

From Bench to Bag: Scale-up & cGMP Manufacture of Emerging Vaccine Technologies

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Introduction

There has been a recent upsurge in the interest in using viral vectors such as adenoviruses, in the field of viral vaccines and gene therapy. It is estimated that there are currently 377 clinical trials world wide conducted in association with this class of therapeutic product¹. Adenoviral vectors have been traditionally manufactured using classical purification techniques such as density gradient ultracentrifugation and tangential flow filtration. These methodologies are laborious, time consuming and scale-limiting. The recent increase in demand, for adenoviral vectors coupled with the relatively high titres needed for pre-clinical and clinical trials has fuelled the requirement for a new approach to production.



Figure 1. Pilot scale-up chromatography.

Eden's development strategy has focused on the identification of a platform process which could be readily applied to any adenoviral vector with minimal process development requirements, with the eventual goal being a generic process which could be rapidly deployed to facilitate any Ad5 manufacturing campaign.

Process development



The purification process consists of a cell harvest, lysis and clarification strategy followed by a DNA reduction step using Benzonase Endonuclease and buffer exchange/ concentration by tangential flow filtration. The main purification steps utilise the Biomonolith system (a strong anion exchange chromatography column) followed by a second, polishing size exclusion chromatography column. The final formulation process step is executed by concentration and buffer exchange using tangential flow filtration followed by a final 0.22µm bioburden reduction filtration.

Figure 2. Size exclusion chromatography development.

Key advantages of platform chromatography

By adopting this novel, platform chromatography based approach, Eden's purification strategy has the significant advantages of:

- Rapid application for initial process sighting studies – plug and play technology to significantly reduce development timelines
- Robust and reproducible process – successfully applied to a number of suspension cell lines including HEK.293, A549 and PER.C6
- Linear scalability – successfully scaled from 100 mL to 25 L
- Significant savings on capital equipment outlay
- cGMP compliant – applied successfully for the clinical production of adenoviral vectors

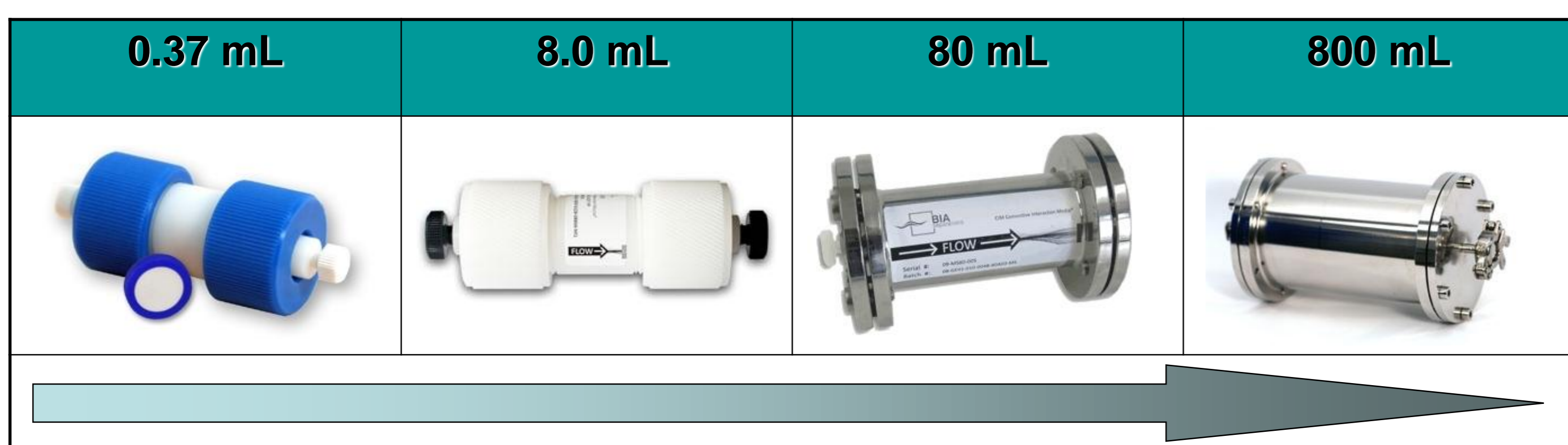


Figure 3. Linear scale up through monolith chromatography².

References

1. <http://www.wiley.co.uk/genotherapy/clinical>
2. Reproduced with kind permission from BIA Separations (www.BIAseparations.com)

Process exemplification and scale up

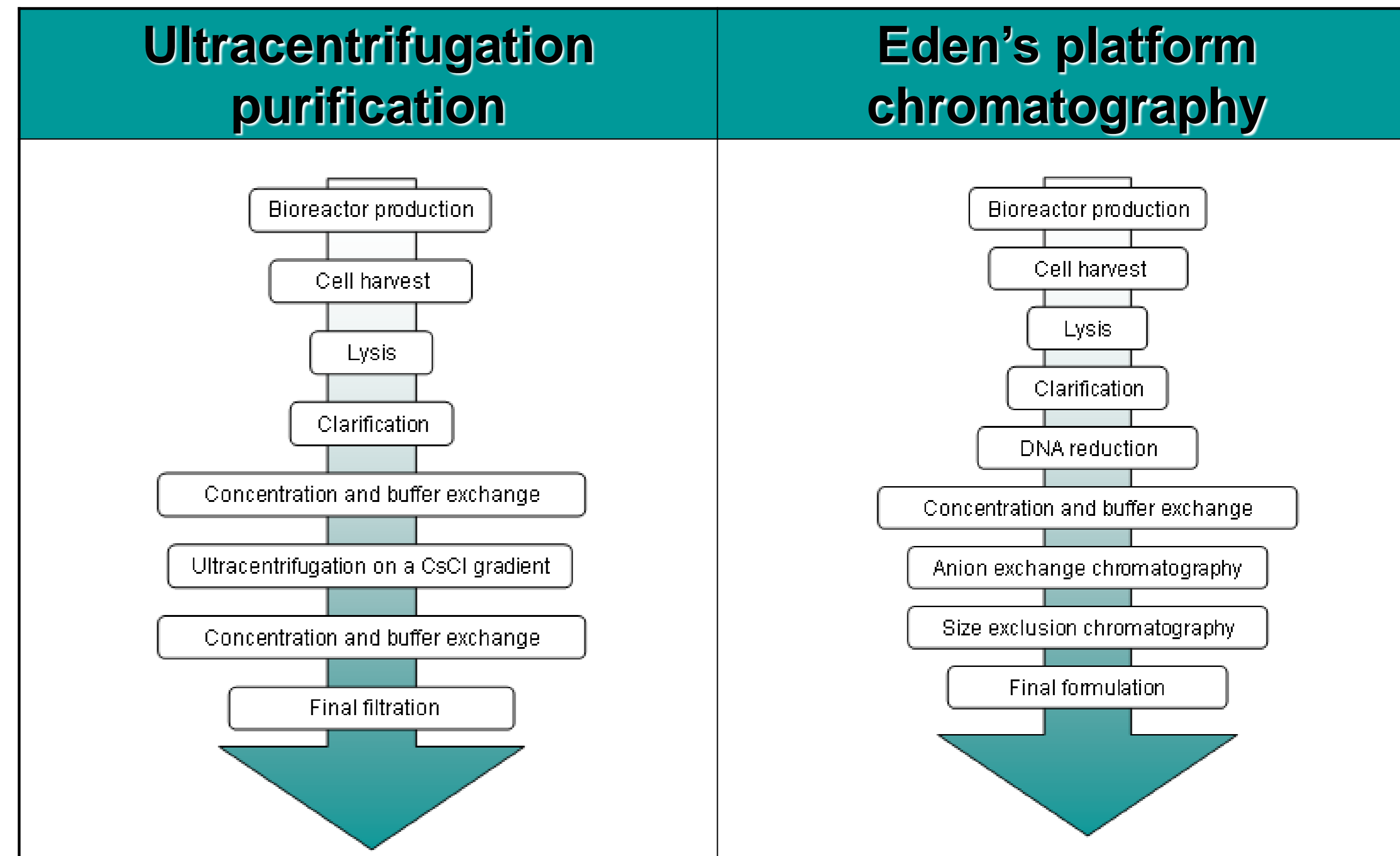


Figure 4. Classical vs. chromatographic purification.

So far Eden's purification process has been successfully applied to wide ranging feed streams including:

- ☑ 125 mL to 1 L Shake flask – small scale process scouting
- ☑ 2.5 L Stirred tank – intermediate process development and exemplification
- ☑ 3.0 L Disposable bag format – intermediate process development and exemplification
- ☑ 20 L Stirred tank – scale-up verification
- ☑ 25 L Disposable bag format – scale-up verification and clinical production

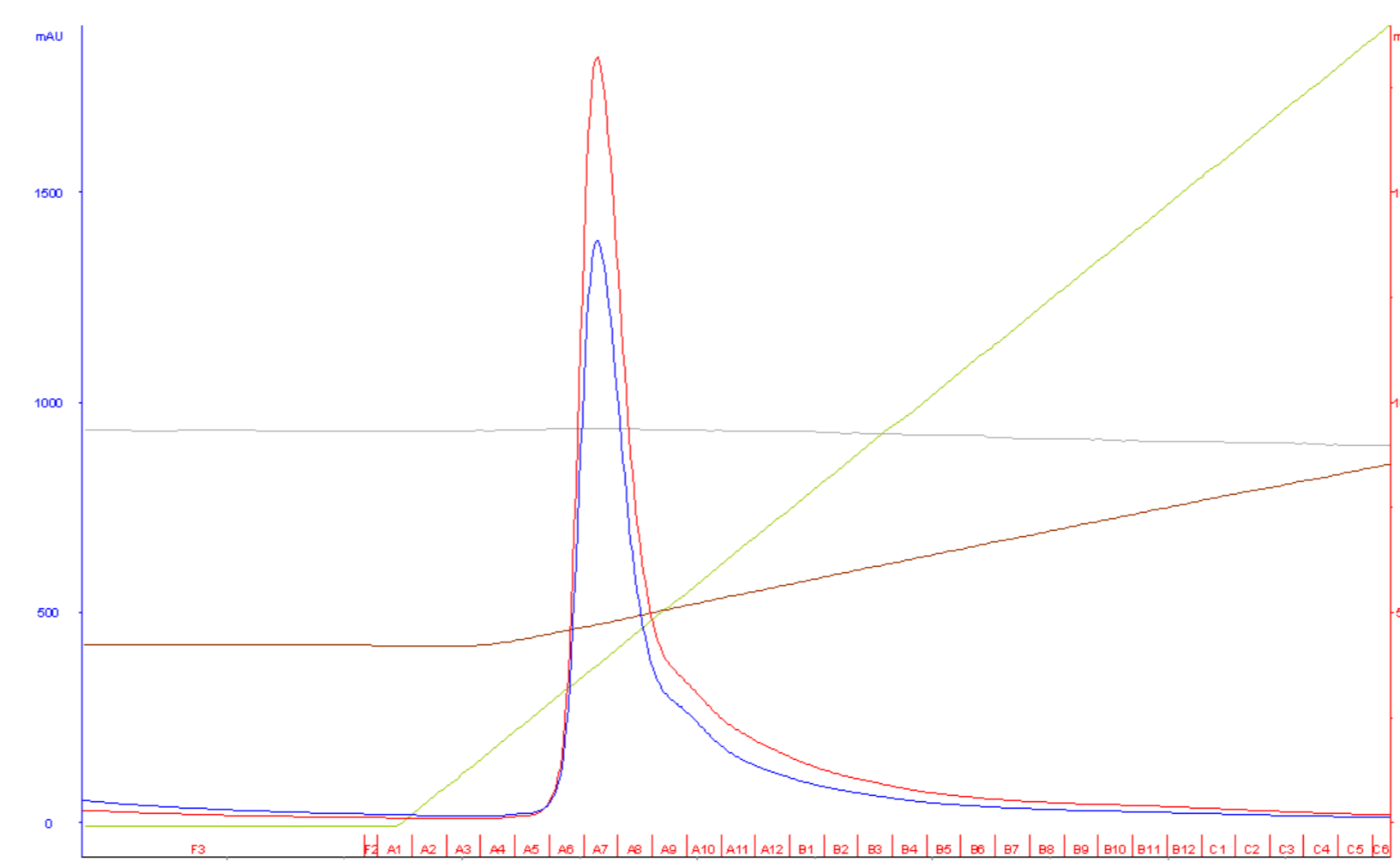


Figure 5. Typical AEX chromatogram.

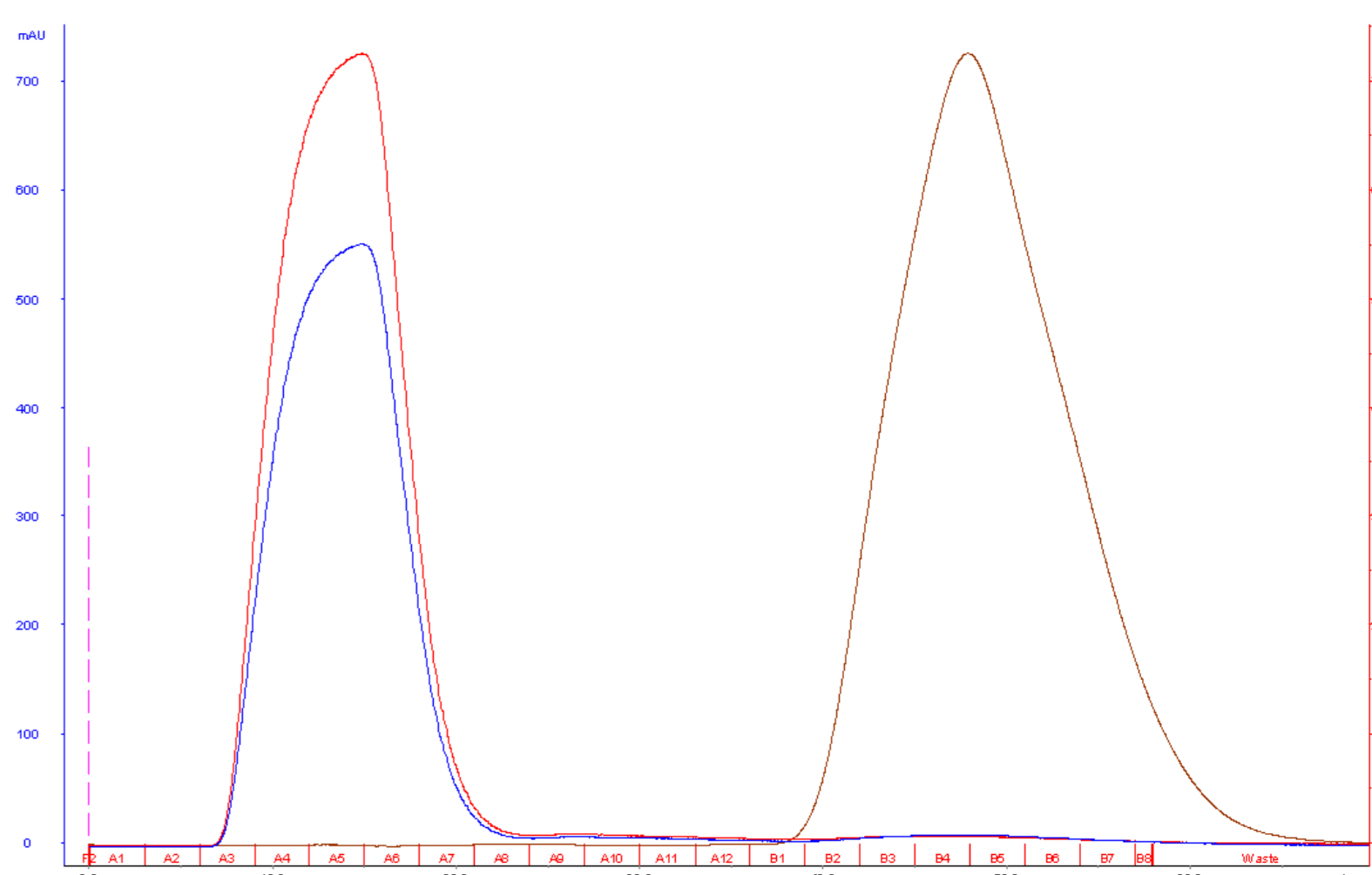


Figure 7. Typical SEC chromatogram.

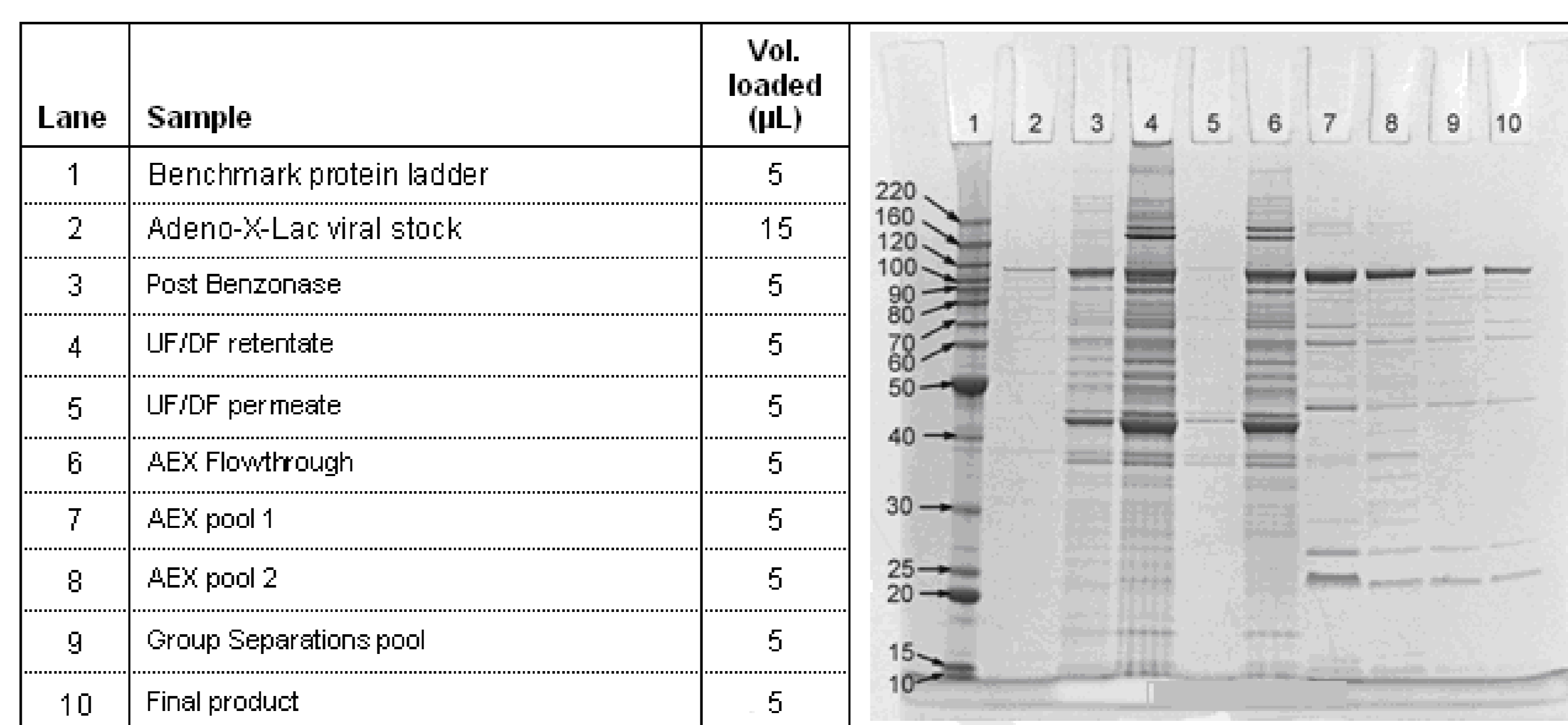


Figure 7. SDS-PAGE analysis through a typical purification process.

Conclusions

A number of Ad5 vectors have been applied to Eden's platform purification strategy with resounding success, requiring little, if any further development to produce virus of sufficient quantity and quality for toxicology and early phase clinical trials. The process has also been exemplified at a range of scales with virus produced from a number of supportive cell lines including HEK293, A549 and PER.C6. To date Eden has been successful in generating highly purified virus for both pre-clinical and clinical trials at a range of scales. Within Eden's cGMP production factuality, from a 25 L disposable bag system it has been possible to purify 1.7×10^{14} total viral particles (1.48×10^{13} infectious) to a high degree suitable for early phase clinical trials.