CPMV-HT Protein Expression System

Mutagenesis of the Cow Pea Mosaic Virus (CPMV) expression system leads to expression levels of 25 to 30% of total soluble protein in plants

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Tech ID: 07.439

Patent Literature

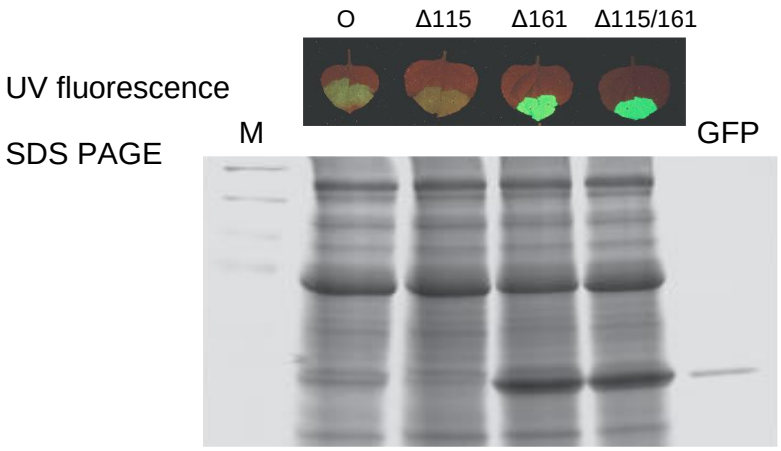
Granted Patents:
US 8,674,084 B2, EP2240589 B1
Also granted in Australia, Canada, China, India, Indonesia, Israel, Japan, Mexico, New Zealand, Russian Federation, Singapore, South Africa and South Korea.

George Lomonossoff and Frank Sainsbury at the John Innes Centre have further developed the Cow Pea Mosaic Virus (CPMV) protein expression systems for plants. The new developments are based on a deleted version of RNA-2 of CPMV where the regions encoding the viral movement protein, both coat proteins and now the upstream start codons within the 5' leader sequence have been removed. The deleted RNA-2 still possess the *cis*-acting sequences which are the elements enhancing translation and thus very high levels of gene amplification are maintained without the concomitant possibility of the modified virus contaminating the environment.

This new system is called CPMV-HT for "hyper-translatable" Cow Pea Mosaic Virus protein expression system. The HT-CPMV system shows dramatic increases in protein levels and thus is an excellent method for the rapid, high-level expression of foreign proteins in plants. The expression system can be used both in stable genetic transformation and transient expression strategies.

The main advantages of HT-CPMV compared to other protein over-expression systems are:

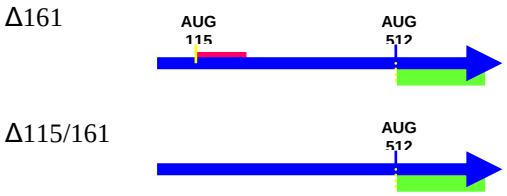
- **Extreme** high level expression of up to 30% total soluble protein
- **Quick** and **easy** to use system
    - Easy cloning
    - Fast expression through agroinfiltration
    - Total time required for expression and protein recovery is only 2 weeks
- Proven to be effective with a **wide range of proteins** (including multimeric and heteromeric proteins, as well as co-expression of multiple proteins)
- **Non-infective** viral-derived expression system



Estimate levels of expression reach 1.5g GFP/Kg leaf tissue

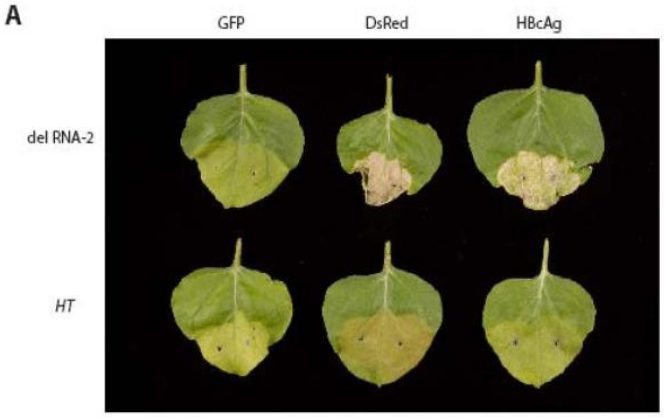
The inventors have found that mutation of the start codon at position 161 in the CPMV RNA-2 vector strongly increases the levels of expression of a protein encoded by a gene inserted after the start codon at position 512. The levels of **protein expression** were **increased about 20-30 fold** compared with expression of the same protein from a CPMV RNA-2 vector with an unaltered start codon at position 161.

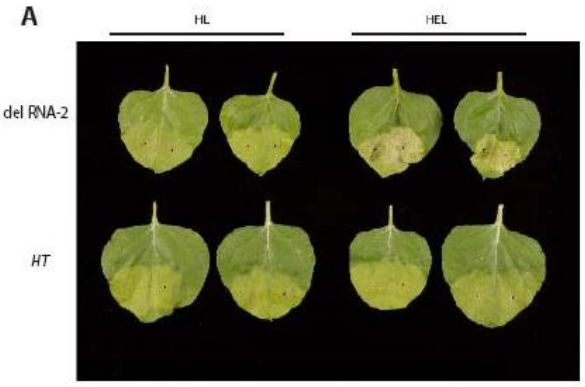
Using GFP to test expression levels in the deleted RNA-2 expression cassette the inventors have demonstrated expression levels reaching approximately **1.5 grams of protein per kg of leaf tissue, corresponding to about 25 to 30% of total soluble protein**. These experiments were conducted by *Agrobacterium*-mediated transient transformation of *Nicotiana benthamiana* plants.

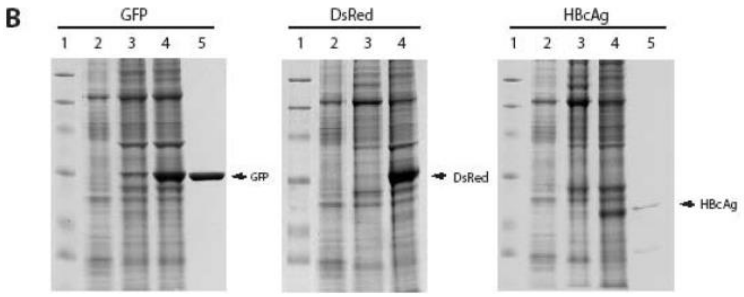


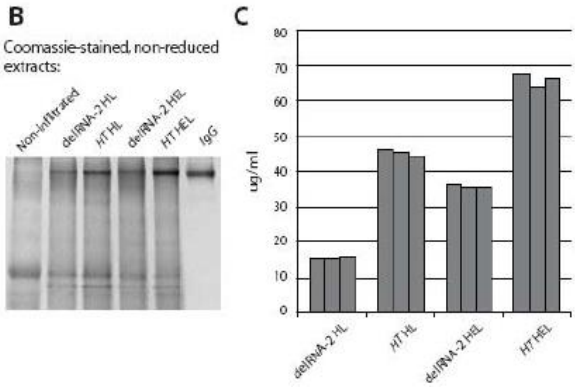
In addition, the inventors have also found that mutation of the "161 start codon" negates the need for maintaining the reading frame alignment between the position of the mutated 161 start codon and the start codon at position 512, thus allowing insertion of sequences of any desired length after the mutated 161 start codon. This is particularly advantageous as it **allows polylinkers of any length** to be inserted into RNA-2 vectors after the mutated start codon, which can then be used to facilitate cloning of a gene of interest into the vector. Furthermore, despite the increase in protein expression, plants transformed with a CPMV RNA-2 vector comprising a mutated 161 start codon are healthy and normal.

Further experiments using the human anti-human Immunodeficiency Virus antibody 2G12 also demonstrated high expression levels - up to **325mg protein per kg** of leaf tissue. Various other proteins have been tested, including DsRed, HBcAg, HL and HEL (see below) and further are currently in development.









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