

Plasmid:        **pHsp70A/RbcS2-cgLuc**

Synthetic gene encoding the extracellular luciferase from *Gaussia princeps* adapted to the nuclear codon usage of *Chlamydomonas reinhardtii* (*cgluc*) under the chimeric HSP70A/RBCS2 promoter in plasmid pHsp70A/RbcS2-Chlamy for Cre/*lox*-mediated plasmid fusion (cloned via *XhoI/BamHI*, *R6ky* origin of replication for maintenance in *E. coli* Pir1-cells (available from Invitrogen), kanamycin resistance).

cgluc-gene:                Genbank EU239244, nucleotides 10-570, 561 bp  
with a recombinant 5`-*XhoI*-restriction site, a recombinant 3`-*SnaBI*-  
and 3`-*BamHI*-restriction sites

```
1 aaactcgaga tgggctgtaa ggtgctgttc gccctgatct gcatcgccgt cgccgagggc
61 aagccgacgg agaacaacga ggacttcaac attgtcgccg tggcgtccaa ctttgccacc
121 accgatctgg acgccgaccg cggcaagctg ccgggcaaga agctgcccct ggaggtcctc
181 aaggaatgag aggcgaacgc ccggaaggcc ggctgcaccc gcggtgcctc gatctgcctg
241 tcccacatca agtgcacgcc gaagatgaag aagttcattc ccggccggtg ccacacctac
301 gaggccgaca aggagtccgc gcagggcggc attggcgagg cgatcgtgga catccccgag
361 attccgggct tcaaggacct ggagccgatg gagcagttca tcgcccaggt ggacctgtgc
421 gtggactgca cgaccggctg tctgaagggc ctggccaatg tgcagtgttc cgacctgttc
481 aagaagtggc tgccccagcg ctgtgccacc ttcgccagca agatccaggg ccagggtggc
541 aagattaagg gcgctggcgg cgatacgtaa ggatcc
```

cgluc-ORF:                MLE + luciferase + T = 189 aa, 20371 Da  
Despite four extra aa due to integration of restriction sites, aa-sequence  
same as wildtype luciferase from *Renilla reniformis* (Genbank:  
AY015993).

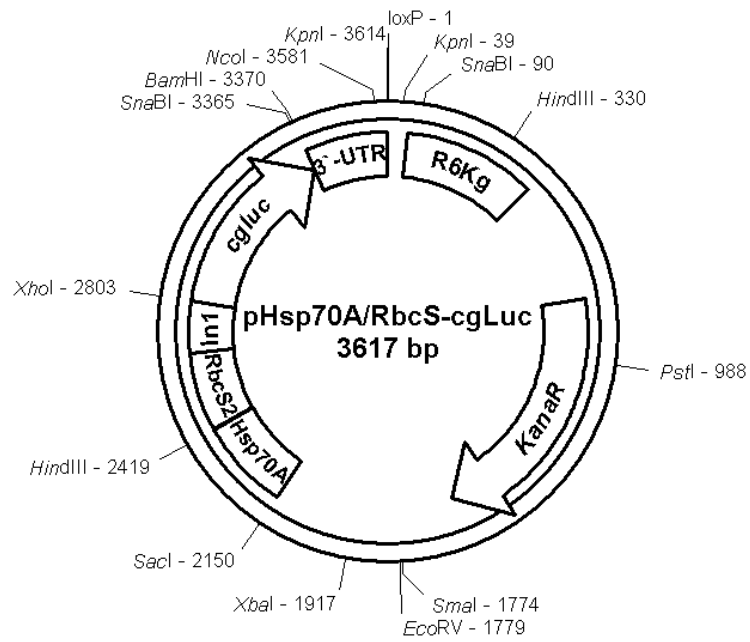
```
1        MLEMGVKVLF ALICIAVAEA KPTENNEDFN IVAVASNFAT TDLADDRGKL
51        PGKKLPLEVL KEMEANARKA GCTRGCLICL SHIKCTPKMK KFIPGRCHTY
101       EGDKESAQGG IGEAIVDIPE IPGFKDLEPM EQFIAQVDLC VDCTTGCLKG
151       LANVQCSDDL KKWLPQRCAT FASKIQGQVD KIKGAGGDT
```

Plasmid-sequence:    pHsp70A/RbcS2-cgLuc        3617 bp  
                          *cgluc*:                        2809-3369  
                          *cgluc*-ORF                    2800-3366

```
1    ATAACTTCGT    ATAATGTATG    CTATACGAAG    TTATGGTACC    GCGGCCGCGT
51    AGAGGATCTG    TTGATCAGCA    GTTCAACCTG    TTGATAGTAC    GTACTAAGCT
101   CTCATGTTTC    ACGTACTAAG    CTCTCATGTT    TAACGTACTA    AGCTCTCATG
151   TTTAACGAAC    TAAACCCCTCA    TGGCTAACGT    ACTAAGCTCT    CATGGCTAAC
201   GTAATAAGCT    CTCATGTTTC    ACGTACTAAG    CTCTCATGTT    TGAACAATAA
251   AATTAATATA    AATCAGCAAC    TTAAATAGCC    TCTAAGGTTT    TAAGTTTTAT
301   AAGAAAAAAA    AGAATATATA    AGGCTTTTAA    AGCTTTTAA    GTTTAAACGGT
351   TGTGGACAAC    AAGCCAGGGA    TGTAACGCAC    TGAGAAGCCC    TTAGAGCCTC
401   TCAAAGCAAT    TTTGTAGTAC    ACAGGAACAC    TTAACGGCTG    ACATGGGAAT
451   TAGCTTCACG    CTGCCGCAAG    CACTCAGGGC    GCAAGGGCTG    CTAAAGGAAG
501   CGGAACACGT    AGAAAGCCAG    TCCGCAGAAA    CGGTGCTGAC    CCCGGATGAA
551   TGTCAGCTAC    TGGGCTATCT    GGACAAGGGA    AAACGCAAGC    GCAAAGAGAA
601   AGCAGGTAGC    TTGAGTGGG    CTTACATGGC    GATAGCTAGA    CTGGGCGGTT
651   TTATGGACAG    CAAGCGAACC    GGAATTGCCA    GCTGGGGCGC    CCTCTGGTAA
701   GGTTGGGAAG    CCCTGCAAAG    TAAACTGGAT    GGCTTTCTTG    CCGCCAAGGA
751   TCTGATGGCG    CAGGGGATCA    AGATCTGATC    AAGAGACAGG    ATGAGGATCG
801   TTTCGCATGA    TTGAAACAAGA    TGGATTGCAC    GCAGGTTCTC    CGGCCGCTTG
851   GGTGGAGAGG    CTATTCGGCT    ATGACTGGGC    ACAACAGACA    ATCGGCTGCT
```

901 CTGATGCCGC CGTGTTCCGG CTGTCAGCGC AGGGGCGCCC GGTTCTTTTT  
 951 GTCAAGACCG ACCTGTCCGG TGCCCTGAAT GAACTGCAGG ACGAGGCAGC  
 1001 GCGGCTATCG TGGCTGGCCA CGACGGGCGT TCCTTGCGCA GCTGTGCTCG  
 1051 ACGTTGTAC TGAAGCGGGA AGGGACTGGC TGCTATTGGG CGAAGTGCCG  
 1101 GGGCAGGATC TCCTGTCATC TCACCTTGCT CCTGCCGAGA AAGTATCCAT  
 1151 CATGGCTGAT GCAATGCGGC GGCTGCATAC GCTTGATCCG GCTACCTGCC  
 1201 CATTCGACCA CCAAGCGAAA CATCGCATCG AGCGAGCACG TACTCGGATG  
 1251 GAAGCCGGTC TTGTGATCA GGATGATCTG GACGAAGAGC ATCAGGGGCT  
 1301 CGCGCCAGCC GAACTGTTCG CCAGGCTCAA GGCGCGCATG CCCGACGGCG  
 1351 AGGATCTCGT CGTGACACAT GGCGATGCCT GCTTGCCGAA TATCATGGTG  
 1401 GAAAATGGCC GCTTTTCTGG ATTCATCGAC TGTGGCCGGC TGGGTGTGGC  
 1451 GGACCGCTAT CAGGACATAG CGTTGGCTAC CCGTGATATT GCTGAAGAGC  
 1501 TTGGCGGCGA ATGGGCTGAC CGCTTCTCG 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 CCCCAGGATA TCCGGATATA GTTCTCTCTT  
 1801 TCAGCAAAA ACCCTCAAG ACCCGTTTAG AGGCCCAAG GGGTTATGCT  
 1851 AGTTATTGCT CAGCGGTGGC AGCAGCCAAC TCAGCTTCCT TTCGGGCTTT  
 1901 GTTAGCAGCC GGATCTTCTA GAATCCCCAG CATGCCCTGCT ATTGTCTTCC  
 1951 CAATCCTCCC CTTTGCTGTC CTGCCCCACC CCACCCCCA GAATAGAATG  
 2001 ACACCTACTC AGACAATGCG ATGCAATTTT CTCAATTTAT TAGGAAAGGA  
 2051 CAGTGGGAGT GGCACCTTCC AGGGTCAAGG AAGGCACGGG GGAGGGGCAA  
 2101 ACAACAGATG GCTGGCAACT AGAAGGCACA GTCGAGGCTG ATAGCGAGCT  
 2151 CGCTGAGGCT TGACATGATT GGTGCGTATG TTTGTATGAA GCTACAGGAC  
 2201 TGATTTGGCG GGCTATGAGG GCGGGGAAG CTCTGGAAGG GCCGCGATGG  
 2251 GGC GCGCGGC GTCCAGAAGG CGCCATACGG CCCGCTGGCG GCACCCATCC  
 2301 GGTATAAAA CCCGCGACCC CGAACGGTGA CCTCCACTTT CAGCGACAAA  
 2351 CGAGCACTTA TACATACGCG ACTATTCTGC CGCTATACAT AACCACTCAG  
 2401 CTAGCTTAAG ATCCCATCAA GCTTGCATGC CGGGCGCGCC AGAAGGAGCG  
 2451 CAGCCAAACC AGGATGATGT TTGATGGGGT ATTTGAGCAC TTGCAACCCT  
 2501 TATCCGGAAG CCCCTGGCC CACAAAGGCT AGGCGCCAAT GCAAGCAGTT  
 2551 CGCATGCAGC CCCTGGAGCG GTGCCCTCCT GATAAACCGG CCAGGGGGCC  
 2601 TATGTTCTTT ACTTTTTTAC AAGAGAAGTC ACTCAACATC TTAANAATGGC  
 2651 CAGGTGAGTC GACGAGCAAG CCCGGCGGAT CAGGCAGCGT GCTTGCAGAT  
 2701 TTGACTTGCA ACGCCCGCAT TGTGTCGACG AAGGCTTTTG GCTCCTCTGT  
 2751 CGCTGTCTCA AGCAGCATCT AACCCTGCGT CGCCGTTTCC ATTTGCAGGA  
 2801 TGCTCGAGAT GGGCGTGAAG GTGCTGTTTCG CCCTGATCTG CATCGCCGTC  
 2851 GCCGAGGCGA AGCCGACGGA GAACAACGAG GACTTCAACA TTGTCGCCGT  
 2901 GCGTCCAAC TTTGCCACCA CCGATCTGGA CGCCGACCGC GGCAAGCTGC  
 2951 CGGCAAGAA GCTGCCCTG GAGTCTCTCA AGGAAATGGA GGCGAACGCC  
 3001 CGGAAGGCCG GCTGCACCCG CGGCTGCCTA ATCTGCCCTGT CCCACATCAA  
 3051 GTGCACGCCG AAGATGAAGA AGTTCATTCC CGGCCGGTGC CACACCTACG  
 3101 AGGCGACAA GGAGTCCGCG CAGGGCGGCA TTGGCGAGGC GATCGTGGAC  
 3151 ATCCCCGAGA TTCCGGGCTT CAAGGACCTG GAGCCGATGG AGCAGTTCAT  
 3201 CGCCAGGTG GACCTGTGCG TGGACTGCAC GACCGCTGT CTGAAGGGCC  
 3251 TGCCCAATGT GCAGTGTTC GACCTGCTCA AGAAGTGGCT GCCCCAGCGC  
 3301 TGTGCCACCT TCGCCAGCAA GATCCAGGGC CAGGTGGACA AGATTAAGGG  
 3351 CGCTGGCGGC GATACGTAAG GATCCCCGCT CCGTGTAAT GGAGGCGCTC  
 3401 GTTGATCTGA GCCTTGCCCC CTGACGAACG GCGGTGGATG GAAGATACTG  
 3451 CTCTCAAGTG CTGAAGCGGT AGCTTAGCTC CCCGTTTCGT GCTGATCAGT  
 3501 CTTTTTCAAC ACGTAAAAAG CGGAGGAGTT TTGCAATTTT GTTGTTGTA  
 3551 ACGATCCTCC GTTGATTTTG GCCTCTTTCT CCATGGGCGG GCTGGGCGTA  
 3601 TTTGAAGCGG GTACCCC

Plasmid-map:



Reference: 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.