

An RNAi System in *Physcomitrellapatens* with an Internal Marker for Silencing Allows for Rapid Identification of Loss of Function Phenotypes

Magdalena Bezanilla, Pierre-François Perroud, Aihong Pan, Paul Klueh, and Ralph S. Quatrano. (2005) *Plant Biol.* 7:251-257.

Abstract

RNAi is a powerful method for generating loss of function mutants, especially for targeting genes belonging to large gene families. We have recently shown that RNAi functions in the moss *Physcomitrella patens*. We obtained stable lines that show constitutive silencing of a nuclearly localized GFP:GUS fusion protein (NLS:GFP:GUS). However lines that display silencing of the protein, do not necessarily have reduced transcript levels. Therefore we developed a system that silences the NLS:GFP:GUS reporter construct at the same time that it silences a gene of interest. We incorporated Gateway® (Invitrogen) recombination cassettes into these vectors to facilitate cloning of many different cDNA sequences. In addition we have generated vectors that contain genomic moss DNA sequence information to increase the production of stable moss lines. We show that transformation with these constructs results in strong silencing within 24 hours and is stable for at least a month after transformation. We incorporated FtsZ2-1, whose loss of function phenotype is known, as a test case for analyzing phenotypes. We show that 100% of regenerating colonies that have silenced GFP exhibit a loss of function FtsZ2-1 phenotype, validating the use of this system to assay phenotypes for plant genes of unknown function.

Figure 2. Moss RNAi Constructs.

All RNAi constructs contain inverted repeats of the target sequence separated by a small loop region. The inverted repeats are preceded by the strong constitutive maize ubiquitin promoter (large black arrow) and followed by the NOS terminator sequence (black box). The thin brown arrows depict the direction of the reading frame of the Gateway® cassette. In all cases arrows within a box denote the orientation of the reading frame for that particular region of sequence. (A) pUFi (plasmid Ubiquitin GFP RNAi) and pUFGi (plasmid Ubiquitin GFP Gateway® RNAi) have GFP sequences to target silencing of NLS:GFP:GUS, with green boxes indicating the GFP sequences. (B) pUGi (plasmid Ubiquitin GUS RNAi) and pUGGi (plasmid Ubiquitin GUS Gateway® RNAi) have GUS sequences to target silencing of NLS:GFP:GUS, with blue boxes indicating the GUS sequence. Vectors in (A) and (B) have pMHUbi as the backbone. (C) The targeting

constructs pTUGi (plasmid Targeting Ubiquitin GUS RNAi) and pTUGGi (plasmid Targeting Ubiquitin GUS Gateway® RNAi) contain the RNAi and Aph [IV] resistance cassettes between two regions of sequence from the *Physcomitrella* Pp108 locus. The grey boxes show the CaMV 35S promoter (arrow denotes direction of transcription), the Aph [IV] gene, which confers hygromycin resistance, and the CaMV 35S terminator sequence. This cassette is placed between two LOX sites, denoted by purple triangles, which allow for subsequent removal of the resistance cassette upon transient expression of CRE. (D) pTEMF_i (plasmid Targeting EM promoter GFP RNAi) is also a targeting vector but expression of the RNAi cassette is driven by the wheat EM promoter depicted by the purple arrow. Vectors in (C) and (D) have pGEM® T-easy as the backbone.

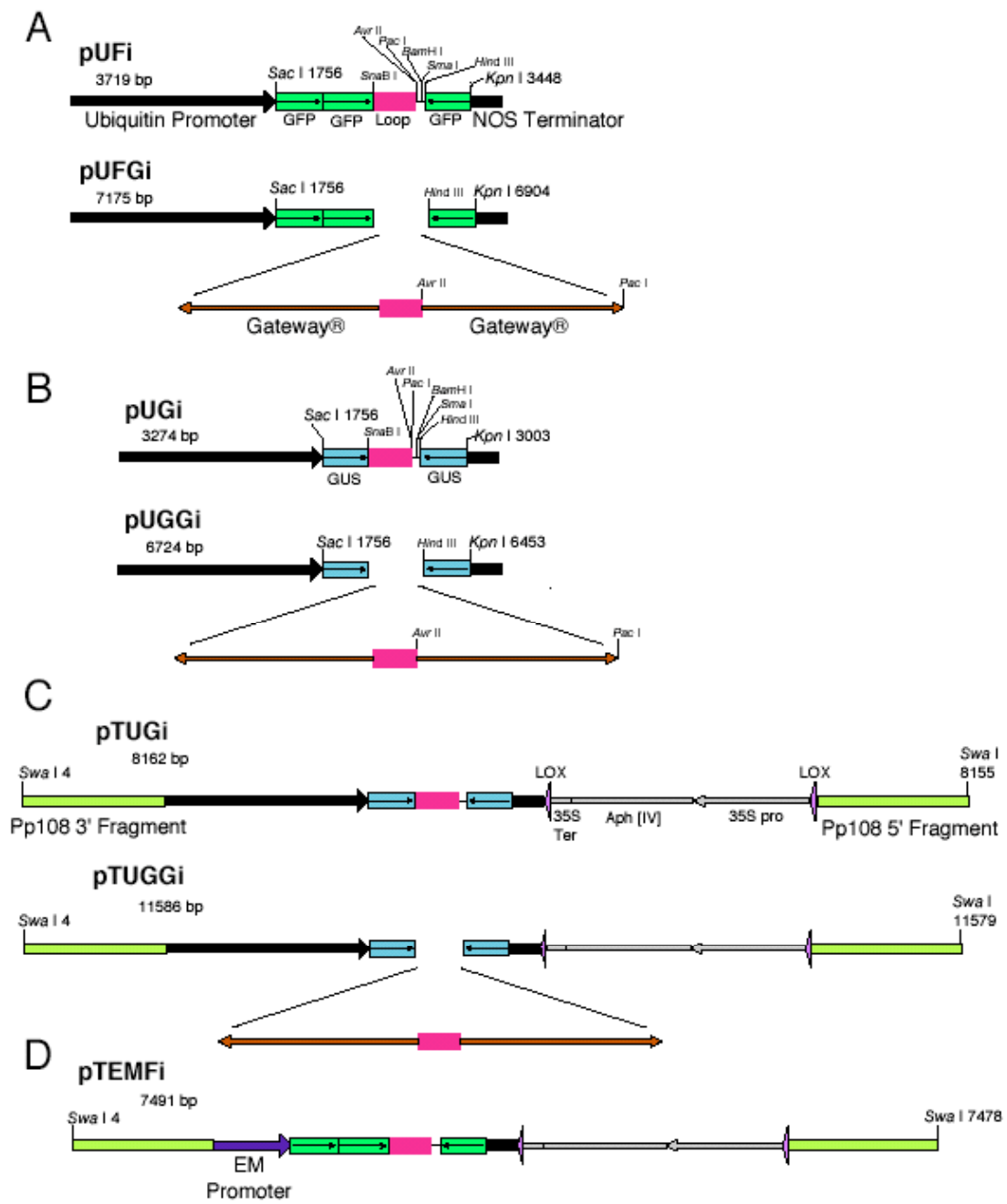


Figure 2