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Technical Note:

Products	GenCheck DNA Extraction Reagent
Note	Evaluation Project
No. / Date	TNG180606 / 6 June 2018

1. Objective

Evaluation of the application of GenCheck DNA Extraction Reagent on various samples and different types of detection platforms i.e. conventional PCR, real-time PCR, DNA sequencing, DNA Strip.

2. General methods

GenCheck can be applied to extract DNA from various materials and does not require a large sample amount. DNA extraction can be done according to the following quick guide:

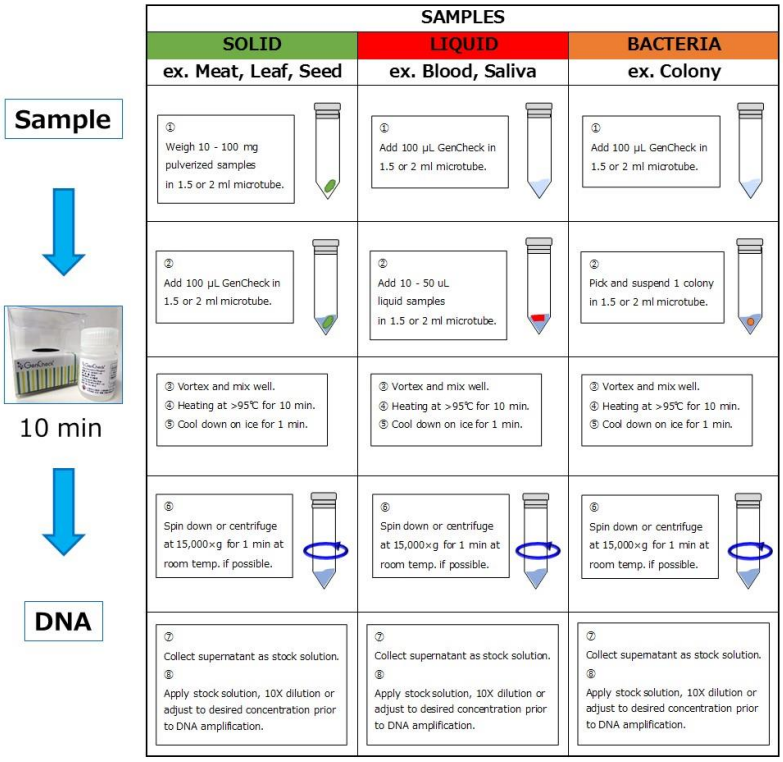


Figure 1. Quick guide of GenCheck DNA Extraction Reagent.

3. DNA concentration results

Table. Examples of DNA concentration measured on stock solution:

3.1 Blood (sheep)

Sample			Stock DNA	
No.	Type	Volume (μL)	DNA Conc. (ng/μL)	Abs 260/280
1	Blood	10	47.5	1.84
2	Blood	10	48	1.86
3	Blood	50	164	1.90
4	Blood	50	168	1.90
5	Blood	100	223	2.16
6	Blood	100	234	2.22

3.2 Saliva (human)

Sample			Stock DNA	
No.	Type	Volume (μL)	DNA Conc. (ng/μL)	Abs 260/280
1	Saliva	10	12.6	0.76
2	Saliva	10	49.5	1.51
3	Saliva	50	44.9	0.60
4	Saliva	50	164	1.34
5	Saliva	100	119	0.72

3.3 Meat (pork and wild boar)

Sample			Stock DNA	
No.	Type	Weight (mg)	DNA Conc. (ng/μL)	Abs 260/280
1	Pork 1	100	518.29	2.57
2	Pork 2	100	1097.24	3.04
3	Wild boar 1	100	625.63	2.7
4	Wild boar 2	100	1049.93	2.72

3.4 Meat (eel)

Sample			Stock DNA	
No.	Type	Weight (mg)	DNA Conc. (ng/μL)	Abs 260/280
1	A. japonica 1	100	961.50	1.45
2	A. japonica 2	100	1052.40	1.67
3	A. japonica 3	100	860.40	1.78
4	A. anguilla 1	100	648.80	2.21
5	A. anguilla 2	100	728.80	1.98
6	A. anguilla 3	100	649.20	2.16
7	A. rostrata 1	100	655.00	1.98
8	A. rostrata 2	100	893.40	2.15
9	A. rostrata 3	100	1008.80	2.08

4. Results on several detection platforms

4.1 Conventional PCR

Table. Outline results including volume of extracted DNA (stock solution and 10X dilution) used in PCR.

No.	Samples		Stock solution 1μL	Stock solution 2.5μL	Stock solution 5μL	10X dilution 1μL	10X dilution 5μL	Primer
	Type	Name						
1	Seeds	Maize (*Zea mays*)	n.d.	+	n.d.	n.d.	n.d.	
		Radish (*Raphanus sativus*)	n.d.	+	n.d.	n.d.	n.d.	
		Rapeseed (*Brassica napus*)	n.d.	+	n.d.	n.d.	n.d.	
		Chinese cabbage (*Brassica rapa subsp. pekinensis*	n.d.	+	n.d.	n.d.	n.d.	Plant foreign material
		Okra (*Abelmoschus esculentus*)	n.d.	+	n.d.	n.d.	n.d.	detection (FASMAC)
		Spinach (*Spinacia oleracea*)	n.d.	+	n.d.	n.d.	n.d.	
		Lettuce (*Lactuca sativa*)	n.d.	+	n.d.	n.d.	n.d.	
		Pea (*Pisum sativum*)	n.d.	+	n.d.	n.d.	n.d.	
2	Bacteria	*Salmonella enterica subsp. enterica* (NFB6001)	+	n.d.	–	n.d.	+	
		Lactobacillus casei (NBRC15883)	+	n.d.	–	n.d.	+	
		Bifidobacterium bifidum (NBRC100015)	+	n.d.	–	n.d.	+	
		Clostridium tertium (NBRC103192)	+	n.d.	–	n.d.	+	
		Bacillus subtilis var. natto	+	n.d.	–	n.d.	+	Bacterial 16S rRNA
		Enterococcus avium (NBRC100477)	+	n.d.	–	n.d.	+	Fungi D2 LSU
		Staphylococcus aureus (ATCC29213)	+	n.d.	–	n.d.	+	
		Streptococcus mutans (NBRC13955)	+	n.d.	–	n.d.	+	
		Aspergillus wentii (NBRC4107)	+	n.d.	–	n.d.	+	
		Saccharomyces cerevisiae (NBRC1136)	+	n.d.	–	n.d.	+	
3	Animall tissue	Tuna	–	n.d.	n.d.	n.d.	+	Fish cocktail
		Mackerel	+	n.d.	n.d.	n.d.	+	
4	Blood	Horse	–	n.d.	n.d.	+	n.d.	ASB2, COR018
		Sheep	n.d.	n.d.	n.d.	n.d.	+	Sheep specific, Matsunaga et al., 1999
5	Saliva	Human	n.d.	n.d.	n.d.	n.d.	+	Human specific, Hasel et al., 2016
		Bacteria	n.d.	n.d.	n.d.	n.d.	+	Bacterial 16S rRNA, Walters et al., 2015

n.d.: no data

Below is results example on DNA electrophoresis:

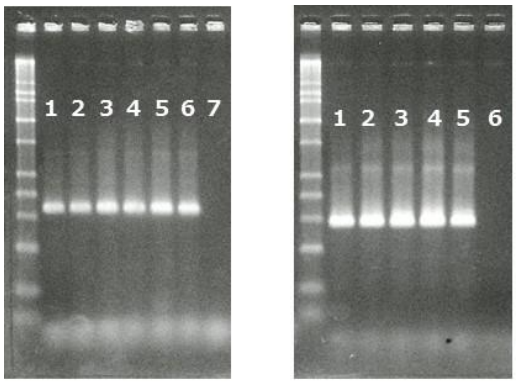


Figure. DNA electrophoresis of PCR products using blood (A) and saliva (B). Sample number is shown according to table provided in 3. DNA concentration results where the last number represents negative controls.

4.2 Qualitative real-time PCR

Intercalating dye (SYBR green) was added on GenCheck Hot Start PCR (FASMAC) including Allergen Checker primer to detect pig and wild boar on LightCycler96 (Roche Molecular Systems, Inc.).

Below is results example of qualitative real-time PCR:

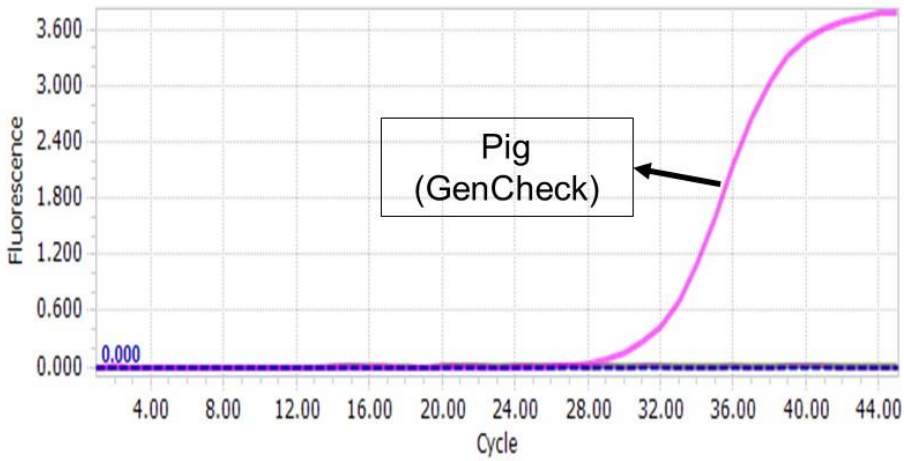


Figure. Porcine DNA detection using real-time PCR

4.3 Quantitative real-time PCR

Taqman universal PCR master mix and hydrolysis probe was applied to quantify DNA copy number of GM soybean RRS.

Below is the results example of quantitative real-time PCR:

Table. Percentage of DNA copy number and comparison of GenCheck to other kits.

DNA Extraction Kit		0.069±0.032%	0.177±0.076%	6.10±1.09%	<0.05%
GenCheck	FASMAC	+	+	+	−
Other products (A)	Other manufacturer	+	+	+	−
Other products (B)	Other manufacturer	−	−	−	−
Other products (C)	Other manufacturer	−	−	−	−

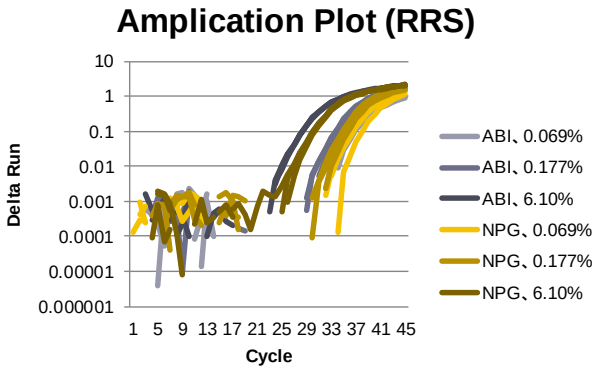
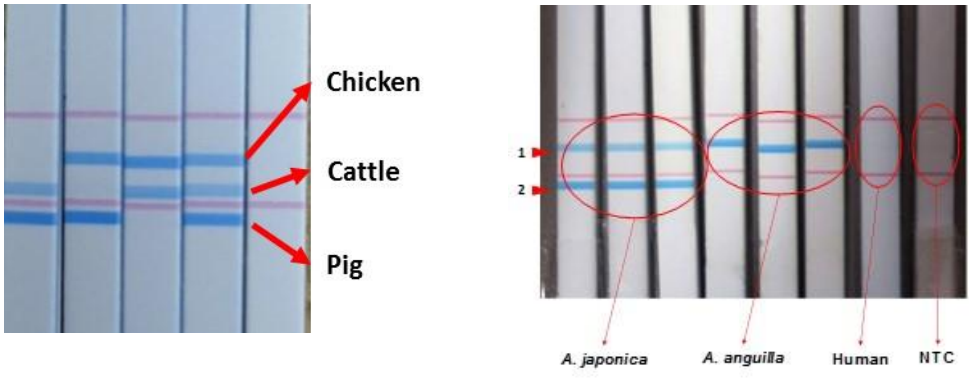


Figure. Amplification plot of GM soybean RRS on real-time PCR

4.4 DNA Strip

We recently developed a new DNA detection platform called as DNA Strip. It combines isothermal DNA amplification and lateral flow DNA Strip. Details of the mechanism can be found in our publication “Single Laboratory Validation of Rapid and Easy DNA Strip for Porcine DNA Detection in Beef Meatballs” in the Journal of AOAC International.

Below is the results example of multiplex detection using DNA Strip.



A B

Figure. Poster presentation at Japanese Society of Animal Breeding and Genetics (A) and Japanese Society of Fisheries Science (B).

4.5 Sanger DNA sequencing

We applied DNA extracted from Japanese eel (*Anguilla japonica*) and confirmed it through conventional PCR and Sanger sequencing.

Below is the results example of Sanger DNA sequencing.

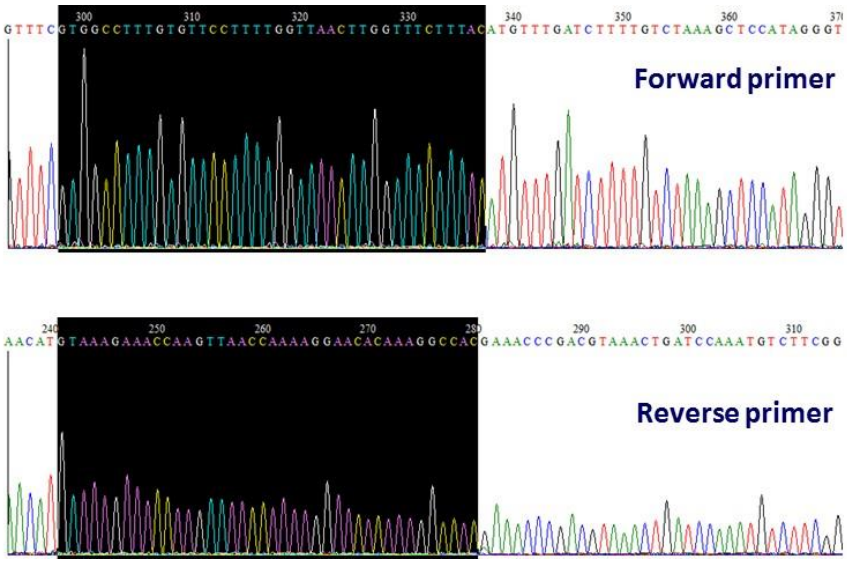
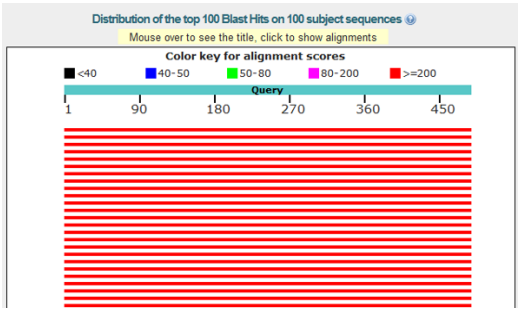


Figure. Raw data of Sanger sequencing of DNA extracted from Japanese eel.



A

Max score	Total score	Query cover	E value	Ident
898	898	100%	0.0	100%
898	898	100%	0.0	100%
898	898	100%	0.0	100%
898	898	100%	0.0	100%
898	898	100%	0.0	100%

B

Figure. Results of sequence alignment search on NCBI BLAST similarity analysis. Figure A and B shows that target of DNA sequencing is 100% identical to sequence database in GenBank.

5. Summary

Our results suggest that GenCheck can be done in short time and is applicable on various samples and different types of detection platforms including both conventional and real-time PCR, DNA Strip, and Sanger DNA sequencing.

6. Reference

a. Hasel P., Dando O., Jiwaji Z., Baxter P., Todd A.C., Heron S., Markus N.M., McQueen J., Hampton D.W., Torvell M., Tiwari S.S., McKay S., Eraso-Pichot A., Zorzano A., Masgrau R., Galea E., Chandran S., Wyllie D.J.A., Simpson T.I., Hardingham G.E. 2017. Neurons and neuronal activity control gene expression in astrocytes to regulate their development and metabolism. Nature communications, 8: 15132.

b. Matsunaga T., Chikuni K., Tanabe R., Muroya S., Shibata K., Yamada J., Shinmura Y. 1999. A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Science 51: 143-148.

c. Walters W., Hyde E.R., Berg-Lyons D., Ackerman G., Humphrey G., Parada A., Gilbert J.A., Jansson J.K., Caporaso J.G., Fuhrman J.A., Apprill A., Knight R. 2015. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems 1:1.