94-230-01p

Notices

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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 94-125-1]

Determination of Nonregulated Status for Additional Calgene, Inc., Genetically Engineered FLAVR SAVR™ Tomato Lines

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: The Animal and Plant Health Inspection Service is announcing that it has added nine additional genetically engineered tomato lines to those subject to its October 19, 1992, interpretive ruling for FLAVR SAVR™ tomatoes, that the subject FLAVR SAVR™ lines need no longer be regulated. The effect of this action is that nine additional delayed softening tomato lines, which have been modified by the incorporation of genetic material described by Calgene, Inc., in its initial request for an interpretive ruling, will no longer be subject to regulation under 7 CFR part 340.

FOR FURTHER INFORMATION CONTACT: Dr. Keith Reding, Biotechnologist; Biotechnology, Biologics, and Environmental Protection; APHIS, USDA, Room 850, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, (301) 436-8761.

SUPPLEMENTARY INFORMATION: On October 19, 1992, the Animal and Plant Health Inspection Service (APHIS) published in the **Federal Register** (57 FR 47608-47616, Docket No. 92-087-2) a notice announcing the issuance of an interpretive ruling that previously field tested lines of the Calgene, Inc., FLAVR SAVR™ tomato do not present a plant pest risk and are not regulated articles under the regulations contained in 7 CFR part 340. This action was in response to a petition submitted by Calgene seeking a determination from APHIS that its FLAVR SAVR™ tomato no longer be deemed a regulated article, based on an absence of plant pest risk. The effect of the action was that previously field tested lines of the FLAVR SAVR™ tomato and their progeny would no longer be regulated under these regulations.

FLAVR SAVR™ tomatoes were defined by Calgene in its initial petition to include any tomatoes transformed with one of seven identified plasmid vectors that all carry an antisense copy of the tomato polygalacturonase gene and a bacterial neomycin phosphotransferase gene with associated regulatory sequences. Calgene's initial request to APHIS in 1992 was for a determination pertaining to all FLAVR SAVR™ transformants produced in tomato using any one of the seven plasmid vectors. Calgene indicated in its petition that data provided to the Agency was representative of the data gathered for all lines tested up to that time. The initial determination announced by APHIS on October 19, 1992, only applied to those lines that had already been field tested. However, APHIS indicated that new lines were likely to exhibit properties similar to those of lines already field tested under permit. The determination also allowed for cross-breeding of the identified FLAVR SAVR™ tomato lines with any other lines or cultivars of tomato without permit. One additional FLAVR SAVR™ tomato line was added to the original determination on October 3, 1994 (59 FR 50220, Docket No. 94-096-1).

The nine additional FLAVR SAVR™ tomato lines that are the subject of this notice were constructed using the plasmid PCGN4109, which contains the promoter/terminator from either PCGN1557 or PCGN1578. These latter two vectors were among the seven included in Calgene's initial petition to APHIS. In our determination on October 19, 1992, the lines using these vectors were not deregulated because they had not been field tested. These lines have been field tested in accordance with APHIS regulations at 7 CFR part 340, and data provided to APHIS indicate that the new transformant lines, produced in a manner identical to the earlier transformant lines, behave similarly to those earlier FLAVR SAVR™ tomato lines to which the determination initially applied. Reports from field trials and other data indicate that the new tomato lines grow normally, exhibit the expected morphological, reproductive, and physiological properties, and do not have unexpected pest or disease susceptibility or symptoms. Therefore, the APHIS determination of nonregulated status for previously field tested FLAVR SAVR™ tomato lines of October 19, 1992, applies as well to the new transformed lines.

Done in Washington, DC, this 14th day of November 1994.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 94-28540 Filed 11-17-94; 8:45 am]

BILLING CODE 3410-34-P

CALGENE

October 27, 1994

Dr. Keith Reding
Biotechnology, Biologics, and
Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
6505 Belcrest Road
Hyattsville, MD 20782

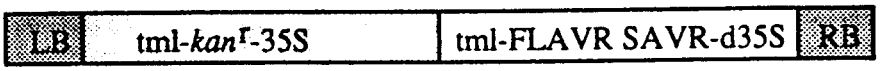
Additional FLAVR SAVR™ Tomato Lines: 4109

Dear Dr. Reding:

Please find attached example data on Southern analysis and PG activity level for two lines 114F 4109a 26 and 114F 4109a 81. The previously submitted Southern and PG data were for lines currently in the field under Notification; once the field test is completed, we will request another addition to the FLAVR SAVR Determination.

pCGN4109a (referred to as pCGN4109) was derived from the binary vector pCGN1558 and FLAVR SAVR tomato lines produced using this construct are the subject of this request. pCGN4109b was another construct that is not under consideration at this time and no data have been submitted to you.

The orientation of the genes is as indicated in the August 17, 1994 petition:



pCGN4109

If you have additional questions, please give me a call 10/28 or Don Emlay next week. Thank you.

Sincerely,

Keith Redenbaugh, Ph.D.
Regulatory Affairs

Received
10/28/94

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Davis, California 95616
Telephone (916) 753-6313
Telex: 350370 CGENE

4109 PG Enzyme Activity

114F 4109a-26

Generation	Genotype	PG Activity*	*kan*^{r} segregation
	114F Control	0.94 - 1.43	
T_1	114F 4109a - 26	0.05 - 0.24	3:1 segregation
T_2	114F 4109a - 26	0.06 - 0.14	Homozygous
T_3	114F 4109a - 26	0.11	Homozygous

141F 4109a-81

Generation	Genotype	PG Activity*	*kan*^{r} segregation
	141F Control	0.89 - 1.34	
T_1	141F 4109a - 81	0.15 - 0.29	3:1 segregation
T_2	141F 4109a - 81	0.02 - 0.09	Homozygous
T_3	141F 4109a - 81	0.17	Homozygous

*Modification of Sheehy, et al. (1988. PNAS 85:8805) PG assay.

4109 Southerns

Southern analysis of 4109a plants for DNA transfer beyond T-DNA borders

The 624a blot has two plants that we wish to commercialize; 114F 4109a-26 and 141 4109a-81. DNA on this blot was digested with restriction enzymes BamHI and BglII. The blot was probed first with a pCGN1532 probe and exposed to film for 7 days (Figure 1). The lane containing control genomic DNA, spiked with one copy of pCGN1436 plasmid DNA (containing the same backbone structure as pCGN4109a), had the 5 bands expected when cut with restriction enzymes BamHI and BglII. A transgenic plant containing transfer beyond the border and included as a control (#5, Figure 1) shows a banding pattern characteristic of a plant having extra DNA. Plants 114F 4109a -26 and 141 4109a-81 did not show any hybridization to the 1532 probe.

This blot was then stripped and reprobed with a kan probe and exposed to film for two days (Figure 2). The lane containing control DNA spiked with one copy of pCGN1436 DNA contained a band that hybridized to the kan probe of a size between 4.4 and 6.6 kb. The expected size would be 5.3 kb. The lane with 114F 4109a-26 had a band that hybridized to kan at about 5 kb and the lane with 141F 4109a-81 had a band that hybridized to kan at about 4 kb. The presence of these bands proves that there was DNA present on the filter from these plants that the pCGN1532 probe should have hybridized to if there was transfer beyond the T-DNA borders.

Figure 1. Southern of tomato lines transformed with 4109a (asPG) and 1436 (asPG). Plant DNA was digested with the restriction enzymes BamHI and BglII. One copy of plasmid DNA from pCGN1436 was spiked into non-transgenic plant DNA as a positive control. Plant 5, a plant transformed with pCGN1436 an example of a plant with transfer of DNA outside the T-DNA borders was also included as a positive control. The blot was probed with pCGN1532 which was linearized with the restriction enzymes BamHI and EcoRI. The blot was exposed to X-Ray film for 7 days. Plants 114F 4109a-26 and 141F 4109a-81 do not have transfer of DNA beyond the T-DNA borders. Lane assignments are as follows:

(1) Molecular markers, (2) Blank lane, (3) Blank lane, (4) pCGN1436 1 copy, (5) 141F 4109a-81 (6) 114F 4109a-26, (7 - 12) pCGN1436 transgenic events 1 -6, (13) Blank lane.

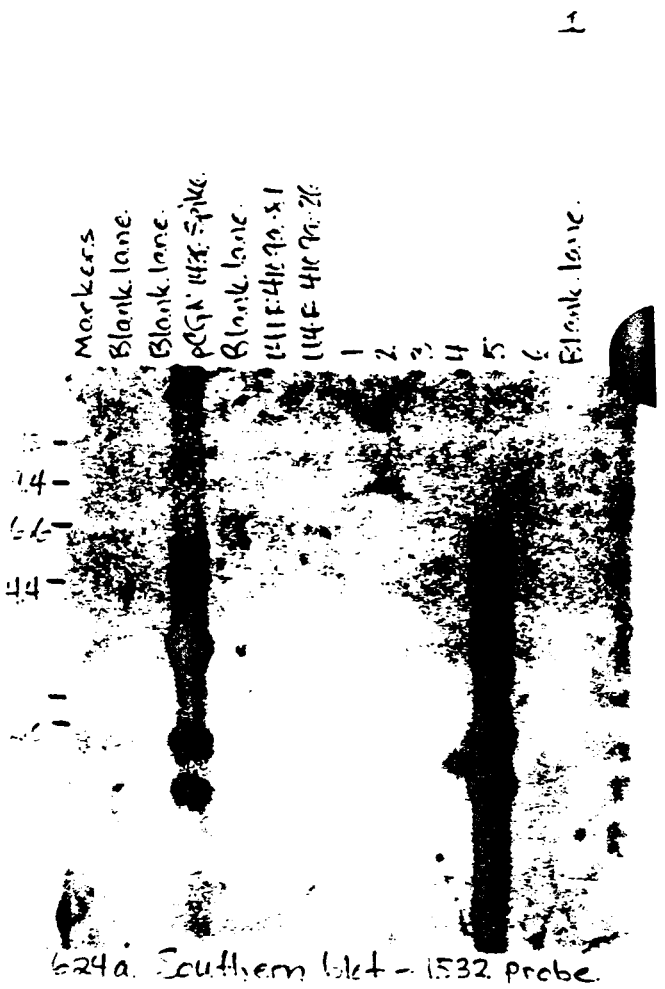
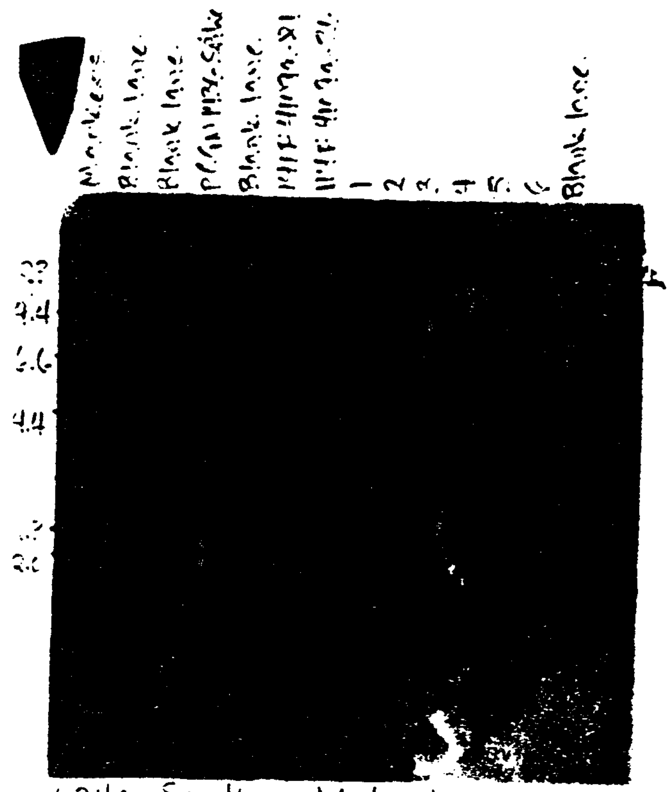


Figure 2 Southern of tomato lines transformed with 4109a (asPG) and 1436 (asPG). Plant DNA was digested with the restriction enzymes BamHI and BglII. One copy of plasmid DNA from pCGN1436 was spiked into non-transgenic plant DNA as a positive control. This blot was previously probed with pCGN1532 and exposed (Figure 1) and then stripped of probe. The blot was then hybridized a second time with *kan*^{r} probe. Filter was exposed to X-Ray film for 2 days. Lane assignments are as follows:

(1) Molecular markers, (2) Blank lane, (3) Blank lane, (4) pCGN1436 1 copy, (5) 141F 4109a-81 (6) 114F 4109a-26, (7 - 12) pCGN1436 transgenic events, (13) Blank lane.



Southern blot - Kan Probe.