NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA2G Robust HotStart ReadyMix with Dye (KK5706)
Manufacturer : KAPA BIOSYSTEMS
Application : PCR of the difficult-to-amplify chloroplast DNA extracted from silica-dried leaves

The data shown below are published through the courtesy of Ms. Setsuko Suzuki, Ecological Genetics Laboratory, Department of Forest Genetics, Forestry and Forest Products Research Institute, Japan.

Experimental conditions

While the enzyme we have used in the past contributed to stable PCR amplification in the case of DNA extracted from raw leaves, it made PCR amplification difficult for the DNA extracted from leaves dried with silica gel.
Therefore, finding that amplification is also available for DNA obtained from dried plant samples as described in the "Product Feedback from Our Customers 2013<11>", we tried to achieve PCR amplification using KAPA2G Robust HotStart ReadyMix with Dye.

DNA extraction method: CTAB Method

We compared the enzyme manufactured by TA(other company) with KAPA2G Robust HotStart ReadyMix.

● **Reaction formula of TA(other company)**

Enzyme of TA	0.05
10×Buffer	1
dNTP Mix	0.8
Primer Forward (2μM)	0.5
Primer Reverse (2μM)	0.5
D.W.	5.15
DNA	2
Total	10μl

● **Reaction formula of KAPA2G Robust HotStart ReadyMix**

2×KAPA	5
Primer Forward (10μM)	0.5
Primer Reverse (10μM)	0.5
D.W.	0
DNA	4
Total	10μl

● **Thermal cycler**
ABI GeneAmp 9700

● **Amplification size**
matK : 850bp
trnQ-rps16 : 1060bp

● **PCR Program of TA(other company)**

94℃	3 : 00	
94℃	0 : 30	40 cycle
50℃	1 : 00	
72℃	1 : 30	
72℃	10 : 00	
4℃	forever	

Total reaction time: About 2.5 to 3 hours

● **PCR Program of KAPA2G Robust HotStart ReadyMix**

〈trnQ-rps16〉

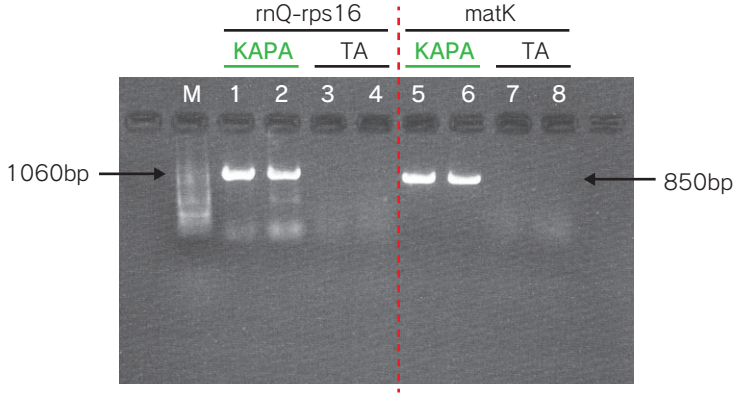
94℃	3 : 00	
94℃	0 : 15	38 cycle
58℃	0 : 15	
72℃	0 : 30	
72℃	10 : 00	
4℃	forever	

〈matK〉

94℃	3 : 00	
94℃	0 : 15	35 cycle
55℃	0 : 15	
72℃	0 : 30	
72℃	10 : 00	
4℃	forever	

Total reaction time: About an hour

Result



M 100bp Ladder

1. Sample 1	KAPA	trnQ-rps16
2. Sample 2	KAPA	trnQ-rps16
3. Sample 1	Enzyme of TA	trnQ-rps16
4. Sample 2	Enzyme of TA	trnQ-rps16
5. Sample 1	KAPA	matK
6. Sample 2	KAPA	matK
7. Sample 1	Enzyme of TA	matK
8. Sample 2	Enzyme of TA	matK

<Customer's comments>

While amplification could be well achieved with whatever Taq in the case of DNA extracted from raw leaves, when we used the DNA extracted from leaves rapidly dried with silica gel immediately after collecting them in the field, no amplification was achieved.
At that time, as we came to know from the Product Feedback of KAPA2G Robust HotStart ReadyMix (KK5706) that amplification could also be achieved by using the DNA obtained from dried samples, we tried KAPA2G Robust HotStart ReadyMix.
As a result, using KAPA kit, we succeeded in the amplification in an amazing manner. We could also obtain the base sequence without any problem.
The short reaction time (about an hour) is wonderful, too, in addition to the stable amplification.
It was also convenient for agarose gel electrophoresis that the kit originally had loading dye as one of its components.

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