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Spearheading Change from Scientists to Patients: The Artificial Intelligence Impact on Cell and Gene Therapy

Abstract

Cell and gene therapies (CGTs) have incredible potential for patients, yet critical construct design problems lead to prohibitively high manufacturing costs and elevated risk of adverse immune responses. To overcome these design issues, we've developed a novel CGT-specific artificial intelligence (AI) solution, FORMsight^{AI}, that proactively identifies and corrects potential issues that decrease construct quality and yield early on in the clinical development process. Here, we discuss our partnership with a publicly traded gene therapy company that was struggling with viral vector truncation during the packaging of its adeno-associated virus (AAV)-based therapeutic. Working with Form Bio's experts and using FORMsight^{AI} resulted in an 18% increase in full reads and a 70% decrease in truncations. These development improvements will increase the accessibility and safety of gene therapies while drastically decreasing R&D timelines, and, ultimately, time-to-market.

Introduction

AAV Viral Vector Impurities Lead to CGT Safety Concerns

There are significant hurdles that stand in the way of rapid, streamlined development and manufacturing of safe, efficacious cell and gene therapeutics. AAV-based vectors have been linked to quality issues due to inefficient manufacturing processes. More specifically, impurities of AAV viral capsid production have been shown to be contaminated with non-therapeutic genomes which lead to higher relative dosing, inducing an inflammatory immune response in patients causing significant adverse events[1] and in some cases death.[2]

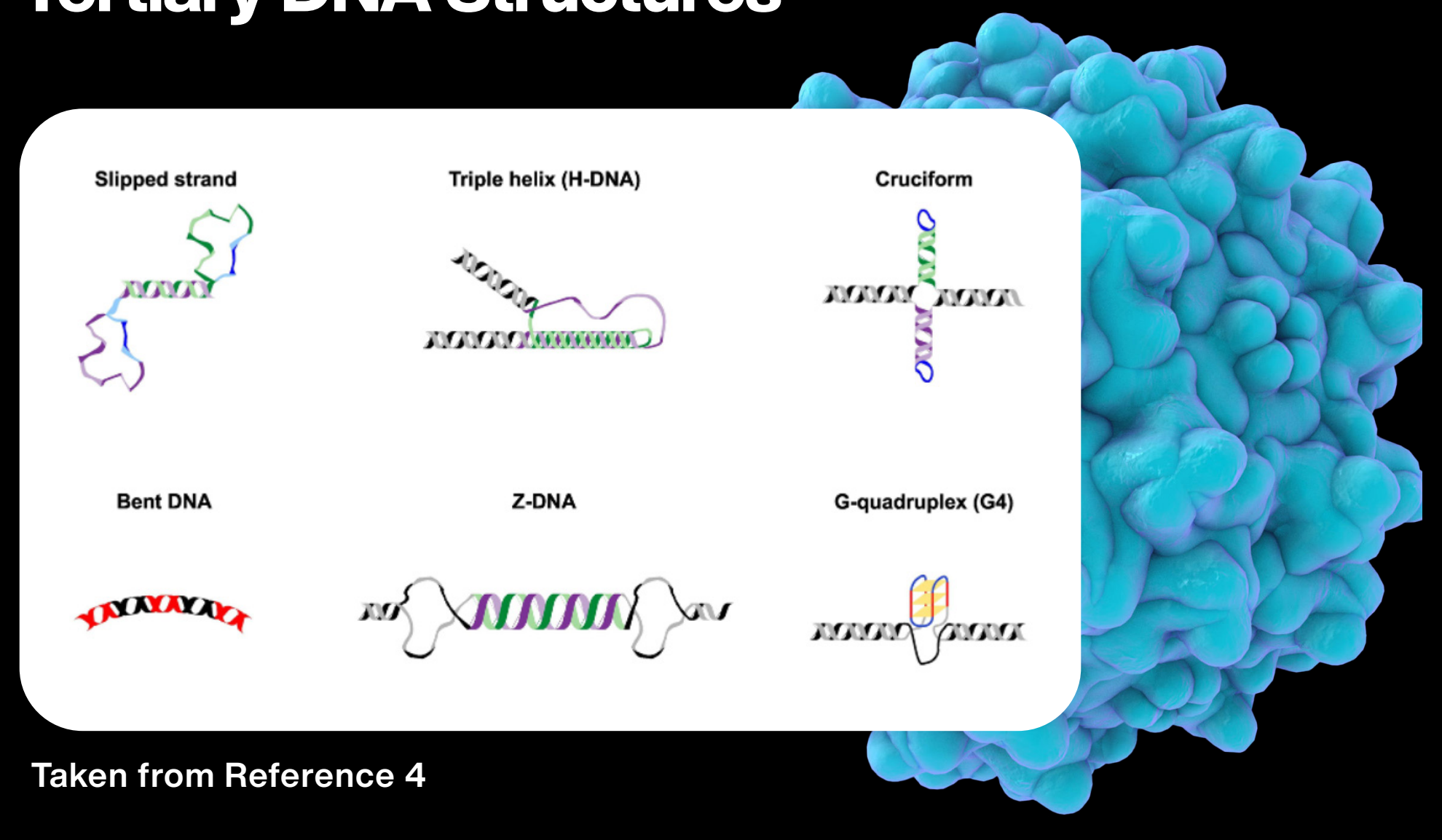
FDA Draft Guidance on AAV-Based Gene Therapies

In 2021, the FDA's Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) shared a report and draft guidance* on safety issues for AAV-based gene therapies. In its report the FDA singled out the importance of screening AAV-based gene therapies for empty capsids.[3]

*SOURCE: PROPOSED DRAFT GUIDANCE FOR FDA CONSIDERATION: TESTING OF ADENO-ASSOCIATED VIRAL (AAV) VECTOR-BASED HUMAN GENE THERAPY PRODUCTS FOR EMPTY CAPSIDS DURING PRODUCT MANUFACTURE. 2021

Empty capsids or capsids with partial genomes are caused by secondary and tertiary DNA structures in the genome that lead to replication errors and increased mutation rates. Minimizing these structures through sequence optimization is reliant on trial and error and not often addressed until the manufacturing phase in the later stage of clinical development.

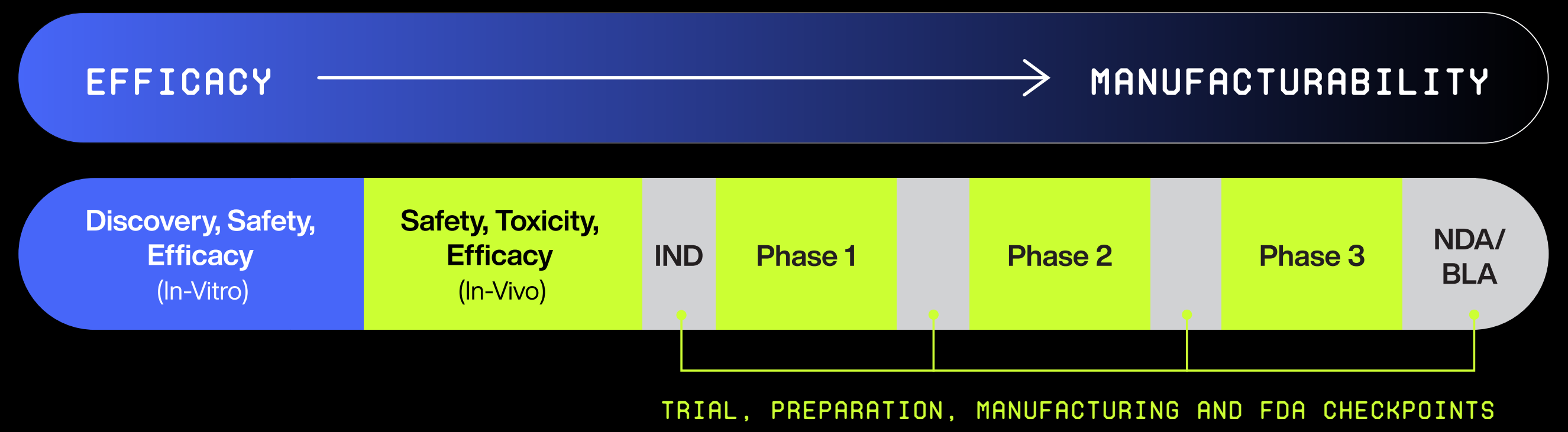
Failures in Replication and Packaging of AAV Viral Vectors as a Source of Impurities Caused by Secondary and Tertiary DNA Structures



Taken from Reference 4

Late Stage Clinical Success Starts with Predicting Potential AAV Construct Failures Early In The Clinical Development Process

As cell and gene therapy development progresses, attention shifts from efficacy alone to manufacturability and cost efficiency.



EFFICACY → MANUFACTURABILITY

Discovery, Safety, Efficacy (In-Vitro) | Safety, Toxicity, Efficacy (In-Vivo) | IND | Phase 1 | Phase 2 | Phase 3 | NDA/BLA

TRIAL, PREPARATION, MANUFACTURING AND FDA CHECKPOINTS

Methods

Form Bio's Transformer Neural Network Architecture Development[5,6,7,8]

INVESTED IN AN ADVANCED AI TECHNOLOGY STACK FOR MODEL TRAINING FOR HYPERPARAMETER OPTIMIZATION AND INFERENCE PERFORMANCE.

TRAINED MACHINE LEARNING MODELS TO IDENTIFY TRUNCATION PROPENSITIES - INCLUDING TRUNCATION DIVERSITY AND MAGNITUDE - BASED ON SPECIFIC, WELL-ESTABLISHED DNA FEATURES.

UTILIZE CODING, TRAINING AND INFERENCE ARCHITECTURE ON PRE-TRAINED MODELS FOR METHYLATION AND VARIANT EFFECT PREDICTION.

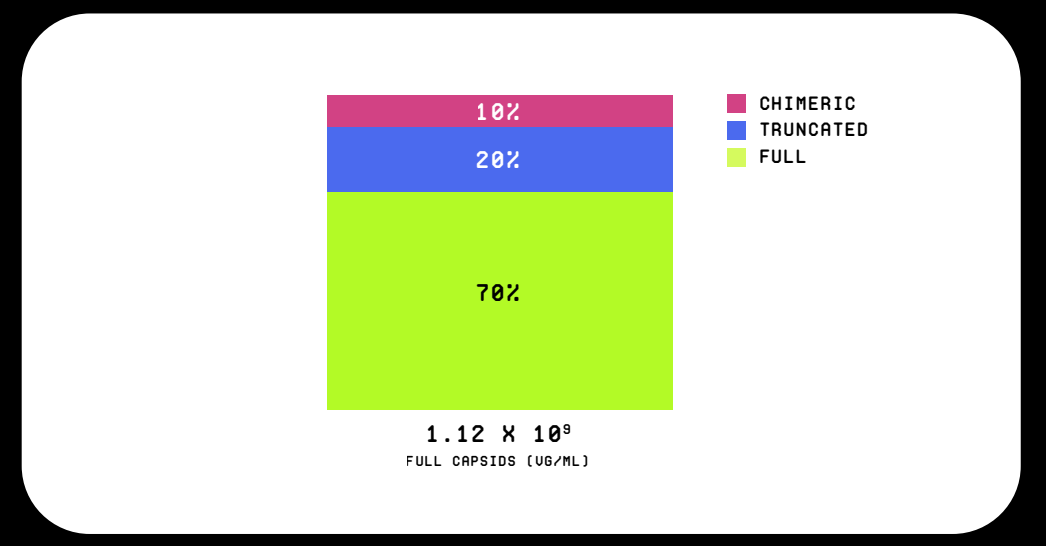
ADAPTED STATE-OF-THE-ART TRANSFORMER NEURAL NETWORK ARCHITECTURE FOR LARGE LANGUAGE MODELS, APPLIED TO DNA AND PROTEIN ANALYSIS.

FORMsight^{AI}

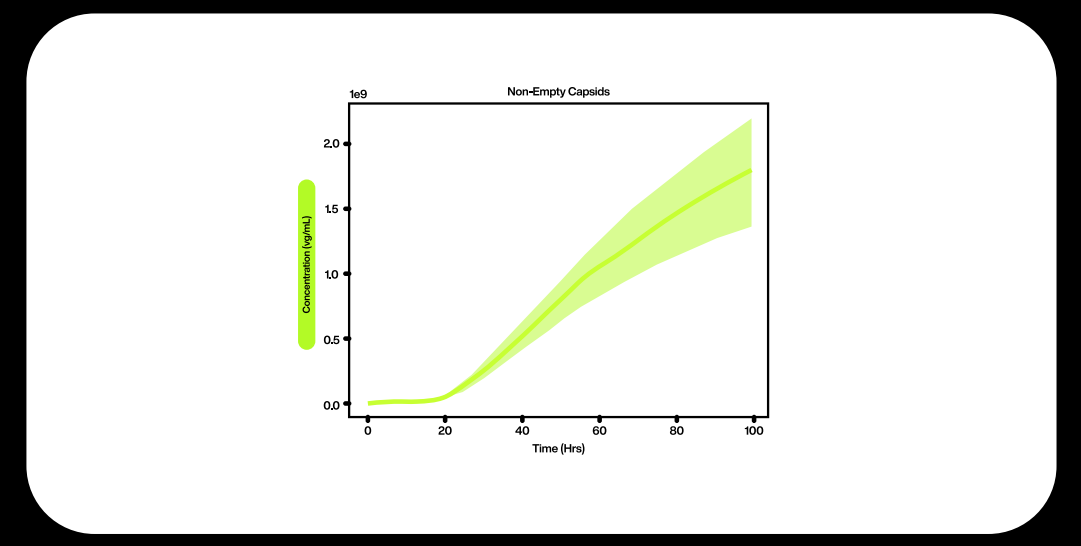
Utility of our breakthrough AI-powered solutions, FORMsight^{AI} SIMULATE and FORMSight^{AI} OPTIMIZE was employed to analyze and predict AAV viral vector propensity for impurities without costly trial and error.

With FORMsight^{AI} SIMULATE we:

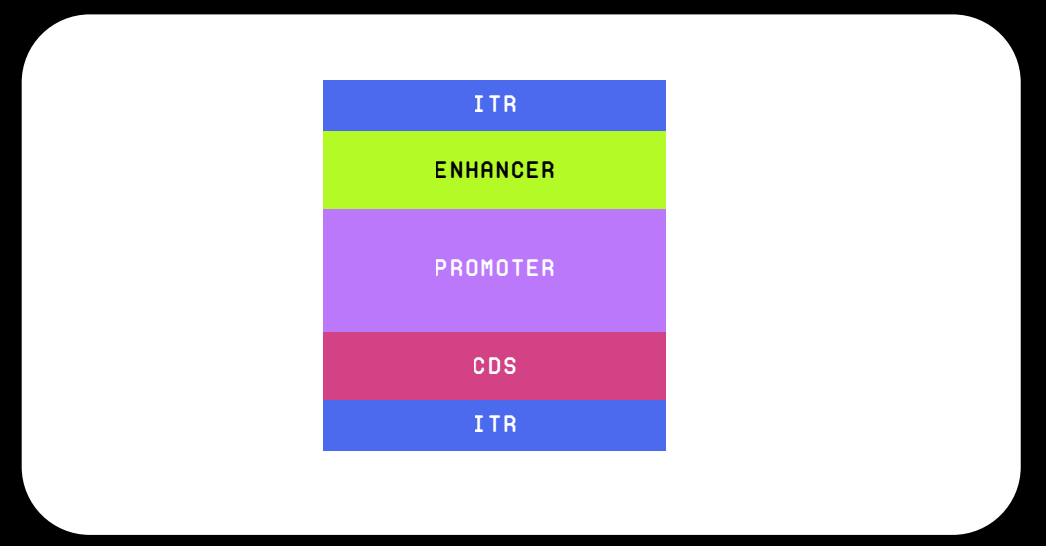
Predicted Capsid Fill Rates by Full, Truncated and Chimeric



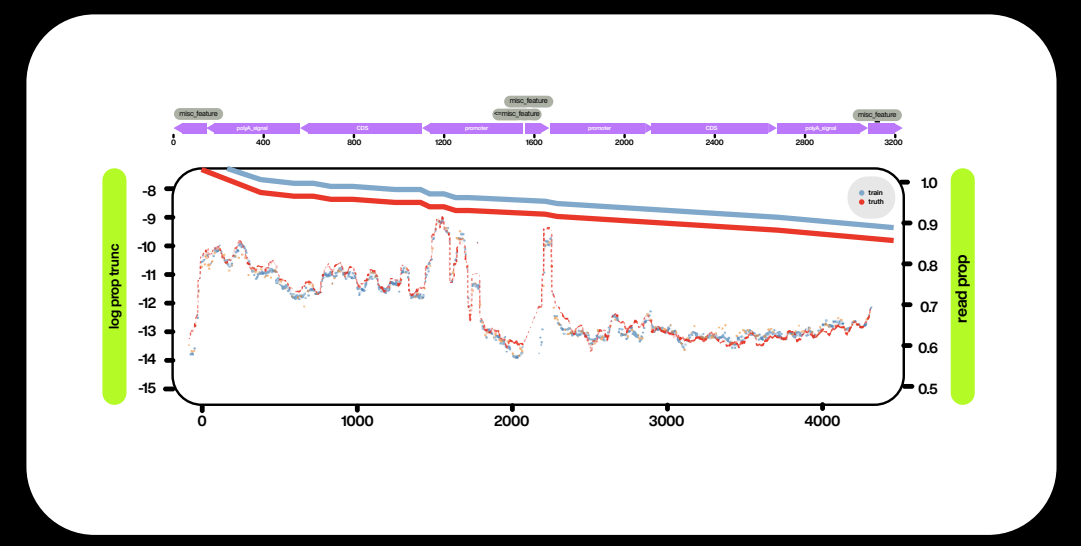
Simulated Capsid Fill Progress Per Hour



Understood Truncation Propensity Contribution by Region



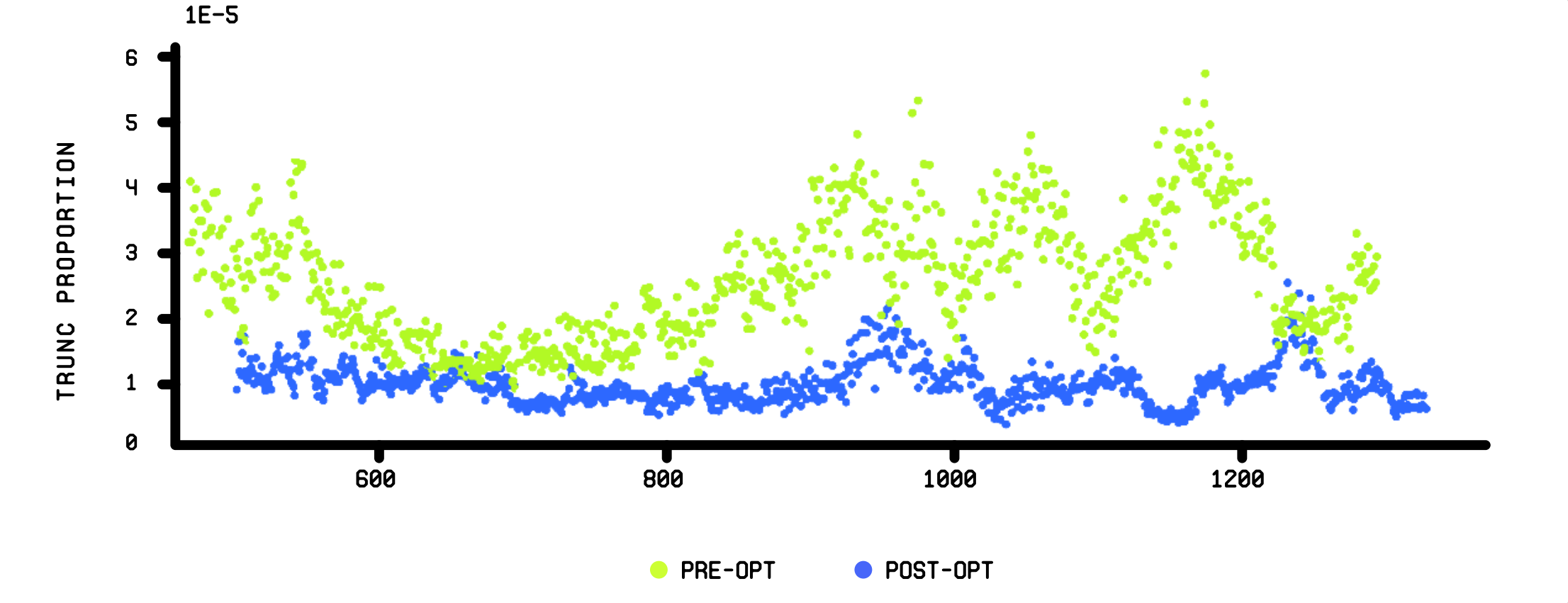
Predicted Truncation Propensities and Hotspots by Structure Type



With FORMsight^{AI} OPTIMIZE we:

- Identified the ideal combination of construct parameters for maximized predicted full reads, minimized predicted truncation propensity and other key factors
- Generated a new construct design blueprint

Example Pre- and Post-Optimization Comparison of Truncation Propensities



Results

FORMsight^{AI} Predicted Failures and Optimized AAV Constructs Driving Clinical Development Success

FORMsight^{AI} was deployed in partnership with a publicly traded gene therapy company to reduce the amount of truncated constructs and resulting manufacturing impurities in an AAV-based therapeutic. Our deep learning model successfully identified three main areas of concern: unoptimized codons, CpG islands, and secondary and tertiary structures. By recognizing these problematic sequences, we accurately predicted truncation and were able to optimize the promoter and coding sequence (CDS) and suggest alternative promoters to minimize truncations ultimately improving manufacturability and yield.

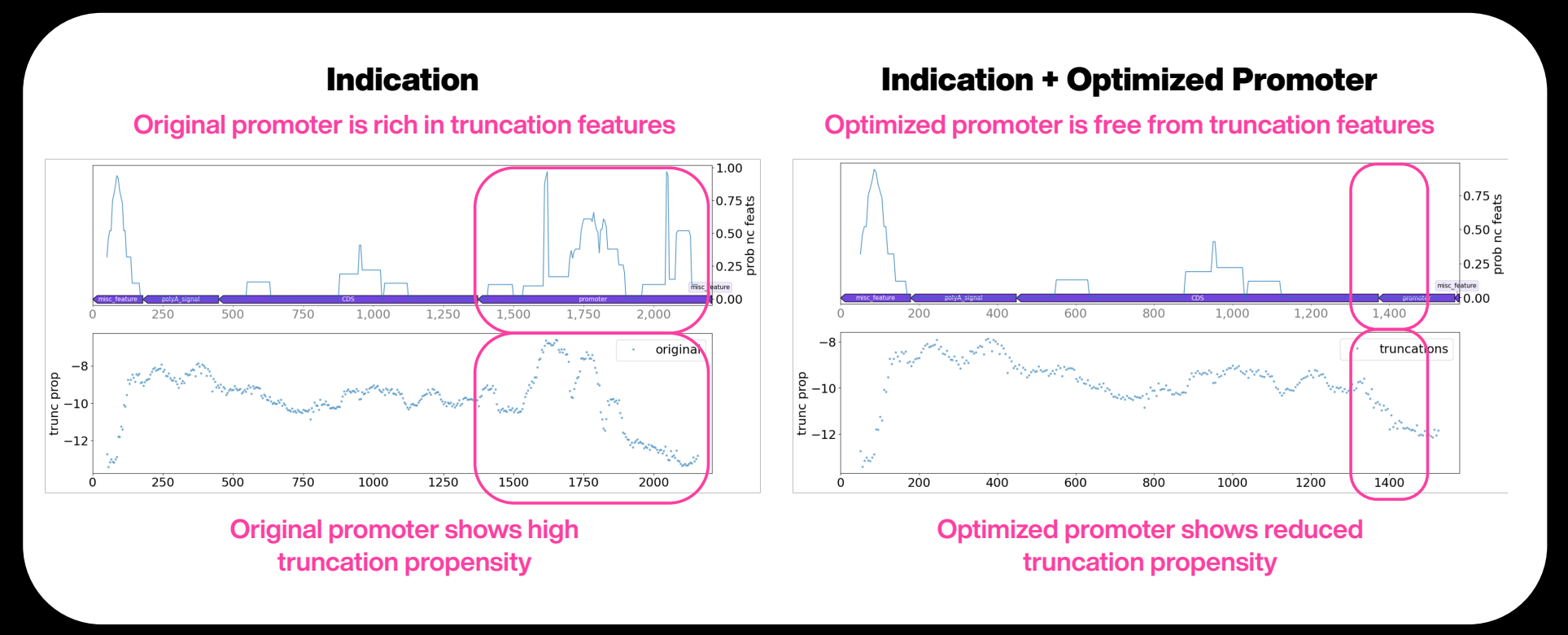


Figure 1: Optimized promoter dramatically reduced secondary and tertiary structures and truncation propensity

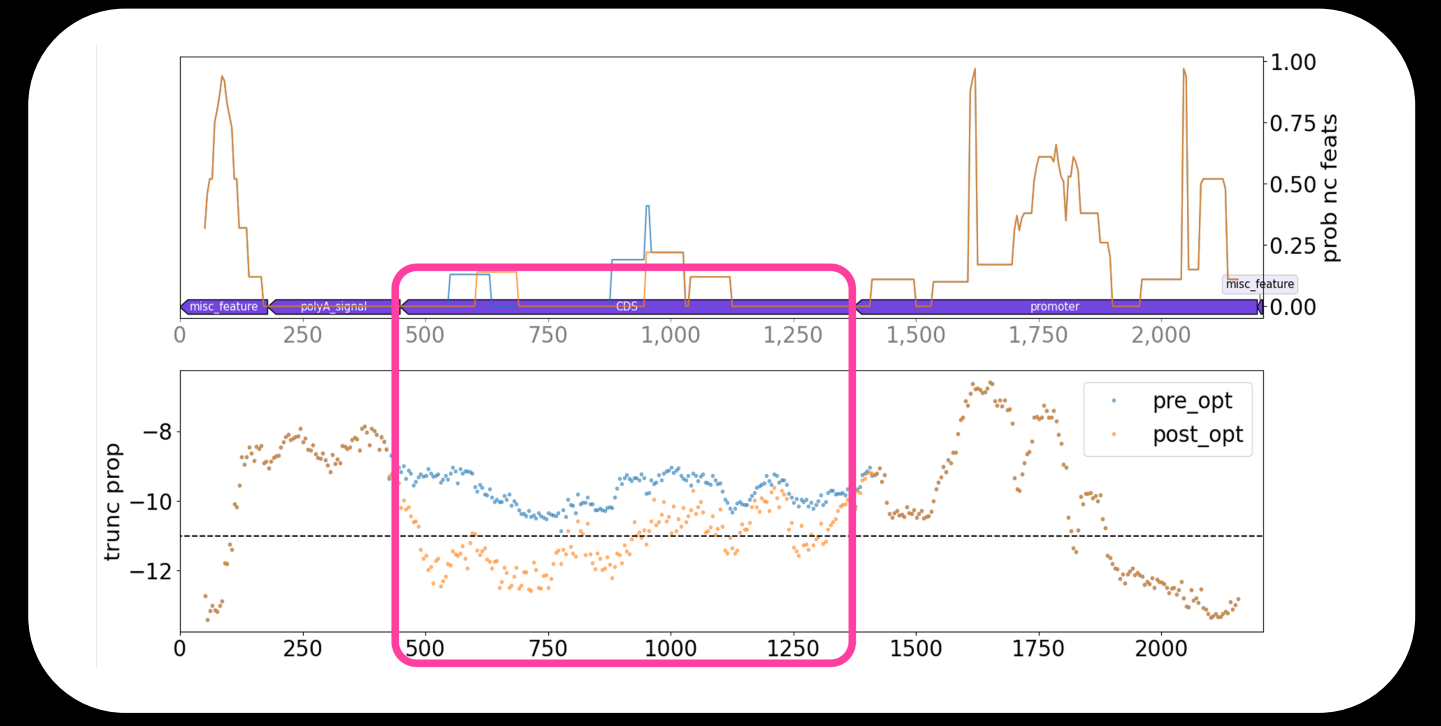


Figure 2: Optimized coding sequence (CDS) reduced truncation propensity

UP TO
18%
INCREASE IN FULL READS

UP TO
70%
DECREASE IN TRUNCATIONS

Figure 3: Using FORMsight^{AI}, our partner increased full reads and decreased truncated reads of their AAV-based gene therapy.

Conclusion

Here, we've trained and deployed a powerful deep learning and optimization framework, FORMsight^{AI}, focused on predicting the truncation propensity of AAV viral vectors and optimizing constructs early in the clinical development pathway for improved late stage manufacturing success. In collaboration with a publicly traded gene therapy company, we've completed a proof-of-concept study that was able to pinpoint problems during viral production, provide optimization guidance, and enable the selection of the most viable constructs to be tested for improved manufacturability. The widespread application of our AI-based solution to make manufacturing easier, faster and less expensive offers exciting possibilities for cell and gene therapy developers to significantly impact the accessibility and the treatment options for patients with life threatening diseases.

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REFERENCES:
1. MAURYA, S., SARANGI, P. & JAYANDHARAN, G.R. SAFETY OF ADENO-ASSOCIATED VIRUS-BASED VECTOR-MEDIATED GENE THERAPY-IMPACT OF VECTOR DOSE. CANCER GENE THER 29, 1305-1306 (2022).
2. HIGH-DOSE AAV GENE THERAPY DEATHS. NAT BIOTECHNOL *NEWS BRIEF* 38, 910 (2020).
3. PROPOSED DRAFT GUIDANCE FOR FDA CONSIDERATION: TESTING OF ADENO-ASSOCIATED VIRAL VECTOR-BASED HUMAN GENE THERAPY PRODUCTS FOR EMPTY CAPSIDS DURING PRODUCT MANUFACTURE. DARK HORSE CONSULTING. MARCH 2023.
4. NIK-ZAINAL S. ET AL., NONCANONICAL SECONDARY STRUCTURES ARISING FROM NON-B DNA MOTIFS ARE DETERMINANTS OF MUTAGENESIS. GENOME RES. SEP;28(9):1264-1271 (2018).
5. NIPKO, J. DEVELOPING MACHINE LEARNING POWERED SOLUTIONS FOR CELL AND GENE THERAPY CANDIDATE VALIDATION. FORM BIO RESOURCE CENTER. PUBLISHED NOVEMBER 2022. ACCESSED APRIL 26, 203
6. KETZ, N. APPLYING DEEPMIND'S TRANSFORMER ARCHITECTURE TO OPTIMIZE AAV CONSTRUCTS. FORM BIO RESOURCE CENTER. PUBLISHED MARCH 8, 2023. ACCESSED APRIL 26, 2023.
7. KETZ, N. UNDERSTANDING HOW BEST TO ALLOCATE RESOURCES WHEN TRAINING LARGE LANGUAGE MODELS IN GENE DEVELOPMENT PROGRAMS. FORM BIO RESOURCE CENTER. PUBLISHED JAN 31, 2023. ACCESSED APRIL 26, 2023.
8. DAY, A. SHORTING GENE THERAPY DEVELOPMENT TIME WITH STRATEGIC USE OF LARGE LANGUAGE MODELS. FORM BIO RESOURCE CENTER. PUBLISHED MARCH 2, 2023. ACCESSED APRIL 26, 2023.

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