VIROVEK

CERTIFICATE OF ANALYSIS

Purified AAV6-CMV-GFP and AAV6-Empty (Lot# 21-623 and Lot#19-596E**)**

Storage Conditions

The AAV vectors should be stored at -80°C for long term usage. When storing for frequent use, 4°C is recommended. It's not recommended to store AAV vectors at -20°C.

Shelf Life

5 years when stored at -80°C. Minimize the freeze and thaw cycle.

Shipping Conditions

Dry ice overnight

Description

AAV6-CMV-GFP was produced in insect Sf9 cells by dual infection with rBV-pFB inCap6-inRep-kozak-updated-hr2 (Clone ID: V290) (Fig. 2) and rBV-V445-pFB-CMV-GFP (Fig. 3). For AAV6-Empty, only rBV-V290 was used. The vectors were purified through 2 rounds of CsCl ultracentrifugations. The CsCl band was removed through buffer exchange with 2 PD-10 desalting columns.

QPCR Titer

2E+13 vg/mL
The titers of AAV6-CMV-Luciferase was determined with QPCR method using primers/probe corresponding to the AAV2 ITR. The titer of AAV6-Empty is determined by nanodrop and SDS-PAGE analysis.

Quality Control Data

The AAV vector was formulated in 1xPBS buffer pH7.4, containing 0.001% pluronic F-68, and sterilized with 0.22μm low protein-binding filter. SDS-PAGE and SimplyBlue Staining (Invitrogen) verified the purity of the vectors (Fig. 1). QPCR analysis determines the titers of the AAV samples.

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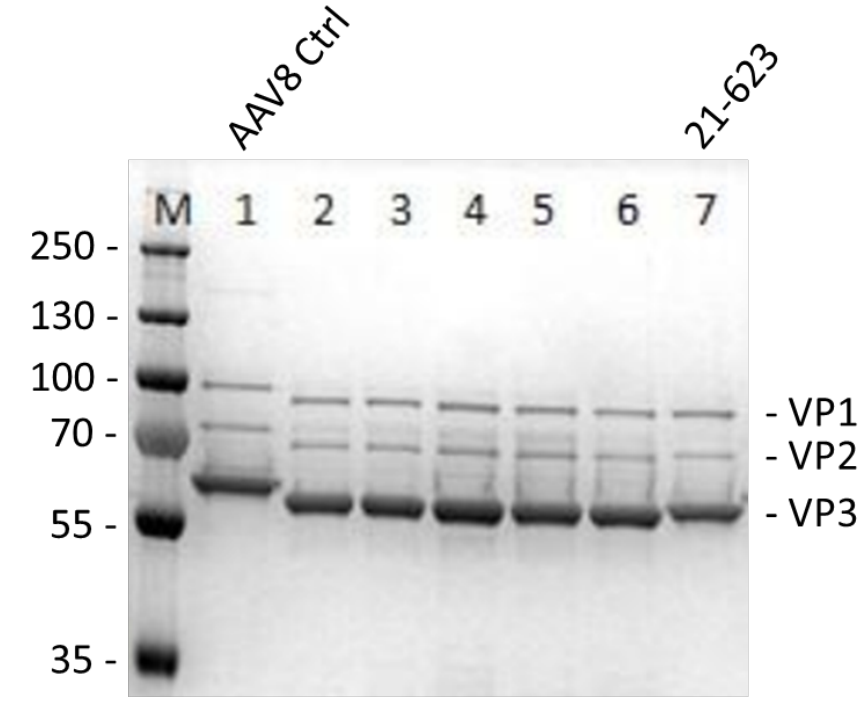

Fig. 1. SDS-PAGE and Instant Blue Staining of purified AAV6-CMV-GFP (Lot#21-623). Lane M, protein ladder; lane 1, AAV8 control; lane 7, AAV6-CMV-GFP (#21-623)

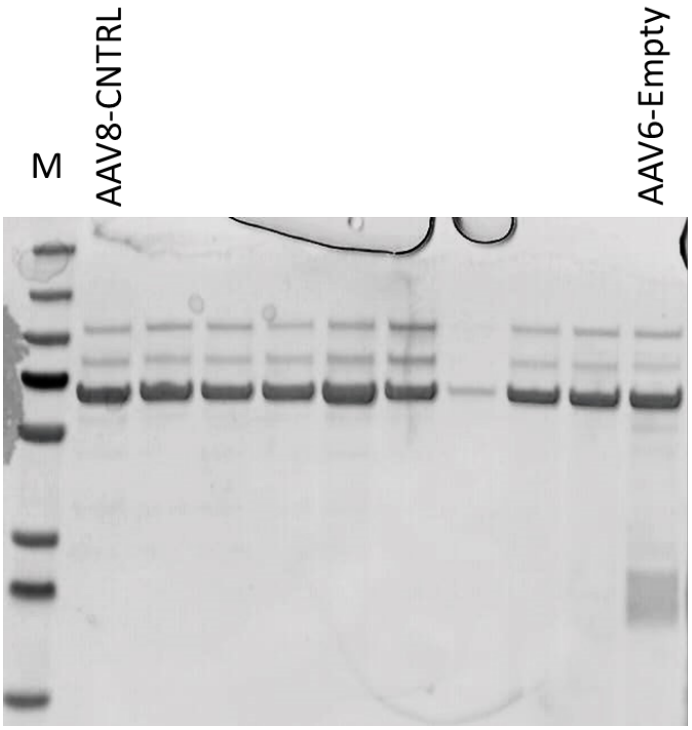

Fig. 2. SDS-PAGE and Instant Blue Staining of purified AAV6-Empty (Lot#19-596E). Lane M, protein ladder; lane 1, AAV8 control; lane 10, AAV6-Empty

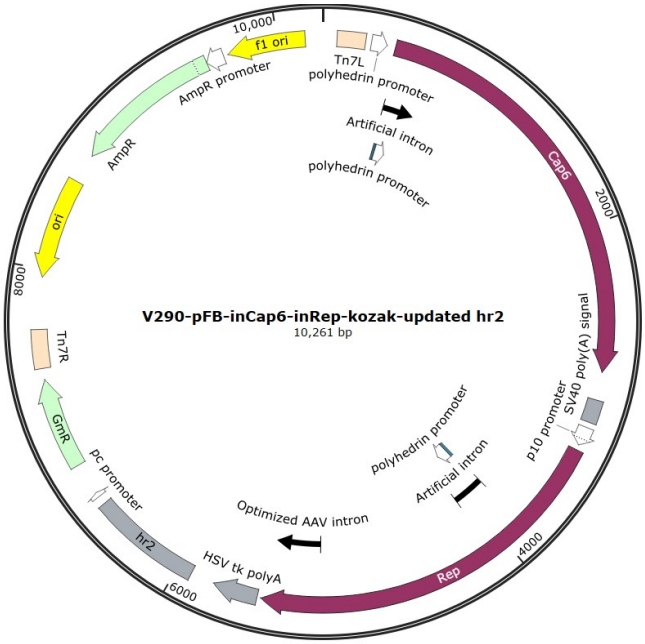

Fig. 3. Diagram of plasmid used to generate rBV-V290-pFB-inCap6-inRep-kozak-updated-hr2

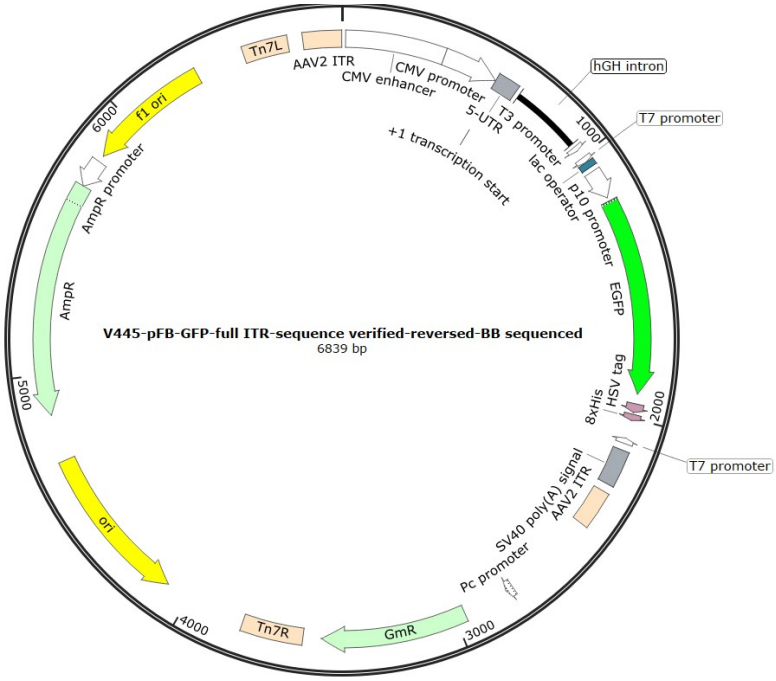

Fig. 4. Diagram of plasmid used to generate rBV-V445-pFB-GFP.

Approved by: *Santanu Raychaudhuri*
Date: January 19, 2023