G0463 pscAAVmcsBgHpA

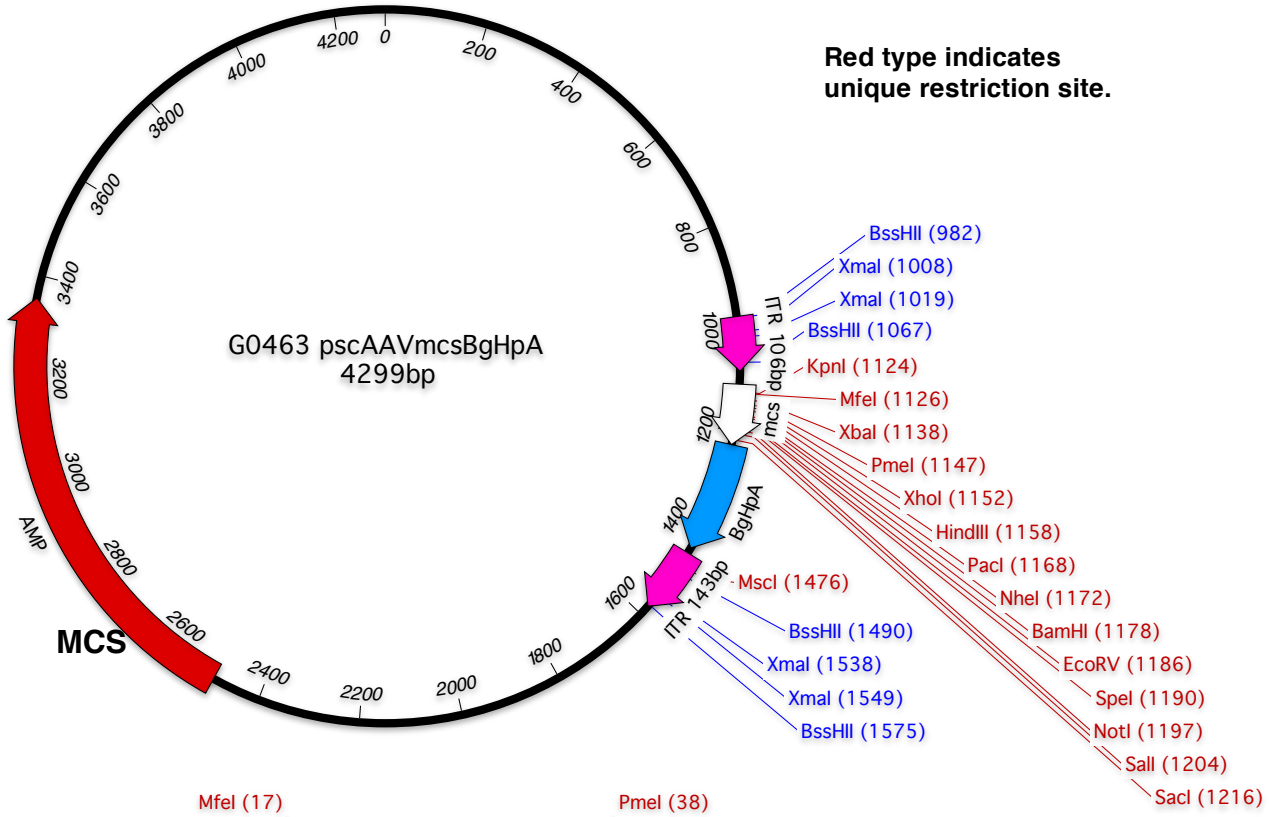
University of Iowa Carver College of Medicine
VIRAL VECTOR CORE

Plasmid Features:

Coordinates	Feature
980-1085	AAV2 ITR 106bp (mutated ITR)
1110-1226	MCS
1227-1440	BgHpA
1453-1595	AAV2 ITR (143bp)
2496-3356	B-lactamase (Ampicillin)

Antibiotic Resistance: Ampicillin. Bacterial Backbone: Brian Kasper.

Note: To check the integrity of the AAV ITR's perform single restriction enzyme digestions with XmaI, BssHII, and MscI



>G0463 pscAAVmcsBgHpA 4299bp

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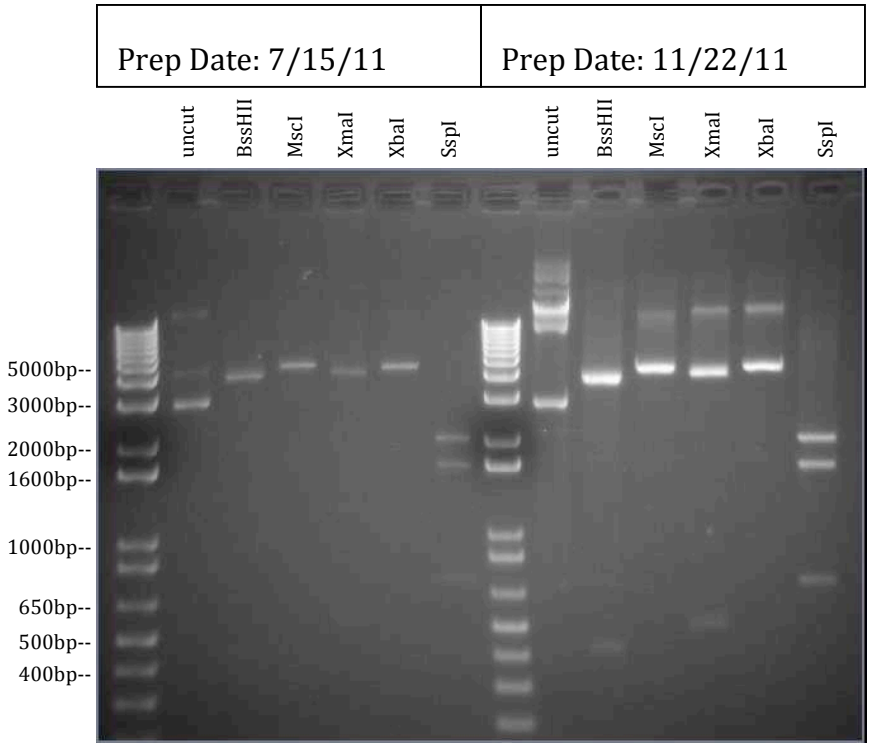
GAAGCGGAAGAGC

Plasmid and ITR Integrity:

pscAAVmcsBgHpA
VVC # G0463
Prep date: 7/15/11 Concentration: 0.8ug/ul
Prep date: 11/22/11 Concentration: 0.7ug/ul

Expected Fragments from digest of insertless plasmid
BssHI: 3706bp, 423bp, 85bp, 85bp
MscI: Linearized 4299bp
XmaI: 3758bp, 519bp, 11bp, 11bp
XbaI: Linearized 4299bp
Ssp1: 1971bp, 1596bp, 711bp, 21bp



Cloning Suggestions for working with Self-Complementary AAV Plasmids:

Advantage of the Self-Complementary AAV Plasmid:
The efficiency of AAV vectors is "hindered by the need to convert the single-stranded DNA (ssDNA) genome into double-stranded DNA (dsDNA) prior to expression. This step can be entirely circumvented through the use of self-complementary vectors, which package an inverted repeat genome that can fold into dsDNA without the requirement for DNA synthesis or base-pairing between multiple vector genomes. The increases in efficiency gained with self-complementary AAV (scAAV) vectors have ranged from modest to stunning, depending on the tissue, cell type, and route of administration." McCarty *Molecular Therapy* (2008) **16** 10, 1648–1656 doi:10.1038/mt.2008.171

Insert Restrictions:
The important trade-off for the efficiency of self-complementary vectors is the loss of half the coding capacity of the vector. The total package size from ITR to ITR should not exceed ~2.2kb in order to maintain the self-complementary package. Though published studies have indicated that there may be some latitude in packaging capacity for the self-complementary vectors, the University of Iowa Gene Transfer Vector Core will only prepare constructs that do not exceed ~2.2kb in the self-complementary backbone. Small protein-coding genes (up to 55 kd), and any currently available RNA-based therapy can be accommodated. Larger constructs likely form single-stranded DNA or a mixture of single-stranded and double-stranded DNA upon packaging thus there is little advantage.

Bacterial Backbone:
The backbone is based on self-complementary AAV vectors kindly provided by Brian Kaspar.

ITRs (Inverted Terminal Repeats):
The yield of dimeric genomes in a scAAV prep is increased dramatically by inhibiting resolution at one terminal repeat. This is accomplished by mutating or deleting the terminal resolution site sequence from one ITR, such that the Rep protein cannot generate the essential ssDNA nick. The self-complementary vector contains one wild-type ITR of 128bp and one mutated ITR of 105bp. For discussion and illustration of this process, **Self-complementary AAV Vectors; Advances and Applications** by Douglas M. McCarty is a good basic reference. *Molecular Therapy* (2008) **16** 10, 1648–1656 doi:10.1038/mt.2008.171.

Recombination and ITR Integrity:
Recombination is a possibility at the Inverted Terminal Repeats (ITRs). The ITRs in the pscAAV plasmids have several convenient restriction sites to determine whether the ITRs are intact without sequencing. Sequencing the ITRs can be very difficult in this plasmid due to their hairpin secondary structure. We recommend doing a single digest each of BssHII, MscI, and XmaI. Determine your fragment sizes for each digest and check carefully to see that you get what you expect and no extraneous bands. Be sure to check your final midi or maxi product (not just your miniprep) as recombination is possible during the amplification process.

We recommend using a stable E. coli strain such as SURE2, Stable2, or Stable3 for transformation of your final plasmid product. These competent cell strains have been engineered to stop unwanted rearrangement events and lack the components of the pathways that catalyze the rearrangement and deletion of nonstandard secondary and tertiary structures, including cruciforms (caused by inverted repeats) and Z-DNA, that occur frequently in eukaryotic cells. Cloning, however, can be difficult in these strains and you may choose to use DH5a competent cells for cloning.

Sequencing pscAAV plasmid ITRs

The backbone and ITRs of these plasmids were sequenced and confirmed by the University of Iowa DNA Facility in November 2011. We do not recommend sequencing the pscAAV plasmid ITRs on a routine basis. They are extremely difficult to read through. We recommend that you sequence your insert from ITR to ITR and analyze the ITRs by digest.

Please contact us with any questions:

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