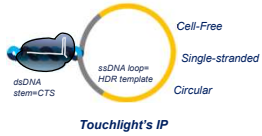
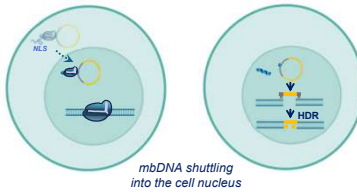




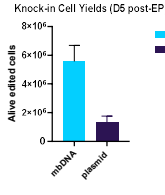
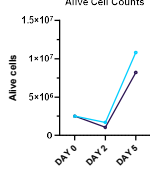
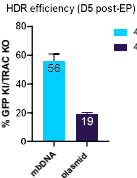
mbDNA Introduction



- 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



mbDNA improves CRISPR HDR



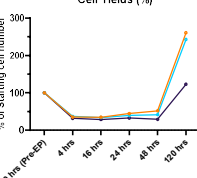
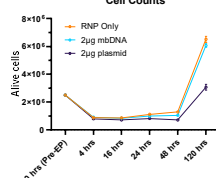
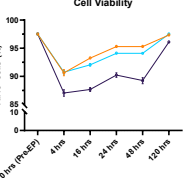
Methods: Cas9 RNP and mbDNA or plasmid encoding a promoterless EGFP-targeting the TRAC locus were electroporated into activated human primary T cells. Targeted insertion was measured by EGFP expression via flow cytometry. KO was measured by anti-TCR staining via flow cytometry.

mbDNA generates **>50% HDR** at the TRAC locus

mbDNA mediates **3x times as much HDR** than plasmid

mbDNA yields **4x more knock-in cell yields** than plasmid

mbDNA supports high cell yields



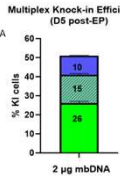
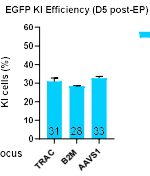
Methods: Cas9 RNP and mbDNA or plasmid encoding a promoterless EGFP-targeting the TRAC locus were electroporated into activated human primary T cells. Cell viability was measured by a live/dead stain via flow cytometry. Cells were counted by flow cytometry. Cell yields were measured as % of electroporated cell numbers (ZE6 cells).

mbDNA electroporation **does not impair T cell expansion**

mbDNA samples **expand as well as no DNA control** (RNP only)

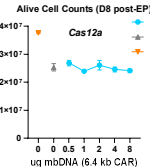
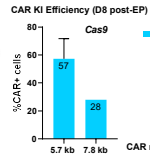
mbDNA ensures **lower initial T cell toxicity** and vastly improved **cell recovery**, yielding **2.5x more total cells**

mbDNA enables efficient multiplexing



mbDNA delivers large cargos, maintaining low T cell toxicity

Collaborator data



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**

Efficient performance with Cas9 and Cas12a

Efficient integration of **cargo larger than AAV's genome**

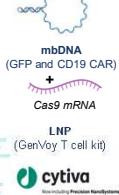
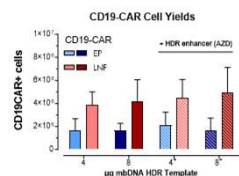
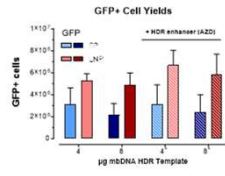
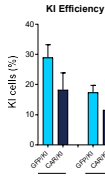
Achieves high **edited cell yields** in T cells

Enables **more edited HSCs** than dsDNA payloads

mbDNA demonstrates Cas9-targeted knock-in both by EP and LNP

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

Data Powered By **elevatebio**



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

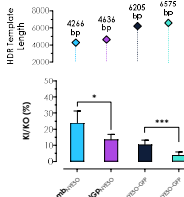
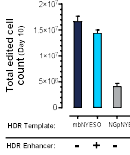
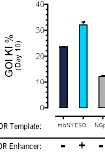
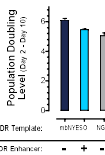
LNP-formulated mbDNA+Cas9 mRNA + sgRNA successfully knock-in two different payloads in T cell ex vivo and achieve 17% GFP KI and 12% CAR KI

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

Improvement over next-gen pDNA with LEG14 nuclease

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

Data Powered By **elevatebio**

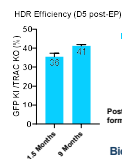
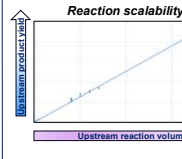
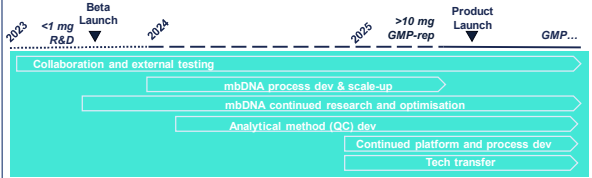


mbDNA **outperforms** NgpDNA with improved targeted TCR insertion of two donor templates with LEG14 nuclease

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

mbDNA supports multi-mg GMP-representative manufacture

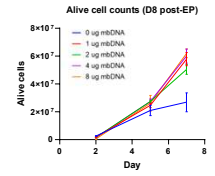
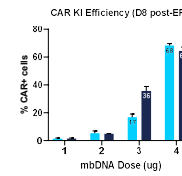


Molecule's stability
mbDNA's performance in T cells maintained in samples over at least 9 months post-formulation

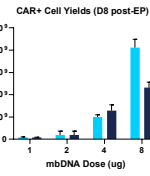
Biophysical assays: concentration, pH and purity data all support stability

mbDNA is a highly efficient CAR HDR template

Collaborator data



Methods: Cas9 RNP and mbDNA from GMP-rep process encoding CAR targeting the TRAC locus were electroporated into activated human primary T cells. Targeted insertion was measured by EGFP expression via flow cytometry.



mbDNA generates **60-70% CAR KI**

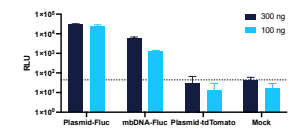
mbDNA samples **expanded similarly to no DNA controls** (RNP only)

Increasing concentrations of mbDNA achieve **increasing knock-in cell counts** due to low toxicity at high doses

Low donor-to-donor variability observed

mbDNA is a functional episomal expression vector

Luciferase expression in HepG2 cells



- 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**

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

SONOTHERA™

Next gen(e) editing tools – alternative DNA architectures for 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 (HTTI), 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	