REGISTRY PARTS (BIOBRICKS)

The registry parts arrived as DNA fragments (synthesized by IDT and Syntezza Bioscience). The DNA fragments were then cloned into the iGEM pSB1C3 vector. After cloning, the constructs were validated by restriction and PCR. As a final stage of validation of the integrity of both the synthesis and cloning process, sequencing was performed on all parts using a forward primer located on the vector.

Results

The sequencing showed that: 1) all parts matched the requested sequence, and 2) cloning frame matched the planned sequence (Figure 1).

Figure 1. Sequencing analysis of cloned Biobricks in pSB1C3 vector. **A**. phTERT; **B**. gMLP; **C**. gUBB; **D**. U6-gMLP; **E**. U6-gUBB.

For more information on submitted registry parts:
http://2015.igem.org/Team:BGU_Israel/Parts

CLONING OF BOOMERANG COMPONENTS

*-cloned by Syntezza Bioscience

For the subcloning, the "master" sequence (hTERT promoter_eGFP_polyA) with appropriate restriction sites was designed and cloned into AAV vector (Figure 2).

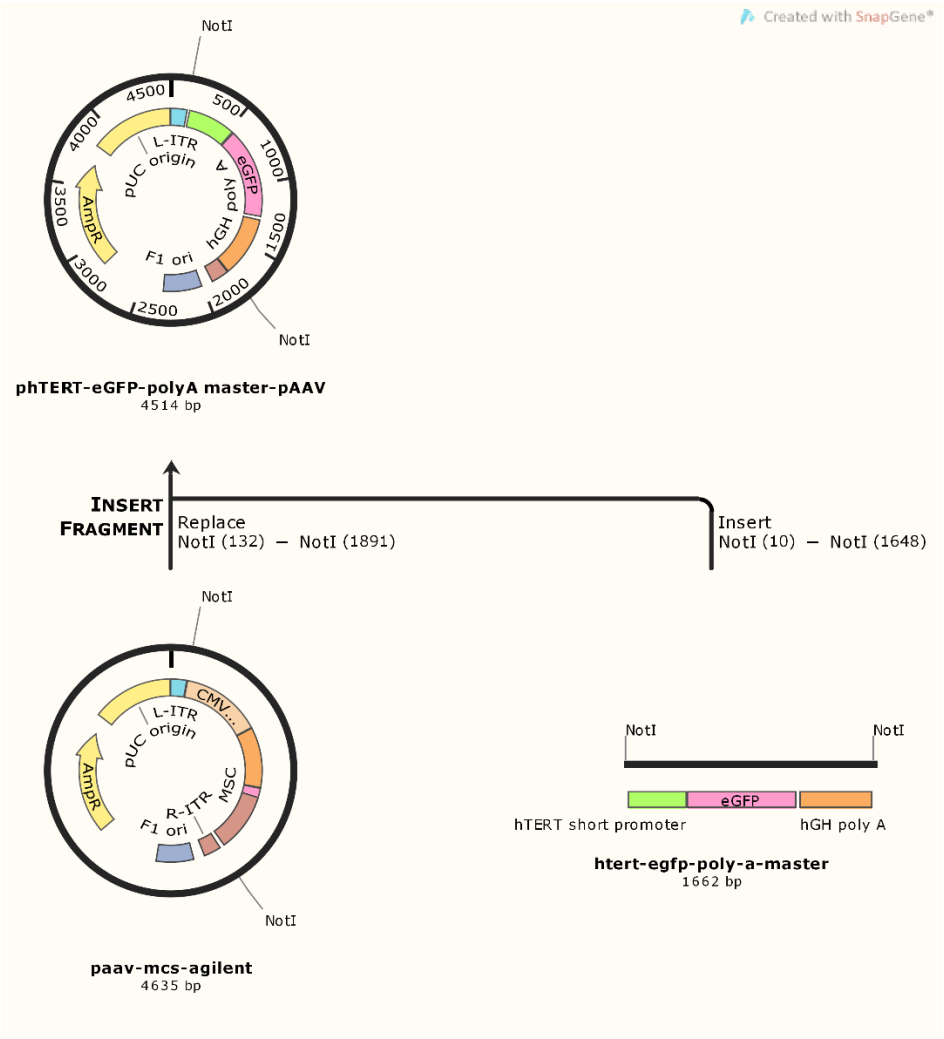
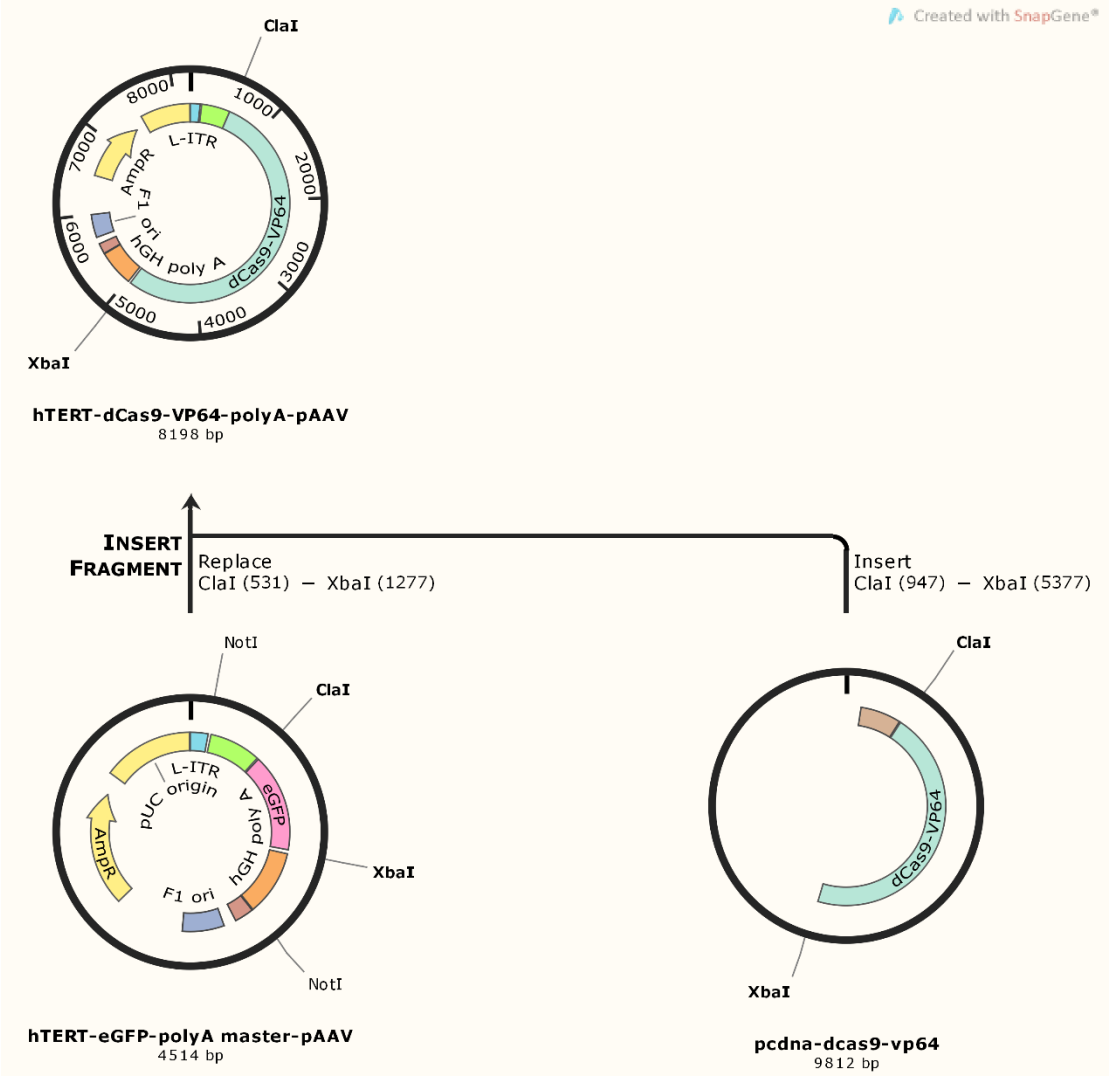


Figure 2. Cloning of "master" template into AAV vector.

All subsequent clonings were performed into this resulting plasmid.

Activation system

*-phTERT-dCas9-VP64**



-CMV-dCas9-VP64

The construct was cloned as shown in Figure 3.

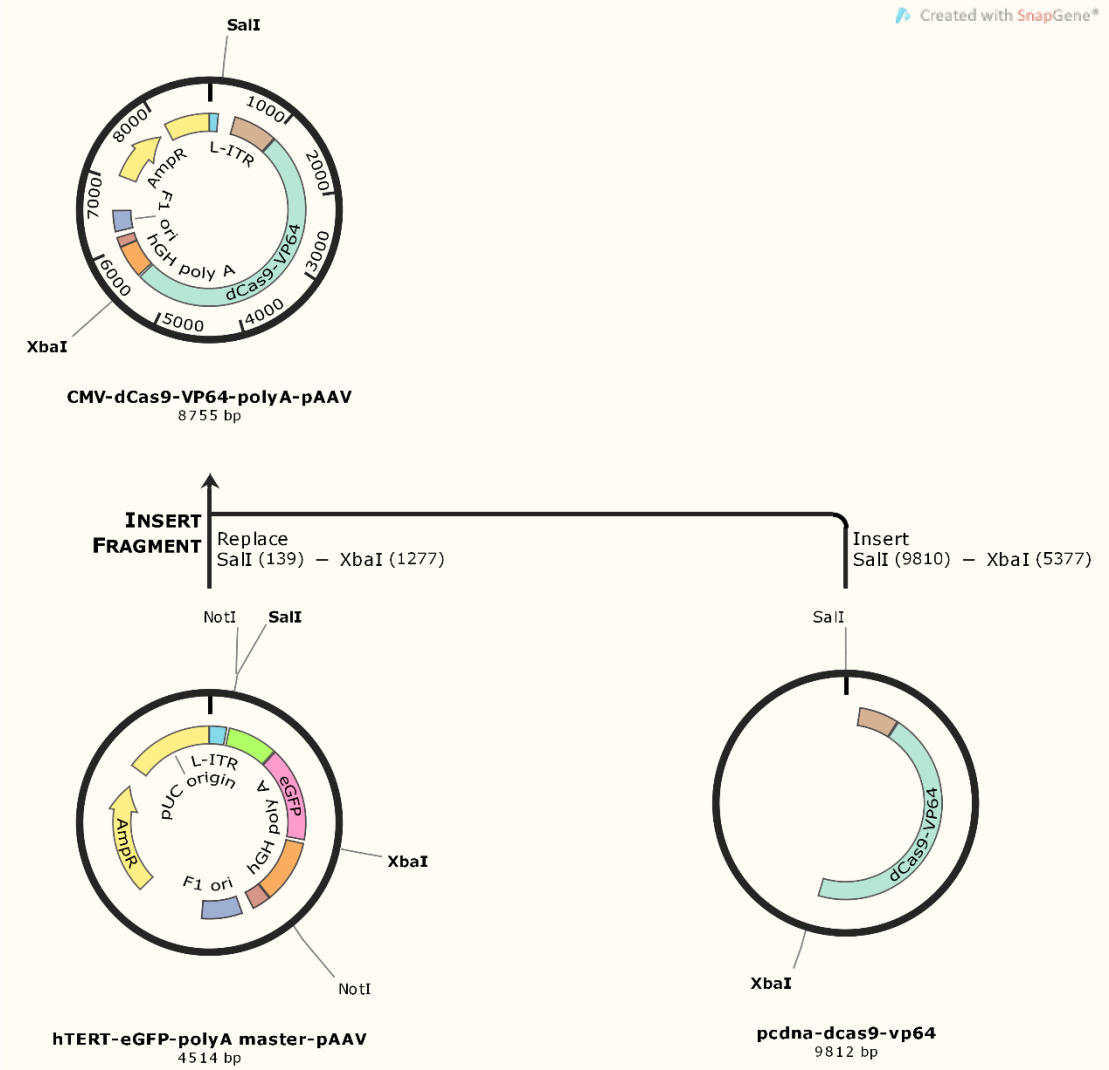


Figure 3. Cloning of CMV-dCas9-VP64 AAV vector.

After the cloning, the plasmid was restricted using a different set of restriction enzymes from the one used for the cloning process.

Results

The restriction pattern matched the expected outcome (Figure 4).

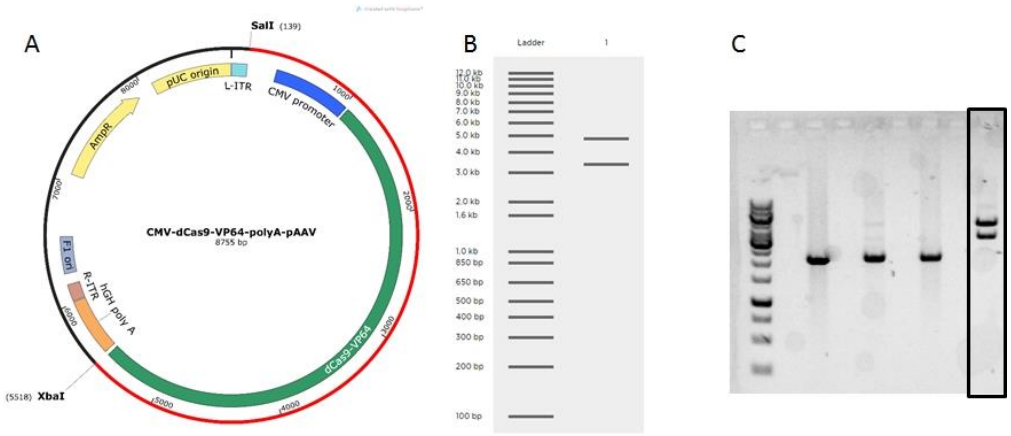
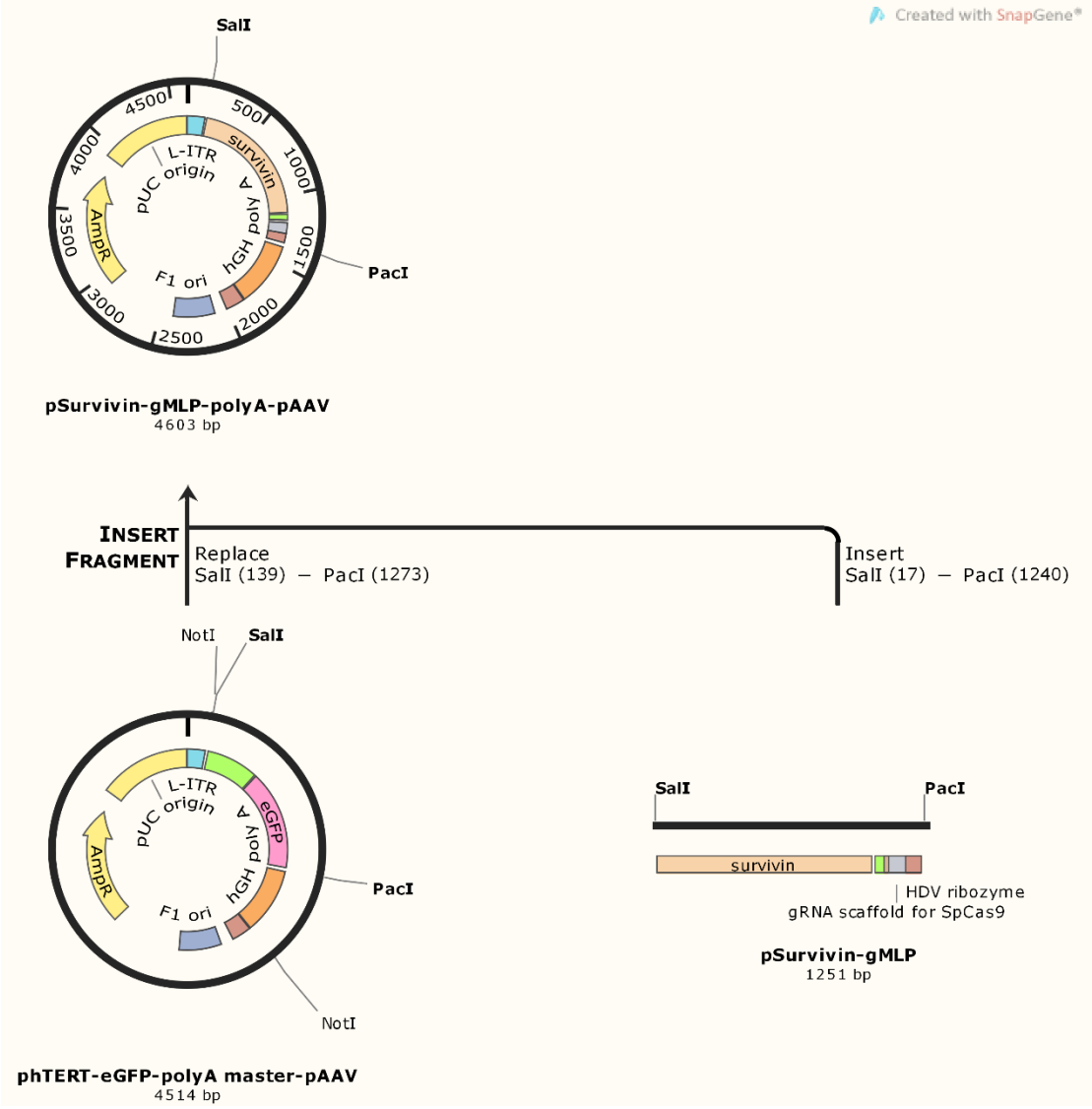


Figure 4. CMV-dCas9-VP64 AAV. **A**. Final plasmid map. **B**. A virtual representation of the restriction outcome with SalI and XbaI. **C**. The actual restriction results.

*-pSurvivin-gMLP**



-U6-gMLP

The construct was cloned as shown in Figure 5.

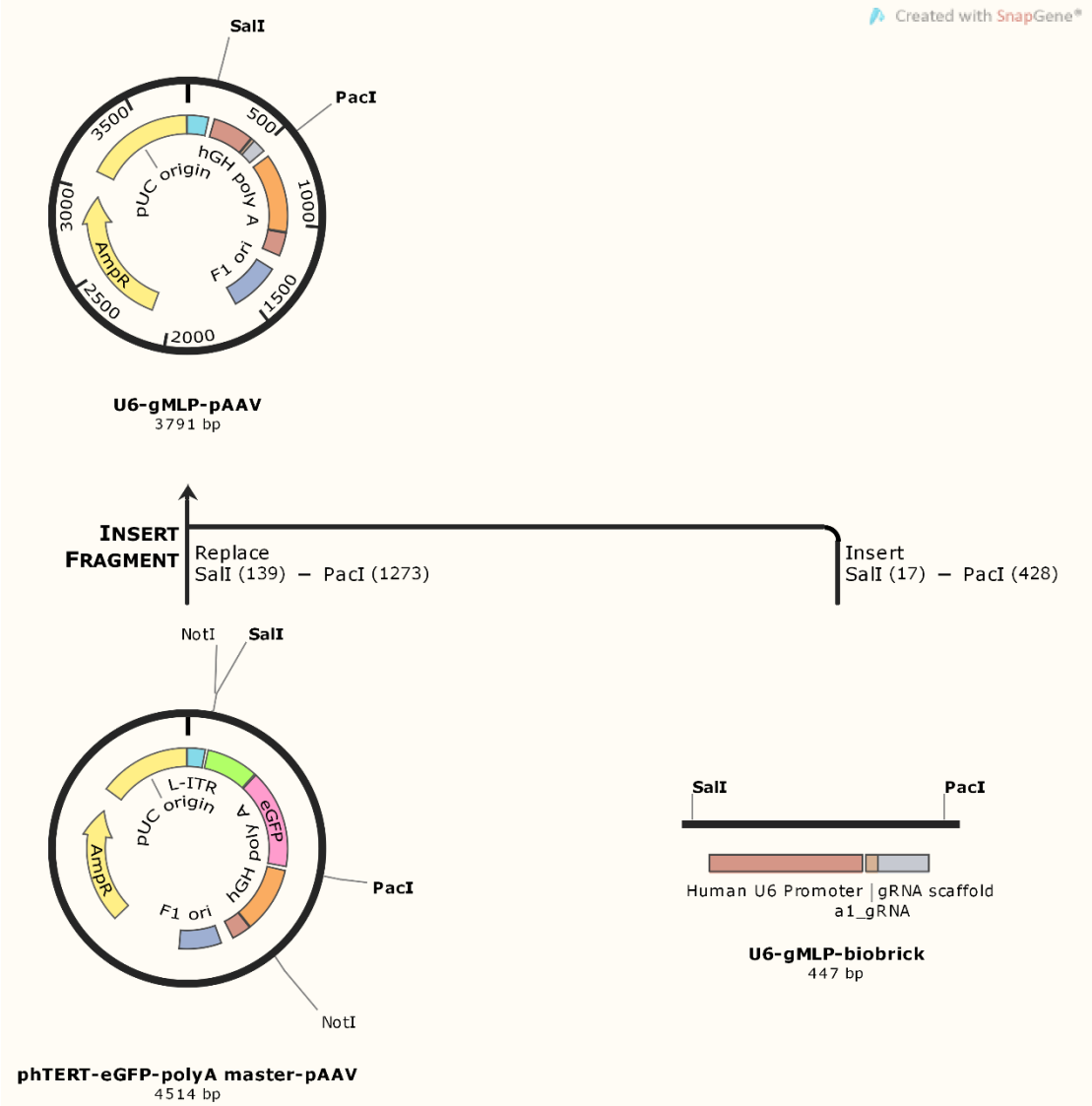


Figure 5. Cloning of U6-gMLP AAV vector.

After the cloning, the plasmid was restricted using a different set of restriction enzymes from the one used for the cloning process.

Results

The restriction pattern matched the expected outcome (Figure 6).

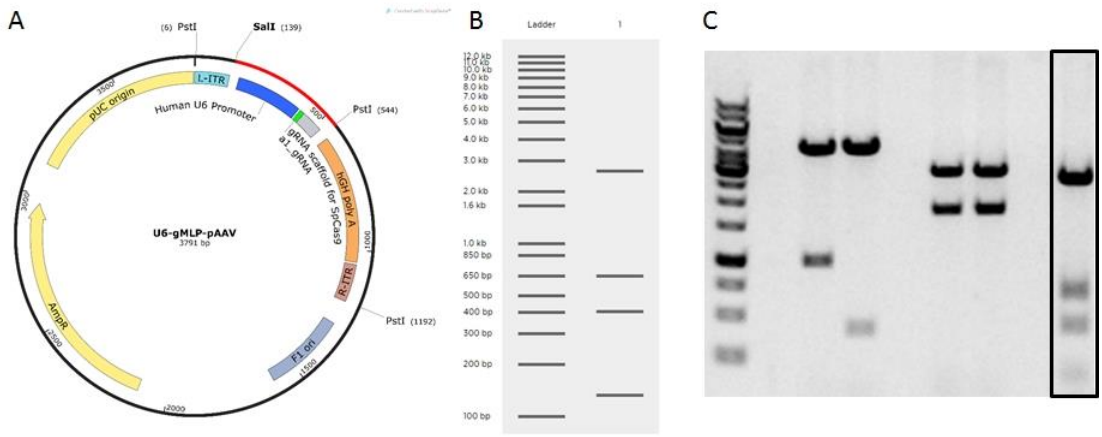
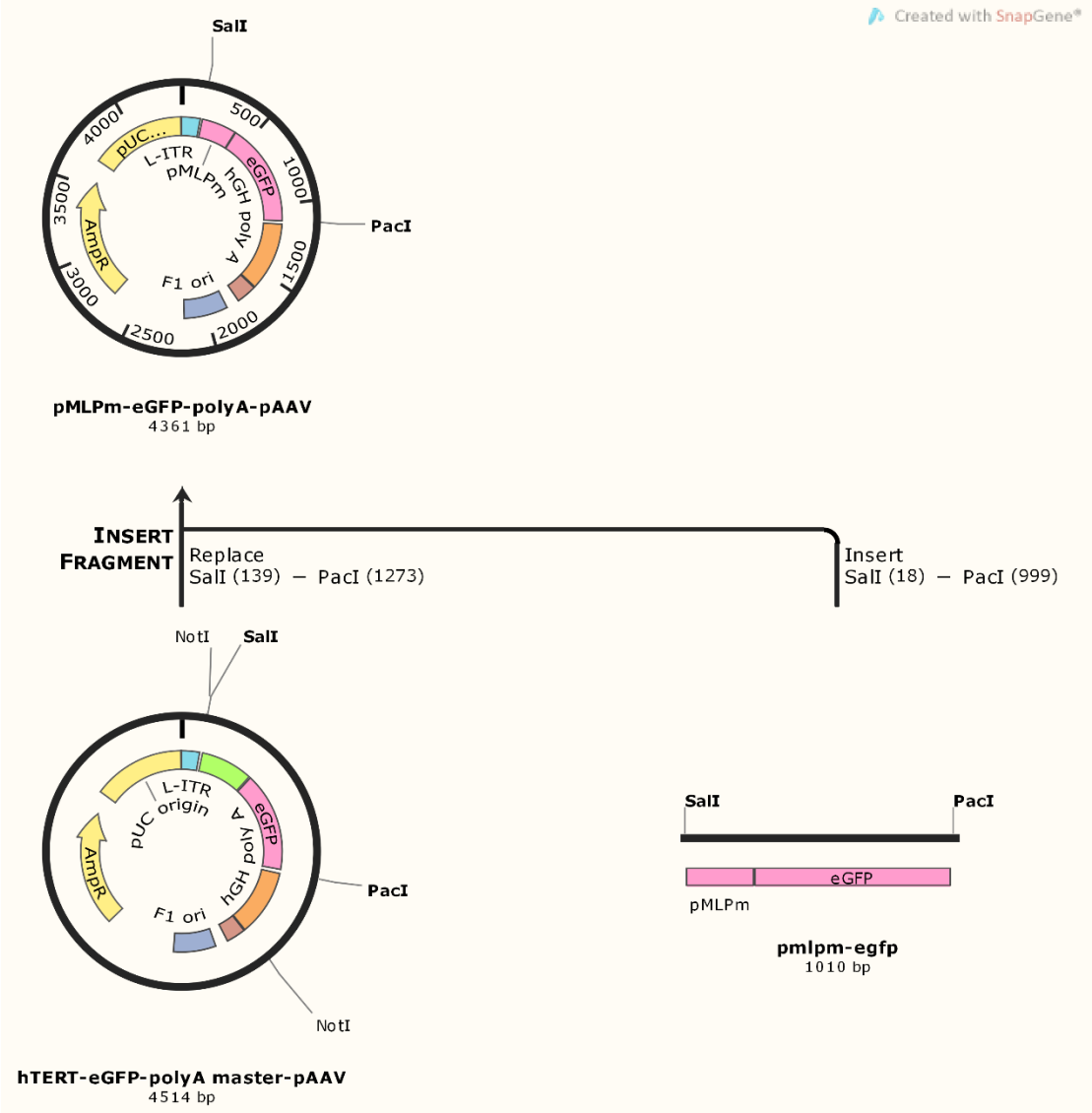


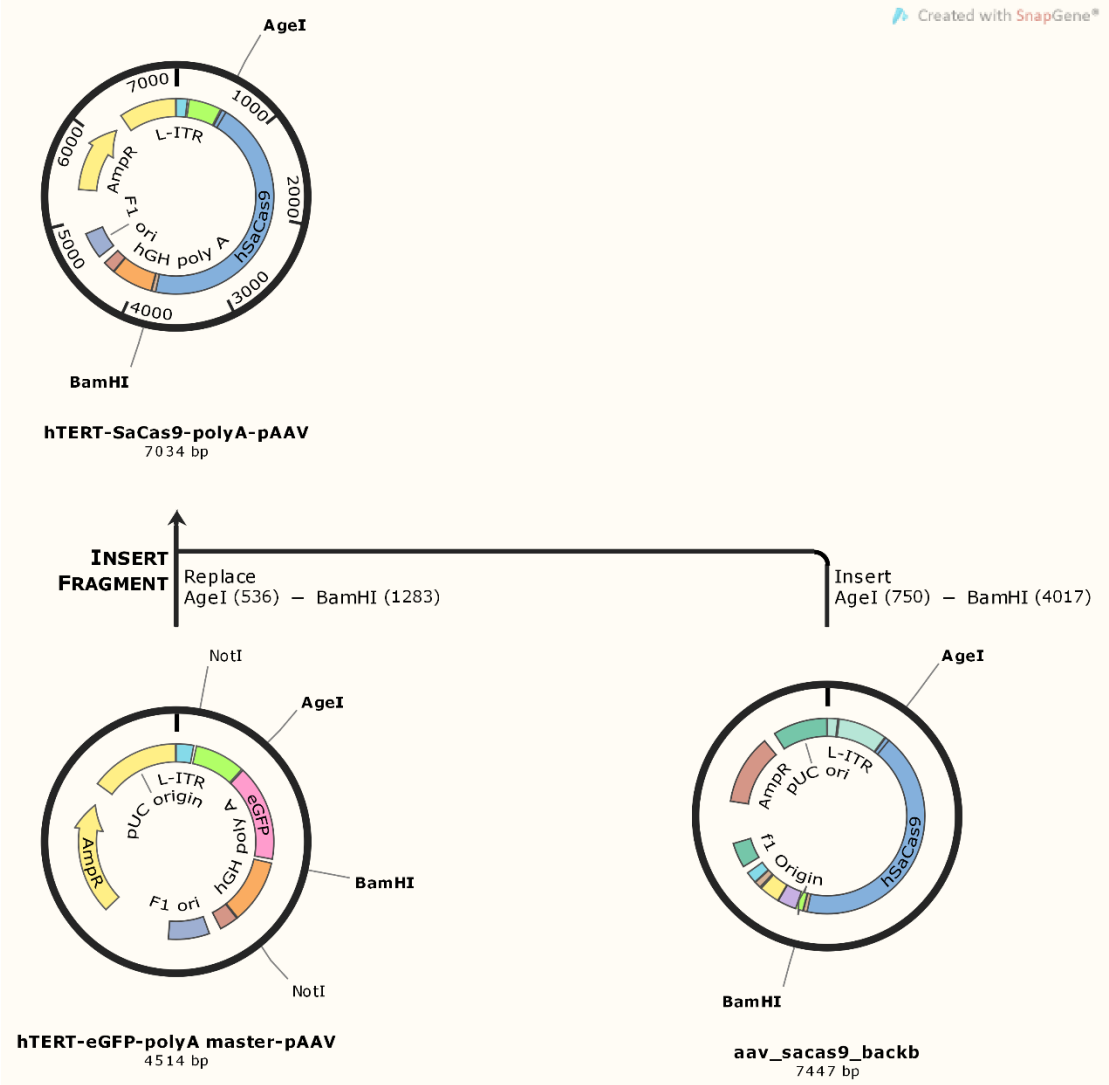
Figure 6. U6-gMLP AAV. **A**. Final plasmid map. **B**. A virtual representation of the restriction outcome with SalI and PstI. **C**. The actual restriction results.

*-pMLPm-eGFP**



Knock-out system

*-phTERT-SaCa9**



-CMV-SaCas9

The construct was cloned as shown in Figure 7.

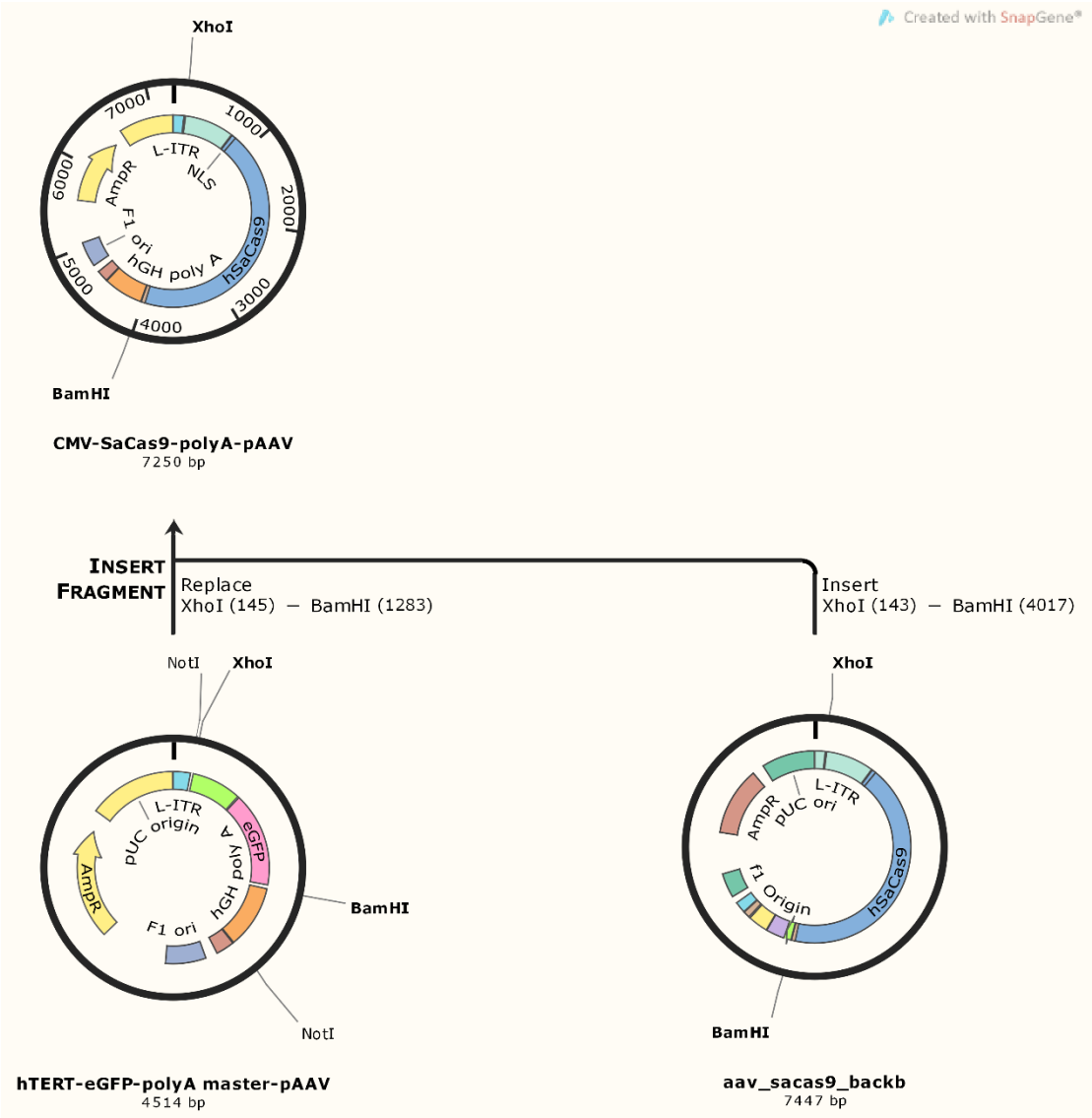


Figure 7. Cloning of CMV-SaCas9 AAV vector.

After the cloning, the plasmid was restricted using a different set of restriction enzymes from the one used for the cloning process.

Results

The restriction pattern matched the expected outcome (Figure 8).

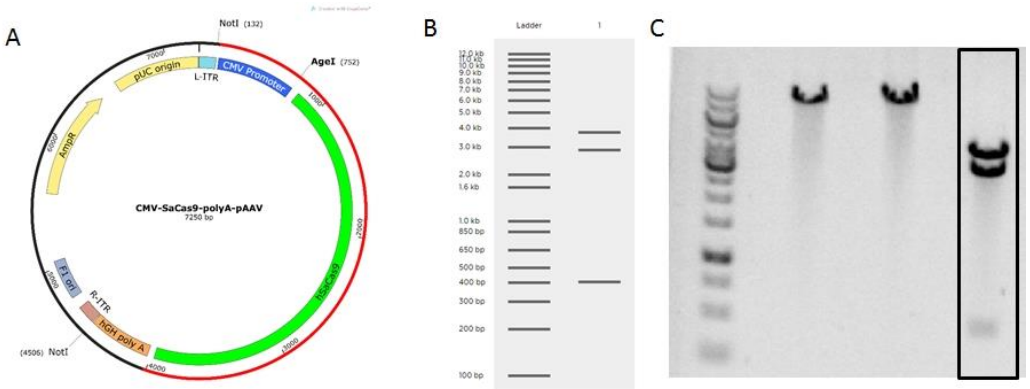
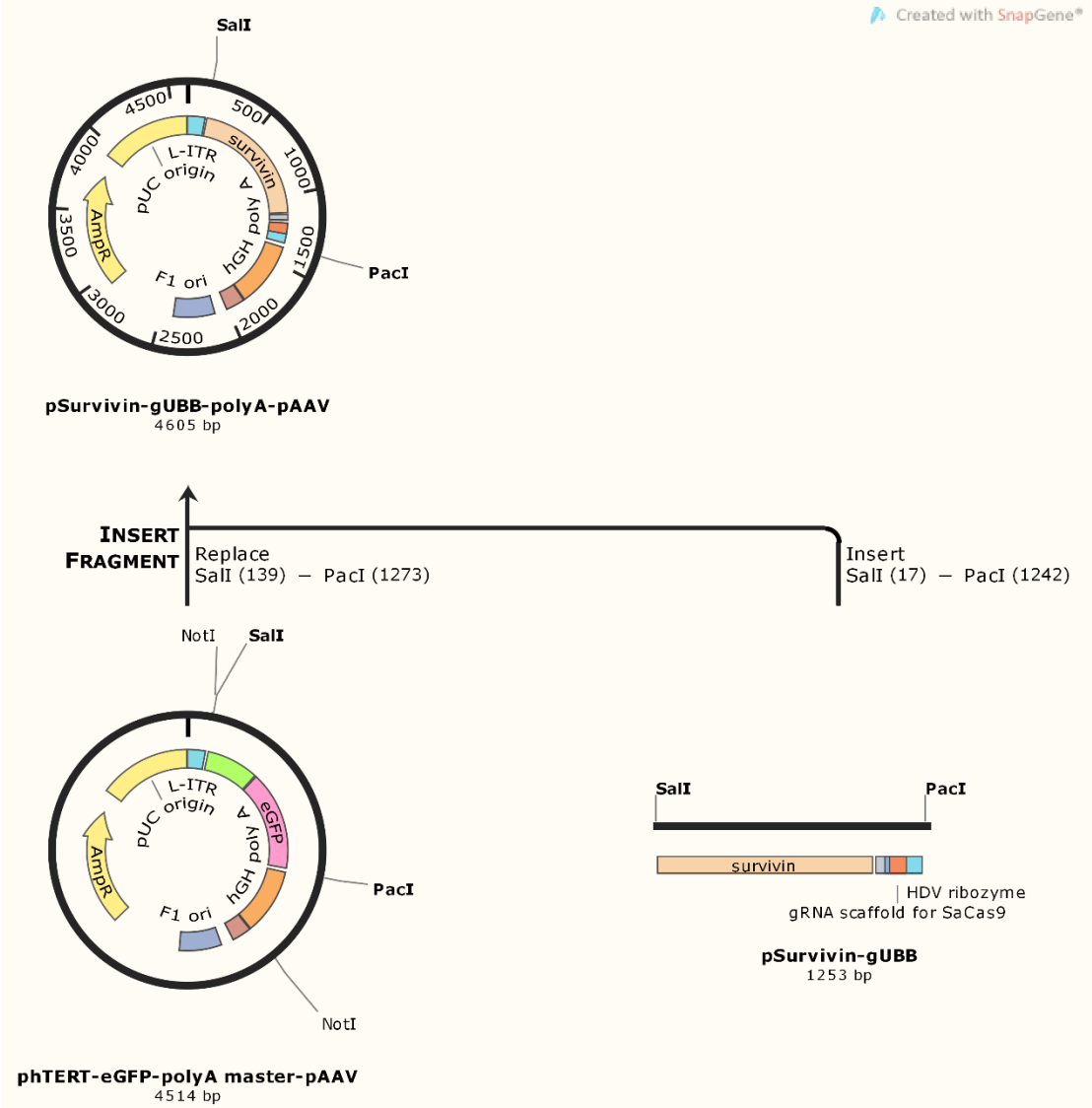


Figure 8. CMV-SaCas9 AAV. **A**. Final plasmid map. **B**. A virtual representation of the restriction outcome with AgeI and NotI. **C**. The actual restriction results.

-pSurvivin-gUBB



-U6-gUBB

The construct was cloned as shown in Figure 9.

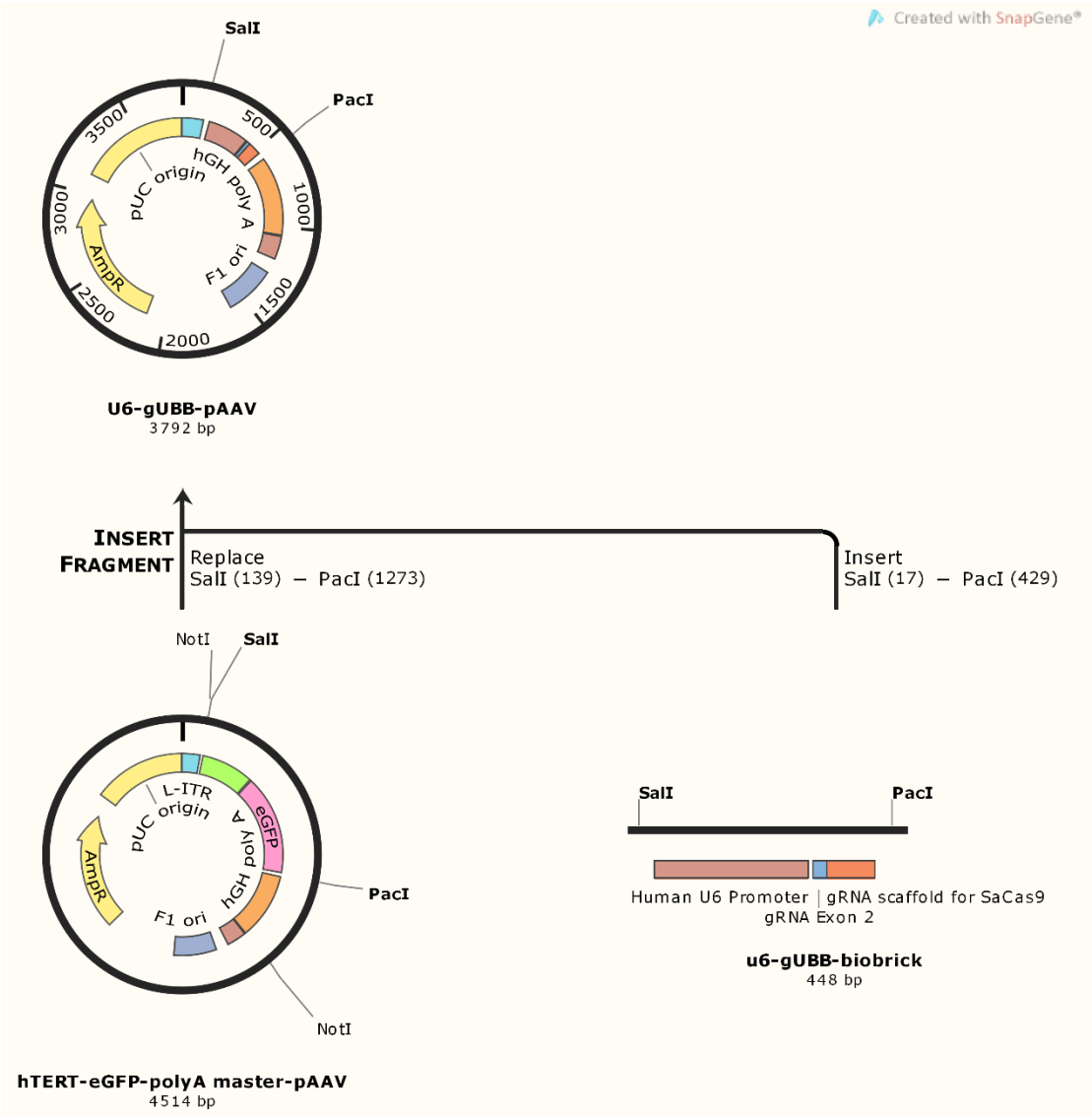


Figure 9. Cloning of U6-gMLP AAV vector.

After the cloning, the plasmid was restricted using a different set of restriction enzymes from the one used for the cloning process.

Results

The restriction pattern matched the expected outcome (Figure 10).

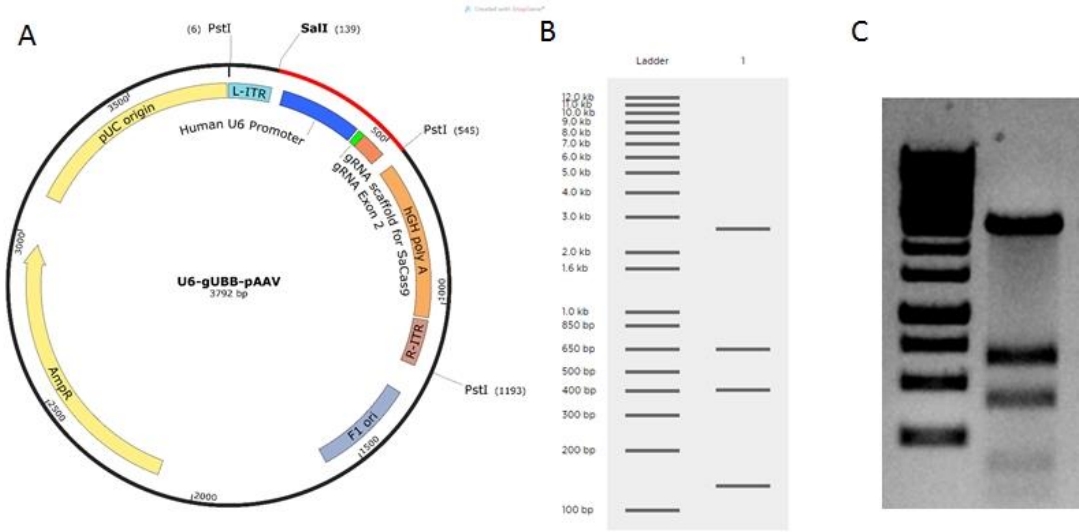


Figure 10. U6-gUBB AAV. **A**. Final plasmid map. **B**. A virtual representation of the restriction outcome with SalI and PstI. **C**. The actual restriction results.

General controls

*-phTERT-eGFP**

This is the "master" template mentioned above.

-pSurvivin-mCherry

The construct was cloned as shown in Figure 11.

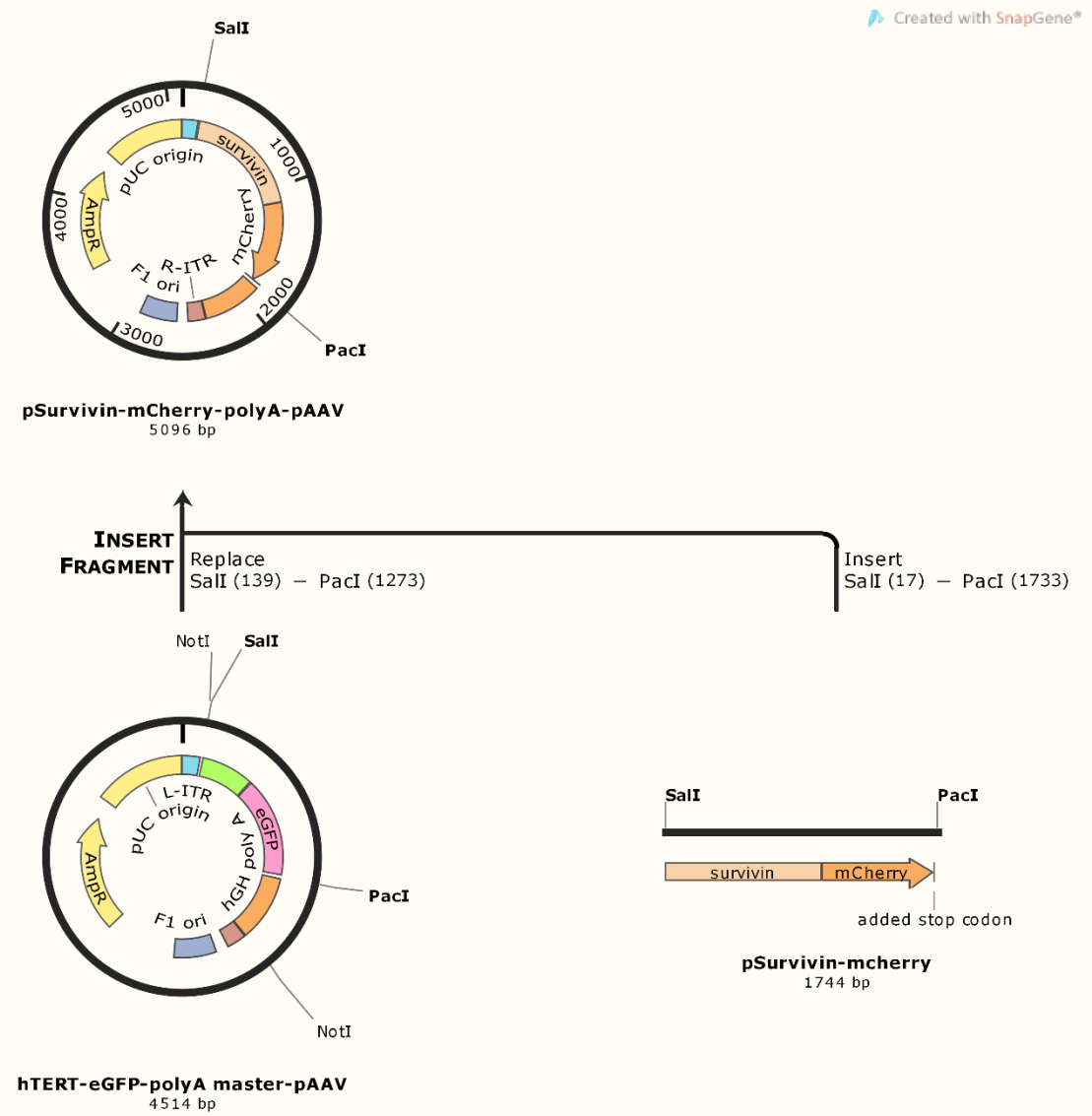


Figure 11. Cloning of pSurvivin-mCherry AAV vector.

After the cloning, the plasmid was restricted using a different set of restriction enzymes from the one used for the cloning process.

Results

The restriction pattern matched the expected outcome (Figure 12).

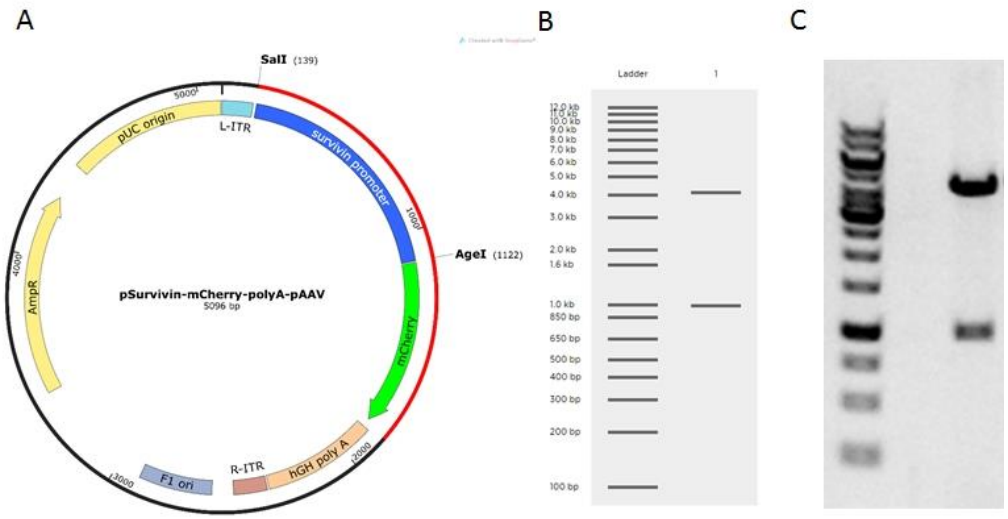


Figure 12. pSurvivin-mCherry AAV. **A**. Final plasmid map. **B**. A virtual representation of the restriction outcome with SalI and AgeI. **C**. The actual restriction results.

-AAV-GFP

The construct was cloned as shown in Figure 13.

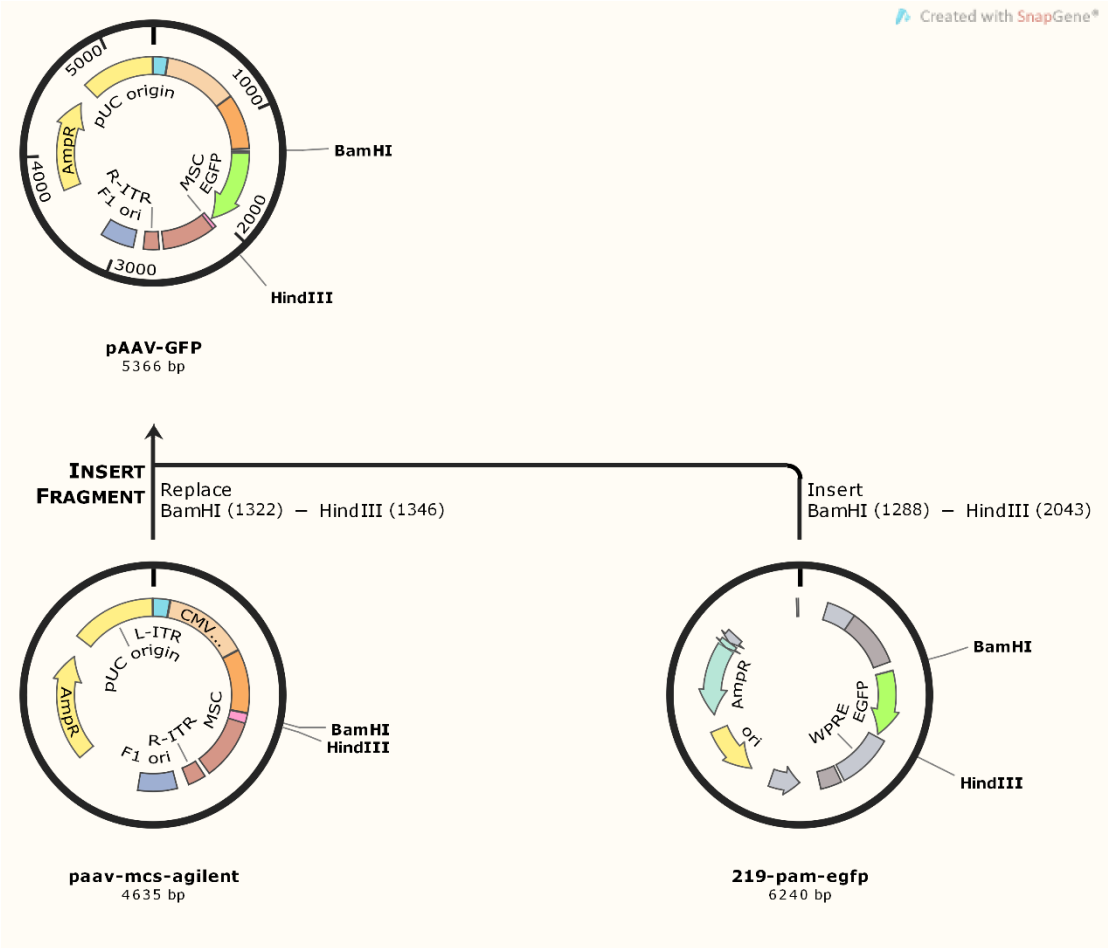


Figure 13. Cloning of GFP AAV vector.

After the cloning, the plasmid was restricted using a different set of restriction enzymes from the one used for the cloning process (BamHI & HindIII).

Results

The restriction pattern matched the expected outcome.

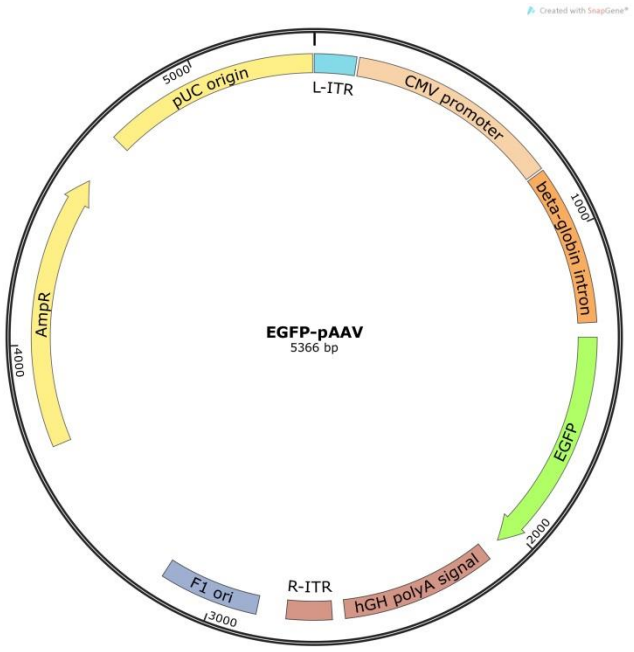


Figure 14. Final plasmid map of eGFP AAV.