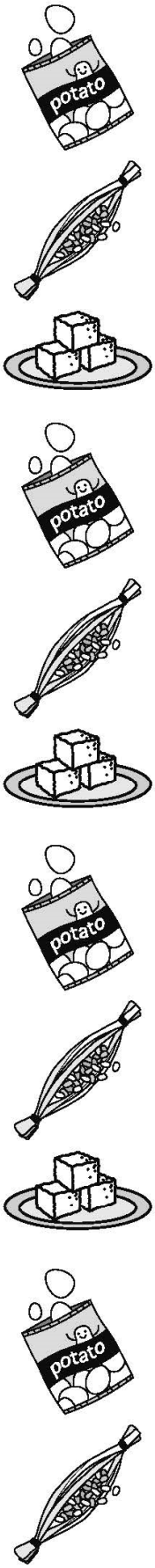


GM quicker 4

- DNA Extraction Kit
for Processed foods -

Manual

Ver. 3.0

Code No. 316-07791

NIPPON GENE CO., LTD.

Contents

I Description ---------- 2

II Product Components ---------- 2

III Storage Conditions ---------- 2

IV Precautions ---------- 3

V Protocol selection related to JAS law display items ---------- 3

VI Protocol ---------- 4

＜Protocol 1: DNA extraction method from dried foods＞ ---------- 5

＜Protocol 2: DNA extraction method from foods containing large amount of water＞ ---------- 6

VII Data Collection ---------- 8

VIII Troubleshooting ---------- 12

IX Related Products ---------- 13

X Reference ---------- 14

I Description

GM quicker 4 is a kit for extracting and purifying DNA in food products. Since this kit applies the principle that DNA is adsorbed to silica in the presence of chaotropic ion, DNA contained in food products can be extracted without using toxic organic solvents such as phenol and chloroform.

The spin column used in this kit ensures the maximum column volume within the range in which the rotor of a common centrifuge can be used, and the enclosed silica gel membrane ensures a high adsorption volume to recover DNA in food products.

Since DNA obtained by this kit can be used for the enzyme reactions such as PCR and LAMP, the application is expected to examine genetically-modified food products, specific raw materials, food microorganisms, raw materials and the like.

II Product Components

GE1 Buffer	100 ml	× 2 *
GE2-M Buffer	20 ml	× 1
GB3 Buffer	12.5 ml	× 3 *
GW Buffer	40 ml	× 1 *
TE (pH8.0)	10 ml	× 1 *
Proteinase K (20 mg/ml)	1 ml	× 1 *
α-Amylase (high concentration)	0.1 ml	× 1 *
RNase A (100 mg/ml)	0.5 ml	× 1 *
Spin Column	50 sets	
Manual	1	

* Each item can be purchased separately. Please see page 13 for more details.

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 Precautions

- This product is a reagent for research and cannot be used for medicinal or other purposes.
- This product should be handled only by persons having a basic knowledge about reagents.
- Handle this product in accordance with the descriptions in the manual.
- We are not responsible for any problems that may arise if product is not handled in accordance with the manual.

Ⅴ Protocol selection related to JAS* law display items (Reference)

* Japanese Agricultural Standards (JAS)

Protocol 1: Dried food products (freeze-dried food and the like)

(1)Frozen and dried bean curd, okara (residue of soybean after soy milk is extracted) and yuba (dried bean curd film) (2) soybean powder (3) broiled soybean (4) dried potato (5) corn snack (6) corn starch (7) popcorn (8) potato snacks (9) potato starch

Protocol 2: Food products having a high water content (liquid food products and food products homogenized by adding sterilized water)

(10) bean curd and fried bean curd (11) natto (fermented soybean) (12) soy milk and the like (13) miso (fermented soybean) (14) baked soybean (15) canned or bottled soybean (16) frozen corn (17) canned or bottled corn (18) frozen potato

Other: Target products difficult to classify due to the variations in form.

For the following items, choose the protocol by consulting the above classification.

Products in which the major raw material is: a material listed in (1)-(18), soybean (for cooking), soybean powder, soybean protein, soybean in pods, soybean sprout, corn flour, corn grits (except corn flakes), corn (for cooking), potato (for cooking), alfalfa, and sugar beet (for cooking).

VI Protocol

<Reagents, instruments etc., necessary in addition to the kit>

- Micropipette
- Pipette tips
- 50 ml Conical tube
- 2.0 ml Microtube
- 1.5 ml Microtube
- Food mill (Cutter mill etc.)
- Centrifuge
- Test tube mixer
- Incubator

<Pretreatment method for food products>

In the food product inspection, to reduce sampling error, it is necessary to homogenize well so that the food product is homogeneous. JAS analytical test handbook "Manual for testing / analyzing genetically modified food products" <3rd Edition> considers methods by which the sample is pulverized after adding sterilized water of the same weight or by which the sample is dried by freeze drying and then pulverized, but mainly describes the methods by which sterilized water is added and the sample is homogenized with a cutter mill or the like.

In this kit, protocol 1 describes the method developed for food products that have been dried by freeze-drying or the like, and protocol 2 describes the method developed for liquid food products and homogenized food products prepared by adding sterilized water to a sample.

<Protocol 1: DNA extraction method from dried foods>

(1) Weigh 1.0 g of the pulverized sample in a 50 ml conical tube, and add 4.0 ml of GE1 Buffer, 10 µl of RNase A, 2 µl of α-Amylase and 20 µl of Proteinase K, respectively. Conduct flash centrifugation (momentary centrifugation) in order to have any sample and Buffer on the sides collect at the bottom of the tube and then mix well with a Test tube mixer (30 seconds or longer). Note 1

(2) Heat at 65°C for 30 min. Stir at the highest speed for 10 sec at 10 minutes intervals with the Test tube mixer.

(3) Add 400 µl of GE2-M Buffer and stir well with the Test tube mixer.

(4) Centrifuge (≥ 4K x g, 10 min, 4°C). Note 2

(5) Transfer 800 µl of the supernatant to a fresh 2.0 ml microtube. Note 3

(6) Add 600 µl of GB3 Buffer and mix well by inverting the tube 10-12 times.

(7) Centrifuge (≥ 10K x g, 5 min, 4°C) and recover as much of the supernatant as possible into a 2.0 ml microtube. Note 4

(8) Transfer 700 µl of the supernatant of (7) to a Spin Column, centrifuge (≥ 10K x g, 1 min, 4°C) and discard the filtrate.

(9) Transfer the entire residual supernatant of (7) to the Spin Column of (8), centrifuge (≥ 10K x g, 1 min, 4°C) and discard the filtrate.

(10) Add 600 µl of GW Buffer to the Spin Column and then centrifuge (≥ 10K x g, 1 min, 4°C) and discard the filtrate.

(11) Transfer the Spin Column to a fresh 1.5 ml microtube.

(12) Add 50 µl of TE (pH8.0) dropwise to the center of the membrane and then leave standing for 3 min at room temperature.

(13) Centrifuge (≥ 10K x g, 1 min, 4°C) and recover the filtrate.

<Protocol 2: DNA extraction method from foods containing large amount of water>

(1) Weigh 1.0 g of the pulverized sample in a 50 ml conical tube, and add 1.0 ml of GE1 Buffer, 10 µl of RNase A, 2 µl of α-Amylase and 20 µl of Proteinase K, respectively. Conduct flash centrifugation (momentary centrifugation) in order to have any sample and Buffer on the sides collect at the bottom of the tube and then mix well with a Test tube mixer (30 seconds or longer). Note 1

(2) Heat at 65°C for 30 min. Stir at the highest speed for 10 sec at 10 minutes intervals with the Test tube mixer.

(3) Add 200 µl of GE2-M Buffer and stir well with the Test tube mixer.

(4) Centrifuge (≥ 4K x g, 10 min, 4°C). Note 2

(5) Transfer 800 µl of the supernatant to a fresh 2.0 ml microtube. Note 3

(6) Add 600 µl of GB3 Buffer and mix well by inverting the tube 10-12 times.

(7) Centrifuge (≥ 10K x g, 5 min, 4°C) and recover as much of the supernatant as possible into a 2.0 ml microtube. Note 4

(8) Transfer 700 µl of the supernatant of (7) to a Spin Column, centrifuge (≥ 10K x g, 1 min, 4°C) and discard the filtrate.

(9) Transfer the entire residual supernatant of (7) to the Spin Column of (8), centrifuge (≥ 10K x g, 1 min, 4°C) and discard the filtrate.

(10) Add 600 µl of GW Buffer to the Spin Column and then centrifuge (≥ 10K x g, 1 min, 4°C) and discard the filtrate.

(11) Transfer the Spin Column to a fresh 1.5 ml microtube.

(12) Add 50 µl of TE (pH8.0) dropwise to the center of the membrane and then leave standing for 3 min at room temperature.

(13) Centrifuge (≥ 10K x g, 1 min, 4°C) and recover the filtrate.

(Note 1) If the stirring is insufficient, the DNA yield is decreased markedly. Vertically apply the tube to the Test tube mixer and make sure to stir for 30 sec. If the stirring is insufficient, further stir for 30-60 sec.

(Note 2) Confirm the maximum revolving speed of the rotor of the refrigerated centrifuge and the maximum g-force resistance of the conical tube to be used.

(Note 3) Recover the supernatant in such a way that precipitates and film-like floating objects are not taken if at all possible.

(Note 4) Recover the supernatant to a fresh 2.0 ml tube in such a way that precipitates and film-like floating objects are not taken if at all possible.

VII Data Collection

1. Absorption spectra of DNA extracted with this kit.

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

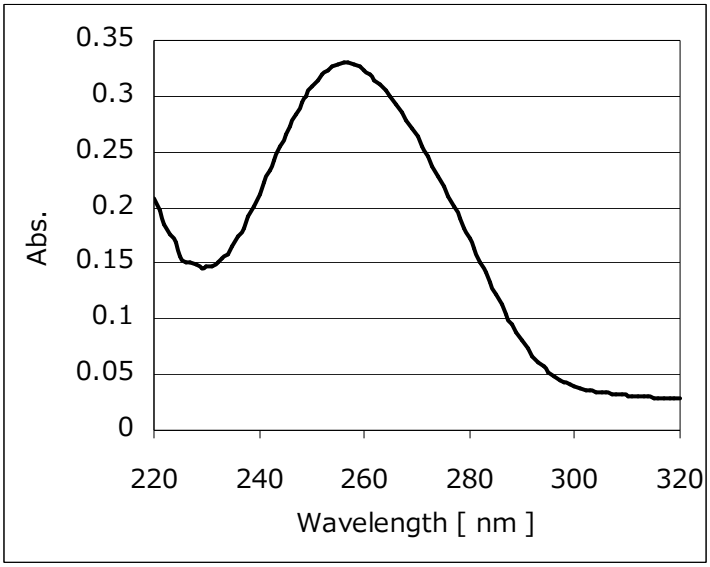


Fig. 1 Absorption spectra of DNA extracted from soybean powder (Protocol 1)

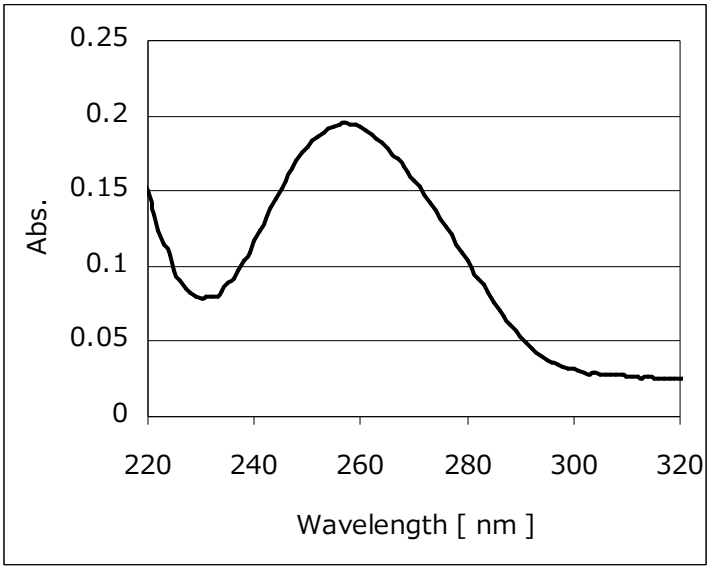


Fig. 2 Absorption spectra of DNA extracted from soy milk (Protocol 2)

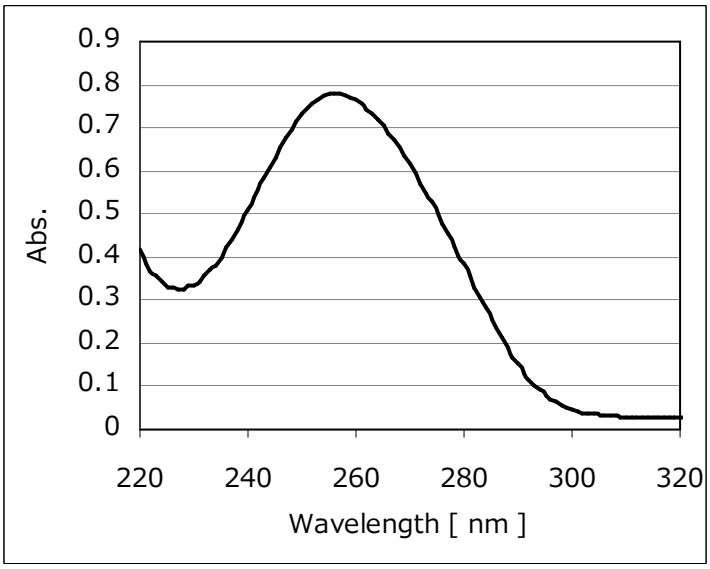


Fig. 3 Absorption spectra of DNA extracted from corn soup powder (Protocol 1)

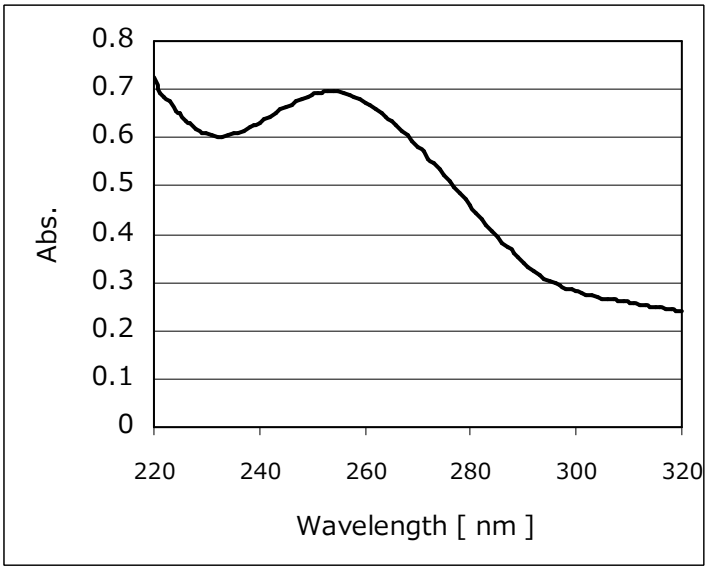
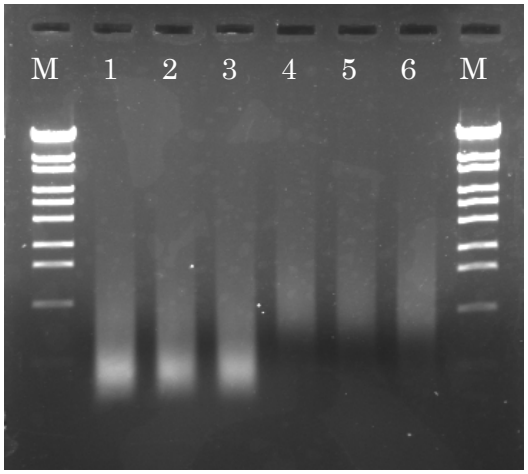


Fig. 4 Absorption spectra of DNA extracted from potato snacks (Protocol 1)

2. Agarose gel electrophoresis of DNA extracted with this kit.

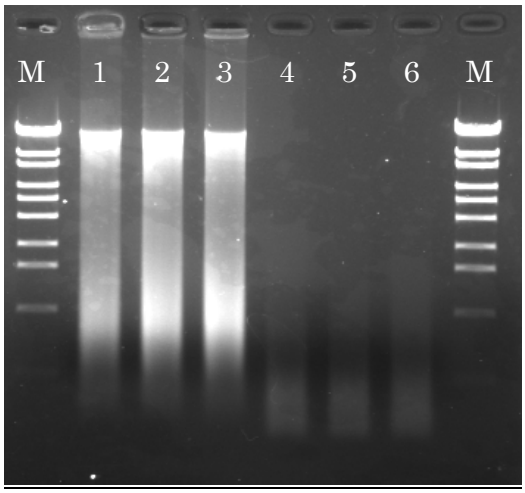
DNA could be extracted from processed food products of soybean (soybean powder: Protocol 1 and soy milk: Protocol 2).



Lane M : OneSTEP Marker 6 (λ/ StyI digest)
Lane 1, 2, 3 : DNA derived from soybean powder
Lane 4, 5, 6 : DNA derived from soy milk

1/10 amount of DNA solution (5 µl) extracted with this kit was electrophoresed in 1% Agarose S.

DNA could be extracted from processed food products of corn (corn soup powder: Protocol 1 and canned whole corn: Protocol 2).

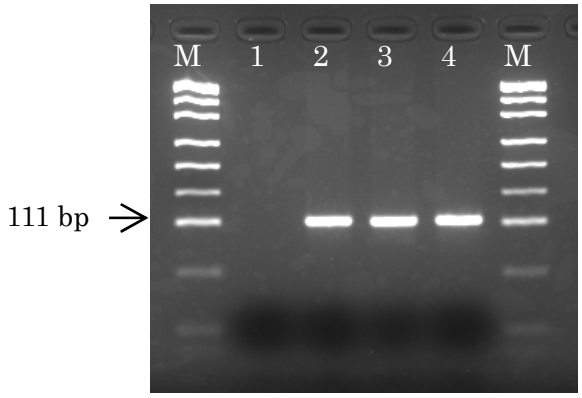


Lane M : OneSTEP Marker 6 (λ/ StyI digest)
Lane 1, 2, 3 : DNA derived from corn soup powder
Lane 4, 5, 6 : DNA derived canned whole corn

1/10 amount of DNA solution (5 µl) extracted with this kit was electrophoresed in 1% Agarose S.

3. Detection of endogenous genes by PCR

PCR was performed using endogenous potato DNA UGPase oligonucleotide (Code No.: 310-05991) and the DNA solution derived from potato snacks extracted with this kit as a template, The PCR conditions were as follows: 95°C, 10 min → (95°C, 30 sec → 60°C, 30 sec → 72°C, 30 sec) x 40 Cycles → 72°C, 7 min.



Lane M : OneSTEP Marker11 (pUC19/MspI digest)

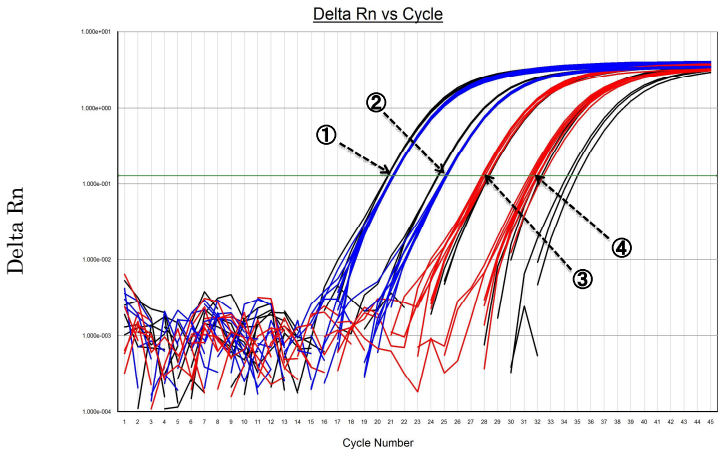
Lane 1 : Negative control (TE solution)

Lane 2-4 : Potato snacks

A portion of the PCR product (5 µl) was electrophoresed in 3% Agarose 21 gel.

4. Detection of endogenous gene by real-time PCR

Quantitative tests of DNA extracted with this kit from rice noodle and rice paper were performed using rice endogenous DNA PLD oligonucleotide set 2 (Code No.: 312-07531) (n=3).



Amplification curve by ABI7500 Fast System (Base Line 3-15, Th.Line=0.128)

(1) Rice noodle DNA (190,000 copies/2.5 µl) (2) 10-fold diluted rice noodle DNA (12,800 copies/2.5 µl) (3) Rice paper DNA (1,840 copies/2.5 µl) (4) 10-fold diluted rice paper DNA (147 copies/2.5 µl)

The processed rice DNA extracted with this kit can be quantitated by real-time PCR.

VIII Troubleshooting

Trouble	Cause	Suggestion
After centrifugation, sufficient amount of the supernatant cannot be recovered.	Not enough amount of GE1 Buffer for the sample.	Reduce the amount of the sample or increase the amount of GE1 Buffer.
	Insufficient centrifugal force	Increase the centrifugal g or the centrifugal time.
	The temperature of the crude extract is not decreased from 65°C to room temperature.	It may be effective to decrease the temperature by cooling in ice cold water for several minutes immediately before the centrifugation to recover the supernatant of (5).
Spin column is clogged.	Precipitates and floating objects on the surface of the supernatant are mixed in.	To avoid the precipitates or floating objects being mixed in, transfer the supernatant of (5) to a 1.5 ml or 2.0 ml microtube and centrifuge again at a higher g, and then recover the supernatant again and add GB3 Buffer.
		Precipitates may float when left standing after centrifugation. When the precipitates are floating, centrifuge again before recovering the supernatant.
Low yield of DNA	Insufficient sample crushing	Crush the sample as fine as possible so as to be homogeneous.
	Low extraction efficiency	Mix well with the Test tube mixer after the addition of GE1 Buffer and enzyme. During the heating reaction, the viscosity is decreased and the efficiency of stirring increases, so mix well.
	Insufficient elution	When centrifugation-elution is performed immediately after the addition of TE (pH8.0) to the Spin Column, the DNA yield may be variable. Allow sufficient elution by standing at room temperature for a few minutes, and then centrifuge.
	Amount of DNA in the sample is small	Use 2 or more samples and treat them separately until the recovery of the supernatant of (7) at the step of centrifugation after the addition of GB3 Buffer. Then, combine the recovered supernatant and apply the whole mixture to a Spin Column in multiple fractions. Thereby, increased DNA yield is expected.
	The temperature of the enzyme treatment is too low or too high.	Adjust the temperature as described in the manual (65°C). Also, in the heating process, it is recommended to use a water bath or a heat block having a good heat conduction efficiency.
	Loss of degrading enzyme activity	Store Proteinase K and α-Amylase at -20°C.
High RNA contamination	RNase A is inactivated	GE1 buffer and RNase A cannot be stored as a mixture. Store them separately.
DNA cannot be amplified by PCR	PCR inhibitor may exist.	Perform PCR after diluting with sterilized water or the like.
		When the coloring to the spin column is due to the dye derived from the sample, it may be effective i) to perform process of (10) of washing with GW Buffer twice, or ii) to wash with 80% ethanol after the washing process of (10).

IX Related Products

Code No.	製品名	包装単位
317-06361	*GM quicker*	50 reactions
310-06591	*GM quicker* 2	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
319-08141	Collection Tube	50 tubes×2

X Reference

1) Yasutaka Minegishi, Junichi Mano, Yasuo Kato, Kazumi Kitta, Hiroshi Akiyama and Reiko Teshima (2013) Japanese Journal of Food Chemistry and Safety, in press

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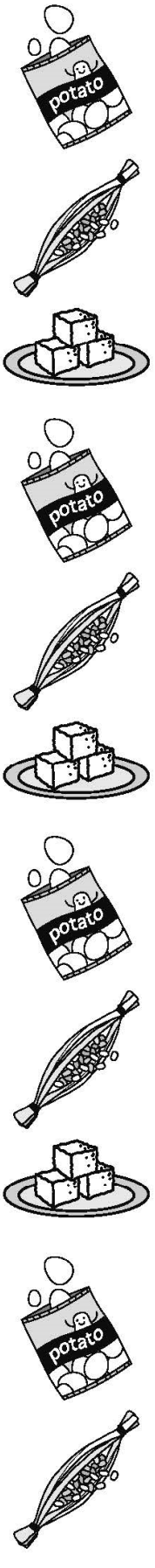

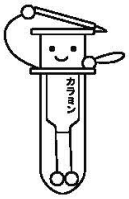

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If you have any questions, please contact us by web form.

http://www.nippongene.com/