

Plasmid:        **pHsp70A/RbcS2-Chlamy**

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. All elements of the *Chlamydomonas* expression cassette for *Renilla-luciferase* can be exchanged using two single cutting restriction enzymes. Note, that contrary to the original publication, the combination of *Bam*HI and *Nco*I should be used to exchange the 3`-UTR, as two *Kpn*I-sites are present.

<u>Plasmid-sequence:</u>	pHsp70A/RbcS2-Chlamy	4025 bp	
	<i>loxP</i> :	1-34	
	<i>crluc</i> expression cassette:	2150-4022	(from pRbcRL(Hsp196))
	( <i>HSP70A</i> -promoter:	2150-2419	( <i>Sac</i> I/ <i>Xho</i> I)
	<i>RBCS2</i> -promoter:	2420-2649	or
	intron1 from <i>RBCS2</i> :	2650-2803	( <i>Xba</i> I/ <i>Xho</i> I)
	<i>crluc</i> :	2804-3778	( <i>Xho</i> I/ <i>Bam</i> HI)
	<i>RBCS2</i> -3`-UTR:	3779-4022	( <i>Bam</i> HI/ <i>Nco</i> I)

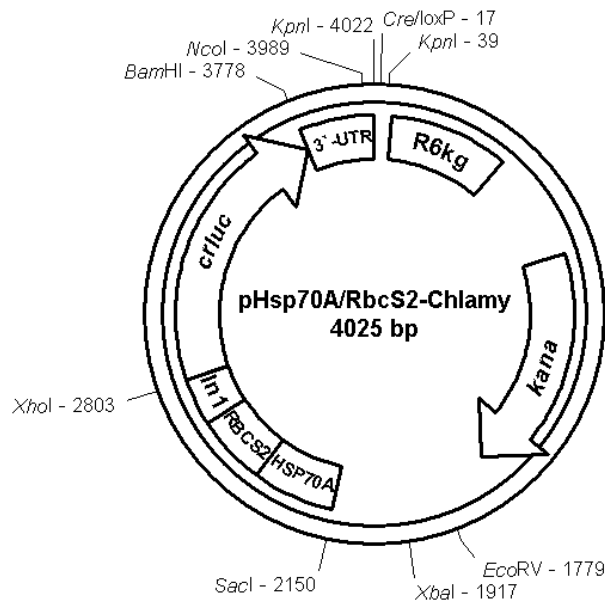
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1  ATAACTTCGT  ATAATGTATG  CTATACGAAG  TTATGGTACC  GCGGCCGCGT
51 AGAGGATCTG  TTGATCAGCA  GTTCAACCTG  TTGATAGTAC  GTACTAAGCT
101 CTCATGTTTC  ACGTACTAAG  CTCTCATGTT  TAACGTACTA  AGCTCTCATG
151 TTTAACGAAC  TAAACCCCTCA  TGGCTAACGT  ACTAAGCTCT  CATGGCTAAC
201 GTACTAAGCT  CTCATGTTTC  ACGTACTAAG  CTCTCATGTT  TGAACAATAA
251 AATTAATATA  AATCAGCAAC  TTAATAGCC  TCTAAGGTTT  TAAGTTTTAT
301 AAGAAAAAAA  AGAATATATA  AGGCTTTTAA  AGCTTTTAAG  GTTTAACGGT
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  GGCTTCTTGT  CCGCCAAGGA
751 TCTGATGGCG  CAGGGGATCA  AGATCTGATC  AAGAGACAGG  ATGAGGATCG
801 TTTTCGCATGA  TTGAACAAGA  TGGATTGCAC  GCAGGTTCTC  CGGCCGCTTG
851 GGTGGAGAGG  CTATTCGGCT  ATGACTGGGC  ACAACAGACA  ATCGGCTGCT
901 CTGATGCCGC  CGTGTTCCGG  CTGTCCAGCGC  AGGGGCGCCC  GGTTCTTTTT
951 GTCAGACCG  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  TTGTTCGATCA  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  CGCTTCCTCG  TGCTTTACGG  TATCGCCGCT
1551 CCCGATTTCG  AGCGCATCGC  CTTCTATCGC  CTTCTTGACG  AGTTCTTCTG
1601 AGCGGGACTC  TGGGGTTCGA  AATGACCGAC  CAAGCGACGC  CCAACCTGCC
1651 ATCACGAGAT  TTCGATTCCA  CCGCCGCCTT  CTATGAAAGG  TTGGGCTTCG
1701 GAATCGTTTT  CCGGGACGCC  GGCTGGATGA  TCCTCCAGCG  CGGGGATCTC
1751 ATGCTGGAGT  TCTTCGCCCA  CCCCAGGATA  TCCGGATATA  GTTCTCCTTT
1801 TCAGCAAAAA  ACCCTCAAG  ACCCGTTTAG  AGGCCCAAG  GGGTTATGCT
1851 AGTTATTGCT  CAGCGGTGGC  AGCAGCCAAC  TCAGCTTCCT  TTCGGGCTTT
1901 GTTAGCAGCC  GGATCTTCTA  GAATCCCCAG  CATGCCTGCT  ATTGTCTTCC
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1951 CAATCCTCCC CCTTGCTGTC CTGCCCCACC CCACCCCCCA GAATAGAATG
2001 ACACCTACTC AGACAATGCG ATGCAATTTT 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 GCCGCGATGG
2251 GGC GCGCGGC GTCCAGAAGG CGCCATACGG CCCGCTGGCG GCACCCATCC
2301 GGTATAAAAG CCCGCGACCC CGAACGGTGA CCTCCACTTT CAGCGACAAA
2351 CGAGCACTTA TACATACCGG ACTATTCTGC CGCTATACAT AACCACTCAG
2401 CTAGCTTAAG ATCCCATCAA GCTTGCATGC CGGGCGCGCC AGAAGGAGCG
2451 CAGCAAACC AGGATGATGT TTGATGGGGT ATTTGAGCAC TTGCAACCCT
2501 TATCCGGAAG CCCCTGGCC CACAAAGGCT AGGCGCCAAT GCAAGCAGTT
2551 CGCATGCAGC CCCTGGAGCG GTGCCCTCCT GATAAACCGG CCAGGGGGCC
2601 TATGTTCTTT ACTTTTTTAC AAGAGAAGTC ACTCAACATC TTAAAATGGC
2651 CAGGTGAGTC GACGAGCAAG CCCGGCGGAT CAGGCAGCGT GCTTGCAGAT
2701 TTGACTTGCA ACGCCCGCAT TGTGTCGACG AAGGCTTTTG GCTCCTCTGT
2751 CGCTGTCTCA AGCAGCATCT AACCTGCGT CGCCGTTTCC ATTTGCAGGA
2801 TGCTCGAGAT GGCCAGCAAG GTGTACGACC CCGAGCAGCG CAAGCGCATG
2851 ATCACCGGCC CTCAGTGGTG GGCTCGCTGC AAGCAGATGA ACGTGCTGGA
2901 CAGCTTCATC AACTACTACG ACAGCGAGAA GCACGCCGAG AACGCCGTGA
2951 TCTTCCTGCA CGGCAACGCC GCCAGCAGCT ACCTGTGGCG CCACGTGGTG
3001 CCCACATCG AGCCCGTGGC CCGCTGCATC ATCCCGACC TGATCGGCAT
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3101 ACAAGTACCT GACCGCCTGG TTCGAGCTGC TGAACCTGCC CAAGAAGATC
3151 ATCTTCGTGG GCCACGACTG GGGCGCCTGC CTGGCCTTCC ACTACAGCTA
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3251 ACGTGATCGA GAGCTGGGAC GAGTGGCCCG ACATCGAGGA GGACATCGCC
3301 CTGATCAAGA GCGAGGAGGG CGAGAAGATG GTGCTGGAGA ACAACTTCTT
3351 CGTGGAGACC ATGCTGCCCA GCAAGATCAT GCGCAAGCTG GAGCCCAGAG
3401 AGTTCGCCGC CTACCTGGAG CCCTTCAAGG AGAAGGGCGA GGTGCGCCGT
3451 CCCACCTGA GCTGGCCTCG CGAGATCCCC CTGGTGAAGG GCGGCAAGCC
3501 CGACGTGGTG CAGATCGTGC GCAACTACAA CGCTACCTG CGCGCCAGCG
3551 ACGACCTGCC CAAGATGTTT ATCGAGAGCG ACCCCGGCTT CTTAGCAAC
3601 GCCATCGTGG AGGGCGCCAA GAAGTTCCCC AACACCGAGT TCGTGAAGGT
3651 GAAGGGCCTG CACTTCAGCC AGGAGGACGC TCCCAGCAG ATGGGCAAGT
3701 ACATCAAGAG CTTTCGTGGAG CGCGTGCTGA AGAACGATC GGCCAGCCAG
3751 CCGGAGCTGG CCCCAGGAG TACGTAAGGA TCCCCTCC GTGTAATGG
3801 AGGCGCTCGT TGATCTGAGC CTTGCCCCCT GACGAACGGC GGTGGATGGA
3851 AGATACTGCT CTCAAGTGCT GAAGCGGTAG CTTAGCTCCC CGTTTCGTGC
3901 TGATCAGTCT TTTTCAACAC GTAAAAAGCG GAGGAGTTTT GCAATTTTGT
3951 TGGTTGTAAC GATCCTCCGT TGATTTTGGC CTCTTCTCC ATGGGCGGGC
4001 TGGCGTATT TGAAGCGGGT ACCCC

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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.
- 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.