pKD46-RecA and pKD46-RecA$_{pa}$ Recombineering Vectors

Instruction Manual

Catalog Numbers

NTC-RV-RecA
NTC-RV-RecApa

Version 2
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General Information

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

Storage: Plasmids should be stored at -20˚C

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pKD46-RecA recombineering vectors

Introduction

Recombineering (**recombi**nogenic eng**ineering**) 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* (Redα) gene product has 5′ to 3′ exonuclease activity, and the *bet* (Redβ) 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 30ºC, and lost at 42ºC (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 DH5α 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 30ºC, and lost at 42ºC
• 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
Escherichia coli recA gene	Improve recombineering in RecA- hosts
rpsL	Streptomycin counterselection-based plasmid elimination in rpsL-streptomycinR hosts
repA101(ts), *oriR101* origin of replication	Plasmid propagation at 30ºC. Temperature sensitive origin is non functional at 42ºC
Ampicillin resistance gene (ampR)	Plasmid selection in *Escherichia coli* 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
Pseudomonas aeruginosa recA gene	Improve recombineering in RecA- hosts
Polylinker 2	Addition of new genes to vector
repA101(ts), *oriR101* origin of replication	Plasmid propagation at 30ºC. Temperature sensitive origin is non functional at 42ºC
Ampicillin resistance gene (ampR)	Plasmid selection in *Escherichia coli* cells

Recombineering Vectors

Vector	RecA	Quantity	Catalog Number	Price
pKD46-recA	*Escherichia coli*	20 µg	NTC-RV-RecA	$320.00
pKD46-recA$_{pa}$	*Pseudomonas aeruginosa*	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 30ºC to prevent inactivation of the temperature sensitive *repA101*(ts), *oriR101* origin of replication. This origin is non functional at 42ºC, allowing plasmid elimination by temperature induction.

Preparation of competent cells for recombineering

Grow transformed cells on LB+ampicillin agar plates overnight at 30ºC. Use cells to inoculate (to an OD_{600} of ~0.05) LB media containing 100 ug/mL ampicillin and 0.2% arabinose (to induce recombineering genes). Grow culture at 30ºC to midlog (0.2-0.6 OD_{600}/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 -80ºC.

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 purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes.

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.

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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.

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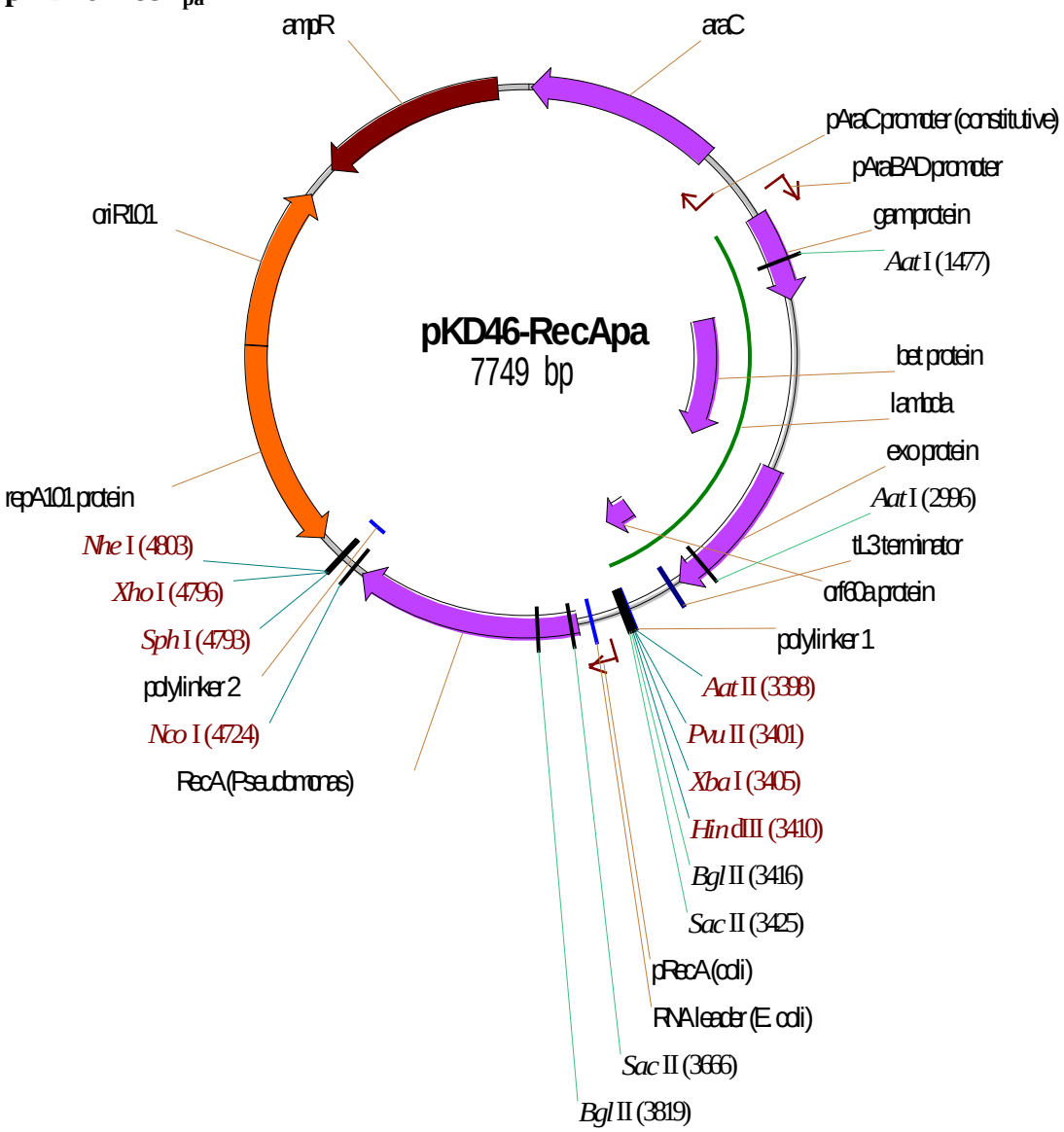
pKD46-RecA$_{pa}$



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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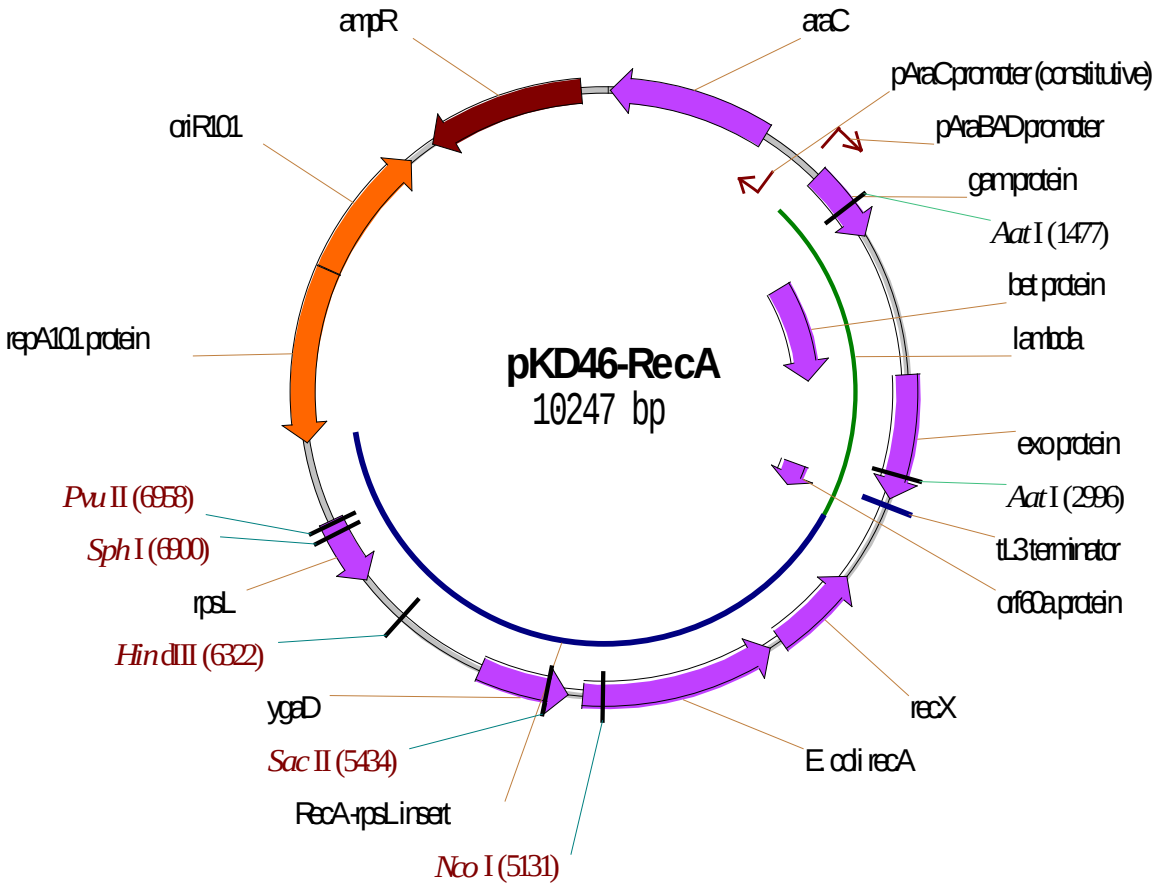
pKD46-RecA



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
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