

**Efficiency of gene targeting in *Physcomitrella patens* I. Factors affecting gene targeting efficiency by direct DNA transfer.**

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The high efficiency of somatic homologous recombination in *Physcomitrella patens* is now a widely appreciated phenomenon that is increasingly being exploited as a tool for gene functional analysis. However, the factors affecting the frequency of gene targeting events have not been systematically investigated. These factors include the nature of targeting constructs, the extent of homology between targeted locus and targeting construct, the location and orientation of selectable marker genes within targeting constructs and the means of DNA delivery.

As a part of the EC Framework V 'Pregene' programme, we are systematically analysing these factors. We have selected two members of small multigene families in *P. patens* as targets to determine the conditions made by these factors to the efficiency of gene targeting. These are i) a member of the RNA-binding protein 'Puf-domain' gene family and ii) a member of the gene family encoding the small rho-1-like GTP binding (*rop*) protein.

By introducing an *nptII* selectable marker cassette into different site within genomic clones of these genes, we have created a series of targeting constructs of varying homology length and 3'/5' symmetry. By analysing the nature of the integration events in several hundred stable transformants we are beginning to define, quantitatively, the relationship between homology length, symmetry and targeting efficiency in *P. patens*.

This work is a part of EC framework V, PREGENE project.