

Plasmid: **pKScGFP**

Synthetic gene encoding the green fluorescent protein (GFP) from *Aequorea victoria* adapted to the nuclear codon usage of *Chlamydomonas reinhardtii* (*cGFP*). In vector pBluescriptII-KS (cloned via *Xho*I/*Bam*HI, ampicillin resistance).

cGFP-gene: Genbank AF188479, nucleotides 1-717, 717bp
with recombinant 5'-XhoI and 3'-BamHI-restriction sites

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1      CTCGAGATGG CCAAGGGCGA GGAGCTGTTC ACCGGTGTGG TCCCCATCCT
51     GGTGGAGCTG GACGGCGACG TGAACGGCCA CAAGTTCTCC GTCTCCGGCG
101    AGGGTGAGGG TGACGCCACC TACGGCAAGC TGACCCTGAA GTTCATCTGC
151    ACCACCGGCA AGCTGCCCCG GCCCTGGCCC ACCCTGGTCA CCACCCTGAC
201    CTACGGTGTG CAGTGCTTCT CCCGCTACCC CGACCACATG AAGCAGCACG
251    ACTTCTCAA GTCCGCCATG CCCGAGGGCT ACGTGCAGGA GCGCACCATC
301    TTCTTCAAGG ACGACGGCAA CTACAAGACC CGCGCCGAGG TCAAGTTCGA
351    GGGCGACACC CTGGTGAACC GCATCGAGCT GAAGGGCATC GACTTCAAGG
401    AGGACGGCAA CATCCTGGGC CACAAGCTGG AGTACAATA CAACTCCCAC
451    AACGTGTACA TCATGGCCGA CAAGCAGAAG AACGGCATCA AGGTGAACTT
501    CAAGATCCGC CACAACATCG AGGACGGCTC CGTGCAGCTG GCCGACCACT
551    ACCAGCAGAA CACCCCATC GCGGATGGCC CCGTGCTGCT GCCCGACAAC
601    CACTACCTGT CCATCCAGTC CGCCCTGTCC AAGGACCCCA ACGAGAAGCG
651    CGACCACATG GTCCTGCTGG AGTTCGTAC CGCTGCCGGC ATCACCACG
701    GCATGGACGA GCTGTACAAG TAAGGATTC
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cGFP-ORF: 238 aa, 26814 Da
aa-sequence same as wildtype GFP from *Aequorea victoria* (Genbank: P42212), but with four changes:

- S2A** for additional *Msc*I-restriction site to produce GFP-fusion proteins
- F64L** for improved fluorescence properties
- S65T** for improved fluorescence properties
- T203I** error during synthesis

Note that only three changes (S2A, F64L, S65T) are mentioned in the original publication (Fuhrmann, M. et al., 1999, Plant J 19, 353-61), although pKScGFP is the original clone.

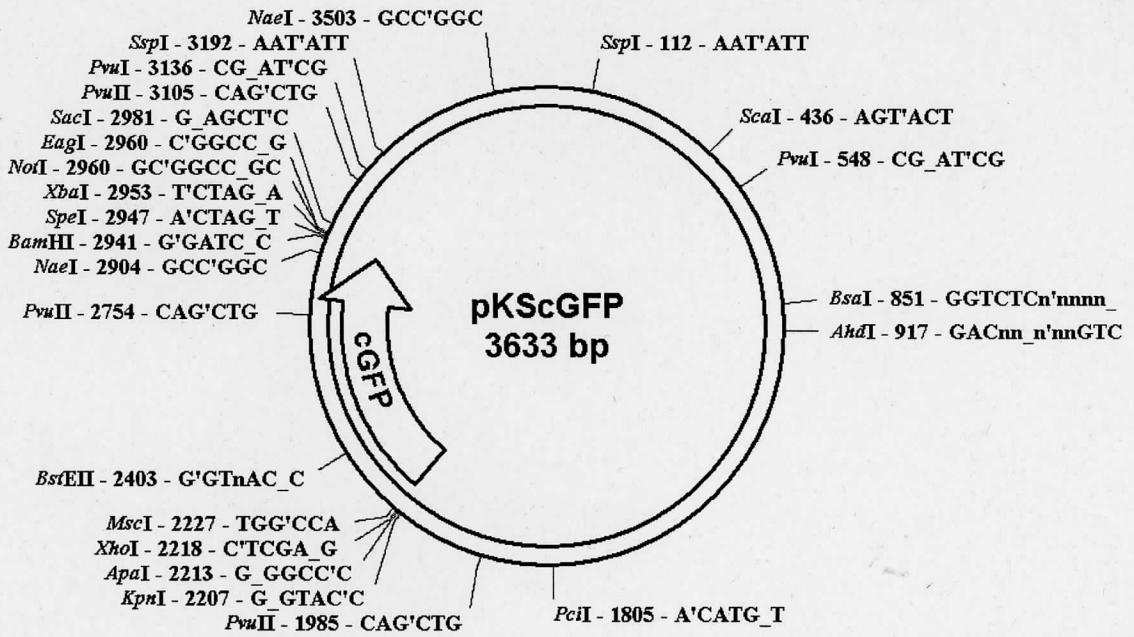
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1      MAKGEELFTG VVPILVELDG DVNGHKFSVS GEGEGDATYG KLTLKFICTT
51     GKLPVPWPTL VTTLTYGVCQ FSRYPDHMKQ HDFFKSAMPE GYVQERTIFF
101    KDDGNYKTRA EVKFECDTLV NRIELKGIDF KEDGNILGHK LEYNYNSHNV
151    YIMADKQKNG IKVNFKIRHN IEDGSVQLAD HYQQNTPIGD GPVLLPDNHY
201    LSIQSALSXD PNEKRDMVL LEFVTAAGIT HGMDELYK*
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Plasmid-sequence: pKScGFP 3633 bp
 cGFP: 2224-2940

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1      GGTGGCACTT TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT
51     CTAATAACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA
101    TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT
151    GTCGCCCTTA TTCCCTTTTT TCGGCATTT TGCCTTCCTG TTTTGTCTCA
201    CCCAGAAACG CTGGTGAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC
251    GAGTGGGTTA CATCGAAGT GATCTCAACA GCGGTAAGAT CCTTGAGAGT
301    TTTGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT
351    ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG CAACTCGGTC
401    GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA
451    GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC
501    CATAACCATG AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG
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551 GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA
601 ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA
651 CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG TTGCGCAAAC
701 TATTAAGTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC
751 TGGATGGAGG CGGATAAAGT TGCAGGACCA CTCTGCGCT CGGCCCTTCC
801 GGCTGGCTGG TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC
851 GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA
901 GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA
951 GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC
1001 AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT
1051 AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAATCCC
1101 TTAACGTGAG TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA
1151 AAGGATCTTC TTGAGATCCT TTTTTCTGCG GCGTAATCTG CTGCTTGCAA
1201 ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTCGCCGG ATCAAGAGCT
1251 ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA
1301 ATACTGTCTT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT
1351 GTAGCACCGC CTACATACTT CGCTCTGCTA ATCCTGTTC ACAGTGGCTG
1401 TGCCAGTGGC GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACTGATG
1451 TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTT GTGCACACAG
1501 CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA
1551 GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC
1601 CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG
1651 GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT
1701 TGAGCGTCGA TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA
1751 ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT
1801 GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT
1851 TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGCAAAGC
1901 GACCGGATCA AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG
1951 CCTCTCCCGG CGCGTTGGCC GATTCAATTA TGCAGCTGGC ACGACAGGTT
2001 TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC
2051 TCACTCATTG GGCACCCAG GCTTTACTT TTATGCTTCC GGCTCGTATG
2101 TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA
2151 CCATGATTAC GCCAAGCGCG CAATTAACCC TCACTAAAGG GAACAAAAGC
2201 TGGGTACCGG GCCCCCCTC GAGATGGCCA AGGGCGAGGA GCTGTTCCAC
2251 GGTGTGGTCC CCATCCTGGT GGAGCTGGAC GGCAGCTGA ACGGCCACAA
2301 GTTCTCCGTC TCCGGCGAGG GTGAGGGTGA CGCCACCTAC GGCAGCTGA
2351 CCTGAAAGTT CATCTGCACC ACCGGCAAGC TGCCCGTACC CTGCCCCACC
2401 CTGGTACCCA CCTGACCTA CGGTGTGCAG TGCTTCTCCC GGTACCCCGA
2451 CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC GAGGGCTACG
2501 TGCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC
2551 GCCGAGGTCA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA
2601 GGGCATCGAC TTCAAGGAGG ACGGCAACAT CCTGGGCCAC AAGCTGGAGT
2651 ACAACTACAA CTCCCACAAC GTGTACATCA TGGCCGACAA GCAGAAGAAC
2701 GGCATCAAGG TGAACCTCAA GATCCGCCAC AACATCGAGG ACGGCTCCGT
2751 GCAGCTGGCC GACCACTACC AGCAGAACAC CCCCATCGGC GATGGCCCCG
2801 TGCTGTGCC CGACAACCAC TACCTGTCCA TCCAGTCCG CCTGTCCAAG
2851 GACCCCAACG AGAAGCGCGA CCACATGGTC CTGCTGGAGT TCGTACCAGC
2901 TGCCGGCATC ACCCACGGCA TGGACGAGCT GTACAAGTAA GGATCCACTA
2951 GTTCTAGAGC GGCCGCCACC GCGGTGGAGC TCCAATTCGC CCTATAGTGA
3001 GTCGTATTAC GCGCGCTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG
3051 AAAACCTTGG CGTTACCCAA CTTAATCGCC TTGCAGACA TCCCCCTTTC
3101 GCCAGCTGGC GTAATAGCGA AGAGGCCCGC ACCGATCGCC CTTCCCAACA
3151 GTTGCGCAGC CTGAATGGCG AATGGAAATT GTAAGCGTTA ATATTTTGT
3201 AAAATTCCGG TTAAATTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG
3251 CCGAAATCGG CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG
3301 TTGAGTGTG TTCCAGTTTG GAACAAGAGT CCACTATTA AGAACGTGGA
3351 CTCCAACGTC AAAGGGCGAA AAACCGTCTA TCAGGGCGAT GCCCCACTAC
3401 GTGAACCATC ACCCTAATCA AGTTTTTTGG GGTTCGAGTG CCGTAAAGCA
3451 CTAAATCGGA ACCCTAAAGG GAGCCCCGA TTTAGAGCTT GACGGGGAAA
3501 GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG
3551 CTAGGGCGCT GGCAAGTGTA GCGGTCACGC TGCAGCTAAC CACCACACC
3601 GCCGCGCTTA ATGCGCCGCT ACAGGGCGG TCA

Plasmid-map:



Reference: Fuhrmann, M., Oertel, W. and Hegemann, P. (1999) A synthetic gene coding for the green fluorescent protein (GFP) is a versatile reporter in *Chlamydomonas reinhardtii*. Plant J 19, 353-64.