



Recombineering Vectors pKD46-RecA and pKD46-RecA_{pa}

Instruction Manual

Catalog Numbers

NTC-RV-RecA
NTC-RV-RecA_{pa}

Version 1
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General Information

Contents: 20 ug each of plasmid vector shipped in 1x TE buffer.

Storage: Plasmids should be stored at -20°C

pKD46-RecA recombineering vectors

Introduction

Recombineering (**recombinogenic engineering**) is a homologous recombination-based technology used to modify DNA. Target DNA molecules (plasmids, BAC vectors or the host chromosome) are precisely altered by homologous recombination in host cells which express recombineering enzymes. Recombineering in *E. coli* often utilize the phage λ Red recombination functions (Murphy, 1998; Datsenko and Wanner, 2000). The λ genes involved in Red recombination are *exo*, *bet*, and *gam*. The *exo* (Red α) gene product has 5' to 3' exonuclease activity, and the *bet* (Red β) gene product is a single-strand DNA binding protein that promotes annealing. The *gam* gene product inhibits the RecBCD nuclease preventing linear DNA (*i.e.* PCR product) degradation.

Recombineering in *E. coli* is typically performed using specialized recombineering plasmids. For example, the pKD46 plasmid was developed for recombineering and contains arabinose-inducible *exo*, *bet*, and *gam* and *orf60a* genes in a conditional temperature sensitive (ts) vector that is maintained at 30°C, and lost at 42°C (Datsenko and Wanner, 2000).

Nature Technology Corporations (NTC's) recombineering vectors are *recA*⁺ derivatives of the popular pKD46 recombineering plasmid (Williams et al., 2009). The addition of *recA* facilitates recombineering in common *recA*⁻ bacterial strains such as DH5 α or DH10B.

These *recA*⁺ recombineering vectors are available in two versions.

pKD46-*recA* encodes constitutively expressed *Escherichia coli recA*⁺ along with wild type *rpsL*. Plasmid borne *rpsL* converts streptomycin resistant *rpsL* strains such as DH10B into streptomycin sensitive strains, allowing counterselection-based plasmid elimination (Imam et al., 2000).

pKD46-*recA*_{pa} encodes constitutively expressed *Pseudomonas aeruginosa recA*⁺. The RecA_{PA} protein induces hyper recombination in *E. coli*, in the absence of SOS induction, and presence or absence of *E. coli* RecA protein (Baitin et al., 2006).

Both vectors have been validated for use in PCR- or genomic DNA-mediated recombineering applications (Williams et al., 2009)

In summary, the pKD46-*recA* and pKD46-*recA*_{pa} vectors offer the following advantages

- Arabinose-inducible *exo*, *bet*, and *gam* and *orf60a* genes
- Conditional temperature sensitive (ts) vector that is maintained at 30°C, and lost at 42°C
- Counterselection against plasmid in streptomycin resistant (*rpsL*-based) host strains (pKD46-*recA*)
- Polylinkers for addition of new genes into vector (pKD46-*recA*_{pa})

pKD46-recA vector features

Feature	Function
Constitutively expressed AraC	Repression of AraBAD promoter
AraBAD promoter	Highly repressed arabinose-inducible promoter
gam, bet, eco genes	Red recombination genes
tL3 terminator	Maintain plasmid stability during arabinose induction
<i>Escherichia coli recA</i> gene	Improve recombineering in RecA- hosts
rpsL	Streptomycin counterselection-based plasmid elimination in rpsL-streptomycinR hosts
<i>repA101</i> (ts), <i>oriR101</i> origin of replication	Plasmid propagation at 30°C. Temperature sensitive origin is non functional at 42°C
Ampicillin resistance gene (ampR)	Plasmid selection in <i>Escherichia coli</i> cells

pKD46-recA_{pa} vector features

Feature	Function
Constitutively expressed AraC	Repression of AraBAD promoter
AraBAD promoter	Highly repressed arabinose-inducible promoter
gam, bet, eco genes	Red recombination genes
tL3 terminator	Maintain plasmid stability during arabinose induction
Polylinker 1	Addition of new genes to vector
<i>Pseudomonas aeruginosa recA</i> gene	Improve recombineering in RecA- hosts
Polylinker 2	Addition of new genes to vector
<i>repA101</i> (ts), <i>oriR101</i> origin of replication	Plasmid propagation at 30°C. Temperature sensitive origin is non functional at 42°C
Ampicillin resistance gene (ampR)	Plasmid selection in <i>Escherichia coli</i> cells

Recombineering Vectors

Vector	RecA	Quantity	Catalog Number	Price
pKD46-recA	<i>Escherichia coli</i>	20 µg	NTC-RV-RecA	\$320.00
pKD46-recA _{pa}	<i>Pseudomonas aeruginosa</i>	20 µg	NTC-RV-RecApa	\$320.00

Transformation and bacterial propagation

pKD46-recA and pKD46-recA_{pa} plasmids are ampicillin resistant, and are selected and propagated on standard *Escherichia coli* media supplemented with ampicillin (50-100 µg/mL).

Note: Growth of pKD46 and derivatives must be performed at 30°C to prevent inactivation of the temperature sensitive *repA101(ts)*, *oriR101* origin of replication. This origin is non functional at 42°C, allowing plasmid elimination by temperature induction.

Preparation of competent cells for recombineering

Grow transformed cells on LB+ampicillin agar plates overnight at 30°C. Use cells to inoculate (to an OD₆₀₀ of ~0.05) LB media containing 100 µg/mL ampicillin and 0.2% arabinose (to induce recombineering genes). Grow culture at 30°C to midlog (0.2-0.6 OD₆₀₀/mL), cool on ice, and made electrocompetent by centrifugation and washing twice in ice cold 10% glycerol (first wash 1x original volume, second wash ½ x original volume). Resuspend the final pellet in ice cold 10 % glycerol (1/200 x original culture volume). Use immediately or store several months at -80°C

References

- Baitin, D.M., Bakhlanova, I.V., Kil, Y.V., Cox, M.M. and Lanzov, V.A. (2006) Distinguishing characteristics of hyperrecombinogenic RecA protein from *Pseudomonas aeruginosa* acting in *Escherichia coli*. *J Bacteriol.* 188, 5812-5820.
- Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA.*, 97, 6640-6645.
- Imam, A.M., Patrinos, G.P., de Krom, M., Bottardi, S., Janssens, R.J., Katsantoni, E., Wai, A.W.K., Sherratt, D.J. and Grosvelf, F.G. (2000) Modification of human β -globin locus PAC clones by homologous recombination in *Escherichia coli*. *Nucl Acid Res.* 28, e65.
- Murphy, K.C. (1998) Use of bacteriophage λ recombination functions to promote gene replacement in *Escherichia coli*. *J Bacteriol.* 180, 2063-2071.
- Williams, J.A., Luke, J. and Hodgson, P. (2009) Strain Engineering by Genome Mass Transfer: Efficient Chromosomal Trait Transfer Method Utilizing Donor Genomic DNA and Recipient Recombineering Hosts. *Mol Biotechnol.* Epub.

Patent and Licensing information

Limited License

Nature Technology Corporation (NTC) grants the end user (purchaser) of the pKD46-recA and pKD46-recA_{pa} expression vectors a nontransferable, non-exclusive license to use the plasmids for non-commercial research purposes only. These vectors are intended for research use only by the purchaser. The vectors cannot be resold, repackaged, or used for the making or selling of any commercial product or service without the written approval of Nature Technology Corporation.

Separate licenses are available from NTC for the express purpose of non-research use or applications of the pKD46-recA and pKD46-recA_{pa} vectors.

Product Use Limitations

The pKD46-recA and pKD46-recA_{pa} vectors are sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use.

Note: This vector has not been completely sequenced. The sequence file was created using information in Genbank sequence databases and published literature.

Patent Information

NTC makes no representations that the use of the pKD46-recA and pKD46-recA_{pa} vectors will not infringe any patent, copyright, trademark, or other proprietary rights.

For more information, please contact:

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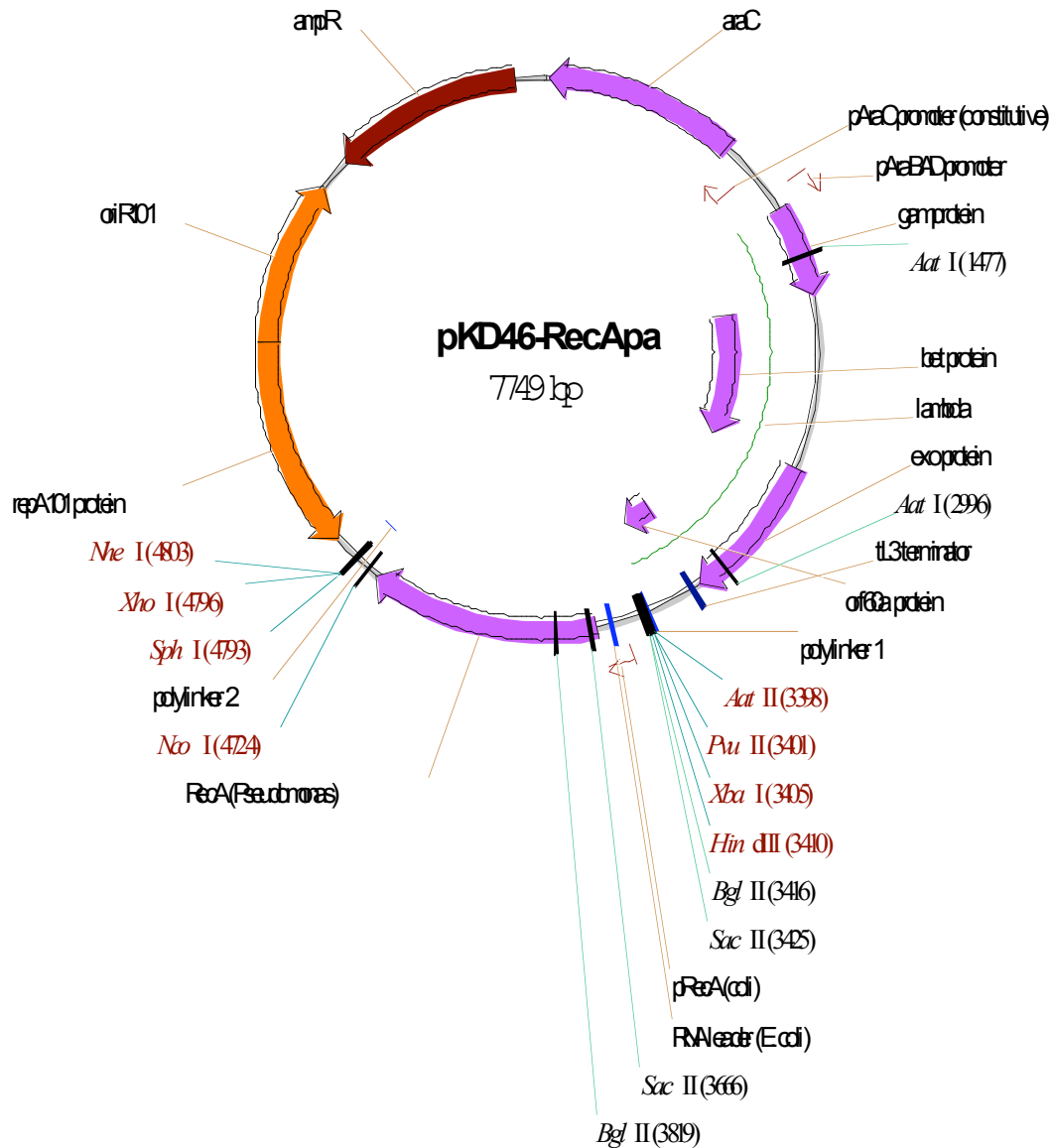
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pKD46-RecA_{pa}



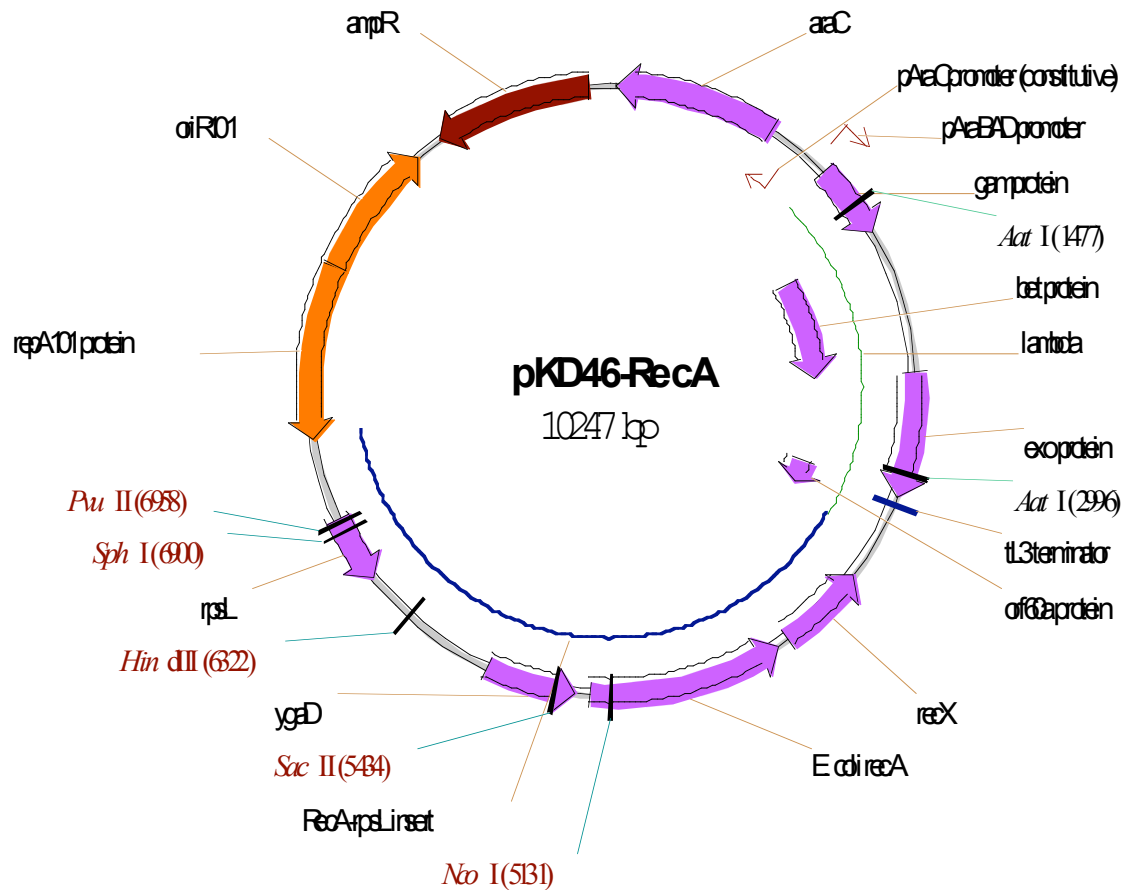
Vector Features

- araC* repressor (of AraBAD promoter): 11-889
- gam* protein: 1244-1660
- bet* protein: 1666-2451
- exo* protein: 2448-3128
- orf60a* protein: 3125-3307
- tL3* terminator: 3170-3226
- Polylinker 1: 3392-3427
- RecA* gene (*Pseudomonas aeruginosa*): 3634-4674
- Polylinker 2: 4723-4807
- repA101*: 4916-5866
- oriR101*: 5870-6610
- AmpR*: 6758-7618

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pKD46-RecA



Vector Features

araC repressor (of *AraBAD* promoter): 11-889
gam protein: 1244-1660
bet protein: 1666-2451
exo protein: 2448-3128
orf60a protein: 3125-3307
tL3 terminator: 3170-3226
RecA gene (*Escherichia coli*): 4177-5238
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