

Engineering the Chloroplast Genome

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Chloroplasts are the site of photosynthesis in plants mostly seen in leaves. Plastids of higher plants are generally semiautonomous. The plastid genome is a circular molecule of double stranded DNA. Despite the small size of plastid genome compared to the nuclear genome, chloroplast DNA makes up as much as 10-20% of the total cellular DNA and contains about 130 genes.

With the introduction of transgenic crops, many fears have arisen regarding harmful side effects. The chloroplast transformation system has many attractive advantages over nuclear transformation, which is very much in vogue currently for generating transgenic plants. Plastids have retained many eubacterial features including gene organization in operons and prokaryotic mechanism of gene expression. Chloroplast genes are inherited maternally, so gene pollution caused by trans gene escape via pollen can be controlled and also the risk of development of weeds resistant to toxin can be reduced. The plant cell has 10000 copies of the plastid genome. Hence many fold expression of the transgene can be expected. It is useful in evolving vaccine producing plants as it enables high level of protein production. Also there is the possibility of producing multiple proteins using polycistronic mRNAs.

Transgene expression is more stable in transplastomic plants than in nuclear transformants because transgenes are integrated into chloroplast genomes by homologous recombination and not affected by gene silencing. Chloroplast transformation permits to insert several transgenes under the control of one promoter. This enables engineering of complex traits.

Stability of chloroplast transformation depends on the integration of foreign DNA into the chloroplast genome by homologous recombination. Hence the introduced gene must be flanked by sequences homologous to the chloroplast genome. Generally homologous sequences of more than 400bp or more on each side of the construct are used. The primary plasmid transformation event involves the change of only a single or at most few plasmid genomes out of the 10000 plastid DNA copies present in the leaf mesophyll cell. Genetic stability of transplastomic cell line and plants require homoplasmy where the transgenic plastid forms the majority in the plant. It can be achieved by allowing for a sufficient number of cell divisions under high selective pressure as exerted by high concentrations of the selecting antibiotic spectinomycin.

Biolistics that involves the introduction of DNA coated particles using a 'gene gun' that can deliver the particles into the cell and is most commonly used method of transforming chloroplasts. It has a high efficiency rate and permits rapid regeneration of transformed



tissue from a variety of explants. Chloroplast specific vectors have also been developed to facilitate the incorporation of the transgene into the chloroplast genome.

In 1989, Maliga and co-workers were the first to succeed with chloroplast transformation in a higher plant. They demonstrated stable transformation in tobacco by engineering chloroplast 16S ribosomal RNA with point mutations that confer resistance to spectinomycin and streptomycin. In addition to introducing resistance genes against insect pests or herbicides, the plastid transformation has the potential to improve crop plants in various other ways such as improved photosynthesis through RuBisco engineering,

improved tolerance to drought, salinity and resistance to bacterial and fungal pathogens.

The benefits of this technology can be harvested not only for the improvement of agriculture, but also for the growth of pharmaceutical industries for the production of vaccines, recombinant proteins and plantibodies through chloroplast transformation of crops.

Suggested Reading

R Bock, Transgenic plastids in basic research and plant biotechnology. *J. Mol. Biol.*, Vol.312, pp.425-438, 2001.

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Information and Announcements

National Board for Higher Mathematics (Department of Atomic Energy) Grants for Participating in ICM 2006, Madrid

The National Board for Higher Mathematics proposes to support participation of a large number of mathematicians in the International Congress of Mathematicians to be held in Madrid, Spain, during August 22-30, 2006.

Applications are invited from mathematicians/college teachers/research scholars for availing of these grants. The grant includes round trip airfare to Madrid, registration fees, and a modest daily allowance. Interested persons can get the application form and related information from any of the following websites:

- 1) <http://www.nbhm.dae.gov.in/> ; 2) http://math.iisc.ernet.in/icm2006_travel_form.html
- 3) <http://www.imsc.res.in/math/> ; 4) <http://www.isid.ac.in/~rlk/nbhm2006.html>

The forms can also be obtained by writing to **ICM 2006 Travel Grants, c/o The Member-Secretary, NBHM, Department of Atomic Energy, Anushakti Bhavan, C.S.M. Marg, Mumbai 400 001**, enclosing a self-addressed envelope with stamp worth Rs.10/-.

The applications should be sent to designated addresses for each region as indicated in the form.

The last date for receiving applications is 31 December 2005.

