Supporting Information for

CRISPR-Cas9 based engineering of actinomycetal genomes

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SUPPLEMENTAL TABLES (Table S1-Table S5)

Table S1 Strains and plasmids used in this study.

Name	Description	Reference
WT	*Streptomyces coelicolor* A3(2)	95 SNPs and 1 deletion of[1]
No Target	WT with pCRISPR-Cas9	This study
Mismatch	WT with sgRNA: ActIorf1-1 NT including its PAM sequence	This study
Δ*actIorf1*-1	WT with pCRISPR-Cas9 carrying sgRNA: ActIorf1-1 NT, 1 bp insertions from the DSB site	This study
Δ*actIorf1*-2	WT with pCRISPR-Cas9 carrying sgRNA: ActIorf1-6 T, 10721 bp deletion around the DSB site	This study
Δ*actvb*-1	WT with pCRISPR-Cas9 carrying sgRNA: Actvb-2 NT, 14716 bp deletion around the DSB site	This study
Δ*actvb*-2	WT with pCRISPR-Cas9 carrying sgRNA: Actvb-5 NT, 37173 bp deletion around the DSB site	This study
Δ*actIorf1*-ligD1-Δ*actIorf1*-ligD8	WT with pCRISPR-Cas9-ScaligD carries sgRNA: ActIorf1-6 T, 8 random red clones	This study
Δ*actvb*-ligD1-Δ*actvb*-ligD8	WT with pCRISPR-Cas9-ScaligD carries sgRNA: Actvb-2 NT, 8 random red clones	This study
orf1 deletion1-orf1 deletion10	WT with *actIORF1* recombination arm in the pCRISPR-Cas9 carrying sgRNA: ActIorf1-6 T, *actIORF1* gene was deleted, 10 random clones	This study
vb deletion1-vb deletion10	WT with *actVB* recombination arm in the pCRISPR-Cas9 carrying sgRNA: Actvb-2 NT, *actVB* gene was deleted, 10 random clones	This study
orf1 knockdown-1	WT with pCRISPR-dCas9 carrying sgRNA: orf1p-S1 T	This study
orf1 knockdown-2	WT with pCRISPR-dCas9 carrying sgRNA: orf1p-S3 T	This study
orf1 knockdown-3	WT with pCRISPR-dCas9 carrying sgRNA: orf1p-S5 T	This study
orf1 knockdown-4	WT with pCRISPR-dCas9 carrying sgRNA: orf1p-A1 NT	This study
orf1 knockdown-5	WT with pCRISPR-dCas9 carrying sgRNA: orf1p-A4 NT	This study
orf1 knockdown-6	WT with pCRISPR-dCas9 carrying sgRNA: orf1p-A5 NT	This study
orf1 knockdown-7	WT with pCRISPR-dCas9 carrying sgRNA: ActIorf1-2T	This study
orf1 knockdown-8	WT with pCRISPR-dCas9 carrying sgRNA: ActIorf1-3T	This study
orf1 knockdown-9	WT with pCRISPR-dCas9 carrying sgRNA: ActIorf1-4T	This study

orf1 knockdown-10	WT with pCRISPR-dCas9 carrying sgRNA: ActIorf1-1NT	This study
orf1 knockdown-11	WT with pCRISPR-dCas9 carrying sgRNA: ActIorf1-7NT	This study
orf1 knockdown-12	WT with pCRISPR-dCas9 carrying sgRNA: ActIorf1-8NT	This study
ET12567/pUZ8002	*Escherichia coli* for conjugation, *dam-13::Tn9 dcm-6 hsdM* Cml^R, carrying helper plasmid pUZ8002	[2]
Mach1™-$T1^R$	*Escherichia coli* for routine cloning, *lacZΔM15 hsdR lacX74 recA endA tonA*	Life Technologies
pGM1190	temperature sensitive plasmid, tsr, aac(3)IV, oriT, *to* terminator, PtipA, RBS, *fd* terminator	[3]
pGM1190-sgRNA	pGM1190 with sgRNA scaffold	This study
pCRISPR-Cas9	pGM1190-sgRNA with *cas9*	This study
pCRISPR-dCas9	pGM1190-sgRNA with d*CAS9* (D10A and H840A)	This study
pCRISPR-Cas9-ScaligD	pCRISPR-Cas9 with a ScaligD expression cassette	This study
pCRISPR-Cas9-orf1-1	pCRISPR-Cas9 carries sgRNA: ActIorf1-1 NT	This study
pCRISPR-Cas9-orf1-2	pCRISPR-Cas9 carries sgRNA: ActIorf1-2 T	This study
pCRISPR-Cas9-orf1-3	pCRISPR-Cas9 carries sgRNA: ActIorf1-3 T	This study
pCRISPR-Cas9-orf1-4	pCRISPR-Cas9 carries sgRNA: ActIorf1-4 T	This study
pCRISPR-Cas9-orf1-5	pCRISPR-Cas9 carries sgRNA: ActIorf1-5 T	This study
pCRISPR-Cas9-orf1-6	pCRISPR-Cas9 carries sgRNA: ActIorf1-6 T	This study
pCRISPR-Cas9-vb1	pCRISPR-Cas9 carries sgRNA: Actvb-1 NT	This study
pCRISPR-Cas9-vb2	pCRISPR-Cas9 carries sgRNA: Actvb-2 NT	This study
pCRISPR-Cas9-vb3	pCRISPR-Cas9 carries sgRNA: Actvb-3 T	This study
pCRISPR-Cas9-vb4	pCRISPR-Cas9 carries sgRNA: Actvb-4 T	This study
pCRISPR-Cas9-vb5	pCRISPR-Cas9 carries sgRNA: Actvb-5 NT	This study
pCRISPR-Cas9-vb6	pCRISPR-Cas9 carries sgRNA: Actvb-6 NT	This study
pCRISPR-Cas9-orf1-6-Tem	pCRISPR-Cas9-orf1-6 with *actIORF1* homologous recombination template	This study
pCRISPR-Cas9-vb2-Tem	pCRISPR-Cas9-vb2 with *actVB* homologous recombination template	This study
pCRISPR-Cas9-ScaligD-orf1-6T	pCRISPR-Cas9-ScaligD carries sgRNA: ActIorf1-6 T	This study
pCRISPR-Cas9-ScaligD-vb2	pCRISPR-Cas9-ScaligD carries sgRNA: Actvb-2 NT	This study
pCRISPR-dCas9-1	pCRISPR-dCas9 carrying sgRNA: orf1p-S1 T	This study
pCRISPR-dCas9-2	pCRISPR-dCas9 carrying sgRNA: orf1p-S3 T	This study
pCRISPR-dCas9-3	pCRISPR-dCas9 carrying sgRNA: orf1p-S5 T	This study
pCRISPR-dCas9-4	pCRISPR-dCas9 carrying sgRNA: orf1p-A1 NT	This study

pCRISPR-dCas9-5	pCRISPR-dCas9 carrying sgRNA: orf1p-A4 NT	This study
pCRISPR-dCas9-6	pCRISPR-dCas9 carrying sgRNA: orf1p-A5 NT	This study
pCRISPR-dCas9-7	pCRISPR-dCas9 carrying sgRNA: ActIorf1-1NT	This study
pCRISPR-dCas9-8	pCRISPR-dCas9 carrying sgRNA: ActIorf1-2T	This study
pCRISPR-dCas9-9	pCRISPR-dCas9 carrying sgRNA: ActIorf1-3T	This study
pCRISPR-dCas9-10	pCRISPR-dCas9 carrying sgRNA: ActIorf1-4T	This study
pCRISPR-dCas9-11	pCRISPR-dCas9 carrying sgRNA: ActIorf1-7NT	This study
pCRISPR-dCas9-12	pCRISPR-dCas9 carrying sgRNA: ActIorf1-8NT	This study

Table S2 Primers used in this study.

Sets	Primer name	Sequence (5'-3')#, *, §	Purpose
1	ActIorf1-F1	CATGCCATGG**GTGGCTCGAAGGAGGCTCGA**GTTTTAGAGCTAGAAATAGC	sgRNAs amplification
2	ActIorf1-F2	CATGCCATGG**AGCTCGATCAAGTCGATGGT**GTTTTAGAGCTAGAAATAGC	
3	ActIorf1-F3	CATGCCATGG**GAAGCGCAGAGTCGTCATCA**GTTTTAGAGCTAGAAATAGC	
4	ActIorf1-F4	CATGCCATGG**CCCCTCGCCCTACCGTTCAC**GTTTTAGAGCTAGAAATAGC	
5	ActIorf1-F5	CATGCCATGG**GCGCGAGTATCTGCTGCTGT**GTTTTAGAGCTAGAAATAGC	
6	ActIorf1-F6	CATGCCATGG**CTGCAACGCGTACCACATGA**GTTTTAGAGCTAGAAATAGC	
7	ActIorf1-F7	CATGCCATGG**TGAGCAGTTCCCAGAACTGC**GTTTTAGAGCTAGAAATAGC	
8	ActIorf1-F8	CATGCCATGG**AGGAGGCTCGAAGGCCGATA**GTTTTAGAGCTAGAAATAGC	
9	ActVB-F1	CATGCCATGG**TCGCCGCAACTGTCGAACAC**GTTTTAGAGCTAGAAATAGC	
10	ActVB-F2	CATGCCATGG**CTGCCATCTTCGAACTCCCT**GTTTTAGAGCTAGAAATAGC	
11	ActVB-F3	CATGCCATGG**TTCCCGGTGTTCGACAGTTG**GTTTTAGAGCTAGAAATAGC	
12	ActVB-F4	CATGCCATGG**ACTGGTCTGCCTGGCTCGTA**GTTTTAGAGCTAGAAATAGC	
13	ActVB-F5	CATGCCATGG**ATCTTCGAACTCCCTAGGCG**GTTTTAGAGCTAGAAATAGC	
14	ActVB-F6	CATGCCATGG**GTCCCGGAGCATTCCCTGGT**GTTTTAGAGCTAGAAATAGC	
15	orf1p-S1 T-F	CATGCCATGG**GTGTTCCCCTCCCTGCCTCG**GTTTTAGAGCTAGAAATAGC	
16	orf1p-S3 T-F	CATGCCATGG**TCCCTCACGCGCTCAGCTTT**GTTTTAGAGCTAGAAATAGC	
17	orf1p-S5 T-F	CATGCCATGG**CTTTGGGCGCCCGGCTCGAG**GTTTTAGAGCTAGAAATAGC	
18	orf1p-A1 NT-F	CATGCCATGG**CCTTCGACCGCCGCTCGAGC**GTTTTAGAGCTAGAAATAGC	
19	orf1p-A4 NT-F	CATGCCATGG**GCCCAAAGCTGAGCGCGTGA**GTTTTAGAGCTAGAAATAGC	
20	orf1p-A5 NT-F	CATGCCATGG**TGAGCGCGTGAGGGACCACG**GTTTTAGAGCTAGAAATAGC	
21	sgRNA-R	ACGCCTACGTAAAAAAAGCACCGACTCGGTGCC	
22	gRNA check-F	ACATGTGCGGTCGATCTT	sgRNAs sequencing
23	gRNA check-R	TACGTAAAAAAAGCACCGAC	
24	orf1-5‘F	TCGTCGAAGGCACTAGAAGGCATCCGCTGAACGAGACCC	*actIORF1*

25	orf1-5‘R	GCTCACGTCGAAGCGGGTGACCACGCAGGACTCCGAAGTC	homologous recombination template construction
26	orf1-3‘F	TCACCCGCTTCGACGTGAG	
27	orf1-3‘R	GGTCGATCCCCGCATATAGGTTCGCCGAGCACCAGGTC	
28	VB-5‘F	TCGTCGAAGGCACTAGAAGGCGACTCGCTCGCCCTGATG	*actVB* homologous recombination template construction
29	VB-5‘R	CACCAACCTGCTCGGGCTGCGCCGTGGAAGTGGGTGTTGAC	
30	VB-3‘F	GCAGCCCGAGCAGGTTGG	
31	VB-3‘R	GGTCGATCCCCGCATATAGGTCCGTTGCGGCGTCCATC	
32	VB-check-F	CGGCTGGTGCGTCAGCAAC	Check *actVB* deletion
33	VB-check-R	ACGTGGCGGGTCGAACGG	
34	ORF1-check-F	CCGCCTTGAGGACCTGTTTG	Check *actIORF1* deletion
35	ORF1-check-R	ACACGCTGACCGACTTGGG	
36	CAS9-check-F	TCCACGAGCACATCGCCAAC	Check *cas9* sub-cloning
37	CAS9-check-R	GACCTTGTAGTCGCCGTAGACG	
36	ScaligD-F	TCGTCGAAGGCACTAGAAGGGCGGTCGATCTTGACGGCTG	ScaligD expression cassette amplification
37	ScaligD-R	GGTCGATCCCCGCATATAGGTGCCGCCGGGCGTTTTTTAT	
38	orf1-6 ligD test-F	CCGCCGACACCCCGATCACC	Check NHEJ for *actIORF1* editing
39	orf1-6 ligD test-R	ACCGCAGCTTCCGCTCCCTG	
40	vb2 ligD test-F	CGAGGTGATCGACGCCAACC	Check NHEJ for *actVB* editing
41	vb2 ligD test-R	TCGCCGAGCAGGATGATGTG	

#: The 20 nt target sequences are shown in bold. The pattern of the sgRNA-F primer is: CATGCCATGG$\mathbf{N_{20}}$GTTTTAGAGCTAGAAATAGC.

*: The overlap sequence for Gibson assembly is shown in red. §: The restriction sites are underlined.

Table S3 sgRNAs used in this study.

sgRNA	The target Sequences	PAM	Purpose
ActIorf1-1 NT	GTGGCTCGAAGGAGGCTCGA	AGG	Gene deletion/expression control
ActIorf1-2 T	AGCTCGATCAAGTCGATGGT	CGG	Gene deletion/expression control
ActIorf1-3 T	GAAGCGCAGAGTCGTCATCA	CGG	Gene deletion/expression control
ActIorf1-4 T	CCCCTCGCCCTACCGTTCAC	AGG	Gene deletion/expression control
ActIorf1-5 T	GCGCGAGTATCTGCTGCTGT	CGG	Gene deletion
ActIorf1-6 T	CTGCAACGCGTACCACATGA	CGG	Gene deletion
ActIorf1-7 NT	TGAGCAGTTCCCAGAACTGC	CGG	Gene expression control
ActIorf1-8 NT	AGGAGGCTCGAAGGCCGATA	CGG	Gene expression control
Actvb-1 NT	TCGCCGCAACTGTCGAACAC	CGG	Gene deletion
Actvb-2 NT	CTGCCATCTTCGAACTCCCT	AGG	Gene deletion
Actvb-3 T	TTCCCGGTGTTCGACAGTTG	CGG	Gene deletion
Actvb-4 T	ACTGGTCTGCCTGGCTCGTA	CGG	Gene deletion
Actvb-5 NT	ATCTTCGAACTCCCTAGGCG	AGG	Gene deletion
Actvb-6 NT	GTCCCGGAGCATTCCCTGGT	CGG	Gene deletion
orf1p-S1 T	GTGTTCCCCTCCCTGCCTCG	TGG	Gene expression control
orf1p-S3 T	TCCCTCACGCGCTCAGCTTT	GGG	Gene expression control
orf1p-S5 T	CTTTGGGCGCCCGGCTCGAG	CGG	Gene expression control
orf1p-A1 NT	CCTTCGACCGCCGCTCGAGC	CGG	Gene expression control
orf1p-A4 NT	GCCCAAAGCTGAGCGCGTGA	AGG	Gene expression control
orf1p-A5 NT	TGAGCGCGTGAGGGACCACG	AGG	Gene expression control

Table S4 List of SNPs in the WT used in this study as compared to the *S. coelicolor* A3(2) reference sequence (Genbank: AL645882)

Position	Gap Index	Base	Reference
62640	0	C	T
104233	0	G	A
549823	0	-	C
603360	0	G	A
619172	0	G	C
619978	0	G	C
646916	0	C	T
657081	0	G	T
921797	0	A	C
1415134	0	G	C
1523375	0	G	T
1523376	0	T	G
1616265	0	G	-
1616552	0	G	T
1622503	0	T	A
1625888	0	A	G
1634118	0	G	C
1634749	0	A	G
1642021	0	A	C
1644238	0	C	A
1644332	0	G	C
1644333	0	C	G
1649111	0	C	G
1649434	0	-	G
1649515	0	-	G
1740776	0	C	T
1765204	0	G	-
2246578	0	G	C
2325801	0	G	C
2326305	0	C	T
2326306	0	T	C
2326307	0	C	T
2458984	0	G	-
2501991	0	C	G
2533425	0	G	A
2852282	0	G	T
2855965	0	C	-
2976040	0	G	T
3095242	0	G	-
3144977	0	-	C
3230998	0	C	A
3230999	0	C	A
3231000	0	C	A
3348737	0	A	G
3348967	0	G	C

3349264	0	C	G
3396367	0	G	T
3460645	0	-	C
3667835	0	C	G
3680393	0	C	A
3958102	0	C	T
4256852	0	C	A
4341687	0	T	G
4397330	0	G	C
4397368	0	-	G
4401621	0	-	C
4726818	0	G	C
4912784	0	G	-
5016898	0	G	C
5044225	0	G	C
5095649	0	C	A
5107398	0	G	C
5479807	0	G	-
5505703	0	T	G
5633841	0	G	C
5644122	0	C	-
5753680	0	-	C
5762044	0	G	-
5818709	0	G	A
5818718	0	A	G
5818720	0	A	T
6008595	0	G	T
6281831	0	G	T
6370280	0	G	-
7044234	0	G	A
7084800	0	A	C
7118314	0	C	G
7146838	0	G	A
7180216	0	C	G
7183704	0	C	G
7203532	0	C	G
7261050	0	C	G
7284887	0	G	T
7364061	0	C	G
7384786	0	T	-
7393003	0	G	T
7552569	0	-	C
7859556	0	A	C
8004829	0	G	A
8044092	0	G	C
8288082	0	G	-
8479534	0	C	T
8594044	0	C	T
8595557	0	G	C
8612266	0	C	A

Table S5 List of mutations detected from whole genome sequencing (the results shown are after subtracted from the WT)*, §.

Name	Position	Mutation	Annotation	Gene	Description
Mismatch	2,474,084	A→C	T8P (ACC→CCC)	SCO2305→	putative ABC transporter ATP-binding subunit
	4,477,934	2 bp→TC	coding (195-196/609 nt)	SCO4084→	hypothetical protein SCD25.20
	8,265,166	G→C	intergenic (+76/-125)	SCO7449→/→SCO7450	putative membrane protein. /putative secreted protein
	8,267,257	G→C	intergenic (+13/+26)	SCO7451→/←SCO7452	conserved hypothetical protein SC5C11.08/putative O-methyltransferase.
No Target	1,645,577	+G	intergenic (-554/+422)	SCO1536←/←SCO1537	conserved hypothetical protein SCL2.26c/putative transport system membrane protein
	1,645,634	A→G	intergenic (-611/+365)	SCO1536←/←SCO1537	conserved hypothetical protein SCL2.26c/putative transport system membrane protein
	2,462,898	(G)12→13	intergenic (-386/+324)	SCO2292←/←SCO2293	secreted endo-1,4-beta-xylanase B (xylanase B)/putative integral membrane protein
	5,093,984	G→C	P550A (CCC→GCC)	SCO4664←	putative integral membrane protein
	6,442,710	(G)9→10	intergenic (-96/+43)	SCO5885←/←SCO5886	putative membrane protein/3-oxoacyl-[acyl-carrier-protein] synthase II
	8,163,408	T→C	T129T (ACA→ACG)	SCO7350←	putative membrane efflux protein.
	2,311,509	(TGA)4→5	coding (176/1638 nt)	SCO2148←	cytochrome B subunit
Δ*actIorf1*-1	2,440,703	A→G	L173P (CTC→CCC)	SCO2271←	hypothetical protein SCC75A.17c.
	7,846,245	A→G	S10P (TCC→CCC)	SCO7056←	putative gntR-family transcriptional regulator
	7,846,250	T→G	D8A (GAC→GCC)	SCO7056←	putative gntR-family transcriptional regulator
	5,529,858	.→A	coding (58/1404 nt)	SCO5087←	actinorhodin polyketide beta-ketoacyl synthase alpha subunit

Δ*actIorf1*-2	2,462,898	(G)12→11	intergenic (-386/+324)	SCO2292←/←SCO2293	secreted endo-1,4-beta-xylanase B (xylanase B)/putative integral membrane protein
	7,846,245	A→G	S10P (TCC→CCC)	SCO7056←	putative gntR-family transcriptional regulator
	8,267,257	G→C	intergenic (+13/+26)	SCO7451→/←SCO7452	conserved hypothetical protein SC5C11.08/putative O-methyltransferase.
	5,527,269	Δ10,721		[SCO5084]–[SCO5096]	11 genes lost, SCO5087 included
Δ*actvb*-1	5,818,673	Δ1 bp	intergenic (+125/-)	SCO5350→/–	hypothetical protein SCBAC5H2.19/–
	7,186,210	Δ9 bp	coding (1379-1387/1998 nt)	SCO6492→	hypothetical protein
	5,532,664	Δ14,716 bp		[SCO5089]–[SCO5105]	17 genes lost, SCO5092 included
Δ*actvb*-2	2,440,703	A→G	L173P (CTC→CCC)	SCO2271←	hypothetical protein SCC75A.17c.
	3,180,456	A→C	intergenic (+74/+48)	SCO2928→/←SCO2929	putative asnC-family transcriptional regulator/putative transposase
	5,513,345	Δ37,173 bp		[SCO5070]–[SCO5107]	38 genes lost, SCO5092 included

*: Strains in this table correspond to the strains and data in Figure 3 of the main text.

§: In the last four strains of this table, the sgRNA targeted two different genes in the actinorhodin cluster for deletion. The result was a distribution of random sized deletions, these four mutations are shown in red.

SUPPORTING FIGURES

Figure S1-S2

Figure S1. No actinorhodin production was observed when Cas9 and the target sgRNAs were co-expressed. (A) The clones were transferred from the induced ISP2 agar plate to a new ISP2 agar plate without antibiotics, and the photo was taken after 7 days incubation at 30 °C; (B) The same sets of clones shown in (A) were inoculated in ISP2 liquid medium without antibiotics. After 7 days shaking at 30 °C, the cultures were centrifuged at 8000 g for 5 minutes at room temperature, the supernatants were transferred into new tubes, and the photo was taken. The numbers are correspond to the labels of (A).

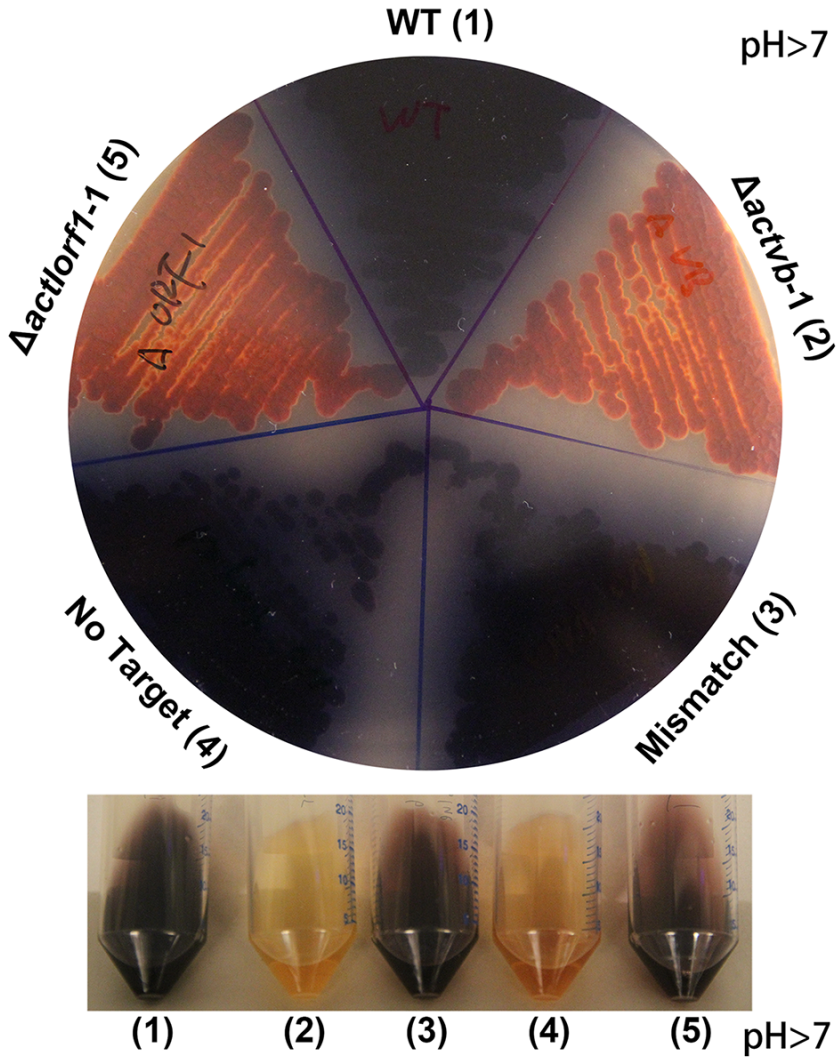
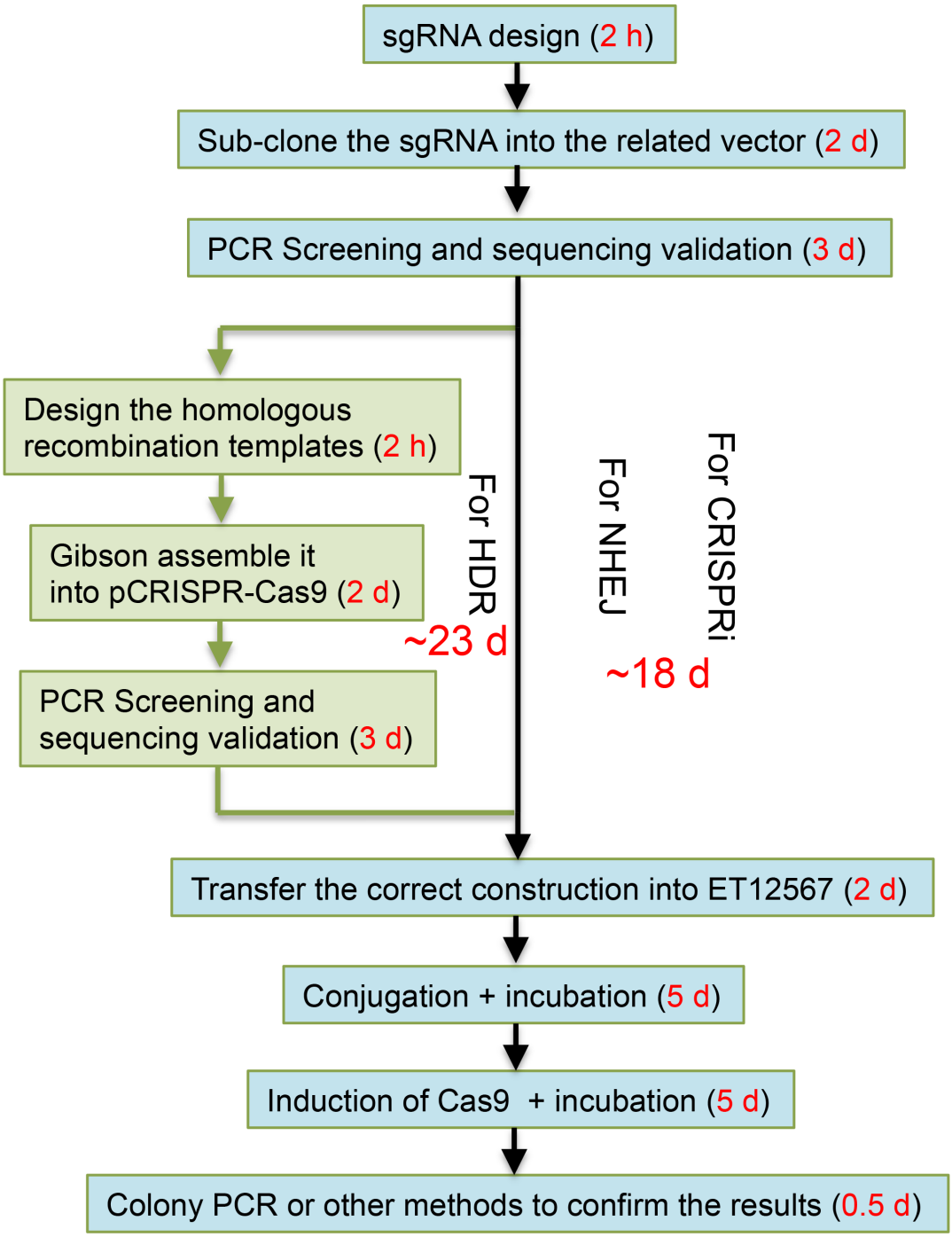


Figure S2. A workflow for the CRISPR-Cas9 system.



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