

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.