Potency development for an *in vivo* AAV gene editing therapy

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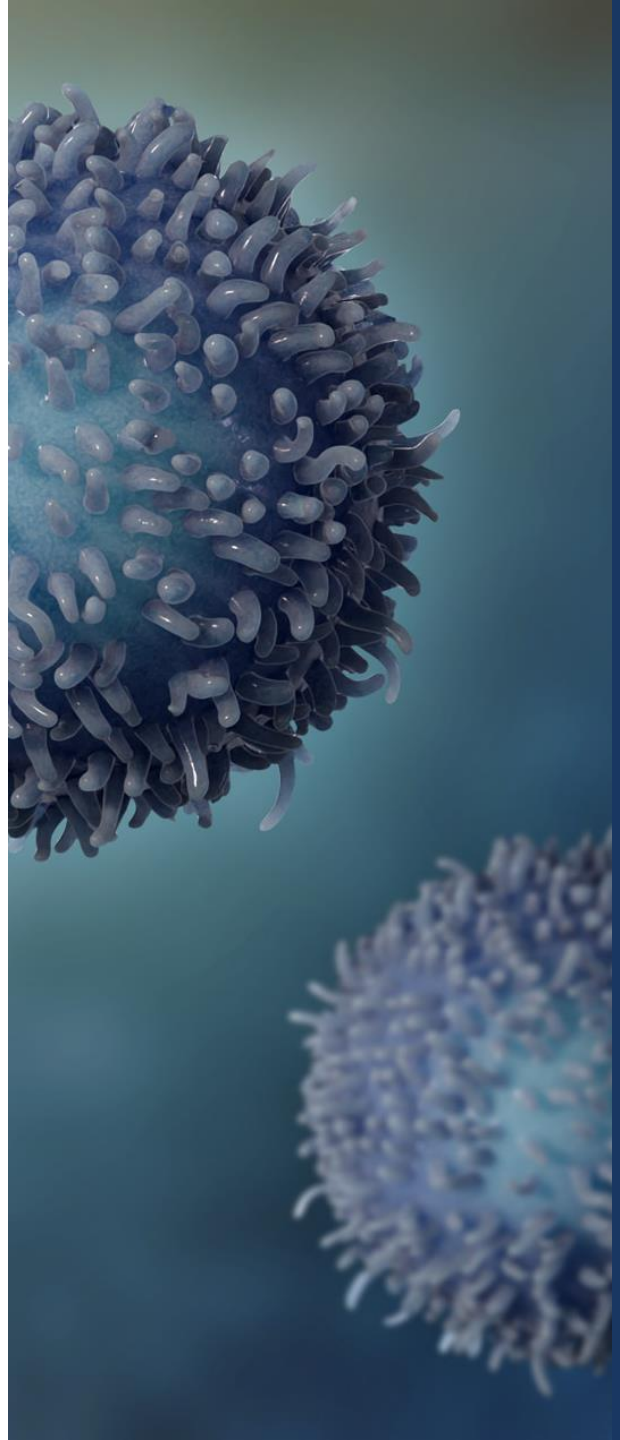

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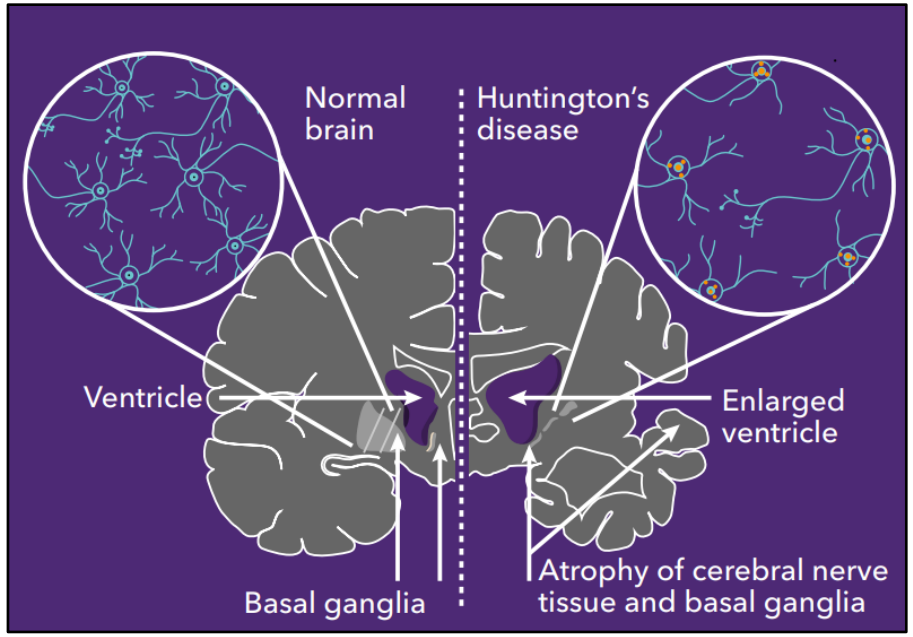
Accelerating the genetic medicine industry through a new approach to design, manufacture and develop genomic medicines

- ✓ End-to-end process development and cGMP manufacturing
- ✓ Expert analytical team with experience across HSCs, T cells, B cells, NKs and gene therapy modalities
- ✓ Experienced in early-stage assay development through late-stage assay validation and commercialization
- ✓ Experienced in supporting global drug development programs with in-house regulatory expertise
- ✓ Deep subject matter expertise in a variety of analytical platforms including but not limited to, flow cytometry, molecular biology, cell-based assays including potency, immunoassays, biophysical methods and microbiology

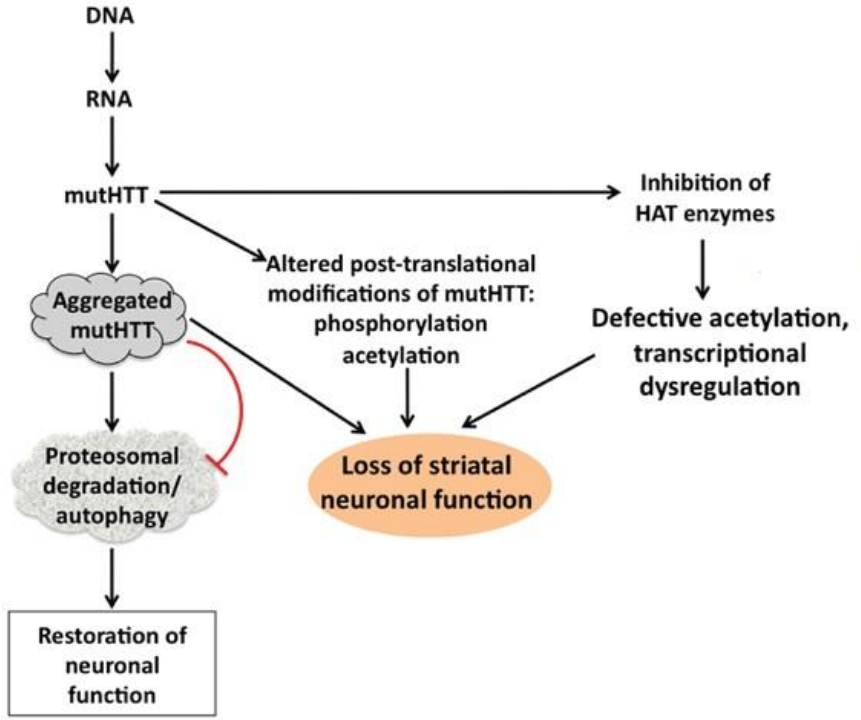
Contents

Huntington's Disease (HD) pathogenesis

- HD is an inherited, fatal neurological condition caused by **mutation of the HTT gene**.
  - CAG trinucleotide repeat expansions lead to **polyglutamine (polyQ)** tracts of variable length in the protein.
- mutHTT protein forms **intracellular aggregates** in neurons, and aggregation correlates with polyQ expansion and neurotoxicity.
- Histopathological hallmarks: neuronal loss in the striatum (**motor and reward systems**); enlarged lateral ventricles (cerebrospinal fluid). Degeneration occurs in the cerebral cortex (**cognition**) during progressed stages of HD.



Huntington's Disease Brain Pathology (Bio-Techne)



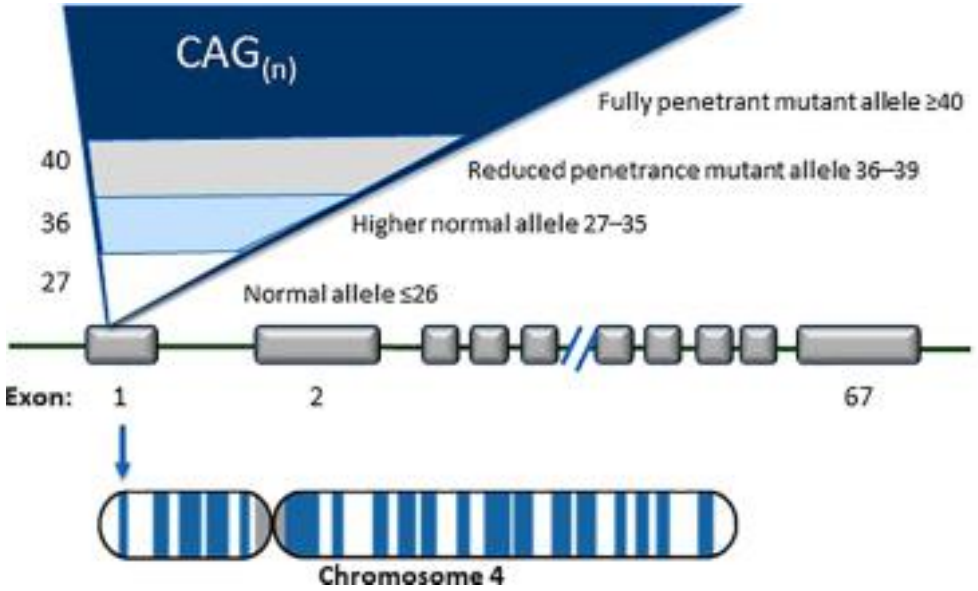
Huang *et al.* Experimental and Therapeutic Medicine. 2016

HD genetics overview

Individuals with Huntington disease (HD) are mostly **heterozygous for the CAG repeat**:

- having one wild-type (wtHTT), and
- one abnormally expanded mutant huntingtin gene (mutHTT) allele

Length of the polyQ correlates with phenotypic dysfunction



Description of gene	CAG repeat range	Risk of HD	Risk of HD in next generation
Normal	≤26	No HD	No
Higher normal	27–35	No HD	Possible
Reduced penetrance	36–39	Possible HD	Yes
Full penetrance	≥40	Definite HD	Yes

Gatto *et al.* Clinical Parkinsonism & Related Disorders. 2020

Specific SNPs are co-located with the mutHTT allele and can be targeted while preserving wild-type *HTT* (wtHTT) allele (Claassen et al. Neurology Genetics. 2020).

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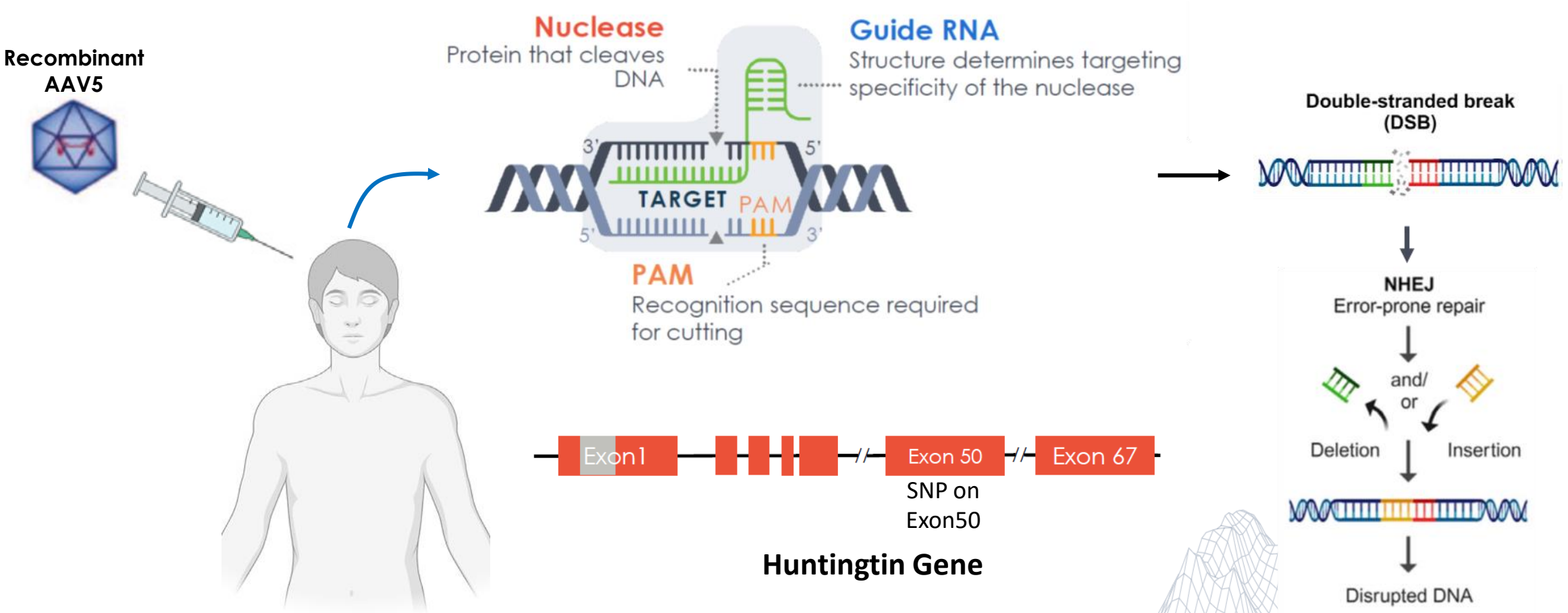
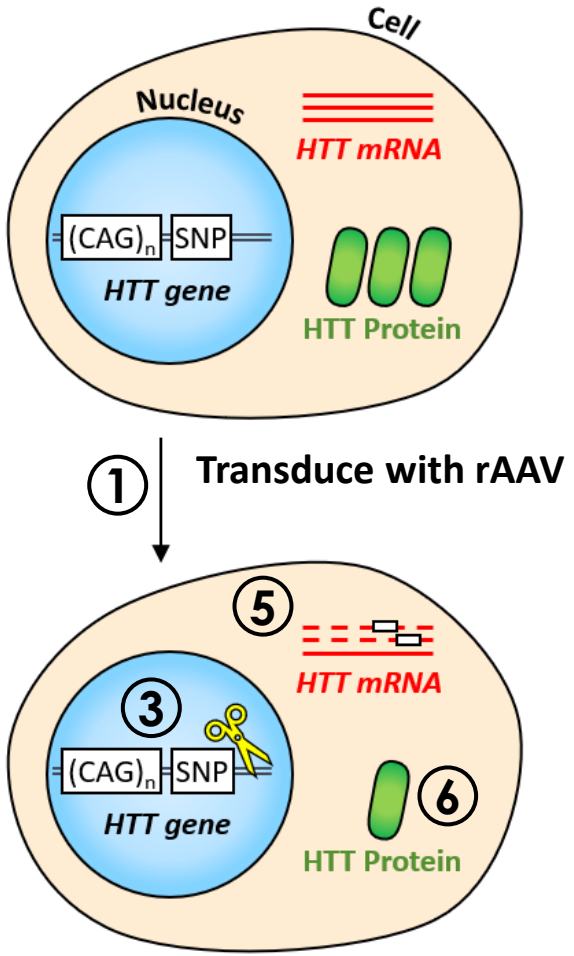
LETI-101: An Investigational *In Vivo* Gene Therapy for HD



Adapted from Palacios *et al.* Frontiers In Genome Editing. 2024

Molecular events leading to intended therapeutics activity

1. **Transduce** cells with AAV encoding nuclease and guide RNA.
2. Cells **express** the RNP complex in nucleus.
3. Nuclease cleaves DNA, leading to **double-stranded breaks**.
4. DNA repair mechanisms are activated, producing nucleotide insertions/deletions (**INDELs**) of random size at the target site.
5. INDELs can generate frameshift mutations, resulting in **premature termination codons** in the transcribed HTT mRNA.
6. mRNA is degraded by the **nonsense-mediated decay pathway** within the cell, ultimately leading to a loss of mutant HTT protein.



Strategy for potency development

A potency assay must ensure intended therapeutic effect measuring the biological activity (FDA 2023)

A potency assay should assess MoA, therapeutic activity, or intended biological effect (FDA 2011)

The biological cascade for the rAAV5 product is as follows:

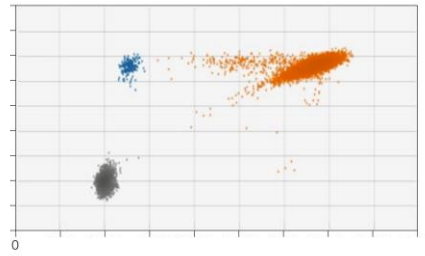
STEPS FOR EXPRESSION

AAV Vector Transduces Cells ▷ DNA Delivery to Nucleus ▷ RNA Transcription ▷ Protein Translation ▷ RNP Complex Assembles in Nucleus

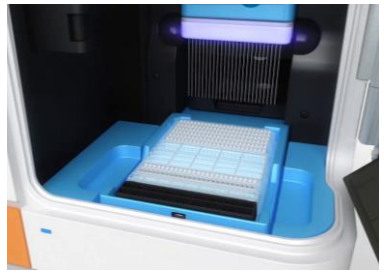
STEPS FOR ACTIVITY

DNA Double-Stranded Break ▷ INDEL (PTC) ▷ mRNA Nonsense-Mediated Decay ▷ Loss of mHTT Protein ▷ Biological Intervention ▷ Intended Therapeutic Effect

ddPCR-Based Drop-Off Assay (NHEJ Detection)



Capillary or ELISA-Based Immunoassay



%Editing Potency
(Focus of this talk)

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Attributes of a suitable cell line for indel potency

Critical Attributes	Challenge
Permissive to AAV5 transduction	AAV5 serotype has poor *in vitro* cell line transduction
Contains target SNP for RNP target engagement	SNP must be experimentally determined for each cell line; cells may not contain target SNP

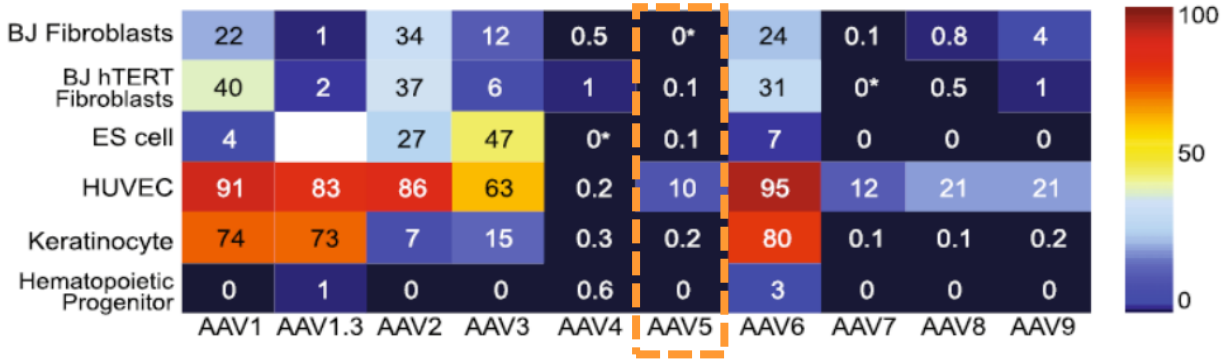
Other Preferred Attributes:

- ❑ Easy to grow and maintain in culture
- ❑ Commercially available
- ❑ Less lot-to-lot variability; established cell line preferred
- ❑ Representative of the target tissue or a representative surrogate

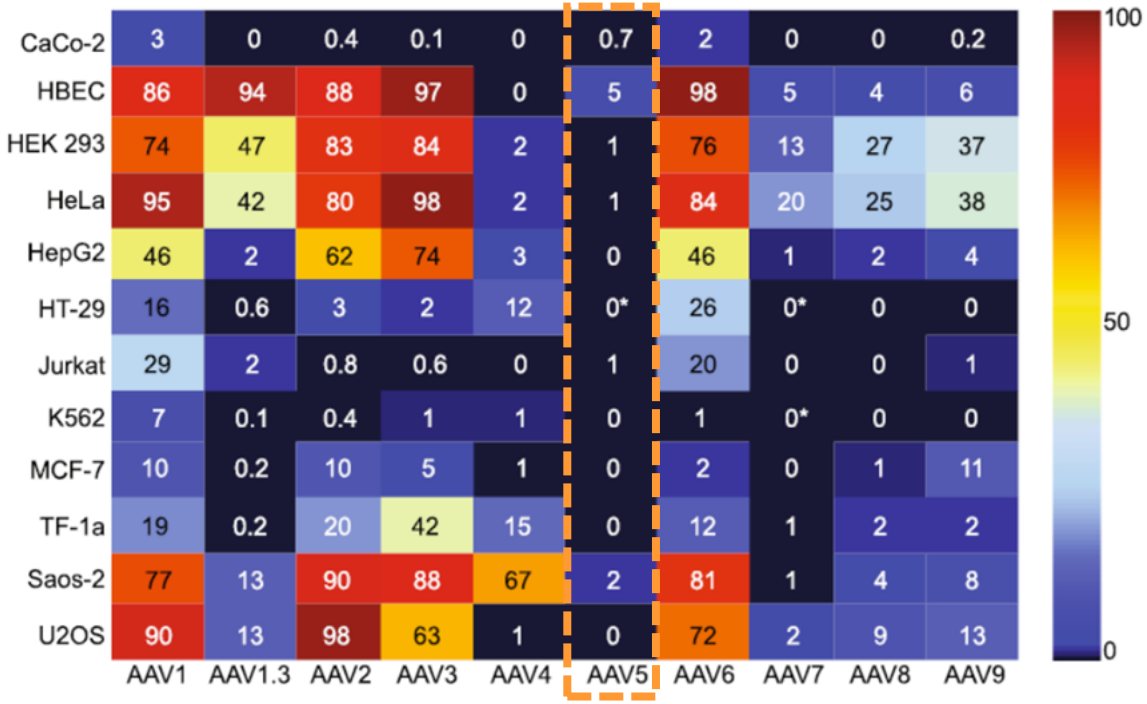
Permissibility of AAV serotypes in human cells

- In general, AAV5 demonstrates poor transduction efficiency in primary or immortalized human cells in culture
- Different AAV serotypes have evolved distinctive interactions with the same receptor
- AAV5 is known to use o-linked sialic acid as the primary receptor and PDGFR as a co-receptor (Kaludov *et al.* 2001; Seiler *et al.* 2006; Wu *et al.* 2006; Di Pasquale *et al.* 2003)
- AAV5 also interacts with PKD domain (PKD1) of AAVR to promote transduction (Di Pasquale *et al.* 2017)

AAV Transduction of human primary cells



AAV Transduction of human immortalized cells



AAV serotypes expressing GFP reporter were used for transducing at a MOI of 10^5 vg/ cell followed by flow cytometry analysis.

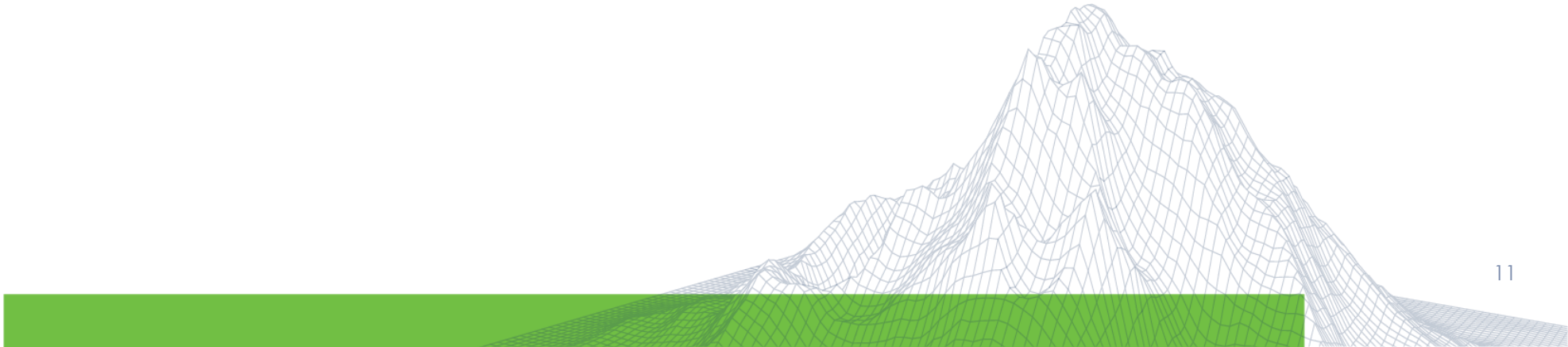
The number in the box is the actual percentage of GFP positive cells with that serotype.

** = Transduction less than 0.01% but greater than 0.0%*

Data adapted from Ellis *et al.* Virology Journal. 2013

Screening strategy for a suitable cell line for potency

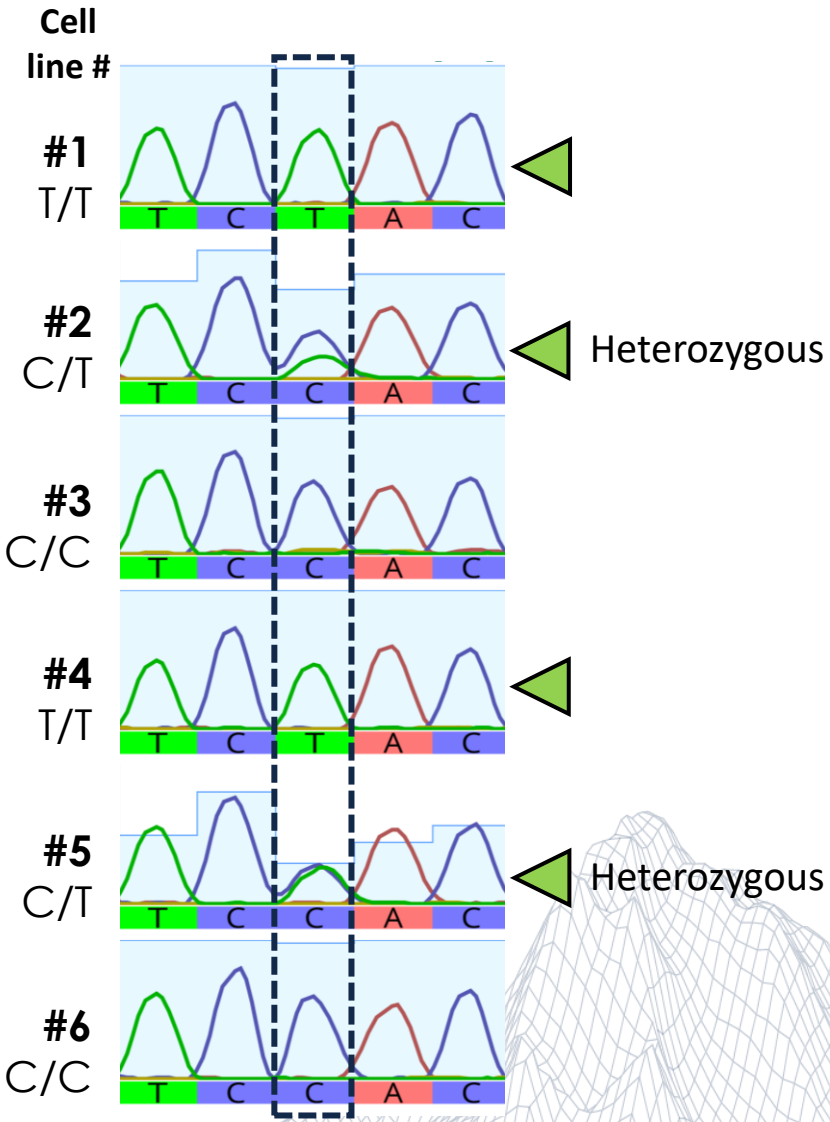
	Attributes	Screening Strategy
1.	Contains target SNP for RNP target engagement	Screen for the specific SNP by sanger sequencing
2.	AAV5 transduction efficiency	Screen candidate cells using AAV5-GFP as surrogate by flow cytometry
3.	Easy to grow and maintain in culture	Check cell viability and population doubling



1. SNP screening in different cell lines by sanger sequencing

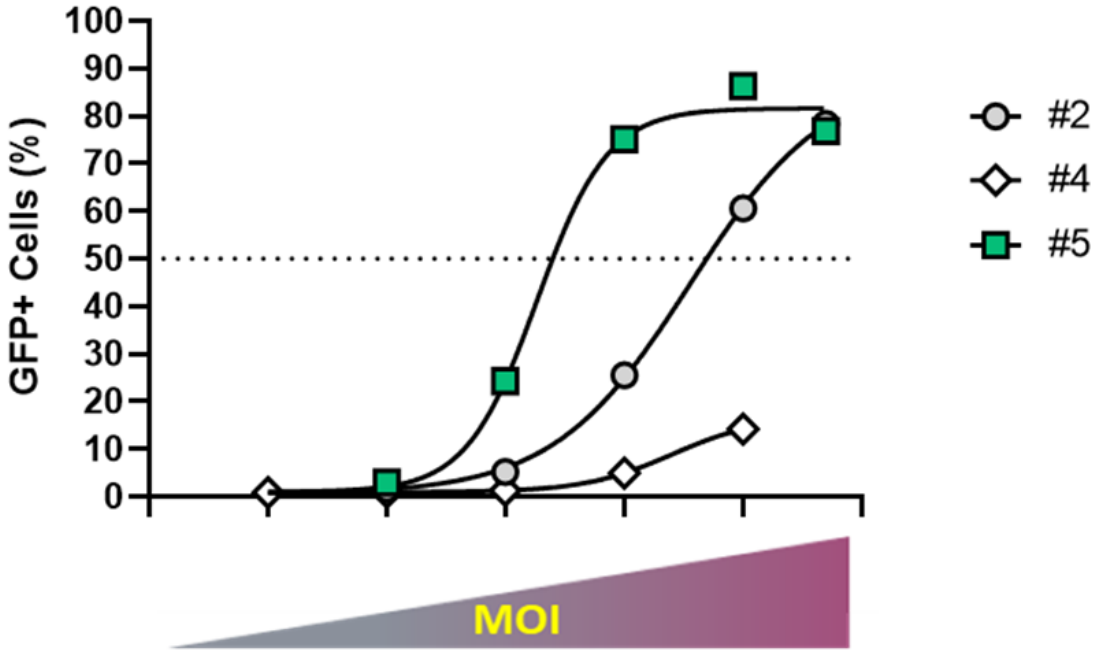
SNP analysis:

At least one ‘T’ allele is required for target engagement.

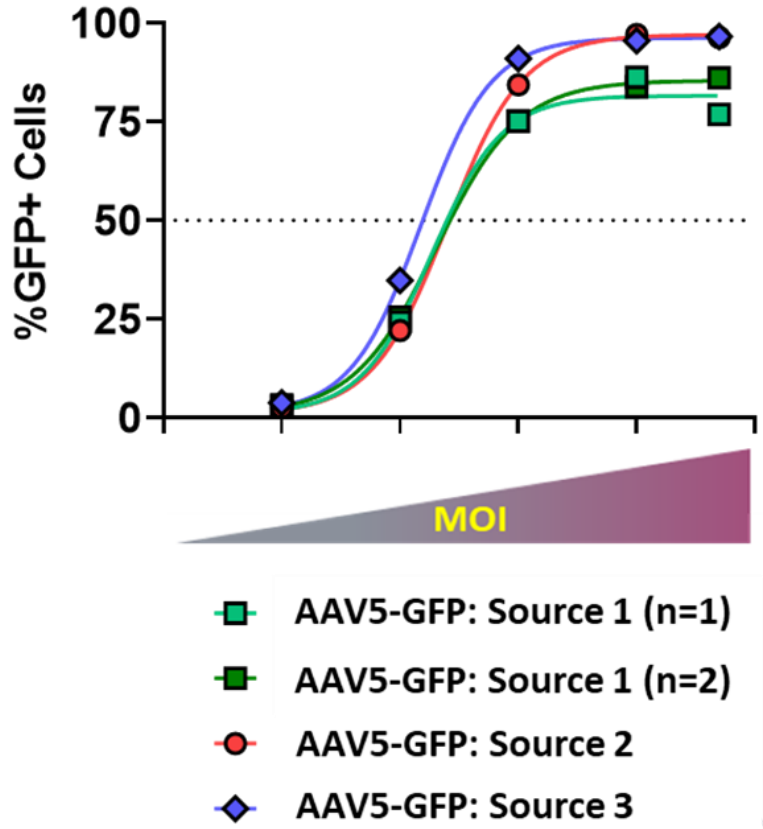


2. AAV5 transduction efficiency screening

Candidate cells were transduced with AAV5-GFP (ElevateBio) and assessed by flow

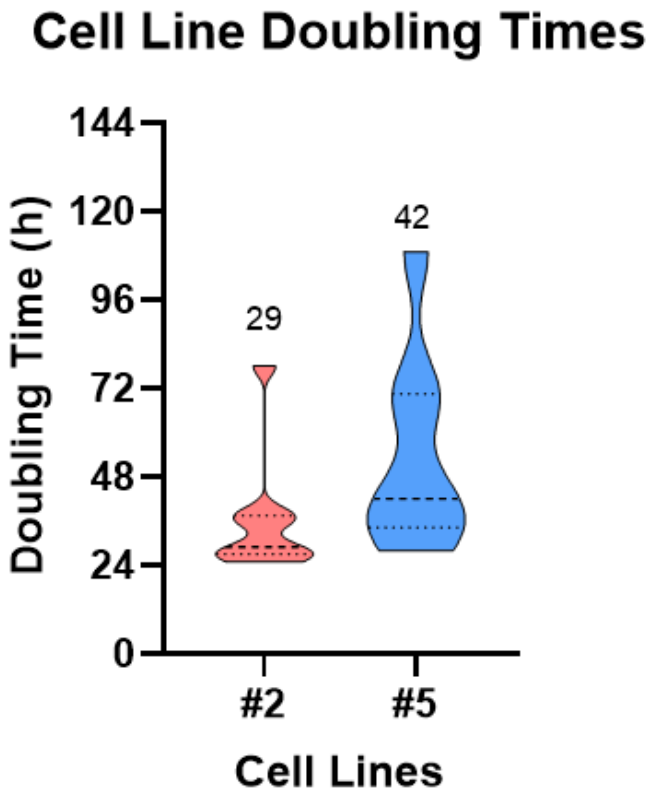


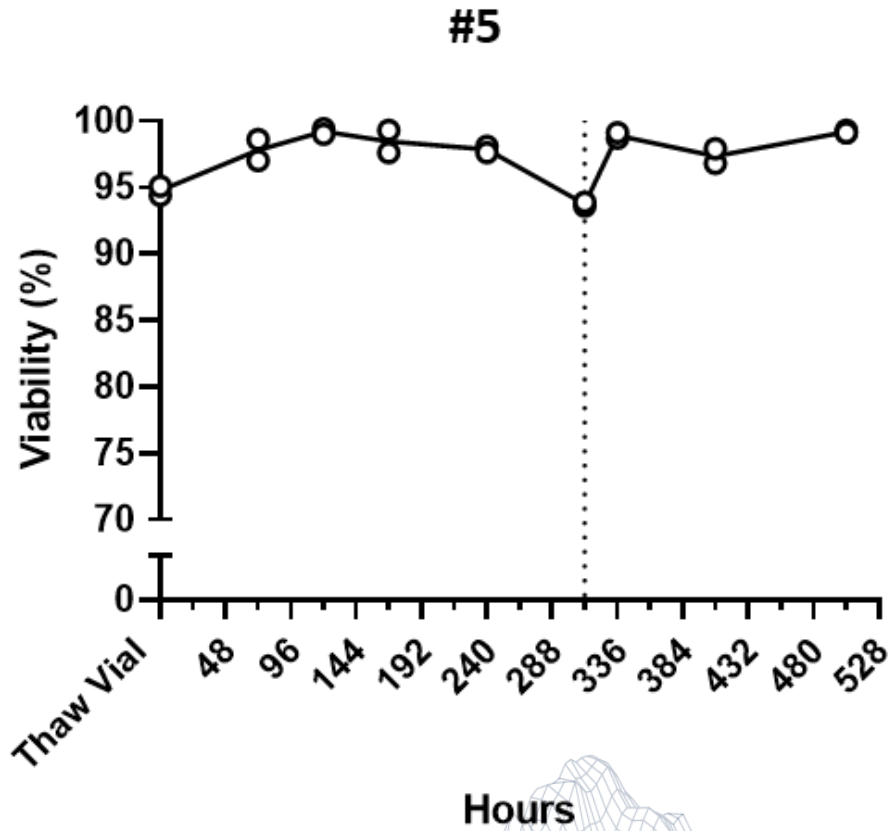
Transduction efficiency in Cell line #5: Repeatability with AAV5-GFP from 3 different sources



Cell line #5 showed the best transduction efficiency

3. Growth in culture





Candidate #5: Established cell line, easy to culture and maintain

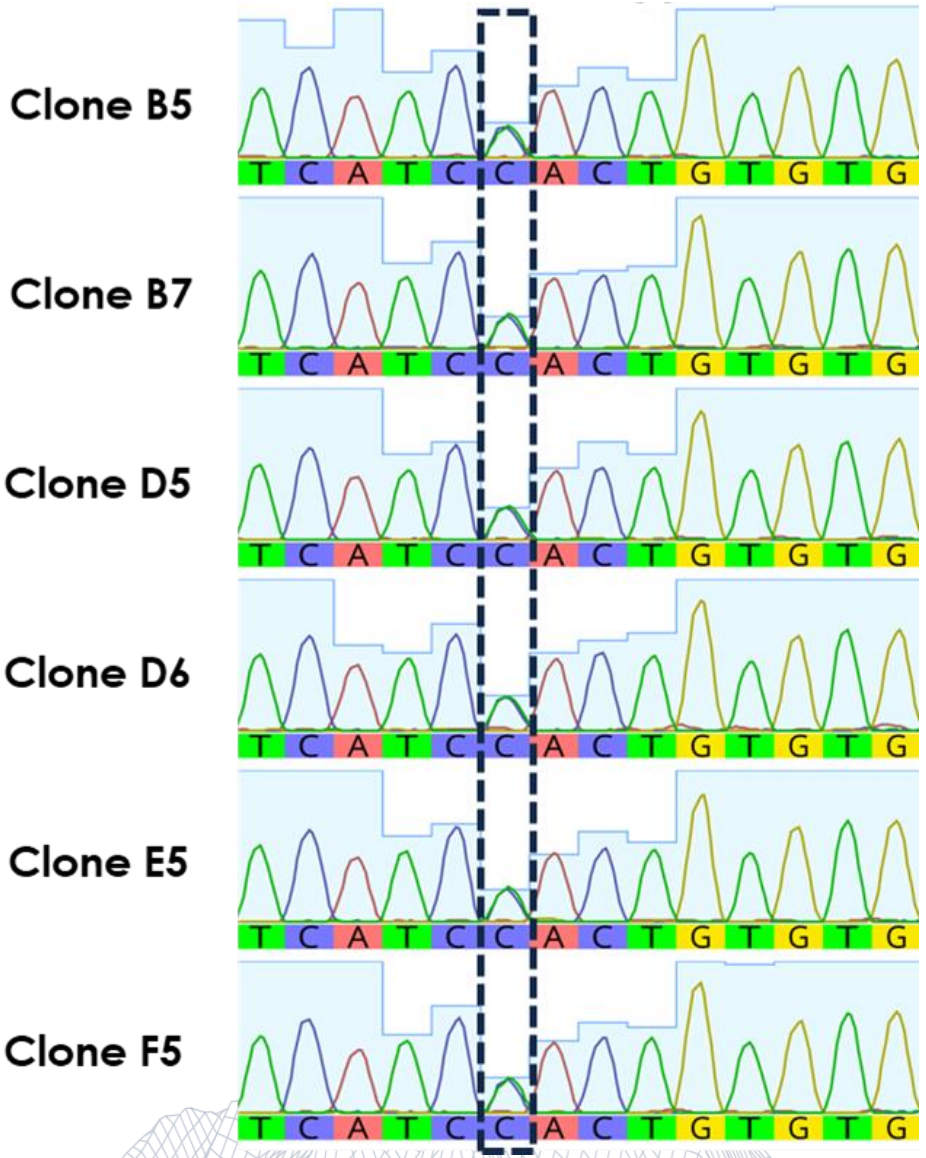
Clonal purity of cell line #5

Could #5 be a mixed population instead of a true heterozygous cell line?

i. Single cell clones were generated

ii. Clonal populations of cells were isolated and cultured over the course of 35 days

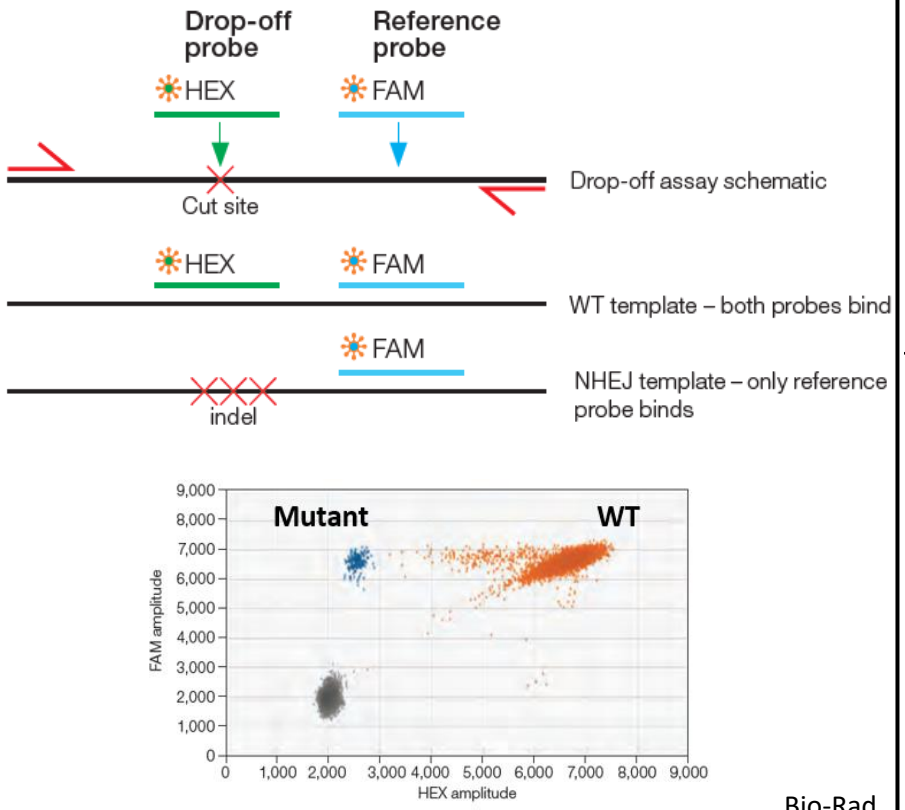
iii. 6 clones were sequenced to confirm that the clones were **heterozygous C/T** and <u>not</u> a mixed population of homozygous cells

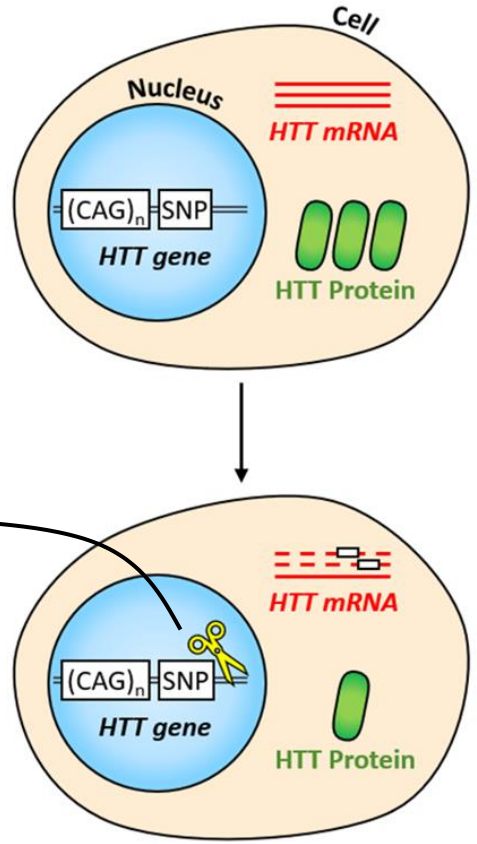


Indel potency design: Measuring %Editing by ddPCR

Measure Genome Editing

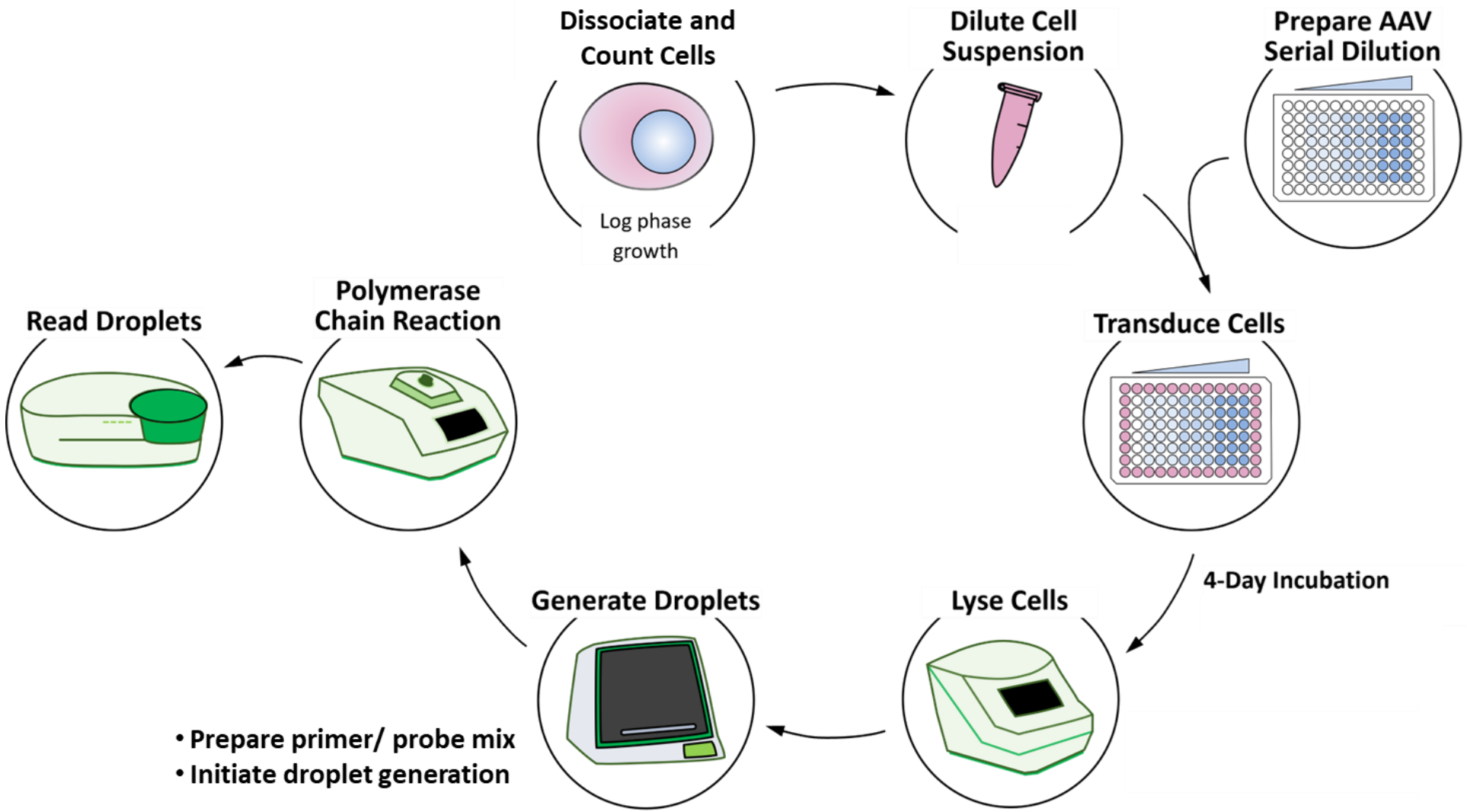
INDEL detection by ddPCR with reference and drop-off probes.



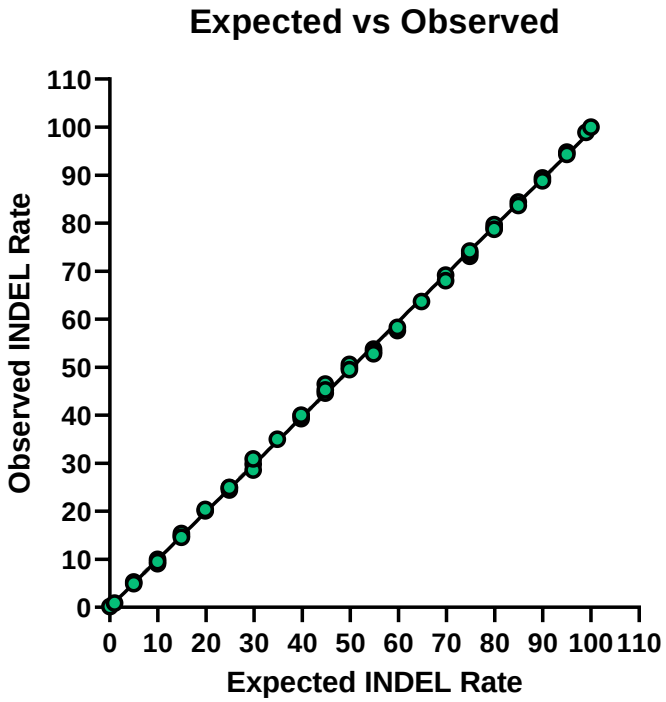


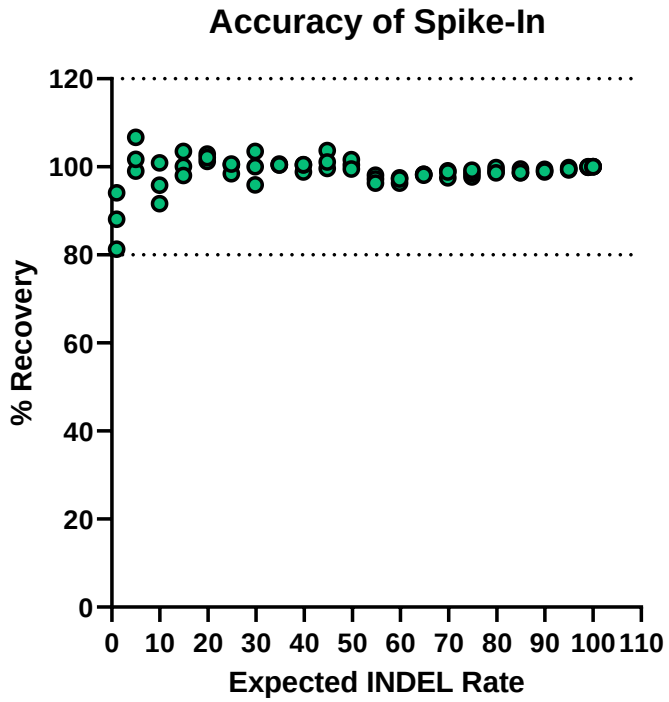
- ddPCR primers and probes were designed for a NHEJ drop-off assay
- Editing will prevent the NHEJ/drop-off probe from binding
- Reference probe binds to all alleles
- gBlocks were designed to mimic INDELs expected from nuclease cutting

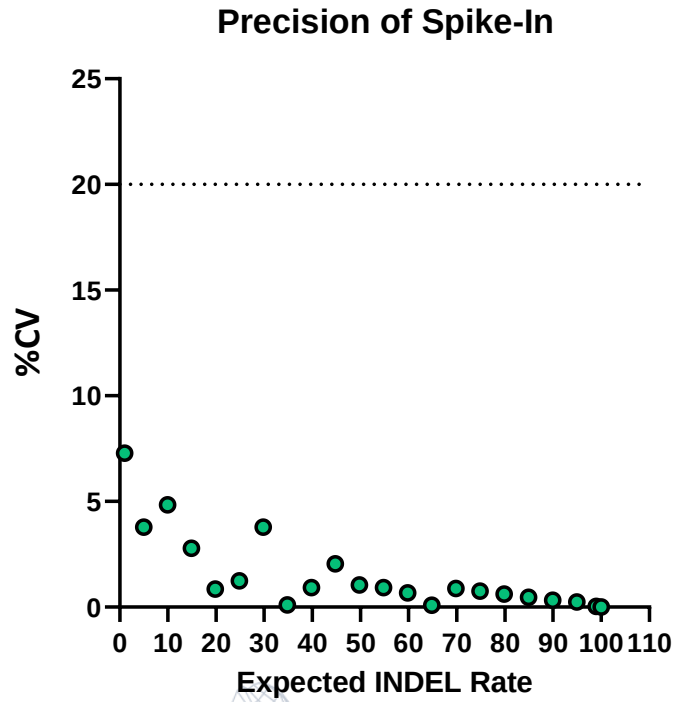
ddPCR method overview



Assay performance using synthetic DNA



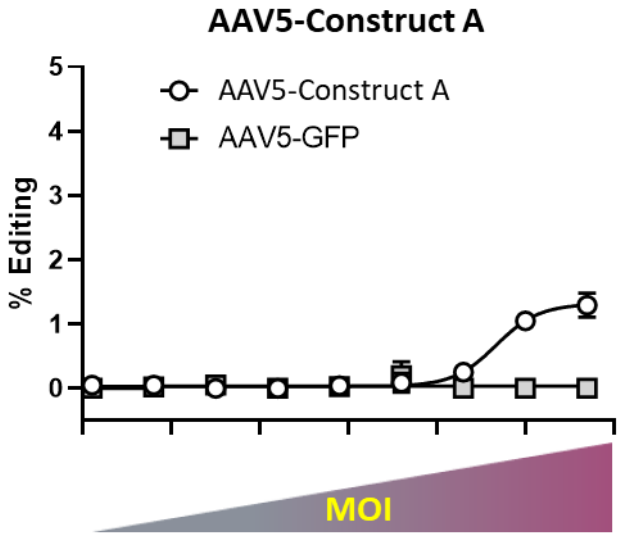


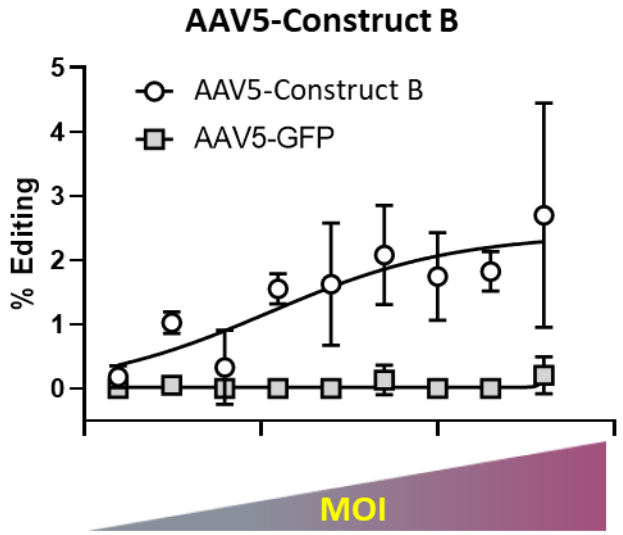


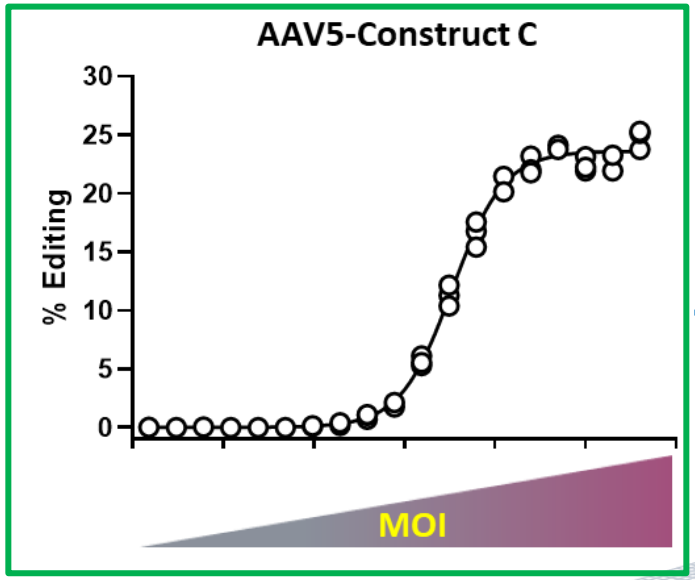
	Slope
Observed INDEL Rate	0.9911

	R squared
Observed INDEL Rate	0.9995

Determining %Editing of different AAV constructs

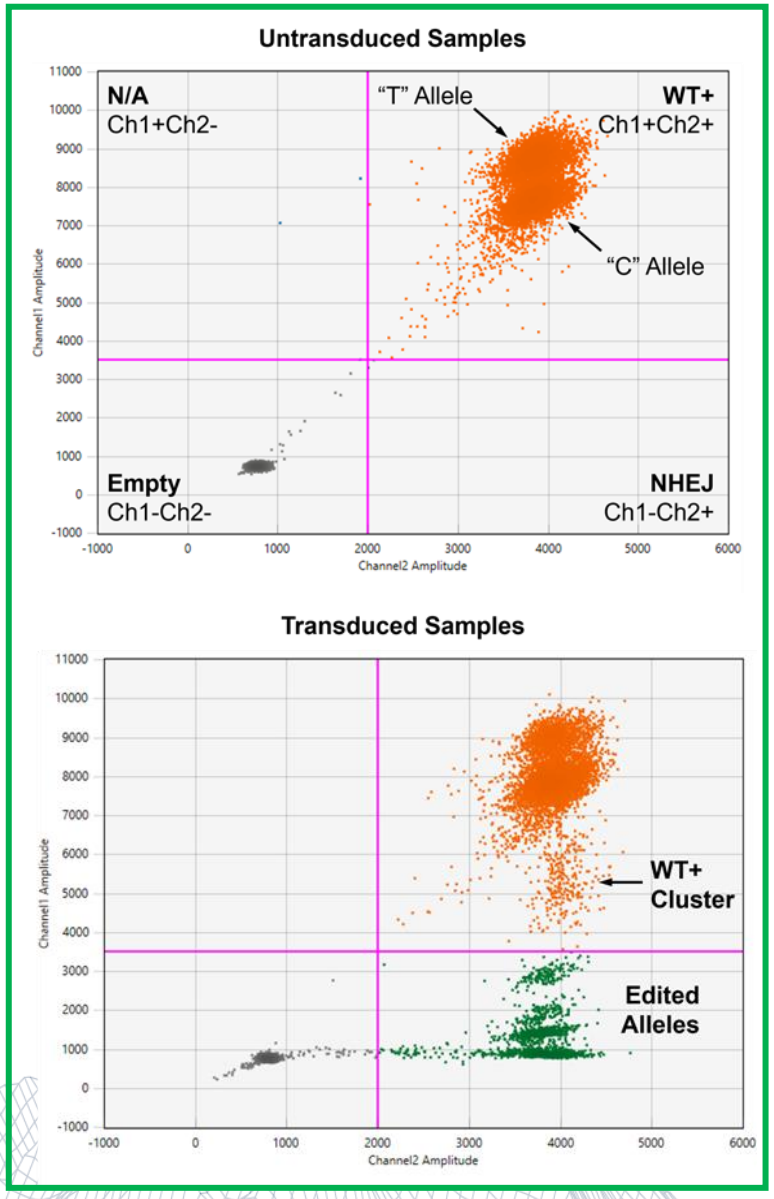






Target cells are **C/T heterozygous**:

So, **Max %editing is 50%** based on accessible target engagement



Summary

- A suitable cell line was identified for the Indel detection potency assay:
  - Showed >80% transduction efficiency by flow cytometry
  - Contained the unique SNP for target engagement
- A potency method measuring INDEL by ddPCR was developed for an *in vivo* gene editing therapy delivered by AAV:
  - Initial method development using synthetic g-block DNA
  - The assay showed good accuracy and precision
  - The assay performance was confirmed using the identified cell line

ACKNOWLEDGEMENTS

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