

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

--^a Did not test this antibiotic

ND^b 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 γ β α -kan	Red α , Red β and Red γ	pHERD30T	kanamycin resistance gene	<i>araC</i> P _{BAD} promoter	<i>rhaR-rhaS</i> P _{Rha} promoter
pHERD30T-BAD-plu γ β α -kan	Plu α , Plu β and Plu γ				
pHERD30T-BAD-TE _{Psy} -kan	RecT _{Psy} and RecE _{Psy}				
pHERD30T-BAD-plu γ TE _{Psy} -kan	RecT _{Psy} , RecE _{Psy} and Plu γ				
pHERD30T-BAD-red γ TE _{Psy} -kan	RecT _{Psy} , RecE _{Psy} , and Red γ				
pHERD30T-BAD-BAS-kan	Alpha, Beta and SSB from phage_AB31				
pHERD30T-BAD-plu γ BAS-kan	Alpha, Beta, SSB from phage_AB31 and Plu γ				
pHERD30T-BAD-red γ BAS-kan	Alpha, Beta, SSB from phage_AB31 and Red γ				

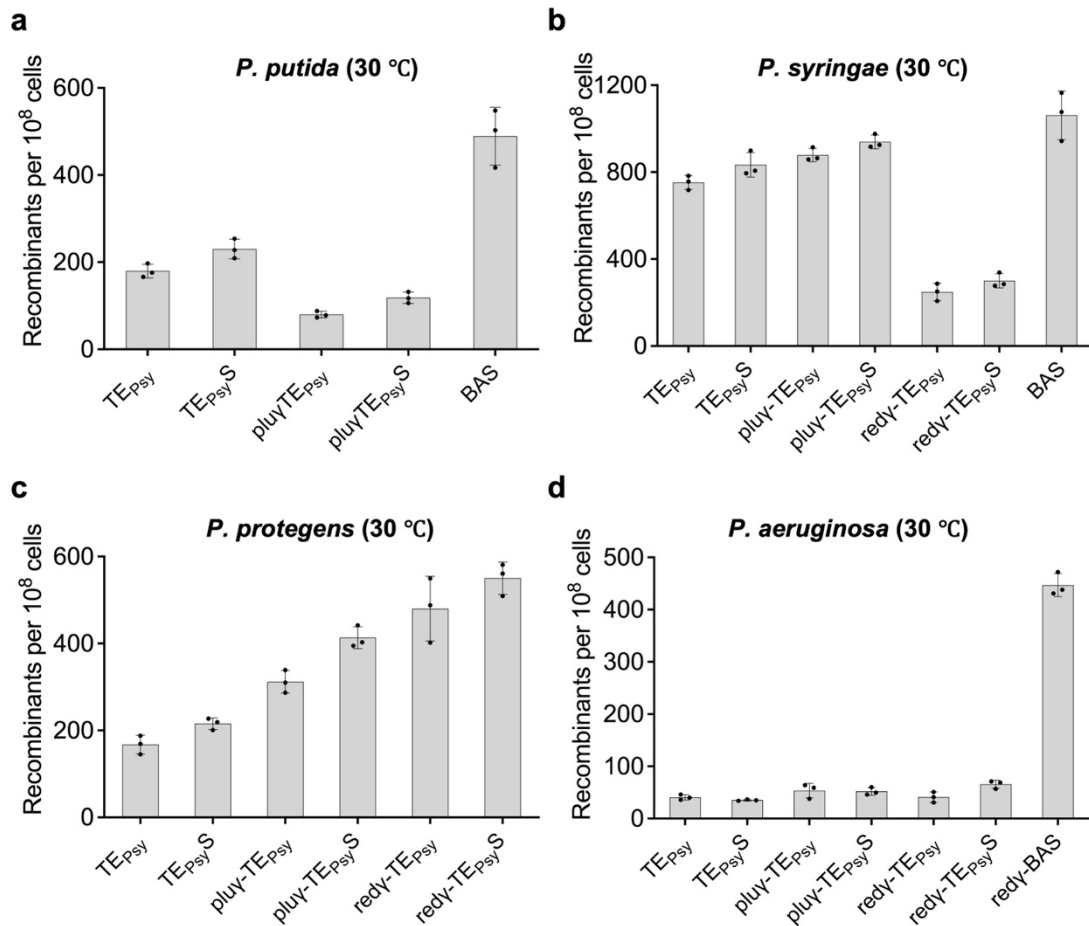
Supplementary Table 3 *Pseudomonas* strains used in this protocol.

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	1
<i>P. syringae</i> DC3000	An agriculturally important plant pathogen could cause bacterial speck of tomato	30	2
<i>P. protegens</i> pf5	A plant commensal bacterium that inhabits the rhizosphere and produces secondary metabolites that suppress soilborne plant pathogens	30	3
<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	4

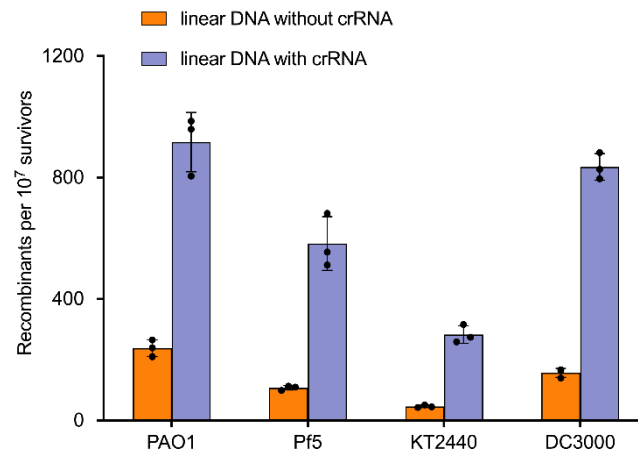
Supplementary Table 4 Plasmids used in this protocol.

Plasmids	Addgene number	Characteristics	Step number	Sequence	references
pHERD30T-BAD-GFP-Rha-Cas-kan	201836	<i>gfp</i> under BAD promoter, <i>cas</i> under Rha promoter	Step 39	Supplementary note 1	⁵
pHERD30T-BAD-red γ β α -Rha-Cas-kan (Red γ β α -Cas3)	194866	<i>red$\gamma$$\beta$$\alpha$</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 2	⁵
pHERD30T-BAD-plu γ β α -Rha-Cas-kan (Plu γ β α -Cas3)	194867	<i>plu$\gamma$$\beta$$\alpha$</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 3	⁵
pBBR1-Rha-TE _{Psy} -kan	201841	<i>recTE_{Psy}</i> under Rha promoter		Supplementary note 4	⁵
pBBR1-Rha-TE _{Psy} S-kan	201842	<i>recTE_{Psy}SSB</i> under Rha promoter		Supplementary note 5	This study
pBBR1-Rha-plu γ TE _{Psy} -kan	201837	<i>recTE_{Psy}</i> and <i>pluγ</i> under Rha promoter		Supplementary note 6	⁵
pBBR1-Rha-plu γ TE _{Psy} S-kan	201838	<i>recTE_{Psy}SSB</i> and <i>pluγ</i> under Rha promoter		Supplementary note 7	This study
pBBR1-Rha-red γ TE _{Psy} -kan	201839	<i>recTE_{Psy}</i> and <i>redγ</i> under Rha promoter		Supplementary note 8	⁵
pBBR1-Rha-red γ TE _{Psy} S-kan	201840	<i>recTE_{Psy}SSB</i> and <i>redγ</i> under Rha promoter		Supplementary note 9	This study
pHERD30T-BAD-TE _{Psy} -Rha-Cas-kan (TE _{Psy} -Cas3)	194868	<i>recTE_{Psy}</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 10	⁵
pHERD30T-BAD-plu γ TE _{Psy} -Rha-Cas-kan (Plu γ TE _{Psy} -Cas3)	194869	<i>recTE_{Psy}</i> and <i>pluγ</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 11	⁵
pHERD30T-BAD-red γ TE _{Psy} -Rha-Cas-kan (Red γ TE _{Psy} -Cas3)	194870	<i>recTE_{Psy}</i> and <i>redγ</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 12	⁵
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	⁵
pHERD30T-BAD-plu γ BAS-Rha-Cas-kan (Plu γ BAS-Cas3)	194872	BAS gene and <i>pluγ</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 14	⁵
pHERD30T-BAD-red γ BAS-Rha-Cas-kan (Red γ BAS-Cas3)	194873	BAS gene and <i>redγ</i> under BAD promoter, <i>cas</i> under Rha promoter	Steps 10, 14	Supplementary note 15	⁵
pR6K-Tps-genta-tet-T7RP	201843	PCR template to amplify lox71-genta ^R -lox66 or <i>genta^R</i> gene	Steps 27B(i), 41	Supplementary note 16	⁵

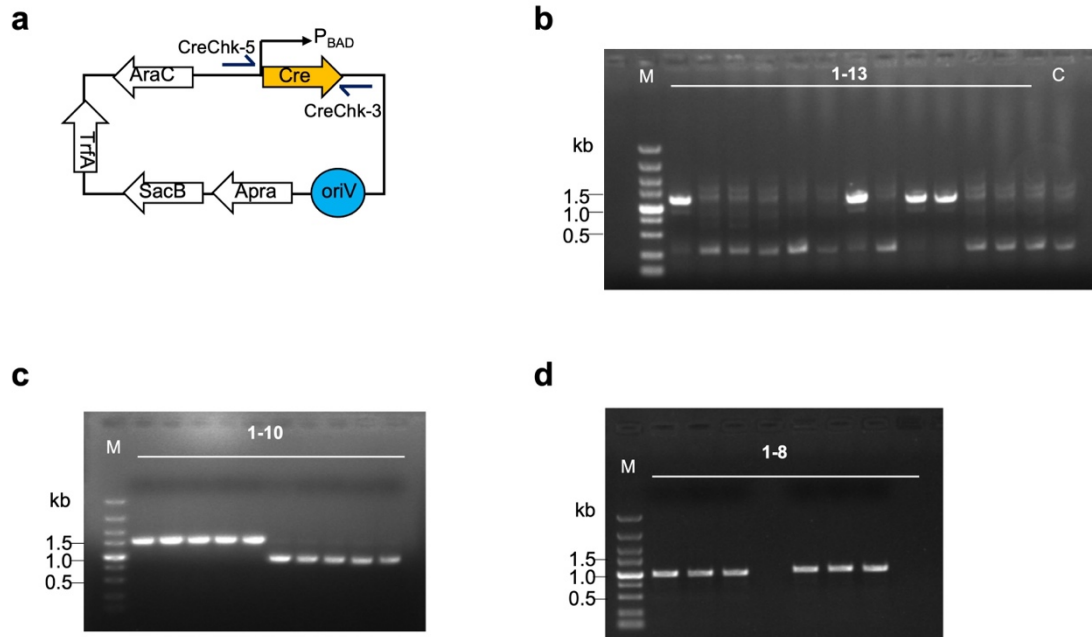
pR6K-SacB-genta	201844	PCR template to amplify the SacB-genta ^R cassette	Step 27A(i)	Supplementary note 17	This study
pRK2-BAD-Cre-SacB-apra	201845	Express the site-specific recombinase (Cre) and add sucrose to quench the plasmid under the SacB counter selection pressure	Step 27B(iv)	Supplementary note 18	⁵
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-red γ β α -kan-amp-ccdB	201847	<i>red$\gamma$$\beta$$\alpha$</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 20	This study
pHERD30T-BAD-plu γ β α -kan-amp-ccdB	201848	<i>plu$\gamma$$\beta$$\alpha$</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 21	This study
pHERD30T-BAD-TE _{Psy} -kan-amp-ccdB	201849	<i>recTE_{Psy}</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 22	This study
pHERD30T-BAD-plu γ TE _{Psy} -kan-amp-ccdB	201850	<i>recTE_{Psy}</i> and <i>pluγ</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 23	This study
pHERD30T-BAD-red γ TE _{Psy} -kan-amp-ccdB	201851	<i>recTE_{Psy}</i> and <i>redγ</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-plu γ BAS-kan-amp-ccdB	201853	BAS gene and <i>pluγ</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 26	This study
pHERD30T-BAD-red γ BAS-kan-amp-ccdB	201854	BAS gene and <i>redγ</i> under BAD promoter, <i>cas</i> under Rha promoter and amp-ccdB cassette	Step 1	Supplementary note 27	This study



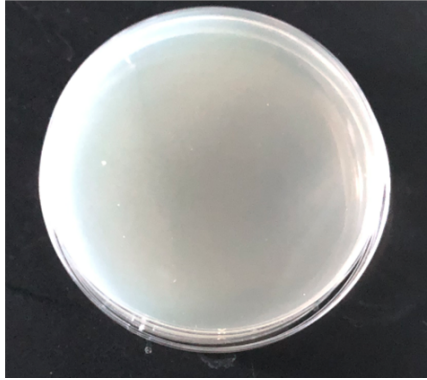
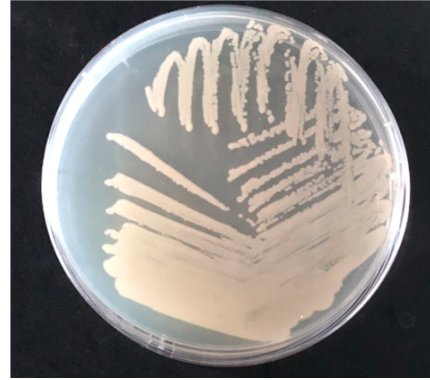
Supplementary Figure 1. The effect of SSB protein on recombination efficiency in *Pseudomonas*. (a) Results from the recombineering assay in *P. putida* KT2440 upon the expression of TE_{Psy}, TE_{Psy}SSB, PluγTE_{Psy}, PluγTE_{Psy}SSB and BAS at 30 °C. (b) Results from the recombineering assay in *P. Syringae* DC3000 upon the expression of TE_{Psy}, TE_{Psy}SSB, PluγTE_{Psy}, PluγTE_{Psy}SSB, RedγTE_{Psy}, RedγTE_{Psy}SSB and BAS at 30 °C. (c) Results from the recombineering assay in *P. protegens* pf5 upon the expression of TE_{Psy}, TE_{Psy}SSB, PluγTE_{Psy}, PluγTE_{Psy}SSB, RedγTE_{Psy} and RedγTE_{Psy}SSB at 30 °C. (d) Results from the recombineering assay in *P. aeruginosa* PAO1 upon the expression TE_{Psy}, TE_{Psy}SSB, PluγTE_{Psy}, PluγTE_{Psy}SSB, RedγTE_{Psy}, RedγTE_{Psy}SSB and RedγBAS at 30 °C. Data represent the mean ± SD from three independent experiments in each case. Statistical source data are provided in Supplementary Data 1.



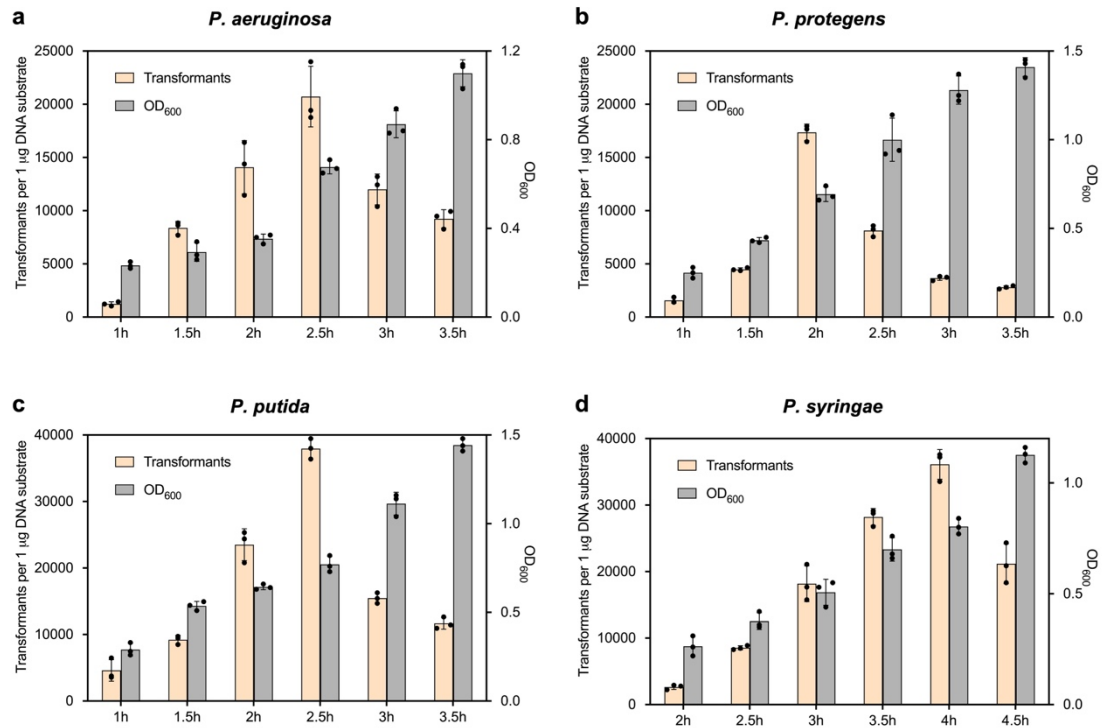
Supplementary Figure 2. Recombineering efficiency test in *Pseudomonas* using Red γ BAS-Cas3 for *P. aeruginosa* PAO1, Red γ TE_{Psy}-Cas3 for *P. protegens* Pf5, BAS-Cas3 for *P. putida* KT2440, and BAS-Cas3 for *P. syringae* DC3000. Data represent the mean $\pm$ SD from three independent experiments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



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.

a**b**

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-Apr^r (a) and plasmid quenching (b).



Supplementary Figure 5. Optimization of transformation in *Pseudomonas*. The optical density (OD) at 600 nm (OD₆₀₀) and number of transformants after electroporation of pHERD30T-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 $\pm$ 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).
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 4. Stover, C.K. et al. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**, 959-964 (2000).
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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-red γ β α -Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-red γ β α -kan 14928 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-red γ β α -kan

ACCESSION pHERD30T-BAD-red γ β α -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="red γ " /note="gam protein from <i>E. coli</i> , recBCD inhibitor "
CDS	13458..14243

CDS
/label="redβ"
/note=" ssDNA annealing protein from *E. coli*"
14240..14920
/label="redα"
/note=" exonuclease from *E. coli*"

ORIGIN

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Supplementary note 3: Complete sequence of the pHERD30T-BAD-plu γ β α -Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-plu γ β α -kan 14899 bp DNA circular SYN 21-MAR-2019
 DEFINITION pHERD30T-BAD-plu γ β α -kan
 ACCESSION pHERD30T-BAD-plu γ β α -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..13290 /label="plu γ " /note="gam protein from Photorhabdus, recBCD inhibitor"
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CDS /label="pluβ"
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 /gene="pluα"
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ORIGIN

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Supplementary note 4: Complete sequence of the pBBR1-Rha-TE_{Psy}-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-TE_{Psy}-kan 8921 bp DNA circular SYN 21-MAR-2019
 DEFINITION pBBR1-Rha-TE_{Psy}-kan
 ACCESSION pBBR1-Rha-TE_{Psy}-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	6059..6946 /label="recTpsy" /note="ssDNA annealing protein, recombinase from Pseudomonas syringae"

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_{Psy}S-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-TE_{Psy}S-kan 9387 bp DNA circular SYN 21-MAR-2019
DEFINITION pBBR1-Rha-TE_{Psy}S-kan
ACCESSION pBBR1-Rha-TE_{Psy}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_{Psy}-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-pluγTE_{Psy}-kan 9192 bp DNA circular SYN 21-MAR-2019
DEFINITION pBBR1-Rha-pluγTE_{Psy}-kan
ACCESSION pBBR1-Rha-pluγTE_{Psy}-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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CATGTGCTTCCGCATGCGGCCACCTCGACCAGCGCCTTGGTCCTAGACGGCGACAGCC
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GTCTCGATGGAGTACTTCCCGGTGAACGGTTCGGGCGGCGAAACTGGTCGCCGGCTGG
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GTTGGGAAGACTCCGGACGCGCTGCCAGCGCTGCGCATCGAAGTAACCGGAATGGTC
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AACTGACCTGCAGACCGACCAGCACTTCGCTGATGCAGAGAAAACAGTGAAGTGGT
GTGGCGAGGTCGAGGAGCGCCTGGAAGCAGCCAAGCAACATGCGCTGAGCCAGACG
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ATCGTTCTGAAAGCGAAAGCAGCGCTGCGTGATCATCTGGACAAGATCAACACCTCG
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CGCGCCCCGCGCCGCAAGCTGCTGAGGCATCCAAACCAGTCGAACAACCAGCTCCGAG
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GAACGTCACCGACCTTCATGCGCTGGTAAAAGCCGTCTACGAAGGTGCTGCGCCGAT
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GACTTCCAAATGGACGGAGTAACCATCACGCAGGTGGCAGCATGATGTACAAGTAAG
CTTACCGGTTTATTGACTACCGGAAGCAGTGTGACCGTGTGCTTCTCAAATGCCTGAG
GCCAGTTTGCTCAGGCTCTCCCCGTGGAGGTAATAATTGACGATATGATCATTTATTC
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ATTCAGGTCGAGGTGGCCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACA
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TGCTGCGCAACCCAAGTG

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Supplementary note 7: Complete sequence of the pBBR1-Rha-pluγTE_{Psy}S-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-pluγTE_{Psy}S-kan 9658 bp DNA circular SYN 21-MAR-2019
DEFINITION pBBR1-Rha-pluγTE_{Psy}S-kan
ACCESSION pBBR1-Rha-pluγTE_{Psy}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="pluγ" /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
CTACCGGCGCGGCAGCGTGACCCGTGTCGGCGGCTCCAACGGCTCGCCATCGTCCAG
AAAACACGGCTCATCGGGCATCGGCAGGCGCTGCTGCCC GCGCCGTTCCCATTCCTCC

GTTTCGGTCAAGGCTGGCAGGTCTGGTTCCATGCCCCGAATGCCGGGCTGGCTGGGC
GGCTCCTCGCCGGGGCCGGTCGGTAGTTGCTGCTCGCCCGGATACAGGGTCGGGATG
CGGCGCAGGTTCGCCATGCCCCAACAGCGATTTCGTCTGGTCGTGATCAACCACCA
CGGCGGCACTGAACACCGACAGGCGCAACTGGTCGCGGGGCTGGCCCCACGCCACGC
GGTCATTGACCACGTAGGCCGACACGGTGCCGGGGCCGTTGAGCTTCACGACGGAGA
TCCAGCGCTCGGCCACCAAGTCCTTGACTGCGTATTGGACCGTCCGCAAAGAACGTCC
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GCCACGAGGTGATGCAGCAGCATTGCCGCCGTGGGTTTCCTCGCAATAAGCCCCGGCC
CACGCCTCATGCGCTTTGCGTTCCGTTTGACCCAGTGACCGGGCTTGTTCTTGGCTTG
AATGCCGATTTCTCTGGACTGCGTGGCCATGCTTATCTCCATGCGGTAGGGTGCCGCA
CGGTTGCGGCACCATGCGCAATCAGCTGCAACTTTTCGGCAGCGCGACAACAATTAT
GCGTTGCGTAAAAGTGGCAGTCAATTACAGATTTTCTTTAACCTACGCAATGAGCTAT
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CGGGGTTCCCCCACGCCTTCGGCGCGGCTCCCCCTCCGGCAAAAAGTGGCCCCCTCCGG
GGCTTGTTGATCGACTGCGCGGCCTTCGGCCTTGCCCAAGGTGGCGCTGCCCCCTTGG
AACCCCCGCACTCGCCGCCGTGAGGCTCGGGGGGCAGGCGGGCGGGCTTCGCCTTCG
ACTGCCCCCACTCGCATAGGCTTGGGTCGTTCCAGGCGCGTCAAGGCCAAGCCGCTGC
GCGGTGCTGCGCGAGCCTTGACCCGCCTTCCACTTGGTGTCCAACCGGCAAGCGAA
GCGCGCAGGCCCGCAGGCCGGAGGCTTTTCCCAGAGAAAATTAAAAAAATTGATGGG
GCAAGGCCCGCAGGCCGCGCAGTTGGAGCCGGTGGGTATGTGGTCGAAGGTGGGTAG
CCGGTGGGCAATCCCTGTGGTCAAGCTCGTGGGCAGGCGCAGCCTGTCCATCAGCTTG
TCCAGCAGGGTTGTCCACGGGCCGAGCGAAGCGAGCCAGCCGGTGGCCGCTCGCGGC
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CCGGAGAGCAAGCCCGTAGGGCGCCGACGCCCGTAGGCGGTCACGACTTTGCGAA
GCAAAGTCTAGTGAGTATACTCAAGCATTGAGTGGCCCCGCCGAGGACCCGCCTTGC
GCTGCCCCCGTCGAGCCGGTTGGACACCAAAAAGGGAGGGGCAGGCATGGCGGCATAC
GCGATCATGCGATGCAAGAAGCTGGCGAAAATGGGCAACGTGGCGGCCAGTCTCAAG
CACGCCTACCGCGAGCGCGAGACGCCAACGCTGACGCCAGCAGGACGCCAGAGAA
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GCCAGAGAAGCGGCGCAAGGACGCTGTGTTGGCGGTGAGTACGTATGACGGCCAG
CCCGGAATGGTGGAAAGTCGGCCAGCCAAGAACAGCAGGCGGCGTTCTTCGAGAAGGC
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Supplementary note 8: Complete sequence of the pBBR1-Rha-red γ TE_{Psy}-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-red γ TE_{Psy}-kan 9351 bp DNA circular SYN 21-MAR-2019
DEFINITION pBBR1-Rha-red γ TE_{Psy}-kan
ACCESSION pBBR1-Rha-red γ TE_{Psy}-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="red γ " /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-red γ TE_{Psy}S-kan plasmid in Genbank format.

LOCUS pBBR1-Rha-red γ TE_{Psy}S-kan 9820 bp DNA circular SYN 21-MAR-2019
DEFINITION pBBR1-Rha-red γ TE_{Psy}S-kan
ACCESSION pBBR1-Rha-red γ TE_{Psy}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="red γ " /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_{Psy}-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-TE_{Psy}-kan 15589 bp DNA circular SYN 21-MAR-2019
 DEFINITION pHERD30T-BAD-TE_{Psy}-kan
 ACCESSION pHERD30T-BAD-TE_{Psy}-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..13923 /label="recTpsy" /note="ssDNA annealing protein, recombinase from Pseudomonas syringae"

CDS

13920..15581

/label="recEpsy"

/note="exonuclease from *Pseudomonas syringae*"

ORIGIN

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Supplementary note 11: Complete sequence of the pHERD30T-BAD-plu γ TE_{Psy}-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD -plu γ TE_{Psy}-kan 15960 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-plu γ TE_{Psy}-kan

ACCESSION pHERD30T-BAD-plu γ TE_{Psy}-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..13290 /label="plu γ " /note="gam protein from Photorhabdus, recBCD inhibitor"
CDS	13307..14194

/label="recTpsy"
/note="ssDNA annealing protein, recombinase from *Pseudomonas syringae*"
CDS 14191..15852
/label="recEpsy"
/note="exonuclease from *Pseudomonas syringae*"

ORIGIN

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Supplementary note 12: Complete sequence of the pHERD30T-BAD-red γ TE_{Psy}-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-red γ TE_{Psy}-kan 16019 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-red γ TE_{Psy}-kan

ACCESSION pHERD30T-BAD-red γ TE_{Psy}-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..13452 /label="plu γ " /note="gam protein from Photorhabdus, recBCD inhibitor"
CDS	13466..14353

/label="recTpsy"
/note="ssDNA annealing protein, recombinase from *Pseudomonas syringae*"
CDS 14350..16011
/label="recEpsy"
/note="exonuclease from *Pseudomonas syringae*"

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 /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"
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CDS	13036..13779 /label="beta" /note="ssDNA annealing protein from Pseudomonas aeruginosa"
CDS	13766..14389

CDS /label="alpha"
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 14412..14855
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ORIGIN

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Supplementary note 14: Complete sequence of the pHERD30T-BAD-pluγBAS-Rha-Cas-kan plasmid in Genbank format.

LOCUS pHERD30T-BAD-pluγBAS-kan 15143 bp DNA circular SYN 21-MAR-2019
DEFINITION pHERD30T-BAD-pluγBAS-kan
ACCESSION pHERD30T-BAD-pluγBAS-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..13290 /label="pluγ" /note="gam protein from Photorhabdus, recBCD inhibitor"
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ORIGIN

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

LOCUS pHERD30T-BAD-red γ BAS-kan 15293 bp DNA circular SYN 21-MAR-2019
 DEFINITION pHERD30T-BAD-red γ BAS-kan
 ACCESSION pHERD30T-BAD-red γ BAS-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..13449 /label="red γ " /note="lamda Red gam protein, recBCD inhibitor"
CDS	13466..14209

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ORIGIN

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GGACGTCAGCGAGGCGATCTGCCTCATCAAGGACTCCCCAACAAATGGAAGACTTGCA
GTCAGCATTTCAGCAATGCCTGGAAAGCCTACAAGACAAAGGGCGCGCGGACAGCT
TACGGCGGCCAAGGACCAGCGGAAGAAAGAACTGCTGGACGCCCCATAGACGTTGA
ATTCGAGGAGACCGGCGATGATCGAGCAGCGTAGTGATGAATGGTTTCGCACAGCGCC
TGGGGCGGGTGACGGCCAGCAAGGTCAAGGATGTGATGGCAAAGGGGGCGCAGTGGC
GCCCCTTCTGCTACCCGCCAGAACTACATGATGCAGCTCCTGTGCGAGCGCCTGACCG
GCAAGCGCGAGGAAGGATTACACGCGCCGCAATGCAGCGTGGTACCGACCTGGAGC
CGATTGCCCGCTCGGCATACGAGTTCAATGCAGGCGTAATGACGATCGAAACAGGCC
TGATCATCCATCCGCGAATCGATGGATTTGGCGCGTCGCCAGATGGCCTCGCGGGGG
AGCATGGGCTCGTCGAGATTAAATGCCCCGTCTACCGCAACCCACATCTACACGATGC
AGTCGGGCAAGCACGACCCACAGTACGAGTGGCAGATGCTCGCCCAAATGTCATGCA

GCGGCCGCGAGTGGGTCTGACTTCGTGAGCTTCGACGACCGTCTGCCTGATGAATTGCA
GTACGTGTGCTTCCGCTACCACCGCGACGAGGAACGCATTTCGCGAGATGGAGTCCGA
GGTTAAAGCGTTCCTGGAAGAGTTGGCAGAGCTTGAACATCAGATGCGAGAACGCAT
GAGGAAAGCAGCATGAGTGCACAGGAGGGGATCCTATGAGAGGCGTTAACAAAGTC
ATTCTGGTTGGCAACGTCGGTGGTGACCCGGAAACTCGCTATATGCCCAACGGCAAT
GCGGTGACCAACATCACCTCGCCACCAGCGAGAGCTGGAAGGACAAGCAGACCGG
CCAGCAACAGGAGCGCACCGAATGGCACCGCGTGGTGTTCTTCGGGAAGTTGGCGGA
GATCGTCGGGCAGCACGTAAAGAAGGGTCAGCAACTGTACGTCGAGGGTTCACTCAG
AACTCGAAAGTGGCAGGCGCAGGACGGTCAGGACCGCTACACAACAGAGATCATCGT
CGACATGCACGGGCAGATGCAGATGTTTGGCGGAAAGCCTGGAAATGAACAGGCAG
CTCAGAGCAGGTCATCTACTCAGCAGCAGAGTGCGCCTCAGCAGCGTAGCGCTCAGG
ATGAATTCGACGACGATATTCCTTTATTAATTAA

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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
rep_origin	1..1047 /label="r6k ori" /note="replication origin"
misc_feature	1494..1527 /label="lox71" /note="mutant lox site"
CDS	1761..2294 /label="gentaR" /note="gentamycin resistance gene "
misc_feature	2312..2345 /label="lox66" /note="mutant lox site"
CDS	2346..2969 /label="tetR" /note="regulator gene of tetO promoter"
CDS	3054..5705 /label="T7P" /note="T7 RNA polymerase"

ORIGIN

ATGGAAAAAAGGAATTTTCGTGTTTTGATAAAATACTGTTTTCTGAAGGGAAAAAAT
ACAGTGGAAGCAAAAACCTGGCTTGATAATGAGTTTCCGGA CTCTGCCCCAGGGAAA
TCAACAATAATTGATTGGTATGCAAAATTC AAGCGTGGTGAAATGAGCACGGAGGAC
GGTGAACGCAGTGGACGCCCCGAAAGAGGTGGTTACCGACGAAAACATCAAAAAAAT
CCACAAAATGATTTTGAATGACCGTAAAATGAAGTTGATCGAGATAGCAGAGGCCTT
AAAGATATCAAAGGAACGTGTTGGTCATATCATTCAATATTTGGATATGCGGAA
GCTCTGTGCAAAATGGGTGCCGCGGAGCTCACATTTGACCAAAAACAACACGTGT
TGATGATTCTGAGCGGTGTTTGCAGCTGTAACTCGTAATACACCCGAGTTTTTCCGTC
GATATGTGACAATGGATGAAACATGGCTCCATCACTACACTCCTGAGTCCAATCGAC
AGTCGGCTGAGTGGACAGCGACCGGTGAACCGTCTCCGAAGCGTGGAAGACTCAAA
AGTCCGCTGGCAAAGTAATGGCCTCTGTTTTTTGGGATGCGCATGGAATAATTTTTAT
CGATTATCTTGAGAAGGGAAAAACCATCAACAGTGA CTATTATATGGCGTTATTGGA
GCGTTTGAAGGTCGAAATCGCGGCAAAACGGCCCCACATGAAGAAGAAAAAAGTGTT
GTTCCACCAAGACAACGCACCGTGCCACAAGTCATTGAGAACGATGGCAAAAATTCA

TGAATTGGGCTTCGAATTGCTTCCCCACCCGCCGTATTCTCCAGATCTGGCCCCCAGC
GACTTTTTCTTGTTCAGACCTCAAAAGGATGCTCGCAGGGAAAAAATTTGGCTGCA
ATGAAGAGGTGATCGCCGAACTGAGGCCTATTTTGAGGCAAAACCGAAGGAGTACT
ACCAAAATGGTATCAAAAAATTGGAAGGTCGTTATAATCGTTGTATCGCTCTTGAAGG
GAACTATGTTGAATAAACAGGTTGGCTGATAAGTCCCCGGTCTGAGCTCGCCATCAGT
TCAACCTGTTGATAGTACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTT
AACGTACTAAGCTCTCATGTTTAACGAACTAAACCCTCATGGCTAACGTACTAAGCTC
TCATGGCTAACGTACTAAGCTCTCATGTTTCACGTACTAAGCTCTCATGTTTGAACAA
TAAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAA
AAAAAGAATATATAAGGCTTTTAAAGCTTTTAAGGTTTAACGGTTGTGGACAACAA
GCCAGGGATGTAACGCACTGAGAAGCCCTTAGAGCCTCTCAAAGCAATTTTCAGTGA
CGAATTCTGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACCTGGCGGGC
TACCGTTTCGTATAATGTATGCTATACGAAGTTATGAAGGCACGAACCCAGTTGACATA
AGCCTGTTTCGGTTCGTAAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCC
AGAACCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGT
TATGACTGTTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACG
CCGTGGGTCGATGTTTGTATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGAT
GTTACGCAGCAGGGCAGTCGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATC
ATTCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATC
TTTTCGGTCGTGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGA
TTACCTCGGGAACCTTGCTCCGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAA
GAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCCAGGTTTGAGCAGCCGCGTA
GTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTG
CCACCGCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGAT
CTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGG
CATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACCTAACAATT
CGTTCAAGCCGAATAACTTCGTATAATGTATGCTATACGAACGGTATTAAGACCCACT
TTCACATTTAAGTTGTTTTTCTAATCCGCATATGATCAATTCAAGGCCGAATAAGAAG
GCTGGCTCTGCACCTTGGTGATCAAATAATTCGATAGCTTGTCGTAATAATGGCGGCA
TACTATCAGTAGTAGGTGTTTCCCTTTCTTCTTTAGCGACTTGATGCTCTTGATCTTCC
AATACGCAACCTAAAGTAAAATGCCCCACAGCGCTGAGTGCATATAATGCATTCTCT
AGTGAAAAACCTTGTTGGCATAAAAAGGCTAATTGATTTTCGAGAGTTTCATACTGTT
TTTCTGTAGGCCGTGTACCTAAATGTACTTTTGCTCCATCGCGATGACTTAGTAAAGC
ACATCTAAAACCTTTTAGCGTTATTACGTAAAAAATCTTGCCAGCTTTCCCCTTCTAAA
GGGCAAAAGTGAGTATGGTGCCTATCTAACATCTCAATGGCTAAGGCGTCGAGCAAA
GCCCGCTTATTTTTTACATGCCAATACAATGTAGGCTGCTCTACACCTAGCTTCTGGGC
GAGTTTACGGGTGTTTAAACCTTCGATTCCGACCTCATTAAGCAGCTCTAATGCGCTG
TTAATCACTTTACTTTTATCTAATCTAGACATCATTAATTCCTAATTTTTGTTGACACTC
TATCATTGATAGAGTTATTTTACCACTCCCTATCAGTGATAGAGAAGAGGCACTAAAT
GAACACGATTAACATCGCTAAGAACGACTTCTCTGACATCGAACTGGCTGCTATCCCG
TTCAACACTCTGGCTGACCATTACGGTGAGCGTTTAGCTCGCGAACAGTTGGCCCTTG
AGCATGAGTCTTACGAGATGGGTGAAGCACGCTTCCGCAAGATGTTTGAGCGTCAAC
TTAAAGCTGGTGAGGTTGCGGATAACGCTGCCGCCAAGCCTCTCATCACTACCCTACT
CCCTAAGATGATTGCACGCATCAACGACTGGTTTGAGGAAGTGAAAGCTAAGCGCGG

CAAGCGCCCGACAGCCTTCCAGTTCCTGCAAGAAATCAAGCCGGAAGCCGTAGCGTA
CATCACCATTAAGACCACTCTGGCTTGCCTAACCAGTGCTGACAATACAACCGTTCAG
GCTGTAGCAAGCGCAATCGGTTCGGGCCATTGAGGACGAGGCTCGCTTCGGTTCGTATC
CGTGACCTTGAAGCTAAGCACTTCAAGAAAAACGTTGAGGAACAACCTCAACAAGCGC
GTAGGGCACGTCTACAAGAAAGCATTATGCAAGTTGTCGAGGCTGACATGCTCTCTA
AGGGTCTACTCGGTGGCGAGGCGTGGTCTTCGTGGCATAAGGAAGACTCTATTCATGT
AGGAGTACGCTGCATCGAGATGCTCATTGAGTCAACCGGAATGGTTAGCTTACACCG
CCAAAATGCTGGCGTAGTAGGTCAAGACTCTGAGACTATCGAACTCGCACCTGAATA
CGCTGAGGCTATCGCAACCCGTGCAGGTGCGCTGGCTGGCATCTCTCCGATGTTCCAA
CCTTGCGTAGTTCCCTCCTAAGCCGTGGACTGGCATTACTGGTGGTGGCTATTGGGCTA
ACGGTCGTCGTCTCTGGCGCTGGTGCCTACTCACAGTAAGAAAGCACTGATGCGCTA
CGAAGACGTTTACATGCCTGAGGTGTACAAAGCGATTAAACATTGCGCAAAACACCGC
ATGGAAAATCAACAAGAAAGTCCTAGCGGTTCGCCAACGTAATCACCAAGTGGAAGCA
TTGTCCGGTCGAGGACATCCCTGCGATTGAGCGTGAAGAACTCCCGATGAAACCGGA
AGACATCGACATGAATCCTGAGGCTCTACCGCGTGGAACGTGCTGCCGCTGCTGT
GTACCGCAAGGACAAGGCTCGCAAGTCTCGCCGTATCAGCCTTGAGTTCATGCTTGAG
CAAGCCAATAAGTTTGCTAACCATAAGGCCATCTGGTTCCTTACAACATGGACTGGC
GCGGTTCGTGTTTACGCTGTGTCAATGTTCAACCCGCAAGGTAACGATATGACCAAAG
GACTGCTTACGCTGGCGAAAGGTAAACCAATCGGTAAGGAAGGTTACTACTGGCTGA
AAATCCACGGTGCAAACCTGTGCGGGTGTGATAAGGTTCCGTTCCCTGAGCGCATCA
AGTTCATTGAGGAAAACACGAGAACATCATGGCTTGCGCTAAGTCTCCACTGGAGA
ACACTTGGTGGGCTGAGCAAGATTCTCCGTTCTGCTTCCTTGCGTTCTGCTTTGAGTAC
GCTGGGGTACAGCACACCGGCCTGAGCTATAACTGCTCCCTTCCGCTGGCGTTTGACG
GGTCTTGCTCTGGCATCCAGCACTTCTCCGCGATGCTCCGAGATGAGGTAGGTGGTCG
CGCGGTTAACTTGCTTCCTAGTGAAACCGTTCAGGACATCTACGGGATTGTTGCTAAG
AAAGTCAACGAGATTCTACAAGCAGACGCAATCAATGGGACCGATAACGAAGTAGTT
ACCGTGACCGATGAGAACACTGGTGAAATCTCTGAGAAAGTCAAGCTGGGCACTAAG
GCACTGGCTGGTCAATGGCTGGCTTACGGTGTTACTCGCAGTGTGACTAAGCGTTCAG
TCATGACGCTGGCTTACGGGTCCAAAGAGTTCGGCTTCGTCAACAAGTGCTGGAAG
ATACCATTACGCCAGCTATTGATTCCGGCAAGGGTCTGATGTTCACTCAGCCGAATCA
GGCTGCTGGATACATGGCTAAGCTGATTTGGGAATCTGTGAGCGTGACGGTGGTAGC
TGCGGTTGAAGCAATGAACTGGCTTAAGTCTGCTGCTAAGCTGCTGGCTGCTGAGGTC
AAAGATAAGAAGACTGGAGAGATTCTTCGCAAGCGTTGCGCTGTGCATTGGGTAACT
CCTGATGGTTTTCCCTGTGTGGCAGGAATACAAGAAGCCTATTCAGACGCGCTTGAACC
TGATGTTCCCTCGGTCAGTTCCGCTTACAGCCTACCATTAACACCAACAAAGATAGCGA
GATTGATGCACACAAACAGGAGTCTGGTATCGCTCCTAACTTTGTACACAGCCAAGA
CGGTAGCCACCTTCGTAAGACTGTAGTGTGGGCACACGAGAAGTACGGAATCGAATC
TTTTGCACTGATTCACGACTCCTTCGGTACCATTCCGGCTGACGCTGCGAACCTGTTCA
AAGCAGTGCGCGAAACTATGGTTGACACATATGAGTCTTGATGTACTGGCTGATTT
CTACGACCAGTTCGCTGACCAGTTGCACGAGTCTCAATTGGACAAAATGCCAGCACTT
CCGGCTAAAGGTAACCTGAACTCCGTGACATCTTAGAGTCGGACTTCGCGTTCGCGT
AAGAATTCGGATCCAGACCGGGGACTTATCAGCCAACCTGTTATGTGGCGCGGTATT
ATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTTCGCCGCATACACTATTCTCAGAAT
GACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTA

AGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTC
TGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATC
ATGTAAATCTGCAGAATTCCAGCACACTGGCGGCCGTTACTAGACCGTCCAGTCTGGC
AGGCCGGAACATCGGTCAGCAGATAGGCTTTACCAGTAAGAAGGAGATATACAT
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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

TCCATGGCCTCCTTAGAACGGAATGTCGTCGTCGAAGCTGTCGTAGTCCTGGGCCGGT
TGCGGCGCCGGTTGCTGCTGCGGGGCCGGATCCATATGTCAGCCGTTAAGTGTTCTCTG
TGTCACTCAAATTGCTTTGAGAGGCTCTAAGGGCTTCTCAGTGC GTTACATCCCTGG
CTTGTTGTCCACAACCGTTAAACCTTAAAAGCTTTAAAAGCCTTATATATTCTTTTTT
TCTTATAAACTTAAACCTTAGAGGCTATTTAAGTTGCTGATTTATATTAATTTTATT
GTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCTTAGTACGTTAGCCATGAG
AGCTTAGTACGTTAGCCATGAGGGTTAGTTTCGTAAACATGAGAGCTTAGTACGTTA
AACATGAGAGCTTAGTACGTGAAACATGAGAGCTTAGTACGTACTATCAACAGGTTG
AACTTAAGGATCCTGCGGCCCCGAGGGCGGCAGGGGGTCTTCGAAGGTCTGCCCCGTGG
CGCGGACGGGCCGCGCCGAAGGGCGTCGCAGCTGAATTACATTCCCAACCGCGTGG
CACAACAACCTGGCGGGCTACCGTTCTGTATAATGTATGCTATACGAAGTTATGAAGGC
ACGAACCCAGTTGACATAAGCCTGTTTCGGTTCGTAAACTGTAATGCAAGTAGCGTATG
CGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTG
GCGGTTTTTCATGGCTTGTTATGACTGTTTTTTTGTACAGTCTATGCCTCGGGCATCCAA
GCAGCAAGCGCGTTACGCCGTGGGTCGATGTTTGATGTTATGGAGCAGCAACGATGT
TACGCAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACAAAGTTAGGTG
GCTCAAGTATGGGCATCATTCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCAT
GCGGGCTGCTCTTGATCTTTTCGGTCGTGAGTTCGGAGACGTAGCCACCTACTCCCAA

CATCAGCCGGACTCCGATTACCTCGGGAAGTTGCTCCGTAGTAAGACATTCATCGCGC
TTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCAG
GTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCAC
CGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCG
CTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTC
TCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTA
CCGCCACCTAACAATTCGTTCAAGCCGAATAACTTCGTATAATGTATGCTATACGAAC
GGTATTAAGACCCACTTTCACATTTAAGTTGTTAACGTAACAGAGACAGCACGTTCTG
CGGGACCTGGTTAGCCTGCGCCAGAACGGAAGTACCGGCCTGCTGCAGAATCTGCGC
GCGAGACATGTTGGAAACTTCGGTCGCGTAGTCGGAATCTTCGATACGGCTACGCGCT
TCAGACAGATTGTTTACGGTATTGCCAGGTTGGTGATAGCAGAGTTGAAACGGTTTT
GTACCGCACCCAGATCAGAGCGCAGCGCATCCACCTGCGCCAGCGCGGCATCAATTT
TCTGCAGCGGGTTTTCGGTGGTTTTAGCGGCTGCTTCCGCCAGCTCTGGTTGTGCTTTG
AAATCATGACCAGCGGCTTTGCGTTCACTATTATTTAGTGAAATGAGATATTATGATA
TTTTCTGAATTGTGATTA AAAAGGCAACTTTATGCCCATGCAACAGAACTATAAAAA
ATACAGAGAATGAAAAGAAACAGATAGATTTTTTTAGTTCTTTAGGCCCCGTAGTCTGCA
AATCCTTTTATGATTTTCTATCAAACAAAAGAGGAAAATAGACCAGTTGCAATCCAAA
CGAGAGTCTAATAGAATGAGGTCGAAAAGTAAATCGCGCGGGTTTGTTACTGATAAA
GCAGGCAAGACCTAAAATGTGTAAAGGGCAAAGTGTATACTTTGGCGTCACCCCTTA
CATATTTTAGGTCTTTTTTTATTGTGCGTAACTAACTTGCCATCTTCAAACAGGAGGGC
TGGAAGAAGCAGACCGCTAACACAGTACATAAAAAAGGAGACATGAACGATGAACA
TCAAAAAGTTTGCAAAACAAGCAACAGTATTAACCTTTACTACCGCACTGCTGGCAG
GAGGCGCAACTCAAGCGTTTGCGAAAGAAACGAACCAAAAGCCATATAAGGAAACA
TACGGCATTTC CATATTACACGCCATGATATGCTGCAAATCCCTGAACAGCAAAAAA
ATGAAAAATATCAAGTTCCTGAGTTCGATTTCGTCCACAATTAAAAATATCTCTTCTGC
AAAAGGCCTGGACGTTTGGGACAGCTGGCCATTACAAAACGCTGACGGCACTGTGCG
AAACTATCACGGCTACCACATCGTCTTTGCATTAGCCGGAGATCCTAAAAATGCGGAT
GACACATCGATTTACATGTTCTATCAAAAAGTCGGCGAAACTTCTATTGACAGCTGGA
AAAACGCTGGCCGCGTCTTTAAAGACAGCGACAAATTCGATGCAAATGATTCTATCCT
AAAAGACCAACACAAGAATGGTCAGGTTACAGCCACATTTACATCTGACGGAAAAAT
CCGTTTATTCTACACTGATTTCTCCGGTAAACATTACGGCAAACAAACACTGACAAC
GCACAAGTTAACGTATCAGCATCAGACAGCTCTTTGAACATCAACGGTG TAGAGGAT
TATAAATCAATCTTTGACGGTGACGGAAAAACGTATCAAAATGTACAGCAGTTCATC
GATGAAGGCAACTACAGCTCAGGCGACAACCATACGCTGAGAGATCCTCACTACGTA
GAAGATAAAGGCCACAAATACTTAGTATTTGAAGCAAACACTGGAAGTGAAGATGGC
TACCAAGGCGAAGAATCTTTATTTAAACAAAGCATACTATGGCAAAGCACATCATTCT
TCCGTCAAGAAAGTCAAAAACCTTCTGCAAAGCGATAAAAAACGCACGGCTGAGTTAG
CAAACGGCGCTCTCGGTATGATTGAGCTAAACGATGATTACACACTGAAAAAAGTGA
TGAAACCGCTGATTGCATCTAACACAGTAACAGATGAAATTGAACGCGCGAACGTCT
TTAAATGAACGGCAAATGGTATCTGTTCACTGACTCCCGCGGATCAAAAATGACGA
TTGACGGCATTACGTCTAACGATATTTACATGCTTGGTTATGTTTCTAATTCTTTAACT
GGCCCATACAAGCCGCTGAACAAAACCTGGCCTTGTGTTAAAAATGGATCTTGATCCTA
ACGATGTAACCTTTACTTACTCACACTTCGCTGTACCTCAAGCGAAAGGAAACAATGT
CGTGATTACAAGCTATATGACAAACAGAGGATTCTACGCAGACAAACAATCAACGTT

TGCGCCTAGCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGTTGTCAAAGACAG
CATCCTTGAACAAGGACAATTAACAGTTAACAAACTGTATCTTTCACATCATAACGCTT
TCTGCACGTTTCAGTGAGTCCAGACCCAGGGTCTGAGAGTTGATCTGCTTCAGATCGAT
ATCGATAGTTTTACCGTCGTTGGCGCCAACCTGGATGGTCAGGGTGTTCCTGCGCC
AGGACTTTCACGCCGTTGAACTGAGTCTGGCCGGATACACGGTCGATTCGTTTCAGGC
GCTGGGTGATTTTCAGCCTGGATGGAGTCGAGGTCAGACTGGGAGTTGGTGCTGTTAG
CAGACTGAACCGCCAGTTCACGCACACGCTGCAGGTTGTTGTTGATTCGTTTCAGCGC
GCCTTCAGTGGTCTGCGCAATGGAGATACCGTCGTTAGCGTTACGGGAAGCCTGAGTC
AGACCTTTGATGTTTCGCGGTGAAACGGTTAGCAATCGCCTGACCTGCCGCATCGTCTT
TCGCGCTGTTGATACGCAGACCAGAAGACAGACGCTCGATAGCGGTGCCAGTGCGG
ACTGGGATTTGTTTCAGGTTATTCTGGGTCAGCAGCGACAGACTGTTAGTGTTGATTAC
TTGTGCCAT

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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
CGCTCACCGGGCTGGTTGCCCTCGCCGCTGGGCTGGCGGCCGTCTATGGCCCTGCAAA
CGCGCCAGAAACGCCGTCTGAAGCCGTGTGCGAGACACCGCGGCCGCCGGCGTTGTGG
ATACCTCGCGGAAACTTGGCCCTCACTGACAGATGAGGGGCGGACGTTGACACTTG
AGGGGCCGACTACCCGGCGCGCGTTGACAGATGAGGGGCAGGCTCGATTTTCGGCC
GGCGACGTGGAGCTGGCCAGCCTCGAAATCGGCGAAAACGCCTGATTTTACGCGAG
TTTCCCACAGATGATGTGGACAAGCCTGGGGATAAGTGCCCTGCGGTATTGACACTTG
AGGGGCGCGACTACTGACAGATGAGGGGCGCGATCCTTGACACTTGAGGGGCAGAGT
GCTGACAGATGAGGGGCGCACCTATTGACATTTGAGGGGCTGTCCACAGGCAGAAAA
TCCAGCATTTGCAAGGGTTTCCGCCCGTTTTTCGGCCACCGCTAACCTGTCTTTTAACC
TGCTTTTAAACCAATATTTATAAACCTTGTTTTTAACCAGGGCTGCGCCCTGTGCGCGT

GACCGCGCACGCCGAAGGGGGGTGCCCCCCTTCTCGAACCCTCCCGGCCCGCTCTCG
AGTTGGCAGCATCACCCATAATTGTGGTTTCAAAATCGGCTCCGTCGATACTATGTTA
TACGCCAACTTTGAAAACAACTTTGAAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGA
ATGCAAGGAACAGTGAATTGGAGTTCGTCTTGTTATAATTAGCTTCTTGGGGTATCTT
TAAATACTGTAGAAAAGAGGAAGGAAATAATAAGTGCAATACGAATGGCGAAAAGC
CGAGCTCATCGGTCAGCTTCTCAACCTTGGGGTTACCCCCGGCGGTGTGCTGCTGGTC
CACAGCTCCTTCCGTAGCGTCCGGCCCCCTCGAAGATGGGCCACTTGGACTGATCGAGG
CCCTGCGTGCTGCGCTGGGTCCGGGAGGGACGCTCGTCATGCCCTCGTGGTCAGGTCT
GGACGACGAGCCGTTTCGATCCTGCCACGTCGCCC GTTACACCGGACCTTGGAGTTGTC
TCTGACACATTCTGGCGCCTGCCAAATGTAAAGCGCAGCGCCCATCCATTTGCCTTTG
CGGCAGCGGGGCCACAGGCAGAGCAGATCATCTCTGATCCATTGCCCTGCCACCTC
ACTCGCCTGCAAGCCCGGTGCCCCGTGTCCATGAACTCGATGGGCAGGTACTTCTCCT
CGGCGTGGGACACGATGCCAACACGACGCTGCATCTTGCCGAGTTGATGGCAAAGGT
TCCCTATGGGGTGCCGAGACACTGCACCATTCTTCAGGATGGCAAGTTGGTACGCGTC
GATTATCTCGAGAATGACCACTGCTGTGAGCGCTTTGCCTTGGCGGACAGGTGGCTCA
AGGAGAAGAGCCTTCAGAAGGAAGGTCCAGTCGGTCATGCCTTTGCTCGGTTGATCC
GCTCCCGCGACATTGTGGCGACAGCCCTGGGTCAACTGGGCCGAGATCCGTTGATCTT
CCTGCATCCGCCAGAGGCGGGATGCGAAGAATGCGATGCCGCTCGCCAGTCGATTGG
CTGAGTTCATATTATTTAGTGAAATGAGATATTATGATATTTTCTGAATTGTGATTAA
AAAGGCAACTTTATGCCCATGCAACAGAACTATAAAAAATACAGAGAATGAAAAG
AAACAGATAGATTTTTTAGTTCTTTAGGCCCGTAGTCTGCAAATCCTTTTATGATTTTC
TATCAAACAAAAGAGGAAAATAGACCAGTTGCAATCCAAACGAGAGTCTAATAGAAT
GAGGTGCAAAAAGTAAATCGCGCGGGTTTGTTACTGATAAAGCAGGCAAGACCTAAAA
TGTGTAAAGGGCAAAGTGTATACTTTGGCGTCACCCCTTACATATTTTAGGTCTTTTTT
TATTGTGCGTAACTAACTTGCCATCTTCAAACAGGAGGGCTGGAAGAAGCAGACCGC
TAACACAGTACATAAAAAAGGAGACATGAACGATGAACATCAAAAAGTTTGCAAAA
CAAGCAACAGTATTAACCTTTACTACCGCACTGCTGGCAGGAGGCGCAACTCAAGCG
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ACACGCCATGATATGCTGCAAATCCCTGAACAGCAAAAAAATGAAAAATATCAAGTT
CCTGAGTTCGATTCGTCCACAATTA AAAATATCTCTTCTGCAAAAGGCCTGGACGTTT
GGGACAGCTGGCCATTACAAAACGCTGACGGCACTGTCGCAAACTATCACGGCTACC
ACATCGTCTTTGCATTAGCCGGAGATCCTAAAAATGCGGATGACACATCGATTTACAT
GTTCTATCAAAAAGTCGGCGAAACTTCTATTGACAGCTGGAAAAACGCTGGCCGCGT
CTTTAAAGACAGCGACAAATTCGATGCAAATGATTCTATCCTAAAAGACCAACACA
AGAATGGTCAGGTTTACGCCACATTTACATCTGACGGAAAAATCCGTTTATTCTACACT
GATTTCTCCGGTAAACATTACGGCAAACAAACACTGACAACTGCACAAGTTAACGTA
TCAGCATCAGACAGCTCTTTGAACATCAACGGTGTAGAGGATTATAAATCAATCTTTG
ACGGTGACGGAAAAACGTATCAAATGTACAGCAGTTCATCGATGAAGGCAACTACA
GCTCAGGCGACAACCATAACGCTGAGAGATCCTCACTACGTAGAAGATAAAGGCCACA
AATACTTAGTATTTGAAGCAAACACTGGAAGTGAAGATGGCTACCAAGGCGAAGAAT
CTTTATTTAACAAGCATACTATGGCAAAAGCACATCATTCTTCCGTCAAGAAAGTCA
AAA ACTTCTGCAAAGCGATAAAAAACGCACGGCTGAGTTAGCAAACGGCGCTCTCGG
TATGATTGAGCTAAACGATGATTACACACTGAAAAAAGTGATGAAACCGCTGATTGC
ATCTAACACAGTAACAGATGAAATTGAACGCGCAACGTCTTTAAAATGAACGGCAA

ATGGTATCTGTTCACTGACTCCCGCGGATCAAAAATGACGATTGACGGCATTACGTCT
AACGATATTTACATGCTTGGTTATGTTTCTAATTCTTTAACTGGCCCATACAAGCCGCT
GAACAAAACCTGGCCTTGTGTTAAAAATGGATCTTGATCCTAACGATGTAAACCTTTACT
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TGACAAACAGAGGATTCTACGCAGACAAACAATCAACGTTTGCGCCTAGCTTCCTGCT
GAACATCAAAGGCAAGAAAACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACA
ATTAACAGTTAACAAATACCTAGATGTGGCGCAACGATGCCGGCGACAAGCAGGAGC
GCACCGACTTCTTCCGCATCAAGTGTTTTGGCTCTCAGGCCGAGGCCACGGCAAGTA
TTTGGGCAAGGGGTCGCTGGTATTCGTGCAGGGCAAGATTCGGAATACCAAGTACGA
GAAGGACGGCCAGACGGTCTACGGGACCGACTTCATTGCCGATAAGGTGGATTATCT
GGACACCAAGGCACCAGGCGGGTCAAATCAGGAATAAGGGCACATTGCCCGGGCGT
GAGTCGGGGCAATCCCGCAAGGAGGGTGAATGAATCGGACGTTTGACCGGAAGGCAT
ACAGGCAAGAACTGATCGACGCGGGGTTTTCCGCCGAGGATGCCGAAACCATCGCAA
GCCGCACCGTCATGCGTGCGCCCCGCGAAACCTTCCAGTCCGTCGGCTCGATGGTCCA
GCAAGCTACGGCCAAGATCGAGCGCGACAGCGTGCAACTGGCTCCCCCTGCCCTGCC
CGCGCCATCGGCCCGCGTGGAGCGTTCGCGTCGTCTCGAACAGGAGGCGGCAGGTTT
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CCGCCGGCGAGGACCTGGCAAAACAGGTCAGCGAGGCCAAGCAGGCCGCGTTGCTG
AAACACACGAAGCAGCAGATCAAGGAAATGCAGCTTTCCTTGTTTCGATATTGCGCCG
TGGCCGGACACGATGCGAGCGATGCCAAACGACACGGCCCCGCTCTGCCCTGTTACCC
ACGCGCAACAAGAAAATCCCGCGCGAGGCGCTGCAAAACAAGGTCATTTTCCACGTC
AACAAGGACGTGAAGATCACCTACACCGGCGTCGAGCTGCGGGCCGACGATGACGA
ACTGGTGTGGCAGCAGGTGTTGGAGTACGCGAAGCGCACCCCTATCGGGCAGCCGAT
CACCTTCACGTTCTACGAGCTTTGCCAGGACCTGGGCTGGTCGATCAATGGCCGGTAT
TACACGAAGGCCGAGGAATGCCTGTGCGCCTACAGGCGACGGCGATGGGCTTCACG
TCCGACCGCGTTGGGCACCTGGAATCGGTGTGCTGCTGCACCGCTTCCGCGTCCTGG
ACCGTGGCAAGAAAACGTCCCGTTGCCAGGTCCTGATCGACGAGGAAATCGTCGTGC
TGTTTGCTGGCGACCACTACACGAAATTCATATGGGAGAAGTACCGCAAGCTGTGCGC
CGACGGCCCGACGGATGTTGACTATTTAGCTCGCACCGGGAGCCGTACCCGCTCA
AGCTGGAAACCTTCCGCCTCATGTGCGGATCGGATTCCACCCGCGTGAAGAAGTGGC
GCGAGCAGGTGCGCGAAGCCTGCGAAGAGTTGCGAGGCAGCGGCCTGGTGGAACAC
GCCTGGGTCAATGATGACCTGGTGCATTGCAAACGCTAGACGCTCAGTGGAACGAGG
TTCATGTGCAGCTCCATCAGCAAAAGGGGATGATAAGTTTATCACCACCGACTATTTG
CAACAGTGCCGTTGATCGTGCTATGATCGACTGATGTCATCAGCGGTGGAGTGCAATG
TCGTTGCGTCGCGGTGCATGGAGCCGGGCCACCTCGACCTGAATGGAAGCCGGCGGC
ACCTCGCTAACGGATTCACCACTCCAAGATGCATAATGTGCCTGTCAAATGGACGAA
GCAGGGATTCTGCAAAACCCTATGCTACTCCGTCAAGCCGTCAATTGTCTGATTCTTA
CCAATTATGACAACTTGACGGCTACATCATTTACTTTTTCTTCACAACCGGCACGGAA
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TCAAAACCAACATTGCGACCGACGGTGGCGATAGGCATCCGGGTGGTGCTCAAAAGC
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GGCGAAAGAACCCCGTATTGGCAAATATTGACGGCCAGTTAAGCCATTTCATGCCAGT
AGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTTCGCGAGCCTCCGGATGAC
GACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATATCACCCGGTCGGCAAA
CAAATTCTCGTCCCTGATTTTTTACCACCCCTGACCGCGAATGGTGAGATTGAGAAT
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TCATTTTGCGCTTCAGCCATACTTTTCATACTCCCGCCATTTCAGAGAAGAAACCAATT
GTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCC
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ACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTAT
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CCGGTCGATGCAACGAGTGATGAGGTTTCGCAAGAACCTGATGGACATGTTTCAGGGAT
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CGGCATGGTGCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTT
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TTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGC
AATGCTGTTTCACTGGTTATGCGGCGGATCCGAAAAGAAAACGTTGATGCCGGTGAA
CGTGCAAAACAGGCTCTAGCGTTCGAACGCACTGATTTTCGACCAGGTTTCGTTCACTCA
TGGAATAAGCGATCGCTGCCAGGATATACGTAATCTGGCATTCTGTTGGGATTGCTTA
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ACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACC
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TCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTTGCCGGGTCAGAAAAA
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GATGGCGATTAGCCAGCCCGCCTAATGAGCGGGCTTTTTTTTGAACAAAATGA

//

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

AGCTGAATTACATTCCCAACCGCGTGGCACAACAACCTGGCGGGCTACCGTTCGTATA
ATGTATGCTATACGAAGTTATGAAGGCACGAACCCAGTTGACATAAGCCTGTTTCGGTT
CGTAAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTTGACC
GAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGTTATGACTGTTTTT
TTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTCGATG
TTTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGCAGG
GCAGTCGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTTCGCACATGTA
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GTTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGAATCCGATTACCTCGGGAA
CTTGCTCCGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTT
GGCGCTCTCGCGGCTTACGTTCTGCCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATA
TCTATGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCAT
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GATTACGGTGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAA
GTGATGCACTTTGATATCGACCCAAGTACCGCCACCTAACAATTCGTTCAAGCCGAAT
AACTTCGTATAATGTATGCTATACGAACGGTATTAAGACCCACTTTCACATTTAAGTT
GTTTCGCGCCGCTGGTGCCGCTGGTTGGACGCCAAGGGTGAATCCGCCTCGATACCCTG
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GCCCAGACGCCTCCCCCGCCCCTTCAGGGGCACAAATGCGGCCCCAACGGGGCCACG
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ACGTGGCCTCACCCCCAATGGTTTACAAAAGCAATGCCCTGGTCGAGGCCGCGTATC
GCCTCAGTGTTACAGGAACAGCGGATCGTTCTGGCCTGTATTAGCCAGGTGAAGAGGA
GCGAGCCTGTCACCGATGAAGTGATGTATTACAGTGACGGCGGAGGACATAGCGACGA
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GAAACGGCGGGAAGTCCGGTTAACCCAAGAGCCCAATGGCAAGGGGAAAAGACCGA
GTGTGATGATTACCGGCTGGGTGCAAACAATCATCTACCGGGAGGGTGAGGGCCGTG
TAGAACTCAGGTTACCAAAGACATGCTGCCGTACCTGACGGAACCTACCAAACAGT
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TTTACGAGCTGCTCATGCAATGGGACAGCATCGGCCAGCGCGAAATAGAAATTGACC
AGCTGCGAAAGTGGTTTCAACTGGAAGGCCGGTATCCCTCGATCAAGGACTTCAAGT
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CAAGCGAAAGGAAACAATGTCGTGATTACAAGCTATATGACAAACAGAGGATTCTAC
GCAGACAAACAATCAACGTTTGC GCCTAGCTTCCTGCTGAACATCAAAGGCAAGAAA
ACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACAATTAACAGTTAACAAATTC
AGGTATCGATACCGTCGACGGGGATTCTTAAGGTATACTTTCCGCTGCATAACCCTG
CTTCGGGGTCATTATAGCGATTTTTTCGGTATATCCATCCTTTTTTCGCACGATATACAG
GATTTTGCCAAAGGGTTCGTGTAGACTTTCCTTGGTGTATCCAACGGCGTCAGCCGGG
CAGGATAGGTGAAGTAGGCCCCACCCGCGAGCGGGTGTTCCTTCTTCACTGTCCCTTAT
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CTTGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGG
CGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA
GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAGGCTC
CCTCGTGCCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCC
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GCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC
TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
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CGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAA
CAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAA
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ATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTT
GGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATT
TCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGC
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ACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTCGAATTGGCCGCGGC
GTTGTGACAATTTACCGAACAACTCCGCGGCCGGGAAGCCGATCTCGGCTTGAACGA
ATTG

Supplementary note 20: Complete sequence of the pHERD30T-BAD-red γ β α -Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-red γ β α -kan-amp-ccdB 16255 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-red γ β α -kan-amp-ccdB

ACCESSION pHERD30T-BAD-red γ β α -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..14788 /label="red γ " /note="gam protein from <i>E. coli</i> , recBCD inhibitor "
CDS	14785..15570 /label="red β " /note=" ssDNA annealing protein from <i>E. coli</i> "
CDS	15567..16247 /label="red α " /note=" exonuclease from <i>E. coli</i> "

ORIGIN

ATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAG
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 CTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCT
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 TTGTATTGTGGGACGCAGATACAGTGTCCTTATACACAAGGAATGTCGAACGTGGCCTCA
 CCCCCAATGGTTTACAAAAGCAATGCCCTGGTCGAGGCCGCGTATCGCCTCAGTGTTTCAGG
 AACAGCGGATCGTTCTGGCCTGTATTAGCCAGGTGAAGAGGAGCGAGCCTGTCACCGATGA
 AGTGATGTATTCAAGTGACGGCGGAGGACATAGCGACGATGGCGGGTGTCCCTATCGAATCTT
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CCTGACGGAAC TCACCAAACAGTTCACCAAATACGCCTTGGCTGACGTGGCCAAGATGGA
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Supplementary note 21: Complete sequence of the pHERD30T-BAD-pluγβ α -Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-pluγβ α -kan-amp-ccdB 16226 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-pluγβ α -kan-amp-ccdB

ACCESSION pHERD30T-BAD-pluγβ α -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="pluγ" /note="gam protein from Photorhabdus, recBCD inhibitor "
CDS	14617..15534 /label="pluβ" /note=" ssDNA annealing protein from Photorhabdus"
CDS	15538..16218 /label="pluα" /note=" exonuclease from Photorhabdus"

ORIGIN

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Supplementary note 22: Complete sequence of the pHERD30T-BAD-TE_{Psy}-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-TE_{Psy}-kan-amp-ccdB 16916 bp DNA circular SYN 21-MAR-2019

DEFINITION pHERD30T-BAD-TE_{Psy}-kan-amp-ccdB

ACCESSION pHERD30T-BAD-TE_{Psy}-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 <i>Pseudomonas syringae</i> "
CDS	15427..16908 /label="recEpsy" /note="exonuclease from <i>Pseudomonas syringae</i> "

ORIGIN

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Supplementary note 23: Complete sequence of the pHERD30T-BAD-pluγTE_{Psy}-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-pluγTE_{Psy}-kan-amp-ccdB 17187 bp DNA circular SYN
21-MAR-2019

DEFINITION pHERD30T-BAD-pluγTE_{Psy}-kan-amp-ccdB

ACCESSION pHERD30T-BAD-pluγTE_{Psy}-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="pluγ" /note="gam protein from Photorhabdus, recBCD inhibitor "
CDS	14634..15521 /label="recTpsy" /note="ssDNA annealing protein, recombinase from Pseudomonas syringae"
CDS	15518..17179 /label="recEpsy" /note="exonuclease from Pseudomonas syringae"

ORIGIN

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Supplementary note 24: Complete sequence of the pHERD30T-BAD-red γ TE_{Psy}-Rha-Cas-kan-amp-ccdB plasmid in Genbank format.

LOCUS pHERD30T-BAD-red γ TE_{Psy}-kan-amp-ccdB 17346 bp DNA circular SYN
21-MAR-2019

DEFINITION pHERD30T-BAD-red γ TE_{Psy}-kan-amp-ccdB

ACCESSION pHERD30T-BAD-red γ TE_{Psy}-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..14779 /label="redY" /note="gam protein from <i>E. coli</i> , recBCD inhibitor "
CDS	14793..15680 /label="recTpsy" /note="ssDNA annealing protein, recombinase from <i>Pseudomonas syringae</i> "
CDS	15677..17338 /label="recEpsy" /note="exonuclease from <i>Pseudomonas syringae</i> "

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"

CDS	13139..14017 /label="araC" /note="regulator gene of L-arabinose operon"
CDS	14363..15106 /label="beta" /note="ssDNA annealing protein from Pseudomonas aeruginosa"
CDS	15093..15716 /label="alpha" /note="exonuclease from Pseudomonas aeruginosa"
CDS	15739..16182 /gene="SSB" /note="single strand binding protein from Pseudomonas aeruginosa"

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" /note="ssDNA annealing protein from Pseudomonas aeruginosa"
CDS	15346..15987 /label="alpha" /note="exonuclease from Pseudomonas aeruginosa"
CDS	16010..16453 /gene="SSB" /note="single strand binding protein from Pseudomonas aeruginosa"

ORIGIN

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

LOCUS pHERD30T-BAD-red γ BAS-kan-amp-ccdB 16620 bp DNA circular SYN
21-MAR-2019

DEFINITION pHERD30T-BAD-red γ BAS-kan-amp-ccdB

ACCESSION pHERD30T-BAD-red γ 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"

CDS	13139..14017 /label="araC" /note="regulator gene of L-arabinose operon"
CDS	14363..14776 /label="redY" /note="gam protein from <i>E. coli</i> , recBCD inhibitor "
CDS	14793..15536 /label="beta" /note="ssDNA annealing protein from <i>Pseudomonas aeruginosa</i> "
CDS	15523..16146 /label="alpha" /note="exonuclease from <i>Pseudomonas aeruginosa</i> "
CDS	16169..16612 /gene="SSB" /note="single strand binding protein from <i>Pseudomonas aeruginosa</i> "

ORIGIN

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