Automated Micro-TFF System Streamlines Purification and Operator Time in a Lean rAAV Manufacturing Operation

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INTRODUCTION

Dyno's ability to leverage machine-learning to accelerate best-in-class capsid discovery and validation relies on the rapid generation of high-quality data across our platform. Our internal high-throughput manufacturing pipeline is focused on generating highly diverse capsid libraries while also building and refining processes that minimize variant dropout, maximize variant yield, and reliably deliver capsid libraries on-time. Towards faster and more reliable production of capsid libraries, we have sought to both increase the amount of process information we capture and to decrease the amount of hands-on time spent and manipulation performed by our process operators.

Low volume (0.5 - 5 mL range) ultrafiltration and diafiltration (UFDF) is a process area with both high operator hands-on time and manipulation due to the use of molecular weight cut-off (MWCO) spin filtration devices. MWCO spin filters have previously been the device of choice as they are simple to implement and relatively inexpensive. Unfortunately, in addition to the substantial hands-on time, these devices provide for no process monitoring and function in a dead-end filtration modality.

We evaluated and then implemented an automated µTFF system for use in downstream purification. Specifically, we characterized the Formulatrix® µPulse®; an automated TFF system capable of operating in our scale of interest, from 100 mL to 0.5 mL, according to critical outcome parameters including step yield, process time, and hands-on time.

METHODS

MWCO UFDF - MWCO spin filters (ThermoFisher Scientific PES 100 kDa 15 mL) were prepared according to manufacturers recommendations. Pre-aliquoted samples were diluted to 1.5X their starting volume. Spin durations were set by operators based on estimated flow rate. Sample was brought to 1/10th of its starting volume. Fifteen volumes of buffer were added, mixed, and processed through. This was repeated 3X, returning the sample volume to 1/10th the starting volume each time. After completion of the buffer exchange steps, sample was harvested by pipetting. The filters were rinsed with buffer that was then pooled with the processed material, then adjusted up to final volume.

µTFF UFDF - Pre-aliquoted starting material was diluted to 1.25X the starting volume. The TFF filter and tubing were prepared utilizing the onboard wash and rinse protocols. Sample was concentrated to 1/20th its pre-dilution starting volume, then buffer exchanged with 20 discrete 2 mL steps with the standard settings. Sample was collected by chasing the system with alternating air and buffer until the sample was 1/10th its starting volume.

RESULTS

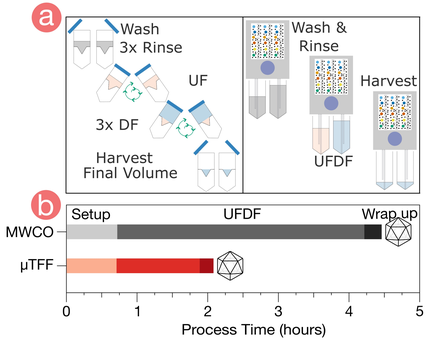

Figure 1: Comparison of the MWCO and µTFF Methods
(a) High-level diagram depicting the major steps in the workflow for MWCO UFDF (left panel) and µTFF UFDF (right panel). (b) Timeline overview highlighting the key process phases in running both processes.

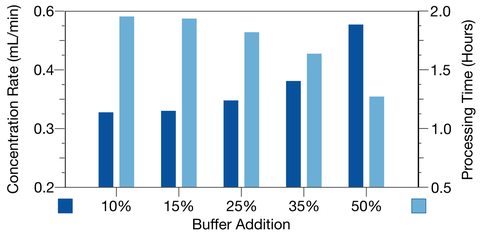

Figure 2: Dilution Yields Increased Ultrafiltration Processing Rate and Reduced total Process Time in µTFF
Aliquots (20 mL) of AAV vector starting material (from iodixanol density gradients) were diluted with increasing amounts of 1X DPBS-based buffer and run on the µTFF system to observe processing time. Concentration rate/mL (dark blue) of starting material was calculated by correcting the raw output data for sample density, buffer density, and the proportional volume of each in the dilution mixture. The total time to concentrate (light blue) our maximum standard harvest volume, 40 mL, was calculated for each dilution condition.

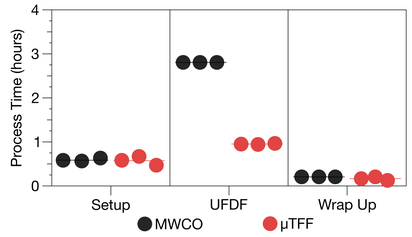

Figure 3 : Automated µTFF Decreases Total Process Time
Three independent operators performed UFDF on independent aliquots of the same AAV sample with both MWCO spin filters and the µTFF. Each experiment began with (21mL µTFF) or (10mL per MWCO filter) of starting material. Sample was diluted with a 1X DPBS-based buffer. Time points were manually noted by operators, and µTFF time points were checked against the system data timestamps. Processing time for each of the three main phases of the operation was compared. Performing UFDF by µTFF resulted in a significantly faster processing rate and shorter overall process.

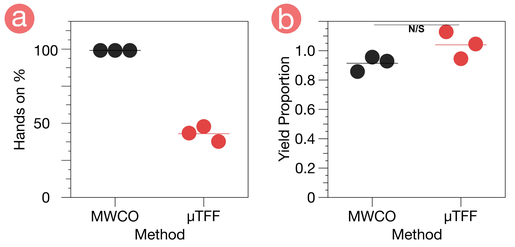

Figure 4 : Automated µTFF Decreases Proportion of Hands-on Time While Maintaining Step-Yield
(a) The MWCO spin filtration devices required constant (100%) hands-on operation, whereas the shorter µTFF process required operators to be present for less than 50% of the run time. With the faster overall run time, this resulted in 1 hour of hands-on time for the µTFF vs. 4 hours for the MWCO spin devices. (b) After the purified product was recovered from and titered for both methods, we observed a high step yield with no significant difference between the two procedures.

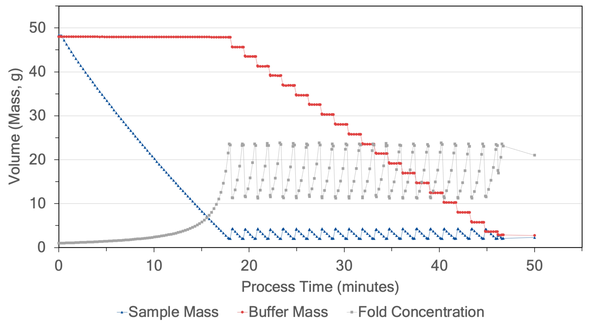

Figure 5: Automated µTFF Captures Rich Process Data
35 mL of post-iodixanol material was processed acording to our new protocol. Run data was captured by the µTFF system in addition to routine operator notes. Scatter plotting data points of sample volume, buffer volume, and concentration factor, display both the data richness and adherence to the programmed settings. Additional raw data such as diaphragm and valve pressures and timing are also captured (not plotted here). MWCO spin filter devices provide no data capture outside of operator notes.

IMPACT

The µPULSE instrument improves the reliability of our capsid manufacturing process, helping ensure stability in our capsid library screening and validation cycle times.

Process Efficiency:

- Total process time was reduced by over 50%.
- Hands-on time of operators was reduced to less than 50% of run time.
- Operators at various skill levels were successfully trained to execution proficiency within a single 4 hour training session.
- µTFF consumable costs did not increase process costs.
- Programmed execution minimizes risk of over-concentration and subsequent loss of AAV.

Automation and Data Capture:

- Automatic and complete data capture enables individual run monitoring as well as run over run trend analysis.
- Automatic and complete data capture also provides data to guide process optimization and troubleshooting.
- Walk-away automation enables our operators to run other processes - better enabling overlapping pooled-batch production strategies to minimize timelines.

Consistent Quality:

- High step yield was retained.
- Fully programmed execution creates uniform consistency of the ultrafiltration and diafiltration steps.
- Approximation of continuous diafiltration has enabled more consistent iodixanol removal below the threshold required for additional QC assays.

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All authors work within Dyno's Manufacturing team; each contributing significantly to method development, planning, analysis, and poster generation. Authors are listed alphabetically by family name. List order has no correlation to contribution.