



The University of Georgia

Department of Crop and Soil Sciences
Institute for Plant Breeding, Genetics & Genomics

1 June 2013

Dr. Michael Gregoire
Biotechnology Regulatory Services
USDA-APHIS
4700 River Road, Unit 98
Riverdale, MD 20737

RE: APHIS confirmation of the regulatory status of soybean engineered for altered flavonoid profiles

Dear Dr. Gregoire:

I am writing to determine whether soybeans we are developing are considered regulated articles as a plant pest according to 7 C.F.R. § 340

The products in question are soybean (*Glycine max* cv. Jack) engineered with Biolistics. The genetic constructs used do not contain any DNA of plant pest/pathogen origin, nor is their behavior altered in any way that would convert them into a plant pest or increase their weediness. They are as follows:

Selectable marker cassette, present in all plants:

- Ubiquitin promoter from potato *Solanum tuberosum* GenBank L22576
- Hygromycin phosphotransferase (*hph*) from *E. coli*: GenBank V01499
- Ubiquitin terminator from potato: GenBank L22576

Plants will also have one of the following constructs:

For overexpression:

- Polyubiquitin promoter (*gmubi*) from soybean, *Glycine max* GenBank EU310508
- Flavonoid glycosyltransferase from soybean, *Glycine max* Glyma07g14530
- Rubisco small subunit terminator from pea, *Pisum sativum* GenBank X00806

For gene silencing:

- Polyubiquitin promoter (*gmubi*) from soybean, *Glycine max* GenBank EU310508
- Arm 1: 150 bp from Flavonoid glycosyltransferase from soybean, *Glycine max*, Glyma07g14530 = GenBank XM_003530115
- Linker: *GusA* gene from *E. coli*, (bases 787-1718) GenBank AF305918
- Arm 2: The same as arm 1, but in antisense orientation
- Rubisco small subunit terminator from pea, *Pisum sativum* GenBank X00806

For gene silencing:

- Polyubiquitin promoter (*gmubi*) from soybean, *Glycine max* GenBank EU310508
- Arm 1: Glyma07g14530 CDS+3'UTR from Flavonoid glycosyltransferase from soybean, *Glycine max*, Glyma07g14530 = GenBank XM_003530115
- Linker: *GusA* gene from *E. coli*, (bases 787-1718) GenBank AF305918
- Arm 2: The same as arm 1, but in antisense orientation
- Rubisco small subunit terminator from pea, *Pisum sativum* GenBank X00806

For gene silencing:

- Polyubiquitin promoter (*gmubi*) from soybean, *Glycine max* GenBank EU310508
- 22 bp tasi recognition target for mi1510 from soybean, *Glycine max*
- 99 bp from Flavonoid glycosyltransferase from soybean, *Glycine max*, Glyma07g14530
- Rubisco small subunit terminator from pea, *Pisum sativum* GenBank X00806

For gene silencing:

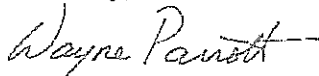
- Polyubiquitin promoter (*gmubi*) from soybean, *Glycine max* GenBank EU310508
- 22 bp tasi recognition target for mi1514 from soybean, *Glycine max*
- 99 bp from Flavonoid glycosyltransferase from soybean, *Glycine max*, Glyma07g14530
- Rubisco small subunit terminator from pea, *Pisum sativum* GenBank X00806

In all cases, the vector backbone is pSmart HC-kan, Lucigen.com, GenBank AF532107

Right now, these soybeans are exclusively for research purposes. We are fully aware that if development of these soybeans were to proceed beyond research, FDA and possibly EPA will need to be consulted.

Thanks in advance for your assistance with the process. Please let me know if I need to provide additional information.

Sincerely,



Wayne Parrott,
Professor