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## IRES ENABLED GENE TRAPPING IN PLANTS

### CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a national phase entry under 35 U.S.C. § 371 of International Application PCT/US02/1 1924, filed April 17, 2002, published in English, which claims benefit of U.S. Provisional Patent Applications 60/284,239, filed Apr. 17, 2001. The disclosures of all of said applications are incorporated by reference herein.

### TECHNICAL FIELD

This invention relates to DNA vectors containing internal ribosome entry site sequences (IRES) functional in plants and uses of the vectors in plants.

### BACKGROUND

The ongoing genomic sequencing project on a number of organisms has resulted in an enormous amount of sequence data being deposited in public databases (Schuler, et al., *Science* 274:540-546 (1996)). Analyzing these data using a variety of bioinformatics tools can result in assigning function or protein identification to a number of these genes. However, true biological function cannot be determined without biological data. In animals and in plants the most successful strategy has been to knock out gene function either randomly through saturation mutagenesis or the use of antisense technology to study phenotype one gene at a time. In these functional screens, mutagenic agents are used to produce a large number of organisms that are analyzed for the specific phenotype or metabolic profile. Matching phenotype with genetic lesion has identified many genes involved in development and metabolism. This approach has been carried out successfully in the fruit fly *Drosophila melanogaster* (Nusslein-Volhard et al., *Nature* 287:795-801 (1980)), the nematode *C. elegans* (Brenner, *Genetics* 77:71-94 (1974)), and in *Arabidopsis thaliana* (Mayer, et al., *Nature* 353:402-407 (1991)).

In the mouse, gene trapping has provided a powerful approach to recover and identify novel phenotypes (Brown, *J Inherit Metab Dis* 21:532-539 (1998)). Ideally, in the process of gene discovery, no assumption should be made about which genes or pathways should be disrupted or examined. This approach, however, has not proven successful over time. With mice, however, the situation has changed dramatically with the advent of embryonic stem (ES) cell lines and the means to generate and select genetic alterations (Evans et al., *Nature* 292:154-156 (1981)). ES cells can be maintained in culture as totipotent cells, that is, cells that can give rise to all types of differentiated cells under proper growth conditions. These cells can also be genetically altered with relative ease (Thomas et al., *Cell* 51:503-512 (1987)). Like the ES cells from mice, plant cells from many plants are totipotent and can be used in similar studies.

Assigning gene function by observation of phenotype due to disruption of a gene in the transformed plant is not always straightforward. When there are multiple copies of a gene in a gene family, the phenotype might not be immediately evident. By determining the spatial and temporal expression of the disrupted gene, further evidence is gained for assigning gene function. This is especially valuable when a simple phenotype is not evident or when relating more complex phenotypes to functions and development of the whole

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organism. In some instances no obvious phenotype may be discerned but spatial and temporal expression of the reporter may provide critical information for defining the function of that genetic locus. The reporter gene is able to provide much higher resolution than gene chips or Northern analysis for tissue specific expression.

Including additional functions to the gene-trapping vector can provide novel tools for gene expression. With recombination sites incorporated into the vector it is possible to insert a gene of interest at this defined location. This may be done in a fashion to simply insert a gene of interest next to, or to replace the reporter gene, or to permit multiple/tandem insertions and replacements. Analysis of expression patterns in phenotypically normal plants will provide "landing sites" for inserting a gene of interest to obtain a highly specific and well-defined pattern of expression. As there are numerous drawbacks to the current random nature of gene insertion during plant transformation, this approach offers significant advantages.

### Gene Trapping

Alternative strategies for identifying gene function were explored in the early 1990s. The approach of "gene trapping" was investigated to screen libraries of random mutants. The principal of gene trapping is essentially the random insertion of a DNA vector and the ensuing disruption of endogenous structural genes. Further improvements to the approach was to include a reporter gene that could readily signal the presence of the vector DNA. The reporter gene mimics the expression of the endogenous gene while mutating the same locus (Evans et al., *Trends Genet.* 13:370-374 (1997)). Large libraries of clones with random integrations can be isolated and stored indefinitely for future analysis. By using PCR (polymerase chain reaction) the sequence of the "trapped" gene can be identified. This technique allows the identification of genes regardless of their level of expression in vivo (Frohman et al., *Proc. Natl. Acad. Sci. USA* 85:8998-9002 (1988)). The ability to mutate, identify phenotype, and analyze expression of a specific gene makes gene trapping a very attractive tool for functional genomics. Gene trapping has been used for disruption and identification of genes in mouse ES cells (Skarnes et al., *Genes Dev.* 6:903-918 (1992)), Zambrowicz, et al., *Nature* 392:608-611 (1998)), genes including those membrane and secreted proteins (Skarnes et al., *Proc. Natl. Acad. Sci. USA* 92:6592-6596 (1995)), genes activated in differentiated mouse ES cells (Salminen et al., *Dev. Dyn.* 212:326-333 (1998)), genes to respond to retinoic acid (Forrester et al., *Proc. Natl. Acad. Sci. USA* 93:1677-1682 (1996)), and genes that are important in the development of the mammalian nervous system (Stoykova et al., *Dev. Dyn.* 212:198-213 (1998)).

### Design of Gene Trap Vectors

Trapping vectors fall into essentially two different categories. The "enhancer-trap" vectors must integrate near an enhancer that activates the reporter gene that is fused to a minimal promoter (Bellen et al., *Genes Dev* 3:1288-1300 (1989)). "Promoter trap" vectors have no 5' expression element in front of the reporter. Gene-trap vectors may contain a splice acceptor (SA) at the 5' end of the reporter gene resulting in the generation of fusion transcripts following integration into the intron of an actively transcribed gene (Skarnes et al., *Genes Dev.* 6:903-918 (1992)), Forrester et al., *Proc. Natl. Acad. Sci. USA* 93:1677-1682 (1996), Brenner et al., *Proc. Natl. Acad. Sci. USA* 86:5517-5521 (1989), von Melchner et al., *Genes Dev.* 6:919-927 (1992), Wurst et al., *Genetics* 139:889-899 (1995)). For functional genomics