

Table S4. Mutations introduced by the CRISPR/Cas9 system

gRNA	donor	#	mutation
<i>ade6</i>	no	1	1 bp insertion
		2	2 bp deletion
		3	2 bp deletion
		4	1 bp insertion
		5	2 bp deletion
		6	2 bp deletion
		7	2 bp deletion
		8	3 bp deletion

gRNA	donor	#	mutation	<i>W104A</i> mutation freq.
<i>swi6</i>	oligo 15 ds gRNA#2	1	1 bp deletion	
		2	mixture of two unknown insertions (100~ bp, 200~ bp)	
		3	5 bp deletion	
		4	8 bp deletion	
		5	<i>W104</i> mutation	1/10 (10 %)
		6	16 bp deletion	TBZ sensitivity 46 % (19/49)
		7	1 bp insertion	
		8	141 bp insertion (salmon DNA)* ¹	
		9	1 bp insertion	
		10	1 bp deletion	

gRNA	donor	#	mutation	<i>W104A</i> mutation freq.
<i>swi6</i>	oligo 20 Fw gRNA #2	1	<i>W104A</i> mutation	
		2	199 bp insertion (salmon DNA)* ²	
		4	<i>W104A</i> mutation	
		5	<i>W104A</i> mutation	4/8 (50 %)
		6	1 bp deletion	TBZ sensitivity 50 % (14/24)
		7	1 bp deletion	
		8	62 bp insertion (no significant similarity)* ³	
		9	<i>W104A</i> mutation	

gRNA	donor	#	mutation	<i>W104A</i> mutation freq.
<i>swi6</i> gRNA #2	oligo 20 Rv	1	1 bp deletion	
		2	<i>W104A</i> mutation + 1 bp deletion	
		3	<i>W104A</i> mutation	
		4	<i>W104A</i> mutation	
		5	73 bp insertion* ⁴	5/10 (50 %)
		6	<i>W104A</i> mutation	TBZ sensitivity 63 % (20/32)
		7	5 bp deletion	
		8	1 bp deletion	
		9	<i>W104A</i> mutation	
		10	<i>W104A</i> mutation	
	oligo 20 Fw +Rv	1	<i>W104A</i> mutation	
		2	5 bp deletion	
		3	5 bp deletion	
		4	<i>W104A</i> mutation	
		5	<i>W104A</i> mutation	
		6	6 bp deletion	8/14 (57 %)
		7	<i>W104A</i> mutation	TBZ sensitivity 68% (27/40)
		8	<i>W104A</i> mutation	
		9	<i>W104A</i> mutation	
		10	<i>W104A</i> mutation	
		11	5 bp deletion	
		12	1 bp deletion	
		13	mixture of <i>wt</i> and ~1000 bp insertion	
		14	<i>W104A</i> mutation	
	oligo 20 ds	1	<i>W104A</i> mutation	
		2	<i>W104A</i> mutation	
		3	<i>W104A</i> mutation	
		4	2 bp deletion	
		5	<i>W104A</i> mutation + 1 bp deletion	7/10 (70 %)
		6	8 bp deletion	TBZ sensitivity 56 % (27/48)
		7	<i>W104A</i> mutation	
		8	<i>W104A</i> mutation	
		9	<i>W104A</i> mutation	
		10	<i>W104A</i> mutation	

gRNA	donor	#	mutation	<i>W104A</i> mutation freq.
<i>swi6</i> gRNA#2	oligo 25 Fw	1	<i>W104A</i> mutation	
		2	<i>W104A</i> mutation	
		3	<i>W104A</i> mutation	5/7 (71 %)
		4	<i>W104A</i> mutation	TBZ sensitivity 54 % (13/24)
		5	<i>W104A</i> mutation	
		6	1 bp insertion	
		7	1 bp deletion	
	oligo 25 Rv	1	132 bp insertion* ⁵	
		2	<i>W104A</i> mutation	
		3	1 bp insertion	
		4	11 bp deletion	4/8 (50 %)
		5	<i>W104A</i> mutation	TBZ sensitivity 58 % (14/24)
		6	6 bp deletion	
		7	<i>W104A</i> mutation	
		8	<i>W104A</i> mutation	
	oligo 25 Fw + Rv	1	<i>W104A</i> mutation	
		2	<i>W104A</i> mutation	
		3	<i>W104A</i> mutation	
		4	<i>W104A</i> mutation	8/9 (89 %)
		7	mixture of <i>wt</i> and ~200 bp insertion	
		8	<i>W104A</i> mutation	TBZ sensitivity 50 % (12/24)
		9	<i>W104A</i> mutation	
		10	<i>W104A</i> mutation	
		11	<i>W104A</i> mutation	
	oligo 25 ds	1	<i>W104A</i> mutation	
		2	<i>W104A</i> mutation	
		3	<i>W104A</i> mutation	
		4	<i>W104A</i> mutation	8/8 (100 %)
		5	<i>W104A</i> mutation	TBZ sensitivity 38 % (15/40)
		6	<i>W104A</i> mutation	
		7	<i>W104A</i> mutation	
		8	<i>W104A</i> mutation	

gRNA	donor	#	mutation	<i>W104A</i> mutation freq.
<i>swi6</i>	PCR fragment	1	<i>W104A</i> mutation	
gRNA#2	575 bp	2	<i>W104A</i> mutation	
		3	<i>W104A</i> mutation	
		4	<i>W104A</i> mutation	
		5	<i>W104A</i> mutation	
		6	<i>W104A</i> mutation	
		7	<i>W104A</i> mutation	16/16 (100 %)
		8	<i>W104A</i> mutation	TBZ sensitivity 50 % (16/32)
		9	<i>W104A</i> mutation	
		10	<i>W104A</i> mutation	
		11	<i>W104A</i> mutation	
		12	<i>W104A</i> mutation	
		13	<i>W104A</i> mutation	
		14	<i>W104A</i> mutation	
		15	<i>W104A</i> mutation	
		16	<i>W104A</i> mutation	
<i>swi6</i>	PCR fragment	1	1 bp deletion	
gRNA#5	575 bp	2	<i>W104A</i> + 5 bp deletion	
		3	1 bp deletion	
		4	1 bp deletion	
		5	<i>W104A</i> mutation	1/14 (7 %)
		6	<i>W104A</i> mutation + 15 bp deletion* ⁶	
		7	1 bp deletion	TBZ sensitivity 61 % (22/36)
		8	<i>W104A</i> mutation + 1 bp deletion	
		9	39 bp deletion	
		10	3 bp deletion	
		11	1 bp deletion	
		12	<i>W104A</i> mutation + 191~ bp salmon DNA insertion* ⁷	
		13	13 bp deletion	
		14	3 bp deletion	

gRNA	donor	#	mutation	<i>S604A</i> mutation freq.
<i>mrcI</i>	oligo 25 Fw	1	2 bp deletion	
		2	<i>S604A</i> mutation	
		3	2 bp deletion	
		4	<i>S604A</i> mutation	
		5	<i>S604A</i> mutation	6/10 (60 %)
		6	2 bp deletion	HU sensitivity 23/24 (96 %)
		7	2 bp deletion	
		8	<i>S604A</i> mutation	
		9	<i>S604A</i> mutation	
		10	<i>S604A</i> mutation	
	oligo 25 Rv	1	<i>S604A</i> + 1 bp deletion	
		2	1 bp deletion	
		3	2 bp deletion	
		4	<i>S604A</i> mutation	
		5	2 bp deletion	2/10 (20 %)
		6	<i>S604A</i> mutation	HU sensitivity 20/24 (83 %)
		7	2 bp deletion	
		8	2 bp deletion	
		9	2 bp deletion	
		10	2 bp deletion	
	oligo 25 Fw + Rv	1	<i>S604A</i> mutation	
		2	<i>S604A</i> mutation	
		3	<i>S604A</i> mutation	
		4	<i>S604A</i> mutation	
		5	<i>S604A</i> mutation	9/10 (90 %)
		6	<i>S604A</i> mutation	HU sensitivity 23/24 (96 %)
		7	<i>S604A</i> mutation + mixture of indels* ⁸	
		8	<i>S604A</i> mutation	
		9	<i>S604A</i> mutation	
		10	<i>S604A</i> mutation	
	oligo 25 ds	1	<i>S604A</i> mutation	
		2	<i>S604A</i> mutation	
		3	<i>S604A</i> mutation	
		4	<i>S604A</i> mutation	
		5	<i>S604A</i> mutation	10/10 (100 %)
		6	<i>S604A</i> mutation	HU sensitivity 20/24 (83 %)
		7	<i>S604A</i> mutation	
		8	<i>S604A</i> mutation	
		9	<i>S604A</i> mutation	
		10	<i>S604A</i> mutation	

gRNA-Cas9	donor	#	mutation	<i>S604A</i> mutation freq.
<i>mrc1</i> (<i>LEU2</i>)	oligo 25 ds	1	1 bp deletion	
		2	<i>S604A</i> mutation	
		3	<i>S604A</i> mutation	
		4	<i>S604A</i> mutation	
		5	2 bp deletion	7/10 (70 %)
		6	<i>S604A</i> mutation	HU sensitivity 39/46 (85 %)
		7	<i>S604A</i> mutation	
		8	<i>S604A</i> mutation	
		9	<i>S604A</i> mutation	
		10	2 bp deletion	
<i>mrc1</i> (<i>ura4⁺</i>)	oligo 25 ds	1	<i>S604A</i> mutation	
		2	<i>S604A</i> mutation	
		3	2 bp deletion	
		4	2 bp deletion	
		5	<i>S604A</i> mutation	8/12 (67 %)
		6	<i>S604A</i> mutation	HU sensitivity 46/47 (98 %)
		7	800~bp insertion	
		8	<i>S604A</i> mutation	
		9	<i>S604A</i> mutation	
		10	<i>S604A</i> mutation	
		11	<i>S604A</i> mutation	
		12	1 bp deletion	

gRNA	donor	#	mutation	mutation freq.
<i>stn1</i>	none	1	wt	6/8 (75 %)
		2	wt	
		3	2 bp deletion	
		4	2 bp deletion	
		5	1 bp deletion	
		6	3 bp deletion	
		7	1 bp deletion	
		8	1 bp deletion	
gRNA	donor	#	insertion	knock-in freq.
<i>stn1</i>	oligo 98 bp Fw	4	101~ bp insertion (salmon DNA)* ⁹	4/24 (17 %)
		13	210~ bp insertion	
		20	460~ bp insertion	
		9	2 × Flag	
		14	2 × Flag	
		18	2 × Flag	
		21	2 × Flag +1 bp deletion	
		24	2 × Flag	
	oligo 98 bp Rv	2	2 × Flag + 8 bp insertion	0/24 (0 %)
		24	77 bp insertion* ¹⁰	
	oligo 98 bp Fw+Rv	1	mixture of two unknown insertions (100~ bp, 200~ bp)	9/11 (82%)
		2	3 × Flag* ¹¹	
		3	2 × Flag* ¹²	
		4	2 × Flag + 3 bp and 8 bp deletions* ¹³	
		5	2 × Flag	
		6	100~ bp insertion	
		7	2 × Flag + 4 bp exchange* ¹⁴	
		8	2 × Flag	
		9	3 × Flag	
		10	2 × Flag	
		11	2 × Flag	
		14	3 × Flag	
		19	3 × Flag	
		24	200~ bp insertion	

gRNA	donor	#	insertion	knock-in freq.
<i>stn1</i>	oligo 98 bp ds	1	2 × Flag	8/9 (89 %)
		4	400~ bp insertion	
		5	3 × Flag	
		17	2 × Flag	
		18	3 × Flag	
		19	3 × Flag	
		20	2 × Flag	
		21	2 × Flag	
		22	2 × Flag + 1 bp deletion	
		24	2 × Flag	

gRNA	donor	#	mutation	mutation freq.
<i>reb1</i>	none	1	2 bp deletion	6/8 (75%)
		2	<i>wt</i>	
		3	2 bp deletion	
		4	1 bp deletion	
		5	<i>wt</i>	
		6	1 bp deletion	
		7	1 bp deletion	
		8	1 bp deletion	

(g)GTGAACAGGGAGTACAGGAGAGGGCTCAGAACGCACCCTTGTTGGGGCCCCAGTGTTGAGGATCAGCGGGG
AGGAGATGTTGTTGCCACCTCACCACTGGGGGCGCCCGTCAGGAAGTCCAGTACCCAGTTGCACAGGGC
GGGGTCGAGACCCAGGGTCTCTCTCTCTCTCTCTCTCTCTCTCT~

mrc1 knock-in

*⁸ mixtures of the *mrc1-S604A* + insertion/deletion at the position near the 3' of the *mrc1-S604A* donor oligonucleotides

1. 3 bp deletion

5'-GTTCAAAACAGTCAACCAAGCGCTGCTCAACTCACAATCGTCGACGCTACATA---TCAACCTC~ -3'

2. insertion of the *mrc1-S604A* donor oligonucleotides (2 bp deletions)

5'-GTTCAAAACAGTCAACCAAGCGCTGCTCAACTCACAATCGTCGACGCT ↓ GTTCA--CAGTCAAC~ -3'

stn1 knock-in

stn1⁺ wt 5'-GGGAATGACCCTAAT ↓ AAATGCTAGAAAATGAAAATCAACTAACG-3'

*⁹ *stn1* 5'-----AATGCTAGAAAATGAAAATCAACTAACG-3'

101~ bp insertion (Partial sequence (black letter) has similarity with salmon DNA)

~GGAATAGACCTAATTGGACAAATGTCAACTATTTTAAACCATGAAAATACAGTTATAAACAGTTTTCCATAAAC
AAGGTCTAAAAACGTGTTTCGATGCAAA

*¹⁰ *stn1*⁺ 5'-GGGAATGACCCTAAT ↓ AAATGCTAGAAAATGAAAATCAACTAACG-3'

74 bp insertion (no significant similarity sequence)

CGTGATATTTTAAACAGTCCATAAAGCACTCAGGACGGCCCTGACAGAGAGAGGGCCGTAGTAGGGTGCTTCC
A

*¹¹ *stn1*⁺ 5'-GGGAATGACCCTAAT ↓ - - ATGCTAGAAAATGAAAATCAACTAACG-3'

3 × Flag insertions (1 × Flag insertion (+ 3 bp (GAC(Asp) : grey letter)) between 2 × Flag sequences)

ATGGACTACAAGGACGACGATGACAAGGATTACAAAGATGACGACGATGACAAGGATTACAAAGATGACGAC
GATAAG

*¹² *stn1*⁺ 5'-GGGAATGACCCTAAT ↓ - - ATGCTAGAAAATGAAAATCAACTAACG-3'

2 × Flag insertion (donor DNA)

ATGGACTACAAGGACGACGATGACAAGGATTACAAAGATGACGACGATAAG

*¹³ *stn1*⁺ 5'-GGGAATGACCCTAAT ↓ - - - - CTAGAAAATGAAAATCAACTAACG-3'

2 × Flag insertion + 3 bp deletion and 8 bp deletions near the 5' of the *stn1*⁺

ATGGACTACAAGGACGACGATGACAAGGATTACAAAGAT - - - GACG - - - - -

*¹⁴ *stn1*⁺ 5'-GGGAATGACCCTAAT ↓ - - ATGCTctagAATGAAAATCAACTAACG-3'

2 × Flag insertion + 4 bp in-frame exchange (small letter) in the *stn1*⁺

ATGGACTACAAGGACGACGATGACAAGGATTACAAAGATGACGACGATAAG

underline: PAM, ↓ : cleavage site, - : deletion, underline: short-homology at the junction of the insertion, shadow: introduced point mutations, underline: first ATG, dot-lines: Flag sequences