(Provisional Translation)

Testing for Foods Produced by Recombinant DNA Techniques

1. Sampling Methods

1.1 Sampling of Foods Produced by Recombinant DNA Techniques

1.1.1 Sampling of Corn Kernels and Soybeans

On the assumption that corn kernels or soybeans produced by recombinant DNA techniques exist inhomogeneously, obtain a representative sample from a lot in accordance with lot size, type of packing (bulk or sacked) and packaging style of the lot. Take notice that corn kernels or soybeans from other lots do not commingle when you obtain a sample. To obtain a sample, use utensils, containers and packaging materials that are disposable or cleaned thoroughly.

Mix a sample well until it becomes homogenous, and weigh out certain amount of grains* that you need for tests. Then, grind them until they become homogeneous with equipments (*e.g.* grinding machine).

*Approximately 500g of corn kernels or soybeans are needed for a quantitative test.

1.1.1.1. Sampling Sacked Grains

Obtain samples according to the following table.

Lot Size	Number of Sacks to be Unpacked	Amount of Sample (Kg)	Number of Samples	
			Qualitative	Quantitative
≦ 15	2	1	1	3
16 ～ 25	3	1	1	3
26 ～ 90	5	1	1	3
91 ～ 150	8	1	1	3
151 ～ 280	13	1	1	3
281 ～ 500	20	1	1	3
501 ～ 1,200	32	1	1	3
1,201 ～ 3,200	50	1	1	3
3,201 ～ 10,000	80	1	1	3
10,001 ～ 35,000	125	1	1	3
35,001 ～ 150,000	200	1	1	3
150,001 ～ 500,000	315	1	1	3
≧500,001	500	1	1	3

1.1.1.2. Sampling Bulk Grains

1.1.1.2.1 Sampling Silos During Loading

When foods are loaded into silos, regard a silo as a lot and obtain a representative sample of a lot with tools (*e.g.* auto-sampler). Draw corn kernels or soybeans for 15 times at appropriate intervals in order that the total amount weighs more than 10 Kg. Then, divide the grains homogeneously and use a portion (more than 1 Kg) as a sample per silo.

Regarding corn kernels or soybeans that have already been loaded into silos, obtain a sample in the same way when they are reloaded into another silo.

1.1.1.2.2 Sampling Barges During Loading

When foods are loaded into barges (including domestic vessels), regard a barge as a lot and obtain a representative sample of a lot with tools (*e.g.* auto-sampler). Draw grains for 15 times at appropriate intervals in order that the total amount weighs more than 10 Kg. Then, divide the grains homogeneously and use a portion (more than 1 Kg) as a sample per barge.

1.1.1.2.3 Sampling Barges After Loading

When foods have already been loaded into barges, regard a barge as a lot and obtain a representative sample of a lot by drawing corn kernels or soybeans from 15 points (every 5 points from the high, middle, and lower layer of the barge) in order that the total amount weighs more than 10 Kg. Then, divide the grains homogeneously and use a portion (more than 1 Kg) as a sample per barge.

1.1.2. Sampling of Papaya

Regarding sampling of papayas, obtain a sample according to the following table.

Lot Size	Number of Packages to be Unpacked	Amount of Sample (Number of Pieces)	Number of Sample
≦ 50	2	2	1
51 ～ 500	3	3	1
501 ～35,000	5	5	1
≧35,001	8	8	1

1.2 Sampling of Processed Foods

Regarding sampling of processed foods, obtain a sample according to the following table.

1.2.1.Ground Corn and Soybean (*e.g.* corn grits, corn flower, corn meal)

Obtain samples according to the table in "1.1.1.1. Sampling Sacked Grains".

1.2.2. Processed Foods other than 1.2.1.

Obtain samples according to the following table.

Lot Size	Number of Packages to be Unpacked	Amount of Sample (g)	Number of Samples	
			Qualitative	Quantitative
≦ 15	2	120	1	3
16 ～ 50	3	120	1	3
51 ～ 150	5	120	1	3
151 ～ 500	8	120	1	3
501 ～ 3,200	13	120	1	3
3,201 ～ 35,000	20	120	1	3
35,001～500,000	32	120	1	3
≧500,001	50	120	1	3

2. Tests for foods produced by recombinant-DNA techniques whose safety assessments are not reviewed

2.1 Test methods

2.1.1 Test of corn (CBH351)

The lateral flow method is used in the test of corn kernels. This method is also used in the test of ground food products such as corn grits, corn flour and corn meal (hereinafter referred to as "corn semi-products"), in which proteins newly expressed by genetic modification are not subjected to physical and chemical changes.

The qualitative PCR method is used for other corn processed food products.

Corn semi-products are tested by the lateral flow method and then subjected to the identification test using the qualitative PCR method.

2.1.1.1 Detection of CBH351 in corn grains

2.1.1.1.1 Lateral flow method

The commercially available kit, Trait Bt9 Corn Grain Test Kit (Part # 7000003) manufactured by Strategic Diagnostics Inc.(SDI), is used. The procedures described below are basically the same as directed in the kit's explanatory leaflet. When an experiment is performed in a laboratory, RO water purified using a reverse osmotic membrane or distilled water should be used unless otherwise specified.

2.1.1.1.1.1 Testing procedures

Grind 800 kernels randomly selected from a sample, and place the ground corn* in an open-mouthed jar with a lid (a jar holds approximately 500 mL). Add 288 mL of water and shake the jar for 10-20 seconds to thoroughly wet all the mixture. If there is no free liquid at this stage, add a small amount of water, shake the jar, and check for the presence of the free liquid. Repeat this procedure until a few mL of free liquid develop. Transfer 0.5 mL of the free liquid to a 1.5-mL sample tube provided with the test kit, and place a Trait Bt9 Test Strip vertically into the sample tube.

* Normally, grind 230 g of corn kernels (if the 230 g of kernels are made up of fewer than 800 kernels, grind 800 kernels).

2.1.1.1.1.2 Judgment of results

10 minutes after placing the test strip*, observe the result window of the test strip. If two red lines appear on the result window, the result is positive; if only a control line appears, the result is negative; and if no line appears, the test is invalid.

* If the strip is placed in the tube for more than 10 minutes, the red lines may deepen and hinder an accurate judgment.

2.1.1.2 Detection of CBH351 in processed corn products

In accordance with "Extraction and purification of DNA from processed food products" (2.2.3), extract one sample in duplicate. Subject the obtained DNA solution to qualitative PCR under the conditions specified below.

2.1.1.2.1 Qualitative PCR

Qualitative PCR is the method to amplify some parts of the extracted DNA by use of primers and to detect the amplified DNA by electrophoresis.

* As a very small amount of template DNA is amplified in PCR reaction, it is very important to

avoid contamination of DNA other than the target DNA (particularly PCR byproducts). Moreover, DNase, secreted from the human skin surface, must be prevented from entering the PCR reaction mixture as it decomposes DNA. Therefore, disposable tubes and tips must be autoclaved at 121°C for 20 minutes or more just prior to use. As for the water used in qualitative PCR, unless otherwise specified, use RO water purified using a reverse osmotic membrane or super-purified water prepared by deionizing distilled water to 17 MΩ/cm by Milli-Q *etc.*, and then autoclaved at 121°C for 20 minutes or more.

2.1.1.2.1.1 PCR amplification

The PCR reaction mixture is prepared as follows in a PCR reaction tube: add 2.5 µL of DNA sample solution adjusted at 10 ng/µL (25 ng as DNA) in an ice bath to a mixture including PCR buffer*1, 0.20 mmol/L dNTP, 3 mmol/L $MgCl_2$, 0.2 µmol/L 5' and 3' primers*2 and 0.625-units Taq DNA polymerase*3, to bring the total volume to 25 µL. Place the reaction tube in a PCR amplifier*4. After starting the reaction by keeping the mixture at 95°C for 10 minutes, maintain the temperature of the mixture at 95°C for 0.5 minutes, at 60°C for 0.5 minutes, and then at 72°C for 0.5 minutes as one cycle, and repeat this cycle for 40 times. Stop the reaction by keeping the mixture at 72°C for 7 minutes and store the mixture at 4°C as the PCR amplified reactant. As the blank reactant, both of the PCR reaction mixtures without primers or the DNA sample solution should be subjected to PCR reaction. To confirm the extraction of DNA, subject a mixture including a positive-control (to detect reference gene) primer pair*5 instead of the primer pairs for detection of CBH351 to PCR reaction for each DNA sample solution.

*1 PCR buffer
Use a PCR Buffer II (manufactured by PE Biosystems) or a buffer producing an equivalent result.

*2 Primer pair for detection of CBH351
F-primer (CaM03-5'): 5'-CCT TCG CAA GAC CCT TCC TCT ATA-3'
R-primer (CBH02-3'): 5'-GTA GCT GTC GGT GTA GTC CTC GT-3'

*3 Taq DNA polymerase
Use an AmpliTaq Gold DNA Polymerase (manufactured by PE Biosystems) or a polymerase producing an equivalent result.

*4 PCR amplifier
Use a GeneAmp PCR System 9600 (manufactured by Perkin Elmer Co., Ltd.) or a system producing an equivalent result.

*5 Primer pair for positive control (to detect reference gene)
F-primer (Zein n-5'): 5'-CCT ATA GCT TCC CTT CTT CC-3'
R-primer (Zein n-3'): 5'-TGC TGT AAT AGG GCT GAT GA-3'

2.1.1.2.1.2. Agarose gel electrophoresis

The PCR amplified reactant is separated by agarose gel electrophoresis to identify the amplified DNA band.

2.1.1.2.1.2.1 Preparation of agarose gel

Add TAE buffer*1 to the required quantity of agarose, and heat the mixture to dissolve the agarose. Then, add 5 µL of ethidium bromide (10 mg/mL)*2 per 100 mL of the above solution, cool the mixture to approximately 50°C, pour it into a gel maker, and cool and solidify it at room temperature to make a gel*3. Although it is desirable to use the gel immediately after

preparation, it can be preserved in the buffer for several days. The concentration of the gel (1.0% - 4.0%) should be decided based on the band length of the goal product, as it depends on the length of the DNA to be electorophoresed.

*1 TAE buffer
Prepare a solution having final concentrations for 40 mmol/L Tris-acetic acid and 1 mmol/L EDTA with distilled water, and use the solution as the TAE buffer.

*2 Ethidium bromide
Ethidium bromide is a fluorescent agent that enters the interstices of the double-stranded DNA and has a strong carcinogenic and toxic effect. Wear gloves and a mask during handling.

*3 Pre-staining
Pre-staining is used here. Without the additional ethidium bromide here, post-staining of gel following completion of electrophoresis can be used as directed in 2.1.1.2.1.2.3.

2.1.1.2.1.2.2 Electrophoresis
Set the prepared gel in an electrophoresis bath filled with TAE buffer. Add 7.5 µL of PCR amplified reactant to a proper quantity of gel-loading buffer, and inject the mixture into the well of gel. If sample injection into the gel takes too long, the DNA will diffuse, resulting in a vague pattern. Next, perform electrophoresis at a constant voltage of 100 V until the BPB in the gel-loading buffer advances to the 1/2-2/3 position of the gel.

2.1.1.2.1.2.3 Staining of gel (post-staining)
When pre-staining is used, the procedures specified in this section are not necessary. Transfer the electrophoresed gel to a container containing a sufficient amount of TAE buffer to soak the gel thoroughly. Add 5 µL of ethidium bromide (10 mg/mL) per 100 mL buffer, place the container on a shaker, and perform staining for 30 minutes while shaking the container mildly.

2.1.1.2.1.3 Gel image analysis
Place the stained electrophoretic gel on a piece of plastic wrap* spread on the stage of a gel image analyzer, and radiate ultraviolet rays (312 nm). Identify the electrophoretic pattern on the display of the gel image analyzer. Judge the presence of the goal band by comparing it with the DNA molecular-weight standard. If the blank reactant displays a corresponding PCR amplified band, perform the experiment again as the procedures following DNA extraction are invalid. Store the electrophoretic results as image data.

* Plastic wrap
Use poly (vinylidene chloride) film, or ultraviolet rays will be absorbed and no clear pattern will be obtained.

2.1.1.2.1.4 Judgment of result
When a 157-bp PCR amplified band is found in the lane for the positive control primer pair (to detect reference gene), and a 170-bp PCR amplified band is found in the lane for the CBH351 detection primer pair, prepare the PCR reaction mixture using an identification primer pair* from the same DNA sample solution, and subject it to PCR amplification. Apply the obtained PCR amplified reactant to agarose gel electrophoresis, and perform gel image analysis. When a 171-bp amplified band is detected, this sample is CBH351-positive. If two DNA extracts of the same sample produce different results, judge the sample to be positive. Unless a counterpart extract displays the expected amplified band in the lane for the control primer pair, perform electrophoresis again. Unless the expected amplified band appears here, the result for the extract is invalid. Judge the result from the counterpart. When both extracts of the same

sample display no amplified band in the lane for the control primer pair, perform a third extraction, PCR reaction, and subsequent procedures, and judge the result. If the third DNA extract displays no PCR amplified band in the lane for the control primer pair, the non-approved food product in this sample is undetectable. The table below shows examples of judgment.

Example of judgment

	Sample number	1	2	3	4	5	6	7	8	9
Extract 1	Control primer	+	+	+	+	+	+	+	+	-
	Detection primer	+	+	+	+	-	-	+	+	/
	Identification primer	+	+	+	+	/	-	-	-	/
Extract 2	Control primer	+	+	+	-	+	-	+	-	-
	Detection primer	+	+	-	-	-	-	+	-	/
	Identification primer	+	-	/	/	/	/	-	/	/
Judgment		**Positive**	**Positive**	**Positive**	**Positive**	**Negative**	**Negative**	**Negative**	**Negative**	/

Sample No. 9 will be subjected to a third extraction.
* "+" means "positive", "-" means "negative", and "/" means "no test."

* The CBH identification primer pair is as follows:
F-primer (Cry9C-5'): 5'-TAC TAC ATC GAC CGC ATC GA-3'
R-primer (35Ster-3'): 5'-CCT AAT TCC CTT ATC TGG GA-3'

2.1.1.3 Detection of CBH351 in corn semi-products (corn grits, corn flour, corn meal *etc.*)

Test the sample by the lateral flow method as directed in 2.1.1.1.1, except using 230 g of the sample without grinding. If the result is positive, extract DNA from the sample in duplicate as directed in 2.2.1 and subject the DNA extract to the qualitative PCR described in 2.1.1.2.1. When the PCR amplified reactant from one extract displays a 157-bp PCR amplified band in the positive-control (detecting reference gene) primer pair lane, and a 170-bp PCR amplified band is found in the CBH351 detection primer lane, judge the sample to be CBH351-positive.

2.1.2 Detection of papaya (55-1)

For raw papayas and their processed food, perform qualitative PCR as described in 2.1.1.2.1, except use the 55-1 detection primer pair (NosC-5', CaMVN-3') that produces (detects) a 207-bp amplified band, the papain primer pair (papain-5', papain-3') that produces (detects) a 211-bp amplified band for positive control (detecting reference gene), and the 55-1 identification primer (for 2nd screening) pair (CaM3-5', GUSn-3') that produces (detects) a 250-bp amplified band.

55-1 Detection primer pair
F-primer (NosC-5'): 5'-TTA CGG CGA GTT CTG TTA GG-3'
R-primer (CaMVN-3'): 5'-CAT GTG CCT GAG AAA TAG GC-3'

Papain-gene detection primer pair
F-primer (papain-5'): 5'-GGG CAT TCT CAG CTG TTG TA-3'
R-primer (papain-3'): 5'-CGA CAA TAA CGT TGC ACT CC-3'

55-1 Identification primer pair
F-primer (CaM 3-5'): 5'-CCT TCG CAA GAC CCT TCC TCT ATA-3'
R-primer (GUS n-3'): 5'-TCG TTA AAA CTG CCT GGC AC-3'

2.1.3 Detection of New Leaf Y potato (including processed food)

Perform Qualitative PCR as directed in 2.1.1.2.1, using a detection primer pair that produces (detects) a 225-bp amplified band for detection of New Leaf Y and a Pss primer pair (detects the Patatin gene universally existing in potato) that produces (detects) a 216-bp amplified band for positive control (to detect reference gene). For the confirmation test of New Leaf Y presence, use a confirmation primer pair that produces (detects) a 161-bp amplified band for detection of PVY-cp gene.

New Leaf Y detection primer pair
F-primer (p-FMV05-5') : 5'-AAA AGA GCT GTC CTG ACA GC-3'
R-primer (PVY02-3') : 5'-TCC TCC TGC ATC AAT TGT GT-3'

Pss primer pair (for detection of the patatin gene)
F-primer (Pss01n-5'): 5'-TGA CCT GGA CAC CAC AGT TAT-3'
R-primer (Pss01n-3'): 5'-GTG GAT TTC AGG AGT TCT TCG A-3'

PVY-cp gene detection primer pair
F-primer (PVY01-5') : 5'-GAA TCA AGG CTA TCA CGT CC-3'
R-primer (PVY01-3') : 5'-CAT CCG CAC TGC CTC ATA CC-3'

2.2 Extraction and purification of DNA

Unless otherwise specified, the water used in the extraction and purification of DNA is RO water purified using a reverse osmotic membrane, or ultra-purified water produced by deionizing distilled water to 17 MΩ /cm using a Milli-Q and autoclaving at 121°C for 20 minutes or more.

2.2.1 Extraction and purification of DNA from corn kernels and soybeans

The CTAB method, which uses a mixture of the surfactant cetyl trimethyl ammonium bromide (CTAB) and phenol/chloroform in extraction and purification, is applicable to various areas, leaves virtually no PCR inhibitors, and provides high-purity DNA. However, it has the disadvantages of using harmful reagents such as phenol and chloroform and requiring complicated purification procedures. Currently marketed DNA extraction kits eliminate such disadvantages. They include a silica-gel membrane type, a silica-base resin type, an ion-exchange resin type and a magnet-adsorptive bead type. All of these allow the extraction and purification of DNA usable in PCR from corn kernels and soybeans. This section describes purification using the CTAB method, a silica-gel membrane-type kit (QIAGEN DNeasy Planet Mini) or a silica-base resin-type kit (Promega Wizard DNA Clean-Up System).

2.2.1.1 CTAB method

Place 2 g of uniformly ground sample into a polypropylene centrifuge tube (50 mL), add 15 mL of CTAB buffer*[1], and homogenize the mixture. Add 30 mL of CTAB buffer while washing the edge of the tube and the tip of the homogenizer, mix them by overturning the tube, and leave the mixture at 55°C for 30 minutes. Stir the mixture, place 600 µL of the homogeneous suspension in a micro centrifuge tube (1.5 mL), add 500 µL of phenol/chloroform mixtures*[2], mix them by overturning, suspend the mixture mildly using a mixer, centrifuge it at 7,500 x g for 15 minutes, and transfer the water layer (upper) to another micro centrifuge tube. Here, be careful not to disturb the medium layer. Add 500 µL of a chloroform/isoamyl alcohol mixtures*[3], mix by overturning, suspend the mixture mildly using a mixer, centrifuge it at 7,500 x g and room temperature for 15 minutes, and transfer the water layer (upper) to another micro centrifuge tube. Add the same volume of isopropyl alcohol (room temperature), mix by overturning, perform centrifugation at 7,500 x g and room temperature for 10 minutes, and

throw away the supernatant by decantation. Gently add 500 µL of 70% ethanol along the wall of the tube, perform centrifugation at 7,500 x g and room temperature for 1 minute, and suck out as much ethanol as possible without touching the precipitate. Then, vacuum-dry the precipitate for 2-3 minutes, being careful not to dry it completely. Add 50 µL of TE buffer*4, mix fully, and leave the mixture at room temperature for 15 minutes. During this process, overturn the tube occasionally to dissolve the precipitate completely. Add 5 µL of RNase A and leave the mixture at 37°C for 30 minutes. After adding 200 µL of CTAB buffer, add 250 µL of chloroform/isoamyl alcohol mixture, mix them by overturning, suspend the mixture mildly using a mixer, centrifuge it at 7,500 x g and room temperature for 15 minutes, and transfer the water layer (upper) to another micro centrifuge tube without disturbing the medium layer. Add 200 µL of isopropyl alcohol, mix by overturning, centrifuge the mixture at 7,500 x g and room temperature for 10 minutes, and throw away the supernatant by decantation. Next, gently add 200 µL of 70% ethanol along the wall, perform centrifugation at 7,500 x g and room temperature for 1 minute, and suck out as much ethanol as possible, without touching the precipitate. Vacuum-dry the precipitate for 2-3 minutes, being careful not to dry it completely. Add 50 µL of water to the precipitate, mix, and leave the mixture at room temperature for 15 minutes. During this process, overturn the tube for mixing occasionally to dissolve the precipitate completely. Use this solution as the DNA sample stock solution.

*1 CTAB buffer
Place 8 mL of 0.5 mol/L EDTA (pH 8.0), 20 mL of 1 mol/L Tris/HCl (pH 8.0), and 56 mL of 5 mol/L salt solution in a beaker, add enough water to bring the volume to approximately 150 mL, add 4 g of cetyl trimethyl ammonium bromide (CTAB) while stirring, and dissolve it completely. Add enough water to bring the total volume to 200 mL, and autoclave the solution. Use the solution as the CTAB buffer.

*2 Phenol/chloroform mixtures
Mix 1 mol/L Tris/HCl (pH 8.0) saturated phenol with chloroform/isoamyl alcohol at 1:1 (v/v), and use this as the phenol/chloroform mixture.

*3 Chloroform/isoamyl alcohol mixtures
Mix chloroform and isoamyl alcohol at 24:1 (v/v), and use this as the chloroform/isoamyl alcohol mixture.

*4 TE buffer
Prepare a solution having final concentrations of 10 mmol/L Tris/HCl (pH 8.0) and 1 mmol/L EDTA (pH 8.0) with water, and use this solution as the TE buffer.

2.2.1.2 Silica-gel membrane-type kit method

Place 2 g of uniformly ground sample in a polypropylene centrifuge tube (50 mL), add 10 mL of AP1 buffer previously warmed at 65°C and 20 µL of RNase A, mix vigorously with a vortex mixer to pulverize lumps of the sample, and leave the mixture at 65°C for 15 minutes. During this process, shake the sample by overturning the centrifugal tube 2-3 times. Add 3250 µL of AP2 buffer, leave the mixture on ice for 5 minutes, and centrifuge it at 3,000 x g or more for 5 minutes. Then, apply 500 µL of the supernatant to a QI Ashredder spin column, and perform centrifugation at 10,000 x g or more for 4 minutes and place the eluate in a micro centrifuge tube (15 mL). After repeating this procedure, add 1.5 times the eluate volume of AP3 buffer/ethanol mixture*1. Apply 500 µL of this solution to a Mini spin column, and perform centrifugation at 10,000 x g or more for 1 minute. Apply 500 µL of the remaining solution to the same Mini spin column, perform centrifugation under the same conditions, and dispose of the eluate. Repeat the same procedures until all of the solution is used. Apply 500 µL of AW

buffer to the column, perform centrifugation at 10,000 x g or more for 1 minute, and dispose of the eluate. Apply the AW buffer again and repeat the same procedures. After disposing of the eluate, subject the Mini spin column to centrifugation at 10,000 x g or more for 15 minutes to dry it. Transfer the Mini spin column to a centrifuge tube of the kit, add 70 µL of previously warmed water, leave the mixture for 5 minutes, and centrifuge it at 10,000 x g or more for 1 minute to elute DNA. Add water again and repeat the same procedures, put the obtained eluates together, and use this as the DNA sample stock solution.

*1 AP3 buffer/ethanol mixture
Mix AP3 buffer and ethanol (96%-100%) at 1:2 and use this as the AP3 buffer/ethanol mixture.

2.2.1.3. Silica-based resin-type kit method

Place 2 g of uniformly ground sample in a polypropylene centrifuge tube (50 mL), add 17.2 mL of extraction buffer*[1], 2 mL of 5 mol/L guanidine–HCl, and 0.8 mL of 20 mg/mL Proteinase K, mix them vigorously using a vortex mixer, and keep the mixture at 55°C - 60°C for 3 hours while shaking the tube. Cool the mixture to room temperature and centrifuge it at 3,000 x g for 10 minutes. If the supernatant is turbid, transfer a portion of it to a micro centrifuge tube (1.5 mL) and further centrifuge it at 14,000 x g for 10 minutes. Place 500 µL of the obtained clear supernatant and 1 mL of DNA Clean-Up Resin in a micro centrifuge tube (1.5 mL), and mix them by overturning. Connect an injection syringe to the upper end of a Mini column, and attach them to a manifold (aspirator*[2]). After confirming that the cock of the manifold is closed, apply the mixture to the Mini column through the injection syringe. Open the cock, remove the solvent completely by aspiration, and add 2 mL of 80% isopropyl alcohol through the syringe to wash the column. Remove the syringe, attach the Mini column to a micro centrifuge tube (1.5 mL), and centrifuge at 1,000 x g and room temperature for 2 minutes to dry the column. Transfer the Mini column to another micro centrifuge tube (1.5 mL), and drip it with 50 µ of water previously warmed to 65°C - 70°C. After 1 minute, centrifuge it at 1,000 x g or more and room temperature for 1 minute to elute the DNA, and use the obtained eluate as the DNA sample stock solution.

*1 Extraction buffer
10 mmol/L Tris-HCl buffer solution (pH 7.5) containing 150 mM sodium chloride (NaCl); 2 mmol/L EDTA; and 1% SDS.

*2 Aspirator
If an aspirator is not available, centrifugation can be substituted.

2.2.2 Extraction and purification of DNA from papaya

Remove the seeds from the papayas, cut the flesh into 10 mm cubes, and freeze-dry them. Grind the frozen flesh using a mixing mill. Perform extraction and purification of DNA on the sample, in accordance with the CTAB or silica-gel membrane-type kit (QIAGEN DNeasy Plant Mini) methods.

2.2.2.1 CTAB method

Place 20 mg of the ground sample in a micro centrifuge tube (1.5 mL), add 150 µL of CTAB buffer, and homogenize the mixture using a micro mixer. Add 450 µL of CTAB buffer while washing the inside of the tube, mix the solution by overturning, and leave it at 55°C for 30 minutes. Add 500 µL of phenol/chloroform mixture, mix the solution by overturning, suspend the mixture mildly using a mixer, centrifuge it at 7,500 x g and room temperature for 15 minutes, and transfer the water layer (upper) to another micro centrifuge tube without disturbing the medium layer. Add 500 µL of chloroform/isoamyl alcohol mixture, mix by overturning,

suspend the mixture mildly using a mixer, centrifuge it at 7,500 x g and room temperature for 15 minutes, and transfer the water layer (upper) to another micro centrifuge tube. Add the same quantity of isopropyl alcohol (room temperature), mix the solution by overturning, centrifuge it at 7,500 x g and room temperature for 10 minutes, and discard the supernatant by decantation. Gently add 500 µL of 70% ethanol along the wall of the tube, centrifuge the precipitate at 7,500 x g and room temperature for 1 minute, and suck out as much of the ethanol as possible without touching the precipitate. Then, vacuum-dry the precipitate for 2-3 minutes, being careful not to dry the precipitate completely. Add 50 µL of TE buffer, mix the solution well, and leave it at room temperature for 15 minutes. During this process, overturn the tube occasionally to completely dissolve the precipitate. Add 5 µL of RNase A, and leave the mixture at 37°C for 30 minutes. After adding 200 µL of CTAB buffer, add 250 µL of chloroform/isoamyl alcohol mixture, mix the solution by overturning the tube, suspend the mixture mildly using a mixer, centrifuge it at 7,500 x g and room temperature for 15 minutes, and transfer the water layer (upper) to another micro centrifuge tube. During this process, be careful not to disturb the medium layer. Add 200 µL of isopropyl alcohol, mix the solution by overturning the tube, perform centrifugation at 7,500 x g and room temperature for 10 minutes, discard the supernatant by decantation, and remove the isopropyl alcohol using a pipette. Gently add 200 µL of 70% ethanol along the tube wall, perform centrifugation at 7,500 x g and room temperature for 1 minute, and suck out as much ethanol as possible without touching the precipitate. Then, vacuum-dry the precipitate for 2-3 minutes, being careful not to dry the precipitate completely. Add 50 µL of water, mix the solution, and leave it at room temperature for 15 minutes. During this process, overturn the tube occasionally to dissolve the precipitate completely. Use the solution as the DNA sample stock solution.

2.2.2.2 Silica-gel membrane-type kit method

Place 80 mg of ground sample in a micro centrifuge tube (2 mL), and perform extraction and purification on the sample using a silica-gel membrane kit (QIAGEN DNeasy Plant Mini) as directed below. Add 400 µL of AP1 buffer previously warmed to 65°C and 4 µL of RNase A of the kit, use a homogenizer to mix them fully and pulverize lumps of the sample, and leave it at 65°C for 15 minutes. During this process, overturn the tube several times to shake it. Then, add 130 µL of AP2 buffer, leave the mixture on ice for 5 minutes, and perform centrifugation at 10,000 x g and room temperature for 5 minutes. Apply the supernatant to a QIAshredder spin column, perform centrifugation at 10,000 x g and room temperature for 2 minutes, and transfer the eluate to a micro centrifuge tube (2 mL). Add 1.5 times the eluate volume of AP3 buffer/ethanol mixture to the centrifuge tube and stir the mixture for 10 seconds using a vortex mixer. Apply 500 µL of the mixture to a Mini spin column, perform centrifugation at 10,000 x g and room temperature for 5 minutes,* and discard the eluate. Apply 500 µL of the remaining mixture to the same Mini spin column, perform centrifugation under the same conditions, and discard the eluate. Repeat the same procedures until all of the mixture is used up. Add 500 µL of AW buffer to the column, perform centrifugation at 10,000 x g and room temperature for 5 minutes, and discard the eluate. Add the AW buffer again and repeat the same procedures. After throwing away the eluate, perform centrifugation at 10,000 x g or more for 15 minutes to dry the Mini spin column. Transfer the Mini spin column to the kit's centrifuge tube, add 50 µL of previously warmed water, leave the tube for 5 minutes, and perform centrifugation at 10,000 x g for 1 minute to elute DNA. Add water again and repeat the same procedures. Put the obtained eluates together, and use this mixture as the DNA sample stock solution.

* If there are deposits in the solution, the column may become clogged. In such a case, prolong the centrifugation time to 10 minutes for complete elution.

2.2.3 Extraction and purification of DNA from processed food

The extraction and purification of DNA from processed food products prescribed in Attached Table 2, which are provided in Article 8 of Notification No. 517 of the Ministry of Agriculture, Forestry and Fisheries (2000), is performed as directed in the JAS analytical test handbook, "Genetically Modified Food Test and Analysis Manual for Individual Products," issued by IAI Center for Food Quality, Labeling, and Consumer Services. Extraction and purification of DNA from tacos and tortillas (restricted to products processed using heat), potato (including processed food) and canned papaya are performed as specified below.

2.2.3.1 Extraction and purification of DNA from tacos and tortillas (restricted to products processed using heat)

Freeze-dry the sample and grind it using a mixing mill *etc*. Place 1 g of the ground sample in a polypropylene centrifuge tube (50 mL), and perform extraction and purification of DNA using an ion-exchange resin type of DNA extraction and purification kit (QIAGEN Genomic-tip), as specified below. Add 4 mL of G2 buffer*[1] to the sample, mix the solution vigorously using a vortex mixer, add a further 4 mL of G2 buffer, 100 µL of Proteinase K*[2], and 10 µL of RNase A, mix the solution by shaking, and leave it at 50°C for 2 hours. During this process, overturn the centrifuge tube 2-3 times to mix. Then, perform centrifugation at 3,000 x g or more and low temperature (4°C) for 15 minutes, transfer the supernatant to a polypropylene centrifuge tube (15 mL), and centrifuge it mildly again. Then, apply the supernatant by dividing it into 2-mL portions in a QIAGEN Genomic-tip 20/G equilibrated with 1 mL of QBT buffer*[1]. Wash the tip 3 times with 2 mL of QC buffer*[1], transfer the tip to another centrifuge tube, add 1 mL of QF buffer*[1] previously warmed to 50°C two times, and elute the DNA. Transfer the eluate to a centrifuge tube, add 0.7 times the eluate volume of isopropyl alcohol, mix the solution fully, centrifuge the mixture at 10,000 x g or more and low temperature (4°C) for 15 minutes, and discard the supernatant. Add 1 mL of 70% ethanol and perform centrifugation at 10,000 x g or more and low temperature (4°C) for 5 minutes. After throwing away the supernatant, dry the precipitate using an aspirator, add 100 µL of water, leave it at 65°C for 5 minutes, dissolve the DNA by pipetting, and use the solution as the DNA sample stock solution.

*1 G2, QBT, QC, and QF buffers are included in the kit. If they are not enough, they can be prepared as directed in the kit's explanatory leaflet.

*2 Use QIAGEN's protainase or that having an equivalent effect.

2.2.3.2 Extraction and purification of DNA from potato (including processed food)

Grind the sample using a mixing mill *etc*. and place 200mg of the ground sample in a polypropylene centrifuge tube (15 mL). If the water content of the sample is high, perform centrifugation at 8,000 x g for 15 minutes and discard the supernatant. Perform extraction and purification of DNA on the sample using a silica-gel membrane-type kit (QIAGEN DNeasy Plant Mini), as directed below.

Add 1.5 mL of AP1 buffer previously warmed to 65°C and 10 µL of RNaseA to the sample, mix them vigorously using a vortex mixer to pulverize lumps of sample, and leave the mixture at 65°C for 15 minutes. During this process, shake the sample by overturning the tube for several times. Then, add 400 µL of AP2 buffer, leave the tube on ice for 5 minutes, and perform centrifugation at 10,000 x g and room temperature for 5 minutes. Transfer the supernatant to another centrifuge tube, apply 500 µL of the supernatant to a QIAshrendder spin column, perform centrifugation at 10,000 x g and room temperature for 2 minutes, and transfer the eluate to the kit's centrifuge tube. Repeat these procedures until all of the supernatant has been applied. Divide the obtained eluate into 2 equal parts, transfer them to separate centrifuge tubes (2 mL), add 1.5 times the eluate quantity of AP3 buffer/ethanol mixture to each tube, and stir them for 10 seconds using a vortex mixer to obtain a solution. Apply 500 µL of the obtained solution to a

Mini spin column, perform centrifugation at 10,000 x g and room temperature for 1 minute, and discard the eluate. Of the remaining solution, apply a further 500 µL to the same Mini spin column, perform centrifugation under the same conditions, and discard the eluate. Repeat the same procedures until all of the solution is used up. Next, add 500 µL of AW buffer to the column, perform centrifugation at 10,000 x g and room temperature for 1 minute, and discard the eluate. Add the AW buffer again and repeat the same procedures. After discarding the eluate, dry the Mini spin column by centrifugation at 10,000 x g or more for 15 minutes. Transfer the Mini spin column to the kit's centrifuge tube, add 50 µL of previously warmed water, leave the column for 5 minutes, and perform centrifugation at 10,000 x g and room temperature for 1 minute to eluate the DNA. Add water again, perform the same procedures, put the obtained eluates together, and use the eluate as the DNA sample stock solution.

2.2.3.3 Extraction and purification of DNA from canned papaya

Wash the sample fully with water, freeze-dry it, and grind it using a mixing mill. Place 2 g of the ground sample in a polypropylene centrifuge tube (50 mL) and perform extraction and purification of DNA on it using an ion-exchange resin-type DNA extraction and purification kit (QIAGEN Genomic-tip), as directed below.

Add 7.5 mL of G2 buffer to the sample, mix them vigorously using a vortex mixer, add further 7.5 mL of G2 buffer, 200 µL of QIAGEN Proteinase K, and 20 µL of RNase A, mix them by shaking, and leave the mixture at 50°C for 2 hours. During this process, overturn the tube 2-3 times to mix. Perform centrifugation at 3,000 x g or more and low temperature (4°C) for 15 minutes, transfer the supernatant to a polypropylene centrifuge tube (15 mL), and centrifuge it mildly again. Apply the supernatant by dividing it into 2-mL portions in a QIAGEN Genomic-tip 20/G equilibrated with 1 mL of QBT buffer. Wash the tip with 2 mL of QC buffer 3 times, transfer it to another centrifuge tube, and add 1 mL of QF buffer previously warmed to 50°C twice, and eluate the DNA. Transfer the eluate to a centrifuge tube, add a 0.7 times the eluate volume of isopropyl alcohol, mix them fully, perform centrifugation at 10,000 x g or more and low temperature (4°C) for 15 minutes, discard the supernatant, add 2 mL of 70% ethanol, and perform centrifugation again at 10,000 x g or more and low temperature (4°C) for 5 minutes. Discard the supernatant, dry the precipitate with an aspirator, add 100 µL of water, leave the solution at 65°C for 5 minutes, dissolve the DNA by pipetting, and use the solution as the DNA sample stock solution.

2.2.4 Determination of the purity of DNA in DNA sample stock solutions and storage of DNA sample solution

Dilute a proper amount of DNA sample by 10 times with TE buffer, determine the UV absorption spectrum of this dilution at 200-300 nm, and record its absorbencies at 260 nm and 280 nm (O.D. 260 and O.D. 280*). Using the value for O.D. 260 as 50 ng/µL DNA, calculate the concentration of DNA and O.D. 260/O.D. 280. If the ratio is 1.7-2.0, the DNA is fully purified. Based on the determined DNA concentration, dilute the DNA stock solution with water to a concentration required for subsequent PCR reaction, dispense the solution in 20 µL portions into micro tubes, freeze it, and store it at −20°C for use as the DNA sample solution. Use the sample solution immediately after melting. The remaining sample should not be used and must be discarded. Unless the concentration of the DNA sample stock solution reaches that required for PCR, use it as the DNA sample solution without dilution.

* Regard the value for O.D. 260 as the absorbance derived from DNA, and the value for O.D. 280 as the absorbance derived from impurities such as proteins.

3. A Method for the foods produced by recombinant-DNA techniques whose safety assessments were reviewed

3.1. Soybeans

3.1.1 ELISA-Method

The ELISA Method is used for detecting CP4EPSPS protein in a sample. Take 0.1 g of a ground sample sieved through a 100-mesh sieve (the mesh length is 150μm), subject them to the GMO Soya RUR Test Kit manufactured by SDI whose method is explained in the leaflet. The method is described as follows:

Measure 0.1 g of both a sample and a standard sample into centrifuging tubes made of polypropylen (50 mL). Add 20 mL of Soya Extraction buffer and, mix for at least 1 minute with a vortex mixer, and then let the mixture settle for five minutes until a clear supernatant appears. Use the supernatant as a sample solution. As the scope of the standard curve is 0-2.5% for SDI Kit, it is necessary to prepare also 1/10 dilution (diluted with Soya Extraction Buffer) in order to interpolate the quantitative value within the scope for a sample solution. Put 100 μL of each sample solution into Wells and incubate them at room temperature for one hour. Then wash the Wells four times with Wash buffer, add 100 μL of the RUR Conjugate 1 and incubate them at room temperature for 30 minutes. Again wash the Wells four times with Wash buffer. Then add 100 μL of the RUR Conjugate 2 , and incubate it at room temperature for 30 minutes, then wash the Wells four times. Add 100 μL of Color Reagent and incubate them at room temperature for 30 minutes. Add 100 μL of the Stop Solution to stop color development. Read the absorbance of the developed color at 450 nm using a microtiter plate reader, and determine the GMO content by using the standard curve of the reference standard sample*. Duplicate the test and average the two data.

*extra-cost options

3.1.2. Quantitative PCR Method

Here the quantitative PCR method is used with Taqman Chemistry. In this method a luminescent origo-nucleotide probe is used. In this method, a luminescent origo-nucleotide probe, which anneals in the DNA sequence between the both primer pairs and has two dyes (reporter and quencher) is used. When this probe is hydrolysed by the 5'-nuclease activity of the DNA polymerase, the reporter dye is separated from the quencher dye and emits luminescence. The strength of luminescence shows exponential increase for the PCR cycle. Therefore, by comparing the PCR cycle number which has reached a certain luminescence, the amount of original DNA can be obtained. The quantitative analysis of GM products is to determine the amount of recombinant gene compared to the internal standard which is an universally present gene in the non-GMOs. In the case of soybeans, use the lectin-gene that is ubiquitous in soybeans as the internal standard. The number of copies of the lectin-gene in the DNA-sample is obtained by quantitative PCR which uses a primer pair (Le1-n02) detecting the lectin-gene and a probe (Le1-Taq). At the same time, the number of copies of the GM DNA recombinant gene in a sample is obtained by another quantitative PCR using a primer pair (RRS-01) and probe (RRS-Taq).

The percentage of GMO in a sample is obtained from the following way.

- Divide the number of recombinant gene copies by the number of the lectin-gene copies.
- Divide this value again by the Internal standard ratio*(which is determined previously), and multiply the result by 100.

In the following paragraphs we will describe the quantitative PCR. As for the water used in Quantitative PCR for 5.1.2., 5.2.2., unless otherwise specified, use water, which is purified RO water purified using a reverse osmotic membrane or deionizing distilled water to 17 MΩ/cm by Milli-Q and is autoclaved at 121°C for 20 minutes or more.

*Internal standard ratio: the ratio of the number of target recombinant gene to the number of internal standard gene (*e.g.* in the case of Soybeans, lectin gene) in seeds detected by a certain quantitative PCR. The internal standard ratio is provided separately for each primer pair and probe.

3.1.2.1 Making the standard solution for the standard curve

Prepare a GM plasmid DNA solution of which the number of copies has already been determined, using salmon sperm DNA solution (5ng/ L), and dilute GM plasmid DNA solution so that plasmid DNA in 2.5 µL makes standard curve (e.g. 10, 125, 1,500, 20,000, 250,000 copies)*. Use the dilution as the standard solution for the standard curve.

* Dilution of the DNA solution

In order to avoid influence of trace DNA absorption to the wall of the centrifuge-tube non-specifically, salmon sperm DNA liquid is used for dilution.

3.1.2.2 Preparation of the solution for the PCR reaction

The composition of 25 µL solution for the PCR reaction is the following:

- 12.5 µL of Universal PCR Master Mix *[1]
- 0.5 mol/L (12.5 pmol) of Primer
- 0.2 mol/L (5pmol) of Probe
- 2.5 µL (50ng) of 20ng/µL DNA sample solution or 2.5 µL of the standard solution for the standard curve, or 2.5 µL of 5ng/µL the salmon sperm DNA solution (blank experimental solution)

In order to minimize the differences between the PCR reactions, prepare PCR reaction solution for 3wells per each DNA sample solution*[2]. Firstly prepare the mixture (Master Mix) of the Universal PCR Master Mix with the primer pair and the probe, which are a little more than quantity required, then mix up Master Mix with the each DNA sample and/or the blank sample in different centrifuge tubes. Divide this mix solution of 25 µL into each well on a plate and put the lid of the plate on. At this time, to be impartial, close alternately from both sides. Then, using a special roller, seal up the wells. Lastly observe the bottom of the wells, and if there are bubbles, knock lightly a rim of the plate to dissolve the bubbles.

*1 Universal Master Mix

This solution has high viscosity. Therefore, when adding to and mixing with other solutions, it is necessary to make sure that they are thoroughly mixed. If not, the PCR reaction possibly might not work well. Therefore after mixing it with a vortex for about 3 seconds just before usage, centrifuge lightly, and use after the solution has amassed at the bottom of the tube. When dividing the mixture into the wells, remember stirring and centrifuging will be difficult after this, put it into the bottom of the well accurately.

*2 Preparation of the solution for the quantitative PCR reaction

Take the reagents from the refrigerator, and if necessary, melt them at room temperature, then keep them on ice. If use the same tip successively for dividing, the usual handling will not work from the second time onward because the air is cooled in the pipette. It is necessary to read up on how to use the pipette at low temperatures in the manual of the pipette before starting the procedure.

3.1.2.3 Quantitative PCR

ABI PRISM7700 or 5700 is used for the quantitative PCR. Set the plate onto the device.

Check that the Cover temperature is around 105°C, begin the reaction and the reading of the data. The conditions for the reaction are as follows: After maintaining it at 50° C for 2 minutes, keep it at 95° C for 10 minutes. After starting the reaction with a hot start method, conduct 40 cycles of amplification reaction (30 seconds at 95° C and 1 minute at 59° C for 1 cycle). After the end of the reaction, analyse the results.

3.1.2.4. Drawing the standard curve

The standard curve for the internal standard gene and the recombinant gene can be obtained from the following way:

On the amplification curve plotting the increasing amount of luminescent signals (ΔRn), select the ΔRn part where luminescent signals from both of the standard solution for the standard curve and DNA sample solution amplify exponentially, and draw a threshold line (Th). At this time, a threshold line (Th) must not cross the non-specific amplified curve from the blank sample solution. The exact drawing of a threshold line (Th) is performed as directed in the JAS analytical test handbook, "Genetically Modified Food Test and Analysis Manual for Individual Products," issued by IAI Center for Food Quality, Labeling, and Consumer Services of MAFF (the Ministry of Agriculture, Forestry and Fisheries). Use the point, where the Th and the luminescent signal of the standard solution for the standard curve cross, as the "threshold cycle" (Ct). Next, plot the Ct value (y-axis) for the logarithm of copies of the (each) standard solutions for standard curve (x-axis). Use the approximate straight line obtained from each Ct as the standard curve.

3.1.2.5. Calculation of the content of the foods produced by recombinant-DNA techniques in samples

Obtain the Ct for unknown DNA sample, by using the Th of the standard curve. For each of the internal standard gene and recombinant gene, calculate the numbers of original genes' copies for all 3 Wells from the standard curve. Use the average of these values is to be the number of copies of the original internal standard gene and original recombinant gene. Calculate the amount of GMO contained in the products from following formula:

The GMO content in the products (%) = [(number of copies of the original recombinant gene) / (number of copies of the original internal standard genes × internal standard ratio)] × 100

In the case of soybeans, relevant GM variety is only "Round Up Ready Soybean", the value calculated from the number of copies of Le1 and RRS genes shows the percentage GMO in the product.

3.1.3 Judgement of result

Extract 3 times for each IP handling samples. If the average of the amount of GMO by ELISA or Quantitative PCR is more than 5%, the IP handling has possibly failed.

3.2 Corn

In case of corn, use only the quantitative PCR, not ELISA to quantify. Because not only there are various GM varieties which have different objective proteins, but also the expression amounts of the proteins in each GM variety are different even if a single kind of protein manifests for each GM variety.

3.2.1 Quantitative PCR method

As there are some GM varieties for corn, firstly subject to the screening, and quantify each GM variety separately based on the screening results, and judge from the result of total of the quantitative value for each GM variety. In the case of corn, use the starch synthase IIb (SSIIb)

gene as the universally present 'internal standard' gene. Calculate the amount of GMO content using following numbers formula as shown in 3.1.2.5.

• The number of copies of starch synthase IIb (SSIIb) gene (obtained from the primer pair SSIIb and probe SSIIb-Taq, which detects starch synthase IIb (SSIIb) gene).

• The number of copies of target recombinant gene (obtained from the primer pair and probe, which detects target recombinant gene,)

3.2.1.1 Screening

3.2.1.1.1. The Quantification of GM varieties inserted 35S promoter derived from the Cauliflower mosaic virus

As the GM varieties, Event 176, Bt11, T25 and Mon810, are inserted the 35S promoter (CaM) derived from the Cauliflower mosaic virus, concerning the mixtures in these varieties, it is possible to infer the approximate amount of GM contained from an index of these DNA contents. The method for the quantification is the same method as the quantitative PCR method described for the soybeans apart from the primer and the probe. Use starch synthase llb (SSllb) gene as internal standard gene, and the primer pair SSllb and the probe SSllb-Taq, which detect starch synthase llb (SSllb) gene. The primer pair and the probe for target gene is P35S-1 and P35S-Taq*, respectively. The amount of GM inserted the 35S promoter (CaM) is calculated using a internal standard ratio that has been defined in the Annex.

*The internal standard ratio in case of using P35S-1 and P35S-Taq, refer to calculated value for Mon810. Mon810 is the GM variety which is produced widest in the United States, there is only one copy of the 35S promoter in a recombinant DNA of Mon810, then there is little possibility in underestimating the amount of GMO in a sample. The concentration of P35S-Taq is 0.1 µmol/L (2.5 pmol).

3.2.1.1.2 Determination of GA21

GA21 GM variety is not inserted CaM gene. Therefore, it is necessary to quantify the content of this variety using the same DNA sample, which is used for the determination of CaM gene. The amount of GA21 is obtained from calculating the number of copies of GA21 with same method as described in 3.2.1.1.1., using GA21-3 and GA21-Taq (GA21 specific primer and probe, respectively), for the same sample as analysing CaM.

3.2.1.1.3 Judgement of the Results

Each sample is extracted once and subject to quantitative PCR. If the sums of the amount of GMO inserted CaM gene sequence and the amount of GA21 is higher than 4.5%, extract more twice. In this case, perform Specific determination of GM corn varieties for each DNA sample solution (from 3 times extraction).

3.2.1.2. Specific determination of GM corn varieties

3.2.1.2.1. Determination of Event 176, Bt 11, T25 and Mon 810

As quantitative primer and probe, use E-176-2 and E 176-Taq, Bt 11-3 and Bt 11-Taq, and T25-1 and T25-Taq, and M810-2 and M810-Taq, for GM varieties of Event 176, Bt 11, T25 and Mon 810, respectively. The number of copies of the varieties Event 176, Bt 11, T25 and Mon 810 are obtained by the same method as described in 3.2.1.1. Then determine the content of Event 176, Bt 11, T25 and Mon 810.

3.2.2 Judgement of the Results

Calculate the total amount for each DNA sample solution of GA21, Event 176, Bt 11, T25 and Mon 810 obtained under 3.2.1.1.2. If the average of each total for a sample is more than 5%, the IP handling of the sample has possibly failed.

(Annex) Internal Ratio

Food	Varieties	Internal Standard Ratio	Remarks
Soybeans	Roundup Ready Soybean	0.95	Le1-n02 and Le1-Taq, RRS-01and RRS-Taq
Corn	Not specified (Screening)	0.39	SSIIb and SSIIb-Taq, P35S-1 and P35S-Taq
Corn	GA21	1.40	SSIIb and SSIIb-Taq, GA21-3 and GA21-Taq
Corn	Event176	2.05	SSIIb and SSIIb-Taq, E176-2 and E176-Taq
Corn	Bt11	0.50	SSIIb and SSIIb-Taq, Bt11-3 and Bt11-Taq
Corn	T25	0.34	SSIIb and SSIIb-Taq, T25-1 and T25-Taq
Corn	Mon810	0.38	SSIIb and SSIIb-Taq, Mon810-2 and Mon810-Taq

(For your information)

1 You can purchase AP1/AP2 buffer and RNase A sepaately used in "2.2.1.2 Silica-gel membrane-type kit method" from QIAGEN (Forefront Towere II. 3-13-1 Kachidoki Chuo-Ward Tokyo 104-0054 Japan, Tel: +81-3-5547-0811, Fax: +81-3-5547-0818).

2 You can purchase GM standard plasmid DNA used in "3.1.2.1 Making the standard solution for the standard curve" from NIPPON Gene (1-29 Tonya-cho Toyama-City Toyama 930-0982 Japan, Tel: +81-76-451-6548, Fax: +81-76-451-6547).

3 You can purchase the primer pairs and the probes used in "3.1.2.2 Preparation of the solution for the PCR reaction" from NIPPON GENE and FASMAC (5-1-3 Midorigaoka, Atsugi-City, Kanagawa 243-0041 Japan, Tel: +81- 46-295-8787, Fax: +81- 46-294-3738).