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

Objective

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

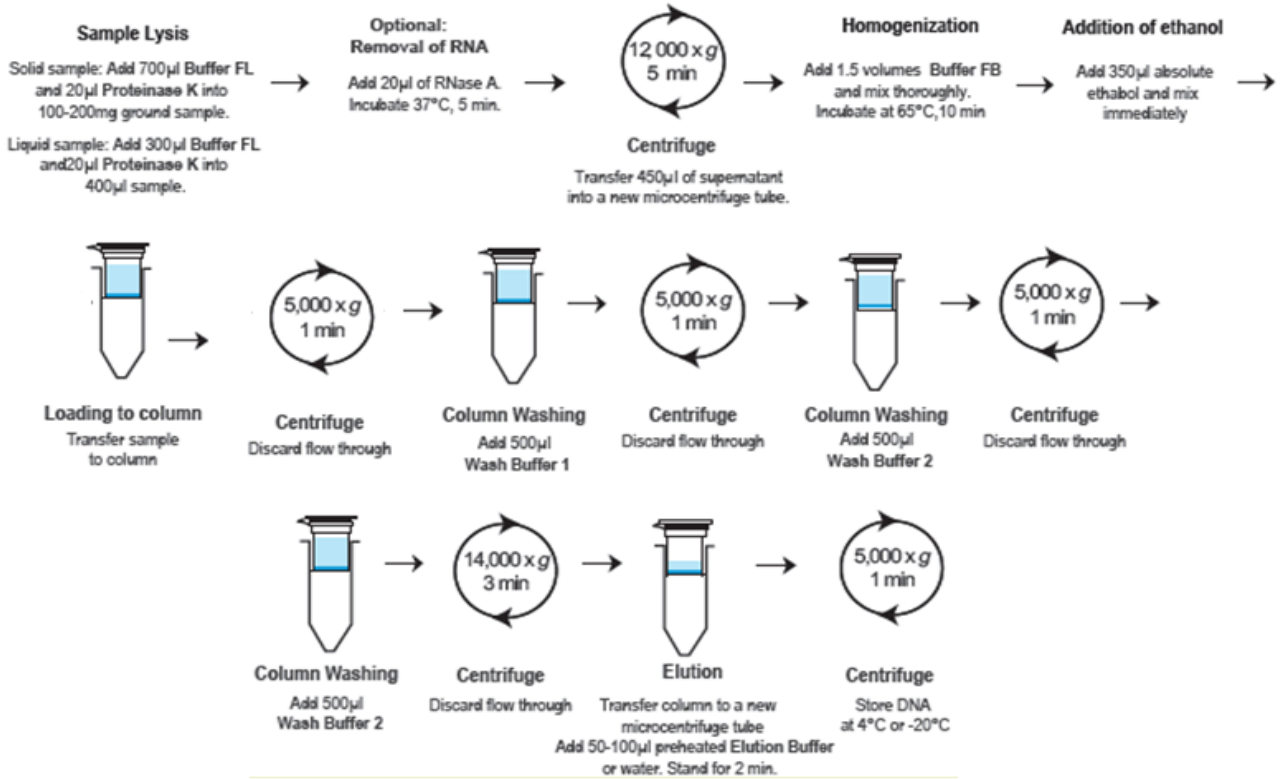
Passing Criteria

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 398bp band size.**
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

- Raw food sample – raw pork
- Processed food sample – pork sausage
- Highly processed food sample – pork cube

Protocol



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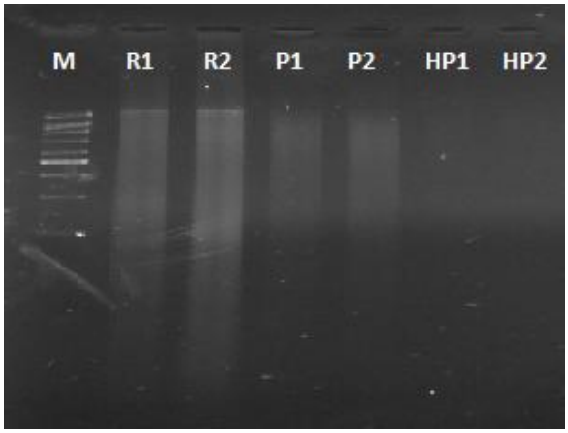
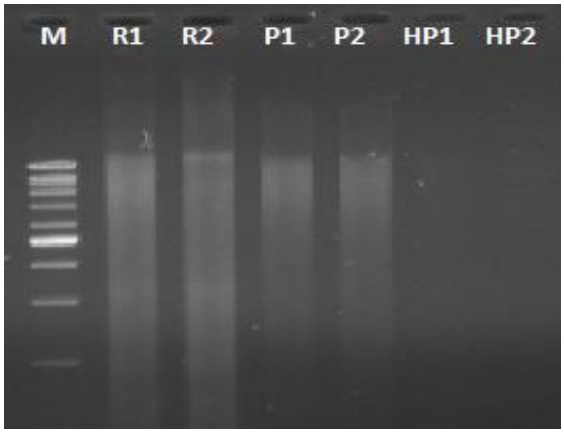
Results



Extraction using cold-stored Proteinase K



Extraction using room temp-stored Proteinase K

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

Legend:

M:	1kb DNA Ladder
R1&R2:	Extracted DNA from raw food sample (raw pork) with more than 50ng/µl
P1&P2:	Extracted DNA from processed food sample (pork sausage) with less than 10ng/µl
HP1&HP2:	Extracted DNA from highly processed food sample (pork cube) with less than 5ng/µl

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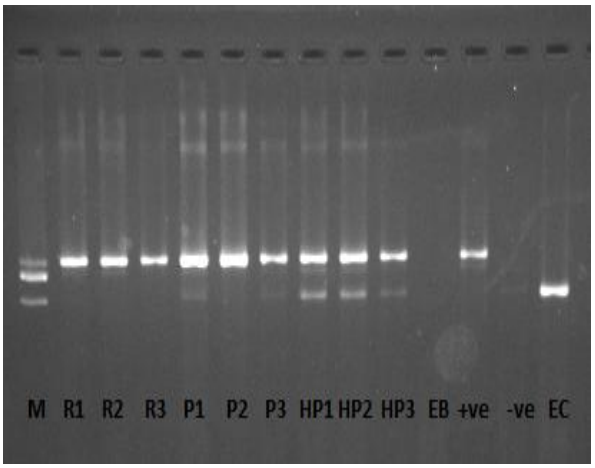
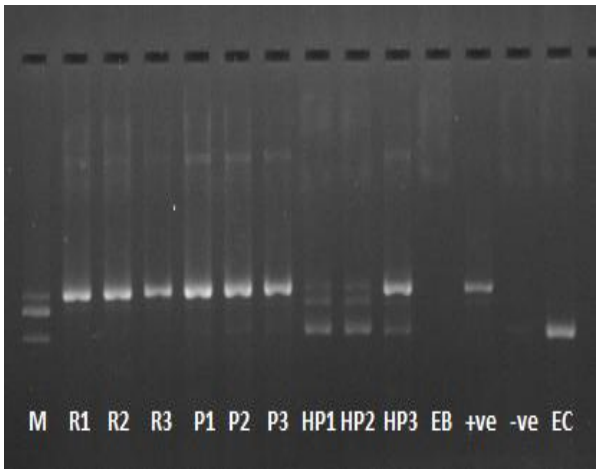
Downstream Application
Conventional PCR and real-time PCR were carried out using the extracted DNA. Both tests were performed using food specific primer.

Conventional PCR Result



Extraction using cold-stored Proteinase K



Extraction using room temp-stored Proteinase K

Figure 3&4: 5µl of the PCR products were loaded into 3% TBE agarose gel. The expected band size of PCR products were 398bp.

Legend:

M:	Porcine marker: plant-303bp; vertebrate-359bp; porcine-398bp
R1,R2,R3:	Amplification using extracted DNA (raw pork)
P1,P2,P3:	Amplification using extracted DNA (pork sausage)
HP1,HP2,HP3:	Amplification using extracted DNA (pork cube)
EB:	Amplification using elution buffer
+ve:	Amplification using pork DNA
-ve:	Amplification with no extracted DNA
EC:	Amplification with external control – plant DNA

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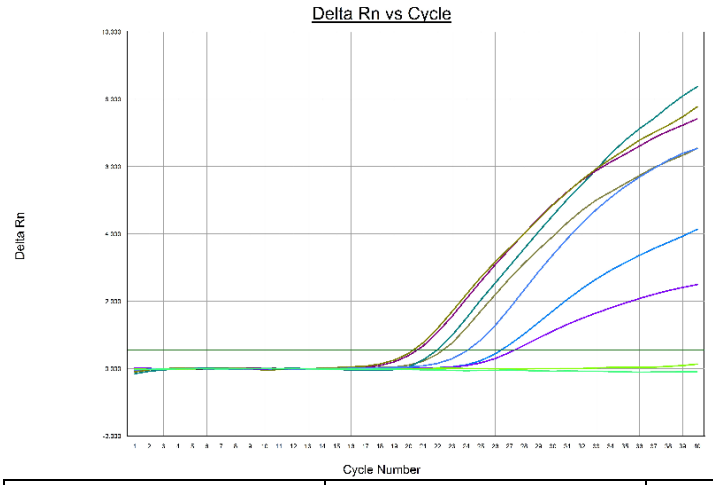
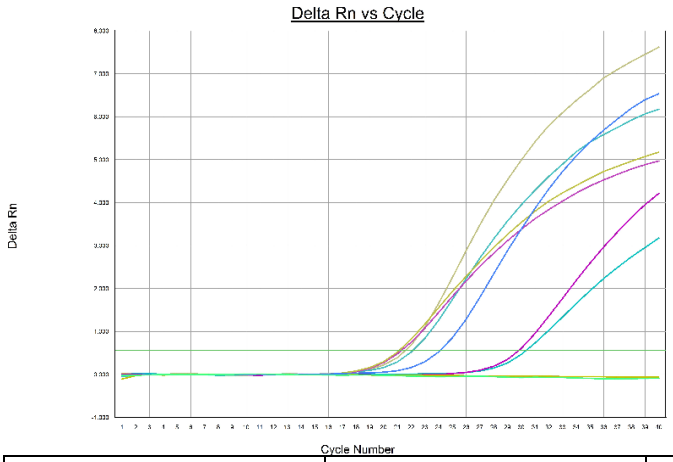
Real-time PCR Result



Graph table for amplification using extracted DNA that used cold-stored Proteinase K in extraction test.

Sample	Ct Value Sample 1	Ct Value Sample 2	Mean of Ct Values
Raw sample	22.3881	21.9988	22.1935
Processed sample	20.6016	20.3398	20.4707
Highly processed sample	26.4343	27.3721	26.9032
Positive control			24.1199
Negative control			Undet.
Elution Buffer			Undet.



Graph table for amplification using extracted DNA that used room temp-stored Proteinase K in extraction test.

Sample	Ct Value Sample 1	Ct Value Sample 2	Mean of Ct Values
Raw sample	21.5973	22.1409	21.8691
Processed sample	21.3152	21.1476	21.2314
Highly processed sample	30.3686	29.8348	30.1017
Positive control			24.1199
Negative control			Undet.
Elution Buffer			Undet.

Difference Ct value between RT and Cold – raw food sample : **0.3244**
Difference Ct value between RT and Cold – processed food sample : **0.7607**
Difference Ct value between RT and Cold – highly processed food sample : **3.1985**

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Conclusion

3 different samples were extracted using GF-1 Food 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 except for high processed food sample. The sensitivity of the conventional and real-time assay was not affected by the use of room temperature-stored Proteinase K.

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