Cat. # R074A

For Research Use

TaKaRa

MightyAmp® Genotyping Kit

Product Manual

v201303Da

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I. Description

The MightyAmp Genotyping Kit offers a simple method of DNA extraction and PCR amplification for animal tissues, such as 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. PCR performed using MightyAmp DNA Polymerase, which facilitates amplification of DNA from crude samples, 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 amplification 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 non-specific amplification.

II. Kit Components

One kit provides enough reagents for 200 reactions ＊1.

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

＊1 : Used at a reaction volume of 50 μl.
＊2 : Mg^{2+} concentration is 4 mM (2X) and dNTP concentration is 800 μM each (2X).

III. Storage

All components except Extraction Buffer : –20℃
Extraction Buffer : Room temperature (about 25℃)

IV. Primer Design

We recommend selecting primer sequences using primer design software such as OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.).

Choose primers with Tm values not less than 60℃, where Tm is calculated using the following formula:

Tm value (℃) = [(nA + nT) x 2] + [(nC + nG) x 4] – 5

For MightyAmp DNA Polymerase, do not use primers containing inosine.

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 (20 mg/ml) 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: Guideline 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: Since a precipitate forms at 4℃, keep at room temperature (about 25℃) during the procedure.

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

V-2. PCR reaction composition (50 μl reaction volume)

	Amount	Final concentration
2X MightyAmp Buffer	25 μl	1X
Primer 1	15 pmol	0.3 μM
Primer 2	15 pmol	0.3 μM
Extraction Buffer Extract＊	≦ 2.5 μl	
MightyAmp DNA Polymerase	1 μl	1.25 U/50 μl
Sterilized water	up to 50 μl	

＊: 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 elongation temperature of 68℃.

［3 step PCR］

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

［Option：2 step PCR］

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

＊: Since 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 in kit) and PCR reaction solution at a ratio of 1:4, load 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 using MightyAmp DNA Polymerase include 1 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, etc.). In addition, cloning into a blunt-ended vector is also possible by 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.

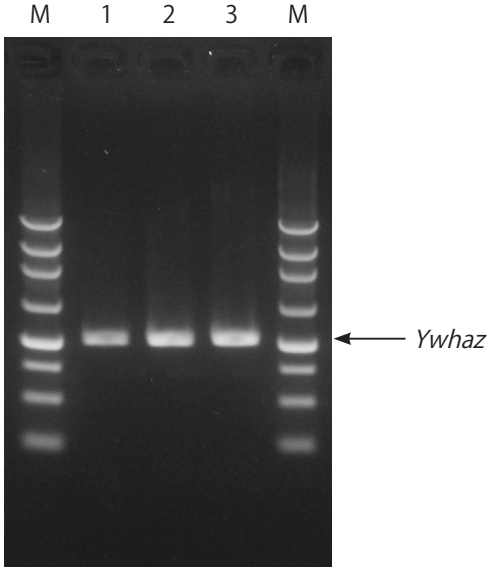
1. DNA extraction from mouse tail and PCR amplification

Method:

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

Results:

The target gene was amplified well from mouse tail using the MightyAmp Genotyping Kit.



PCR conditions

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

Extract volumes for PCR reactions

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

M. 250 bp DNA Ladder

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

Fig. 1. PCR amplification from mouse tail

2. DNA extraction from plant leaf and PCR amplification

Method:

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

Results:

The target gene was amplified well from tomato leaf using the MightyAmp Genotyping Kit.

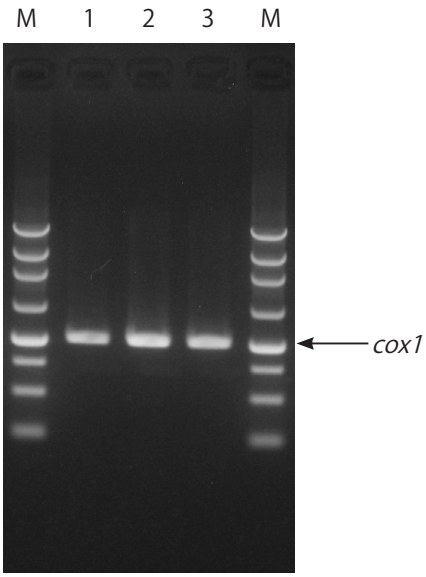


Fig. 2. PCR amplification from tomato leaf

PCR conditions

98℃	2 min.	
↓		
98℃	10 sec.	⎫
60℃	15 sec.	⎬ 30 Cycles
68℃	1 min.	⎭

Extract volumes for PCR reactions

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

M. 250 bp DNA Ladder

＊: We recommend that the extract volume used for the PCR reaction not exceed 1/20 of the PCR reaction 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 reaction solution • Increase or decrease the quantity of tissue sample used for the extract
Non-specific 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.2 (Cat. #R071A)
MightyAmp® for Card (Cat. #R072A)
MightyAmp® for FFPE (Cat. #R073A/B)
TaKaRa PCR Thermal Cycler Dice® Gradient/Standard (Cat. #TP600/TP650)
Agarose L03 [TAKARA] (Cat. #5003/5003B)
T-Vector pMD20 (Cat. #3270)
T-Vector pMD19 (Simple) (Cat. #3271)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)

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[L15] Hot Start PCR
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