Directed Evolution of AAV Targeting Lung Epithelia Using Aerosol Delivery Identifies 4D-A101, a Variant Demonstrating Robust Gene Delivery in Non-Human Primates

Abstract 1336

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[1] 4D Molecular Therapeutics, Emeryville, CA
[2] University of California, Berkeley, CA

4DMT

Disclosures

- Full-time employee at 4D Molecular Therapeutics, Inc.
- Co-founder and owner of shares in 4D Molecular Therapeutics, Inc.
- Inventor on patents and/or pending patent applications related to AAV capsid variants and AAV gene delivery.

Approved & Late-Stage AAV Gene Therapies are NOT Targeted; 4DMT Is Developing Precision-Guided Products

FOUR KEY CHALLENGES FOR CONVENTIONAL AAV VECTORS

Delivery: sub-optimal routes, high doses

Transduction: poor efficacy, limited tissues

Inflammation: toxicity challenges

Antibodies: limit market, efficacy

CURRENT PRODUCTS FOCUS ON "LOW-HANGING FRUIT"

Luxturna (IRD): subretinal surgery

Hemophilia: ≥ 5% correction required

Zolgensma (SMA): patients < 2 years old

"NEXT-GEN" VECTORS

- Unmodified AAV discovered in nature
- Modified natural vectors: rationally designed or engineered (not evolved)
- Selection with small libraries with low diversity
- Selection in mice (not primates)

4DMT PRECISION-GUIDED VECTORS

Delivery: Optimal Route & Lower Doses

Transduction: Highly Efficient

Inflammation: Reduced

Antibody Resistance

PROPRIETARY TARGETED VECTORS FOR RARE & LARGE MARKET DISEASES

- **PRECISION-GUIDED VECTORS**
- **Original AAV directed evolution company**
- **~1 BILLION sequences**
- **37 Capsid Libraries**
- **Selection & validation in PRIMATES**
- **Characterization in human organotypic disease models**

Discovery of Next-Generation AAV Vectors

RATIONALE FOR DIRECTED EVOLUTION

- Differences in capsid protein sequences between serotypes
  - Structural differences in surface loop regions

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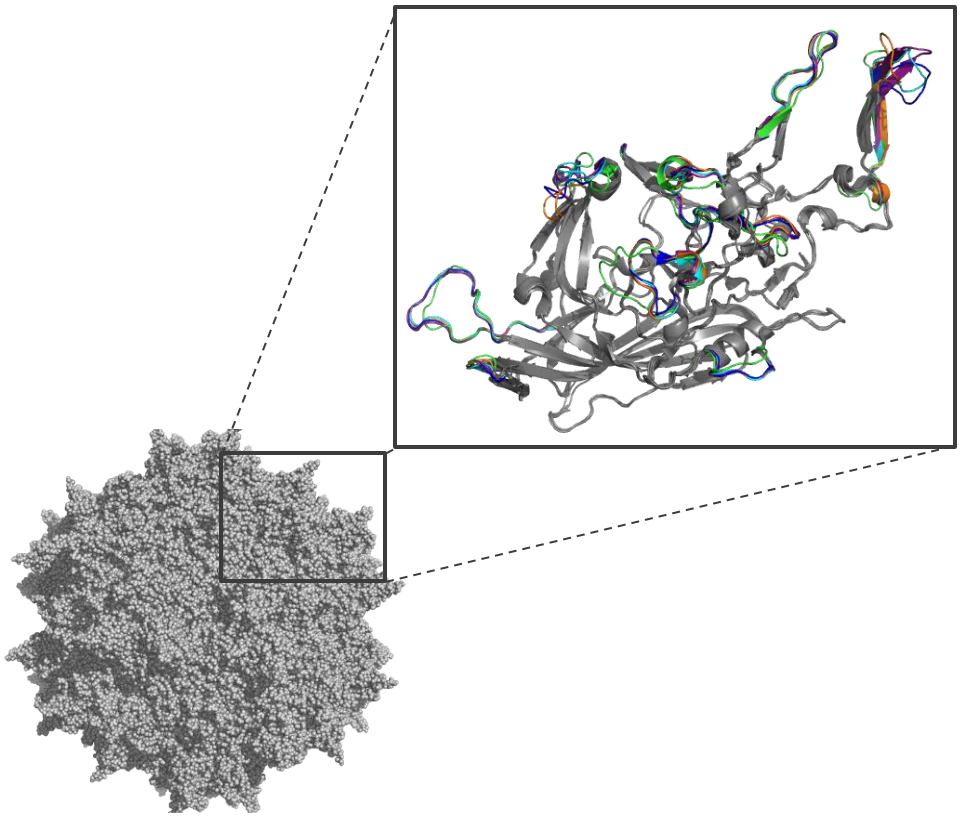

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  - Cell surface receptors utilized
  - Transduction efficiency for various cell types
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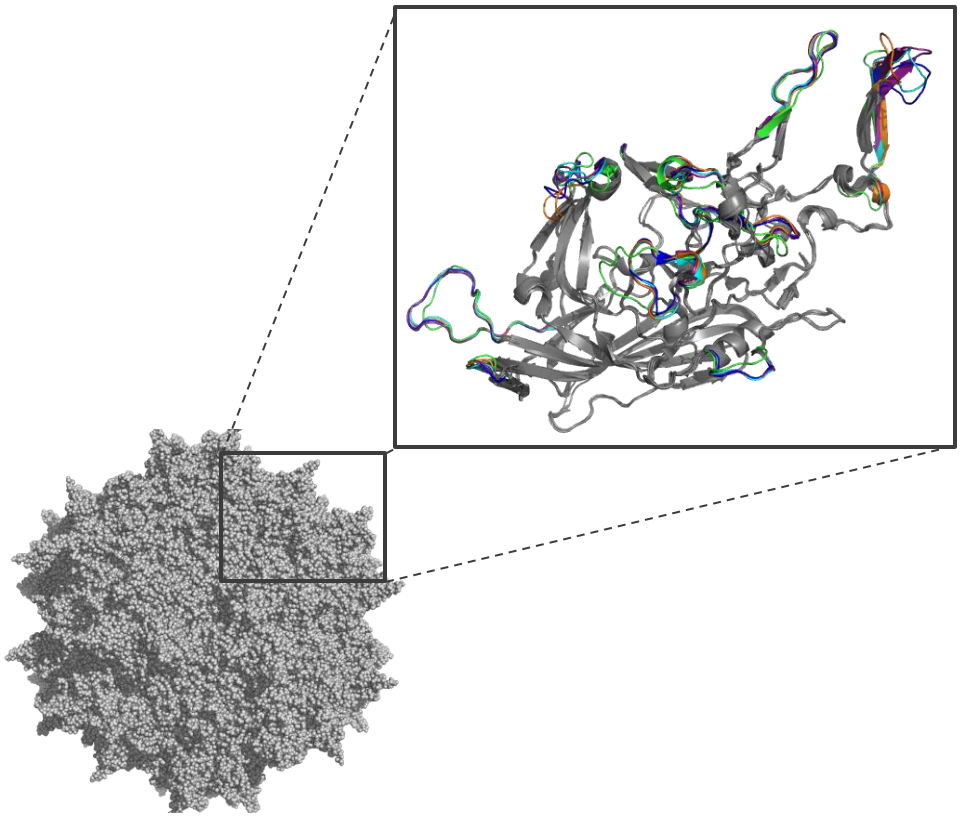
  - Affinity for antibodies



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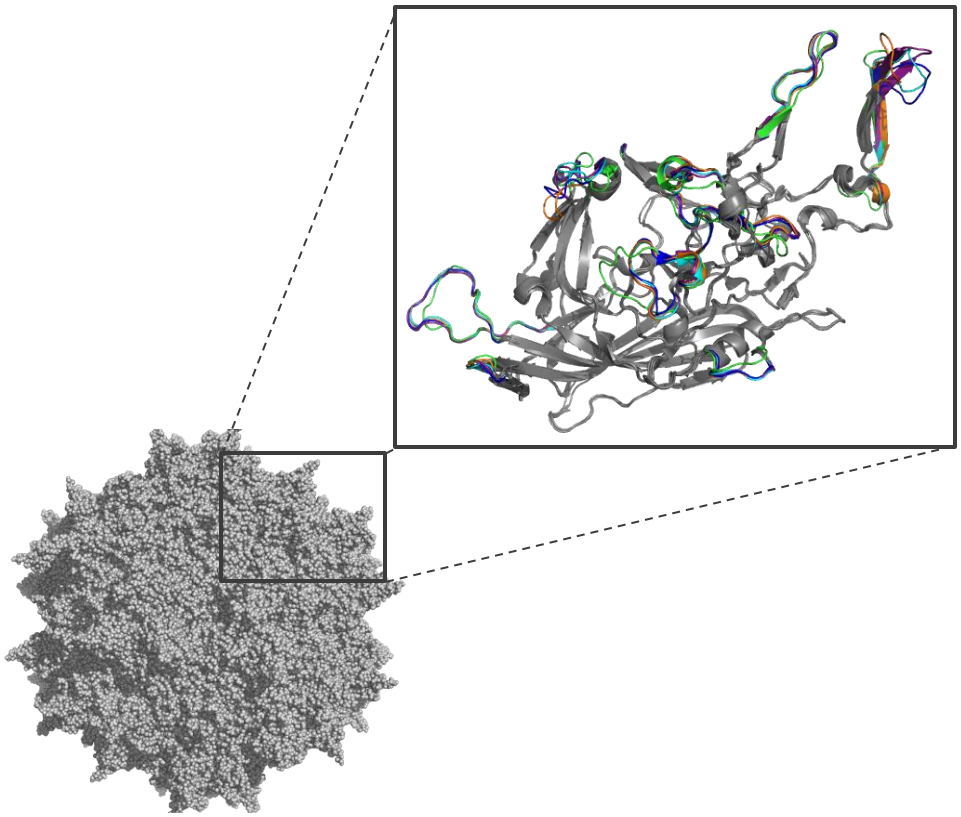
  - *Cap* gene can be mutated to produce capsid protein changes



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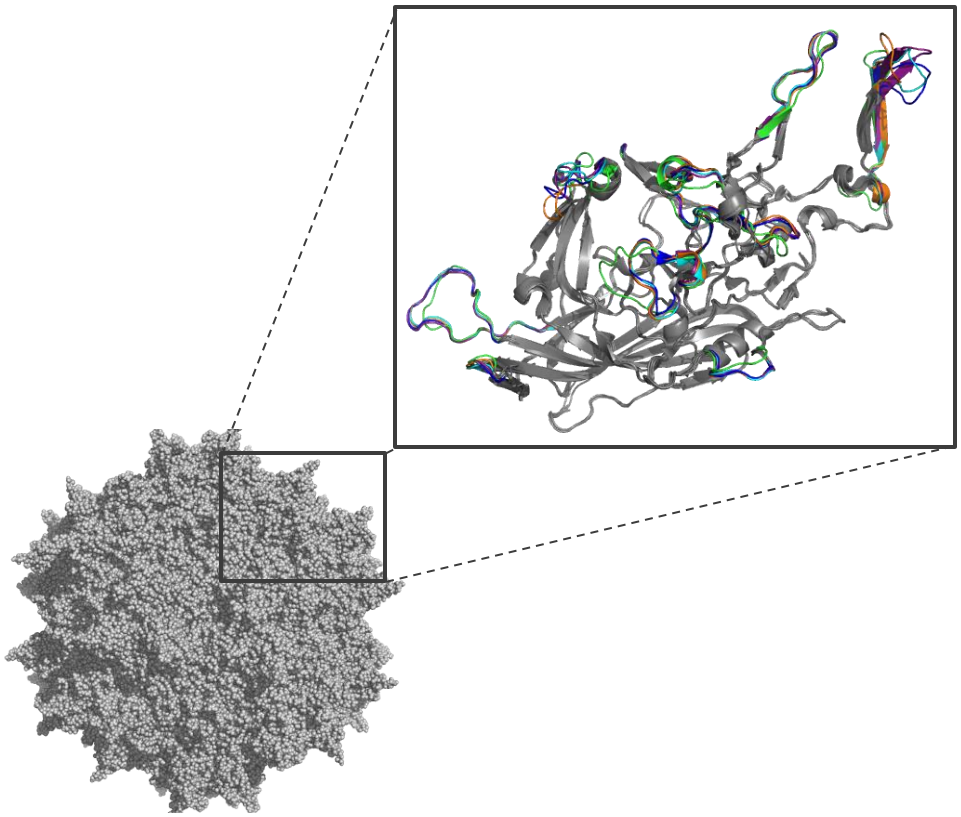
- Problems:
  - Knowledge of structure/sequence to function relationship is incomplete
  - Knowledge of gene delivery "bottleneck" in each situation is incomplete

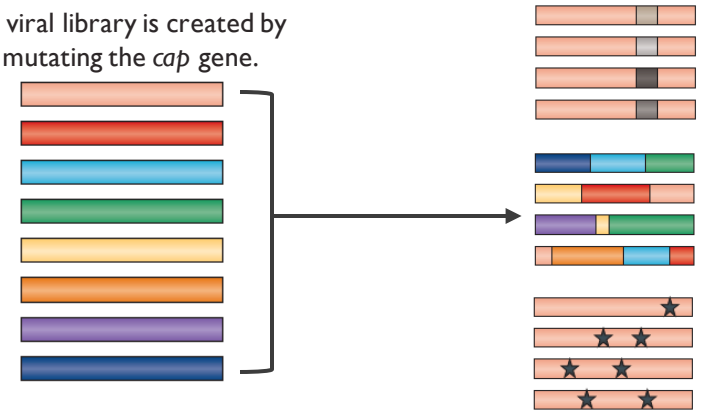


4DMT AAV Capsid Discovery Platform

THERAPEUTIC VECTOR EVOLUTION

Diversity: **>1 BILLION** Vector Variants in 4DMT Libraries (n>35)

A viral library is created by mutating the *cap* gene.



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Kotterman & Schaffer (2014)

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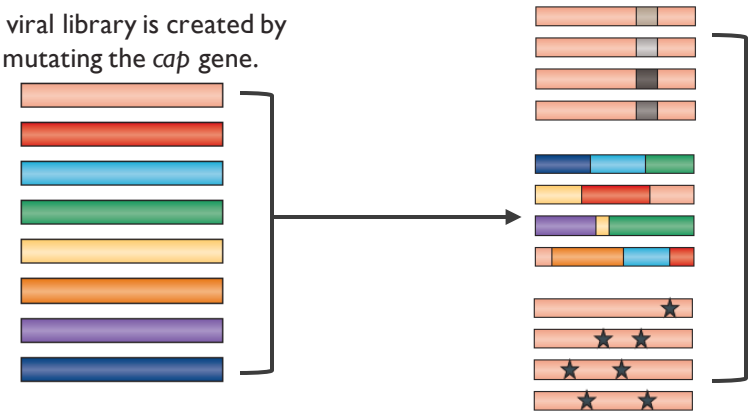
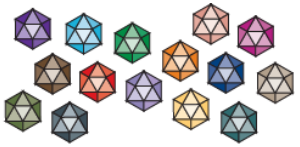


Each virus is composed of a mutant capsid surrounding the *cap* gene encoding that capsid.



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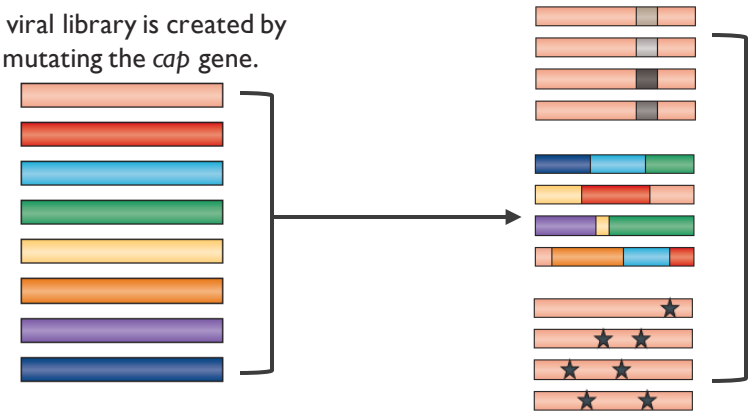

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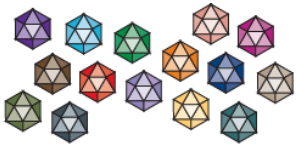

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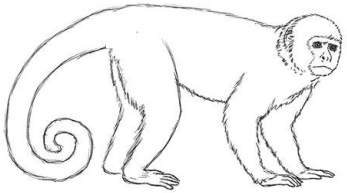

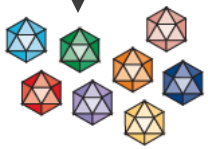

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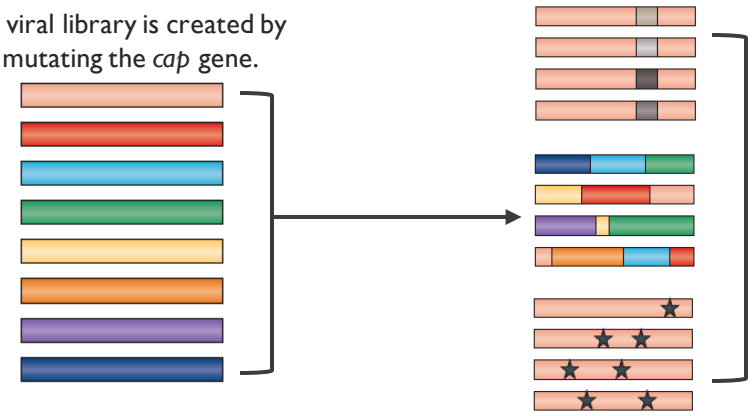

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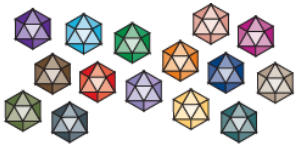

TARGET VECTOR PROFILE
Primates *in vivo*
Human antibodies & organotypic models *ex vivo*

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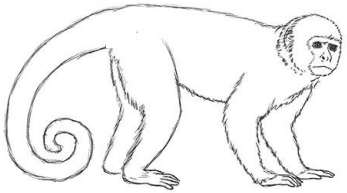

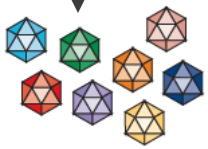

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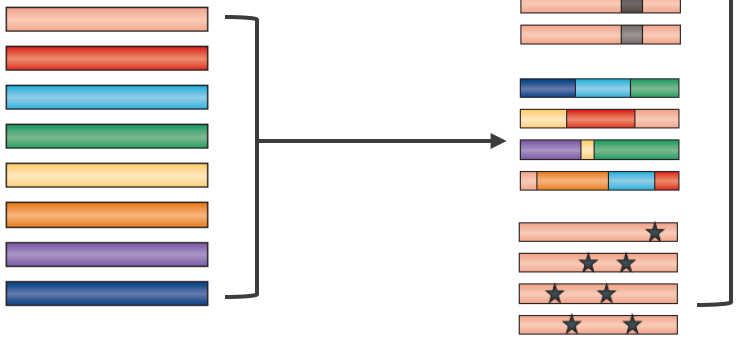

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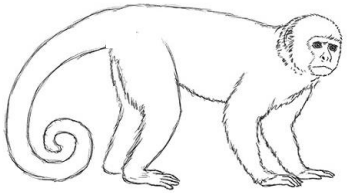

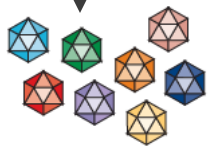

Successful viruses are enriched through repeated selection.

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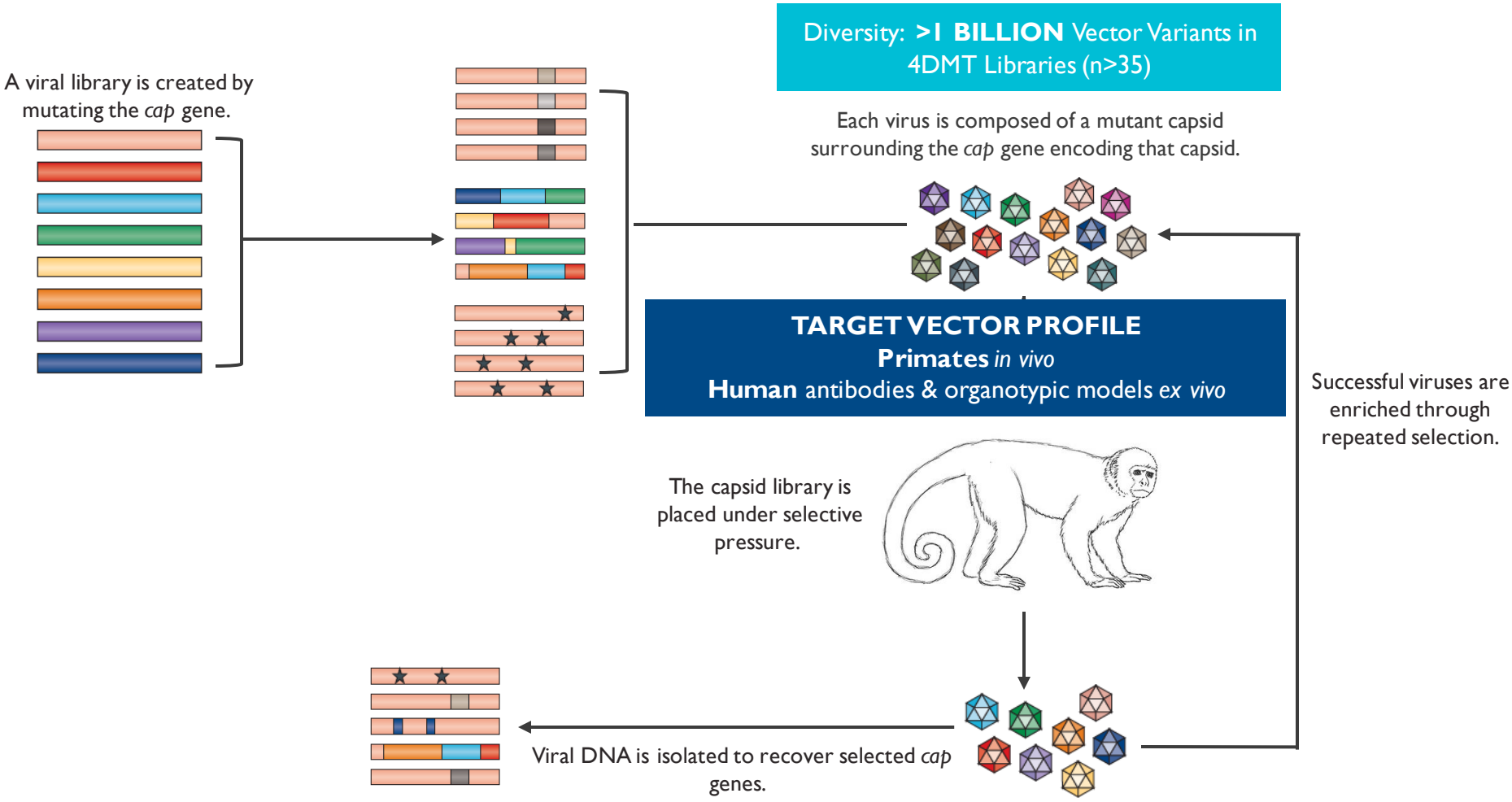

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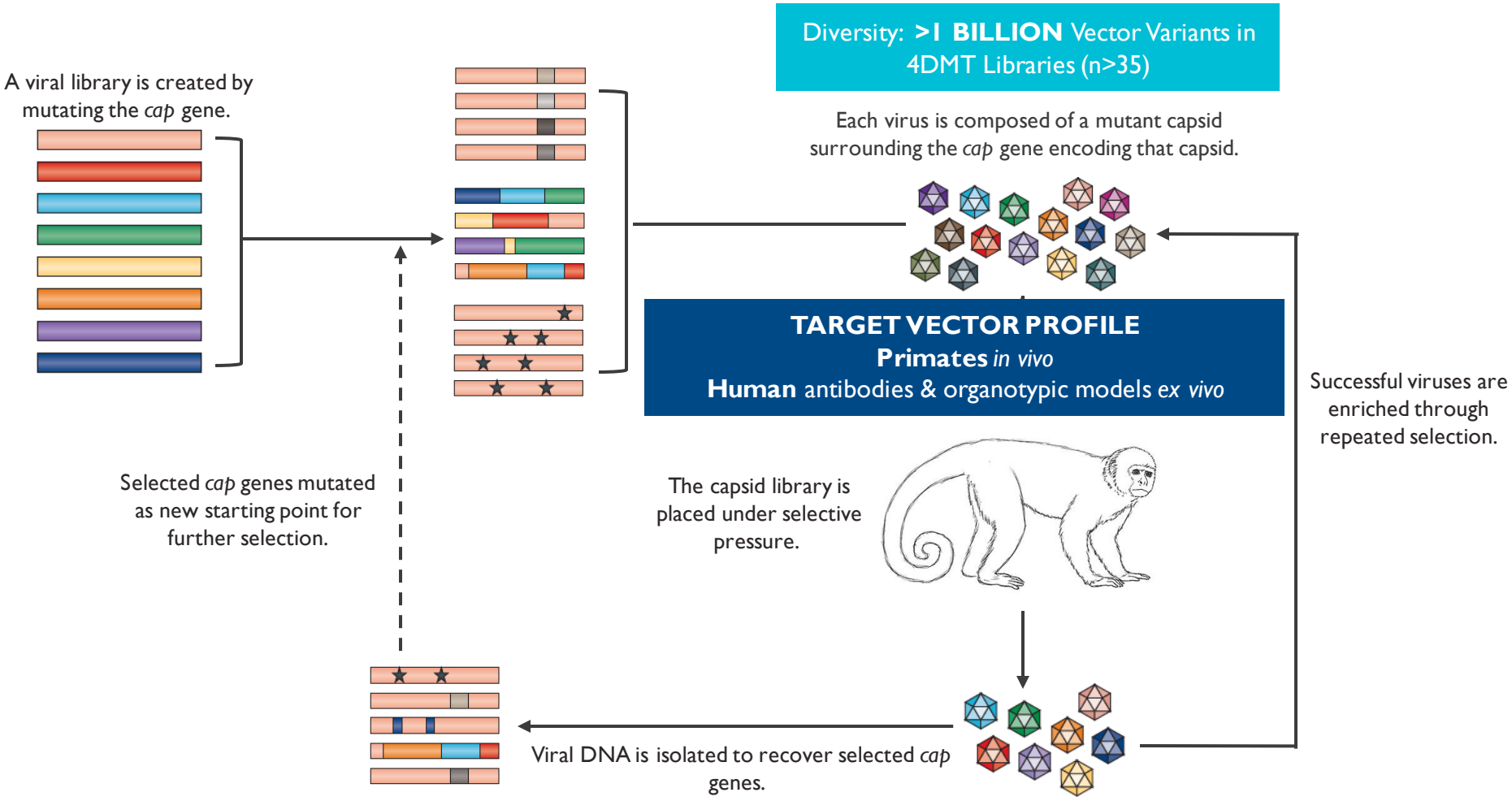

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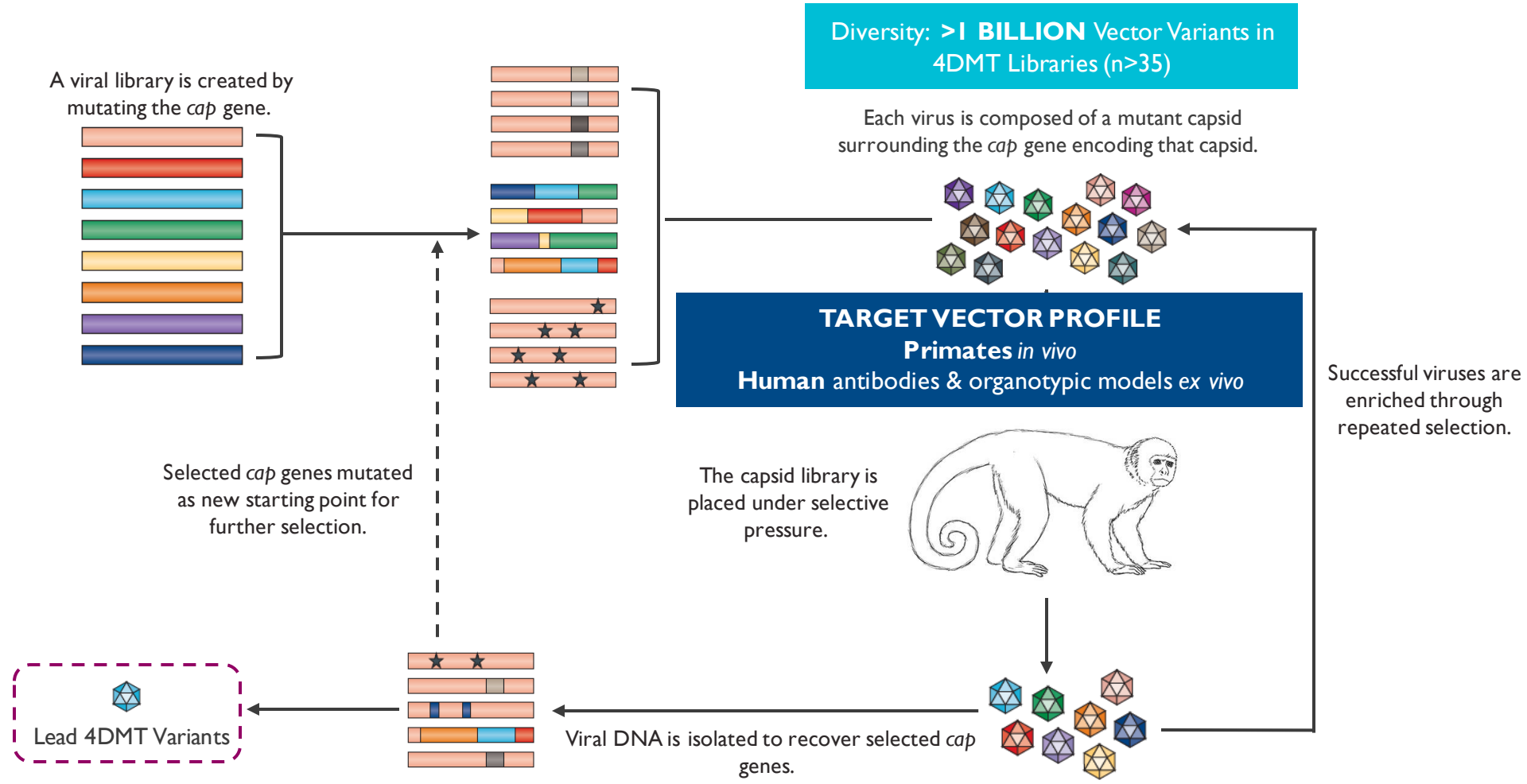

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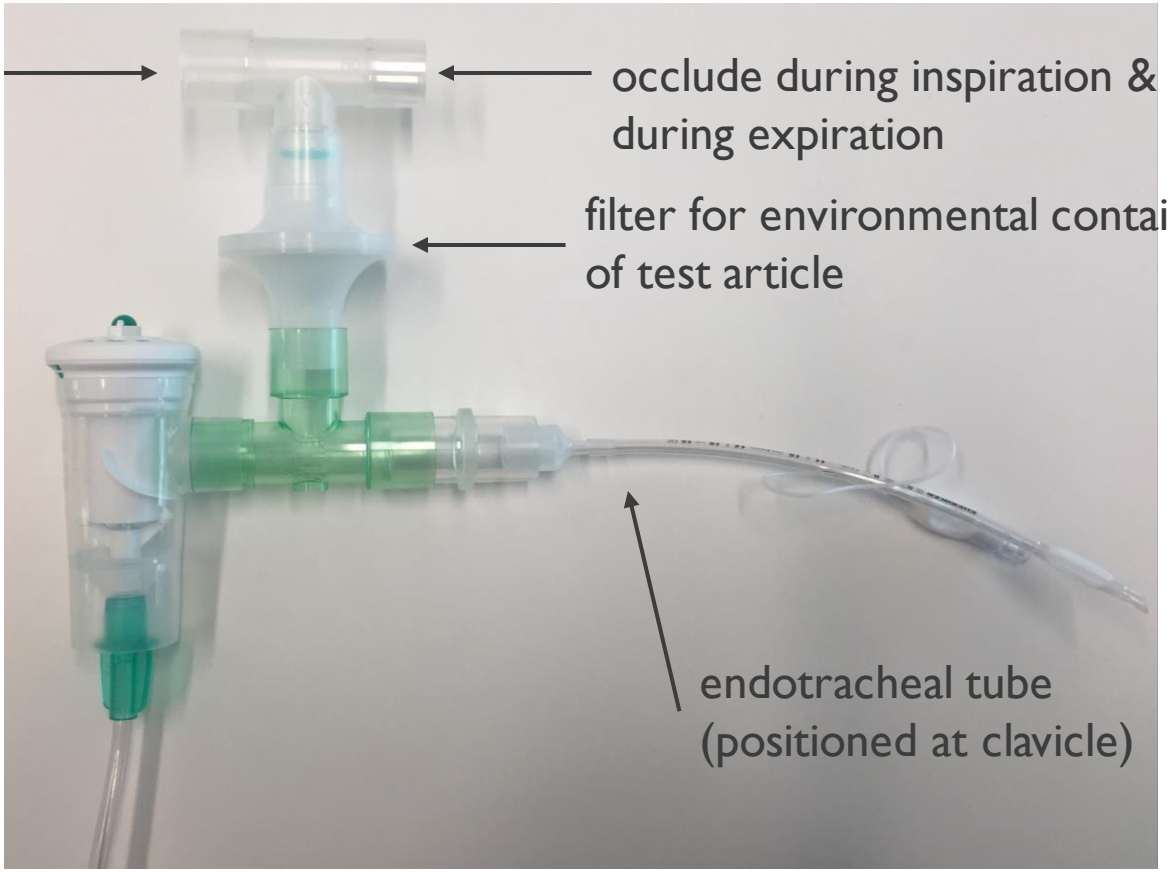
Aeroeclipse II NHP-Adapted Delivery Device

CLINICAL DELIVERY DEVICE ADAPTED FOR USE IN ANETHESIZED NHP



Aeroeclipse II Aerosol Dye Distribution Study

ROBUST DYE DELIVERY THROUGHOUT ALL LUNG LOBES

- Dye distribution after exposure to 5 mL 2% Evans Blue dye in 4DMT formulation buffer
- Dye distribution similar between n = 2 NHPs and throughout all lung lobes
- No dye detected in the esophageal or stomach tissue for either NHP
- Duration of exposure and number of breaths similar between animals
  - NHP #1 (Male) – Exposure: 36 mins, 405 breaths
  - NHP #2 (Female) – Exposure: 32 mins, 406 breaths

Discovery of Lead 4DMT Lung Vector: 4D-A101

8 NOVEL VARIANTS IDENTIFIED, INCLUDING 1 LEAD

~1 billion variants (in library)

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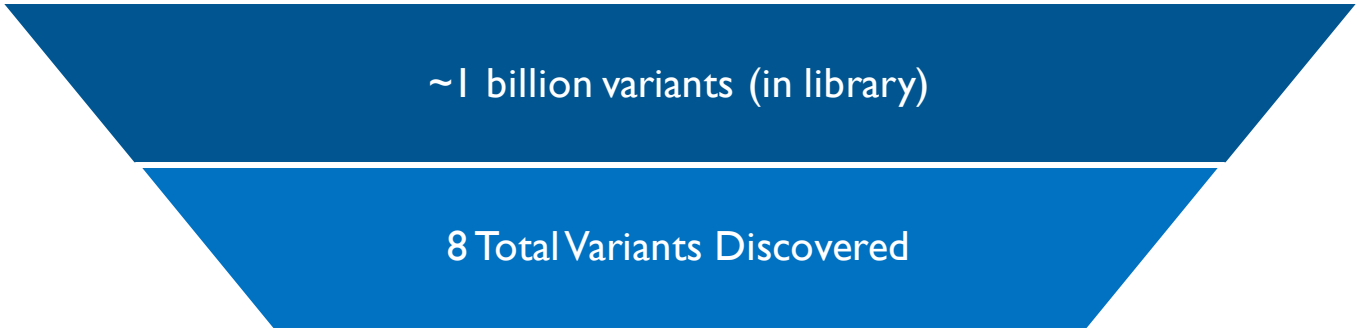

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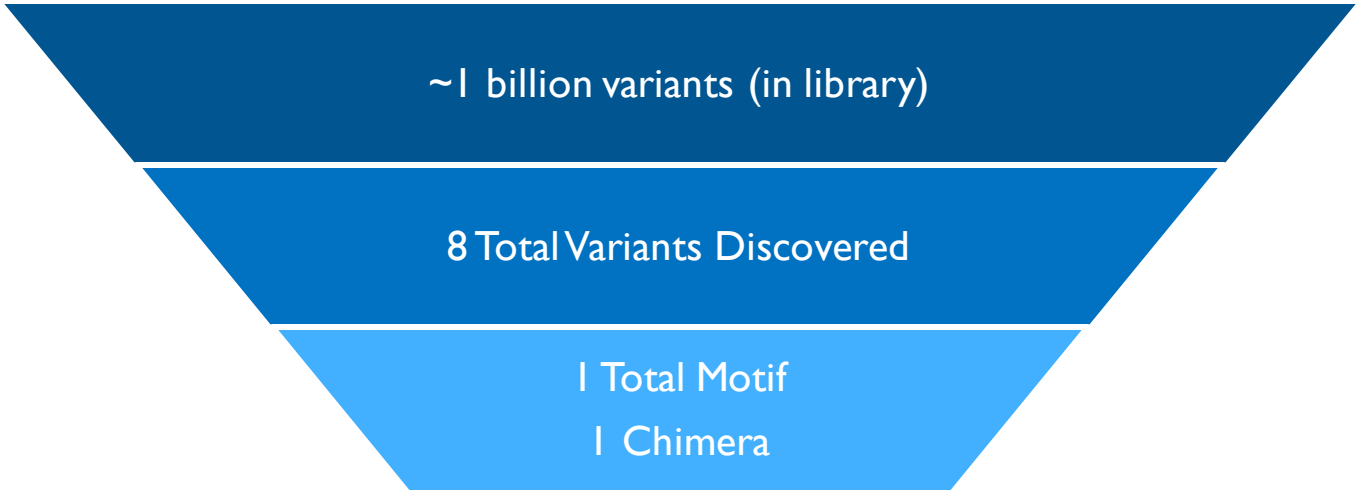

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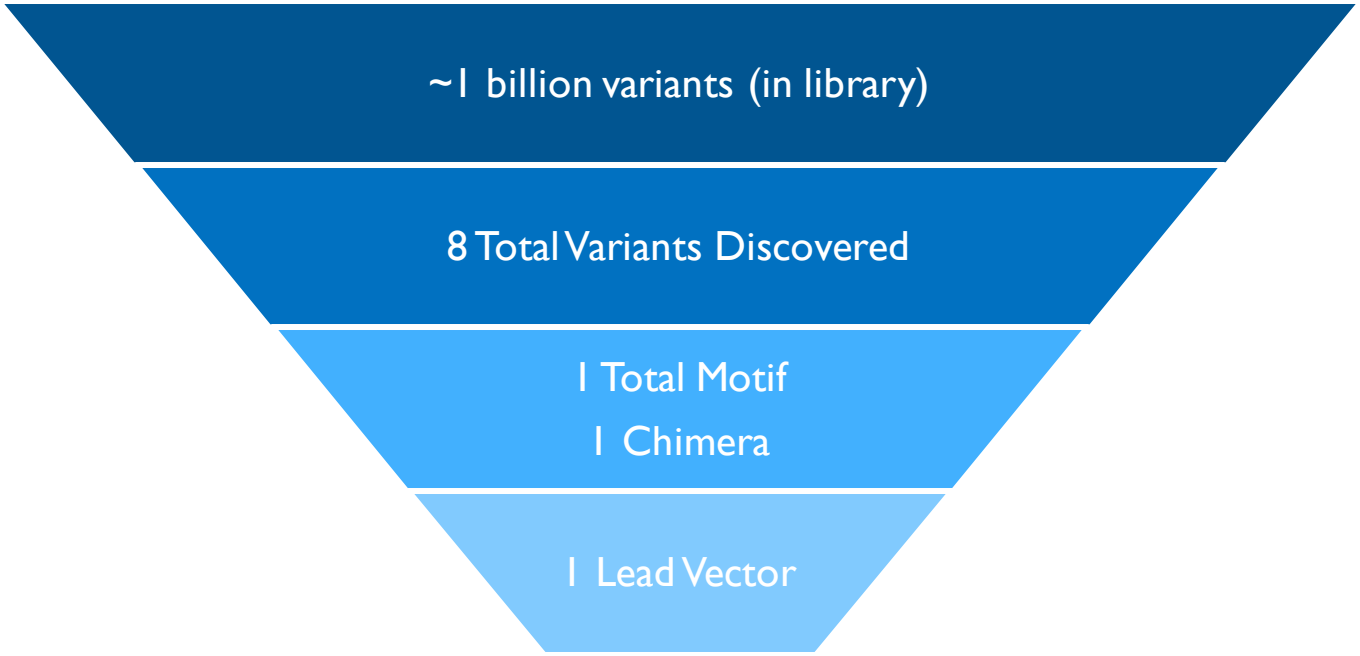

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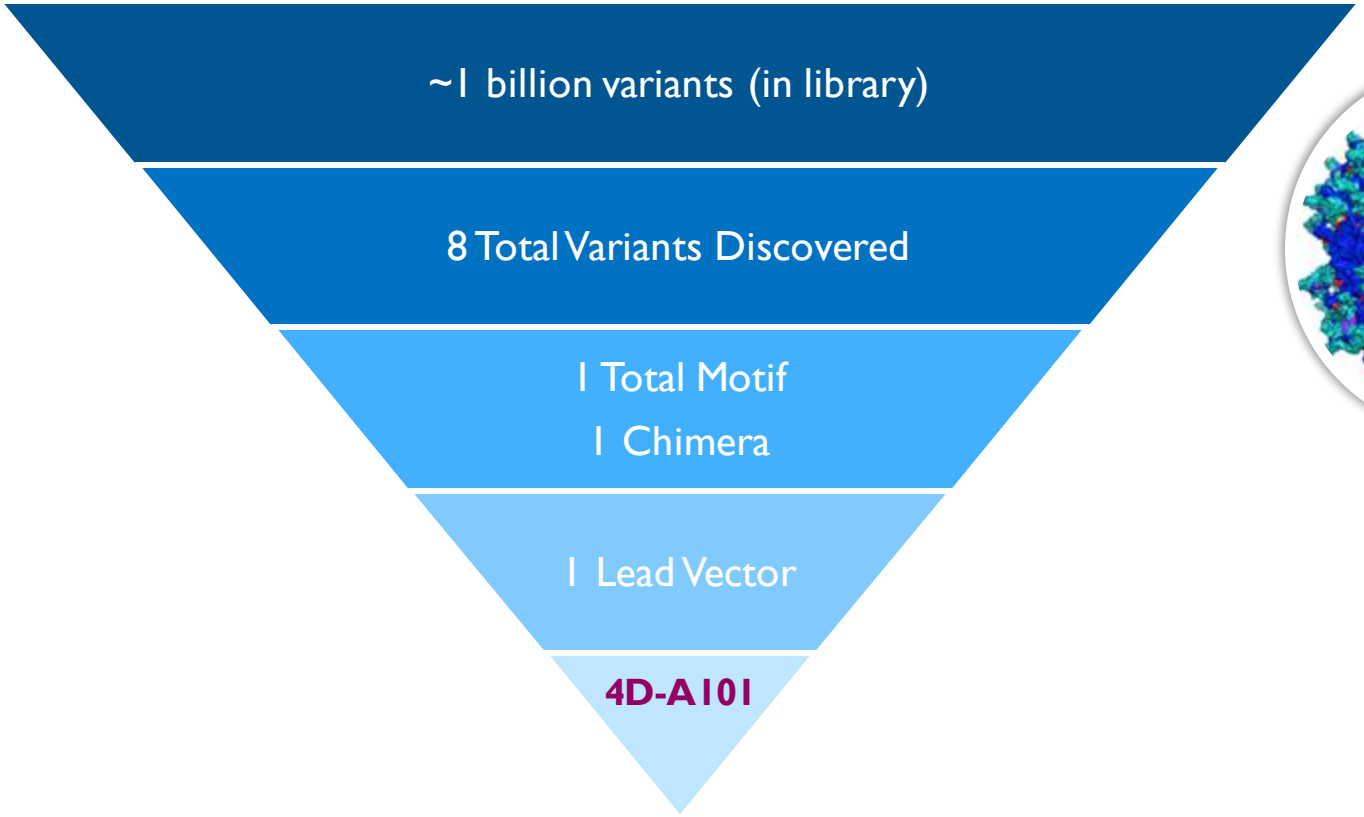

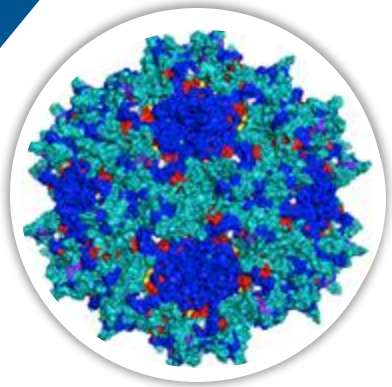

4D-A101

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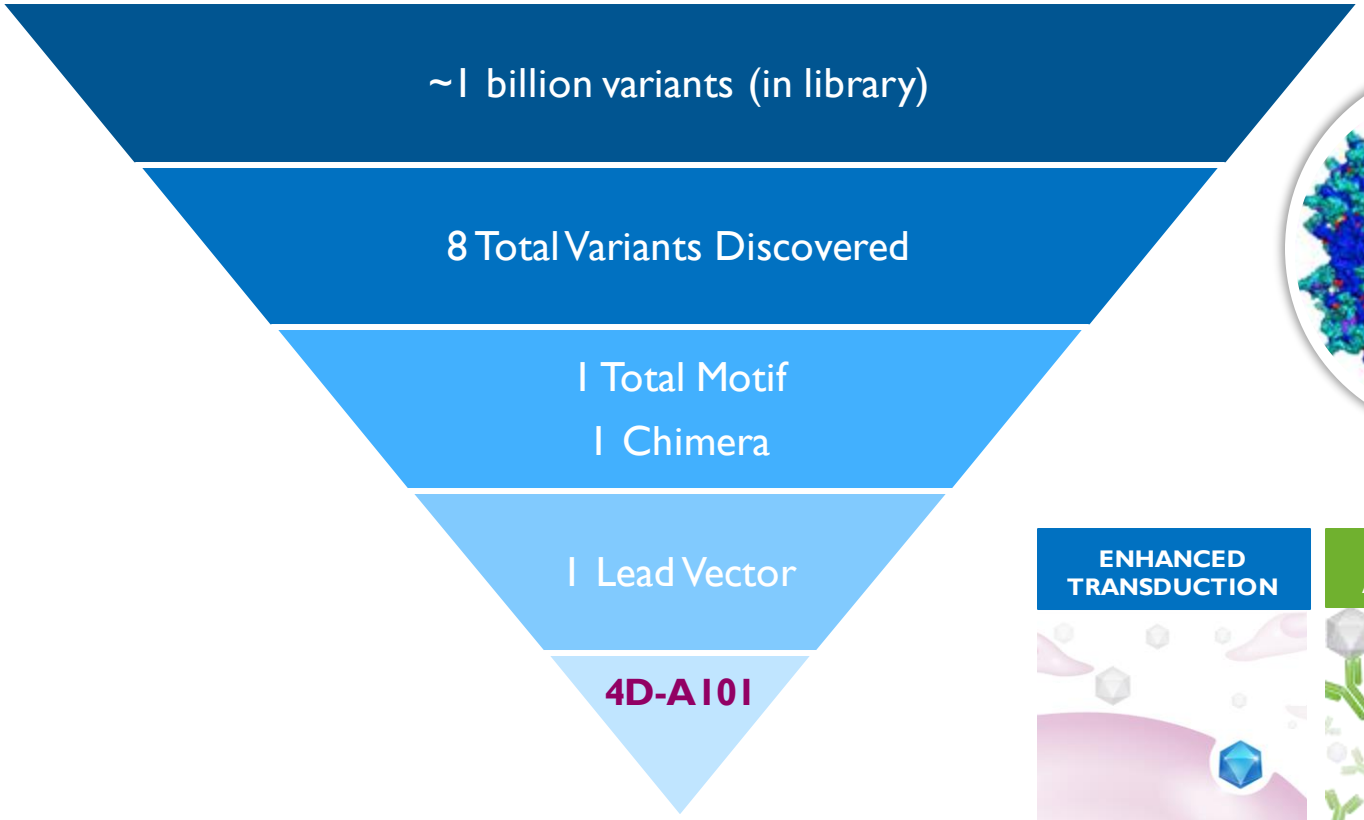

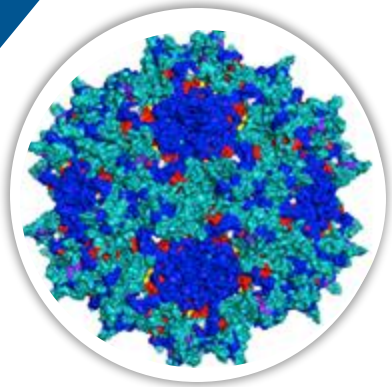

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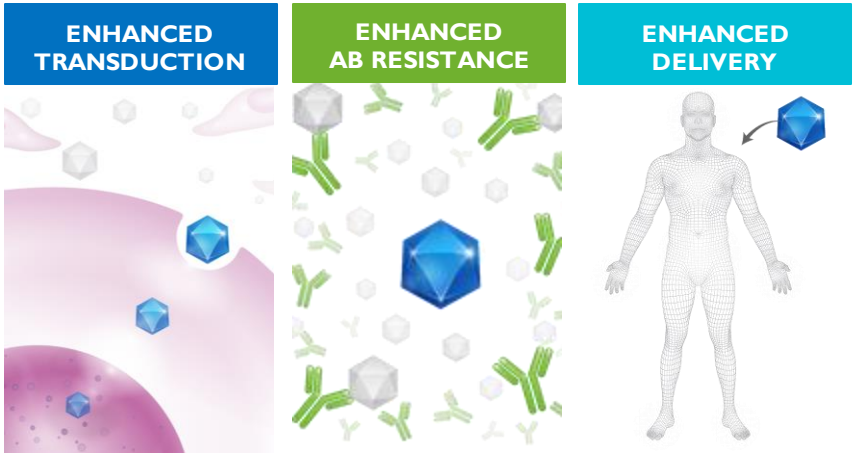


4D-A101 Resists Pre-Existing Human Anti-AAV Antibodies

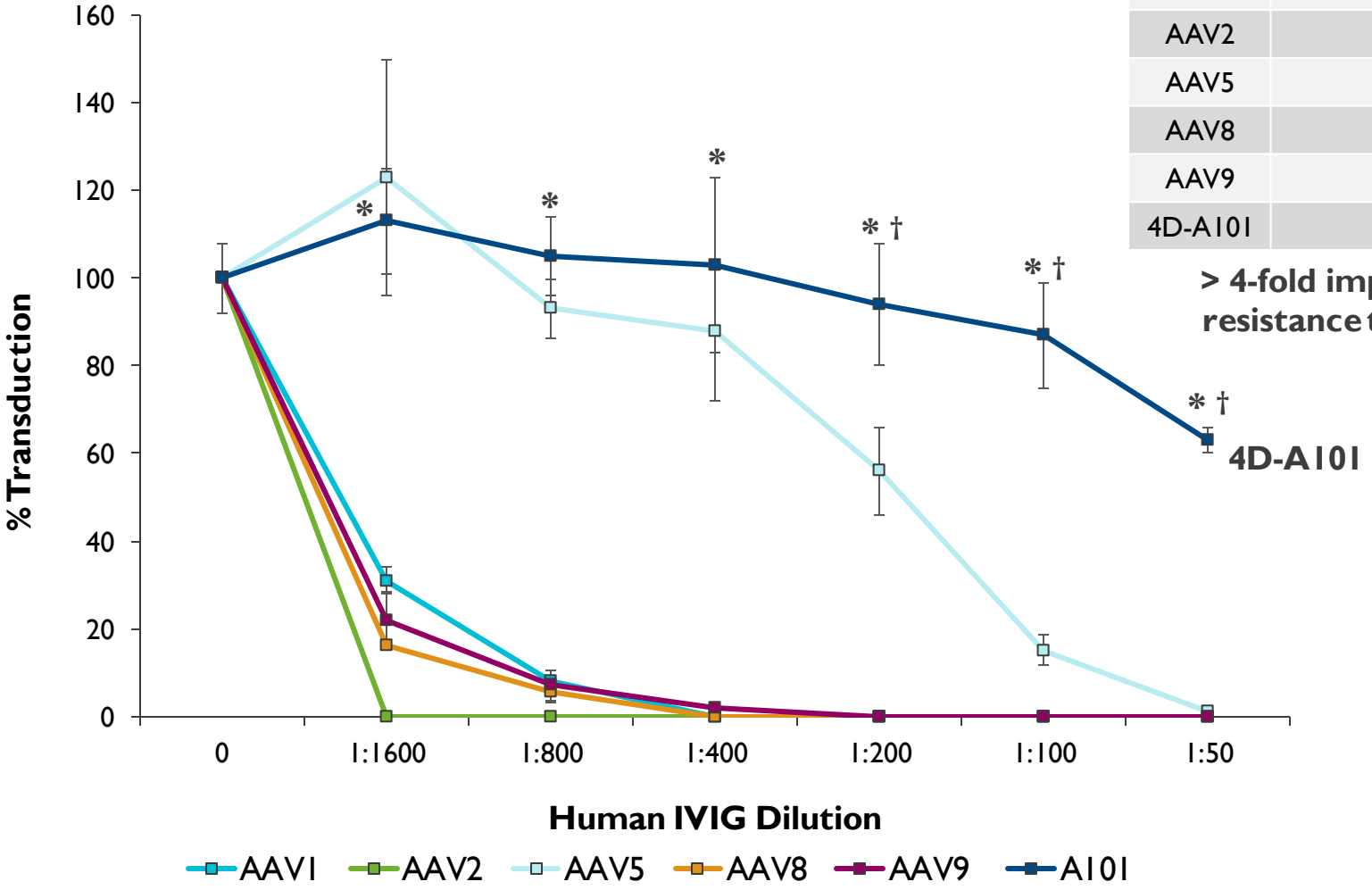
RESISTANCE AT HIGH (1:50) TITERS COMPARED TO WILD-TYPE SEROTYPES

* $p < 0.05$ for 4D-A101 vs AAV1, AAV2, AAV8, and AAV9

† $p < 0.05$ for 4D-A101 vs AAV5

AAV	Neutralizing IVIG Dilution
AAV1	> 1:1600
AAV2	> 1:1600
AAV5	1:200
AAV8	> 1:1600
AAV9	> 1:1600
4D-A101	< 1:50

> 4-fold improvement in resistance to antibodies

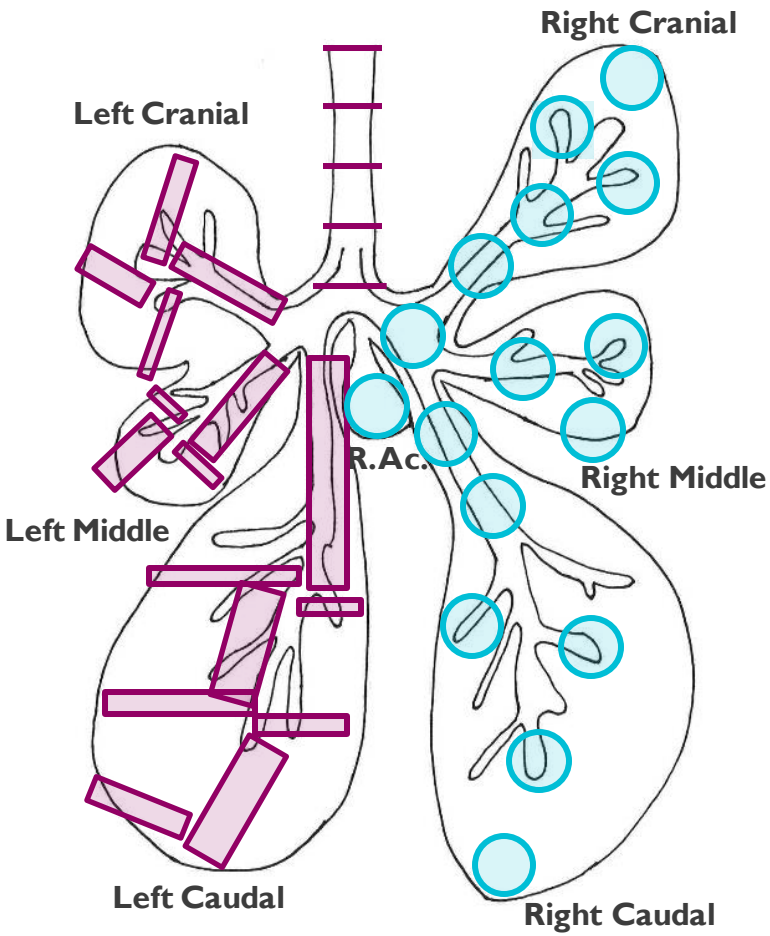


4D-A101.CAG-EGFP In Vivo Characterization

AEROSOLIZED DELIVERY TO NHP LUNG

DOSE	# OF NHP	ROUTE	IN-LIFE
2.8 x 10^{12} vg/kg (~1 x 10^{13} vg)	3	Aerosolized (AeroEclipse II)	8 weeks

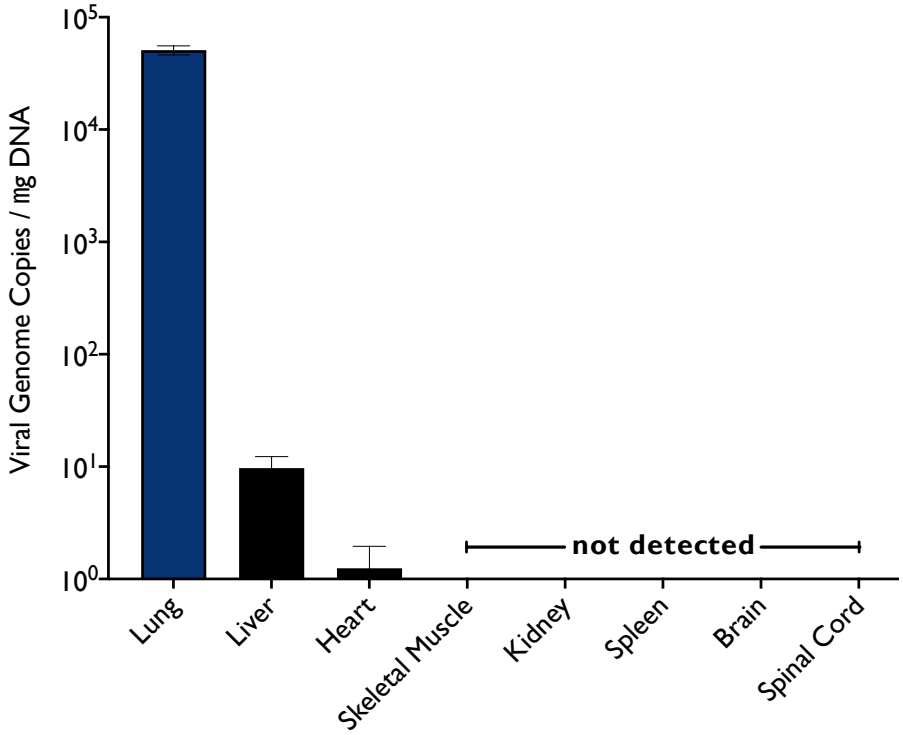
TISSUE COLLECTION	ANALYSIS
▪ Lung ▪ Heart ▪ Liver ▪ Skeletal Muscle (triceps, quadriceps, diaphragm) ▪ Kidney ▪ Spleen ▪ CNS (brain, spinal cord)	▪ qPCR ▪ ELISA (lung & qPCR+ tissues) ▪ Immunofluorescence & H&E (lung & ELISA+ tissues)



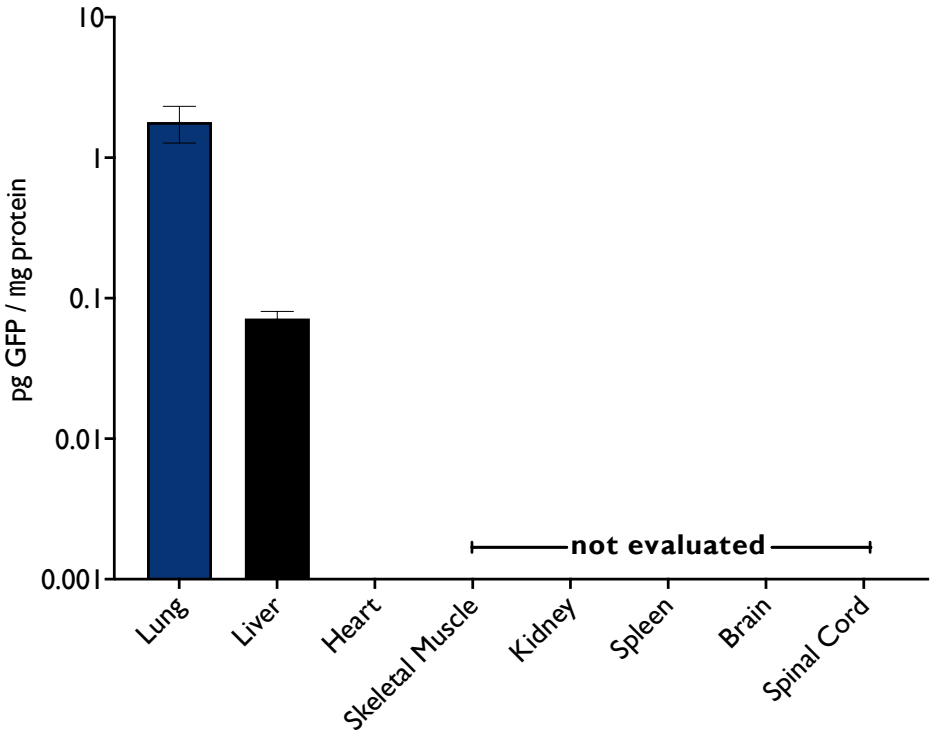
4D-A101 Delivers Payload & Expresses Protein in Lungs

GENOME LOCALIZATION & PROTEIN EXPRESSION IN 100% OF LUNG SAMPLES

GENOME LOCALIZATION (QPCR)



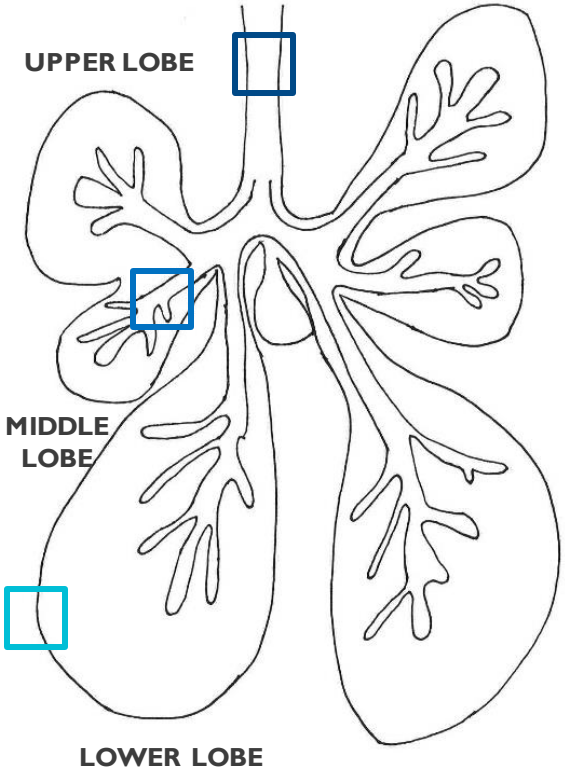
PROTEIN EXPRESSION IN QPCR+ SAMPLES (ELISA)



- Consistent delivery between animals
- 4D-A101 viral genomes & protein expression present in all lung samples
  - Evenly distributed across multiple bronchial levels and alveoli
  - Evenly distributed across cranial, middle, and caudal sections

4D-A101 Protein Expression Distributed Across Lung Regions

REPRESENTATIVE GFP EXPRESSION IN TRACHEA, BRONCHI, & ALVEOLI



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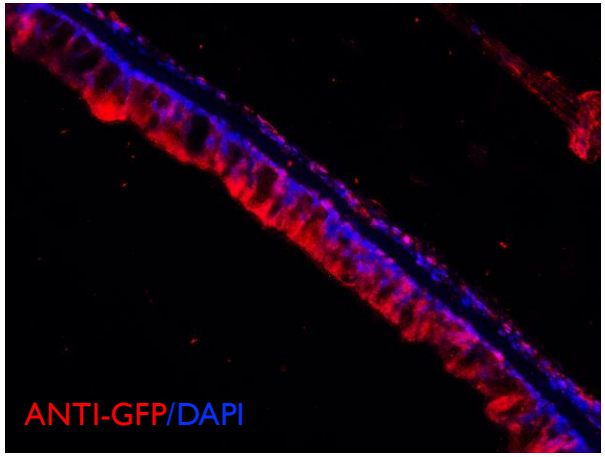
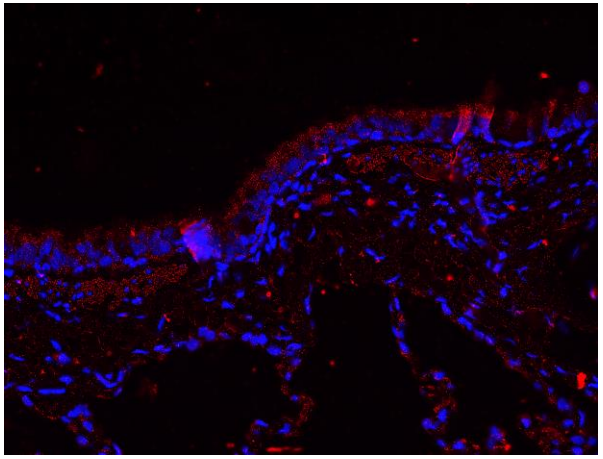
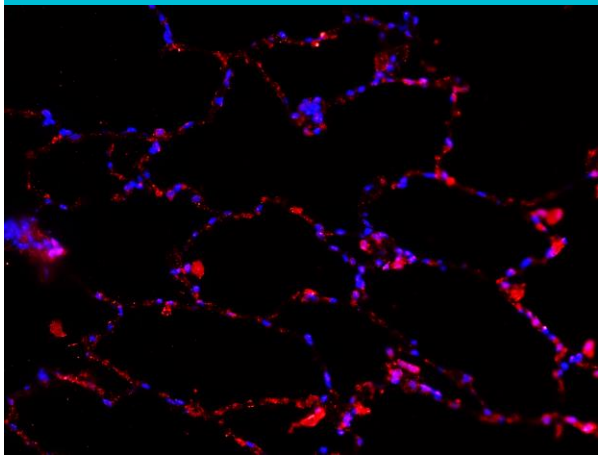


BRONCHI

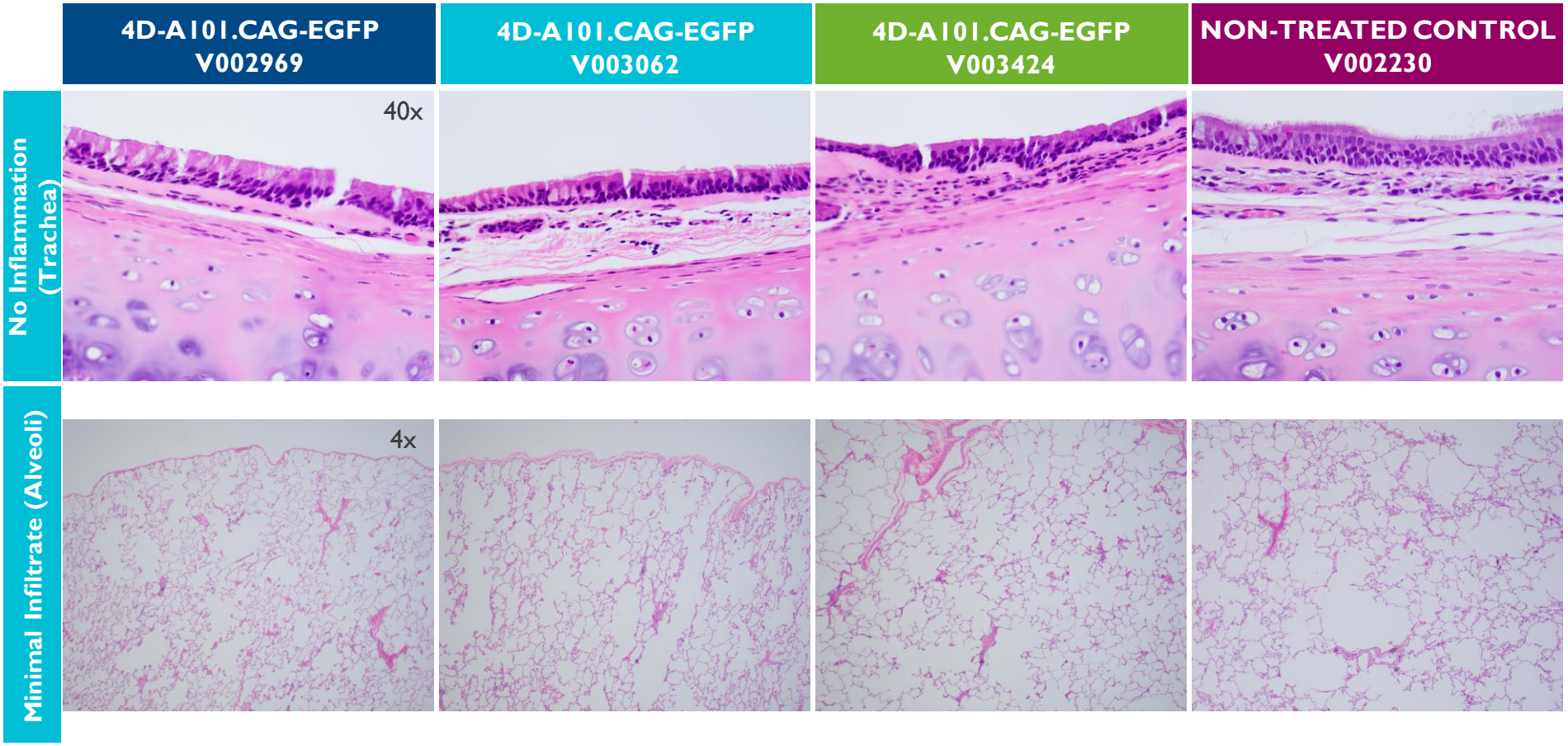


ALVEOLI



4D-A101 Administration is Safe in NHP

NO 4D-A101-RELATED ADVERSE HISTOPATHOLOGY OR CLINICAL PATHOLOGY



- 4D-A101 delivery well-tolerated
- No abnormal hematology or clinical chemistry findings
- No test-article-related adverse histopathology in lungs

Conclusions

- 4D-A101 represents first use of directed evolution in NHP to identify a vector engineered for lung tropism.
- 4D-A101 capsid is significantly more resistant to neutralization by anti-AAV antibodies *in vitro*.
- Delivery by aerosolization results in robust and widespread transduction and transgene expression throughout NHP lung.
- Localized delivery to lung results in minimal systemic exposure.
- Novel 4D-A101 vector represents advantage over existing AAV serotypes for lung gene therapy.

Acknowledgments

- 4DMT Process & Analytical Development
- 4DMT Project Management
- Laurie Tatalick, D.V.M., Ph.D. (Histopathology)