



APPLICATION FOR LICENCE FOR INTENTIONAL RELEASE OF GMOs INTO THE ENVIRONMENT: Application No. DIR 049/2004

SUMMARY INFORMATION

Project Title:	GM cotton Field Trial – Evaluation under field conditions of the cotton rubisco small subunit promoter driving a reporter gene
Applicant:	CSIRO GPO Box 1700 Canberra ACT 2601
Common name of the parent organism:	Cotton
Scientific name of the parent organism:	<i>Gossypium hirsutum</i> L.
Modified trait(s):	Expression of two selectable marker genes (antibiotic resistance) and one reporter gene (enables detection and quantification of gene expression)
Identity of the genetic elements responsible for the modified trait(s):	<ul style="list-style-type: none">• <i>uidA</i> (β-glucuronidase or GUS) from the bacterium <i>Escherichia coli</i> (reporter gene)• <i>nptII</i> and <i>hph</i> genes from the bacterium <i>E. coli</i> (antibiotic resistance)• <i>rbcS</i> (rubisco small subunit) promoter from cotton• 35S promoter from Cauliflower Mosaic virus
Proposed Location(s)	Shire of Narrabri in New South Wales
Proposed Release Size:	One site covering an area of up to 0.1 hectares in each of two summer growing seasons
Proposed Time of Release	October 2004 – May 2006

Introduction

The *Gene Technology Act 2000* (the Act) took effect on 21 June 2001. The Act, supported by the *Gene Technology Regulations 2001*, an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, underpins Australia's nationally consistent regulatory system for gene technology. Its objective is to protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and managing those risks by regulating certain dealings with genetically modified organisms (GMOs).

The Act establishes a statutory officer, the Gene Technology Regulator (the Regulator), to administer the legislation and make decisions under the legislation. The Regulator is supported by the Office of the Gene Technology Regulator (OGTR), an Australian Government regulatory agency located within the Health and Ageing portfolio.

The legislation sets out the requirements for considering applications for licences for dealings with GMOs and the matters that the Regulator must take into account before deciding whether, or not, to issue a licence.

The application and the proposed dealings

The OGTR has received a licence application from CSIRO for the intentional release of genetically modified (GM) cottons into the environment, on a limited scale and under controlled conditions.

CSIRO proposes to study a new promoter that is a candidate for controlling the expression of commercially useful introduced traits in cotton. A promoter is a short regulatory sequence that controls the level and location of expression of the protein encoded by a gene. The new promoter is the Rubisco small subunit (*rbcS*) promoter derived from cotton itself. In unmodified cotton plants, the *rbcS* promoter controls the expression of the Rubisco small subunit gene that is involved in the photosynthetic pathway in plant green tissues. In the GM cotton plants proposed for release, the introduced *rbcS* promoter controls the expression of a reporter gene. The aim of the proposed release is to test the efficacy of the *rbcS* promoter from cotton in controlling the expression of a reporter gene (*uidA* or β -glucuronidase gene) derived from a common gut bacterium (*Escherichia coli*). A reporter gene is a gene that encodes an easily detectable protein and can therefore be used to study the activity of a promoter of interest. The *uidA* reporter gene enables visual identification of plant tissues in which this gene is being expressed and provides an indication of the level of activity of the promoter being tested.

CSIRO proposes to conduct a limited and controlled release of 60 GM cotton lines to compare the performance of two different promoters – the *rbcS* promoter from cotton (30 lines) and a commonly used viral promoter, 35S from Cauliflower Mosaic virus (30 lines). The GM cotton lines also contain either one or two antibiotic resistance genes (*hph* and/or *nptII*) that were used as selectable markers during development of the GM cottons in the laboratory.

The *uidA* reporter gene, the *nptII* and *hph* antibiotic resistance marker genes and the 35S viral promoter were all present in previously approved GM cottons (see below for details). However, DIR 049/2004 is the first application for a licence involving a field trial of GM cotton lines containing the *rbcS* promoter.

The proposed release would be conducted on one site covering an area of up to 0.1 hectares in each of the summer growing seasons of 2005 and 2006 at the Australian Cotton Research Institute (ACRI) in the shire of Narrabri, New South Wales.

CSIRO proposes to harvest and retain a small quantity of seed from the release for possible future trials that would be subject to further approvals, and to destroy excess seed by burning.

The proposed dealings will involve transport of the GM cotton seed to the release site and transport of the harvested GM materials that will not be used for future trials from the release site to a nearby cotton gin. Ginned seed will be transported back to ACRI where it will be burned. Transport of the GM material and cleaning of gin facilities would be in accordance with the guidelines issued by the Regulator.

None of the cotton plants from the release, or their by-products, would be used for animal and human food. However, the applicant proposes to sell lint from the release and from surrounding pollen trap cotton plants. Lint does not contain genetic material or protein.

Previous releases of the GMO

There have been no previous applications to release GM cotton lines containing the *rbcs* promoter. However, various combinations of the 35S promoter and the introduced genes have previously been approved for release. CSIRO conducted a limited and controlled release of GM cotton containing the *uidA* reporter gene controlled by the 35S viral promoter (PR100 and PR100X) under the former voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC). Licences for the intentional release of GM cottons containing the *uidA* reporter gene controlled by the 35S viral promoter, and the *nptII* and *hph* antibiotic resistance marker genes, have been issued under the current regulatory system, as listed in the table below.

Introduced Genes	DIR reference	Applicant	Type of release
<i>35S/uidA/nptII</i>	005/2001	Cotton Seed Distributors Ltd	Limited and controlled
	006/2001	CSIRO	Limited and controlled
	009/2001	Department of Agriculture WA	Limited and controlled
	012/2001	Monsanto	Commercial
<i>nptII</i>	008/2001	Department of Agriculture WA	Limited and controlled
	022/2002	Monsanto	Commercial
	023/2002	Monsanto	Commercial
<i>hph</i>	017/2002	CSIRO	Limited and controlled
	025/2002	CSIRO	Limited and controlled
	034/2003	Syngenta	Limited and controlled
	036/2003	CSIRO	Limited and controlled

The 35S viral promoter was also used to control the expression of other genes such as *cryIAb*, *cryIAc*, *cry2Ab*, *bar* and *cp4 epsps* in GM cottons approved under DIR 015/2002, DIR 016/2002, DIR 035/2003, DIR 036/2003 and DIR 038/2003 for limited and controlled releases.

There have been no reports of adverse effects on human health or the environment resulting from these releases.

Parent organism

The parent organism is cultivated cotton (*Gossypium hirsutum* L.), which is exotic to Australia and is grown as an agricultural crop in New South Wales and Queensland and on a trial basis in Western Australia and the Northern Territory.

Genetic modification and its effect

Thirty GM cotton lines contain the *uidA* reporter gene controlled by the *rbcs* promoter from cotton and two selectable antibiotic resistance marker genes, *nptII* and *hph*. The other 30 GM cotton lines contain the same reporter gene controlled by the commonly used 35S viral promoter from Cauliflower Mosaic virus and one selectable antibiotic resistance marker gene, *nptII*.

The native *rbcs* promoter controls the expression of the Rubisco small subunit gene that encodes a subunit of the ribulose-1,5-bisphosphate carboxylase protein that is involved in the photosynthetic pathway in plant green tissues. It is expected that the *rbcs* promoter from cotton will direct the expression of the *uidA* reporter gene in the photosynthetic green tissues

of GM cotton plants, whereas the 35S viral promoter will have a non-specific action that will result in expression of the *uidA* reporter gene in most tissues of the GM cotton plants.

In addition to the promoters, other short regulatory sequences that control expression of the genes are present in the GM cottons. A short sequence (intron) derived from the Castor bean catalase gene is present within the *uidA* reporter gene construct to prevent expression of the *uidA* gene until after integration into the plant genome. Terminator sequences derived from a common soil bacterium *Agrobacterium tumefaciens* are also present. Although *Agrobacterium tumefaciens* and Cauliflower Mosaic virus are plant pathogens, their regulatory sequences comprise only a small part of their total genomes, and are not in themselves capable of causing disease.

The *uidA* reporter gene encodes the enzyme β -glucuronidase (GUS) that enables visual identification of plant tissues in which this gene is being expressed. The tissue containing the GUS enzyme will turn a dark blue colour after adding a staining solution. This staining also provides an indication of the level of activity of the promoter that is being used to control the expression of the *uidA* reporter gene.

The *nptII* selectable marker gene encodes for an enzyme, neomycin phosphotransferase (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. The *hph* selectable marker gene encodes for a hygromycin phosphotransferase enzyme (HPT) conferring resistance to the antibiotic hygromycin. These marker genes enabled the identification of successfully transformed plants during the stages of developing the GM cottons in the laboratory.

The reporter gene and both antibiotic resistance genes are derived from the common gut bacterium *Escherichia coli*. Although certain strains of *E. coli* are pathogenic, the three genes comprise only a small part of the bacterial genome and are in themselves not able to cause disease.

Method of gene transfer

The gene construct comprising the *uidA* gene, controlled by the *rbcS* promoter, and the *nptII* and *hph* genes were introduced into the cotton on a standard plasmid vector carried by *Agrobacterium tumefaciens*. The *uidA* gene, controlled by the 35S promoter, and the *nptII* gene were introduced into the cotton in the same way. This vector is 'disarmed' since it lacks the genes that encode the tumour-inducing functions of *A. tumefaciens*.

Consultation on preparation of the Risk Assessment and Risk Management Plan

The Regulator has made an initial assessment as to whether the proposed release may pose significant risks to human health and safety or the environment, in accordance with section 49 of the Act. Due to the low risk potential of the GMO, the control measures that will be imposed, and the limited scale and scope of the dealings, **the Regulator has decided that the proposed release does not pose a significant risk to human health and safety or the environment.**

This means that the Regulator is **not required to seek public comment** on the assessment of this proposal until a risk assessment and risk management plan (RARMP) has been prepared. In the interim, copies of the application are available on request from the OGTR. Please quote application number DIR 049/2004.

In preparing the RARMP, the Regulator will seek input from a wide range of key stakeholders and expert groups comprising State and Territory Governments, relevant Australian Government agencies, the Minister for the Environment and Heritage, the Gene Technology

Technical Advisory Committee and the relevant local council, as required by section 50 of the Act. In accordance with section 52 of the Act, the Regulator will again consult with these prescribed agencies and authorities, as well as the public, in finalising the RARMP.

At this stage, the consultation version of the RARMP is expected to be issued for an extended six week consultation period in **July 2004**. The public will be invited to provide submissions on the RARMP via advertisements in the media and direct mail to anyone registered on the OGTR mailing list. Summaries and copies of the RARMP will be available from the OGTR, or on the OGTR website.

If you have any questions about the application or the assessment process, please contact the OGTR at:

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