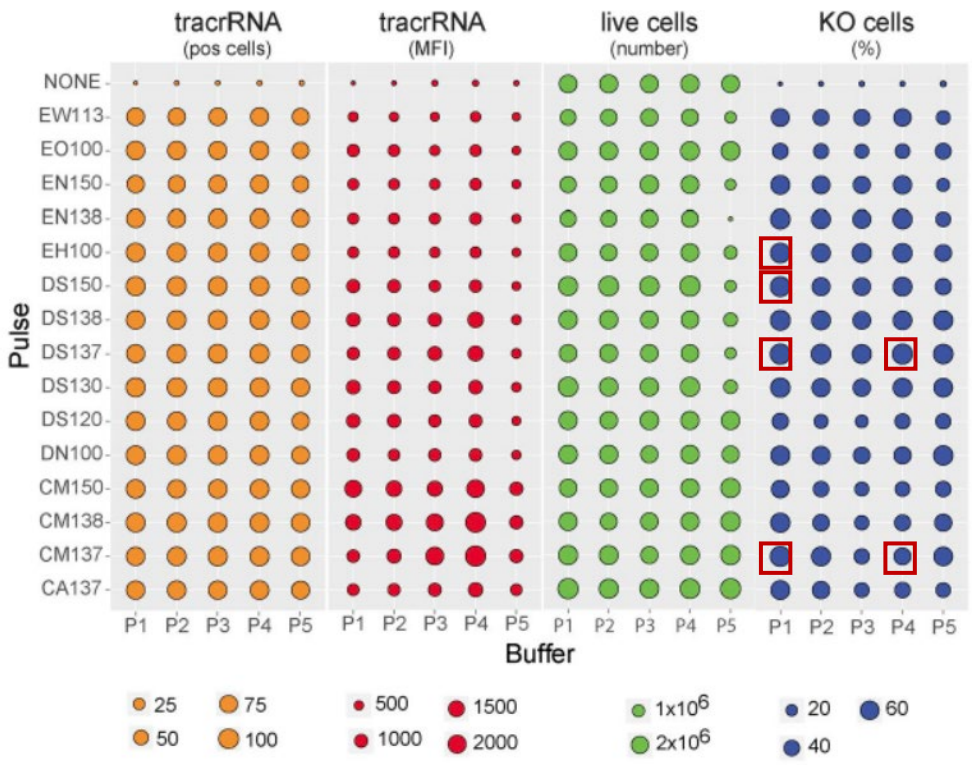
- Nucleofection® of RNPs into resting human CD4+ T cells and activated mouse CD8+ T cells
- Effects of different Nucleofection® Conditions
- Effects of different sgRNAs
- Cas9:sgRNA ratio
- Effects of different Cas9 proteins
- Transfection of multiple gRNAs

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Screening of RNP Transfection Conditions

In activated mouse CD8+ T cells

Lonza
Cell & Gene



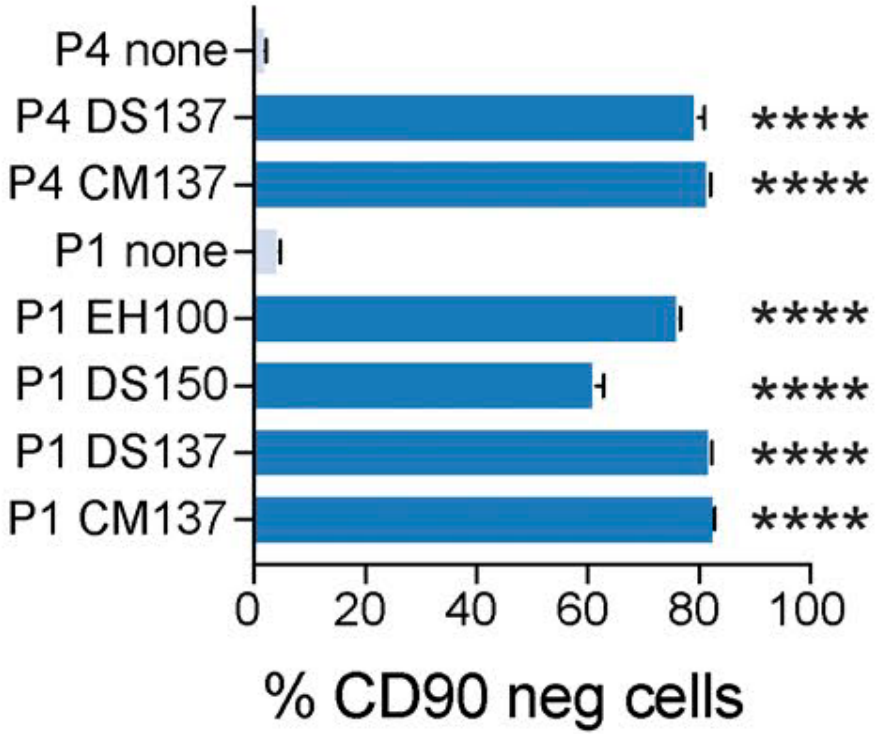
- Screening for mean fluorescence intensity (MFI) and knock-out of CD90 (KO)
- Transfection resulted in functional target gene KO by using specific sgRNA (CD90-negative cells by FACS)

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Screening of RNP Transfection Conditions

In activated mouse CD8+ T cells

Lonza
Cell & Gene



- Verification with selected Nucleofection® Conditions
- Transfection resulted in functional target gene KO by using specific sgRNA (CD90-negative cells by FACS)

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Different sgRNAs Show Different Effects

In activated mouse CD8+ T cells

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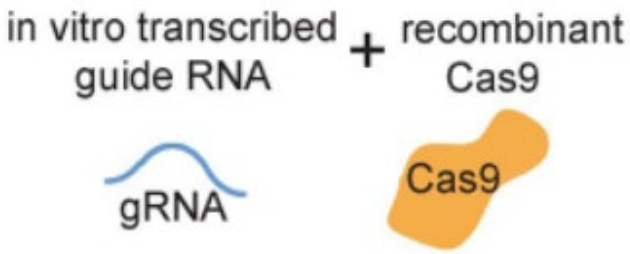

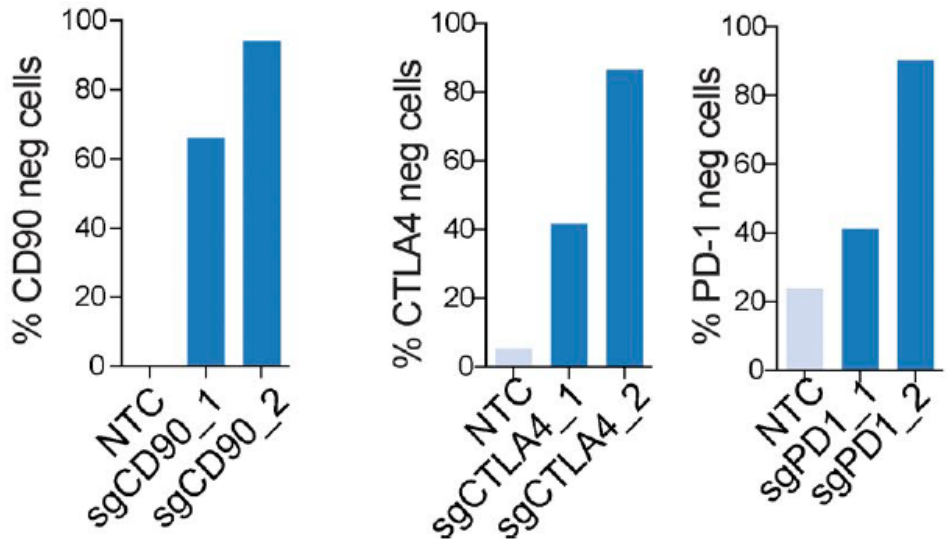

- gRNA targeting different regions can result in different KO efficiency

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Optimization of Cas9 Protein – gRNA Ratio

In activated mouse CD8+ T cells

Lonza
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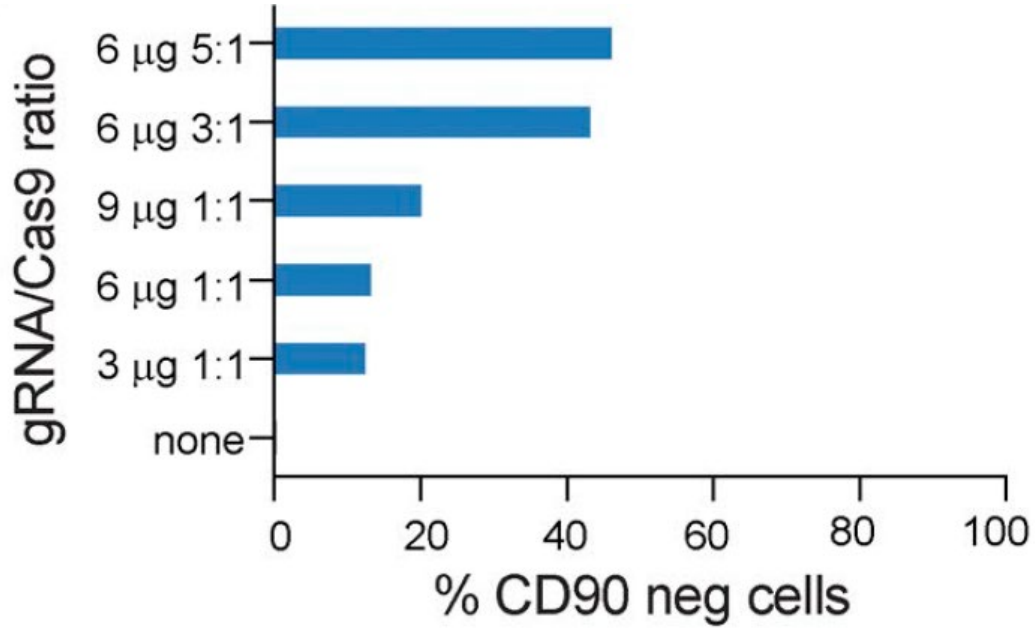

- Fine tuning of total complexing ratio and total RNP amount
- KO efficiency of CD90 in activated mouse CD8+ T cells is highly dependent on the amount and the ratio

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Optimizing Knock-Out Efficiency

Multiple gRNAs in non-activated human CD4+ T cells

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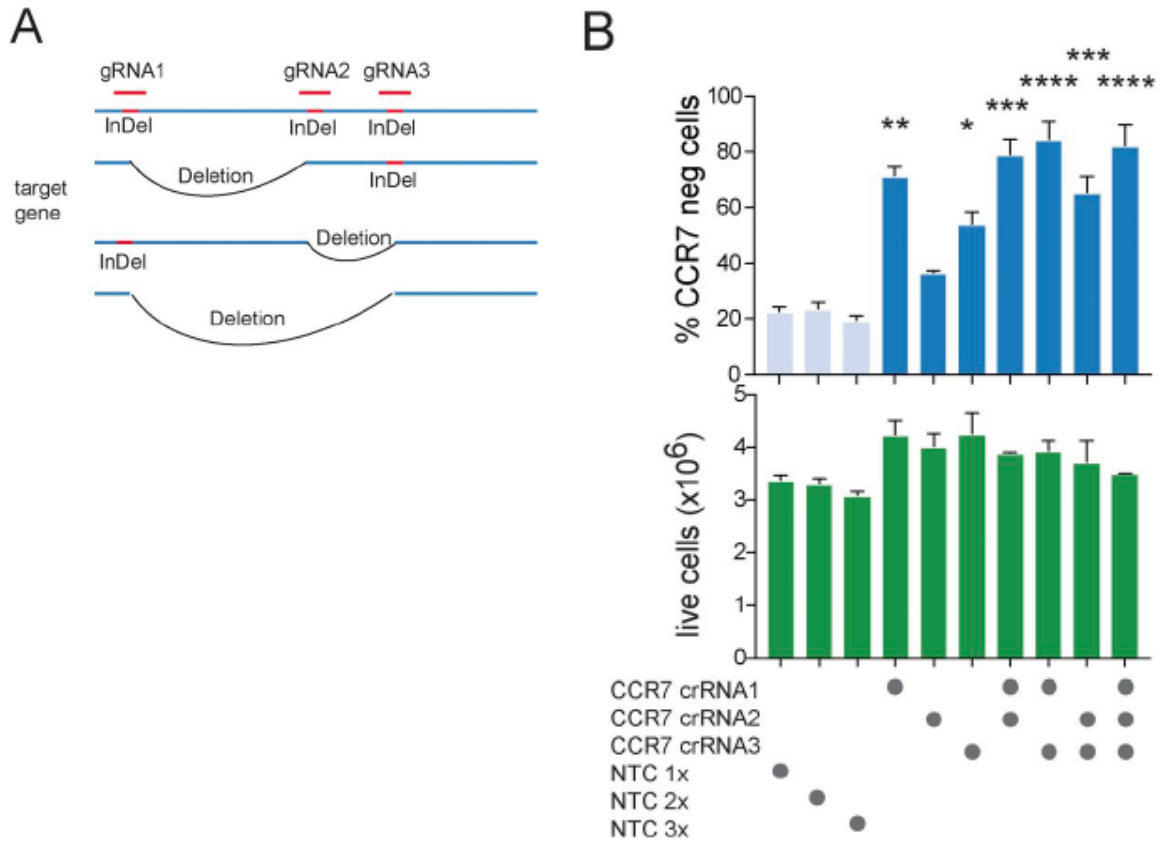
Cell & Gene



Multiple gRNAs targeting the same gene might increase the KO efficiency

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Optimizing Knock-Out Efficiency

Influence of different Cas9 proteins

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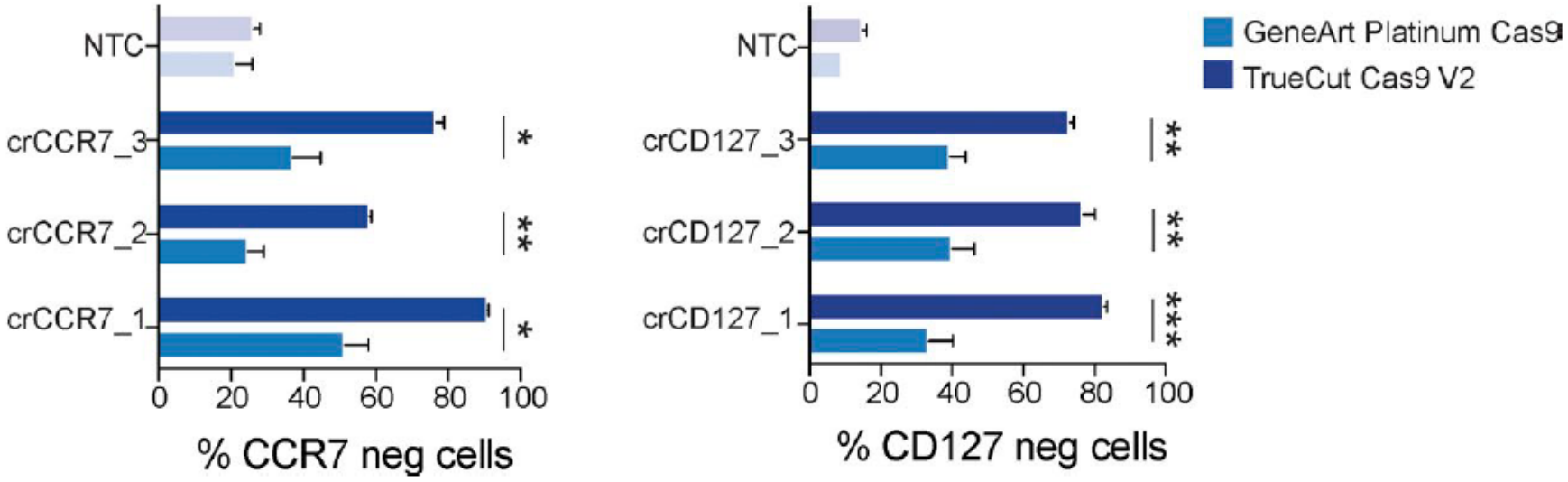


KO efficiency for CCR7 and CD127 in non-activated human CD4+ T cells varies depending on Cas9 protein used

Figure by Seki and Rutz (2018) Optimized RNP transfection for highly efficient CRISPR/Cas9-mediated gene knockout in primary T cells | Journal of Experimental Medicine, under Creative Commons License (Attribution 4.0 International)

Case Study: Immune Cells

Drug discovery screening in primary T cells

Lonza
Cell & Gene

- High-throughput platform for the efficient, multiplex editing of host factors that control HIV infection in primary CD4+ T cells.

CD4+ T-cells

Infection with HIV

+ Cas9 RNPs

Functional genetics of HIV host factors

- Rapid generation of isogenic human cells with ablated candidate factors and identifies gene modifications that provide viral resistance.

Hultquist et al., 2016, Cell Reports, 17, 1438-1452; October 25, 2016
Hultquist et al., 2019, Nature Protocols, January ;14(1):1-27.

Case Study: Immune cells

Nucleofection® of RNPs in Resting T cells

Lonza
Cell & Gene

OPEN

Rapid, efficient and activation-neutral gene editing of polyclonal primary human resting CD4+ T cells allows complex functional analyses

Manuel Albanese[1,2,9,10], Adrian Ruhle[1,2,10], Jennifer Mittermaier[1,2], Ernesto Mejías-Pérez[1,2], Madeleine Gapp[1,2], Andreas Linder[2,3], Niklas A. Schmacke[2], Katharina Hofmann[1,2], Alexandru A. Hennrich[1,2], David N. Levy[4], Andreas Humpe[5], Karl-Klaus Conzelmann[1,2,6], Veit Hornung[2], Oliver T. Fackler[7,8] and Oliver T. Keppler[1,2,6]

- Schematic overview of the pipeline to establish polyclonal KOs in human resting CD4+ T cells

- For further information and detailed protocol see: Case Study and Associated Protocol "Synthetic sgRNAs Enable Researchers to Study Viral Infection in Resting Human CD4+ T Cells" from Synthego and Lonza

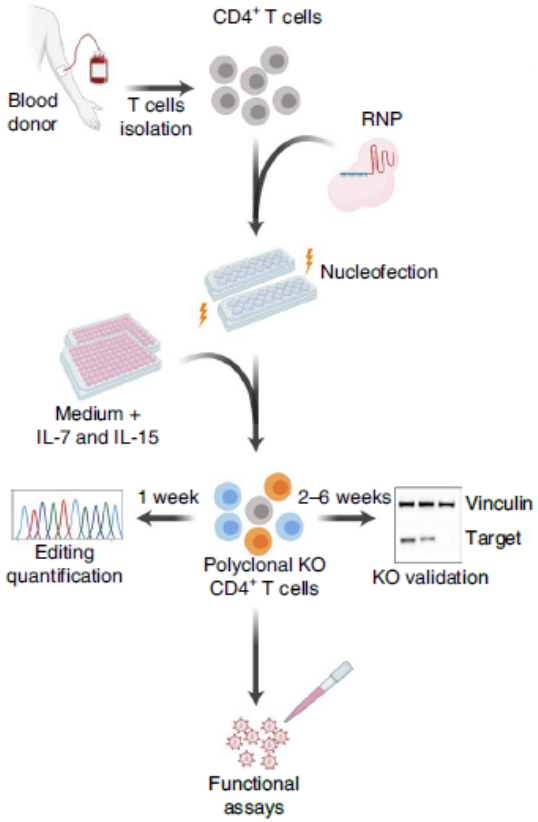
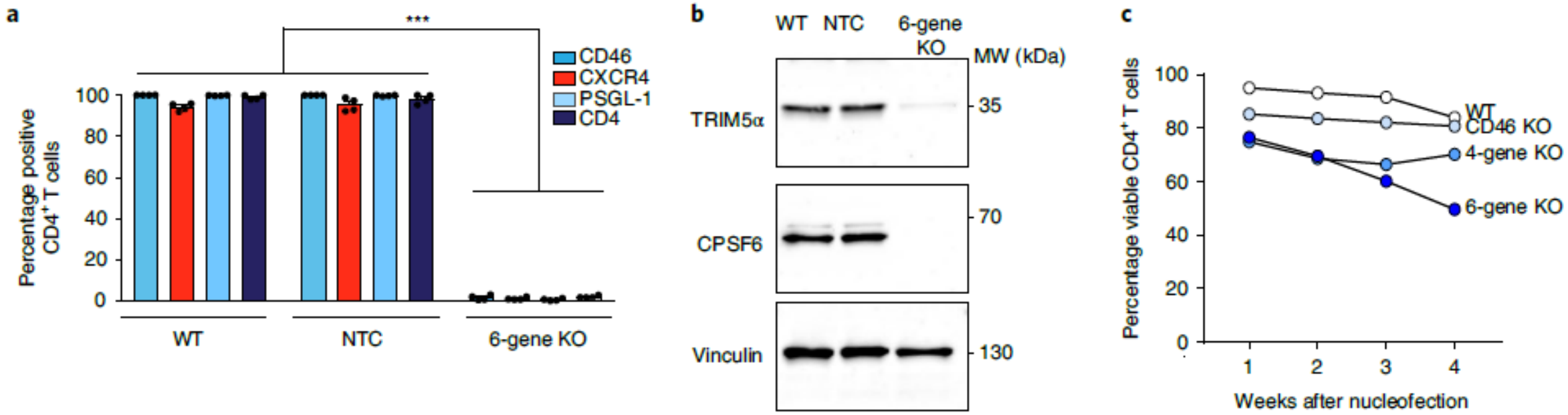


Figure from Albanese *et al.*, (2022) Rapid, efficient and activation-neutral gene editing of polyclonal primary human resting CD4+ T cells allows complex functional analyses | Nature Methods under Creative Commons License (Attribution 4.0 International)

Case Study: Nucleofection® of RNPs in Resting T cells

Six-gene KO following a single RNP Nucleofection® Process

Lonza
Cell & Gene



Simultaneous, polyclonal six-gene KO following a single RNP Nucleofection® Process. Expression of surface receptors was quantified by flow cytometry after 2 weeks (**a**) or immunoblot (**b**) 25d after Nucleofection®

Viability of cells with *CD46*-single KO, four-gene KO (*CD46, CD4, CXCR4* and *PSGL-1*) or six-gene KO (as in **a**) were analyzed. WT cells served as control.

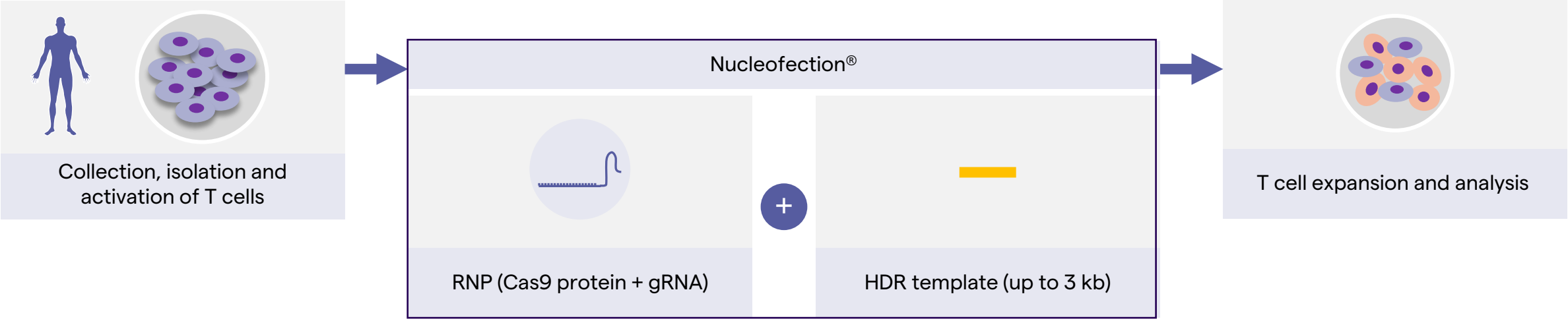
Figures from Albanese *et al.* (2022) Rapid, efficient and activation-neutral gene editing of polyclonal primary human resting CD4+ T cells allows complex functional analyses | Nature Methods under Creative Commons License (Attribution 4.0 International)

For further information and detailed protocol see: Case Study and Associated Protocol "Synthetic sgRNAs Enable Researchers to Study Viral Infection in Resting Human CD4+ T Cells" from Synthego and Lonza

Case Study: Immune Cells

Insertion of large DNA sequences in primary human T cells

Lonza
Cell & Gene



Development of a CRISPR-Cas9 genome-targeting system that does not require viral vectors, allowing **rapid and efficient insertion of large DNA sequences (> 1kb) at specific sites in the genomes of primary human T cells**, while preserving cell viability and function.

Roth, T.L. *et al.* (2018) Reprogramming human T cell function and specificity with non-viral genome targeting | Nature. *Nature* 559, 405–409

Development of a widely adaptable technology to barcode and track **targeted integrations of large non-viral DNA templates (2-3 kb) to perform pooled knock-in screens** in primary human T cells.

Roth, T.L. *et al.* (2020) Pooled Knockin Targeting for Genome Engineering of Cellular Immunotherapies: Cell Cell 181, 728-744

Theo Roth was a speaker at Lonza Virtual Event, Genome Editing of Immune Cells, Sept 2022

Case Study: Immune cells

Efficient gene knock-in in primary human and murine myeloid cells

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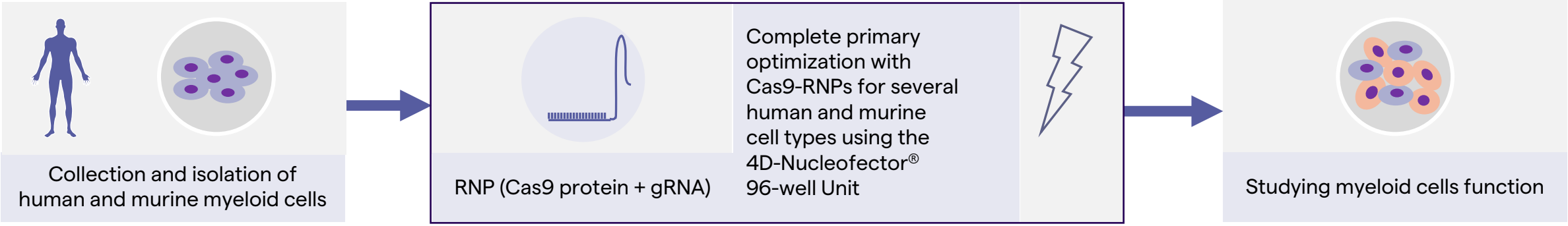
Cell & Gene



- Near population-level genetic KO of single and multiple targets
- Cellular fitness and response to immuno-logical stimuli is not significantly impacted by gene editing
- Enables pathway discovery and drug target validation concerning innate immunity

Freund *et al.* (2020) Efficient gene knockout in primary human and murine myeloid cells by non-viral delivery of CRISPR-Cas9 | Journal of Experimental Medicine. J Exp Med, 217 (7): e20191692.

Genome Editing

Case studies

Lonza
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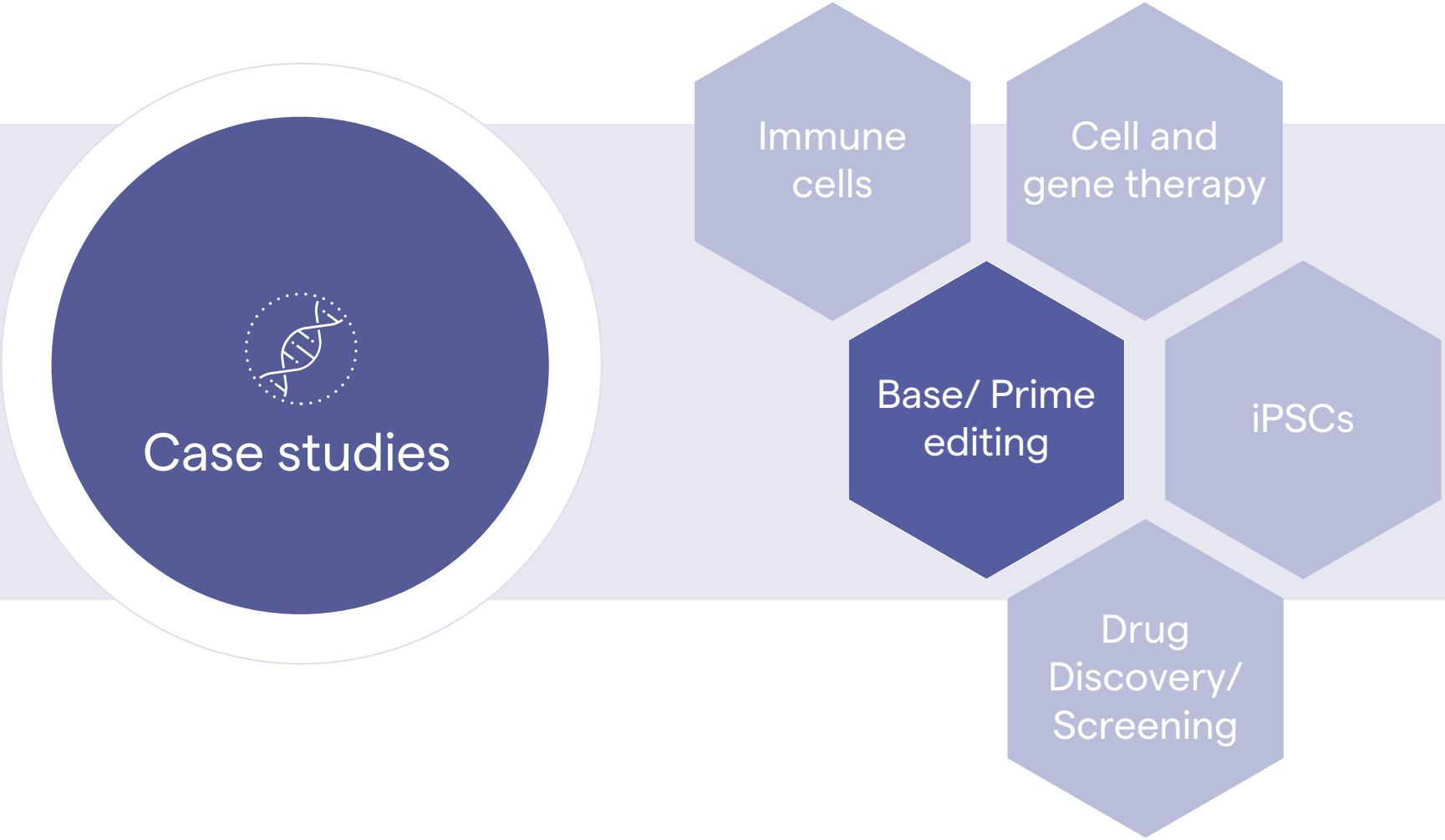


Nucleofector® Technology – Enabling Genome Editing

Base editing

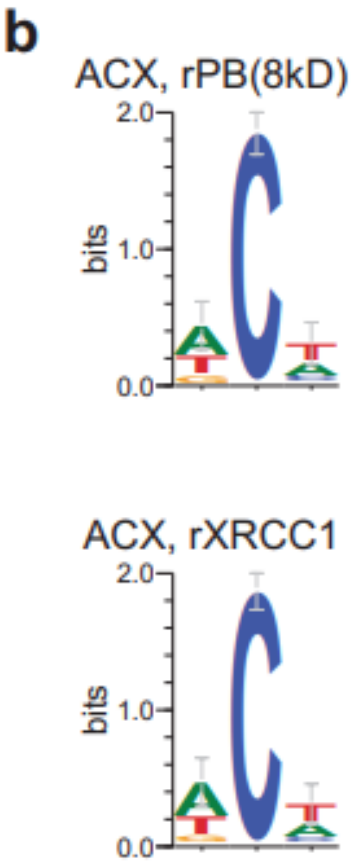
Lonza
Cell & Gene

https://doi.org/10.1038/s41467-021-21559-9 OPEN

Programmable C:G to G:C genome editing with CRISPR-Cas9-directed base excision repair proteins

Liwei Chen[1], Jung Eun Park[1], Peter Paa[1], Priscilla D. Rajakumar[1], Hong-Ting Prekop[1], Yi Ting Chew[1], Swathi N. Manivannan[1] & Wei Leong Chew[1]✉

- Development of „a class of C:G to G:C Base Editors to create single-base genomic transversions in human cells“
- „(..) Nickase-Cas9 fused to a cytidine deaminase and base excision repair proteins“



- DNA WebLogo created with target motifs in which C:G at position 6 was edited to G:C (n=2)
- Genes linked to dyslipidemia, hypertrophic cardiomyopathy, and deafness were targeted, showing the therapeutic potential of these base editors in interrogating and correcting human genetic diseases.

Citation and figure adapted from Chen *et al.* (2021) Programmable C:G to G:C genome editing with CRISPR-Cas9-directed base excision repair proteins | Nature Communications 12, 1384 under Creative Commons License (Attribution 4.0 International)

Case Study: Base editing

Potential base editing strategies for future clinical applications

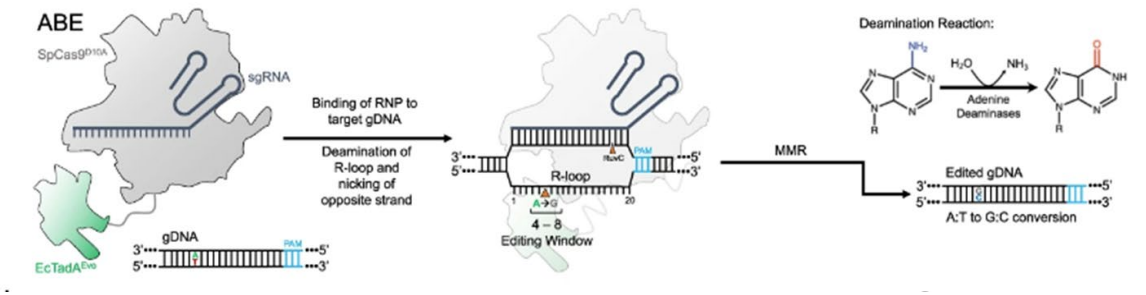
Lonza
Cell & Gene

Article
https://doi.org/10.1038/s41467-022-34479-z

Adenine base editing efficiently restores the function of Fanconi anemia hematopoietic stem and progenitor cells

Received: 8 April 2022
Accepted: 26 October 2022
Published online: 12 November 2022

Sebastian M. Siegner[1,3], Laura Ugalde[2,3], Alexandra Clemens[1,3], Laura Garcia-Garcia[2], Juan A. Bueren[2], Paula Rio[2], Mehmet E. Karasu[1] & Jacob E. Corn[1]



C•G → T•A

A•T → G•C

C•G → G•C

- Relies on introducing single base changes without DNA cleavage
- The deaminated base (U or I) gets changed to T or G and leads to the final mutation as C to T (CBE) or A to G (ABE)
- Effective ABE editing in HPSCs from FA patients leads to restored FA pathway funtion
- Indicates potential of base editing strategies for future clinical applications

Figure adapted from Siegner *et al.* (2022) Adenine base editing efficiently restores the function of Fanconi anemia hematopoietic stem and progenitor cells | Nature Communications. Nat Commun 13, 6900 and Kluesner *et al.* (2021) CRISPR-Cas9 cytidine and adenosine base editing of splice-sites mediates highly-efficient disruption of proteins in primary and immortalized cells | Nature Communications Nat Commun 12, 2437 under Creative Commons License (Attribution 4.0 International)

Case Study: Base editing

Gene editing strategy for IPEX patients

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IPEX syndrome

Severe pediatric disease with limited treatment options; „caused by mutations in the FOXP3 gene, which plays critical role in immune regulation“

IPEX syndrome is an ideal candidate for a therapeutic approach

CRISPR-based gene correction in HSPCs or T cells permitting regulated expression of FOXP3 protein

Persistent FOXP3 protein expression and reserved Tregs phenotype and function in FOXP3 edited Tregs

Gene editing strategy is suitable for IPEX patients with diverse mutations

“These results demonstrate the feasibility of gene correction, which will be instrumental for the development of therapeutic approaches for other genetic autoimmune diseases”

IPEX = immune dysregulation polyendocrinopathy enteropathy X-linked; FOXP3 = fork-head box protein 3
Goodwin *et al.*, (2020) CRISPR-based gene editing enables FOXP3 gene repair in IPEX patient cells | Science Advances Vol 6, Issue 19, 6 May 2020

Genome Editing

Case studies

Lonza
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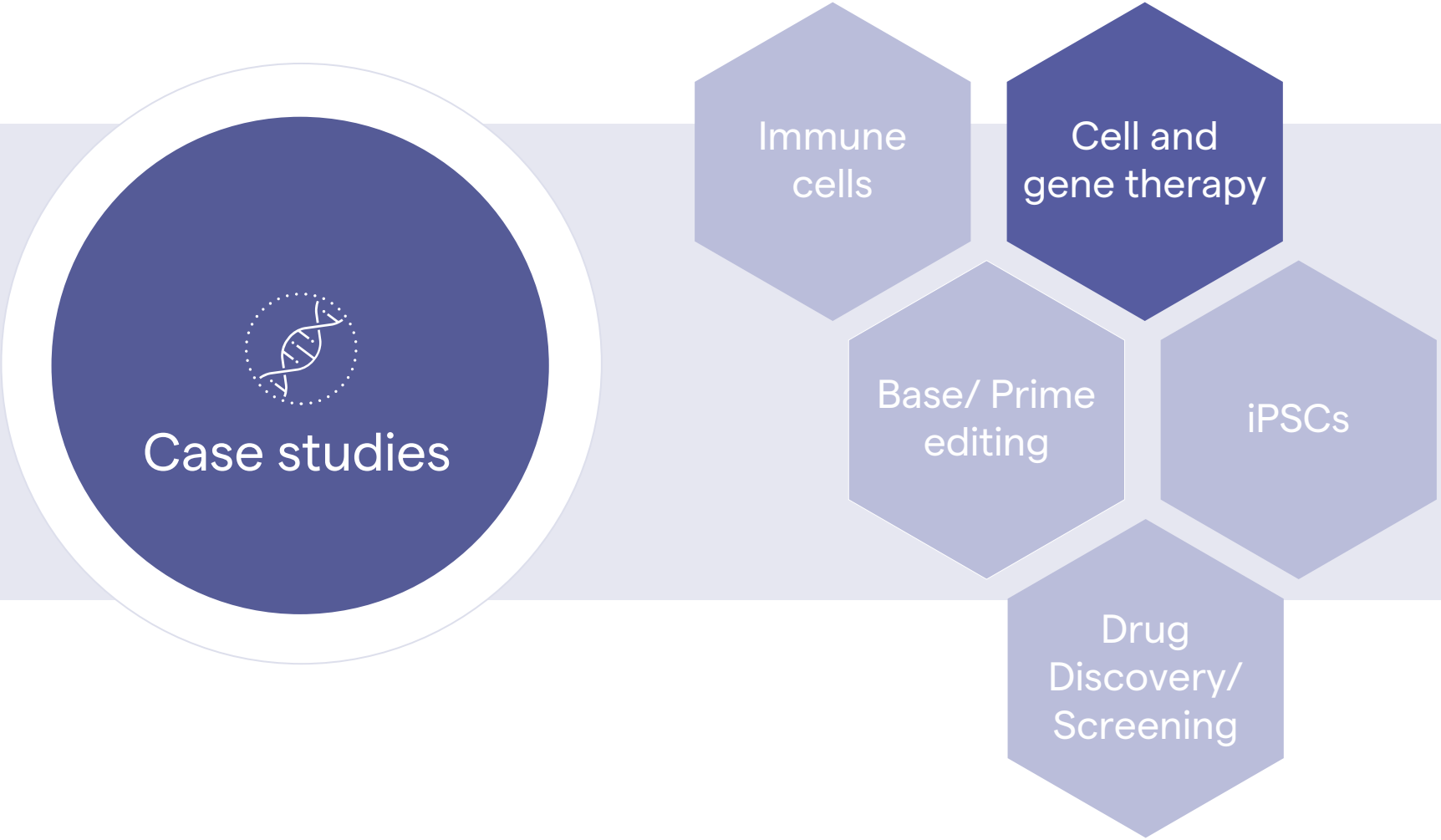

Gene Modification

Different methods relevant for cell and gene therapies

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Non-viral Gene Modification

Transposon based

(Sleeping Beauty, PiggyBAC®)

Transposon based genomic integration

Targeted gene editing

(ZFN, TALEN, CRISPR)

Targeted gene editing technologies (CRISPR/ Cas9, ZFN and TALEN)

All three can be delivered in different modalities (pDNA, mRNA, Protein or RNPs)

CRISPR/Cas9-mediated HBB Gene Editing in HSPCs

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β-haemoglobinopathies

(sickle cell disease, β-thalassaemia)
Caused by mutations in the HBB gene

CRISPR/Cas9-mediated HBB gene editing in HSPCs

Cas9 gene-editing system combining Cas9 RNPs & adeno-associated viral vector delivery of a homologous donor to achieve homologous recombination at the HBB gene

Electroporation of 80 million CD34+ HSPCs using the **4D-Nucleofector® LV-Unit**

Purification of a population of HSPCs with > 90% targeted integration

Advancing the development of next-generation therapies for β-haemoglobinopathies

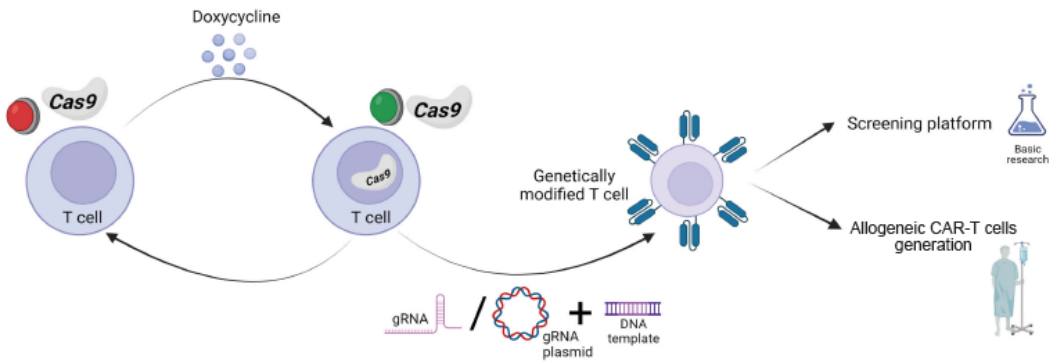
Dever *et al.*, (2016) CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells | Nature. Nature 539, 384-389
For further reading: Lattanzi *et al.*, (2021) Development of β-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease | Science Translational Medicine Sci Transl Med. Jun 16; 13(598);

Inducible Cas9 T cells: An Innovative Platform for Allogeneic CAR-T cell Generation

Marian Nicola[1], Ariel Gilert[1], Christian Fischer[2], Alessandro Di Cara[2], Frida Grynspan[1]
[1]Lonza Biologics R&D, CIC Haifa, IL; [2]Lonza Biologics R&D, Basel, CH

Background

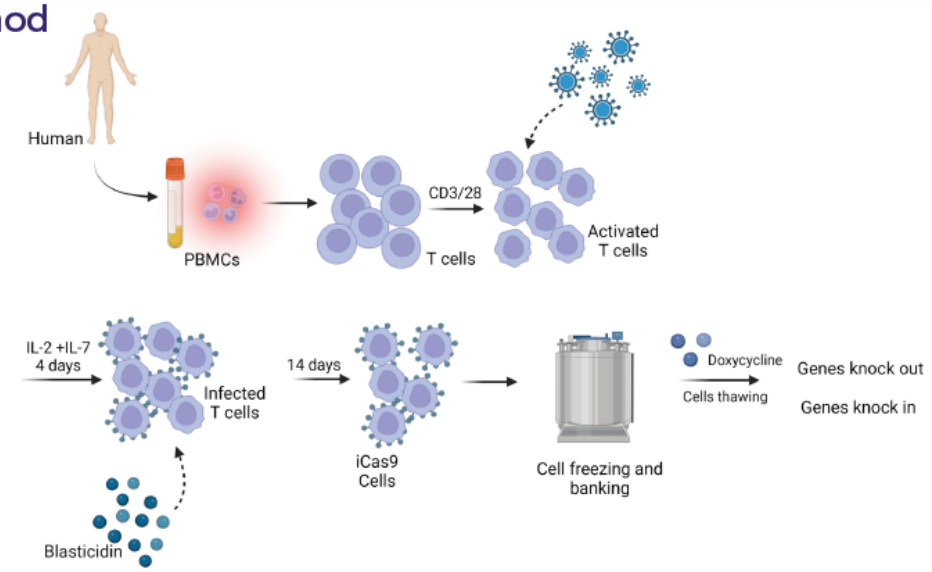
- CAR-T cells show great promise in treating many types of cancers
- Genetically engineered allogeneic T cells have the potential to provide universal CAR-T cells to treat large number of patients
- CRISPR/Cas9 is used for generating allogeneic T cells



Why is it Necessary?

The use of autologous CAR-T cells requires individual manufacturing for each patient which is challenging in terms of quality, mass production and logistics. Allogeneic CAR-T cells have the potential to address these challenges

Method



Inducible Cas9 T Cells

Combining viral and non-viral approaches

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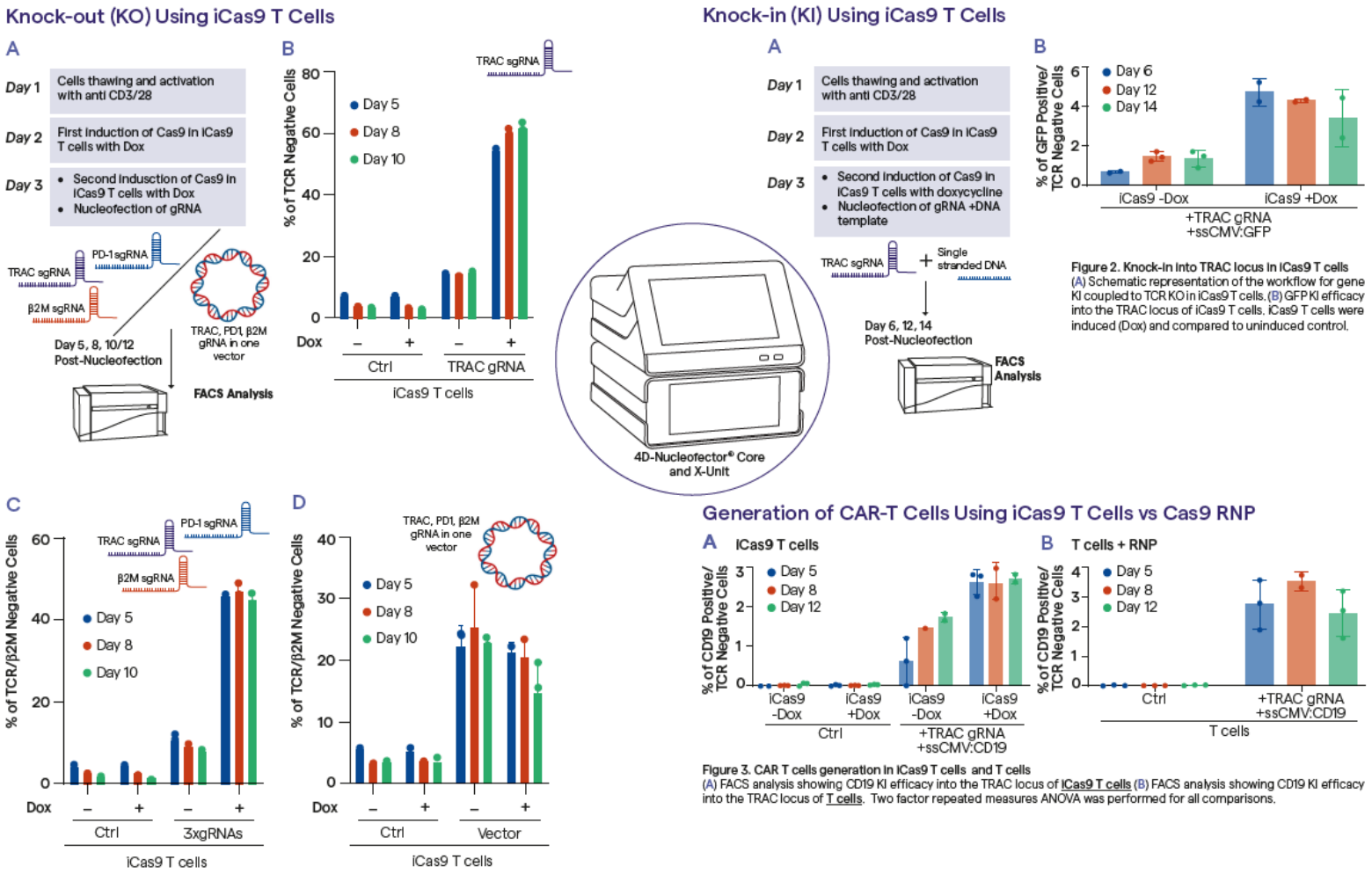


Safety evaluation of CRISPR/Cas9 Genome Editing

A study in HSPCs

Lonza
Cell & Gene

Article
https://doi.org/10.1038/s41467-022-32233-z

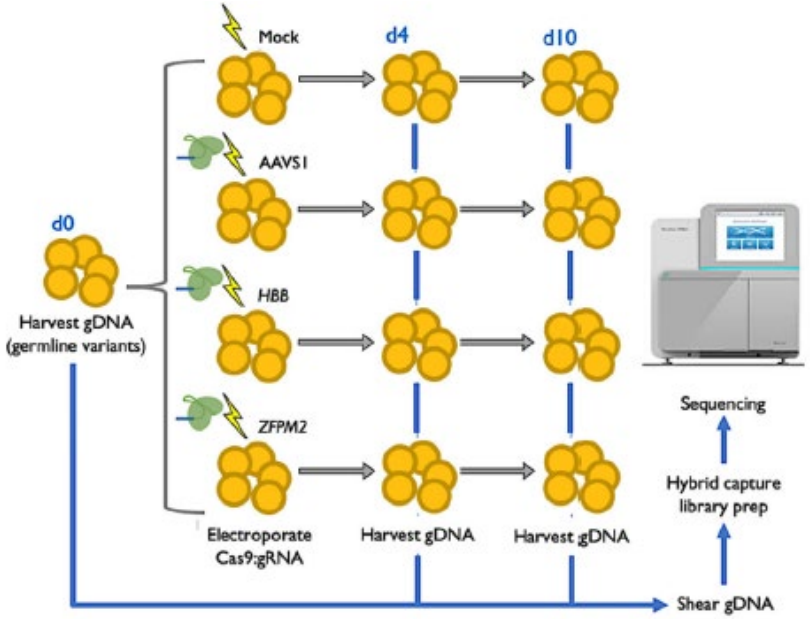
Ultra-deep sequencing validates safety of CRISPR/Cas9 genome editing in human hematopoietic stem and progenitor cells

Received: 17 December 2021
Accepted: 22 July 2022
Published online: 11 August 2022

M. Kyle Cromer[1,2,4], Valentin V. Barsan[2,4], Erich Jaeger[3], Mengchi Wang[3], Jessica P. Hampton[2], Feng Chen[3], Drew Kennedy[3], Jenny Xiao[3], Irina Khrebtukova[3], Ana Granat[3], Tiffany Truong[3] & Matthew H. Porteus[2]

„As CRISPR-based therapies enter the clinic, evaluation of safety remains a critical and active area of study."

Nucleofection® of RNPs in primary human hematopoietic stem and progenitor cells (HSPCs) using the 4D-Nucleofector® X Unit



Employing a „clinical next generation sequencing (NGS) workflow to achieve high sequencing depth and detect ultra-low frequency variants across exons of genes associated with cancer, all exons, and genome wide"

Citation and figure adapted from Cromer *et al.*, 2022, Ultra-deep sequencing validates safety of CRISPR/Cas9 genome editing in human hematopoietic stem and progenitor cells | Nature Communications under Creative Commons License (Attribution 4.0 International)

4D-Nucleofector® LV Unit

Commercial license

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Who needs the license?	• A license is required by the account who owns and uses the equipment(s), not by the account who finally uses the generated product (if that differs)
When does payment start?	• Payment starts upon commercial application of the equipment
Coverage	• Any equipment used by the account is covered • Any product (used for research or clinical applications*) or service is covered
Fees	• Fees are to be negotiated • Payments can be one-time or annual

*Clinical use requires the approval from the appropriate regulatory authorities

Publications and Trials

With 4D-Nucleofector® LV Unit

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Cell & Gene

Publications

- Nawandar et al. (2022) Human anelloviruses produced by recombinant expression of synthetic genomes. bioRxiv preprint
- Ottoviano et al. (2022) Phase 1 clinical trial of CRISPR-engineered CAR19 universal T cells for treatment of children with refractory B cell leukemia. Sci. Transl. Med. 14, eabq3010
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- Georgiadis et al (2021) Base-edited CAR T Cells for combinational therapy against T cell malignancies. Leukemia 35(12):3466-3481
- Lattanzi et al. (2021) Development of β-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. Science Translational Vol. 13, Issue 59
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- Brownrigg et al. (2020) Closed-system transposon-mediated manufacture of GMP grade CAR T-cells via the Lonza Nucleofector LV XL. Cytotherapy 22: S382
- Mendt et al. (2018) Generation and testing of clinical-grade exosomes for pancreatic cancer. JCI Insight 3(8). *JCI Insight*. 2018;3(8):e99263
- Schiroli et al. (2017) Preclinical modeling highlights the therapeutic potential of hematopoietic stem cell gene editing for correction of SCID-X1. Sci Transl Med 9(411):eaan0820
- Dever et al. (2016) CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature.539(7629):384-389

Clinical trials

- 2 ongoing
- Further in preparation/IND filing

4D-Nucleofector® LV Unit

1 mL Nucleocuvette® Cartridge

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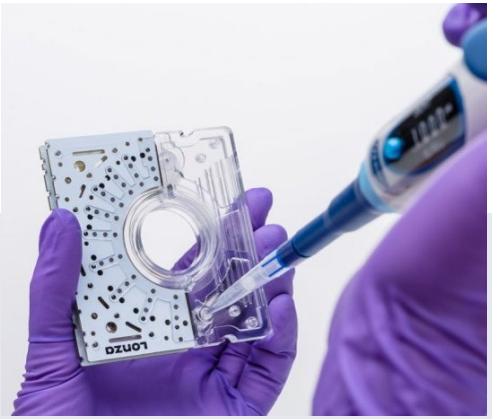
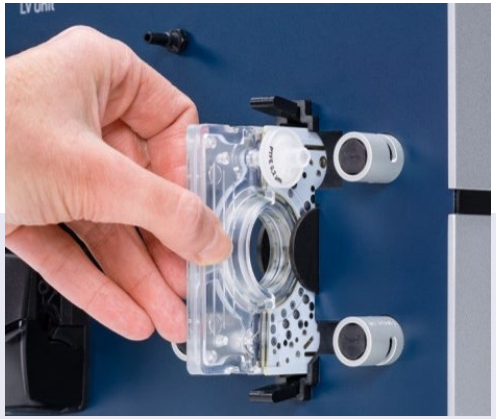
Cell & Gene

- 1 mL filling volume
- Up to 1 x 10^8 cells
- Manual filling via sterile injection port
- Provided together with cell-specific Nucleofector® Solution
- Video tutorial: Large volume transfection for cell and gene therapy | Lonza



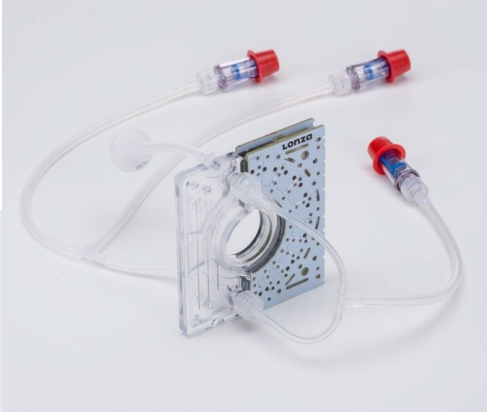


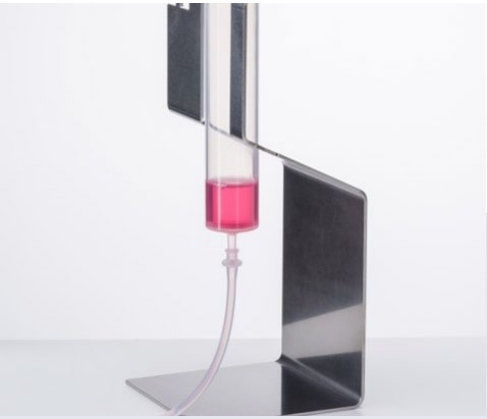
4D-Nucleofector® LV Unit

1 mL Nucleocuvette® Cartridge

Lonza
Cell & Gene

- Up to 20 x 1 mL filling volume
- Up to 1 x 10^9 cells
- Automatic filling via reservoirs (or bags)
- Provided together with cell-specific Nucleofector® Solution
- Video tutorial: Large volume transfection for cell and gene therapy | Lonza





Genome Editing

Case studies

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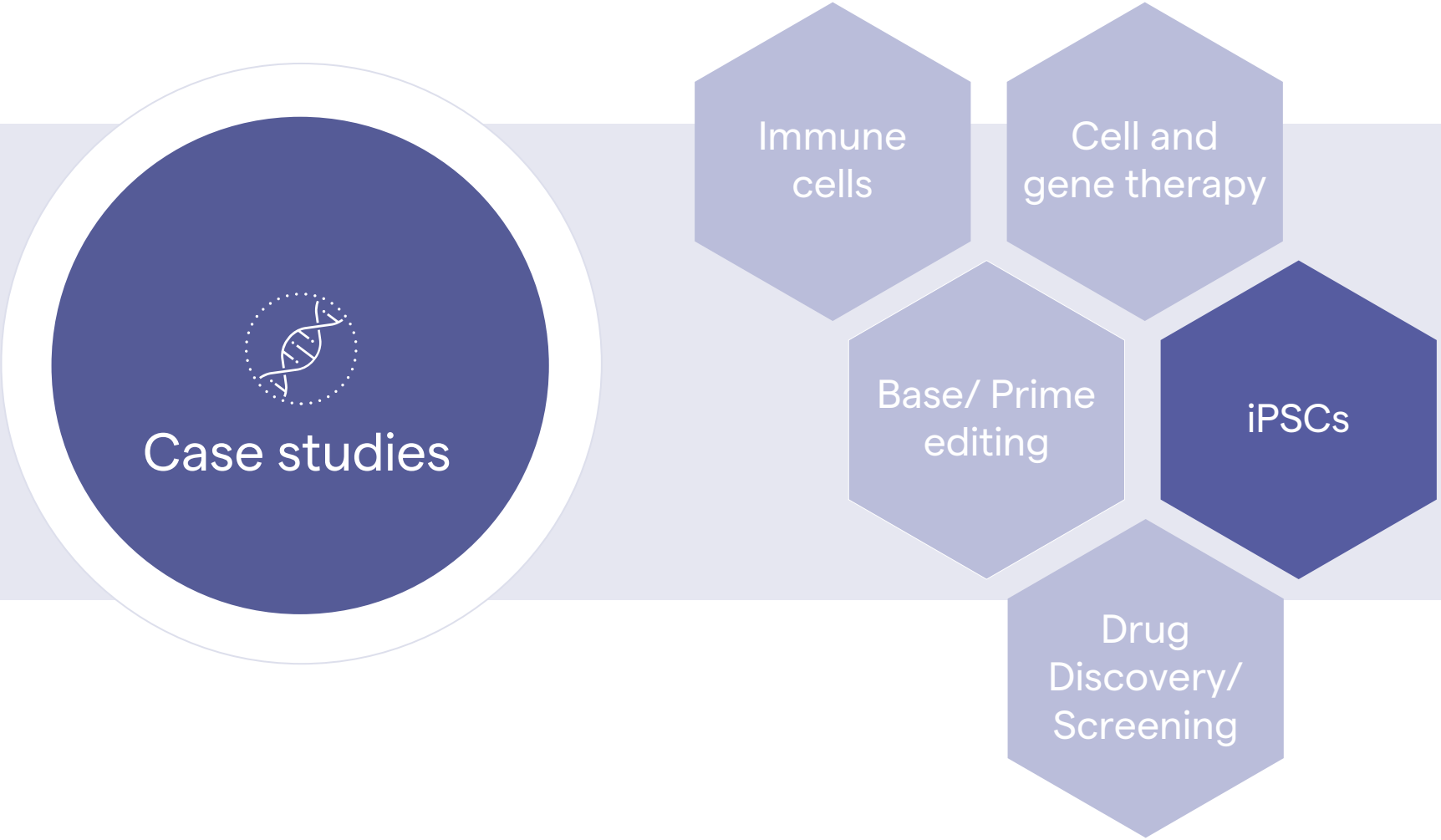

Transfection of Human Induced Pluripotent Stem Cells

Technical Reference Guide and Citations

Lonza
Cell & Gene

BioResearch

Transfection of Human Induced Pluripotent Stem Cells using Nucleofector™ Technology

Technical Reference Guide

Table 1. Optimization scheme for 4D-Nucleofector™ System

P3 Solution (Strip 1, n=2)		P4 Solution (Strip 2, n=2)	
CA-137	CA-137	CA-137	CA-137
CB-150	CB-150	CB-150	CB-150
CD-118	CD-118	CD-118	CD-118
CE-118	CE-118	CE-118	CE-118
CM-113	CM-113	CM-113	CM-113
DC-100	DC-100	DC-100	DC-100
DN-100	DN-100	DN-100	DN-100
No program	No program	No program	No program

- Yang *et al.*, (2013) Optimization of scarless human stem cell genome editing | Nucleic Acids Research | Oxford Academic (oup.com). Nucleic Acids Res 41:9049-61
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- Chavez *et al.*, (2015) Highly efficient Cas9-mediated transcriptional programming | Nature Methods. Nat Meth 12(4): 326–328
- Grobarczyk *et al.*, (2015) Generation of Isogenic Human iPS Cell Line Precisely Corrected by Genome Editing Using the CRISPR/Cas9 System | SpringerLink. Stem Cell Rev and Rep 11:774–787
- Tanudjojo *et al.*, (2021) Phenotypic manifestation of α-synuclein strains derived from Parkinson's disease and multiple system atrophy in human dopaminergic neurons | Nature Communications Nat Comm 12, 3817

Nucleofection® of iPSC-derived Dopaminergic Neurons

Tanudjojo et al. Nat.Comm. 2021

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- Induced α-synuclein aggregation in human iPSC-derived dopaminergic neurons using fibrils from Parkinson's disease
- Identified 56 proteins differentially interacting with aggregates triggered by brain-amplified fibrils, e.g. DJ-1 protein
- Knock-out of DJ-1 in iPSC-derived dopaminergic neurons enhance fibril-induced aggregation and neuronal death
- DJ-1, the SNCATRIP iPSC clone was transfected with two sgRNAs and CRISPR Hifi Cas9 protein (IDT), 1.5 Mio. Cells, P3 Solution, Nucleofector® Program CA-167

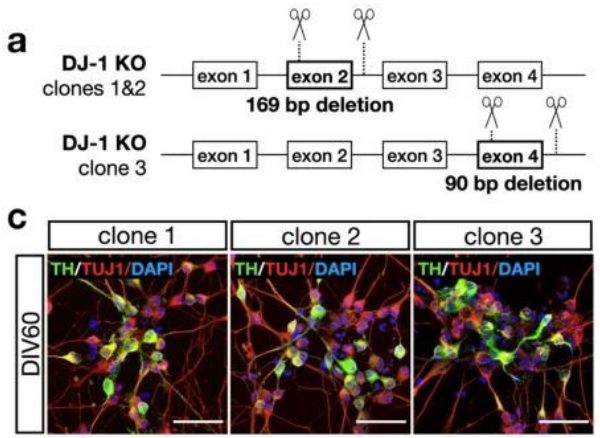
Article | Open Access | Published: 21 June 2021

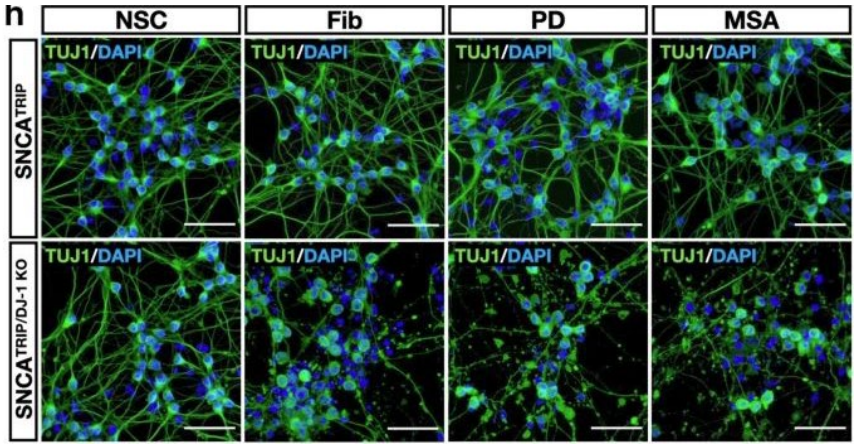
Phenotypic manifestation of α-synuclein strains derived from Parkinson's disease and multiple system atrophy in human dopaminergic neurons

Benedict Tanudjojo, Samiha S. Shaikh, Alexis Fenyi, Luc Bousset, Devika Agarwal, Jade Marsh, Christos Zois, Sabrina Heman-Ackah, Roman Fischer, David Sims, Ronald Melki & George K. Tofaris

Nature Communications 12, Article number: 3817 (2021) | Cite this article

9155 Accesses | 24 Citations | 31 Altmetric | Metrics





Citation and figure adapted from Tanudjojo *et al.*, (2021) Phenotypic manifestation of α-synuclein strains derived from Parkinson's disease and multiple system atrophy in human dopaminergic neurons | Nature Communications under Creative Commons License (Attribution 4.0 International)

Genome Editing

Case studies

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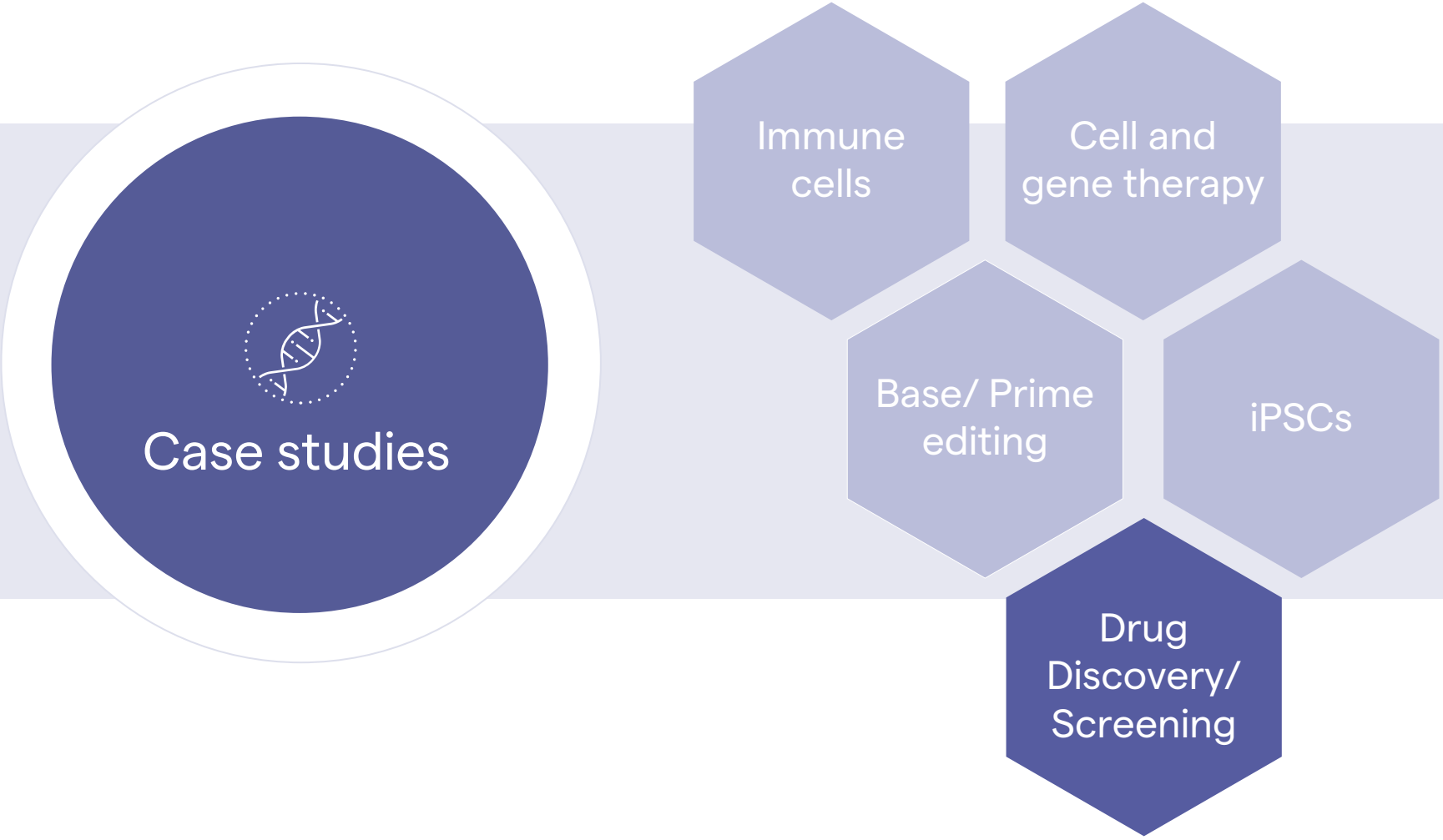

Case Study: Arrayed CRISPR screening in primary SAECs

Workflow: 384-well Nucleofection® Process

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Cell & Gene

"Small airway epithelial cells (SAECs) play a central role in the pathogenesis of lung diseases and are now becoming a **crucial cellular model for target identification and validation in drug discovery.** However, primary cell lines such as SAECs are often difficult to transfect using traditional lipofection methods; (...)"

"Here we have established a **robust, scalable, and automated arrayed CRISPR nuclease (CRISPRn) screening workflow** for SAECs which can be combined with a myriad of disease-specific endpoint assays."

Dickson *et al.* (2023) Highly scalable arrayed CRISPR mediated gene silencing in primary lung small airway epithelial cells - SLAS Discovery Volume 28, Issue 2, P29-35

High Throughput Nucleofector® Systems (96-well and 384-well)

Partial automation vs. full automation

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Partial automation:

- Plate handling only (filling of plates, transfer into Nucleofector® System); manual control of Nucleofector® System
- **Does not require software integration**

Full automation:

- Controlling the Nucleofector® System via the LH System
- **Does require software integration**

High Throughput Nucleofector® Systems (96-well and 384-well)

Full automation

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4D-Nucleofector® 96-well Unit:

- **Does require software integration**

- To automate the 4D-Nucleofector® 96-well Unit we offer the **4D-Nucleofector® Automation Package** (SBA-3001)
  - Serial number specific automation software
  - Automation Server Interface documentation (API)
  - CAD files (4D-Nucleofector® Core and 96-well Unit)
  - Simulation software can be provided to support the full integration

384-well HT Nucleofector® System:

- **Does require software integration**

- Automation Package included

Arrayed CRISPR Screening in COVID Research

For further reading

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Wang et al., Cell, 2021:
Genetic Screens Identify Host Factors for SARS-CoV-2 and Common Cold Coronaviruses: Cell

- **Aim:** Identifying viral entry factors for SARS-CoV-2 and other coronaviruses
- **Method:** Genome wide CRISPR Screens in relevant cell lines
- **Result:** Insights on the coronavirus life cycle with potential impact on the development of host-directed therapies

Gordon et al., Science, 2020:
Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms | Science

- **Aim/ Method:** Carry out "comparative viral-human protein-protein interaction and viral protein localization analysis" for SARS-Cov-2, SARS-CoV-1 and MERS-CoV and "subsequent functional genetic screening" to identify "host factors that functionally impinge on coronavirus proliferation"
- **Result:** Identification of „important molecular mechanisms and potential drug treatments that merit further molecular and clinical study"

Arrayed CRISPR Screening in Drug Discovery

For further reading

Lonza
Cell & Gene

Shan Yu et al., ACS Chemical Biology, 2022:
Genome-wide CRISPR Screening to Identify Drivers of TGF-β-Induced Liver Fibrosis in Human Hepatic Stellate Cells

- **Aim:** Identification of new and better druggable targets for treatment of liver fibrosis
- **Method:** Arrayed genome-wide CRISPR/Cas9 screen in Hepatic stellate cells
- **Result:** Identification of novel and previously known regulators of liver fibrosis

Supporting Nucleofection® Experiments

Lonza resources

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Bioscience Homepage & Product Pages

Electroporation and Nucleofector® Technology | Lonza

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US

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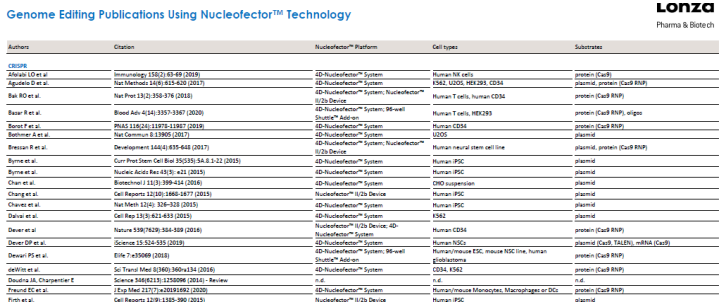
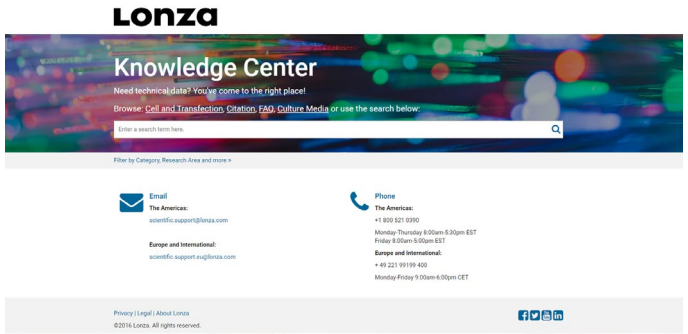
Protocols, Citation List, Bench guides, Brochures, White Paper, Toss Sheets, etc.



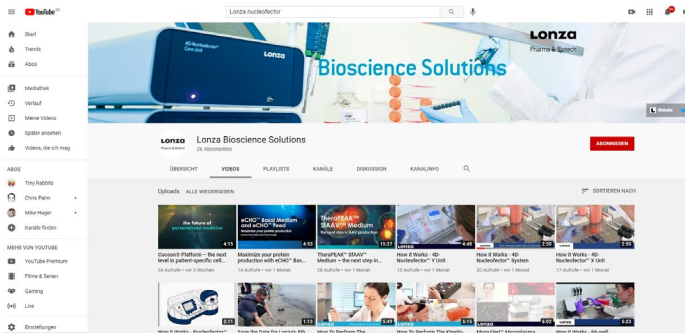
Authors	Citation	Nucleofector™ Platform	Cell types	Substrates
CRISPR				
Alfalahi LO et al	Immunology 158(2):63-69 (2019)	4D-Nucleofector™ System	Human NK cells	protein (Cas9)
Agudelo D et al.	Nat Methods 14(8):815-820 (2017)	4D-Nucleofector™ System	K562, U2OS, HEK293, CD34	plasmid, protein (Cas9 RNP)
Bai RO et al.	Nat Prot 13(2):350-376 (2018)	4D-Nucleofector™ System; Nucleofector™ II/2b Device	Human T cells, human CD34	protein (Cas9 RNP)
Bazar R et al.	Blood Adv 4(14):3357-3367 (2020)	4D-Nucleofector™ System; 96-well Shuttle™ Add-on	Human T cells, HEK293	protein (Cas9 RNP), oligo
Borot F et al.	PNAS 116(24):11973-11987 (2019)	4D-Nucleofector™ System	Human CD34	protein (Cas9 RNP)
Bothmer A et al.	Nat Commun 8:13905 (2017)	4D-Nucleofector™ System	U2OS	plasmid
Bressan R et al.	Development 144(4):635-648 (2017)	4D-Nucleofector™ System; Nucleofector™ II/2b Device	Human neural stem cell line	plasmid, protein (Cas9 RNP)
Byrne et al.	Curr Prot Stem Cell Biol 35(S35):5A.8.1-22 (2015)	4D-Nucleofector™ System	Human iPSC	plasmid
Byrne et al.	Nucleic Acids Rec 43(9): e21 (2015)	4D-Nucleofector™ System	Human iPSC	plasmid
Chan et al.	Biotechnol J 11(3):399-414 (2016)	4D-Nucleofector™ System	CHO suspension	plasmid
Chang et al.	Cell Reports 12(10):1660-1677 (2015)	Nucleofector™ II/2b Device	Human iPSC	plasmid
Chaves et al.	Nat Meth 12(4): 326-328 (2015)	4D-Nucleofector™ System	Human iPSC	plasmid
Dalvai et al.	Cell Rep 13(3):621-633 (2015)	4D-Nucleofector™ System	K562	plasmid
Dever et al	Nature 539(7629):504-509 (2016)	Nucleofector™ II/2b Device; 4D-Nucleofector™ System	Human CD34	protein (Cas9 RNP)
Dever DP et al.	Science 15:524-535 (2019)	4D-Nucleofector™ System	Human NSCs	plasmid (Cas9, TALEN), mRNA (Cas9)
Dewari PS et al.	Elife 7:e35069 (2018)	4D-Nucleofector™ System; 96-well Shuttle™ Add-on	Human/mouse ESC, mouse NSC line, human glioblastoma	protein (Cas9 RNP)
deWitt et al.	Sci Transl Med 8(360):360ra134 (2016)	4D-Nucleofector™ System	CD34, K562	protein (Cas9 RNP)
Doudna JA, Charpentier E	Science 346(6213):1258096 (2014) - Review	n.d.	n.d.	n.d.
Fraund EC et al.	J Exp Med 217(7):e20191692 (2020)	4D-Nucleofector™ System	Human/mouse Monocytes, Macrophages or DCs	protein (Cas9 RNP)
Firth et al.	Cell Reports 12:91:1585-390 (2015)	Nucleofector™ II/2b Device	Human iPSC	plasmid

Lonza Knowledge Center



www.knowledge.lonza.com

Videos on YouTube



4D-Nucleofector® Platform - The Story Of - YouTube

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Thank You

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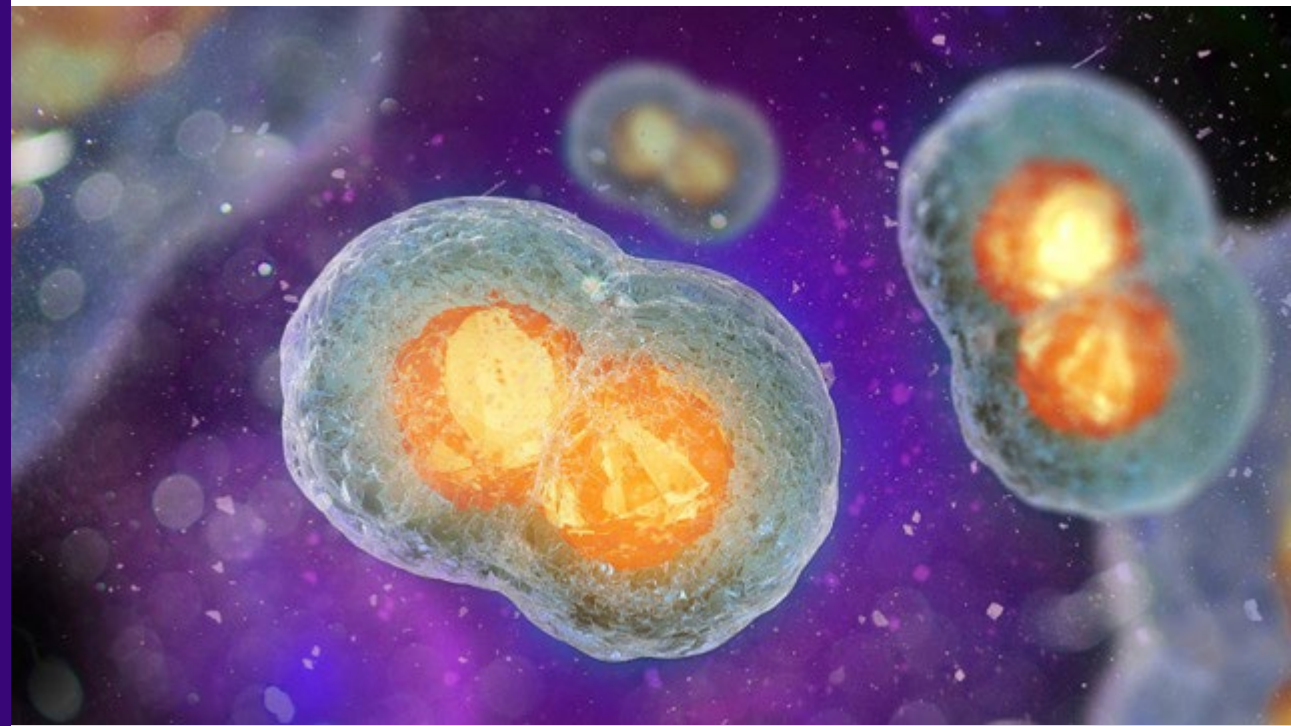
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