

**An AFLP-based genetic linkage map of *Physcomitrella*
Yasuko Kamisugi and Andrew C. Cuming.**

**Centre for Plant Science, Faculty of Biological Sciences,
Leeds University, Leeds LS2 9JT, UK.**

Tel: +44-113-3433096

Fax: +44-113-3433144

e-mail: a.c.cuming@leeds.ac.uk

Genetic analysis provides one of the most powerful tools for discovering the molecular basis of complex developmental traits in living organisms. Through the identification and analysis of mutants that perturb a particular phenotype, the investigator can determine the number of genes that contribute to that trait, by complementation testing, their relationship to one another in a genetic network of interactions, through combinatorial analyses of epistasis, and the underlying molecular nature of the genes and enzymes involved through the cloning and sequence analysis of the mutated genes. Moreover, this experimental approach is philosophically satisfying as it requires no *a priori* knowledge or speculation concerning the nature of the genes involved.

While *Physcomitrella* provides an excellent model for the assignment of gene function through “reverse genetics”, its full potential will only be realised once efficient protocols for forward genetic analysis (moving from phenotype to gene sequence) can be realised. The first requirement for such an approach is the construction of a well-marked genetic linkage map that will enable the map-based cloning of randomly generated phenotypic mutants in unknown genes.

To this end we have generated a genetic linkage map based on Amplified Fragment Length Polymorphisms (AFLPs) – molecular markers ideally suited to high-throughput applications. We generated a population of *ca.* 2000 recombinant haploid progeny by crossing a transgenically marked Gransden2004 plant with the Villersexel-K3 accession, and selected 188 progeny as a mapping population. For the analysis we used fluorescently labelled AFLP primer pairs for fragment resolution using an ABI3130 capillary sequencer. An initial screen of 224 primer combinations was undertaken with the parental genotypes before selecting 39 combinations for the genotyping of the mapping population. In excess of 10,000 genetic loci were interrogated and 1561 polymorphic loci were identified and genotyped.

Using the genetic analysis software JoinMap, we have constructed a linkage map in which 1317 markers have been placed on 26 linkage groups with a total genetic map length of 4461cM. The next challenge is to integrate the genetic map with the physical map (the genome sequence). This will be undertaken through a combination of bioinformatic and experimental identification of the sequences of AFLP fragments, and incorporation of additional genetic markers - in particular the gene-anchored SSR markers identified in Freiburg and SNP loci derived from the analysis of Villersexel cDNA sequences.

Financial support from the Gatsby Charitable Foundation is gratefully acknowledged.