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Complementary Gene Editing Technologies Enhance Rapid Generation of Stable Cell Lines Producing Enveloped Virus-Like Particles

Corey Brizzee, PhD
Director of Gene Editing

Demeetra's Core Technologies

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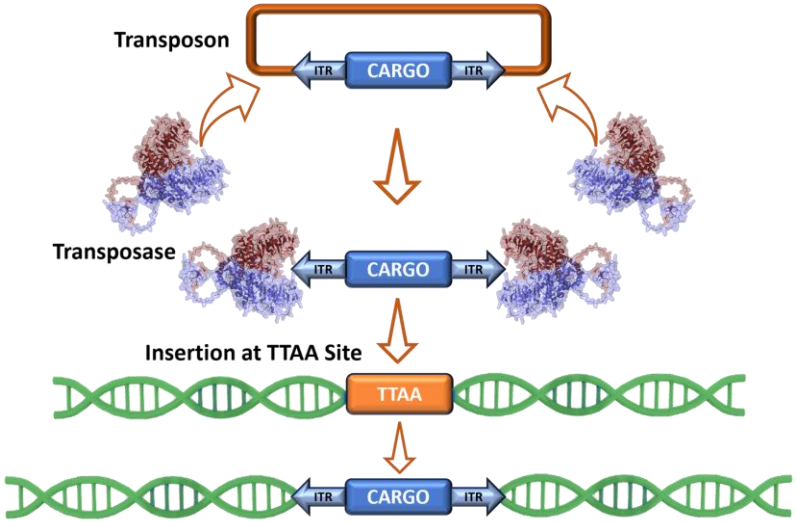
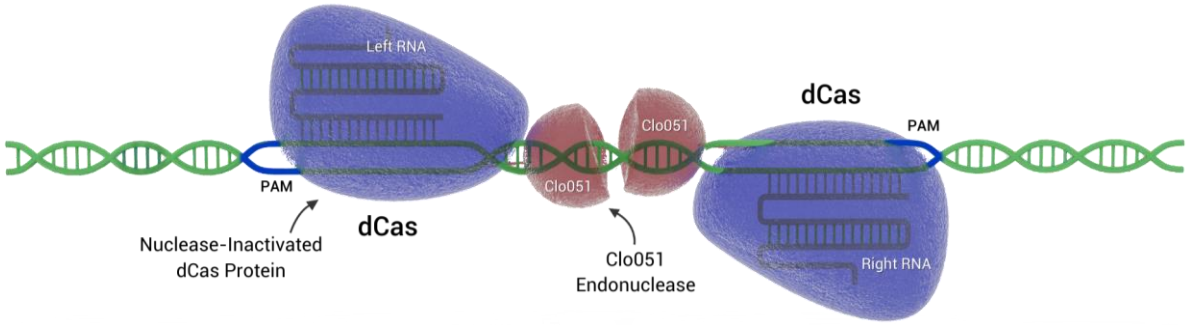
piggyBac Transposase/transposon



- Transposase integrates cargo at highly expressed sites – open chromatin and non-coding regions
- Used as a genome surveyor tool

Cas-CLOVER Targeted Nuclease



- High efficiency targeted dimeric nuclease
- High fidelity: cuts only when Clo051 nuclease dimerizes
- Larger deletions with overhanging staggered cuts – high knock-in efficiency

Bioproduction Platforms for Rapid Response to Pandemics

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Overall Goals:

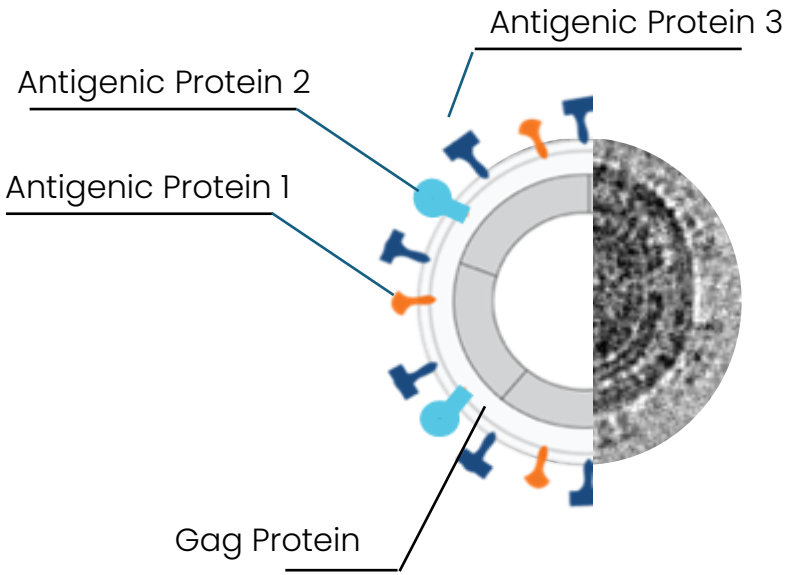
1) Stable Cell Line expressing Core GAG protein
   - Increased levels of GAG correlate to higher eVLP production
2) Rapid **and** Tailored expression of Antigenic Proteins
   - Specific ratios of expression lead to better efficacy in animal studies

To achieve these goals:

1) piggyBac transposase to identify novel sites in genome for a wide range of GAG expression
2) Targeted knock in of VLP cargo elucidates site-specific expression contribution of novel sites compared to known genomic safe harbors

eVLPs

Enveloped Virus-Like Particles



A perfect match for enveloped viruses

CEPI VBI VACCINES

Novel High-Expressing Site Identified via piggyBac Genome Mapping

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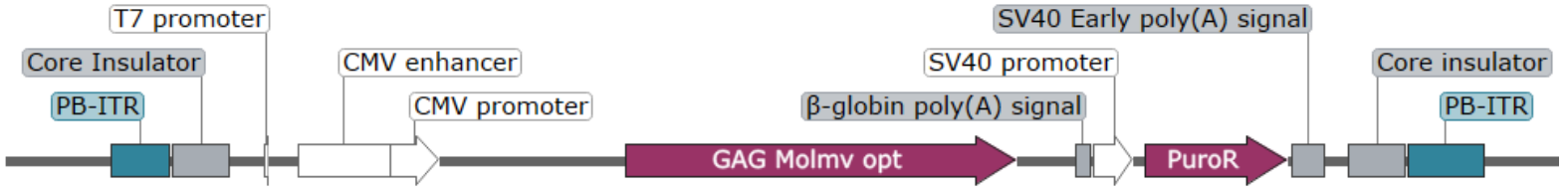
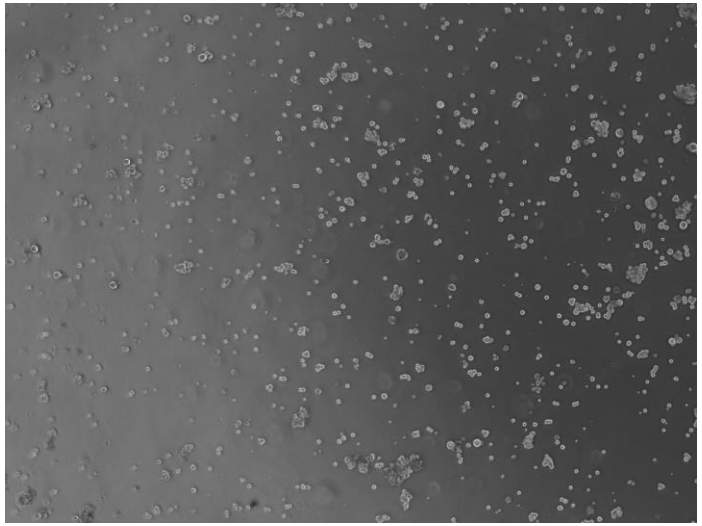
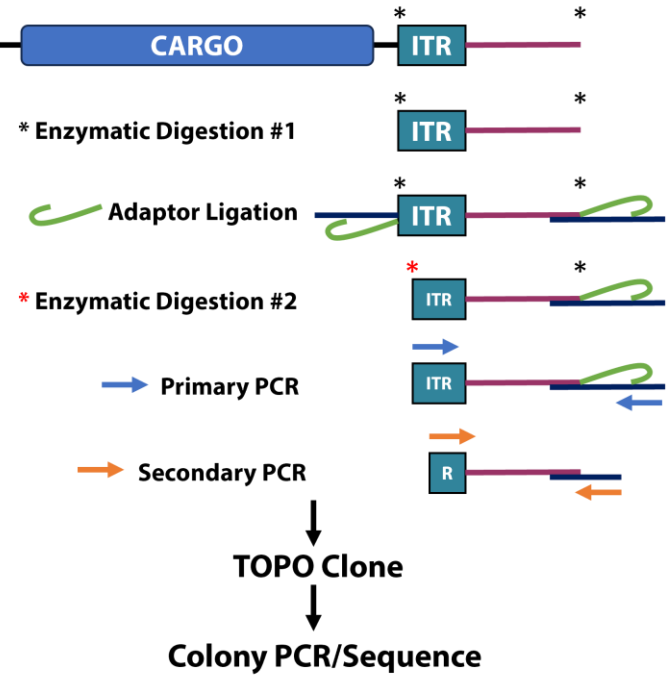


HEK293SF Single-Cell Clonal Population





Clone Site Mapping and GAG expression

Clone #	Total Integrations	[GAG] (ng/ml)	Genomic Locations
1 (b2)	4	0.0629	Chr.8 (73,738,168) Chr.10 (11,461,767) Chr.17 (21,100,584) Chr.22 (41,468,704)
3 (b4)	4	0.0670	
4 (b5)	4	0.1596	
6 (b7)	4	0.1238	
7 (b8)	4	0.0373	
8 (b9)	1	0.1001	Chr.2 (proprietary)

Cas-CLOVER Targeted Knock-In Concept and Workflow

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Novel site in Chromosome 2 was compared to known Safe-Harbor sites, ***Rogi1***(high) and ***GSH31***(medium), from Aznauryan et al. 2022

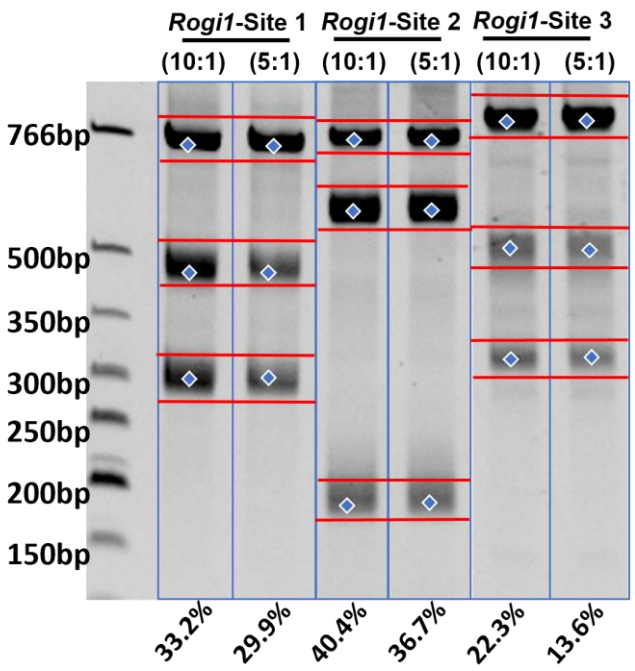
- Typically design three guide pairs per target region
- Test with various ratios of guides to Cas-CLOVER

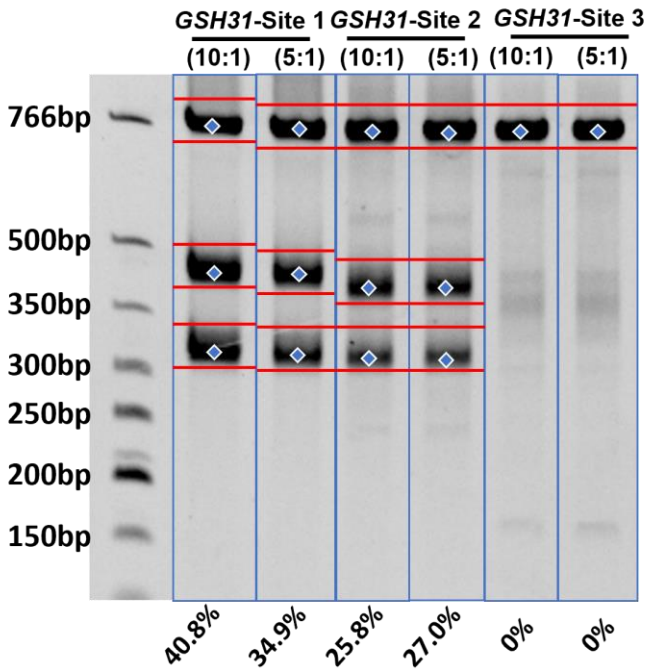
Cell Reports Methods

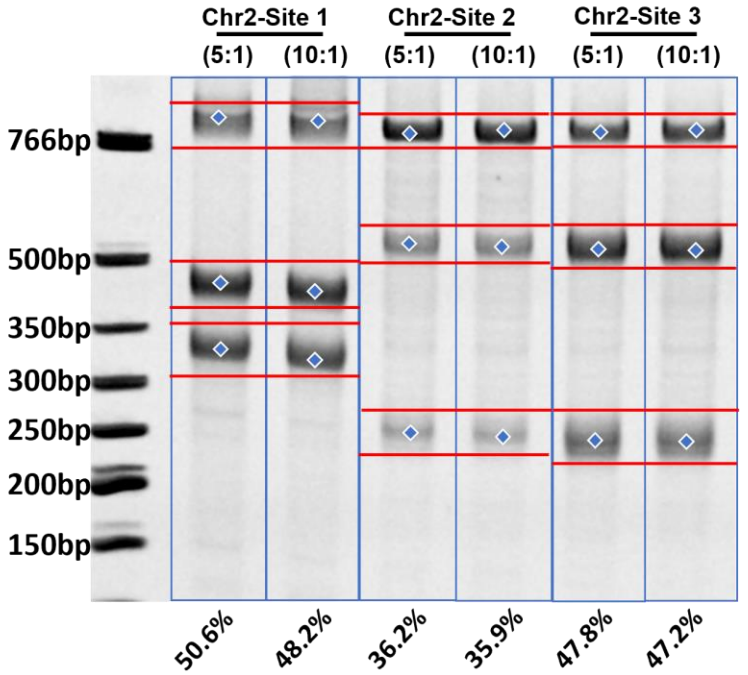
CellPress
OPEN ACCESS

Discovery and validation of human genomic safe harbor sites for gene and cell therapies

Erik Aznauryan,[1,2,3,4] Alexander Yermanos,[1,5,6] Elvira Kinzina,[7] Anna Devaux,[8] Edo Kapetanovic,[1] Denitsa Milanova,[3,4] George M. Church,[3,4,9] and Sai T. Reddy[1,9,10,*]







At Demeetra, we simultaneously test guide while performing all knock-ins to save 5-7 days.

Site-Specific Knock In of eVLP Cargo Validates Novel Chromosome 2 Site

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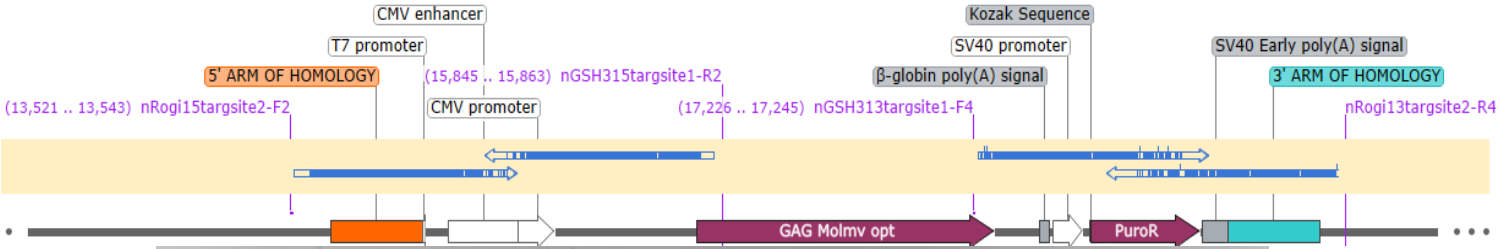

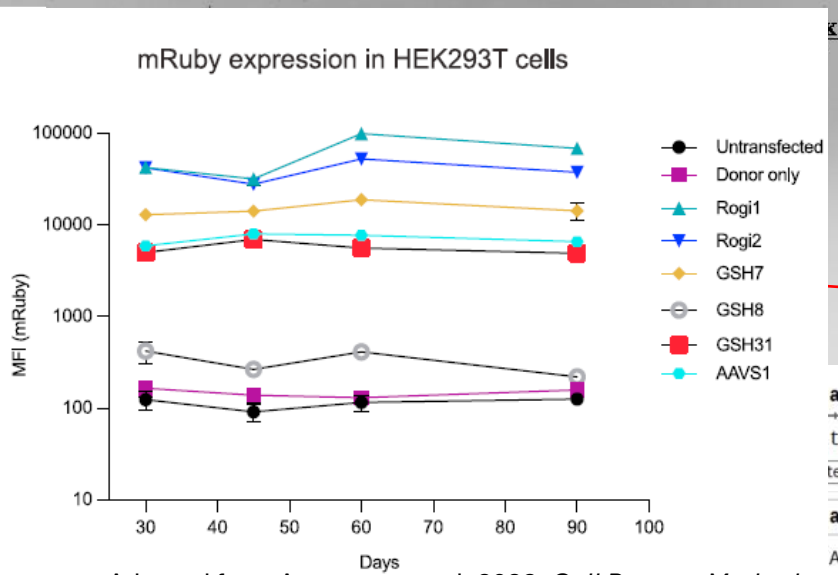

Adapted from Aznauryan et al. 2022, *Cell Reports Methods*

Sample Identification	VCD (E+6 cells/mL)	Viability (%)	[Gag] (ng/mL)
GSH31	2.08	95	3724
Chromosome 2	1.58	97	3535
Rogi1	1.83	92	<LOD at 1/100

Current Work and Future Platforms

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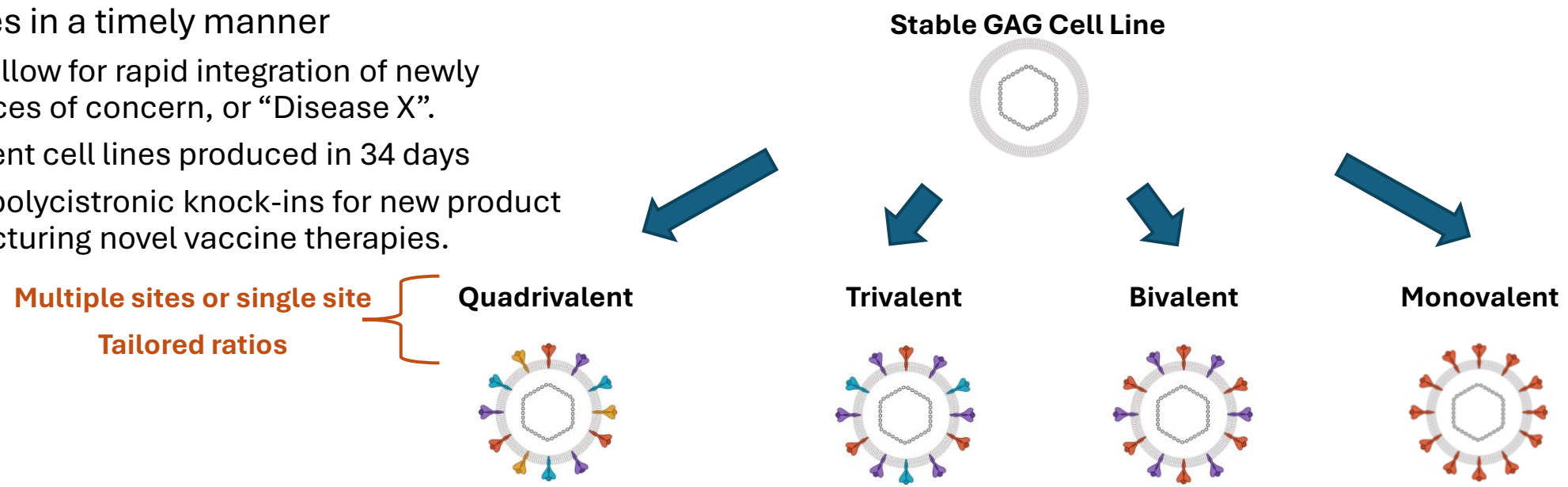
Develop Stable Cell Lines in a timely manner

- Leverage strategies to allow for rapid integration of newly identified future variances of concern, or “Disease X”.
- Newly developed Bivalent cell lines produced in 34 days
- Future bicistronic and polycistronic knock-ins for new product candidates for manufacturing novel vaccine therapies.



Questions?