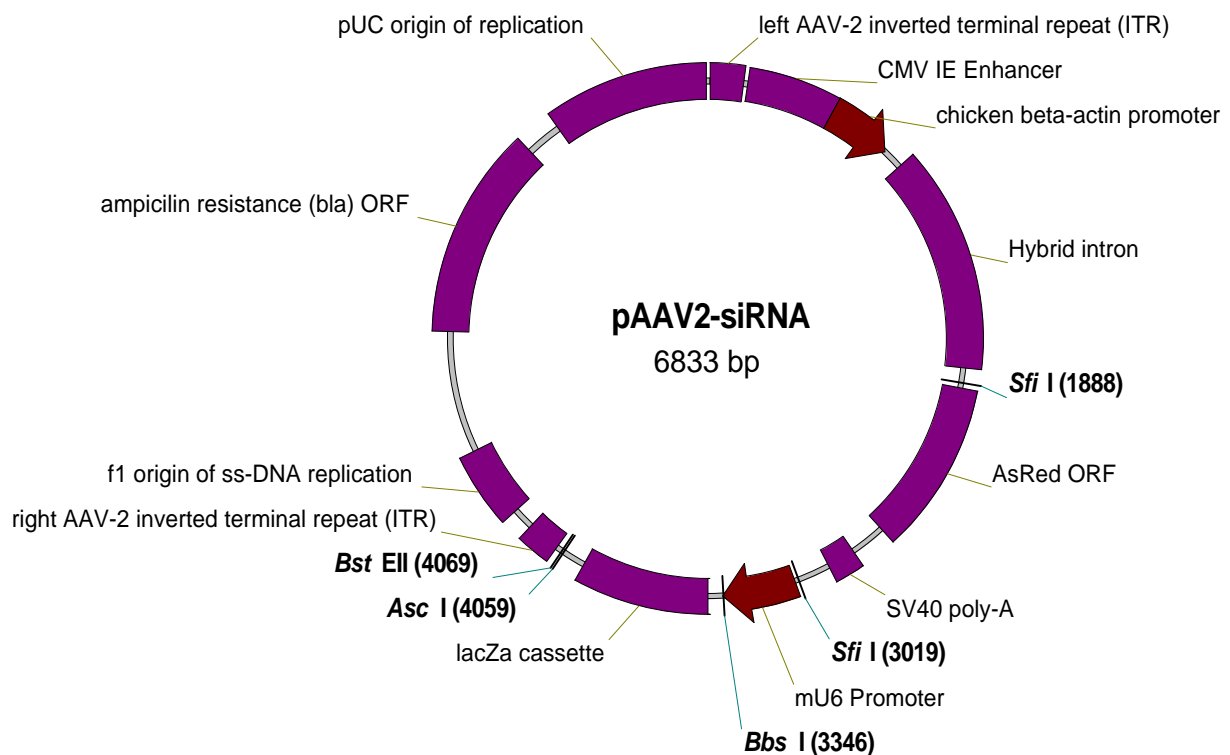


pAAV-siRNA

Catalog Number: 0914



Left ITR: 1-141

CMV IE enhancer/chicken β -actin promoter: 160-816

Hybrid intron: 905-1823

AsRed ORF: 1910-2605

SV40 poly-A: 2757-2878

mU6 promoter: 3035-3348

lacZ α cassette: 3412-3959

Right ITR: 4096-4236

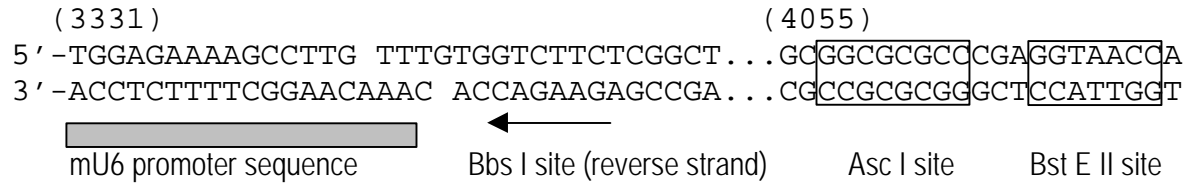
f1 origin: 4328-4634

ampicillin resistance (bla) ORF: 5153-6007

pUC origin of replication: 6161-6828

Note:

1. AsRed ORF and SV40 poly-A sequence can be removed by Sfi I digestion, end blunting, and re-ligation.
2. siRNA insert can be subcloned between Bbs I site and either one of the Bst E II and Asc I sites.
3. lacZ α expression cassette provides convenient blue-white selection when subcloning siRNA insert behind mU6 promoter. Bacterial strains that complement lacZ α function such as DH5 α or DH10B should be used.
4. Detail of pAAV-siRNA subcloning site:



5. The siRNA insert designed to clone into pAAV2-siRNA plasmid needs to have compatible ends with Bbs I site as well as either the Asc I or Bst E II site indicated above. The TTTT sequence is also required for appropriate transcription termination since it is not included in the plasmid.
6. Suggested sequencing primer for siRNA insert:

5' -gagactataaatatcccttgag-3' (3313-3335)