

## **T1: RNAi in the moss *Physcomitrella patens***

Magdalena Bezanilla, Aihong Pan, and Ralph S. Quatrano

Department of Biology, Washington University, 1 Brookings Drive, Saint Louis, MO 63130

E-mail: manena@biology.wustl.edu

The moss *Physcomitrella patens* performs efficient homologous recombination, which allows for the study of individual gene function by generating gene disruptions. Yet if the gene of study is essential, gene disruptions cannot be isolated in the predominantly haploid *Physcomitrella*. Additionally, disruption of a gene does not always generate observable phenotypes due to redundant functions from related genes. However, RNA interference (RNAi) can provide mutants for both of these situations. We show that RNAi disrupts gene expression in *Physcomitrella*, adding a significant tool for the study of plant gene function. To assay for RNAi in moss, we constructed a line (NLS-4) expressing a nuclearly localized GFP:GUS fusion reporter protein. We targeted the reporter protein with two RNAi constructs, GUS-RNAi and GFP-RNAi, expressed transiently by particle bombardment. Transformed protonemal cells are marked by co-bombardment with dsRed2, which diffuses between the nucleus and cytoplasm. Cells transformed with control constructs have nuclear/cytoplasmic red fluorescence and nuclear green fluorescence. In cells transformed with GUS-RNAi or GFP-RNAi constructs, the nuclear green fluorescence was reduced on average nine-fold as soon as 48 hours after transformation. Moreover, isolated lines of NLS-4 stably transformed with GUS-RNAi construct have silenced nuclear GFP, indicating that RNAi is propagated stably. Thus, RNAi adds a powerful tool for functional analysis of plant genes in moss.