Rapid Enzymatic Synthesis and Scaling of DNA Gene Therapy Molecules

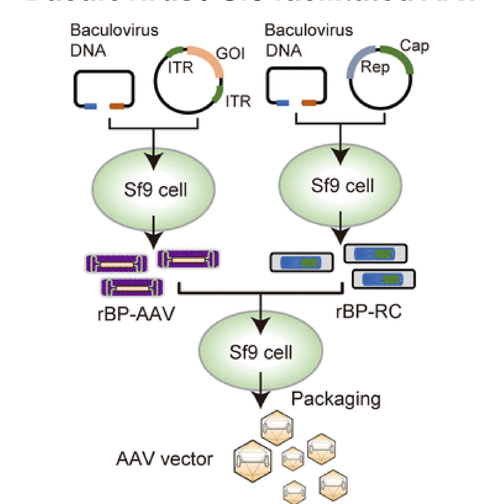
Daniel Blackstock, John Durant, Joris Cipi, Anthony Dawson, Shawna Downing, David Turkington, Adam Villa, Russell Monds, Matthew Stanton
Generation Bio, Cambridge, MA 02142, U.S.A.

Introduction

Gene therapy production is traditionally reliant on cells

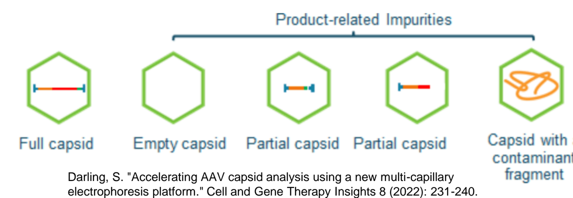
AAV / viral vectors (encapsidated DNA)
- DNA payload and capsid delivery vector produced simultaneously intracellularly
- Technology Limitations
  - Long production times (weeks/months)
  - Low yields (<10^{14} vg/L or <0.25 mg/L (DNA))
  - Propensity for product quality difficulties
  - Mix of partial, empty, full capsid vectors
  - Small-to-large scale variability
  - Production barriers (consistency, yields, and timelines) restrictive to stable preclinical / clinical development

Baculovirus / Sf9 facilitated AAV



Ohmori, T. "Advances in gene therapy for hemophilia: basis, current status, and future perspectives." Int J Hematol 111, 31–41 (2020).

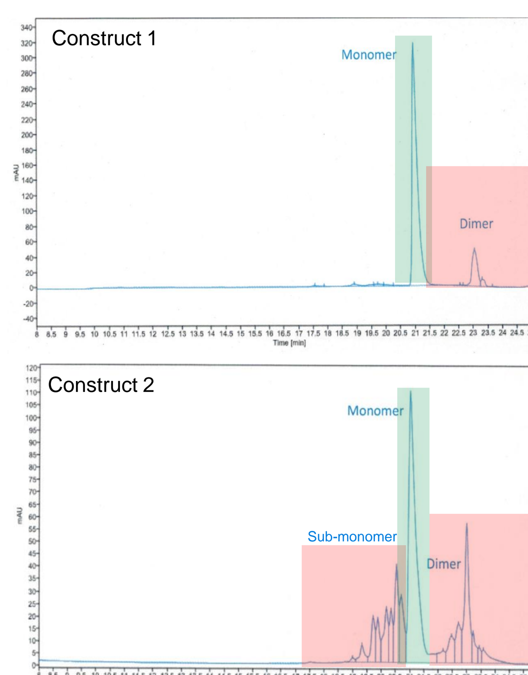
Non-viral (capsid free DNA)
- LNP mediated delivery
- Separates DNA drug substance from delivery vector for step wise control
  - Eliminates cell controlled encapsidation



Darling, S. "Accelerating AAV capsid analysis using a new multi-capillary electrophoresis platform." Cell and Gene Therapy Insights 8 (2022): 231-240.

GBIO v1 (old) non-viral DNA process
- Baculovirus / Sf9 facilitated production
- Standing complications:
  - Construct-to-construct variability
  - Extraneous DNA products
  - Low yields (1-10 mg/L)
  - Long production times (weeks/months)

GBIO v1 (old) DNA production – Baculovirus / Sf9 derived



How to address and overcome common gene therapy quality and manufacturing limitations?

Objective & Approach

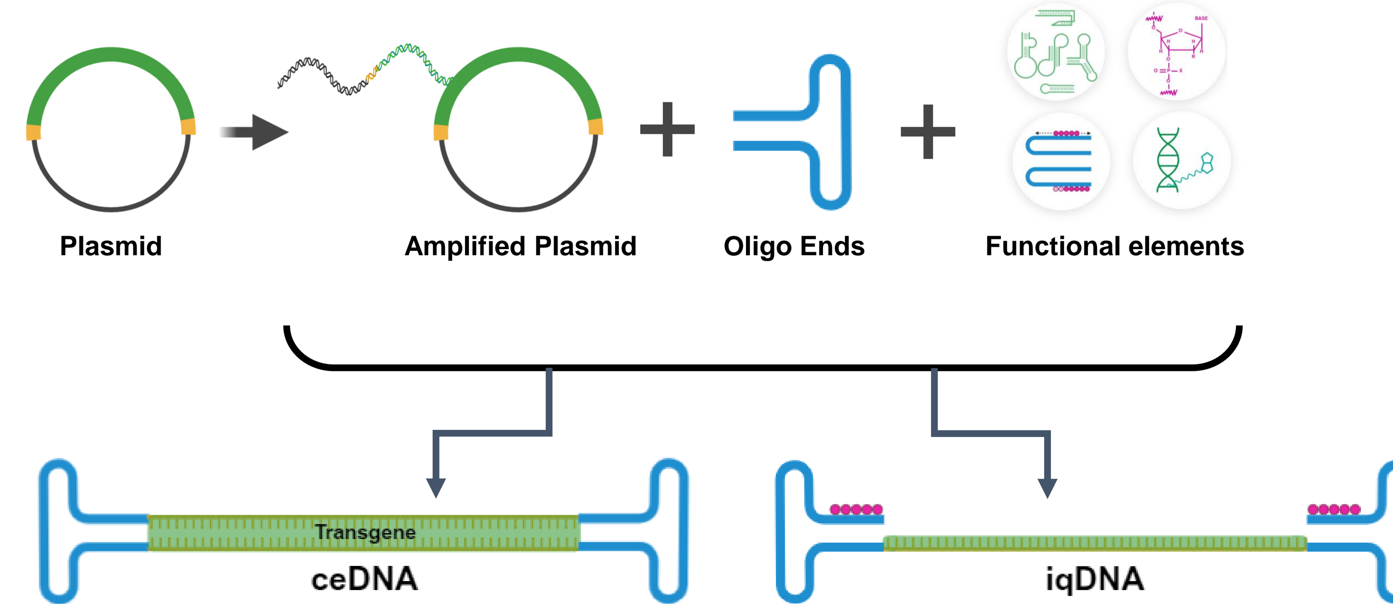
Develop a cell-free, enzymatic DNA production method

Goals
- Simplify system, remove complex (cellular & viral) components responsible for unrestrained byproduct formation [enhance process control]
- Eliminate cell growth times for rapid turnaround of DNA

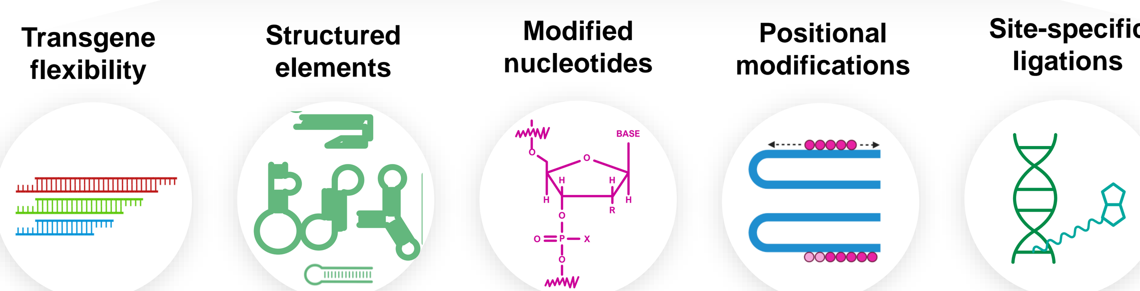
Design Considerations
- Synthetic nature to facilitate heightened DNA functionality
  - Engineerable / modular design and components
- Platform
  - Build based on standard inputs and constituents
- Minimize number of enzymes to drive process simplicity
  - Fewer steps and components = robustness and reduced costs
- Empower scalability
  - Take advantage of highly processive nature of enzymes

Design

Rapid Enzymatic Synthesis (RES)

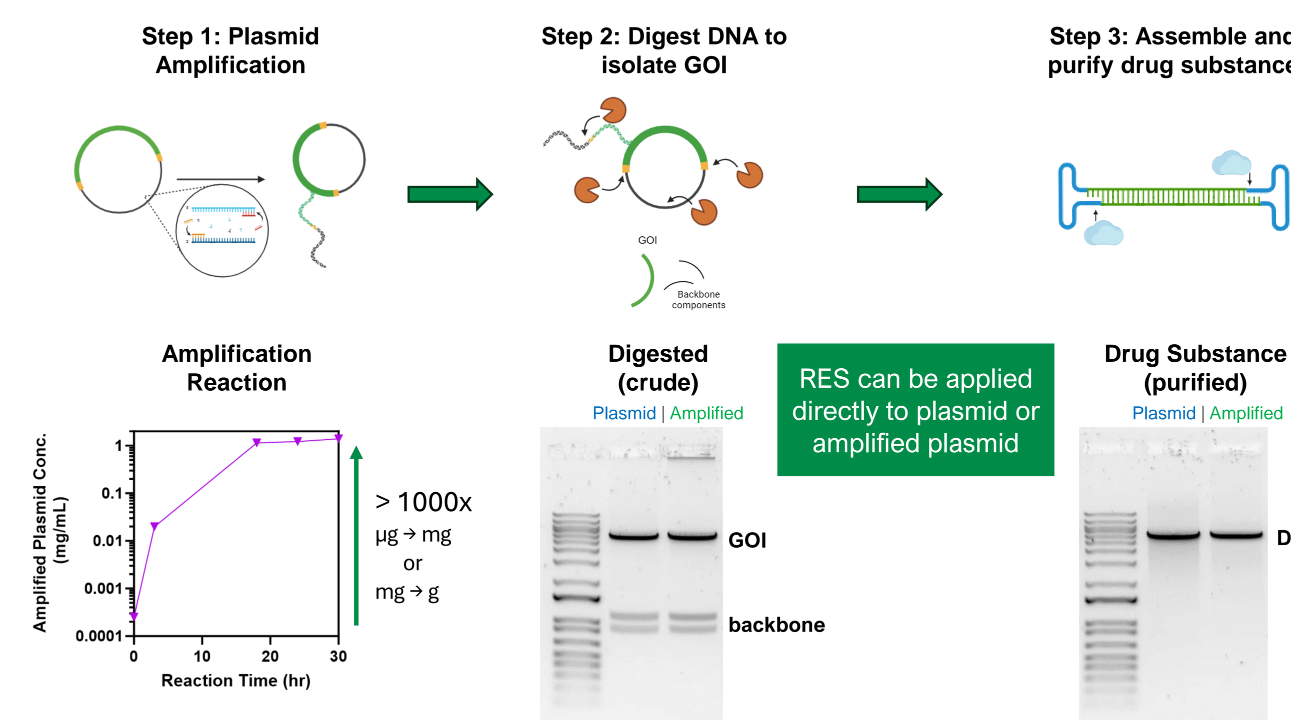


Learn more about iqDNA at Russel Mond's poster #1294

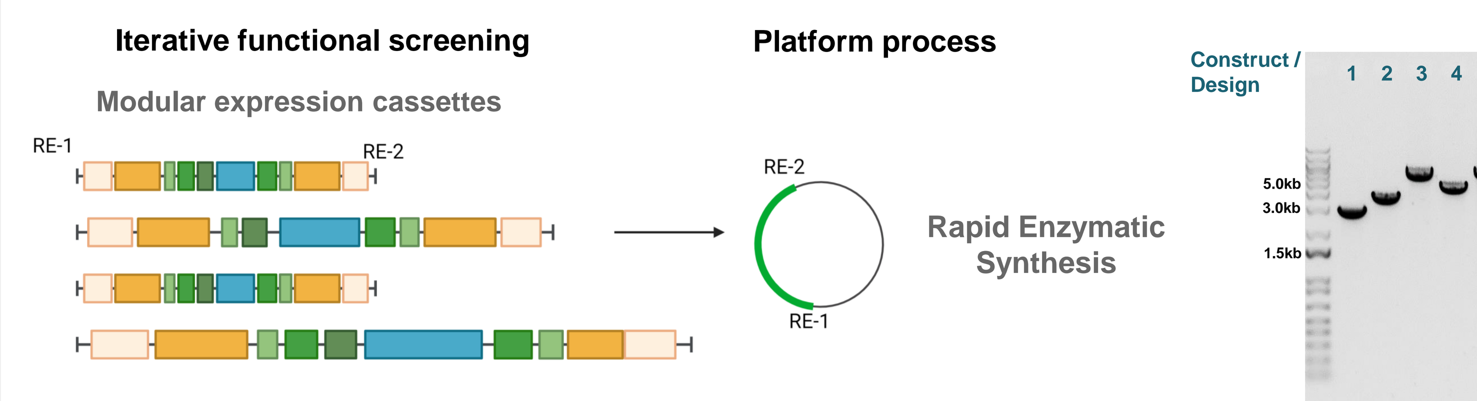


Experimental & Results

Characterizing and applying RES method



Demonstrating platform application

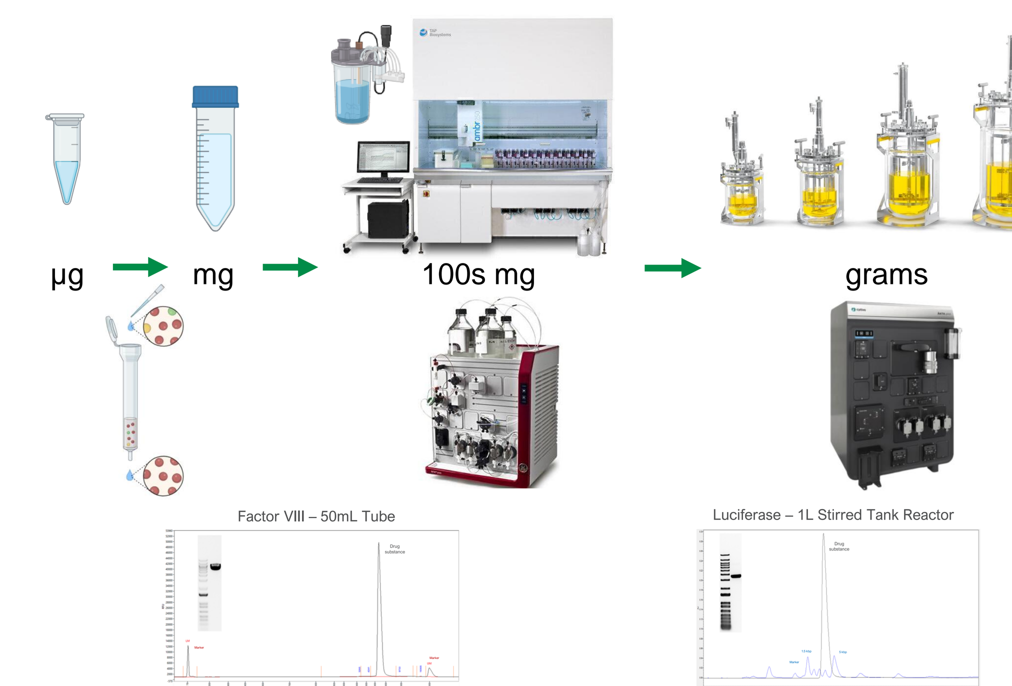


Experimental & Results Continued

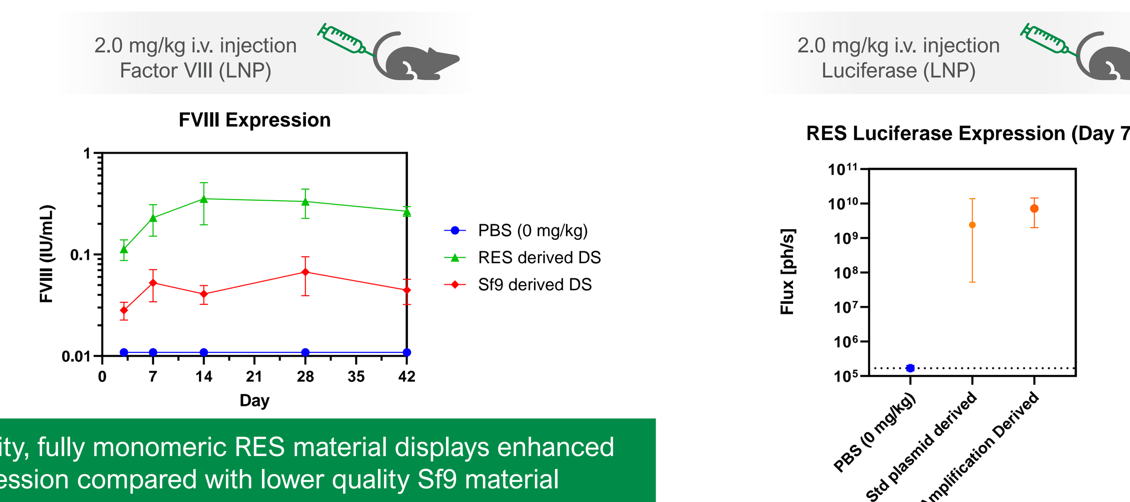
Accelerated and high-quality DNA production across scales

Plasmid for initial screening — Amplification for rapid scaling

Process	Starting Scale (plasmid)			Amplification Scale (utilizes plasmid template from starting scale)					
	microgram - milligram			100 milligrams			1 gram		
	Plasmid receipt time	Processing time	Volumetric scale	Plasmid Amplification time	Processing time	Volumetric scale	Plasmid Amplification time	Processing time	Volumetric scale
RES	~ 2 wk	3 d	1 – 10 mL	2 d	4 d	~ 100 mL	2 d	4 d	~ 1 L
Sf9 Cell Based	~ 3 wk (bacmid)	3 - 4 wk	100 - 1000 mL	N/A	5 - 6 wk	> 10 L	N/A	6 - 7 wk	> 100 L



Expression validation using RES derived DNA



Conclusion & Discussion

Rapid enzymatic synthesis (RES) demonstrated to be exceptional method for DNA gene therapy production

- Platform application
  - Standardized enzymes, recognition motifs, reaction conditions, and processing methods
- Synthetic makeup provides valuable engineerability and modular functionality
- Dramatically enhanced product quality control compared to cell-based methods
- Heightened R&D preclinical development enablement
  - At least 10-fold increase in screening / throughput compared with Sf9 process due to >100-fold reduction in volumetric scale
  - Plasmid receipt to purified drug substance in ~1 week (across all scales)
- Ultra-fast scalability at minimized production volumes
  - High volumetric (g/L) yields; traditional stirred tank reactor compatibility
  - ~1 week turn-around of gram quantities of drug substance with only µg/mg starting plasmid required