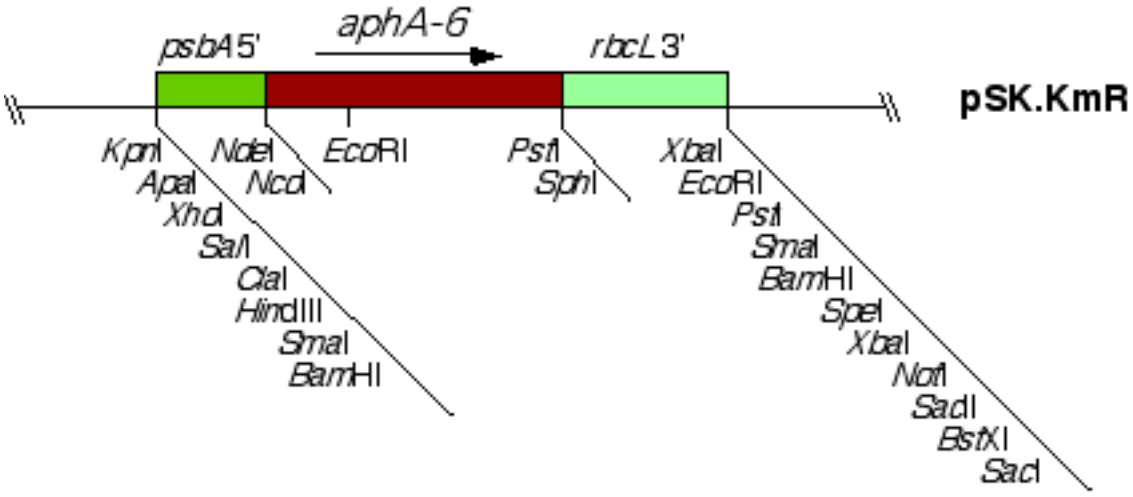
The aphA-6 cassette

We have developed a series of chloroplast expression vectors and used these to produce a new dominant marker for chloroplast transformation in *Chlamydomonas reinhardtii*.

Bateman JM and Purton S (2000) Tools for chloroplast transformation in Chlamydomonas: expression vectors and a new dominant selectable marker. 263: 404-410.

The marker is based on the bacterial *aphA-6* gene that encodes an aminoglycoside phosphotransferase (APH(3')) and mediates resistance to kanamycin and amikacin [Martin et al. (1988) Molec. Microbiol 2:615-625]. As shown in the figure below, the coding region of *aphA-6* is fused to the promoter and 5' UTR of the *C. reinhardtii* chloroplast gene *psbA* and the 3' UTR of *rbcL*. The cassette is flanked by various RE sites to facilitate cloning. The cassette has been inserted in the vector pBluescript SK- to give the plasmid pSK.KmR.



The DNA sequence of the cassette (1,658 bp), from the *Kpn*I site to the *Sac*I site within the pBluescript MCS, is given below. The codon region of the *aphA-6* gene is in UPPERCASE.

ggtaccgggccccccctcgaggtcgacggtatcgataagcttgatcccgggatccaagaaaagtgagctattaacgcgtc
ctattttaatactccgaaggaggcagttggcaggcaactgccactgacgtcccgtaagggtaaggggacgtccactggcg
tcccgtaaggggaaggggacgtaggtacataaatgtgctaggtaactaacgtttgatttttgtggtataatatatgtac
catgcttttaatagaagcttgaatttataaattaaaatatttttacaatattttacggagaaattaaaactttaaaaaaa
ttaacatATGACCATGGAATTACCAAATATTATTCAACAATTTATCGGAAACAGCGTTTTAGAGCCAAATAAAATTGGTC
AGTCGCCATCGGATGTTTATTCTTTTAATCGAAATAATGAAACTTTTTTTCTTAAGCGATCTAGCACTTTATATACAGAG
ACCACATACAGTGTCTCTCGTGAAGCGAAAATGTTGAGTTGGCTCTCTGAGAAATTAAAGGTGCCTGAACTCATCATGAC
TTTTCAGGATGAGCAGTTTGAATTCATGATCACTAAAGCGATCAATGCAAAACCAATTTCAGCGCTTTTTTTAACAGACC

AAGAATTGCTTGCTATCTATAAGGAGGCACTCAATCTGTTAAATTCAATTGCTATTATTGATTGTCCATTTATTTCAAAC
ATTGATCATCGGTTAAAAGAGTCAAAATTTTTTATTGATAACCAACTCCTTGACGATATAGATCAAGATGATTTTGACAC
TGAATTATGGGGAGACCATAAAACTTACCTAAGTCTATGGAATGAGTTAACCGAGACTCGTGTTGAAGAAAGATTGGTTT
TTTCTCATGGCGATATCACGGATAGTAATATTTTTATAGATAAATTCAATGAAATTTATTTTTTAGATCTTGGTCGTGCT
GGGTTAGCAGATGAATTTGTAGATATATCCTTTGTTGAACGTTGCCTAAGAGAGGATGCATCGGAGGAAACTGCGAAAAT
ATTTTTAAAGCATTTAAAAAATGATAGACCTGACAAAAGGAATTATTTTTTAAAACTTGATGAATTGAATTGAttccaag
cattatctaaaatactctgcaggcatgcaagctagcttgtactcaagctcgtaacgaaggtcgtgaccttgctcgtgaag
gtggcgacgtaattcgttcagcttgtaaatggtctccagaacttgctgctgcatgtgaagtttggaaagaaattaaattc
gaatttgatactattgacaaactttaatttttattttttcatgatgtttatgtgaatagcataaacatcgtttttatttttt
atggtgtttaggttaaatacctaaacatcattttacatttttaaaattaagttctaaagttatcttttgtttaaatttgc
ctgtctttataaattacgatgtgccagaaaaataaaatcttagctttttattatagaatttatctttatgtattatattt
tataagttataataaaagaaatagtaacatactaaagcggatgtagcgcgtttatcttaacggaagtctagaggcatcga
attcctgcagcccgggggatccactagttctagagcggccgccaccgcggtggagctc

Note: Although the psbA::aphA-6::rbcL construct is functional in *E. coli*, conferring kanamycin resistance @20µg/ml, we have found that the marker (unlike an equivalent psbA::aadA::rbcL construct) does not work well in the initial selection of recombinants (*i.e.* when cloning the cassette into a plasmid it is difficult to select for transformants on Km-containing medium). A better strategy is to select for *E. coli* transformants using the marker carried on your plasmid (*e.g.* ampicillin resistance) and then score for transformant colonies that are also resistant to 20µg/ml kanamycin.

Saul Purton
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