

Monitoring Orf Virus Production with PATfix® Analytical Method

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Introduction

Orf virus (ORFV) is a promising vaccine vector candidate, capable of inducing a strong, long-lasting immune response without integrating into the host genome. Its broad host range and ability to express diverse antigens make it a valuable tool for recombinant vaccines and oncolytic therapies. However, effective analytical methods for characterizing ORFV materials, particularly for complex upstream samples, containing a mixture of viral particles, host cell components and process-related impurities, are limited. Additionally, traditional analytics often focus on detecting only one parameter at a time, requiring multiple assays to build a complete picture of viral production.

To address this gap, our study focused on integrating the liquid chromatography PATfix® analytical method as a multi-parameter analytical tool to monitor and guide upstream process. The method allows fast and reliable profiling of both viral product and impurities, enabling insights into how process parameters, such as cell line, media, cell density at infection (CDAI), multiplicity of infection (MOI) and nuclease treatment influence virus production. Our approach emphasizes upstream optimization to enhance viral yield while reducing the downstream purification burden as well.

Experimental approach

The PATfix system (Figure 1) allows rapid and informative analysis of in-process ORFV harvest samples, using anion exchange CIMac QA-0.1 (6 µm) monolithic analytical column (Sartorius BIA Separations). For PATfix analytics samples were thawed, centrifuged and diluted in running buffer. The total runtime is approximately 22 minutes per samples, with data being analysed using the PATfix Software.

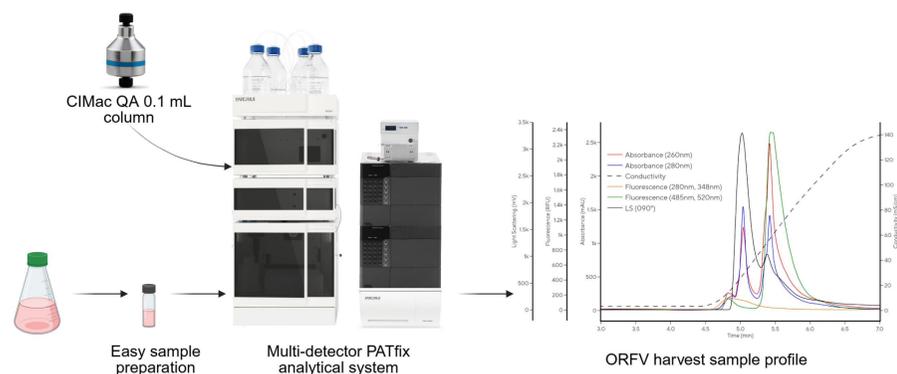


Figure 1: Schematic presentation of PATfix system setup and sample characterization using PATfix Software.

The multiple-detector PATfix technology enables simultaneous detection of absorbance (monitored at 260 nm and 280 nm), two different fluorescence signals (set to Ex 280 nm and Em 348 nm wavelengths for tryptophan fluorescence detection and Ex 285 nm and Em 520 nm wavelengths for dsDNA detection using PicoGreen dye). Additionally, the light scattering emitted/scattered from the sample light scattering is measured at a 90° angle using multi-angle light scattering (MALS) detector. The latter is used for ORFV quantification.

With the aim of obtaining high virus yield, we have tested a broad range of different upstream variables, changing the cell line, cell culture media, production conditions etc.. The model virus used was ORFV strain D1701-V-GFP, a recombinant expressing green fluorescent protein. The samples were analysed with our analytical method and based on the results we were able to determine the best production conditions.

Comparing different upstream strategies

After securing some starting process conditions, we have investigated the impact of different CDAI. Suspension HEK293-F cells were seeded at different CDAI in chemically defined medium one day prior infection. The infection was performed using MOI 0.5 to maintain consistency across conditions. Harvest samples were collected at 72 hours post-infection and frozen. Results of the experiment are shown in Figure 2.

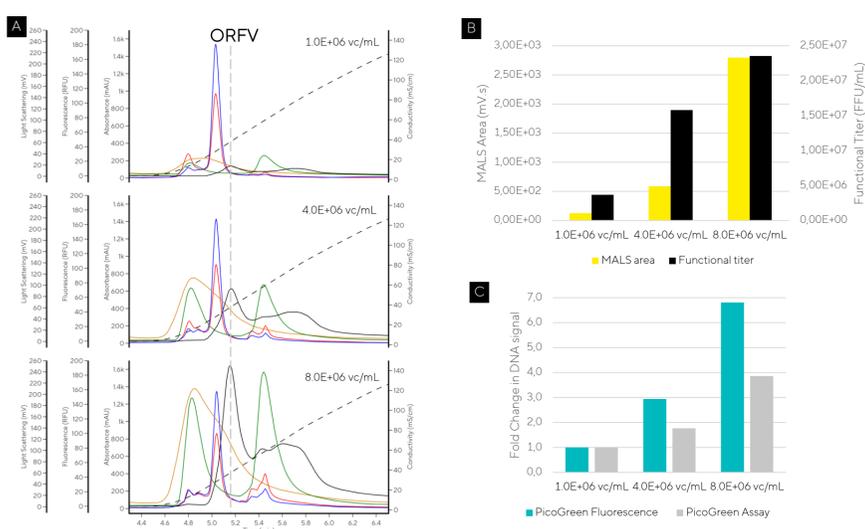


Figure 2: Effect of different CDAI on virus yield and impurity profile. A) PATfix Chromatogram, zoom in to the elution gradient of harvest samples, B) MALS signal area compared with functional titer, measured with foci-forming assay, C) fold increase in PicoGreen fluorescence signal and PicoGreen assay, reflecting accumulation of residual host cell DNA. vc - viable cells, FFU - foci-forming unit

Changes in CDAI influenced the chromatography profile, both in the distribution and intensity of virus peak and impurity signals. With increasing CDAI, more ORFV was produced (from low to high CDAI, 1 log in viral MALS area) but at the same time, also the residual DNA peak was significantly higher with chosen CDAI of 8.0E+06 vc/mL.

References:

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- Rzih, H. J., Rohde, J., & Amann, R. (2016). Generation and selection of Orf virus (ORFV) recombinants. In *Methods in Molecular Biology* (Vol. 1349).

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Cell lysis

To enhance virus release we tested the effect of a lysis step prior harvest. Samples were subjected to a mild lysis treatment, and the resulting harvest samples were analyzed.

As shown in Figure 3, the introduction of lysis significantly increased the virus-associated peak, indicating an improvement in virus recovery. In fact, functional titer measurement confirmed more than a 6-fold increase compared to a non-lysed control sample. The virus peak not only increased but also shifted to an earlier retention time. Since the separation is based on net charge of the biomolecules, this shift may reflect a change in the surface characteristics of the virus particles. The lysis buffer can interact with the viral envelope, reducing its net charge and decrease binding strength to the anion-exchange column.

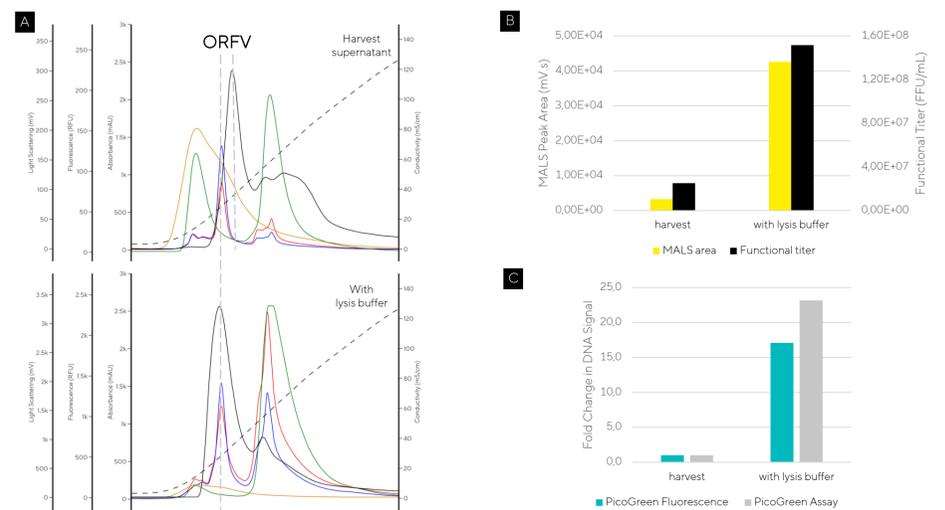


Figure 3: Effect of lysis step on virus recovery and elution profile. A) PATfix Chromatogram, zoom in to the elution gradient of harvest samples, with and without addition of lysis buffer, B) MALS signal area compared to the functional titer, measured with foci-forming assay, C) fold increase in PicoGreen fluorescence signal and PicoGreen assay.

Nuclease treatment optimization

With the help of PATfix analysis, we were able to recognize the need for a nuclease treatment step. The analysis of the current process revealed elevated fluorescence signals - particularly from the DNA-specific PicoGreen detector - indicating a significant presence of residual host cell nucleic acids.

We performed an experiment comparing three upstream harvests that were identical in all parameters except for nuclease treatment: 1) no nuclease, 2) low nuclease concentration, and 3) high nuclease concentration was added. The results are shown in Figure 3.

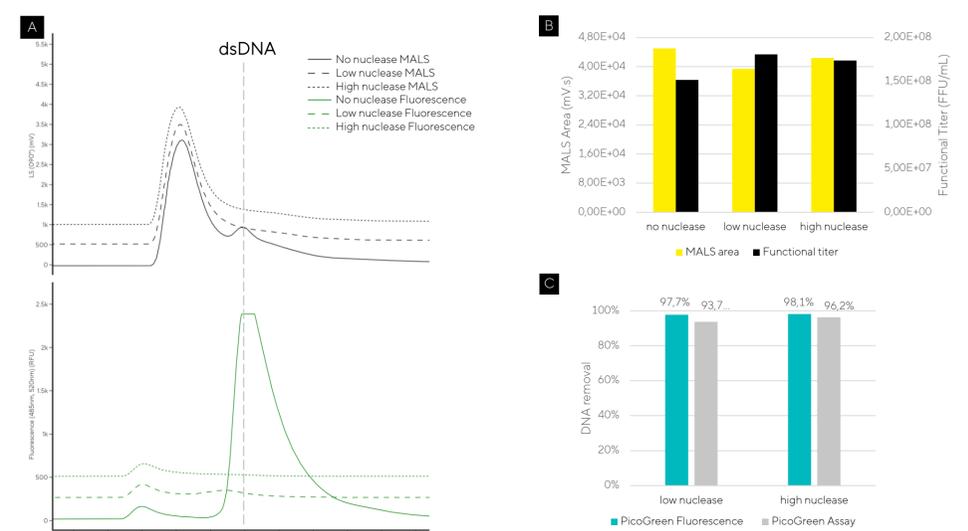


Figure 4: Comparing different nuclease treatment strategies. A) PATfix Chromatogram, zoom in to the elution gradient of harvest samples, B) MALS signal area compared with functional titer, measured with foci-forming assay, C) percentage of DNA removal achieved with nuclease treatment, assessed by both PicoGreen fluorescence signal reduction and PicoGreen assay.

Addition of nuclease in low concentration for 1 hour at 37 °C led to a reduction of DNA (93.7 % DNA removal as measured by the PicoGreen assay) and improved purity, without compromising viral particle yield.

Conclusion

This systematic approach demonstrates how PATfix multi-signal monitoring can guide upstream process design, identifying conditions that maximize virus yield. This workflow serves as a foundation for further enhancements in viral vector production, as the resulting process is more compatible with downstream purification.

Contribution of the analytical method:

- easy-to-use
- fast (22 minutes per sample)
- easy sample preparation (only centrifugation and dilution needed)
- low limit of quantification (2.0E+04 FFU)
- small volume needed (0.5 mL injection volume)
- equipped with software to control the system and analyze the samples
- can be used on complex and purified samples
- orthogonal to molecular biology methods

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