Vector optimization for non-viral antibody gene transfer and expression of anti-SARS-CoV2, human monoclonal antibodies in mice

Nathaniel Silver, Sid Jindal, Nicholas Parsonnet, Luke Hamm, Matt Manganiello, Henry Sun, Debra Klatte, Phillip Samayoa, Doug Kerr
Generation Bio, 301 Binney Street, 4th Floor, Cambridge, MA 02142

Since their initial approval over 30 years ago, monoclonal antibodies (mAbs) have seen great success as a therapeutic class. However, their high cost of production as well as the need for frequent administration has limited their widespread use in areas outside of oncology and autoimmune diseases. Antibody gene transfer (AGT) provides an alternative means of delivering specific mAbs, wherein an antibody is vectorized and produced in vivo. This may enable patients to produce their own biotherapeutic for an extended period. AAV-based delivery of vectorized antibodies has demonstrated a capacity to produce efficacious levels of antibodies in a variety of pre-clinical, disease models. However, there remain significant limitations to AAV that have limited its use for AGT in the clinic. In particular, its genetic cargo capacity of ~4.7kb limits encoding of multiple polypeptides, immunogenicity of the viral capsid restricts dosing to a single administration, and costly, scale-limited manufacturing processes prevent use in wide-spread diseases like HIV, Influenza, or COVID19, particularly in the prophylactic setting where they might be the most impactful. Non-viral gene therapy is an attractive solution to address these limitations and potentially enable prophylactic use of AGT on a much broader scale than is possible with passive administration of recombinant mAb or AAV based delivery.

Generation Bio has developed a non-viral gene therapy platform to deliver and durably express therapeutic proteins systemically in vivo. It is comprised of ceDNA, an engineered, double-stranded, linear, covalently closed-ended DNA construct, formulated in a lipid nanoparticle delivery system, ctLNP. The ctLNP delivery system has been designed for hepatocyte-specific delivery by using a biological targeting ligand and does not contain any component of the viral capsid, allowing repeated administration. Studies in immunocompetent mice have demonstrated that systemic administration of a single dose of ctLNP-formulated ceDNA results in delivery to hepatocytes and durable transgene expression. Administration of a second dose further increased expression.

Here we present the use of our non-viral gene therapy platform to express a monoclonal, SARS-CoV2 neutralizing antibody (Vir Biotechnology) as well as our vector optimization approach to maximize expression. We compare bicistronic, furin/2A-peptide based expression cassettes to paired heavy and light chain vectors as well as multi-promoter, scarless designs. While larger in size, we find that the latter two vector formats, in particular bidirectional promoter cassettes, significantly improve expression of antibodies in vivo relative to the bicistronic designs commonly used in AAV vectors to encode multiple peptides. Further optimization of regulatory as well as heavy and light chain sequences enhanced ceDNA-based expression, achieving therapeutically relevant levels of systemic mAb expression in mice. These data demonstrate early capabilities of our non-viral AGT platform and provide a path for non-viral delivery of antibodies for disease prevention and treatment.

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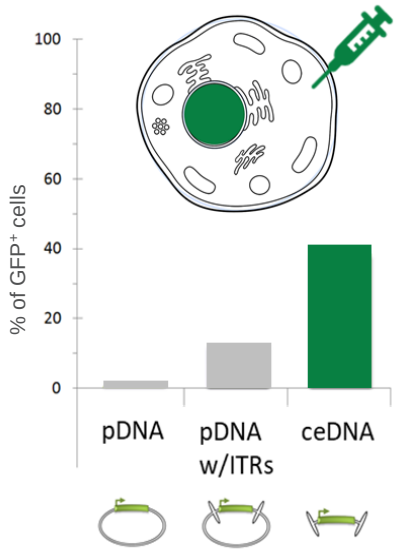
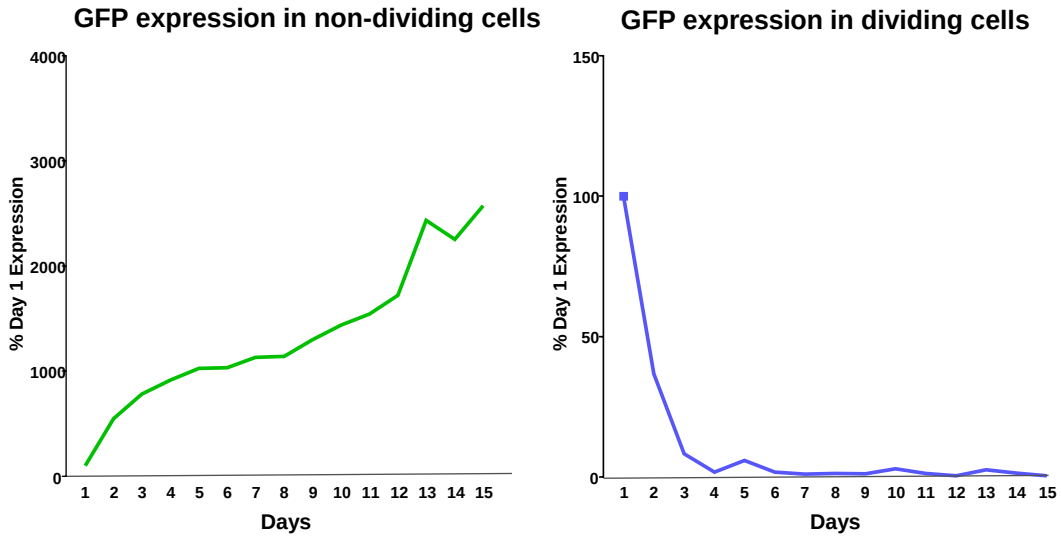
ceDNA is a closed-ended, linear, duplex DNA vector whose structure imparts key features for non-viral gene therapy

ceDNA ITR structure is key to accessing the nucleus



ceDNA expression is consistent with non-integrating episomes



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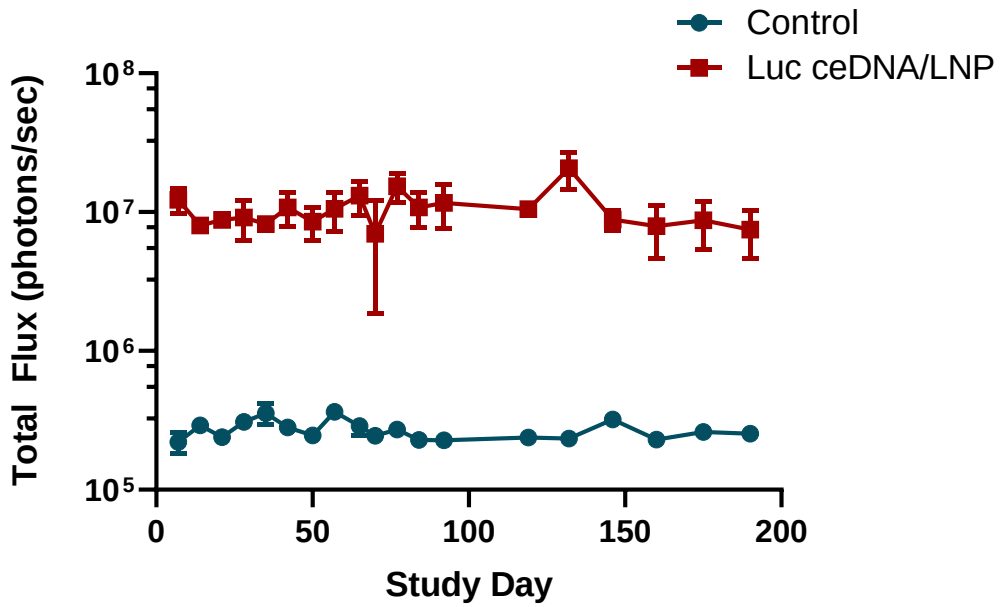
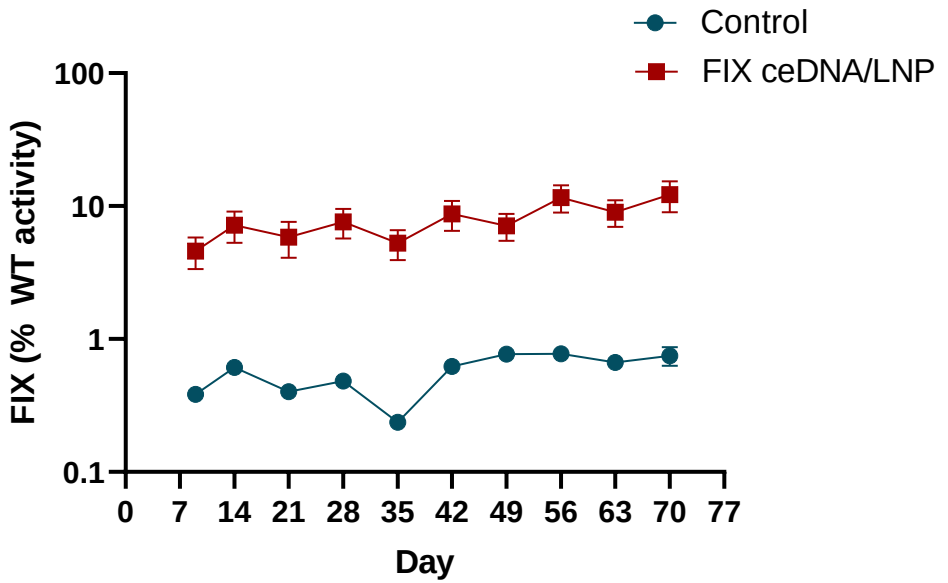
ceDNA-LNP shows durable expression in immunocompetent mice after a single IV administration

Luciferase



- Single IV administration at study day 0
- Total flux measured by IVIS *in vivo* imaging

Factor IX



- Single IV administration at study day 0
- Factor IX activity calculated from protein ELISA

FIX used as surrogate for durability and redosing in wildtype mice because this human protein does not raise neutralizing antibodies in mice, unlike human FVIII

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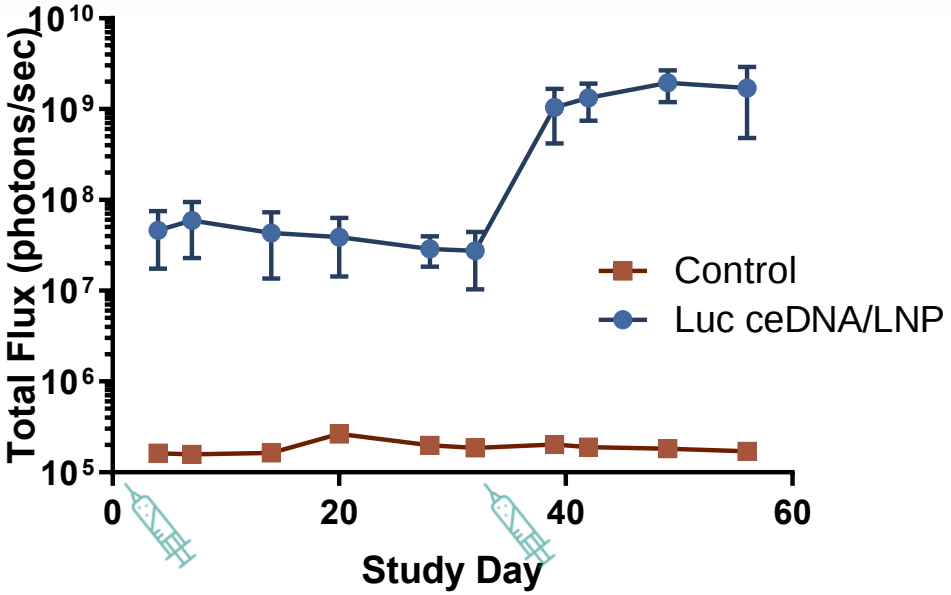
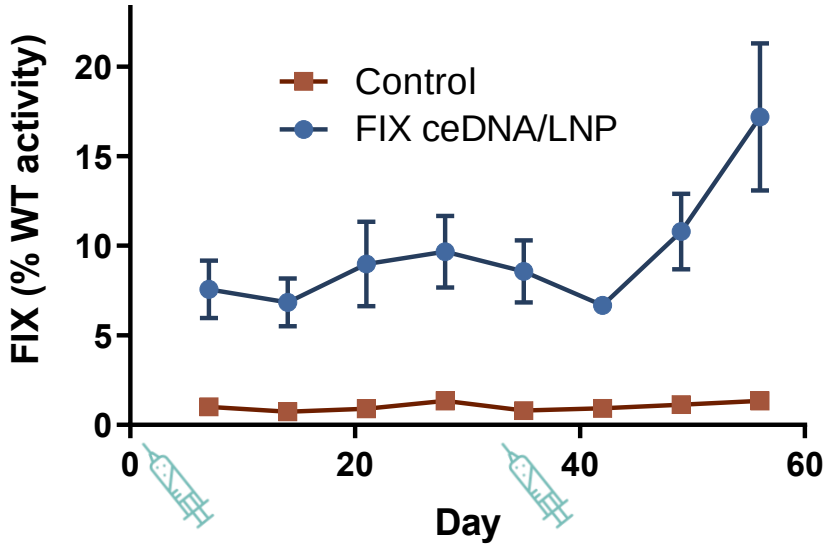
ceDNA-LNP redosing achieves increased expression in immunocompetent mice

Luciferase



- Single IV administration at study day 0
- Re-dosed at day 35 with 10X higher dose
- Total flux measured by IVIS *in vivo* imaging

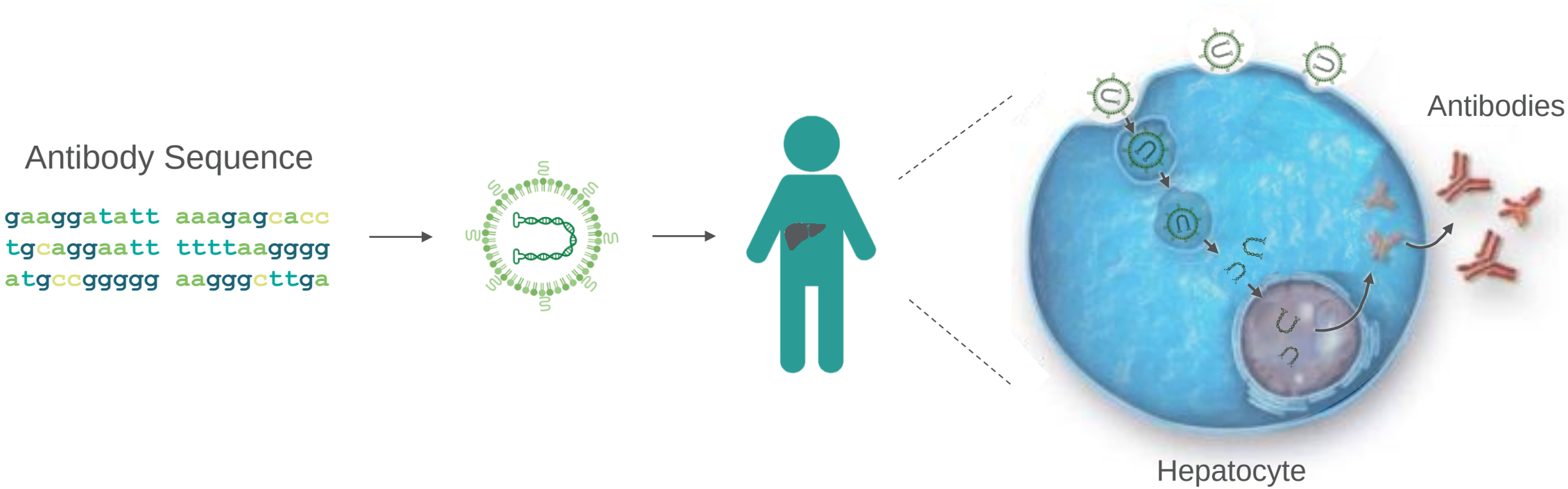
Factor IX



- Single IV administration at study day 0
- Re-dosed at day 36 at same dose level
- Factor IX activity calculated from protein ELISA

FIX used as surrogate for durability and redosing in wildtype mice because this human protein does not raise neutralizing antibodies in mice, unlike human FVIII

ceDNA Antibody Gene Transfer (AGT) enables persistent, hepatic expression and secretion of monoclonal antibodies



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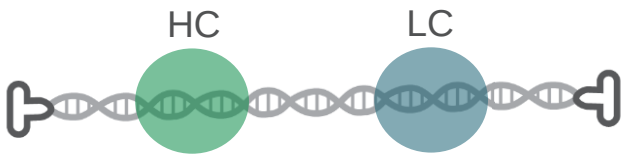
Liver specific, dual ORF and dual vector designs encoding a SARS-CoV2 neutralizing antibody yield highest expressing constructs

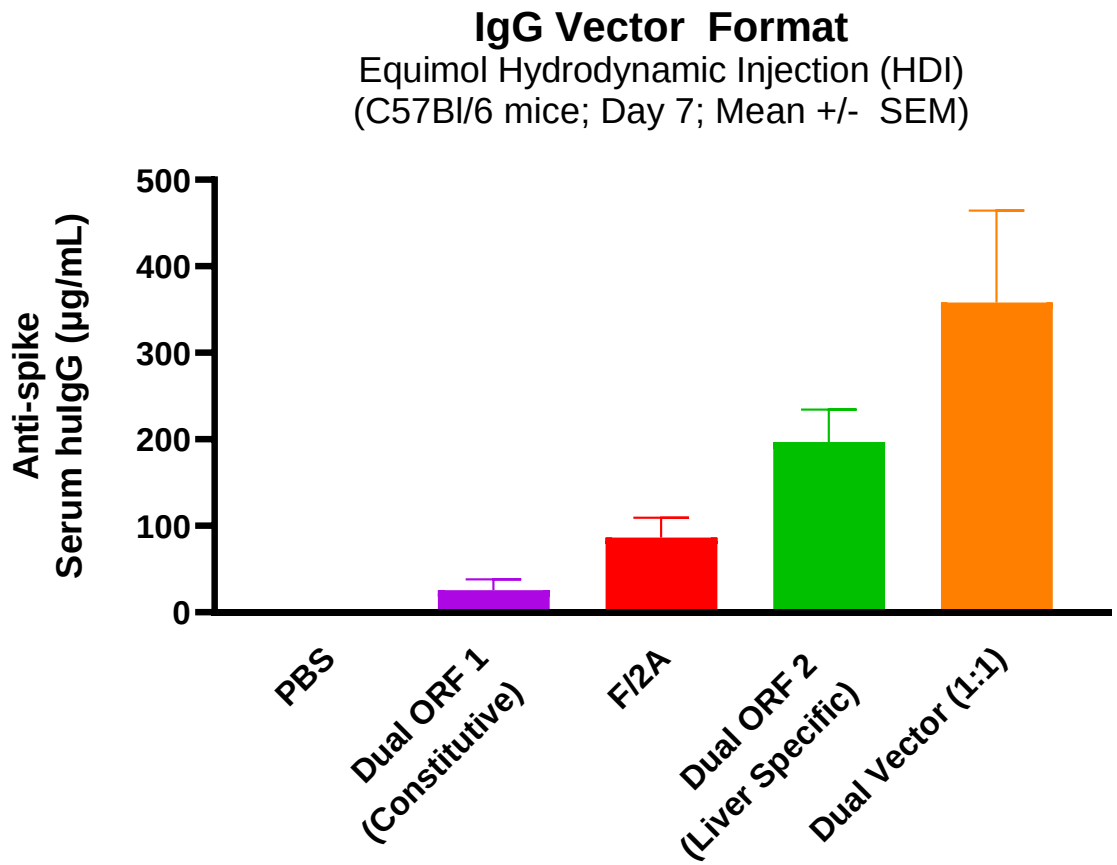
Vector Format Optimization

Bicistronic Cassettes

Dual ORF cassettes



Dual vectors



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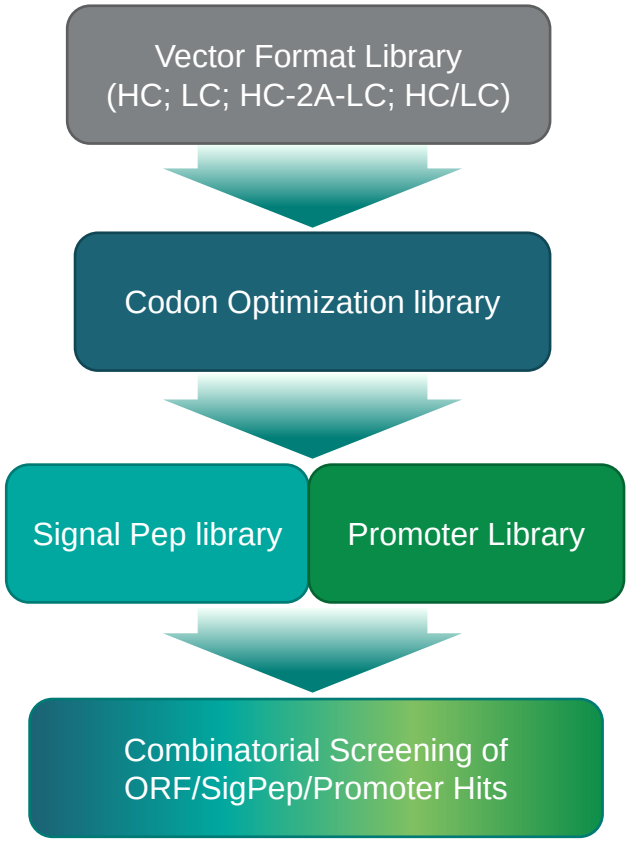
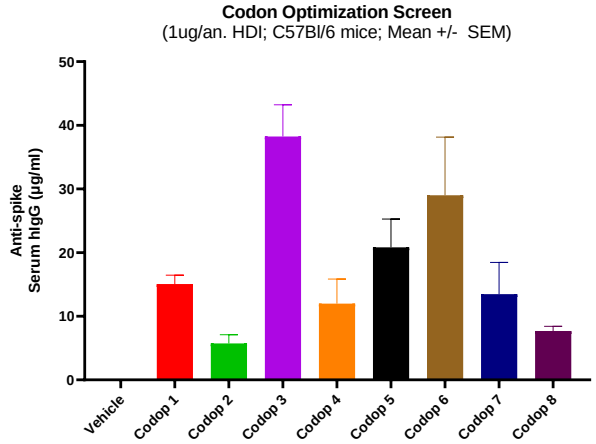
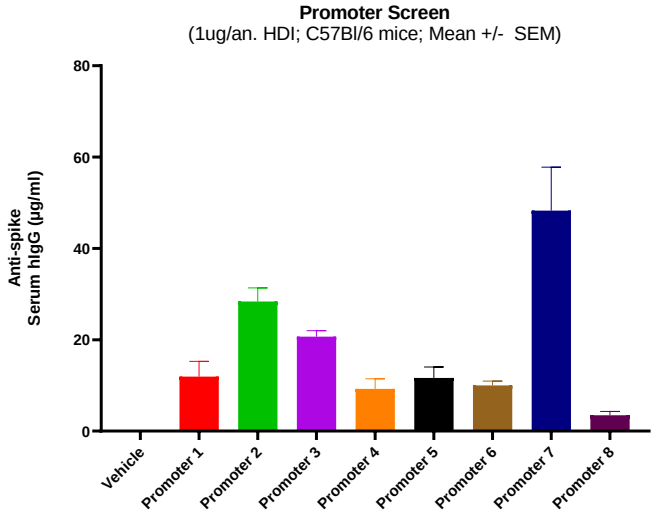
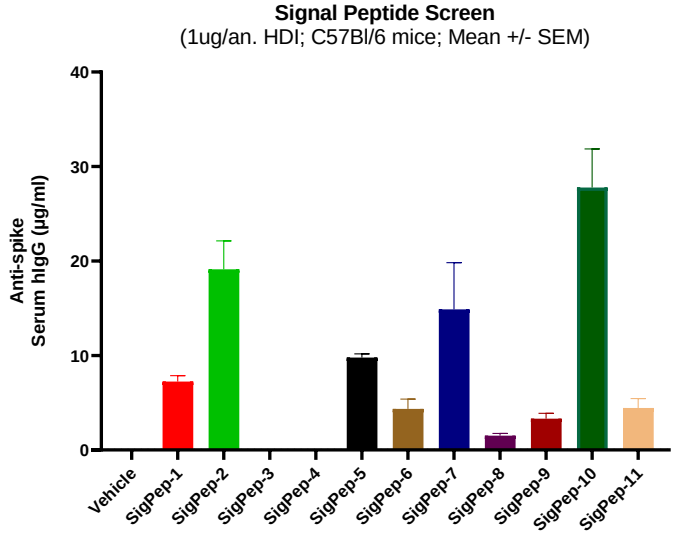
ceDNA vector optimization across promoter, ORF codon usage, and signal peptides identified high expressing dual vector and dual ORF designs

Vector Cassette Optimization









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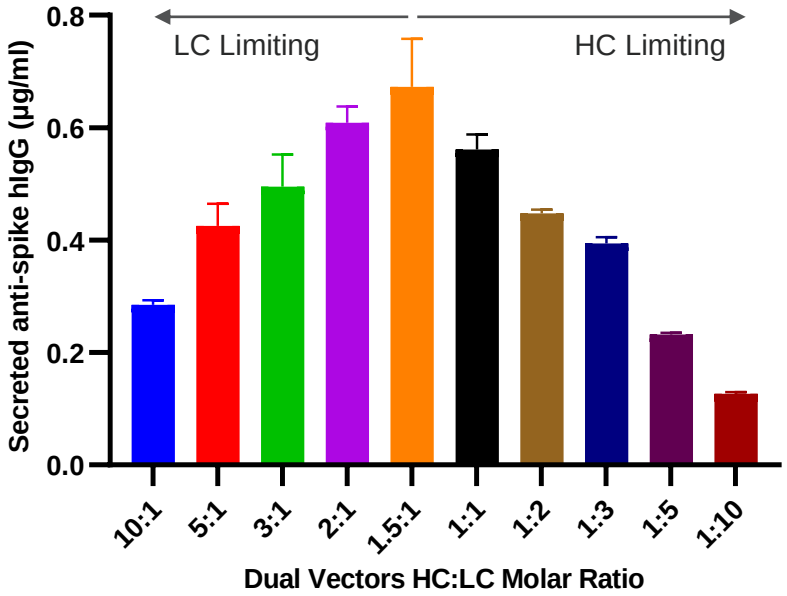
Screening of Heavy and Light chain ratios suggests hepatic IgG expression with ceDNA is enhanced with higher HC:LC molar ratios

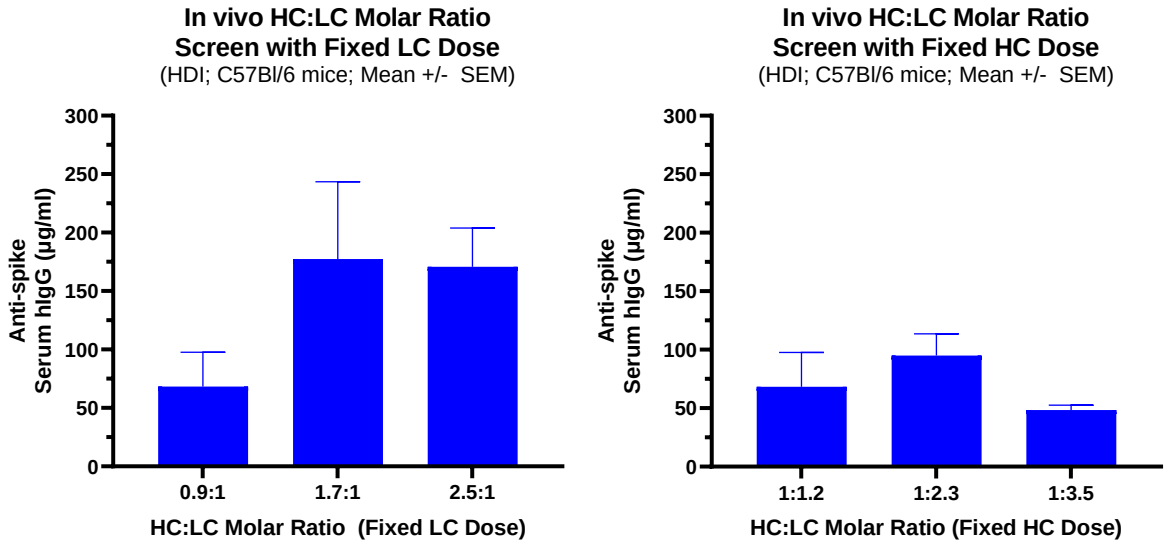
In vitro Screen of HC:LC Ratios



3:2 HC to LC molar ratio is optimal for dual vector ceDNA construct pairs

HepG2 cells; supernatant collected at 72hr

In vivo Fixed HC/LC Dose Screen of HC:LC Ratios



At a fixed dose of LC or HC ceDNA, increasing dose of HC improves expression while increase LC dose has limited effect

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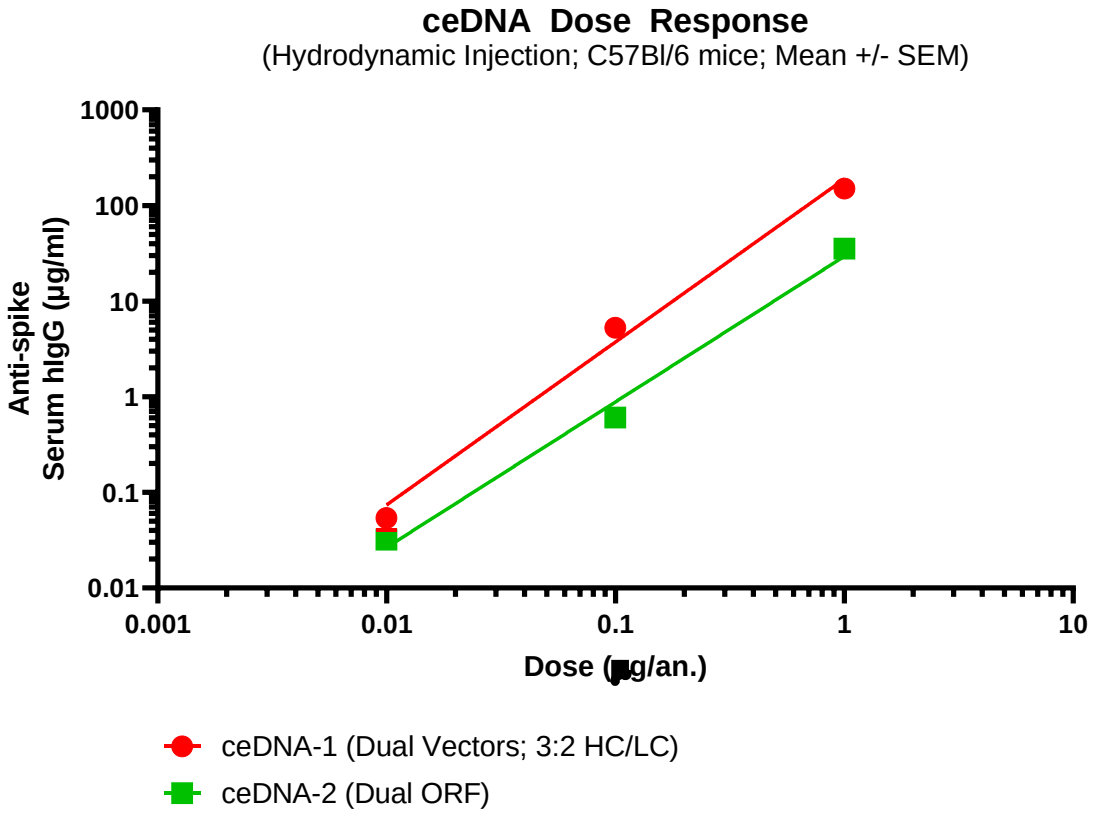
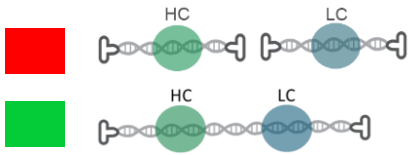
ceDNA format of optimized designs show dose dependent expression, with best dual vector designs yielding 5-10x higher activity relative to dual ORF designs





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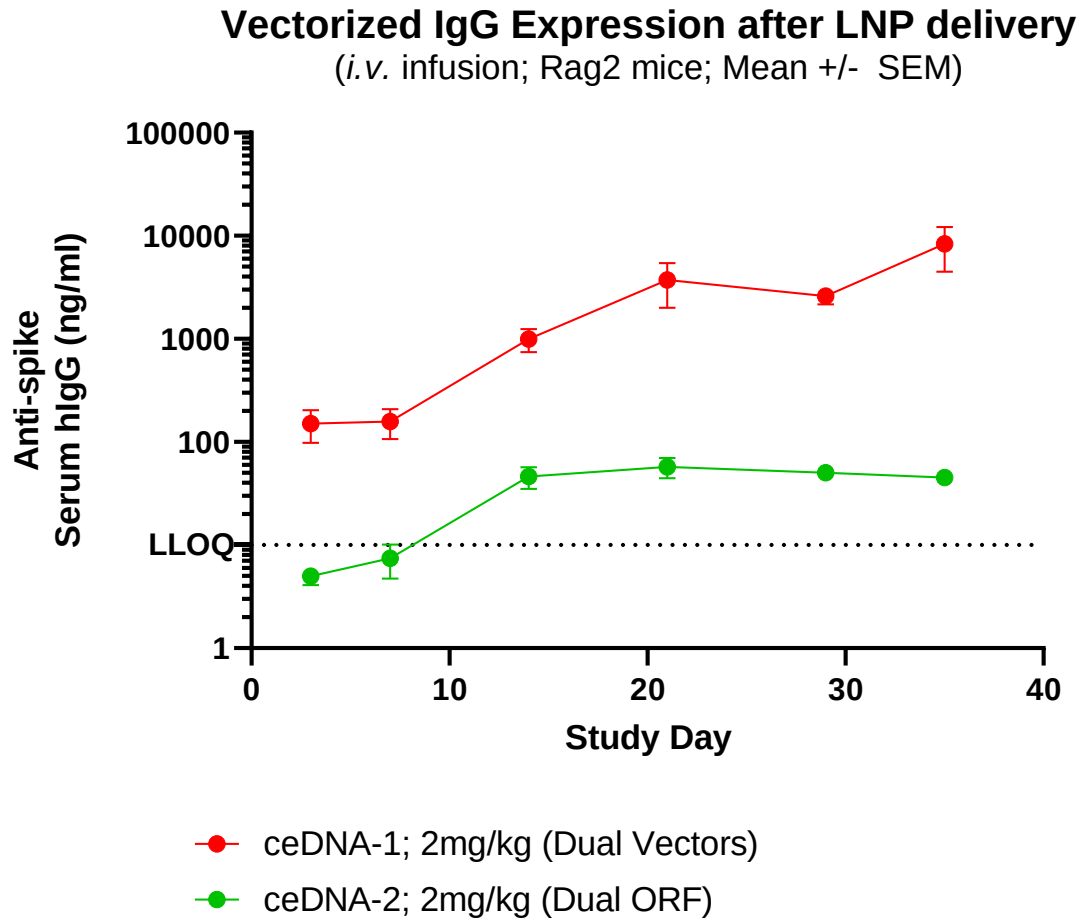
LNP delivery of dual vectors achieved persistent, therapeutically relevant concentrations of anti-SARS-CoV2 hIgG in mice



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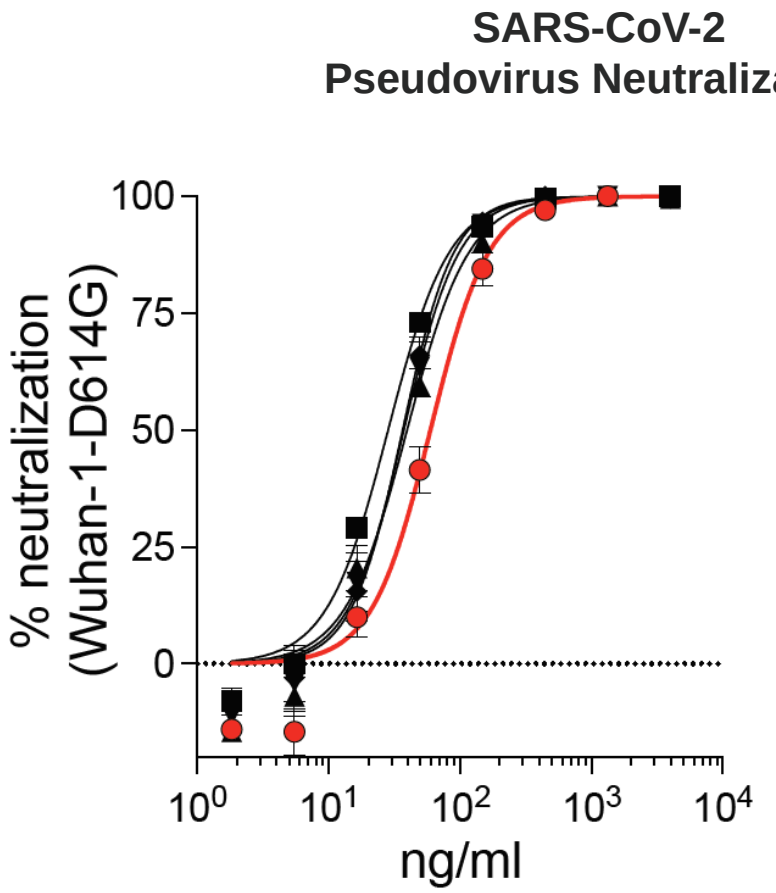
Serum from ceDNA treated animals shows potent neutralization of SARS-CoV-2 pseudovirus, equivalent to the recombinantly produced antibody



	IC50 (ng/ml)
rAb	59.58
An. 1	28.39
An. 2	39.59
An. 3	37.42
An. 4	36.62

Serum neutralizing activity of individual animals treated with ceDNA encoding anti-SARS-CoV2 mAb (HDI; C57Bl/6 mice) or recombinant mAb spiked into mouse serum at a comparable dilution

All neutralization assays performed by collaborators at VIR Biotechnology (VeroE6 cells; CoV2 Spike (D19) pseudotyped VSV-Luc)

Evolution of gene therapy manufacturing – novel enzymatic process with potential for improved scaling and cost effectiveness

Benefit for antibody gene transfer, with potential to impact millions of patients

	UPSTREAM	DOWNSTREAM PURIFICATION	FORMULATION	PAYLOAD/PURITY	PAYLOAD SCALE
AAV	STEP 1 Sf9 cell production and then encapsidation 	STEP 2 Budded virus harvested: Scale limited due to capsid instability  *Mixture of full/empty capsids, complete and incomplete genomes*	STEP 3 Multi-column purification: full versus empty only 	 **INCONSISTENT** *Payload & Quality*	**Tens of thousands** *10s of Thousands*
ceDNA 	STEP 1 Suspension Sf9 cell production 	STEP 2 ceDNA harvested from cells 	STEP 3 ceDNA drug substance purified  STEP 4 High-purity ceDNA packaged 	 **CONSISTENT** *Payload & Quality*	**Millions** **Hundreds of Millions**

STEP 1 **Enzymatic ceDNA manufacture** (cell free) One day process from pDNA

Data demonstrate early capabilities of non-viral AGT platform and provide a path for non-viral delivery of antibodies for disease prevention and treatment

- Encoding HC and LC on separate ceDNA vectors and delivering both in a 3:2 molar ratio is optimal format for hepatic expression
- Combinatorial optimization of regulatory sequences and heavy and light chain codons of anti-SARS-CoV2 mAb resulted in highly expressing constructs
- LNP co-formulation and delivery of paired vectors achieved therapeutically relevant levels of mAb in mice (~8ug/ml)
- Antibodies produced in situ retained binding and functional activity, and demonstrated comparable ex vivo neutralization activity relative to recombinant control
- Non-viral delivery of ceDNA encoding neutralizing, monoclonal antibody has potential for persistent expression and long-term protection against SARS-CoV2 with a single dose
- Enzymatic synthesis may further unlock scaled manufacturing, potentially enabling AGT to reach global populations for SARS-CoV2 and future pandemics

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