

Comparison Test of Performance of Proteinase K Stored Cold and Stored Room Temp. Bacterial 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 bacterial DNA extraction test.

Passing Criteria

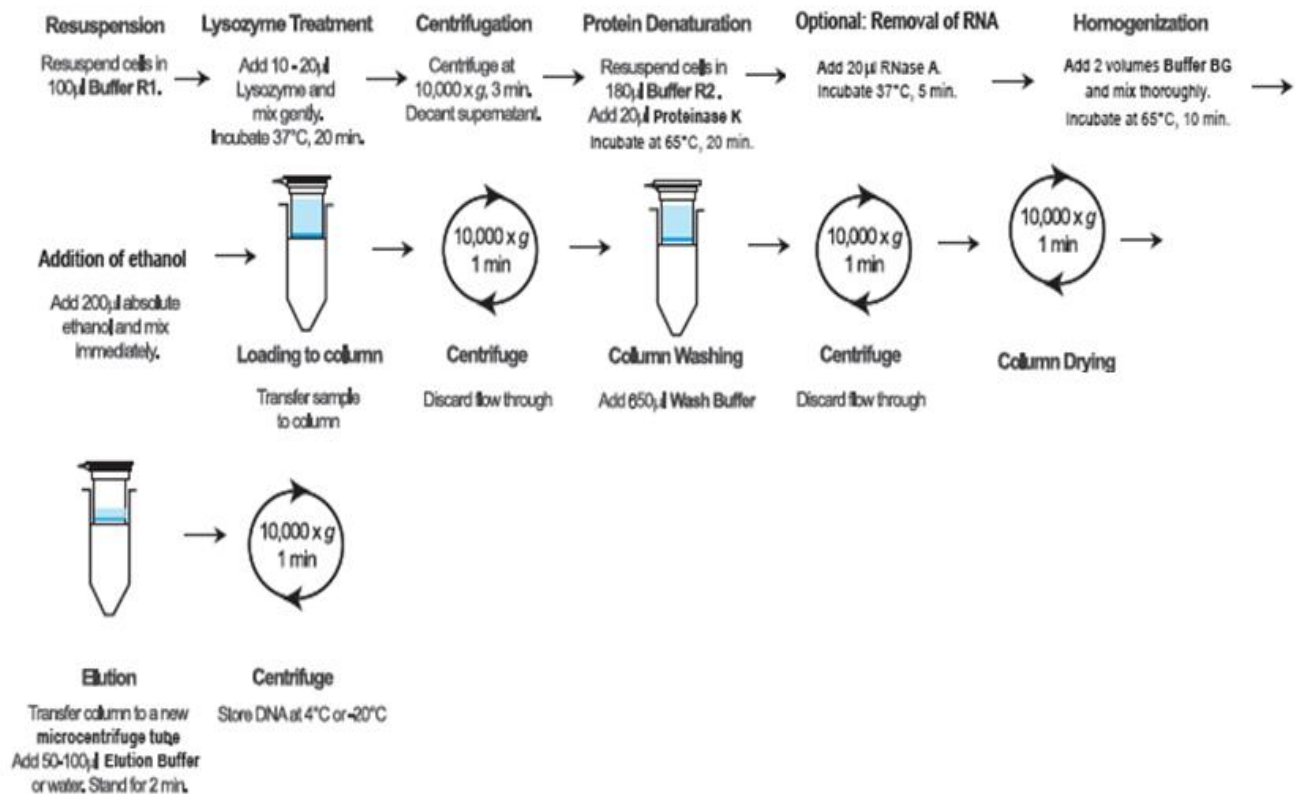
The reading of nucleic acid is detected and correspondence to absorbance value limit for A260 wavelength. Corresponding absorbance value limits for A260 is within the **range of 0.01 to 1.6 Abs** and for **A260/280 is greater than 1.7**.

The amplification of extracted DNA using conventional PCR showed **positive results with 1.5kb 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

- Bacteria TOP10F⁺
- Bacteria DH5 α
- Bacteria *Thermus aquaticus*

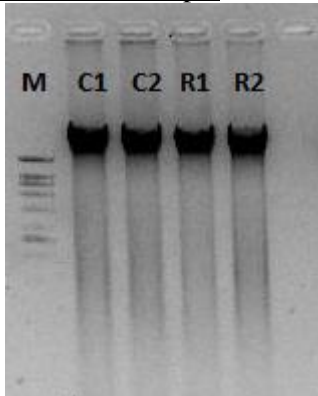
Protocol



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Results

Bacteria TOP10F' Sample



Legend:

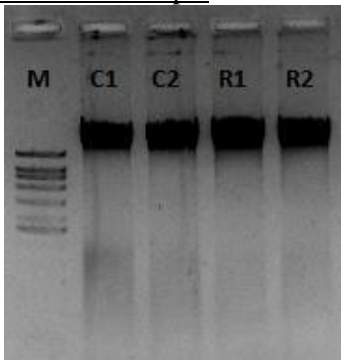
C1&C2: Extracted DNA with more than 300ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 300ng/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.

Bacteria DH5α Sample



Legend:

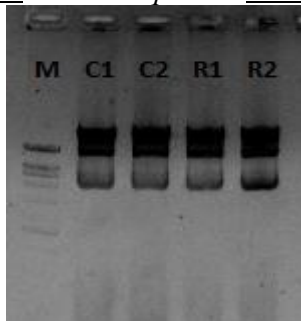
C1&C2: Extracted DNA with more than 300ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 300ng/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.

Bacteria *Thermus aquaticus* Sample



Legend:

C1&C2: Extracted DNA with more than 350ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 350ng/μl; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

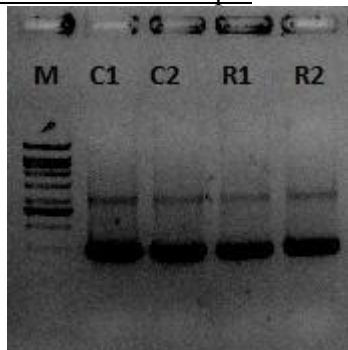
Figure 3: 2μl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

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Downstream Application

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

Bacteria TOP10F⁺ Sample



Legend:

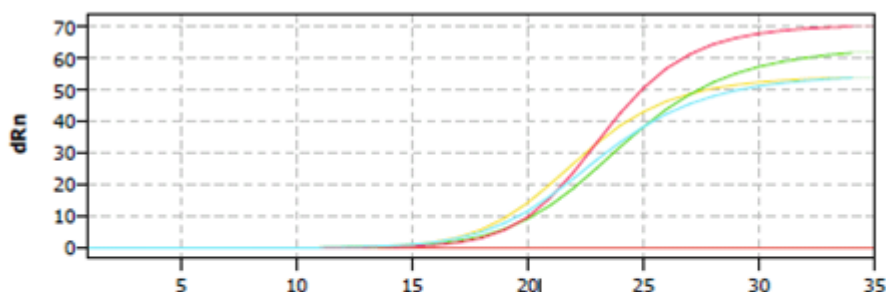
M: 1kb DNA ladder

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 1.5kb.

GOI



| Well | | Sample n | Sample t | Gene | Ct | Mean Ct | Conc. Std | Mean Co | Std.Dev. | Std.Dev. |
|------|--|-----------|----------|------|-------|---------|-----------|---------|----------|----------|
| G6 | | RT TOPF | Unknown | | 19.24 | 19.24 | | | 0 | |
| G5 | | RT TOPF | Unknown | | 19.74 | 19.74 | | | 0 | |
| G3 | | Cold TOPF | Unknown | | 18.82 | 18.82 | | | 0 | |
| G4 | | Cold TOPF | Unknown | | 19.85 | 19.85 | | | 0 | |
| G2 | | -ve | Unknown | | No Ct | | | | | |

| | |
|---|--------------|
| Mean Ct value for RT TOP10F | 19.490 |
| Mean Ct value for Cool TOP10F | 19.335 |
| Difference Ct value between RT and Cool | 0.155 |

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 0.155.

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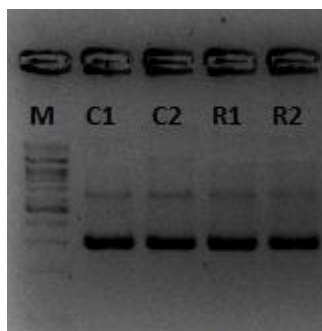
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Bacteria DH5 α Sample



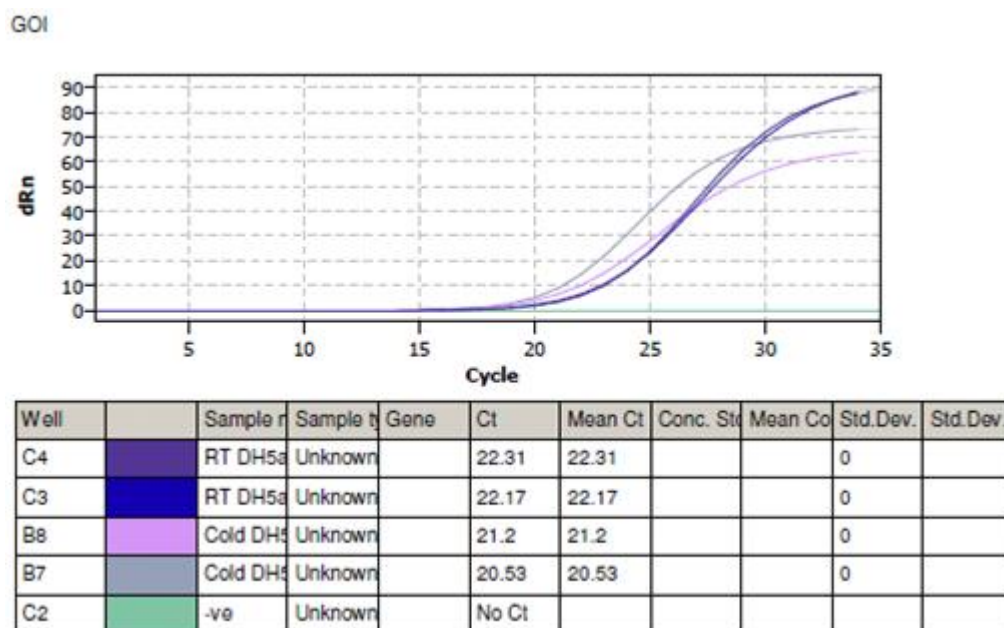
Legend:

M: 1kb 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

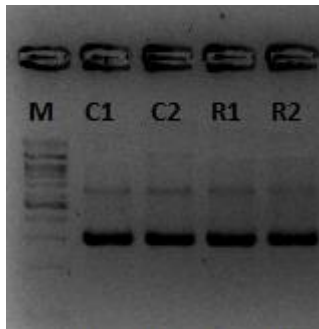
Figure 6: 2 μ l of extracted DNA was used for amplification. 5 μ l of PCR product was loaded into 1% TBE gel. The expected band size is 1.5kb.



| | |
|---|--------------|
| Mean Ct value for RT DH5 α | 22.240 |
| Mean Ct value for Cool DH5 α | 20.865 |
| Difference Ct value between RT and Cool | 1.375 |

Figure 7: 2 μ l 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 1.375.

Bacteria *Thermus aquaticus* Sample



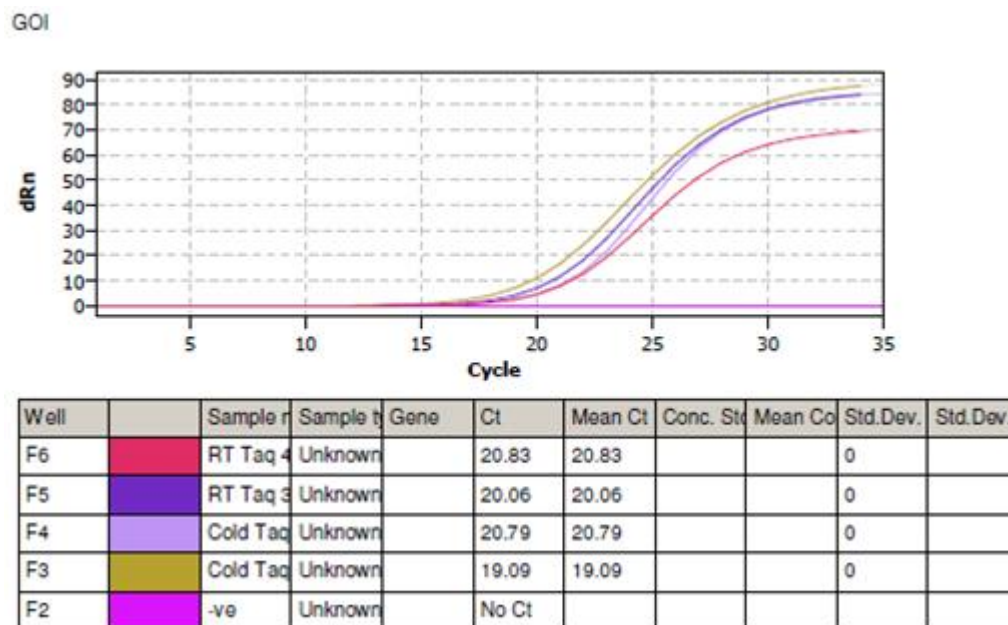
Legend:

M: 1kb 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 1.5kb.



| | |
|---|--------------|
| Mean Ct value for RT Eggplant | 20.445 |
| Mean Ct value for Cool Eggplant | 19.940 |
| Difference Ct value between RT and Cool | 0.505 |

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 0.505.



Conclusion

3 different bacteria samples were extracted using GF-1 Bacterial 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 2Ct 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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29th June 2016

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