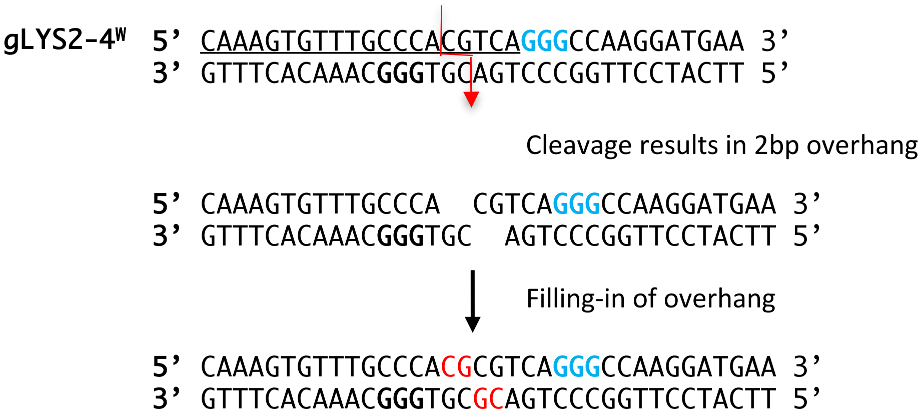
Supplemental Information Appendix

Suppl. Figure 1. 2bp overhang results in templated insertions



Suppl. Figure 2. Pol4 dependence of Indels from Cas9 gRNAs g*MAT*α-1, g*MAT*α-2 gRNA g*MAT***a**-1 and g*MAT***a**-2

A.

gMATα-1

5’ CAAAATGCAGCACGGAATATGGG 3’

Mutation	Indel	Frequency
CACGGAAATATGGG	+1	8
CACGGATATATGGG	+1	6
CACGG-ATATGGG	-1	4
CACGGAAAATATGGG	+2	1

pol4Δ

Mutation	Indel	Frequency
CACGG-ATATGGG	-1	9
CACGGA--------	-8	8
CACGGAA------	-6	2
CACG-AATATGGG	-1	1

gMATα-2

5’ TATTACTCACAGTTTGGCTCCGG 3’

Mutation	Indel	Frequency
GTTTGGCCTCCGG	+1	10
GTTTGGGCTCCGG	+1	4
GTTTAGGGCTCCGG	+2	4
GTTTGGCCCTCCGG	+2	1
GTTTGGGGCTCCGG	+2	1

pol4Δ

Mutation	Indel	Frequency
GTTTGG-TCCGG	-1	18
GTTTGGGCTCCGG	+1	2
GTTTG-CTCCGG	-1	4
GTTTGGC--CGG	-2	1

B.

gMAT**a**-1

5’ TTTATAAAATTATACTGTTGCGG 3’

Mutation	Indel	Frequency
ATACTGGTTGCGG	+1	4
ATACTGTGTTGCGG	+2	4
ATAC---TGCGG	-3	2
ATACTGTTTGCGG	+1	3
ATACTGTTTTTGCGG	+3	2
ATACTTTGTTGCGG	+2	1
ATACTGGGGTTGCGG	+3	1
ATA---TTGCGG	-3	1

pol4Δ

Mutation	Indel	Frequency
ATACT-TTGCGG	-1	10
ATA-------------C	-13	3
ATAC---TGCGG	-3	3
ATACT--TGCGG	-2	2
ATA---TTGCGG	-3	1
TTT----------TAA	-650	1

gMAT**a**-2

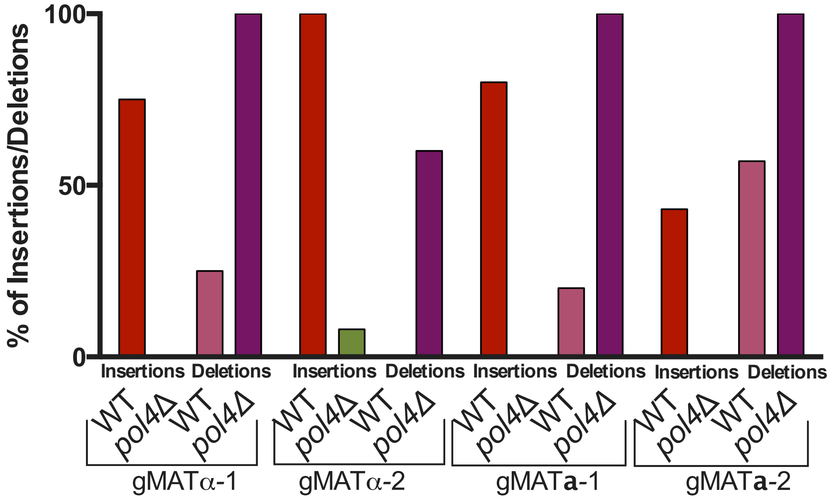
5’ ATCAATATCACCCCAAGCACGGG 3’

Mutation	Indel	Frequency
CCCCA-GCACGGG	-1	16
CCCCAAAGCACGGG	+1	10
CCCCAAAAGCACGGG	+2	1
CCCCAACCGCACGGG	+2	1

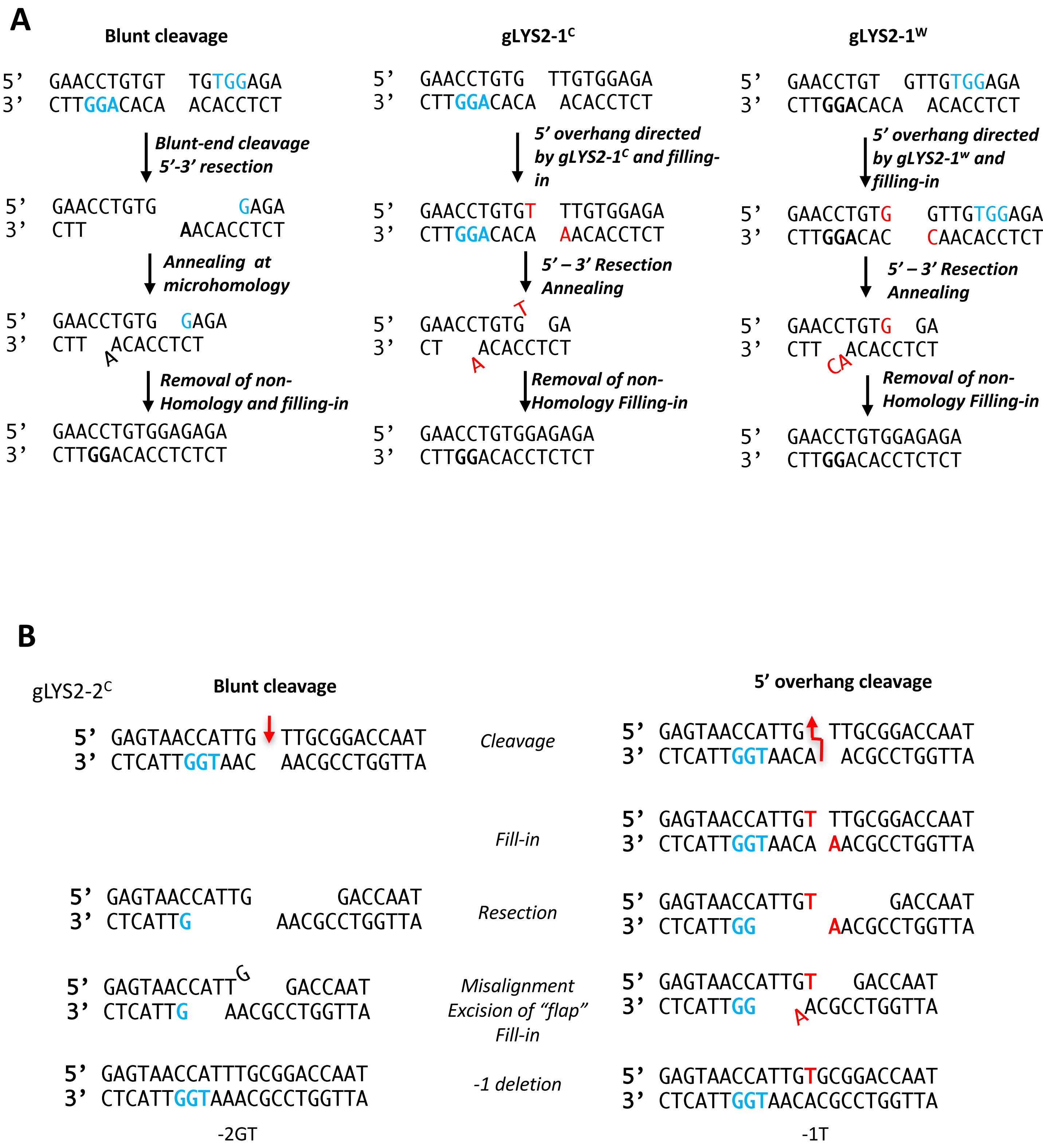
pol4Δ

Mutation	Indel	Frequency
CCCCA-GCACGGG	-1	16

C.



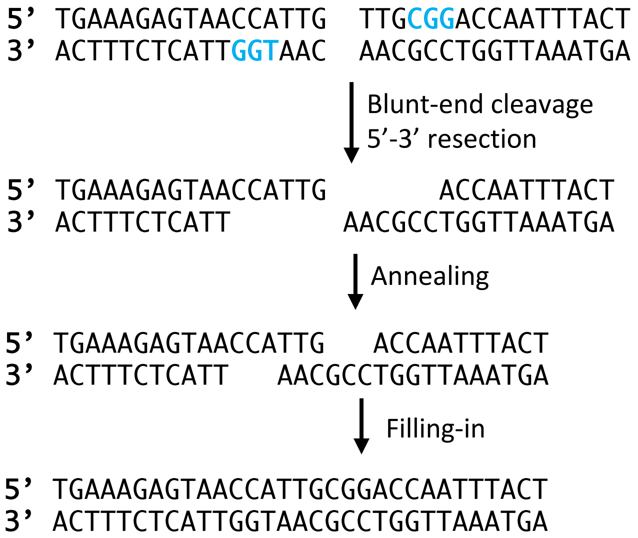
Suppl. Figure 3. Deletions in *LYS2* with blunt cleavage versus 5’ overhang cleavage.



Suppl. Figure 4

A

TTG deletion after blunt-end cleavage



5’ TGAAAGAGTAACCATTG TTGCGGACCAATTTACT
3’ ACTTTCTCATTGGTAAC AACGCCTGGTTAAATGA

Blunt-end cleavage
5’-3’ resection

5’ TGAAAGAGTAACCATTG ACCAATTTACT
3’ ACTTTCTCATT AACGCCTGGTTAAATGA

Annealing

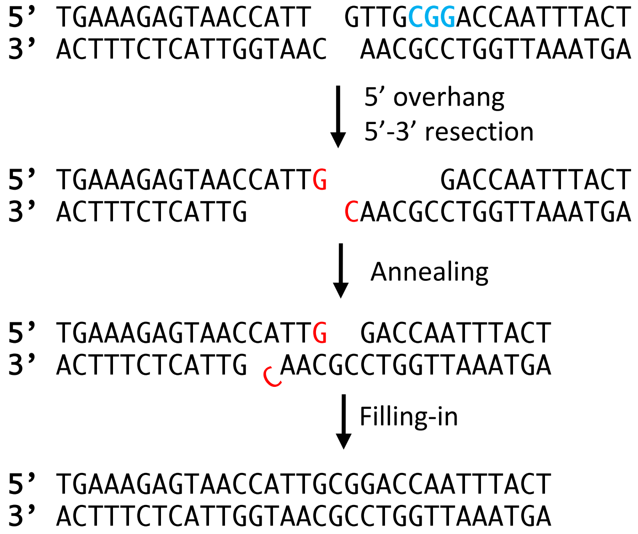
5’ TGAAAGAGTAACCATTG ACCAATTTACT
3’ ACTTTCTCATT AACGCCTGGTTAAATGA

Filling-in

5’ TGAAAGAGTAACCATTGCGGACCAATTTACT
3’ ACTTTCTCATTGGTAACGCCTGGTTAAATGA

B

TTG deletion mediated by gLYS2-2^{W} with 5’ overhang



5’ TGAAAGAGTAACCATT GTTGCGGACCAATTTACT
3’ ACTTTCTCATTGGTAAC AACGCCTGGTTAAATGA

5’ overhang
5’-3’ resection

5’ TGAAAGAGTAACCATTG GACCAATTTACT
3’ ACTTTCTCATTG CAACGCCTGGTTAAATGA

Annealing

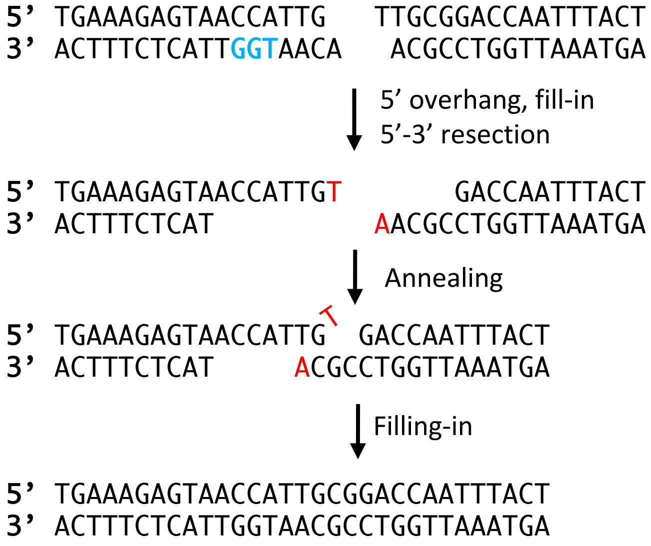
5’ TGAAAGAGTAACCATTG GACCAATTTACT
3’ ACTTTCTCATTG C AACGCCTGGTTAAATGA

Filling-in

5’ TGAAAGAGTAACCATTGCGGACCAATTTACT
3’ ACTTTCTCATTGGTAACGCCTGGTTAAATGA

C

TTG deletion mediated by gLYS2-2^{C} with 5’ overhang



5’ TGAAAGAGTAACCATTG TTGCGGACCAATTTACT
3’ ACTTTCTCATTGGTAACA ACGCCTGGTTAAATGA

5’ overhang, fill-in
5’-3’ resection

5’ TGAAAGAGTAACCATTGT GACCAATTTACT
3’ ACTTTCTCAT AACGCCTGGTTAAATGA

Annealing

T
5’ TGAAAGAGTAACCATTG GACCAATTTACT
3’ ACTTTCTCAT ACGCCTGGTTAAATGA

Filling-in

5’ TGAAAGAGTAACCATTGCGGACCAATTTACT
3’ ACTTTCTCATTGGTAACGCCTGGTTAAATGA

Suppl. Figure 5. Effect of Mre11 on Cas9-mediated NHEJ

gMATα-1

5’ CAAAATGCAGCACGGAATATGGG	**Indel**	**Frequency**
mre11Δ		
CAAAATGCAGCACGGA-TATGGG	-1	6
CAAAATGCAGCACGGAATATGGG	No change	2
mre11Δ + mre11--3		
CAAAATGCAGCACGG-ATATGGG	-1	8
CAAAATGCAGCACGGAAATATGGG	+1	3
CAAAATGCAGCACGGAAAATATGGG	+2	2
CAAAATGCAGCACG--ATATGGG	-2	1
CAAAATGCAGCA-------TGGG	-7	1
CAAAATGCAGCACGGATATATGGG	+1	1
CAAAATGCAGCA-----TATGGG	-5	1
CAAAATGCAGCACGGATAATATGGG	+2	1
CAAAATGCAGCACGGATATGATATGGG	+4	1
CAAAATGCAGCACGGAAAAAAAATATGGG	+6	1
mre11Δ + mre11-4		
CAAAATGCAGCACGGA-TATGGG	-1	4
CAAAATGCAGCACGGAAATATGGG	+1	2

Suppl. Figure 6. Templated Insertions in *CAN1*

gRNA CAN1 Target

5′ GATACGTTCTCTATGGAGGATGG 3′

5′ CTATGGAGGATGG	**Indel**	**Frequency**	**Microhomology**
CTATGGAAGGATGG	+1	29%	-
CTATGG-------	-7	24%	TGG
CTATGG-GGATGG	-1	10%	-
CTATGGAGGGATGG	+1	10%	-
CTATGGAAAAAGGATGG	+4	5%	-
CTATGGAAAGGATGG	+2	2%	-
CTATGG--GATGG	-2	2%	-
CTATGGATGGATGG	+1	2%	-
CTATGGA--ATGG	-2	2%	-
CTATGGAG-ATGG	-1	2%	-
CTATGGACCGGATGG	+2	2%	-
CTATGGATTGGATGG	+2	2%	-
CTATGGAAAAAAGGATGG	+5	2%	-
CTATGGATTTTTGGATGG	+5	2%	-

n =41

Suppl. Table 1: gRNA Chromosomal Target Sequences used in this paper

gRNA	**Chromosomal Target**	**PAM**	**Strand**
*MAT*α gRNA #1	5’ CAAAATGCAGCACGGAATAT 3’	GGG	
*MAT*α gRNA #2	5’ TATTACTCACAGTTTGGCTC 3’	CGG	
*MAT***a** gRNA #1	5’ TTTATAAAATTATACTGTTG 3’	CGG	
*MAT***a** gRNA #2	5’ ATCAATATCACCCCAAGCAC 3’	GGG	
gCAN1-1W	5’ TCACAAACACACCACAGACG 3’	TGG	Non-transcribed
gCAN1-1C	5’ ATGGTATTGACCCACGTCTG 3’	TGG	Transcribed
gLYS2-1W	5’ CCCAGAGAGAACCTGTGTTG 3’	TGG	Non-transcribed
gLYS2-1C	5’ TGTTGGAGTCTCCACAACAC 3’	AGG	Transcribed
gLYS2-2W	5’ TGAAAGAGTAACCATTGTTG 3’	CGG	Transcribed
gLYS2-2C	5’ AGTAAATTGGTCCGCAACAA 3’	TGG	Non-transcribed
gLYS2-3W	5’ TGCGGAAGACGCCAAGAAAT 3’	TGG	Transcribed
gLYS2-3C	5’ TAGCGTCTCAACCAATTTCT 3’	TGG	Non-transcribed
gLYS2-4W	5’ CAAAGTGTTTGCCCACGTCA 3’	GGG	Transcribed
gLYS2-4C	5’ TTCATCCTTGGCCCTGACGT 3’	GGG	Non-transcribed
CAN1	5’ GATACGTTCTCTATGGAGGA 3’	TGG	Non-transcribed

Supplemental Table 2

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Frequency	% Normalized
1w	WT	NA	CCCTTCCCAGAGAGAACCTGTGTTG**TGG**AGACTCCAACACTAAA	-	-	-	-
1w	Lys2-	-14	CCCTTCCCAGAGAGAACCT--------------CCAACACTAAA	2	CT	22	20.4%
1w	Lys2-	-5	CCCTTCCCAGAGAGAACCTGTG-----**G**AGACTCCAACACTAAA			14	13.0%
1w	Lys2-	+1	CCCTTCCCAGAGAGAACCTGT**G**GTTG**TGG**AGACTCCAACACTAAA			9	8.3%
1w	Lys2-	-2	CCCTTCCCAGAGAGAACCTGT--TG**TGG**AGACTCCAACACTAAA	2	GT	7	6.5%
1w	Lys2-	-16	CCCTTCCCAGAGAGA----------------CTCCAACACTAAA	4	GAGA	9	8.3%
1w	Lys2-	+2	CCCTTCCCAGAGAGAACCTGTGGG**TT**TTG**TGG**AGACTCCAACACTAAA			5	4.6%
1w	Lys2+	bps G-> T	CCCTTCCCAGAGAGAACCTGTGTT**t****TGG**AGACTCCAACACTAAA			5	4.6%
1w	Lys2-	+1	CCCTTCCCAGAGAGAACCTGTG**A**TTG**TGG**AGACTCCAACACTAAA			5	4.6%
1w	Lys2-	+1	CCCTTCCCAGAGAGAACCTGTG**T**TTG**TGG**AGACTCCAACACTAAA			3	2.8%
1w	Lys2-	-1	CCCTTCCCAGAGAGAACCTGT-TTG**TGG**AGACTCCAACACTAAA			3	2.8%
1w	Lys2-	+2	CCCTTCCCAGAGAGAACCTGT**GG**GTTG**TGG**AGACTCCAACACTAAA			2	1.9%
1w	Lys2+	-3	CCCTTCCCAGAGAGAACCTGTGT--**-GG**AGACTCCAACACTAAA	3	TGT	2	1.9%
1w	Lys2-	-17	CCCTTCCCAGAGAGAACC-----------------AACACTAAA	2	CC	2	1.9%
1w	Lys2-	+11	CCCTTCCCAGAGAGAACCTGTGT**GTGGGTGTGGG**TG**TGG**AGACTCCAACACTAAA			1	0.9%
1w	Lys2-	+4	CCCTTCCCAGAGAGAACCTGTG**AAAA**TTG**TGG**AGACTCCAACACTAAA			1	0.9%
1w	Lys2+	+3	CCCTTCCCAGAGAGAACCTGT**GGG**GTTG**TGG**AGACTCCAACACTAAA			1	0.9%
1w	Lys2-	-40	CCCTTCCCAGAGAGAACC--GTT	2	CC	1	0.9%
1w	Lys2-	-37	CCCT-------------------------------------AAA	2	CT	1	0.9%
1w	Lys2-	-29	CCCT-----------------------------CCAACACTAAA	2	CT	1	0.9%
1w	Lys2-	-29	C-----------------------------ACTCCAACACTAAA			1	0.9%
1w	Lys2+	-28	CCCTTCC----------------------------AACACTAAA	3	TCC	1	0.9%
1w	Lys2+	-18	CCCTTCCCAGAGA------------------ACTCCAACACTAAA	2	GA	1	0.9%
1w	Lys2-	-16	CCCTTCCCAGAGAGAACC----------------CAACACTAAA	1	C	1	0.9%
1w	Lys2-	-16	CCCTTCCCAGAGAGAACCT----------------AACACTAAA			1	0.9%
1w	Lys2-	-16	CCCTTCCCAGAGAGAAC----------------TCCAACACTAAA	1	C	1	0.9%
1w	Lys2+	-15	CCCTTCCCAGAGAGAACC---------------CCAACACTAAA			1	0.9%
1w	Lys2+	-15	CCCTTCCCAGAGAGAACCT---------------CAACACTAAA			1	0.9%
1w	Lys2-	-14	CCCTTCCCAGAGAGAACCTG--------------CAACACTAAA			1	0.9%
1w	Lys2-	-14	CCCTTCCCAGAGAGAAC--------------CTCCAACACTAAA	2	CT	1	0.9%

1w	Lys2-	-13	CCCTTCCCAGAGAGA-------------AGACTCCAACACTAAA	1	A	1	0.9%
1w	Lys2-	-13	CCCTTCCCAGAGAGAACCTG-------------CCAACACTAAA			1	0.9%
1w	Lys2-	-1	CCCTTCCCAGAGAGAACCTGTGT-G**TGG**AGACTCCAACACTAAA			1	0.9%
1w	Lys2+	bps T -> G	CCCTTCCCAGAGAGAACCTG**g**GTTG**TGG**AGACTCCAACACTAAA			1	0.9%
						108	100.0%

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Frequency	% Normalized
1c	WT	NA	GAGAA**CCT**GTGTTGTGGAGACTCCAACACTAAATTCCGACA				
1c	Lys2-	-16	GAGA----------------CTCCAACACTAAATTCCGACA	4	GAGA	43	42.2%
1c	Lys2-	-14	GAGAA**CCT**--------------CCAACACTAAATTCCGACA	2	CT	28	27.5%
1c	Lys2+	-3	GAGAA**CCT**GTGT---GGAGACTCCAACACTAAATTCCGACA	3	TGT	6	5.9%
1c	Lys2-	+1	GAGAA**CCT**GTG**T**TTGTGGAGACTCCAACACTAAATTCCGACA			7	6.9%
1c	Lys2-	+1	GAGAA**CCT**GTG**G**TTGTGGAGACTCCAACACTAAATTCCGACA			3	2.9%
1c	Lys2+	+3	GAGAA**CCT**GTG**TTT**TTGTGGAGACTCCAACACTAAATTCCGACA			4	3.9%
1c	Lys2-	-17	GAGAA**CC**-----------------AACACTAAATTCCGACA	2	CC	2	2.0%
1c	Lys2-	-2	GAGAA**CCT**GT--TGTGGAGACTCCAACACTAAATTCCGACA	2	GT	2	2.0%
1c	Lys2-	-1	GAGAA**CCT**GTG-TGTGGAGACTCCAACACTAAATTCCGACA	1	T	2	2.0%
1c	Lys2-	+2	GAGAA**CCT**GTG**TT**TTGTGGAGACTCCAACACTAAATTCCGACA			2	2.0%
1c	Lys2+	+3	GAGAA**CCT**GTGT**GGG**TGTGGAGACTCCAACACTAAATTCCGACA			1	1.0%
1c	Lys2-	+2	GAGAA**CCT**GTG**GG**TTGTGGAGACTCCAACACTAAATTCCGACA			1	1.0%
1c	Lys2-	+1	GAGAA**CCT**GTG**A**TTGTGGAGACTCCAACACTAAATTCCGACA			1	1.0%
						102	100.0%

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Frequency	% Normalized
2w	WT	NA	TCGAATGAAAGAGTAACCATTGTTG**CGG**ACCAATTTACTCAATA				
2w	Lys2+	-3	TCGAATGAAAGAGTAACCATTG---**CGG**ACCAATTTACTCAATA	3	TTG	61	58.1%
2w	Lys2-	+1	TCGAATGAAAGAGTAACCATT**G**GTTG**CGG**ACCAATTTACTCAATA			10	9.5%
2w	Lys2-	+1	TCGAATGAAAGAGTAACCATTG**T**TTG**CGG**ACCAATTTACTCAATA			9	8.6%
2w	Lys2-	+1	TCGAATGAAAGAGTAACCATTG**A**TTG**CGG**ACCAATTTACTCAATA			5	4.8%
2w	Lys2-	+2	TCGAATGAAAGAGTAACCATTG**TT**TTG**CGG**ACCAATTTACTCAATA			6	5.7%
2w	Lys2+	+3	TCGAATGAAAGAGTAACCATTG**TTT**TTG**CGG**ACCAATTTACTCAATA			3	2.9%
2w	Lys2-	+2	TCGAATGAAAGAGTAACCATT**GG**GTTG**CGG**ACCAATTTACTCAATA			2	1.9%
2w	Lys2-	-17	TCGAATGAAAGA-----------------CCAATTTACTCAATA	2	GA	1	1.0%
2w	Lys2-	-1	TCGAATGAAAGAGTAACCATTGTT-**CGG**ACCAATTTACTCAATA			1	1.0%

2w	Lys2-	+4	TCGAATGAAAGAGTAACCATTG**TTTT**TTG**CGG**ACCAATTTACTCAATA			1	1.0%
2w	Lys2+	+3	TCGAATGAAAGAGTAACCATTG**GTG**TTG**CGG**ACCAATTTACTCAATA			1	1.0%
2w	Lys2+	+3	TCGAATGAAAGAGTAACCATTG**ATA**TTG**CGG**ACCAATTTACTCAATA			1	1.0%
2w	Lys2-	+2	TCGAATGAAAGAGTAACCATTG**CC**TTG**CGG**ACCAATTTACTCAATA			1	1.0%
2w	Lys2-	+1	TCGAATGAAAGAGTAACCATTG**AA**TTG**CGG**ACCAATTTACTCAATA			1	1.0%
2w	Lys2-	+1	TCGAATGAAAGAGTAACCATTG**C**TTG**CGG**ACCAATTTACTCAATA			1	1.0%
2w	Lys2+	bps T -> G	TCGAATGAAAGAGTAACCATTGT**g**G**CGG**ACCAATTTACTCAATA			1	1.0%
						105	100.0%

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Frequency	% Normalized
2c	WT	NA	TATTCGAATGAAAGAGTAA**CCA**TTGTTGCGGACCAATTTACTCAATA	-			
2c	Lys2-	+1	TATTCGAATGAAAGAGTAA**CCA**TTG**T**TTGCGGACCAATTTACTCAATA			55	35.5%
2c	Lys2-	-1	TATTCGAATGAAAGAGTAA**CCA**TTG-TGCGGACCAATTTACTCAATA	1	T	23	14.8%
2c	Lys2+	-3	TATTCGAATGAAAGAGTAA**CCA**TTG---CGGACCAATTTACTCAATA	3	TTG	15	9.7%
2c	Lys2-	+1	TATTCGAATGAAAGAGTAA**CCA**TTG**G**TTGCGGACCAATTTACTCAATA			12	7.7%
2c	Lys2-	+2	TATTCGAATGAAAGAGTAA**CCA**TTG**TT**TTGCGGACCAATTTACTCAATA			11	7.1%
2c	Lys2+	+3	TATTCGAATGAAAGAGTAA**CCA**TTG**TTT**TTGCGGACCAATTTACTCAATA			8	5.2%
2c	Lys2-	-13	TATTCGAATGAAAGAGTAA**CCA**-------------ATTTACTCAATA	4	ACCA	8	5.2%
2c	Lys2-	+1	TATTCGAATGAAAGAGTAA**CCA**TTG**C**TTGCGGACCAATTTACTCAATA			4	2.6%
2c	Lys2-	+2	TATTCGAATGAAAGAGTAA**CCA**TTG**GG**TTGCGGACCAATTTACTCAATA			3	1.9%
2c	Lys2+	+3	TATTCGAATGAAAGAGTAA**CCA**TTG**AAA**TTGCGGACCAATTTACTCAATA			2	1.3%
2c	Lys2-	-13	TATTCGAATGAAAGAGTAA**CCA**TT-------------TTACTCAATA	1	T	2	1.3%
2c	Lys2-	-40	TATTCGAATGAAAGAGTAA**CCA**----TGCGGACCAATTTACTCAATA	1	T	1	0.6%
2c	Lys2-	+1	TATTCGAATGAAAGAGTAA**CCA**TTG**A**TTGCGGACCAATTTACTCAATA			1	0.6%
2c	Lys2+	-6	TATTCGAATGAAAGAGTAA**CCA**TTG------ACCAATTTACTCAATA	1	G	1	0.6%
2c	Lys2+	+3	TATTCGAATGAAAGAGTAA**CCA**TTG**CCC**TTGCGGACCAATTTACTCAATA			1	0.6%
2c	Lys2+	+3	TATTCGAATGAAAGAGTAA**CCA**TTG**GGG**TTGCGGACCAATTTACTCAATA			1	0.6%
2c	Lys2+	+3	TATTCGAATGAAAGAGTAA**CCA**TTGT**ATT**TGCGGACCAATTTACTCAATA			1	0.6%
2c	Lys2-	+2	TATTCGAATGAAAGAGTAA**CCA**TTG**AA**TTGGGACCAATTTACTCAATA			1	0.6%
2c	Lys2-	+2	TATTCGAATGAAAGAGTAA**CCA**TTG**CT**TTGGGACCAATTTACTCAATA			1	0.6%
2c	Lys2+	+3	TATTCGAATGAAAGAGTAA**CCA**TTG**TTG**TTGCGGACCAATTTACTCAATA			1	0.6%
2c	Lys2+	bps C-> A	TATTCGAATGAAAGAGTAA**CCA**TTGTTG**a**GGACCAATTTACTCAATA			1	0.6%
2c	Lys2-	-1	TATTCGAATGAAAGAGTAA**CCA**TT-TTGCGGACCAATTTACTCAATA			1	0.6%
2c	Lys2+	bps G-> A	TATTCGAATGAAAGAGTAA**CCA**TT**a**TTGCGGACCAATTTACTCAATA			1	0.6%

						155	100.0%
gRNA	**Lys2+/-**	**Insertion(+) Deletions(-)**	**Sequence**	**Microhomology**		**Frequency**	**% Normalized**
3w	WT	NA	TCACTGCAAATTATGCGGAAGACGCCAAGAAAT**TGG**TTGAGACGCTACCAAG				
3w	Lys2-	-1	TCACTGCAAATTATGCGGAAGACGCCAAG-AAT**TGG**TTGAGACGCTACCAAG	1	A	36	56.3%
3w	Lys2-	-20	TCACTGCAAATTATGCGGAAGACGC--------------------TACCAAG	2	GC	7	10.9%
3w	Lys2+	bps A ->G	TCACTGCAAATTATGCGGAAGACGCCAAG**g**AAT**TGG**TTGAGACGCTACCAAG			6	9.4%
3w	Lys2+	bps G -> A	TCACTGCAAATTATGCGGAAGACGCCAA**a**AAAT**TGG**TTGAGACGCTACCAAG			4	6.3%
3w	Lys2+	bps G -> T	TCACTGCAAATTATGCGGAA**t**ACGCCAGGAAAT**TGG**TTGAGACGCTACCAAG			2	3.1%
3w	Lys2-	-19	TCACTGCAAATTATGCGGAAG-------------------GACGCTACCAAG			1	1.6%
3w	Lys2+	-3	TCACTGCAAATTATGCGGAAGACGCCAAG---T**TGG**TTGAGACGCTACCAAG			1	1.6%
3w	Lys2+	-3	TCACTGCAAATTATGCGGAAGACGCCAA---AT**TGG**TTGAGACGCTACCAAG	1	A	1	1.6%
3w	Lys2-	-1	TCACTGCAAATTATGCGGAAGACGCCAAGAAA-**TGG**TTGAGACGCTACCAAG	1	T	1	1.6%
3w	Lys2+	+3	TCACTGCAAATTATGCGGAAGACGCCAAG**AAA**AAAT**TGG**TTGAGACGCTACCAAG			1	1.6%
3w	Lys2-	+2	TCACTGCAAATTATGCGGAAGACGCCAAG**AA**AAAT**TGG**TTGAGACGCTACCAAG			1	1.6%
3w	Lys2-	+2	TCACTGCAAATTATGCGGAAGACGCCAAGA**TT**AT**TGG**TTGAGACGCTACCAAG			1	1.6%
3w	Lys2+	bps AA->CC	TCACTGCAAATTATGCGGAAGACGCC**cc**GAAAT**TGG**TTGAGACGCTACCAAG			1	1.6%
3w	Lys2+	bps G-> A	TCACTGCAAATTATGC**a**GAAGACGCCAAGAAAT**TGG**TTGAGACGCTACCAAG			1	1.6%
						64	100.0%
gRNA	**Lys2+/-**	**Insertion(+) Deletions(-)**	**Sequence**	**Microhomology**		**Frequency**	**% Normalized**
3c	WT	NA	ATGCGGAAGACG**CCA**AGAAATTGGTTGAGACGCTACCAAGTTCGTA				
3c	Lys2-	+1	ATGCGGAAGACG**CCA**AGA**A**AATTGGTTGAGACGCTACCAAGTTCGTA			28	50.9%
3c	Lys2-	-1	ATGCGGAAGACG**CCA**AGA-ATTGGTTGAGACGCTACCAAGTTCGTA			21	38.2%
3c	Lys2-	-1	ATGCGGAAGACG**CCA-**GAAATTGGTTGAGACGCTACCAAGTTCGTA			1	1.8%
3c	Lys2+	bps G->A	ATGCGGAAGACG**CCA**AGAAATTGGTTGA**a**ACGCTACCAAGTTCGTA			1	1.8%
3c	Lys2-	-20	ATGCGGAAGACG**C**--------------------TACCAAGTTCGTA	1	C	1	1.8%
3c	Lys2-	-20	ATGCGGAAGACG**CCA**--------------------CCAAGTTCGTA	1	A	1	1.8%
3c	Lys2-	-2	ATGCGGAAGACG**CCA**AGA--TTGGTTGAGACGCTACCAAGTTCGTA	1	A	1	1.8%
3c	Lys2+	bps A->C	ATGCGGAAGACG**CCc**AGAAATTGGTTGAGACGCTACCAAGTTCGTA			1	1.8%
						55	100.0%
gRNA	**Lys2+/-**	**Insertion(+) Deletions(-)**	**Sequence**	**Microhomology**		**Frequency**	**% Normalized**

4w	Lys2+ WT	NA	TTTCAAAGTGTTTGCCCACGTCA**GGG**CCAAGGATGAAG				
4w	Lys2+	bps A->C	TTTCAAAGTGTTTGCCC**c**CGTCA**GGG**CCAAGGATGAAG			25	18.8%
4w	Lys2-	-1	TTTCAAAGTGTTTGCCCAC-TCA**GGG**CCAAGGATGAAG			25	18.8%
4w	Lys2+	-12	TTTCAAAGTGTTTGCC------------AAGGATGAAG	3	GCC	18	13.5%
4w	Lys2-	+1	TTTCAAAGTGTTTGCCCAC**G**GTCA**GGG**CCAAGGATGAAG			17	12.8%
4w	Lys2-	-11	TTTCAAAGTGTTTGCCCA-----------AGGATGAAG	3	CCA	16	12.0%
4w	Lys2-	+2	TTTCAAAGTGTTTGCCCACG**CG**TCA**GGG**CCAAGGATGAAG			6	4.5%
4w	Lys2-	+1	TTTCAAAGTGTTTGCCCAC**C**GTCA**GGG**CCAAGGATGAAG			5	3.8%
4w	Lys2-	-16	TTTCAAAG----------------**GG**CCAAGGATGAAG	1	G	4	3.0%
4w	Lys2-	-1	TTTCAAAGTGTTTGCC-ACGTCA**GGG**CCAAGGATGAAG			3	2.3%
4w	Lys2+	bps G->T	TTTCAAAGTGTTT**t**CCCACTTCA**GGG**CCAAGGATGAAG			3	2.3%
4w	Lys2+	-30	TTTCAAAGTGTTTGC------------------------------A	3	TGC	1	0.8%
4w	Lys2+	-12	TTTCAAAGTGTT------------**GG**CCAAGGATGAAG			1	0.8%
4w	Lys2-	-11	TTTCAAAGTGTTTGCCCA-----------AGGATGAAG	3	CCA	1	0.8%
4w	Lys2-	-10	TTTCAAAGTGT----------CA**GGG**CCAAGGATGAAG	1	T	1	0.8%
4w	Lys2-	-7	TTTCAAAGTGTTTGCC-------**GGG**CCAAGGATGAAG	2	CA	1	0.8%
4w	Lys2+	-6	TTTCAAAGTGTTTGCC------A**GGG**CCAAGGATGAAG	1	C	1	0.8%
4w	Lys2-	-2	TTTCAAAGTGTTTGCCCA--TCA**GGG**CCAAGGATGAAG			1	0.8%
4w	Lys2-	-1	TTTCAAAGTGTTTGCCCA-GTCA**GGG**CCAAGGATGAAG			1	0.8%
4w	Lys2-	+2	TTTCAAAGTGTTTGCCCAC**GG**GTCA**GGG**CCAAGGATGAAG			1	0.8%
4w	Lys2-	+1	TTTCAAAGTGTTTGCCCACG**C**TCA**GGG**CCAAGGATGAAG			1	0.8%
4w	Lys2-	+1	TTTCAAAGTGTTTGCCCACG**A**TCA**GGG**CCAAGGATGAAG			1	0.8%
4w	Lys2+	bps T->G	TTTCAAAGTGTTTGCCCACG**g**CA**GGG**CCAAGGATGAAG			1	0.8%
						133	100.0%

gRNA	**Lys2+/-**	**Insertion(+) Deletions(-)**	**Sequence**	**Microhomology**		**Frequency**	**% Normalized**
4c	Lys+ WT	NA	AAAGTGTTTG**CCC**ACGTCAGGGCCAAGGATGAAGAAGCT				
4c	Lys2-	+1	AAAGTGTTTG**CCC**ACG**G**TCAGGGCCAAGGATGAAGAAGCT			7	12.5%
4c	Lys2-	-1	AAAGTGTTTG**CCC**ACG-CAGGGCCAAGGATGAAGAAGCT	.		5	8.9%
4c	Lys2+	-12	AAAGTGTTTG**CC**------------AAGGATGAAGAAGCT	3	GCC	4	7.1%
4c	Lys2-	-11	AAAGTGTTTG**CCC**A-----------AGGATGAAGAAGCT	3	CCA	4	7.1%
4c	Lys2+	bps C->A	AAAGTGTTTG**CCC**ACGT**a**AGGGCCAAGGATGAAGAAGCT			4	7.1%
4c	Lys2+	bps G ->T	AAAGTGTTTG**CCC**AC**t**TCAGGGCCAAGGATGAAGAAGCT			4	7.1%

4c	Lys2-	-13	AAAGTGTT-------------GCCAAGGATGAAGAAGCT	1	G	3	5.4%
4c	Lys2-	+1	AAAGTGTTTG**CCC**ACG**T**TCAGGGCCAAGGATGAAGAAGCT			2	3.6%
4c	Lys2+	bps T->A	AAAGTGTTTG**CCC**ACGaCAGGGCCAAGGATGAAGAAGCT			2	3.6%
4c	Lys2+	bps T ->C	AAAGTGTTTG**CCC**ACGcCAGGGCCAAGGATGAAGAAGCT			2	3.6%
4c	Lys2+	-12	AAAGTGTTTG**CCC**------------AGGATGAAGAAGCT	1	A	1	1.8%
4c	Lys2-	-11	AAAGTGTTTG**CCC**ACG-----------GATGAAGAAGCT	1	G	1	1.8%
4c	Lys2-	-5	AAAGTGTTTG**CCC**A-----GGGCCAAGGATGAAGAAGCT	2	CA	1	1.8%
4c	Lys2+	-3	AAAGTGTTTG**CCC**AC---AGGGCCAAGGATGAAGAAGCT	1	C	1	1.8%
4c	Lys2-	-1	AAAGTGTTTG**CC**-ACGTCAGGGCCAAGGATGAAGAAGCT	1	C	1	1.8%
4c	Lys2-	-1	AAAGTGTTTG**CCC**AC-TCAGGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2-	-1	AAAGTGTTTG**CCC**ACGTC-GGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2-	+2	AAAGTGTTTG**CCC**ACG**GG**TCAGGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2-	+2	AAAGTGTTTG**CCC**ACG**TT**TCAGGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps GTC ->TTT	AAAGTGTTTG**CCC**ACtttAGGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps A ->C	AAAGTGTTTG**CCC**ACGTCAGGGCCAAGGcTGAAGAAGCT			1	1.8%
4c	Lys2+	bps G->A	AAAGTGTTTG**CCC**ACGTCAaGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps G ->T	AAAGTGTTTG**CCC**ACGTCAGGGCCAAtGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps G->C	AAAGTGTTTG**CCC**ACGTCAGGGCCAAcGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps G ->A	AAAGTGTTTG**CCC**ACaTCAGGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps G ->A	AAAGTGTTTG**CCC**ACGTCAaGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps G ->T	AAAGTGTTTG**CCC**ACGTCAtGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps C ->G	AAAGTGTTTG**CCC**AgGTCAGGGCCAAGGATGAAGAAGCT			1	1.8%
4c	Lys2+	bps C ->A	AAAGTGTTTG**C**a**C**ACGTCAGGGCCAAGGATGAAGAAGCT			1	1.8%
						56	100.0%

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Frequency	% Normalized
pol4							
2w	WT	NA	TTCGAATGAAAGAGTAACCATTGTTG**CGG**ACCAATTTACTCAATA				
2w	Lys2+	-3	TTCGAATGAAAGAGTAACCATTG---**CGG**ACCAATTTACTCAATA	3	TTG	103	94.5%
2w	Lys2+	-3	TTCGAATGAAAGAGTAACC---GTTG**CGG**ACCAATTTACTCAATA			3	2.8%
2w	Lys2-	-10	TTCGAATGAAAGAGTAACCATTG----------ATTTACTCAATA			3	2.8%
						109	100.0%

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology	Frequency	% Normalized
pol4						
2c	WT	NA	AAAGAGTAA**CCA**TTGTTGCGGACCAATTTACTCAATA			
2c	Lys2-	-1	AAAGAGTAA**CCA**TTGT-GCGGACCAATTTACTCAATA	1 T	45	64.3%
2c	Lys2-	-13	AAAGAGTAA**CCA**-------------ATTTACTCAATA		11	15.7%
2c	Lys2-	+1	AAAGAGTAA**CCA**TTG**T**TTGCGGACCAATTTACTCAATA		5	7.1%
2c	Lys2-	-13	AAAGAGTAA**CCA**TT-------------TTACTCAATA	1 T	4	5.7%
2c	Lys2+	-3	AAAGAGTAA**CCA**TTGT---GGACCAATTTACTCAATA		3	4.3%
2c	Lys2-	-14	AAAGAGTAA**CCA**TT--------------TACTCAATA	2 TT	1	1.4%
2c	Lys2+	bps G ->A	AAAGAGTAA**CCA**TTGTTGaGGACCAATTTACTCAATA		1	1.4%
					70	100.0%

gRNA	CAN1+/-	Insertion(+) Deletions(-)	Sequence	Microhomology	Frequency	% Normalized
1w	**WT**		TGAAGCTTCACAAACACACCACAGACG**TGG**GTCAATACCATT			
1w	Can1-	+1	TGAAGCTTCACAAACACACCACAG**A**ACG**TGG**GTCAATACCATT		14	23%
1w	Can1-	+1	TGAAGCTTCACAAACACACCACA**G**GACG**TGG**GTCAATACCATT		10	17%
1w	Can1-	+1	TGAAGCTTCACAAACACACCACAG**T**ACG**TGG**GTCAATACCATT		8	13%
1w	Can1+	-21	TGAAGCTTCACAAA---------------------TACCATT		4	7%
1w	Can1-	+2	TGAAGCTTCACAAACACACCACAGA**AA**CG**TGG**GTCAATACCATT		3	5%
1w	Can1+	+3	TGAAGCTTCACAAACACACCACAGA**AAA**CG**TGG**GTCAATACCATT		3	5%
1w	Can1-	-1	TGAAGCTTCACAAACACACCACAG-CG**TGG**GTCAATACCATT		3	5%
1w	Can1+	1bps	TGAAGCTTCACAAACACACCACAG**c**CG**TGG**GTCAATACCATT		2	3%
1w	Can1-	+4	TGAAGCTTCACAAACACACCACAG**AAAA**ACG**TGG**GTCAATACCATT		2	3%
1w	Can1-	+2	TGAAGCTTCACAAACACACCACAG**TT**ACG**TGG**GTCAATACCATT		2	3%
1w	Can1-	+2	TGAAGCTTCACAAACACACCACAG**CC**ACG**TGG**GTCAATACCATT		2	3%
1w	Can1-	-2	TGAAGCTTCACAAACACACCA--GACG**TGG**GTCAATACCATT		1	2%
1w	Can1-	-2	TGAAGCTTCACAAACACACCACA--CG**TGG**GTCAATACCATT		1	2%
1w	Can1-	+2	TGAAGCTTCACAAACACACCACAG**TA**ACG**TGG**GTCAATACCATT		1	2%
1w	Can1-	-19	TGAAGCTTCACAAACACACCA-------------------T		1	2%
1w	Can1-	-1	TGAAGCTTCACAAACACACCACA-ACG**TGG**GTCAATACCATT		1	2%
1w	Can1+	+3	TGAAGCTTCACAAACACACCACAGA**TTT**CG**TGG**GTCAATACCATT		1	2%
1w	Can1+	+3	TGAAGCTTCACAAACACACCACAGA**CCC**CG**TGG**GTCAATACCATT		1	2%

						60	100%
gRNA	**CAN1+/-**	**Insertion(+) Deletions(-)**	**Sequence**	**Microhomology**		**Frequency**	**% Normalized**
1c	**WT**		TGAAGCTTCACAAACACACCACAGACGTGGGTCAATACCAT				
1c	Can1-	+1	TGAAGCTTCACAAACACA**CCA**CAG**A**ACGTGGGTCAATACCATT			64	70%
1c	Can1+	-3	TGAAGCTTCACAAACACA**CCA**CAG---TGGGTCAATACCATT	1	G	9	10%
1c	Can1-	-1	TGAAGCTTCACAAACACA**CCA**CAG-CGTGGGTCAATACCATT			2	2%
1c	Can1+	1bps	TGAAGCTTCACAAACACA**CCA**CAG**c**CGTGGGTCAATACCATT			2	2%
1c	Can1-	+1	TGAAGCTTCACAAACACA**CCA**CAGA**C**CGTGGGTCAATACCATT			2	2%
1c	Can1-	-2	TGAAGCTTCACAAACACA**CCA**CA--CGTGGGTCAATACCATT	1	A	2	2%
1c	Can1-	-1	TGAAGCTTCACAAACACA**CCA**CA-ACGTGGGTCAATACCATT			1	1%
1c	Can1-	-1	TGAAGCTTCACAAACACA**CCA**CAGACG-GGGTCAATACCATT			1	1%
1c	Can1+	1bps	TGAAGCTTCACAAACACA**CCA**CAGACG**g**GGGTCAATACCATT			1	1%
1c	Can1-	+1	TGAAGCTTCACAAACACA**CCA**CAG**T**ACGTGGGTCAATACCATT			1	1%
1c	Can1-	+2	TGAAGCTTCACAAACACA**CCA**CAG**AA**ACGTGGGTCAATACCATT			1	1%
1c	Can1+	+3	TGAAGCTTCACAAACACA**CCA**CAG**ATA**ACGTGGGTCAATACCATT			1	1%
1c	Can1-	+1	TGAAGCTTCACAAACACA**CCA**CAGA**T**CGTGGGTCAATACCATT			1	1%
1c	Can1-	+2	TGAAGCTTCACAAACACA**CCA**CAGA**TT**CGTGGGTCAATACCATT			1	1%
1c	Can1-	+2	TGAAGCTTCACAAACACA**CCA**CAG**GG**ACGTGGGTCAATACCATT			1	1%
1c	Can1+	-18	TGAAGCTTCACAAACA-----------------ATACCATT	2	CA	1	1%
1c	Can1-	-19	TGAAGCTTCACAAACACA**CCA**-------------------TT	4	ACCA	1	1%
						92	100%

Supplemental Table 3

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Average Frequency	Sample 1	Sample 2	Sample 3	Frequency 1	Frequency 2	Frequency 3
1w	Lys- WT F	NA	CCCTTCCCAGAGAGAACCTGTGTTG**TGG**AGACTCCAACACTAAA			0.4%	60	31	252	0.27%	0.15%	0.72%
1w	Lys2-	-14	CCCTTCCCAGAGAGAACCT--------------CCAACACTAAA	2	CT	20.5%	4120	3955	8480	18.55%	18.78%	24.29%
1w	Lys2-	-2	CCCTTCCCAGAGAGAACCTGT--TG**TGG**AGACTCCAACACTAAA	2	GT	16.6%	3925	3315	5683	17.67%	15.74%	16.28%
1w	Lys2-	+1	CCCTTCCCAGAGAGAACCTGT**G**GTTG**TGG**AGACTCCAACACTAAA			16.2%	4147	3814	4174	18.67%	18.11%	11.95%
1w	Lys2-	-5	CCCTTCCCAGAGAGAACCTGTG-----**G**AGACTCCAACACTAAA	3	GTG	9.9%	1885	2223	3762	8.49%	10.55%	10.77%
1w	Lys2-	-1	CCCTTCCCAGAGAGAACCTGT-TTG**TGG**AGACTCCAACACTAAA			6.2%	1366	1196	2312	6.15%	5.68%	6.62%
1w	Lys2-	+2	CCCTTCCCAGAGAGAACCTGTGGG**TT**TTG**TGG**AGACTCCAACACTAAA			4.4%	742	1255	1352	3.34%	5.96%	3.87%
1w	Lys2-	-17	CCCTTCCCAGAGAGAACC-----------------AACACTAAA	2	CC	3.8%	928	717	1313	4.18%	3.40%	3.76%
1w	Lys2-	+1	CCCTTCCCAGAGAGAACCTGTG**T**TTG**TGG**AGACTCCAACACTAAA			3.6%	885	834	1041	3.98%	3.96%	2.98%
1w	Lys2+	-3	CCCTTCCCAGAGAGAACCTGTGT---**GG**AGACTCCAACACTAAA	3	TGT	2.9%	658	485	1201	2.96%	2.30%	3.44%
1w	Lys2+	-15	CCCTTCCCAGAGAGAACC---------------CCAACACTAAA			2.6%	598	349	1226	2.69%	1.66%	3.51%
1w	Lys2-	-16	CCCTTCCCAGAGAGA----------------CTCCAACACTAAA	4	GAGA	2.5%	676	478	763	3.04%	2.27%	2.19%
1w	Lys2-	+2	CCCTTCCCAGAGAGAACCTGT**GG**GTTG**TGG**AGACTCCAACACTAAA			2.4%	667	505	634	3.00%	2.40%	1.82%
1w	Lys2-	+1	CCCTTCCCAGAGAGAACCTGTG**A**TTG**TGG**AGACTCCAACACTAAA			2.2%	440	530	699	1.98%	2.52%	2.00%
1w	Lys2-	-16	CCCTTCCCAGAGAGAACCT----------------AACACTAAA			1.8%	387	352	725	1.74%	1.67%	2.08%
1W	Lys2+	+3	CCCTTCCCAGAGAGAACCTGTGGG**TTT**TTG**TGG**AGACTCCAACACTAAA			0.9%	151	231	304	0.68%	1.10%	0.87%
1w	Lys2-	-1	CCCTTCCCAGAGAGAACCTGTGT-G**TGG**AGACTCCAACACTAAA	1	G	0.9%	170	133	409	0.77%	0.63%	1.17%
1w	Lys2+	-15	CCCTTCCCAGAGAGAACCT---------------CAACACTAAA			0.6%	90	190	131	0.41%	0.90%	0.38%
1w	Lys2-	-16	CCCTTCCCAGAGAGAACC----------------CAACACTAAA	1	C	0.4%	67	118	156	0.30%	0.56%	0.45%
1w	Lys2+	+3	CCCTTCCCAGAGAGAACCTGT**GGG**GTTG**TGG**AGACTCCAACACTAAA			0.3%	51	132	53	0.23%	0.63%	0.15%
1w	Lys2-	-14	CCCTTCCCAGAGAGAACCTG--------------CAACACTAAA			0.3%	185	10	21	0.83%	0.05%	0.06%
1w	Lys2-	-13	CCCTTCCCAGAGAGAACCTG-------------CCAACACTAAA	1	T	0.2%	1	115	0	0.00%	0.55%	0.00%
1w	Lys2-	-29	C-----------------------------ACTCCAACACTAAA			0.2%	2	12	131	0.01%	0.06%	0.38%
1w	Lys2+	-28	CCCTTCC----------------------------AACACTAAA	3	TCC	0.1%	0	48	52	0.00%	0.23%	0.15%
1w	Lys2-	+4	CCCTTCCCAGAGAGAACCTGTG**AAAA**TTG**TGG**AGACTCCAACACTAAA			0.1%	0	20	14	0.00%	0.09%	0.04%
						100.00%	**22150**	**21031**	**34666**	**100.0%**	**100.00%**	**100.00%**

	Lys2+/-	**Insertion(+) Deletions(-)**	**Sequence**	**Microhomology**		**Average Frequency**	**Sample 4**	**Sample 5**	**Sample 6**	**Frequency 4**	**Frequency 5**	**Frequency 6**
1c	Lys+ WT R	NA	GAGAA**CCT**GTGTTGTGGAGACTCCAACACTAAATTCCGACA			3.7%	613	787	373	3.56%	4.17%	3.37%
1c	Lys2-	-14	GAGAA**CCT**--------------CCAACACTAAATTCCGACA	2	CT	27.7%	4423	5344	3211	25.68%	28.30%	29.05%
1c	Lys2-	+1	GAGAA**CCT**GTG**T**TTGTGGAGACTCCAACACTAAATTCCGACA			21.1%	3637	4131	2228	21.11%	21.87%	20.16%
1c	Lys2-	-1	GAGAA**CCT**GTG-TGTGGAGACTCCAACACTAAATTCCGACA	1	T	9.0%	1625	1545	1035	9.43%	8.18%	9.36%
1c	Lys2-	+1	GAGAA**CCT**GTG**G**TTGTGGAGACTCCAACACTAAATTCCGACA			7.0%	1366	1182	747	7.93%	6.26%	6.76%
1c	Lys2+	-3	GAGAA**CCT**GTGT---GGAGACTCCAACACTAAATTCCGACA	3	TGT	5.7%	1241	940	555	7.20%	4.98%	5.02%
1c	Lys2-	-2	GAGAA**CCT**GT--TGTGGAGACTCCAACACTAAATTCCGACA	2	GT	5.0%	877	823	612	5.09%	4.36%	5.54%
1c	Lys2-	-5	GAGAA**CCT**GTG-----GAGACTCCAACACTAAATTCCGACA	2	TG	3.9%	704	747	390	4.09%	3.96%	3.53%
1c	Lys2-	-16	GAGA----------------CTCCAACACTAAATTCCGACA	4	GAGA	3.3%	458	755	373	2.66%	4.00%	3.37%
1c	Lys2-	-23	GAGAA**CCT**-----------------------AATTCCGACA			3.3%	458	755	373	2.66%	4.00%	3.37%
1c	Lys2+	-15	GAGAA**CC**---------------CCAACACTAAATTCCGACA	13549		2.4%	407	491	255	2.36%	2.60%	2.31%
1c	Lys2-	-16	GAGAA**CCT**----------------AACACTAAATTCCGACA	9996	0.737766625	2.2%	289	429	294	1.68%	2.27%	2.66%
1c	Lys2-	+2	GAGAA**CCT**GTG**TT**TTGTGGAGACTCCAACACTAAATTCCGACA			1.6%	270	307	162	1.57%	1.63%	1.47%
1c	Lys2-	+2	GAGAA**CCT**GTG**GG**TTGTGGAGACTCCAACACTAAATTCCGACA			1.4%	278	299	118	1.61%	1.58%	1.07%
1c	Lys2-	+1	GAGAA**CCT**GTG**A**TTGTGGAGACTCCAACACTAAATTCCGACA			0.6%	140	11	107	0.81%	0.06%	0.97%
1c	Lys2+	+3	GAGAA**CCT**GTG**TTT**TTGTGGAGACTCCAACACTAAATTCCGACA			0.6%	161	52	50	0.93%	0.28%	0.45%
1c	Lys2-	-17	GAGAA**CC**-----------------AACACTAAATTCCGACA	2	CC	0.4%	55	83	32	0.32%	0.44%	0.29%
1c	Lys2-	-14	GAGAA**CCT**G--------------CAACACTAAATTCCGACA			0.3%	77	50	31	0.45%	0.26%	0.28%
1c	Lys2+	-15	GAGAA**CC**-------------------CACTAAATTCCGACA			0.3%	33	56	52	0.19%	0.30%	0.47%
1c	Lys2+	-28	----------------------------AACACTAAATTCCGACA	3	TCC	0.3%	52	71	29	0.30%	0.38%	0.26%
1c	Lys2+	+3	GAGAA**CCT**GTGT**GGG**TGTGGAGACTCCAACACTAAATTCCGACA			0.1%	31	7	13	0.18%	0.04%	0.12%
1c	Lys2+	-15	GAGAA**CCT**---------------CAACACTAAATTCCGACA			0.1%	22	17	5	0.13%	0.09%	0.05%
						100.00%	**16613**	**18099**	**10681**	**100.0%**	**100.00%**	**100.00%**

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Reads	% Normalized
4W	**Lys2+ WT**	NA	TCAAAGTGTTTGCCCACGTCA**GGG**CCAAGGATGAAGAAGCTGCATT			**22**	**0.65%**
4w	Lys2-	+1	TCAAAGTGTTTGCCCAC**G**GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			756	22.26%
4w	Lys2-	-11	TCAAAGTGTTTGCCCA-----------AGGATGAAGAAGCTGCATT	2	CA	697	20.52%
4w	Lys2-	-1	TCAAAGTGTTTGCCCAC-TCA**GGG**CCAAGGATGAAGAAGCTGCATT			669	19.70%
4w	Lys2+	-12	TCAAAGTGTTTGCC------------AAGGATGAAGAAGCTGCATT	2	CC	355	10.45%
4w	Lys2-	+2	TCAAAGTGTTTGCCCA**CG**CGTCA**GGG**CCAAGGATGAAGAAGCTGCATT			198	5.83%
4w	Lys2-	-16	TCAAAG---------------**-GG**CCAAGGATGAAGAAGCTGCATT	1	G	108	3.18%
4w	Lys2-	-10	TCAAAGTGT----------CA**GGG**CCAAGGATGAAGAAGCTGCATT	2	GT	82	2.41%
4w	Lys2-	+1	TCAAAGTGTTTGCCCACG**T**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			43	1.27%
4w	Lys2+	1bps C->T	TCAAAGTGTTTGCCCA**t**GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			42	1.24%
4w	Lys2-	-1	TCAAAGTGTTTGCC-ACGTCA**GGG**CCAAGGATGAAGAAGCTGCATT	1	C	33	0.97%
4w	Lys2+	-30	TCAAAGTGTTTGC------------------------------ATT	1	C	32	0.94%
4w	Lys2+	-12	TCAAAGTGTTTGCCC------------AGGATGAAGAAGCTGCATT	1	A	30	0.88%
4w	Lys2-	-11	TCAAAGTGTTTGCCCACG-----------GATGAAGAAGCTGCATT	1	G	30	0.88%
4w	Lys2-	+2	TCAAAGTGTTTGCCCACG**TG**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			29	0.85%
4w	Lys2-	+2	TCAAAGTGTTTGCCCAC**GG**GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			28	0.82%
4w	Lys2-	-5	TCAAAGTGTTTGCCCA-----**GGG**CCAAGGATGAAGAAGCTGCATT	2	CA	26	0.77%
4w	Lys2-	-2	TCAAAGTGTTTGCCCA--TCA**GGG**CCAAGGATGAAGAAGCTGCATT			19	0.56%
4w	Lys2-	+1	TCAAAGTGTTTGCCCAC**C**GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			19	0.56%
4w	Lys2+	-6	TCAAAGTGTTTGCCCAC----**--G**CCAAGGATGAAGAAGCTGCATT	1	G	15	0.44%
4w	Lys2+	-18	TCA------------------**GGG**CCAAGGATGAAGAAGCTGCATT	1	A	13	0.38%
4w	Lys2-	-4	TCAAAGTGTTTGCC----TCA**GGG**CCAAGGATGAAGAAGCTGCATT			11	0.32%
4w	Lys2-	-22	TCAAAGTGTT----------------------GAAGAAGCTGCATT	1	T	11	0.32%
4w	Lys2-	+1	TCAAAGTGTTTGCCCACG**C**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			11	0.32%
4w	Lys2-	-7	TCAAAGTGTTTGC-------A**GGG**CCAAGGATGAAGAAGCTGCATT	1	C	10	0.29%
4w	Lys2+	-21	TCAAAGTGTTTG---------------------AAGAAGCTGCATT	2	TG	10	0.29%
4w	Lys2-	-25	TCAAAGTG-------------------------AAGAAGCTGCATT	2	TG	10	0.29%

4w	Lys2+	-6	TCAAAGTGTTTGCC------A**GGG**CCAAGGATGAAGAAGCTGCATT	1	C	9	0.27%
4w	Lys2-	+1	TCAAAGTGTTTGCCCAC**T**GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			9	0.27%
4w	Lys2-	-10	TCAAAGTGTTTGCCC----------CAAGGATGAAGAAGCTGCATT	1	C	8	0.24%
4w	Lys2-	+1	TCAAAGTGTTTGCCCACG**A**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			7	0.21%
4w	Lys2-	-1	TCAAAGTGTTTGCCCA-GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			6	0.18%
4w	Lys2+	-6	TCAAAGTGTTTG------TCA**GGG**CCAAGGATGAAGAAGCTGCATT	1	G	6	0.18%
4w	Lys2+	1bps G->A	TCAAAGTGTTTGCCCAC**a**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			6	0.18%
4w	Lys2+	1bps G->T	TCAAAGTGTTTGCCCAC**t**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			6	0.18%
4w	Lys2+	-3	TCAAAGTGTTTGCCCACG---**GGG**CCAAGGATGAAGAAGCTGCATT			4	0.12%
4w	Lys2+	-12	TCAAAGTG------------A**GGG**CCAAGGATGAAGAAGCTGCATT			4	0.12%
4w	Lys2+	1bps G->T	TCAAAGTGTTT**t**CCCACGTCA**GGG**CCAAGGATGAAGAAGCTGCATT			4	0.12%
4w	Lys2+	1bps C->A	TCAAAGTGTTTGCC**a**ACGTCA**GGG**CCAAGGATGAAGAAGCTGCATT			3	0.09%
4w	Lys2-	-2	TCAAAGTGTTTGCCC--GTCA**GGG**CCAAGGATGAAGAAGCTGCATT	1	C	2	0.06%
4w	Lys2-	-5	TCAAAGTGTTTGC-----TCA**GGG**CCAAGGATGAAGAAGCTGCATT			2	0.06%
4w	Lys2-	-8	TCAAAGTGTTTGCCCAC--------CAAGGATGAAGAAGCTGCATT	1	C	2	0.06%
4w	Lys2-	-1	TCAAAGTGTTTGCCCACG-CA**GGG**CCAAGGATGAAGAAGCTGCATT			2	0.06%
4w	Lys2+	-9	TCAAAGTGTT---------CA**GGG**CCAAGGATGAAGAAGCTGCATT	1	T	2	0.06%
4w	Lys2+	+3	TCAAAGTGTTTGCCCAC**GGG**GTCA**GGG**CCAAGGATGAAGAAGCTGCATT			2	0.06%
4w	Lys2-	-2	TCAAAGTGTTTGCCCAC--CA**GGG**CCAAGGATGAAGAAGCTGCATT			1	0.03%
4w	Lys2-	+2	TCAAAGTGTTTGCCCACG**TT**TCA**GGG**CCAAGGATGAAGAAGCTGCATT			1	0.03%
4w	Lys2+	1bps T->G	TCAAAGTGTTTGCCCAC**g**GCA**GGG**CCAAGGATGAAGAAGCTGCATT			1	0.03%
						3396	**100.00%**

gRNA	Lys2+/-	Insertion(+) Deletions(-)	Sequence	Microhomology		Frequency	% Normalized
4C	**Lys2+ WT**	NA	TCAAAGTGTTTG**CCC**ACGTCAGGGCCAAGGATGAAGAAGCTGCATT			***95***	***6.25%***
4c	Lys2-	-11	TCAAAGTGTTTG**CCC**A-----------AGGATGAAGAAGCTGCATT	1	A	213	14.00%
4c	Lys2+	1bps C->A	TCAAAGTGTTTG**CCC**ACGT**a**AGGGCCAAGGATGAAGAAGCTGCATT			183	12.03%

4c	Lys2-	+1	TCAAAGTGTTTG**CCC**ACG**G**TCAGGGCCAAGGATGAAGAAGCTGCATT			149	9.80%
4c	Lys2+	-12	TCAAAGTGTTTG**CCC**AC------------GATGAAGAAGCTGCATT	1	G	147	9.66%
4c	Lys2+	1bps C->A	TCAAAGTGTTTG**CCC**A**a**GTCAGGGCCAAGGATGAAGAAGCTGCATT			114	7.50%
4c	Lys2+	1bps C->T	TCAAAGTGTTTG**tCC**ACGTCAGGGCCAAGGATGAAGAAGCTGCATT			107	7.03%
4c	Lys2+	-12	TCAAAGTGTTTG**CC-**-----------AAGGATGAAGAAGCTGCATT	2	CC	69	4.54%
4c	Lys2+	1bps G-> C	TCAAAGTGTTTG**CCC**A**c**CTCAGGGCCAAGGATGAAGAAGCTGCATT			50	3.29%
4c	Lys2+	+3	TCAAAGTGTTTG**CCC**AC**GGG**GTCAGGGCCAAGGATGAAGAAGCTGCATT			47	3.09%
4c	Lys2+	6bps ATGAAGA->AGGTGAT	TCAAAGTGTTTG**CCC**ACGTCAGGGCCAAGGA**ag**G**tgat**AGCTGCATT			39	2.56%
4c	Lys2+	2bps CAA->GAG	TCAAAGTGTTTG**CCC**ACGTCAGGGC**gag**GGATGAAGAAGCTGCATT			37	2.43%
4c	Lys2-	+2	TCAAAGTGTTTG**CCC**ACGTTTCA**GG**GCCAAGGATGAAGAAGCTGCATT			35	2.30%
4c	Lys2+	1bps A->T	TCAAAGTGTTTG**CCCt**CGTCAGGGCCAAGGATGAAGAAGCTGCATT			31	2.04%
4c	Lys2+	1bps G->T	TCAAAGTGTTTG**CCC**AC**t**TCAGGGCCAAGGATGAAGAAGCTGCATT			20	1.31%
4c	Lys2+	1bps C->T	TCAAAGTGTTTG**CtC**ACGTCAGGGCCAAGGATGAAGAAGCTGCATT			19	1.25%
4c	Lys2-	+1	TCAAAGTGTTTG**CCC**ACG**A**TCAGGGCCAAGGATGAAGAAGCTGCATT			18	1.18%
4c	Lys2+	1bps G-> C	TCAAAGTGTTTG**CCC**ACGTCAGG**c**CCAAGGATGAAGAAGCTGCATT			18	1.18%
4c	Lys2+	1bps G-> C	TCAAAGTGTTTG**CCC**ACGTCA**c**GGCCAAGGATGAAGAAGCTGCATT			18	1.18%
4c	Lys2-	-1	TCAAAGTGTTTG**CC-**ACGTCAGGGCCAAGGATGAAGAAGCTGCATT			14	0.92%
4c	Lys2-	-1	TCAAAGTGTTTG**CCC**ACGTCAGGGCC-AGGATGAAGAAGCTGCATT			13	0.85%
4c	Lys2+	4bps CCCAC->GGGAA	TCAAAGTGTTTG**ggg**A**a**CGTCAGGGCCAAGGATGAAGAAGCTGCATT			10	0.66%
4c	Lys2+	1bps C->G	TCAAAGTGTTTG**CCC**ACGT**g**AGGGCCAAGGATGAAGAAGCTGCATT			10	0.66%
4c	Lys2+	1bps T->G	TCAAAGTGTTTG**CCC**ACG**g**CAGGGCCAAGGATGAAGAAGCTGCATT			10	0.66%
4c	Lys2-	-1	TCAAAGTGTTTG**CCC**ACG-CAGGGCCAAGGATGAAGAAGCTGCATT	1	G	8	0.53%
4c	Lys2-	-2	TCAAAGTGTTTG**CCC**AC--CAGGGCCAAGGATGAAGAAGCTGCATT			7	0.46%
4c	Lys2-	-1	TCAAAGTGTTTG**CCC**ACGTCA-GGCCAAGGATGAAGAAGCTGCATT	1	G	6	0.39%
4c	Lys2+	7bps GTCAGGG->ACCACAC	TCAAAGTGTTTG**CCCac**AC**cac**ACCCAAGGATGAAGAAGCTGCATT			6	0.39%
4c	Lys2+	1bps G-> T	TCAAAGTGTTTG**CCC**ACGTCA**t**GGCCAAGGATGAAGAAGCTGCATT			6	0.39%
4c	Lys2+	-12	TCAAAGTGTTTG**CCC**------------AGGATGAAGAAGCTGCATT	1	A	5	0.33%
4c	Lys2-	+2	TCAAAGTGTTTG**CCC**ACG**CG**TCAGGGCCAAGGATGAAGAAGCTGCATT			4	0.26%

4c	Lys2-	-16	TCAAAG----------------GGCCAAGGATGAAGAAGCTGCATT			2	0.13%
4c	Lys2-	+1	TCAAAGTGTTTG**CCC**AC**C**GTCAGGGCCAAGGATGAAGAAGCTGCATT			2	0.13%
4c	Lys2-	+1	TCAAAGTGTTTG**CCC**AC**T**GTCAGGGCCAAGGATGAAGAAGCTGCATT			2	0.13%
4c	Lys2+	1bps C->A	TCAAAGTGTTTG**CCC**ACGTCAGGGC**a**AAGGATGAAGAAGCTGCATT			2	0.13%
4c	Lys2+	1bps C->T	TCAAAGTGTTTG**CCC**A**t**GTCAGGGCCAAGGATGAAGAAGCTGCATT			2	0.13%
4c	Lys2+	1bps G->A	TCAAAGTGTTTG**CCC**AC**a**TCAGGGCCAAGGATGAAGAAGCTGCATT			2	0.13%
4c	Lys2+	-6	TCAAAGTGTTTG**CCC**AC------GCCAAGGATGAAGAAGCTGCATT	1	G	1	0.07%
						1521	**100.00%**

Suppl. Table 4: Percent of survivors with one or more bases inserted.
Sequence data are presented in Suppl. Table 2. Data for LYS2-1 and LYS2-4 reflect Illumina sequencing with thousands of reads, whereas those for LYS2-2, LYS2-3 and CAN1-1 are based on approximately 100 individual sequences.

A Percent insertions

Bases Inserted	gLYS2-1^{W}	gLYS2-1^{C}	gLYS2-2^{W}	gLYS2-2^{C}	gLYS2-3^{W}	gLYS2-3^{C}	gLYS2-4^{W}	gLYS2-4^{C}	gCAN1-1^{W}	gCAN1-1^{C}
+1 predicted	16	21	10	36	0	51	22	0	23	70
+1 other	2	8	14	11	0	0	3	11	27	4
+2	7	2	10	10	3	0	8	3	13	3
+3	1	1	5	9	2	0	0	3	9	1

B Percent homonucleotide insertions

Bases Inserted	gLYS2-1^{W}	gLYS2-1^{C}	gLYS2-2^{W}	gLYS2-2^{C}	gLYS2-3^{W}	gLYS2-3^{C}	gLYS2-4^{W}	gLYS2-4^{C}	gCAN1-1^{W}	gCAN1-1^{C}
+2	100	100	100	100	100	0	12	100	85	100
+3	100	100	60	89	100	0	0	100	100	100