

Plasmid: **pKS-aph7⁻-lox**

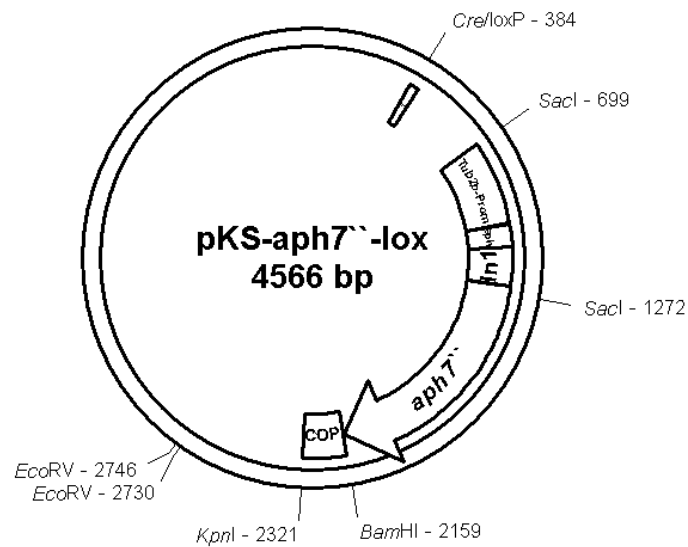
A *loxP*-site was introduced into a Klenow-treated *PsiI* site of vector pBluescriptII-KS (ampicillin resistance) and a *PciI* restriction site was destroyed and replaced by an *EcoRV* site (double insertion). An expression cassette for *Chlamydomonas reinhardtii* comprising the beta2-tubulin-promoter, the *aph7⁻*-gene from *Streptomyces hygroscopicus* (containing a copy of intron1 from the *RBCS2* gene) and the 3'-UTR from the Chlamyopsin1-gene was inserted via *SacI/KpnI*.

<u>Plasmid-sequence:</u>	pKS-aphVIII-lox	4566 bp	
	<i>loxP</i> :	368-402	
	beta2-tubulin-promoter:	699-1012	(<i>SacI/SalI</i>)
	<i>aph7⁻</i> (from pHyg3):	1013-2158	(<i>XhoI/BamHI</i>)
	(intron1 from <i>RBCS2</i>):	1099-1243)	
	<i>COP1</i> -3'-UTR:	2159-2321	(<i>BamHI/KpnI</i>)

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1 CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG
51 CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTTCG
101 TTTCTTCCCT TCCTTTCTCG CCACGTTCCG CGGCTTTCCC 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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551 GCCTCTTCGC TATTACGCCA GCTGGCGAAA GGGGGATGTG CTGCAAGCGG
601 ATTAAGTTGG GTAACGCCAG GGTTTTCCCA GTCACGACGT TGTA AAAACGA
651 CGGCCAGTGA GCGCGGTAA TACGACTCAC TATAGGGCGA ATTGGAGCTC
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4501 TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGTTCC GCGCACATT
4551 CCCCAGAAAAG TGCCAC

Plasmid-map:



Reference: Berthold, P., Schmitt, R., and Mages, W. (2002) An engineered *Streptomyces hygroscopicus* aph7^{-lox} gene mediates dominant resistance against hygromycin B in *Chlamydomonas reinhardtii*. *Protist* 153(4), 401-12.

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.