Authorized host vector system

Rhodococcus Expression Vectors

pTip, pNit, pCpi Vector Series

pTip Vector Series（Product No. : RE-0001 ～ RE-0008）
pNit Vector Series（Product No. : RE-0009 ～ RE-0016）
pCpi Vector Series（Product No. : RE-0017 ～ RE-0024）

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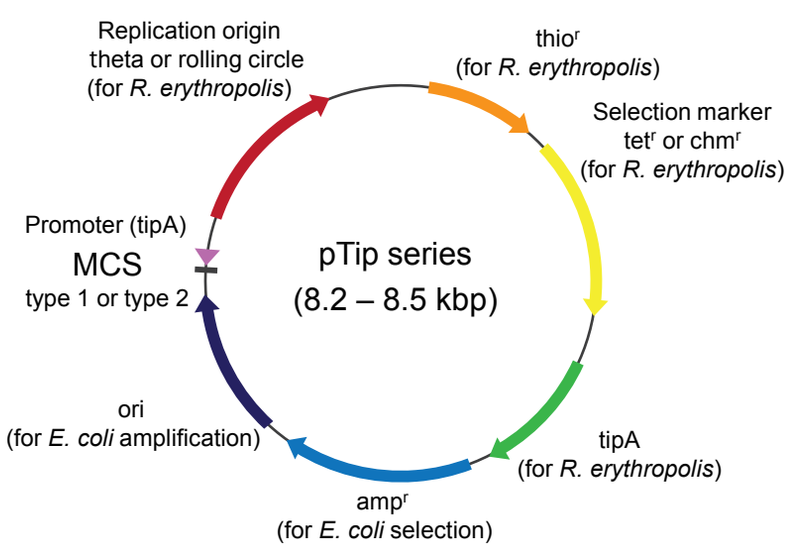
1. Description

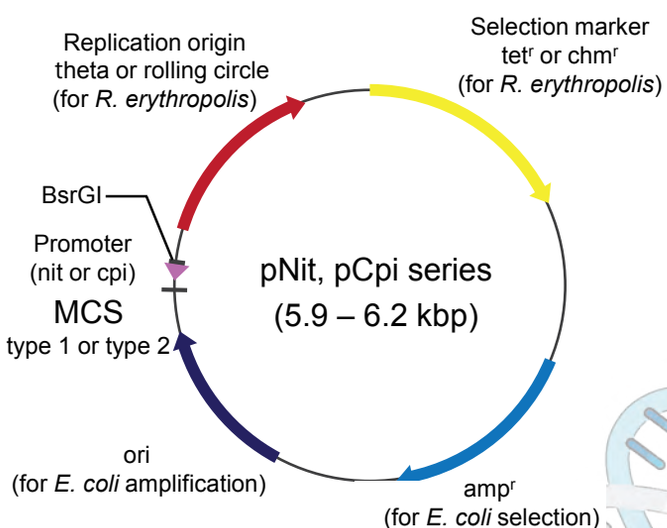
Regulation of expression level and co-expression are workable with the combination of three promoters (thiostrepton-induced pTip, moderate expression pNit, and high expression pCpi promoters) and two replication origins (theta and rolling circle-types). *Rhodococcus erythropolis* L88 Competent Cells (Product No. RE-L88) is applicable to expression of induced gene. The competent cells can grow and express recombinant protein at 4 to 30 ℃. It is therefore expected to successfully express recombinant protein, which is difficult in *E. coli*, such as lethal protein, protein inhibiting cell proliferation, and protein forming inclusion body. Transformation efficiency is 1×104 colonies/μg at least. pTip, pNit, pCpi Vector Series are shuttle vectors and can be applied to *E. coli* in cloning and vector construction; copy number is 300-500 and it has ampicillin resistance (50 μg/ml). pTip Vector Series have a thiostrepton-induced promoter, which expresses recombinant protein in a dose dependent manner. In most cases, maximum efficiency is achieved under recommended conditions. Since two His-tag sequences are in multi cloning sites, it is easy to design His-tagged recombinant protein and refine it.

2. Content

Mass :	10 μg	Volume :	100 μl	Concentration :	100 ng/μl
Solvent :	T10E1	Storage :	-20 °C		

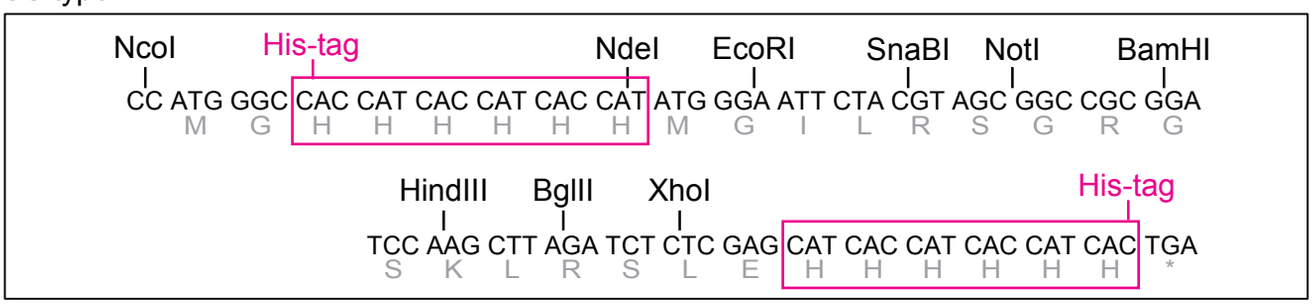
3. Vector Maps



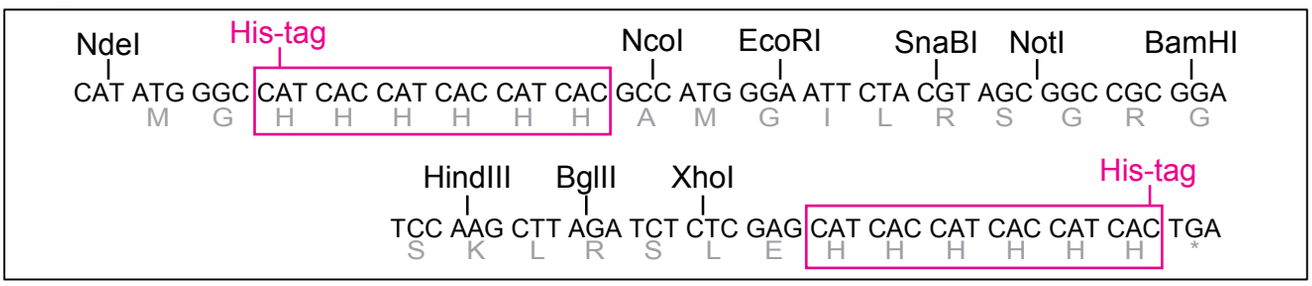


4. Multi Cloning Sites

MCS type 1



MCS type 2



5. List of Vectors

pTip Vector series

Name	Promoter	Selection	Replication	MCS	Size (bp)	Accession	Product code
pTipQT1	tipA (inducible)	tetracycline	theta type	type1	8207	AB127592.1	RE-0001
pTipQT2	tipA (inducible)	tetracycline	theta type	type2	8211	AB127593.1	RE-0002
pTipRT1	tipA (inducible)	tetracycline	rolling circle	type1	8275	AB127596.1	RE-0003
pTipRT2	tipA (inducible)	tetracycline	rolling circle	type2	8279	AB127597.1	RE-0004
pTipQC1	tipA (inducible)	chloramphenicol	theta type	type1	8343	AB127590.1	RE-0005
pTipQC2	tipA (inducible)	chloramphenicol	theta type	type2	8388	AB127591.1	RE-0006
pTipRC1	tipA (inducible)	chloramphenicol	rolling circle	type1	8452	AB127594.1	RE-0007
pTipRC2	tipA (inducible)	chloramphenicol	rolling circle	type2	8456	AB127595.1	RE-0008

pNit Vector series

Name	Promoter	Selection	Replication	MCS	Size (bp)	Accession	Product code
pNitQT1	nit(constitutive)	tetracycline	theta type	type1	5984	AB127600.1	RE-0009
pNitQT2	nit(constitutive)	tetracycline	theta type	type2	5988	AB127601.1	RE-0010
pNitRT1	nit(constitutive)	tetracycline	rolling circle	type1	6058	AB127604.1	RE-0011
pNitRT2	nit(constitutive)	tetracycline	rolling circle	type2	6062	AB127605.1	RE-0012
pNitQC1	nit(constitutive)	chloramphenicol	theta type	type1	6153	AB127598.1	RE-0013
pNitQC2	nit(constitutive)	chloramphenicol	theta type	type2	6157	AB127599.1	RE-0014
pNitRC1	nit(constitutive)	chloramphenicol	rolling circle	type1	6227	AB127602.1	RE-0015
pNitRC2	nit(constitutive)	chloramphenicol	rolling circle	type2	6231	AB127603.1	RE-0016

pCpi Vector series

Name	Promoter	Selection	Replication	MCS	Size (bp)	Accession	Product code
pCpiQT1	Cpi(constitutive)	tetracycline	theta type	type1	5923	-	RE-0017
pCpiQT2	Cpi(constitutive)	tetracycline	theta type	type2	5926	-	RE-0018
pCpiRT1	Cpi(constitutive)	tetracycline	rolling circle	type1	5997	-	RE-0019
pCpiRT2	Cpi(constitutive)	tetracycline	rolling circle	type2	6000	-	RE-0020
pCpiQC1	Cpi(constitutive)	chloramphenicol	theta type	type1	6092	-	RE-0021
pCpiQC2	Cpi(constitutive)	chloramphenicol	theta type	type2	6095	-	RE-0022
pCpiRC1	Cpi(constitutive)	chloramphenicol	rolling circle	type1	6166	-	RE-0023
pCpiRC2	Cpi(constitutive)	chloramphenicol	rolling circle	type2	6169	-	RE-0024

6. Operation Procedure using *Rhodococcus erythropolis* L88

Expression of Recombinant Protein (pTip Vector Series)

<seed culture>

1. Inoculate single colony in LB medium with an antibiotic (tetracycline: 5 µg/ml, chloramphenicol: 20 µg/ml).
2. Incubate it with shaking at 28 °C until stationary phase (2-3 days in general).

<Main culture>

1. Inoculate 1 ml seed culture medium well suspended to 9 ml fresh LB medium (baffled flask is desirable).
2. Incubate it with shaking at 28 °C until OD600 reaches to 0.6 - 0.8.
3. Add thiostrepton (final concentration: 0.1 to 0.2 µg/ml). Thiostrepton stock solution (10 mg/ml in DMSO) can be stored at -20 °C.
4. Incubate it with shaking at 28 °C for 16 hours.

Expression of Recombinant Protein (pNit and pCpi Vector Series)

<seed culture>

1. Inoculate single colony in LB medium with an antibiotic (tetracycline: 5 µg/ml, chloramphenicol: 20 µg/ml).
2. Incubate it with shaking at 28 °C until stationary phase (2-3 days in general).

<Main culture>

1. Inoculate 1 ml seed culture medium well suspended to 9 ml fresh LB medium (baffled flask is desirable).
2. Incubate it with shaking at 28 °C for 24-48 hours.

Cell Disruption

1. Centrifuge 10 ml culture medium for 10 minutes at 3,000 x *g* at 4 °C.
2. Discard supernatant. Freezing and thawing of pellet increases efficiency of bacteriolysis.
3. Suspend pellet in 1ml phosphate buffer (pH 8.0).
4. Add lysozyme (final concentration: over 1 mg/ml).
   Efficiency of bacteriolysis is increased in a dose dependent manner.
5. Incubate on ice for 1 hour.
6. Disrupt cells through sonication or glass bead milling. Setting condition is dependent on each device.
   Avoid high temperature.
7. Centrifuge at 20,000 x *g* at 4 °C and recover the supernatant as a disrupted cell suspension.

Precautions

a. Since transformation efficiency of *R. erythropolis* L88 is lower than *E. coli*, these competent cells are not applicable to cloning and vector construction. It is recommended to construct vector in *E. coli* and then transform *R. erythropolis* L88 by using the plasmid.

b. These products may be unstable according to strain of *E. coli*. DH5α and XL1-blue are recommended.

c. Use ampicillin (50 µg/ml) for host *E. coli*.

d. The above procedure is only one example.
   It is possible to scale up/down culture according to the requirement of recombinant protein.

e. In regard to co-expression, it is recommended to transform with two vectors, which have a different replication origin, each other one by one, not simultaneously.

7. References

1) Nakashima and Tamura. "A novel system for expressing recombinant proteins over a wide temperature range from 4 to 35 ℃." Biotechnology and bioengineering 86.2 (2004): 136-148.
2) TOMOHIRO TAMURA, NORIKO TAMURA. "*Development of a Host-Vector System in Rhodococcus Species*" Journal of Environmental Biotechnology 7.1 (2007): 3-10.
3) Nakashima and Tamura. "Isolation and characterization of a rolling-circle-type plasmid from *Rhodococcus erythropolis* and application of the plasmid to multiple-recombinant-protein expression." Applied and environmental microbiology 70.9 (2004): 5557-5568.
4) Kagawa et al. "Identification of a methanol-inducible promoter from *Rhodococcus erythropolis* PR4 and its use as an expression vector." Journal of bioscience and bioengineering 113.5 (2012): 596-603.

8. Cautions

Reselling or modification of these products and producing any commercial products from these products are not permitted.

These products are for research use only.

These products, their main structures, their derivative and what is produced through them cannot be transferred to a third party.

It is necessary to submit "*Rhodococcus erythropolis* Protein Expression System License Agreement" when you order these products.

9. Related product

Rhodococcus erythropolis L88 Competent Cells (Product No. RE-L88)

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