GM quicker

- GMO DNA Extraction Kit for Grain -

Manual

Ver. 4.0

Code No. 317-06361

NIPPON GENE CO., LTD.

Contents

Ⅰ Description

GM quicker is a kit to extract DNA from grains, such as maize and soybean. This kit uses the principle called Boom Technology that DNA is adsorbed by silica under the Chaotropicion existence. Neither phenol nor chloroform is used in the extract operation.

The method of using DNA becomes widespread in the inspection of Genetically Modified Organisms (GMO). Current DNA extraction kits were not necessarily efficient in the extraction from grains, such as maize and soybean because their extractions object had been leaf.

In this kit, the object is focused on the grains so that high refinement level DNA can be extracted in only about 35 minutes. This kit is designed considering operation problems such as the crossing contaminations of the samples for the inspection. The silicagel membrane included in the spin column to be used with this kit has enough capacity to adsorb sufficient DNA and enables high elution efficiency.

DNA obtained with GM quicker can be used for PCR and the restriction enzyme reaction.

Ⅱ Product Components

GE1 Buffer	100 ml	× 3
GE2 Buffer	37.5 ml	× 1
GB3 Buffer	12.5 ml	× 1
GW Buffer	40 ml	× 1
TE（pH8.0）	10 ml	× 1
RNase A(100 mg/ml)	0.5 ml	× 2
Spin Column	50 sets	
Manual	1	

＊ DNA extraction can be done 50 times for the sample 1g of the maize powder.
If the extraction should be done 50 times for the sample 1g of the soybean powder, GE1 Buffer, GE2 Buffer, and RNase A would be insufficient. Please purchase them separately.

Related Products

314-06371	GE1 Buffer	500 ml
311-06381	GE2 Buffer	200 ml
318-06391	RNase A （100 mg/ml）	0.5 ml×5

Ⅲ Storage Conditions

All the kit components can be stored at room temperature (15-25°C). RNase A is stable for longer periods when stored at 4°C or -20°C. GW Buffer includes ethanol, keep the buffer bottle tightly closed after use.

Ⅳ Directions

- This kit should be used for research only and not used for medicine or any other purpose.
- Please do not handle this kit without basic knowledge of the reagent.
- Please handle this kit according to the manual.
- NIPPON GENE is not responsible for any troubles caused by handling different from the manual.

Ⅴ Protocol

＜Reagents and machine necessary beside this kit＞

- Ethanol
- Isopropanol
- Ice
- Pipette
- Pipette-tip
- 50 ml tube
- 2 ml tube
- 1.5 ml tube
- Food mill
- Centrifuge
- Vortex mixer

＜Maize DNA extraction Protocol＞

① Crush the maize seeds by the food mill, etc. to prepare the sample of maize powder

② Weigh out 1.0 g of the maize powder, and transfer to a 50 ml tube. Add 6 ml of GE1 Buffer and 20 µl of RNase A. Mix well by vortexing for 30 seconds(※1)

③ Incubate at room temperature for 10 minutes.

④ Add 750 µl of GE2 Buffer,(※2) and mix well by inverting 10-12 times.

⑤ Chill on ice for 10 minutes.

⑥ Centrifuge at ≧5,000 x g for 10 minutes at 4℃.(※3)

⑦ Transfer 4 ml of supernatant to a fresh 50 ml tube.(※4)

⑧ Transfer 400 µl of supernatant from the 50 ml tube to a fresh 1.5 ml tube. Store the rest of supernatant at 4℃.(※5)

⑨ Add 50µl of GB3 Buffer.

⑩ Add 200 µl of ethanol, and mix well by inverting 10-12 times.(※6)

⑪ Move all the mixture made in ⑩ to Spin Column, and centrifuge at ≧13,000 x g, for 30 seconds at 4℃. Discard the liquid in the collection tube.

⑫ Add 600 µl of GW Buffer to Spin Column, and centrifuge at ≧13,000 x g for 60 seconds at 4℃. Discard the liquid in the collection tube.

⑬ Remove the Spin Column to a fresh 1.5 ml tube.

⑭ Add 50 µl of TE (pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑮ Centrifuge at ≧13,000 x g for 60 seconds at 4℃, and collect the DNA solution.

＜100 mg of Maize DNA extraction protocol＞

① Crush the maize seeds by the food mill, etc. to prepare the sample of maize powder

② Weigh out 100 mg of the maize powder, and transfer to a 2 ml tube. Add 600µl of GE1 Buffer and 4 µl of RNase A. Mix well by vortexing for 30 seconds(※1)

③ Incubate at room temperature for 5 minutes.

④ Add 75 µl of GE2 Buffer,(※2) and mix well by inverting 10-12 times.

⑤ Chill on ice for 5 minutes.

⑥ Centrifuge at ≧13,000 x g for 5 minutes at 4℃.(※3)

⑦ Transfer 400 µl of supernatant from the 50 ml tube to a fresh 1.5 ml tube.(※4)

⑧ Add 50 µl of GB3 Buffer.

⑨ Add 200 µl of ethanol, and mix well by inverting 10-12 times.(※6)

⑩ Move all the mixture made in ⑨ to Spin Column, and centrifuge at ≧13,000 x g, for 30 seconds at 4℃. Discard the liquid in the collection tube.

⑪ Add 600 µl of GW Buffer to Spin Column, and centrifuge at ≧13,000 x g for 60 seconds at 4℃. Discard the liquid in the collection tube.

⑫ Remove the Spin Column to a fresh 1.5 ml tube.

⑬ Add 50 µl of TE(pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑭ Centrifuge at ≧13,000 x g for 60 seconds at 4℃, and collect the DNA solution.

＜Soybean DNA extraction protocol＞

① Crush the soybeans by the food mill, etc. to prepare the sample of soybean powder

② Weigh out 1.0 g of the soybean powder, and transfer to a 50 ml tube. Add 12 ml of GE1 Buffer and 40 µl of RNase A. Mix well by vortexing for 30 seconds.(※1)

③ Incubate at room temperature for 10 minutes.

④ Add 1.5 ml of GE2 Buffer,(※2) and mix well by inverting 10-12 times.

⑤ Chill on ice for 10 minutes.

⑥ Centrifuge at ≧5,000 x g for 10 minutes at 4℃.(※3)

⑦ Transfer 8 ml of supernatant to a fresh 50 ml tube.(※4)

⑧ Transfer 700 µl of supernatant from the 50 ml tube to a fresh 2 ml tube. Store the rest of supernatant at 4℃.(※5)

⑨ Add 250 µl of GB3 Buffer.

⑩ Add 250 µl of isopropanol, and mix well by inverting 10-12 times.(※6)

⑪ Move 600 µl of the mixture made in ⑩ to Spin Column, and centrifuge at ≧13,000 x g, for 30 seconds at 4℃. Discard the liquid in the collection tube.

⑫ Add the remaining mixture made in ⑩ to the same Spin Column, and centrifuge at ≧13,000 x g, for 30 seconds at 4℃. Discard the liquid in the collection tube.

⑬ Add 600 µl of GW Buffer to the Spin Column, and centrifuge at ≧13,000 x g for 60 seconds at 4℃. Discard the liquid in the collection tube.

⑭ Remove the Spin Column to a fresh 1.5 ml tube.

⑮ Add 50 µl of TE(pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑯ Centrifuge at ≧13,000 x g for 60 seconds at 4℃, and collect the DNA solution.

＜100 mg of Soybean DNA extraction protocol＞

① Crush the soybeans by the food mill, etc. to prepare the sample of soybean powder

② Weigh out 100 mg of the soybean powder, and transfer to a 2 ml tube. Add 600 µl of GE1 Buffer and 4 µl of RNase A. Mix well by vortexing for 30 seconds(※1)

③ Incubate at room temperature for 5 minutes.

④ Add 75 µl of GE2 Buffer,(※2) and mix well by inverting 10-12 times.

⑤ Chill on ice for 5 minutes.

⑥ Centrifuge at ≧13,000 x g for 5 minutes at 4℃.(※3)

⑦ Transfer 400 µl of supernatant from the 50 ml tube to a fresh 1.5 ml tube.(※4)

⑧ Add 150 µl of GB3 Buffer.

⑨ Add 150 µl of isopropanol, and mix well by inverting 10-12 times.(※6)

⑩ Move all the mixture made in ⑨ to Spin Column, and centrifuge at ≧13,000 x g, for 30 seconds at 4℃. Discard the liquid in the collection tube.

⑪ Add 600 µl of GW Buffer to Spin Column, and centrifuge at ≧13,000 x g for 60 seconds at 4℃. Discard the liquid in the collection tube.

⑫ Remove the Spin Column to a fresh 1.5 ml tube.

⑬ Add 50 µl of TE(pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑭ Centrifuge at ≧13,000 x g for 60 seconds at 4℃, and collect the DNA solution.

※1 If mixing is insufficient, DNA yield is decreased remarkably. If the 50 ml tube is not vertical, the mixture becomes foamy, which decreases mixing efficiency. The 50 ml tube should be vertical to the vortex mixer and mix well for 30 seconds. If mixing is insufficient, mix for 30-60 another seconds.

※2 Please add GE2 Buffer even though the foam generated by ② remains. Because the viscosity of the mixture has risen, please invert carefully until GE2 Buffer is mixed well.

※3 Please confirm the maximum speed of centrifuge rotor and the maximum centrifugation stability (x g) of 50 ml tube. If the 50 ml tube is centrifuged at its maximum g, an adapter should be used and please confirm that the tube is not damaged by preliminary examination.

※4 Please avoid taking neither precipitate nor float.

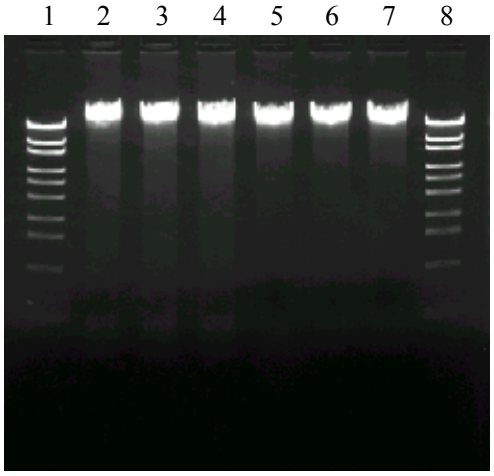
※5 The rest of the supernatant can be used within 24 hours. However, white precipitation would appear in the supernatant stored at 4℃. In this case, please mix by vortexing at room temperature to dissolve before the operation is started.

※6 Add GB3 Buffer before isopropanol, and then mix. If the precipitation appears, mix well by inverting until the liquid becomes clear.

Ⅵ Data Collection

1. DNA extraction from Maize and Soybean

DNA could be extracted from both of the seeds of Maize and Soybean with this kit.



Lane 1, 8 : OneSTEP Marker 6(λ / StyI digest)
Lane 2～4 : Maize genomic DNA
Lane 5～7 : Soybean genomic DNA

＊ 1/25 of the extracted DNA was run on a 1% Agarose S gel.

2. Absorption spectrum of Maize and Soybean DNA

The absorption peak in the vicinity of A_{260} shows that DNA extracted with this kit is high purity.

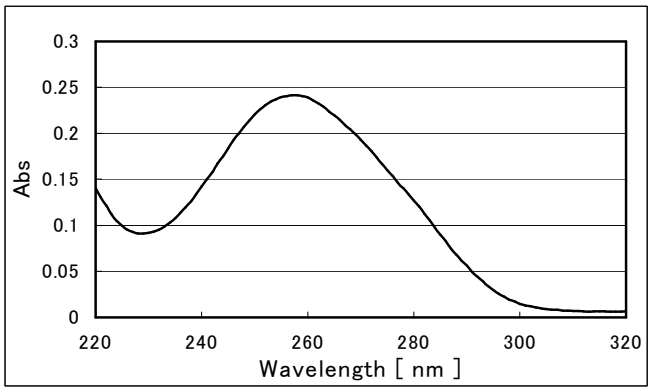


Fig.1 UV scan of Maize DNA extracted with this kit.

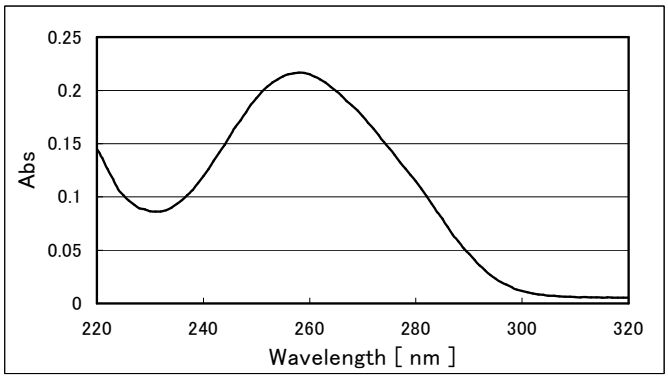
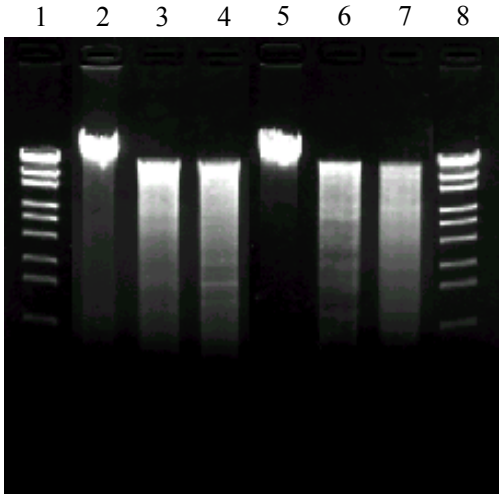


Fig.2 UV scan of Soybean DNA extracted with this kit.

3. Restriction enzyme digestion of Maize and Soybean DNA

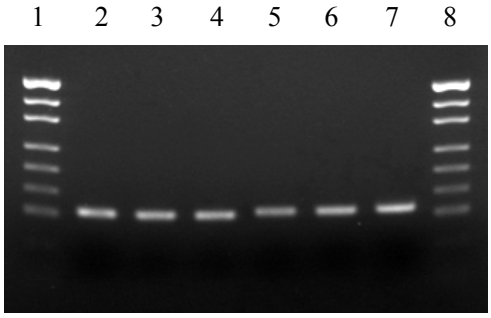
DNA extracted with this kit was digested by EcoRI and HindIII.



Lane 1. 8：OneSTEP Marker6 (λ / StyI digest)
Lane 2　：Maize genomic DNA intact
Lane 3　：Maize genomic DNA digest / EcoRI
Lane 4　：Maize genomic DNA digest / HindIII
Lane 5　：Soybean genomic DNA intact
Lane 6　：Soybean genomic DNA / EcoRI
Lane 7　：Soybean genomic DNA / HindIII

4. Detection of Maize Starch Synthase Ⅱ b (*SSIIb*) and Soybean Lectin (*LeI*) genes by PCR

PCR was performed using primers for detection of *SSIIb* and *LeI*.The PCR condition followed the Japanese Ministry of Agriculture, Forestry and Fisheries guideline, “Japanese Agricultural Standard (JAS) analysis manuals”.



Lane 1, 8　：OneSTEP Marker 11(pUC19/MspI digest)
Lane 2～4：Maize *SSIIb* (114 bp)
Lane 5～7：Soybean *Le1* (118 bp)

＊5μl of PCR products were run on a 3% Agarose 21 gel.

Ⅶ Troubleshooting

Trouble	Cause	Suggestion
Low DNA yield	Insufficient crush	Please crush the samples as in pieces as possible. It is desirable that the sizes of the samples are well matched, so please pass the samples through a sieve if necessary.
	Extraction efficiency is low.	Please mix well by vortexing after adding GE1 Buffer and RNase A. Especially, in the case for soybean, it is easy to become lumpy, and the surface of the powder does not come in contact with extraction buffer.
		If the 50 ml tube is not vertical, the mixture becomes foamy, and mixing efficiency would be decreased. The 50ml tube should be vertical to the vortex mixer and mix well.
	Insufficient elution	If the centrifugation for elution were performed immediately after adding TE to the Spin Column, DNA yield would be low. Please incubate for 3 minutes at room temperature.
RNA in the elute	No activation of RNase	RNase A cannot be stored after it is mixed with GE1 Buffer. Please store them separately.
A large amount of white precipitate that appeared after adding GB3 Buffer remains even if isopropanol or ethanol is added.	Insufficient icing	Please enlarge the contact area of ice and 50 ml tube to raise the cooling efficiency. I the case for soybean, mix well because the mixture becomes viscous after adding GE2 Buffer. (The white precipitate would remain in the extraction from other grains except maize and soybean. In the cases, mix well after adding GE2 Buffer, and then centrifuge the mixture. The supernatant can be transferred to the Spin Column.)
Low $OD_{260/280}$	Taking precipitate or float to the Spin Column.	The oil membrane might appear to the liquid level for maize after centrifugation in 50 ml tube. Inserting 1 ml tip vertically in the liquid level and then lifting it slowly could remove the oil material. Please careful not to touch the precipitant by the tip when the supernatant is collected.

Ⅷ Related Products

Code No.	Product Name	Size
310-06591	*GM quicker 2*	50 reactions
311-07241	*GM quicker 3*	50 reactions
316-07791	*GM quicker 4*	50 reactions
319-07161	*GM quicker 96*	96well-plate×4
317-07341	On-Site Column Set for *GM quicker*	20 reactions
314-06371	GE1 Buffer	500 ml
311-06381	GE2 Buffer	200 ml
318-06391	RNase A（100 mg/ml）	0.5 ml×5
311-06641	GW Buffer	40 ml×2
318-06651	GE2-K Buffer	100 ml
315-06661	GB3 Buffer	12.5 ml×2
312-06671	*GM quicker 2* Enzyme Set (Proteinase K 2 ml, α-Amylase 0.2 ml)	1 Set
316-90025	TE (pH8.0)	500 ml
318-90105	Distilled Water, Deionized, Sterile	500 ml
312-01193	Agarose S	100 g
313-03242	Agarose 21	25 g
312-06512	Agarose XP	25 g
311-02682	Agarose X	25 g
311-05281	OneSTEP Marker 6	1,500 μl
318-05791	OneSTEP Marker 4	375 μl
312-05831	OneSTEP Marker 11	375 μl
319-08141	Collection Tube	50 tubes×2

Ⅸ Simple protocol

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[Maize]

Weigh out 1.0g of powder sample and transfer to 50ml tube

GE1 Buffer 6ml
RNase A (100mg/ml) 20 μl

Vortex mixer (30sec)
* When the tube (50ml) is vertically appropriated to the vortex mixer, mixing it firmly for 30 seconds.
If the mixing is insufficient, you should mix it for another 30-60 seconds. DNA yeild is decreased remarkably if the mixing operation is insufficient.

Incubate at R.T. for 10min

GE2 Buffer 750 μl

Invert the tube
10～12 times to mix well

Chill on ice for 10min

Centrifuge
≧5,000×*g*、10min、4℃

Transfer 4ml of supernatant to a fresh 50ml tube and keep in the refrigerator

* Collect the supernatant. Avoid taking neither precipitation nor the float.

Transfer 400 μl of supernatant to a fresh 1.5ml tube

GB3 Buffer 50 μl
Ethanol 200 μl
* First adding the GB3 Buffer,then the ethanol is added.

Invert the tube
10～12 times to mix
* If the deposit is made and makes the liquid cloudy, mixing it until the liquid becomes clear.

Transfer mixture from the 1.5ml tube to a Spin Column

Centrifuge
≧13,000×*g*、30sec、4℃
* 12000 rpm ; when using the rotor with radius 8.2cm.

Discard the liquid in the collection tube

GW Buffer 、600 μl

Centrifuge
≧13,000×*g*、60sec、4℃

Discard the liquid in the collection tube

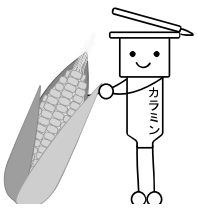
Transfer Spin Column to a fresh 1.5ml tube

TE 50 μl

Incubate at R.T.for 3min

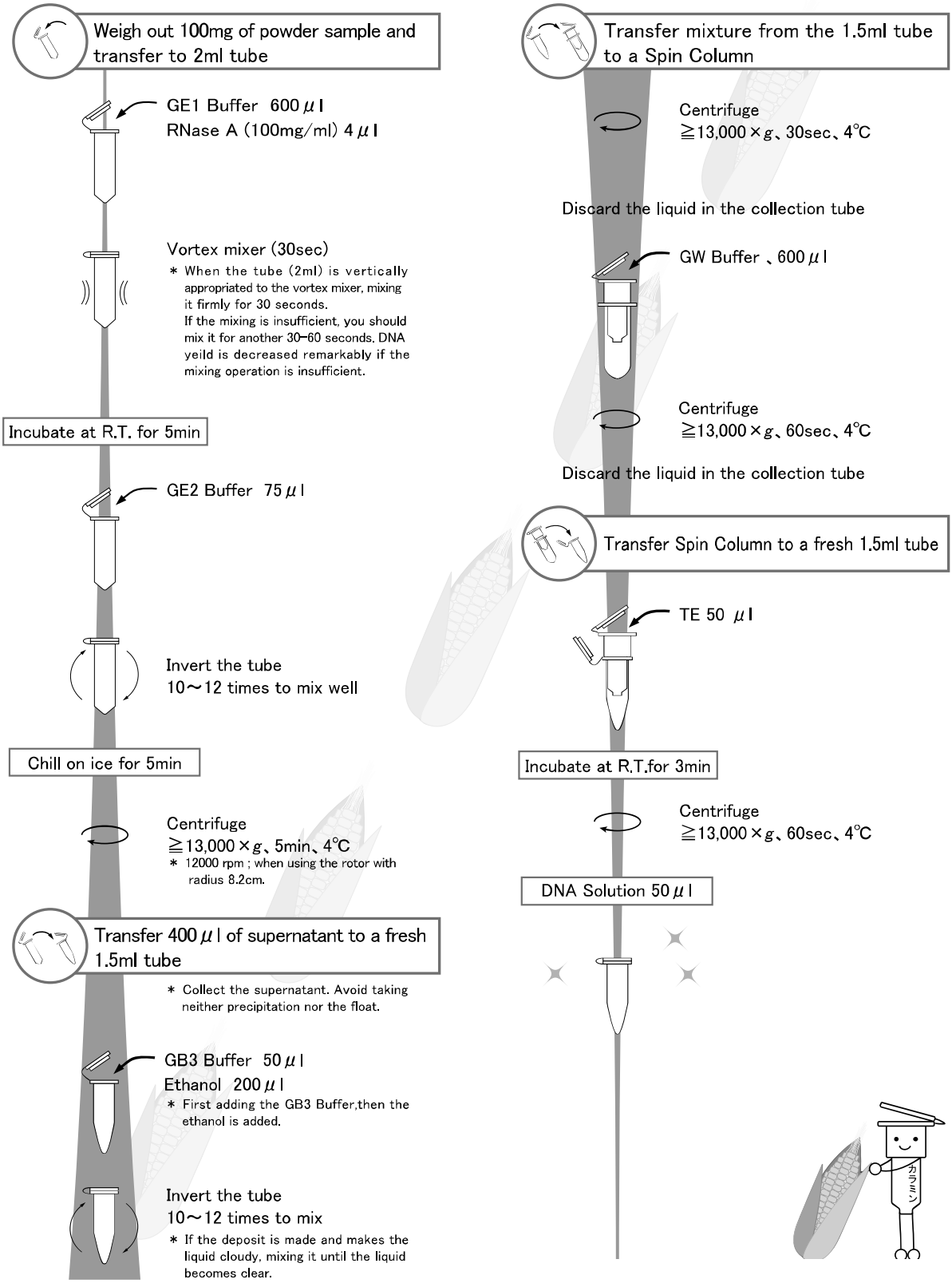
Centrifuge
≧13,000×*g*、60sec、4℃

DNA Solution 50 μl



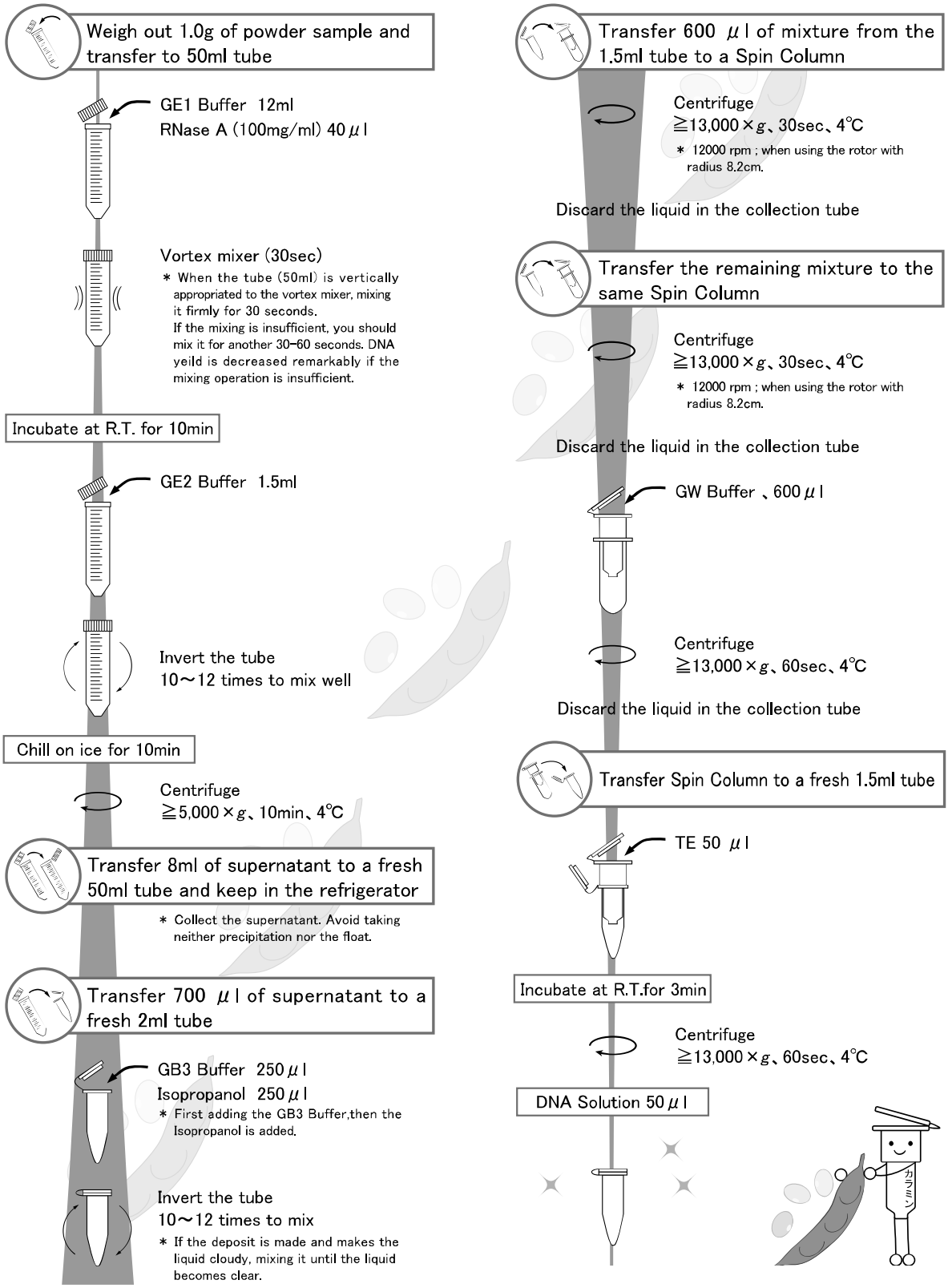
NIPPON GENE [Simple protocol] *GM quicker* - GMO DNA Extraction Kit for Grain -

[Maize] (Small-Scale Extraction)



NIPPON GENE [Simple protocol] *GM quicker* – GMO DNA Extraction Kit for Grain –

[Soybean]



NIPPON GENE [Simple protocol] *GM quicker* - GMO DNA Extraction Kit for Grain -

[Soybean] (Small-Scale Extraction)

Weigh out 100mg of powder sample and transfer to 2ml tube

GE1 Buffer 600 μl
RNase A (100mg/ml) 4 μl

Vortex mixer (30sec)
* When the tube (2ml) is vertically appropriated to the vortex mixer, mixing it firmly for 30 seconds.
If the mixing is insufficient, you should mix it for another 30-60 seconds. DNA yeild is decreased remarkably if the mixing operation is insufficient.

Incubate at R.T. for 5min

GE2 Buffer 75 μl

Invert the tube
10～12 times to mix well

Chill on ice for 5min

Centrifuge
≧13,000×*g*、5min、4℃
* 12000 rpm ; when using the rotor with radius 8.2cm.

Transfer 400 μl of supernatant to a fresh 1.5ml tube
* Collect the supernatant. Avoid taking neither precipitation nor the float.

GB3 Buffer 150 μl
Isopropanol 150 μl
* First adding the GB3 Buffer,then the Isopropanol is added.

Invert the tube
10～12 times to mix
* If the deposit is made and makes the liquid cloudy, mixing it until the liquid becomes clear.

Transfer mixture from the 1.5ml tube to a Spin Column

Centrifuge
≧13,000×*g*、30sec、4℃

Discard the liquid in the collection tube

GW Buffer 、600 μl

Centrifuge
≧13,000×*g*、60sec、4℃

Discard the liquid in the collection tube

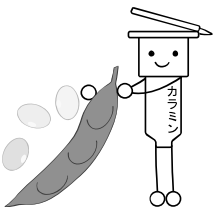
Transfer Spin Column to a fresh 1.5ml tube

TE 50 μl

Incubate at R.T.for 3min

Centrifuge
≧13,000×*g*、60sec、4℃

DNA Solution 50 μl



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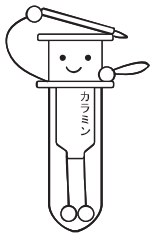

NIPPON GENE CO., LTD.

If you have any questions, please contact us by web form.

http://www.nippongene.com/