5' AGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCG男CTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGC 3'

$3^{\prime}$ TCAGCTGGACGTCCGTACGTTCGAACCQTGACCGGCAGCAAAATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCG 5' $\frac{1}{3} \frac{1}{3}$


NS 3' ${ }^{\text {CCTAGGAGATCTCAGCTGGACGTCCGTACGTTCGAACCGTGACCGGCAGCAAAATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCG }}{ }^{\text {1364: } 50 \text { HOR-NS NotI }}$
5) TACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGA GTCCGTACGTTCGAACCGTGACCGGCAGCAAATOTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCGTGTAGGGGGAAAGCGGTCGACCGC



Gggatcctctagagtcgacetgcaggcatgcaagct tgecactgeccet
$1 \mathrm{~B} \rightarrow$ gGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT gggatcctctagagtcgacctgcaggcatgcaagcttggcactggccgt

1B $\rightarrow$ ggGatcctctagagtcgacctgcaggcatgcaagct tggcactgeccgt
$2 \mathrm{~B} \rightarrow$ gGGatCCTCTAGAGTCGACCTGCAGGCATGCAAGCTIGGCACTGGCCGT
1W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
$2 W \rightarrow$ ggGatcctctagagtcgacctgcaggcatgcaagcttggcactggccgt
$3 W \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
$4 W \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
$5 W \rightarrow$ gGGatCcictagagtcgacctgcaggcatgcaagct ggcactgeccgi
6W $\rightarrow$ GgGatcctctagagtcgacctgcaggcatgcaagcttggcactgeccgt
7W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
8W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
9W $\rightarrow$ gGGatcctctagagtcgacctocaggcatgcaagct ggcactgeccgt
10W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
11W $\rightarrow$ ggGatcctctagagtcgacctgcaggcatgcaagcttggcactgeccet
$12 \mathrm{~W} \rightarrow$ GgGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
13W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
14W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
15W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
16W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
$17 \mathrm{~W} \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
$18 \mathrm{~W} \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT
20W $\rightarrow$ Gggatcctctagagtcgacctgcaggcatgcaagct tggcactggccgt
$21 \mathrm{~W} \rightarrow$ gGGatcctctagagtcgacctgcaggcatgcaagcttggcactgeccog
$22 W \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT gggatcctctagagtcgacctgcaggcatgcaagcttggcactggccgt 2W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT 3W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT $4 \mathrm{~W} \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT

16W $\rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTG6CCGT
17W $\rightarrow$ G6GATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTG6CCGT 18W $\rightarrow$ gGgatcctctagagtcgacctgcaggcatgcaagcttggcactgeccgi 19W $\rightarrow$ GgGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT 20W $\rightarrow$ GgGatcctctagagtcgacctgcaggcatgcaagct tggcactgeccgt $21 W \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT $22 W \rightarrow$ GGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTGGCGGCCGC-

tacaacgicgtgactggeaanaccctegcgitacccaac
TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACCCAAG TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACCCAAG TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC tacaacgicgigactggeanaaccctggcgitacccaac TACAACGTCGTGAGTGGGAAAACCCTGGCGITACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC AAAAGCGGCCGCTITITACAACGTCGTGACTGGGAAAACCCTGGCGTTACGCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TAAAAGCGGCCGCTITTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGITACGCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACT6GGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTG6CGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC AAAAGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TAAAAGCGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TA-AACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAAC

5' 'gaccatgattacgattcgagctggotacggggatcctctagagtcgacctgcagocatocagectrgeactgecga 3. ACTGGTACTAATGCTTAAGCTCGAGCCATGGGCCCCTAGGAGATCTCAGCTGGAGTCCGTACGTTCGAACGGTGACGGGCAGCAA


Sgtacceggegatcctctagagicgacctgcagecatgcangcttsgcactgeccgt

28 -gciaccegggatcctctagagtcgacctocagecatgcaaccttogcactgcccg

$2 \mathrm{~W} \rightarrow$ - gGtacccgggatcctctagagtcgacctacaggcatgcaagcttgacactggccet
 5W $\rightarrow$ gGTacceggelatccteragagicgacctgcagecatgcaagctisgcactgeccgr
 7W $\rightarrow$ egtacceggegatcctctagagtcgacctgcagscatgcaagcttagcactegccet
 $11 \mathrm{~W} \rightarrow$ gotacccoggeatcetctagagtegacctgcagscatgcaagettagcactgeccet $12 \mathrm{~W} \rightarrow$ : 06 tacccgogeatcctctagagtcgacctochgecatgcaagcttsgcactogcce 13W $\rightarrow$ GGTACGCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT 15W $\rightarrow$ - getacceggebatcctctagagtcgacctgcagbcatgcaagcttgacactgeccet
$16 \mathrm{~W} \rightarrow$
$17 \mathrm{~W} \rightarrow$
$18 \mathrm{~W} \rightarrow$ 19W $\rightarrow$
$20 \mathrm{~W} \rightarrow$ cctctagagtcgacctocagecatgcanocttabeactogccgt getaccegggatcctctagagtcgacctgcagecatgcaagettggcactggcegt еCTCTAGAOTCOACCTOCAGSCATGCAAOCTTOBCACTOOCCGT ;gtacccgggatectcragagtegacetgcaggeatgcaagettggcactggceot bogtacccgeggatcctctagagtcoacctochgecatgcaaccttgacactgeccot sgctaccegggatectctagagtecacctocagecatgcaacettggeacteccegt - agitaccogggatectctagagtcgacetgcaggeatgcaagcttgacactgeccgt :gctaccegggatectctagagtegacctgcaggeatgcaagettggcactggcegt gotacccgggatcctctagagtcgacctgcagecatgcaagcttsgcactgoccot cctagcggggeatcctctagagtceacctocagecatgcaagcttgecactgeccg pgetaccegggatcctctagagtegacctgcagbcatgcaagettggcactgeccet :00taccggogoatcctctagagtcgacgtocagscatocanocttobcactogccot jgetacccgggang ctctagagtcgacctecaggcatgcaagcttgecactegccgt jegtaccegggatcctctagagtcoacctgcagecatocaagcttggeactegcegt :ggtaccegggatectctagagtegacctgcaggcatgcaagcttgocactgecegt gotaccegggatcctetagagtegacctgcaggcatgcaagcttageactgecegt getaccegggeatcetctagagicgacctgcagccatgcaacettgecacteccegt gotacecgggatcctctagagtcgacttgcagecatgcagocttgocactgeccet gctaccegggeatcctctagagtcgacctgcaggcatgcaagcttsgeactgecegt -getacceggegañectctagagtcgacctgcagecatgcabocttsgicactogccgt gGtacceggeganicctctagagtcgacctgcagecatgcaaccttsgeactgeccgt
cctctagagtcgacctgcaggcatgcaagcttgacactgeccgt -


tacaacgicgtgactgegaamaccctegcgithacccaactitaatcgecilgcagcal
tacaacgtcetgactgeganaaccctoocgttacccaacttantcoccttgcabcac TACAACOTCGTGACTGCOAAAACCCTGGCOTTACCCAACTTABTCGCCTTGCAGCA tacaacgicgtgactgegahaaccctgecettacccaactlaatcgectigcagcac
 tacaacgicgtgactgegahaaccctgecgttacccaacttaatcgecttgcagcac TACAACOTCGTGACTGGGARAACCCTOGCGTTACCCAACTTAATCGCCTTGCAGCAA tacaacgicgtgactggeahaaccetgecgttacccaacitaatcgectigcagcac TACAACGTCGTGACTGGGAAAACCCTGECGTTACCCAACTTAATCGCCTTGCAGCAS tacaacgicetgactgcgaanccetgccettacccaactiaatcgectigeagcal tacaacgtcetgactgeganaaccctegcgttacccaactiantgectigcagcat tacaacgtcgtgactgegaanaccetcecettacccaacttantcgccttgcagcat tacaacetcetgactggeaanaccctgocgttacccaacttantcgcctigcagcag tacaacgTcg tgactggganahccctcgcgttacccaacttantcaccttgcagcal tacaacetcgtgactgegahaaccctgecgttacccaacttantcgcctigeagcar TACAACOTCGTGACTGGGAAAACCCTGOCQTTACCCAACTTAATCOCCTYGCAGCAA tacaacgicglgactgggahaaccctggcgttacccaactitantcgcctigcagcal TAGAACGTCETGACTGCOAAAACCCTOOCOTTACGCAACTTAATGGCCTTGCAGCAS tacaacgtcgtgactgegahaaccetgecgttacccaactiantcgectl gcagcac tachacgecgegactggganaaccctogcgttacccaacttantcgcct tocagcas TACAACGTCGTGACTGCGAAAACCCTGCCGTTACCCAACTTAATCGCCT1GCAGCAA tacaacgicgTgactgegahanccctgecgTtacccaacttantcocctigcagcac tacaacgTCgTgactggearanccctcocgttacceancttaatcgcctigcageal tacaacgtcgtaactgggahaaccctgecgttacccaacttantcgccifgcagcal TA--ACGTCGTGACTGOGABAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAS tacaacetcgtgactgegahaaccetgecgttacccaactitatcgcctigeagcac tacancetcetgactgcoahancoctoocgttacccancttantcgecttocabcac thageggccgetti tacaacgtcgtgactggeahaaccctgecgttacccaactiaatcgectigchgcat TAAARGCOGCCGCTITTACAACOTCGTGACTGGOARAACCCTCOCGTTACCCAACTTAATCGCCTTGCAGCAS tacaacgicg tactggeaahaccetgecgttacceancitaatcgectigcagcac TACAACGTCGTGACTGGGAAAACOCTGECGTTACCCAACTTAATCOCCTTGCAGCAC CACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAA tacaacgicgtgactgggaanaccctgocg tiacccaacttantcgecttgcagcac TACAACGTCGTGACTGGCAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAS tacaacgicgtgactggganaaccctggcgttacccaactiantcgectigcagcas TACAACGTGGTGACTGGGAAAACGCTGGCGTTACCCAACTTAATGGCCTTGGAGCAC tacaacetcetgactgegaanaccctgecgttacccaacttantcgectigcagcal TACAACGTGGTGACTGGGAAAACCCTGGCGTTACGCAACTTAATCGCCTTGGAGCAL tacaacgtcetgactgggahaacectgecgttacccaactiaatcgectigcagcac tagnacgtcgtgactacganaacgetgecgttacgcaacttantggccttgcageac

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, $5^{\prime}$ 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 lac $Z$ gene, contains an eight base NOTI site in the center, and is positioned in a $3^{\prime}$ to $5^{\prime}$ orientation. The purple box highlights the correct integration of a NOTI 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.
$5^{\prime}$
IGGATCCTCTAGAGTCGACCTGCAGGCATGGAAGCTTGGCACTGGCCSTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTI : ССTAGGAGATCTCAGCTGGACGTCCGTACGTTCGAACCGTGACCGOAGCAAAATOTTGCAOCACTGACCCTTTGGGACCGCAA


ECCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCG TTTTACAACGTCGTGACTGGGAAAACCCTGGCI CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCC CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCO CCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGGCGTCO ccoggatcctctagagtcgacctocaggcatgcaagcttggcactggccetco :ccgggatcctctagagtcgacctocaggcatbcaagcttggcactggccetco ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccetco ccggggatcctctagagtcgacctocaggcatgcaagcttggcactggccetcccgggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetcqgeggccec ecggggatcctctagagtcgacctocaggcatgcaagcttggcactggccetc ccggggatcctctagagtcgacctgcaggcatecaagcttggcactgeccotco ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetco ocggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetco ecggggatcotctagagtcgacctgcaggcatgcaagcttggcactgeccotco ccoggatcctctagagtcgacctocaggcatgcaagcttggcactggccetcogeggccge ccgggGatcctctagagtcgacctocaggcatgcaagcttggcactgeccetco ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccgtco ccggggatcctctagagtcgacctocaggcatgcaagcttggcactggccgtco ecggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccetco ocgggGatcctctagagtcgacctgcaggcatgcaagcttggcactggccetc geggecoc ccggggatcotctagagtcgacctocaggcatgcaagcttggcactgeccetco ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccgtco cCGgGGatcctctagagtcgacctgcaggcaigcaagcttggcactgeccgico ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccgtco ccgggontcctctagagtcgacctgcaggcatgcaagcttggcactgeccetco ccggggatcctictagagtcgacctgcaggcatgcaagcttggcactggccgtc ccgggatcctctagagtcgacctgcaggcatgcaagcttgegactggccetcegcgeccgc ccgeggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetc dgcgeccge ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetco ccgggatcctctagagtcgacctgcaggcatgcaagcttggcactggccgtco ccggggatcctctagagtcgacctocaggcatgcaagcttggcactgeccetce ccggggintcctctagagtcgacctgcaggcatgcaagcttggcactgeccetce ccggggatcctctagagtcgacctocaggcatgcaagcttggcactgeccetco iccggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccetc ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccetc ccgggeatcctctagagtcgacctgcaggcatgcaagcttggcactgeccetce ccggggatcctctagagtcgacctocaggcatgcaagettggcactggccetco ccggggatcctctagagtcgacctgcaggcatccaagcttggcactgeccetco iccggGgatcctctagagtcgacctgcaggcatgcaagcttggcactgeccgtco cCgGgGatcctctagagtcgacctgcaggcatgcaagcttggcactggccetc ccggggatcctctagagtcgacctgcaggcatgcaagcttggcactgeccetcl cCgGgGatcctctagagtcgacctgcaggcatgcaagcttggcactggccetcl ccggggatcctctagagtcgacctgcaggcaigcaagcttggcactggccetc gcgeccec cCggggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetco ccgGgGatcctctagagtcgacctgcaggcatgcaagcttggcactggccetc geggccec ccgggeatcctctagagtcgacctgcaggcatgcaagcttggcactggccetce ccggggatcctictagagtcgacctgcaggcatgcaagcttggcactggccetcl ccgggatcctctagagtcgacctgcaggcatgcaagcttggcactggccetc geggcegc

TTTTACAACGTCGTGACTGGGAAAACCCTGGCI TTTTACAACGTCGTGACTGGGAAAACCCTGGCI TTTTACAACGTCGTGACTGGGAAAACCCTGGCI TTTTACAACGTCGTGACTGGGAAAACCCTGGCI -......... CAACGTCGTGACTGGGAAAACCCTGGCi rTTTACAACGTCGTGACTGGGAAAACCCTGGC। TTTTACAACGTCGTGACTGGGAAAACCCTGGC -...ACAACGTCGTGACTGGGAAAACCCTGGCI -...ACAACGTCGTGACTGGGAAAACCCTGGCi ICAACGTCGTGACTGGGAAAACCCTGGCi CAACGTCGTGACTGGGAAAACCCTGGCI CAACGTCGTGACTGGGAAAACCCTGGCI TTTTACAACGTCGTGACTGGGAAAACCCTGGCI $\cdots$.........AACGTCGTGACTGGGAAAACCCTGGCI ACAACGTCGTGACTGGGAAAACCCTGGCI CAACGTCGTGACTGGGAAAACCCTGGGI -...ACAACGTCGTGACTGGGAAAACCCTGGCi TTTTACAACGTCGTGACTGGGAAAACCCTGGGI -...ACAACGTCGTGACTGGGAAAACCCTGGCI - CAACGTCGTGACTGGGAAAACCCTGGC ACAACGTCGTGACTGGGAAAACCCTGGCI CAACGTCGTGACTGGGAAAACCCTGGC। CAACGTCGTGACTGGGAAAACCCTGGCI ACAACGTCGTGACTGGGAAAACCCTGGC। TTTACAACGTCGTGACTGGGAAAACCCTGGC TTTTACAACGTCGTGACTGGGAAAACCCTGGC FAACGTCGTGACTGGGAAAACCCTGGC ACAACGTCGTGACTGGGAAAACCCTGGC ACAACGTCGTGACTGGGAAAACCCTGGC CAACGTCGTGACTGGGAAAACCCTGGC ACAACGTCGTGAGTGGGAAAACCCTGGC GAACGTCGTGAGTGGGAAAACCCTGGC GACTGGGAAAACCCTGGC ICAACGTCGTGACTGGGAAAACCCTGGC ACAACGTCGTGACTGGGAAAACCCTGGC CAACGTCGTGACTGGGAAAACCCTGGC CAACGTCGTGACTGGGAAAACCCTGGC CAACGTCGTGACTGGGAAAACCCTGGC ..-ACAACGTCGTGACTGGGAAAACCCTGGC TTTTACAACGTCGTGACTGGGAAAACCCTGGC ITTTACAACGTCGTGACTGGGAAAACCCTGGC
 TTTTACAACGTCGTGACTGGGAAAACCCTGGC -...ACAACGTCGTGACTGGGAAAACCCTGGC $\cdots$.......AACGTCGTGACTGGGAAAACCCTGGC TTTTACAACGTCGTGACTGGGAAAACCCTGGC





tCgagctcegtacceggegatcctic tagabtcgacctocaggcatgcangettogeactgeccotce
$12 \mathrm{~W} \rightarrow$
1w
2w $=$
$3 \mathrm{~W} \rightarrow$
4 w
SW
5W－
$6 \mathrm{~W} \rightarrow$
7W $\rightarrow$
8w－
9W $\rightarrow$
10W $\rightarrow$
$11 W \rightarrow$
$12 \mathrm{w}=$
$13 \mathrm{~W} \rightarrow$
$14 \mathrm{~W} \rightarrow$
$15 \mathrm{~W} \rightarrow$
$16 \mathrm{~W} \rightarrow$
17w $\rightarrow$
18W $\rightarrow$
19W $\rightarrow$
20W $\rightarrow$
21w $\rightarrow$
$22 \mathrm{~W} \rightarrow$
23W $\rightarrow$
$24 \mathrm{~W} \rightarrow$
25W $\rightarrow$
26W $\rightarrow$
27w $\rightarrow$
28W $\rightarrow$
29W $\rightarrow$

## $30 \mathrm{~W} \rightarrow$

## $31 w^{-}$

32 W
tceagctcgetacccgggatcetc tagagtceacctecaggcatgcaagettogcactggecgtco rceagotcogtacccoggatcotc tagagtcgacctbcagocatgcangettogeactgaccotce ceagctcgetacccogegatcctc tagagtcgacctgcaggcatocangcttogcactgaccetco rcoagctcogtacccgogatcctc tagabtcgacctocagocatocaagctrogcactgoccotce icgagctcgetaccegggeatcctc tagabtcgacetgcaggcatgcaagcttggcactggccotco CGagctcgetacccggegatcctc tagagtcgacctbcaggcatgcangcttggcactggcegtce GGAGCTGQGTACCCGGGGABCCTC TAGAGTCOACCTGCAGGCATGCAAGCTTGGCACTGGCGGTCC
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IITAGAACGTCGTGACTGGGAAAACCCTGGCGTTACEGAAGTTAATCGCGTTGCAGGACATCCCCCTTTCECCAI TTTACAACGTCGTGACTGGGAAACCCTGGCCTTACCCAACTTAATCGCOTTGCAGCACATCCCCOTTTCGCCAS FITTACAACGTCGTOACTGGGAAAACCCTGGCOTTACCCAACTTAATCGCCTTGCAOCACATCCCCCTTTCGGCAG TTTTACAACGTCGTGACTGGEAAAACCGTGGCOTTACCCAACTTAATCGCCTTGCAGCACATCCCOCTTTCGCCAS TTTTACAACGTCQTGACTG日QAAAACGCTG日COTTACGCAACTTAATCOCOTTGCABCACATCCGCCTTTCOCCAS

 －．．．．CaACOTCGTGACTGGGAAAACCCTGGCOTTACCCAACTTAATCGCCTTGCAGCACATCCCOCTTTCGCCAS
 －．．．－chacetcgtgactggbaaanccctogcettacccaacttaatcgccttgcagcacatccccctttcgccas
 CHTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCECTTTCGCCAS －．．．－cancctcotgactogeaanaccetgocottacccaacttantcoccttocagcacatccccettrcoccas －．．．－caAcGTCGTGACTGGGAAAACCCTGOCETTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAS

 ．．．．．GAACGTCQTGACTGQGAAAACCCTGGCOTTACCCAACTTAATCGCCTTGCAGCACATCCCOCTTTCGCCAS

 ．．．．．．caACgTCGTGACTGGGAAAACCCTGGCOTIACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC
 －．．．．．．．aAcctcgteactggganaaccetcgcottacccaacttantceccttgcagcacatccccctttcoccar －．－ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAS

 CTTTACAACGTCGTGACTGGOAAAACCCTGGCGTTACCCAACTTAATCCCCTTGCAGCACATCCCOCTTTCOCCAA
 －．－－CAACETCGTGACTG日GAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAC

 －－ancaacgtcgtgactgggaanaccetggcgttacccaacttaatcgcettgeagcacatccccetticgecag －．．－．CaAcgtcgtcactgegaahaccctggcgttacccaacttantcgcettgcagcacatccccctttcgccag


 ．－．－ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG TEWCAACGTCGTGACTGGGAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCOCCTTTCGCCAG ．－．－ACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATGGCCTTGCAGCACATCCCCCTTTCGCCAG …－EAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG －～नACAACGTCGTGACTGGGAAAACCCTGGCETTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
 ．－．－．БAACGTCGTGACTGOGAAAACCCTGGCGTTACCCAACTTAATCOCCTTGCAGCACATCCCCCTTTCOCCAG －．－－）CAACGTCGTGACTGGGAAAACCGTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG ．．．．．F：AaCgTCGTGACTGEGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCGCCCTITCGCCAG ．．．．．FAACGTCGTGACTGGGAAAACCCTGECBTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG ．．．．．CAACGTCGTGACTGGGAAAACCCTGCCGTTACCCAACTTAATCGCCTTGCAGCACATCCOCCTTTCGCCAG T－ACAACGTCGTGACTGOGAAAACCCTOOCOTTACCCAACTTAATCOCCTTGCAOCACATCCCOCTTTCGCCAB


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 lac $Z$ gene, contains an eight base NOTI site in the center, and is positioned in a $3^{\prime}$ to $5^{\prime}$ orientation. The purple box highlights the correct integration of a NOTI 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 |  |
| $\begin{aligned} & \text { Cas12a } 1364 \\ & \text { 20HDR-NS } \end{aligned}$ | 42 | 22 | 64 |  | $\begin{aligned} & \text { Cas9 } 1364 \\ & \text { 20HDR-NS } \end{aligned}$ | 20 | 41 | 61 |  |
| $\begin{aligned} & \text { Cas12a } 1364 \\ & 50 H D R-N S \end{aligned}$ | 34 | 28 | 62 |  | $\begin{aligned} & \text { Cas9 1364 } \\ & \text { 50HDR-NS } \end{aligned}$ | 3 | 58 | 61 |  |
| Column Totals | 76 | 50 | 126 |  | Column Totals | 23 | 99 | 122 |  |
| P-Value $<0.05$ |  |  |  | 0.2748 | P-Value <0.05 |  |  |  | 0.0001 |
| *Cas12a 20HDR-NS vs. Cas12a 75HDR-NS |  |  |  |  | *Cas9 20HDR-NS vs. Cas9 75HDR-NS |  |  |  |  |
|  | HDR | Indel | Total |  |  | HDR | Indel | Total |  |
| $\begin{aligned} & \text { Cas12a } 1364 \\ & \text { 20HDR-NS } \end{aligned}$ | 42 | 22 | 64 |  | Cas9 1364 <br> 20HDR-NS | 20 | 41 | 61 |  |
| $\begin{aligned} & \text { Cas12a } 1364 \\ & 75 H D R-N S \end{aligned}$ | 26 | 30 | 56 |  | $\begin{aligned} & \text { Cas9 } 1364 \\ & \text { 75HDR-NS } \end{aligned}$ | 2 | 61 | 63 |  |
| Column Totals | 68 | 52 | 120 |  | Column Total | 22 | 102 | 124 |  |
| P-Value <0.05 |  |  |  | 0.0428 | P-Value <0.05 |  |  |  | 0 |
| Cas12a 50HDR-NS vs. Cas12a 75HDR-NS |  |  |  |  | Cas9 50HDR-NS vs. Cas9 75HDR-NS |  |  |  |  |
|  | HDR | Indel | Total |  |  | HDR | Indel | Total |  |
| $\begin{aligned} & \text { Cas12a } 1364 \\ & 50 H D R-N S \end{aligned}$ | 34 | 28 | 62 |  | $\begin{aligned} & \text { Cas9 } 1364 \\ & \text { 50HDR-NS } \end{aligned}$ | 3 | 58 | 61 |  |
| $\begin{aligned} & \text { Cas12a } 1364 \\ & 75 H D R-N S \end{aligned}$ | 26 | 30 | 56 |  | $\begin{aligned} & \text { Cas9 1364 } \\ & \text { 75HDR-NS } \end{aligned}$ | 2 | 61 | 63 |  |
| 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.

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

| Cas12a 1364 20HDR-NS |  |  |  |  |  | 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 1364 75HDR-NS |  |  |  |  |  | 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. 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.

