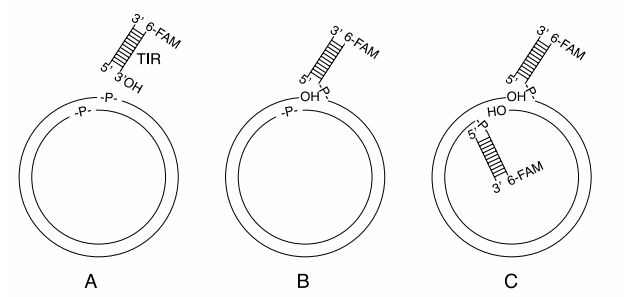

Figure S1: Single and double integration of a fluorescent oligonucleotide duplex derived from the terminal inverted repeat (TIR) of the *A. boonei* casposon.

Starting from a covalently closed, circular plasmid (A), integration of the duplex on one strand generates a nicked, relaxed form of the plasmid (B); tandem integration of a second duplex on the other strand generates staggered cuts on both strands and linearizes the plasmid (C).

Table S1. Strains

Name	Genotype	Origin
Escherichia coli TOP10	F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ *lac*X74 *rec*A1 *ara*D139 Δ(*araleu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Invitrogen (ThermoFisher Scientific)
SoluBL21(DE3)	F- *ompT hsdS*B (rB-mB-) *gal dcm* (DE3) + uncharacterized mutations increasing the solubility of recombinant proteins	amsbio

Table S2. Plasmids

Name	Features	Origin
pETM-11	Expression vector with T7 promoter, encoding N-terminal His-tag and Tev protease processing site	EMBL Protein Expression and Purification Facility
pETM-11 Cas1	Expression vector for the production of Cas1	This work
pMA-Target	Plasmid with a 265 nt insert carrying the original target site prior to integration of the *A. boonei* casposon	This work
pMA-ΔTarget	Deletion of nt 11-265 of the pMA-Target insert	This work
pMA-T46-265	Segment 46-265 of the pMA-Target insert	This work
pMA-T82-265	Segment 82-265 of the pMA-Target insert	This work
pMA-T126-265	Segment 126-265 of the pMA-Target insert	This work
pMA-T1-140	Segment 1-140 of the pMA-Target insert	This work
pMA-T1-176	Segment 1-176 of the pMA-Target insert	This work
pMA-T1-221	Segment 1-221 of the pMA-Target insert	This work
pMA-T118-148	Segment 118-148 of the pMA-Target insert	This work
pMA-T82-140	Segment 82-140 of the pMA-Target insert	This work
pMA-T103-140	Segment T103-140 of the pMA-Target insert	This work
pMA-T82-125	Segment T82-125 of the pMA-Target insert	This work
pMA-T82-104	Segment T82-104 of the pMA-Target insert	This work
pMA-Casp-kana	Mini-casposon encoding kanamycin resistance	This work

Table S3. Oligonucleotides

Name	Sequence	Use
6-FAM-LE26r	6-FAM-TTAAGAGGGGATGTATATATATCCCC	Integration of fluorescent duplex
LE-26	GGGGATATATATACATCCCCTCTTAA	
LE41	GGGGATATATATACATCCCCTCTTAAGTTCCCTTTTAGATC	Amplification of mini-casposon
RE41	GGGGATATATATATATCCCCTCTTAAGTTCCCTTTTAAGCT	
533r	GCATCCATGTTGGAATTTAATCG	Sequencing insertion site of mini-casposon
Seq3	CGCTGAGCAATAACTAGCATAACC	
LE32	GGGGATATATATACATCCACTCTTAAGTTCCC	Mapping of oligonucleotide
LE32r	GGGAACTTAAGAGTGGATGTATATATATCCCC	

1179	GACTTGGTTGAGTACTCACCAGTCACAG	duplex integration
1737r	GCTTAATCAGTGAGGCACCTATCTC	

Table S4. Alignment of the sites bordering the left and right TIR after integration by de-tagged and His-tagged casposase into pMA-Target of the mini-casposon encoding kanamycin resistance

Numbering of the residues refers to the numbering of the sequence of the recipient plasmid. The distal ends of the inserted casposon are shown in blue; the TSD segment is shown in green; additional nucleotides added by the *E. coli* host upon filling the duplicated single-strand gaps created by the integration are shown in black; duplicated segments originating from integration at sites different form the original *A. boonei* target site are shown in red; upstream and downstream sequences immediately flanking the TSD are shown in orange and magenta, respectively

Original sequence found in the *A. boonei* genome

```
                TSD1        Left TIR    Right TIR      TSD2
CCGGGCGGCCCCACTACGAGGAG---GGGGATATA... ATATCCCCCCCCACTACGAGGAGACACCT
```

Canonical integrations by de-tagged Cas1 into pMA-Target

```
     510            525
CCGGGCGGCCCCACTACGAGGAG-----GGGATATA...ATATCCCCGCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCC--CCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAG----GGGGATATA...ATATCCCCA-CCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCC-ACCCACTACGAGGAGACACCT
```

Uncanonical integrations catalyzed by de-tagged Cas1 into pMA-Target

```
    1866            1882
AGTGGTCCTGCAACTTTATCCGCC---GGGGATATAT...ATATCCC-TGCAACTTTATCCGCCTCCA

                    1882
AGTGGTCCTGCAACTTTATCCGCC---GGGGATATAT...ATATCCCCACATCTCATCTGTAACATCA

                                          1117
   Overlapping sequences              ...ATATCCCCAGTAGGTCGTTCGCTCCAAG

                    471                                        525
GGGTTCCTTTACCTCCTGCAACCAAGAGGGGATATAT...ATATCCCCACCCACTACGAGGAGACACC

        1646          1661
AAACTTGGTCTGACAGTTACCAATGCTGGGGATATAT...ATATCCCCAACAGTTACCAATGCTTAAT
```

Canonical integrations catalyzed by tagged Cas1 into pMA-Target

```
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCTCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCC-ACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGCT--GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGACCTGGGGATATA...ATATCCCCTCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCTCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCGCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCGCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGC-----GGATATA...ATATCCCC-CCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCGCCCACTACGAGGAGACACCT
```

CCGGGCGGCCCCACTACGAGGAGC---GGGGATATA...ATATCCCC-CCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCC-CCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGC---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGG---GGGGATATA...ATATCCCCTCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCCACCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGG---GGGGATATA...ATATCCCCTCCCACTACGAGGAGACACCT
CCGGGCGGCCCCACTACGAGGAGT---GGGGATATA...ATATCCCC--CCACTACGAGGAGACACCT

Non-canonical integration catalyzed by tagged Cas1 into pMA-Target

665 687
ACAAGACTGGCCTCATGGGCCTTCCGGGGATA... ATATCCCCGACTGGCCTCATGGGCCTTCCGCT

222 525
GGGCCTCTTCGCTATTACGCCAGTGGGGATATA......ATATCCCCACCCACTACGAGGAGACACCT

Table S5. Alignment of the sites bordering the left and right TIR after integration by de-tagged and His-tagged casposase into pMA-ΔTarget of the mini-casposon encoding kanamycin resistance

Numbering of the residues refers to the numbering of the sequence of the recipient plasmid. The distal ends of the inserted casposon are shown in blue; additional nucleotides added by the *E. coli* host upon filling the duplicated single-strand gaps created by the integration are shown in black; duplicated segments, which originate from integration at sites different form the original *A. boonei* target, site are shown in red.

Integrations catalyzed by de-tagged Cas1 into pMA-ΔTarget

513 528
GGGCGCTCTCCGCTTCCTCGCTCGGGGATATA...ATATCCCCATCCGCTTCCTCGCTCACTGACTCG

1261 1275
GCTCAGTGGAACGAAAACTCACCGGGGATATA...ATATCCCCAGAACGAAAACTCACGTTAAGGGAT

483 498
ACATGGTCATAGCTGTTTCCTTGGGGATATA...ATATCCCCACATAGCTGTTTCCTTGCGTATTGGG

1243 1260
ATCTACGGGGTCTGACGCTCAGTGGGGATATA...ATATCCCCGGGTCTGACGCTCAGTGGAACGAAA

649 2070
CTCCGCCCCCCTG...GTTCTTTGGGGATATAT...ATATCCCCACCCCTG...GTTCTTCGGGGCGA

1226 1241
TAGAAGATCCTTTGATCTTTTCT-GGGGATATA...ATATCCCCCCTTTGATCTTTTCTACGGGGTCT

289 444
ACGACGTTGTAAA...GCTTTCTGGGGATATAT...ATATCCCCAGTTGTAAA...GCTTTCTCAGTC

1649 1662
TGTTGCCGGGAAGCTAGAGTAAAGGGGATATAT...ATATCCCCAGAAGCTAGAGTAAGTAGTTCGCC

2350 2364
ATAGGGGTTCCGCGCACATTTCTGGGGATATAT...ATATCCCCATCCGCGCACATTTCCCCGAAAAG

Integrations catalyzed by tagged Cas1 into pMA-ΔTarget

628 643
TTGCTGGCGTTTTTCCATAGGCTGGGGATATA...ATATCCCCGGTTTTTCCATAGGCTCCGCCCCC

1772 1776
CTCCGGTTCCCAACGATCAAGGGGGGATATA...ATATCCCCAGGCGAGTTACATGAT

```
    623            638
CCGGTTGCTGGCGTTTTTCCATTGGGGATATA...ATATCCCCACTGGCGTTTTTCCATAGGCTCC

    990           1536
GCCACTGGTAACAG...AGAACTGGGGATATA...ATATCCCCAGTAACAG...AGAACCACGCTCAC

   2349           2364
ATAGGGGTTCCGCGCACATTTCTGGGGATATA...ATATCCCCATTCCGCGCACATTTCCCCGAAAAG

    1776          1790
ATCAAGGCGAGTTACATGATCCTGGGGATATA...ATATCCCCAGAGTTACATGATCCCCCATGTT

    1261          1275
ACTCAGTGGAACGAAAACTCACTGGGGATATA...ATATCCCCGAACGAAAACTCACGTTAAGGGATT

  246             2048
GTGCTAGGCGATTA...AAGTGTGGGGATATA...ATATCCCCAGGCGATTA...AAGTGCTCATCAT
```