Assessment of Two HEK293 Cell Line Cloning Strategies to Improve AAV Yield

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EXECUTIVE SUMMARY

- We implemented two strategies to identify high producing clones for Voyager's engineered AAV capsids, using different parental HEK cell lines.
- The first approach, we single-cell cloned from a proprietary clonal cell line and we finally got top clones that improves titers and drug quality.
- The second approach, we cloned from a non-clonal HEK-293 cell line using mini-pooled approach, and a lead cell pool was identified and further adapted to five different media conditions, showing comparable titers to the lead clone from the prior approach.

RESULTS

Approach I: Single-cell Cloning from a Clonal Cell line

Figure 1. **Finalized Process for Developing Internal Cell Lines from VYGR-293 Cells**

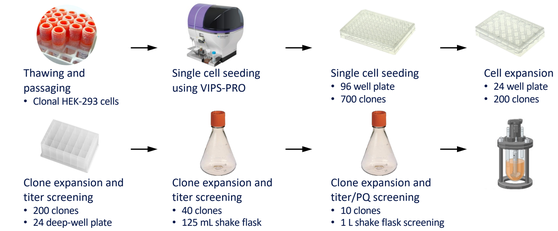


Figure 2. **24DWP Screening of Top Clones by Transgene #1 and #2**

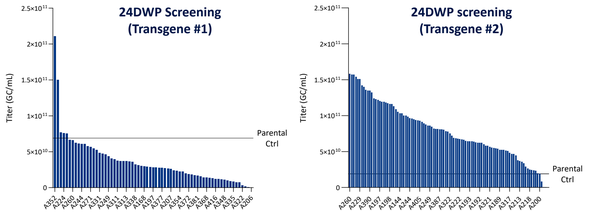
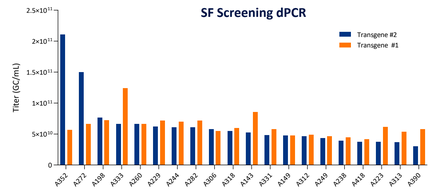
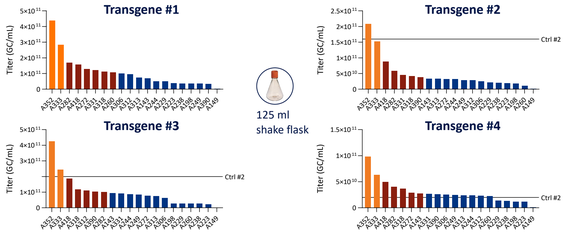


Figure 3. **Shake Flask Screening: Observe Different Trend in Two Transgenes**



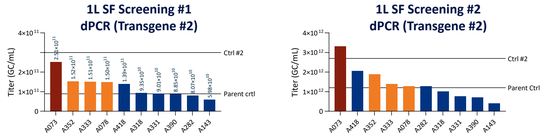
- Different rankings were observed during the screening, indicating that cells might take some time to adapt to suspension culture.

Figure 4. **Shake Flask Screening: 4 Different Transgenes Exhibit Different Yield Profile**



- Two lead clones A333 and A352 showed highest titers across all transgenes.

Figure 5. **1L Shake Flask Screening: Top 10 Clones were Screened Using Transgene #2**



- The VYGR293 clones (A073) showed very comparable titers as two commercial cell lines.

Figure 6. **%Full is Similar Among All Single Cell Clones**

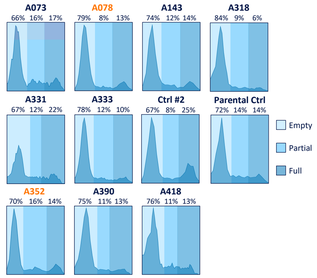


Figure 7. **Clones Showed Different Degree of Clumpiness**

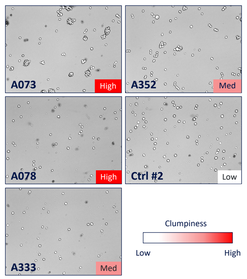
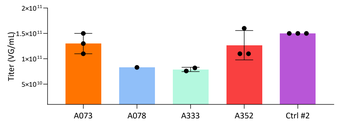


Figure 8. **Performance Across Cell Lines in 2L Bioreactors (Transgene #2)**



- Internal cell lines reached 1.6E11 vg/mL at harvest with minimal optimization.
- Three cells lines < 1E11 vg/mL, 3 cells lines > 1 E11 vg/mL (benchmark).
- A352 selected for program, and process optimization is in progress for further titer improvements.

Table 1. **AUC Shows High Partials and Low Fulls for All Samples**

	Empty (%)	Partial (%)	Full (%)	LOC (%)	HOC (%)
Crtl #1	41.98	11.75	10.76	32.9	2.61
Crtl #2	42.61	9.39	9.05	17.94	21
A078	59.94	10.01	7.17	22.89	0
A073	53.49	14.92	11.4	1.31	18.02
A333	66.28	11.27	7.39	6.34	8.72
A352	54.86	18.98	13.16	10.95	2.05

Table 2. **hcDNA (Normalized to titer)**

	AC Eluate	Total (ng/mL)	Encapsidated DNA (ng/mL)	Free (ng/mL)	Total DNA (ng)/1E13
Crtl #1	5.86e+11	108.91	103.89	5.02	1858.532
Crtl #2	1.41e+12	105.92	105.17	0.75	751.2057
A078	6.93e+11	79.89	79.89	0	1152.814
A073	1.30e+12	200.43	197.55	2.88	1541.769
A333	9.98e+11	171.95	171.68	0.27	1722.946
A352	1.36e+12	72	68.27	3.73	529.4118

Approach II: Mini-pool Cloning from Non-clonal Cell line

Figure 9. **Cloning from Non-clonal Cell Line Using the Mini-pool Strategy**

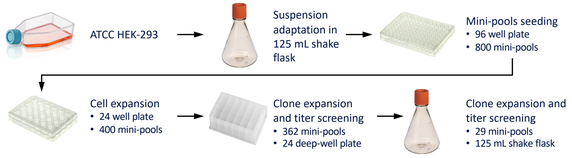


Figure 10. **Suspension Adaptation: Adapt Non-clonal Cell Line into Suspension Form and Monitor the Doubling Time**

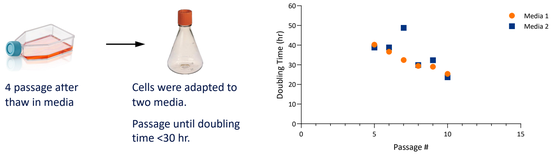
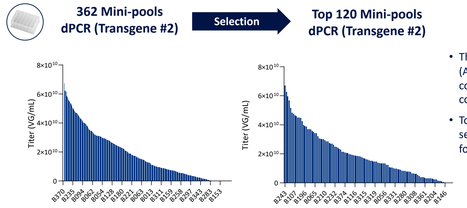
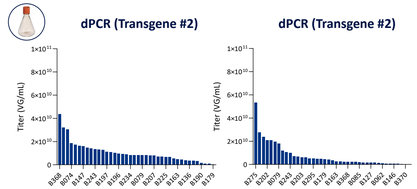


Figure 11. **24 DWP Screening: 362 Mini-pools were Screened Initially, and Removed Low Producers for the Subsequent Screening**



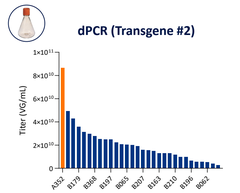
- The VYGR293 clones (A073) showed very comparable titers as two commercial cell lines.
- Top 40 mini-pools were selected to passage in SF for screening in SF.

Figure 12. **24 DWP Screening: 40 mini-pools from DWP were Picked for Screening in Two Separate SF Experiments**



- Cells were adapted three passages in SF before used for screening.
- The rank of two experiment had some discrepancies.
- Top 29 mini-pools were picked for 3rd round of SF screening. A sufficiently large set of mini-pools was progressed to a third screen so the inconsistency in the ranking across experiments was mitigated.

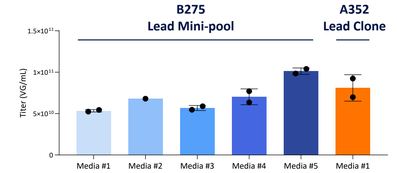
Figure 13. **125 mL SF Screening: Top 29 Mini-pools were Picked and Screened for the 3rd Time to Pick Top 10 Mini-pools**



	3rd SF	2nd SF	1st SF
B275	1	1	2
B165	2	12	32
B179	3	19	40
B250	4	8	23
B074	5	29	4
B368	6	25	1
B243	7	10	10
B236	8	9	24
B197	9	14	13
B203	10	13	33

- Top 10 mini-pools were picked and banked for future single cell cloning.
- Mini-pool B275 was picked as the lead clone for medium screening in the next step.

Figure 15. **Mini-pool Approach Produced Pool with Similar Titers as the Cloning Process Starting from a Single Clone**



- To select a medium that yields the most VG from B275 also reduce the COGs, which will be used in the future single-cell cloning stage.
- B275 produced ~1E11 VG/mL in Prime media.

NEXT STEPS

- Compare the product quality of the mini-pool with the clones from a single cell. Bioreactor study to check their performance.
- Perform single-cell cloning to mini-pools to further identify higher AAV producers.

CONCLUSIONS

- Clonal HEK-293 cell lines can be further single cell cloned to isolate high AAV producer.
- Starting from more genetically diverse HEK-293 cell lines yield better chance of isolating high AAV producers.
- Mini-pool approach produced pools with similar titers as the cloning process starting from a single clone.

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