Plasmid: **pRbcRL(Hsp196)**

Synthetic gene encoding luciferase from *Renilla reniformis* adapted to the nuclear codon usage of *Chlamydomonas reinhardtii* (*cluc*). Together with other genetic elements in vector pBluescriptII-KS (cloned via *Sac*I/*Kpn*I, ampicillin resistance).

cluc-gene: Genbank AY004213, nucleotides 11-943, 933bp
with a recombinant 5`-*Xho*I-restriction site, a shortened **HSV**-tag and recombinant 3`-*SnaB*I- and 3`-*Bam*HI-restriction sites

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1       CTCGAGATGG CCAGCAAGGT GTACGACCCC GAGCAGCGCA AGCGCATGAT
51      CACCGGCCCT CAGTGGTGGG CTCGCTGCAA GCAGATGAAC GTGCTGGACA
101     GCTTCATCAA CTACTACGAC AGCGAGAAGC ACGCCGAGAA CGCCGTGATC
151     TTCCTGCACG GCAACGCCGC CAGCAGCTAC CTGTGGCGCC ACGTGGTGCC
201     CCACATCGAG CCCGTGGCCC GCTGCATCAT CCCCGACCTG ATCGGCATGG
251     GCAAGAGCGG CAAGAGCGGC AACGGCAGCT ACCGCCTGCT GGACCACTAC
301     AAGTACCTGA CCGCCTGGTT CGAGCTGCTG AACCTGCCCA AGAAGATCAT
351     CTTCGTGGGC CACGACTGGG GCGCCTGCCT GGCCTTCCAC TACAGCTACG
401     AGCACCAGGA CAAGATCAAG GCCATCGTGC ACGCCGAGAG CGTGGTGGAC
451     GTGATCGAGA GCTGGGACGA GTGGCCCGAC ATCGAGGAGG ACATCGCCCT
501     GATCAAGAGC GAGGAGGGCG AGAAGATGGT GCTGGAGAAC AACTTCTTCG
551     TGGAGACCAT GCTGCCCAGC AAGATCATGC GCAAGCTGGA GCCCGAGGAG
601     TTCGCCGCCT ACCTGGAGCC CTTCAAGGAG AAGGGCGAGG TGCGCCGTCC
651     CACCCTGAGC TGGCCTCGCG AGATCCCCCT GGTGAAGGGC GGCAAGCCCG
701     ACGTGGTGCA GATCGTGCGC AACTACAACG CCTACCTGCG CGCCAGCGAC
751     GACCTGCCCA AGATGTTCAT CGAGAGCGAC CCCGGCTTCT TCAGCAACGC
801     CATCGTGGAG GGCGCCAAGA AGTTCCCCAA CACCGAGTTC GTGAAGGTGA
851     AGGGCCTGCA CTTCAGCCAG GAGGACGCTC CCGACGAGAT GGGCAAGTAC
901     ATCAAGAGCT TCGTGGAGCG CGTGCTGAAG AACGATACGG CCAGC**CAGCC**
951     **GGAGCTGGCC CCGGAGGAT**A CGTAAGGATC C
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cluc-ORF: MLE + luciferase + AS + HSV-tag + T = 325 aa, 37458 Da
aa-sequence same as wildtype luciferase from *Renilla reniformis* (Genbank: M63501), but with three changes:

T2A	for additional *Msc*I-restriction site to produce luciferase-fusion proteins
E310D	for additional restriction site used for cloning
Q311T	for additional restriction site used for cloning

Note that the ORF encodes for additional aa:

MLE	if start-ATG upstream of the *Xho*I-restriction site is used
AS	aa-spacer
QPGLAPED	shortened HSV-tag
T	extra aa due to *SnaB*I-restriction site

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1       MLEMASKVYD PEQRKRMITG PQWWARCKQM NVLDSFINYY DSEKHAENAV
51      IFLHGNAASS YLWRHVVPHI EPVARCIIPD LIGMGKSGKS GNGSYRLLDH
101     YKYLTAWFEL LNLPKKIIFV GHDWGACLAF HYSYEHQDKI KAIVHAESVV
151     DVIESWDEWP DIEEDIALIK SEEGEKMVLE NNFFVETMLP SKIMRKLEPE
201     EFAAYLEPFK EKGEVRRPTL SWPREIPLVK GGKPDVVQIV RNYNAYLRAS
251     DDLPKMFIES DPGFFSNAIV EGAKKFPNTE FVKVKGLHFS QEDAPDEMGK
301     YIKSFVERVL KN**DTASQPEL APEDT***
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Plasmid-sequence: pRbcRL(Hsp196) 4731 bp
cluc: 1316-2248
cluc-ORF 1307-2284

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1       CTGACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG
51      CGCAGCGTGA CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC
101     TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC CGTCAAGCTC
151     TAAATCGGGG GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC
201     GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG GGCCATCGCC
251     CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA
301     GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT
351     TCTTTTGATT TATAAGGGAT TTTGCCGATT TCGGCCTATT GGTTAAAAAA
401     TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATTAACGC
451     TTACAATTTC CATTCGCCAT TCAGGCTGCG CAACTGTTGG GAAGGGCGAT
501     CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG GGGATGTGCT
551     GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG
601     TAAAACGACG GCCAGTGAGC GCGCGTAATA CGACTCACTA TAGGGCGAAT
651     TGGAGCTCGC TGAGGCTTGA CATGATTGGT GCGTATGTTT GTATGAAGCT
701     ACAGGACTGA TTTGGCGGGC TATGAGGGCG GGGGAAGCTC TGGAAGGGCC
751     GCGATGGGGC GCGCGGCGTC CAGAAGGCGC CATACGGCCC GCTGGCGGCA
801     CCCATCCGGT ATAAAAGCCC GCGACCCCGA ACGGTGACCT CCACTTTCAG
851     CGACAAACGA GCACTTATAC ATACGCGACT ATTCTGCCGC TATACATAAC
901     CACTCAGCTA GCTTAAGATC CCATCAAGCT TGCATTCCGG GCGCGCCAGA
951     AGGAGCGCAG CCAAACCAGG ATGATGTTTG ATGGGGTATT TGAGCACTTG
1001    CAACCCTTAT CCGGAAGCCC CCTGGCCCAC AAAGGCTAGG CGCCAATGCA
1051    AGCAGTTCGC ATGCAGCCCC TGGAGCGGTG CCCTCCTGAT AAACCGGCCA
1101    GGGGGCCTAT GTTCTTTACT TTTTTACAAG AGAAGTCACT CAACATCTTA
1151    AAATGGCCAG GTGAGTCGAC GAGCAAGCCC GGCGGATCAG GCAGCGTGCT
1201    TGCAGATTTG ACTTGCAACG CCCGCATTGT GTCGACGAAG GCTTTTGGCT
1251    CCTCTGTCGC TGTCTCAAGC AGCATCTAAC CCTGCGTCGC CGTTTCCATT
1301    TGCAGGATGC TCGAGATGGC CAGCAAGGTG TACGACCCCG AGCAGCGCAA
1351    GCGCATGATC ACCGGCCCTC AGTGGTGGGC TCGCTGCAAG CAGATGAACG
1401    TGCTGGACAG CTTCATCAAC TACTACGACA GCGAGAAGCA CGCCGAGAAC
1451    GCCGTGATCT TCCTGCACGG CAACGCCGCC AGCAGCTACC TGTGGCGCCA
1501    CGTGGTGCCC CACATCGAGC CCGTGGCCCG CTGCATCATC CCCGACCTGA
1551    TCGGCATGGG CAAGAGCGGC AAGAGCGGCA ACGGCAGCTA CCGCCTGCTG
1601    GACCACTACA AGTACCTGAC CGCCTGGTTC GAGCTGCTGA ACCTGCCCAA
1651    GAAGATCATC TTCGTGGGCC ACGACTGGGG CGCCTGCCTG GCCTTCCACT
1701    ACAGCTACGA GCACCAGGAC AAGATCAAGG CCATCGTGCA CGCCGAGAGC
1751    GTGGTGGACG TGATCGAGAG CTGGGACGAG TGGCCCGACA TCGAGGAGGA
1801    CATCGCCCTG ATCAAGAGCG AGGAGGGCGA GAAGATGGTG CTGGAGAACA
1851    ACTTCTTCGT GGAGACCATG CTGCCCAGCA AGATCATGCG CAAGCTGGAG
1901    CCCGAGGAGT TCGCCGCCTA CCTGGAGCCC TTCAAGGAGA AGGGCGAGGT
1951    GCGCCGTCCC ACCCTGAGCT GGCCTCGCGA GATCCCCCTG GTGAAGGGCG
2001    GCAAGCCCGA CGTGGTGCAG ATCGTGCGCA ACTACAACGC CTACCTGCGC
2051    GCCAGCGACG ACCTGCCCAA GATGTTCATC GAGAGCGACC CCGGCTTCTT
2101    CAGCAACGCC ATCGTGGAGG GCGCCAAGAA GTTCCCCAAC ACCGAGTTCG
2151    TGAAGGTGAA GGGCCTGCAC TTCAGCCAGG AGGACGCTCC CGACGAGATG
2201    GGCAAGTACA TCAAGAGCTT CGTGGAGCGC GTGCTGAAGA ACGATACGGC
2251    CAGCCAGCCG GAGCTGGCCC CGGAGGATAC GTAAGGATCC CCGCTCCGTG
2301    TAAATGGAGG CGCTCGTTGA TCTGAGCCTT GCCCCCTGAC GAACGGCGGT
2351    GGATGGAAGA TACTGCTCTC AAGTGCTGAA GCGGTAGCTT AGCTCCCCGT
2401    TTCGTGCTGA TCAGTCTTTT TCAACACGTA AAAAGCGGAG GAGTTTTGCA
2451    ATTTTGTTGG TTGTAACGAT CCTCCGTTGA TTTTGGCCTC TTTCTCCATG
2501    GGCGGGCTGG GCGTATTTGA AGCGGGTACC CAGCTTTTGT TCCCTTTAGT
2551    GAGGGTTAAT TGCGCGCTTG GCGTAATCAT GGTCATAGCT GTTTCCTGTG
2601    TGAAATTGTT ATCCGCTCAC AATTCCACAC AACATACGAG CCGGAAGCAT
2651    AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGCTAACTC ACATTAATTG
2701    CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG
2751    CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC GTATTGGGCG
2801    CTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC
2851    GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA
2901    TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
2951    CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC
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3001   CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC
3051   CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT
3101   GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC
3151   TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG TAGGTATCTC
3201   AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
3251   CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA
3301   ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG
3351   ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG
3401   GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC
3451   TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
3501   CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
3551   GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT
3601   CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA
3651   TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT
3701   TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT
3751   GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC
3801   ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
3851   ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG
3901   CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA
3951   AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG
4001   GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG
4051   CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA
4101   TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
4151   GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA
4201   AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT
4251   CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC
4301   AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC
4351   CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG
4401   CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC
4451   GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT
4501   CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG
4551   CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT
4601   CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC
4651   TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
4701   GTTCCGCGCA CATTTCCCCG AAAAGTGCCA C
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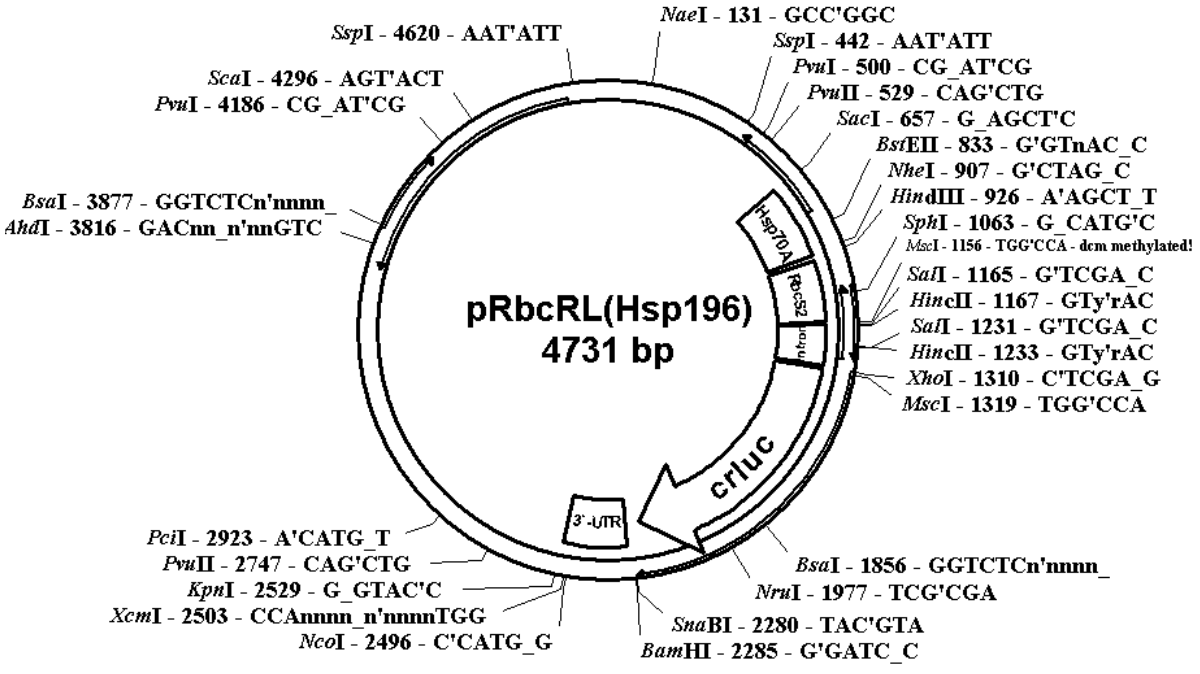
Plasmid-map:



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