

DNA sequences for gene expression

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Gene structure studies tell us that in order for genes to be processed to mRNA and proteins, specific DNA sequences need to be in place. The beauty of genetic manipulation is that chimeric genes and promoters can be put together to obtain the desired expression. In DNA plasmid construction, there are basic requirements for the gene to be expressed in plants, animals or microorganisms.

Types of Expression vectors

Genes can be expressed depending on the kind of insert the construct has: cDNA, transcriptional fusion, and translational fusion. Gene discovery through mRNA differential display allows gene isolation via reverse transcriptase for a cDNA product. A cDNA contains its own promoter and terminator. Depending on its size, it can be inserted into an expression vector for testing in transgenic plants. A transcriptional fusion makes use of some gene coding sequences with their own promoter and AUG site in one plasmid construct. The product will be a series of independent proteins. This is the usual way of designing plasmid constructs in crop genetic engineering. Gene coding sequence will be that for selectable marker gene, the economically important gene, and the reporter gene. The translational fusion in a plasmid construct is when two or more genes are placed successively in one construct, in the same direction, with one AUG site and one terminator site. The translation product is a fused protein. This kind of construct is used for studying proteins and its interactions with other proteins.

Promoters

Promoters regulate gene expression both quantitatively and qualitatively. Promoters are multi-step structures that contain specific cis-acting sequences with a modular organization. These are regulatory regions that induce gene transcription spatially, temporally, and quantitatively in vegetative and reproductive parts at different inductive conditions. Thus, promoters can be tissue-specific, inducible, strong promoters, or constitutive (expressed in all plant parts). The promoter sequences of the plants are composed of the TATA box and the CG rich regions at the 5' untranslated region. This is where transcription factor proteins bind so that the RNA polymerase can recognize the start site for

transcription. Genetic manipulation of the 5' untranslated region can be done to obtain the desired expression. Addition of enhancer sequences and introns in this region can enhance expression

The cauliflower mosaic virus (CaMV) 35S promoter has often been used as a strong and constitutive promoter for the expression of many foreign genes in transgenic plants of many plant species, especially dicots. Although the widely used CaMV 35S promoter is active in monocot cells, its relative strength is substantially less than in dicot cells. The CaMV35S promoter is found to be inactive in some cell types, e.g. pollen. The genes for rice actin and maize ubiquitin have been investigated as potentially useful alternatives to the CaMV35S. There are two polyubiquitin genes isolated and sequenced, both containing an intron in their 5'-untranslated regions, expressed constitutively at 25°C in maize seedlings and are inducible to higher levels upon heat shock. Transient expression in maize protoplasts from the *Ubi I* promoter, first exon and first intron (UBI) was more than 10-fold higher than expression from the CaMV35S promoter, using chloramphenicol acetyl transferase (CAT) as the reporter gene. The high activity of the maize *Ubi 1* promoter has now been documented in transient and/ or stable transformation in monocots including rice, wheat, barley, sugarcane, maize, and *Panicum, lemna*. The original intron present in the 5' untranslated region of the *Ubi-1* gene was retained in all the constructs used by Christensen and Quail (1996) because of numerous studies showing that introns strongly enhance transgene expression in cereals. There is also evidence that the maize *Ubi-1* intron is spliced correctly in transgenic rice cells. An additional potentially useful feature of the *Ubi-1* promoter is that it is stress-inducible. Both thermal and mechanical stresses have been shown to cause strong enhancement of the *Ubi-gus* transgene activity in transformed rice. It is possible that this fact may result in stronger expression of selectable marker fusion genes during the early stages of transformation, where recipient cells are exposed to a variety of stresses such as high osmotic pressures, particle bombardment and growth on toxic compounds. The stress-inducibility of the *Ubi-1* promoter might also be useful for driving conditional expression of genes that confer tolerance or resistance to various biotic and abiotic stresses such as pathogen attack, heat, and water deficit.

Use of introns in the coding sequence

Introns or the non-coding regions are usually spliced out after mRNA transcription just before translation process. Evidence shows that adding a couple of intron sequences in between gene coding sequences such as in hygromycin and β -glucuronidase gene enhance their expression in transgenic plants.

Multiple Gene Strategies

A current strategy to develop useful genetically modified crops is to pyramid several resistance genes into one plant, termed as multiple gene strategies. Rice has been improved to contain the *Bt* gene for stemborer resistance, *Xa21* gene for bacterial blight resistance and the chitinase gene for sheath blight resistance (Datta et al, 2001).

Anti-sense technology

Genes coding for certain proteins and enzymes can be down regulated by using the anti-sense technology. The reverse sequence of the code is introduced and the resulting mRNA product in the transgenic plant hybridizes with the natural mRNA, inactivating it and slows down its biological function. This technology has been used in the delayed ripening of tomato Flavr Savr and papaya.