

HMEJ-based safe-harbor genome editing enables efficient generation of cattle with increased resistance to tuberculosis

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Supporting Tables

Table S1. Sequences of primers for qPCR.

Gene name	Primer	Sequence (5'-3')	Product length
<i>ROSA26</i>	Forward	GCGTTTGTACTACTTGCTCATC	243 bp
	Reverse	AAGAGGGTCAGTAGAATCCCA	
β -actin	Forward	ATCACCATCGGCAATGAGCGGTTC	217 bp
	Reverse	CGGATGTCGACGTCACACTTCATGA	
<i>THUMPD3</i>	Forward	GCAAAATCAGCAAAGACCGG	129 bp
	Reverse	TGAACTGGTAATCCTTAAACTCCTG	
<i>SETD5</i>	Forward	AGCAAGCTCCTGAGAAAGTAAC	200 bp
	Reverse	CTTCAACCTTCTATCTTCCCG	
<i>LHFPL4</i>	Forward	GCCACGGTCTACAAGATCTG	204 bp
	Reverse	GATGAGGGCGTTGAGGATG	

Table S2. Sequences of primers for promoter fragments amplification.

Vectors	Primer	Sequence (5'-3')	Product length
pGL4.10-2007/+505	Forward	CTCGCTAGCAGATTCTAATCCCATAACGAGC	2512 bp
	Reverse	CCCAAGCTTCCACCCTACCTACCTAAGCAC	
pGL4.10-1807/+505	Forward	CTCGCTAGCAAACACTGATATAAAACTAATC	2312 bp
pGL4.10-1607/+505	Forward	CTCGCTAGCTAAAGGAAATTTGCAGAGAACC	2112 bp
pGL4.10-1407/+505	Forward	CTCGCTAGCAGAGCTGGCTGCAGCATC	1912 bp
pGL4.10-1207/+505	Forward	CTCGCTAGCGGAAAGAGAATTGATAAAGGC	1712 bp
pGL4.10-1007/+505	Forward	CTCGCTAGCTAATGTCCTTAGTGTGGGAAACG	1512 bp
pGL4.10-807/+505	Forward	CTCGCTAGCGAGGAGAGCAGTGAGAGG	1312 bp
pGL4.10-607/+505	Forward	CTCGCTAGCAGGGGGATGAGAAGGG	1112 bp
pGL4.10-407/+505	Forward	CTCGCTAGCGAGGCAGCAGGACTCGAGTTA	912 bp

Table S3. Sequences of primers for promoter fragments amplification.

Vectors	Primer	Sequence (5'-3')	Product length
pGL4.10-1007/+505	Forward	CTCGCTAGCAGATTCTAATCCCATAACGAGC	1512 bp
	Reverse	CCCAAGCTTCCACCCTACCTACCTAAGCAC	
pGL4.10-1007/+405	Reverse	CCCAAGCTTAAACACGCCATTTGTG	1412 bp
pGL4.10-1007/+305	Reverse	CCCAAGCTTCTCCGTCCGATTCGA	1312 bp
pGL4.10-1007/+205	Reverse	CCCAAGCTTTTCAGCGTAACAAACGC	1212 bp
pGL4.10-1007/+105	Reverse	CCCAAGCTTCGGACCCCTCCACCA	1112 bp
pGL4.10-1007/+5	Reverse	CCCAAGCTTCTCCGCTGATTGGCAG	1012 bp

Table S4. Primer sequences for sgRNA cloning.

Number	Top Guide oligo	Bottom Guide oligo
11	CACCGTGTCTCGAGTCTCGATTATGGG	AAACCCATAATCGAGACTCGACAC
34	CACCGAGCGAAACCACTGCGCGGGT	AAACACCCGCGCAGTGGTTTCGCTC
43	CACCGTCCATGTCTCGAGTCTCGATTA	AACTAATCGAGACTCGACATGGAC
44	CACCGCCATGTCTCGAGTCTCGATTAT	AAACATAATCGAGACTCGACATGGC
45	CACCGTTGCAGTCTCGCGCCGATTTT	AAACAAAATCGGCGCGAGCTGCAAC

Table S5. Sequences of primers for Junction PCR.

Primer	Sequence (5'-3')	Product length
Lj-S	GCCAATCAGCGGAAGCC	1824 bp
Lj-A	TTCACCTTGATGCCGTTCTT	
Rj-S	ATAATCAGCCATAACCACA	2833 bp
Rj-A	TTCCAGCCTACCAACA	
Rj-S'	CAGGCAGATTCTTTACCG	2261 bp
Rj-A'	CAAGTCACTCATTCCGTTT	

Table S6. Sequences of sgRNA11 potential on/off target sites.

Name	On-target site	Potential off-target site
sgRNA11-OT1		TtaaGgGTCTtGATTATGGGTGG
sgRNA11-OT2		TGTgGAGaaTaGATTATGGGTGG
sgRNA11-OT3		TtTaGAtgCTtGATTATGGGGGG
sgRNA11-OT4	TGTCGAGTCTCGATTATGGGNGG	TGatGtGaCTgGATTATGGGGGG
sgRNA11-OT5		gGTCagtTCcCGATTATGGGCGG
sgRNA11-OT6		TGTgGAGagTgGATTATGGGTGG
sgRNA11-OT7		TGTCGtGgtTaGATTATGGGAGG
sgRNA11-OT8		TGTgGttgCTgGATTATGGGAGG

Table S7. Primer sequences for amplification of potential off target region.

Name	Primer	Sequence (5'-3')	Product length
sgRNA11-OT1	Forward	ACCTGGTCTTCTGCTTCA	597 bp
	Reverse	TCCTCTTTCCTTCTCCCT	
sgRNA11-OT2	Forward	AGGAGGATGGAAGTGAA	550 bp
	Reverse	TACTTGGGTGTCTCATAAA	
sgRNA11-OT3	Forward	CACAGAATCGCCTCC	638 bp
	Reverse	ATGCCTCAAAGAATGG	
sgRNA11-OT4	Forward	TGTCTCAGAAACCCTACAA	515 bp
	Reverse	GGAGTCTACAGGCACCAT	
sgRNA11-OT5	Forward	CCTGCACCCTAATAGATGG	458 bp
	Reverse	GACGAATGCCTCCGACT	
sgRNA11-OT6	Forward	AACACTGGGCATCTGG	430 bp
	Reverse	GGGAGGAATGGGCTA	
sgRNA11-OT7	Forward	TACACTAAATCACGCAGAC	375 bp
	Reverse	AGTGGCAGGGATGG	
sgRNA11-OT8	Forward	GGGAAGATGAATGAAAGCA	687 bp
	Reverse	CATTACCAGCAGTTAGGGA	

Supplementary Figures

Mouse	TTTGGTGTGGGAAAAGCAGCAGCCATCTGAGATAGGAACTGGA AAAACAGAGGAGAGCGGTT CAGGAAGATTATGGAG-----GGGAGGACTGGGCCCCACGAGCG--ACCAGAGTT
Bovine	----GTGTGGGAAACGAAGCAATCATCTGCGATGGGACCCAGGACGAGGGGGAAGCGTCCAGGAACATTCGGGGTGGGGTGGGGAGATCGAAGCCCCCCGAGAGGGATCAGAGTT
Pig	----GTGTGGGAAAGGAAGCAATCATCTGCAATAGGGACCTTAGGACGAGAGAAAGCGTCCAGGAACATTC TTGGA--GGG--GGGAGATCGAGGGCCCCA-GAGCG--ACCAGAGTT
Mouse	GTCACAAGGCCGCAAGAACAGGGGAGGTGGGG--GCTCAGGGACAGAAAAAAA--GTATG---TGTATTTTGAGAGCAGGGT--TGGGAGCCTCTCTGAAA--AGGGTATAAACGTGG
Bovine	GTCACAAGGCCGCGCGAACCGGGGTGGGGTGGCGTTTGGGAGGGGAAAAAAA--GTGTGCTGTGTATTTTGAGAGGAGAGCAGTGAGAGGCCATCTCTCAAGTAAAAGGTAACCGTGG
Pig	GTCACAAGGCCGCGCGAACCGGGGTGGGGTGGGGTTTGGGAGGGGAAAAAAAAGTGTG---TGTGTATTTGAGGAGGGCGCGAGAGGCCATTCTCAAGTAAAAGGTAACCGTGG
Mouse	AGTAGGCAATACCAGGCAAAAAGGGAGACCAGAGTAGGGGGAGGGGAAGTCTGACCCAGGAAGACATTA AAAAGGTAGTGGGTCGACTAGATGAGGAGCCTTCTCTCTG
Bovine	AGTAGGCAATCCCGGG--AAAAGGGTGAAGAGGCGTGGGGGAGGGGAAGCTCTGACCCAGGAAGACGTGAAAAGGTAGTGGGTCGACGAGATTACGGATGGGGCTCTCGCC
Pig	AGTAGGCAATTCACAGG--AAAAGGGTGAAGAGGCGTGGGGGAGGGGAAGCTCTGACCCAGGAAGACATGAAAAGGTAGTGGGTCGACTAGATTAGGAGGGGGCTCTCGCC
Mouse	GGCAAGAGCGGTGCAATGGTGTAAAGG--TAGCTGAGAAGACAAAAGGGCAAGCATCTCTGCTACCAGGCTGGGAGGCCAGGCCACGCCGAGAGAGGGAACGAGGGAG
Bovine	TGGAAAGGGTGCACACGGTGTGTAGGGGCGGGCAGGGGATGAGAAGGGCAACATCTCTACTGAGAGCCTGGGAGGGCCAGGCCACCTCC--GAGAGCAACCGCGGGAG
Pig	TGGAAAGAGGGGTACAGTGGTGTGGGGG--GCGAGGGGGATGGGAGGGGCAAGCATCTCTGCTGAGAGCGGGGAGGGCCAGGCCACCTCC--GAGAGCAACCGCGGGAGG
Mouse	ACTGAGG---TGACCTTCTTTCCCGGGGCCCGGTCTGTGGTTCGGTCTCTTTTCTGTGGACCTTACCTTGACCCAGGCGTCCGGGGCTGGGCCCGGGCTGCGGCGCACG
Bovine	ACGGAGGAGGTGACCTTCCCTCCCGGGGCCCGGTCTGAGGGTAGGTTCTCTTTTCTGTGGACCTTACCTTGTCCAGGC-----CTGGGCCCGGGCTGCGGCGCACG
Pig	ACGGAGGAGGTGACCTTCCCTCCCGGGGCCCGGTGTGAGGGAGGTTCTCTTTTCTGTGCGACCTTACCTTGTCCAGGC-----CTGGGCCCGGGCTGCGGCGCACG
Mouse	GCACCTCCCGGAGGCAGCGACTCGAGTTAGGCCCAAAGCGCGGCCACGGCGTTTCTGGCCGGGAATGGCCGTACCCGTGAGGTGGGGTGGGGGACAGAAAAGGGGAGCGGAGCCC
Bovine	GCACTCCCGGAGGCAGCGACTCGAGTTAGGCCCAAAGCGCGGCCACGGCGTTTCTGGCCGGGAATGGCCGTCCCGTGAAGTGGGGTGGGGACAGAAA--GGCGAGCGAGCCA
Pig	GCACCTCCCGTAGGCAGCGACTCGAGTTAGGCCCAAAGCGCGGCCACGGCGTTTCTGGCCGGGAATGGCCGTCCCGTGAAGTGGGGTGGGGGACAGAAA--GGCGAGCGAGCCA
Mouse	GAGGCGGGAGGGGGA--GGGCCAGGGCGGAGGGG--CGGCCACTACTGTGTGGCGACTGGCGGGACTAGGGTGCCTGAGTCTCTGAGCGCAGCGGGCGGGCCGCCCTCC
Bovine	AGGGCGGGAGGGGGAAGGGCCAGGGAGGGGGG--CGGCCACTACTGTGTGGCGACTGGCGGGACTGGGGTGCCTGAGTCTCTGAGCGCAGCGGGCGGGCCGCCCTCC
Pig	AGGGCGGTGAGGGGA--GGGCCAGGGAGGGGGGGCGGCCACTACTGTGTGGCGACTGGCGGGACTGGGGTGCCTGAGTCTCTGAGCGCAGCGGGCGGGCCGCCCTCC
Mouse	CCGGCGGGCAGCGGGCCAGCGGGC-----AGCTCACTAGCCCGCTGCCGAGCGGAAACGCCACTGACCGCACGGGATTCCAGTGCCGGCCAGGGGACCGGGGACAGC
Bovine	CCGGCGGGCAGCGGGCCAGCGGGTAGC-----AGCTCACTAGCCCGCTGCCGAGCGGAAACGCCACTGACCGCACGGGATTCCAGTGCCGGCCAGGGGACCGGGGACAGC
Pig	CCGGCGGGCAGCGGGCCAGCGGGCAGCAGTCACTAGCCCGCTGCCGAGCGGAAACGCCACTGACCGCACGGGATTCCAGTGCCGGCCAGGGGACCGGGGACAGC
Mouse	CCCCCTCCCGCGGCCATTGGCTCTCCGCCACCGCCACACTTATTGGCCGTGCCCGCCAATCAGCGGAGCTGCCGGGGCCGCTAAAGAAGAGGCTGTGCTTTGGGGCTCCG
Bovine	CCCCCTCCCGCGGCCATTGGCCCTCCGCCACCGCCCGCACCTATTGGCCAAGTCTGTCGAATCAGCGGAAGCCGCCGGGGCCACTAGAAGAAGGCTGTGCTTTGGGGCTCCG
Pig	CCCCCTCCCGCGGCCATTGGCCCTCCGCCACCGCTCTGCACTTGGCCAGTCCCGCCAATCAGCGGAAGCCGCCGGGGCCGCTAGAAGAAGGCTGTGCTTTGGGGCTCCG
Mouse	GCTCCTCAGAGACCTCGGCTAG
Bovine	GCTCCTCAGAGACCTCGGCTAG
Pig	GCTCCTCAGAGACCTCGGCTAG

Figure S1. Comparison of mouse, bovine and pig *ROSA26* promoter and exon 1 sequences showed high sequence conservation among these species. The top arrow and the bottom arrow denote the 5' start of the mouse and pig *ROSA26* transcript, respectively; the same site of the middle sequence is assumed to be the start of the 5' bovine transcript.

3' RACE

GCCTCGGCTAGGTAGGGGAGCGGAACTCTGGTGAGGGGGTCCGGCGGATTGGTG
GGGATGGGTGGCTGAGGTCGTCTGGCCGGTACCTGGGGGTCGCTTTCCCCGGTGGGA
AGAGGGGAGAATAGCGTTTGTACACTTGCTCATCTTGTCCCAGTCATACCATTGGCTT
TCTCCTCTCCTGTTTTTGGTATCCCGGTGAGTCATGAAACCAGACAGGTTTCACCACCA
ATTAAGGCTACCCAGCTCGAGCATAGGCTTCACTCTTGCCCAGAAATGCATTTATTCCT
CTTTTATGGATATTCTGGAGTCTTTACCTTGATTTTCATTTAATTTTTTAACCTCAGCTGG
GATTCTACTGACCCTCTTAATAGTCCAGATGATCTTGACGACTGCTTTGCTGAGAACCG
GACGTGAGGTTTCAGCAACATCTCTTTTATATCCTTAGAATAACCTTTCAACCCATTTTCAT
TGATATGCTTATGAGTTAGTAATCAAGCTCAGTTGCCATAAGGCTAGTATCCTTCGAACT
AGGATCTCTTGTCTCTGGTATCTGCTGATACAACTTTCATATGTGTCCAGGACAGTAGTT
CTCATACAAAGATAACAGCATGGAAGTAACCGATCCAACCTCCTTTACTGCCTGGTAACT
ACTGACAGGATGCGTTCCATCATCACAAATGTGATGTACAAGGTCCCTCAATGGACTAA
CCTCACCTTAACAGCCTTTTTGTTTGTGACAGTTTTCCACATACACACCCAAACAATATT
ATTGGACCTCTTTGTAGGGGTGGTTCCTCCTGGAGTGCTACCCTTGATAGTCCTTACCC
TTCCAATAAAGACTGTAAAACCTC

Figure S2. Identification of the bROSA26 non-coding RNA. The partial sequence of the bROSA26 predicated exon 1 was marked with gray shadow. Primer 3' RACE was marked in red.

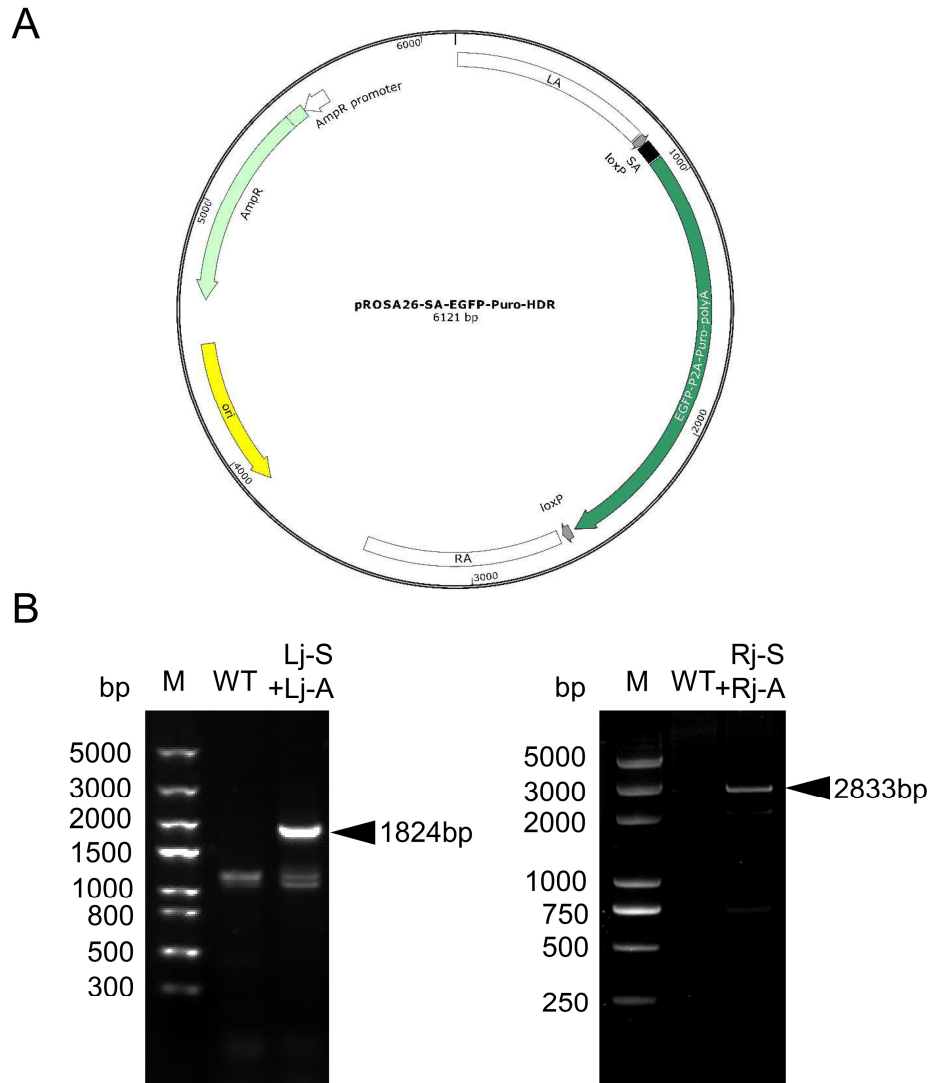
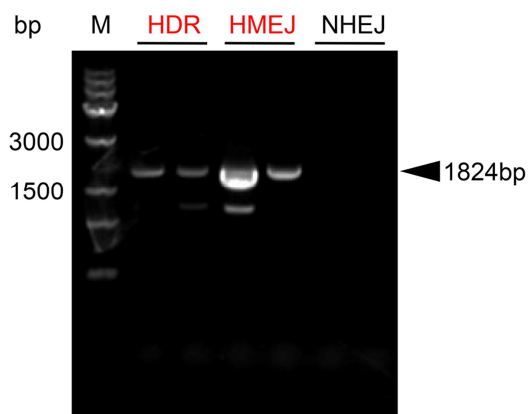


Figure S3. Insertion and selection of the reporter genes transgenic colonies by HDR-based method. (A) Schematic representation of the HDR-mediated gene targeting vector. (B) The 5' (left, 1824-bp) and 3' junction (right, 2833-bp) PCR analyses confirming correct joining between genome and HDR-based donor plasmids. “WT” represents wild-type cells (non-transfected BFFs). M, marker.

A



B

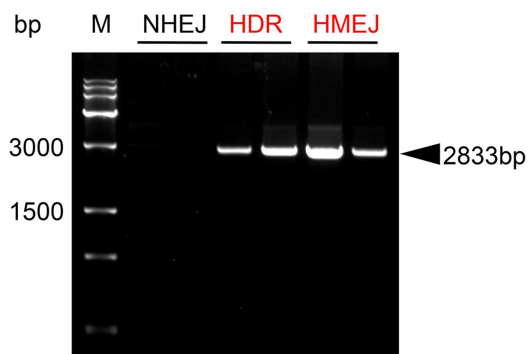


Figure S4. Representative junction PCR of the reporter gene transgenic colonies by HMEJ-, NHEJ- and HDR-based method. The 5' (left, 1824-bp) and 3' junction (right, 2833-bp) PCR analyses confirming correct joining in HMEJ and HDR groups. M, marker.

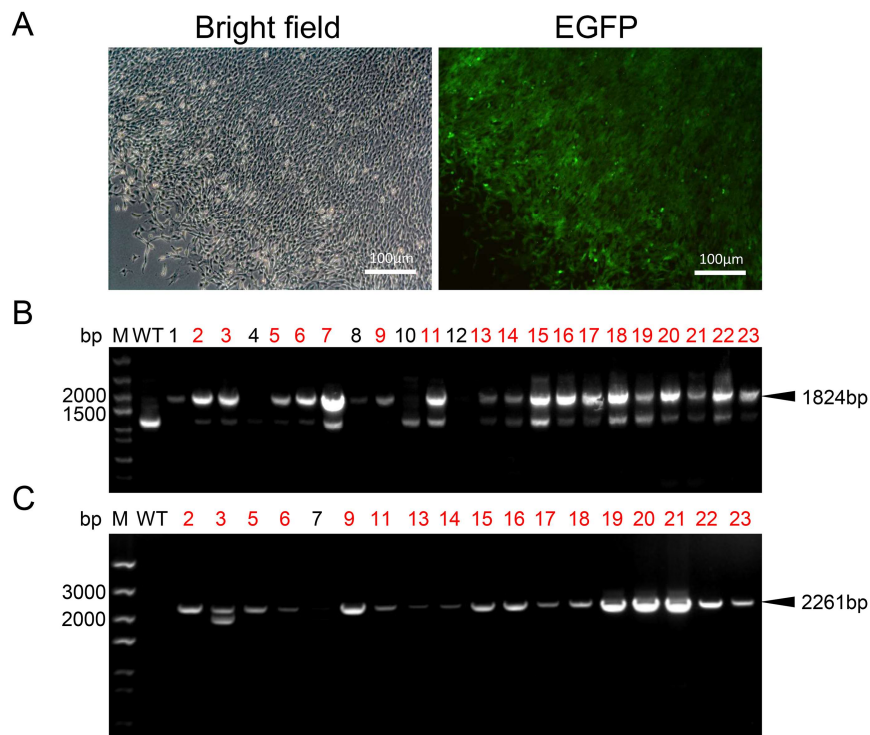


Figure S5. Photographs of *NRAMP1* gene KI colony and representative junction PCR results of puromycin-resistant colonies. (A) Stably transfected cells by HMEJ-mediated *NRAMP1* gene KI after positive drug selection under a fluorescence stereomicroscope. (B, C) Representative 5' junction (B, 1824-bp), 3' junction (C, 2261-bp) PCR results of puromycin-resistant colonies. Red fonts represent positive results. "WT" represents wild-type cells (non-transfected BFFs). M, marker.

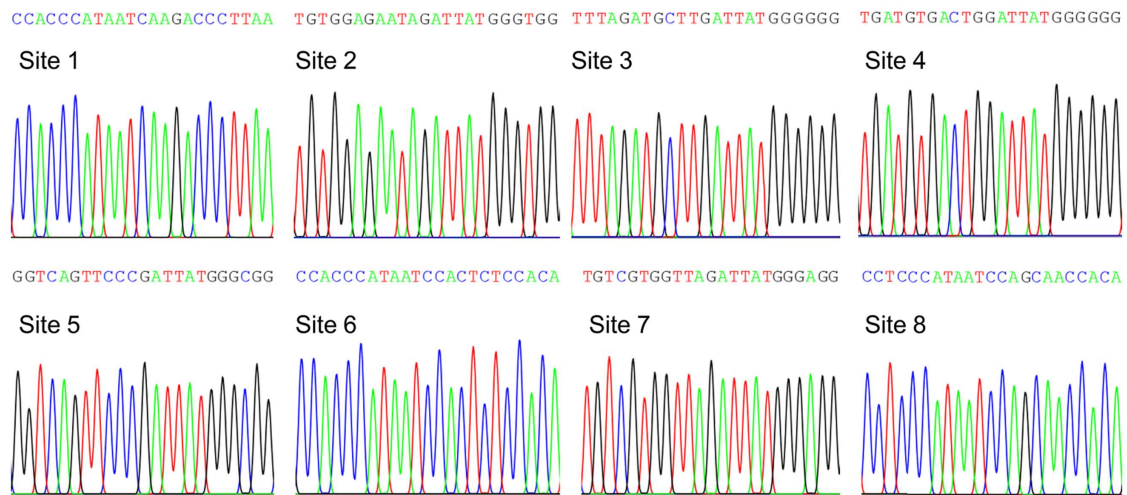


Figure S6. Sanger sequencing results of eight potential off-target sites in gene-edited cattle.