

Plasmid: **pKS-aphVIII-lox**

A *loxP*-site was introduced into a Klenow-treated *PsiI* site of vector pBluescriptII-KS (ampicillin resistance) and a *PciI* restriction site was destroyed and replaced by an *EcoRV* site (double insertion). An expression cassette for *Chlamydomonas reinhardtii* comprising the beta2-tubulin-promoter, the *aphVIII*-gene from *Streptomyces rimosus* and the 3'-UTR from the Chlamyopsin1-gene was inserted via *SacI/KpnI*.

<u>Plasmid-sequence:</u>	pKS-aphVIII-lox	4308 bp	
	<i>loxP</i> :	368-402	
	beta2-tubulin-promoter:	699-1012	(<i>SacI/SalI</i>)
	<i>aphVIII</i> (from pSII105):	1013-1833	(<i>XhoI/BamHI</i>)
	<i>COP1</i> -3'-UTR:	1902-2063	(<i>BamHI/KpnI</i>)

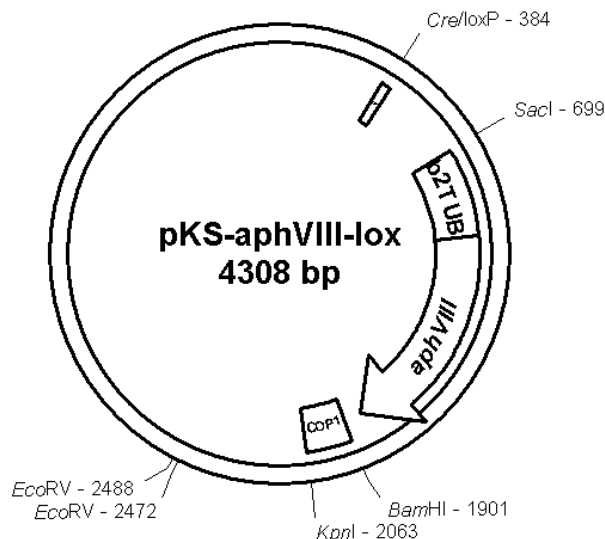
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101 TTTCTTCCCT  TCCTTTCTCG  CCACGTTTCGC  CGGCTTTCCT  CGTCAAGCTC
151 TAAATCGGGG  GCTCCCTTTA  GGGTTCGGAT  TTAGTGCTTT  ACGGCACCTC
201 GACCCCAAAA  AACTTGATTA  GGGTGATGGT  TCACGTAGTG  GGCCATCGCC
251 CTGATAGACG  GTTTTTTCGCC  CTTTGACGTT  GGAGTCCACG  TTCTTTAATA
301 GTGGACTCTT  GTTCCAAACT  GGAACAACAC  TCAACCCTAT  CTCGGTCTAT
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451 TTTAACAAAA  ATTTAACGCG  AATTTTAACA  AAATATTAAC  GCTTACAATT
501 TCCATTCGCC  ATTCAGGCTG  CGCAACTGTT  GGGAAGGGCG  ATCGGTGCGG
551 GCCTCTTCGC  TATTACGCCA  GCTGGCGAAA  GGGGGATGTG  CTGCAAGGCG
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651 CGGCCAGTGA  GCGCGCGTAA  TACGACTCAC  TATAGGGCGA  ATTGGAGCTC
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2001 ACCCGAACAG  ATTGATAACC  GCCTTGGCAT  TTCCTGTCAG  AATGTAACGT
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4251 TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA
4301 AGTGCCAC

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Plasmid-map:



Reference: Sizova, I., Fuhrmann, M., and Hegemann, P. (2001) A *Streptomyces rimosus* aphVIII gene coding for a new type phosphotransferase provides stable antibiotic resistance to *Chlamydomonas reinhardtii*. *Gene* 277, 221-9.

Heitzer, M. and Zschoernig, B (2007) Construction of modular tandem expression vectors for the green alga *Chlamydomonas reinhardtii* using the Cre/lox-system. *Biotechniques* 43(3), 324-32.