TOUCHLIGHT

DNA is our DNA™

Reimagining DNA Payloads: Enzymatic Circular DNA for the Next Generation of Cell and Gene Therapies

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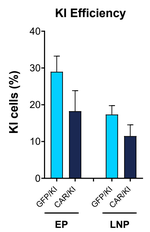
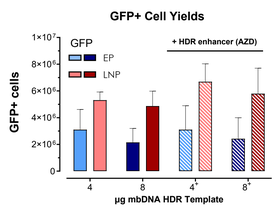
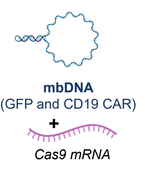
mbDNA demonstrates editing by both by EP and LNP

ElevateBio Authors: Aaron Hoover, Sharlene Amador, Chesney Michels, Jeff Cram, Mike Paglia



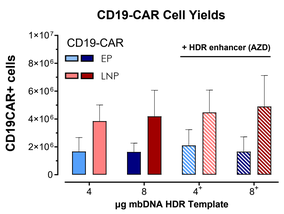




LNP
(GenVoy T cell kit)

Data Powered By elevatebio

Learn more



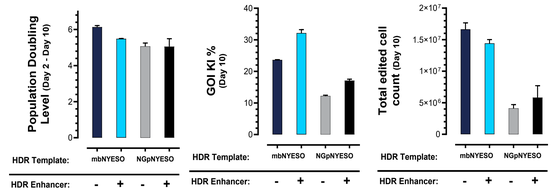
The superior expansion of **Cytiva LNP-treated cells resulted in 1.7 to 3.0-fold higher total edited cell yields** compared to EP-treated cells

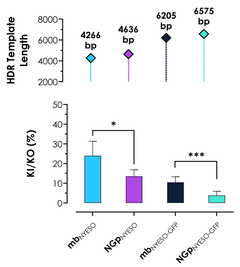
LNP-formulated mbDNA+Cas9 mRNA + sgRNA successfully knock-in two different payloads in T cell *ex vivo* and achieve **17% GFP KI and 12% CAR KI**

It is possible that higher KI efficiency can be achieved through process optimization

Improvement over next-gen pDNA with LEG14 nuclease

ElevateBio Authors: Aaron Hoover, Sharlene Amador, Chesney Michels, Jeff Cram, Mike Paglia





mbDNA outperforms NGpDNA with improved targeted TCR insertion of two donor templates with LEG14 nuclease

With **~4.5kb** template, mbDNA achieved a **1.8-fold increase** in HDR

With **~6.5kb** template, mbDNA achieved a **2.8-fold increase** in HDR

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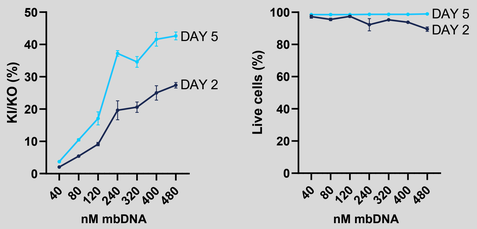
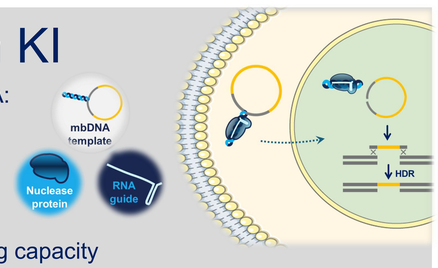
mbDNA is tolerated well and has high KI

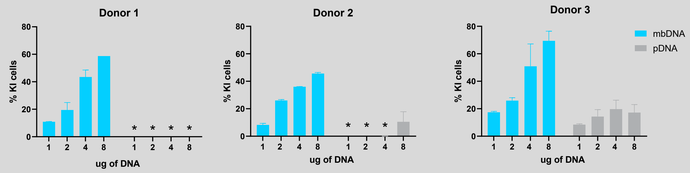


Toxic dose limit not found

- Synthetic **circular** ssDNA:
  - Improved stability
  - Structural advantage
- Gene-length:
  - **Multi-kb** sequences
  - **Beyond viral** packaging capacity
- Improved **manufacturability & scalability** over existing long ssDNA
  - Leveraging Touchlight's established **enzymatic technology** and GMP manufacturing capabilities

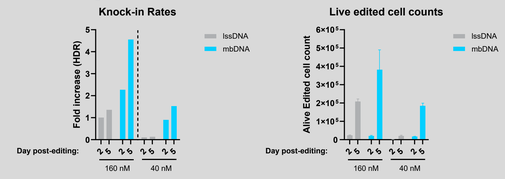


Low donor-to-donor variability



Consistent knock in across 3 donors

Outperforms ssDNA



Higher KI rates and live edited cell counts

Multiple scales and batches performed similarly

Highly efficient editing produces CAR T cells
- 60-75% CAR KI achieved

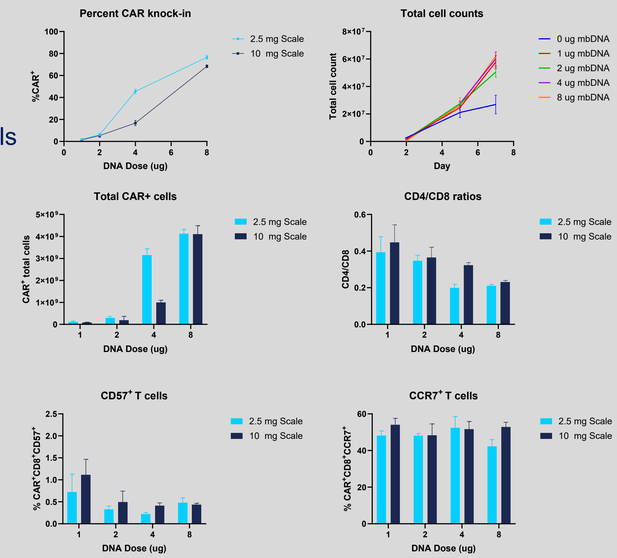
Electroporation did not impair T cell expansion
- mbDNA samples expanded similarly to mock controls

High number of CAR⁺ T cells generated
- **>1E9** CAR+ cells produced

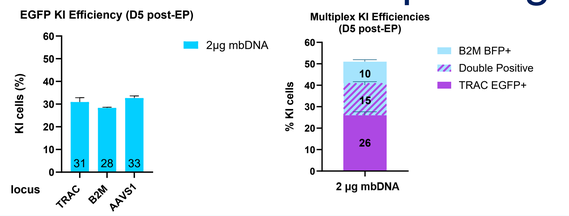
Expected CD4:CD8 ratio maintained at all doses
- Low donor to donor variability observed

Low levels of terminally differentiated T cells were quantified post-electroporation
- CAR⁺CD57⁺% was low across conditions

T cells retain capacity for proliferation and survival post-electroporation
- CAR⁺CD8⁺CCR7⁺% maintained across conditions



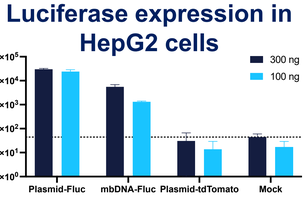
Enables efficient multiplexing



mbDNA delivers **similar knock-in** at the **TRAC, B2M and AAVS1 loci** in T cells

Consistent editing at **multiple loci** allows efficient **multiplexing** and generation of **double-positive T cells**

mbDNA is a functional episomal expression vector



Authors: Elizabeth Del Greco, Margarita Krivega, Julia Poniatowski and Ivan Krivega
SonoThera, Inc.

- A **non-integrative mbDNA** generates firefly luciferase **transgene expression** sufficiently above background in the liver cell line HepG2
- This suggests mbDNA could be used as a vector for **episomal expression** in Non-Viral Gene Therapy (NVGT), helping to **improve toxicities from double-stranded DNA** *ex vivo/in vivo*

SONOTHERA™

Non-HDR Applications

Advantages:
Reduced immunogenicity

Cell-free
- Enzymatic manufacture
- High purity
- allows for high sequence complexity and secondary structures

Full sequence flexibility
- No backbone
- No manufacturing-imposed motifs

Architecture	Unique Feature	Application
Megabulb DNA (mbDNA)	Double stranded DNA stem with single stranded circle 'bulb'	Homology-directed repair (HDR), episomal expression
Double-stranded circle (dscDNA)	Fully double stranded circular molecule where every base pair is user defined	Transposases, recombinases, integrases, homology-independent targeted integration (HITI), episomal expression
Single-stranded circle (sscDNA)	Fully single stranded circular molecule where every base pair is user defined	
Hybrid single-stranded circle (hsscDNA)	User defined double stranded and single stranded regions Phosphorothioates to protect DNA ends from degradation	