Optimization and scale-up of AAV2-AQP1 Production using TransIT-VirusGEN

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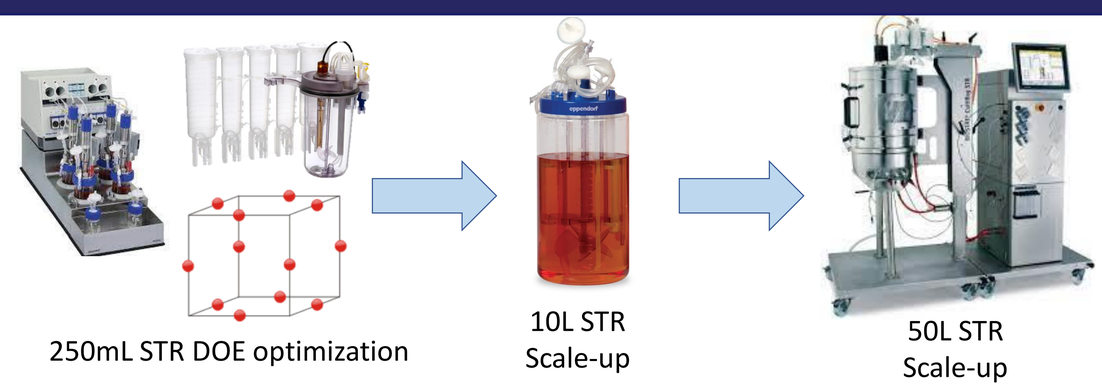
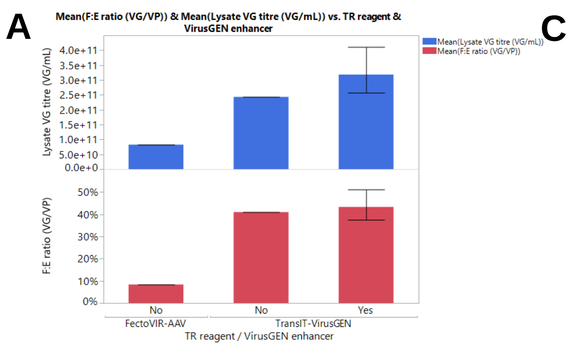
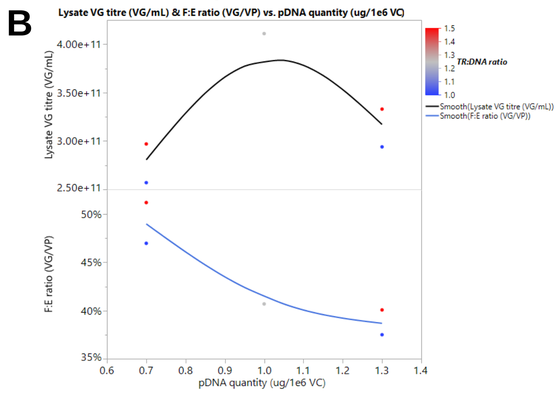
Introduction

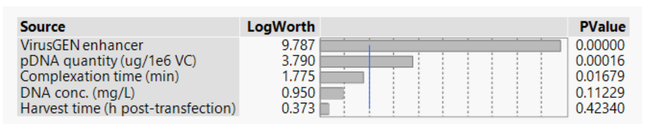
Transient transfection in AAV production is a complex and multimodal operation requiring multi-stage optimization to yield robust and predictable outcomes at manufacturing scale. Transfection optimization and scale-up to 50L STR were performed on AAV2-AQP1 using TransIT-VirusGEN, aiming for a minimum USP yield of $2x10^{11}$ VG/mL and 30% full capsids before polishing unit operations, to enable future commercial supply at 200L scale.



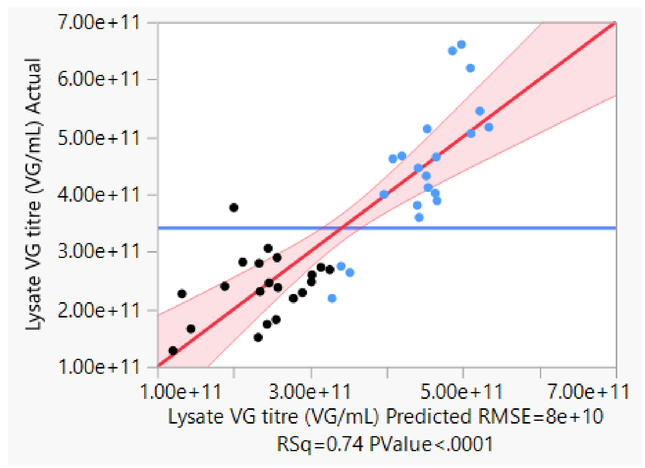
Results: DoE optimization of TransIT-VirusGEN system







Source	LogWorth	PValue
VirusGEN enhancer	9.787	0.00000
pDNA quantity (ug/1e6 VC)	3.790	0.00016
Complexation time (min)	1.775	0.01679
DNA conc. (mg/L)	0.950	0.11229
Harvest time (h post-transfection)	0.373	0.42340



Results: Reproducible performance across scale

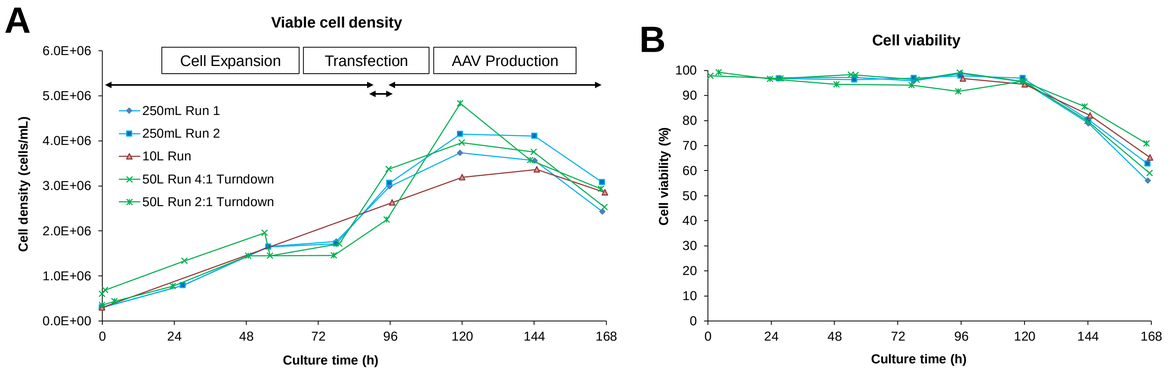


Figure 3. Comparison of cell growth across process scales with TransIT. HEK293 cells were cultured in STRs; 250mL (n=2), 10L (n=1) and 50L (n=2). 50L STR cultures were run at a 4:1 and 2:1 turndown (TD) ratios, inoculated at $6.0x10^5$ VC/mL and $3.5x10^5$ VC/mL respectively. Cultures were subsequently triple-transfected with TransIT-VirusGEN+VirusGEN Enhancer and harvested by chemical lysis at 72h post-transfection.

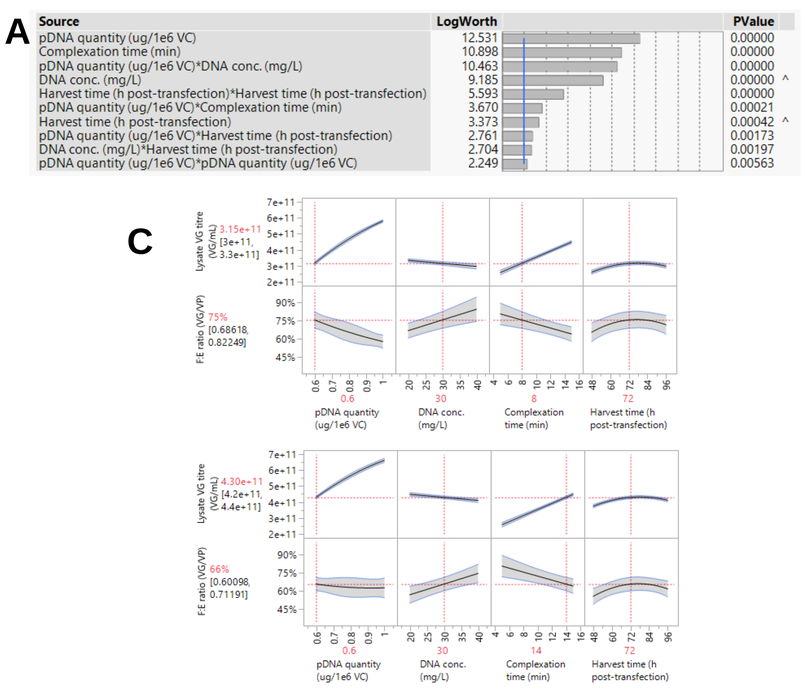
(A) Viable cell density. All cultures reached 2.3-3.4x10^6 cells/mL at transfection (96h post-inoculation). Cell growth trends post-transfection were largely similar across process scales. The cell expansion strategy for 4:1 TD ratio was adapted to reach the same VCD range and working volume at transfection as the 2:1 TD ratio condition, by means of different feed volumes.

(B) Cell viability. All conditions were healthy at ≥90% cell viability at transfection. Conditions across all scales maintained a cell viability ≥50% until harvest point.

Figure 1. (A) Comparison of TransIT-VirusGEN vs. FectoVIR-AAV transfection. HEK293 cells were cultured in 250mL STRs to 2.3-3.0x10^6 viable cells/mL and triple-transfected with an optimized FectoVIR-AAV protocol or with TransIT-VirusGEN+VirusGEN Enhancer, testing ranges of pDNA quantity (0.7-1.3 µg/10^6 VC) and TransIT:DNA ratio (1:1-1.5:1) in a full-factorial design. The centre point was also tested without VirusGEN Enhancer. Cells were lysed 4 days post-transfection. All TransIT conditions resulted in superior VG titre and VG/VP ratio compared to FectoVIR-AAV, respectively 3.7-fold and 5.2-fold higher. VirusGEN Enhancer resulted in a 1.7-fold increase in VG titre but no improvement in VG/VP ratio compared to the center point transfected without VirusGEN Enhancer.

(B) Effect of pDNA quantity and TransIT:DNA ratio. An optimum VG production was observed at 1.0 µg pDNA/10^6 VC. A decrease in VG/VP ratio was observed with increasing pDNA quantity, suggesting further possible optimization of pDNA quantity to reach an optimum balance between productivity and full:empty ratio. TransIT:DNA ratio had a minimal effect on VG titre and VG/VP ratio across the range of 1:1 to 1.5:1, therefore it was fixed at 1.25:1 in the next optimization rounds.

(C) Linear effects for VG titre. HEK293 cells were cultured to 2.2-2.6x10^6 cells/mL prior to transfection with TransIT-VirusGEN. A 5-parameter custom DOE was designed with JMP 13, testing VirusGEN Enhancer presence (Yes/No), pDNA quantity (0.6-1.0 µg/10^6 VC), [DNA] during complexation (20-40mg/L), complexation time (5-15min) and harvest timing (48-96h post-transfection). A standard least squares model was calculated for VG titre (R^2 = 0.74, p-value < 0.0001) and showed that VirusGEN Enhancer presence overpowered VG production outcome. Consequently, subsequent modelling focused solely on the conditions containing VirusGEN Enhancer.



Source	LogWorth	PValue
pDNA quantity (ug/1e6 VC)	12.531	0.00000
Complexation time (min)	10.898	0.00000
pDNA quantity (ug/1e6 VC)*DNA conc. (mg/L)	10.463	0.00000
DNA conc. (mg/L)	9.185	0.00000 ^
Harvest time (h post-transfection)*Harvest time (h post-transfection)	5.593	0.00000
pDNA quantity (ug/1e6 VC)*Complexation time (min)	3.670	0.00021
Harvest time (h post-transfection)	3.373	0.00042 ^
pDNA quantity (ug/1e6 VC)*Harvest time (h post-transfection)	2.761	0.00173
DNA conc. (mg/L)*Harvest time (h post-transfection)	2.704	0.00197
pDNA quantity (ug/1e6 VC)*pDNA quantity (ug/1e6 VC)	2.249	0.00563

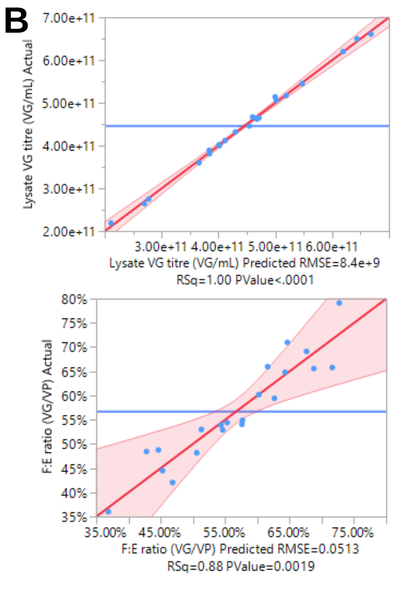


Figure 2. (A) Least Squares Fit Effect Summary. A standard least squares model was calculated for VG titre and F:E ratio using JMP 13 with conditions containing VirusGEN Enhancer only (n=7 at 48h, 72h and 96h harvest time points). All linear effects were found to be significant, with pDNA quantity being the most important and harvest timing the least important factor.

(B) VG titre and F:E ratio Actual by Predicted plots. VG titre and F:E ratio Actual by Predicted plots showed excellent correlation and significance (VG titre: R^2=1.00, p-value<0.0001 ; F:E ratio: R^2=0.88, p-value=0.0019).

(C) Prediction profilers. The entire design space tested met minimum USP VG titre ≥2x10^{11} VG/mL, however an increase in VG titre was associated with a decrease in F:E ratio for all factors except Harvest time. Operating space selection therefore focused on maintaining VG/VP ratio above 60% whilst allowing robust operations at scale. A low pDNA quantity was preferred to maximize F:E ratio whilst minimizing cost-of-goods. In addition, a complexation time range of 8-14 min was chosen to make the transfection operation practical at large scale, with a minimal compromise on product quality. [DNA] during complexation had a moderate effect on F:E ratio and was fixed at the center point at 30mg/L. Target harvest time was fixed at 72h post-transfection where it showed an optimum in both VG titre and F:E ratio.

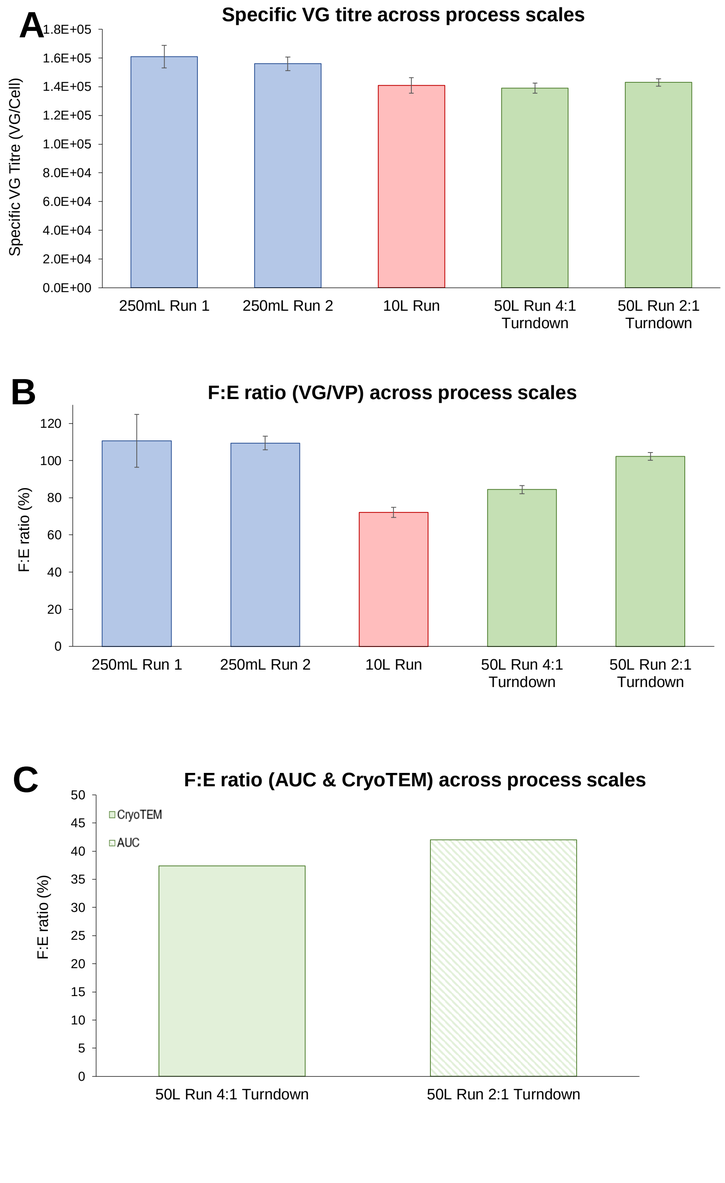


Figure 4. (A) VG titre across process scales transfected using TransIT.
All cultures reached 2.3-3.4x10^6 VC/mL and transfected with TransIT-VirusGEN + VirusGEN Enhancer, using the DOE optimized parameters. All cultures were harvested at 72h post-transfection. VG titre in lysate, normalised to viable cell density at transfection, showed reproducible performance above 130,000 VG/cell, translating to a USP yield between 3.2 and 4.8x10^{14} VG/L in the lysate; 1.6-2.4-fold above the minimum USP yield requirement.

(B) Full:Empty capsids ratio by VG/VP titre. Full:Empty capsids ratio was maintained above 60% across all scales evaluated as measured by VG/VP titre, consistent with DoE operating space optimisation.

(C) Full:Empty capsids ratio by AUC and CryoTEM. Consistent F:E capsid ratios of 37-42% by AUC/Cryo-TEM in affinity chromatography eluates were obtained in 50L runs at 2:1 and 4:1 TD ratio, confirming the suitability of the transfection setpoints determined by DoE and showing a reproducible scale-up performance using TransIT-VirusGEN+VirusGEN Enhancer transfection system.

Conclusion

Production of AAV2-AQP1 using TransIT-VirusGEN has yielded 3.7-fold and 5.2-fold increases in viral genome titer and full:empty capsids ratio respectively, over a previously optimized FectoVIR-AAV based process. Scale-up to 10L and 50L STRs of different operational approaches has been successful and yielded reproducible viral productivity and product quality across 250mL to 50L scales. Additionally, no modifications were required from our existing baseline upstream process platform to integrate TransIT-VirusGEN, which makes this transfection reagent a platform of choice for the future scale up of AAV2-AQP1 production to 200L bioreactors.