

Supplemental Figures

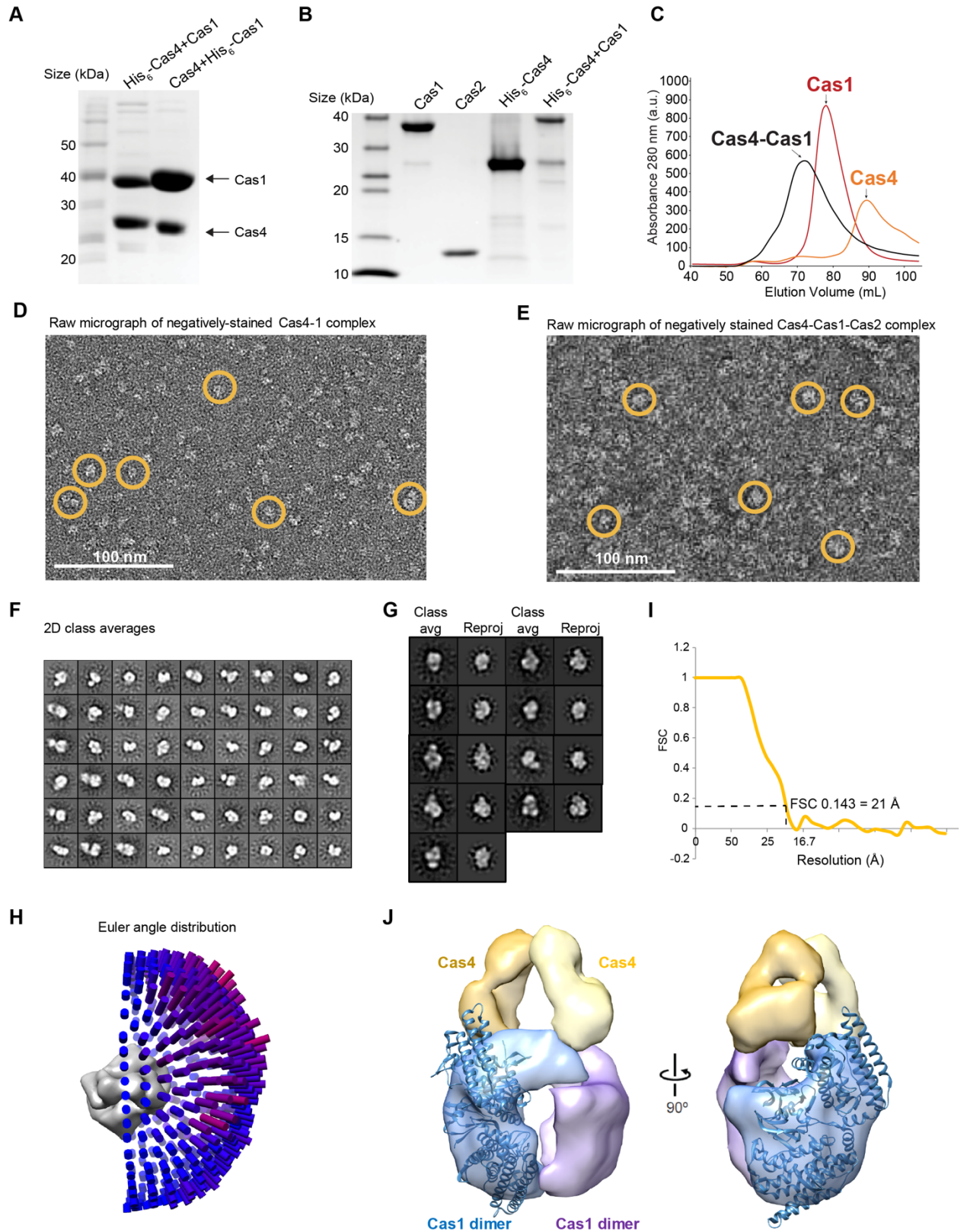


Figure S1, related to Figure 1. Molecular architecture of the Cas4-Cas1 complex.

(A) Co-expressed and purified Cas4-Cas1 complex on 12% SDS PAGE gel stained with Coomassie blue. His₆-Cas4 with untagged Cas1 or His₆-Cas1 with untagged Cas4 were co-expressed and complex was purified by nickel affinity chromatograph and size-exclusion chromatography. The stoichiometry of the two preparations are different due to incomplete separation of Cas4-His₆-Cas1 complex from free His₆-Cas1 on size-exclusion column. Therefore, His₆-Cas4-Cas1 was used for all biochemistry and structural studies. (B) Coomassie-blue stained SDS/PAGE gel of purified proteins used in this study. (C) Size-exclusion chromatography (SEC) of co-purified Cas4-Cas1 complex with individually purified Cas1 and Cas4. (D) Representative raw micrograph of negatively stained Cas4-Cas1 complex. Scale bar indicates 100 nm. Several particles are outlined with yellow circles. (E) Representative raw micrograph of negatively stained Cas4-Cas1-Cas2 complex. Scale bar indicates 100 nm. Several particles are outlined with yellow circles. (F) Reference-free 2D class averages of Cas4-Cas1 complex. The width of the boxes is ~230 Å. (G) Reference-free 2D class averages of Cas4-Cas1 complex (first, third, and fifth columns) matched to reprojections of the final reconstruction (second, fourth, and sixth columns). Again, the width of the boxes is ~230 Å. (H) Euler angle distribution of particles for the final C2 3D reconstruction. (I) Fourier shell correlation (FSC) curve for the final reconstruction, showing the resolution to be ~21 Å using the 0.143 gold standard FSC criterion (J) The crystal structure of Cas1 from *Archaeoglobus fulgidus* (PDB ID: 4N06) is docked into one Cas1 dimer in the final Cas4-Cas1 reconstruction. The models are colored as follows: gold, first Cas4; yellow, second Cas4; blue, first Cas1 dimer; purple, second Cas1 dimer.

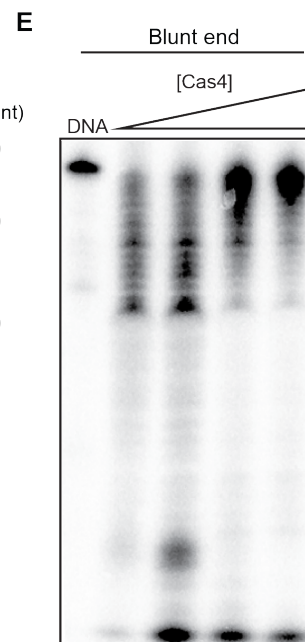
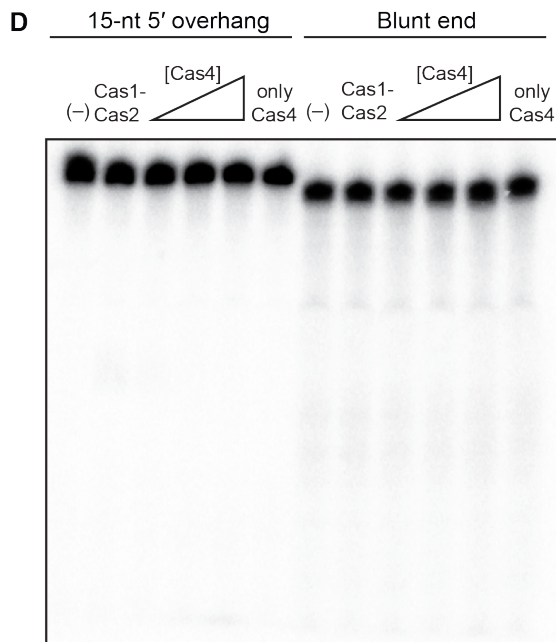
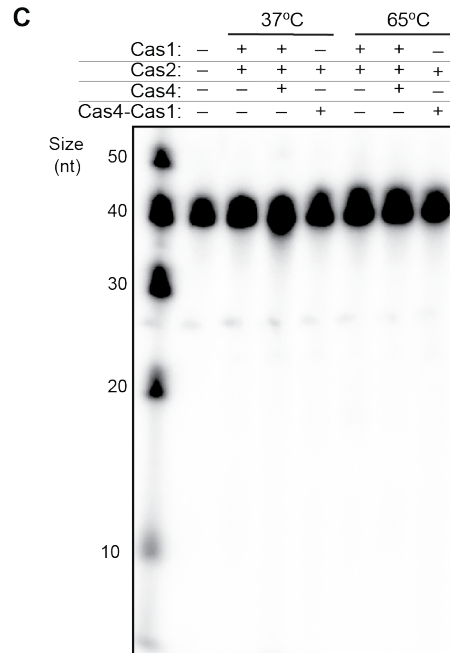
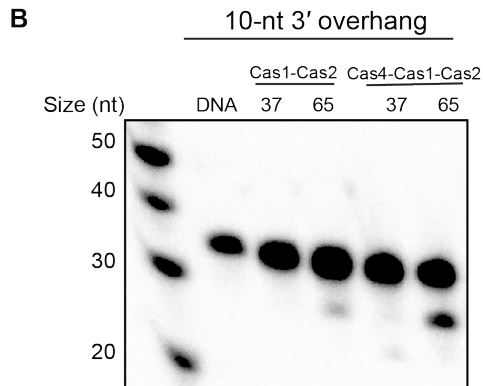
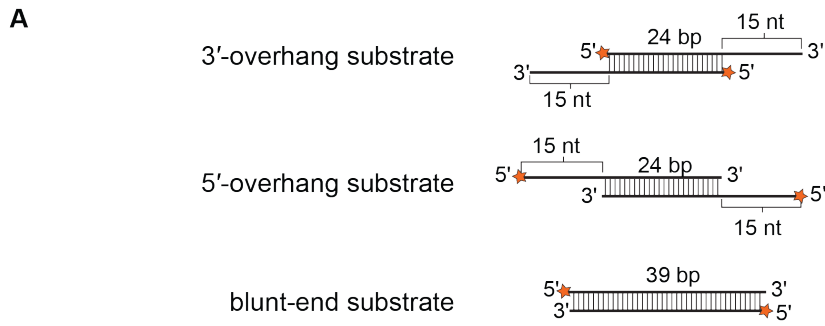


Figure S2, related to Figure 2. Single-stranded 3' overhangs are required for processing prespacers.

(A) Substrates used to test processing activity. Star indicates sites of ^{32}P radiolabel. (B) Processing assay of 3'-overhang DNA (15-nt overhangs with 24-bp duplex) incubating Cas1+Cas2 or Cas4-Cas1+Cas2 at 37 or 65°C, as indicated. (C) Processing assay using blunt end or 5'-overhang DNA as substrates. The concentration of Cas4 was titrated (100, 500 and 1000 nM) in the presence of Cas1+Cas2 complex. (D) Processing assay using 5'-overhang DNA as substrates at 37 or 65°C, as indicated. (E) Cas4 exonuclease assay using blunt end DNA. The concentration of Cas4 was titrated from 2 μM -20 μM .

A

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BhaCas4 -----MASNEEDRYLMLSLGLQHFQF  KRQWALIHIEQQW--- 34
SsoCas4 -----MIT-----EFLKKKLEEHLSHVKEENTIYVTDLVR  PRRVRYESEYKELAIS 48
PcaCas4 MELLSPKPLCSVVNCEDELEKLDHVSALNELRREQEIPKLLPGIYAHRYDFRRVSPSIINDFEY  PRLLWVQHKLGLKLLS 80
XooCas4 -----MDDADLIPLSALQHLYL  PRQCALIHVEQQW--- 31
HvoCas4 -----MSSTDVV-----EYV-QDERDPSRSPNVPI TGLMVQYHYV  KRELWFMANGID I--- 49
CjeCas4 -----MNSSLHNIRFTGTQINYPFL  KKKLWYFSHDIQM--- 34
          :
          * :

BhaCas4 -----EENVRTIEGQHLHKKADQPFMKEK--RGSKLTVRAMPIQSKNLQISGIC  VVEFVQDSEGIELSGVSGSYKAFPV  YKR 111
SsoCas4 QVYAPSAI LGDILHLGLSVLKGNFNAETEVE TLREINVGKVVYKIKGRADAI IRNDNGKS-----IVIE  IKT 116
PcaCas4 EKSVVSI IRGRILHERYERLLSQYENVVAEY-----KVEIG-----DLVGVV  D LVI-KRGGEY-----IPV  EIK- 138
XooCas4 --AENRQTAEGRLLHQRADAPQAERR--RGV-RTVTAMPLLALELGITGKA  VVEFHRDGDG-----EVAFP  VYKR 98
HvoCas4 DRETTNIQRGTHVDET-----SYGTSRR-----SFMIDNRIQL  D I--LDSGD-----V-M  EVKV 95
CjeCas4 EQNSDAVYLQKLIHET-----SYEREKK-----EIDIDDTIKI  F--IGNDR-----VIH  EVKK 81
          * :
          * :

          motif II
          motif III

BhaCas4 -----GKPKKGDEDIV  LVAQAMCLEEMLVCRIDKGYLFYNEIKHRVEVPITDALRDKVQMAKEMHHYENRHTPKV-K 185
SsoCas4 SRSDKGLPLIHHK--M  LQIYLWLFSA-EK-----GILVYITPDRIAEYEINEPLDEATIVRLAEDTIMLQNSPRFN--- 185
PcaCas4 ---TGFSKEAHK--T  LQIYISMLKA-RF-----GYLVYRNHVEVVRHN-----DAALDVLLKIREILSAREAPP--- 197
XooCas4 ---GHPKVHRADEV  LCAQALCLEHMLGRAVPTGALFYGQTRRRKDVAFDAALRALTLDTIADTRAMLHSDSTPAARY 173
HvoCas4 S-----SALFKPAR--M  LLYLWYLR- IHDIDKDGVLAYPTERRKRES-VVLDETTAEVESTVRGVLDVVGRDSDPPQLE 167
CjeCas4 S-----DKVEEPI--W  LKYYIWLKQ-KGADGITGKINYPKLRKTLD-VFLEPEDEEKIQSILKEIQGIINTELPPAVE 153
          * :
          * :

          motif QxxxY

BhaCas4 TGPFC  NNC  SLQSI  LPKLMNKRSVKRYIEGRlse--- 219
SsoCas4 --W  ECKY  CIFSVI  CPAKLT----- 202
PcaCas4 --AK  CNS  IFKPI  CKNLL----- 213
XooCas4 DAKR  CDA  SLIDV  CQPRLLDRGSVDQWVRRQLNADED 210
HvoCas4 KKP  Y  CGT  LYQDL  CWM----- 183
CjeCas4 RMM  K  CRN  C  SYGDI  C  WV----- 169
          * :
          * :

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B

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BhaCas1 MKKLLNNTLYVTQPTYLSLD-----GDNVVLKE---QEKLGRPLPHNLEAIVGFGY----- 49
EcoCas1 -----MTWLPNPIPLKDRVSMIFLQYGQIDVIDGAPVLI-DK---TGIRTHIPVGSVACIMLEPG----- 57
SpyCas1 MAGWRTVVVNTHSKLSYKNNHLIPKDAYKTELIHLSEIDI-----LLLET---TD-----IVLSTMLVKR----- 57
XooCas1 MRRQLNNTLYATTDGAWLRKD-----GANIVMEVE---RQERARLPVHMLESVLCIGR----- 49
PA14_33350 MDDI-----SPSELKTIHLSKRANLYYLQHCRLVLRV-NGGRVEYVTDEGRSHYWNIPANTTSLLLGTGTSITQA 69
          * :
          * :

BhaCas1 ----TGASPALMGYCAERNISI-----TFLTNGRFLARVVGESRGNVLRKTKYRISENDQESTKIARNFITGK 115
EcoCas1 ----TRVSHAAVRLAAQVGTLL-----VWYGEAGVRV-----Y-ASGQPPGAR-SDK-----L 99
SpyCas1 ----LVDENVLVIFCDDKRLPT-----AMLMPF-----YGRHDSLQLGK-QMSWSET--VKSQVWTTI  IAQK 113
XooCas1 ----VAVSPQLLGFCEHGISI-----CYLTPQGRFLARVEGVPVSGNVLLRRAQYRRSDDPAGCAAIVRHLLAGK 115
PaeCas1 AMRELARAGVLVGFCCGGGTPLFSANEVDVEVSWLTPQSEYRPTLEYLQ-----RWVGF-WFDEEK-RLVAARHFQRAR 140
          * :
          * :

BhaCas1 --VYNSKWMLERMTREHPLR-VNVEQFKATSQLLSVMMQEIRNCDSLES LR-----GW  GQAAINYNKVFQDMILQ 183
EcoCas1 --LYQAKLALD-----EDLRLKVVYRKM-----FELRFGEPAARRSVEQLR-----GI  GSRVRATYALLA----- 153
SpyCas1 ---ILNQSCYLG-----ACS YFEK-----SQSIMDLYHGLENFDPSS-NR  GHAARIYFNTL F----- 161
XooCas1 ---IHNQRAVLRGWRDHGDCLTDVAAFQHSLKRLKRIPQRVLVETD VDLR-----GL  EGAAQSYFVGFVQQLVRA 184
PaeCas1 LERIRHWSLEDRLDAGFAVDATA-----VAVEDSARALEQAPNHEHLLTE  EARLSKRLFKLAAQA--- 204
          * :
          * :

BhaCas1 QKEEFAFHGRSRPPP---KDNVNAMLSFAYTLLANDVAAALETVGLDAYVGF  HQ-DRPGRASLALDLMEELRGLYADR 258
EcoCas1 KQYGV TWNGRRYDPKDWKGDITNQCISAATSCLYGVTEAAILAAGYAPAI  GFVHT-GKPLSFVYDIADI  IKPDTVVPKA 232
SpyCas1 ---GNDFS-----RDLEHPINAGLDYGYTL LLSMFAREVVVSGCMTQFGLK  HA-NQFNQNFASDIMEPFRPLVDKI 229
XooCas1 DKPLLRFPGGRNRRPPP---RDAFNALLSFLYTL LTHDCRSALSVGLDPAVG  FLHR-DRPGRPSLALDLAAEFRLPLGER 259
PaeCas1 TRY-----GEFVRAKRGSGGDPANRFLDHGNYLAYGLAATATWV  LGIPHGLAVL  HGKTRRGGVLVFDVADLIKDSLILPQA 279
          * :
          * :

BhaCas1 FVLSLINRKEMTADG-FYKKENGAVLMTDEARKTF-LKAWQTKKQEKITH  P YLGEKMSWGLVPYVQALLARFLRG---- 332
EcoCas1 FEIARRNPGEPE--D-REV-----RLA-----CRDIFRSSKTLAKLI  PLIEDVLAAGEIQPPAPP 283
SpyCas1 VYENRNQFPFKIKRELFTLFSDTFSYNGKEMYL TN I  SDYT-----KVV  KALNNEGKGV 285
XooCas1 LALS LINRRQLNERD-FQVFDNGAVLLKDDSRKTV-LIAYQERKRE  QLQHAFLGEKVDIGLLPFVQAQLLARHLRG---- 333
PaeCas1 FLSAMRGDEEQDFRQ---ACLDNL---SRAQALDFMIDTLKDV  AQ-----RSTVSA----- 324
          * :
          * :

BhaCas1 -----DLDEYPPFLWK 343
EcoCas1 EDAQPVAIPLPVSLGDAGHRSS---- 305
SpyCas1 E-----PRI- 289
XooCas1 -----DLDGYPAPFWK 344
PaeCas1 ----- 324

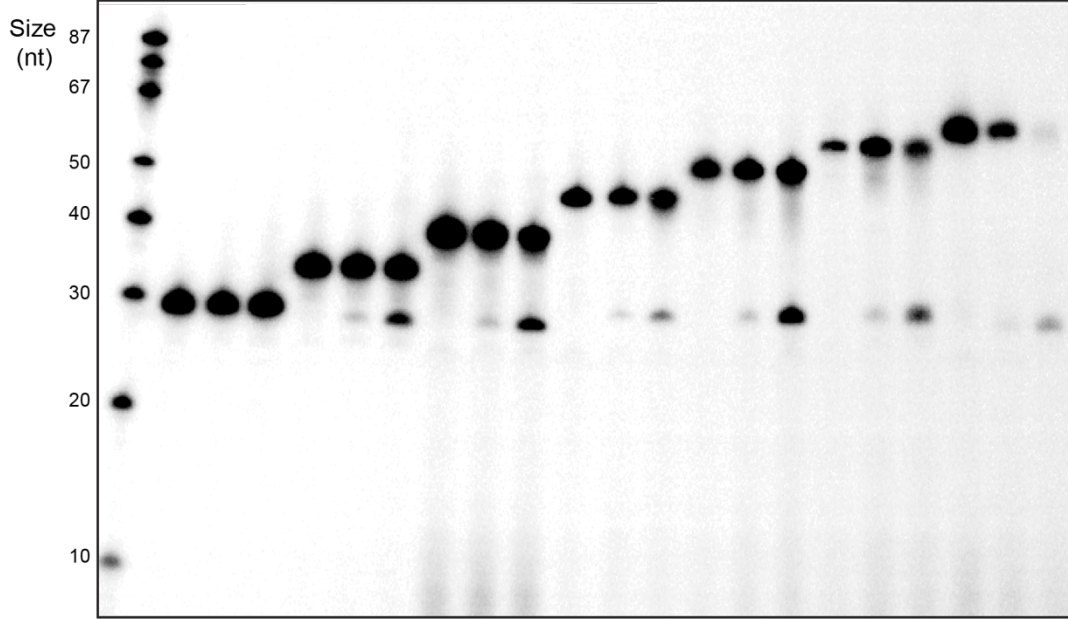
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Figure S3, related to Figures 2 and 4. Sequence alignments of Cas4 and Cas1.

(A) Sequence alignment of Cas4 orthologs. Cys residues involved in the Fe-S cluster are highlighted in yellow and residues involved in RecB-like motifs are highlighted in Red. Homologs from *Bacillus halodurans* (accession code: Q9KFY0), *Sulfolobus solfataricus* (Q97TX9), *Pyrobaculum calidifontis* (A3MTK6), *Xanthomonas oryzae pv. oryzae* (A0A0K0GPX6), *Haloferax volcanii* (D4GQN9), and *Candidatus Jettenia caeni* (I3IP77) were used for alignments. The conserved residues are labeled with asterisks and similar residues are labeled with dots. (B) Sequence alignment of Cas1 orthologs. Conserved catalytic residues are boxed in red. Homologs from *Bacillus halodurans* (Q9KFX9), *Escherichia coli* (Q46896), *Streptococcus pyogenes serotype M1* (Q99ZW1), *Xanthomonas oryzae pv. oryzae* (A0A0K0GQ44), and *Pseudomonas aeruginosa* (Q02ML7) were used for alignments. The conserved residues are labeled as asterisks and highly conserved residues are labeled as dots.

A

Overhang length (nt):	5			10			15			20			25			30			35		
Cas1	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-
Cas2	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Cas4-Cas1	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+

**B**

Duplex length (bp):	16			20			22			24			26			30			34		
Cas1	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-
Cas2	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Cas4-Cas1	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+

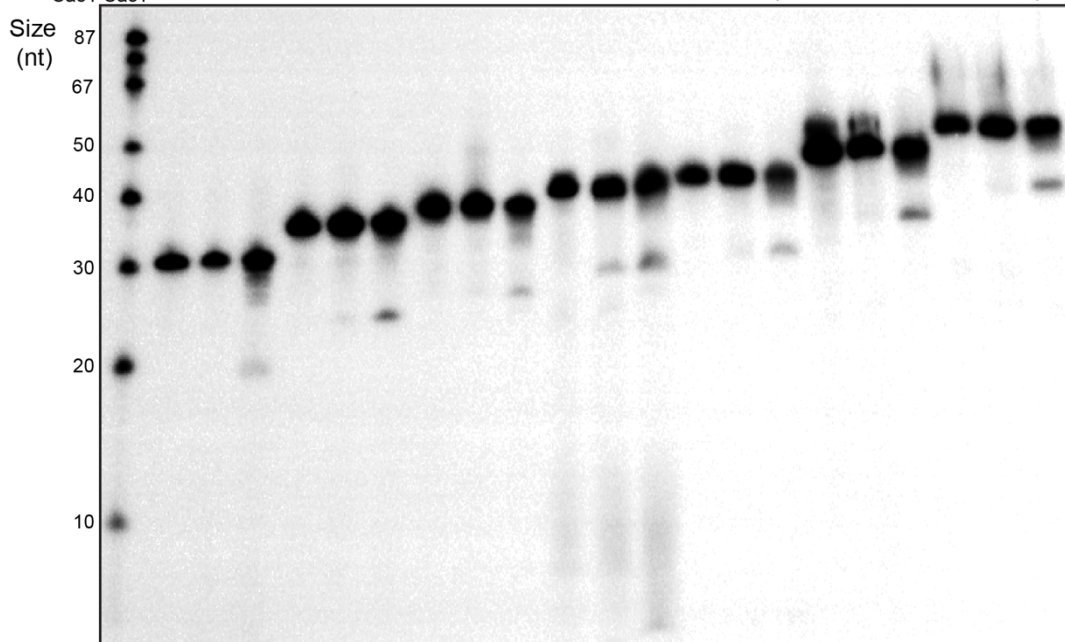


Figure S4, related to Figure 2. Cas4-Cas1-Cas2 processes prespacers with varying overhang and duplex length.

(A) Processing assay using different 3'-overhang lengths with constant duplex length (24 bp). (B)

Processing assay using different duplex lengths with constant 3'-overhang length (15 nt).

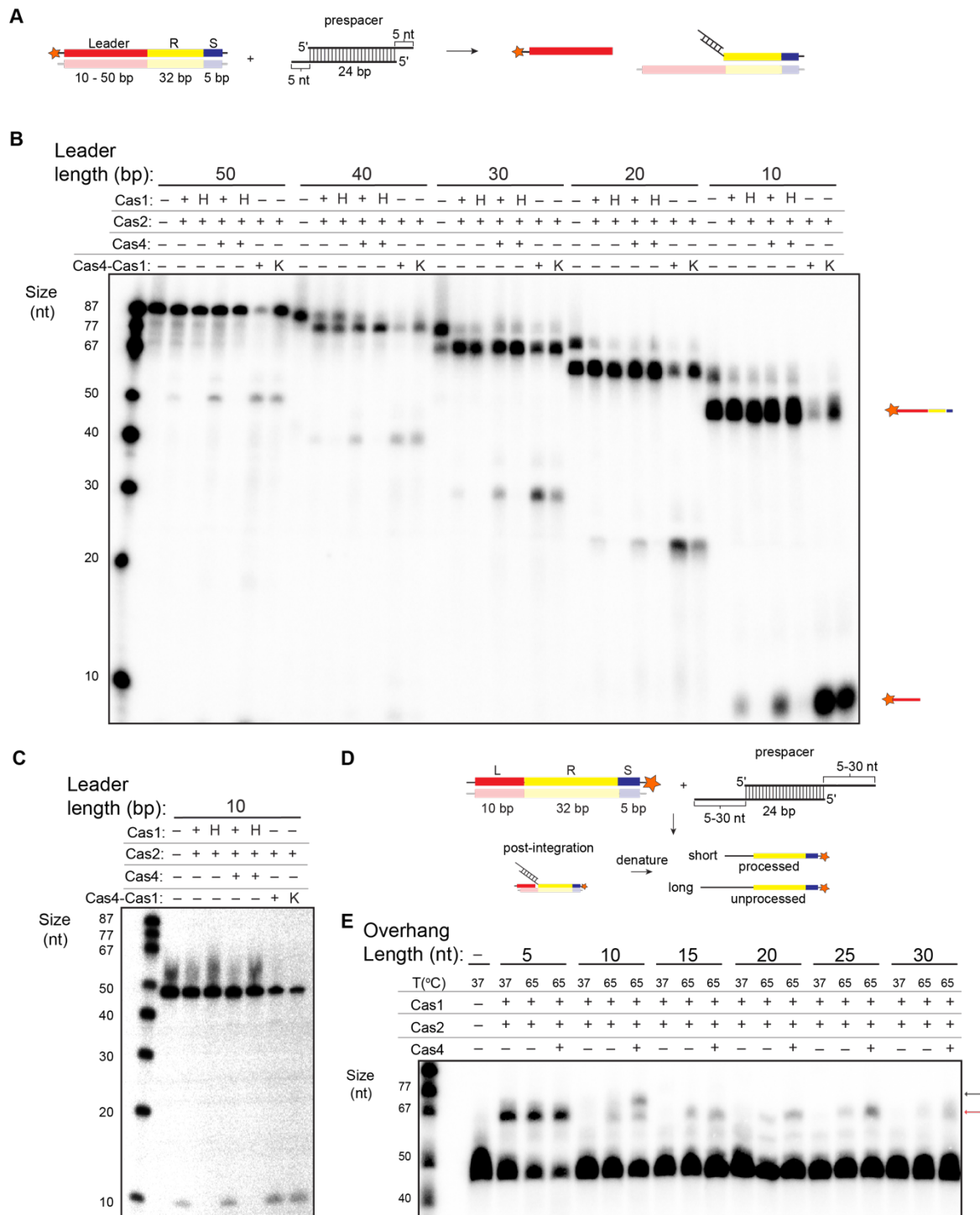


Figure S5, related to Figure 4. Integration assays using minimal CRISPR.

(A) Schematic view of integration assay using a 5'-radiolabeled minimal CRISPR with the 5-nt 3' overhang prespacer. Red, leader; yellow, repeat; blue, spacer; star is radiolabel at indicated position.

(B) Integration assay using the short linear CRISPRs with different leader lengths. (C) Cleavage assay using the minimal CRISPR (10-bp leader) in the absence of prespacer. (D) Schematic view of integration assay using 3'-radiolabeled minimal CRISPR and prespacers with different 3' overhang length. (E) Integration assay in the absence or presence of Cas4 at 37 or 65°C, as indicated. Red arrow indicates the integrated products of processed prespacers while black arrow indicates the integrated products of unprocessed prespacers.

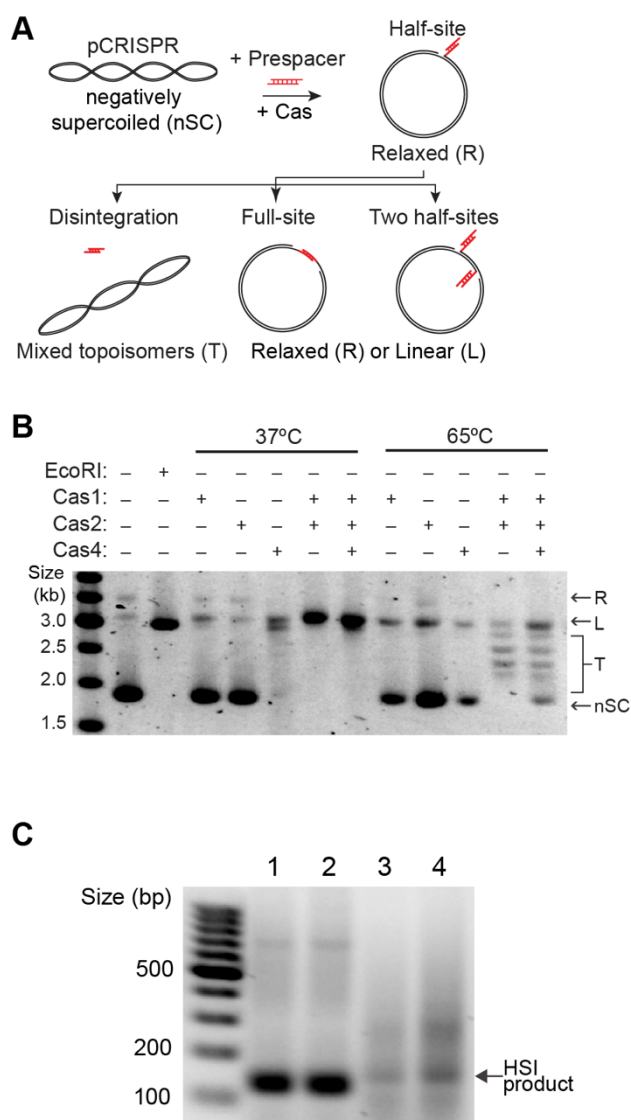


Figure S6, related to Figure 5. Cas1-Cas2 and Cas4-Cas1-Cas2 integrate prespacers into pCRISPR.

(A) Schematic view of integration assay and possible products using a supercoiled plasmid and the pre-processed prespacer. (B) Integration assay using Cas1, Cas2, Cas4 individually or in complex with prespacer at 37 or 65° C. The prespacer is a 24-bp duplex flanked by 5-nt 3' overhangs. EcoRI digested plasmid was used for a linear standard. (C) PCR products of the half-site integrated (HSI) products of Cas1-Cas2 in the presence of Cas4 using a prespacer with degenerate sequences on 15 nt 3' overhangs. The numbers indicate the four different events that are depicted in Figure 5A.

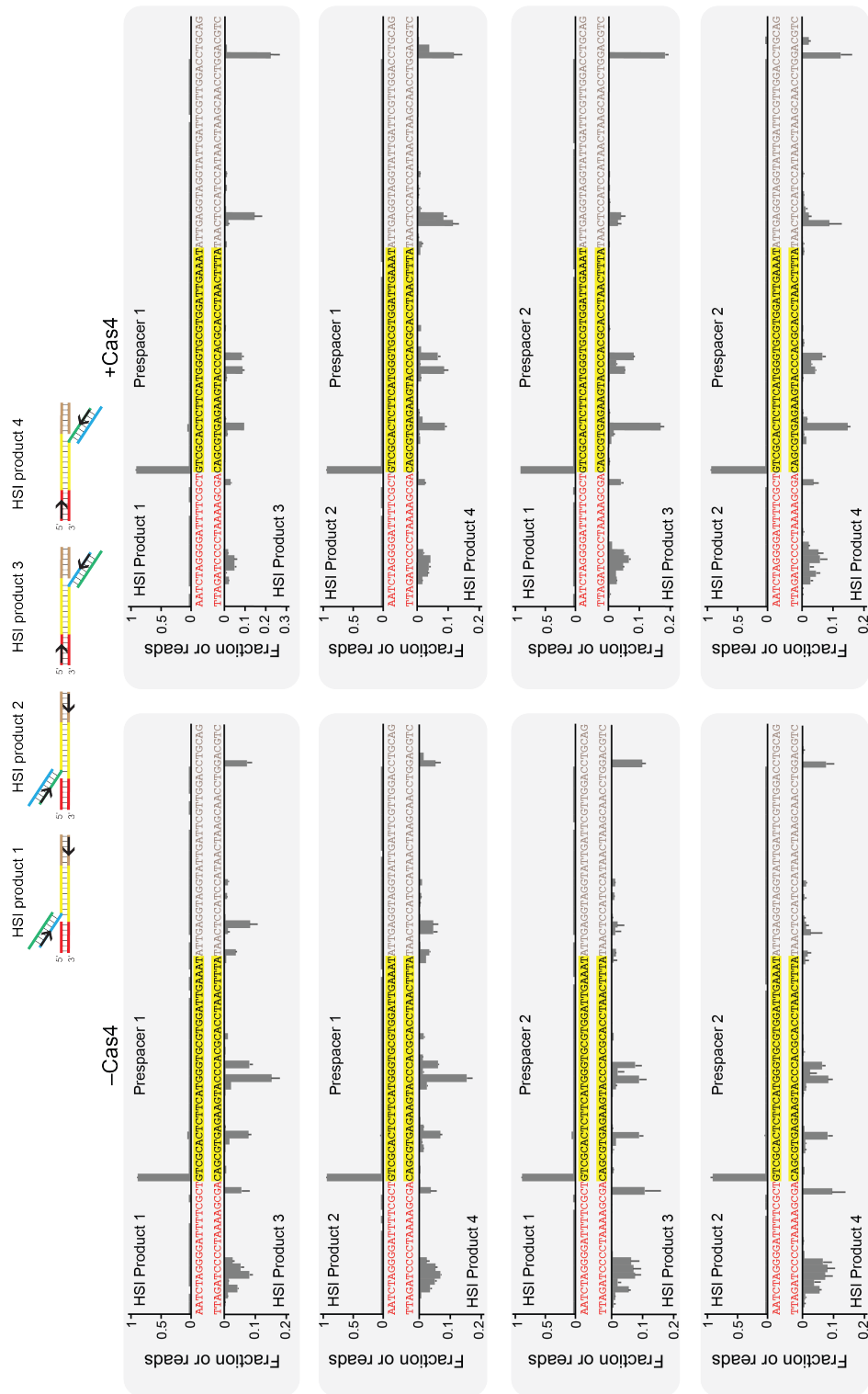


Figure S7, related to Figure 5. Integration sites for all conditions and pacers tested in HSI sequencing experiment. The average fraction of read counts at each start site from three separate replicates are plotted, with error bars representing standard deviation. Red: leader, yellow: repeat.

Table S1. Related to STAR Methods. Primers used in this study.

Name	Sequence (5' → 3')	Description
1	GTCG GGATCC ATGGCCAGTAATGAAGAAGACCG	Cas4 BamHI forward primer
2	TGTGT CTCGAG TCATTGCTCAGTCTCCCCTC	Cas4 XhoI reverse primer
3	GTCG GGATCC ATGAAAAAGCTATTAACACTCTATATGTGAC	Cas1 BamHI forward primer
4	TGTGT CTCGAG CTAATTCCACAGAAATGGCGG	Cas1 XhoI reverse primer
5	GTCG GGATCC ATGCTTGTTTTAATTACGTATGATGTCC	Cas2 BamHI forward primer
6	TGTGT CTCGAG TTAAAAGATAAGAGGGTCCTCTAAATCG	Cas2 XhoI reverse primer
7	TGTGT GAATTC TGGTGCGAACCTCAAGC	pCRISPR EcoRI forward primer
8	GTCG GGATCC GGGTCGGATGATGTCGC	pCRISPR BamHI reverse primer
9	CGCCATAAAACCGACATAAGCATCAAG	Cas1/Cas4-Cas1 H234A forward primer
10	CAAGACCGTCCTGGCC	Cas1/Cas4-Cas1 H234A reverse primer
11	CGCGTATTCAACAGGAAATGCC	Cas4-Cas1 K110A forward primer
12	CGAGGGAAGCCAAAG	Cas4-Cas1 K110A reverse primer
13 ^a	CGTAGCTGAGGACCAC	Forward primer against top strand of prespacer for detecting HSI products
14 ^a	CTGTTCTGGTGGTCCTC	Forward primer against bottom strand of prespacer for detecting HSI products
15 ^a	GCCAAGCTTGCATGC	Reverse primer against pCRISPR for detecting HSI products integrated in the plus strand
16 ^a	ATTCCCTATTTTATCAAAGTGATTTTC	Reverse primer against pCRISPR for detecting HSI products integrated in the minus strand

^aFor HSI product sequencing experiments, restriction enzyme sites (low-throughput sequencing, Figure 5B-C) or barcodes (high-throughput sequencing, Figures 5D and S6C) were added to the 5'-end of primers.

Table S2. Related to STAR Methods. Substrate oligonucleotides used in this study. Bold indicates repeat sequences, RC indicates the complementary strand of the previous strand.

Sequence (5' → 3')	Description	Figure
CTGTTCTGGTGGTCCTCAGCTACG TTTTG	5 nt 3'-overhang prespacer	4B, S4A, S5B, S5E, S6B
CGTAGCTGAGGACCACCAGAACAG TTTTG	RC	
AATTCCTATTTTATCAAAGTGATTTTCTAGAATCTAGGGGATTTTCGCTG TCGCACTCTTCATGGGTGCGTGGATTGAAATATTGA	50 bp leader	S5B
TCAATATTTCAATCCACGCACCCATGAAGAGTGCGACAGCGAAAATCCCCTAGAT TCTAGAAAATCACTTTGATAAAAATAGGGAATT	RC	
TTTATCAAAGTGATTTTCTAGAATCTAGGGGATTTTCGCTG TCGCACTCTTCATGG GTGCGTGGATTGAAATATTGA	40 bp leader	S5B
TCAATATTTCAATCCACGCACCCATGAAGAGTGCGACAGCGAAAATCCCCTAGAT TCTAGAAAATCACTTTGATAAA	RC	
TGATTTTCTAGAATCTAGGGGATTTTCGCTG TCGCACTCTTCATGGGTGCGTGG TTGAAATATTGA	30 bp leader	S5B
TCAATATTTCAATCCACGCACCCATGAAGAGTGCGACAGCGAAAATCCCCTAGATT CTAGAAAATCA	RC	
GAATCTAGGGGATTTTCGCTG TCGCACTCTTCATGGGTGCGTGGATTGAAATA TTGA	20 bp leader	S5B
TCAATATTTCAATCCACGCACCCATGAAGAGTGCGACAGCGAAAATCCCCTAGATT C	RC	
GATTTTCGCTG TCGCACTCTTCATGGGTGCGTGGATTGAAATATTGA	10 bp leader	S5B-C
TCAATATTTCAATCCACGCACCCATGAAGAGTGCGACAGCGAAAATC CGTAGCTGAGGACCACCAGAACAG CTCA G	RC	
CTGTTCTGGTGGTCCTCAGCTACG CTCA G	5 nt 3'-overhang prespacer	5C
TTTTTTTTAAGTTTT CTGTTCTGGTGGTCCTCAGCTACG	RC	
TTTTTTTTAAGTTTT CTGTTCTGGTGGTCCTCAGCTACG	15 nt 5'- overhang prespacer	S2C, S2D
TTTTTTTTAAGTTTT CGTAGCTGAGGACCACCAGAACAG	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTGAATTTTTTTTT	Blunt end DNA	S2D, S2E
AAAAAAAAATTCAAACCTGTTCTGGTGGTCCTCAGCTACG	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTGAATTTTTTTTT	15 nt 3' overhang prespacer	2B-C, 2E-F, S2B, 3C, 4B-C, S4A, S5E
CTGTTCTGGTGGTCCTCAGCTACG TTTTGAATTTTTTTTT	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTGAATTTTTTTTTTTTTTTTTTTTT	25 nt overhang prespacer	2E-F, S4A, S5E
CTGTTCTGGTGGTCCTCAGCTACG TTTTGAATTTTTTTTTTTTTTTTTTTTT	RC	
CTGTTCTGGTGGTCCTCAGCTACG TTTTGAATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	35 nt overhang prespacer	2E-F, S4A
CGTAGCTGAGGACCACCAGAACAG TTTTGAATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	RC	
CTGTTCTGGTGGTCCTCAGCTACG TTTTGAATTT	10 nt 3'- overhang prespacer	S4A, S5E
CGTAGCTGAGGACCACCAGAACAG TTTTGAATTT	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTGAATTTTTTTTTTTTTTTTT	20 nt 3'- overhang prespacer	S4A, S5E
CTGTTCTGGTGGTCCTCAGCTACG TTTTGAATTTTTTTTTTTTTTTTT	RC	

CGTAGCTGAGGACCACCAGAACAG TTTTGAATTTTTTTTTTTTTTTTTTTTTTTTTTTTT	30 nt 3'-overhang prespacer	S4A, S5E
CTGTTCTGGTGGTCCTCAGCTACG TTTTGAATTTTTTTTTTTTTTTTTTTTTTTTTTTTT	RC	
CGTAGCTGAGGACCAC CTCA GAA CTGATCGT	16 bp duplex prespacer	S4B
GTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
CGTAGCTGAGGACCACCAGA CTCA GAA CTGATCGT	20 bp duplex prespacer	S4B
TCTGGTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
CGTAGCTGAGGACCACCAGAAC CTCA GAA CTGATCGT	22 bp duplex prespacer	S4B
GTTCTGGTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
CGTAGCTGAGGACCACCAGAACAG CTCA GAA CTGATCGT	24 bp duplex prespacer	S4B
CTGTTCTGGTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
CGTAGCTGAGGACCACCAGAACAGTA CTCA GAA CTGATCGT	26 bp duplex prespacer	S4B
TACTGTTCTGGTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
CGTAGCTGAGGACCACCAGAACAGTAGTCG CTCA GAA CTGATCGT	30 bp duplex prespacer	S4B
CGACTACTGTTCTGGTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
CGTAGCTGAGGACCACCAGAACAGTAGTCGGCTC CTCA GAA CTGATCGT	34 bp duplex prespacer	S4B
GAGCCGACTACTGTTCTGGTGGTCCTCAGCTACG CTCA GAA CTGATCGT	RC	
GATTTTCGCTGTCGCACTTTCATGGGTGCGTGGATTGAAATA	10 bp leader for 3'-end labeling	4B-C, S5E
TCAATATTTCAATCCACGCACCCATGAAGAGTGCGACAGCGAAAATC	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTNNNTTTTTTTT	HSI-PS1	5, S7
CTGTTCTGGTGGTCCTCAGCTACG TTTTNNNTTTTTTTT	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTCNNNTTTTTTTT	HSI-PS2	5, S7
CTGTTCTGGTGGTCCTCAGCTACG TTTTCNNNTTTTTTTT	RC	
CGTAGCTGAGGACCACCAGAACAG TTTTTCTTTTTTTT	15 nt 3'-overhang prespacer with TTC PAM	3C
CTGTTCTGGTGGTCCTCAGCTACG TTTTTCTTTTTTTT	RC	