

Comparison Test of Performance of Proteinase K Stored Cold and Stored Room Temp. Tissue DNA Extraction Test

Objective

To compare the performance of cold-stored Proteinase K (-20°C) and room temperature-stored Proteinase K (25-28°C) used in tissue DNA extraction test.

Passing Criteria

The reading of nucleic acid is detected and correspondence to absorbance value limit for A260 wavelength. Corresponding absorbance value limits for A260 is within the **range of 0.01 to 1.6 Abs** and for **A260/280 is greater than 1.7**.

The amplification of extracted DNA using conventional PCR showed **positive results with 350bp band**.

The amplification of extracted DNA using real-time PCR showed positive results with the **difference of Ct value between two Proteinase Ks less than 3**.

Samples

- Beef tissue sample (tripe meat)
- Chicken tissue sample (chicken breast)
- Pork tissue sample (lean meat)

Protocol

A. DNA Extraction from Cultured Animal Cells

Centrifugation and resuspension

Pellet cells at 800 x g for 5 min. Add 200µl of PBS and resuspend completely

Cell lysis

Add 20µl of Proteinase K
Add 2µl of Lysis Enhancer.
Add 200µl of Buffer TB. Mix by pulsed-vortexing

B. DNA Extraction from Animal Tissue

Tissue preparation

Cut tissue into small pieces or grind into fine powder in liquid nitrogen

Tissue lysis

Add 250µl Buffer TL.
Add 20µl Proteinase K.
Mix by pulsed-vortexing.
Add 12µl Lysis Enhancer.
Incubate 65°C, 1 - 3 hr.

Optional: Removal of RNA

Add 20 l RNase A.
Incubate 37°C, 10 min.

Homogenization

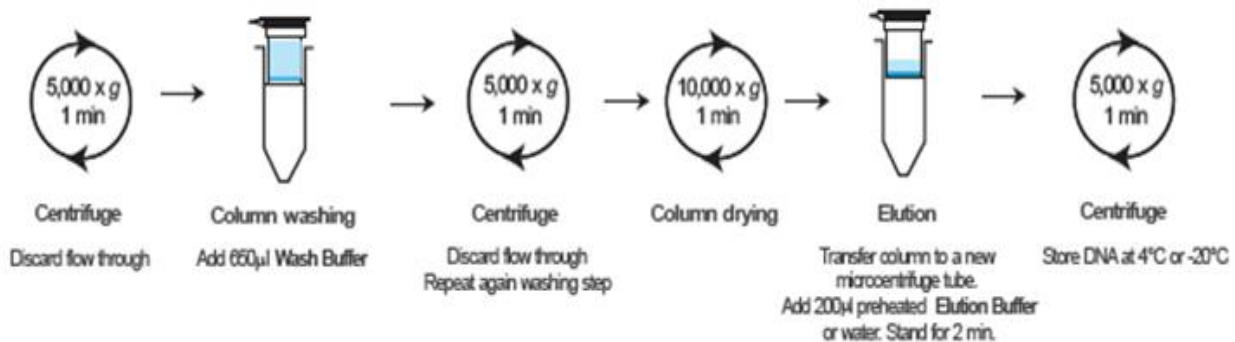
Add 2 volumes Buffer TB and mix thoroughly.
Incubate 65°C, 10 min.

Addition of ethanol

Add 200µl absolute ethanol and mix immediately.

Loading to column

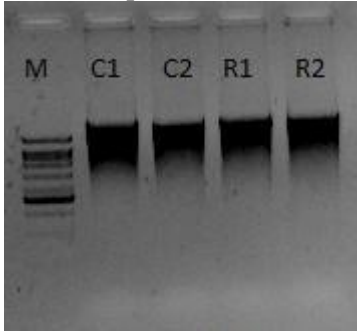
Transfer sample to column



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Results

Beef Tissue Sample



Legend:

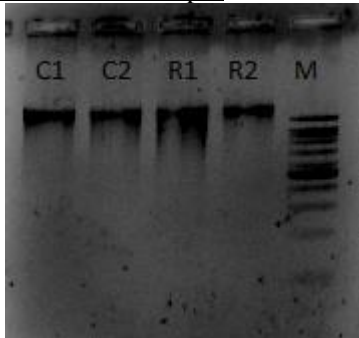
C1&C2: Extracted DNA with more than 50ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 50ng/μl; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

Figure 1: 2μl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Chicken Tissue Sample



Legend:

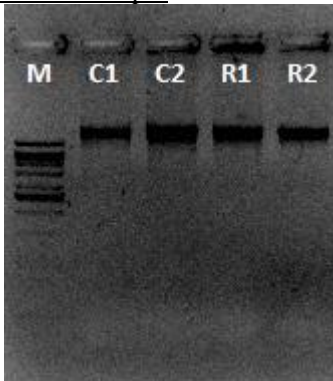
C1&C2: Extracted DNA with more than 30ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 30ng/μl; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

Figure 2: 2μl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Pork Tissue Sample



Legend:

C1&C2: Extracted DNA with more than 30ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 30ng/μl; extraction using room temperature stored Proteinase K

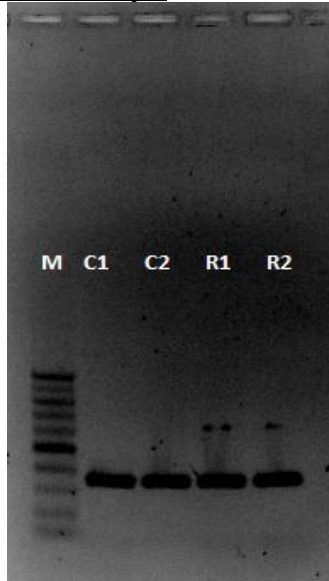
M: VC 1kb DNA ladder

Figure 3: 2μl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

Downstream Application

Conventional PCR and real-time PCR were carried out using the extracted DNA. Both tests were performed using tissue universal primer.

Beef Tissue Sample



Legend:

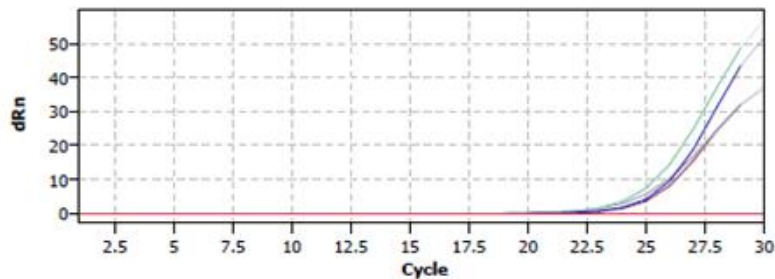
M: 100bp DNA ladder

C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction

R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction

Figure 4: 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.

GOI



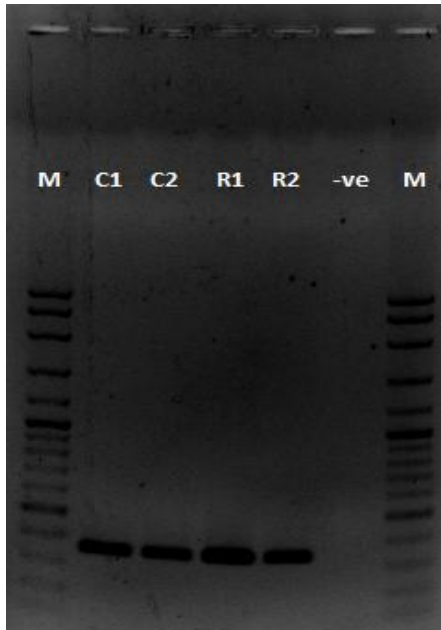
Well	Sample r	Sample t	Gene	Ct	Mean Ct	Conc. Std	Mean Co	Std.Dev.	Std.Dev.
C3	RT Beef	Unknown		24.89	24.89			0	
C2	RT Beef	Unknown		24.1	24.1			0	
B7	Cool Beef	Unknown		24.37	24.37			0	
B6	Cool Beef	Unknown		25.06	25.06			0	
D8	-VE	Unknown		No Ct					

Mean Ct value for RT Beef	24.495
Mean Ct value for Cool Beef	24.715
Difference Ct value between RT and Cool	0.220

Figure 5: 2µl of extracted DNA was used for real-time amplification. According to the graph and table on top, the difference in Ct value between two different Proteinase Ks is 0.220.

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Chicken Tissue Sample



Legend:

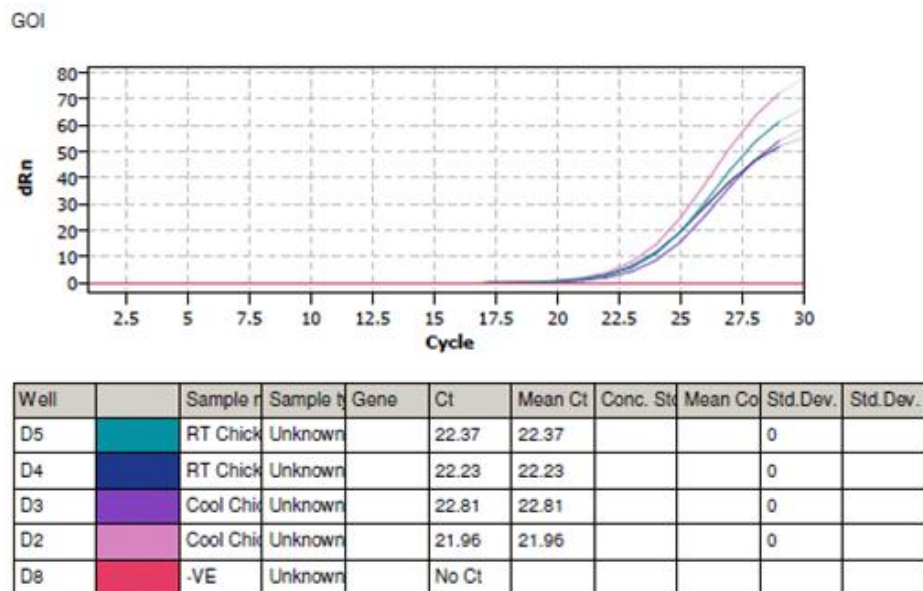
M: 100bp plus DNA ladder

C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction

R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction

-ve: Amplification product with no extracted DNA

Figure 6: 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.

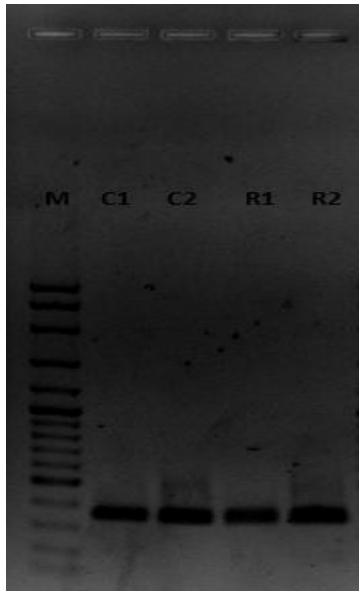


Mean Ct value for RT Chicken	22.300
Mean Ct value for Cool Chicken	22.385
Difference Ct value between RT and Cool	0.085

Figure 7: 2µl of extracted DNA was used for real-time amplification. According to the graph and table showed on top, the difference in Ct value between two different Proteinase Ks is 0.085

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Pork Tissue Sample



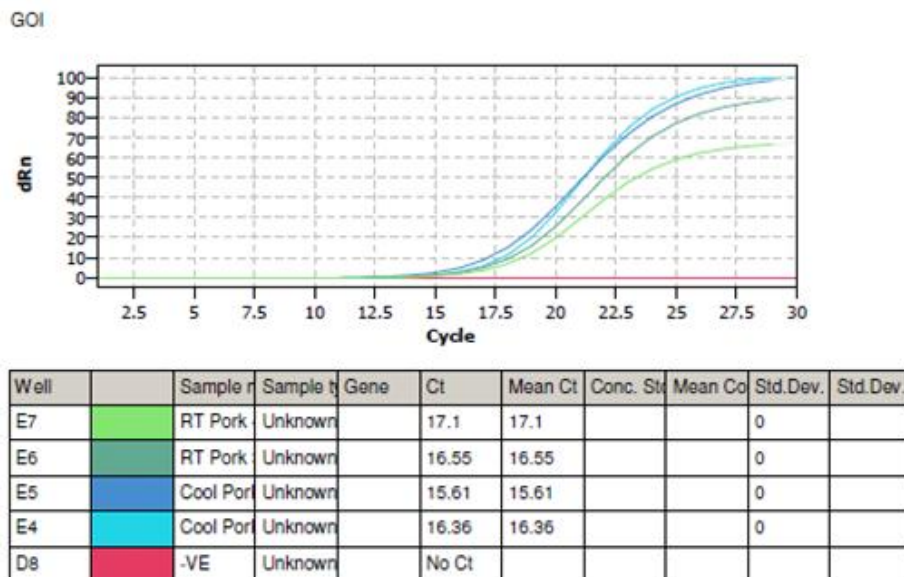
Legend:

M: 100bp plus DNA ladder

C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction

R1&R2: Amplification product using extracted DNA which used room temperature-stored Proteinase K in extraction

Figure 8: 2µl of extracted DNA was used for amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 350bp.



Mean Ct value for RT Pork	16.825
Mean Ct value for Cool Pork	15.985
Difference Ct value between RT and Cool	0.84

Figure 9: 2µl of extracted DNA was used for real-time amplification. According to the graph and table showed on top, the difference Ct value between two different Proteinase Ks is 0.84.

Conclusion

3 different tissue samples were extracted using GF-1 Tissue DNA Extraction kit. From the gel photos, there was no significant difference showed in the performance of Proteinase K that was stored in either cold or room temperature condition as the results of amplifications of extracted DNA using conventional PCR showed no significant different for bands; and using real-time PCR showed that all differences between the two Proteinase Ks are within 1Ct value. The sensitivity of the conventional and real-time assay was not affected by the use of room temperature-stored Proteinase K.

Prepared by,
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