BioBricks: GMK_TK30

Investigator: Bea

Cloning strategy for preparation of inserting sr39 and subclone it into pAAV_iGEM_MCS

1. Design Primers for inserting prefix and suffix to fusion enzyme mgmk_tk30 in RFC25
2. Perform PCR to insert mgmk_tk30 in RFC25 standard
   a. *Remember: do not use NgoMIV and PstI for cloning!!*
3. Ligation of PCR product into pAAV_iGEM_MCS with **XbaI** and **AgeI**
4. pAAV_iGEM_mgmk_tk30 ready for cell culture

Primers

➔ **New order because of wrong primers** (the old primer contained the start- and stop from the coding sequence of the enzyme. These are not needed in the iGEM standard).

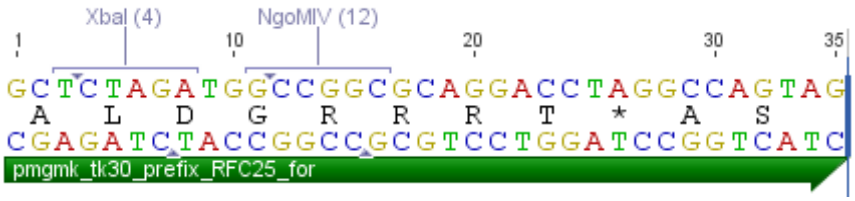
mGMK_TK30 - Prefix-Primer (in RFC25)

Forward: pMGMK_TK30_ prefix_RFC25_for

XbaI NgoMIV 53°C, 19bp, 63%GC

5'- GC TCTAGA TG GCCGGC GCAGGACCTAGGCCAGTAG-3` **Total: 73°C, 35bp, 66%GC**

Extra bases



mGMK_TK30 - Suffix-Primer (in RFC25)

Reverse: pMGMK_TK30 _suffix_RFC25_rev

AgeI 52°C, 19bp, 63%GC

5´- CTG ACCGGT GTTAGCCTCCCCCATCTCC -3´ **Total: 64°C, 28 bp, 64%GC**

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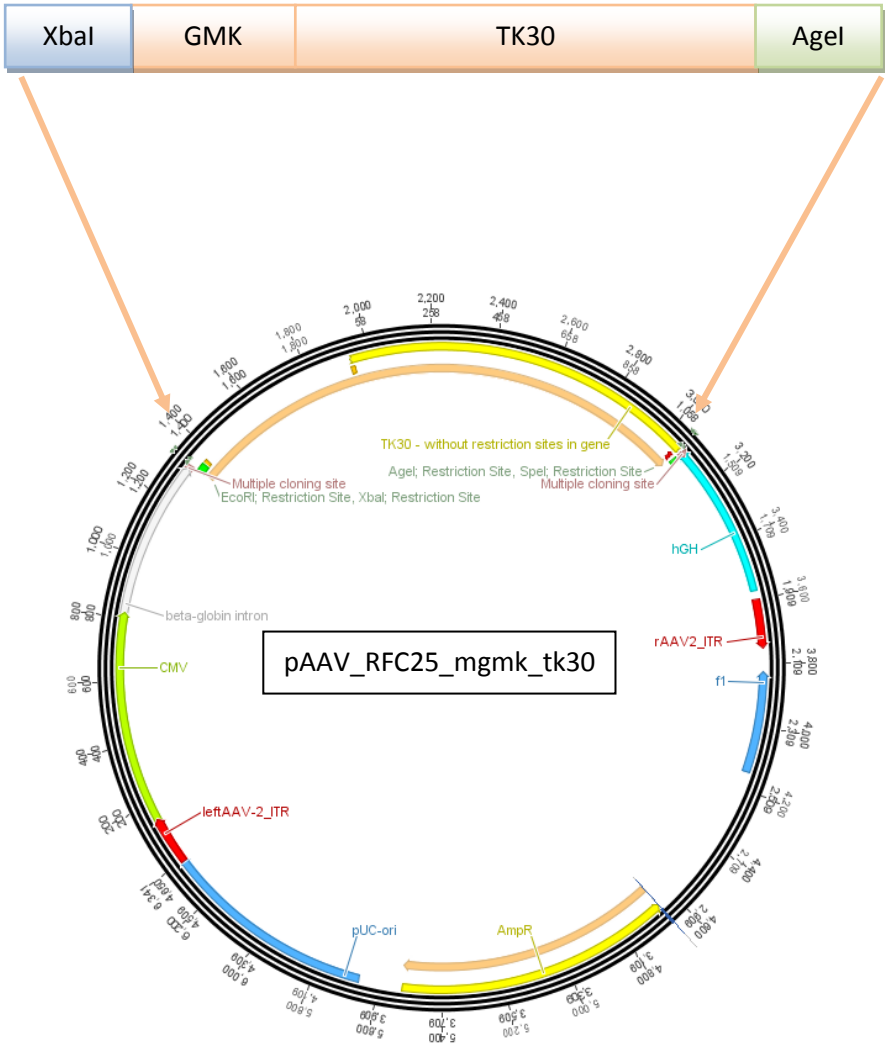
Cloning strategy

Digest vector pAAV_RFC25 with XbaI and AgeI

1. Digest PCR product with XbaI and AgeI
2. Ligate PCT product and vector



Remember:

Extra bases if restriction enzymes cut near end (5´and 3´)

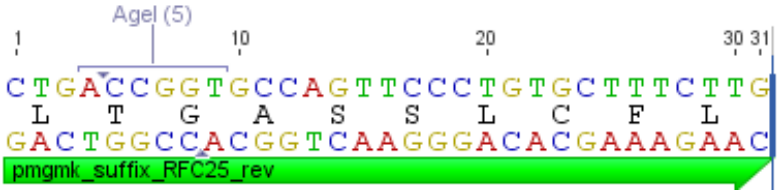
http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/cleavage_oligonucleotides.asp

mGMK – Suffix Primer (in RFC25)

Reverse: pMGMK_suffix_RFC25_rev

AgeI 22p, 57°C, 55%

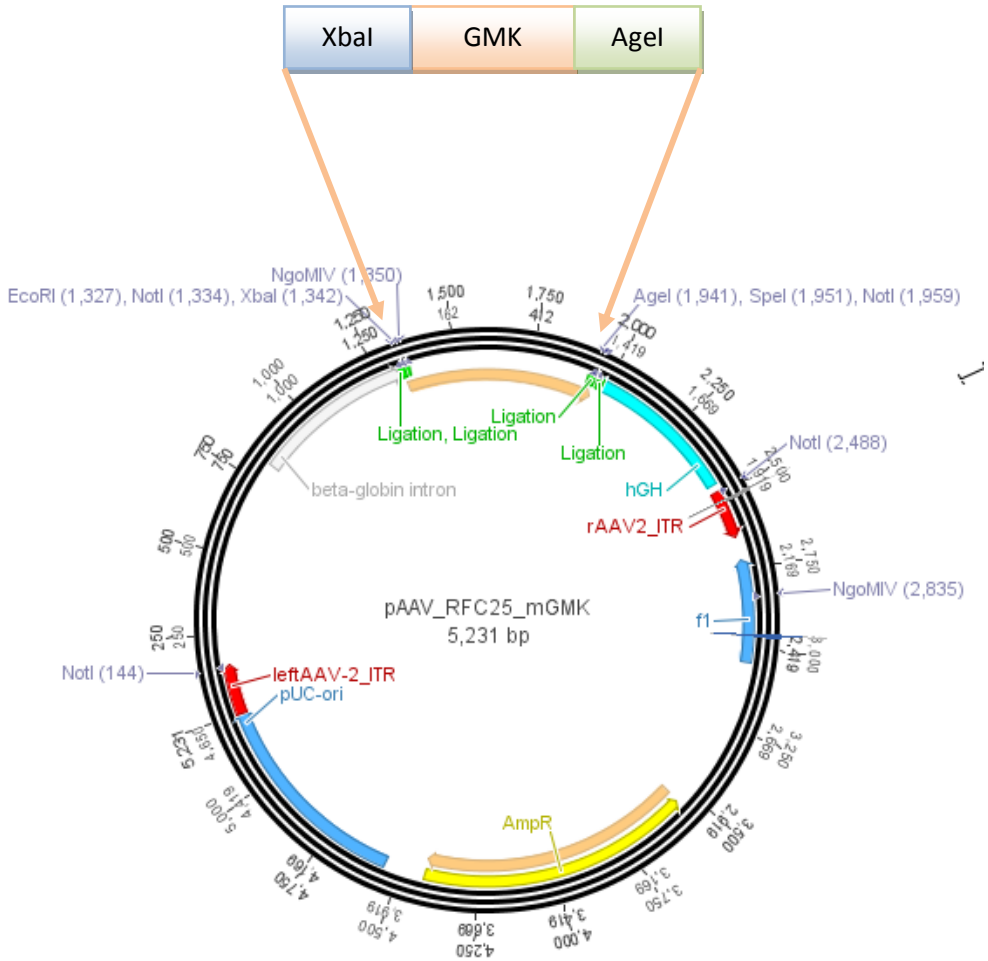
5´-CTG ACCGGT GCCAGTTCCCTGTGCTTTCTTG-3´ **Total: 67°C, 31bp, 58%GC**



Cloning strategy:

Ligation of PCR product mGMK with RFC25 add-ons into pAAV_RFC25

- Digest with XbaI and AgeI and ligate into vector which was digested with XbaI and AgeI as well



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Gene synthesis of TK30:

Amino acid composition in mutant and wt TK

	152	159	160	161	168	169
wtTK	A	L	I	F	A	L
Tk30	V	I	L	A	Y	F
Sr39	A	I	F	L	F	M

Tk30: containing no restriction sites in the coding sequence. Prefix (NgoMIV) and Suffix (AgeI and SpeI) in RFC25 standard.

Basepairs: 1129 bp

5´-

NgoMIV

GCCGGCGCGTCTGCGTTCGACCAGGCTGCGCGTTCTCGCGGCCATAGCAACCGACGTACGGCGTTGCGCCCT**C**
GGCGGCAGCAAGAAGCCACGGAAGTCCGCCCGGAGCAGAAAATGCCCACGCTACTGCGGGTTTATATAGAC
GGTCCCCACGGGATGGGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTCGCGCGACGATATCGTC
TACGTACCCGAGCCGATGACTTACTGGCGGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACAC
AACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACA
ATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGAGGCTGGGAGCTCACATG
TCCCGCCCCCGGCCCTCACCATTTTGGCTGACCGCCATCCCATCGCCTATTTCCTGTGCTACCCGGCCGCGCGGT
ACCTTATGGGCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTGGCCCTCATCCCGCCGACCTTGCCCGGCACC
AACATCGTGCTTGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAAACGCCAGCGCCCCGGCGAG
CGGCTGGACCTGGCTATGCTGGCTGCGATTCGCCGCGTTTACGGGCTACTTGCCAATACGGTGCGGTAT**CTGC**
AATGCGGCGGGTCGTGGCGGGAGGACTGGGGACAGCTTTCGGGGACGGCCGTGCCGCCCCAGGGTGCCGA
GCCCCAGAGCAACGCGGGCCCACGACCCCATATCGGGGACACGTTATTTACCCTGTTTCGGGCCCCCGAGTTG
CTGGCCCCCAACGGCGACCTGTATAACGTGTTTGCCTGGGCCTTGGACGTCTTGGCCAAACGCCTCCGTTCCAT
GCACGTCTTTATCCTGGATTACGACCAATCGCC**CGCCGG**GTGCCGGGACGCCCTGCTGCAACTTACCTCCGG
GATGGTCCAGACCCACGTCACCACCCCCGGCTCCATACCGACGATATGCGACCTGGCGCGCACGTTTGCCCGG
GAGATGGGGGAGGCTAACACCGGTTAATACTAGT-3´

AgeI SpeI

Left ITR:

5'-
GAATTCGCGGCCGCTTCTAGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCA
AAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGC
CAACTCCATCACTAGGGGTTCCTTACTAGTAGCGGCCGCTGCAG -3`

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Cloning strategy:

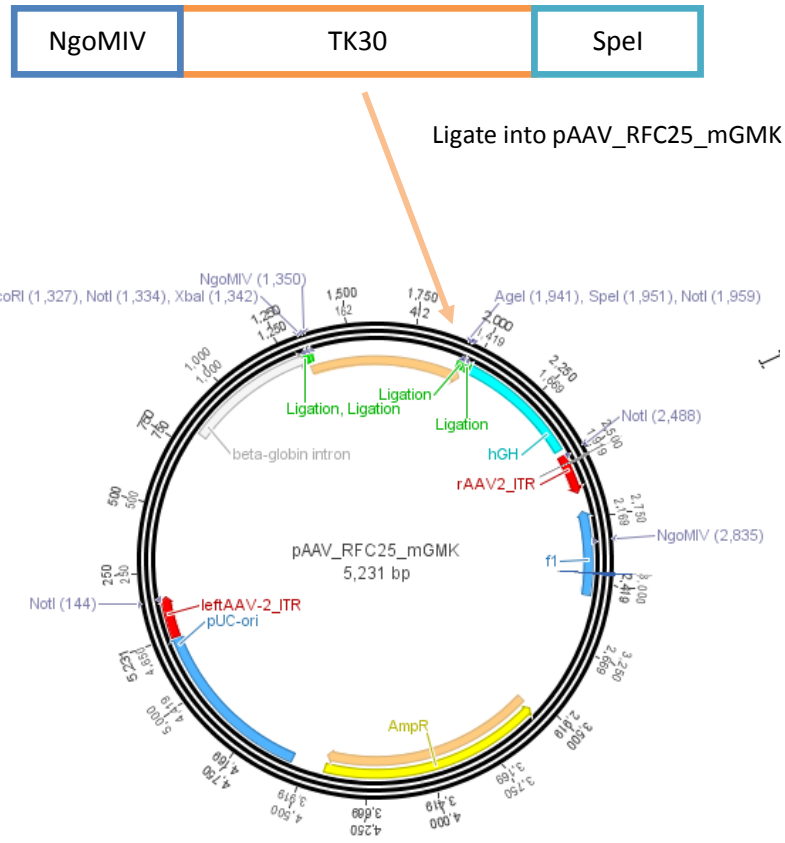
Obtained construct of gene synthesis

NgoMIV - TK30 - AgeI-SpeI----EcoRI- NotI-XbaI- ITR - SpeI-NotI-PstI

3. Digest with NgoMIV and SpeI to obtain TK30 which can be separated on an agarose gel
4. Digest with EcoRI and PstI to obtain ITR which can be separated on an agarose gel

Ligation

1. Cut pAAV_RFC25_mGMK with AgeI and SpeI
2. Ligation leads to a scar between mGMK and tk 30 because of fusing tk30 to mGMK.



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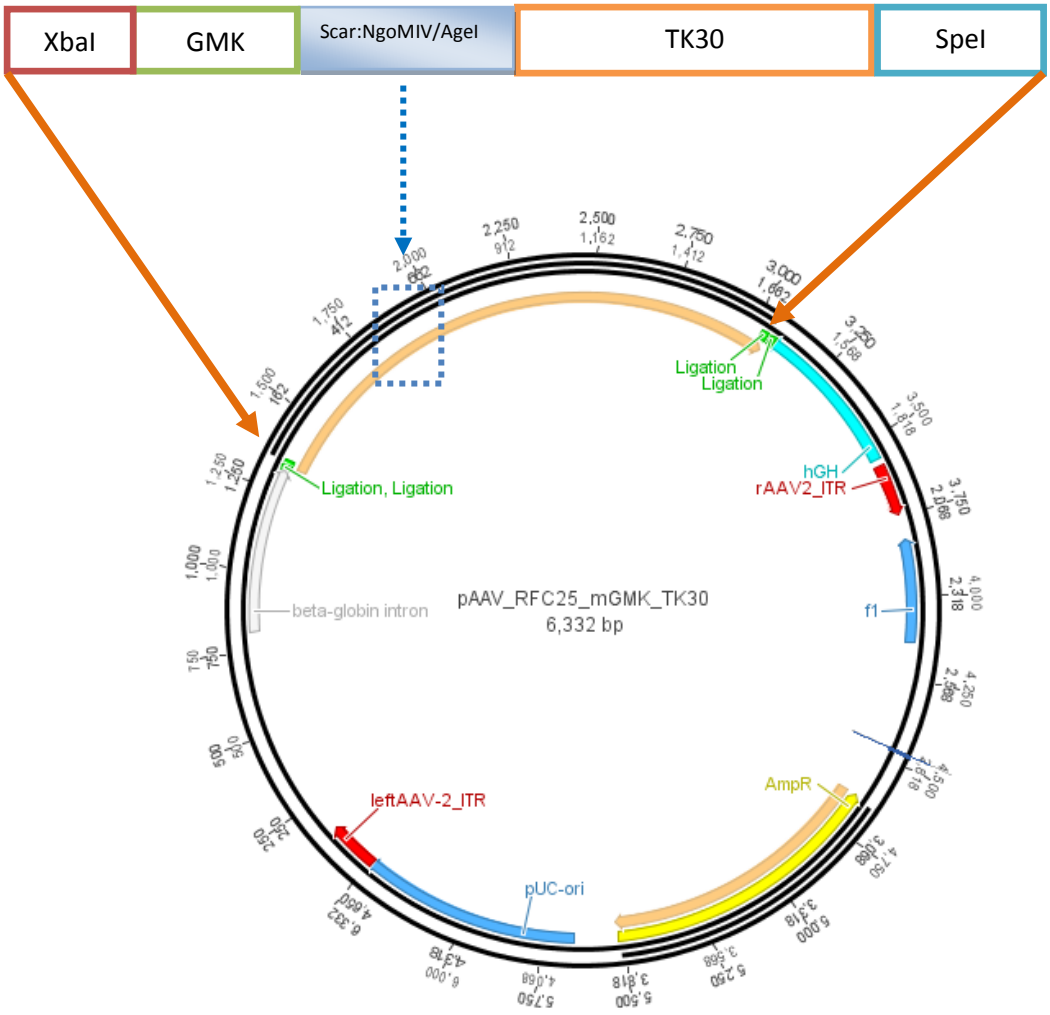
Final product: pAAV_RFC25_mGMK_tk30 (without restriction sites in gmk_tk construct)



For sending it to parts registry:

1. Use *pSB1C3_CFP* for submitting BioBricks to partsregistry
2. Digest pSB1C3 with XbaI and AgeI or EcoRI and SpeI
3. Digest pAAV_RFC25_mGMK_TK30 with XbaI and AgeI or EcoRI and SpeI
4. Ligation of insert (mGMK_tk30 into pSB1C3) into pSB1C3

Gene synthesis of sr39:

Idea:

Amino acid composition in mutant TKs and wt TK differ in only five or six amino acids which are located nearby. Fore gene synthesis of sr39 there is no need to order the whole gene. Therefore only a part of the sr39 gene which contains desired mutations is synthesized and has been ordered.

	152	159	160	161	168	169
wtTK	A	L	I	F	A	L
Tk30	V	I	L	A	Y	F
Sr39	A	I	F	L	F	M

Part of sr39

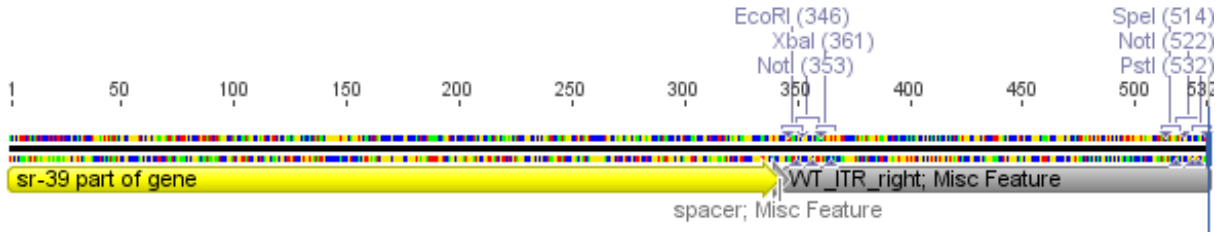
Bases: 340 bp

NruI

5´TCGCGAACATCTACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTA
ATGACAAGCGCCCAGATAACAATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGG
GGGAGGCTGGGAGCTCACATGCCCCGCCCCCGGCCCTCACCATTTTCCTGGACCGCCATCCCATCGCCTTCATG
CTGTGCTACCCGGCCGCGCGGTACCTTATGGGCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTGGCCCTCA
TCCCGCCGACCTTGCCCGGCACCAACATCGTGCTTGGGGCCCT**TCCGGA** -3´

BspEI

Ordered gene:



Cloning strategy:

5. Cut pAAV_iGEM_GMK_TK30 with *NruI* and *BspEI* which cut in TK30 sequence in pAAV_RFC25_mGMK_TK30 once
6. Digest sr39 part of gene with NruI and *BspEI* and separate it from the right ITR
7. Insert **part of sr39** gene into digested pAAV_iGEM_GMK_TK30

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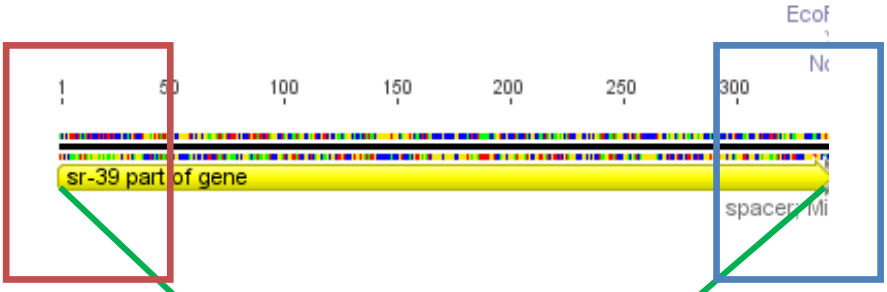
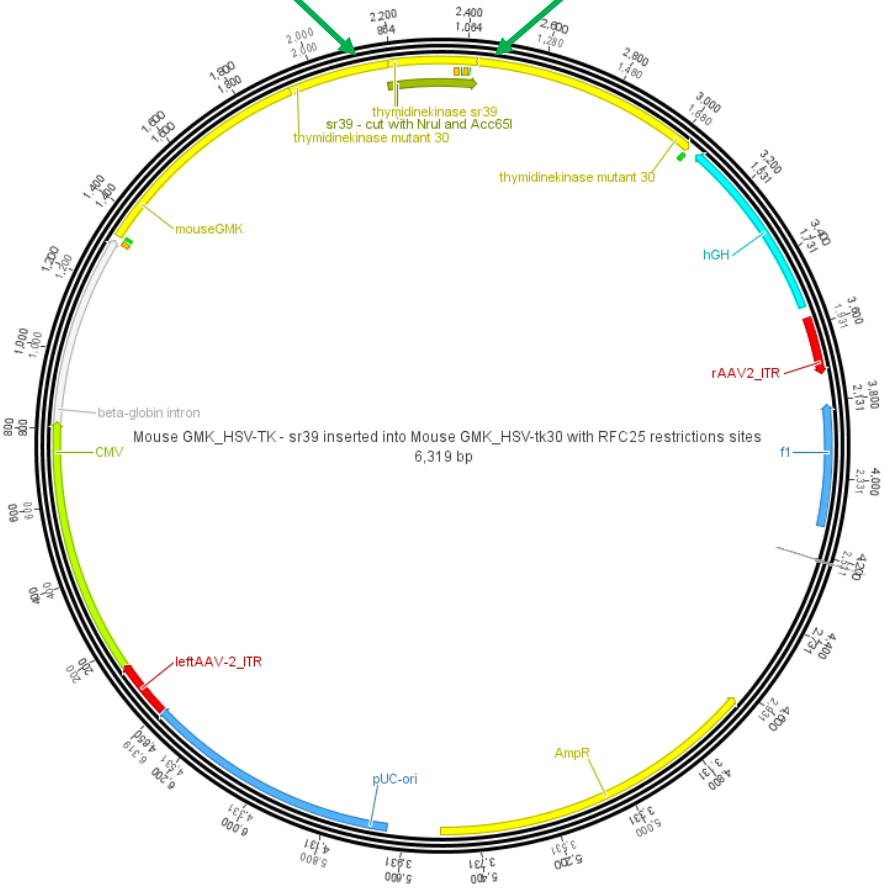
NruI (blunt cutter) BspEI (cutter with overhang)





→ Ligation of sr39 with overhangs for restriction enzymes NruI and BspEI into mGMK_TK30 which has been cut with *Nru**I*** and *BspEI*