

# Precise genome engineering in *Pseudomonas* using phage-encoded homologous recombination and the Cascade–Cas3 system

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**Supplementary Table 1** Antibiotic concentrations used in four *Pseudomonas* strains.

Strain	Antibiotic ( $\mu\text{g mL}^{-1}$ ) [km]	Kanamycin [genta]	Gentamycin [apra]	Apramycin [tet]	Tetracycline [amp]	Ampicillin [cm]	Chloramphenicol [cm]
<i>P. aeruginosa</i> PAO1	300	15	60	50	ND <sup>b</sup>	90	
<i>P. protegens</i> pf5	10	15	20	35	ND	ND	
<i>P. putida</i> KT2440	15	10	40	-- <sup>a</sup>	300	ND	
<i>P. syringae</i> pv. tomato str. DC3000	15	3	20	3	200	ND	

--<sup>a</sup> Did not test this antibiotic

ND<sup>b</sup> Can not use this antibiotic

**Supplementary Table 2** Recombineering systems used in this protocol.

Recombineering systems	Recombinases	Vector	Antibiotic resistance gene	Promoter for guide RNA and Cascade-Cas3 system	Promoter for recombinases
pHERD30T-BAD-red $\gamma$ $\beta$ $\alpha$ -kan	Red $\alpha$ , Red $\beta$ and Red $\gamma$				
pHERD30T-BAD-plu $\gamma$ $\beta$ $\alpha$ -kan	Plu $\alpha$ , Plu $\beta$ and Plu $\gamma$				
pHERD30T-BAD-TE <sub>Psy</sub> -kan	RecT <sub>Psy</sub> and RecE <sub>Psy</sub>				
pHERD30T-BAD-plu $\gamma$ TE <sub>Psy</sub> -kan	RecT <sub>Psy</sub> , RecE <sub>Psy</sub> and Plu $\gamma$	pHERD30T	kanamycin resistance gene	<i>araC</i> P <sub>BAD</sub> promoter	<i>rhaR-rhaS</i> P <sub>Rha</sub> promoter
pHERD30T-BAD-red $\gamma$ TE <sub>Psy</sub> -kan	RecT <sub>Psy</sub> , RecE <sub>Psy</sub> , and Red $\gamma$				
pHERD30T-BAD-BAS-kan	Alpha, Beta and SSB from phage_AB31				
pHERD30T-BAD-plu $\gamma$ BAS-kan	Alpha, Beta, SSB from phage_AB31 and Plu $\gamma$				
pHERD30T-BAD-red $\gamma$ BAS-kan	Alpha, Beta, SSB from phage_AB31 and Red $\gamma$				

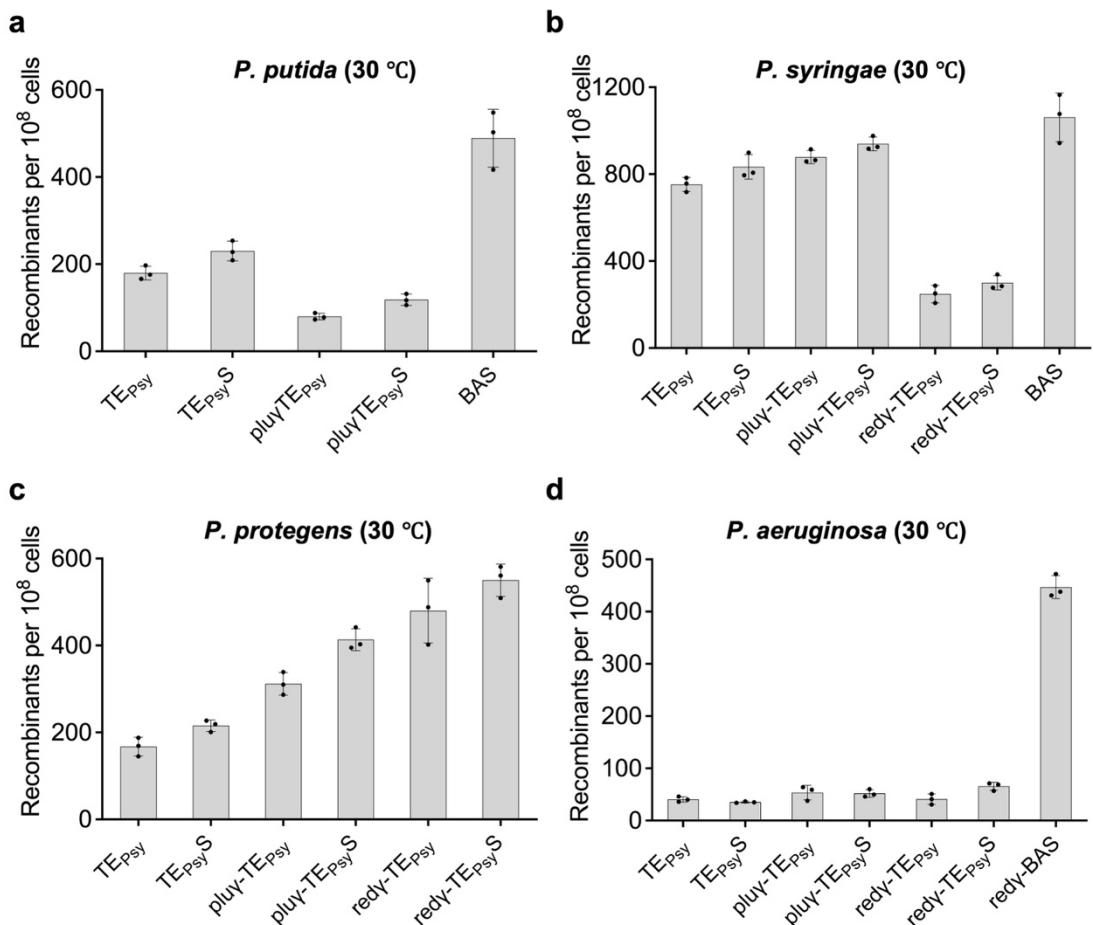
**Supplementary Table 3** *Pseudomonas* strains used in this protocol.

Strains	Description	Optimum growth temperature (°C)	Source
<i>P. putida</i> KT2440	A metabolically versatile saprophytic soil bacterium that has been certified as a biosafety host for the cloning of foreign genes	30	<sup>1</sup>
<i>P. syringae</i> DC3000	An agriculturally important plant pathogen could cause bacterial speck of tomato	30	<sup>2</sup>
<i>P. protegens</i> pf5	A plant commensal bacterium that inhabits the rhizosphere and produces secondary metabolites that suppress soilborne plant pathogens	30	<sup>3</sup>
<i>P. aeruginosa</i> PAO1	A ubiquitous environmental bacterium that is one of the top three causes of opportunistic human infections. A major factor in its prominence as a pathogen is its intrinsic resistance to antibiotics and disinfectants.	37	<sup>4</sup>

**Supplementary Table 4** Plasmids used in this protocol.

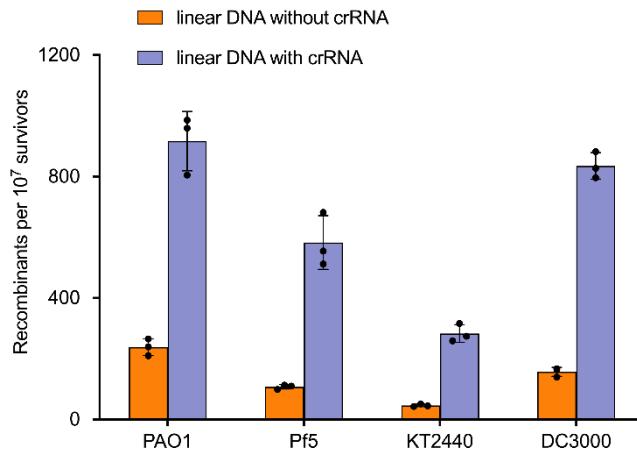
<b>Plasmids</b>	<b>Addgene number</b>	<b>Characteristics</b>	<b>Step number</b>	<b>Sequence</b>	<b>references</b>
pHERD30T-BAD-GFP-Rha-Cas-kan	201836	<i>gfp</i> under BAD promoter, <i>cas</i> under Rha promoter	Step 39	Supplementary note 1	<sup>5</sup>
pHERD30T-BAD-redy $\beta$ $\alpha$ -Rha-Cas-kan (Redy $\beta$ $\alpha$ -Cas3)	194866	<i>redy<math>\beta</math><math>\alpha</math></i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 2	<sup>5</sup>
pHERD30T-BAD-pluy $\beta$ $\alpha$ -Rha-Cas-kan (Pluy $\beta$ $\alpha$ -Cas3)	194867	<i>pluy<math>\beta</math><math>\alpha</math></i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 3	<sup>5</sup>
pBBR1-Rha-TE <sub>Psy</sub> -kan	201841	<i>recTE<sub>Psy</sub></i> under Rha promoter		Supplementary note 4	<sup>5</sup>
pBBR1-Rha-TE <sub>PsyS</sub> -kan	201842	<i>recTE<sub>PsyS</sub></i> under Rha promoter		Supplementary note 5	This study
pBBR1-Rha-pluyTE <sub>Psy</sub> -kan	201837	<i>recTE<sub>Psy</sub></i> and <i>pluy</i> under Rha promoter		Supplementary note 6	<sup>5</sup>
pBBR1-Rha-pluyTE <sub>PsyS</sub> -kan	201838	<i>recTE<sub>PsyS</sub></i> and <i>pluy</i> under Rha promoter		Supplementary note 7	This study
pBBR1-Rha-redyTE <sub>Psy</sub> -kan	201839	<i>recTE<sub>Psy</sub></i> and <i>redy</i> under Rha promoter		Supplementary note 8	<sup>5</sup>
pBBR1-Rha-redyTE <sub>PsyS</sub> -kan	201840	<i>recTE<sub>PsyS</sub></i> and <i>redy</i> under Rha promoter		Supplementary note 9	This study
pHERD30T-BAD-TE <sub>Psy</sub> -Rha-Cas-kan (TE <sub>Psy</sub> -Cas3)	194868	<i>recTE<sub>Psy</sub></i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 10	<sup>5</sup>
pHERD30T-BAD-pluyTE <sub>Psy</sub> -Rha-Cas-kan (PluyTE <sub>Psy</sub> -Cas3)	194869	<i>recTE<sub>Psy</sub></i> and <i>pluy</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 11	<sup>5</sup>
pHERD30T-BAD-redyTE <sub>Psy</sub> -Rha-Cas-kan (RedyTE <sub>Psy</sub> -Cas3)	194870	<i>recTE<sub>Psy</sub></i> and <i>redy</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 12	<sup>5</sup>
pHERD30T-BAD-BAS-Rha-Cas-kan (BAS-Cas3)	194871	BAS gene under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 13	<sup>5</sup>
pHERD30T-BAD-pluyBAS-Rha-Cas-kan (PluyBAS-Cas3)	194872	BAS gene and <i>pluy</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 14	<sup>5</sup>
pHERD30T-BAD-redyBAS-Rha-Cas-kan (RedyBAS-Cas3)	194873	BAS gene and <i>redy</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 15	<sup>5</sup>
pR6K-Tps-genta-tet-T7RP	201843	PCR template to amplify lox71-genta <sup>R</sup> -lox66 or genta <sup>R</sup> gene	Steps 27B(i), 41	Supplementary note 16	<sup>5</sup>

pR6K-SacB-genta	201844	PCR template to amplify the SacB-genta <sup>R</sup> cassette	Step 27A(i)	Supplementary note 17	This study
pRK2-BAD-Cre-SacB-apra	201845	Express the site-specific recombinase (Cre) and add sucrose to quench the plasmid under the SacB counter selection pressure	Step 27B(iv)	Supplementary note 18	<sup>5</sup>
pHERD30T-SacB-genta	201846	the counter-selection marker ( <i>sacB</i> ) to counterselect the plasmid after adding sucrose	Step 27A(xii)	Supplementary note 19	This study
pHERD30T-BAD-redy $\beta$ α-kan-amp-ccdB	201847	<i>redy</i> $\beta$ α under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 20	This study
pHERD30T-BAD-pluy $\beta$ α-kan-amp-ccdB	201848	<i>pluy</i> $\beta$ α under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 21	This study
pHERD30T-BAD-TE <sub>Psy</sub> -kan-amp-ccdB	201849	<i>recTE</i> <sub>Psy</sub> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 22	This study
pHERD30T-BAD-pluyTE <sub>Psy</sub> -kan-amp-ccdB	201850	<i>recTE</i> <sub>Psy</sub> and <i>pluy</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 23	This study
pHERD30T-BAD-redyTE <sub>Psy</sub> -kan-amp-ccdB	201851	<i>recTE</i> <sub>Psy</sub> and <i>redy</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 24	This study
pHERD30T-BAD-BAS-kan-amp-ccdB	201852	BAS gene under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 25	This study
pHERD30T-BAD-pluyBAS-kan-amp-ccdB	201853	BAS gene and <i>pluy</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 26	This study
pHERD30T-BAD-redyBAS-kan-amp-ccdB	201854	BAS gene and <i>redy</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 27	This study



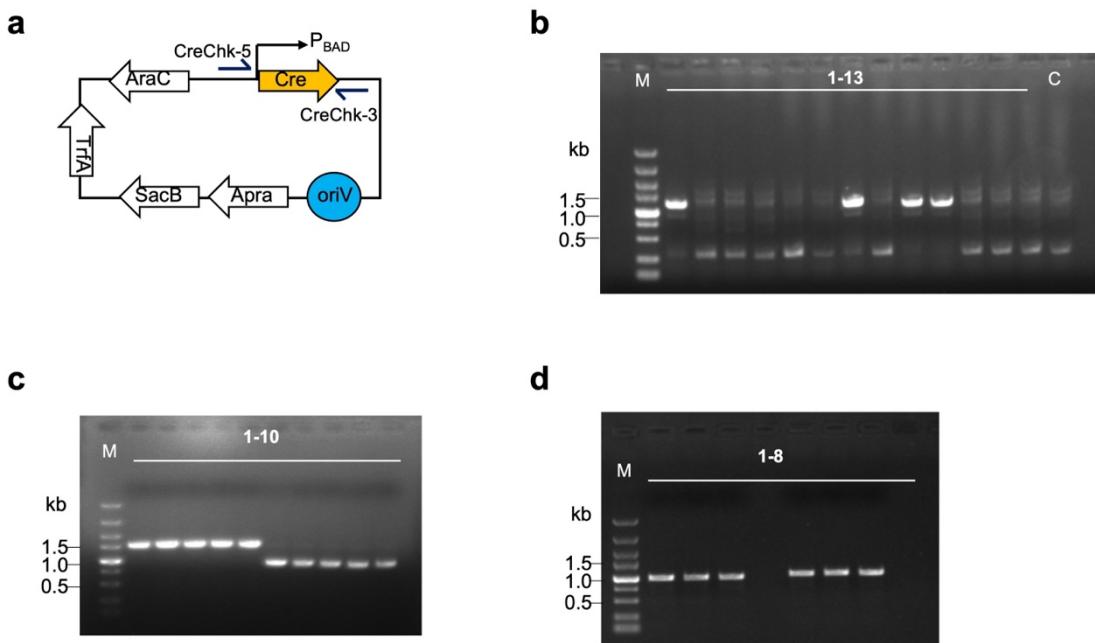
**Supplementary Figure 1.** The effect of SSB protein on recombination efficiency in *Pseudomonas*.

**(a)** Results from the recombineering assay in *P. putida* KT2440 upon the expression of TE<sub>Psy</sub>, TE<sub>Psy</sub>SSB, Plu $\gamma$ TE<sub>Psy</sub>, Plu $\gamma$ TE<sub>Psy</sub>SSB and BAS at 30 °C. **(b)** Results from the recombineering assay in *P. Syringae* DC3000 upon the expression of TE<sub>Psy</sub>, TE<sub>Psy</sub>SSB, Plu $\gamma$ TE<sub>Psy</sub>, Plu $\gamma$ TE<sub>Psy</sub>SSB, RedyTE<sub>Psy</sub>, RedyTE<sub>Psy</sub>SSB and BAS at 30 °C. **(c)** Results from the recombineering assay in *P. protegens* pf5 upon the expression of TE<sub>Psy</sub>, TE<sub>Psy</sub>SSB, Plu $\gamma$ TE<sub>Psy</sub>, Plu $\gamma$ TE<sub>Psy</sub>SSB, RedyTE<sub>Psy</sub> and RedyTE<sub>Psy</sub>SSB at 30 °C. **(d)** Results from the recombineering assay in *P. aeruginosa* PAO1 upon the expression TE<sub>Psy</sub>, TE<sub>Psy</sub>SSB, Plu $\gamma$ TE<sub>Psy</sub>, Plu $\gamma$ TE<sub>Psy</sub>SSB, RedyTE<sub>Psy</sub>, RedyTE<sub>Psy</sub>SSB and RedyBAS at 30 °C. Data represent the mean ± SD from three independent experiments in each case. Statistical source data are provided in Supplementary Data 1.

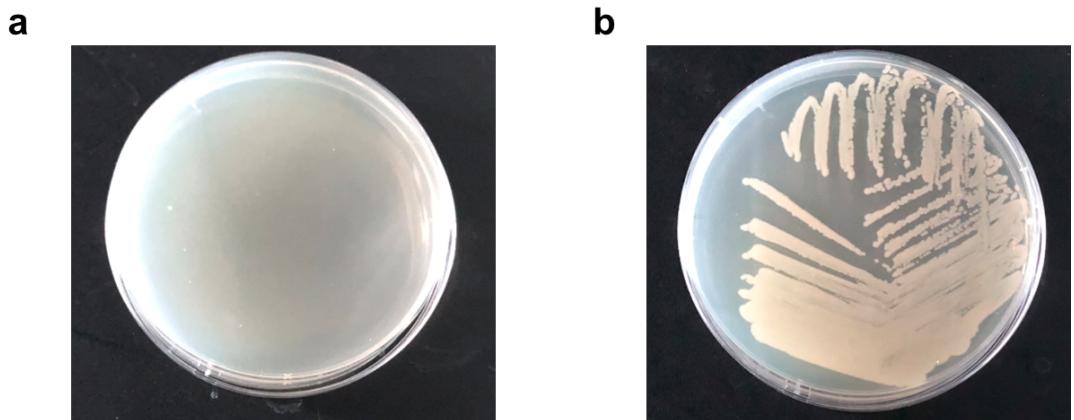


**Supplementary Figure 2.** Recombineering efficiency test in *Pseudomonas* using RedyBAS-Cas3 for *P. aeruginosa* PAO1, RedyTE<sub>Psy</sub>-Cas3 for *P. protegens* Pf5, BAS-Cas3 for *P. putida* KT2440, and BAS-Cas3 for *P. syringae* DC3000. Data represent the mean  $\pm$  SD from three independent experiments in each case. Statistical source data are provided in Supplementary Data 1.

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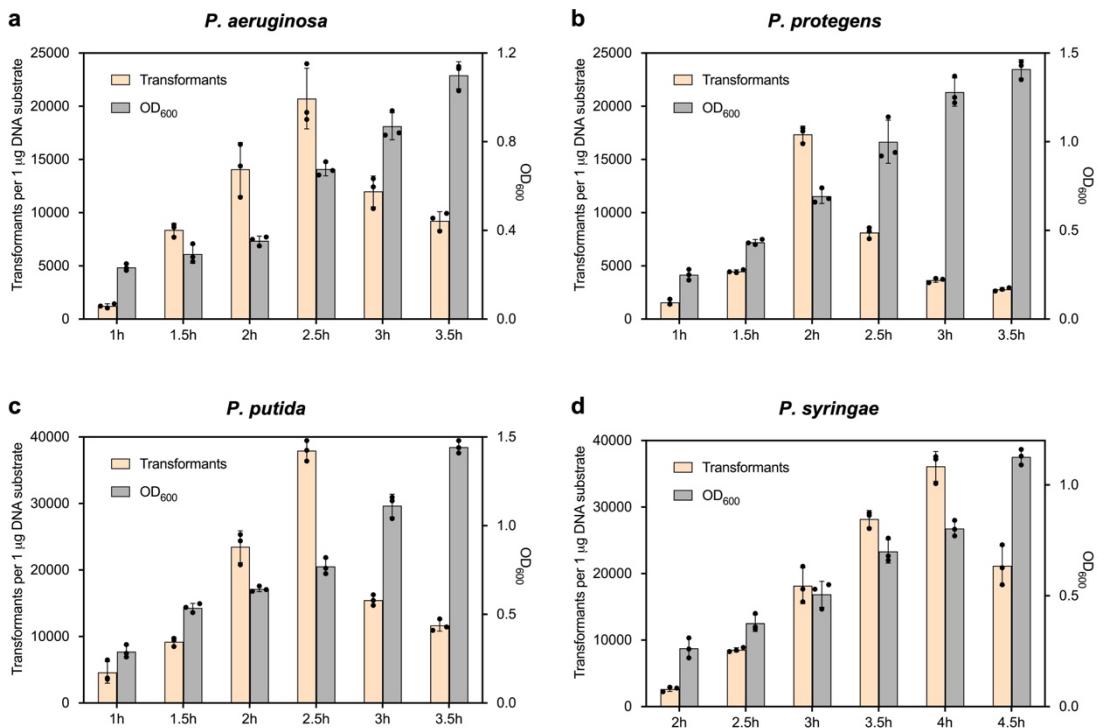


**Supplementary Figure 3.** PCR verification of genome engineering via site-specific recombinase (Cre) in *P. putida* KT2440. **(a)** Diagram of the expression plasmid pRK2-BAD-Cre-SacB-apra. **(b)** Insertion of the lox72-Genta-lox66 cassette into the *lysR* target using primer pairs KTlysChk-5/GenChk-5 gives a PCR product of 1082 bp. Lane M is the Takara DL5000 marker. Lane C is the wild-type strain, used as a negative control. Lanes 1–13 are recombinants. Clones 1, 7 and 10 are correct. **(c)** Colony PCR verification using primer pairs KTlysChk-5/KTlysChk-3 before Cre induction (lanes 1–5) gives a PCR product of 1590 bp, and after Cre induction (lane 6–10) gives PCR product of 784 bp. All clones are correct. **(d)** Colony PCR verification using primer pairs CreChk-5/CreChk-3 after Cre expression plasmid counter-selection. Clones 4 and 8 are correct.



**Supplementary Figure 4.** SacB function test on LB plate containing 10 % sucrose. *P. putida* mutant colony harbouring the Cre expression plasmid, pRK2-BAD-Cre-SacB-Apra (**a**) and plasmid quenching (**b**).

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**Supplementary Figure 5.** Optimization of transformation in *Pseudomonas*. The optical density (OD) at 600 nm (OD<sub>600</sub>) and number of transformants after electroporation of pHED30T-BAD-GFP-Rha-Cas-kan in *P. aeruginosa* PAO1 (a), *P. protegens* Pf5 (b), *P. putida* KT2440 (c), and *P. syringae* DC3000 (d). Data represent the mean ± SD from three independent experiments in each case. Statistical source data are provided in Supplementary Data 1.

## Reference

1. Nelson, K.E. et al. Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ Microbiol* **4**, 799-808 (2002).
2. Buell, C.R. et al. The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Proc Natl Acad Sci U S A* **100**, 10181-10186 (2003).
3. Paulsen, I.T. et al. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat Biotechnol* **23**, 873-878 (2005).
4. Stover, C.K. et al. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**, 959-964 (2000).
5. W, Z. et al. Cascade-Cas3 facilitates high accuracy of genome engineering in *Pseudomonas* using phage-encoded homologous recombination. *Engineering Microbiology* **2**, 10046 (2022).

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## Supplementary note

Supplementary note 1: Complete sequence of the pHERD30T-BAD-GFP-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-GFP-kan 13796 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-GFP-kan  
ACCESSION pHERD30T-BAD-GFP-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label="pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4468..6702 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	6753..7427 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	7424..9187 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	9199..10068 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"
CDS	11812..12690 /label="araC" /note="regulator gene of L-arabinose operon"
CDS	13036..13788

/label="eGFP"  
/note="green fluorescent protein"

## ORIGIN

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Supplementary note 2: Complete sequence of the pHERD30T-BAD-redy $\beta$  $\alpha$ -Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-redy $\beta$  $\alpha$ -kan 14928 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-redy $\beta$  $\alpha$ -kan  
ACCESSION pHERD30T-BAD-redy $\beta$  $\alpha$ -kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

CDS 258..1052 /label="kanR" /note="kanamycin resistance gene"  
rep\_origin 1093..2291 /label=" pHERD30T ori" /note="replication origin"  
CDS 2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"  
CDS 3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"  
Promoter 4196..4314 /label="Rha promoter"  
CDS 4468..6702 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"  
CDS 6753..7427 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"  
CDS 7424..9187 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"  
CDS 9199..10068 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"  
CDS 11812..12690 /label="araC" /note="regulator gene of L-arabinose operon"  
CDS 13036..13461 /label="redy" /note="gam protein from *E. coli*, recBCD inhibitor "  
CDS 13458..14243

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CDS 14240..14920  
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## ORIGIN

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Supplementary note 3: Complete sequence of the pHERD30T-BAD-plu $\gamma$  $\beta$ -Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-plu $\gamma$  $\beta$ -kan 14899 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-plu $\gamma$  $\beta$ -kan  
ACCESSION pHERD30T-BAD-plu $\gamma$  $\beta$ -kan  
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SOURCE  
ORGANISM  
FEATURES Location/Qualifiers  
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ORIGIN

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Supplementary note 4: Complete sequence of the pBBR1-Rha-TE<sub>Psy</sub>-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-TE<sub>Psy</sub>-kan 8921 bp DNA circular SYN 21-MAR-2019  
DEFINITION pBBR1-Rha-TE<sub>Psy</sub>-kan  
ACCESSION pBBR1-Rha-TE<sub>Psy</sub>-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

CDS 258..1052 /label="kanR"  
/note="kanamycin resistance gene"  
rep\_origin 1093..2291 /label="pHERD30T ori"  
/note="replication origin"  
CDS 2292..3140 /label="rhaR"  
/note="regulator gene of L-rhamnose operon"  
CDS 3214..4050 /label="rhaS"  
/note="regulator gene of L-rhamnose operon"  
Promoter 4196..4314 /label="Rha promoter"  
CDS 4468..6702 /label="cas3"  
/note="CRISPR-Cas3 system Cas3 protein"  
CDS 6753..7427 /label="cas5"  
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CDS 7424..9187 /label="cas8"  
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CDS 9199..10068 /label="cas7"  
/note="CRISPR-Cas3 system Cas7 protein"  
CDS 11812..12690 /label="araC"  
/note="regulator gene of L-arabinose operon"  
CDS 6059..6946 /label="recTpsy"  
/note="ssDNA annealing protein, recombinase from Pseudomonas syringae"

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CDS 6943..8604  
/label="recEpsy"  
/note="exonuclease from Pseudomonas syringae"

ORIGIN

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Supplementary note 5: Complete sequence of the pBBR1-Rha-TE<sub>Psy</sub>S-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-TE<sub>Psy</sub>S-kan 9387 bp DNA circular SYN 21-MAR-2019  
DEFINITION pBBR1-Rha-TE<sub>Psy</sub>S-kan  
ACCESSION pBBR1-Rha-TE<sub>Psy</sub>S-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

rep\_origin 1..675  
/label="pBBR1 ori"  
/note="replication origin"

CDS 1657..2655  
/label="mob"  
/note="replication protein"

CDS 2913..3707  
/label="kanR"  
/note="kanamycin resistance gene"

CDS 4006..4944  
/label="rhaR"  
/note="regulator gene of L-rhamnose operon"

CDS 4928..5764  
/label="rhaS"  
/note="regulator gene of L-rhamnose operon"

promoter 6002..6033  
/label="Rha promoter"

CDS 6059..6946  
/label="recTpsy"  
/note="ssDNA annealing protein from Pseudomonas syringae"

CDS 6943..8604  
/label="recEpsy"  
/note="exonuclease from Pseudomonas syringae"

CDS 8618..9061  
/gene="SSB"  
/note="single strand binding protein from Pseudomonas aeruginosa"

ORIGIN

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Supplementary note 6: Complete sequence of the pBBR1-Rha-pluγTE<sub>Psy</sub>-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-pluγTE<sub>Psy</sub>-kan 9192 bp DNA circular SYN 21-MAR-2019

DEFINITION pBBR1-Rha-pluγTE<sub>Psy</sub>-kan

ACCESSION pBBR1-Rha-pluγTE<sub>Psy</sub>-kan

KEYWORDS

SOURCE

ORGANISM

FEATURES Location/Qualifiers

rep\_origin 1..675

/label="pBBR1 ori"

/note="replication origin"

CDS 1657..2655

/label="mob"

/note="replication protein"

CDS 2913..3707

/label="kanR"

/note="kanamycin resistance gene"

CDS 4006..4944

/label="rhaR"

/note="regulator gene of L-rhamnose operon"

CDS 4928..5764

/label="rhaS"

/note="regulator gene of L-rhamnose operon"

promoter 6002..6033

/label="Rha promoter"

CDS 6059..6313

/label="pluγ"

/note="gam protein from *Photorhabdus*, recBCD inhibitor"

CDS 6330..7217

/label="recTpsy"

/note="ssDNA annealing protein, recombinase from *Pseudomonas syringae*"

CDS 7214..8875

/label="recEpsy"

/note="exonuclease from *Pseudomonas syringae*"

ORIGIN

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//

Supplementary note 7: Complete sequence of the pBBR1-Rha-pluyTE<sub>Psy</sub>S-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-pluyTE<sub>Psy</sub>S-kan 9658 bp DNA circular SYN 21-MAR-2019

DEFINITION pBBR1-Rha-pluyTE<sub>Psy</sub>S-kan

ACCESSION pBBR1-Rha-pluyTE<sub>Psy</sub>S-kan

KEYWORDS

SOURCE

ORGANISM

FEATURES Location/Qualifiers

rep\_origin 1..675

/label="pBBR1 ori"

/note="replication origin"

CDS 1657..2655

/label="mob"

/note="replication protein"

CDS 2913..3707

/label="kanR"

/note="kanamycin resistance gene"

CDS 4006..4944

/label="rhaR"

/note="regulator gene of L-rhamnose operon"

CDS 4928..5764

/label="rhaS"

/note="regulator gene of L-rhamnose operon"

promoter 6002..6033

/label="Rha promoter"

CDS 6059..6313

/label="pluy"

/note="gam protein from *Photorhabdus*, recBCD inhibitor"

CDS 6330..7217

/label="recTpsy"

/note="ssDNA annealing protein from *Pseudomonas syringae*"

CDS 7214..8875

/label="recEpsy"

/note="exonuclease from *Pseudomonas syringae*"

CDS 8889..9332

/gene="SSB"

/note="single strand binding protein from *Pseudomonas aeruginosa*"

ORIGIN

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Supplementary note 8: Complete sequence of the pBBR1-Rha-redyTE<sub>Psy</sub>-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-redyTE<sub>Psy</sub>-kan 9351 bp DNA circular SYN 21-MAR-2019

DEFINITION pBBR1-Rha-redyTE<sub>Psy</sub>-kan

ACCESSION pBBR1-Rha-redyTE<sub>Psy</sub>-kan

KEYWORDS

SOURCE

ORGANISM

FEATURES Location/Qualifiers

rep\_origin 1..675

/label="pBBR1 ori"

/note="replication origin"

CDS 1657..2655

/label="mob"

/note="replication protein"

CDS 2913..3707

/label="kanR"

/note="kanamycin resistance gene"

CDS 4006..4944

/label="rhaR"

/note="regulator gene of L-rhamnose operon"

CDS 4928..5764

/label="rhaS"

/note="regulator gene of L-rhamnose operon"

promoter 6002..6033

/label="Rha promoter"

CDS 6059..6475

/label="redy"

/note="lambda Red gam protein, recBCD inhibitor"

CDS 6489..7376

/label="recTpsy"

/note="ssDNA annealing protein, recombinase from Pseudomonas syringae"

CDS 7373..9034

/label="recEpsy"

/note="exonuclease from Pseudomonas syringae"

ORIGIN

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Supplementary note 9: Complete sequence of the pBBR1-Rha-redyTE<sub>Psy</sub>S-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-redyTE<sub>Psy</sub>S-kan 9820 bp DNA circular SYN 21-MAR-2019

DEFINITION pBBR1-Rha-redyTE<sub>Psy</sub>S-kan

ACCESSION pBBR1-Rha-redyTE<sub>Psy</sub>S-kan

KEYWORDS

SOURCE

ORGANISM

FEATURES Location/Qualifiers

rep\_origin 1..675

/label="pBBR1 ori"

/note="replication origin"

CDS 1657..2655

/label="mob"

/note="replication protein"

CDS 2913..3707

/label="kanR"

/note="kanamycin resistance gene"

CDS 4006..4944

/label="rhaR"

/note="regulator gene of L-rhamnose operon"

CDS 4928..5764

/label="rhaS"

/note="regulator gene of L-rhamnose operon"

promoter 6002..6033

/label="Rha promoter"

CDS 6059..6475

/label="redy"

/note="lambda Red gam protein, recBCD inhibitor"

CDS 6489..7376

/label="recTpsy"

/note="ssDNA annealing protein from Pseudomonas syringae"

CDS 7373..9034

/label="recEpsy"

/note="exonuclease from Pseudomonas syringae"

CDS 9051..9494

/gene="SSB"

/note="single strand binding protein from Pseudomonas aeruginosa"

ORIGIN

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Supplementary note 10: Complete sequence of the pHERD30T-BAD-TE<sub>Psy</sub>-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-TE<sub>Psy</sub>-kan 15589 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-TE<sub>Psy</sub>-kan  
ACCESSION pHERD30T-BAD-TE<sub>Psy</sub>-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

CDS 258..1052 /label="kanR" /note="kanamycin resistance gene"  
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CDS 2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"  
CDS 3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"  
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CDS 4468..6702 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"  
CDS 6753..7427 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"  
CDS 7424..9187 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"  
CDS 9199..10068 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"  
CDS 11812..12690 /label="araC" /note="regulator gene of L-arabinose operon"  
CDS 13036..13923 /label="recTpsy" /note="ssDNA annealing protein, recombinase from Pseudomonas syringae"

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CDS 13920..15581  
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ORIGIN

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Supplementary note 11: Complete sequence of the pHERD30T-BAD-pluγTE<sub>Psy</sub>-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD -pluγTE<sub>Psy</sub>-kan 15960 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-pluγTE<sub>Psy</sub>-kan  
ACCESSION pHERD30T-BAD-pluγTE<sub>Psy</sub>-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
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CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4468..6702 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
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CDS	7424..9187 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	9199..10068 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"
CDS	11812..12690 /label="araC" /note="regulator gene of L-arabinose operon"
CDS	13036..13290 /label="pluγ" /note="gam protein from Photorhabdus, recBCD inhibitor"
CDS	13307..14194

/label="recTpsy"  
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CDS 14191..15852  
/label="recEpsy"  
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ORIGIN

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Supplementary note 12: Complete sequence of the pHERD30T-BAD-redyTE<sub>Psy</sub>-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-redyTE<sub>Psy</sub>-kan 16019 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-redyTE<sub>Psy</sub>-kan  
ACCESSION pHERD30T-BAD-redyTE<sub>Psy</sub>-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

CDS 258..1052  
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/note="kanamycin resistance gene"  
rep\_origin 1093..2291  
/label=" pHERD30T ori"  
/note="replication origin"  
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Supplementary note 13: Complete sequence of the pHERD30T-BAD-BAS-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-BAS-kan 14863 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-BAS-kan  
ACCESSION pHERD30T-BAD-BAS-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

CDS 258..1052  
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Supplementary note 14: Complete sequence of the pHERD30T-BAD-pluyBAS-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-pluyBAS-kan 15143 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-pluyBAS-kan  
ACCESSION pHERD30T-BAD-pluyBAS-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

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Supplementary note 15: Complete sequence of the pHERD30T-BAD-redyBAS-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-redyBAS-kan 15293 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-redyBAS-kan  
ACCESSION pHERD30T-BAD-redyBAS-kan  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

CDS 258..1052  
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ORIGIN

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Supplementary note 16: Complete sequence of the pR6K-Tps-genta-tet-T7RP plasmid in Genbank format.

LOCUS pR6K-Tps-genta-tet-T7RP 6103 bp DNA circular SYN 21-MAR-2019  
DEFINITION pR6K-Tps-genta-tet-T7RP  
ACCESSION pR6K-Tps-genta-tet-T7RP  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

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ORIGIN

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AACGTACTAACGCTCTCATGTTAACGAACAAACCCATGGCTAACGTTAACGCT  
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CGTGACCTTGAAGCTAACGACTTCAAGAAAAACGTTGAGGAACAACTCAACAAAGCGC  
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Supplementary note 17: Complete sequence of the pR6K-SacB-genta plasmid in Genbank format.

LOCUS pR6K-SacB-genta 4216 bp DNA circular SYN 21-MAR-2019  
DEFINITION pR6K-SacB-genta  
ACCESSION pR6K-SacB-genta  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
rep_origin	95..468 /label="r6k ori" /note="replication origin"
misc_feature	596..629 /label="lox71" /note="mutant lox site"
CDS	863..1396 /label="gentaR" /note="gentamycin resistance gene "
misc_feature	1414..1447 /label="lox66" /note="mutant lox site"
CDS	1813..3663 /label="SacB" /note="SacB gene "

#### ORIGIN

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TCTTATAAAACTAAAACCTTAGAGGCTATTAAAGTTGCTGATTATATTAAATT TATT  
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CGGAGGCAGGGCATTGCCACC CGCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCG  
CTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCAGTGGCTC  
TCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTGATATGACCCAAGTA  
CCGCCACCTAACAAATTGTTCAAGCGAATAACTCGTATAATGTATGCTATACGAAC  
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GCGAGACATGTTGGAAACTTCGGTTCGCGTAGTCGGAATCTCGATACGGCTACGCGCT  
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TTGACGGCATTACGTCTAACGATATTACATGCTGGTTATGTTCTAATTCTTAACT  
GGCCCATACAAGCCGCTGAACAAAACCTGGCCTTGTGTTAAAATGGATCTGATCCTA  
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CAGACTGAACCGCCAGTTCACGCACACGCTGCAGGTTGTTGATTTCAGCGC  
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Supplementary note 18: Complete sequence of the pRK2-BAD-Cre-SacB-apra plasmid in Genbank format.

LOCUS pRK2-BAD-Cre-SacB-apra 7524 bp DNA circular SYN 21-MAR-2019  
DEFINITION pRK2-BAD-Cre-SacB-apra  
ACCESSION pRK2-BAD-Cre-SacB-apra  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES Location/Qualifiers

rep_origin	1..630 /label="oriV" /note="replication origin"
promoter	631..824 /label="kan promoter"
CDS	842..1618 /label="apraR" /note="apramycin resistance gene "
CDS	1619..3469 /label="sacB" /note="sacB gene"
CDS	3768..4916 /label="TrfA" /note="replication protein"
CDS	5227..6105 /label="araC" /note="regulator gene of L-arabinose operon"
promoter	6133..6381 /label="BAD promoter"
CDS	6382..7482 /label="Cre" /note="site-specific recombinase"

#### ORIGIN

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//

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Supplementary note 19: Complete sequence of the pHERD30T-SacB-genta plasmid in Genbank format.

LOCUS pHERD30T-SacB-genta 5756 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-SacB-genta

ACCESSION pHERD30T-SacB-genta

KEYWORDS

SOURCE

ORGANISM

FEATURES Location/Qualifiers

CDS 312..845

/label="genta"

/note="gentamycin resistance gene"

rep\_origin 1392..2162

/label="pHERD30T ori"

/note="replication origin"

CDS 2595..4013

/label="sacB"

/note="sacB gene"

ORIGIN

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Supplementary note 20: Complete sequence of the pHERD30T-BAD-red $\gamma$  $\beta$  $\alpha$ -Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-red $\gamma$  $\beta$  $\alpha$ -kan-amp-ccdB 16255 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-red $\gamma$  $\beta$  $\alpha$ -kan-amp-ccdB  
ACCESSION pHERD30T-BAD-red $\gamma$  $\beta$  $\alpha$ -kan-amp-ccdB  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS	13139..14017
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	/note="regulator gene of L-arabinose operon"
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	/label="redy"
	/note="gam protein from <i>E. coli</i> , recBCD inhibitor "
CDS	14785..15570
	/label="red $\beta$ "
	/note=" ssDNA annealing protein from <i>E. coli</i> "
CDS	15567..16247
	/label="reda"
	/note=" exonuclease from <i>E. coli</i> "

#### ORIGIN

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Supplementary note 21: Complete sequence of the pHERD30T-BAD-plu $\gamma$  $\beta$  $\alpha$ -Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-plu $\gamma$  $\beta$  $\alpha$ -kan-amp-ccdB 16226 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-plu $\gamma$  $\beta$  $\alpha$ -kan-amp-ccdB  
ACCESSION pHERD30T-BAD-plu $\gamma$  $\beta$  $\alpha$ -kan-amp-ccdB  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS	13139..14017 /label="araC" /note="regulator gene of L-arabinose operon"
CDS	14363..14617 /label="pluy" /note="gam protein from Photorhabdus, recBCD inhibitor "
CDS	14617..15534 /label="pluβ" /note=" ssDNA annealing protein from Photorhabdus"
CDS	15538..16218 /label="plua" /note=" exonuclease from Photorhabdus"

#### ORIGIN

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Supplementary note 22: Complete sequence of the pHERD30T-BAD-TE<sub>Psy</sub>-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-TE<sub>Psy</sub>-kan-amp-ccdB 16916 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-TE<sub>Psy</sub>-kan-amp-ccdB

ACCESSION pHERD30T-BAD-TE<sub>Psy</sub>-kan-amp-ccdB

KEYWORDS

SOURCE

ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS 13139..14017  
/label="araC"  
/note="regulator gene of L-arabinose operon"  
CDS 14363..15250  
/label="recTpsy"  
/note="ssDNA annealing protein, recombinase from Pseudomonas syringae"  
CDS 15427..16908  
/label="recEpsy"  
/note="exonuclease from Pseudomonas syringae"

ORIGIN

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Supplementary note 23: Complete sequence of the pHERD30T-BAD-pluγTE<sub>Psy</sub>-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-pluγTE<sub>Psy</sub>-kan-amp-ccdB 17187 bp DNA circular SYN  
21-MAR-2019  
DEFINITION pHERD30T-BAD-pluγTE<sub>Psy</sub>-kan-amp-ccdB  
ACCESSION pHERD30T-BAD-pluγTE<sub>Psy</sub>-kan-amp-ccdB  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS 13139..14017  
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/label="pluy"  
/note="gam protein from Photorhabdus, recBCD inhibitor "  
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#### ORIGIN

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Supplementary note 24: Complete sequence of the pHERD30T-BAD-redyTE<sub>Psy</sub>-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-redyTE<sub>Psy</sub>-kan-amp-ccdB 17346 bp DNA circular SYN  
21-MAR-2019  
DEFINITION pHERD30T-BAD-redyTE<sub>Psy</sub>-kan-amp-ccdB  
ACCESSION pHERD30T-BAD-redyTE<sub>Psy</sub>-kan-amp-ccdB  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS 13139..14017  
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CDS 14363..14779  
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/note="gam protein from *E. coli*, recBCD inhibitor "  
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/label="recEpsy"  
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#### ORIGIN

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Supplementary note 25: Complete sequence of the pHERD30T-BAD-BAS-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-BAS-kan-amp-ccdB 16190 bp DNA circular SYN 21-MAR-2019  
DEFINITION pHERD30T-BAD-BAS-kan-amp-ccdB  
ACCESSION pHERD30T-BAD-BAS-kan-amp-ccdB  
KEYWORDS  
SOURCE  
ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label="pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

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CDS 14363..15106  
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#### ORIGIN

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Supplementary note 26: Complete sequence of the pHERD30T-BAD-pluγBAS-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-pluγBAS-kan-amp-ccdB 16461 bp DNA circular SYN

21-MAR-2019

DEFINITION pHERD30T-BAD-pluγBAS-kan-amp-ccdB

ACCESSION pHERD30T-BAD-pluγBAS-kan-amp-ccdB

KEYWORDS

SOURCE

ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS 13139..14017  
/label="araC"  
/note="regulator gene of L-arabinose operon"  
CDS 14363..14617  
/label="pluy"  
/note="gam protein from Photorhabdus, recBCD inhibitor "  
CDS 14634..15377  
/label="beta"  
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CDS 15346..15987  
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/note="single strand binding protein from Pseudomonas aeruginosa"

#### ORIGIN

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Supplementary note 27: Complete sequence of the pHERD30T-BAD-redyBAS-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-redyBAS-kan-amp-ccdB 16620 bp DNA circular SYN

21-MAR-2019

DEFINITION pHERD30T-BAD-redyBAS-kan-amp-ccdB

ACCESSION pHERD30T-BAD-redyBAS-kan-amp-ccdB

KEYWORDS

SOURCE

ORGANISM

FEATURES	Location/Qualifiers
CDS	258..1052 /label="kanR" /note="kanamycin resistance gene"
rep_origin	1093..2291 /label=" pHERD30T ori" /note="replication origin"
CDS	2292..3140 /label="rhaR" /note="regulator gene of L-rhamnose operon"
CDS	3214..4050 /label="rhaS" /note="regulator gene of L-rhamnose operon"
Promoter	4196..4314 /label="Rha promoter"
CDS	4462..5332 /label="amp" /note="ampicillin resistance gene"
CDS	5371..5676 /label="ccdB" /note="a bacterial toxin that poisons DNA gyrase"
CDS	5795..8029 /label="cas3" /note="CRISPR-Cas3 system Cas3 protein"
CDS	8080..8754 /label="cas5" /note="CRISPR-Cas3 system Cas5 protein"
CDS	8751..10514 /label="cas8" /note="CRISPR-Cas3 system Cas8 protein"
CDS	10526..11395 /label="cas7" /note="CRISPR-Cas3 system Cas7 protein"

CDS 13139..14017  
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/label="beta"  
/note="ssDNA annealing protein from *Pseudomonas aeruginosa*"  
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/gene="SSB"  
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#### ORIGIN

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