A robust and flexible baculovirus-insect cell system for AAV vector production with improved yield, capsid ratios and potency

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Introduction

Manufacturing of recombinant adeno-associated viruses (rAAV) for gene and cell therapy applications has increased significantly and spurred development of improved mammalian and insect cell-based production systems. We developed a baculovirus-based insect cell production system- the Sangamo (SGMO) Helper- with a novel gene architecture and greater flexibility to modulate the expression level and content of individual Rep and Cap proteins. In addition, we incorporated modifications to the rAAV6 capsid sequence that improves yield, capsid integrity and potency:

- Production of rAAV6 using the SGMO Helper has improved yields compared to the Bac-RepCap helper from the Kotin lab.
- SGMO Helper-derived rAAV6 is resistant to a previously described proteolytic cleavage unique to baculovirusinsect cell production systems and has improved capsid ratios and potency, in vitro and in vivo, compared to rAAV6 produced using Bac-RepCap.
- AAV production using the SGMO Helper is scalable using bioreactors, with similar improvements in yield, capsid ratio and in vitro potency.

Our studies demonstrate that the SGMO Helper is an improved platform for rAAV manufacturing to enable delivery of cutting-edge gene and cell therapies.

Gene Architecture of the SGMO Helper



Production of rAAV6 using the SGMO Helper

Experiment	Sample	Purified AAV titer (vg/mL)	Purified AAV yield (vg)	Harvest yield (vg/mL culture)	VPI	VP2	VP3
	Bac-RepCap	2.23E+13	2.45E+13	1.23E+11	l I	2.1	45
	SGMO Helper	5.99E+13	8.99E+13	4.50E+11	l l	1.1	21
2	Bac-RepCap	2.25E+13	2.70E+13	1.35E+11	l l	2.1	60
	SGMO Helper	7.97E+13	1.20E+14	6.00E+11	l l	1.3	24
3	Bac-RepCap	9.46E+12	9.46E+12	4.73E+10	l I	2.1	67
	SGMO Helper	6.21E+13	8.69E+13	4.35E+11	l I	l I	19
4	Bac-RepCap	1.83E+13	1.65E+13	8.25E+10	l l	1.8	60
	SGMO Helper	8.40E+13	1.09E+14	5.45E+11	l I	0.8	17
Mid-scale	Bac-RepCap	3.56E+13	3.98E+14	2.65E+11	I.	1.7	82
production	SGMO Helper	4.85E+13	9.89E+14	6.59E+11	I	1.2	24
In vivo study	Bac-RepCap	1.37E+13	3.01E+13	1.51E+11	I	1.5	59
	SGMO Helper	3.47E+13	1.15E+14	5.75E+11	I.	0.9	27

Modifications to rAAV6 capsids produced in insect cells



Testing rAAV6 produced using the SGMO Helper



Testing of AAV6 produced using either Bac-RepCap or the SGMO Helper. (A) Western blot analysis of cell lysates collected at Day 6 harvest using an antibody specific to either Rep or Cap. Naïve Sf9 cells were run as a negative control for antibody recognition. (B) SDS-PAGE and Coomassie Blue stained gel of purified AAV6 from 4 independent production runs. The individual Cap (VP1, VP2 and VP3) proteins are indicated on the panel. The proteolytic cleavage fragment observed with AAV6 produced using Bac-RepCap is indicated by an asterisk. AAV8 produced using the 293 production system (293 Reference) was included for comparison. (C) In vitro potency of AAV6 produced using 293 cells or Sf9 cells (Bac-RepCap and SGMO Helper) on HepG2 cells. HepG2 cells were transduced using the indicated MOI and, after 5 days, cell supernatants were assayed for α -gal A activity. The nmol/hr/mL α -gal A activity was calculated based on fluorescent activity using a standard curve. Each virus sample was assessed for potency in triplicate, with each of the replicates being assayed in duplicate. (D) In vitro potency of AAV6 produced using 293 cells or Sf9 cells (Bac-RepCap and SGMO Helper) on HuH7 cells, as described in (C). (E) In vitro potency of AAV6 produced using 293 cells or Sf9 cells (Bac-RepCap and SGMO Helper) on HepG2 cells, as described in Figure 1B.

Presented at the ASGCT 26th Annual Meeting, 2023

2217 (A) The AAV6 VPI sequence was modified to contain the AAV2 phospholipase A2 (PLA2) domain, which improved rAAV6 yield, and a short fragment of VPI from AAV9 at the indicated nucleotide coordinates, which prevents proteolytic cleavage of rAAV6. (B) In vitro potency of AAV6 produced using 293 cells or Sf9 cells (Bac-RepCap and PLA2 transplant) on HepG2 cells. HepG2 cells were transduced using an MOI of IE+06 and, after 5 days, cell supernatants were assayed for huFIX expression. The ng/mL huFIX quantity was calculated using a standard curve. (C) SDS-PAGE and potency testing of AAV6 produced using the Bac-RepCap or the Bac-RepCap containing sequence from AAV9 corresponding to a proteolytic cleavage site. The individual Cap (VPI, VP2 and VP3) proteins are indicated on the panel. The proteolytic cleavage fragment observed with AAV6 produced using the Bac-RepCap is indicated by an asterisk. In vitro potency of AAV6 produced using 293 cells or Sf9 cells (Bac-RepCap and AAV9 transplant) was performed as in (**B**).

Testing rAAV6 from scaled-up production using the SGMO Helper



Testing of AAV6 produced at 3L scale using either Bac-RepCap or the SGMO Helper. (A) SDS-PAGE and Coomassie Blue stained gel of purified AAV6 from 3L production run. The individual Cap (VPI, VP2 and VP3) proteins are indicated on the panel. The proteolytic cleavage fragment observed with AAV6 produced using Bac-RepCap is indicated by an asterisk. AAV8 produced using the 293 production system (293 Reference) was included for comparison. (B) Electropherogram from CE-SDS analysis of rAAV6 produced in scaled up bioreactors using either the Bac-RepCap or SGMO Helper. The VP1, VP2, VP3, truncated VP3 and VP1/2 cleavage fragments are indicated. (C) In vitro potency of AAV6 produced using 293 cells or at 3L scale in Sf9 cells (Bac-RepCap and SGMO Helper) on HepG2 cells was performed as described in Figure 2B.

In vivo testing of rAAV6 produced using the SGMO Helper



Testing of AAV6 produced using Bac-RepCap and the SGMO Helper for in vivo potency. (A) SDS-PAGE and Coomassie Blue stained gel of purified AAV6. The individual Cap (VP1, VP2 and VP3) proteins are indicated on the panel. The proteolytic cleavage fragment observed with AAV6 produced using Bac-RepCap is indicated by an asterisk. AAV8 produced using the 293 production system (293 Reference) was also included for comparison. (B) In vitro potency of AAV6 produced using 293 cells or Sf9 cells (Bac-RepCap and SGMO Helper) on Hepal-6 cells. Hepal-6 cells were transduced using the indicated MOI and, after 5 days, cell supernatants were assayed for α -Gal A activity and analyzed as previously described in Figure 2B. (C) Mouse plasma levels of α -Gal A activity from mice IV dosed with 5E+12 vg/kg or 2E+13 vg/kg of 293 AAV6 or Sf9 AAV6 produced using Bac-RepCap or the SGMO Helper. 28 days post-administration, mice were euthanized and plasma samples were collected to assay for α -Gal A activity. The nmol/hr/mL α -Gal A activity was calculated based on fluorescent activity using a standard curve.

Conclusion

- Bac-RepCap.
- vitro potency.

References

Smith, R. H., Levy, J. R., and Kotin, R. M. (2009). A simplified baculovirus-AAV expression vector system coupled with one-step affinity purification yields high-titer rAAV stocks from insect cells. Mol. I her. 17, 1888–96 Kohlbrenner, E., Aslanidi, G., Nash, K., Shklyaev, S., Campbell-Thompson, M., Byrne, B. J., Snyder, R. O., Muzyczka, N., Warrington Jr, K. H., and Zolotukhin, S. (2005). Successful production of pseudotyped rAAV vectors using a modified baculovirus expression system. Mol. Ther. 12, 1217-25. Galibert, L., Savy, A., Dickx, Y., Bonnin, D., Bertin, B., Mushimiyimana, I., van Oers, M. M., and Merten, O.-W. (2018). Origins of truncated supplementary capsid proteins in rAAV8 vectors produced with the baculovirus system. PLoS. One. 13, e0207414–. Oyama, H., Ishii, K., Maruno, T., Torisu, T., and Uchiyama, S. (2021). Characterization of Adeno-Associated Virus capsid proteins with two types of VP3-related components by capillary gel electrophoresis and mass spectrometry. Hum. Gene. Ther. 32, 1403–1416.



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