

Plasmid: pAES14

This plasmid contains a kanamycin resistance marker and an *R6ky* origin of replication for maintenance in *E.coli* Pir1-cells (available from Invitrogen), a *loxP*-site allows plasmid-fusion by Cre-recombinase. The codon adapted *crluc* gene for *Renilla* -luciferase (AY004213) is divided by intron sequences 2 and 3 of the *Chlamydomonas reinhardtii* *RBCS2* gene (Genbank: X04472) inserted into unique *NruI*- and *SnaBI*-sites. Together with one copy of *RBCS2* intron 1 present in the 5'-untranslated region of the promoter, all three introns occur once and in their physiological order. The extracellular expression of luciferase is mediated by the first 21 amino acids of the *ARS2* gene of *Chlamydomonas reinhardtii* (AF333184). A C-terminal His₆-tag allows purification from the culture medium.

crluc-ORF: MLE + *Ars2* + luciferase + TH₆T = 325 aa, 37458 Da
aa-sequence same as for intracellular *Renilla*-luciferase in pRbcRL(Hsp186)

Note that the ORF encodes for additional aa:

MLE if start-ATG upstream of the *XhoI*-restriction site is used

MGALAVFAVACLA AVASVAHA *ARS2* signal peptide

ADLQ extra aa due to cloning and *PstI*-restriction site

THHHHHHT His₆-tag

Introns were inserted between aa 247/248 and 349/350

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1      MLEMGALAVF AVACLAAVAS VAHAADLQAS KVYDPEQRKR MITGPQWWAR
51     CKQMNVLDSF INYYDSEKHA ENAVIFLHGN AASSYLWRHV VPHIEPVARC
101    IIPDLIGMGK SGKSGNGSYR LLDHYKYLTA WFELLNLPKK IIFVGHWDWGA
151    CLAFHYSYEH QDKIKAIVHA ESVVDVIESW DEWPDIEEDI ALIKSEEGEK
201    MVLENNFFVE TMLPSKIMRK LEPEEFAAYL EPFKEKGEVR RPTLSWPREI
251    PLVKGKGPDV VQIVRNYNAY LRASDDLPKM FIESDPGFFS NAIVEGAKKF
301    PNTEFVKVKG LHFSQEDAPD EMGKYIKSFV ERVLKNDTAS QPELAPEDTT
351    HHHHHHT*
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Plasmid-sequence: pAES14 4690 bp
loxP: 1-34
HSP70A-promoter: 2150-2419
RBCS2-promoter: 2420-2649
intron1 from *RBCS2*: 2650-2803
crluc: 2804-4434
leader from *ARS2* 2809-2871
intron2 from *RBCS2*: 3543-3871
intron3 from *RBCS2*: 4175-4412
His₆-tag 4417-4424
RBCS2-3'-UTR: 4460-4680

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1  ATAACTTCGT ATAATGTATG CTATACGAAG TTATGGTACC GCGGCCGCGT
51 AGAGGATCTG TTGATCAGCA GTTCAACCTG TTGATAGTAC GTECTAAGCT
101 CTCATGTTTC ACGTACTAAG CTCTCATGTT TAACGTACTA AGCTCTCATG
151 TTTAACGAAC TAAACCTCA TGGCTAACGT ACTAAGCTCT CATGGCTAAC
201 GTECTAAGCT CTCATGTTTC ACGTACTAAG CTCTCATGTT TGAACAATAA
251 AATTAATATA AATCAGCAAC TTAAATAGCC TCTAAGGTTT TAAGTTTTAT
301 AAGAAAAAAA AGAATATATA AGGCTTTTAA AGCTTTTAAG GTTTAACGGT
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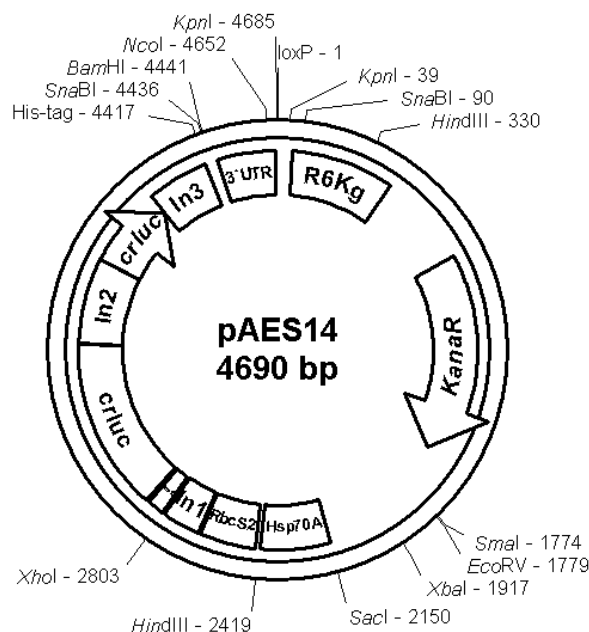
351 TGTGGACAAC AAGCCAGGGA TGTAACGCAC TGAGAAGCCC TTAGAGCCTC
401 TCAAAGCAAT TTTGAGTGAC ACAGGAACAC TTAACGGCTG ACATGGGAAT
451 TAGCTTCACG CTGCCGCAAG CACTCAGGGC GCAAGGGCTG CTAAAGGAAG
501 CGGAACACGT AGAAAGCCAG TCCGCAGAAA CGGTGCTGAC CCCGGATGAA
551 TGTCAGCTAC TGGGCTATCT GGACAAGGGA AAACGCAAGC GCAAAGAGAA
601 AGCAGGTAGC TTGCAGTGGG CTTACATGGC GATAGCTAGA CTGGGCGGTT
651 TTATGGACAG CAAGCGAACC GGAATTGCCA GCTGGGGCGC CCTCTGGTAA
701 GGTGGGAAG CCCTGCAAAG TAAACTGGAT GGCTTCTTG CCGCCAAGGA
751 TCTGATGGCG CAGGGGATCA AGATCTGATC AAGAGACAGG ATGAGGATCG
801 TTTCGCATGA TTGAACAAGA TGGATTGCAC GCAGGTTCTC CGGCCGCTTG
851 GGTGGAGAGG CTATTCGGCT ATGACTGGGC ACAACAGACA ATCGGCTGCT
901 CTGATGCCGC CGTGTTCGGG CTGTCAGCGC AGGGGCGCCC GGTTCTTTTT
951 GTCAAGACCG ACCTGTCCGG TGCCCTGAAT GAACTGCAGG ACGAGGCAGC
1001 GCGGCTATCG TGGCTGGCCA CGACGGGCGT TCCTTGCGCA GCTGTGCTCG
1051 ACGTTGTCAC TGAAGCGGGA AGGGACTGGC TGCTATTGGG CGAAGTGCCG
1101 GGGCAGGATC TCCTGTCATC TCACCTTGCT CCTGCCGAGA AAGTATCCAT
1151 CATGGCTGAT GCAATGCGGC GGCTGCATAC GCTTGATCCG GCTACCTGCC
1201 CATTGACCA CCAAGCGAAA CATCGCATCG AGCGAGCACG TACTCGGATG
1251 GAAGCCGGTC TTGTGATCA GGATGATCTG GACGAAGAGC ATCAGGGGCT
1301 CGCGCCAGCC GAACTGTTTCG CCAGGCTCAA GGCGCGCATG CCCGACGGCG
1351 AGGATCTCGT CGTGACACAT GGCGATGCCT GCTTGCCGAA TATCATGGTG
1401 GAAAATGGCC GCTTTTCTGG ATTCATCGAC TGTGGCCGGC TGGGTGTGGC
1451 GGACCGCTAT CAGGACATAG CGTTGGCTAC CCGTGATATT GCTGAAGAGC
1501 TTGGCGGCGA ATGGGCTGAC CGTTCCTCG TGCTTTACGG TATCGCCGCT
1551 CCCGATTGCG AGCGCATCGC CTTCTATCGC CTTCTTGACG AGTTCTTCTG
1601 AGCGGGACTC TGGGGTTCGA AATGACCGAC CAAGCGACGC CCAACCTGCC
1651 ATCACGAGAT TTCGATTCCA CCGCCGCCTT CTATGAAAGG TTGGGCTTCG
1701 GAATCGTTTT CCGGGACGCC GGCTGGATGA TCCTCCAGCG CGGGGATCTC
1751 ATGCTGGAGT TCTTCGCCCC CCCCGGGATA TCCGGATATA GTTCCTCCTT
1801 TCAGCAAAA ACCCCTCAAG ACCCGTTTAG AGGCCCAAG GGGTTATGCT
1851 AGTTATTGCT CAGCGGTGGC AGCAGCCAAC TCAGCTTCTT TTCGGGCTTT
1901 GTTAGCAGCC GGATCTTCTA GAATCCCCAG CATGCCGTGCT ATTGTCTTCC
1951 CAATCCTCCC CTTTGCTGTC CTGCCCCACC CCACCCCCCA GAATAGAATG
2001 ACACCTACTC AGACAATGCG ATGCAATTC CTCATTTTAT TAGGAAAGGA
2051 CAGTGGGAGT GGCACCTTCC AGGGTCAAGG AAGGCACGGG GGAGGGGCAA
2101 ACAACAGATG GCTGGCAACT AGAAGGCACA GTCGAGGCTG ATAGCGAGCT
2151 CGCTGAGGCT TGACATGATT GGTGCGTATG TTTGTATGAA GCTACAGGAC
2201 TGATTTGGCG GGCTATGAGG GCGGGGGAAG CTCTGGAAGG GCCCGGATGG
2251 GCGCGCGGCG GTCCAGAAGG CGCCATACGG CCCGCTGGCG GCACCCATCC
2301 GGTATAAAA CCCCGCACCC CGAACGGTGA CCTCCACTTT CAGCGACAAA
2351 CGAGCACTTA TACATACGCG ACTATTCTG CGTATACAT AACCCTCAG
2401 CTAGCTTAAG ATCCCATCAA GCTTGCATGC CGGGCGCGCC AGAAGGAGCG
2451 CAGCCAAACC AGGATGATGT TTGATGGGGT ATTTGAGCAC TTGCAACCCT
2501 TATCCGGAAG CCCCTGGCC CACAAAGGCT AGGCGCCAAT GCAAGCAGTT
2551 CGCATGAGC CCCTGGAGCG GTGCCCTCCT GATAAACC GG CAGGGGGCC
2601 TATGTTCTTT ACTTTTTTAC AAGAGAAGTC ACTCAACATC TTAAAATGGC
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2701 TTGACTTGCA ACGCCCGCAT TGTGTCGACG AAGGCTTTTG GCTCCTCTGT
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2851 GTGGCGTCGG TTGCGCATGC GGCCGACCTG CAGGCCAGCA AGGTGTACGA
2901 CCCCAGCAG CGCAAGCGCA TGATCACCGG CCTCAGTGG TGGGCTCGCT
2951 GCAAGCAGAT GAACGTGCTG GACAGCTTCA TCAACTACTA CGACAGCGAG
3001 AAGCACGCCG AGAACGCCGT GATCTTCTTG CACGGCAACG CCGCCAGCAG
3051 CTACCTGTGG CGCCACGTGG TGCCCCACAT CGAGCCCCTG GCCCGCTGCA
3101 TCATCCCCGA CCTGATCGGC ATGGGCAAGA GCGGCAAGAG CGGCAACGGC
3151 AGCTACCGCC TGCTGGACCA CTACAAGTAC CTGACCGCCT GGTTTCGAGCT
3201 GCTGAACCTG CCCAAGAAGA TCATCTTCGT GGGCCACGAC TGGGGCGCCT
3251 GCCTGGCCTT CCACTACAGC TACGAGCACC AGGACAAGAT CAAGGCCATC
3301 GTGCACGCCG AGAGCGTGTT GGACGTGATC GAGAGCTGGG ACGAGTGGCC

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3351 CGACATCGAG GAGGACATCG CCCTGATCAA GAGCGAGGAG GGCGAGAAGA
3401 TGGTGCTGGA GAACAAC TTCGTGGAGA CCATGCTGCC CAGCAAGATC
3451 ATGCGCAAGC TGGAGCCCGA GGAGTTCGCC GCCTACCTGG AGCCCTTCAA
3501 GGAGAAGGGC GAGGTGCGCC GTCCCACCCT GAGCTGGCCT CGGTGAGCTT
3551 GCGGGGTTGC GAGCAACACT CCAGCAACGA ACAGTGCCCA AGTCAGGAAT
3601 CTGCAGTCAG CCTGGGCTTT CGGCGGCTTT TTCTTGGGCA AACAGCTTGC
3651 ACTCATGCCA GCGCGGCTTG TCCAGCCTCA CTTGAGCTTT CCAGCTGCTA
3701 CCAGCCGGGC TATACGACAG CGACAGAGCC ATAGCGTGG AATCACTTAT
3751 TGGGTTGCCG AAGTAGCGGT CGGAGCGTGA GTTCTTGGTC AAGCCGCCCC
3801 TTATCCGGTT CCTGTCCGTG TCTTTGTCCC TCGTTCACCC TTCGCGGCAC
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3901 CCGACGTGGT GCAGATCGTG CGCAACTACA ACGCCTACTT GCGCGCCAGC
3951 GACGACCTGC CCAAGATGTT CATCGAGAGC GACCCCGGCT TCTTCAGCAA
4001 CGCCATCGTG GAGGGCGCCA AGAAGTTCCC CAACACCGAG TTCGTGAAGG
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4601 TTTGCAATTT TGTTGGTTGT AACGATCCTC CGTTGATTTT GGCCTCTTTC
4651 TCCATGGGCG GGCTGGGCGT ATTTGAAGCG GGTACCCGAC

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Plasmid-map:



Reference: Fuhrmann, M., Hausherr, A., Ferbitz, L. Schodl, T., Heitzer, M. and Hegemann, P (2004) Monitoring dynamic expression of nuclear genes in *Chlamydomonas reinhardtii* using a synthetic luciferase reporter gene. *Plant Mol Biol* 55, 869-81.

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.