# CRISPR/Cas9 mediated targeting of multiple genes in *Dictyostelium*

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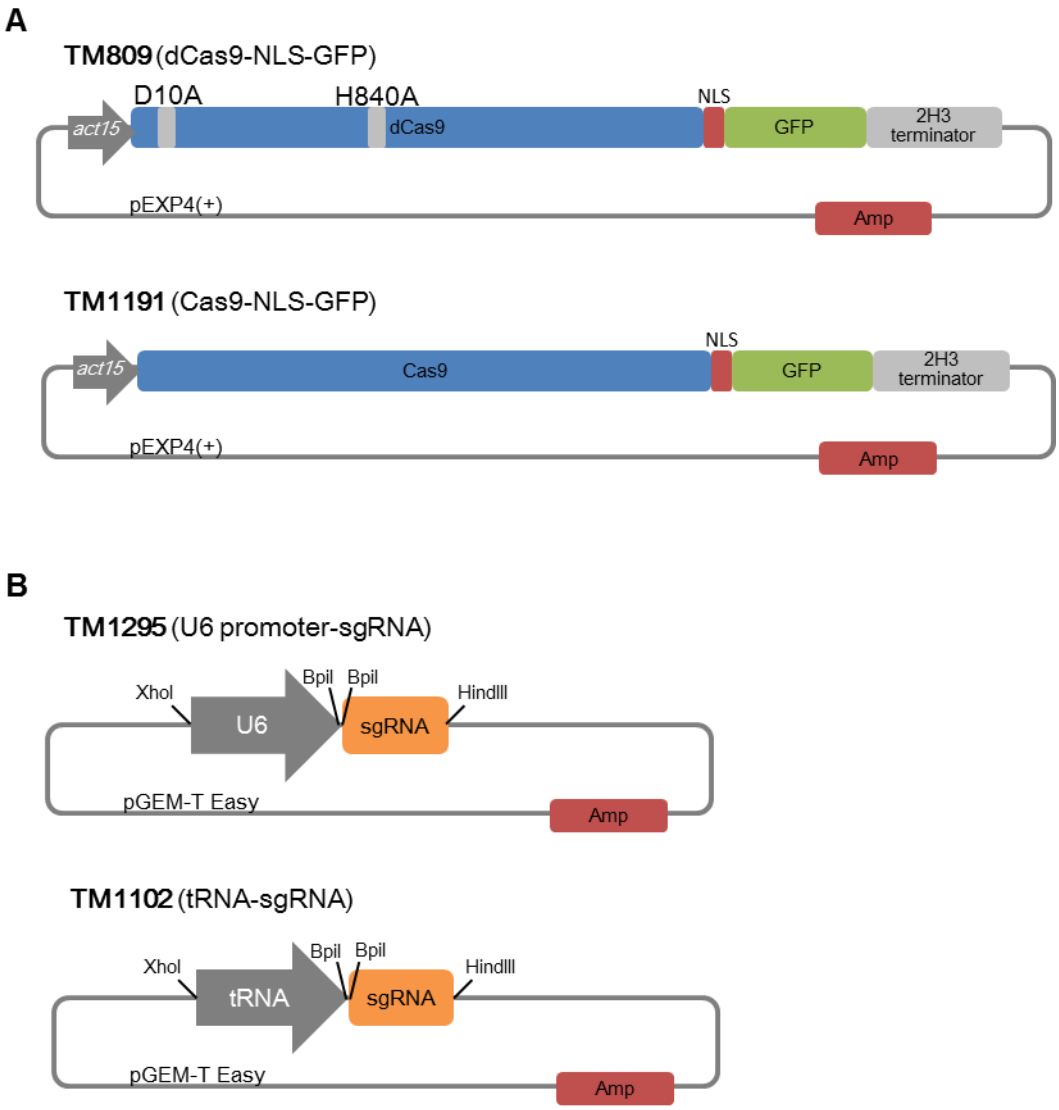
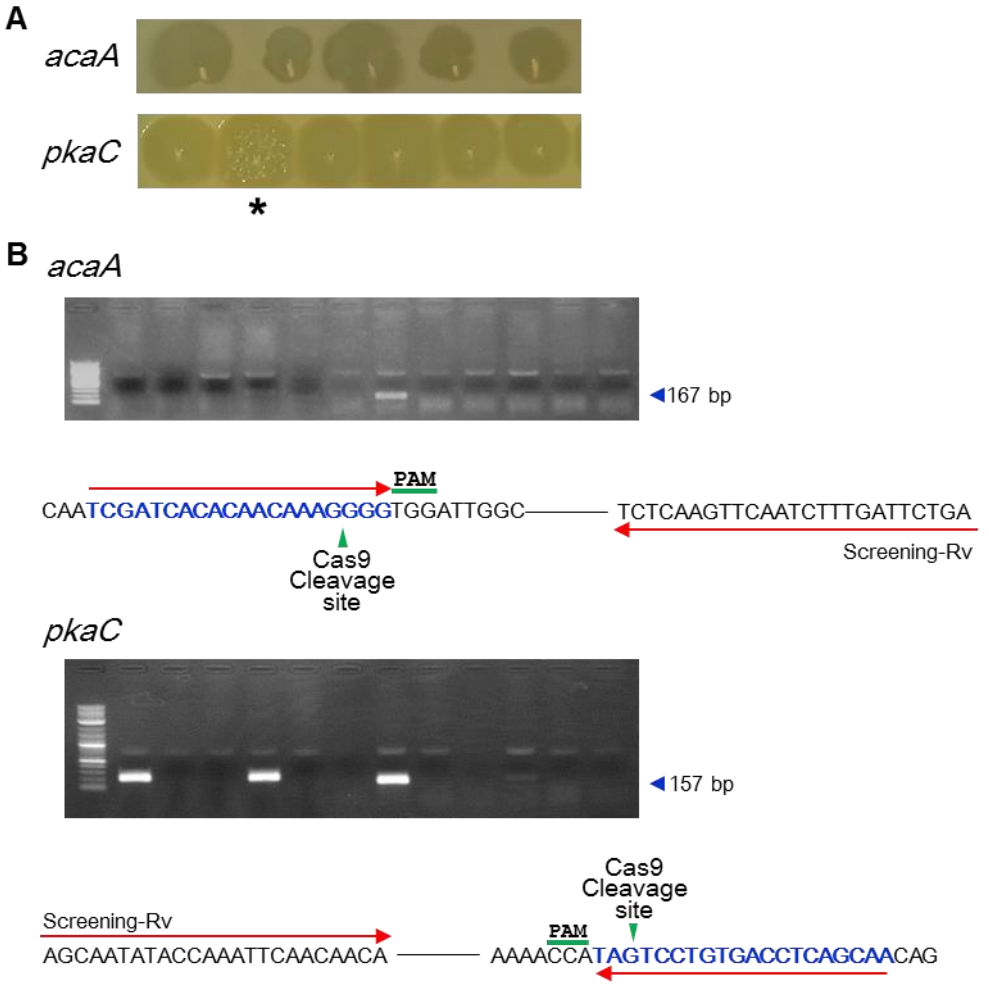


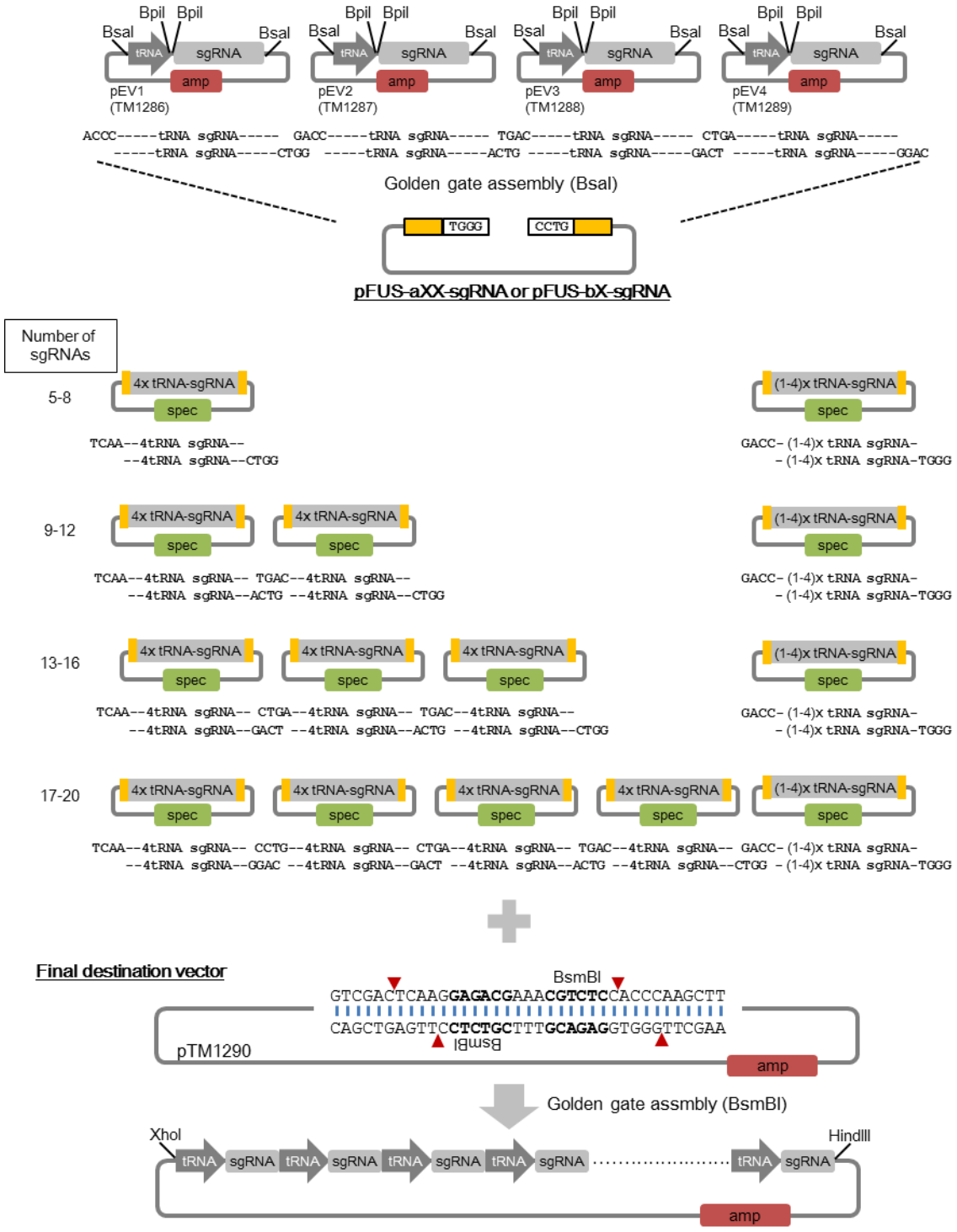

Supplementary Figure S1. Optimised Cas9 expression vectors in *Dictyostelium*.

(A) The dCas9 and Cas9 expression vectors modified from the previous vector system, pSLQ1658-dCas9-EGFP. (B) An sgRNA expression vector under the control of U6 or tRNA. U6, U6 promoter; tRNA, isoleucine tRNA.




Supplementary Figure S2. Analysis of *acaA* and *pkaC* mutants.

(A) Aggregation phenotype of *acaA* and *pkaC* mutants. Single clones were seeded on a bacteria lawn on SM agar plates and imaged after 3-4 days. The asterisk indicates aggregation-positive clone and rest of the clones are aggregation-negative. (B) PCR analysis of individual aggregation-negative clones. The positions of PCR primers relative to the Cas9 cleavage site are presented by red arrows. One of the primers was designed to span the cleavage site, thus no PCR amplifications is expected in mutated clones. Blue arrowheads indicate PCR products observed with wild type cells. The size of the PCR products are 167 bp and 157 bp, respectively. Gel images were cropped, but no other bands were present.




Supplementary Figure S3. Schematic diagram to construct multiplex sgRNA expression vector using Golden Gate assembly.

The target sequence of the individual locus was synthesised and inserted into the Golden Gate entry

vectors (pEV) digested by BpiI. The cloned fragment in the pEV is flanked by BsaI sites and can be sub-cloned into the second module vectors (pFUS) obtained from the Platinum Gate TALEN kit. Once the desired numbers of fragments are constructed, second steps of Golden Gate assembly are performed by BsmBI digestion and ligation step.

Supplementary Table S1. Primer sequences used for cloning and sequencing

| Gene | Description | Sequence |
|---|---|---|
| tdTomato | sgRNA sense | AGCAATGTCCCAGGCGAAGGGCAG |
| tdTomato | sgRNA antisense | AAACCTGCCCTTCGCCTGGGACAT |
| tdTomato | mutation detection Fw | TTGTTGGTTGCTTTATTTTATTTGA |
| tdTomato | mutation detection Rv | AGCAAATCAAAATAATCTGGAAGT |
| *acaA* | sgRNA sense | AGCATCGATCACACAACAAAGGGG |
| *acaA* | sgRNA antisense | AAACCCCCTTTGTTGTGTGATCGA |
| *acaA* | Screening-Rv | TCAGAATCAAAGATTGAACTTGAGA |
| *acaA* | Screening-Fw | GGTGGTCATATTAATCCCATGC |
| *pkaC* | sgRNA sense | AGCATTGCTGAGGTCACAGGACTA |
| *pkaC* | sgRNA antisense | AAACTAGTCCTGTGACCTCAGCAA |
| *pkaC* | Screening-Rv | AGCAATATACCAAATTCAACAACA |
| *pkaC* | Screening-Fw | CTGGCCAAGATATGAAATAGAGG |
| *pikA* | sgRNA sense | AGCAAGTGGAGGACATGTAAATAA |
| *pikA* | sgRNA antisense | AAACTTATTTACATGTCCTCCACT |
| *pikA* | Screening-Rv | GCAGATTCATCACTACCACCA |
| *pikA* | Screening-Fw | ACCCACCCACAACAGATGA |
| *pikB* | sgRNA sense | AGCAAGTTCAACATTGATGTACAA |
| *pikB* | sgRNA antisense | AAACTTGTACATCAATGTTGAACT |
| *pikB* | Screening-Rv | TTCAATTGCTGGTGGTGGTA |
| *pikB* | Screening-Fw | GTGGTGGTAGTAACCTCAGTGTAAA |
| *pikC* | sgRNA sense | AGCAAAATACAGTTGTGCAAGGTT |
| *pikC* | sgRNA antisense | AAACAACCTTGCACAACTGTATTT |
| *pikC* | Screening-Rv | TGGTTGTTGGAAGTATTGAGGA |
| *pikC* | Screening-Fw | TCAATGGTTGCCTTGACATT |
| *pikF* | sgRNA sense | AGCATGAAAAAACAGCTGTTGAAT |
| *pikF* | sgRNA antisense | AAACATTCAACAGCTGTTTTTTCA |
| *pikF* | Screening-Rv | AAATGAACGTGGTGGTACAGC |
| *pikF* | Screening-Fw | CTGAATCAGATGATGAAGGTGA |
| *pikG* | sgRNA sense | AGCACATTAAGTTTAGTTTCTAAA |
| *pikG* | sgRNA antisense | AAACTTTAGAAACTAAACTTAATG |
| *pikG* | Screening-Rv | GAAAAGTCTTATAATTCAAGTTCACCA |
| *pikG* | Screening-Fw | CATACTATTTGGTCCACTATTCGTT |