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	5' 4 3' 1	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG C CGCCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG C CAGCTGGACGTCCGTACGTTCGAACCGTGACCGGCAGC		CTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGC 3'					
	5	u Ser Thr Cys Arg His Ala Ser Leu Ala Leu Ala Val V	30 j	Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg					
			0	lacZo -					
			PAM 1364						
1B	+	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT TTACAACGTCGTGAC					
2B	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT TTACAACGTCGTGAC					
1W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT <mark>GTAAAAGCGGCCGCCGACGGCCGCTT</mark> TTACAACGTCGTGACT					
2W	+	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACT					
3W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT <mark>GTAAAAGCGGCCGCCGACGGCCGCTT</mark> TTACAACGTCGTGACT					
4W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT <mark>GTAAAAGCGGCCGCCGACGGCCGCTT</mark> TTACAACGTCGTGACT					
5W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TIGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACT					
6W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TIGTAAAAGCGGCCGTCGGCGGCCGCTTTTACAACGTCGTGACT					
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8W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TIGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACT					
9W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		I GTAAAAGCGGCCGCCGACGGCCGCTT					
10W	-	AGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	60660066	TTACAACGICGIGACT					
11W	-	AGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		I GTAAAAGCGGCCGCCGACGGCCGCTT					
12W	-	ABTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		I GTAAAAGCGGCCGCCGACGGCCGCTT					
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111/	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
1214		AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	60660060	TT TTACAACGTCGTGACT					
13W	1	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
14W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
2W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TIGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACT					
7W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
9W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
10W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
11W	-	GTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
12W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
13W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
14W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
15W	+	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	60660060	TT TTACAACGTCGTGACT					
16W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
17W	+	GTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		T GTAAAAGCGGCCGCCGACGGCCGCTT TACAACGTCGTGACT					
18W	-	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					
19W	+	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT <mark>GTAAAAGCGGCCGCCGACGGCCGCTT</mark> TACAACGTCGTGACT					
20W	+	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	60660060	TT TTACAACGTCGTGACT					
21W	-	GTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	60660060	TT TTACAACGTCGTGACT					
22W	+	AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TT TTACAACGTCGTGACT					

B

NS 3' CCTAGGRAGATCTCAECTGGACGTCCGTACGTTCGAACCGGCACCGGCAGGGAAAATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCG 5'

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B	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
2B	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
B	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
ZB	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
zw	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
SW	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
5W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
5W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
7W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
BW	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
low	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
1W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
L2W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
L3W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
14W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
15W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
16W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
17W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
18W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
20W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
21W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG	C
22W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
2W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
3W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
5W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	c
SW	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
7W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
BW	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
11W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
12W	+	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
L3W	-	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
L4W	-	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
15W	-	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
16W	-	GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	CI
1.7W	-	BUSATULTUTAGAGTUGACUTUGAGGATUGAAGUTTUGAGCACTGGCCGT	c
ISW	-	BUSATULTITAGAUTUGALUTUGAUGUATUGAAUUTTUGGCACTGGCCGT	C
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20W	1	OBDATECTETAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT	C
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CO		тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CO		тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG		TTGTAAAAGCGGCCGCCGACGGCCGCTTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG		TTGTAAAAGCGGCCGCTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
co	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CO	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CO		TTGTAAAAGCGGCCGCCGACGGCCGCTTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG		TTGTAAAAGCGGCCGCCGACGGCCGCTTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
co		TTGTAAAAGCGGCCGCCGACGGCCGCTTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG		TTGTAAAAGCGGCCGCCGACGGCCGCCTTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тттт	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	GCGGCCGC	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG	60660060	тт т	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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Ce	60660060	ттт	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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CG		T	TAMAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG		TIGTAAAAGCGGCCGCTT T	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
CG		TUSAAAAGCGGCCGCTT	TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC
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> TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA OCGGCCGCTTT TACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAI TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAL TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA GCCGCTTT TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAI AGCGGECCCTTTTACAACGTCGTGACTGCGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA

		GGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
18	+	GETACCCEESEATCCTCTAGASTCGACCTGCAGGCATGCAAGCTTEGCACTGGCCGT
2B	+	GGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
2B	+	GGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
1W	+	GETACCCGESEATCCTCTABASTCGACCTECAGECATECAAECTTEECACTEGCCET
2W	+	GGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
4W	+	GGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
5W	+	DIGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
6W	+	COGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
7W	+	GGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
8W	+	GGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
11W	+	DEGTACCCEGESEATCCTCTAGASTCGACCTGCAGECATGCAAGCTTGSCACTGECCET
12W	+	GETACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
13W	+	SETACCCEGEGEATCCTCTAGAETCGACCTGCAGECATGCAAGCTTGECACTEGCCET
15W	+	GETACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
16W	+	CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
17W	+	CONTACCCGOGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
18W	+	CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
19W	+	DEGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
20W	+	COGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
2W	+	CONTACCCGOGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
3W	+	SGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
4W	+	DEGTACCCGGEGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
5W	+	CONTACCCOGGOATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
6W	+	CONTACCCGOGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
7W	+	GGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
8W	+	DEGTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
9W	+	COGTACCCGGGGGANCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
10W	+	CONTRACTOR C
11W	+	DIGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
13W	+	DEGTACCCGGEGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
14W	+	DEGTACCCGGEGEATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGECACTGGCCGT
15W	+	DEGTACCCGGEGGATCCTCTAGAGTCGACCTGCAGECATGCAAGCTTGGCACTGGCCGT
16W	+	CONTACCCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
17W	+	CONTRACCOSSERANCETETAGASTEGACCTSCAGSCATSCAASETTGSCACTSSCCST
18W	+	DESTACCCGEGGAACCTCTAGASTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
19W	+	CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
20W	+	DEGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
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CI		TGTAAAAGCGGCCGCTT	T.
C	aceaccec]	T	т
C	sceecee	т	т
C		TGTAAAAGCGGCCGCCGACGGCCGCTT	T.
C	acesccec)	T	т
C	ecesceec	T	т
C	acsaccac	т	т
C	scasces	T	т
C		TGTAAAAGCGGCCGCCGACGGCCGCTT	h.
C	sesseese	T	т
C		TGTAAAAGCGGCCGCCGACGGCCGCTT	r.
C	6C66CC6C	T	Т
C	ecesces)	T	т
c		TGTAAAAGCGGCCGCTT	h.
C		TGTAAAAGCGGCCGCTT	T.
C		TGTAAAAGCGGCCGCTT	T.
C		T	т
C		TGTAAAAGCGGCCGCTT	т
C	acascese	T	т
C		T	TAAGI
c		T	TAAAI
C	acascese	T	т
C	aceseces	т	т
C		TGTAAAAGCGGCCGCTT	T.
c		TGTAAAAGCGGCCGTCGGCGGCCGCTT	h.
c		TGTAAAAGCGGCCGCCGACGGCCGCTT	7
C		TGTAAAAGCGGCCGCTT	T.
C		TGTAAAAGCGGCCGCTT	T.
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C		TGTAAAAGCGGCCGCTT	T
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Figure S1A-C: Additional data for Figure 3A-C - Symmetrical Homology Arm Lengths using CRISPR/Cas12a. Each image illustrates Cas12a in blue with the PAM site in red and a staggered red arrow indicating the 5 base, 5' overhang created after cleavage occurs. (A-C) Additional data for Symmetrical Homology Arm Length of 20, 50, and 75 base pairs (48, 108, and 158 bases total). The nonsense symmetrical oligonucleotide with lengths of 20, 50, or 75 base pairs on either side of the cut site is located above the *lacZ* gene, contains an eight base *NOT*I site in the center, and is positioned in a 3' to 5' orientation. The purple box highlights the correct integration of a *NOT*I site while bases highlighted in red outside of the purple box, indicate unintended integration or deletion events. The total number of colonies and the category for each event can be found in the tables located in Figure 3A-C. All blue colonies were found to harbor wild-type sequences and are represented by several blue colonies (labeled 1B and 2B) in all images. A

		NS 3' TTCGAACCGTGACC	GGCAGCAAAATGTTGCAGCACTGACC										
		1364: 20 HDR-NS											
		5 GGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCAC	TGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTT										
		3' CCTAGGAGATCTCAGCTGGACGTCCGTACGTTCGAACCGTG	ACC66CAGCAAAATGTT6CAGCACT6ACCCTTTT666ACC6CAA										
		15 20 25 ly Asp Pro Leu Glu Ser Thr Cys Arg His Ala Ser Leu Ala L	eu Ala Val Val Leu Gin Arg Arg Asp Trp Glu Asn Pro Gly Val										
			Cas9 PAM Cas9-1364										
		CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG TTTTACAACGTCGTGACTGGGAAAACCCTGGC										
1B	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG TTTTACAACGTCGTGACTGGGAAAACCCTGGC										
2B	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG TTTTACAACGTCGTGACTGGGAAAACCCTGGC										
1B	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
2B	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG TTTTACAACGTCGTGACTGGGAAAACCCTGGC										
1W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCO										
2W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCO										
3W	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTC TTTTACAACGTCGTGACTGGGAAAACCCTGGC										
4W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTC0GCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
5W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTC0ACAACGTCGTGACTGGGAAAACCCTGGC										
5W	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
7W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
BW	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
we	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTC0 CAACGTCGTGACTGGGAAAACCCTGGC										
10W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
121	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
13W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
14W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
151	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
1611/	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
1714	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
1 014/	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
1011/	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
2014/	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
2000	-	CCGGGGNTCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
22144	Ξ	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
1W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTC4GCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
2W	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
3W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
4W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
SW	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
6W	-	CCGGGGNTCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
7W	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
BW	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
WP	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
100	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
11W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
12W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
13W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
14W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
15W	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
16W	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
170	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
18W	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG TGACTGGGAAAACCCTGGC										
190	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
200	-	CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG											
211	-	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGG	CCGTCG										
2214	1	CCGGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAGGCTTGGCACTGG	CCGTCGGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGC										
-244	-												

NS	3' cctaggagatctcagctggacgtccgtacgttcgaaccgtgaccggc	AGCAAAAT	GTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCG 5'							
	1364: 50 HDR-NS Noti	60000000								
5'	ACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG	TCGTTTT	CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTT 3	ŕ.						
3'	TGGGCCCCTAGGAGATCTCAGCTGGACGTCCGTACGTTCGAACCGTGACCGCG	AGCAAAAT	GTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCGTGTAGGGGGGAAA 5	1						
	I Pro Gly Asp Pro Leu Glu Ser Thr Cys Arg His Ala Ser Leu Ala Leu Ala 1	Val Val Leu	Gin Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gin Leu Asn Arg Leu Ala Ala His Pro Pro Ph Lozzo →							
	Cas9 PAM	G	s9-1364							
	ACCTGCAGGCATGCAAGCTTGGCACTGGCCGCCG TTTTACAACGTCGTGACTGGGAAAACCCTGGCGT									
			TITTACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	28 ACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TITTACAACGTCGTGACTGGGAAAAACCCTGGCGT1							
	1B = GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	28 GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	1W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	2W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	6W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	7W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	8W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	9W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	10W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	11W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	12W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	13W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	14W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	15W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	16W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT1							
	17W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	18W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	19W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	20W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	21W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TGACTGGGAAAACCCTGGCGT1							
	22W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	sceeccec	TTTTACAACGTCGTGACTGGGAAAACCCTGGCGT1							
	1W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	60660060	TTTTACAACGTCGTGACTGGGAAAACCCTGGCGT							
	2W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	3W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	4₩ → SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	5W - GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	6W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	7W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	GCGGCCGC	TTTTACAACGTCGTGACTGGGAAAACCCTGGCGT							
	8W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	9W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	10W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	11W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	12W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TT-ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	13W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	14W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	15W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	16W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	17W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	18W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	19W - BACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							
	20W - SACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	CCTGCAGG	CATECAASCIISUCACIGGCCGTCGGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGCGT							
	21W - SACCIGCAGGCATGCAAGCTIGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGT							
	22W - FACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGT							

NS

3	AATGCTTAAGCTCGAGCCATGGGCCCCCTAGGAGGTCTCGAGCTGGCGCCGCGCGCG	
	U 1364: 75 HDR-NS <u>GECCÉGCE</u>	
5'	ATTACGAATTCGAGCTCGGGAACCCGGGGATCCTCTAGAGTCGACCTGGCATGCAAGCTTGGCACTGGCACTGGCACTGGCACTGGCGTGACCGGGGAAAACCCTGGCGTACCCGACTTAATCGCCTTGCAGCACAACCTTGCGCAGCTTGCGCAGCTGGCACGACCTGGCGTGACCGGGGAAAACCCTGGCGTGACCGGGGAAAACCCTGGCGTGACGACGTCGGCGTGACGGCGTGACGGGGAAAACCCTGGCGTGACGGGGAAAACCCTGGCGTGACGACGTCGGCGTGACGGCGTGGCGGGGGGGG	3'
3'	TAATSCTTAAGCCCARGEGCATGBGCCCCTAGBAGATCTCAGCTGGACGTCCGTACGTCGAACCGTGACCGTGAGCGACGACGACGACCGTGACCGTGACGGCCGACGACGACGACGACGACGACGACGACGACGAC	5'
	lac2o →	E.

Cas9 PAM Cas9-1364

		TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
18	+	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC
2B	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
1B	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
28	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTTTACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC
1W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC
2W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	1	CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
3W	+	CGAGCTCGGTACCCGGGGGANCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTCGCCA
4W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC
5W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
6W	+	CGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
7W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGTATGCAAGCTTGGCACTGGCCGTCG	\$cesecee	TTTTACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
8W	+	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGGCGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTCGCCA
9W	+	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
10W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTEEACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
11W	+	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTCGCCA
12W	+	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
1W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC
2W	+	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
3W	+	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	1	CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTCGCCA
4W	-	TCGAGCTCGGTACCCGGGGGANCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
5W	+	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
6W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
7W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGGCGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
8W	+	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TTICACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
9W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	aceaccec	TTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
10W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
11W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
12W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
13W	+	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACGTCGTGGTCGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
14W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
15W	-	TCGAGCTCGGTACCCGGSGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
16W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGGCTGGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
17W	-	TEGAGETEGGTACCEGGGGGATECTE	TAGAGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTEGTEGTEGEGGGAGAAAACCETEGEGETTACCCAACTTAATEGEETTEGEGGGAGACATECECETTTEGECAG
18W	-	TCGAGCTEGGTACCEGGGGATECTE	TAGAGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGICGICGIGACIGGGAAAACCCIGGCGITACCCAACITAAICGCCIIGCAGCACAICCCCCIIICGCCAG
19W	-	TEGAGETEGGTACCEGGGGATECTE	TAGAGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	1	
2000	-		TABASTCORCCTOCROOCATOCARGCTTOGCACTOGCCOTCO		
21W	-		TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		
2200	-		TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		
2300	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		
2497		TCOACCTCOCTACCCOOCCATCCTC	TABADTCOACCTOCADOCATOCAADCTTOOCACTOCCOTCO		
2500	Ξ.	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTEGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		
26W	1	TCGASCTCSSTACCCSSSSATCCTC	TAGAGTEGACCTBCAGGCATSCAAGCTTBGCACTBGCCGTCG		CAACGICGIGACIGGGAAAAACCCIGGCGIIACCCAACIIAATCGCCIIGCGGGCGCAICCCCCIIICGCCAG
2814	1	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
201	1	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		CAACGTCGTGACTGGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTCGCCAG
30%	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		TEACAACGTCBTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTBCAGCACATCCCCCTTTCBCCAG
31W	-	TCGAGCTCGGTACCCGGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG	1	CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTCGCCAG
32W	-	TCGAGCTCGGTACCCGGGGATCCTC	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG		ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG

Figure S2A-C: Additional data for Figure 4. A-C - Symmetrical Homology Arm Lengths using CRISPR/Cas9. Symmetrical Homology Arm Lengths using CRISPR/Cas9.Each image illustrates the location of Cas9 in green and the PAM site in red. A solid red line indicates the blunt ended cleavage site. (A-C) Symmetrical Homology Arm Lengths of 20, 50, and 75 base pairs (48, 108, and 158 bases total). The nonsense symmetrical oligonucleotide with lengths of 20, 50, or 75 base pairs on either side of the cut site is located above the *lacZ* gene, contains an eight base *NOT*I site in the center, and is positioned in a 3′ to 5′ orientation. The purple box highlights the correct integration of a *NOT*I site and bases highlighted in red outside of the purple box indicate unintended integration or deletion events for each image. The total number of colonies and the category for each event can be found in the tables located in Figure 4A-C. All blue colonies were found to harbor wild-type sequences and are represented by the four blue colonies (labeled 1B and 2B) in all images.



Figure S3A-B: CRISPR/Cas control reactions with and without cell-free extract. A) Cas12a 1364 reaction with 50 base homology arm lengths. B) Cas9 1364 reaction with 20 base homology arm lengths. The left image contains two Cas12a plates and the right image contains two Cas9 plates. The top plates for each encompass reactions with cell-free extract while the bottom plate reactions do not contain cell-free extract. The top plates for both Cas12a and Cas9 display blue and white colonies; (all white colonies are circled in black) and only blue colonies are present on the bottom plates.



Figure S4A-B: Comparison of Direct *In Vitro* **Product Sequencing via Sanger and Next-Generation Sequencing.** For both figures, DNA was isolated and PCR-amplified directly after recircularization, with no bacterial transformation. **A)** Amplicons were sequenced via Sanger sequencing, and resultant indels were deconvoluted using TIDE. The grey bar at 0 represents wild-type, perfectly repaired sequences. Orange bars represent statistically significant indels, as determined by pearson's r via TIDE. Black bars represent non-significant background. **B)** Amplicons were sequenced via Illumina TruSeq next-generation sequencing, and resultant indels were determined using CRIS.py. Each bar consists of stacked sequences containing all sequence variations of the appropriate indel length in blue, orange, and cyan (in order of contribution percentage). The grey bar at 0 represents wild-type sequences. The horizontal orange bar represents an axis break from 14,000 to 60,405 reads.

Table S1: Fisher's exact test comparing 20, 50, and 75 base homology arms within CRISPR/Cas12a and within CRISPR/Cas9.

Cas12a 20HDR-NS vs. Cas12a 50HDR-NS					*Cas9 20HDR-NS vs. Cas9 50HDR-NS				
	HDR	Indel	Total			HDR	Indel	Total	
Cas12a 1364	42	22	64		Cas9 1364	20	41	61	
20HDR-NS					20HDR-NS				
Cas12a 1364	34	28	62		Cas9 1364	3	58	61	
50HDR-NS					50HDR-NS				
Column Totals	76	50	126		Column Totals	23	99	122	
P-Value < 0.05				0.2748	P-Value < 0.05				0.0001
*Cas12a 20	HDR-NS	5 vs. Casi	12a 75HD	R-NS	*Cas9 20HI	DR-NS v	rs. Cas9	75HDR-	NS
	HDR	Indel	Total			HDR	Indel	Total	
Cas12a 1364	42	22	64		Cas9 1364	20	41	61	
20HDR-NS					20HDR-NS				
Cas12a 1364	26	30	56		Cas9 1364	2	61	63	
75HDR-NS					75HDR-NS				
Column Totals	68	52	120		Column Total	22	102	124	
P-Value <0.05				0.0428	P-Value <0.05				0
Cas12a 501	HDR-NS vs. Cas12a 75HDR-NS				Cas9 50HDR-NS vs. Cas9 75HDR-NS				
	HDR	Indel	Total			HDR	Indel	Total	
Cas12a 1364	34	28	62		Cas9 1364	3	58	61	
50HDR-NS					50HDR-NS				
Cas12a 1364	26	30	56		Cas9 1364	2	61	63	
75HDR-NS					75HDR-NS				
Column Totals	60	58	118		Column Totals	5	119	124	
P-Value < 0.05				0.461	P-Value < 0.05				0.6772

Table S1: Fisher's exact test comparing 20, 50, and 75 base homology arms within CRISPR/Cas12a and within

CRISPR/Cas9. Fisher's exact test was used to compare the three lengths of homology arms within each nuclease. Statistically significant conditions within the comparisons are highlighted in red along with the associated P-value. The total number of precise HDR events and Indels are indicated along with the column totals. The P-value was set to < 0.05 for all comparisons.

	(Cas12a 136	4 20HDR-N	S				Cas9 1364	20HDR-NS		
Trial	Colony Color	Total	HDR	Indel	WT	Trial	Colony Color	Total	HDR	Indel	WT
1	White	20	4	16	0	1	White	18	11	7	0
	Blue	19	0	0	19		Blue	20	0	0	20
2	White	22	20	2	0	2	White	21	3	18	0
	Blue	2	0	0	2		Blue	2	0	0	2
3	White	22	18	4	0	3	White	22	6	16	0
	Blue	2	0	0	2		Blue	2	0	0	2
	Cas12a 1364 50HDR-NS							Cas9 1364	50HDR-NS		
Trial	Colony Color	Total	HDR	Indel	WT	Trial	Colony Color	Total	HDR	Indel	WT
1	White	20	11	9	0	1	White	20	0	20	0
	Blue	16	0	0	16		Blue	21	0	0	21
2	White	21	12	9	0	2	White	19	1	18	0
	Blue	2	0	0	2		Blue	2	0	0	2
3	White	21	11	10	0	3	White	22	2	20	0
	Blue	2	0	0	2		Blue	2	0	0	2
	(Cas12a 136	4 75HDR-N	S		Cas9 1364 75HDR-NS					
Trial	Colony Color	Total	HDR	Indel	WT	Trial	Colony Color	Total	HDR	Indel	WT
1	White	20	10	10	0	1	White	19	1	18	0
	Blue	20	0	0	20		Blue	20	0	0	20
2	White	16	10	6	0	2	White	12	0	12	0
	Blue	2	0	0	2		Blue	2	0	0	2
3	White	20	6	14	0	3	White	32	1	31	0
	Blue	2	0	0	2		Blue	2	0	0	2

Table S2: CRISPR/Cas12a and CRISPR/Cas9 20, 50, and 75 homology arm length triplicate comparison within each nuclease.

Table S2: CRISPR/Cas12a and CRISPR/Cas9 20, 50, and 75 homology arm length triplicate comparison within each nuclease. The table illustrates the total number of blue and white colonies sequenced for each homology arm length experiment (20, 50, or 75) and the triplicates performed within each experiment conducted for Cas12a (left) and Cas9 (right). The table displays which colonies were found to harbor homology-directed repair, insertions or deletions (Indels), or wild-type events. All blue colonies were found to harbor wild-type sequences. Each homology arm length shows comparable numbers of both blue and white colonies for all experiments within each nuclease.