Project Progress Summary

Section 1: PROJECT IDENTIFICATION Information to be provided for project identification	**NOT CONFIDENTIAL**

Title of the project: Precision Engineering of Plant Genes		
Acronym of the project: PREGENE		
Type of contract R&D		**Total project cost** (in euro) 2923835 €
Contract number **QLK3-CT-2000-00365**	**Duration** (in months) 40 Months	**EU contribution** (in euro) 1836153 €
Commencement date **1st March 2001**	**Period covered by the progress report** **1st March 2002 – 28 February 2003**	
PROJECT COORDINATOR		
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Key words (5 maximum - Please include specific keywords that best describe the project.). recombination, gene targeting, precision engineering , crop plants, Physcomitrella.		
World wide web address: **http://www.pregene.de/**		
List of participants Provide all partners' details including their legal status in the contract i.e.,contractor, assistant contractor (to which contractor?).		
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Section 2: Project Progress Report **NOT CONFIDENTIAL**

(2 pages maximum.. Use short sentences. Be factual. Avoid technical terms as much as possible)

Objectives:

The ultimate goal of the consortium is to develop gene targeting as a tool that allows precision engineering of plant genes. Since gene targeting is fundamentally dependent on the process of homologous recombination, the Consortium focused on the investigation of fundamental aspects of homologous recombination with relevance for gene targeting

Results and Milestones:

The underlying process of gene targeting is homologous recombination. Somatic tissues of higher plants have an inefficient apparatus for homologous recombination. Other cells, like those of the moss *Physcomitrella patens*, have high efficiencies of homologous recombination. The difference in the recombination apparatus may be the presence of an active homologous recombination pathway or a relatively inactive illegitimate recombination pathway. To correlate these differences with the efficiencies of gene targeting, selected genes representative for the homologous and illegitimate pathways of recombination are analysed.

The RecA protein and its eukaryotic homologue RAD51 has a central function in the homologous pathway of recombination. For the first time, such a gene was isolated form a gene targeting efficient higher eukaryotic organism, the moss *Physcomitrella patens*. The moss has two, highly homologous, genes. The existence of two genes raises the question about their function. They could have the same or a similar function and therefore be redundant or each of them might specialised in function during evolution. The expression pattern of these genes suggest a specialised function. One gene is specialised for dividing cells while the other one seems to have a role in all cells. Nevertheless, both RAD51 proteins are *bona fide* RAD51 proteins and have biochemical activities comparable to those of their relatives from bacteria, RecA, or other eukaryotes, like the RAD51 protein from yeast and humans However, the proteins are not identical. The two *Physcomitrella* RAD51 proteins differ in essential biochemical characteristics and confer different phenotypes when expressed in yeast. These features together with the specialisation in expression suggest that the *RAD51* genes have functionally diverged, but serve redundant or overlapping functions Nevertheless, these features cannot readily explain the high efficiency of gene targeting in Physcomitrella since both proteins are more similar to a relative from an gene targeting inefficient system, the human RAD51 protein than the one from an efficient system, yeast.

A prerequisite for the analysis of gene targeting in plants is the availability of appropriate assay systems. A variety of such systems were developed within this project and two gene targeting assay systems for higher, diploid plants like *Arabidopsis thaliana* and three systems for *Physcomitrella* are available now. Using these systems, the efficiency of gene targeting in Physcomitrella was shown to be largely independent from the target locus. However, the length and symmetry of homology of repair construct to target locus sequences are important parameters. In addition, the high efficiency of Physcomitrella is not dependent on the transformation method and two different methods resulted in comparable gene targeting frequencies.

In the next phase of the project, selected recombination genes will be transferred from

Physcomitrella to the gene targeting inefficient system *Arabidopsis* and their effect on homologous recombination gene targeting analysed using the newly developed higher plant assays. I addition, these systems lay the foundation to analyse gene targeting in alternative systems, like tobacco microspores. Microspores have some features in common with *Physcomitrella*; they are haploid and the cells are in a developmental stage relatively similar to *Physcomitrella* cells. The microspore transformation system was established and gene targeting will be analysed in the next phase of the project. In addition, the recombination apparatus of such cells will be analysed and the tools necessary have been established. Gene targeting technology is well established in vertebrate cell cultures and such and alternative system was used to analyse to analyse the usefulness of oligonucleotide/recombinase complexes to improve gene targeting. However, oligonucleotides were rather inefficient in introducing targeted modifications into a test gene. Moreover, the recombinase in the complex did not stimulate this efficiency Therefore other approaches need to be analysed.

In addition to its focus on the recombination apparatus, the project also pays attention to other components that play a role in recombination. One of them is chromatin and two plants genes with a role in chromatin were analysed within the frame of this project. A defect in both leads to sensitivity to DNA damaging agents and elevated levels of homologous recombination. The first one is the previously described structural maintenance of chromatin protein gene *MIM*. The other one is the *BRU1* gene that was newly discovered in this period. Further analysis of BRU1 will shed light on the role of chromatin in homologous and non-homologous recombination in plants.

One of the aims of the project was the transfer of technology from model systems to crop plants. In this respect we planned to analyse gene targeting and homologous recombination in a crop plant. As a first step, a system to analyse homologous recombination was transferred to rape seed. The establishment of this system necessitated the development of more efficient transformation protocols and the development of new selectable marker genes. In the next step, homologous recombination will be analysed in this species and compared to established models

To summarise the second phase of the project, important aspects of the homologous recombination apparatus of an gene targeting efficient organism, the moss *Physcomitrella* were investigated. This work resulted in new insights into the relationship of homologous recombination to gene targeting. In addition, new chromatin factors that influence homologous recombination were identified in *Arabidopsis*. Moreover, all the systems necessary for future analysis were established and the transfer of knowledge from model to crop plants was initiated.

Benefits and Beneficiaries:

Tools that allow precision engineering of genes are not yet available in plants. The tool of choice is gene targeting. Today’s transformation methods cannot control the position at which a transgene integrates into the genome of a plant. However, the quality of transgene expression is influenced by the position at which the transgene integrates into the genome and the complexity of the integrated DNA. In addition, additional copies integrated somewhere in the genome often lead to transgenic organisms that show a complexity unacceptable for commercial application. By contrast, gene targeting allows the precise and predictable integration of a single transgene at a predetermined position. Therefore gene targeting is of fundamental importance for plant science, plant breeding, and agriculture.