

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 *XhoI/BamHI*, 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  AGCTGCCCGT  GCCCTGGCCC  ACCCTGGTCA  CCACCCTGAC
201    CTACGGTGTG  CAGTGCTTCT  CCCGCTACCC  CGACCACATG  AAGCAGCACG
251    ACTTCTTCAA  GTCCGCCATG  CCCGAGGGCT  ACGTGCAGGA  GCGCACCATC
301    TTCTTCAAGG  ACGACGGCAA  CTACAAGACC  CGCGCCGAGG  TCAAGTTCGA
351    GGGCGACACC  CTGGTGAACC  GCATCGAGCT  GAAGGGCATC  GACTTCAAGG
401    AGGACGGCAA  CATCCTGGGC  CACAAGCTGG  AGTACAAC TA  CAACTCCCAC
451    AACGTGTACA  TCATGGCCGA  CAAGCAGAAG  AACGGCATCA  AGGTGAACTT
501    CAAGATCCGC  CACAACATCG  AGGACGGCTC  CGTGCAGCTG  GCCGACCACT
551    ACCAGCAGAA  CACCCCCATC  GGCGATGGCC  CCGTGCTGCT  GCCCGACAAC
601    CACTACCTGT  CCATCCAGTC  CGCCCTGTCC  AAGGACCCCA  ACGAGAAGCG
651    CGACCACATG  GTCCTGCTGG  AGTTCGTCAC  CGCTGCCGGC  ATCACCCACG
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 *MscI*-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.

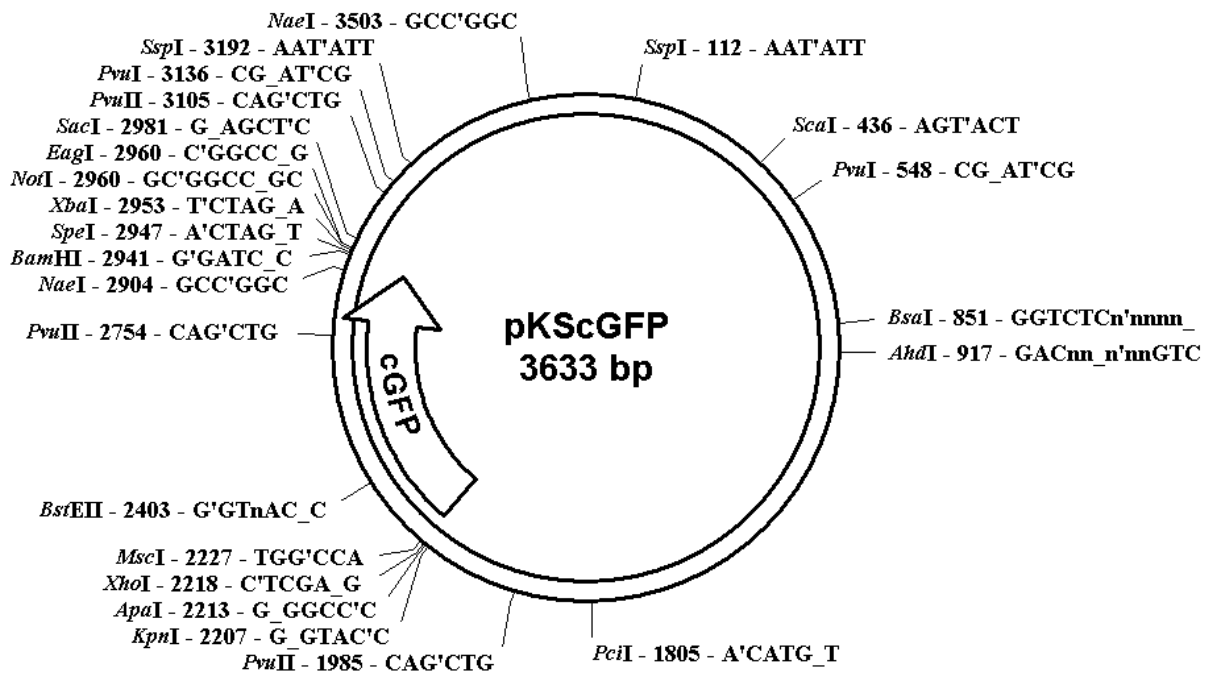
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1      MAKGEELFTG  VVPILVELDG  DVNGHKFSVS  GEGEGDATYG  KLTLKFICTT
51     GKLPVPWPTL  VTTLTYGVQC  FSRYPDHMKQ  HDFFKSAMPE  GYVQERTIFF
101    KDDGNYKTRA  EVKFECDTLV  NRIELKGIDF  KEDGNILGHK  LEYNYNSHNV
151    YIMADKQKNG  IKVNFKIRHN  IEDGSVQLAD  HYQQNTPIGD  GPVLLPDNHY
201    LSIQSALSKD  PNEKRDHMLV  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     CTAAATACAT  TCAAATATGT  ATCCGCTCAT  GAGACAATAA  CCCTGATAAA
101    TGCTTCAATA  ATATTGAAAA  AGGAAGAGTA  TGAGTATTCA  ACATTTCCGT
151    GTCGCCCTTA  TTCCCTTTTT  TGCGGCATTT  TGCCTTCTCG  TTTTGTCTCA
201    CCCAGAAACG  CTGGTAAAAG  TAAAAGATGC  TGAAGATCAG  TTGGGTGCAC
251    GAGTGGGTTA  CATCGAACTG  GATCTCAACA  GCGGTAAGAT  CCTTGAGAGT
301    TTTGCGCCCG  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 GCTTTTTTTCG ACAACATGGG GGATCATGTA
601 ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA
651 CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG TTGCGCAAAC
701 TATTAACTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC
751 TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT 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 TAAAACCTCA TTTTAAATTT
1051 AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC
1101 TTAACGTGAG TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA
1151 AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA
1201 ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTGTCGGG ATCAAGAGCT
1251 ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA
1301 ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT
1351 GTAGCACC GC CTACATACT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC
1401 TGCCAGTGGC GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT
1451 TACCGGATAA GGCAGCGCG TCGGGCTGAA CGGGGGGTTT GTGCACACAG
1501 CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA
1551 GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAAGCG 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 ACCGAGGAGC
1901 GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG
1951 CCTCTCCCCG CGCGTTGGCC GATTCAATTA TGCAGCTGGC ACGACAGGTT
2001 TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC
2051 TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG
2101 TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA
2151 CCATGATTAC GCCAAGCGCG CAATTAACCC TCACTAAAGG GAACAAAAGC
2201 TGGGTACCGG GCCCCCCCTC GAGATGGCCA AGGGCGAGGA GCTGTTACC
2251 GGTGTGGTCC CCATCCTGGT GGAGCTGGAC GCGACGTGA ACGGCCACAA
2301 GTTCTCCGTC TCCGGCGAGG GTGAGGGTGA CGCCACCTAC GGCAAGCTGA
2351 CCCTGAAGTT CATCTGCACC ACCGGCAAGC TGCCCGTGCC CTGGCCCACC
2401 CTGGTCACCA CCCTGACCTA CCGTGTGCAG TGCTTCTCCC GCTACCCCGA
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 TGCTGCTGCC CGACAACCAC TACCTGTCCA TCCAGTCCGC CCTGTCCAAG
2851 GACCCCAACG AGAAGCGCGA CCACATGGTC CTGCTGGAGT TCGTCACCGC
2901 TGCCGGCATC ACCCACGGCA TGGACGAGCT GTACAAGTAA GGATCCACTA
2951 GTTCTAGAGC GGCCGCCACC GCGGTGGAGC TCCAATTCGC CCTATAGTGA
3001 GTCGTATTAC GCGCGCTCAC TGGCCGTCGT TTTACAACGT CGTGA CTGGG
3051 AAAACCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCTTTC
3101 GCCAGCTGGC GTAATAGCGA AGAGGCCCGC ACCGATCGCC CTTCCCAACA
3151 GTTGCGCAGC CTGAATGGCG AATGGAAATT GTAAGCGTTA ATATTTTGT
3201 AAAATTCGCG TTAAATTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG
3251 CCGAAATCGG CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG
3301 TTGAGTGTTG TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA
3351 CTCCAACGTC AAAGGGCGAA AAACCGTCTA TCAGGGCGAT GGCCCACTAC
3401 GTGAACCATC ACCCTAATCA AGTTTTTTTG GGTGAGGTG CCGTAAAGCA
3451 CTAAATCGGA ACCCTAAAGG GAGCCCCGA TTTAGAGCTT GACGGGGAAA
3501 GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG
3551 CTAGGGCGCT GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC
3601 CCCGCGCTTA ATGCGCCGCT ACAGGGCGCG 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.