EzWay™ Mouse Tail Direct PCR Kit

1. Catalog No. K0568600

2. No. of Applications 1 Set
40 reactions at 50ul PCR Volume / 80 reactions at 25ul PCR volume

3. Storage 1 year at -20°C, 1 month at 4°C
(The product is able to be shipped on blue ice and should be stored immediately at -20°C.)

4. Contents

Component	Cat. No	Volume
2X EzWay™ Direct Taq PCR MasterMix	K0568010	1.0 ml
2.5X Mouse Tail Direct Lysis Buffer	K0568610	1.5 ml X 3
4X Magic Buffer	K0561031	1.0 ml
Positive Control	-	20 ul
Control Primer	-	20 ul

Note:

a. MasterMix and Magic Buffer only can be purchased separately.
b. Positive Control is the mixture of the plasmid containing the part of the mouse tyrosinase gene and 1X Lysis Buffer, therefore it can be amplified by the combination of MasterMix and Control Primer to ensure all reagents are working properly.
c. Control primer amplifies 298bp of the mouse tyrosinase gene.

5. Description

EzWay™ Mouse Tail Direct PCR Kit enables DNA amplification directly from a mouse tail without DNA extraction. Just cut the mouse tail and incubate it in the lysis buffer provided in the kit for 10 minutes and then use 1ul of lysate for PCR. It doesn't require overnight incubation or Proteinase K (PK) treatment.

- Genotyping of transgenic mouse using tail or ear tissue
- Fast and simple method for genotyping without DNA extraction
- Just 10 minutes incubation required
- No need to add Proteinase K
- Saving enormous amounts of material and time
- Optimized MasterMix type containing EzWay™ Taq PCR enzyme, dNTP, Direct PCR Buffer, $MgCl_2$, Red dye and additives
- Direct loading of PCR products without adding red dye

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Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

6. Sample Treatment

1. Dilute the 2.5X Mouse Tail Direct Lysis Buffer into 1X concentration with DW. (For example, to make 100 ul of 1X Lysis Buffer, add 40 ul of Lysis Buffer to 60 ul DW.)

   Note:
   a. Due to high viscosity of 2.5X Lysis Buffer, it should be handled with care.
   b. 1X Lysis solution should be made freshly before use.
   c. If starting material is not enough, reduce lysis volume proportionally.

2. Cut mouse tail with 1-2 mm length (or 5-10 mg), and add 100ul of diluted 1X Lysis Buffer. Incubate the tube at 60°C for 10 min in water-bath/rotary hybridization oven or PCR thermal cycler (Vortex at times will be helpful for lysis). The mouse tail may not be completely digested for 10 min incubation, but DNA molecules from the tail tissue are released.

   Note:
   a. Mouse tail lysate can be stored for 1 year at -20°C (or 1 month at 4°C) without any loss of effectiveness.
   b. For rat tail, weigh the rat tail cut, and then use roughly calculated volume of lysis solution proportional to mouse scaled weight.

3. Vortex and spin down briefly.

4. Directly use 1ul supernatant of crude lysate per 20 ul PCR reaction.

   Note:
   a. Mouse Tail Direct Lysis Buffer contains highly concentration EDTA and detergents, so higher amounts of lysate may affect the ability of the buffer to neutralize the inhibitor

7. PCR Amplification

1. Prepare the Mix according to the table below.

Component	Final Concentration	Volume/reaction	
2X EzWay™ Direct Taq PCR MasterMix	1X	12.5 ul	25 ul
5' Primer	0.1 - 0.5 uM	Variable	Variable
3' Primer	0.1 - 0.5 uM	Variable	Variable
4X Magic Buffer	0.-25% (v/v)	0-5 ul	0-10 ul
Distilled water	-	Variable	Variable
Template	-	~1 ul	1-2 ul
Total reaction volume		**25 ul**	**50 ul**

Note:
a. EzWay™ Direct Taq PCR MasterMix contains 3.0 mM $MgCl_2$ (final 1.5 mM $MgCl_2$). Generally, 1.5 mM $MgCl_2$ may give satisfactory PCR results, but for higher $MgCl_2$ than 1.5 mM, add $MgCl_2$ separately.
b. Magic Buffer will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure. We recommend that the volume added should not exceed 25 % (v/v) of final PCR volume.

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2. Mix gently.

3. When using a thermal cycler without a heated lid, add approximately 100ul of mineral oil on top of the mixture.

4. Perform thermal cycling.

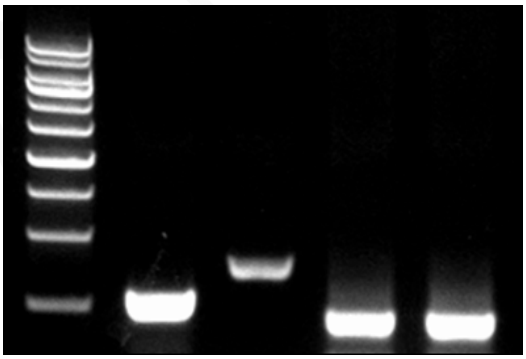
Step		Temp.	Time	Cycles
Initial Denaturation		95°C	2-5 min	1
Cycling	Denaturation	94°C	0.5-1 min	35-45
	Annealing	50-68°C	0.5-1 min	
	Extension	72°C	1-5 min (~1kb/imin)	
Final Extension		72°C	10 min	1

Note:
a. Primers should be 15 to 30 bases in length and near 50% G+C content.
b. Magic Buffer will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure. We recommend that the volume added should not exceed 25 %(v/v) of final PCR volume.
c. If chemically modified hot start Taq PCR enzyme is used, the best initial denaturation time is 10-15 minutes at 95°C.

5. The amplified DNA can be detected by various electrophoresis techniques. The most common techniques are agarose or polyacrylamide gel electrophoresis (**TAE gel/buffer system**) depending on the size of the amplicon.

   Note: Do not use TBE buffer because of band smearing and poor resolution.

8. Data



WT KO WT KO

X Gene GAPDH

PCR was performed with 1 ul mouse tail lysate in 25 ul EzWay™ Direct Taq PCR MasterMix.
Lane 1 & 3 was lysate from WT mouse and lane 2 & 4 was lysate from KO mouse

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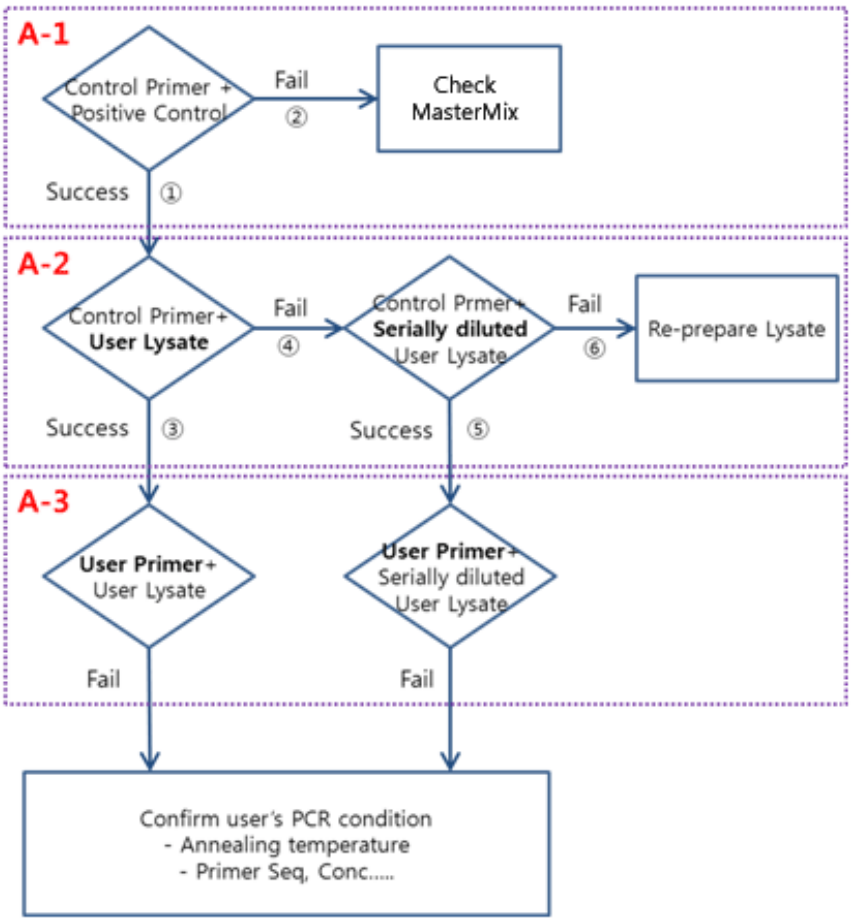
9. Troubleshooting

If the target amplification were failed, check PCR mixture, user's lysate and primers sequentially as the following steps.

Fig 1. Troubleshooting checking guide



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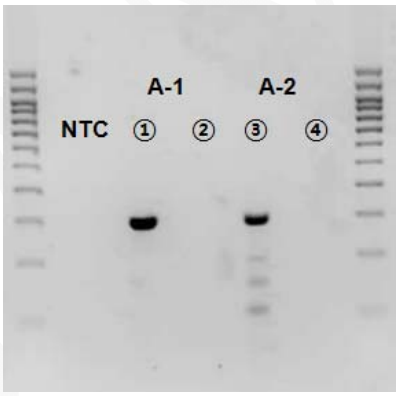
1. Perform the following experiment to confirm the performance of **Direct Taq PCR MasterMix** and/or **user's mouse tail lysate**.

A-1		A-2	
Component	Volume/reaction	Component	Volume/reaction
Direct PCR Mix	12.5 ul	**Direct PCR Mix**	12.5 ul
Primer mix		**Primer mix**	
25x Control Primer	1 ul	25x Control Primer	1 ul
Distilled water	10.5 ul	Distilled water	10.5 ul
Template		**Template**	
Positive Control	1 ul	User's mouse tail lysate	1 ul
Total volume	25 ul	Total volume	25 ul

Step		**Temp.**	**Time**	**Cycles**
Initial Denaturation		95°C	5 min	1
Cycling	Denaturation	95°C	30 sec	35
	Annealing	50°C	30 sec	
	Extension	72°C	30 sec	
Final Extension		72°C	7 min	1

2. Run the PCR products at 2% agarose gel electrophoresis (TAE- is recommended). 298bp size band will be detected with EtBr staining.



1) If no band were seen at A-1 (② in the Fig 1.), Direct Taq PCR MasterMix might have a problem. Check the expiry date and storage condition of the kit.

2) If A-1 is normal, but there is no band at A-2 (④ in the Fig 1), **Mouse tail lysate** might be one of the causes. **Then, Make a serial dilution** of the mouse tail sample and repeat A-2 experiment. If failed (⑥ in the Fig 1), **make the lysate freshly** and repeat A-2 experiment.

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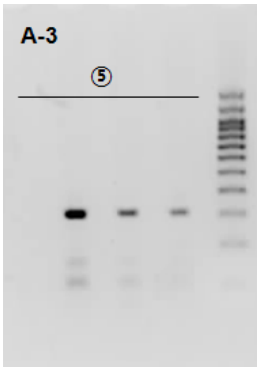

3) If band were seen at A-2 (③ & ⑤), proceed A-3 experiment with the following experiment to confirm whether **user's primer is workable or not.**

A-3	
Component	Volume/reaction
Direct PCR Mix (validated)	12.5 ul
Primer mix	
User's primer	0.1~0.5μM final
Distilled water	10.5 ul
Template	
User's mouse tail lysate (validated)	1 ul
Total volume	25 ul

* Perform the proper PCR cycle for user's primer or determine the appropriate PCR condition.

4) Nevertheless, if no band were seen at A-3 experiment, doubt user's primer itself, especially annealing temperature or cycle number. Therefore, increasing the cycle number or fitting the annealing temperature by gradient PCR is highly recommended.

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10. Related Products

Cat. No.	Product	Size
K0568001	EzWay™ Direct PCR Buffer (5X)	500 ul
K0568002	EzWay™ Direct PCR Buffer Set	1 Set
K0568010	EzWay™ Direct Taq Master Mix (2X)	1 ml
K0568020	EzWay™ Direct Taq Master Mix w/o dye (2X)	1 ml
K0568210	EzWay™ Direct Hot Taq PCR MasterMix	1 ml
K0568500	EzWay™ Direct ApoE Genotyping Kit	50 Tests
K0568700	EzWay™ Plant Direct PCR Kit	1 Kit

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