

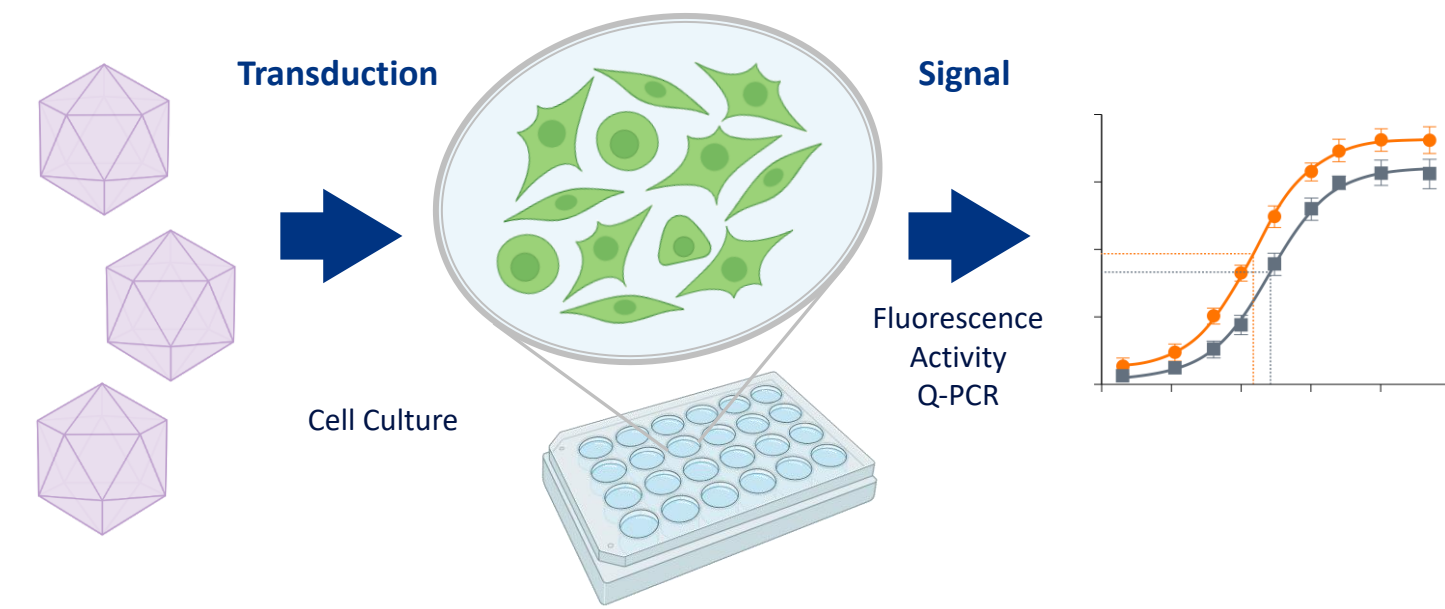
# Exploiting an AAV Capsid Specific Receptor to Develop Stable Cell Lines for Transduction Based Assays for Gene Therapies

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## EXECUTIVE SUMMARY

- Certain cell types required for *in vitro* transduction-based assays may be poorly transduced by engineered AAV capsids, requiring a high multiplicity of infection to demonstrate good signal.
- To resolve this issue, AAV receptors have been engineered into mammalian cells to improve transduction and performance in transduction assays.
- Voyager's discovery of receptors specific for the TRACER™ capsids provides an exciting new avenue to improve transduction and develop proprietary cell lines for assays.
- Voyager's Team has developed engineered HEK293T cell lines expressing an AAV capsid specific receptor as well as a capsid non-specific AAV receptor that have improved transduction and can reduce time for potency assay development.
- Single cell cloning was completed for the engineered HEK293T cell line expressing the AAV capsid specific receptor to yield a cell line with further improved transduction over the non-clonal pool.

Figure 1. Transduction Assays: An Essential Aspect of AAV Characterization



- Selection of well transduced cell line that generates measurable signal is key
- Cell line selection contributes significantly to the assay development lead time

Figure 2. Selected Receptors and Testing of Improvement in Transduction Efficiency

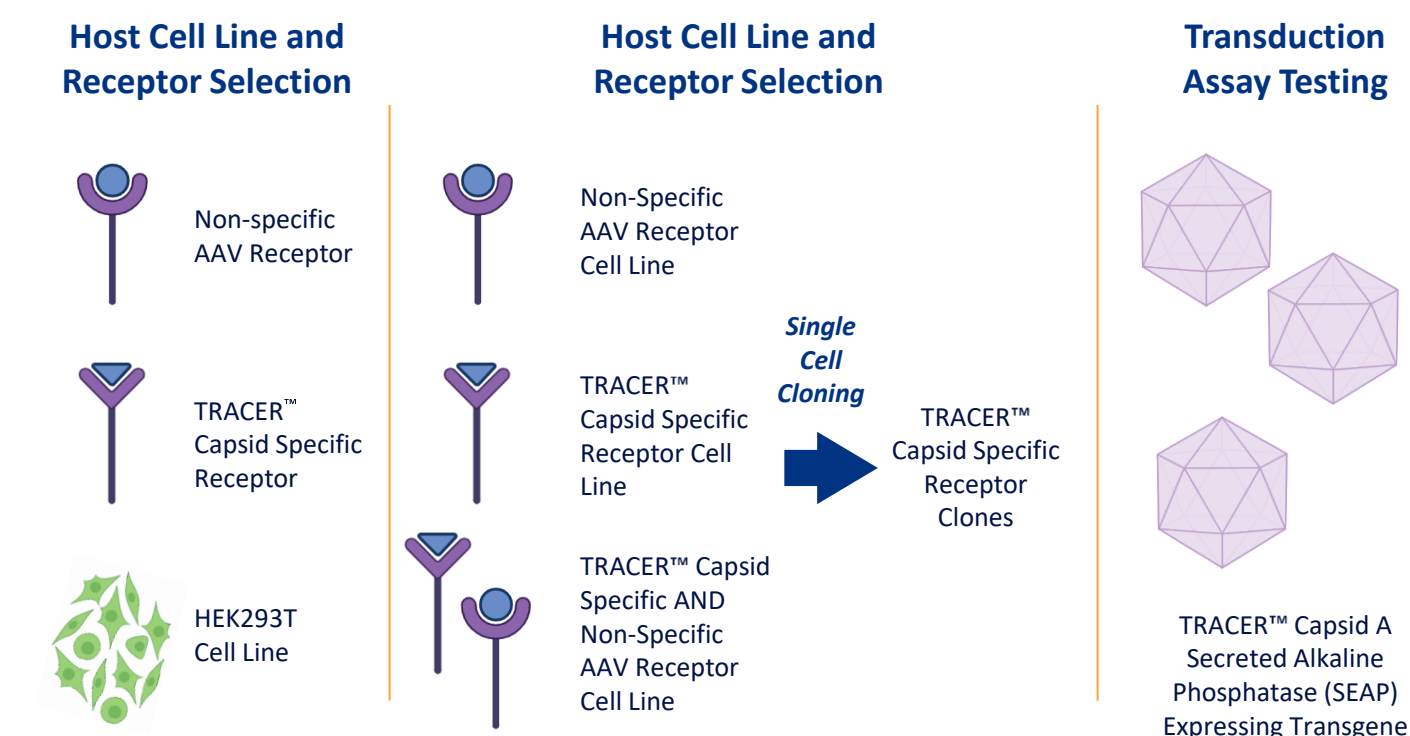


Figure 3. Developing HEK293T-Capsid Specific Receptor/AAV Receptor Stable Pools for Potency Assay

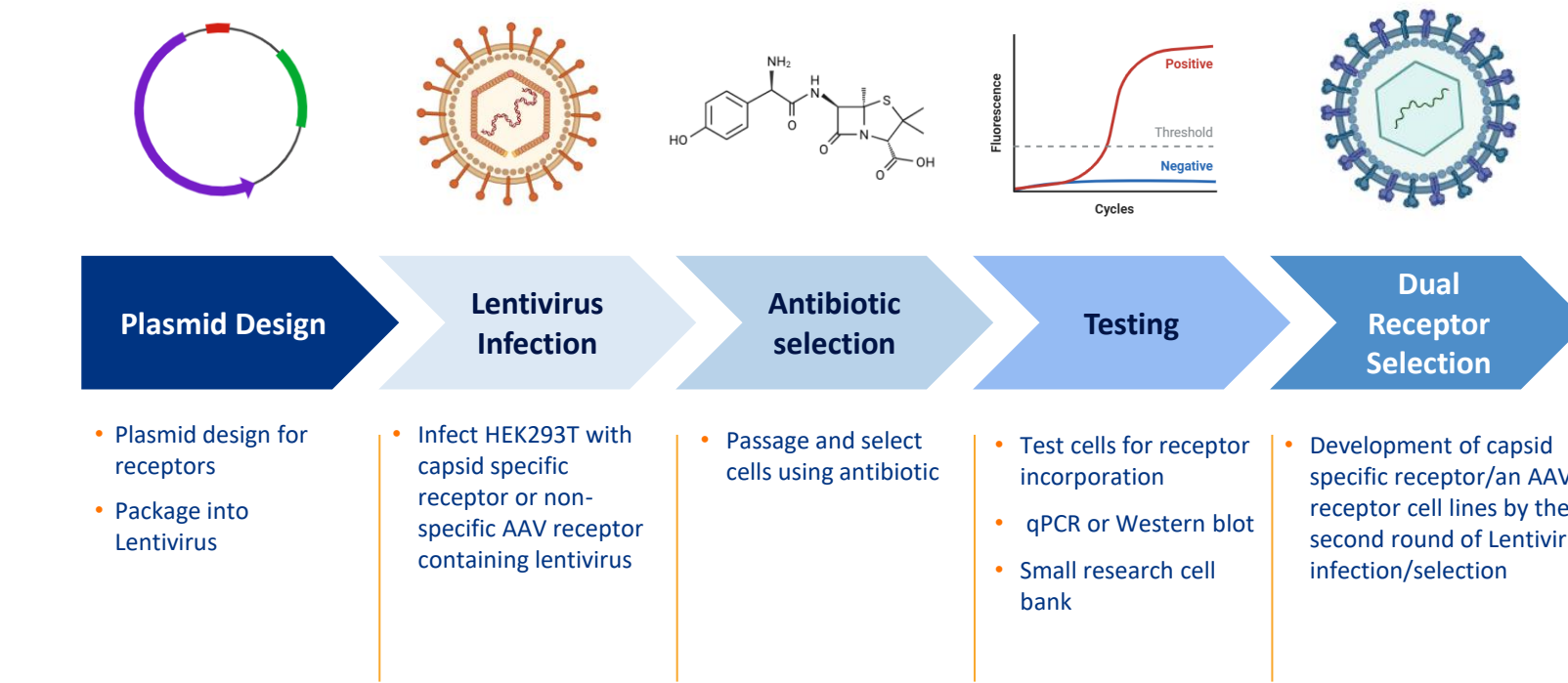


Figure 4. HEK293T Cell Line Expressing Capsid Specific Receptor Showed Improved Transduction Efficiency and SEAP Expression

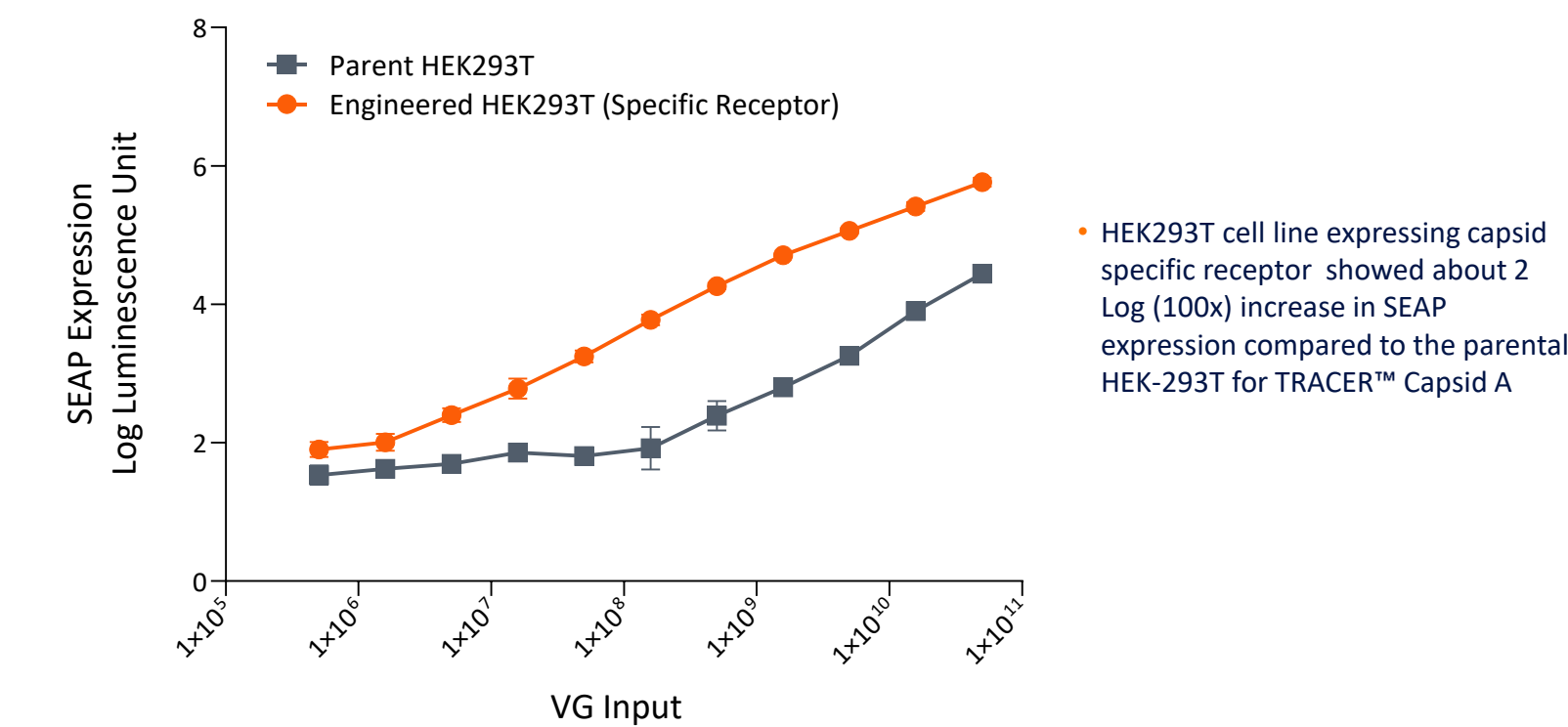


Figure 5. Co-expression of Capsid Specific and Non-Specific (AAV) Receptor Further Improved Transduction Efficiency and SEAP Expression

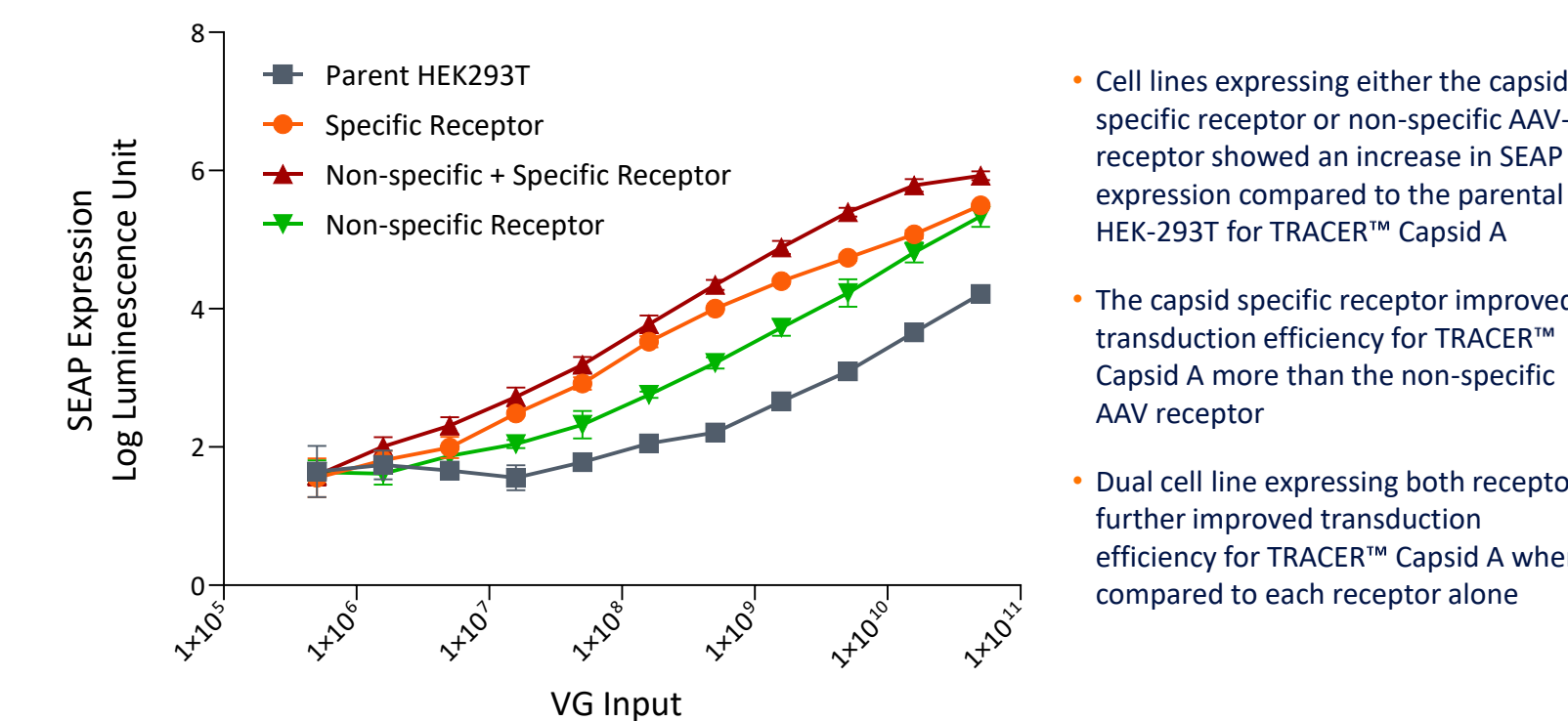


Figure 6. Capsid Specific Receptor Improved Transduction Efficiency to a Greater Extent than Non-Specific AAV Receptor for TRACER™ Capsid A

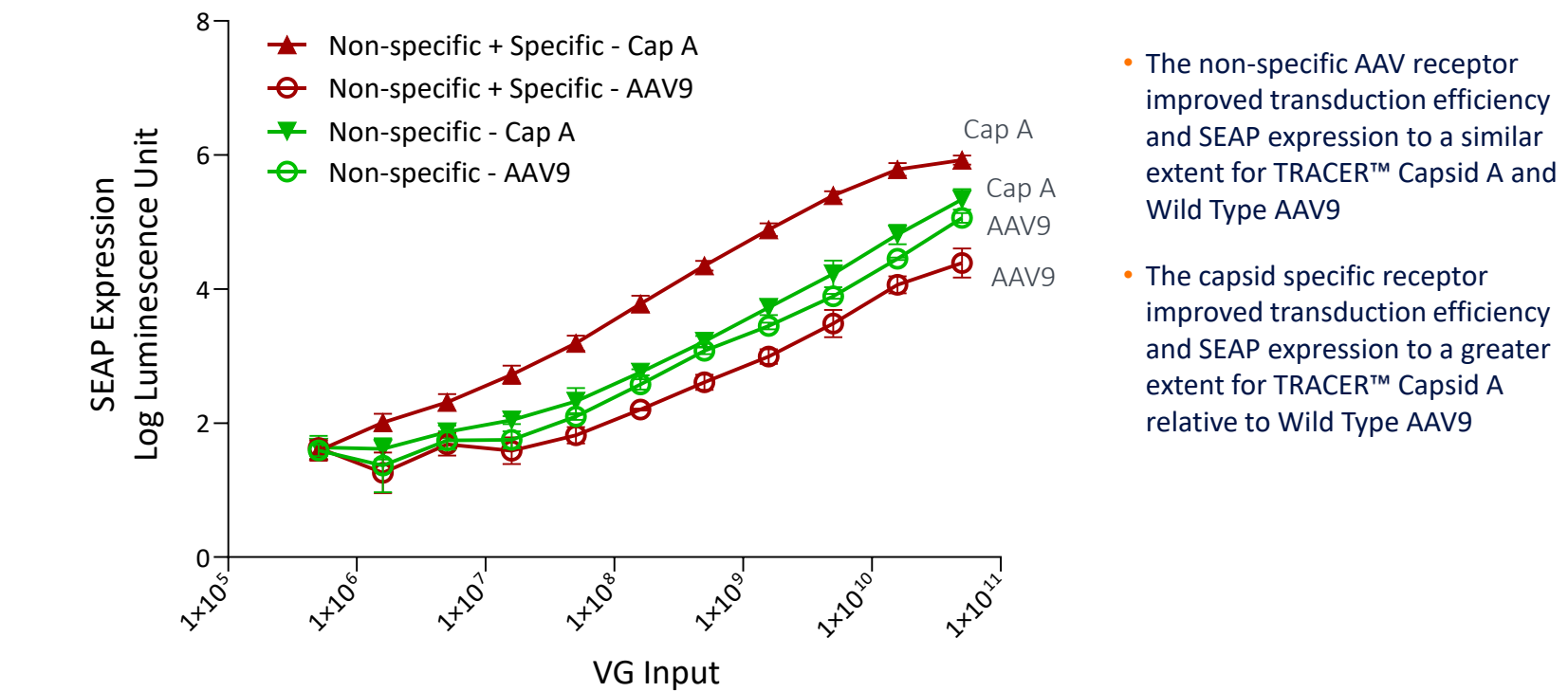


Figure 7. Single Cell Cloning was Conducted on the HEK293T Cell Line Expressing Capsid Specific Receptor to further improve Transduction Efficiency

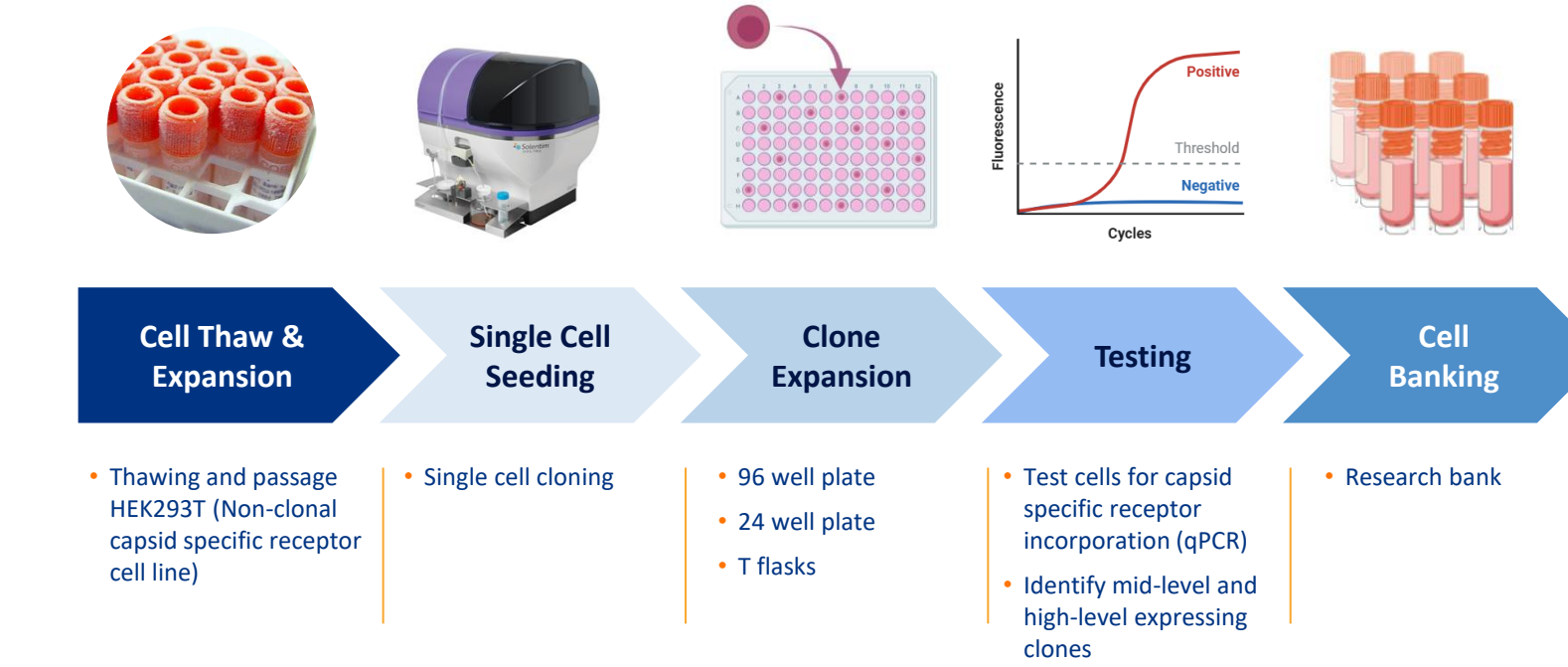


Figure 8. Clonal HEK293T-capsid Specific Receptor Cell Lines with Improved Capsid Specific Receptor Expression Levels were Identified

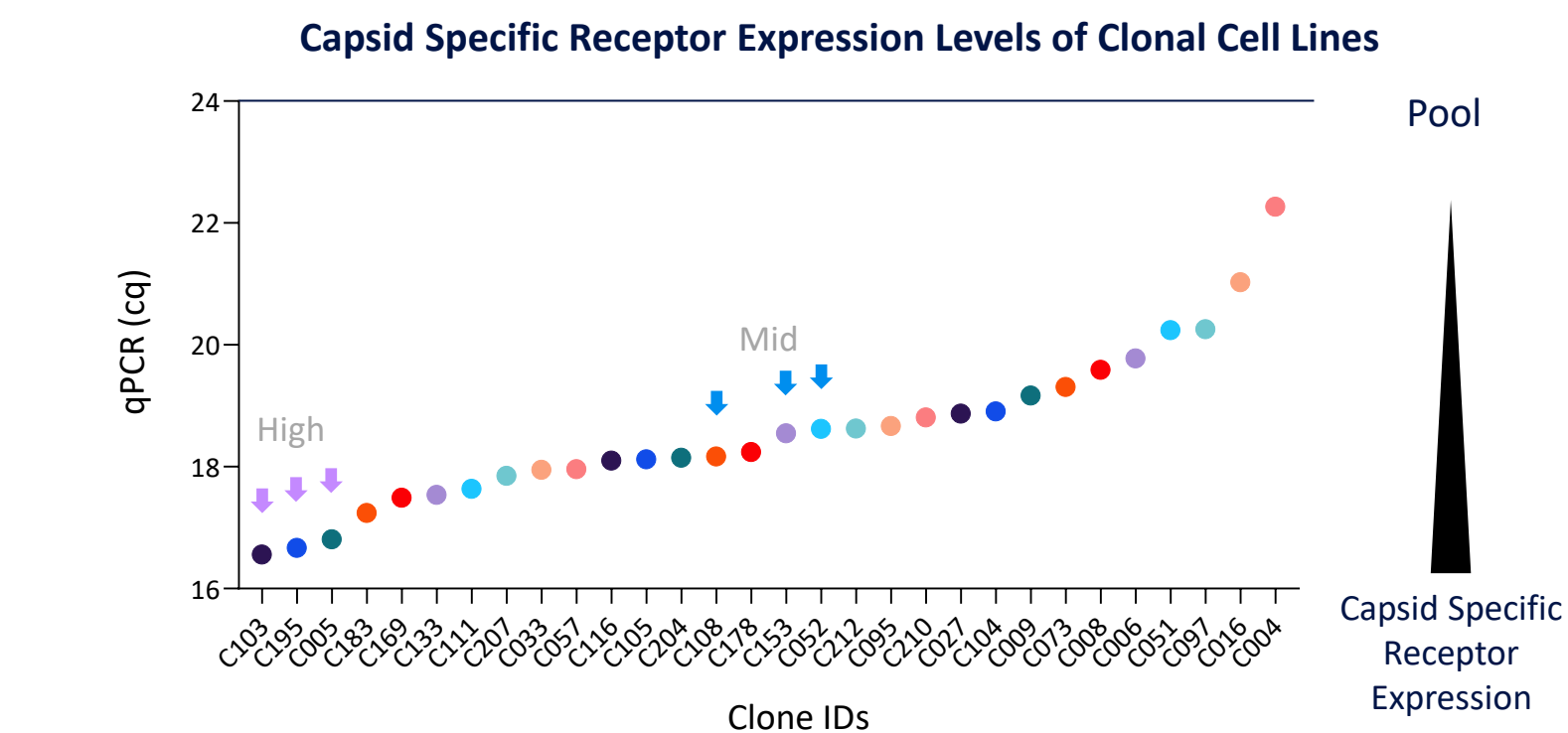


Figure 9. Clones Expressing Capsid Specific Receptor at the Mid-Level Showed Highest Transduction Efficiency Based on SEAP Expression

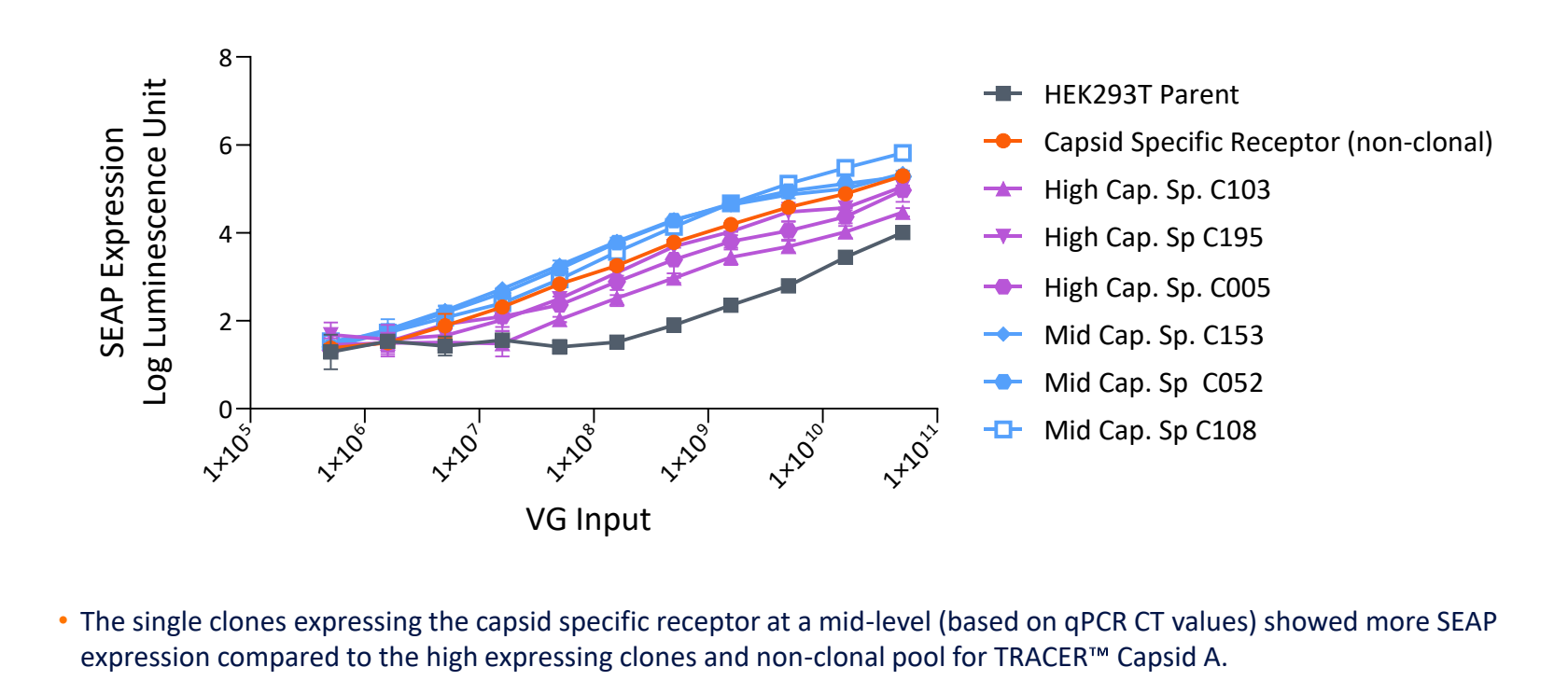
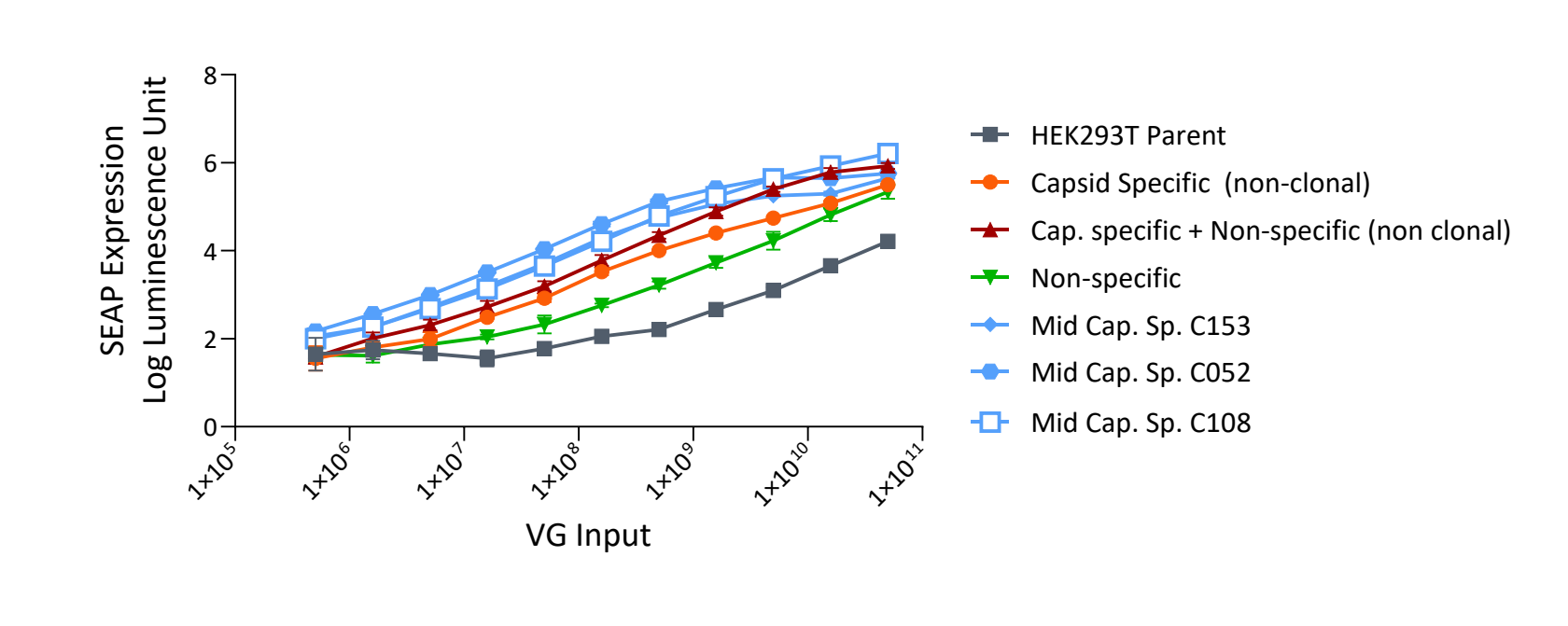


Figure 10. Clonal Cell Lines Expressing the Capsid Specific Receptor Showed Transduction Efficiency Comparable to Non-clonal Cell Line Expressing both the Capsid Specific and Non-specific AAV Receptor



## CONCLUSIONS

- Separate stable cell lines overexpressing a TRACER™ capsid specific receptor or a non-specific AAV receptor were created using lentivirus vectors; TRACER™ Capsid A packaging a secreted alkaline phosphatase (SEAP) transgene was used to assess improvement in transgene efficiency.
- Non-clonal HEK293T cells expressing capsid specific receptor showed increased transduction efficiency compared to parent cell line - as demonstrated by increased SEAP transgene expression.
- Non-clonal HEK293T cells expressing capsid specific receptor showed increased transduction efficiency for TRACER™ Capsid A compared to the non-capsid specific AAV receptor.
- Single cell cloning was conducted to develop clonal HEK293T cell lines expressing capsid specific receptor.
- Clonal HEK293T cell lines expressing capsid specific receptor showed greater transduction efficiency compared to the non-clonal cell lines for TRACER™ Capsid A.

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