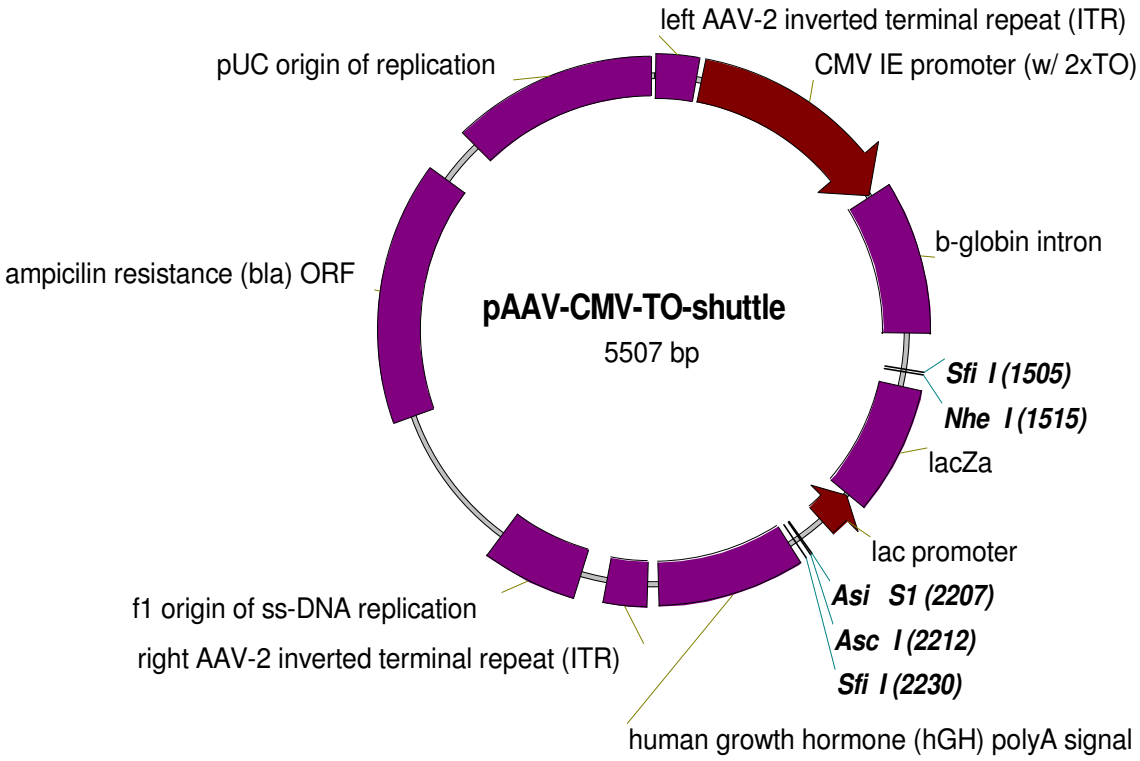
Plasmid Map

pAAV-CMV-TO-shuttle

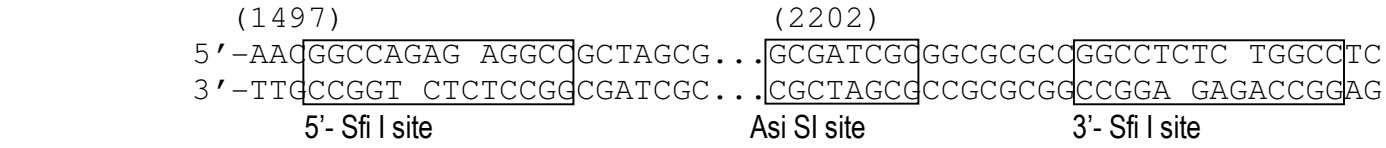
Catalog Number: 0918



Left ITR: 1-141
CMV IE Enhancer/Promoter (w/ 2xTO): 160-880
b-globin intron: 887-1379
LacZα cassette (complementary): 1565-2112 (including lac promoter and lacZα)
Human growth hormone poly-A: 2252-2730
Right ITR: : 2770-2910
f1 origin: 3002-3308
ampicillin resistance (bla) ORF: 3827-4681
pUC origin of replication: 4835-5502

Note:

1. Two copies of tet operator elements (TO) are inserted within the promoter region of the CMV promoter/IE enhancer. They allow suppression of gene expression by co-expressed tet-repressor (TetR). This feature is helpful when the transgene shows toxicity to the AAV packaging cells.
2. lacZα expression cassette provides convenient blue-white selection when subcloning transgene insert behind CMV promoter. Bacterial strains that complement lacZα function such as DH5α or DH10B should be used.
3. Transgene can be cloned between Nhe I site at 5' and either Asc I or Asi SI site at 3'.
4. Detail of Sfi I subcloning sites:



5. Above Sfi I sites can be used to subclone blunt-ended fragment. Such fragment can be first ligated with Sfi I adaptors, then ligate with Sfi I-digested 4782bp fragment of the shuttle plasmid. Please contact Applied Viromics for detail.
6. This shuttle plasmid allows transgene as large as 2800bp to be packed into AAV vector.