

Comparison Test of Performance of Proteinase K Stored Cold and Stored Room Temp. Plant 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 plant 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**.

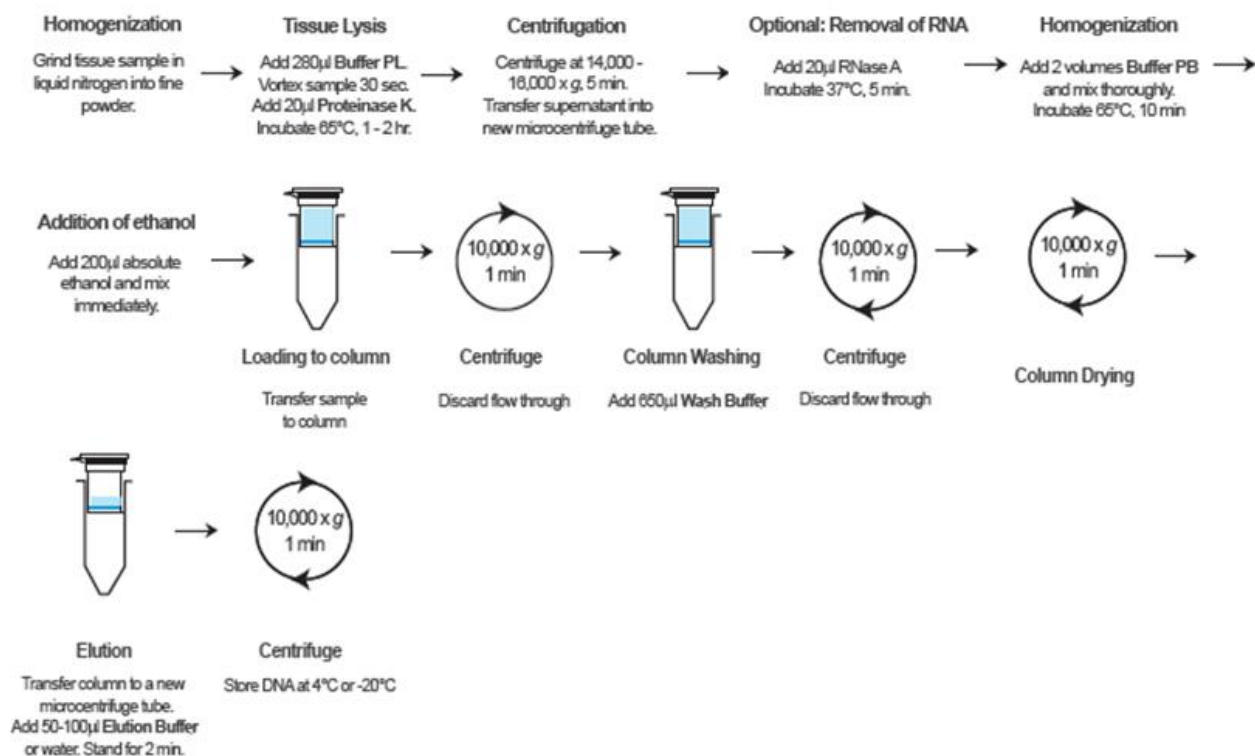
The amplification of extracted DNA using conventional PCR showed **positive results with 1kb band size**.

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

- Ironweed sample
- Tomato jelly sample
- Eggplant sample

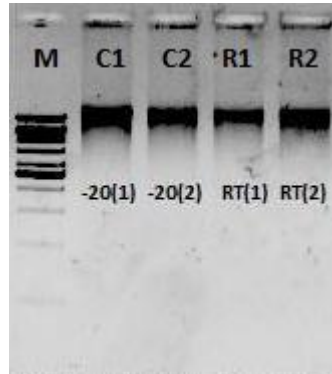
Protocol



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Results

Ironweed Sample



Legend:

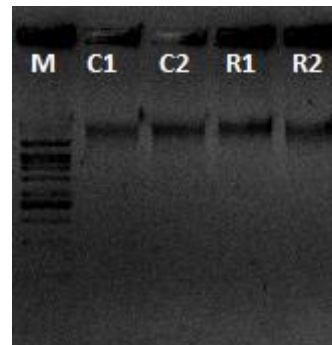
C1&C2: Extracted DNA with more than 30ng/μl; extraction using cold stored Proteinase K

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

M: VC 1kb DNA ladder

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

Tomato Jelly Sample



Legend:

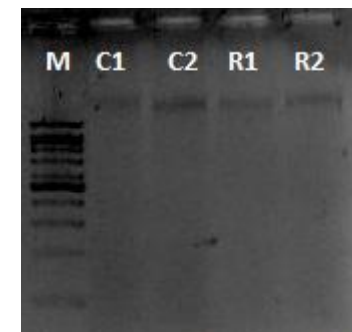
C1&C2: Extracted DNA with more than 10ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 10ng/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.

Eggplant Sample



Legend:

C1&C2: Extracted DNA with more than 60ng/μl; extraction using cold stored Proteinase K

R1&R2: Extracted DNA with more than 60ng/μl; extraction using room temperature stored Proteinase K

M: VC 1kb DNA ladder

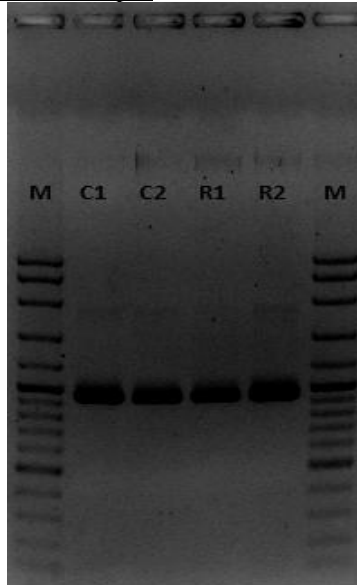
Figure 3: 2μl of the extracted DNA was loaded into 1% TBE agarose gel. The expected band size of extracted DNA is more than 10kb.

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Downstream Application

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

Ironweed Sample



Legend:

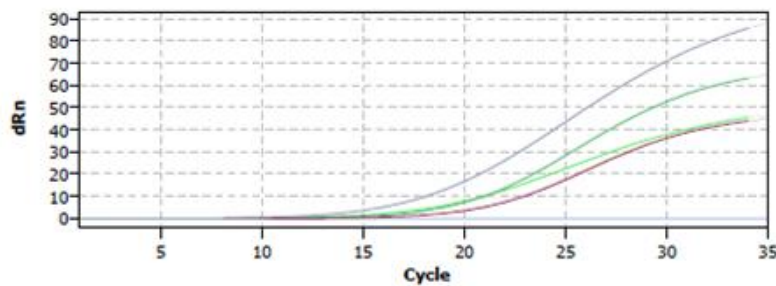
M: 100bp plus DNA ladder

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

GOI



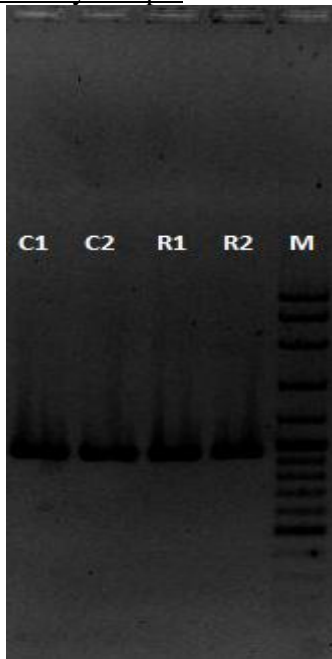
Well	Sample n	Sample t	Gene	Ct	Mean Ct	Conc. St	Mean Co	Std.Dev.	Std.Dev.
B7	RT IW 4	Unknown		18.17	18.17			0	
B6	RT IW 3	Unknown		23.02	23.02			0	
B5	Cool IW	Unknown		21.05	21.05			0	
B4	Cool IW	Unknown		21.04	21.04			0	
B3	-ve	Unknown		No Ct					

Mean Ct value for RT IW	20.595
Mean Ct value for Cool IW	21.045
Difference Ct value between RT and Cool	0.45

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 0.45.

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Tomato Jelly Sample



Legend:

M: 100bp plus DNA ladder

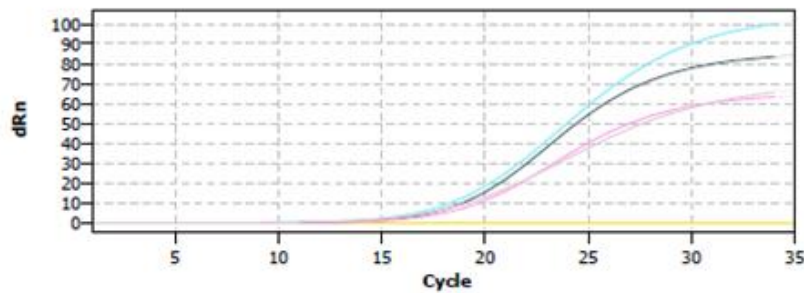
C1&C2: Amplification product using extracted DNA which used cold-stored Proteinase K in extraction

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.

GOI



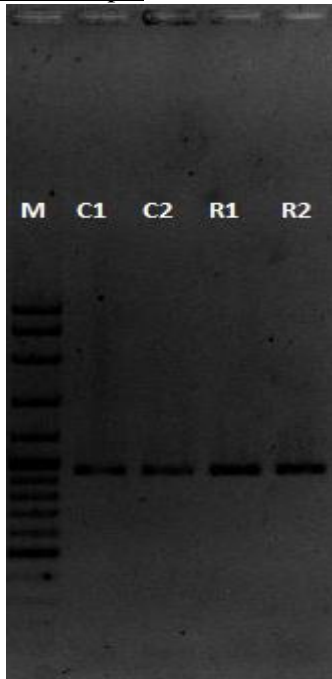
Well	Sample n	Sample t	Gene	Ct	Mean Ct	Conc. St	Mean Co	Std.Dev.	Std.Dev.
G9	RT Toma	Unknown		19.21	19.21			0	
G8	RT Toma	Unknown		19.73	19.73			0	
G7	Cool Tom	Unknown		18.98	18.98			0	
G6	Cool Tom	Unknown		18.36	18.36			0	
G3	-ve	Unknown		No Ct					

Mean Ct value for RT Tomato	19.470
Mean Ct value for Cool Tomato	18.670
Difference Ct value between RT and Cool	0.800

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 0.800.

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Eggplant Sample



Legend:

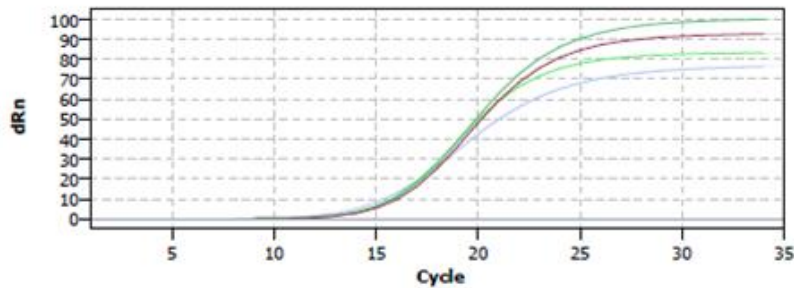
M: 100bp plus DNA ladder

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.

GOI



Well	Sample r	Sample t	Gene	Ct	Mean Ct	Conc. St	Mean Co	Std.Dev.	Std.Dev.
B6	RT E.Pla	Unknown		14.76	14.76			0	
B5	RT E.Pla	Unknown		14.49	14.49			0	
B4	Cold E.Pi	Unknown		14.46	14.46			0	
B3	Cold E.Pi	Unknown		13.91	13.91			0	
B7	-ve	Unknown		No Ct					

Mean Ct value for RT Eggplant	14.610
Mean Ct value for Cool Eggplant	14.185
Difference Ct value between RT and Cool	0.425

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 0.425.

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Conclusion

3 different plant samples were extracted using GF-1 Plant 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,
Vivantis Technical Team
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Vivantis Technologies Sdn Bhd 587389-D

Revongen Corporation Center

No12A, Jalan TP5, Taman Perindustrian UEP,
47600 Subang Jaya, Selangor Darul Ehsan, Malaysia.

E : info@vivantechnologies.com
W: www.vivantechnologies.com

T: +6 03 8025 1603
F: +6 03 8025 1637/1354