Development and Characterization of a Rhabdovirus-Free Clonal Sf9 Cell Line for Enhanced AAV Production in the Baculovirus Expression Vector System

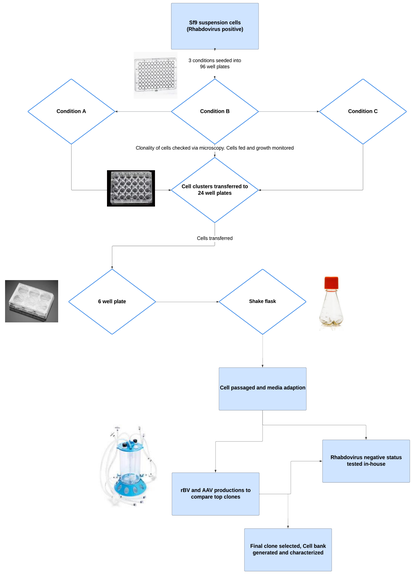
Yingyu (Gina) Zhang, Michelle Martin, Brendan Leung, Chinmay Patkar, Christopher Casey, Jeffrey Kan, Linrui Li, Justin Issacson, Helen Feng, Ting Yang, Robert Lu, Yiling Fang

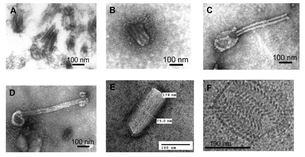
Frontera Therapeutics, Inc. Bedford, MA

Abstract

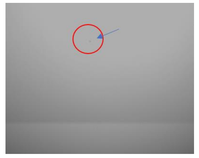
The widespread use of *Spodoptera frugiperda* 9 (Sf9) cell lines in biological product manufacturing has revealed the presence of a novel rhabdovirus identified in 2014. While downstream purification successfully eliminates rhabdovirus, the desire for a virus-free cell line prompted the development of an in-house rhabdovirus-free clonal Sf9 cell line. The rigorous characterization employing a comprehensive panel of safety assays, established in house, confirmed the absence of rhabdovirus. Evaluation of the cell line's performance in baculovirus generation and Adeno-Associated Virus (AAV) production demonstrated superior yields and product quality compared to the conventional Sf9 cells, spanning multiple AAV serotypes. The successful development of this rhabdovirus-free cell line marks a significant advancement, ensuring heightened safety and efficiency in future process development and Good Manufacturing Practice (GMP) productions.

Rhabdovirus-Free Sf9 Cell Cloning Process

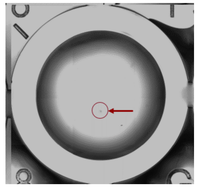




Sf9-Rhabdovirus observed under EM (*Reference: J of Virology 2014 Jun; 88(12):6576-85*)



Day 1: Single cell circled in red

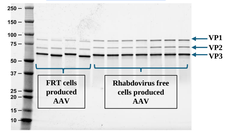


Day 14: Full well with cell clone circled in red

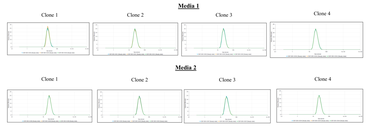
Top Clone Selection Process

Product Quality Attributes	Clone 1		Clone2		Clone 3		Clone 4	
	Serotype A	Serotype B	Serotype A	Serotype B	Serotype A	Serotype B	Serotype A	Serotype B
Lysate vector titer (vg/mL)	7.8E+11	4.9E+11	1.3E+11	4.9E+11	8.0E+11	2.8E+11	8.8E+11	6.1E+11
% Monomer (SEC-HPLC)	100.0%	99.1%	100.0%	97.0%	100.0%	98.9%	100.0%	99.3%
Infectious titer (ip/mL)	1.6E+10	1.5E+10	1.8E+10	1.2E+10	2.5E+10	1.3E+10	1.8E+10	8.4E+09
Res HCP (ng/mL)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Res Sf9 DNA ng/1E13 vg	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	19.2	<LOQ	<LOQ

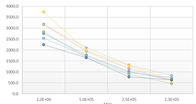
Protein Composition



Dynamic Light Scattering



In vitro Potency

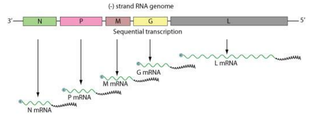


Rhabdovirus Genome RNA Testing Results for Top Clones

Sample Description	Region 1	Region 2	Region 3	Region 4	Region 5
Clone 1	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clone 2	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clone 3	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Clone 4	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
FRT Cell Line (Positive Control)	22.0	21.0	23.3	23.0	21.3

Cell Line Characterization

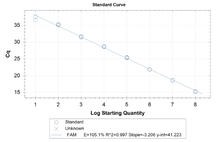
Rhabdovirus Genome RNA Detection—RT-qPCR Method



- Primer sets targeting various genes have all been evaluated
- Primer sets chosen for final method are specific to one of these genes.

Image Source: https://rhabdoviruses.wordpress.com/

Linearity



- Synthesized RNA
- Standard curve range: 10 to 1×10^8 copies
- $R^2 > 0.97$

Intermediate Precision

N	Sample Type	%CV
17	Formulation Buffer + Spike	10%
36	FRT cells + PC	7%

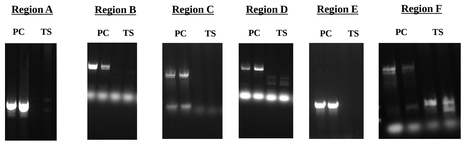
Repeatability

N	Sample Type	%CV
6	Formulation Buffer + Spike	10%
4	Medium + Spike	2%
6	FRT cells + PC	7%

Specificity

Template	Cq
Region 1	Not detected
Region 2	Not detected
Region 3	Not detected
Region 4	9
Region 5	Not detected
Various AAV serotypes plasmids	Not detected
FRT Cells	14

Rhabdovirus Genome RNA Detection—PCR method



PC: Rhabdovirus Positive FRT cell line
TS: Rhabdovirus-free cell line Final Clone

Results have shown that the new cell line is free of any of the rhabdovirus genome sequences

Final Cell Bank Full Panel Testing

Category	Assay	Specification
Characterization	Viable cell density (at thaw)	>1E7 cells / vial
	Viability (3 days post-thaw)	>=90%
Identity	Identification (Barcode)	Sf9 cells
Safety	Sterility	No growth
	Bacteriostasis and fungistasis	No inhibition
	Mycoplasma and mycoplasmastasis	Not detected: No inhibition
	Spiroplasmas	Not detected
	Mycobacteria	Not detected; No inhibition
	In vitro adventitious agents (MRC-5, Vero 76, BHK-21, Sf9)	Not detected
	In vivo adventitious virus detection of inapparent viruses	Not detected
	Rhabdovirus	Report results
	Bovine adventitious viruses	Not detected
	Porcine adventitious viruses	Not detected
	Retroviruses (TEM)	Not detected
	Bovine polyomavirus (BPyV) DNA detection by qPCR	Not detected
	Porcine circoviruses type 1 and 2 (PCV1 and 2) DNA detection by qPCR	Not detected
	Nodavirusn TNCL RNA detection	Not detected

- **All results have met cell bank release specification**
- **The new cell bank has confirmed to be rhabdovirus-free**
- **The new cell line has shown better AAV production capability**
- **AAV produced by the new cell line has higher safety standard without the concern of rhabdovirus contamination**

Acknowledgement

Special thanks to previous Frontera Therapeutics colleagues: Dr. **Chia Chu and Grace Eppolito** for initiating the project and preliminary exploration of the method, **Benjamin Baynes** for the extensive cell culture and banking work following initial cell cloning, **Dr. Long Chen** for providing the media adapted starting cell line and confirmation of Rhabdovirus genome positive status of such cell line, **Sabrina Moison** for downstream purification support of clone selection and **Dr. Stanley Chung** for consultation at early stages.