Evaluation of Alternative Process Cell Lysis Reagents as Replacements for Triton X-100 for AAV Production

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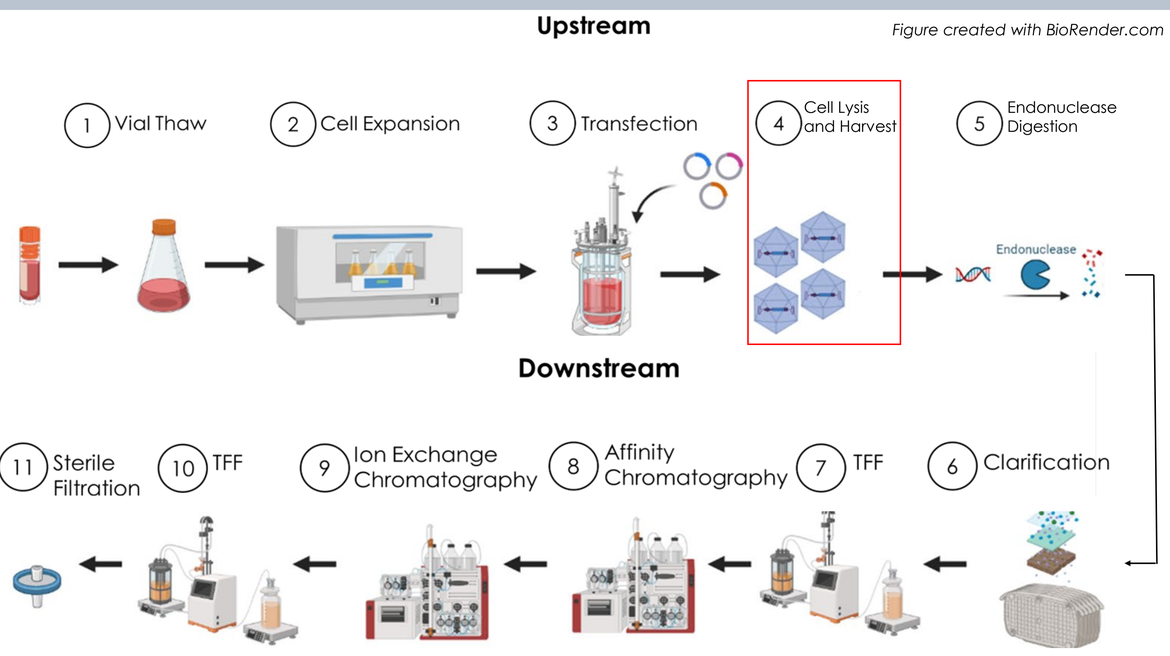
Abstract

Adeno-Associated Vectors (AAV) are utilized in cell and gene therapy as a vehicle to deliver genetic material into target cells. Cell lysis is a critical step since most of the virus is produced intracellularly and retained within the production cells. Both physical and chemical lysis methods are employed to lyse the cells. These commonly include the use of microfluidizers that apply high pressure to shear apart the cells and the use of chemical detergents such as Triton X-100 to disrupt the cell membrane. However, the use of Triton X-100 in the manufacturing of cellular and genetic medicines within the European Union (EU) was banned in 2021 by the European Chemical Agency as part of its REACH initiative due to human health and environmental safety concerns surrounding its endocrine disrupting properties. In order to treat patients within the EU, alternative chemical lysis methods to Triton X-100 are required. In this study we evaluated two GMP-friendly alternative chemicals to replace TritonX-100. Both alternatives effectively lysed the cells, meeting criteria of less than 10% cell viability and comparable genome titer in upstream cell lysates. However, chemical 1 demonstrated effective cell lysis at a lower concentration and shorter time and therefore, was selected as an alternative lysis to Triton. The alternative lysis was evaluated for harvesting AAV5 and AAV6 showing a reduction of cell viability to 2% post lysis in both cases and a genomic titer recovery of 80% for AAV5 and 97% for AAV6. The scalability of the chemical lysis was evaluated at 2L scale. The post lysis cell viability and genomic titer recovery results were compared, and no statistically significant difference was found between results obtained using Triton and the alternative chemical (pValue >0.05). To ensure the effect of the alternative lysis in downstream unit operations, an evaluation was performed to compare downstream processing genomic titers and in-process impurities between Triton and alternative chemical. The results showed both genomic titers and impurities for crude lysate through affinity chromatography in process pools were comparable between Triton and the alternative chemical.

PROCESS OVERVIEW



SCHEMATIC MAP of GOI PLASMID

Results

Time Course Lysis Study

Fig 1A.

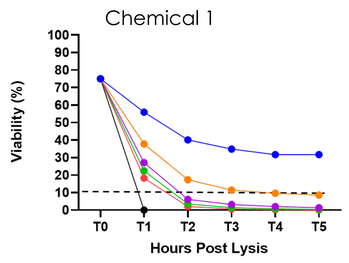


Fig 1B.

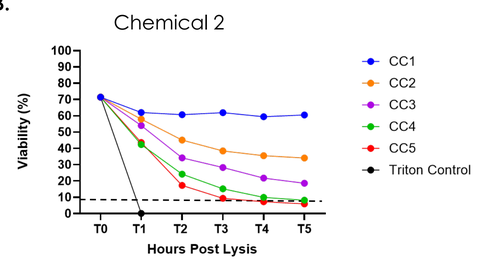


Fig 1C.

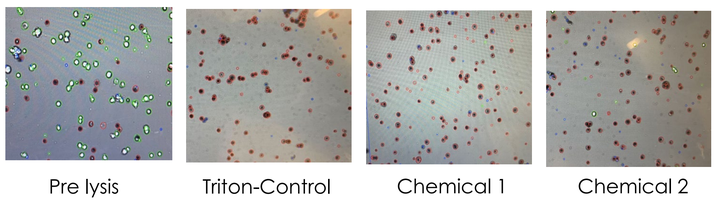


Fig 1. Post Lysis Cell Viability. Plot of cell viability for harvest material lysed with chemical 1 (1A) and chemical 2 (1B) at different concentrations over various post lysis incubation time. Dotted lines show the criteria of <10% post lysis viable cell. (1C) Pre lysis and post lysis viable cells. Pictures were taken for Triton-control at T1, Chemical 1 at T2 (CC3) and Chemical 2 at T4 (CC4).

Time Course Lysis Study and AAV Yield

Fig 2A.

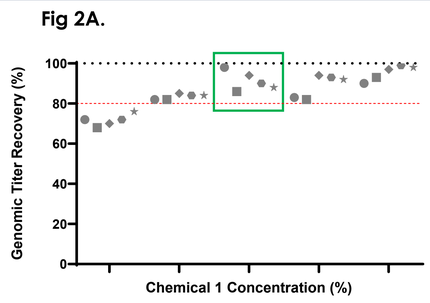


Fig 2B.

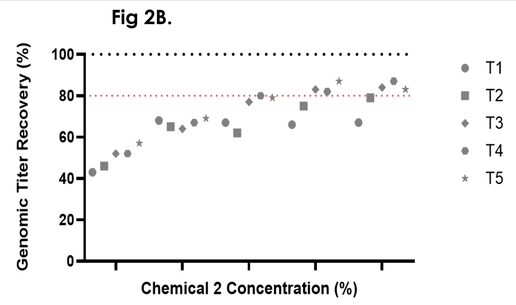


Fig 2. Genomic Titer Recovery of Chemical 1 and Chemical 2 in Comparison with Triton. Plot of crude lysate genomic titers taken at different time course post lysis treatment using (2A) Chemical 1 and (2B) Chemical 2 at five different concentrations. The red line presents the >80% genomic titer recovery in comparison with Triton (control) of 100%. The green box represents the consistency of genomic titer recovery >80% at the same concentration of chemical 1 over different post lysis incubation times.

Assessment of Top Lysis Conditions for AAV5 and AAV6

Fig 3A.

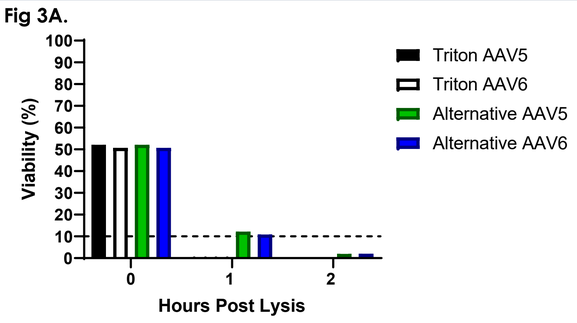


Fig 3B.

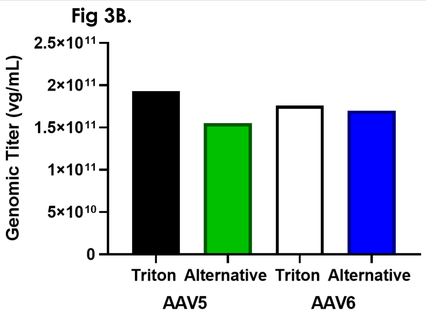
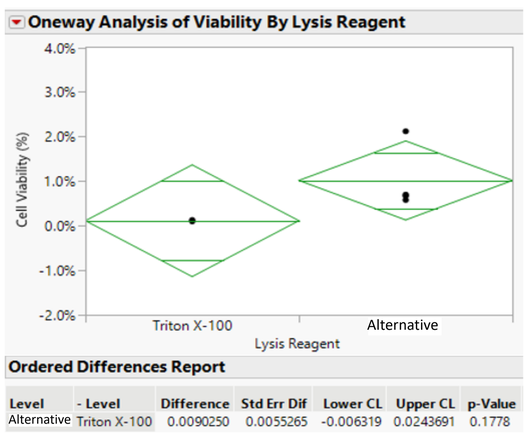


Fig 3. Comparison of Cell Viabilities and Genomic Titers for AAV5 and AAV6. Plot of (3A) cell viabilities and (3B) crude lysate genomic titers for AAV5 and AAV6 material lysed using alternative (AAV5 in green, AAV6 in blue) and Triton X-100 control (black). The dotted line represents the 10% cell lysis efficiency criteria.

Comparison of Post Lysis Cell Viability and Genomic Titer at 2L Scale

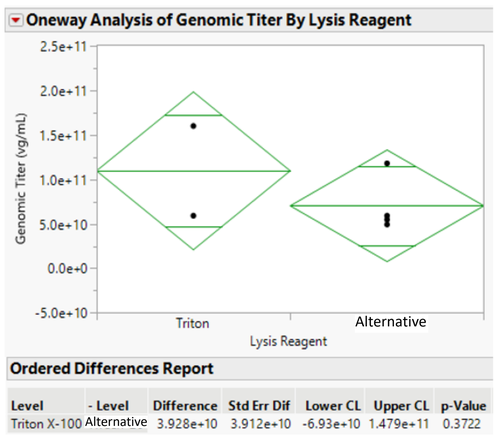
Fig 4A.



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Alternative	Triton X-100	0.0090250	0.0055265	-0.006319	0.0243691	0.1778

Fig 4B.



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Triton X-100	Alternative	3.928e+10	3.912e+10	-6.93e+10	1.479e+11	0.3722

Fig 4.Comparison of Post Lysis Cell Viability and Genomic Titer at 2L Scale. Plot of (4A) cell viabilities and (4B) genomic titers of 2L STR runs lysed with Triton X-100 and alternative chemical.

Comparison of Post Lysis Cell Viability and Genomic Titer at 2L Scale

Fig 5A.

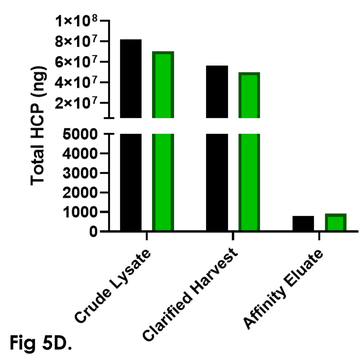


Fig 5B.

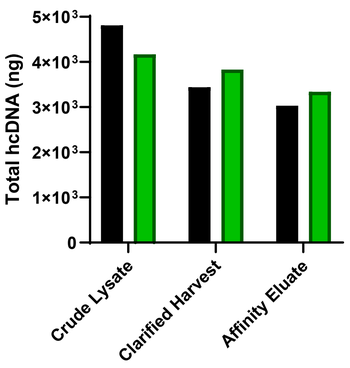


Fig 5C.

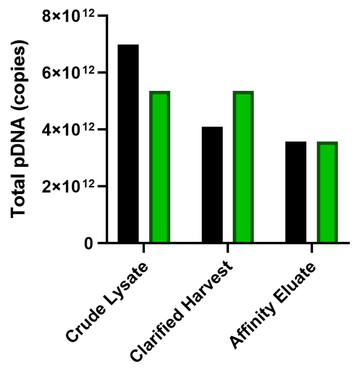


Fig 5D.

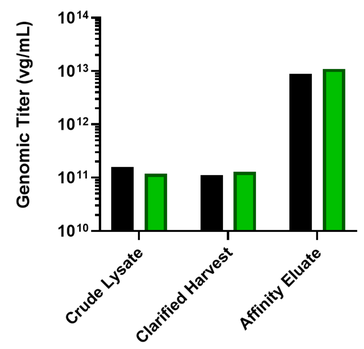


Fig 5. Comparison of Impurities and Genomic Titers in In-Process Pools for Triton and Alternative Chemical. Plot comparing total (5A) host cell protein (HCP), (5B) host cell DNA (hcDNA), (5C) plasmid DNA (pDNA), and (5D) genomic titers of the crude lysate, clarified harvest, and affinity eluate in-process pools for AAV5 harvest material lysed with Triton (black) and alternative chemical (green).

Conclusion

- Alternative lysis chemicals were evaluated to replace Triton-X100 and fit for requirement in EU.
- The alternative lysis is efficient for harvesting different serotypes without any effect on the AAV genomic titers.
- The alternative lysis method is scalable and GMP-friendly.
- The alternative chemical has no negative impact in the subsequent downstream unit operations.

Learn More

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