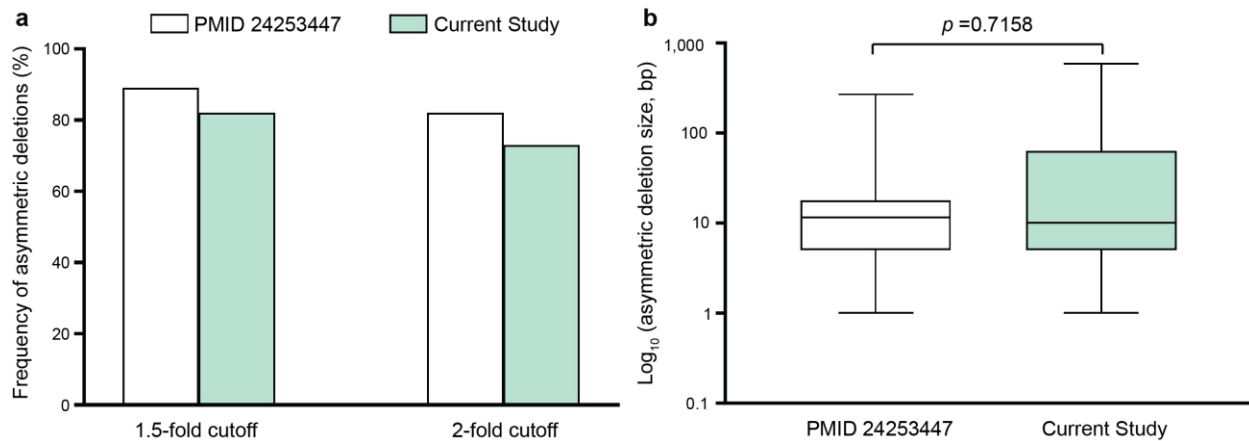
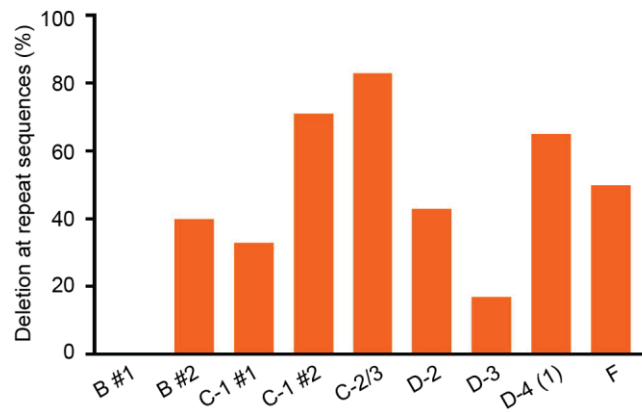


Site of interest	Targeted loci in the mouse genome	Targeted genomic sites
CTCF binding site		D-2 D-3 D-4(1), D-4(2) D-1/3/4 D-1/2/3/4 D-1/2/3/4/5
		E-1 E-2 E-3 E-4 E-5
NFIB binding site		F

Supplementary Figure 1 Schematic diagram of CTCF and NFIB binding sites targeted by CRISPR/Cas9. Five CTCF binding sites in *Wap* locus (D), five CTCF sites in *Csn* locus (D) and one NFIB binding site in *Csn* locus (E) were targeted. The five sites in *Wap* locus were targeted separately or in combination as illustrated on the right panel. CTCF and NFIB binding sites in *Csn* locus were separately targeted. Arrowheads indicate the coding genes. The genomic coordinates and the distances of target sites to the respective gene are also shown.

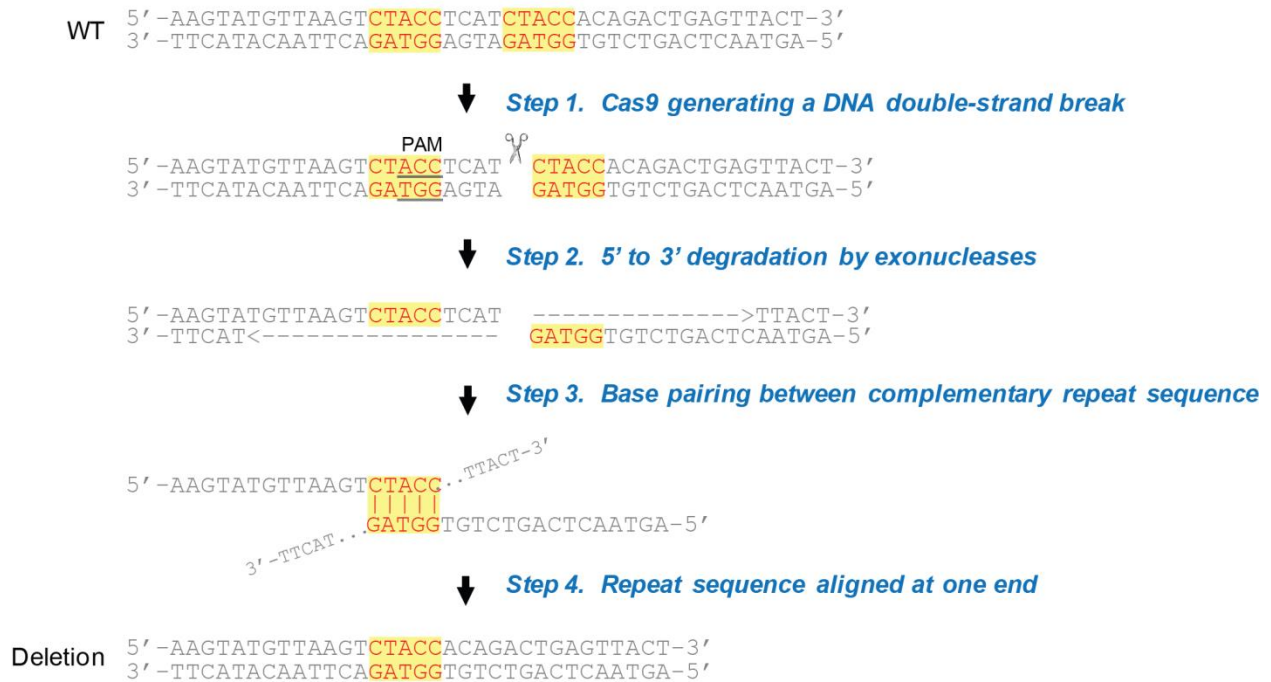


Supplementary Figure 2 Comparison of asymmetric deletions between a prior study (PMID 24253447) and the current study. (a) Frequency of asymmetric deletions observed in a prior study and the current study. Deletion sequences were obtained from the founder sequences of prior study (Supplementary Table 3 and 4)¹ and the current study. Subsequently, asymmetric deletions were examined with two cutoffs, 1) ≥ 1.5 -fold difference between the upstream and downstream of Cas9 cutting site and 2) ≥ 2 -fold difference between each side. The frequency of asymmetric deletions was prevalent in both studies (1.5-fold cutoff: 89% vs 82%, 2-fold cutoff: 82% vs 73%). In a prior study, deletion sequences of total 84 founders from two targeted genomic sites were analyzed. In the current study, deletion sequences of total 139 founders from nine targeted genomic sites were analyzed. Only deletions obtained from a single sgRNA injection were analyzed to avoid the effect of multiple variables. Deletions obtained from a single sgRNA injection in a prior study, Deletions targeting *Foxn1* and *Prkdc*; Deletions obtained from a single sgRNA injection in the current study, Deletions targeting TF binding site B, C-1, C-2/3, D-2, D-3, D-4(1), and F. (b) Deletion sizes of asymmetric deletions (1.5-fold cutoff) obtained from a prior study and the current study. Although the median deletion size of asymmetric deletions from the current study (10 bp) was similar to that from a prior study (11.5 bp), the top 50% of asymmetric deletion sizes was bigger in the current study (up to 600 bp). Median, middle bar inside the box; IQR, 50% of the data; whiskers, 1.5 times the IQR.



Supplementary Figure 3 Frequency of single sgRNA-imposed deletions found at repeat sequences. More than 80% of deletions were found at the repeat sequences with a single sgRNA targeting the genomic site C-2 in mice carrying a C-3 deletion (C-2/3). B #1, $n=8$; B #2, $n=10$; C-1 #1, $n=6$; C-1 #2, $n=7$; C-2/3, $n=12$, D-2, $n=40$; D-3, $n=12$; D-4(1), $n=17$; F, $n=10$.

Repeat sequences align at one end

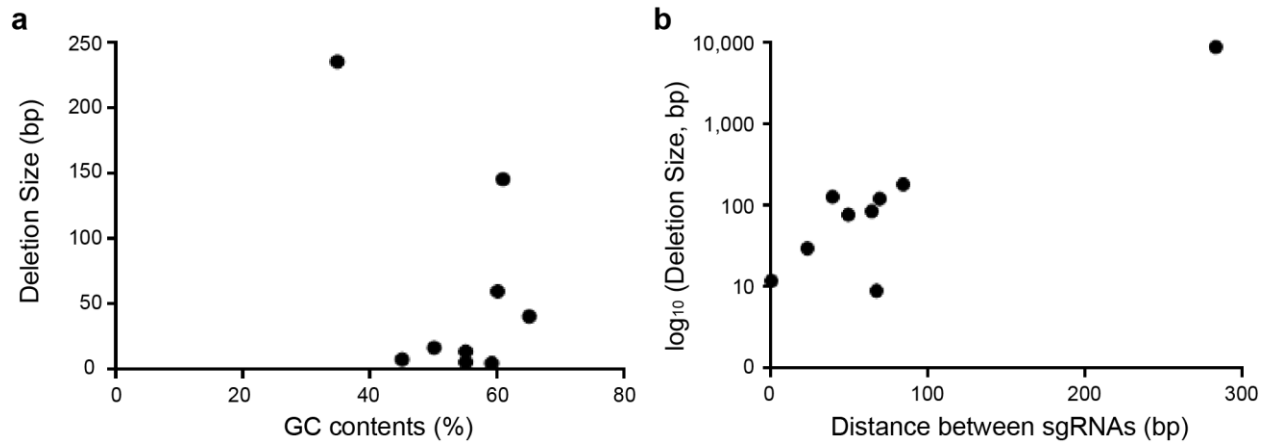


Supplementary Figure 4 Flow of proposed mechanism that can create repeat sequences aligned at one end. After Cas9 creates the blunt end cut 3 bp upstream of the PAM sequence, 5' to 3' exonucleases generate a deletion toward 3' direction. Complementary repeat sequences generate the base pairing and DNA polymerase fills in the gap. Only a single unit of repeat sequence is retained.

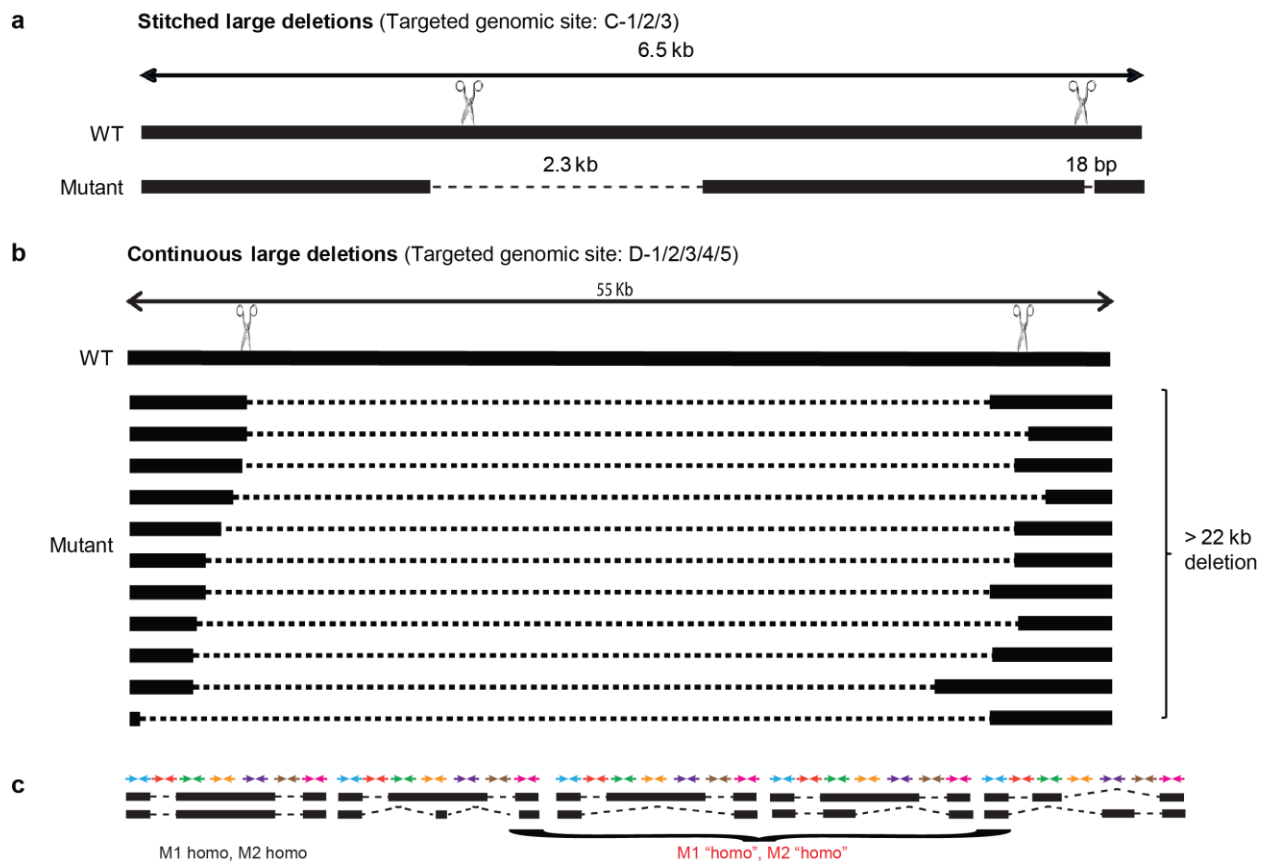
Deletion size (eg. site D-3)



Supplementary Figure 5 Deletion sizes obtained from founders that were targeted at genomic site D-3 with a single sgRNA. More than 80% of deletions were larger than the average deletion size (49 bp). Among them, approximately 40% of deletions exceeded 200 bp.



Supplementary Figure 6 Deletion sizes in relation to the GC content of sgRNAs and the distances between sgRNAs. (a) Scatterplot showing the deletion sizes dependent on the GC content of sgRNAs from injection of single sgRNAs. (b) A scatterplot depicting the deletion sizes dependent on the distances between each sgRNAs from co-injection of more than one sgRNAs targeting a single site.



Supplementary Figure 7 Representative examples of large deletions obtained from the simultaneous injection of four sgRNAs corresponding to two distant sites and the strategy to identify compound large deletions. (a) Stitched large deletions in mice from site C-1/2/3. (b) Continuous large deletions in mice from site D-1/2/3/4/5. (c) Diagram depicting the genotyping method used to identify compound large deletions. Serial primers spanning the target region were used for the PCR genotyping.

Supplementary Table 1 List of studies on CRISPR/Cas9 genome editing in the mouse germline and studies with microhomology-based deletions

1. Deletion at repeat sequences

PMID	System (<i>in vivo</i> mouse or <i>in vitro</i> cell culture)	Deletion at repeat sequences: Repeat sequences aligned at 1) one end (or microhomology-mediated end joining) 2) both ends
Current study	in vivo mouse	1, 2
26887046	in vivo mouse	1 *no statistical analysis was done.
24972169, 24973878, 25201414, 25757625, 26787905, 27301063	in vitro cell culture	1
23709638, 27543296	Drosophila	1
26318124	Zebrafish	1
26199327, 25550322, 26573820	parasite	1

2. Large deletion by CRISPR-single sgRNA injection *in vivo* mouse

PMID	Large deletion (> 400 bp)
Current study	single (600 bp)
26887046	900 bp *no statistical analysis was done.

** 1 out of 18 refs (including PMID 24494965, 26501274, 23545779, 24253447, 24372541, 24675426, 25538209, 25819794, 26160899, 26442875, 26456390, 26713866, 26857612, 26936824, 26964564, 27139777, and 27246398) provided the information of large deletion generated by single sgRNA injection.

3. Large deletion by CRISPR-multiple sgRNA injection *in vivo* mouse

PMID	Large deletion (> 400 bp)
Current study	multiple (24 kb)
27119535, 23992847, 24984260, 26501274, 27151215, 27318086, 27904015	< 1kb
25757625, 25897126	1.2 kb
27210041	2.2 kb
27234594	4 kb
23997119	10 kb
25137067	23 kb
25803037, 26742453	65 kb
26620761	95 kb
27396308	0.5 Mb

** 17 out of 36 refs (including PMID 23643243, 23929337, 24284873, 24315440, 25666713, 25997509, 26053263, 26216318, 26537785, 26817415, 26876963, 27223891, 27224051, 27258160, 27387532, 27483347, 27515175, 27530713, and 27685656) provided the information of large deletion generated by multiple sgRNA injection.

Supplementary Table 2. Summary of embryo microinjection of sgRNAs and Cas9 mRNA

1. Targeting individual genomic sites with single sgRNAs

Targeting strategies	Targeted genomic sites	sgRNA	Embryos injected	Pups born	Frequency of mutations (%)
One-step	B	1	36	18	44
		2	176	55	31
	C-1	1	150	14	79
		2	93	13	62
	D-2	1	150	57	84
	D-3	1	101	31	39
	D-4 (1)	1	27	43	53
F	1	191	40	25	
Two-step	C-2/3	1 (targeting site 2 in site C-3 mutants)	68	20	60

2. Targeting individual genomic sites with more than one sgRNA

Targeting strategies	Targeted genomic sites	sgRNA	Embryos injected	Pups born	Frequency of mutations (%)
One-step	A	1, 2	183	46	33
	C-1/3	1, 2	15	6	100
	D-4 (2)	1, 2	84	17	100
	E1	1, 2	75	75	9
	E2	1, 2	50	50	14
	E3	1, 2, 3	41	41	41
	E4	1, 2, 3	32	12	92
	E5	1, 2, 3	80	30	100
Two-step	D-1/2/3/4	1, 2 (targeting site 2 in site C-1/3/4 mutants)	38	4	50

3. Targeting more than one genomic site with several sgRNAs

Targeting strategies	Targeted genomic sites	sgRNA	Embryos injected	Pups born	Frequency of mutations (%)
One-step	D-1/3/4	1, 2, 3, 4	78	27	7
Two-step	C-1/2, C-1/2/3	1, 2, 3, 4 (targeting site 2 and 3 in site C-1 mutants)	90	23	91
	D-1/2/3/4/5	1, 2, 3, 4 (targeting site 2 and 5 in site D-1/3/4 mutants)	27	10	100

Supplementary Table 3. Frequency of insertions found in each targeted site

1. Targeting individual genomic sites with single sgRNAs

Targeting strategies	Targeted genomic sites	sgRNA	Insertion	Total founders	Insertion frequency (%)
One-step	B	1	0	18	0
		2	1	55	2
	C-1	1	1	14	7
		2	0	13	0
	D-2	1	12	57	21
	D-3	1	0	31	0
	D-4 (1)	1	2	43	5
F	1	0	40	0	
Two-step	C-2/3	1 (targeting site 2 in site C-3 mutants)	0	20	0
	Average				4

2. Targeting individual genomic sites with more than one sgRNA

Targeting strategies	Targeted genomic sites	sgRNA	Insertion	Total founders	Insertion frequency (%)
One-step	A	1, 2	4	46	9
	C-1/3	1, 2	4	6	67
	D-4 (2)	1, 2	0	17	0
	E-1	1, 2	1	75	1
	E-2	1, 2	0	50	0
	E-3	1, 2, 3	0	41	0
	E-4	1, 2, 3	1	12	8
	E-5	1, 2, 3	1	30	3
Two-step	D-1/2/3/4	1, 2 (targeting site 2 in site C-1/3/4 mutants)	0	4	0
	Average				10

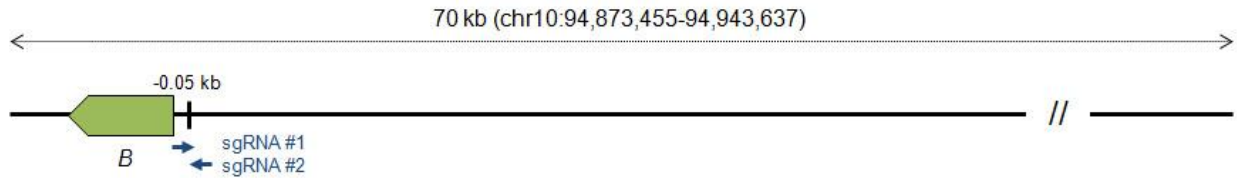
3. Targeting more than one genomic site with several sgRNAs

Targeting strategies	Targeted genomic sites	sgRNA	Insertion	Total founders	Insertion frequency (%)
One-step	D-1/3/4	1, 2, 3, 4	0	27	0
Two-step	C-1/2, C-1/2/3	1, 2, 3, 4 (targeting site 2 and 3 in site C-1 mutants)	4	23	17
	D-1/2/3/4/5	1, 2, 3, 4 (targeting site 2 and 5 in site D-1/3/4 mutants)	0	10	0
	Average				6

Supplementary Table 4. Frequency of misleading genotypes linked to large deletions

Targeted genomic sites	Mosaic large deletions (%)	Founders w/ deletion	Founders w/ mosaic large deletions	Number of alleles	Deletion size (bp)
C-1/2/3	10	20	2	2 3	10/ 338(w/ +800 bp) 6/ 446/ 2,300
D-1/2/3/4/5	33	9	3	2 3 4	0/ 23,339 240/ 22,284/ 23,987 118/ 22,147/ 23,287/ 23,520
D-1/2/3/4	100	1	1	2	2,504/ 15,506

'Gi dd`Ya YbUfmiBchY`%`HUF[YH]b[`]bX]j]Xi U`[Ybca]Wg]hYg`k]H `g]b[`Y`g[FB5 g"
a) Genomic site B (A STAT5 binding site in Socs2 promoter)



1) sgRNA sequence

sgRNA	sgRNA sequence	Co-injection (Yes/No)
sgRNA #1	5'-CCGCTTTCCAGGAAC TTTCC-3'	No
sgRNA #2	5'-GGTCACGTGAGCGGATTCC-3'	

2) Target site

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site

GCAAGAGTTAACCGTCTGTCCCCGGAGCCGAGTGTGCGCGCGACTTCCCAGCCCCGACGCGCCTCCCTCGTCCCCCGC
CTCCCGCCCCCTCGCGCCCCGCCCGCGCGGAGG**CCGCTTTCCAGGAAC TTTCCAGGAAT**CCCGCCTCACGTGAC
CGAGCCCCCGCCAGCGCTTTAAAGAGGCCCGCGCGCTAATGCGCAGGCTTCAAGGGAGCCGGGTGGAGAGAGCCG
GTGCGGGTGCTGGTGGTGGTGGCTGCGGGGTGGAAGCCTGCGACTCCGCCCTCCTCTGACCCTGCACCTCGGGGTGC
TCGGGAACAAGGAGAAGTCCCCAGCCAATGCCTATTAAGCCACCAGTACCTGCCCTGTGTCTGCAACAGCTG
AGCACCTCGGTCCC CGAATAGCAGCCCCCTCTTC

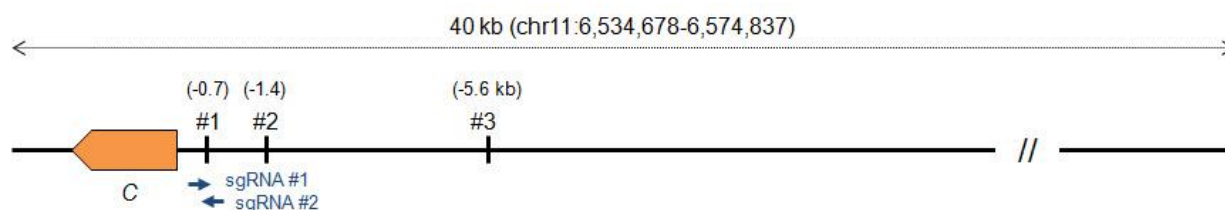
3) Mutated sequence

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **...** red dotted line: Cas9 cutting site (between 3rd and 4th nucleotide upstream of NGG site)
- **---**: deletion sequence, **red:** insertion or point mutation
- **+1:** putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- **- bp:** deletion size, **+ bp:** insertion size, **-:** unknown (only sequenced from one end)
- **A:** asymmetric deletion, **S:** symmetric deletion, **5':** 5'-deletion, **3':** 3'-deletion
- **Green:** repeat sequence aligned at one end (or microhomology), **Pink:** repeat sequence aligned at both ends

No.	Sequence (w/ sgRNA #1)	Mutation start point	Mutation size (bp)	Direction of deletion
WT	GGCCGCT TTCCAGGAAC T TTCCAGGAAT TCCGCCTCACGTGACCGCAG			
6995	GGCCGCT TTCCAGGAA -- TTCCAGGAAT TCCGCCTCACGTGACCGCAG	-3	-3	A(5')
6997	CCCGG----- TTCCAGGAAT TCCGCCTCACGTGACCGCAG	-27	-27	A(5')
6998	GGCCGCT TTCCAGGAA -- TTCCAGGAAT TCCGCCTCACGTGACCGCAG	-3	-3	A(5')
6999	GGCCGCT TTCCAGGAA -- TTCCAGGAAT TCCGCCTCACGTGACCGCAG	-3	-2	A(5')
7000	GGCCGCT TTCCAGGAA -- TTCCAGGAAT TCCGCCTCACGTGACCGCAG	-3	-2	A(5')
7005	GGCCGCT TTCCAGGAAC T--- AGGAAT TCCGCCTCACGTGACCGCAG	-1	-4	A(3')
7007	GGCCGCT TTCCAGGAA -- TTCCAGGAAT TCCGCCTCACGTGACCGCAG	-3	-2	A(5')
6980	CCCGG----- TTCCAGGAAT -----CCGCA	-29	-51	S (29:22)

No.	Sequence (w/ sgRNA #2)	Mutation start point	Mutation size (bp)	Direction of deletion
WT	GAGGCCGCTTTCCAGGAACTTTCCAGGAAATCCGCCTCACGTGACCGC			
93	GAGGCCGCTTTCCAGGAACTTT-----CTTTA	-33	-39	A(5')
94	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
95	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
96	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
124	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
126	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
127	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
128	CCTCG-----...-----CGCAG	-27	-64	S ^(37:27)
130	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
131	GAGGCCGCTTTCCAGGAACTTTCCAGGAA--AGCCTCAC...TGACCGC	-2	-3, +1	A(5')
134	GCTCG-----...-----CGC	-34	-88	A(3') ^(54:34)
137	GCCCC-----...-----CGCAG	-27	-58	A(3') ^(42:16)
143	GAGGCCGCTTTCCAGGAACT--TCCGCCTCACGTGACCGC	-1	-9	A(3')
145	CCCCG--...--TCCAGGAACTTT-----GCCTCACGTGACCGC	+18; -4	-43; -8	A(3') S
147	GAGGCCGCTTTCCAGGAACTTT-----GCCTCACGTGACCGC	-4	-8	S
148	GAGGCCGCTTTCCAGGAACTTTCCAGGAA-----	-	-	A(5')
149	CCCCG-----...-----CTAAT	-54	-85	A(5') ^(31:54)

b) Genomic site C-1 (A STAT5 binding site in *Wap* enhancer)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
C-1	sgRNA #1 5'-TGGAAGGCCACTTCCCAGAA-3'	No
	sgRNA #2 5'-GGCACAGTATGGGCCCTTCT-3'	

2) Target site

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- **Underline:** sgRNA #2 binding site

TTCTCCTGGTGCCTTTTCTGGAGTTTTCTAAAAATTCTTCTAAGAGTGTGGAGGCGCTTGCTCCATGTAGAGAGTGTG
GGGCATACATTGAAAAGGAGCTGCCTGTGCAGAGCACAT**TGGAAGGCCACTTTCCAGAA**GGGCCATACTGTGCCAA
GGGTGTGCATGGGCAGGAAGAGATGTCCTGCTGGGCACTGTGCCCCATTGTTCTCGGGACATTTCTTGATTTCTCTT
GCAAGGAAGGGGATGGGATGACCCAGAGTGACTGTGTGCTTACTGACTGTGAGCGCCAGCCTGTGGTGCAGGCCATA
CTCCTTTGTCTTCACTGGTGGCCCATGGCCTCTTTGAATACGAAGTGTGAGGGGAAGCCCTCTCAGGCTGTAACCTC
G

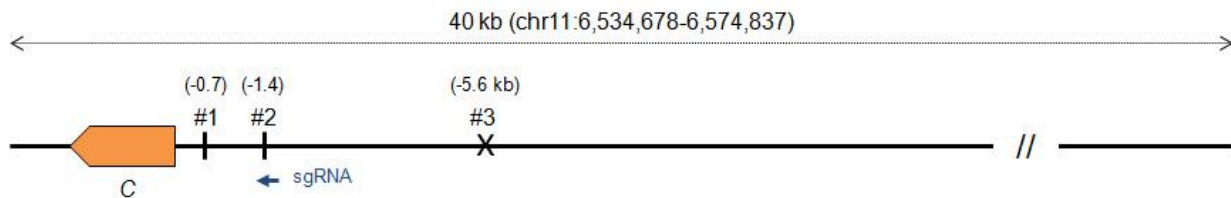
3) Mutated sequence

- **Blue**: Target sequence (GAS motif)
- **Yellow**: Target NGG sequence complemented to sgRNA
- **...** red dotted line: Cas9 cutting site (between 3rd and 4th nucleotide upstream of NGG site)
- **---**: deletion sequence, **red**: insertion or point mutation
- **+1**: putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- **- bp**: deletion size, **+ bp**: insertion size, **-**: unknown (only sequenced from one end)
- **A**: asymmetric deletion, **S**: symmetric deletion, **5'**: 5'-deletion, **3'**: 3'-deletion
- **Green**: repeat sequence aligned at one end (or microhomology), **Pink**: repeat sequence aligned at both ends

No.	Sequence (w/ sgRNA #1)	Mutation start point	Mutation size (bp)	Direction of deletion
WT	ATTGGAAGGCCAC TTCCCAGAA GGG CCCATACTGTGCCAAGGGTGTC			
76	ATTGGAAGGCCAC TTCCCAGAA TGG GCCATACTG...CCAAGGGTGTC	+4	+1	
77	ATTGGAAGGCCAC TTCCCAGAA G -----	+5	-	A(3')
78	ATTGGAAGGCCAC TTCCCAGAA -----	+4	-	A(3')
80	ATTGGAAGGCCAC TTCCC -----	+1	-	A(3')
81	ATTGGA AGC ----- GG GCCATACTGTGCCAAGGGTGTC	-10	-15	A(5')
84	ATTGGAAGGCCAC TTCCCAGAA -----	+4	-	A(3')
85	ATTGGAAGGCCAC ----- GGG CCCATACTGTGCCAAGGGTGTC	-6	-12	S
106	ATTGGAAGGCCA ----- GG GCCATACTGTGCCAAGGGTGTC	-7	-11	A(5')
107	ATTGGAAGGCCAC TTCCC ----- TACTGTGCCAAGGGTGTC	-1	-11	A(3')
108	ATTGGAAGGCCAC TT GG ----- GG AACTGTGCCAAGGGTGTC	-2	-11	A(3')
110	AT ----- ----- CATACTGTGCCAAGGGTGTC	-17	-25	A(5')

No.	Sequence (w/ sgRNA #2)	Mutation start point	Mutation size (bp)	Direction of deletion
WT	AGAGCACATTGGAAGGCCAC TTCCCAGAA GGG GCCATACTGTGCCAA			
87	AGAGCACATTGGAAGGCC CA ----- CA TACTGTGCCAA	-6	-15	A(3')
88	AGAGCACATTGGAAGGCCAC TTCC GG ----- TACTGTGCCAA	-8	-10	A(5')
91	AGAGCACATTGGAAGG ----- GG CCATACTGTGCCAA	-5	-17	A(3')
112	AGAGCACATTGGAAGGCC CA ----- GG A	-18	-27	A(5')
113	AGAGCACATTGGAAGGCCAC TTCC ----- GG GCCATACTGTGCCAA	-2	-7	A(3')
115	AGAGCACATTGGAAGGCCAC TTCC GG ----- GG GGGAA	-224	-225	A(5')
116	TGCCT ----- ... ----- TCTCT	-90	-122	A(5') ^(32:90)
118	AGAGCACATTGGAAGGCCAC TTCCCAGAA -----	-	-	A(5')

c) Genomic site C-2/3 (A STAT5 binding site in *Wap* enhancer)



** The sgRNA targeting site C-2 was injected into zygotes from mice carrying deletion of C-3.

1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
C-2	5'-CCTAAGACACAGGGCCTTCT-3'	No

2) Target site

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Underline:** sgRNA binding site

GGCTACTGCCATGCTTGTGTTAGTCAAGACTAAGTCATACCTATGCTCTGCCAATGCTCTGCTTCCACCCCTGTTCC
 CATTTCGCCTTTGCTGGAACAGAAGGAGGGCCACCAGGAAAGGGCTGGCTAGGGTTGCTTGGCTGCTTGAGT**TTCC**
CAGAAGGCCCTGTGTCTTAGGAGCCAGAGCTCTGTTCTCTCTGCATCTTGCTGGGGCCCATGCACTCTGTGTCTTAT
 GCCACTGGCTGTTATTCTTGCCTATGTACCA

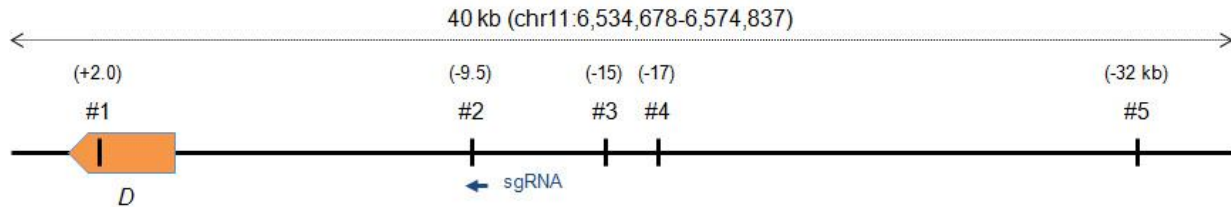
3) Mutated sequence

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **... red dotted line:** Cas9 cutting site (between 3rd and 4th nucleotide upstream of NGG site)
- **---** : deletion sequence, **red:** insertion or point mutation
- **+1:** putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- **- bp:** deletion size, **+ bp:** insertion size, **-:** unknown (only sequenced from one end)
- **A:** asymmetric deletion, **S:** symmetric deletion, **5':** 5'-deletion, **3':** 3'-deletion
- **Green:** repeat sequence aligned at one end (or microhomology), **Pink:** repeat sequence aligned at both ends

No.	Sequence (site C-2)	Mutation start point	Mutation size (bp)	Direction of deletion
WT	GGTTGCTTGGCTGCTTGAGT TTCC AGA AGGCCCTGTGTCTTAGGAG			
1398	GGTTGCTTGGCTGCTTGAGT TTCC ----- --- TGTGTCTTAGGAG	-6	-9	A(5')
1400	GGTTGCTTGGCTGCTTGAGT TTCC ----- CCC TGTGTCTTAGGAG	-3	-6	S
1401	GGTTGCTTGGCTGCTTGAGT TTCC AG AGGCCCTGTGTCTTAGGAG	+1	-1	A(3')
1403	GGTTGCTTGGCTGCTTGAGT TTCC AG AGGCCCTGTGTCTTAGGAG	+1	-1	A(3')
1404	GGTTGCTTGGCTGCTTGAGT TTCC ----- CC TGTGTCTTAGGAG	-4	-8	S
1413	GGTTGCTTGGCTGCTTGAGT TTCC ----- CCC TGTGTCTTAGGAG	-3	-6	S
1416	GGTTGCTTGGCTGCTTGAGT TTCC ----- --- TGTGTCTTAGGAG	-6	-9	A(5')
1417	GGTTGCTTGGCTGCTTGAGT TTCC AGA AGGCCCTGTGTCTTAGGAG	-1	-1	A(5')
1418	GGTTGCTTGGCTGCTTGAGT TTCC AGA ---CCCTGTGTCTTAGGAG	-3	-7	A(5')
1420	GGTTGCTTGGCTGCTTGAGT TTCC ----- --- CTGTGTCTTAGGAG	-5	-8	A(5')
1421	GGTTGCTTGGCTGCTTGAGT TTCC ----- CCC TGTGTCTTAGGAG	-3	-6	S
1422	GGTTGCTTGGCTGCTTGAGT TTCC ----- CCC TGTGTCTTAGGAG	-3	-6	S

No.	Sequence (site C-3)	Mutation start point	Mutation size (bp)
WT	GGTCGGAAGCCAGAAAGG TTCTAGGA ATGAAGACATTACTCTGAATT		
All	GGTCGGAAGCCAGAA----- --- ---TGAAGACATTACTCTGAATT	-6	-11

d) Genomic site D-2 (A CTCF binding site in *Wap* locus)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
D-2	5'-TAACTCATATGGGCCAGTGG-3'	No

2) Target site

- **Blue:** Target sequence (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Underline:** sgRNA binding site

GCTGGATAGCTCTTGCCAGGTGTTTCTTCTGCTGAGAAATGATGAGCGGCCCTGACACCTCAAAGCCGCTTTGCTCCT
 TTGCACATGGATCTCAGAAAAAGCCTCTGAGTTTCCCATATGAACTCAGGAGACCCAACCCAACCCAACCCAACCCA
 ACCCAACCCAACCCAACCCAACCCAACCAAACACCCAAACCGAGAGAAATAACAAAACGGCTCACAGCTCCCTCCACT
 GGCCCATATGAGTTACTGCAGCTGCTTTATCAAAAAACAGCTTCTGGGTCCACAGCAGAGCCAGGCTTTCTTCCAAC
 CTTTCTAGGTCAAAGCCAAGTCTGAGCTCCAGTCTTTTCTTCTGCTTTCTCCACGATACATGTCATCCCTGTTGA
 CCCAGCTTGTTTCAGCCACACCGGTTTCTGATGGCCCTCTCCTGCAGGAACCTC

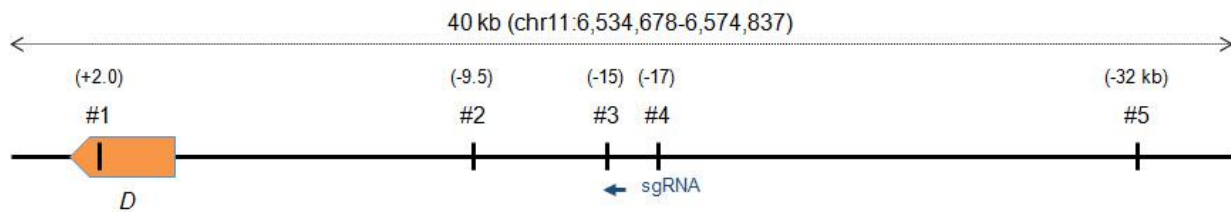
3) Mutated sequence

- **Blue:** Target sequence (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **...** red dot line: Cas9 cutting site (between 3rd and 4th nucleotide upstream of NGG site)
- **---**: deletion sequence, **red**: insertion or point mutation
- **+1**: putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- **- bp**: deletion size, **+ bp**: insertion size, **-**: unknown (only sequenced from one end)
- **A**: asymmetric deletion, **S**: symmetric deletion, **5'**: 5'-deletion, **3'**: 3'-deletion
- **Green**: repeat sequence aligned at one end (or microhomology), **Pink**: repeat sequence aligned at both ends

No.	Sequence	Mutation start point	Mutation size (bp)	Direction of deletion
WT	ACAAAACGGCTCACAGCTCCCTCCACTGGCCCATATGAGTTACTGCA			
9011	ACAAAACGGCTCACAGCTCCCTCCACTGGCCCATATGAGTTACTGCA	+2	+1	
9012	ACAAAACGGCTCACAGCTCCCTCCA---CCATATGAGTTACTGCA	-2	-5	A(3')
9013	ACAAAACGGCTCACAGCT---CCATATGAGTTACTGCA	-2	-12	A(3')
9016	ACAAAACGGCTCACAGCTC---CTGGCCCATATGAGTTACTGCA	+4	-6	A(3')
9018	ACAAAACGGCTCACAGCTCCCTCCA-TA;TGGCCCATATGAGTTACTG	+1	-1, +2	A(3')
9019	ACAAAACGGCTCACAGCTCCCTCCA-----TATGAGTTACTGCA	-5	-8	A(5')
9020	ACAAAACGGCTCACAGCTCCCTCCA---CCATATGAGTTACTGCA	-2	-5	A(3')
9021	ACAAAACGGCTCACAGCTCCCTCCACTTGGCCCATATGAGTTACTGCA	+3	+1	
9022	ACAAAACGGCTCACAGCTCCCTCCACTGGCCCATATGAGTTACTGCA	+3	+1	

9024	ACAAAACGGCTCACAGCTCCCTCCA-----TATGAGTTACTGCA	-5	-8	A(5')
9025	ACAAAACGGCTCACAGCTCCCTCC-----CCATATGAGTTACTGCA	-2	-6	A(3')
9026	ACAAAACGGC-----GGCCATATGAGTTACTGCA	+2	-17	A(3')
9027	CCAAC-----...-----CCATATGAGTTACTGCA	-2	-68	A(3')
9028	ACAAAACGGCTCACAGCTCCCTCCAC-----CCATATGAGTTACTGCA	-2	-4	S
9031	ACAAAACGGCTCACAGCTCCCTCA-----TATGAGTTACTGCA	-5	-8	A(5')
9033	ACAAAACGGCTCACAGCTCCCTCA-----TATGAGTTACTGCA	-5	-8	A(5')
9034	ACAAAACGGCTCACAGCTCCCTCCA-----ATGAGTTACTGCA	-6	-9	A(5')
9035	ACAAAACGGCTCACAGCTCCCTCCCTGCCCCATATGAGTTACTGCA	+4	-1	A(3')
9037	ACAAAACGGCTCACAGCTCCCTCCATCTGGCCCCATATGAGTTACTGC	+3	+1	
9039	ACA---AAAC-----AAAAC	-32	-53	A(5') ^(21:32)
9040	ACAAAACGGCTCACAGCTCCCTCCATCT-----GGCCCCATATGAGTTACT	-2	+1, -2	A(5')
9041	ACAAAACGGCTCACAGCTCCCTCCA-----TATGAGTTACTGCA	-5	-8	A(5')
9042	ACAAAACGGCTCACAGCTCCCTCCA-TGCCCCATATGAGTTACTGCA	+3	-1	A(3')
9044	ACAAAACGGCTCACAGCTCCCTC-----TGCCCCATATGAGTTACTGCA	+3	-3	A(3')
9045	ACAAAACGGCTCACAGCTCCCTCC-----CATATGAGTTACTGCA	-3	-7	S
9046	CCAAC-----...-----TGAGTTACTGCA	-7	-73	A(3')
9047	CCAAC-----...-----AGCTC	-70	-177	S ^(76:101)
9048	ACAAAACGGCTCACAGCTCCCTCCA-----CCAATATGAGTTACTGCA	-2	-5	A(3')
9049	ACAAAACGGCTCACAGCTCCCTCCCTGCCCCATATGAGTTACTGCA	+4	-1	A(3')
9050	ACAAAACGGCTCACAGCTCCCTCCATCTGGCCCCATATGAGTTACTGC	+3	+1	
9052	ACA-----...-----CCATATGAGTTACTGCA	-2	-27	A(3')
9053	ACAAAACGGCTCACAGCTCC-----ATATGAGTTACTGCA	-4	-11	A(3')
9054	ACAAAACGGCTCACAGCTCC-----CCATATGAGTTACTGCA	-2	-9	A(3')
9056	ACAAAACGGCTCACAGCTCCCTCC-----CCATATGAGTTACTGCA	-2	-6	A(3')
9139	ACAAAACGGCTCACAGCTCCCTCC-----ATATGAGTTACTGCA	-4	-8	S
9140	ACAAAACGGCTCACAGCTCCCTCC-----CCATATGAGTTACTGCA	-2	-6	A(3')
9141	ACAAAACGGCTCACAGCTCCCTCCACATGGCCCCATATGAGTTACTGC	+2	+1	
9142	ACAAAACGGCTCACAGCTCCCTCCA-----CCATATGAGTTACTGCA	-2	-5	A(3')
9143	ACAAAACGGCTCACAGCTCCCTCC-----...-----TCGG	-41	-46	A(5')
9144	ACAAAACGGCTCACAGCTCCCTCCATA-TGGCCCCATATGAGTTACTG	+1	+2, -1	A(3')
9145	ACAAAACGGCTCACAGCTCCCTCCATA-TGGCCCCATATGAGTTACTG	+1	+2, -1	A(3')
9146	ACAAAACGGCTCACAGCTCCCTCC-----ATATGAGTTACTGCA	-4	-8	S
9147	ACAAAACGGCTCACAGCTCCCTCC-----ATATGAGTTACTGCA	-4	-8	S
9149	ACAAAACGGCTCACAGCTCCCTCCATCTGGCCCCATATGAGTTACTGC	+3	+1	
9150	ACAAAACGGCTCACAGCTCC-----CCATATGAGTTACTGCA	-2	-9	A(3')
9151	ACAAAACGGCTCACAGCTCCCTCCACTGGCCCCATATGAGTTACTGC	+3	+1	
9152	ACAAAACGGCTCACAGCT-----...-----ACAGC	-35	-45	A(5')
9153	ACAAAACGGCTCACAGCTCCCTCCA-----CCAATATGAGTTACTGCA	-2	-5	A(3')

e) Genomic site D-3 (A CTCF binding site in *Wap* locus)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
D-3	5'-CTTCTGGTGGGCACAGGTAC-3'	No

2) Target site

- **Blue:** Target region (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Underline:** sgRNA binding site

CCGGCCCCAGGTTTGTTTTTTCAGGACCCTCTTGGTGGCTCACAACTTTCTGGAACATCATAGTTGCAAGGGGATCTGAC
 ATCCTCTTCTGGTGGGCACAGGTACTGCATGCATGTGGTACACTGTCATACATGTAGACAAATCAACCAGATACATA
 AGTTAGAAATGAAGAACTGGGCTGGTGAGATGGCTCAGTGGGTAAGAGTACCCGACTGCTCTTC

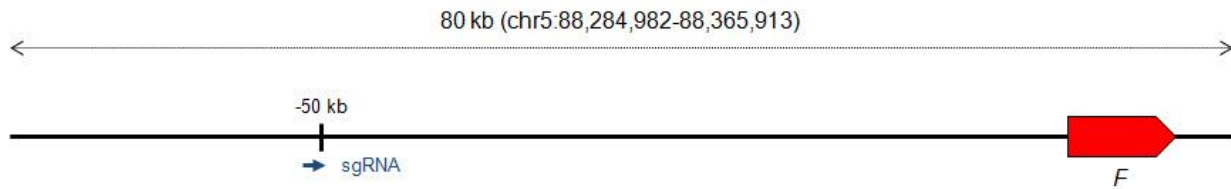
3) Mutated sequence

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **...** red dotted line: Cas9 cutting site (between 3rd and 4th nucleotide upstream of NGG site)
- **---**: deletion sequence, **red**: insertion or point mutation
- **+1:** putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- **- bp:** deletion size, **+ bp:** insertion size, **-:** unknown (only sequenced from one end)
- **A:** asymmetric deletion, **S:** symmetric deletion, **5':** 5'-deletion, **3':** 3'-deletion
- **Green:** repeat sequence aligned at one end (or microhomology), **Pink:** repeat sequence aligned at both ends

No.	Sequence	Mutation start point	Mutation size (bp)	Direction of deletion
WT	TAGTTGCAAGGGGATCTGACATCCTCTTCTGGTGGGCACAGGTACTG			
1257	TAGTTGCAAGGGGATCTGACAT---...-----GAGATG	-96	-102	A(5')
1260	TAGTTGCAAGGGGATCTGAC---...-----GAAACT	-82	-90	A(5')
1263	TAGTTGCAAGGGGAT---...-----GGGCTG	-82	-93	A(5')
1269	TAGTTGCAAGGGGATCTGACA---...-----GACAAA	-52	-59	A(5')
1275	TAGTTGCAAGGGGATCTG---...-----GGGCACAGGTACTG	-5	-15	A(3')
1277	TAGTTGCAAGGGGATCTGACAT---...-----GGTGGGCACAGGTACTG	-2	-8	A(3')
1278	TAGTTGCAAGGGGATCTCAC---...-----CATGTC	-123	-131	A(5')
1283	TAGTTGCAAGGGGATCTGACAT---...-----AACCAC	-178	-184	A(5')
1284	CCCTCCTCCT---...-----GGCACAGGTACTG	-6	-258	A(3')
1285	TAGTTGCAAGGGGATCTGACATCCTCTT---...-----AACTAA	-295	-295	A(5')
1286	TAGTTGCAAGGGGATCTGACATC---...-----ATGAAG	-281	-286	A(5')
1289	TAGTTGCAAGGGGATCTGACA---...-----TACAGT	-229	-236	A(5')

9040	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9041	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9042	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9044	TTCTGCAAGTATGTTAAGTCTA CCTCAT -----	-	-	A(5')
9049	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9051	TTCTGCAAGTATGTTAAGTCTA CCTCAT -----	-	-	A(5')
9053	TTCTGCAAGTATGTTAAGTCTA CCTCAT CT TACCACAGACTGAGTTA	-3	+1	
9054	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9056	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9139	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S
9140	TTCTGCAAGTATGTTAAGTCTA CC ----- CAGACTGAGTTAC	-6	-10	A(5')
9143	TTCTGCAAGTATGTTAAGTCTA CC ----- CAGACTGAGTTAC	-6	-10	A(5')
9145	TTCTGCAAGTATGTTAAGTCTA CCT ----- CAGACTGAGTTAC	-6	-9	A(5')
9147	TTCTGCAAGTATGTTAAGT TACC --- ACAGACTGAGTTAC	-5	-9	S

g) Genomic site F (An NFIB binding site in *Csn3* enhancer)



1) sgRNA sequence

sgRNA sequence	Co-injection (Yes/No)
5'- AACAACTAACTCTTGAAT-3'	No

2) Target site

- **Blue:** Target sequence (NFIB binding motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Underline:** sgRNA binding site

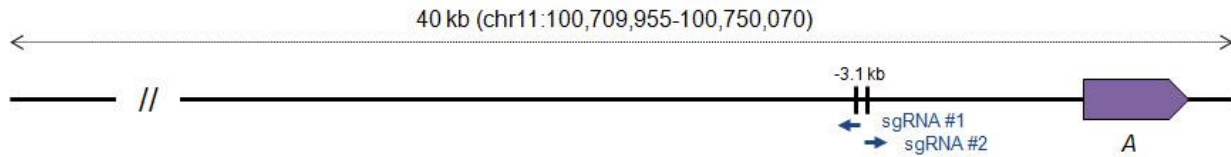
CTACATGCTTAGCGTTTGTTCATTTAAGAAAAATGATTAAGATTAATTTTCAGTGATGGGCAGCAGTTATTCATAAT
CAAAGCCCTGATGGTAAAAGCCTGCTGAGCTGTAATAAATGACCTATAAACTGAAGCCGTGTCCTTCTGAGGAGCTC
TCTGGAATCATTGATGTGTAGATAACATAGATGGAGGGGGAAAAAACAACAACCTAACTCTTGAAT **TGGCAAG**
CTATCTAAATAACTGGGACAACCTGCAGTTCAGTPTTGCCTCTGGGTACACAGGTGGCTGGCCAGTGGCCAGTCCTTT
TCAGCTCTGGCTGGCAATGTCATTGTCCTGAGGGAATTCAGGAAGGCAGTGGCTTCGGTTGGGTTAGATGGACTTCT

3) Mutated sequence

- **Blue:** Target sequence (NFIB binding motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **...** red dot line: Cas9 cutting site (between 3rd and 4th nucleotide upstream of NGG site)
- **---** : deletion sequence, **red:** insertion or point mutation
- **+1:** putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- **- bp:** deletion size, **+ bp:** insertion size, **-:** unknown (only sequenced from one end)
- **A:** asymmetric deletion, **S:** symmetric deletion, **5':** 5'-deletion, **3':** 3'-deletion
- **Green:** repeat sequence aligned at one end (or microhomology), **Pink:** repeat sequence aligned at both ends

No.	Sequence	Mutation start point	Mutation size (bp)	Direction of deletion
WT	CAACAACCTAACTCTTGA ^{AAT} TGGCAAGCTATCTAAATAACTGGGAC			
1	TCCTT-...-----GCTATCTAAATAACTGGGAC	-78	-87	A(5')
2	CAACAACCTAACTCTT ^{TT} GGCAAGCTATCTAAATAACTGGGAC	-2	-6	A(3')
4	CAACAACCT-----...-ATACTAAA	-9	-449	A(3')
13	CAACAACCTAACTCT ^{TT} GGGTATAT	-3	-385	A(3')
14	CAACAACCTAACT-----...-TGTCATTG	-5	-109	A(3')
18	CAA ^{CAA} -----GCTATCTAAATAACTGGGAC	-12	-21	S ^(12:9)
20	CAACAACCTAACTCTT ^{GA} CTTCTCAC	+1	-159	A(3')
21	CAA-----...-GCTTTATA	-15	-585	A(3')
26	CAACAACCTAACTCTT-----...-GTGGAAT	-2	-364	A(3')
31	CAACAACCTAACTCT ^{TT} GTTCTCTC	-3	-199	A(3')

Gi dd`Ya YbHfmiBcH`&`HUf[Yh]b[`]bX]j]Xi U`[Ybca]Wg]hYg`k]h `a cfY`h Ub`cbY`g[FB5`r`
a) Genomic site A (A STAT5 binding site in *Stat5* enhancer)



1) sgRNA sequence

sgRNA	sgRNA sequence	Co-injection (Yes/No)
sgRNA #1	5'-TCCTTTGTTTCTGGGAATAG-3'	Yes
sgRNA #2	5'-TGAAGAGTGTAGAGACCAG-3'	

2) Target site

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site

TGTGTGCCTAGAGTACCTGACCTGCTGGTCTCTCATCTGGGTAGGTGGGCATGGCAGCTGGCAAAGAAAGCAAGGTT
 CCTAGCCTCCACTGCACCACCCAGGCTAGTCCACAGCCTCCCTCCCATC**CCTCTATTCCAGAAACAAGGACAGT**
 CACTGTGCCTCCGGCAGGGTAGCCTTGTGTTCTCTTGGTGAGTTCTCTGTAAATTGACT**TTCTCTGAAGAGTGT**TAGA
 GATGTTCCAGGGGACAGAGATGGAAAGGGGGCTTACCCCAATCCCAAGGCTCCTTACCCTTCTCTGCATTTACAGA
 GAACACACACACACACACACACACACACACACACACACACATTCCCCGACTCTGGCCTATTCTGGCTTCAT
 GATC

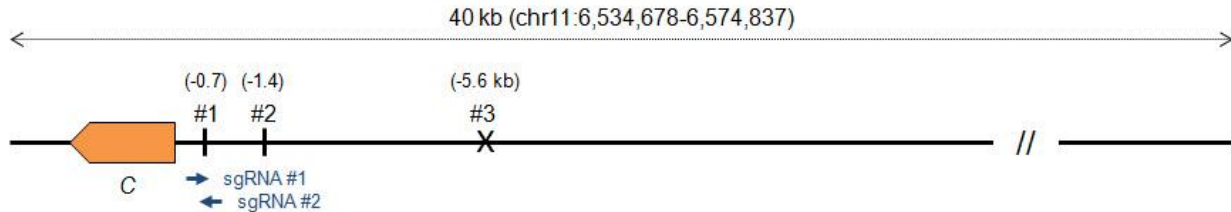
3) Mutated sequence

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Mutation start point	Mutation size (bp)
WT	<i>sgRNA #1</i> 66 bp <i>sgRNA #2</i> 14 bp CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGAAGAG ...CAG GGGACAG		
7001	CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGA ...AG-----ACAG	-1 (sgRNA #2)	-7
7009	CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGAAGAG ...-AGGGGACAG	+1 (sgRNA #2)	-1
7011	CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGA ...GACCC...GGGACAG	-1 (sgRNA #2)	+1
7012	CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGAAGAG ...-AGGGGACAG	+1 (sgRNA #2)	-1
7014	CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGAAGAG ...-AGGGGACAG	+1 (sgRNA #2)	-1
7020	CAT CCT CTA-----AC...AC TTCTCTGAAGAG ...CAGGGGACAG	-9 (sgRNA #1)	-9
7079	CAT CCT CTA TTCCAGAAAC ...AC TT -----...-----AC	-19 (sgRNA #2)	-47
7080	CAT CCT CTA TTCCAGAAAC ...AC TTCTCTGAAGAG TG-...-----ACAG	-8 (sgRNA #2)	-14
7082	CAT CCT CTA TTCCAGAA ...AC TTCTCTGAAGAG ...CAGGGGACAG	-3 (sgRNA #1)	+1
7090	CAT CCT CTA TTCCAGAAAC ...AC TTCT ...AG- TGTT CC...GGGACAG	-5 (sgRNA #2)	-1, +4
7094	CAT CCT CTA TTCCAGAAAC ...AC TTCTCT ...AGA-----AGGGGACAG	-3 (sgRNA #2)	-4
7098	CAT CC ----- AGAAAC ...AC TTCTCTGAAGAG ...CAGGGGACAG	-5 (sgRNA #1)	-9

7103	CATC CCT CTA TTCCCAGAA AC...AC TTCTCTGAAG ...AC-AG AGG ACAG	+1 (sgRNA #2)	-1, +1
7104	CATC CCT CTA TTCCCAGAA AC...AC TTCTCTGAAGA ...GA-... --- ...-TG	-4 (sgRNA #2)	-14
7106	CATC CC ----- AGAA AC...AC TTCTCTGAAG AG...CAG GGG ACAG	-5 (sgRNA #1)	-9

b) Genomic site C-1/3 (STAT5 binding sites in *Wap* enhancer)



**** sgRNAs targeting C-1 were injected into zygotes from mice carrying deletion of C-3.**

1) sgRNA sequence

Target site		sgRNA sequence	Co-injection (Yes/No)
C-1	sgRNA #1	5'-TGGAAGGCCACTTCCCAGAA-3'	Yes
	sgRNA #2	5'-GGCACAGTATGGGCCCTTCT-3'	

2) Target site

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site

TTCTCCTGGTGCTTTTCTGGAGTTTTCTAAAAATTCTTCTAAGAGTGTGGAGGCGCTTGCTCCATGTAGAGAGTGTCC
GGGCATACATTGAAAAGGAGCTGCCTGTGCAGAGCACAT**TGGAAGGCCACTTCCCAGAA**GGGCCCATACTGTGCCAA
GGGTGTCATGGGCAGGAAGAGATGTCCTGCTGGGCACCTGTGCCCATTTGTTCTCGGGACATTTTCCTTGATTTCTCTT
GCAAGGAAGGGGATGGGATGACCCAGAGTGACTGTGTGCTTACTGACTGTGCAGCGCCAGCCTGTGGTGCAGGCCATA
CTCCTTTGTCTTCACTGGTGGCCCATGGCCTCTTTGAATACGAAGTGTGAGGGGAAGCCCTCTCAGGCTGTAACCTC
G

3) Mutated sequence

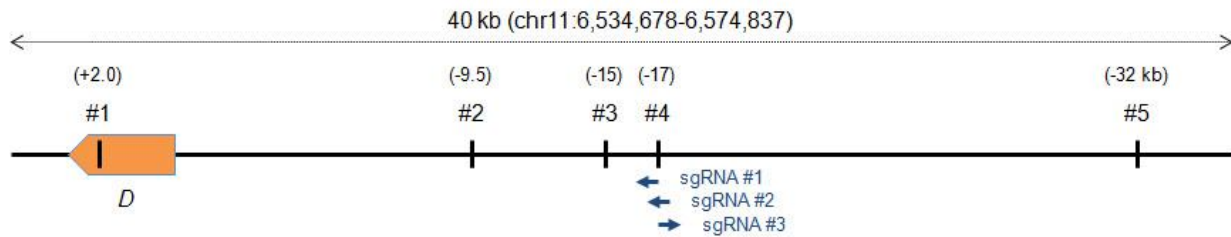
- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **---**: deletion sequence, **red:** insertion or point mutation
- **+1:** putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- **- bp:** deletion size, **+ bp:** insertion size, **-:** unknown (only sequenced from one end)

No.	Sequence (site C-1)	Mutation start point	Mutation size (bp)
WT	CACATTGGAAGGCCAC <i>sgRNA #1</i> TTCCCAGAA <i>sgRNA #2</i> GGG CCCATACTGTGCCAAGGGT		
3325	CACATTGGAAGGCCAC TTCCC AA AG GGGCCCATACTGTGCCAAGGGT	+1 (sgRNA #2)	-1, +1
3326A	CACATTGGAA----- --- ----- --- GGCCCATACTGTGCCAAGGGT	+4 (sgRNA #2)	-16
3326B	CACATTGGAAAG----- --- ----- --- GCCCATACTGTGCCAAGGGT	+5 (sgRNA #2)	-15
3326C	CACATTGGAAGGCCAC TTCCC AA G.. GGGCCCATACTGTGCCAAGGGT	-1 (sgRNA #2)	+1

3327A	CACATTGGAAGGCCA--- --- --- --- ---CCATACTGTGCCAAGGGT	-5 (sgRNA #1)	-14
3327B	CACATTGGAAGGCCAC TTCCCAAGAGGG CCATACTGTGCCAAGGGT	+1 (sgRNA #2)	+2
3328A	CACATTGGAA----- --- --- --- ---GGCCATACTGTGCCAAGGGT	+4 (sgRNA #2)	-16
3328B	CACATTGGAAGG----- --- --- --- ---GCCATACTGTGCCAA-GGT	+12 (sgRNA #1) +22 (sgRNA #1)	-15 -1
3329A	CACATTGGAAGGCCA--- --- --- --- ---CCATACTGTGCCAAGGGT	+7 (sgRNA #2)	-14
3329B	CACATTGGAAGGCCAC TTCCAGAGGG CCATACTGTGCCAAGGGT	+3 (sgRNA #2)	-1
3330	CACATTGGAAGGCCAC TTCCCAAG..GGG CCATACTGTGCCAAGGGT	-1 (sgRNA #2)	+1

No.	Sequence (site C-3)	Mutation start point	Mutation size (bp)
WT	GGTCGGGAAGCCAGAAGG TTCTAGGAAT TGAAGACATTACTCTGAATT		
All	GGTCGGGAAGCCAGAA----- --- ---TGAAGACATTACTCTGAATT	-6	-11

c) Genomic site D-4 (2) (A CTCF binding site in *Wap* locus)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
D-4	sgRNA #1 5'-GACTTAACATACTTGCAGAA-3'	Yes
	sgRNA #2 5'-AACTCAGTCTGTGGTAGATG-3'	
	sgRNA #3 5'-CTGAGGCAGTCTCGTTGGAT-3'	

2) Target site

- **Blue:** Target sequence (CTCF binding region)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- **Underline:** sgRNA #2 binding site
- **Bold and Underline:** sgRNA #3 binding site

ACATTTTAAAAAGAAGACAGAATTGGATAGTGAGTTGTAGGCTGGGTCCAGAAACATTCCTTTATGTAATTCT**CCCTT**
CTGCAAGTATGTTAAGTCTA**CT**CATCTACCACAGACTGAGTTACTTAAATGTTATTCCAGCAGGGGGCGCCAAATC
ATCAGGAATGCCT**CTGAGGCAGTCTCGTTGGAT****GGG**AGAAGAAACTAATCTCTGATGCTTCTTGAGTGTGTGTGTGT
GTGTGTGTGTGTGTGTGTATGTGTGATAGGTCTGGTTAGTAT

3) Mutated sequence

- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)
-

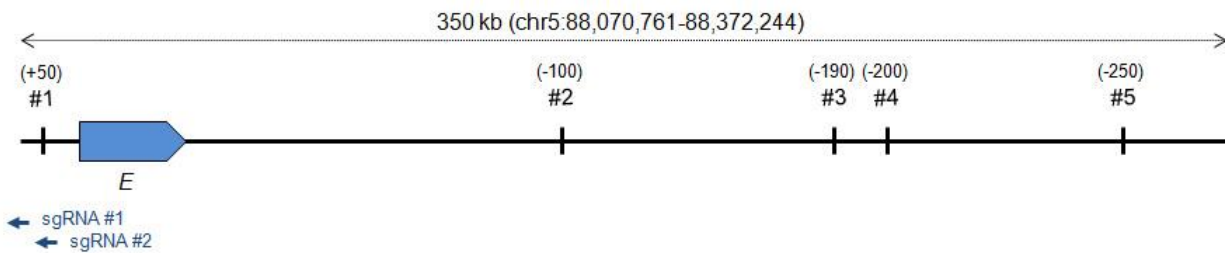
CAACCCAACCCAACCCAACCCAACCAAAACACCCAACCGAGAGAAATAACAAAACGGCTCACAGCTCCCTCCACTGGC
 CCATATGAGTTACTGCAGCTGCTTTATCAAAAAACAGCTTCTGGGTCCACAGCAGAGCCAGGCTTTCTTCCAACCTT
 TCTAGGTCAAAGCCAAGTCCTGAGCTCCAGTTCTTTTCTTGTCTTCTCCACGATACATGTCATCCCTGTTGACCC
 AGCTTGTTTCAGCCACACCGGTTTCTGATGGCCCTCTCCTGCAGGAACCTCTCGCTGAGGTCAAGTGACTACA

3) Mutated sequence

- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Mutation start point	Mutation size (bp)
WT	<i>sgRNA #1</i> 283 bp <i>sgRNA #2</i> ATATGAACTCAGGAGACCCAACC...CCTCTCCTGCAGGAACCTCTCGC		
2392A	AAG---...-----ATA	-1,627	-2,504
2392B	AGC---...-----GTG	-1,084	-15,506

e) Genomic site E-1 (A CTCF binding site in *Csn1s1* locus)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
E-1	sgRNA #1 5'-CAGTTCAGTGGTTAATCAAG-3'	Yes
	sgRNA #2 5'-ACACTAATTTGACCAGTAG-3'	

2) Target site

- **Blue:** Target sequence (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site

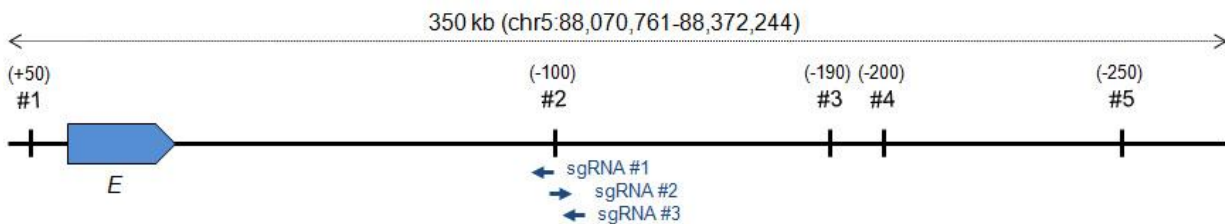
CCTGTTGCTGGACCTTCTTTTCTCAGGCTCCTCTCCATTTCCATCCCTGTAATTCTTTTCAGACAGGAACATTATGG
 GTCAGAATTTTGATAGTTCACAATTTATGCAAAAGAGGTGAGCATTGTGGAGCTTGCCAATTGATTAACCACTGAAC
 TGGACATGCAAGATGTGGCTGCCTCTACTGGTCAAAATTAGTGTTCCACAGCTGGATGGTTCAGTCCCTTTTTTGGG
 GGGGGGGGGGTTGAAATTAGACATTTTTTAAATTTACATTTCAAATTTTATCCCCTTTTCTGGTTTCCCCCATATC
 CCTGCTCCCTCCACCTGCCTCACTGCCCTAGCATTTCCCTATATTAGAGCATTGAGCCTCCACAAAACCAAGGGCTT
 CTCTTCC

3) Mutated sequence

- **Blue:** Target sequence (CTCF binding motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Mutation start point	Mutation size (bp)
WT	<i>sgRNA #1</i> 20 bp <i>sgRNA #2</i> AGCTTG CCA CTTGATTAAC...ATGTGGCTG CCCT CTACTGGTCAAAAT		
8960	AGC...G... CCA CTTGATTAAC...ATGTGGCTG CCCT CTACTGGTCAAAAT	+10 (sgRNA#1)	+10
8966	GTGGA- --- -----TTAA...ATGTGGCTG CCCT C- --- ...--AAAATT	+11 (sgRNA#1) +2 (sgRNA#2)	-13 -8
8968	AGCTTG CCA CT- --- -GAACT...ATGTGGCTG CCC - --- ...--TCACAG	+1 (sgRNA#1) +4 (sgRNA#2)	-12 -21
8969	AGCTTG- --- -----CCAC...ATGTGGCTG CCCT CTACTGGTCAAAAT	+6 (sgRNA#1)	-12
8970	CACA- --- -----TGG...ATGTGGCTG CCCT CT- --- ...--AAAATT	+39 (sgRNA#1) +1 (sgRNA#2)	-54 -7
8972	AGCTTG CCA CTTGATTAAC...ATGTGGCTG CCCT CT- --- GGTCAAAAT	+1 (sgRNA#2)	-3
8973	AGCTTG CCA CTTGA- --- -ACTGAA...GGCTG CCC - --- ...--TTTTTG	-3 (sgRNA#1) +4 (sgRNA#2)	-6 -44

f) Genomic site E-2 (A CTCF binding site in *Csn1s1* locus)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
E-2	sgRNA #1 5'-GATACAGATTTTAAGTGCCT-3'	Yes
	sgRNA #2 5'-GTTTTATTTTGTCCAGGAGA-3'	
	sgRNA #3 5'-TTATTTGATTGCTGGCTAAG-3'	

2) Target site

- **Blue:** Target sequence (CTCF binding region)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site
- **Bold and Underline:** sgRNA #3 binding site

CTTTTCCACTGAGACCCAACCATACATTCCAAGTAGGAGGGAATGGGATCCAATGGCAGTGAACAGAGACTGAGTCA
 GCCGCTGTTGCACTTGTTAGGGGAGTTATATGAACATTAACTACGTATTTGCTACAGTTGTGTACAGGGTCTAGGC
 CCAGATCCTGCATGTTTTCTGGTTGGTGGTGT**CCT**AGGCACTTAAATCTGTATCTGAAGTAGAAACGTTTTAGTGT

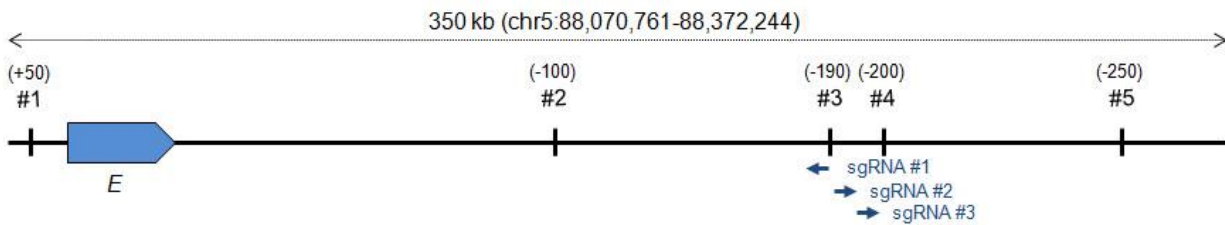
TTTATTTTGTCCAGGAGATGGCAGAGTTGTGATGCAAATACTGTACATTAGTTAGTAAATCTCACCTCTTAGCCAGC
 AATCAAATAATAGGCCTTTTTGCCCCCCCCCCACAGCAGAACAAGTTATTTAATGTATAACTCCTTGCAATAGTAA
 CCTTTCATTTTGAGGATTTTCGTTTATGAGCAATGAAGTTGCATCAATCTGATCCCCCTCTTTCCTCTCCCACCTCAA
 ATCCAAGACTGTTTATTCTTTAATCAATTGTTTCATAGAAAGCATATACAGGCATGTATGTATGTATGTATGTATGT

3) Mutated sequence

- **Yellow**: Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red**: insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Start point	Deletion size (bp)
WT	<i>sgRNA #1</i> 48 bp <i>sgRNA #2</i> 31 bp <i>sgRNA #3</i> TGGTGT CCT AGGCAC...AGGAGATGGCAGAGT...ATCTCA CCT CTTAGC		
467	TGGTGT CCT AGG---...---AGATGGCAGAGT...ATCTCA CCT CTTAGC	-1 (sgRNA#1)	-54
469	GTCTA---...---TCTG...AGA---...---GT...ATCTCA CCT CTTAGC	+41 (sgRNA#1) +4 (sgRNA#2)	-56 -7
470	TGGTGT CCT AGGCA---...---...TGATG...TCTCA CCT CTTAGC	-3 (sgRNA#1)	-66
480	TGGTGT C ---...---TGTA...AGATGGCAGAGT...GTTA---...---TTT	+5 (sgRNA#1) +16 (sgRNA#3)	-16 -40
485	TGGTGT CC ---...---...---...---TTGC	+4 (sgRNA#1)	-139
488	TGGTGT CC ---...---...---...---CTCT	+4 (sgRNA#1)	-253
489	TGGTGT CCTA ---...---...---...---TTGC	+2 (sgRNA#1)	-232

g) Line E-3 (A CTCF binding site in *Csn1s1* locus)



1) sgRNA sequence

Target site	sgRNA sequence	Co-injection (Yes/No)
#3	sgRNA #1 5'-ACAAATTGTCTGCTGAAGGC-3'	Yes
	sgRNA #2 5'-CCTCCTCTAGAGCGGAAACA-3'	
	sgRNA #3 5'-AAATATTGTTCCGGTGCCTCA-3'	

2) Target site

- **Blue**: Target sequence (CTCF binding site)
- **Yellow**: Target NGG sequence complemented to sgRNA
- **Bold**: sgRNA #1 binding site
- Underline: sgRNA #2 binding site
- **Bold and Underline**: sgRNA #3 binding site

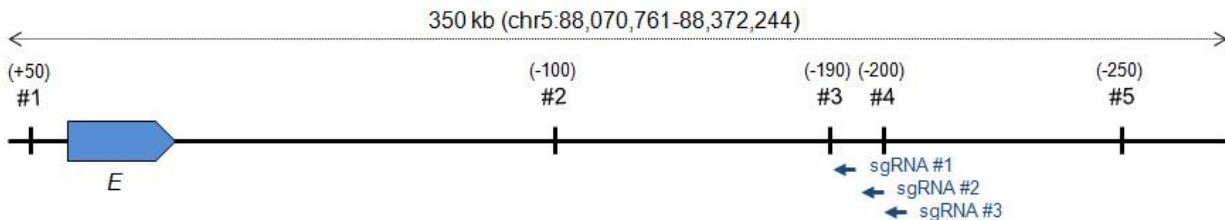
CAGATGGCGATGTTTCTGGCTACGTACCCAGAAACCTTCTCCAGACCCGAAATTACAGAGGCTGTCAAGACACTGGT
 CTCAATAGTAACACACATTTCTCATTTGCCTGTTTGATCCTTCAAATTTAAGTGACTCTAACCATTTATGATGACGAT
 TCTTTCAAGCA **CCTGCCTTCAGCAGACAATTTGTTGGACCTTCCTGTAGGTGGTAGTGAAGGGCTTCTAGATTTTGC**
ACCTCCTCTAGAGCGGAAACAAGGTAATTTCAAGTGCTAGAA**AAATATTGTTTCGGTGCCTCAAGG**TCAGCACTTACTA
 GCAGGATAGACTGAGCTAACTGTTGTTTCTGGGTATCATTTTGGTCCCATA

3) Mutated sequence

- **Blue:** Target sequence (CTCF binding region)
- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Start point	Deletion size (bp)
WT	<i>sgRNA #1</i> 72 bp <i>sgRNA #2</i> 25 bp <i>sgRNA #3</i> CAAGCACCTGCCTTC...GAAACAAGGTAATTT...GCCTCAAGGTCAGCA		
9427	GCTG-...-----...-----...-----TCAAGGTCAGCA	+107 (sgRNA#1)	-225
9428	TTAA-...-----...-----...-----...-CTTA	+43 (sgRNA#1)	-123
9429	CAAGCACCT...CAGCA...-----...-----...-CTTA	-8 (sgRNA#1)	-123
9430	CAAGCACCTGCCTTC...GTAGT...-----...-----...-CTTA	-38 (sgRNA#2)	-90
9432	CAAGCACCTGCCTTC...GAA-----...-----...-CTCAAGGTCAGCA	+1 (sgRNA#2)	-39
9433	CAAGCACCT...TAGA-----...-----...-----...-GGTA	-24 (sgRNA#2)	-156
9434	C-----...-TTTTG...AGGTAATTT...GCCTCAAGGTCAGCA	+11 (sgRNA#1)	-65
9435	CAAGCACCT...AGCA-----...-----...-----...-CTTA	-8 (sgRNA#1)	-123
9436	CAAGCACCTGCCTTC...CCTC-----...-----...-AAAGTCAGCA	-10 (sgRNA#2)	-52
9482	CAAGCACCTGCCTTC...GCAC-----...-----...-----...-TTAC	-16 (sgRNA#2)	-69
9483	CAAGCACCTGC...AGCG-----CAAGGTAATTT...GCCTCAAGGTCAGCA	-3 (sgRNA#2)	-4
9484	CAAGCACCT...AGCAG-----...-----...-----...-AAAT	-9 (sgRNA#1)	-195
9485	CAAGCACCTG-...-TTTGT...ACAAGGTAATTT...GCCTCAAGGTCAGCA	+2 (sgRNA#1)	-14
9486	CAAGCACCTG-----...-----...-TAATTT...GCCTCAAGGTCAGCA	+2 (sgRNA#1)	-86
9487	CAAGCACCTGCCTTC...GAA-CAAGGTAATTT...GCCTCAAGGTCAGCA	+1 (sgRNA#2)	-1
9489	CAAGCACCTGCCTTC-...-----...-TTT...GCCTCAAGGTCAGCA	-4 (sgRNA#1)	-84
9503	CAAGCACCTGCC...GCGGA-----GGTAA...TCGGT-...-----CAGCA	-1 (sgRNA#2) -3 (sgRNA#3)	-5 -10

h) Line E-4 (A CTCF binding site in *Csn1s1* locus)



1) sgRNA sequence

Target site		sgRNA sequence	Co-injection (Yes/No)
#4	sgRNA #1	5'-AGGGCGCTGTTTGCCACAA-3'	Yes
	sgRNA #2	5'-TCTCTGAGCACTACCACACG-3'	
	sgRNA #3	5'-GGCACAGTGAGTCCCATTG-3'	

2) Target site

- **Blue:** Target sequence (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site
- **Bold and Underline:** sgRNA #3 binding site

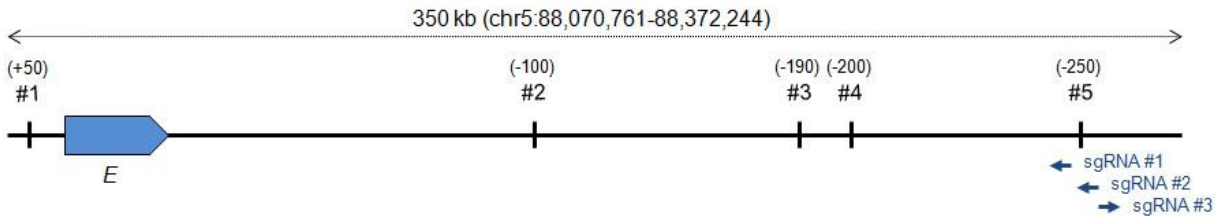
GTGCCTGGCAAACACAGAAGTGGATGCTCACAGTCAGCTACTGGATGGAACATAGGGCCCCCAATGGAGGAGCTAGA
 GAAAGTACCCAAGGAGCTAAAGGGGTCTGCAACCCTATTTGGTGGAAACAATATGAAGTTTGCATTTTTATAAGGG
 GAATAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAAGACCAATTGTGGCAAACAGCGCCTCGTGTGGT
 AGTGCTCAGAGACGTGCATCACTAATCAACAACCTTGGAGCTGTTGAAGGAGCTGATACTTAGCCAGGTTT**GAGGGT**
 GACAGCTAAGCAGCAC**CCT**CAAATGGGACTCACTGTGCCATTTTCGGTACAAAAACTGACTTAGGTCCTTAAAATG
 CATATCAGTTCAACATTCTAAATTGCTGCACACAGTCACTCAGTAGAGGCTCTCATGTATGATAAAAATCTACTTCT
 TTTCTTTTTCTTTTTCTTTTTCTTTTTCTTTTTCTTTTTCTTTTTCTTTTTCTTTTTCTTTTCAATTAGCAATGT

3) Mutated sequence

- **Blue:** Target sequence (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Start point	Deletion size (bp)
WT	<i>sgRNA #1</i> 4 bp <i>sgRNA #2</i> 89 bp <i>sgRNA #3</i> AAAGACCAATTGTGG...CAGCG CCT CGTGTG...CAGCAC CCT CAAATGG		
9490	AAAGACCAATT-----...----- CCT -----...----- CCT ...-TATTC	+1 (sgRNA#1)	-381
9491	AAAGACCAATTG---...----- CCT -----...----- CCT ...-GGACT	-1 (sgRNA#1)	-126
9492	AAAGAC C - T ...TTGCAA...GCG CCT CGT A ...G-...-C...A- CCT ...-CTGTG	+4 (sgRNA#1) -3 (sgRNA#1) -1 (sgRNA#2) -4 (sgRNA#2) +7 (sgRNA#3)	-6 +5 +1 -6 -17
9494	AAAGACCAATTGTGGC-...----- CCT -----...----- CCT ...-ACAAA	-5 (sgRNA#1)	-146
9495	AAAGACCAATTG---...----- CCT -----...----- CCT ...-AAAAT	-1 (sgRNA#1)	-241
9496	AAAGACCAATT-----...----- CCT -----...----- CCT ...-ATTCA	+1 (sgRNA#1)	-382
9497	AAAGA-----...----- CCT -----...----- CCT ...-CCATT	+6 (sgRNA#1)	-145
9498	AAAGAC C -----...----- CCT ...-TAGTG...----- CCT CAAATGG	+4 (sgRNA#1)	-27
9499	AAAGACCAATT-----...----- CCT -----...----- CCT ...-TCGGT	+1 (sgRNA#1)	-145
9500	AAAGAC-----...----- CCT -----...----- CCT ...-ATGCA	+5 (sgRNA#1)	-180
9501	AAAGACCAATTG---...----- CCT -----...----- CCT ...-AGGCT	-1 (sgRNA#1)	-224

i) Line E-5 (A CTCF binding site in *Csn1s1* locus)



1) sgRNA sequence

Target site		sgRNA sequence	Co-injection (Yes/No)
#5	sgRNA #1	5'-TCCAGACTGGAAGTATGATCAA-3'	Yes
	sgRNA #2	5'-ATGCTCAAATTACCTAAAG-3'	
	sgRNA #3	5'-GCTCTGATTGCTTGAATTGA-3'	

2) Target site

- **Blue:** Target sequence (CTCF binding site)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- Underline: sgRNA #2 binding site
- **Bold and Underline:** sgRNA #3 binding site

GACAGTATTTATAGCCTGAGGAATCCAAGGCAATATTTAGCAGGGAAGATGTGAAAAGGGTAATGTTTGAAGTATAA
 ATGGAATTTTCACTTTCTGGTGTGAGCCGTGTAAGTACCAATTTGCATACCGTTTTAATATCTGAATTATATT
 TTGTTT**CCT**TTGATCAGTTCCAGTCTGGAGAAAGTCTAAGTCCAAGAGCT**CCT**CTTTAGGTAATTTTGGCATAA
 AGATGTCTGGAGCTCTGATTGCTTGAATTGA**AGG**TACCAAAGTGTGATGTTACTTTAACATAGATTACAGAGACATG
 AAAGTATAAAAATCACAGTGACATCGTAAATAATACATGATAATCATGAATAAGAAGACAAGGAAGAAAACATAACA
 TACATGGAATATTGCTTTAACACGATTAATGAGAAATTAGAAACAATCTGTTAACTAGAAATTTGTCATTTAAAATA

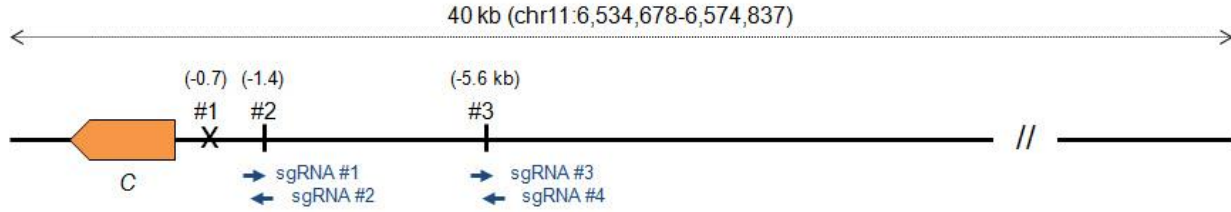
3) Mutated sequence

- **Blue:** Target sequence (CTCF binding motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence	Start point	Deletion size (bp)
WT	<i>sgRNA #1</i> 31 bp <i>sgRNA #2</i> 41 bp <i>sgRNA #3</i> TTGTTT CCT TTGATC...GAGCT CCT CTTTAG...AATTGA AGG TACCAA		
9517	TTGTTT CCT TTG---...----- --- -----...--TTGA AGG TACCAA	-1 (sgRNA#1)	-92
9519	TTGTT --- ---...-CCAGT...CT CCT CTTT...AA AA ATTGA AGG TACCAA	+7 (sgRNA#1) -2 (sgRNA#3)	-14 +2
9520	TTGTTT CCT TTG---...----- --- -----...--TTGA AGG TACCAA	-1 (sgRNA#1)	-92
9521	TTGTTT CCT TTG---...----- --- -----...--TTGA AGG TACCAA	-1 (sgRNA#1)	-92
9522	TTGTTT CCT TTG---...----- --- -----...--TTGA AGG TACCAA	-2 (sgRNA#1)	-92
9523	TTGTTT CCT TTGATCA-...----- --- -----...-----GA AGG TACCAA	-5 (sgRNA#1)	-90
9525	TTGTTT CC -...----- --- -----...----- --- ...-TGTC	+4 (sgRNA#1)	-111

Supplementary Note 3. Targeting more than one genomic site with several sgRNAs.

a) Genomic site C-1/2 and C-1/2/3 (STAT5 binding sites in *Wap* enhancer)



**** sgRNAs targeting site C-2 and C-3 were injected into zygotes from mice carrying deletion of C-1.**

1) sgRNA sequence

Target site		sgRNA sequence	Co-injection (Yes/No)
C-2	sgRNA #1	5'-GGCTGCTTGAGTTTCCCAGA-3'	Yes
	sgRNA #2	5'-CCTAAGACACAGGGCCTTCT-3'	
C-3	sgRNA #3	5'-TCGGGAAGCCAGAAGTTCT-3'	Yes
	sgRNA #4	5'- TCTTCATTCTAGAACCTTC-3'	

2) Target site

a. Site C-2

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #1 binding site
- **Underline:** sgRNA #2 binding site

GGCTACTGCCATGCTTGTGTTAGTCAAGACTAAGTCATACCTATGCTCTGCCAATGCTCTGCTTCCACCCCTGTTCC
 CATTTCGCCTTTGCTGGAACAGAAGGAGGGCCACCAGGAAAGGGCTGGCTAGGGTTGCTT**GGCTGCTTGAGTTTCC**
CAGAAGGCCCTGTGTCTTAGGAGCCAGAGCTCTGTTCTCTCTGCATCTTGCTGGGGCCCATGCACTCTGTGTCTAT
 GCCCACTGGCTGTTATTCTTGCCTATGTACCA

b. Site C-3

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- **Bold:** sgRNA #3 binding site
- **Underline:** sgRNA #4 binding site

ACTCAAGGGAACCTAGCCTTGAGGGCCAGGGTGACACTGGTCAGTTGAGGACATGTGTTTCTGTGTGGTGGGGAAGT
 GTAGGGAGGACAGAGAGGGCCCTGGCTGAACAAGGAGTTCATGAGGTGCACACATCTCCTCAGAGCCTGCAGGCCAGG
TCGGGAAGCCAGAAGTTCTAGGAATGAAGACATTACTCTGAATTCACCAGGCCAGCGGCAAGGGGCATGGGGTTC
 TTATGCCCTGGGGTGTCTGGTTCTTTGGCCTGCCAGTCTTGAGCCTGTGTGCTTCCAGTATGCGTAGGCTCTTTCC
 TGCCCCATCAACT

3) Mutated sequence

- **Blue:** Target sequence (GAS motif)
- **Yellow:** Target NGG sequence complemented to sgRNA
- --- : deletion sequence, **red:** insertion or point mutation
- +1: putative Cas9 cutting site (3 nucleotide upstream of the nearest target NGG sequence)
- - bp: deletion size, + bp: insertion size, -: unknown (only sequenced from one end)

No.	Sequence (site C-1)	Mutation start point	Mutation size (bp)
WT	ATTGGAAGGCCAC TTCCAGA GGGCCCACTACTGTGCCAAGGGTGTC		

All	ATTGGAAGGCC----- --- ---CATACTGTGCCAAGGGTGTG	-8	-16
-----	---	----	-----

No.	Sequence (site C-2 & C-3)	site	Mutation start point	Mutation size (bp)
WT	<i>C-2 (sgRNA #1, #2)</i> GTTGCT...TTGAGT TTCCC AGAAGGCC...CGGGAAGCCAGAAGG TTCTAGG AATGA <i>C-3 (sgRNA #3, #4)</i>			
251	GTTGC-...----- --- ---GCCC...CGGGAAGCCA----- AGG AATGA	C-2 C-3	-2 -6	-24 -9
253	GTTGC-...----- --- ---GCCC...CGGGAAGCCAGAAG----- --- GAATGA	C-2 C-3	-2 -2	-24 -7
254	GTTGCT...TTGAGT TTCCC ACAGAAGGC...GGT-...----- --- TTCTAGG AATGA	C-2 C-3	+2 -16	+2 -14
255	GTTGCT...GG----- --- ---C...CGGGAAGCCAG--GG TTCTAGG AATGA	C-2 C-3	-5 -5	-23 -2
256	GTTGCT...TTGAG-- --- ---AGAAGGCC...CGGGAAGCCA----- TTCTAGG AATGA	C-2 C-3	+4 -6	-6 -5
257	GTTGCT...TTGAGT TTCCC AGAAGGCC...CGGGAAGCCAGAA TG- TCTAGG AATGA	C-2 C-3	-3	+1 /-1
258	GTTGC-...----- --- ---GCCC...CG----- --- GAATGA	C-2 C-3	-2 -14	-24 -18
259	GTTGCT...TTGAG-- --- ---GTGTC...CGGGAAGCCAGAA----- --- AATGA	C-2 C-3	-7 -3	-16 -9
260	GTTGC-...----- --- ---CC...CGGGAAGCCA----- --- GAATGA	C-2 C-3	-4 -6	-26 -11
261	GTTGCT...GGC-...----- --- ---GGCCC...GG----- --- GAATGA	C-2 C-3	-1 -22	-18 -27
262	GTTGCT...TTGA-- --- ---CAGAAGGCC...CGG----- TTCTAGG AATGA	C-2 C-3	+5 -13	-6 -12
263	GTTGC-...----- --- ---GCCC...CGGGAAGCCA----- TTCTAGG AATGA	C-2 C-3	-2 -6	-24 -6
264	GTTGCT...TTGAGT TTCCC AGAAGGCC...CGGGAAGCCAGAA----- --- GAATGA	C-2 C-3	-3	-8
265	GGCC...GCTTG----- --- ---AAGGCC...AGGC----- --- ...-CAC	C-2 C-3	-8 -21	-10 -338, +800
266	GTTGCT...TTGAG-- --- ---CCC...CGGGAAG----- AGG AATGA	C-2 C-3	-3 -9	-12 -12
267	TGTC-...----- --- ---...GAAG...TCGG-... --- ...-AG	C-2 C-3	-464 -13	-2,300 -18
268	GTTGCT...TTGA-- --- ---AGGCC...GGTCGGAAGC-...-- TTCTAGG AATGA	C-2 C-3	+1 -8	-10 -7
269	GAAGCT...TTGAGT TTCCC AGAAGGCC...GGTCGGAAGCCAG-...-- --- GAATGA	C-2 C-3	-5	-10
526	GTTGC-...----- --- ---GCCC...GCCAG GTACA...CCTA TTCTAGG AATGA	C-2 C-3	-2 -198	-24 +196
527	GTTGCT...GGC-...----- --- ---CC...GGTCGGG...CAGAAG TTCTAGG A-TGA	C-2 C-3	-4 +8	-21 -1
528	GTTGC-...----- --- ---GCCC...GGTCGGG...CAGAAG-- --- GAATGA	C-2 C-3	-2 -1	-24 -6

Supplementary Reference

1. Sung YH, *et al.* Highly efficient gene knockout in mice and zebrafish with RNA-guided endonucleases. *Genome Res* **24**, 125-131 (2014).