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Development of a Novel Recombinant Adeno-Associated Virus Production System Using Human Bocavirus 1 Helper Genes

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Previously, we have demonstrated that HBoV1 genes *NP1*, *NS2*, and *BocaSR* are the minimal components for WT AAV replication in 293 cells. Here, we compare the helper functions from HBoV1 with that from adenovirus for the production of rAAV in 293 cells. We demonstrate that triple plasmids transfection of 1) a cloned HBoV1 minigenome (pBocaHelper) that expresses HBoV1 genes *NP1*, *NS2*, and *BocaSR*, 2) pAAV transfer plasmid, and 3) pAAVRepCap supports rAAV production in 293 cells but with a production yield of 1-2 log lower than that using pAdHelper. To optimize this novel rAAV production system, we examined the expression of AAV proteins and the replication of rAAV genome supporting by the transfection of pBocaHelper. We found that the low vector production is largely due to inefficient expression of the AAV Rep52 and capsid proteins, as well as reduced rAAV genome replication. When the AAV2 P19 promoter in pAAVRep was replaced by or the AAV capsid proteins were expressed under the stronger CMV promoter, the enhanced expression of Rep52 and capsid proteins significantly improved the rAAV production by pBocaHelper, approaching a level of 50-70% of that produced by pAdHelper. Through further dissection of the helper functions from pAdHelper, we found that an addition of the Ad *E2A* gene with pBocaHelper significantly increased rAAV DNA replication. As a result, the rAAV vector production reached a level of two times higher than that using pAdHelper in 293 cells in the context of overexpression of capsid proteins. Based on the above findings, we combined HBoV1 *NP1* and *NS2* genes with pAdHelper to create a pSuperHelper, for which overexpression AAV genes *rep* and *cap* by the CMV promoter is not necessary. rAAV production from the new system using the pSuperHelper is ~2-fold greater than that from the conventional production system using pAdHelper.