

## Large-scale functional genomics of *Physcomitrella patens*

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The moss *Physcomitrella patens* is an attractive model system for plant biology and functional genome analysis. It shares many biological features with higher plants but has the unique advantage of an efficient homologous recombination system for its nuclear DNA. This allows precise genetic manipulations and targeted knockouts to study gene function, an approach that due to the very low frequency of targeted recombination events is not routinely possible in any higher plant.

We have developed a high-throughput system for generating a large number (40,000 to date) of *Physcomitrella* mutants via two distinct approaches, based on PEG-mediated transformation of protoplasts. The forward route uses transformation with a gene disruption library produced by transposon mutagenesis of a large number of anonymous cDNA clones; it is expected to yield a saturated *Physcomitrella* mutant collection with insertion mutations in most expressed genes. The reverse route uses knockout DNA constructs specific for defined candidate genes. Quality control with respect to stable integration of the *nptII* marker gene and ploidy level is performed for each transformant. An immediate phenotypic analysis of transformants is made possible by the predominance of the haploid gametophytic state in the life cycle of the moss. Our data show that among the first set of 16,000 transformants, 98% have integrated the *nptII* cassette into the genome. Phenotypic screens revealed that on average 16% of the transformants differed from the wild-type in a variety of developmental, morphological and physiological characteristics, suggesting a highly efficient mutagenesis.