CMC Strategies for AAV Gene Therapies for Highly Prevalent Diseases

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Background

- Gene therapy products constitute introduction of normal or modified genetic material (usually DNA) into a patient to produce a therapeutic protein to alleviate a genetic deficiency.
- Biological research applications and potential treatments for genetic and prevalent diseases are now being made possible by gene therapy.
- The number of clinical trials and approved gene therapy products using recombinant adeno-associated viral (rAAV) vectors are rapidly increasing, highlighting the need to standardize (and improve) manufacturing and purification of these vectors within Chemistry, Manufacturing, and Controls (CMC) to meet quantitative and qualitative requirements of regulatory agencies.
- We describe a new strategy for recombinant Bacmid (rBacmid) preparation that yielded increased rBacmid purity, together with a new process that generated higher titers for AAV2.7m8, an engineered AAV vector capable of pan-retinal tropism via a single in-office intravitreal administration.

Methods

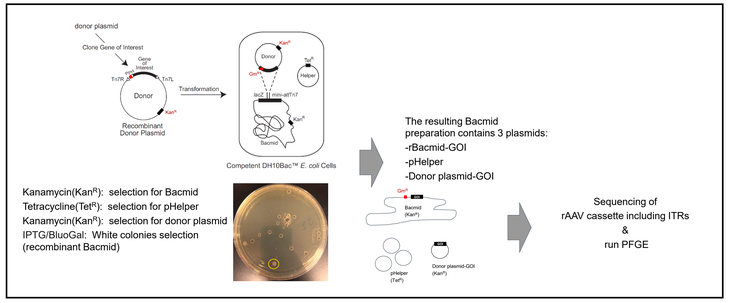


Figure 1. rBacmid preparation in the standard rAAV production process in insect cells. Donor plasmid into which the gene of interest is cloned is flanked by the left and right arms of Tn7, and contains a Gentamicin resistance gene (Gm^R), GC-rich regions between ITRs and Gm^R sequence and in some cases using Kan^R in the backbone of the donor plasmid. The donor plasmid is transformed into the competent cells (DH10Bac E.coli cells) that is used as the host for the donor plasmid.
DH10Bac cells enclose a Bacmid that contains a low-copy number mini-F replicon, Kanamycin resistance marker and a segment of DNA encoding the LacZα peptide into which the attachment site for the bacterial transposon, Tn7 (mini-attTn7) has been inserted. Insertion of the mini-attTn7 does not disrupt the reading frame of the LacZα peptide with a mini-attTn7 target site and a helper plasmid. Once the donor plasmid is transformed into DH10Bac cells, transposition occurs between the mini-Tn7 element on the donor plasmid and the mini-attTn7 target site on the bacmid to generate a recombinant bacmid. This transposition reaction occurs in the presence of transposition proteins supplied by the helper plasmid which encodes the transposase and confers resistance to tetracycline.

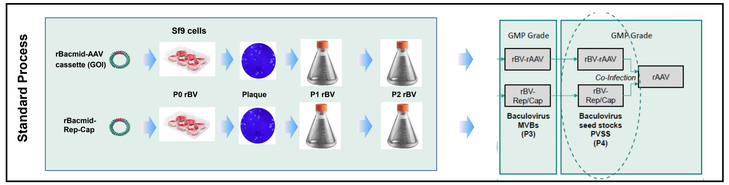
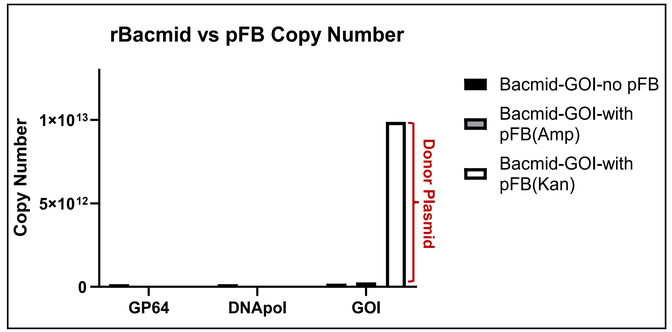


Figure 2. rBacmid preparation in the standard rAAV production process in insect cells.
Low quality rBacmid in the standard rAAV production process in insect cells necessitates P0 (Passage 0), P1, P2 and P3 and P4 rBV (recombinant baculovirus) to obtain sufficient rBV titer.

Results



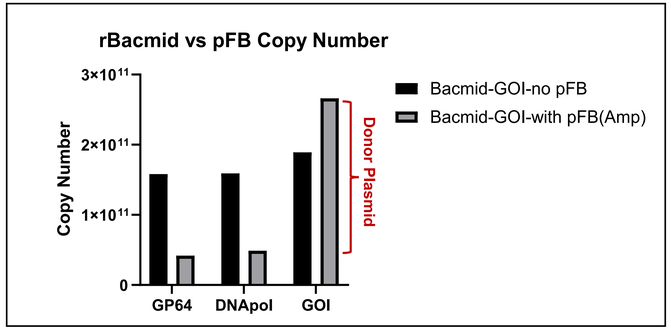


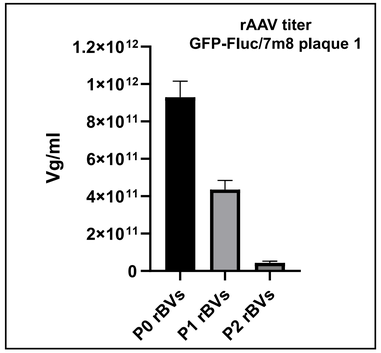
Figure 3. Improved purity of rBacmid with addition of new selection marker. The level of donor plasmid (pFB) in three different rBacmid Preps through ddPCR was evaluated twice. (1) rBacmid with no measurable pFB by addition of streptomycin-selective marker on top of Amp^R, (2) rBacmid with pFB which contains Amp^R and (3) rBacmid with pFB which contains Kan^R. GOI: Gene of Interest; DNApol: DNA polymerase.

Table 1. Evaluation of the ratio of GOI to rBacmid backbone (DNApol or GP64). There was no detection of pFB in our first prep. In the second prep, pFB/rBacmid was 4.6. In the last prep, pFB/rBacmid was 261 indicating that we have 261 copies of pFB vs 1 copy of rBacmid.

	Preps	Ratio GOI/DNApol	Donor plasmid/rBacmid (folds)
1	(New) Bacmid-GOI-no pFB	1.19	0
2	Bacmid-GOI-with pFB (Amp)	5.45	4.6
3	Bacmid-GOI-with pFB (Kan)	309.18	261

Table 2. 7m8 rBaculovirus stability during different passages in two 7m8 plaques.

rBV-7m8	Plaque 1 (7m8/GP64)	Plaque 2 (7m8/GP64)
P0	0.71	0.71
P1	0.31	0.81
P2	0.2	0.54



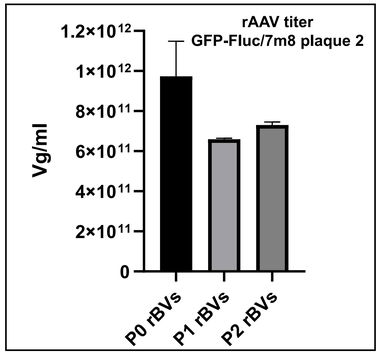


Figure 4. Vector genome (Vg) titer of rAAV generated with two different 7m8 plaques (plaque 1 & 2) and the same GOI. rBaculovirus stability was evaluated following different passages to determine the impact on rAAV titer.

After generating P0 rBV, the plaque purification assay was performed. Ten different plaques were picked for GOI (GFP-Fluc) and 7m8 capsid. The insertion sequence/backbone ratio was checked in 10 different plaques by ddPCR.
Two different 7m8 plaques (plaque 1 and 2) were chosen to generate rAAV with the same GOI plaque for P0, P1 and P2 rBVs.
rAAV titer with Plaque 1-7m8 decreased by 95% however with plaque 2-7m8, rAAV titer decreased by approximately 25%. (Figure 4).

Summary

- **High quality rBacmid enables lower passage number to generate high-titer rBVs (Figure 5)**
  - Replace Kan^R with Amp^R + $Strep^S$ in donor plasmid backbone
- **Product purity can be increased by reducing levels of residual DNA (Figure 5)**
  - Remove Gm^R & GC-rich regions from the donor plasmid
  - Replace Kan^R with Amp^R + $Strep^S$ in donor plasmid backbone
  - Use Sf-RVN (Sf-rhabdovirus negative) cells instead of Sf9 cells
- **Process changes can yield higher titer rAAV (Figure 6)**
  - Use Sf-RVN cells with optimal media instead of Sf9 cells
  - Add feed to the media during rAAV production
  - Use low MOI (0.001) for rAAV production

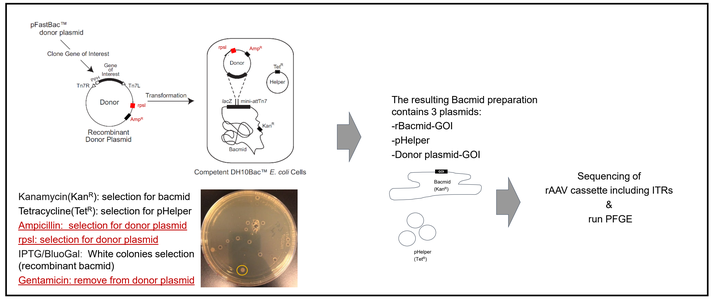


Figure 5. New strategy of rBacmid preparation.

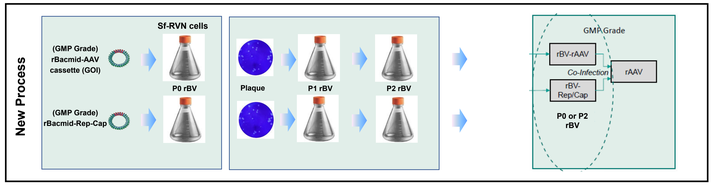


Figure 6. New process of rAAV production in Sf-RVN cells with optimal media.

Conclusion

Using high quality rBacmid, sufficient rBV titer can be generated with earlier passages to retain higher GOI. Higher product purity and greater yields can meet the gene therapy product demand for treatment of prevalent disease.

Acknowledgements: *we would like to thank GlycoBac for providing us Sf-RVN cell (Maghodiaa AB, et al (Protein Expr Purif. 2016 June ; 122: 45–55)).*