Transposagen AAVS1 Safe Harbor Kit

- **AAVS1 XTN/CRISPR**
- **Donor Vector**

The Safe Harbor Kit is designed to facilitate targeting of your gene of choice into the AAVS1 site in the human genome. This locus is considered to be "safe harbor" for integration of transgenes and has been shown to allow expression of a variety of expression cassettes that have been inserted there (*Nature Reviews Cancer* **12**, 51-58 (January 2012).
Site specific nucleases are used to make a double stranded break in the locus, promoting homologous repair with the provided vector. Each vector contains an MCS with convenient cloning site for your gene of interest. Puromycin resistance and either GFP or thymidine kinase genes are also available in select vectors (maps shown below).
If desired, the cassette can be removed using piggyBac transposase (sold separately) with very minimal sequence left behind.

Contents:

- AAVS1 Nuclease: One of the following
  -2 vials Plasmid DNA: Forward and Reverse XTN TAL nuclease plasmids
  -1 vial AAV CRISPR/CAS single shot plasmid
  -2 vials Plasmid DNA: dCAS9-FOK1 and AAV gRNA vector
- One vial donor vector

AAVS1_XTN-1

TAL Nuclease recognition sequences

Blue= TAL Binding sites

Red= Cut region

5' TCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCCCA **3'**

AAVS1_Crispr

Nuclease target sequence

5' GGGGCCACTAGGGACAGGATTGG 3'

AAVS1_NextGen CRISPR

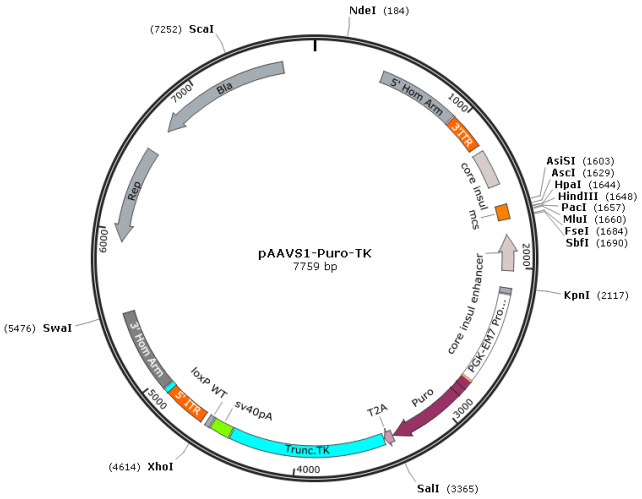
Nuclease target sequence

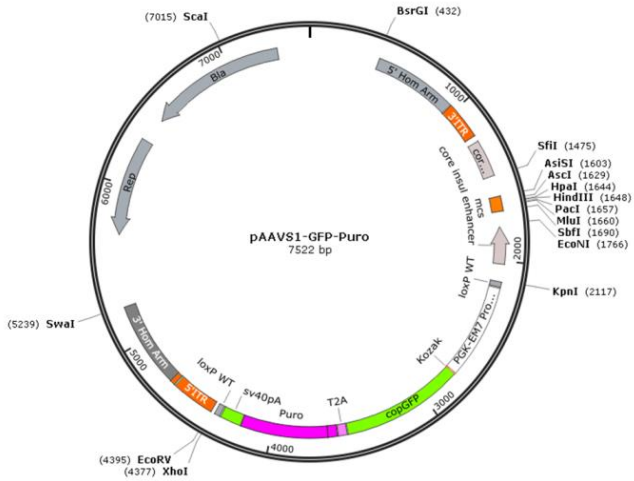
Blue= CRISPR Binding sites

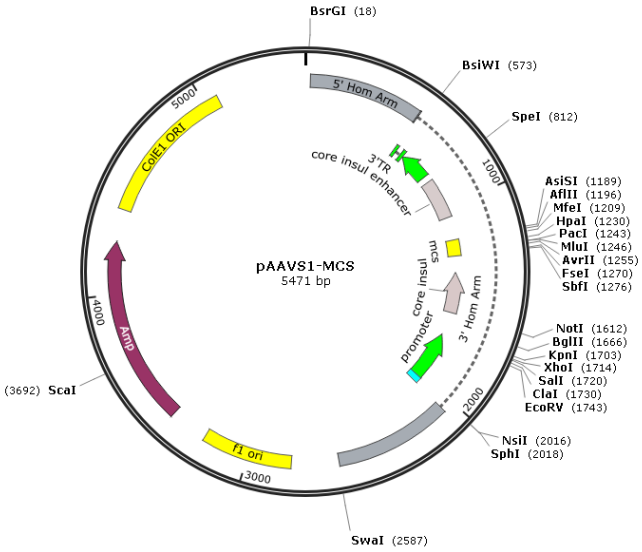
Red= Cut region

5' CCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTT 3'

Available Donor Vectors







Brief Protocols

Safe Harbor Targeting

Transfect your cells with appropriate DNA amounts based on established lab protocols or manufacturers recommendations (for example lipofection reagents or nucleofection reagents) with the following:

1. 1:1 ratio of AAV nuclease: Safe Harbor Vector (note for NextGen CRISPRs, use 5:1 ratio of dCAS9-Fok: gRNA plasmid)
2. Safe Harbor Vector Only
3. No reagents (or fluorescent control with no antibiotic resistance)

-Plate cells in regular media
-Wait 3 days, then apply puromycin selection (if appropriate) at concentrations you have previously determined to be appropriate for killing untransfected cells.
-Plate cells at varying densities in 10 cm plate or other appropriate flask to allow selection of puro resistant colonies. Alternatively, sort for single cells into 96 well plates. Wait for all untransfected cells to die. Resistant colonies should begin to appear around day 7-10 but may vary depending on cell type and doubling time. Cells with no reagents should all die.
-Check for proper targeting using primers that anneal inside the targeting cassette and outside the arms of homology (shown below).

Importantly- this vector does not use a splice acceptor to drive the expression of the puromycin cassette. Therefore, random integration will also result in puromycin resistant colonies. Comparison to vector only should be indicative of targeting efficiency.

Troubleshooting/Variations/Considerations

- Different cell types randomly integrate DNA at varying rates. Comparison of targeting to the vector only control will indicate the frequency of random integration.
- Varying the ratio of nuclease to vector may be necessary
- Targeted GFP intensity (if applicable) will be much lower than transient levels.

Targeting Primers

59 degree anneal/standard PCR protocols

5'AAVS1 targCheckF1	TTCCGGAGCACTTCCTTCT	707bp
5'AAVS1 targCheckR1	CCGATAAAACACATGCGTCA	
3'AAVS1 targCheckF1	CACGCGGTCGTTATAGTTCA	855bp
3'AAVS1 targCheckR1	CGGAGGAATATGTCCCAGAT	