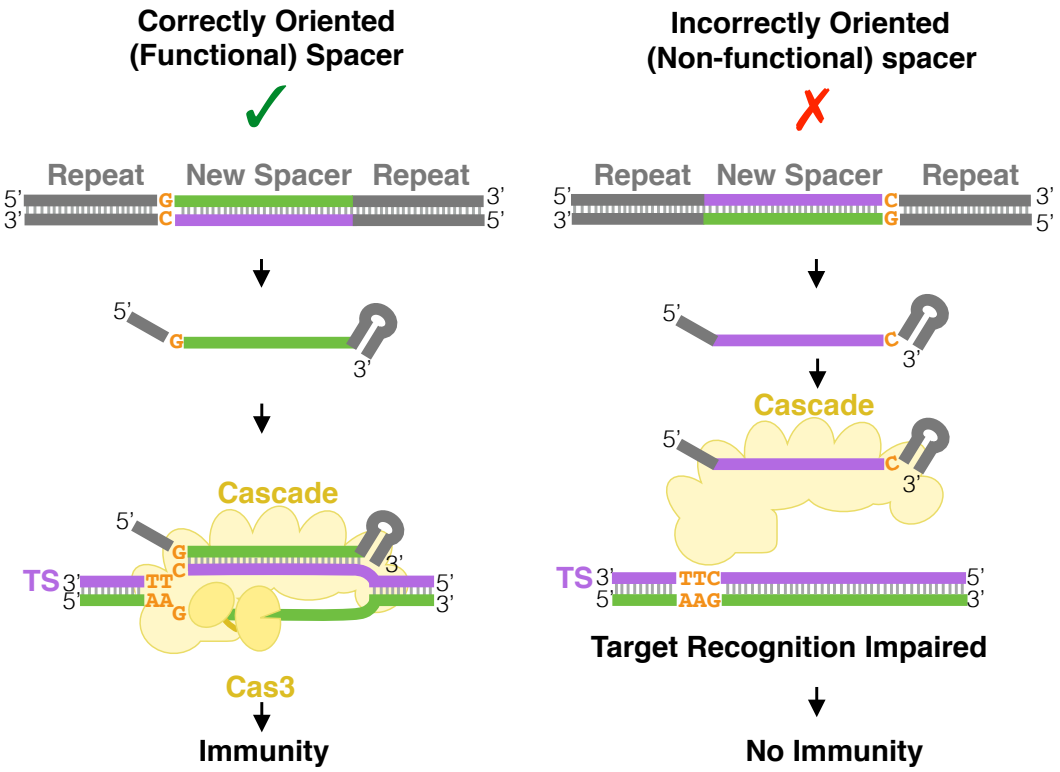
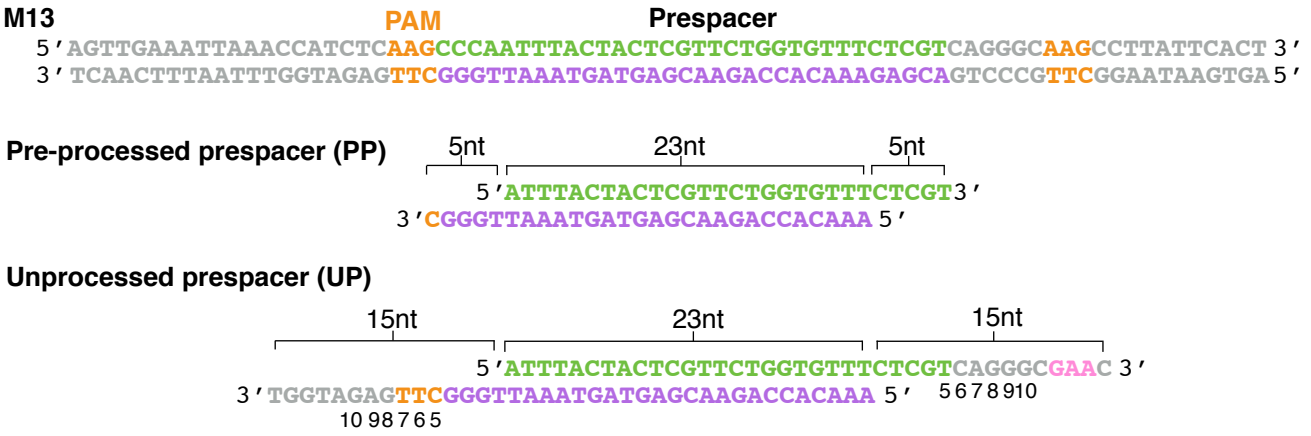
Supplemental Figure 1



Impact of Spacer Orientation on Target Recognition and Immunity
Schematic depicting the consequence of incorrectly oriented spacers on target recognition and immunity. Only the portion of a CRISPR array with a newly incorporated spacer is shown. The strand deriving from the CTT sequence of a prespacer is colored purple and it's complementary strand is colored green. Upon integration in the correct orientation (left), the new spacer is transcribed into a crRNA that is functional for recognition of the target strand (TS) by Cascade, the surveillance complex in type I systems, and subsequent degradation by the helicase-nuclease Cas3. If the new spacer is incorrectly oriented (right), the resulting crRNA will not be complementary to the target strand, thus impairing target recognition and subsequent immunity.

Supplemental Figure 2

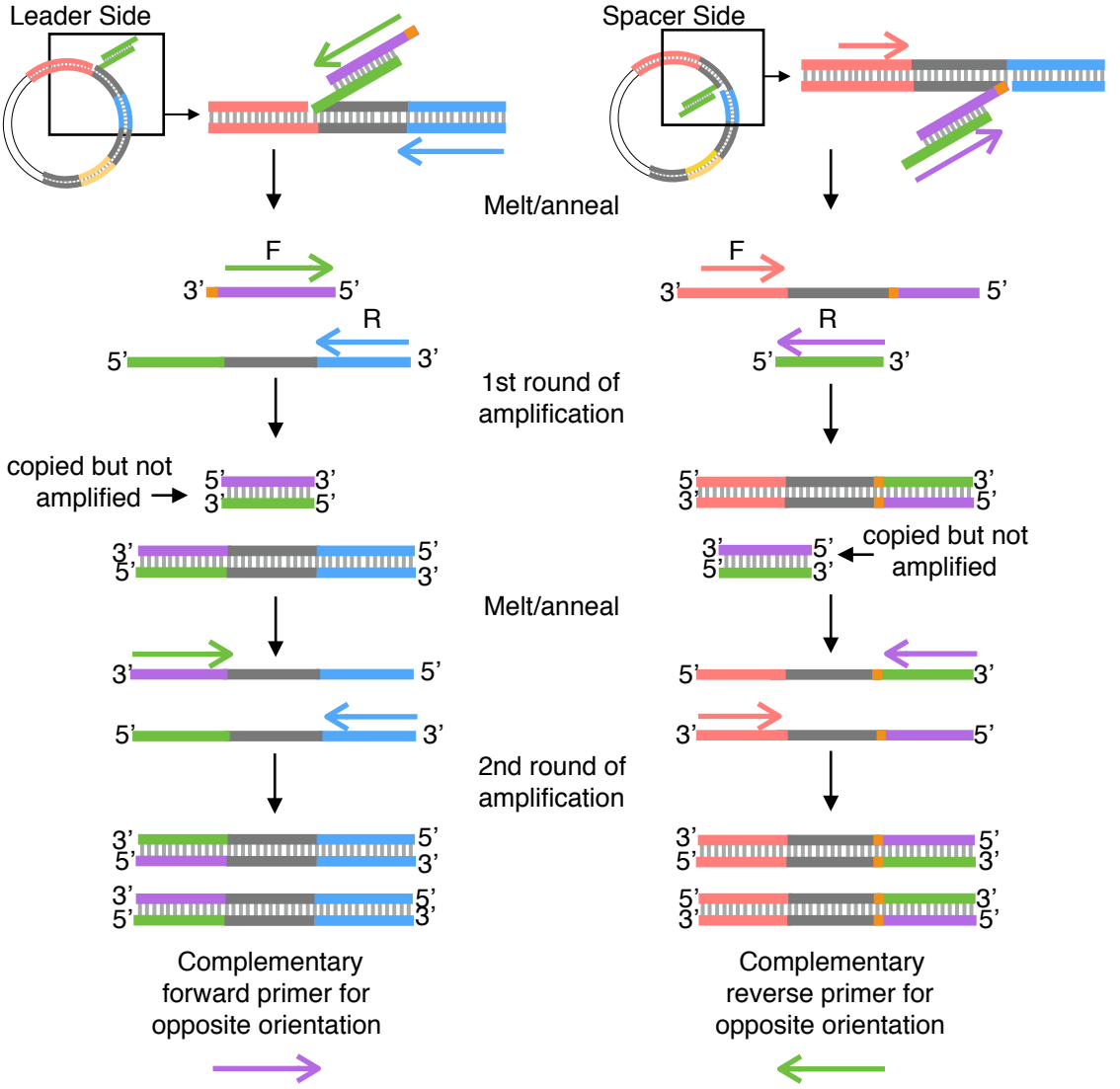
Region of the M13 Phage genome containing the PAM and Prespacer



M13 Phage Derived Prespacers

Region of the M13 phage genome from which the most abundant spacer acquired following M13 bacteriophage infection is derived. DNA strand that is flanking the CTT sequence, denoted as PAM strand in text, is colored purple and complementary strand, denoted as non-PAM strand, is colored green. PAMs in the genome sequence are colored orange. The AAG (orange) in the 3' overhang of the non-PAM strand of the unprocessed prespacer is mutated to GAA (pink) to eliminate any potential contact by Cas1-Cas2. Numbers on the overhang indicate overhang length.

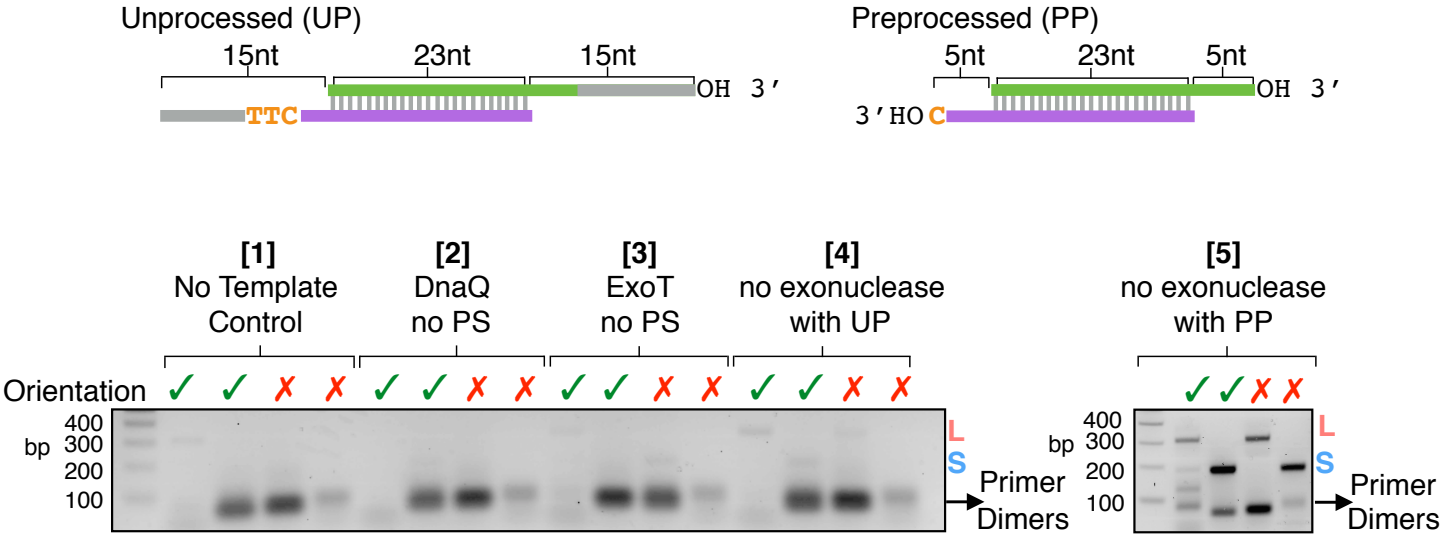
Supplemental Figure 3



PCR amplification of half-site integration products

Left: PCR amplification steps using primers that amplify leader-side integration events in the correct orientation. For the incorrect orientation, a complementary forward primer was used. Right: Steps for amplification of spacer-side integration events in the correct orientation. A complementary reverse primer was used for the incorrect orientation. The actual spacer side primer used for PCR and qPCR binds to spacer 4 and spacer 2 respectively, not spacer 1, as shown here for simplicity.

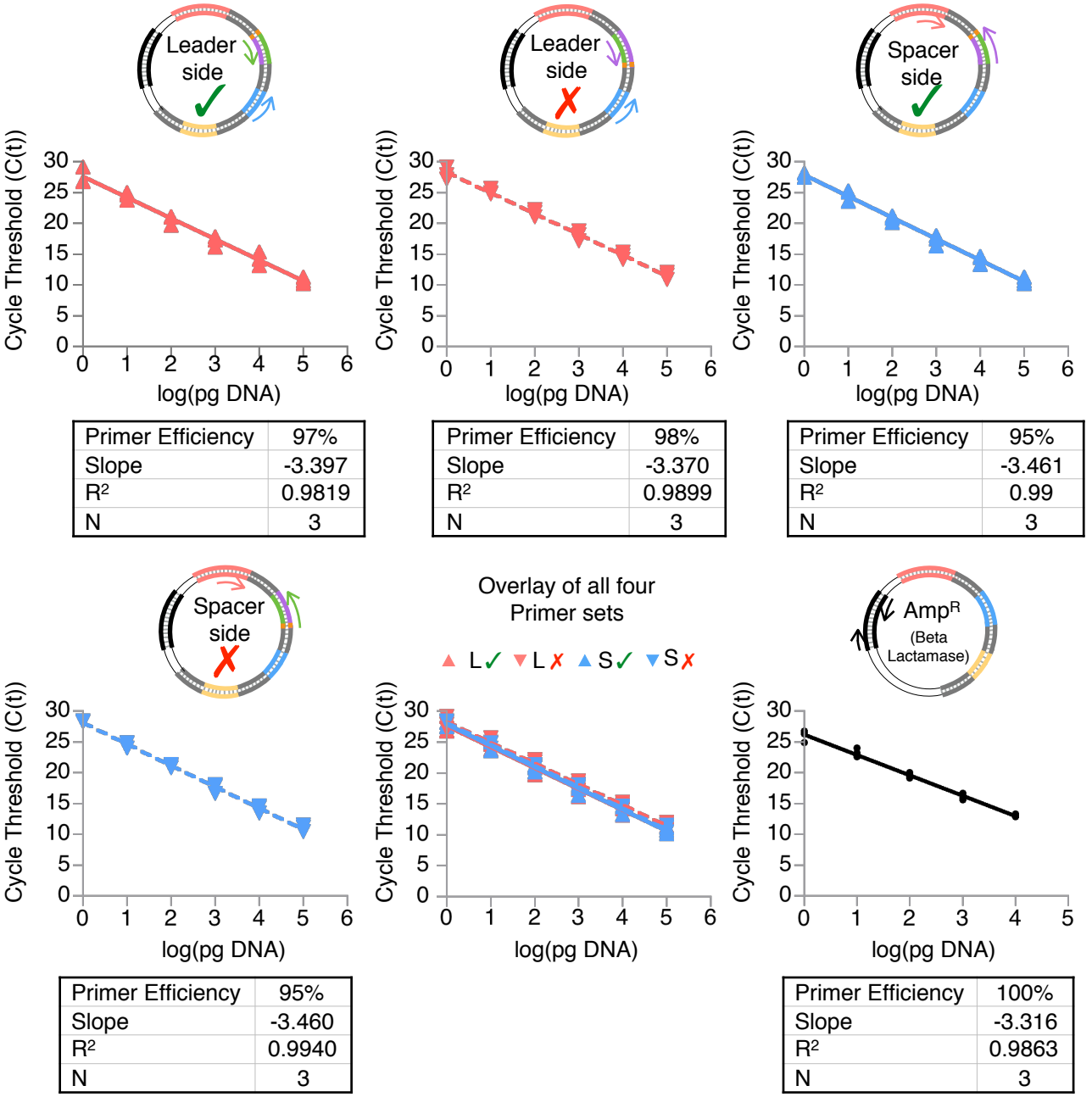
Supplemental Figure 4



PCR amplification of background controls

To ensure that amplification is specific to integration events, open circle species of control reactions were gel extracted and PCR amplified with the same primers used in Figure 2C. [1] PCR reactions with water. [2] control reactions containing Cas1-Cas2, IHF and DnaQ in the absence of prespacer (PS). [3] control reactions containing Cas1-Cas2, IHF and ExoT in the absence of PS. [4] control reactions containing Cas1-Cas2, IHF and UP in the absence of exonuclease. [5] control reactions containing Cas1-Cas2, IHF and PP in the absence of exonuclease.

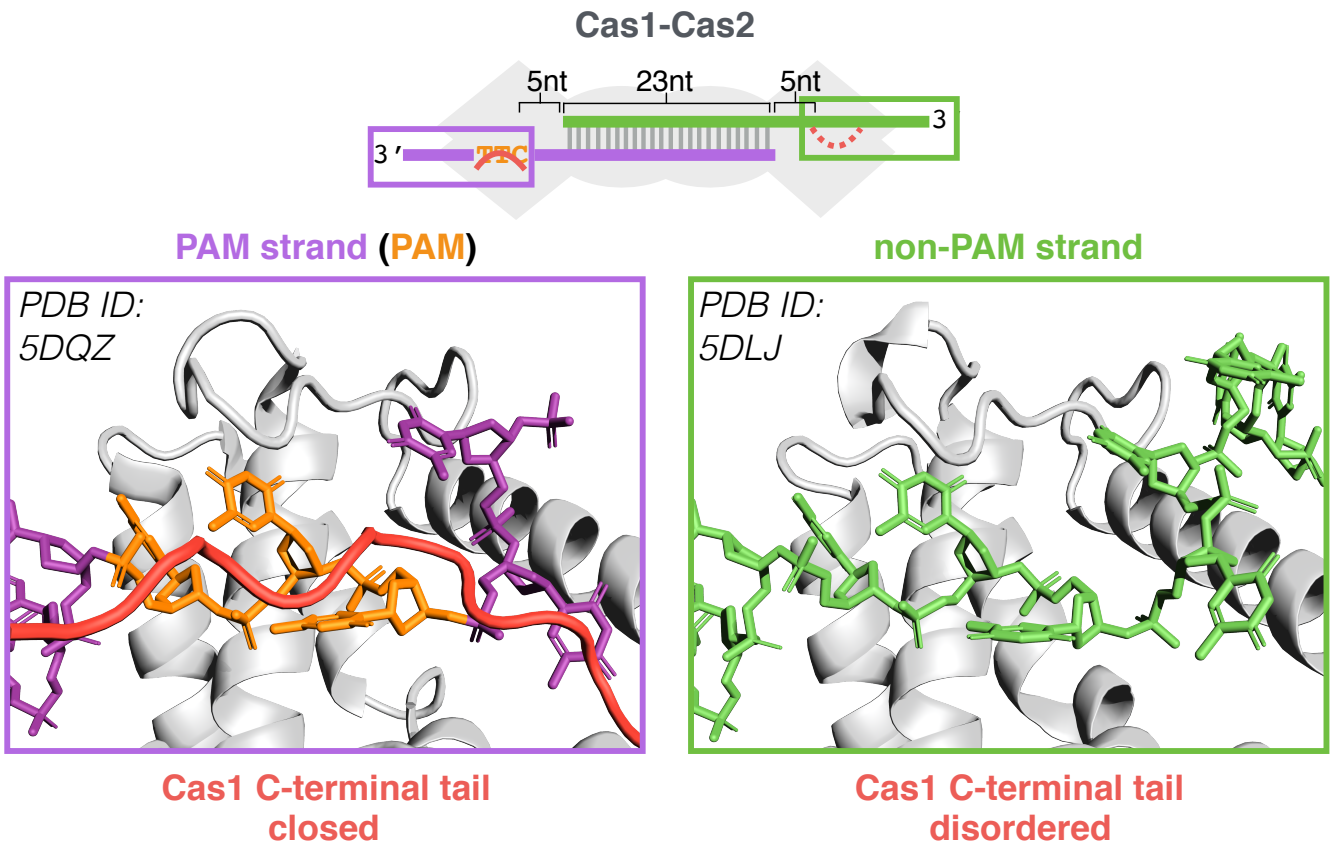
Supplemental Figure 5



Standard Curves for qPCR

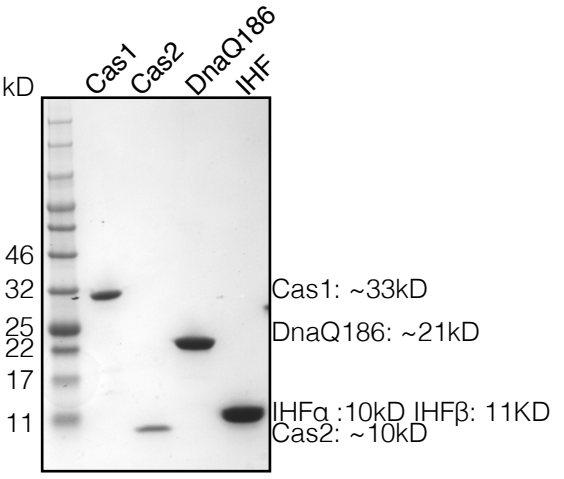
Standard curves used to check primer efficiency of all primer sets used for qPCR, relating pg amounts of DNA to Ct values. Relative amounts of each half-site integration product were normalized to template amounts of the beta lactamase gene. Plasmids above each graph depict templates and primer sets used for each PCR reaction. Average values of 3 replicates are shown in the tables below the graph.

Supplemental Figure 6



PAM Recognition by Cas1 C-terminal Tail
Zoomed-in view of the C-terminal tail of Cas1 in complex with DNA either containing PAM (left; colored purple) or lacking PAM (right; colored green). When bound to DNA containing the PAM sequence, the C-terminal tail is well ordered and involved in specific contacts with the PAM sequence, CTT, but when bound to DNA lacking a PAM sequence, the C-terminal tail is disordered. A schematic of the Cas1-Cas2 complex bound to the unprocessed prespacer is shown above for reference. Dark orange solid and dashed lines on the 3' overhangs correspond to ordered and disordered c-terminal tails respectively.

Supplemental Figure 7



Purified Proteins used in Integration Assays
SDS-PAGE of Cas1, Cas2, DnaQ186, and IHF. L indicates Ladder. Approximate size of each protein is indicated on the right of the gel.

Table 1. Prespacer oligonucleotides used in this study

Overhang Length (nt) (Description)	Non-PAM Strand (5'->3')	PAM Strand (5'->3')	Figures
5 (Preprocessed)	ATTTACTACTCGTTCTGGTGTTTCTCGT	AAACACCAGAACGAGTAGTAAATTGGGC	1, 2, 3, 4, 5
15 (Unprocessed)	ATTTACTACTCGTTCTGGTGTTTCTCGT CAGGGCGAAC	AAACACCAGAACGAGTAGTAAATTGGGC TTGAGATGGT	1,2, 3, 4, 5
15 (No PAM)	ATTTACTACTCGTTCTGGTGTTTCTCGT CAGGGCGAAC	AAACACCAGAACGAGTAGTAAATTGGGG AAGAGATGGT	2
4	ATTTACTACTCGTTCTGGTGTTTCTCG	AAACACCAGAACGAGTAGTAAATTGGG	3, 4
6	ATTTACTACTCGTTCTGGTGTTTCTCGT C	AAACACCAGAACGAGTAGTAAATTGGGC T	3, 4
7	ATTTACTACTCGTTCTGGTGTTTCTCGT CA	AAACACCAGAACGAGTAGTAAATTGGGC TT	3, 4, 5
8	ATTTACTACTCGTTCTGGTGTTTCTCGT CAG	AAACACCAGAACGAGTAGTAAATTGGGC TTG	3, 4
9	ATTTACTACTCGTTCTGGTGTTTCTCGT CAGG	AAACACCAGAACGAGTAGTAAATTGGGC TTGA	3, 4, 5
10	ATTTACTACTCGTTCTGGTGTTTCTCGT CAGGG	AAACACCAGAACGAGTAGTAAATTGGGC TTGAG	3, 4,

Table 2. Oligonucleotides used in Radiation Integration Assay (Figures 2 and 5)

Description	5'->3' Sequence
Leader-Repeat-Spacer Oligo Target F	GTTGGTAGATTGTGACTGGCTTAAAAAATCATTAATTAATAATAGGTTATGTTTAGAGTGT TCCCCGCGCCAGCGGGGATAAACCGAGCA
Leader-Repeat-Spacer Oligo Target R	TGCTCGGTTTATCCCCGCTGGCGCGGGGAACACTCTAAACATAACCTATTATTAATTAATG ATTTTTTAAGCCAGTCACAATCTACCAAC
Leader-Side Expected Product Marker	ATTTACTACTCGTTCTGGTGTTTCTCGTGTGTTCCCCGCGCCAGCGGGGATAAACCGAGCA
Spacer Side Expected Product Marker	AAACACCAGAACGAGTAGTAAATTGGGCGGTTTATCCCCGCTGGCGCGGGGAACACTCTAA ACATAACCTATTATTAATTAATGATTTTTTAAGCCAGTCACAATCTACCAAC

Table 3. Primers used for End-point PCR (Figures 2 and S3)

Description	Forward Primer (5'-> 3')	Reverse Primer (5'-> 3')
Leader-side correctly oriented M13 prespacer	TTTAAGAAGGAGATATAGATCCCCAATT TACTACTCGTTC	TTATGGAGTTGGGATCTTATTACAGTGTCA ACAATCGTTCCC*
Leader-side incorrectly Oriented M13 Prespacer	TTTAAGAAGGAGATATAGATCACGAGAA ACACCAGAACGAG	TTATGGAGTTGGGATCTTATTACAGTGTCA ACAATCGTTCCC*
Spacer-Side Correctly Oriented M13 Prespacer	TTTAAGAAGGAGATATAGATCTGGATGT GTTGTTTGTGTGA	TTATGGAGTTGGGATCTTATTAACGAGAAA CACCAGAACGAG
Spacer-Side Incorrectly Oriented M13 Prespacer	TTTAAGAAGGAGATATAGATCTGGATGT GTTGTTTGTGTGA	TTATGGAGTTGGGATCTTATTACCCAATTT ACTACTCGTTC

All primers contain LIC2 handle sequences for potential cloning purposes

* Primer complementary to spacer 4

Table 4. Primers used for qPCR (Figures 2, S3, and S5)

Description	Forward Primer (5'->3')	Reverse Primer (5'->3')
Leader-side correctly oriented M13 prespacer	TACTCGTTCTGGTGTTTCTCGT	CGTTTTTGGAATTTACAGCGAGG *
Leader-side incorrectly Oriented M13 Prespacer	CGAGAAACACCAGAACGAGTAG	GTTTTTGGAATTTACAGCGAGGC *
Spacer-Side Correctly Oriented M13 Prespacer	AGTTGGTAGATTGTGACTGGCT	ACGAGAAACACCAGAACGAGT
Spacer-Side Incorrectly Oriented M13 Prespacer	AGTTGGTAGATTGTGACTGGCT	GCCCAATTTACTACTCGTTCTGG

* Primer complementary to spacer 2

Table 5. Oligonucleotides used for cloning

Insert	Strain	Vector	Forward Primer (5'->3')	Reverse Primer (5'->3')
Cas1	K12 (MG1655)	pHAT4	GTGTGTCCATGGGTATGACC TGGCTTCCCCTTAATC	GTGTGTGAATTCTCAGCTACTC CGATGGCCTGCATC
Cas2	K12 (MG1655)	pMAT11	GTGTGTCCATGGGAAGTATG TTGGTCGTGGTC	GTGTGTGAATTCTCAAACAGGT AAAAAAGACACC
IHFα-IHFβ	K12 (MG1655)	pET His6 TEV LIC cloning vector (1B)	TACTTCCAATCCAATGCAAT GACCAAGTCAGAATTGA	TTATCCACTTCCAATGTTATTA TTACTCGTCTTTGGGCGAA
DnaQ186	K12 (MG1655)	pET His6 Sumo TEV LIC cloning vector (1S)	CAACAGCAGACGGGAGGTAT GAGCACTGCAATTACACG	GCGAGAACCAAGGAAAGGTTAT TAAGCCATCGACGTTTGACCAC
CRISPR array	BL21(AI)	pET LIC cloning vector (2A-T)	TTTAAGAAGGAGATATAGAT CGATTAAGTACTCTTTAACA TAATG	TTATGGAGTTGGGATCTTATTA ACGTGGATATGTTGCTTATTAC