ARTICLE

# Selection and design of new primers for multiplex polymerase chain reaction (PCR) detection of transgenes in genetically modified (GM) soybean (*Glycine max L.*) Roundup Ready Soybean GTS 40-3-2

**Cynthia T. Hedreyda*** **and Jennifer L. Roxas**

National Institute of Molecular Biology and Biotechnology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines 1101

The approval for planting and direct use of several genetically modified crops in the Philippines is expected to result in requests for the certification that seeds are GM seeds for their added value or request for proof that seeds are non-GM for those who are opposed to GM food. The study was conducted to develop alternative PCR–based procedures that produce clearly distinct size amplicons that could be used to distinguish transgenes in herbicide tolerant GM Roundup Ready Soybean GTS 40-3-2. Initial multiplex PCR experiments using primers designed and reported in earlier studies could detect a 475-bp soybean *lectin* gene fragment and the transgenes *CamV 35S* promoter /*EPSPS* (315-bp) and *nos* terminator (151-bp) from GM soybean (*Glycine max L.*) Roundup Ready GTS 40-3-2. Detection limits for the 315-bp *CamV35S/EPSPS* and the 151-bp *nos* terminator fragments by the multiplex PCR procedure were 0.5% and 0.3%, respectively. New sets of detection primers were designed in this study based on sequences of amplicons from the initial multiplex PCR experiment in order to obtain additional primers that could be used for transgene detection. The alternative multiplex PCR using the new sets of primers and GM soybean Roundup Ready GTS 40-3-2 DNA template, resulted in different sized amplicons for the native soybean *lectin* gene fragment (430-bp), the *CamV* promoter/*CP4-EPSPS* (a modified form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase, 300-bp), and the *nos* terminator gene (173-bp). The new detection procedure amplified both transgenes in Roundup Ready GTS 40-3-2 soybean seed samples with distinct and clear bands when using at least 1.0% wt/wt GM soybean Roundup Ready GTS 40-3-2.

**KEYWORDS**

*Glycine max L.,* transgenic, *CamV/ mEPSPS,* multiplex PCR, *nos* terminator, lectin

*Corresponding author
Email Address: chedreyda@mbb.upd.edu.ph
Submitted: September 5, 2010
Revised: October 28, 2010
Accepted: October 29, 2010
Published: November 25, 2010
Editor-in-charge: Eduardo A. Padlan
Reviewers:
Sevilla D. Detera-Wadleigh
Virginia D. Monje

## INTRODUCTION

Several plant species important in food production has been precisely engineered to contain transgenes that enable them to express specific valuable traits including herbicide tolerance of soybean (*Glycine max L.*). The first genetically modified soybeans were planted in the United States in 1996 (GMO Compass, 2010). More than ten years later, GM soybean is now the principal biotech crop with 65.8 million hectares or 53% of global biotech area planted with the GM crop (James 2008).

About 85% of soybean produced in the United States is genetically modified (GMO Compass, 2010) with the Philippines as one of the leading importers of soybean meal. In addition to this, there are several other GM crops approved for planting and direct use in the Philippines (James 2008). With the introduction of GM crops in the country, it is expected that there will be requests for the certification that seeds are GM seeds for their added value or request for proof that seeds are non-GM for those who are opposed to GM food. This study was focused on GM soybean and experiments were conducted to come up with alternative reliable and sensitive protocols to detect transgenes in herbicide tolerant GM Roundup Ready Soybean GTS 40-3-2.

Monsanto Company's Roundup Ready® GTS 40-3-2 soybean is the most widely used herbicide-tolerant soybean. The soybean line GTS 40-3-2 was developed to allow the use of glyphosate, which is the active ingredient in the herbicide Roundup® for weed control (GM Crop Database, 2004). This GTS 40-3-2 soybean line contains a modified form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (*m-epsps*) that allows the host plant to survive the otherwise lethal application of glyphosate. Other foreign genes introduced in one plasmid with *mepsps,* are the cauliflower mosaic virus (*CamV 35S*) promoter, chloroplast transit peptide (CTP4) coding sequence from *Petunia hybrid*, and a nopaline synthase (*nos*) transcriptional termination element from *Agrobacterium tumefaciens* (GM Crop Database, 2004).

Commercially available detection kits are already available but quite expensive. Previous studies reported protocols that included transgene-targeted PCR to detect GM soybean in plant and food samples (Shirai et al. 1998; Lin et al. 2001; James et al. 2003; Randhawa and Firke 2005). This study reports the experiments conducted to develop detection protocols for foreign genes inserted into GM soybean Roundup Ready GTS 40-3-2. The approach included the literature search for reported gene sequences inserted into the soybean plant and the conduct of multiplex PCR using previously reported as well as newly designed primers targeting a native soybean *lectin* gene and transgenes for modified 5-enolpyruvyl-3-phosphoshikimic acid synthase (*m-epsps*) and nopaline synthase terminator genes (*nos*). The *lectin* gene is present in both GM and non-GM samples and detection of the gene ensures that soybean template DNA is present. Detection of more than one transgene minimizes the possibility of obtaining false positive or negative results. The expected sizes of amplicons in two alternative protocols described in this study vary significantly, resulting in distinct and easily distinguishable bands representing the transgenes. The study also reports the conduct of experiments to determine the smallest concentration (wt/wt) of GM Roundup Ready GTS 40-3-2 soybean that could be detected by the multiplex PCR procedures.

## MATERIALS AND METHODS

Ground GM glyphosate-tolerant Roundup Ready GTS 40-3-2 seed were kindly provided by Monsanto Company, USA. The non-transgenic PSBSy2 soybean requested from the Institute of Plant Breeding, UP Los Baños (IPB, UPLB) in Laguna, was used for extraction of DNA template for non-GM control.

### Preparation of soybean seeds

Ground soybean seed samples containing different weight percentages (100%, 10%, 1%, 0.5%, 0.3%, and 0.1% GM seeds) of transgenic Roundup Ready GTS 40-3-2 were prepared. For example, 100% GM samples contain 100g ground GM seeds, 10% GM preparation contained 10g ground GM seeds plus 90g non-GM PSBSy2 soybean seeds, 1.0% GM sample contained 1.0g ground GM seeds and 99.0g non-GM PSBSy2 soybean seeds. Pure non-GM ground PSBSy2 soybean seeds were used for 0% GM.

Ten grams of soybean seeds were sterilized by rinsing with sterile distilled water twice, incubating the seeds for 45 minutes in a 100-mL solution containing 2.75% (w/v) sodium hypochlorite or 50% of commercially available Zonrox (Green Cross, Inc., Parañaque City, Philippines) and 200 mL Joy detergent (Procter & Gamble Philippines, Inc., Makati City, Philppines), and rinsing again in sterile distilled water four times. Sterilized seeds were incubated at 60°C until dehydrated. Homogenization of the seeds was performed using a Braun Model Type 4184 homogenizer (Braun: Naucalpan, Mexico) after which ground samples were sieved prior to use for DNA extraction.

### DNA extraction from seeds

The Nucleospin® Plant Kit (BD Biosciences: California, USA) was used to extract DNA from soybean seed samples following manufacturer's protocol. Visualization of DNA extracts was done by resolving aliquots of DNA extracts in agarose gel electrophoresis, subsequent staining with ethidium bromide, and observation under ultraviolet (UV) light in a UV transilluminator (UVP: California, USA).

**Table 1.** Primers used to detect soybean (*Glycine max L.*) lectin gene.

| Code | Primers | Sequence | Target | Reference |
|---|---|---|---|---|
| A | Lec 1-5 | GGCTCATAACACACTCTATTATTGT | 818 bp Lectin | Matsuoka et al. (2002) |
| B | Lec 1-3 | TGATGGATCTGATAGAATTGACGTT | | |
| C | GmLec F | GTGCTACTGACCAGCAAGGcAAACTCAGCG | 164 bp Lectin | Vallonhofer et al. (1999) |
| D | GmLec R | GAGGGTTTTGGGGTGCCGTTTTCG | | |

**Table 2.** Primers used to detect transgenes in GM soybean Roundup Ready GTS 40-3-2.

| Code | Primers | Sequence | Target | Reference |
|---|---|---|---|---|
| E | 35S1 | GCT CCT ACA AAT GCC ATC A | CamV 35S (195 bp) | Pietsch et al. (1997) |
| F | 35S2 | GAT AGT GGG ATT GTG CGT CA | | |
| G | CMO1 | CAC TACAAATGCCATCATTGCGATA | CamV 35S (220 bp) | Matsuoka et al. (1999) |
| H | CMO2 | CTTATATAGAGGAAGGGTCTTGCGA | | |
| I | RRS1 | TGA TGT GAT ATC TCC ACT GAC G | CamV/EPSPS (172 bp) | Sanders et al. (1987) |
| J | RRS2 | TGT ATC CCT TGA GCCATG TTG T | | Gasser et al. (1988) |
| K | NOS ter 3-5' | GTCTTGCGATGATTATCATATAATTTCTG | NOS (151 bp) | Matsuoka et al. (2002) |
| L | NOS ter 3-3' | CGC TAT ATT TTG TTT TCT ATC GCG T | | |

**Table 3.** New primers designed in this study that target a lectin gene and transgenes in GM Roundup Ready Soybean GTS 40-3-2

| Primer | Sequence 5' ⟶ 3' | Target gene / amplicon size |
|---|---|---|
| *lecSf* | ACT GAC CAG CAA GGC AAA CT | *lectin* gene (430-bp) |
| *lecSr* | TTT GGT GGA TCC CAA GAG TC | |
| *CaCTPf* | CAA ATG CCA TCA TTG CGA TA | *CamV/ mEPSPS*, 300 bp |
| *CaCTPr* | GAG CCA TGT TGT TAA TTT GTG C | |
| *NosSf* | GAA TCC TGT TGC CGG TCT T | *nos* terninator (173-bp) |
| *NosSr* | AGT TTG CGC GCT ATA TTT TG | |

**Selection of PCR primers that target a native soybean gene and transgenes**

Relevant gene sequences submitted in the database or reported in earlier publications (Gasser et al. 1988; Matsuoka et al. 1999, 2002; Pietsch et al. 1997; Sanders et al. 1987; Vallonhofer et al. 1999) for a native *lectin* gene and transgenes in Roundup Ready soybean GTS 40-3-2 were used in the initial experiments in order to test multiplex PCR detection and to generate sequences that will be used for designing new primers. All primers used in the study were synthesized by Genset Singapore Biotech. Pte Ltd (Singapore, Singapore).

**Multiplex PCR detection of native soybean *lectin* gene and two transgenes from Roundup Ready GTS 40-3-2**

The initial multiplex PCR was performed to detect a native soybean *lectin* gene and transgenes, *m-epsps* and *nos* terminator in Roundup Ready® GTS 40-3-2 (*Glycine max* L.) using previously reported primers (GmLec F/Lec1-3, CMO1/RRS2, and NOS ter3-5'/NOS ter 3-3'). The initial multiplex PCR reaction (20 µL totalµL )contained the following components in the reaction mixture: 1X PCR buffer, 2.0 mM $MgCl_2$, 0.3 mM of each dNTPs, 1.0 µM 3 each of 3 forward and 3 reverse primers, 0.03 U/reaction of Platinum Taq High Fidelity Polymerase (Invitrogen Life Technologies, USA), and 1 µL of template DNA. Forty cycles of PCR was conducted with an initial denaturation at 95°C for 2 minutes, denaturation at 95°C for 1 minute, annealing at 60°C for 20 seconds, extension at 68°C for 40 seconds, and final extension at 68°C for 3 minutes.

The reaction mixture for the alternative multiplex PCR using newly designed primers (Table 3) contained the same components as the initial multiplex PCR but the PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 40 seconds, annealing at 56°C for 40 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 3 minutes. In both sets of experiments the non-transgenic PSBSy2 soybean DNA template was used as 0% GM sample or no GM control.

**Cloning and Sequencing of Multiplex PCR Target Genes**

The amplicons representing the lectin gene and the transgenes that were generated from the initial multiplex PCR was cloned into the pCR® 4-TOPO® vector using TOPO® TA Cloning Kit (Invitrogen Life Technologies: California, USA). Two clones containing the expected product size were selected by colony PCR and/or *Eco*R1 digestion according to manufacturer's instruction. Mini-preparations of the recombinant plasmids containing the desired insert were isolated using the Nucleospin® Miniprep Kit (BD Biosciences: California, USA) and subjected to sequencing using the ABI 377 Automated Sequencer (Perkin Elmer, USA) of NIMBB, UP Diliman.

**Detection limit of transgenes in Roundup Ready GTS 40-3-2**

DNA extracts from known weight percentages of GM Roundup Ready soybean GTS 40-3-2, 100%, 10%, 1%, 0.5%, 0.3%, and 0.1% GM seeds, were used as templates for the first set of multiplex PCR. DNA extracts with 100%, 5%, 2%, 1%, 0.5%, 0.1%, and less than 0.03% GM seeds were used for the second set of multiplex PCR using new primers designed in this study. The DNA template extracted from samples containing the smallest weight percentage of GM seeds that generated visible amplicons in PCR was determined in order to obtain the transgene detection limit.

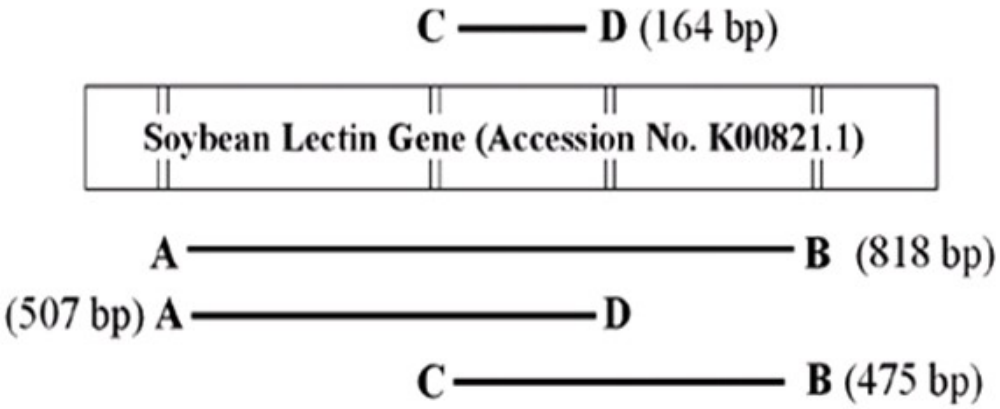


**Figure 1.** Schematic diagram of soybean lectin gene showing the relative annealing sites of *lectin* gene-targeted primers and the expected PCR amplicons using different primer combinations from Table 1.

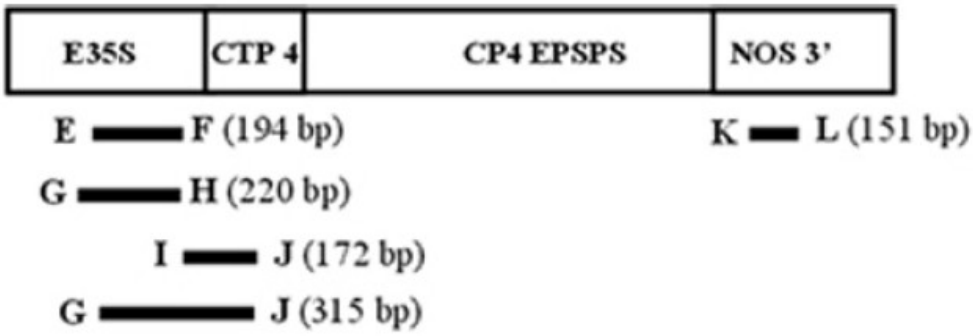


**Figure 2.** Schematic diagram of transgene in GM soybean Roundup Ready GTS 40-3-2 showing the relative annealing sites of transgene-targeted primers and the expected PCR amplicons.

## RESULTS

PCR primers that target a native soybean *lectin* gene and transgenes present in GM Roundup Ready soybean GTS 40-3-2 were designed and reported in previous studies (Tables 1 and 2; Gasser et al. 1988; Matsuoka et al. 1999, 2002; Pietsch et al. 1997; Sanders et al. 1987; Vallonhofer et al. 1999). The relative annealing sites of the primers to the target genes as well as the expected size of amplicons are illustrated in Figures 1 and 2. These primers were tested in preliminary single locus PCR (data not shown) and three pairs of primers, *GmLec F/Lec1-3, CMO1/RRS2,* and *NOS ter3-5'/NOS ter 3-3'*, were selected for the initial multiplex PCR.

**Cloning and Sequencing of Multiplex PCR Target Genes**

Multiplex PCR performed with Roundup Ready GTS 40-3-2 DNA template and primer pairs *GmLec F/Lec1-3, CMO1/RRS2,* and *NOS ter3-5'/NOS ter 3-3'*, generated the expected fragments of the *lectin* gene (475-bp), *CamV/EPSPS* (315-bp), and *nos* terminator (151-bp; Figure 3, lane 5) respectively. Only the 475-bp native soybean *lectin* gene fragment was amplified in PCR using DNA templates from the non-GM PSBSy2 seeds (Figure 3, lanes 3 & 4).

The sequences obtained for each insert fragment was subjected to homology search using Blastn (http://www.ncbi.nlm.nih.gov/BLAST/). The 475-bp fragments from PSBSy2 and GTS 40-3-2 have 99% identity with soybean *lectin* gene (GenBank Accession No. K00821.1). The two *lectin* gene fragments, however, have two (2) nucleotide differences found at bases 61 and 368 (or bases 1452 and 1159 of the GenBank *lectin* coding sequence). Bases 3 to 252 of the 315-bp fragment were 100% homologous to *CamV 35S* promoter sequences (GenBank Accession No. V00141.1), while the bases 271 to 315 of the fragment had 100% identity with *Petunia hybrida* 5-enolpyruvylshikimate 3-phosphate synthase mRNA (GenBank Accession No. M21084.1). The 151-bp fragment was 100% identical to *nos* terminator of *Agrobacterium tumefaciens* gene Ti plasmid (GenBank Accession No. AJ237588.1).

**Multiplex PCR using new primers designed in this study**

Three new sets of primers *lecSf/ lecSfr*, *CaCTPf/ CaCTPr*, and *NosSf/ NosSr* (Table 3) that target the soybean *lectin* gene, *CamV* promoter/*CP4-EPSPS*, and the *nos* terminator gene (respectively) in GM Roundup Ready soybean GTS 40-3-2, were designed based on nucleotide sequences of amplicons from the first multiplex PCR. Relative annealing sites of the new primers, regions flanking bases 1043 to 1472 (GenBank

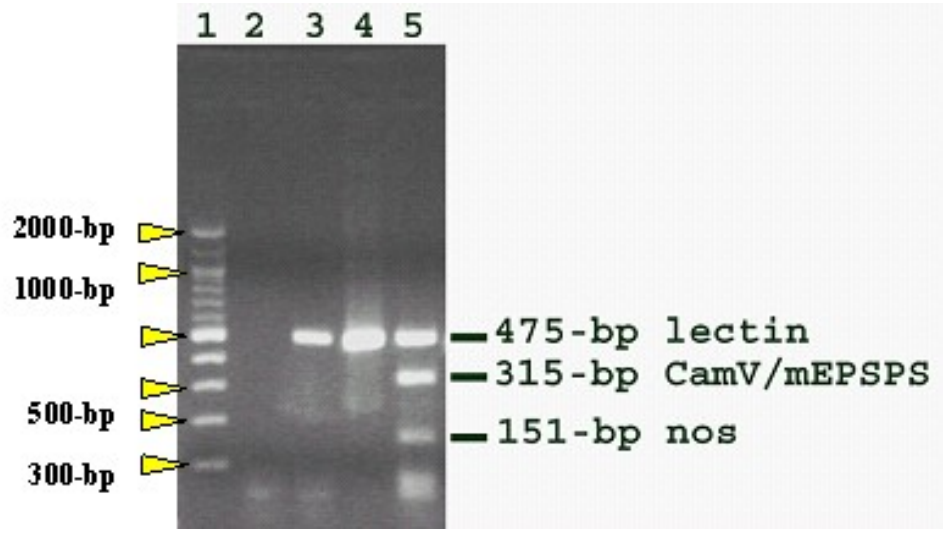


**Figure 3.** Detection of transgenes in Roundup Ready Soybean GTS 40-3-2 using multiplex PCR. Lanes 2-5 contain amplicons from PCR using the following DNA templates, no DNA, non-GM PSBSy2 DNA, non-GM PSBSy2 DNA, and Roundup Ready Soybean GTS40-3-2 DNA, respectively. Lane 1 contains 100 bp ladder (Invitrogen).

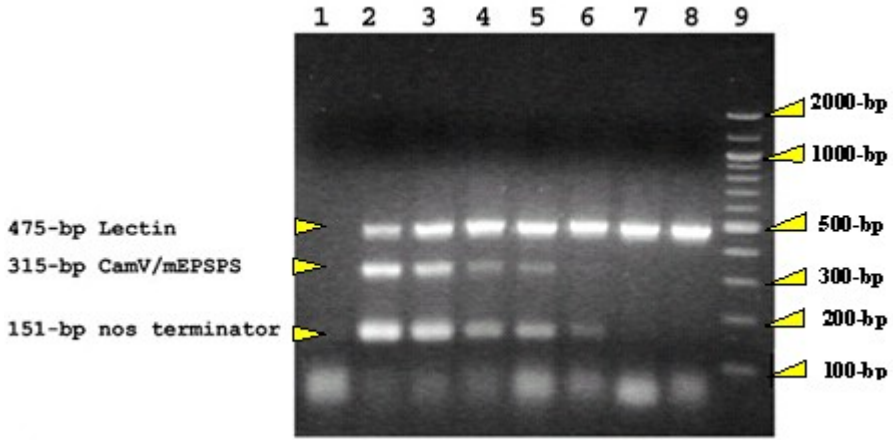


**Figure 4.** Detection limit of a lectin gene and transgenes *CamV/mEPSPS* and *nos* terminator in Roundup Ready Soybean GTS 40-3-2 by multiplex PCR. Lanes 2-7, PCR using DNA templates from samples containing 100%, 10%, 1%, 0.5%, 0.3%, and 0.1% GM Roundup Ready Soybean GTS 40-3-2, respectively; lane 8, PCR using 0%GM Roundup Ready Soybean GTS 40-3-2 or 100% nonGM PSBSy2. Lane 1 is the no DNA template control and lane 9 contains 100 bp ladder (Invitrogen).

Accession no. K00821:Soy bean lectin gene), bases 8-317 (GenBank Accession no. AY592954.1:*Glycine max* transgenic chloroplast CP4 EPSPS fusion protein precursor) and bases 9-181 (GenBank Accession no. AF465641.1: *Agrobacterium tumefaciens nos* gene, terminator region), are shown in Figure 5. Multiplex PCR using new sets of primers (Figure 6) amplified the *lectin* (430-bp), *m-esps* (300-bp), and the *nos* terminator (173-bp) gene fragments and the identity of the amplicons were confirmed through sequence analysis.

**Detection limit of target genes in multiplex PCR**

The multiplex PCR using primer pairs *GmLec F/Lec1-3, CMO1/RRS2, and NOS ter3-5'/NOS ter 3-3'* could produce the 315-bp *CamV/EPSPS* fragment if the template DNA was prepared from a seed sample containing at least 0.5% GM Roundup Ready GTS 40-3-2. The 151-bp *nos* transgene fragment could be detected if the DNA template was prepared from samples with as low as 0.3% GM soybean (Figure 4). The multiplex PCR detection limit of the same transgenes in Roundup Ready soybean GTS 40-3-2 using new primer pairs *lecSf/ lecSfr*, *CaCTPf/ CaCTPr*, and *NosSf/ NosSr* is 1.0% GM seeds for both transgenes. Faint bands, however, were also visible when 0.5% GM soybean was used. Because the *lectin* gene is present in both GM and non-GM soybean sample, the gene could be amplified from all samples.

## DISCUSSION

The study focused on the selection and design of primers for multiplex PCR to develop additional detection procedures for a native soybean *lectin* gene and three transgenes in genetically modified soybean Roundup Ready GTS 40-3-2. The *lectin* gene detection ensures that the template contains soybean DNA and prevents false negative amplification as a result of absence of correct template DNA. Review of literature for gene sequences inserted into Roundup Ready soybean GTS 40-3-2 and literature search for

primers that target the transgenes were necessary to select primers that will provide enough disparity in amplicon sizes for multiplex PCR. The primers that amplify a 164-bp *lectin* and the 172-bp *CamV/EPSPS* gene fragments (Tables 1 and 2), for example, were not selected for the initial multiplex PCR because the primer used to detect the transgene *nos* terminator produce a 151-bp fragment with just about 20 base pair size difference from both. The primer resulting in a smallest sized amplicon of 475-bp for the *lectin* gene fragment was chosen because smaller amplicons require lesser dNTP's and are amplified relatively faster.

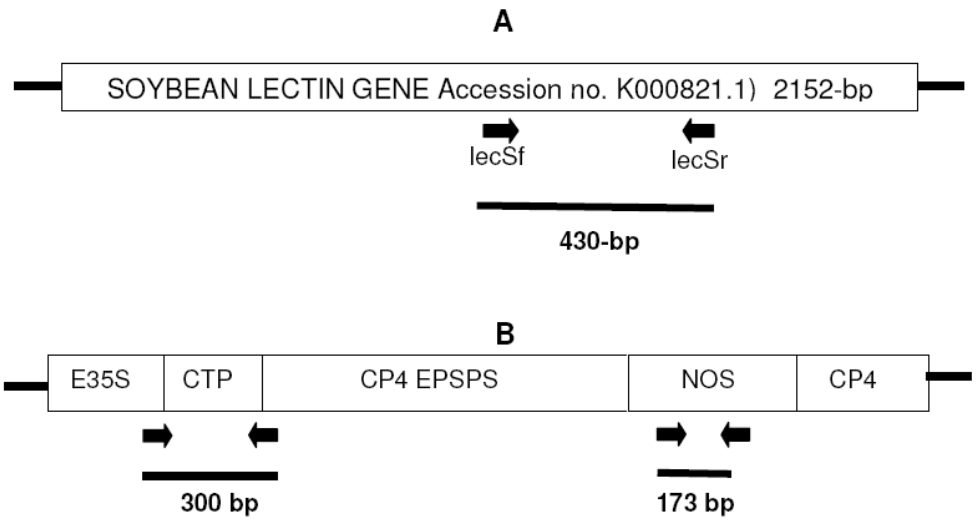


**Figure 5.** Schematic diagram showing the relative annealing sites of lectin-targeted (**A**) and transgene-targeted (**B**) primers and the expected PCR amplicons.

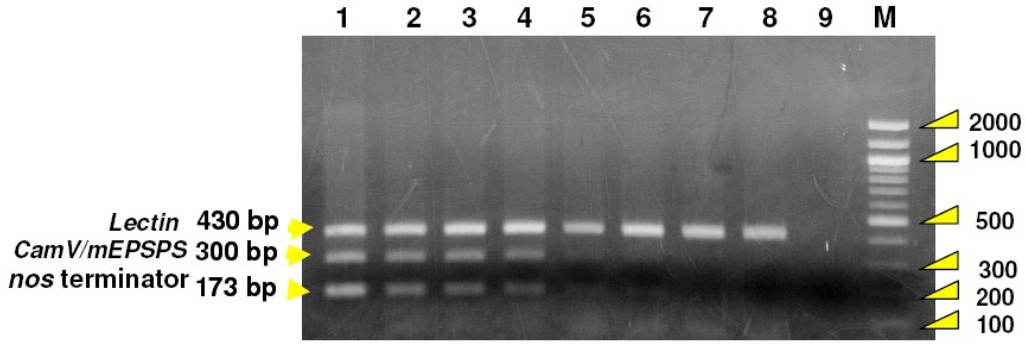


**Figure 6.** Multiplex PCR using new primers targeting lectin gene and transgenes (*CamV promoter/mEPS* and *nos* terminator) of GM Roundup Ready Soybean GTS 40-3-2. Lanes 1-7, DNA template prepared from samples with 100%, 5%, 2%, 1%, 0.5%, 0.1%, and less than 0.03% GM Roundup Ready Soybean GTS 40-3-2 seeds, respectively; lane 8, DNA template from non GM PSBSY2 soybean; and lane 9, no DNA template control. M is the 100bp ladder (New England Biolabs® Inc.)

In this study, the multiplex detection protocols developed used two primer sets to detect the three (3) regions of the transgene cassette in Roundup Ready Soybean GTS 40-3-2 (Figures 2 and 5), namely the *CamV 35S* promoter, modified *EPSPS*, and *nos* terminator. The use of one set of primers targeting a region that traverses two parts of the cassette, the *CamV 35S* promoter and regions of the *EPSPS* coding sequence, offers the advantage of a more economical and accurate detection of the material. The EPSPS, which is involved in the biosynthesis of aromatic amino acids, is present in all plants, bacteria and fungi. The use of a primer set designed to amplify the transgenic m-EPSPS fragment alone, therefore, may be able to detect the native form of EPSPS even in non-GM samples.

Two multiplex PCR detection protocols described in this study could detect all target genes from Roundup Ready Soybean GTS 40-3-2. The first protocol uses transgene-targeted primers selected from the literature with optimized multiplex PCR conditions. New primers were designed and tested in this study to come up with an alternative protocol for detection of the same transgenes. Availability of alternative primers for detection will be advantageous in cases when some primers will not work for reasons not easily identifiable in evaluating actual seed samples. In this study, both multiplex PCR protocols conducted can accurately identify the presence of as little as 0.5 % to 1.0% Roundup Ready GTS 40-3-2 (Figures 4 and 6). The *nos* transgene fragment is still detectable at 0.3 % GM in the initial multiplex PCR, probably because the 151-bp *nos* amplicon was more efficiently amplified being the smallest target gene fragment.

The multiplex PCR detection procedures are now available in the institute for research or routine detection services with comparable precision as expensive kits imported from other countries. The data from this research could be used by

government agencies in further developing protocols needed for regulation of genetically modified crops. The data from the research could also be the basis for more work using the same approach in developing detection protocols for other transgenes inserted in other crops.

## CONCLUSION

This report describes the multiplex PCR procedures that were developed using primers previously reported in the literature or new primers designed in the study. Amplified target genes that could be detected and distinguished from multiplex PCR profiles include the native soybean *lectin* gene that should be amplified if soybean DNA template is present, the *CamV/EPSPS* and the *nos* terminator transgenes, that are amplified if samples contain about 1.0% GM Roundup Ready Soybean GTS 40-3-2.


## ACKNOWLEDGMENTS

The authors would like to thank the Department of Agriculture and the National Institute of Molecular Biology and Biotechnology UP Diliman for funding the research, the Monsanto Company, USA for providing ground seeds of GM Roundup Ready GTS 40-3-2, and Ms. Diana Rose E. Rañoa for the conduct of multiplex PCR experiments using new sets of primers.


## NO CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among authors and institutions and individuals mentioned above in the conduct of this study and the preparation and submission of this manuscript.

## CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Dr. Cynthia Hedreyda is the project leader who proposed and conceptualized the research and the one who wrote the paper. Ms. Jennifer L. Roxas performed the search for transgenes and transgene-targeted primers and conducted the optimized multiplex PCR experiments.

## REFERENCES


Gasser CS, Winter JA, Hironaka CM, Shah DM. J Biol Chem 1988; 263:4280-4287.

GM Crop Database, 2004 *GM Crop Database*. April 2004. AGBIOS. 3 April 2004 (http://www.agbios.com/dbase.php)

GMO compass (http://www.gmo-compass.org/eng/grocery_shopping/crops/19.genetically_modified_soybean.html)

James D, Schmidt AM, Wall E, Green M, Masri S. Reliable detection and identification of genetically modified maize, soybean, and canola by multiplex

PCR analysis. J Agric Food Chem 2003; 51.5829-5834.

James C. Global Status of Commercialized Biotech/GM Crops: 2008. ISAAA Brief No. 39. ISAAA, Ithaca, NY

Lin HY, Chiang JW, Shih DYC. Detection of genetically modified soybeans by PCR method and immunoassay kits. J Food Drug Anal 2001; 9(3):160-166.

Matsuoka T, Kawashima Y, Akiyama H, Miura H, Goda Y, Sebata T, Isshika K, Toyoda M, Hino A. J Food Hyg Soc Japan 1999; 40:149-157.

Matsuoka T, Kuribara H, Takubo K, Akiyama H, Miura H, Goda Y, Kusakabe Y, Isshika K. Toyoda M, Hino A. J Food Hyg Soc Japan 2002; 50:2100-2109.

Pietsch K, Waiblinger HU, Brodmann P, Wurz A. Screeningverfahren zur Identifizierung gentechnisch veraenderter pflanzlicher Lebensmittel. Deutsche Lebensmittel-Rundschau 1997; 93:35-38.

Randhawa GJ, Firke PK. Detection of transgenes in genetically modified soybean and maize using polymerase chain reaction. Indian J Biotech 2006; 5:510-513.

Sanders PR, Winter JA, Barnason AR, Rogers SG, Fraley RT. Nucl Acids Res 1987; 15:1543-1558.

Vallonhofer S, Burg K, Schmidt J, Kroath H. Genetically modified organisms in food screening and specific detection by polymerase chain reaction. J Agric Food Chem 1999; 47:5038-5043.