



Vector Report

Quote: LFKI-170817-ACD-01
Project: GRM7 conditional knockin

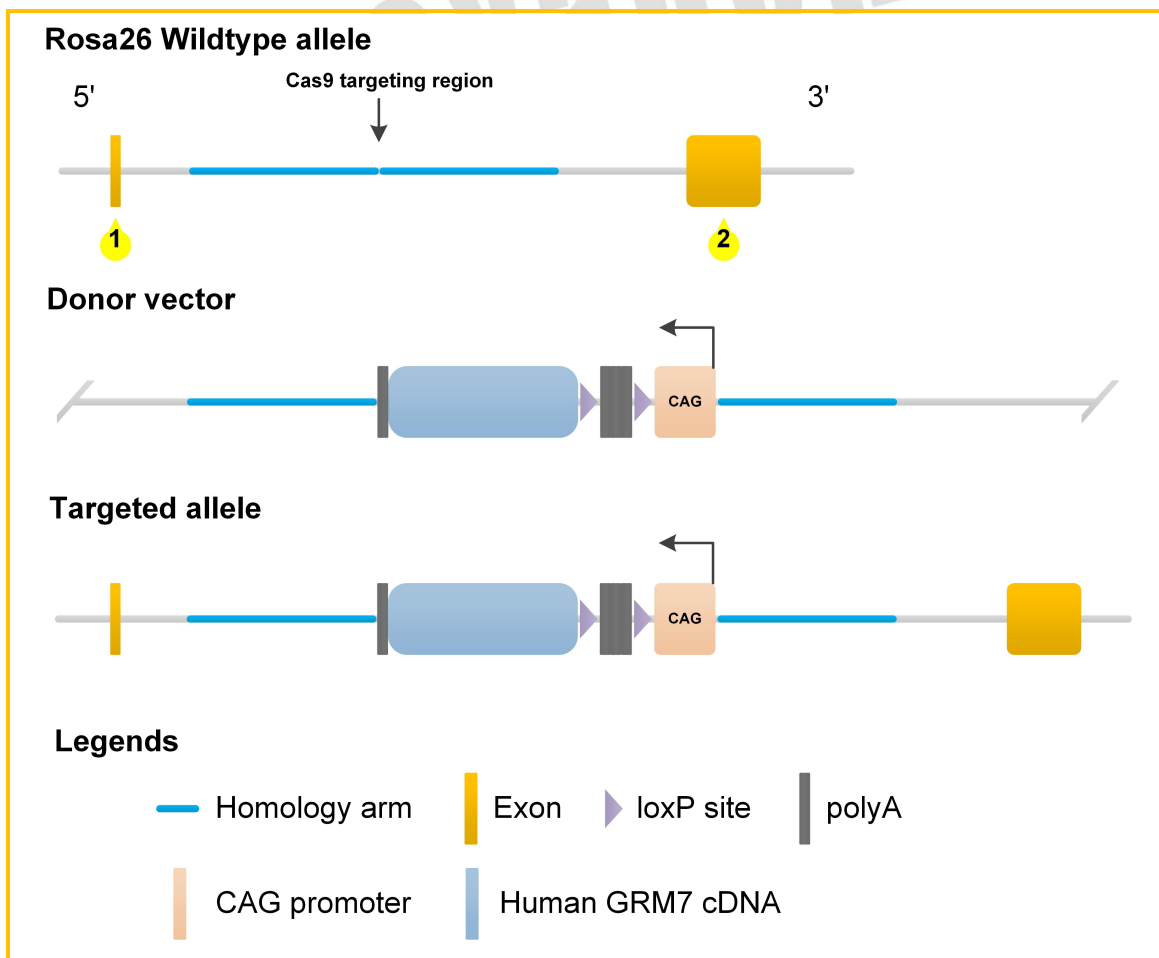
- Confidential -

1. Objective

To create a human GRM7 conditional knockin at the locus of ROSA26 in C57BL/6 mice by CRISPR/Cas-mediated genome engineering.

2. Summary

- The mouse ROSA26 gene (GenBank accession number: NR_027008.1) is located on mouse chromosome 6. The human GRM7 gene (GenBank accession number: NM_181874.2) is located on human chromosome 3.
- For the KI model, the “CAG-loxP-Stop-loxP-human GRM7 cDNA-polyA” cassette was cloned into intron 1 of ROSA26 in reverse direction. The expression of human GRM7 cDNA cassette will be dependent on the expression of Cre recombination.
- Cas9 and gRNA will be co-injected into fertilized eggs with donor vector for KI mice production.
- The pups will be genotyped by PCR followed by sequencing of PCR product.

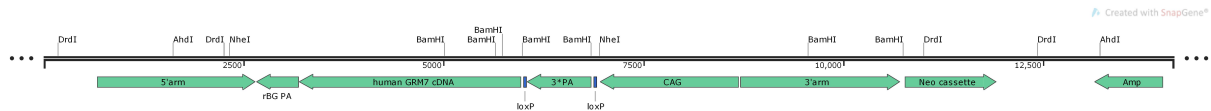


3. Method

Mouse genomic fragments containing homology arms (HAs) were amplified from BAC clone by using high fidelity Taq DNA polymerase, and were sequentially assembled into a targeting vector together with recombination sites and selection markers shown below.

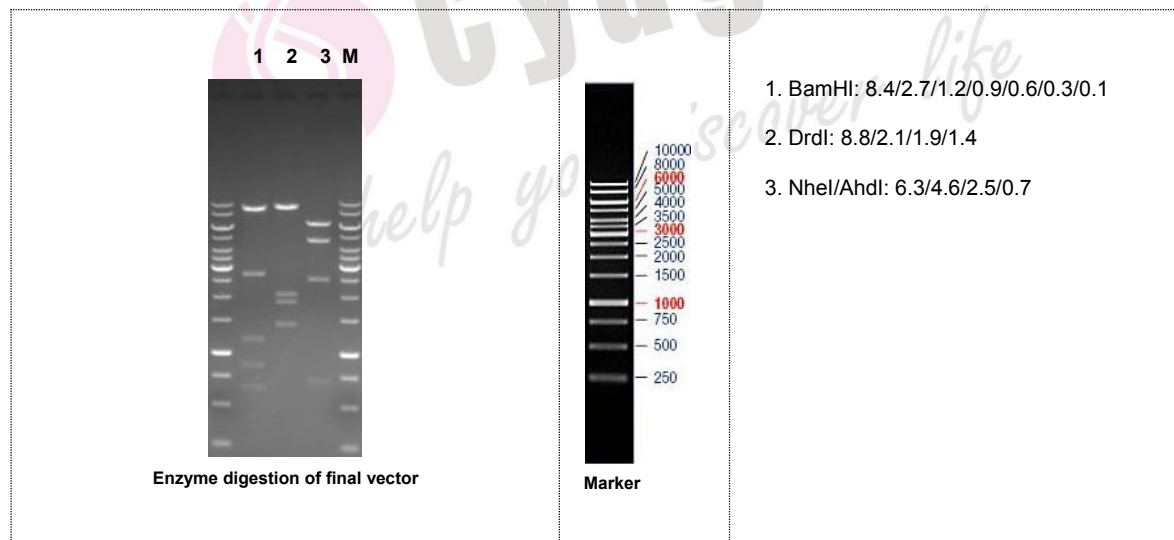
Diagram

Linearized targeting vector



4. Result

Your targeting vector was digested by restriction enzymes for confirmation purposes. Units below are all in kilo-base pair (kb).



5. Sequence of the Final Targeting Vector

	Homology arms	KI region	loxP sites	Sequence confirmed regions
1	CTAAAT	TGTAAG	CGTTAA	TATTTT GTTAAA ATTCGC GTTAAA TTTTGT TTAAAT CAGCTC
61	ATTTTT	TAACCA	ATAGGC	CGAAAT CGGCAA AATCCC TTATAA ATCAAA AGAATA GACCGA
121	GATAGG	GTTGAG	TGTTGT	TCCAGT TTGGAA CAAGAG TCCACT ATTAAA GAACGT GGACTC
181	CAACGT	CAAAGG	GCGAAA	AACCGT CTATCA GGGCGA TGGCCC ACTACG TGAACC ATCACC
241	CTAATC	AAGTTT	TTTGGG	GTCGAG GTGCCG TAAAGC ACTAAA TCGGAA CCCTAA AGGGAG
301	CCCCCG	ATTTAG	AGCTTG	ACGGGG AAAGCC GGCGAA CGTGGC GAGAAA GGAAGG GAAGAA
361	AGCGAA	AGGAGC	GGGCGC	TAGGGC GCTGGC AAGTGT AGCGGT CACGCT GCGCGT AACCAC
421	CACACC	CGCCGC	GCTTAA	TGCGCC GCTACA GGGCGC GTCCCA TTCGCC ATTCAG GCTGCG
481	CAACTG	TTGGGA	AGGGCG	ATCGGT GCGGGC CTCTTC GCTATT ACGCCA GCTGGC GAAAGG
541	GGGATG	TGCTGC	AAGGCG	ATTAAG TTGGGT AACGCC AGGGTT TTCCCA GTCACG ACGTTG
601	TAAAAC	GACGGC	CAGTGA	ATTGTA ATACGA CTCACT ATAGGG CGAATT GGGTAC GCGGCC
661	GCATTC	TGGTAC	CGACAA	GAATTC CCACAG ATTTTC GGTTTT GTCGGG AAGTTT TTTAAT
721	AGGGGC	AAATAA	GGAAAA	TGGGAG GATAGG TAGTCA TCTGGG GTTTTA TGCAGC AAAACT
781	ACAGGT	TATTAT	TGCTTG	TGATCC GCCTCG GAGTAT TTTCCA TCGAGG TAGATT AAAGAC
841	ATGCTC	ACCCGA	GTTTTA	TACTCT CCTGCT TGAGAT CCTTAC TACAGT ATGAAA TTACAG
901	TGTCGC	GAGTTA	GACTAT	GTAAGC AGAATT TTAATC ATTTTT AAAGAG CCCAGT ACTTCA
961	TATCCA	TTTCTC	CCGCTC	CTTCTG CAGCCT TATCAA AAGGTA TTTTAG AACACT CATTTT
1021	AGCCCC	ATTTTC	ATTTAT	TATACT GGCTTA TCCAAC CCCTAG ACAGAG CATTGG CATTTT
1081	CCCTTT	CCTGAT	CTTAGA	AGTCTG ATGACT CATGAA ACCAGA CAGATT AGTTAC ATACAC
1141	CACAAA	TCGAGG	CTGTAG	CTGGGG CCTCAA CACTGC AGTTCT TTTATA ACTCCT TAGTAC
1201	ACTTTT	TGTTGA	TCCTTT	GCCTTG ATCCTT AATTTT CAGTGT CTATCA CCTCTC CCGTCA
1261	GGTGGT	GTTCCA	CATTTG	GGCCTA TTCTCA GTCCAG GGAGTT TTACAA CAATAG ATGTAT
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1381	ATTTAT	AACTG	AGCTAT	TAACCA TTAATG GTTTCC AGGTGG ATGTCT CCTCCC CCAATA
1441	TTACCT	GATGTA	TCTTAC	ATATTG CCAGGC TGATAT TTTAAG ACATTA AAAGGT ATATTT
1501	CATTAT	TGAGCC	ACATGG	TATTGA TTAATG CTTACT AAAATT TTGTCA TTGTAC ACATCT
1561	GTAAAA	GGTGGT	TCCTTT	TGGAAT GCAAAG TTCAGG TGTTTG TTGTCT TTCCTG ACCTAA
1621	GGTCTT	GTGAGC	TTGTAT	TTTTTC TATTTA AGCAGT GCTTTC TCTTGG ACTGGC TTGACT
1681	CATGGC	ATTCTA	CACGTT	ATTGCT GGTCTA AATGTG ATTTTG CCAAGC TTCTTC AGGACC
1741	TATAAT	TTTGCT	TGACTT	GTAGCC AAACAC AAGTAA AATGAT TAAGCA ACAAAT GTATTT
1801	GTGAAG	CTTGGT	TTTTAG	GTTGTT GTGTTG TGTGTG CTTGTG CTCTAT AATAAT ACTATC
1861	CAGGGG	CTGGAG	AGGTGG	CTCGGA GTTCAA GAGCAC AGACTG CTCTTC CAGAAG TCCTGA
1921	GTTCAA	TTCCCA	GCAACC	ACATGG TGGCTC ACAACC ATCTGT AATGGG ATCTGA TGCCCT
1981	CTTCTG	GTGTGT	CTGAAG	ACCACA AGTGTA TTCACA TTAAAT AAATAA ATCCTC CTTCTT
2041	CTTCTT	TTTTTT	TTTTTT	AAAGAG AATACT GTCTCC AGTAGA ATTTAC TGAAGT AATGAA
2101	ATACTT	TGTGTT	TGTTCC	AATATG GTAGCC AATAAT CAAATT ACTCTT TAAGCA CTGGAA
2161	ATGTTA	CCAAGG	AACTAA	TTTTTA TTTGAA GTGTAA CTGTGG ACAGAG GAGCCA TAACTG
2221	CAGACT	TGTGGG	ATACAG	AAGACC AATGCA GACTTT AATGTC TTTTCT CTTACA CTAAGC
2281	AATAAA	GAAATA	AAAATT	GAACCT CTAGTA TCCTAT TTGTTT AAAGCT CTAGCT TTACTT
2341	AACTTT	TGTGCT	TCATCT	ATACAA AGCTGA AAGCTA AGTCTG CAGCCA TTAATA AACATG
2401	AAAGCA	AGTAAT	GATAAT	TTTGGA TTTCAA AAATGT AGGGCC AGAGTT TAGCCA GCCAGT
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2701	TAGTCA	GGAGAG	GAGGAA	AAATCT GGCTAG TAAAAC ATGTAA GGAAAA TTTTAG GGATGT
2761	TAAAGA	AAAAAA	TAACAC	AAAACA AAATAT AAAAAA AATCTA ACCTCA AGTCAA GGCTTT
2821	TCTATG	GAATAA	GGAATG	GACAGC AGGGGG CTGTTT CATATA CTGATG ACCTCT TTATAG
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3121	TTTGTG	AGCCAG	GGCATT	GGCCAC ACCAGC CACCAC CTTCTG ATAGAG AGCCTG CACCTG
3181	AGGACA	ATTTTA	ATTAAC	TATACT GTTGGT GGGATA GTGTAC CAAGTA ACAGAC TTTTGT
3241	ACACTC	TTTCTT	ACTGGT	GGTATA CAGTTG TTTGGG TCTACG TTTTCA CAGAGC TCGGTC
3301	TTTGCC	TCACCG	TTGGGT	CTGTCA CTGGGT TTGTGT GACAGC CTCGAT GACATG GTGGCT
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3541 AAAAAA ATTGGA ATGAAG GCAAGC CATACT ATACAT GTCGTG TACATA GTGAAT CCAATG
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12121	CGGGAA	ACCTGT	CGTGCC	AGCTGC	ATTAAT	GAATCG	GCCAAC	GCGCGG	GGAGAG	GCGGTT
12181	TGCGTA	TTGGGG	GCTCTT	CCGCTT	CCTCGC	TCACTG	ACTCGC	TGCGCT	CGGTCG	TTCGGC
12241	TGCGGG	GAGCGG	TATCAG	CTCAGT	CAAAGG	CGGTAA	TACGGT	TATCCA	CAGAAT	CAGGGG
12301	ATAACG	CAGGAA	AGAACA	TGTGAG	CAAAAG	GCCAGC	AAAAGG	CCAGGA	ACCGTA	AAAAGG
12361	CCGCGT	TGCTGG	CGTTTT	TCCATA	GGCTCC	GCCCCC	CTGACG	AGCATC	ACAAAA	ATCGAC
12421	GCTCAA	GTCAGA	GGTGGC	GAAACC	CGACAG	GACTAT	AAAGAT	ACCAGG	CGTTTC	CCCCTG
12481	GAAGCT	CCCTCG	TGCGCT	CTCCTG	TTCCGA	CCCTGC	CGCTTA	CCGGAT	ACCTGT	CCGCCT
12541	TTCTCC	CTTCGG	GAAGCG	TGGCGC	TTTCTC	ATAGCT	CACGCT	GTAGGT	ATCTCA	GTTCCG
12601	TGTAGG	TCGTTT	GCTCCA	AGCTGG	GCTGTG	TGCACG	AACCCC	CCGTTT	AGCCCG	ACCGCT
12661	GCGCCT	TATCCG	GTAACCT	ATCGTC	TTGAGT	CCAACC	CGGTAA	GACACG	ACTTAT	CGCCAC
12721	TGGCAG	CAGCCA	CTGGTA	ACAGGA	TTAGCA	GAGCGA	GGTATG	TAGGCG	GTGCTA	CAGAGT
12781	TCTTGA	AGTGGT	GGCCTA	ACTACG	GCTACA	CTAGAA	GAACAG	TATTTG	GTATCT	GCGCTC
12841	TGCTGA	AGCCAG	TTACCT	TCGGAA	AAAGAG	TTGGTA	GCTCTT	GATCCG	GCAAAC	AAACCA
12901	CCGCTG	GTAGCG	GTGGTT	TTTTTG	TTTGCA	AGCAGC	AGATTA	CGCGCA	GAAAAA	AAGGAT
12961	CTCAAG	AAGATC	CTTTGA	TCTTTT	CTACGG	GGTCTG	ACGCTC	AGTGGA	ACGAAA	ACTCAC
13021	GTTAAG	GGATTT	TGGTCA	TGAGAT	TATCAA	AAAGGA	TCTTCA	CCTAGA	TCCTTT	TAAATT
13081	AAAAAT	GAAGTT	TTAAAT	CAATCT	AAAGTA	TATATG	AGTAAA	CTTGGT	CTGACA	GTTACC
13141	AATGCT	TAATCA	GTGAGG	CACCTA	TCTCAG	CGATCT	GTCTAT	TTCGTT	CATCCA	TAGTTG
13201	CCTGAC	TCCCCG	TCGTGT	AGATAA	CTACGA	TACGGG	AGGGCT	TACCAT	CTGGCC	CCAGTG
13261	CTGCAA	TGATAC	CGCGAG	ACCCAC	GCTCAC	CGGCTC	CAGATT	TATCAG	CAATAA	ACCAGC
13321	CAGCCG	GAAGGG	CCGAGC	GCAGAA	GTGGTC	CTGCAA	CTTTAT	CCGCCT	CCATCC	AGTCTA
13381	TTAATT	GTTGCC	GGGAAG	CTAGAG	TAAAGTA	GTTTCGC	CAGTTA	ATAGTT	TGCGCA	ACGTTG
13441	TTGCCA	TTGCTA	CAGGCA	TCGTGG	TGTCAC	GCTCGT	CGTTTG	GTATGG	CTTCAT	TCAGCT
13501	CCGGTT	CCCAAC	GATCAA	GGCGAG	TTACAT	GATCCC	CCATGT	TGTGCA	AAAAAG	CGGTTA
13561	GCTCCT	TCGGTC	CTCCGA	TCGTTG	TCAGAA	GTAAGT	TGGCCG	CAGTGT	TATCAC	TCATGG
13621	TTATGG	CAGCAC	TGCATA	ATTCTC	TTACTG	TCATGC	CATCCG	TAAGAT	GCTTTT	CTGTGA
13681	CTGGTG	AGTACT	CAACCA	AGTCAT	TCTGAG	AATAGT	GTATGC	GGCGAC	CGAGTT	GCTCTT
13741	GCCCCG	CGTCAA	TACGGG	ATAATA	CCGCGC	CACATA	GCAGAA	CTTTAA	AAGTGC	TCATCA
13801	TTGGAA	AACGTT	CTTCGG	GGCGAA	AACCTCT	CAAGGA	TCTTAC	CGCTGT	TGAGAT	CCAGTT
13861	CGATGT	AACCCA	CTCGTG	CACCCA	ACTGAT	CTTCAG	CATCTT	TTACTT	TCACCA	GCGTTT
13921	CTGGGT	GAGCAA	AAACAG	GAAGGC	AAAATG	CCGCAA	AAAAGG	GAATAA	GGGCGA	CACGGA
13981	AATGTT	GAATAC	TCATAC	TCTTCC	TTTTTC	AATATT	ATTGAA	GCATTT	ATCAGG	GTTATT
14041	GTCTCA	TGAGCG	GATACA	TATTTG	AATGTA	TTTAGA	AAAATA	AACAAA	TAGGGG	TTCCCG
14101	GCACAT	TTCCCC	GAAAAG	TGCCAC						

BAC Modification (Recombineering)

Project Name: pStart-K-ITR-hGRM7 promoter>LoxP/rBG pA:PGK pA:BGH pA/LoxP:hGRM7[NM_181874.2]:SV40 pA

Project Tracking Number: BAC41-170519-1004

Objective

Clone the fragment containing LoxP/rBG pA:PGK pA:BGH pA/LoxP:hGRM7[NM_181874.2]:SV40 pA into pStart-K-ITR plasmid, and transfer the promoter region of human GRM7 gene from BAC clone RP11-244G3 to pStart-K-ITR backbone to drive the transcription of the fragment.

Project Details

1. The BAC of Interest

a. Basic information

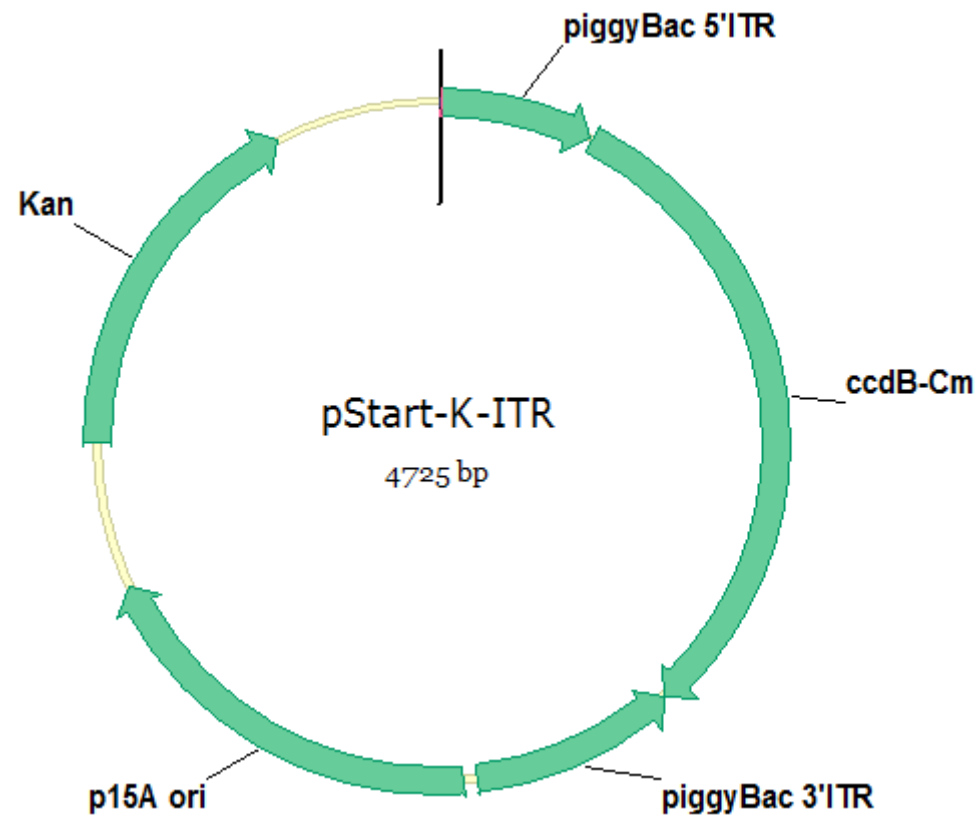
BAC Clone ID	RP11-244G3
Cloning Vector	pBACe3.6
Cloning Site	EcoRI
Insert Length	155,943 bp

b. Genomic region carried on the BAC



Note: BAC clone RP11-244G3 contains human GRM7 gene and the upstream intergenic region.

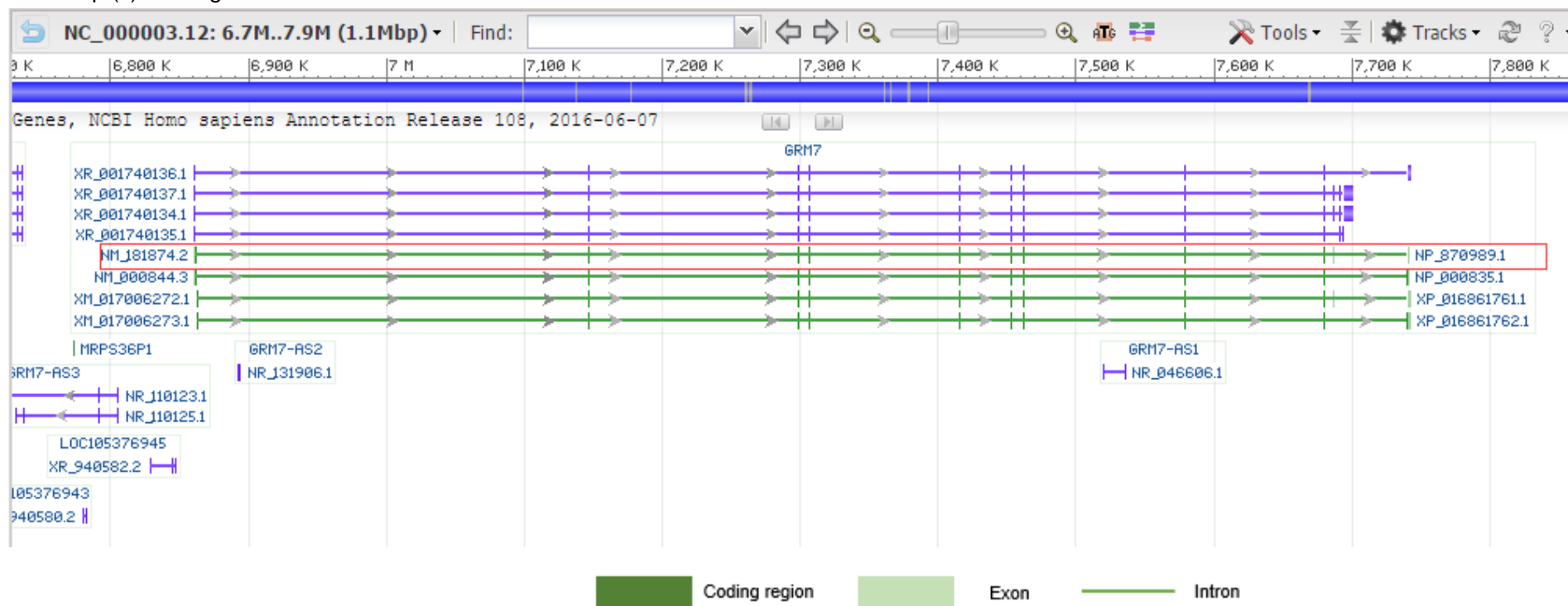
2. pStart-K-ITR backbone



3. The gene of interest (GOI)

Gene Symbol	GRM7
Gene Full Name	glutamate metabotropic receptor 7
Gene ID	2917
Species	Human
Location	GRCh38.p7, Chromosome 3, NC_000003.12 (6861097..7743032)
Exon Count	11

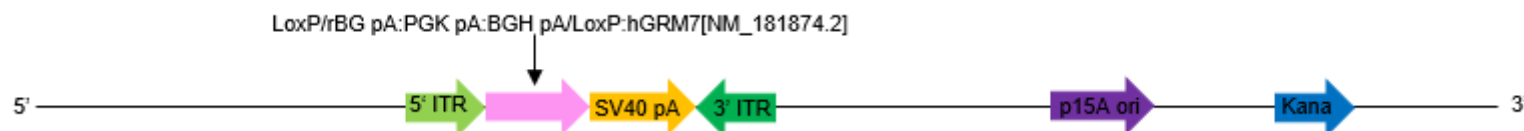
Transcript(s) of the gene:



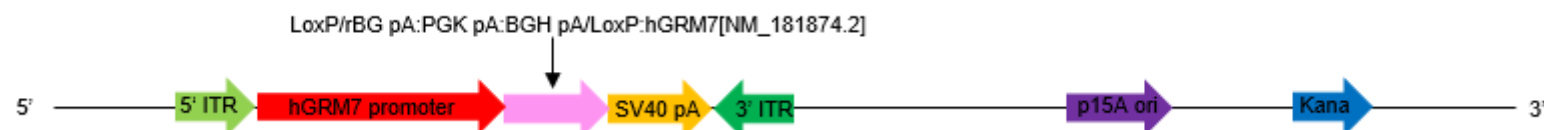
Note: This BAC modification strategy is based on transcript NM_181874.2.

4. BAC modification strategy

- A fragment containing LoxP/rBG pA:PGK pA:BGH pA/LoxP:hGRM7[NM_181874.2]:SV40 pA will be cloned into pStart-K-ITR vector (between the two ITRs) to obtain an intermediate vector.



- The promoter region of Npff on the BAC will be transferred to the intermediate vector through gap-repair cloning method, and inserted right before LoxP/rBG pA:PGK pA:BGH pA/LoxP:hGRM7[NM_181874.2]:SV40 pA and downstream of 5' ITR.



Note: The two ITR elements are recognition sequences of the piggyBac transposase (PBase). If the modified BAC is used to generate transgenic animals using the PiggyBac-on-BAC approach as described on Cyagen's website (<http://www.cyagen.com/us/en/service/piggybac-on-bac-transgenic-mouse.html>), the region from 5' ITR to 3' ITR on the BAC, which contains the GOI, will be transposed into random TTAA sites of the host genome mediated by PBase, with one copy per integration site.

5. Validation

The modified BAC clone will be validated by PCR and sequencing.

Deliverable

Deliverable	E. coli stock
BAC Clone Name	pStart-K-ITR-hGRM7 promoter>LoxP/rBG pA:PGK pA:BGH pA/LoxP:hGRM7[NM_181874.2]:SV40 pA
Plasmid Copy Number	Low
Antibiotic Resistance	Kanamycin (conc. 50 ug/ml)
Cloning Host Strain	DH10B