Cat. # R074A

For Research Use

TaKaRa

MightyAmp™ Genotyping Kit

Product Manual

v201807Da

Table of Contents

I. Description

The MightyAmp Genotyping Kit offers a simple method of DNA extraction and PCR amplification for animal tissues (e.g., mouse tail), and plant tissues.
The Extraction Buffer in combination with Proteinase K included in the kit allow direct DNA extraction from plant and animal tissues. MightyAmp DNA Polymerase enables analysis of a wide range of target DNA (regardless of GC composition) from crude extracts containing PCR-inhibiting compounds that cause difficulty when using conventional PCR polymerases. MightyAmp DNA Polymerase is well suited for amplification of DNA up to approximately 2 kb in length, and shows good yield even with small template quantities. The 5X Loading Dye (included) is formulated to reduce the effect of crude-sample-derived contaminants, and facilitates rapid, easy analysis of PCR products by agarose gel electrophoresis.

MightyAmp DNA Polymerase is designed for hot-start PCR. It includes monoclonal antibodies to inhibit polymerase activity at temperatures up to 98℃, thereby preventing nonspecific amplification.

II. Components (50-μl reactions, for 200 reactions)

(1)	MightyAmp DNA Polymerase (1.25 U/μl)	200 μl
(2)	2X MightyAmp Buffer (Mg^{2+}, dNTP plus)*	1.25 ml x 4
(3)	5X Loading Dye	1 ml x 3
–	Extraction Buffer	20 ml
(5)	Proteinase K	200 μl

* Mg^{2+} concentration is 4 mM in 2X buffer. Each dNTP is provided at 800 μM in 2X buffer.

III. Storage

All other components : -20 ℃
Extraction Buffer : Room temperature (15 - 25℃)

IV. Primer Design

We recommend designing primer sequences using software such as OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.).
Select primers with Tm values > 60℃, where the Tm is calculated using the following formula:

Tm (℃) = [(the number of As and Ts) x 2] + [(the number of Gs and Cs) x 4] - 5

V. Protocol

V-1. DNA extraction from tissue samples

Perform the procedure at room temperature. A thermal cycler may be used for the reaction.

1. Add the tissue sample＊1 to a PCR tube containing 100 μl of Extraction Buffer.
   Note: If the Extraction Buffer forms a precipitate, heat (up to 60℃) and stir gently to dissolve the precipitate.
2. Add 1 μl of Proteinase K to the tube.
3. After incubating for 5 minutes at 60℃, heat at 98℃ for 2 minutes, then cool to room temperature＊2.
4. Centrifuge briefly at room temperature. Use the supernatant (Extraction Buffer Extract) as template for PCR＊3.

＊1 Guidelines for sample amounts for various tissue types:
- Mouse tail ≦ 2 mm
- Mouse ear ≦ 5 mm^2
- Mouse organs ≦ 30 mm^3
- Plant leaves ≦ 5 mm^2

＊2 Because a precipitate forms at 4℃, keep at room temperature (about 25℃) during the procedure.

＊3 For storage, transfer the supernatant to a new tube. Store at -20℃. After returning to room temperature, confirm the absence of a precipitate before using as template for PCR.

V-2. PCR reaction composition

Reagents	Amount	Final conc.
2X MightyAmp Buffer	25 μl	1X
Primer 1	15 pmol	0.3 μM
Primer 2	15 pmol	0.3 μM
Extraction Buffer Extract＊4	≦ 2.5 μl	
MightyAmp DNA Polymerase	1 μl	1.25 U/50 μl
Sterile purified water	up to 50 μl	
Total	50 μl	

＊4 Use the extract at room temperature. In addition, we recommend that the volume of extract used for PCR not exceed 1/20 of the total reaction volume. Place other reagents on ice while performing the procedure.

V-3. PCR conditions

Standard conditions: 3-step PCR with an elongation temperature of 68℃.

[3-step PCR]

98℃	2 min ＊5	
↓		
98℃	10 sec	30 - 40 cycles
60℃	15 sec	
68℃	1 min/kb	

[Optional: 2-step PCR]

98℃	2 min ＊5	
↓		
98℃	10 sec	30 - 40 cycles
68℃	1 min/kb	

＊5 Because hot-start antibodies are included, perform the initial denaturation for 2 minutes at 98℃ to inactivate the antibodies.

V-4. Electrophoresis analysis

After mixing the 5X Loading Dye (included) and PCR solution at a ratio of 1 : 4, load the samples in an agarose gel and perform electrophoresis.
When performing electrophoresis on PCR products amplified using MightyAmp DNA Polymerase, we recommend the use of TAE Buffer. When TBE buffer is used, the migration pattern may be distorted.

VI. 3'-A Overhang of PCR Products

Almost all PCR products amplified with MightyAmp DNA Polymerase include single A nucleotide added at the 3' terminal end. This facilitates direct cloning of amplification products into T-vectors (e.g., pMD20: Cat. #3270, pMD19 (Simple): Cat. #3271). In addition, cloning into a blunt-ended vector is also possible by first blunting and phosphorylating with the Mighty Cloning Reagent Set (Blunt End) (Cat. #6027).

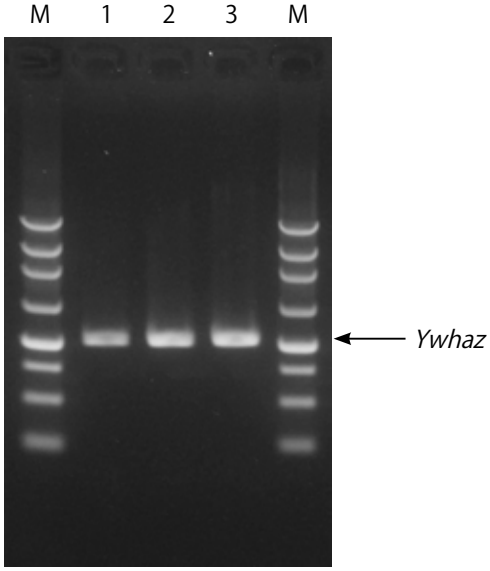
VII. Experimental Example

An example is shown below of PCR amplification using DNA extracted from various biological tissues.

1. DNA extraction and PCR amplification from mouse tail

[Method]
DNA was extracted from approximately 2-mm mouse tail tips, according to the protocol in Section V-1, "DNA extraction from tissue samples." After centrifugation, aliquots of the supernatant were added to 50-μl PCR mixes and PCR amplification of a murine *Ywhaz* gene about 1 kb in length was performed. 4 μl of each PCR solution was mixed with 5X Loading Dye (included) and analyzed by electrophoresis.

[Results]
The target gene amplified well from mouse tail using the MightyAmp Genotyping Kit.



PCR conditions

98℃	2 min	
↓		
98℃	10 sec	] 30 Cycles
60℃	15 sec	
68℃	1 min	

Extract volumes for PCR

1. 1 μl of extract (1/50 of the PCR)
2. 2.5 μl of extract (1/20 of the PCR)
3. 5 μl of extract (1/10 of the PCR)*

M. 250 bp DNA Ladder

* We recommend that the extract volume used for the PCR not exceed 1/20 of the total PCR volume.

Figure 1. PCR amplification from mouse tail.

2. DNA extraction and PCR amplification from plant leaf

[Method]
DNA was extracted from 2-mm diameter tomato leaf samples according to the protocol in Section V-1, "DNA extraction from tissue samples." After centrifugation, aliquots of the supernatant were added to 50-μl PCR mixes and PCR amplification of a tomato *cox1* gene about 1 kb in length was peformed. 4 μl of each PCR solution was mixed with 5X Loading Dye (included) and analyzed by electrophoresis.

[Results]
The target gene amplified well from tomato leaf using the MightyAmp Genotyping Kit.

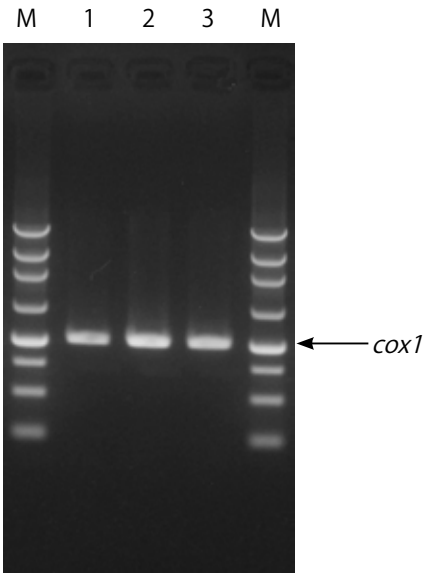


Figure 2. PCR amplification from tomato leaf.

PCR conditions

98℃	2 min	
↓		
98℃	10 sec	30 Cycles
60℃	15 sec	
68℃	1 min	

Extract volumes for PCR

1. 1 μl of extract (1/50 of the PCR)
2. 2.5 μl of extract (1/20 of the PCR)
3. 5 μl of extract (1/10 of the PCR)＊

M. 250 bp DNA Ladder

＊ We recommend that the extract volume used for the PCR not exceed 1/20 of the total PCR volume.

VIII. Troubleshooting

Observation	Possible cause	Solutions
• No amplification • Inefficient amplification	Primer Tm	Design primers according to Section IV, "Primer Design"
	2-step PCR	Try 3-step PCR
	3-step PCR	Try 2-step PCR (Amplification may be improved by using 2-step PCR instead of 3-step PCR.)
	Annealing temperature	Try varying annealing temperature by conducting trials and lowering in 2℃ increments per trial
	Cycle number	Increase the number of cycles (up to 40 cycles)
	Sample quantity and preparation method	• Increase or decrease the volume of extract added to the PCR solution • Increase or decrease the quantity of tissue sample used for the extract
Nonspecific amplification	Primer Tm	Design primers according to Section IV, "Primer Design"
	3-step PCR	Try 2-step PCR
	Cycle number	Set the number of cycles to 25 - 30 cycles.

IX. Related Products

MightyAmp™ DNA Polymerase Ver.3 (Cat. #R076A/B)＊
MightyAmp™ DNA Polymerase Ver.2 (Cat. #R071A/B)＊
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)＊
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)
Agarose L03 [TAKARA] (Cat. #5003/B)
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)
T-Vector pMD20 (Cat. #3270)
T-Vector pMD19 (Simple) (Cat. #3271)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)

＊ Not available in all geographic locations. Check for availability in your area.

MightyAmp, Thermal Cycler Dice, and PrimeGel are trademarks of Takara Bio Inc.