Genome editing across Dictyostelia species enables comparative functional genetics of social amoebas

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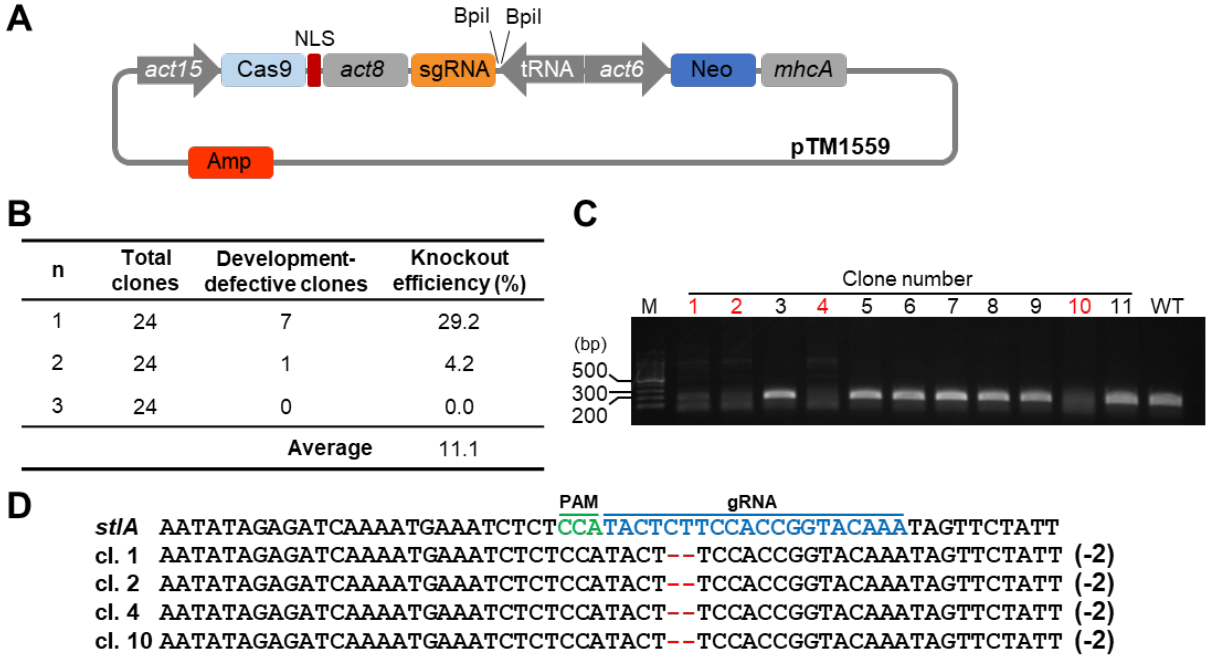
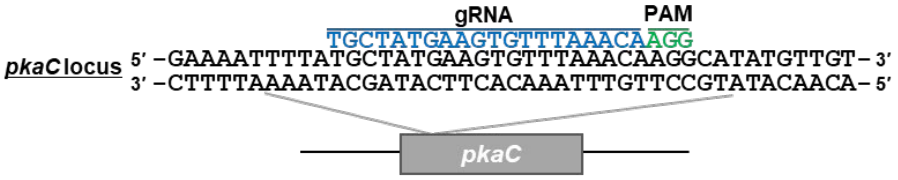


n	Total clones	Development-defective clones	Knockout efficiency (%)
1	24	7	29.2
2	24	1	4.2
3	24	0	0.0
		Average	11.1

Supplementary Figure 1. Knockout of *stlA* using a conventional transient expression CRISPR vector in *P. violaceum*.

(A) Schematic diagram of the Cas9 all-in-one vector (pTM1559) used for transient expression. (B) Proportion of clones exhibiting the development-defective phenotype. In each of three independent experiments, 24 clones were examined for aggregation phenotype. (C) Mutation detection PCR performed on 11 randomly selected clones. Clones carrying mutations at the target site show weaker bands than wild-type (WT) due to inhibited amplification. Clones highlighted in red represent PCR-positive candidates. (D) Sequence analysis of the genomic region containing the target site. The target sequence is shown in blue, the PAM sequence in green, and mutations in red. Numbers in parentheses indicate the size of the deletion.

A



B

n	Total clones	Development-defective clones	Knockout efficiency (%)
1	24	10	41.7
2	24	11	45.8
3	24	9	37.5
		Average	41.6

C

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                    gRNA                PAM
pkaC  TTATGCTATGAAGTGTTTAAACAAGGGCT
cl. 1 TTATGCTATGAAGTGTTTAA-CAAGGGCT (-1)
cl. 2 TTATGCTATGAAGTGTTTAA-CAAGGGCT (-1)
cl. 3 TTATGCTATGAAGTGTTTAA-CAAGGGCT (-1)
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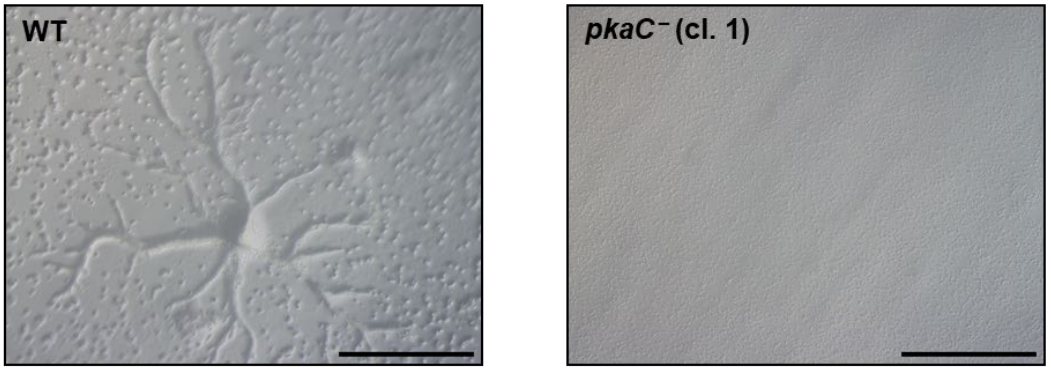
D



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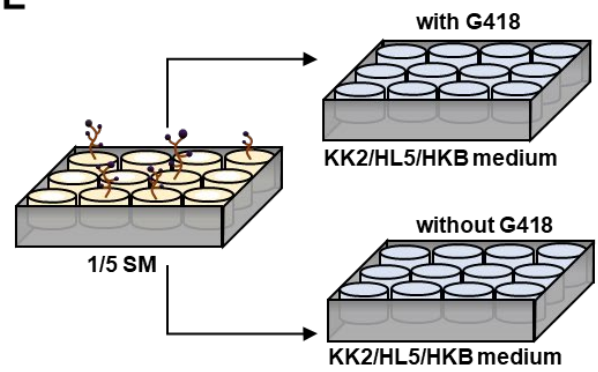
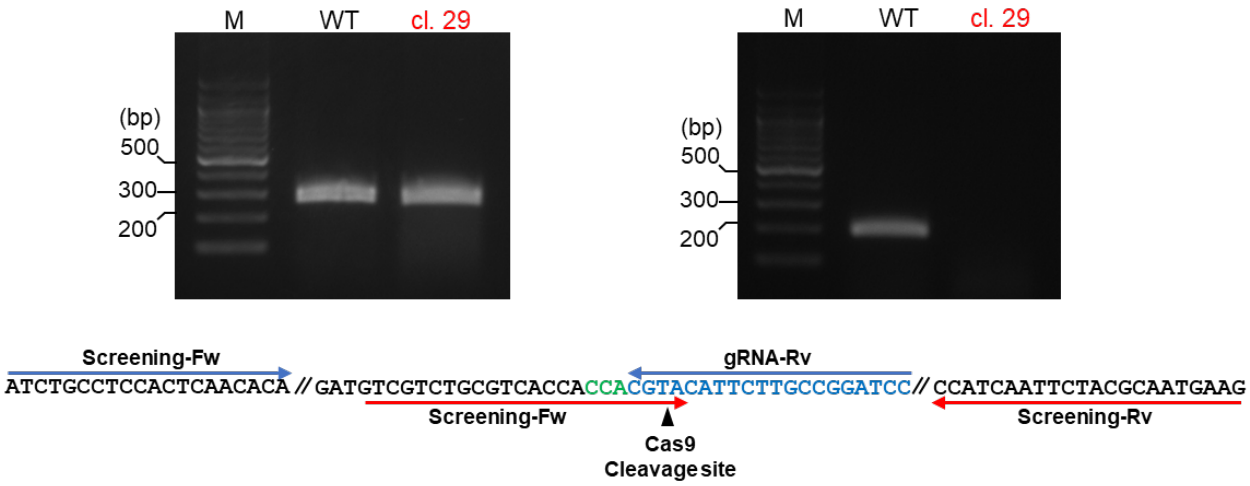


n	Total clones	Drug-resistant clones	Plasmid retention (%)
1	24	1	4.2
2	24	0	0.0
3	22	1	4.5
		Average	2.9

Supplementary Figure 2. Analysis of *pkaC* knockout efficiency and G418 resistance retention in *P. violaceum*.

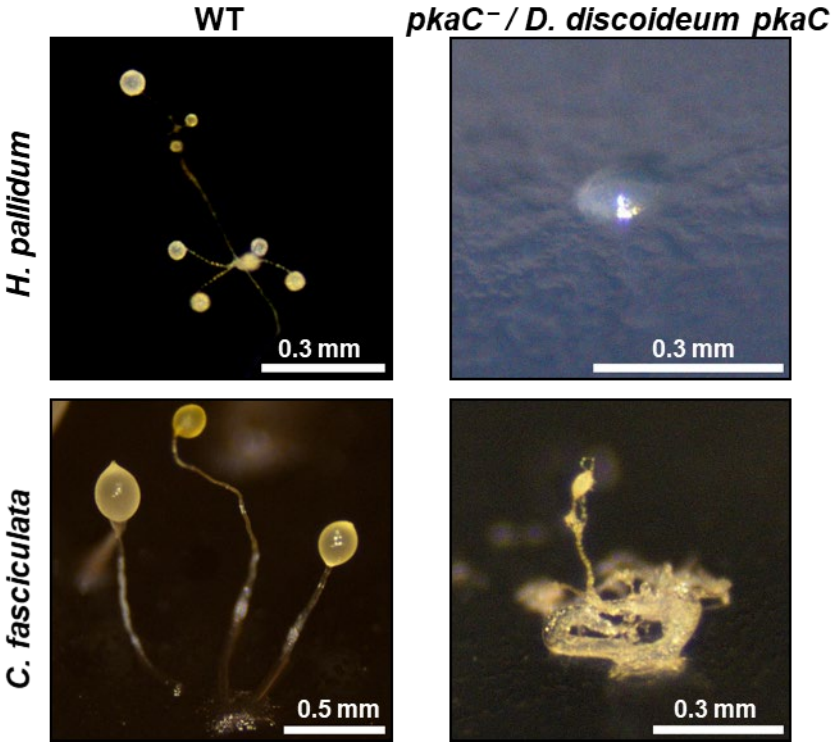
(A) Schematic representation of the target region. (B) Proportion of clones exhibiting development-defective phenotypes. (C) Sequence analysis of the genomic region containing the target site. (D) Developmental phenotypes of parental and *pkaC* knockout mutant at 7 h after starvation. Scale bar: 0.5 mm. (E) Analysis of drug resistance retention in the isolated clones. The proportion of clones able to proliferate under selection was used to determine whether drug resistance was retained.



Supplementary Figure 3. Mutation detection PCR in *H. pallidum*.

(Left) Conventional mutation-detection PCR. The target region was amplified using primers derived directly from the oligonucleotides used for gRNA construction, as indicated by blue arrows.

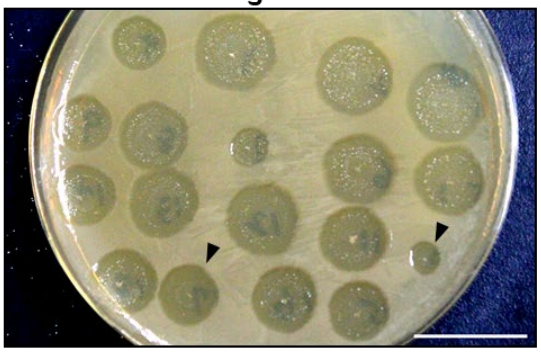
(Right) Improved mutation-detection PCR. The target region was amplified using a primer with a one-nucleotide overhang adjacent to the Cas9 cleavage site together with the mutation detection primer, as indicated by red arrows.



Supplementary Figure 4. Cross-species complementation of *pkaC* in *H. pallidum* and *C. fasciculata*.

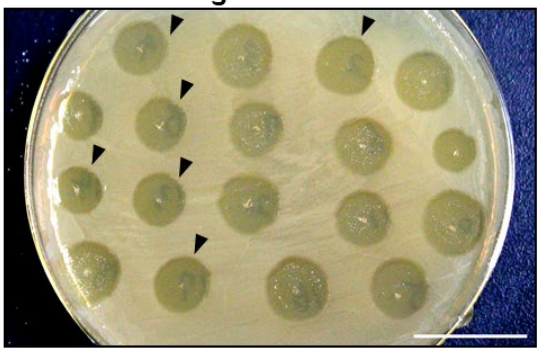
Developmental phenotypes of WT and *D. discoideum* PkaC-rescued strains. In *H. pallidum*, WT cells formed normal fruiting bodies within 24 h, whereas the rescued strain arrested at the mound stage. In *C. fasciculata*, wild-type cells formed normal fruiting bodies within 24 h, whereas the rescued strain produced small and misshapen fruiting bodies after 48 h.

A without Donor oligo



n	Total clones	Development-defective clones	Knockout efficiency (%)
1	36	3	8.3
2	36	1	2.7
3	36	8	22.2
		Average	11.0

B with Donor oligo



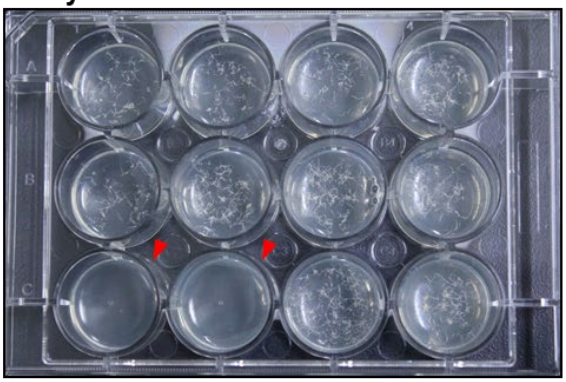
n	Total clones	Development-defective clones	Knockout efficiency (%)
1	36	9	25.0
2	36	11	30.5
3	36	11	30.5
		Average	28.6

Supplementary Figure 5. Comparison of gene disruption efficiency with and without donor oligos.

(A) Phenotypes of mutants generated without donor oligos. Candidate mutants are indicated by black arrows heads. Scale bar: 2 cm. The proportion of clones showing developmental defects among 36 analyzed clones was used to calculate the disruption efficiency. (B) Phenotypes of mutants generated with donor oligos.

A

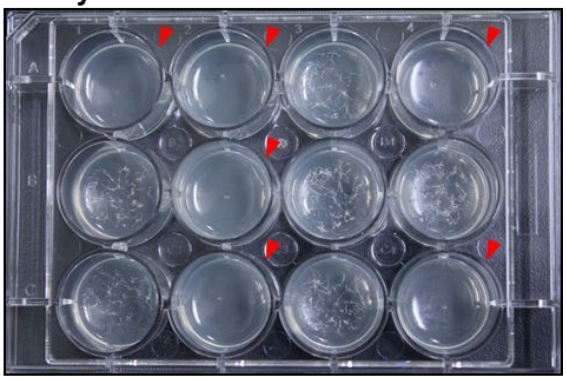
4 days



n	Total clones	Development-defective clones	Knockout efficiency (%)
1	36	0	0.0
2	36	5	13.9
3	36	4	11.1
		Average	8.3

B

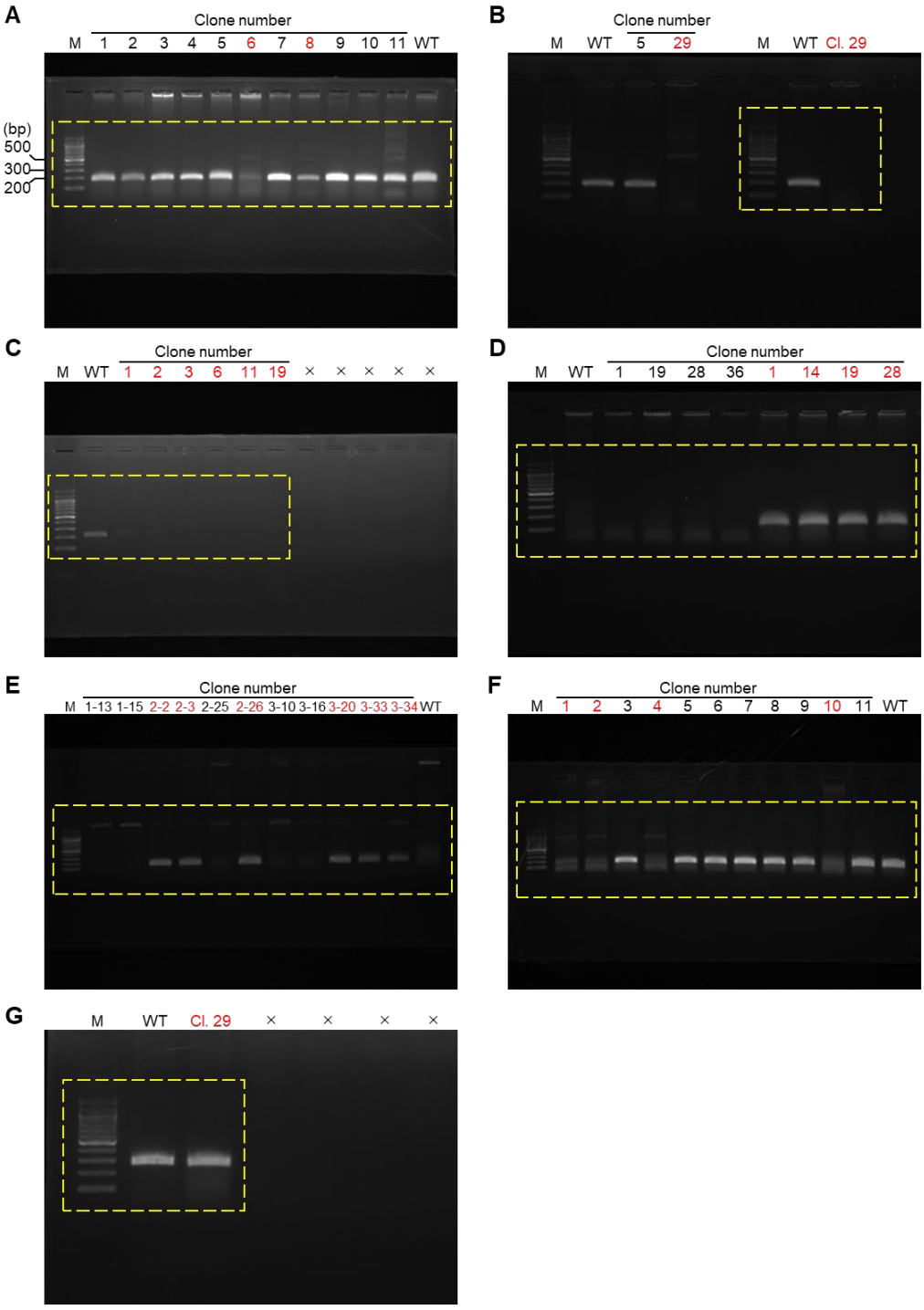
6 days



n	Total clones	Development-defective clones	Knockout efficiency (%)
1	36	2	5.6
2	36	9	25.0
3	36	11	30.6
		Average	20.4

Supplementary Figure 6. Donor DNA-mediated *pkaC* disruption in *H. pallidum* under different G418 selection periods.

(A) 4 days of drug selection. Candidate mutants are indicated by red arrowheads. (B) 6 days of drug selection.



Supplementary Figure 7. Raw gel electrophoresis images corresponding to the Figures in the paper.

(A) The original gel for Fig. 1D. (B) The original gel for Fig. 2C and Fig. S3. (C) The original gel for Fig. 3C. (D) The original gel for Fig. 4C. (E) The original gel for Fig. 5C. (F) The original gel for Fig. S1C. (G) The original gel for Fig. S3.

Supplementary Table S1. Summary of gene knockout conditions and efficiencies.

Gene	Species	Vector	Donor	Selection	Agg. defective	Figure
pkaC	*D. discoideum*	Transient	–	1 day	55/108	4
pkaC	*D. discoideum*	Transient	+	1 day	91/108	4
pkaC	*D. discoideum*	Transient	–	–	12/108	S5
pkaC	*D. discoideum*	Transient	+	–	31/108	S5
stlA	*P. violaceum*	Transient	–	1 day	8/72	S1
stlA	*P. violaceum*	Extrachromosomal	–	4 days	21/108	1
pkaC	*P. violaceum*	Extrachromosomal	–	4 days	30/72	S2
pkaC	*H. pallidum*	Extrachromosomal	–	4 days	1/108	2
pkaC	*H. pallidum*	Extrachromosomal	+	4 days	9/108	5
pkaC	*H. pallidum*	Extrachromosomal	+	6 days	22/108	S6
pkaC	*C. fasciculatum*	Extrachromosomal	–	4 days	21/72	3

Supplementary Table S2. Primer sequences used for cloning and sequencing.

Gene	Description	Sequence
pkaC (D. discoideum)	gRNA sense	AGCATTGCTGAGGTCACAGGACTA
pkaC (D. discoideum)	gRNA antisense	AAACTAGTCCTGTGACCTCAGCAA
pkaC (D. discoideum)	Screening-Rv	CTGGCCAAGATATGAAATAGAGG
stlA (*P. violaceum*)	gRNA sense	AGCATTTGTACCGGTGGAAGAGTA
stlA (*P. violaceum*)	gRNA antisense	AAACTACTCTTCCACCGGTACAAA
stlA (*P. violaceum*)	Screening-Fw	TAACTTGTGCTTGGGAGGCT
stlA (*P. violaceum*)	Sequencing-Rv	CAAGCGGTATCAATGGCCAA
pkaC (*P. violaceum*)	gRNA sense	AGCATGCTATGAAGTGTTTAAACA
pkaC (*P. violaceum*)	gRNA antisense	AAACTACTCTTCCACCGGTACAAA
pkaC (*P. violaceum*)	Screening-Rv	AACACCTCTCCACCAGCTAC
pkaC (*P. violaceum*)	Sequencing-Fw	TCCCAACTCCATCCAATGCT
pkaC (*H. pallidum*)	gRNA sense	AGCAGGATCCGGCAAGAATGTACG
pkaC (*H. pallidum*)	gRNA antisense	AAACCGTACATTCTTGCCGGATCC
pkaC (*H. pallidum*)	Screening-Fw	ATCTGCCTCCACTCAACACA
pkaC (*H. pallidum*)	Sequencing-Rv	CGTTCCGGTTCCAATGACTC
pkaC (*H. pallidum*)	Screening-Fw	GTCGTCTGCGTCACCACGTA
pkaC (*H. pallidum*)	Screening-Rv	CTTCATTGCGTAGAATTGATGG
pkaC (*H. pallidum*)	Sequencing-Fw	ATCTGCCTCCACTCAACACA
pkaC (*C. fasciculata*)	gRNA sense	AGCAATGTGTGCATCGTCTTAAAG
pkaC (*C. fasciculata*)	gRNA antisense	AAACCTTTAAGACGATGCACACAT
pkaC (*C. fasciculata*)	Screening-Fw	CCGATGGTCTTATACACCCCTTTA
pkaC (*C. fasciculata*)	Screening-Rv	ATAGGACATGGGTGGCAGAG
pkaC (*C. fasciculata*)	Sequencing-Fw	CATCAACATCATACAGGCATTTC
pkaC (*C. fasciculata*)	Sequencing-Rv	GAATTTAAATGTTCTACCTGCTTTAGT
pkaC (*C. fasciculata*)	Screening-Fw	GTGCCAGATTACGCCTCTCT
pkaC (*C. fasciculata*)	Screening-Rv	CTGGCCAAGATATGAAATAGAGG
HA tag	Screening-Fw	GTGCCAGATTACGCCTCTCT

Supplementary Table S3. CRISPR/Cas9 vectors used in this study.

Plasmid	Target gene	Backbone	Transient / Extrachromosomal	References
pTM1225	*pkaC (D.discoideum)*	pTM1285	Transient	(Sekine et al., 2018)
pTM2743	*stlA (P. violaceum)*	pTM1599	Transient	This study
pTM2541	*stlA (P. violaceum)*	pTM1660	Extrachromosomal	This study
pTM2782	*pkaC (H. pallidum)*	pTM1660	Extrachromosomal	This study
pTM2795	*pkaC (C. fasciculata)*	pTM1660	Extrachromosomal	This study

Supplementary Table S4. Sequences for donor oligonucleotides.

Target gene	Sequence
pkaC (D. discoideum)	TAGTAGTGGAGATAATAAAAACCATAGTTACCCCTACGACGTGCCAGATTACGCCTCTC
	TGTAATCCTGTGACCTCAGCAACAGACCGTTTAA
pkaC (H. pallidum)	CCATCGATGTCGTCTGCGTCACCACGTATACCCCTACGACGTGCCAGATTACGCCTCT
	CTGTAATCATTCTTGCCGGATCCGCTCCTCACTCA