

Molecular analysis of tagged *Physcomitrella patens* mutants

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Functional genomics relies on efficient and fast re-isolation of tagged genomic loci. For *Physcomitrella patens* three protocols (iPCR, RAGIL, and Trap-cloning) have been established and optimised for the analysis on integration mutants. iPCR is based on restriction and circular religation of genomic DNA, followed by PCR amplification of DNA regions flanking the marker gene. RAGIL, the Rapid Amplification of Genomic Integration Loci is a derivative of RACE protocols. While iPCR and RAGIL rely on PCR-techniques, Trap-cloning is a plasmid rescue approach to clone and screen for marker gene flanking DNA sequences. All protocols have been tested and applied to targeted knockout mutants of *Physcomitrella* and mutants from our mutant collection and all proved to be applicable for mutant analysis in *Physcomitrella*. Best results have been obtained by Trap-cloning that has been used successfully to re-isolate large flanking sequences (up to 9kb). To date we re-isolated 21 genomic integration loci, homologous and non-homologous. Mutant analysis of tagged mutants is faster than in *Arabidopsis* and in contrast to this model plant a gene/function correlation can be rapidly confirmed in *Physcomitrella* by targeted knockout.

This work is a joint project with BASF Plant Science GmbH.