

Optimization of DNAI1 mRNA Constructs to Treat Primary Ciliary Dyskinesia

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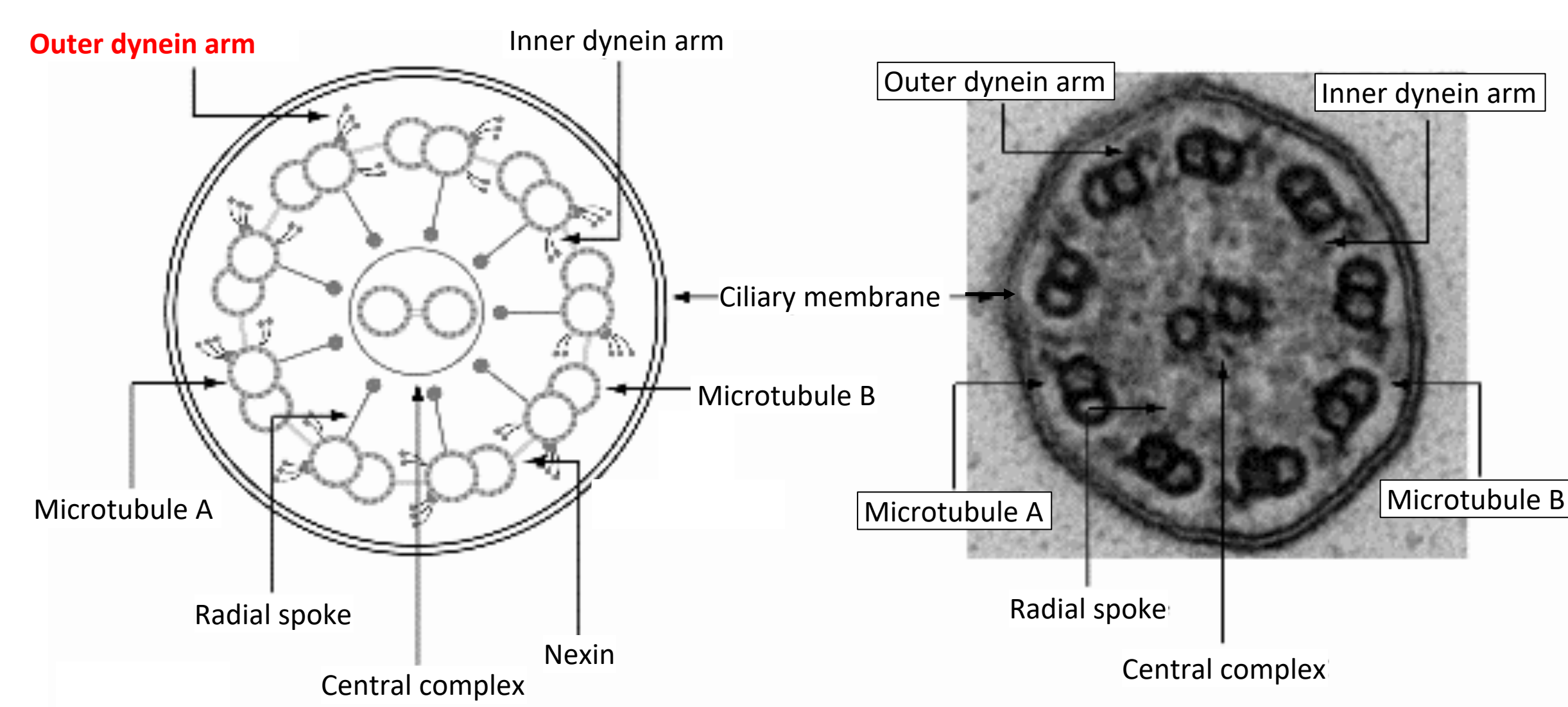
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Introduction

Primary ciliary dyskinesia (PCD) is a genetic respiratory disease caused by inherited mutations in more than 40 different genes. Bi-allelic mutations in any of the PCD genes result in loss of ciliary activity and mucociliary clearance. Currently, there are no disease-modifying therapies available, and treatments are limited to palliative care for the management of symptoms. Thus, there is an unmet medical need for therapeutic approaches to treat the underlying causes of PCD.

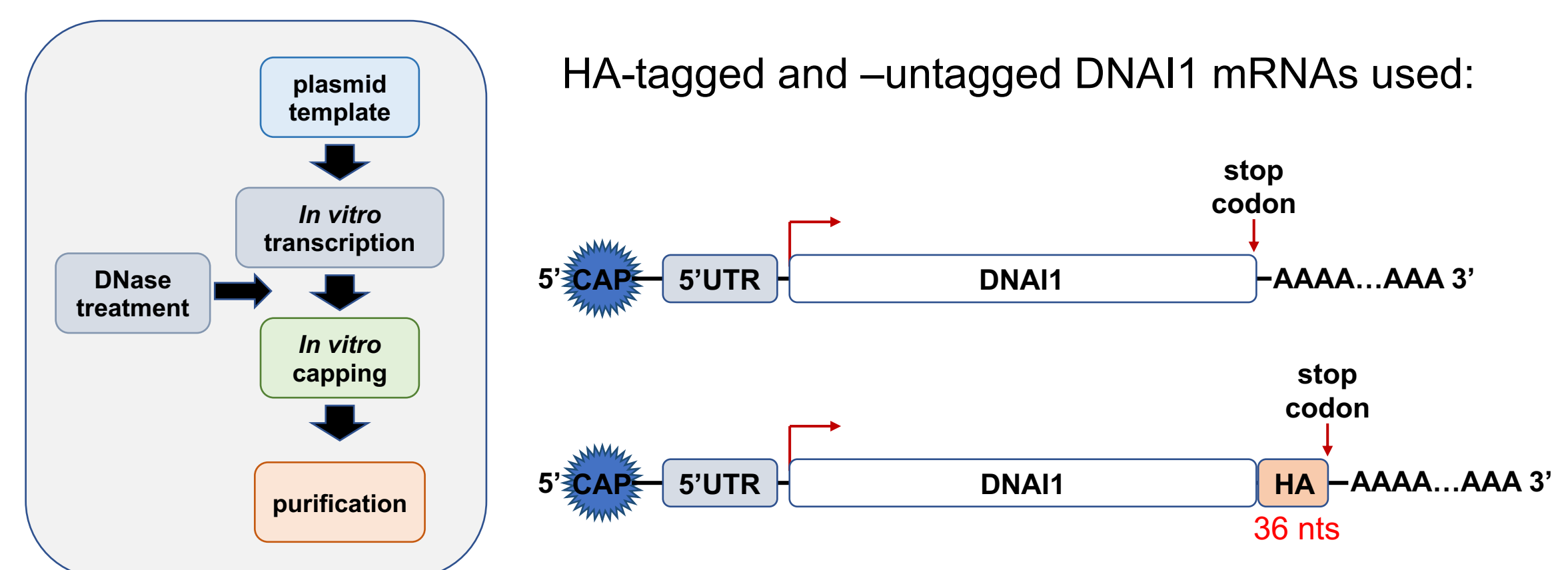
ReCode Therapeutics is developing an mRNA-based approach for the treatment of PCD caused by mutations in DNAI1 (Dynein Axonemal Intermediate Chain 1), an integral component of dynein arms that is essential for ciliary movement. Conceptual advantages over DNA-based gene therapy include the absence of a nuclear localization requirement and the negligible possibility of genomic integration. However, compared with DNA, RNA is intrinsically less stable and susceptible to transesterification which cleaves the backbone at the phosphodiester bond. This represents a significant challenge during the synthesis and purification of RNA and restricts *in vivo* uses as a source to produce therapeutically valuable proteins.

Mutations in DNAI1 impair ciliary movement



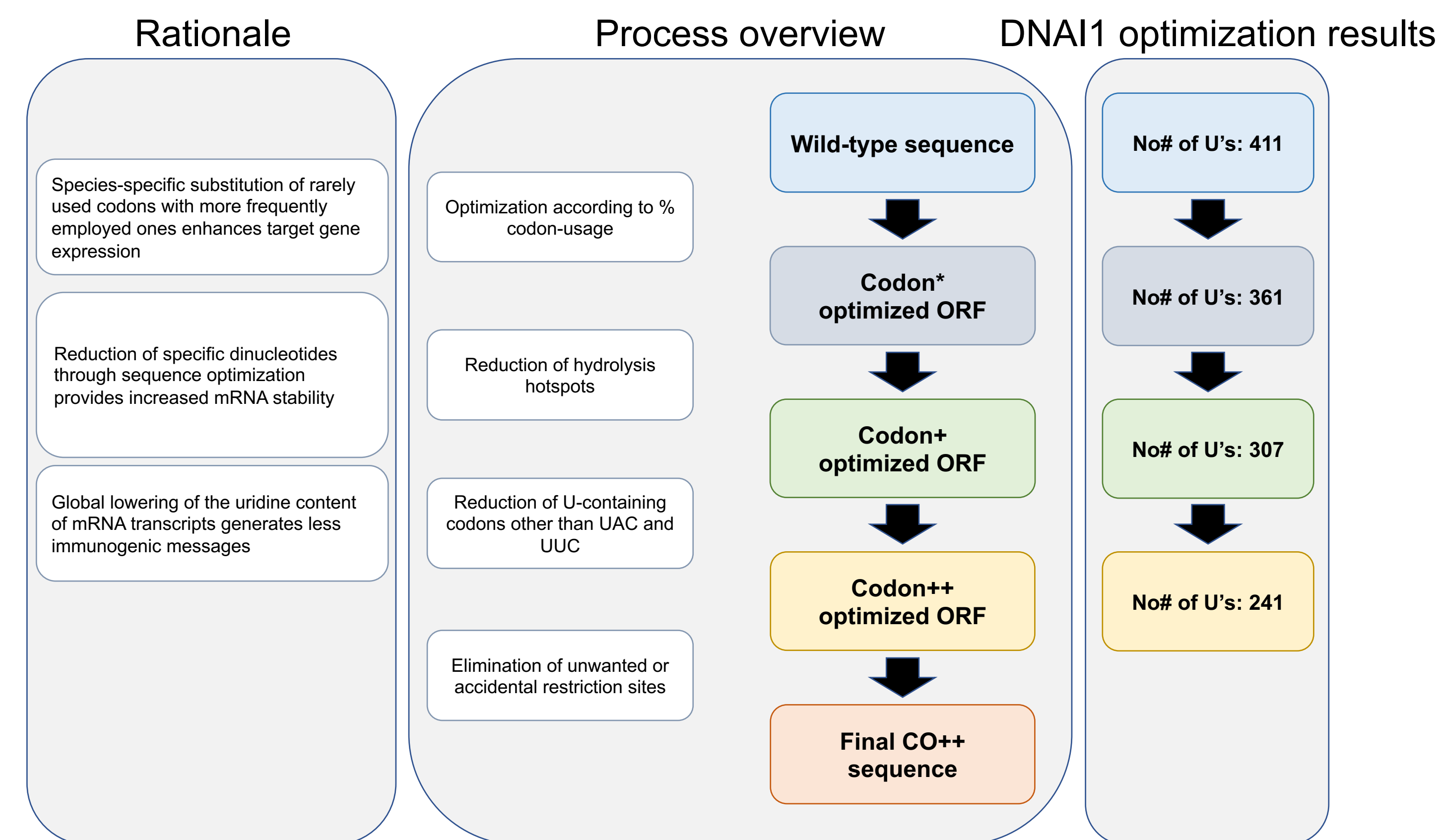
- DNAI1 (699 amino acids), a dynein axonemal intermediate-chain 1 protein, is an integral component of the **outer dynein arm** complex that is essential for ciliary movement.
- Dynein arms are located along the length of central microtubule doublets and hydrolyze ATP to generate ciliary movement.
- DNAI1 is expressed in ciliated cells lining the airways of nasal cavity, middle ear, paranasal sinuses, lower respiratory tract, fallopian tubes, and ventricles in the brain.

mRNA Production Workflow and Constructs

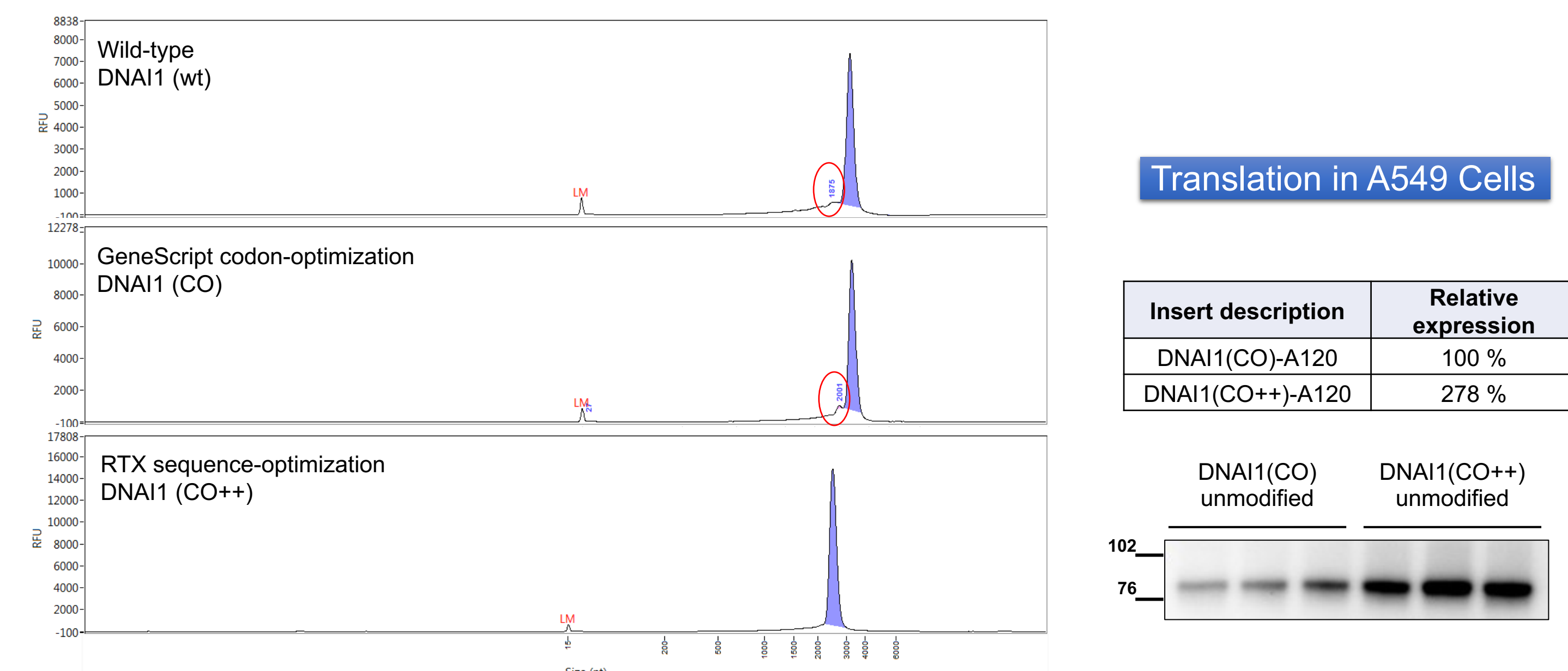


- Cap 1 (m7GpppGm) – enzymatic, post-transcriptional capping approach
- short 5'-UTR with Kozak sequence (GCC ACC AUGx)
- optimized DNAI1 open reading frame (ORF) sequence
- 3'-UTR: poly(A₁₂₀) tail encoded in template

mRNA Sequence Optimization Process

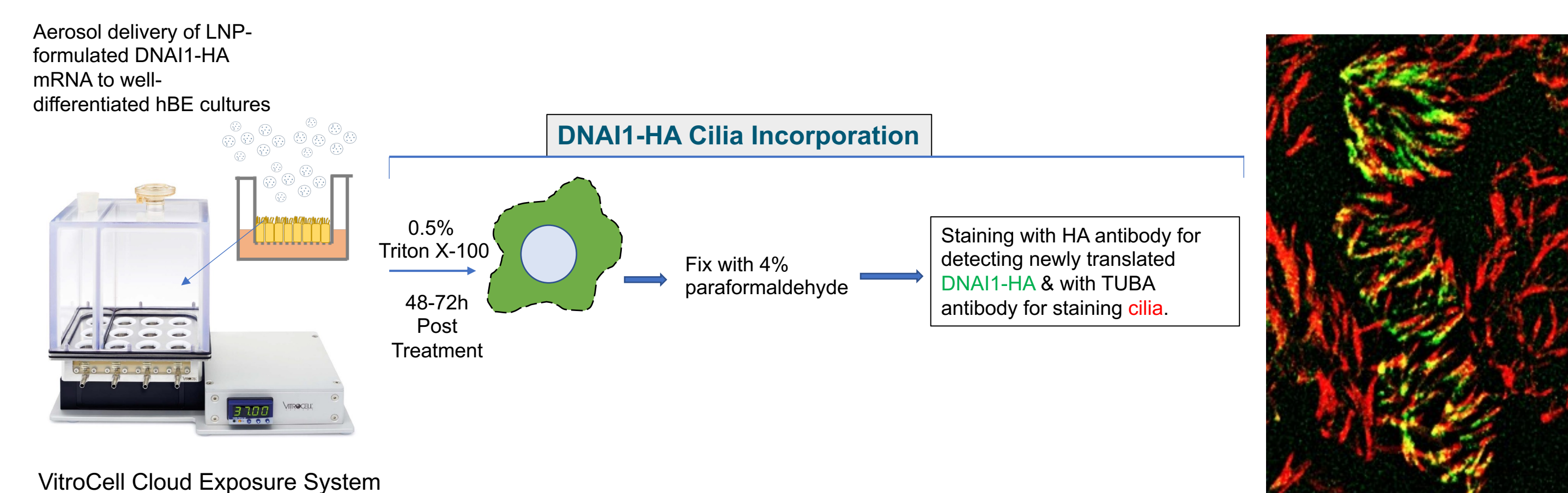


Optimized mRNA sequence improves quality and translation efficiency

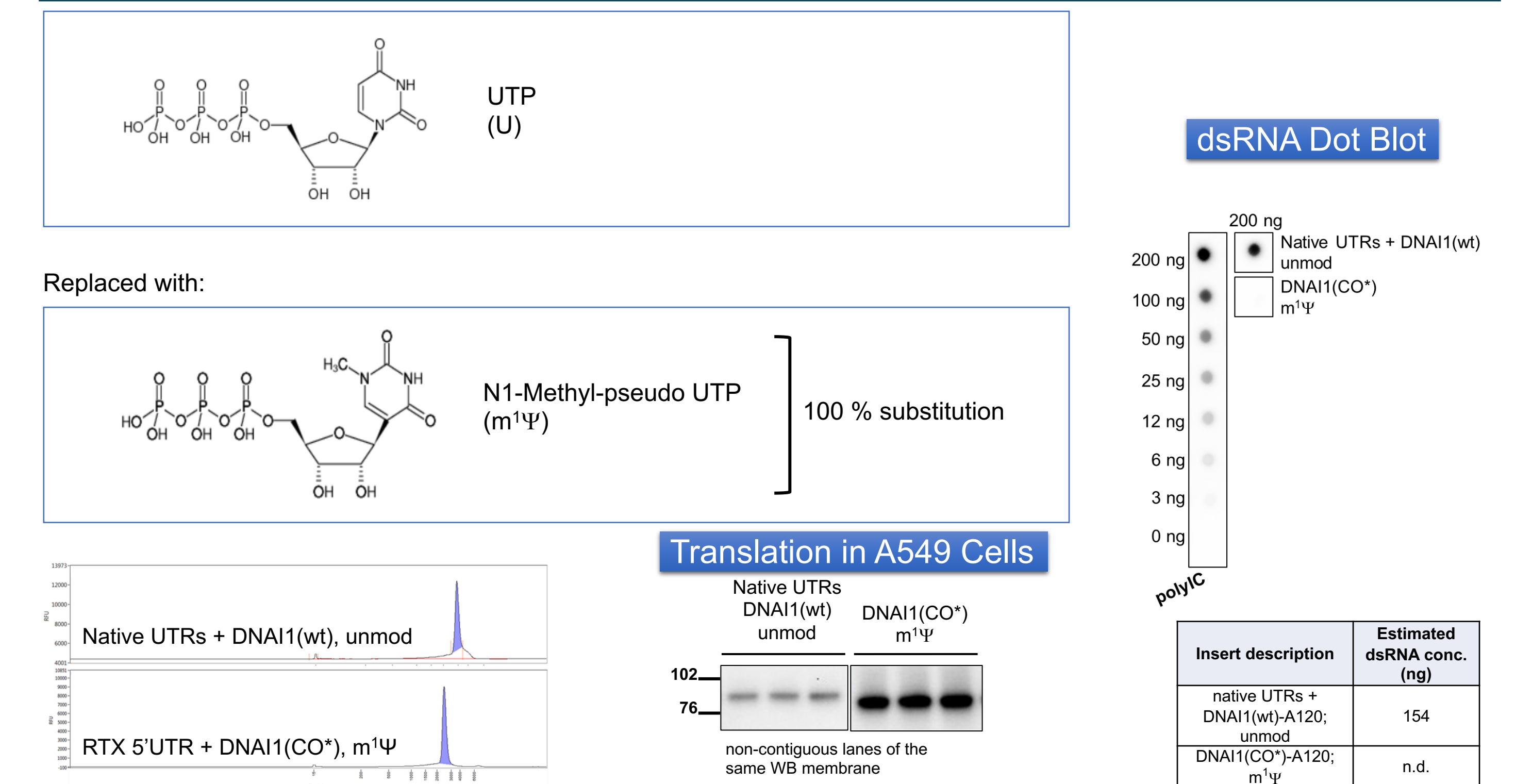


- Incorporation of optimized ORF provided higher quality mRNA (higher fraction of full-length mRNA)
- CO++ sequence optimization provided higher levels of expression in A549 cells

Nebulization of differentiated human bronchial epithelial (hBE) cells grown at an air-liquid interface employing LNP-formulated DNAI1-HA

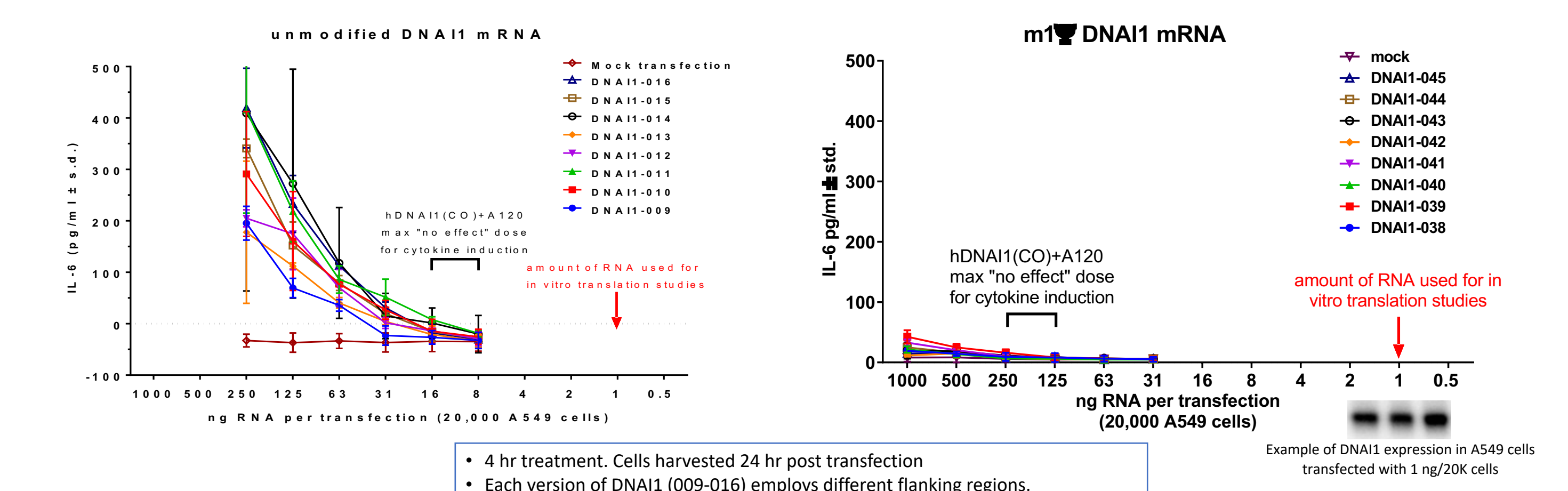


Incorporation of modified nucleotides into mRNA



- 100% m¹Ψ incorporation provided higher quality mRNA (less dsRNA, higher fraction of full-length mRNA)
- 100% m¹Ψ provided highest levels of expression and lowest immunoreactivity in A549 cells
- 100% m¹Ψ in conjunction with codon-optimization increased mRNA stability, quality and production yield (by 50% compared to unmodified/RTX codon optimized)

Immunoreactivity: Unmodified vs. m¹Ψ-containing DNAI1 transcripts



- 5'- and 3'-UTR flanking regions had negligible effect on IL-6 cytokine production
- Similar results compared with IL-6 response obtained with a broader panel of cytokines
- Incorporation of modified nucleotides significantly reduces immunoreactivity
- m¹Ψ mRNA versions elicit no detectable cytokine response at doses 125-250x higher than the doses required for maximum protein production

Summary & Conclusions

- Nucleotide usage schemes aiming to reduce the number of more reactive dinucleotides were adopted. In parallel, we reduced the global U-content in RNA transcripts. Full-length mRNA levels were noticeably improved in sequence-optimized mRNA (CO++) and western blot results indicated an increase in DNAI1 protein levels when compared to cells transfected with wild-type DNAI1 mRNA.
- Incorporation of N1-methyl-pseudouridine (m¹Ψ) was evaluated. An additional enhancement in DNAI1 protein levels was observed. Moreover, incorporation of m¹Ψ reduced dsRNA below detectable levels and significantly reduced *in vitro* cytokine responses.

These results support development of inhaled mRNA as a promising disease-modifying therapy for PCD.

Disclosures:

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