T6: The parameters of gene targeting in Physcomitrella patens: a systematic analysis

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We have undertaken a systematic analysis of the factors affecting the frequency of homologous-recombination-mediated gene targeting in Physcomitrella patens. Using a PCR-based method we have analysed the fate of transforming DNA in 1700 stably transformed lines, for three different loci, obtained using PEG-mediated DNA uptake by protoplasts.

• There is a strong statistical correlation between the length of homologous DNA in the transforming constructs and the frequency with which gene targeting occurs.

• Where targeting has occurred preferentially at one end of the targeting construct, there is a strong statistical correlation with the length of homology contained in this sequence.

• For allele replacement to occur with reasonable frequency, a minimum homology length of ca. 800bp is recommended, and the frequency with which allele replacement occurs is strongly statistically correlated with the length of the shorter homologous sequence flanking the selectable marker.

• Transformation by microprojectile bombardment also results in gene targeting. However the frequency of targeting events obtained is significantly lower than that obtained by PEG-mediated protoplast transfection. The use of linear DNA appears to be critical to obtain allele replacements by microprojectile bombardment.