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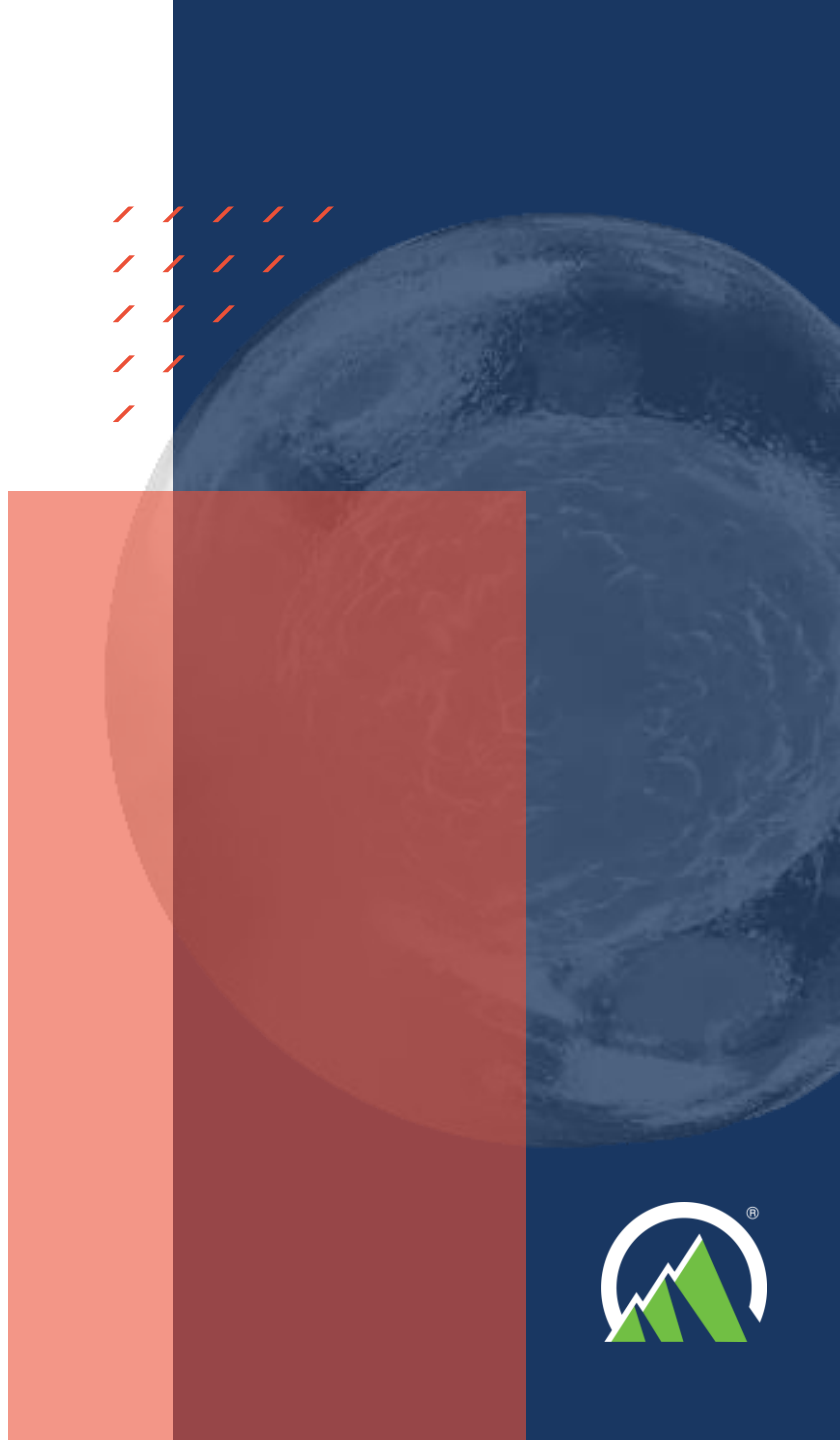
A Novel Approach to Treating Huntington's Disease Using AAV to Deliver Gene Editing Tools to the Brain



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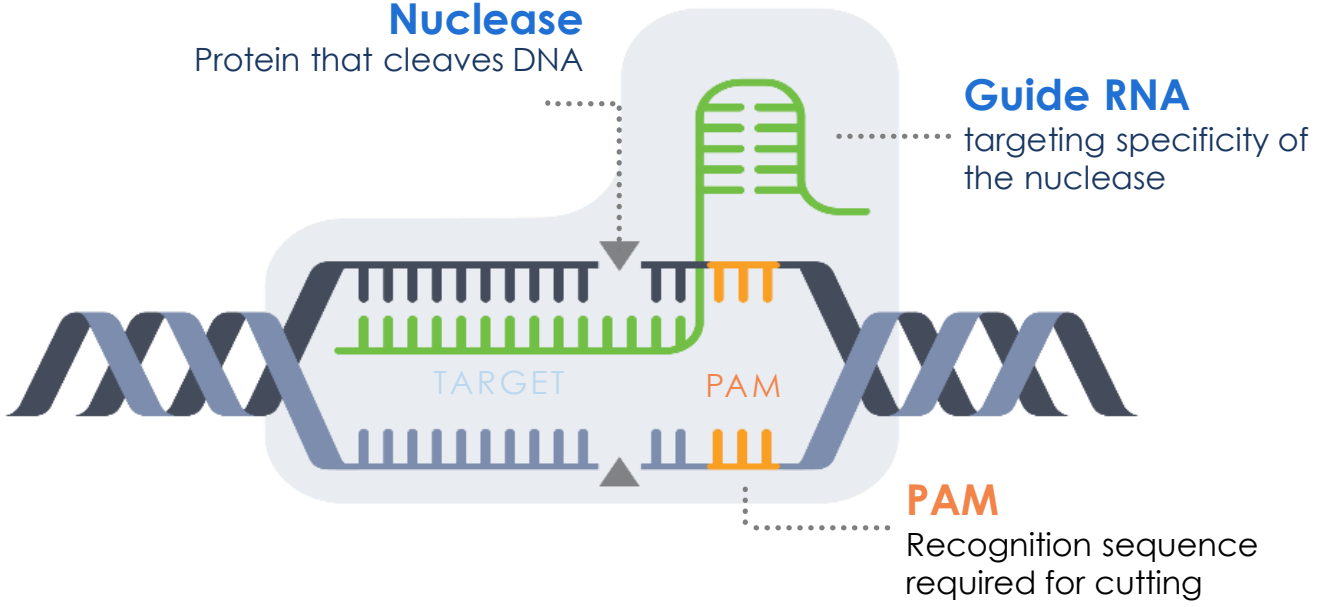
Contents

Life Edit is powered by a robust library of RNA-guided nucleases

Life Edit Genes (LEGs)



- **Smaller LEGs (~800 - ~1,100 aa) facilitate easier delivery**
- **Unique and diverse PAM recognition sites**
- **Flexible targeting strategies**
- **Robust portfolio of patents granted globally covering our lead RGNs, adenine deaminases, RT editors and base editors**

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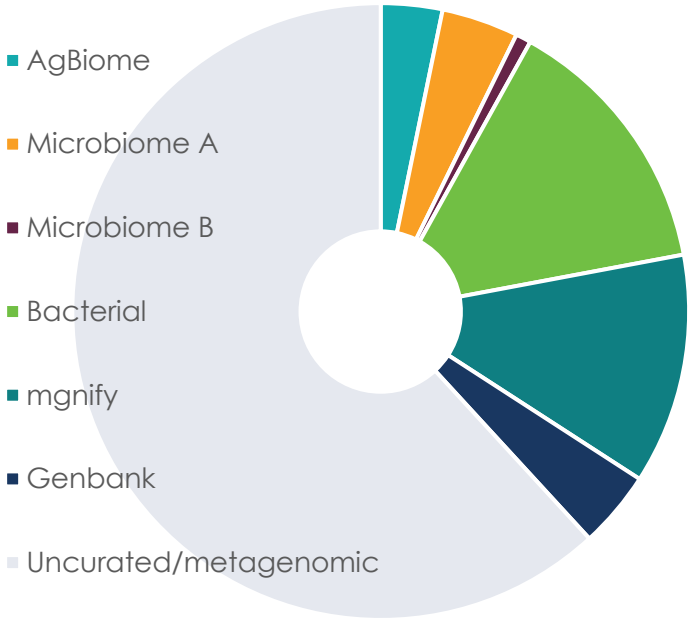
Life Edit has a Proprietary Library of Evolutionarily Distant Nucleases with PAM Sequence Diversity



Over 2M CRISPR-Cas systems

All known Cas subtypes

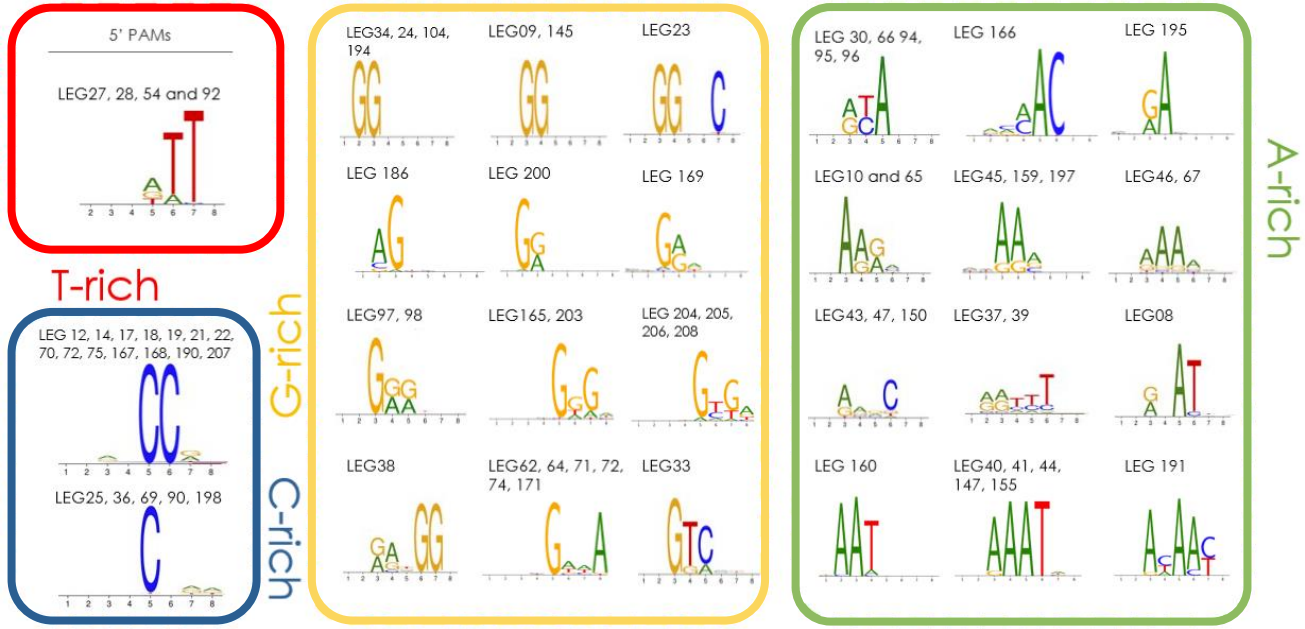
- Focus on Type II and Type V

Cataloged to capture annotations and *gene context*

10+ billion unique proteins (and counting)

Microbial database from multiple sources

Example PAM sequences



Collection of nucleases with diverse PAM sequences

Founded on exclusive access to gene editing systems identified in a proprietary microbe collection for use in human therapeutics, and **expanded** by mining additional genomic data sources

Collection enables the ability to **find additional enzymatic activities** to build future editing systems (e.g., proprietary base editors, transposases, others)

Huntington's Disease (HD) and Treatment Approach

HD patients have severe, progressive, neurodegenerative disease, with no available disease modifying therapies

- HD is a rare, progressive, neurodegenerative disorder
  - Typical onset at 30-50 years; time from symptom emergence to death is ~10-30 years
  - Degeneration and atrophy of the striatum, and later the cerebral cortex

Targeting mutant huntingtin

- HD is caused by the expansion of CAG repeats in the huntingtin gene (*HTT*)
  - Healthy brain: 10-35 CAG repeats; HD pts: 40 to >120 CAG repeats
- Expansion of CAG repeats leads to the production of the mutant HTT protein (mHTT) which ultimately leads to neuronal cell death
- Maintaining wtHTT is a high clinical priority
  - wtHTT protein supports a wide range of homeostatic functions including transcriptional regulation, axonal transport, endosomal trafficking, and vesicular recycling

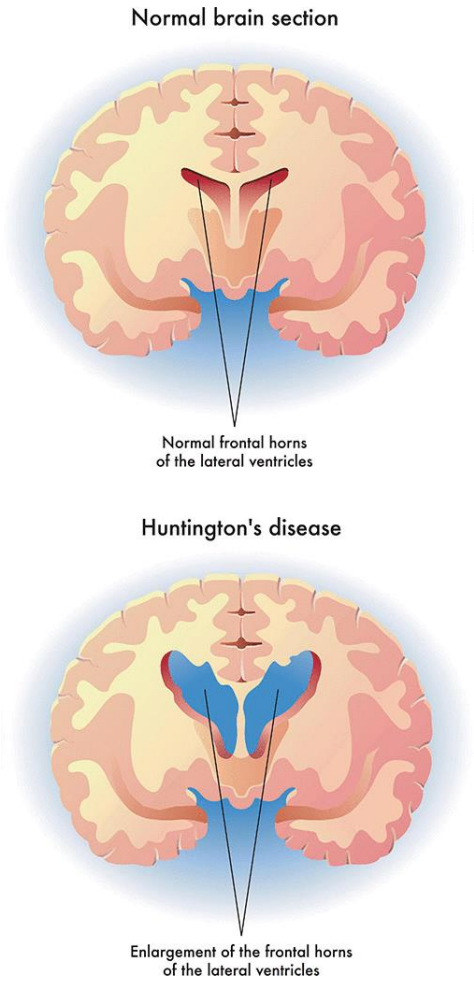
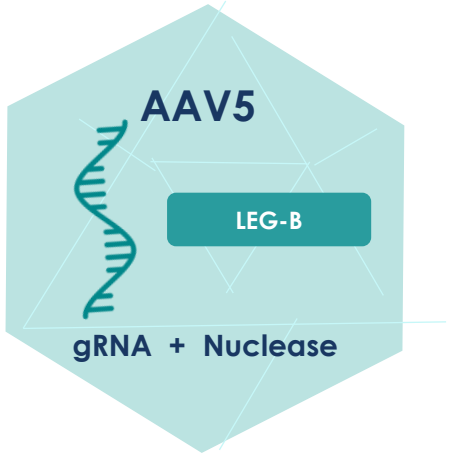
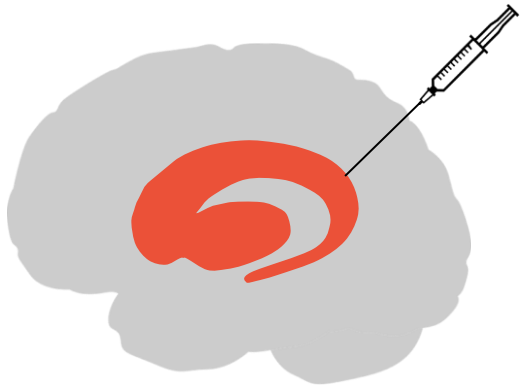


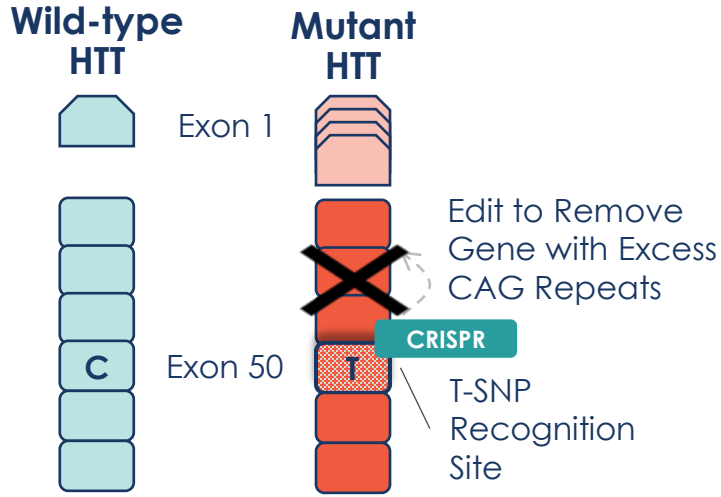
Image credit: Health Direct

LETI-101: A precision Editing Approach as Potential One-Time Treatment for HD

NOVEL CRISPR SYSTEM	TARGETED CNS DELIVERY	ALLELE-SELECTIVE EDITING







Proprietary, compact CRISPR system, packaged in AAV5 vector

One-time, bilateral intrastriatal administration

Potent and selective reduction in mutant while preserving wild-type; selective approach made possible by diverse genomic recognition sites

LETI-101 OFFERS POTENTIAL FOR A DURABLE, **ONE-TIME TREATMENT** WITH AN IMPROVED SAFETY PROFILE THROUGH SELECTIVE TARGETING

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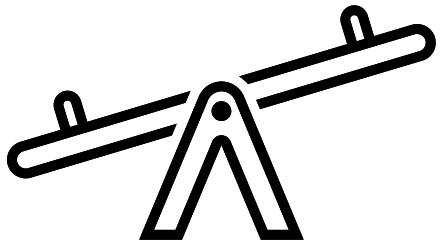
Collaboration on LETI-101 Candidate Selection in R&D

RESEARCH

Can it treat the disease?

Nuclease + sgRNA encoded in an AAV5 vector results in ≥ 40% reduction in mHTT protein in striatum of BACHD mice



DEVELOPMENT

Can we manufacture it?

AAV5 vector with ≥1.00e11 vg/mL from HEK suspension cells and ≥15% full capsids

				Research Batches		Development Batches	
Vector Construct	sgRNA Promotor	Nuclease NLS	Transgene Size	Genome Titer vg/mL	% Capsid Protein : Genome Titer	Upstream Genome Titer vg/mL	% Full Capsid after Affinity Capture
1	A	A	X	9.95e12	60	1.20e11	7.2
2	A	A	X – 98	8.96e12	58	1.12e11	10.8
3	B	A	X – 167	1.22e13	56	1.17e11	11.8
4	B	B	X – 146	1.25e13	54	1.62e11	12.9

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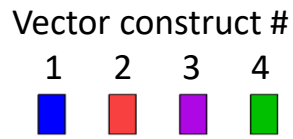
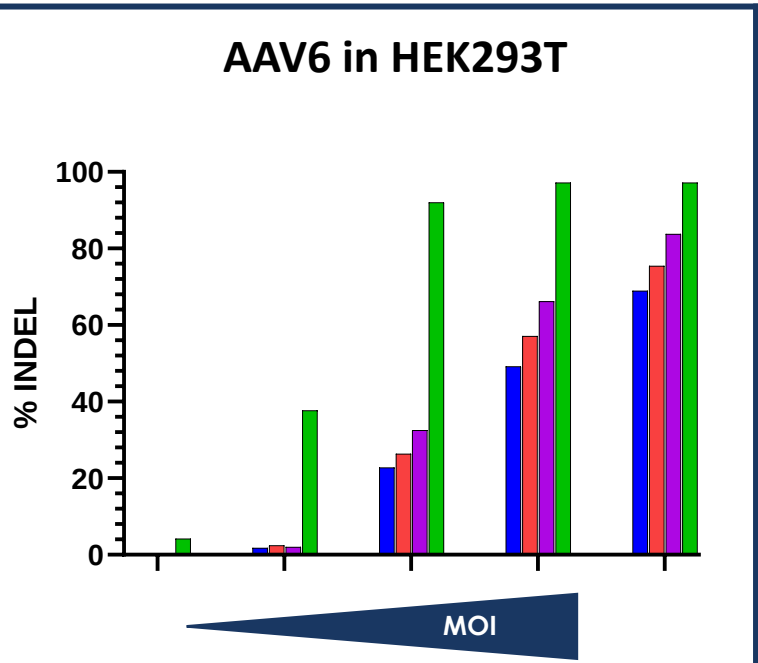
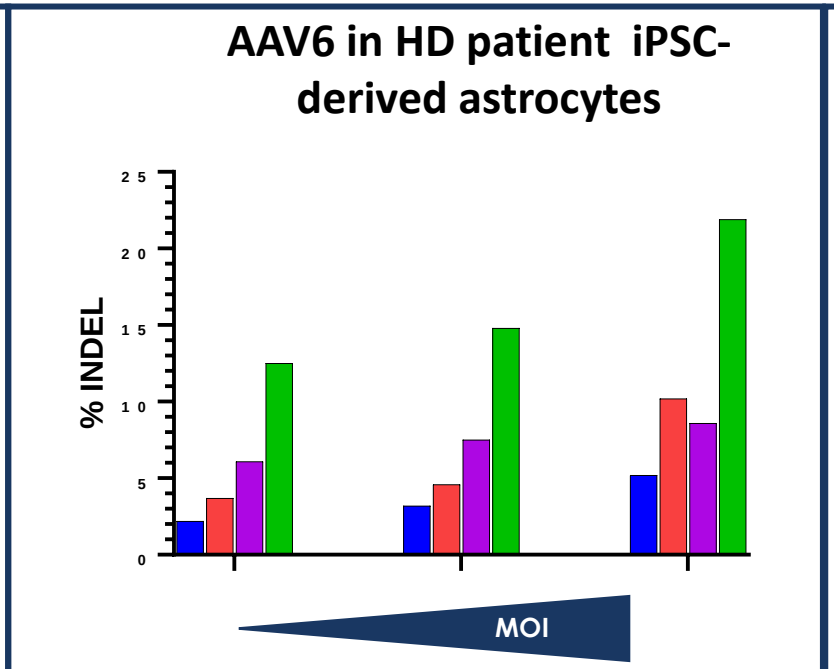
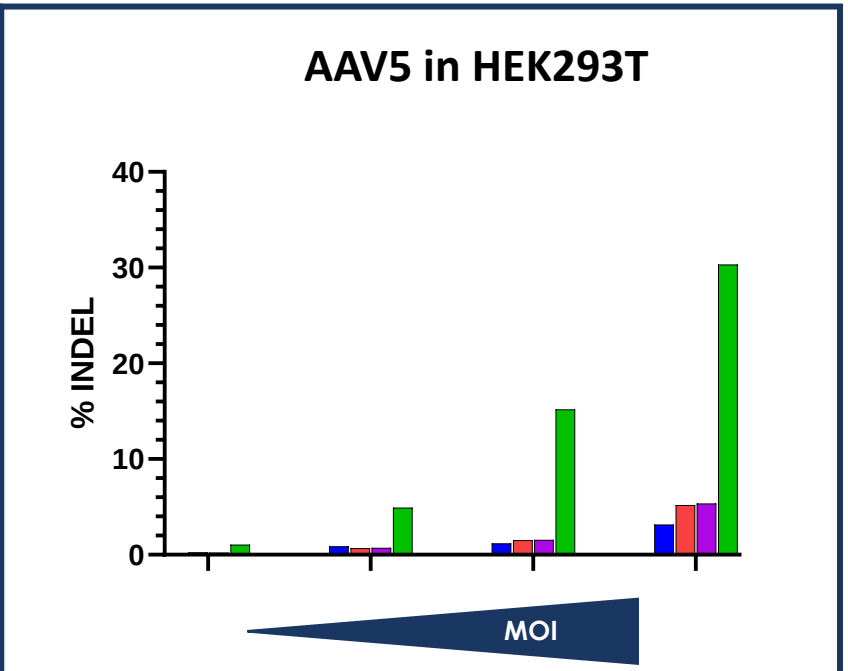
Vector Construct Screening in Research – *In Vitro*









- Construct #4 resulted in improved on-target editing rates relative to previous constructs
- The same rank order was observed among constructs with AAV5 as with AAV6, and in both HEK293t and HD patient iPSC-derived astrocytes

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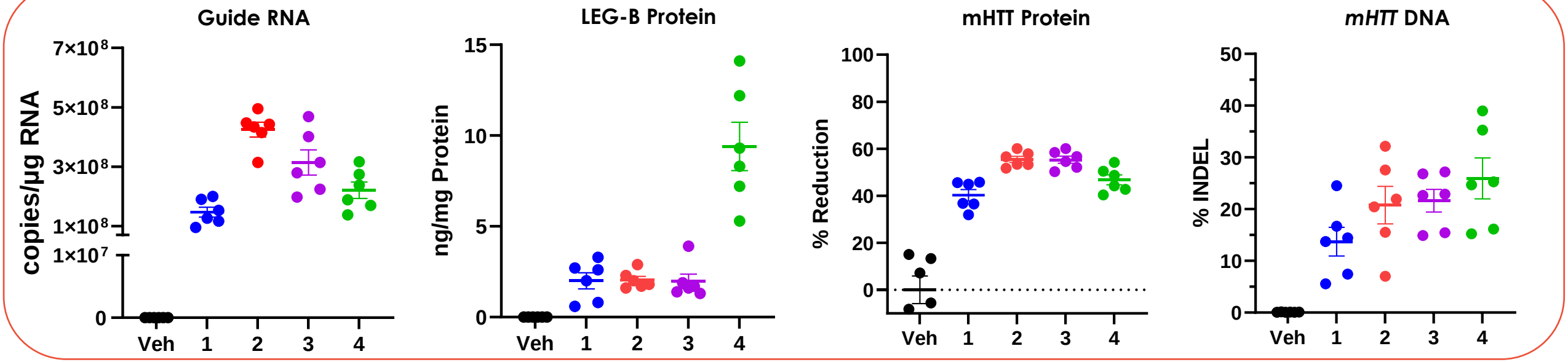
Vector construct screening in Research – BACHD mice



Each point represents individual mice with mean ± SD shown

Study Design	
	❖ **BACHD** transgenic mice (carry HTT rs362331'T' SNP)
	❖ Intrastriatal injections of **AAV5** with 4 vector construct designs at single dose
	❖ 6-weeks in-life duration → brain tissues harvested and striatum analyzed

- **NLS option B exhibited higher LEG-B protein expression**
- **High editing activity levels maintained with all design iterations**

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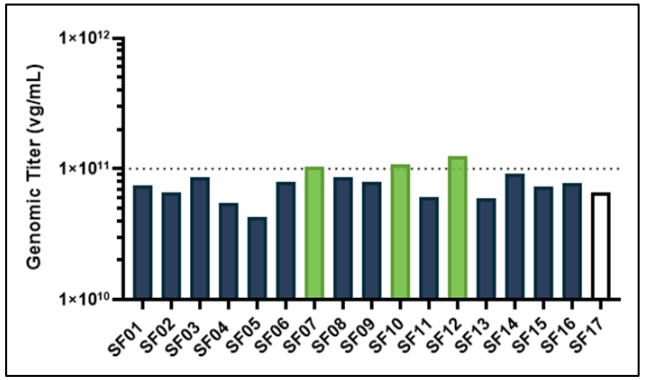
DoE optimization of AAV transfection conditions

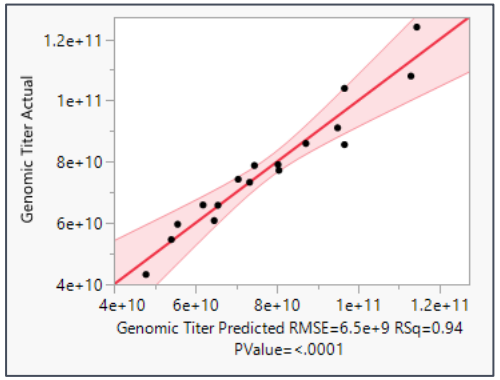
AAV upstream titer

(DoE process inputs)

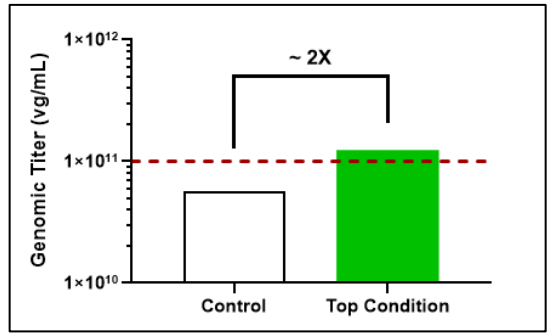
DNA/cell
Cell Density
DNA/Reagent Ratio



AAV5 transfection optimization



Predictive Model

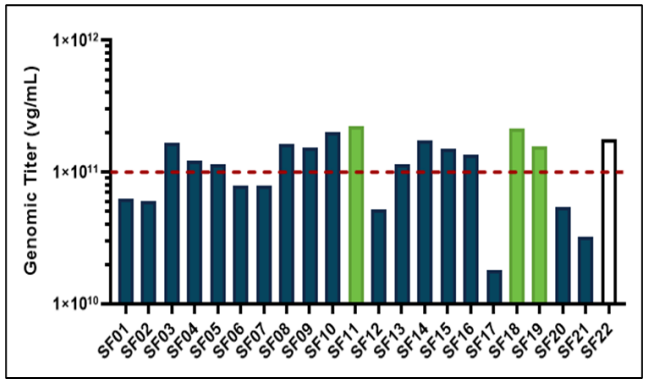


~2X titer improvement

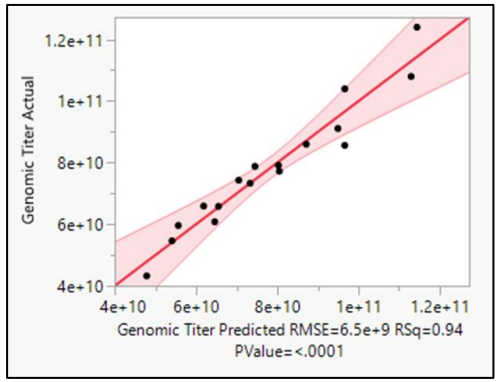
AAV upstream % Full

(DoE process inputs)

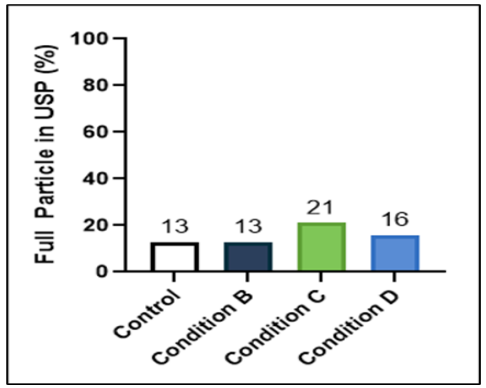
3- Plasmid ratios
- RepCap
- Helper
- GOI



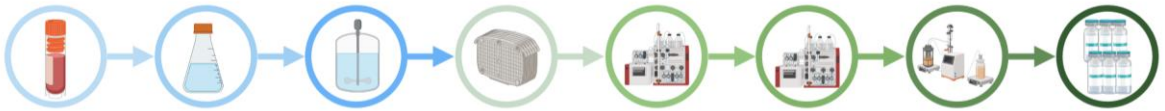
AAV5 transfection optimization



Predictive Model



~2X % Full AAV capsid improvement



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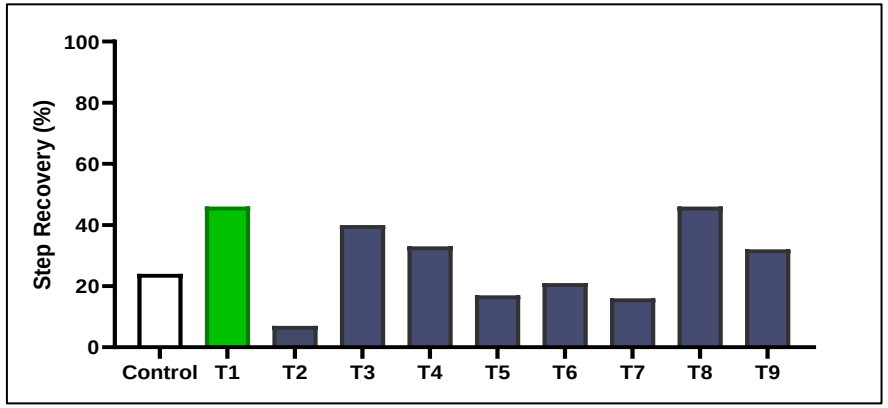
DoE optimization of AAV AEX downstream process

Chromatography parameters

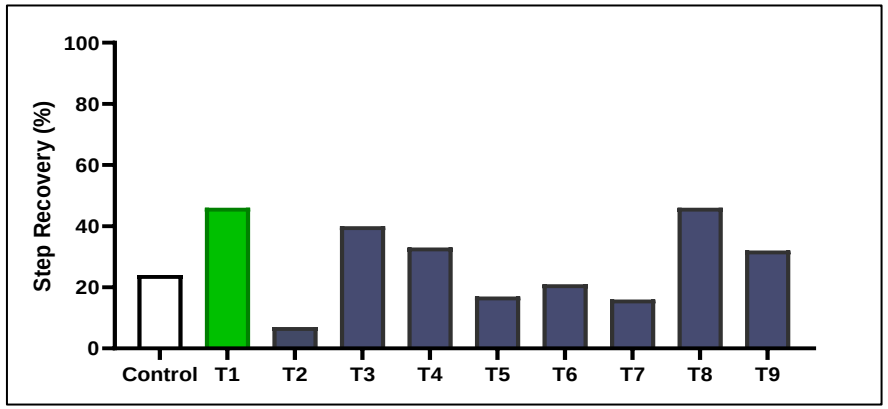
(DoE process inputs)

DoE 1 Elution
Salt type
pH
Supplements

DoE 2 Binding
pH
Conductivity

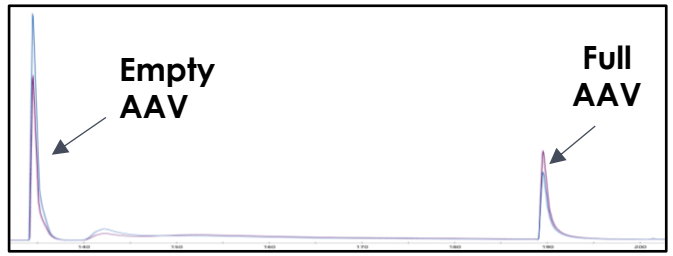


AAV5 DoE 1 Elution parameter optimization



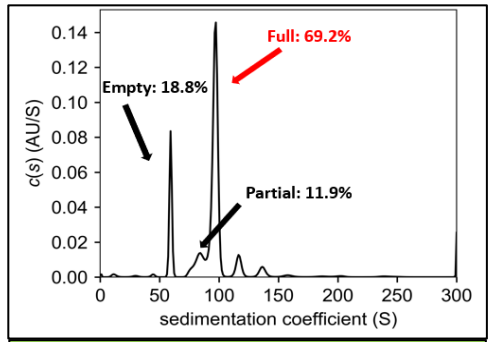
AAV5 DoE 2 Loading adjustment

Downstream AEX %Full optimization



AAV5 - AEX 2-step elution profile

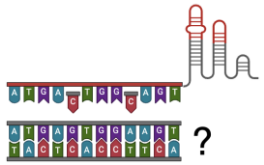
AAV5 AUC

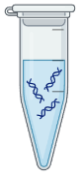


Full content 69.2%

Off-Target Analysis Reveals Exquisite Specificity of LETI-101

Off-Target Identification Strategy





Bioinformatic Prediction

Predicts off-targets based on homology to guide

Population Variation Analysis

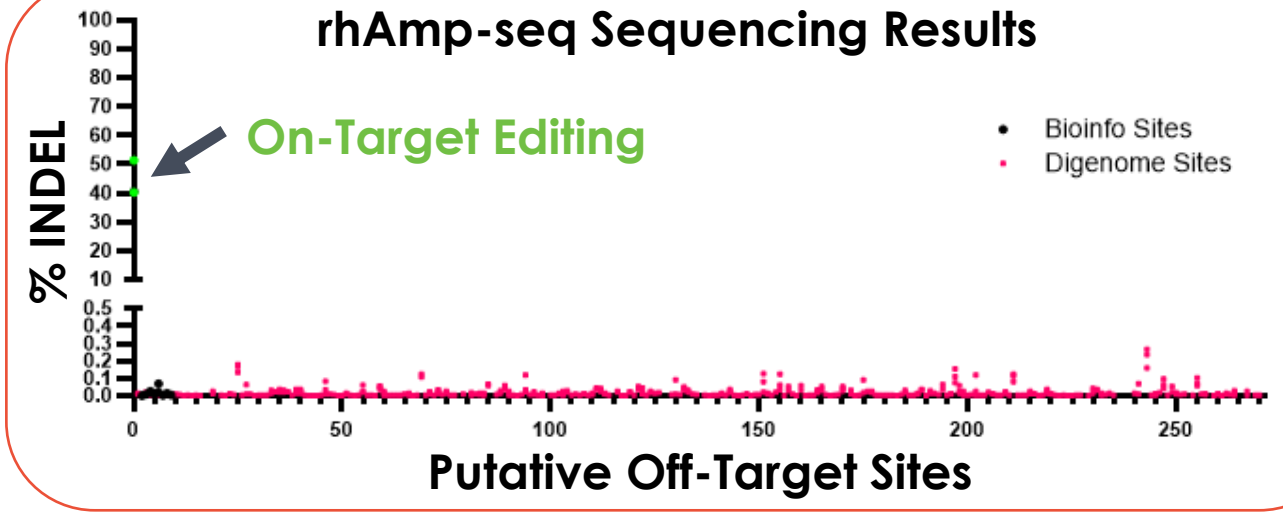
Identifies SNPs that could pose off-target risks

Digenome-Seq

Unbiased biochemical method to identify putative off-targets

~300 putative off-target sites identified and profiled via amplicon sequencing using genomic material from HD patients edited with mRNA/RNA delivery

Off-Target Results Compiled Across 3 HD Fibroblast lines



Indels

No off-target editing observed at sequenced sites & no off-target liabilities identified

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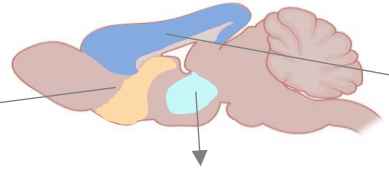
LETI-101 Activity Increases with Dose and Time in BACHD Transgenic Rodent Model



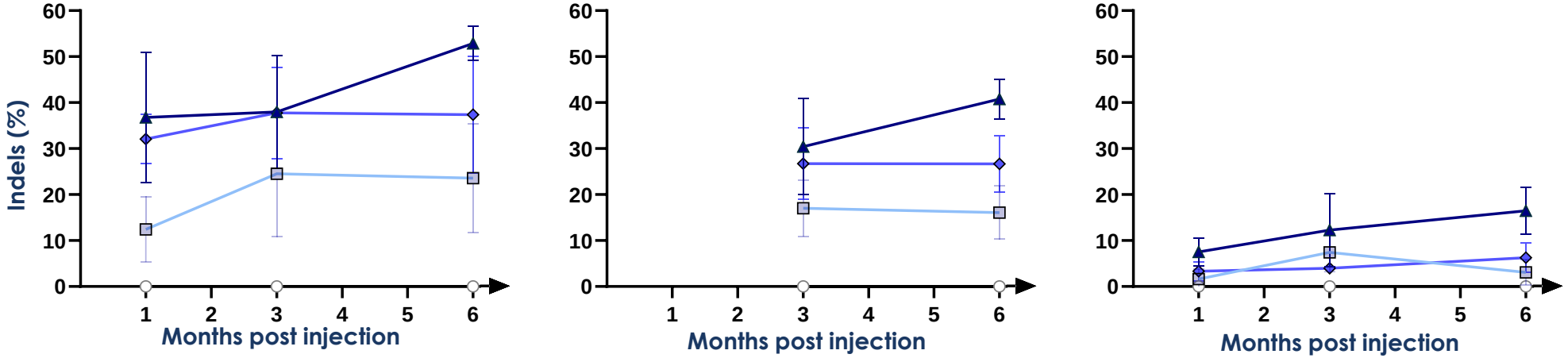
Striatum

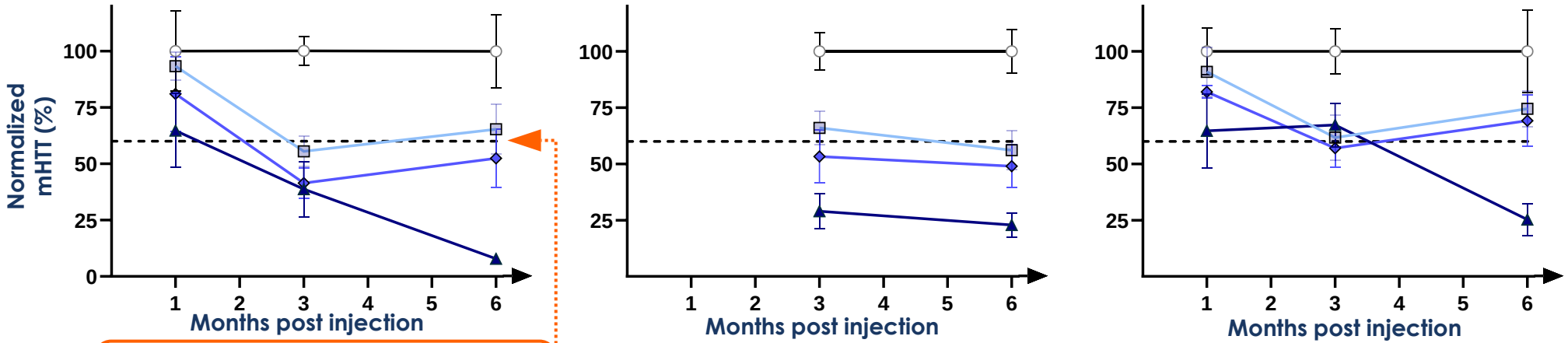
Thalamus

Cortex

Indels

mHTT Protein





Potentially clinically relevant threshold of 40% reduction

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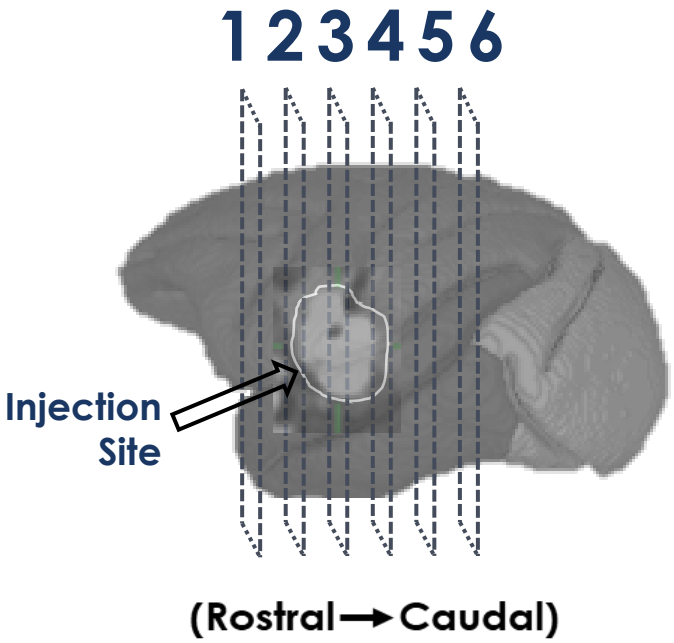
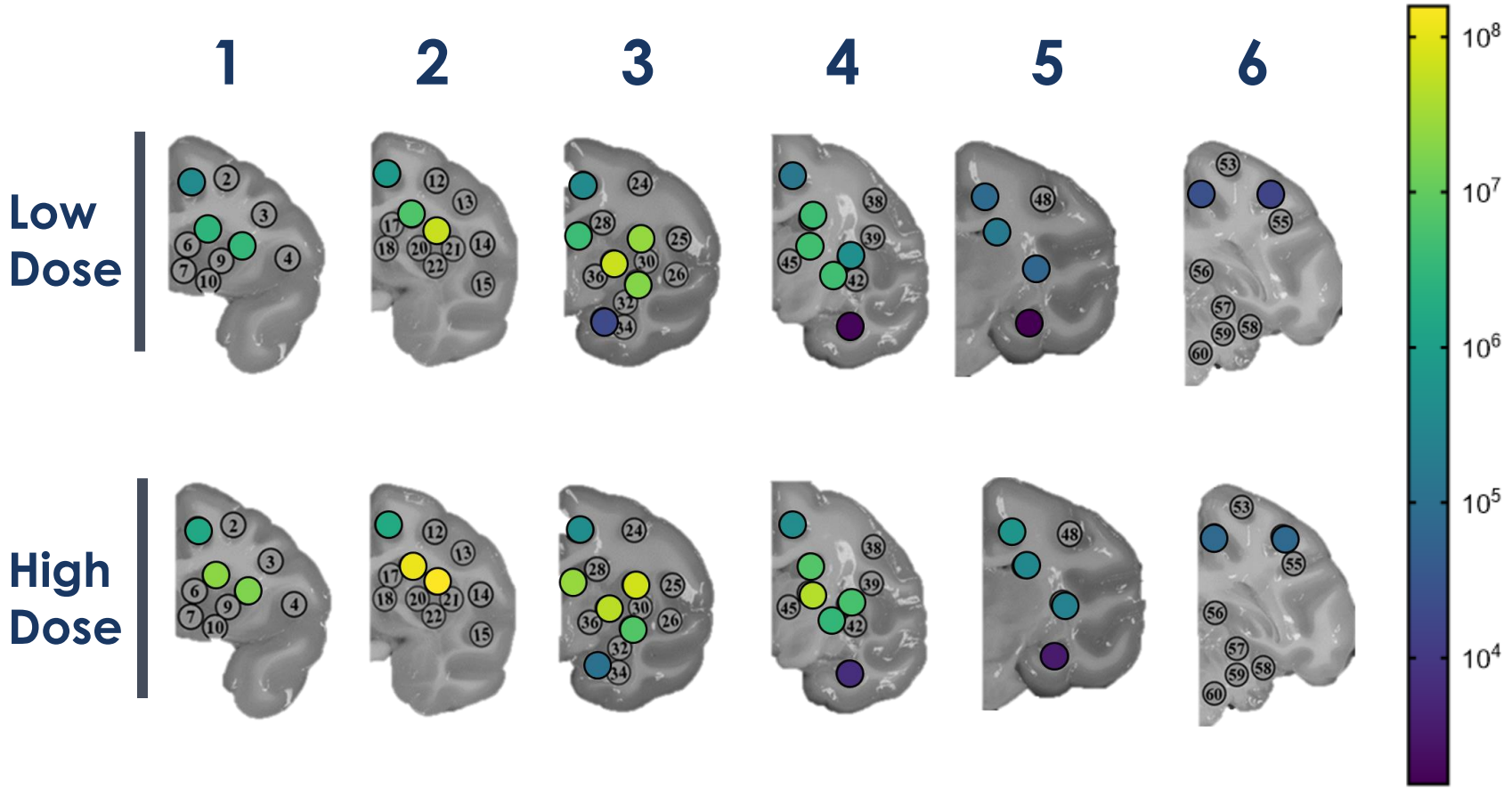
One-Month Biodistribution in NHP: gRNA Highly Expressed in Striatum and Across Brain Regions





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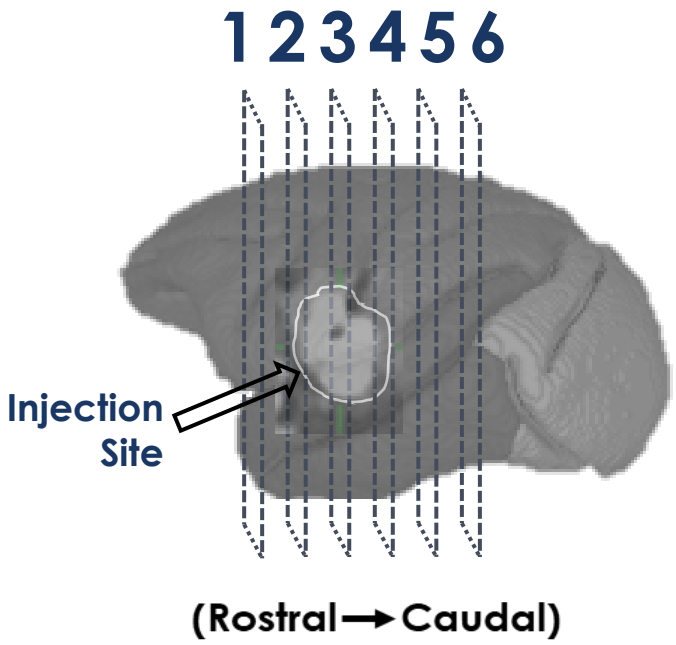
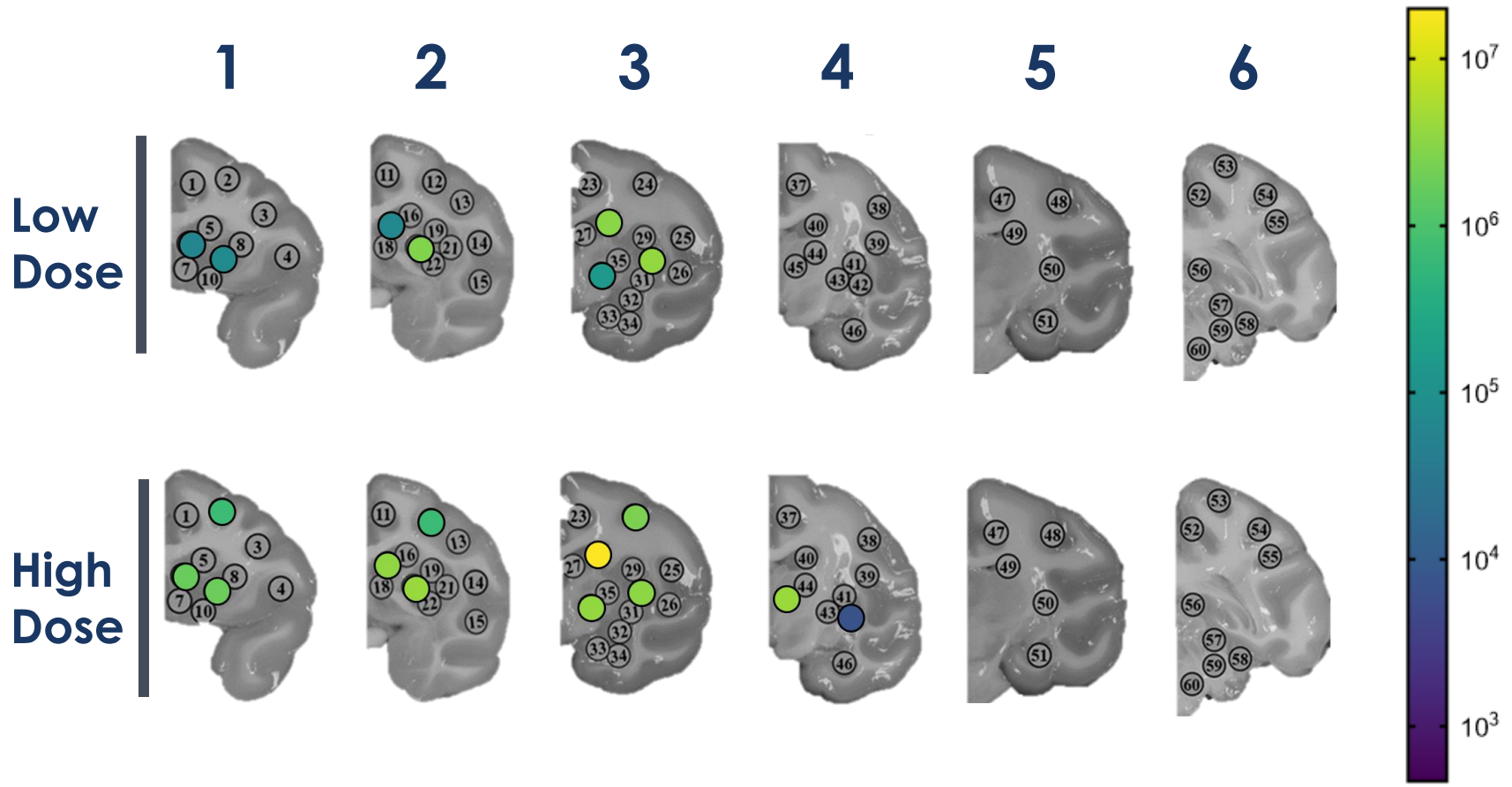
Each circle represents an average across NHPs from low dose (n=2) or high dose (n=3)

One-Month Biodistribution in NHP: LEG-B Protein Highly Expressed in Striatum and Across Brain Regions





pg/µg Total Protein

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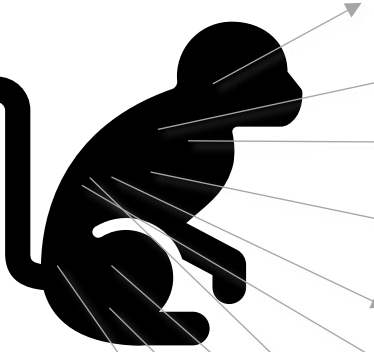
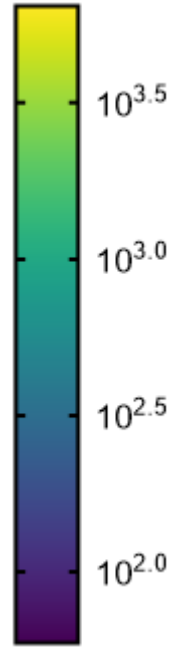
Each circle represents an average across NHPs from low dose (n=2) or high dose (n=3)

Minimal Systemic Biodistribution of Vector with None Detected in Gonads



	Low Dose			High Dose		
	Vector Genomes	LEG-B mRNA	gRNA	Vector Genomes	LEG-B mRNA	gRNA
Lymph Node	○	○	○	●	●	●
Heart	○	○	N/A	○	○	N/A
Lung	○	○	N/A	●	○	N/A
Liver	●	●	●	●	●	●
Kidney	●	○	○	●	●	○
Spleen	●	●	○	●	●	●
Adrenal Gland	○	○	N/A	●	○	●
Gonads	○	○	N/A	○	○	N/A
Muscle	○	○	N/A	○	○	N/A
Sciatic Nerve	○	○	N/A	○	○	N/A



Copies/µg DNA or RNA

NHP in Low dose: male n=1 and female n=1. High dose : male n=1 and female n=2.
Variable tissue sample n based on availability

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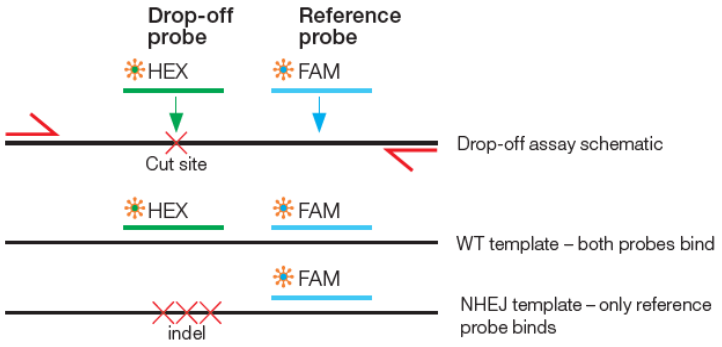
Droplet Digital PCR (ddPCR) Assay for INDEL Formation

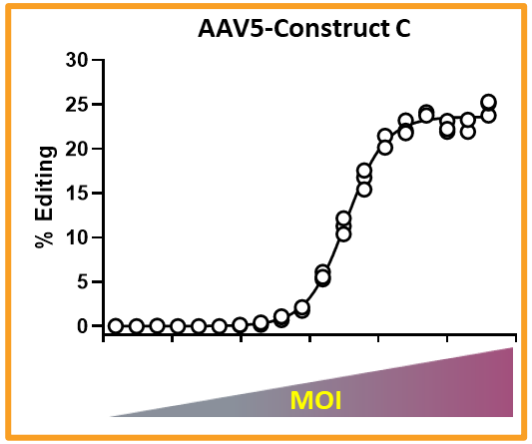
6 human CNS-origin cell lines screened for C/T SNP → 2 were C/T heterozygous (confirmed by cloning) → 1 was transducible with AAV5-GFP and LETI-101

Easy to grow and maintain in culture
- 42-hour doubling time
- ≥ 95% viability

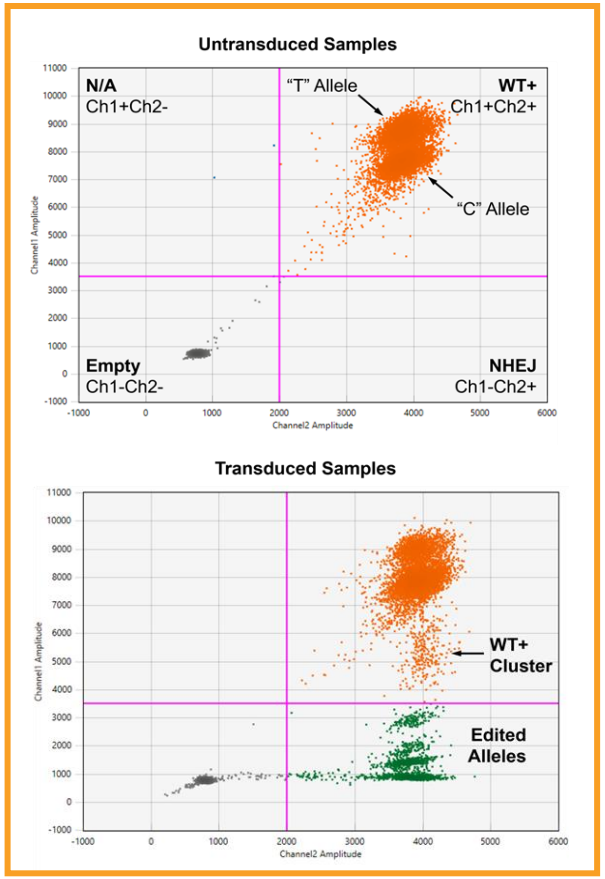
How to measure % Editing by ddPCR

- ddPCR primers and probes were designed for a NHEJ drop-off assay
- Editing will prevent the NHEJ/drop-off probe from binding
- Reference probe binds to all alleles
- gBlocks were designed to mimic INDELs expected from nuclease cutting





Target cells are **C/T heterozygous** → **Max % Editing is 50%** based on accessible target engagement



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Biotech Can Integrate CMC Efforts Early and De-Risk Clinical Trials and Commercialization

LETI-101: A Precision Editing Approach to Treating Huntington's Disease

RESEARCH

- LETI-101 (LEG-B-SGN) delivered by RNA in **patient-derived cells** resulted in allele-selective editing of *mHTT* gene and reduction of mutant HTT protein
- LETI-101 delivered intrastriatally in **BACHD transgenic mice** resulted in dose-dependent vector disposition, transgene expression, and clinically relevant reduction of mHTT protein in striatum
- LETI-101 delivered intrastriatally in **NHP** resulted in dose-dependent vector biodistribution and transgene expression across brain regions that are critically vulnerable in HD. A **NOAEL of 1.13 x 10^{13} vg** (the highest dose evaluated) was obtained

DEVELOPMENT

- **Screening of research constructs** and further optimization of **plasmid ratios** resulted in **21% Full** capsids, exceeding the ≥15% Full requirement
- Construct providing **highest % Full capsids** ALSO **edited better** *in vitro* and *in vivo* and had **higher productivity**. Productivity was further increased by optimizing transfection conditions, resulting in **1.62e11 vg/mL** in crude harvest
- Potency assay developed for early phase in C/T SNP heterozygous CNS-derived tissue culture cell line using ddPCR to detect % Editing

Completing late-stage discovery while evaluating manufacturability saved time and improved yield and editing outcomes

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Life Edit Therapeutics Met with MHRA in September 2024 to Review LETI-101

- Preclinical data package well received; deemed "sufficient and comprehensive" including off-target characterization strategy
- Concurrence with overall clinical trial design and CMC strategy

THANK YOU FOR YOUR ATTENTION!

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