GM quicker 2

- GMO DNA Extraction Kit
for Rice, Canola, and Potato -

Manual

Ver. 3.0

Code No. 310-06591

NIPPON GENE CO., LTD.

Contents

Ⅰ Description

GM quicker 2 is a kit to extract DNA from rice and other grains. 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 40 minutes. This kit is designed for not only nonglutinous rice but also glutinous rice by using α-amylase, which enables the inspection of unknown sample of rice. It is also possible to extract DNA for the inspection of a grain of rice or rape seed. The silica gel 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	40 ml	× 1
GE2-K Buffer	5 ml	× 1
GB3 Buffer	12.5 ml	× 1
GW Buffer	40 ml	× 1
TE（pH 8.0）	10 ml	× 1
Proteinase K (20 mg/ml)	1 ml	× 1
α-Amylase	0.1 ml	× 1
RNase A (100 mg/ml)	0.5 ml	× 1
Spin Column	50 sets	
Manual	1	

Related Products: refer to p. 18.

Ⅲ Storage Conditions

Proteinase K and α-Amylase should be stored at -20°C. The remaining 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 besides this kit＞

- Isopropanol
- Pipette
- Pipette-tip
- 2 ml tube
- 1.5 ml tube
- Food mill
- Centrifuge
- Vortex mixer
- Pestle

＜Rice DNA extraction Protocol＞

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

* Another method: Put 20 grains of rice into 2.0 ml tube, and add 700 µl of GE1 Buffer. Incubate at room temperature for 20 minutes. Crush the rice by a pestle. Add 20 µl of Proteinase K, 2 µl of α-Amylase, and 10 µl of RNase A. Mix by vortexing for 30 seconds. Follow the procedure of ③.

② Weigh out 0.5 g of the rice powder, and transfer to a 2.0 ml tube. Add 700 µl of GE1 Buffer, 20 µl of Proteinase K, 2 µl of α-Amylase, and 10 µl of RNase A . Mix well by vortexing for 30 seconds.(*1)

* It is not necessary to add α-Amylase if the rice sample does not include glutinous rice obviously.

③ Incubate at 60℃ for 15 minutes.

* Incubate at 65℃ for 15 minutes if the sample does not include glutinous rice.

④ Add 85 µl of GE2-K Buffer,(*2) and mix well by vortexing.

⑤ Centrifuge at ≧13000 x g for 5 minutes at room temperature.(*3)

⑥ Transfer 400 µl of supernatant to a fresh 1.5 ml tube.(*4)

⑦ Add 150 µl of GB3 Buffer and then add 150 µl of isopropanol. Mix well by inverting 10-12 times.(*5)

⑧ Transfer all the mixture made in ⑦ to Spin Column, and centrifuge at 13000 x g, for 30 seconds at room temperature. Discard the liquid in the collection tube.

⑨ Add 650 µl of GW Buffer to Spin Column, and centrifuge at 13000 x g for 60 seconds at room temperature. Discard the liquid in the collection tube.

⑩ Remove the Spin Column to a fresh 1.5 ml tube.

⑪ Add 50 µl of TE (pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑫ Centrifuge at 13000 x g for 60 seconds at room temperature, and collect the DNA solution.

＜A grain of Rice DNA extraction protocol＞

① Wrap a grain of rice in aluminum foil and crush it by a hammer, etc. to prepare the sample of rice powder.

* Another method: Put a grain of rice into 1.5 ml tube and add 250 µl of GE1 Buffer. Incubate at room temperature for 20 minutes. Crush the rice by a pestle. Add 10 µl of Proteinase K, 2 µl of α-Amylase, and 5 µl of RNase A. Mix by vortexing for 30 seconds. Follow the procedure of ③.

② Transfer the rice powder to a 1.5 ml tube. Add 250 µl of GE1 Buffer, 10 µl of Proteinase K, 2 µl of α-Amylase, and 5 µl of RNase A. Mix well by vortexing for 30 seconds.(*1)

* It is not necessary to add α-Amylase if the rice sample does not include glutinous rice.

③ Incubate at 60℃ for 15 minutes.

* Incubate at 65℃ for 15 minutes if the sample does not include glutinous rice.

④ Add 40 µl of GE2-K Buffer,(*2) and mix well by vortexing.

⑤ Centrifuge at ≧13000 x g for 5 minutes at room temperature.(*3)

⑥ Transfer 200 µl of supernatant to a fresh 1.5 ml tube.(*4)

⑦ Add 75 µl of GB3 Buffer and then add 75 µl of isopropanol. Mix well by inverting 10-12 times.(*5)

⑧ Transfer all the mixture made in ⑦ to Spin Column, and centrifuge at 13000 x g, for 30 seconds at room temperature. Discard the liquid in the collection tube.

⑨ Add 650 µl of GW Buffer to Spin Column, and centrifuge at 13000 x g for 60 seconds at room temperature. Discard the liquid in the collection tube.

⑩ Remove the Spin Column to a fresh 1.5 ml tube.

⑪ Add 50 µl of TE (pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑫ Centrifuge at 13000 x g for 60 seconds at room temperature, and collect the DNA solution.

＜Rape seed DNA extraction Protocol＞

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

② Weigh out 0.2 g of the rape seed powder, and transfer to 2.0 ml tube. Add 800 µl of GE1 Buffer, 20 µl of Proteinase K, and 10 µl of RNase A. Mix well by vortexing for 30 seconds. (*1)

③ Incubate at 65℃ for 15 minutes.

④ Add 100 µl of GE2-K Buffer,(*2) and mix well by vortexing.

⑤ Centrifuge at ≧13000 x g for 5 minutes at room temperature.(*3)

⑥ Transfer 350 µl of supernatant to a fresh 1.5 ml tube.(*4)

⑦ Add 130 µl of GB3 Buffer.

⑧ Add 130 µl of isopropanol and mix well by inverting 10-12 times.(*5)

⑨ Transfer all the mixture made in ⑧ to Spin Column, and centrifuge at 13000 x g, for 30 seconds at room temperature. Discard the liquid in the collection tube.

⑩ Add 650 µl of GW Buffer to Spin Column, and centrifuge at 13000 x g for 60 seconds at room temperature. Discard the liquid in the collection tube.

⑪ Remove the Spin Column to a fresh 1.5 ml tube.

⑫ Add 50 µl of TE (pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑬ Centrifuge at 13000 x g for 60 seconds at room temperature, and collect the DNA solution.

＜A grain of Rape seed DNA extraction Protocol＞

① Put a grain of rape seed into 1.5 ml tube, and crush it by a pestle.

② Add 250 µl of GE1 Buffer, 10 µl of Proteinase K, and 5 µl of RNase A. Mix well by vortexing for 30 seconds.(*1)

③ Incubate at 65℃ for 15 minutes.

④ Add 40 µl of GE2-K Buffer,(*2) and mix well by vortexing.

⑤ Centrifuge at ≧13000 x g for 5 minutes at room temperature.(*3)

⑥ Transfer 200 µl of supernatant to a fresh 1.5 ml tube.(*4)

⑦ Add 75 µl of GB3 Buffer.

⑧ Add 75 µl of isopropanol and mix well by inverting 10-12 times.(*5)

⑨ Transfer all the mixture made in ⑧ to Spin Column, and centrifuge at 13000 x g, for 30 seconds at room temperature. Discard the liquid in the collection tube.

⑩ Add 650 µl of GW Buffer to Spin Column, and centrifuge at 13000 x g for 60 seconds at room temperature. Discard the liquid in the collection tube.

⑪ Remove the Spin Column to a fresh 1.5 ml tube.

⑫ Add 50 µl of TE (pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑬ Centrifuge at 13000 x g for 60 seconds at room temperature, and collect the DNA solution.

＜Potato DNA extraction Protocol＞

* When DNA is extracted from frozen dry potato, Proteinase K and α-Amylase would not be added. Please follow the rice protocol (p. 4).

① Cut raw potato into 3 mm cubes by knife.

② Weigh out 0.3 g of the potato cut small, and transfer to a 1.5 ml tube. Add 500 µl of GE1 Buffer and 4 µl of RNase A(100 mg/ml). Crush well by a pestle.(*6)

③ Mix well by vortexing for 30 seconds.

④ Add 85 µl of GE2-K Buffer,(*2) and mix well by vortexing.

⑤ Centrifuge at ≧13000 x g for 5 minutes at room temperature.(*3)

⑥ Transfer 400 µl of supernatant to a fresh 1.5 ml tube.(*4)

⑦ Add 150 µl of GB3 Buffer.

⑧ Add 150 µl of isopropanol, and mix well by inverting 10-12 times.(*5)

⑨ Transfer all the mixture made in ⑧ to Spin Column, and centrifuge at 13000 x g, for 30 seconds at room temperature. Discard the liquid in the collection tube.

⑩ Add 600 µl of GW Buffer to Spin Column, and centrifuge at 13000 x g for 60 seconds at room temperature. Discard the liquid in the collection tube.

⑪ Remove the Spin Column to a fresh 1.5 ml tube.

⑫ Add 50 µl of TE (pH8.0) to the Spin Column, and incubate at room temperature for 3 minutes.

⑬ Centrifuge at 13000 x g for 60 seconds at room temperature, and collect the DNA solution.

(*1) If mixing is insufficient, DNA yield is decreased remarkably. If the 2.0 ml or 1.5 ml tube is not vertical, the mixture becomes foamy, which decreases mixing efficiency. The 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-K Buffer even though the foam generated by ② remains. Because the viscosity of the mixture has risen, please invert carefully until GE2-K Buffer is mixed well.

(*3) Please confirm the maximum speed of centrifuge rotor and the maximum centrifugation stability (x g) of 50 ml tube.

(*4) Please avoid taking neither precipitate nor float.

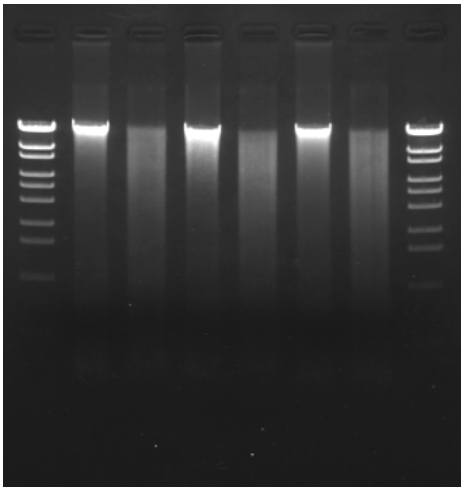
(*5) Add GB3 Buffer before ethanol or isopropanol, and then mix. If the precipitation appears, mix well by inverting until the liquid becomes clear.

(*6) Crush the potato sample after adding GE1 Buffer. If the sample were crushed by food mill before adding GE1 Buffer, a band of extracted genomic DNA by agarose gel electrophoresis would be smear.

Ⅵ Data Collection

1. DNA extraction from Rice and Restriction Enzyme digestion

DNA could be extracted from rice with this kit. And DNA was digested by EcoRI.



Lane 1, 8：OneSTEP Marker 6(λ / StyI digest)
Lane 2 ：*Koshihikari* genomic DNA intact
Lane 3 ：*Koshihikari* genomic DNA/ EcoRI digest
Lane 4 ：Thai rice genomic DNA intact
Lane 5 ：Thai rice genomic DNA/ EcoRI digest
Lane 6 ：Glutinous rice genomic DNA intact
Lane 7 ：Glutinous rice genomic DNA/ EcoRI digest

＊400 ng of the extracted DNA was run on a 1% Agarose S gel.

2. Absorption spectrum of Rice DNA

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

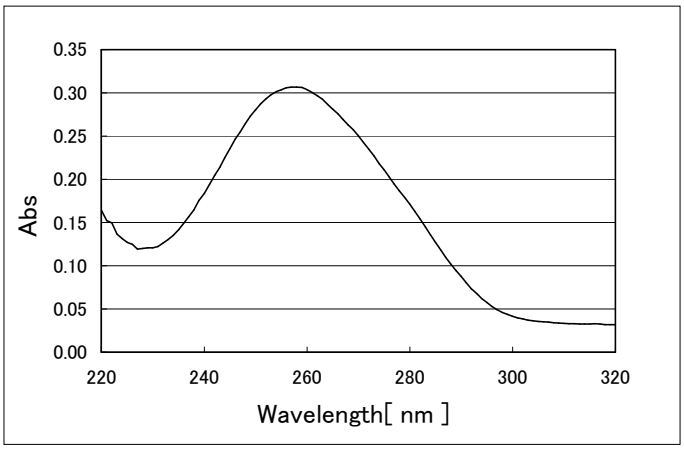


Fig.1 UV scan of *Koshihikari* rice DNA extracted with this kit.

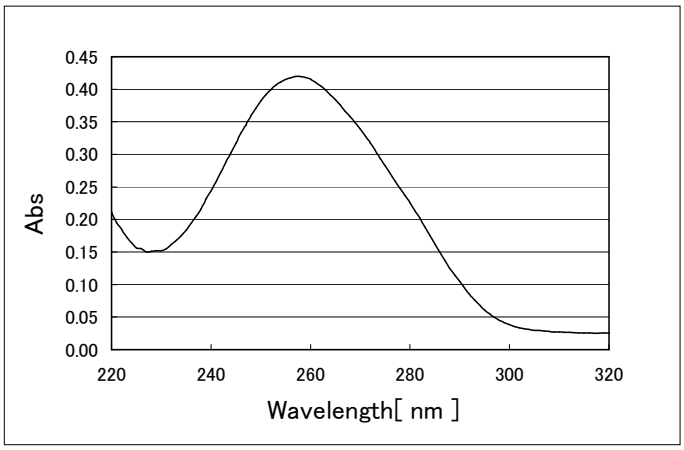


Fig.2　UV scan of Thai rice DNA extracted with this kit.

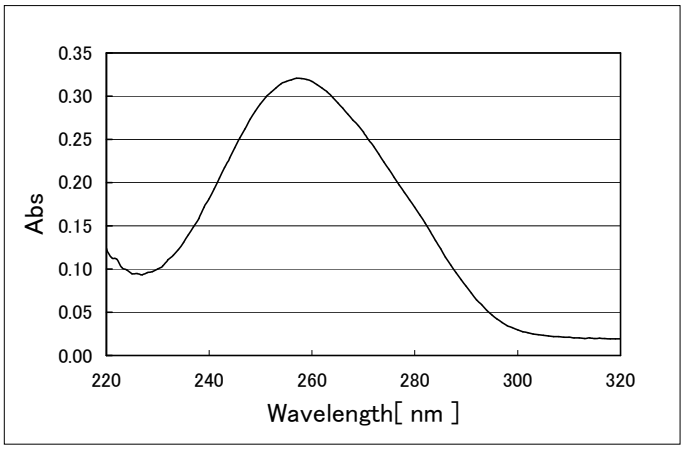
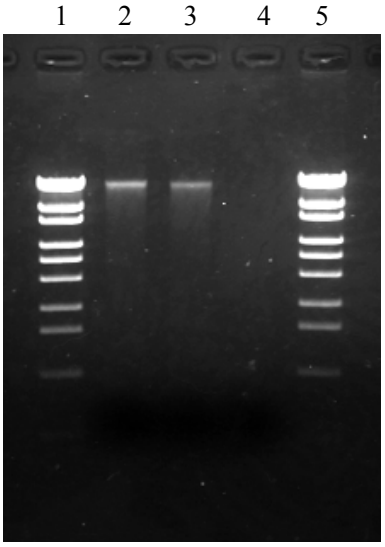


Fig.3　UV scan of Glutinous rice DNA extracted with this kit.

3. DNA extraction from a grain of Rice

DNA was extracted from a grain of rice.

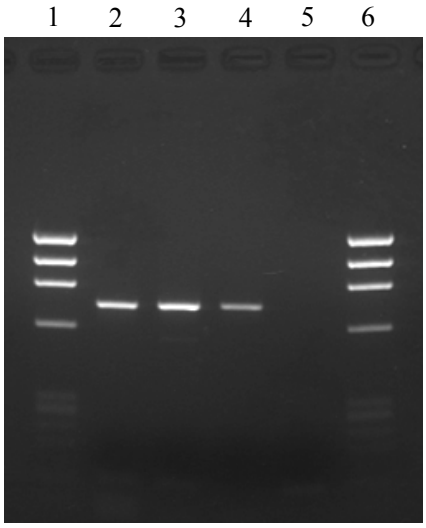


Lane 1, 5 : OneSTEP Marker 6 (λ / StyI digest)
Lane 2 : *Koshihikari* unpolished rice genomic DNA
Lane 3 : *Koshihikari* polished rice genomic DNA
Lane 4 : *Koshihikari* boiled rice genomic DNA

＊20 µl of the extracted DNA 50 µl was run on a 1% Agarose S gel.

4. PCR of DNA extraction from a grain of Rice

PCR could be performed using 0.1 µl of the DNA extracted from a grain of rice as a template without any blocking.

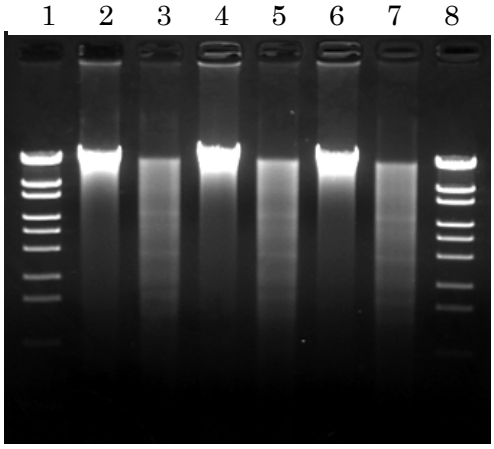


Lane 1, 6 : OneSTEP Marker 4 (*φ*X174/ HaeIII digest)
Lane 2 : Template was *Koshihikari* unpolished rice DNA.
Lane 3 : Template was *Koshihikari* polished rice DNA.
Lane 4 : Template was *Koshihikari* boiled rice DNA.
Lane 5 : No Template Control

＊10 µl of PCR products was run on a 2% Agarose S gel.

5. DNA extraction from Rape seed and Restriction Enzyme digestion

DNA could be extracted from rape seed with this kit. And DNA was digested by EcoRI.



Lane 1, 8 : OneSTEP Marker 6(λ/ StyI digest)
Lane 2, 4, 6 : Rape seed genomic DNA intact
Lane 3, 5, 7 : Rape seed genomic DNA/ EcoRI digest

＊400 ng of the extracted DNA was run on a 1% Agarose S gel.

6. Absorption spectrum of Rape seed DNA

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

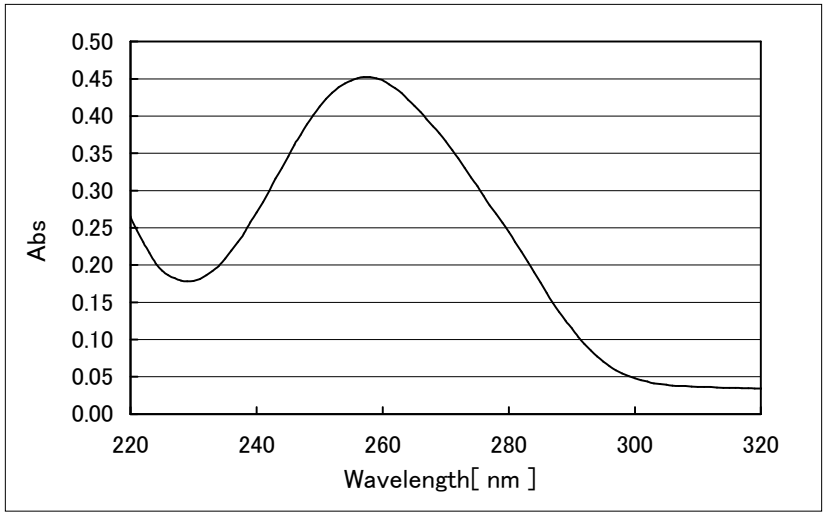
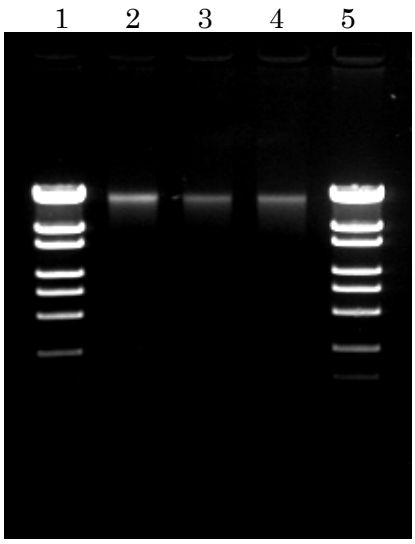


Fig.4 UV scan of Rape seed DNA extracted with this kit.

7. DNA extraction from a grain of Rape seed

DNA was extracted from a grain of Rape seed.



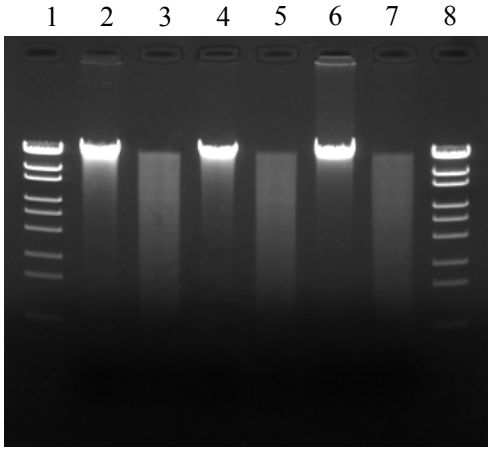
Lane 1, 5 : OneSTEP Marker6 (λ / StyI digest)

Lane 2, 3, 4 : Rape seed genomic DNA

＊15 µl of the extracted DNA 50 µl was run on a 1% Agarose S gel.

8. DNA extraction from Potato and Restriction Enzyme digestion

DNA could be extracted from raw Potato with this kit. And DNA was digested by EcoRI.



Lane 1, 8 : OneSTEP Marker 6 (λ / StyI digest)
Lane 2 : *Danshaku* genomic DNA intact
Lane 3 : *Danshaku* genomic DNA/ EcoRI digest
Lane 4 : *May Queen* genomic DNA intact
Lane 5 : *May Queen* genomic DNA/ EcoRI digest
Lane 6 : Frozen dry potato genomic DNA intact
Lane 7 : Frozen dry potato genomic DNA/EcoRI digest

＊200 ng of the extracted DNA was run on a 1% Agarose S gel.

9. Absorption spectrum of Potato DNA

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

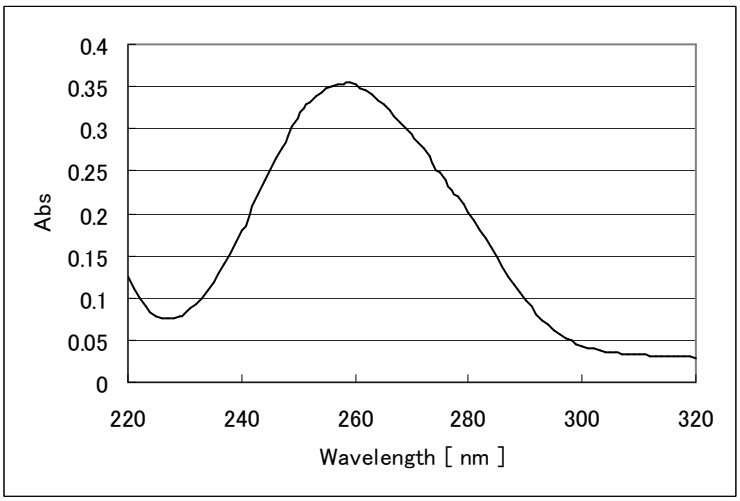


Fig.5 UV scan of *May Queen* potato DNA extracted with this kit.

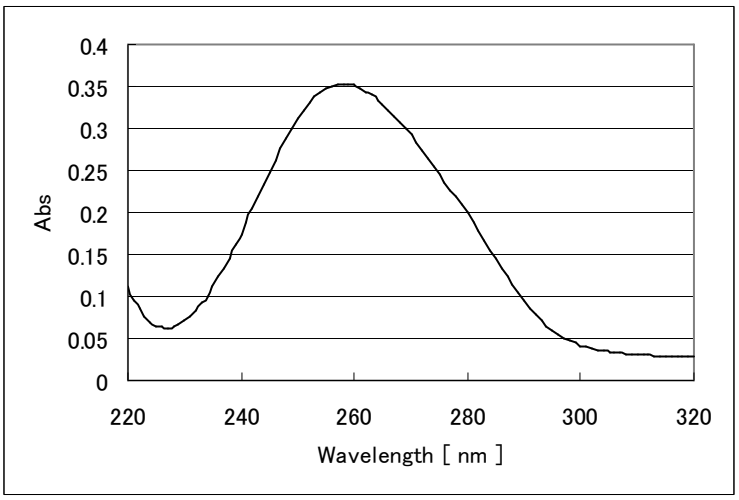
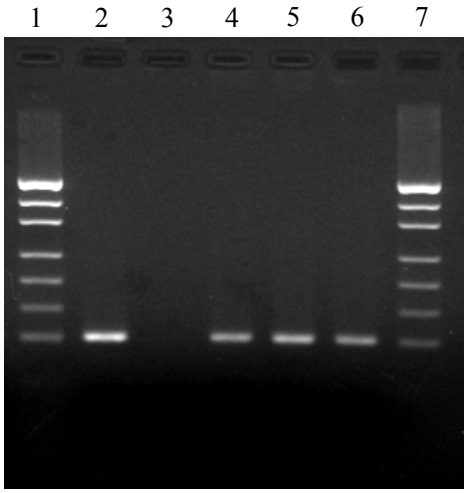


Fig.6 UV scan of *Danshaku* potato DNA extracted with this kit.

10. PCR of DNA extracted from Potato

PCR could be performed using 25 ng of the DNA extracted from potato as a template for detection of UGP gene without any blocking.



Lane 1,7: OneSTEP Marker 11 (φX174/ HaeIII digest)
Lane 2 : Positive Control
Lane 3 : No template Control
Lane 4 : Template was *Danshaku* potato DNA.
Lane 5 : Template was *May Queen* potato DNA.
Lane 6 : Template was frozen dry potato DNA.

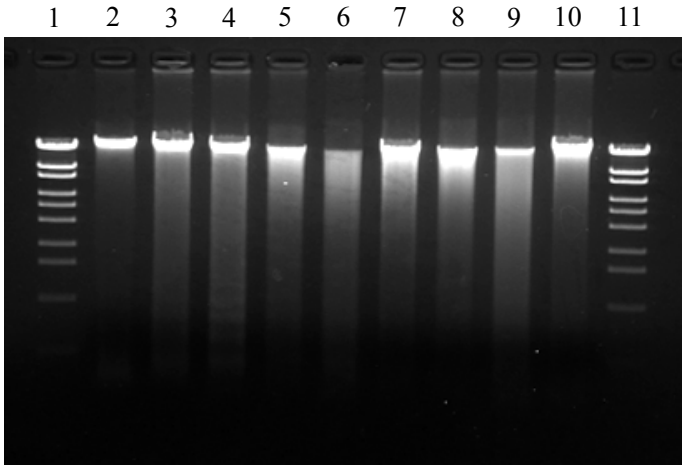
＊ 5 µl of PCR products was run on a 3% Agarose 21 gel.

＊The amplicons were detected clearly.

＊Positive Control : GM Potato Positive Control Plasmid (for qualitative analysis)
Code No. 317-06501

11. DNA extraction from other cereals

DNA was extracted from following cereals.



Lane 1, 11：OneSTEP Marker 6 (λ / StyI digest)
Lane 2： Soy bean genomic DNA
Lane 3： Maize genomic DNA
Lane 4： Wheat genomic DNA
Lane 5： Japanese millet genomic DNA
Lane 6： Millet genomic DNA
Lane 7： Foxtail millet genomic DNA
Lane 8： Hatomugi genomic DNA
Lane 9： Katamaru wheat genomic DNA
Lane 10：Amaranths genomic DNA

＊ 400 ng of the extracted DNA was run on a 1% Agarose S gel.

＊ The amount of powder sample for extraction.

Soy bean ：50 mg

Maize, Wheat, Katamaru wheat, and Amaranths ：200 mg

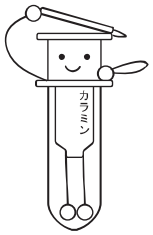
Japanese millet, Millet, Foxtail millet, and Hatomugi ：300 mg

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.
		If the 2.0 ml or 1.5 ml tube is not vertical, the mixture becomes foamy, and mixing efficiency would be decreased. The 50 ml tube should be vertical to the vortex mixer and mix well.
	Insufficient elution	If the centrifugation for elution was performed immediately after adding TE to the Spin Column, DNA yield would be low. Please incubate for 3minutes at room temperature.
	Centrifugation at below 15℃	Please centrifuge at room temperature (25-15℃).
	Incubate at above 70℃ after adding α-Amylase and Proteinase K.	Please incubate at temperature described in the manual.
RNA in the eluate	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 mix	Mix well after adding GE2-K Buffer. (The white precipitate would remain in the extraction. In the cases, mix well after adding GE2-K 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.	Please careful not to touch the precipitant by the tip when the supernatant is collected.

Ⅷ Related Products

Code No.	Product Name	Size
317-06361	*GM quicker*	50 reactions
311-07241	*GM quicker 3*	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
310-06513	Agarose XP	100 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



NIPPON GENE CO., LTD.

If you have any questions, please contact us by web form.
http://www.nippongene.com/