EzWay™ Plant Direct PCR Kit

1. Catalog No. K0568700

2. No. of Applications 1 Set
40 reactions at 50ul PCR Volume / 80 reactions at 20ul 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 Plant Direct Lysis Buffer		1.5 ml
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 plant chloroplast 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 301bp of the plant chloroplast gene.

5. Description

EzWay™ Plant Direct PCR Kit enables DNA amplification directly from plant leaves without DNA extraction. Punch to isolate disks of leaf tissue and incubate it in the lysis buffer provided in the kit for 10 minutes and then use 1ul of lysate for PCR. Or just leaf itself (when using 0.5 mm punched leaf disc) can be used as a template directly without lysis step. Direct Taq PCR MasterMix in this kit is a convenient mixture of Taq enzyme, dNTP, $MgCl_2$, dye, additives and Direct PCR Buffer, so you can just add the leaf extract and primers, and then go for PCR. This kit will save your enormous amounts of material, cost and time from conventional PCR methods using mechanical disruption, organic extraction, column DNA purification, or alcohol precipitation. Also, it reduces risk of handling errors and contamination.

- Amplification of genomic DNA from plant leaves without DNA extraction
- Just 10 minutes incubation required
- No need to add Proteinase K
- Simple and fast PCR procedure requiring minimal handling
- 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

6. Sample Treatment

[Direct Method]

1. Using Harris Uni-Core punch (or Harris Micro punch), cut leaf disc from plant and add one piece to 50 ul PCR reaction directly without lysis, and vortex briefly. Although you may use any size of disc between 0.5 mm and 2 mm, we recommend the 0.5 mm disc.

[Lysis Method]

1. Determine the total number of samples and calculate the required amount of lysis solution. Dilute 2.5X Plant Direct Lysis Buffer with autoclaved water to make final 1X lysis solution (For example, to make 20 ul of 1X Lysis Buffer, add 8 ul of Lysis Buffer to 16 ul DW.), and dispense 20 ul of 1X lysis solution to each tube.

   Note:
   a. Due to high viscosity of 2.5X Plant Direct 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. Take small piece from the plant leaf using 2 mm Harris Uni-Core punch (or Harris Micro punch) or scalpel, and put into tube containing 20 ul of 1X lysis solution. Vortex and incubate the tubes at 85°C for 10 min. The plant leaf may not be completely digested for 10 min incubation, but DNA release is sufficient for PCR.

   Note:
   a. Plant leaf lysate can be stored for 1 year at -20°C (or 1 month at 4°C) without any loss of effectiveness.
   b. If the puncher or scalpel is to be reused, must clean the cutting edge to prevent cross contamination.

3. Vortex and spin down briefly.

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

   Note:
   a. Plant 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.

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℃	5 min	1
Cycling	Denaturation	94℃	0.5-1 min	35-45
	Annealing	50-68℃	0.5-1 min	
	Extension	72℃	1-5 min (~1kb/imin)	
Final Extension		72℃	10 min	1

Note:

a. Primers should be 15 to 30 bases in length and near 50% G+C content.

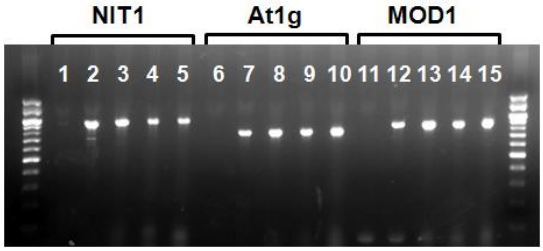
b. Magic Buffer will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure. We recommend that the volume added should not exceed 25 %(v/v) of final PCR volume.

c. If chemically modified hot start Taq PCR enzyme is used, the best initial denaturation time is 10-15 minutes at 95°C.

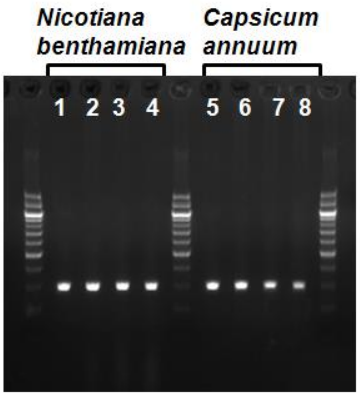
5. The amplified DNA can be detected by various electrophoresis techniques. The most common techniques are agarose or polyacrylamide gel electrophoresis (**TAE gel/buffer system**) depending on the size of the amplicon.

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

8. Data



PCR was performed with 1 ul *Arabidopsis* leaf lysate using the 1X Plant Direct Lysis Buffer solution in 20 ul PCR. And, pieces of *Arabidopsis* leaf with 2 mm Harris Punch were placed directly into 20 ul PCR.
Lane 1, 6 and 11: negative control
Lane 2, 7 and 12: 1uL lysate
Lane 3, 8 and 13: one piece,
Lane 4, 9 and 14: two pieces
Lane 5, 10 and 15: three pieces

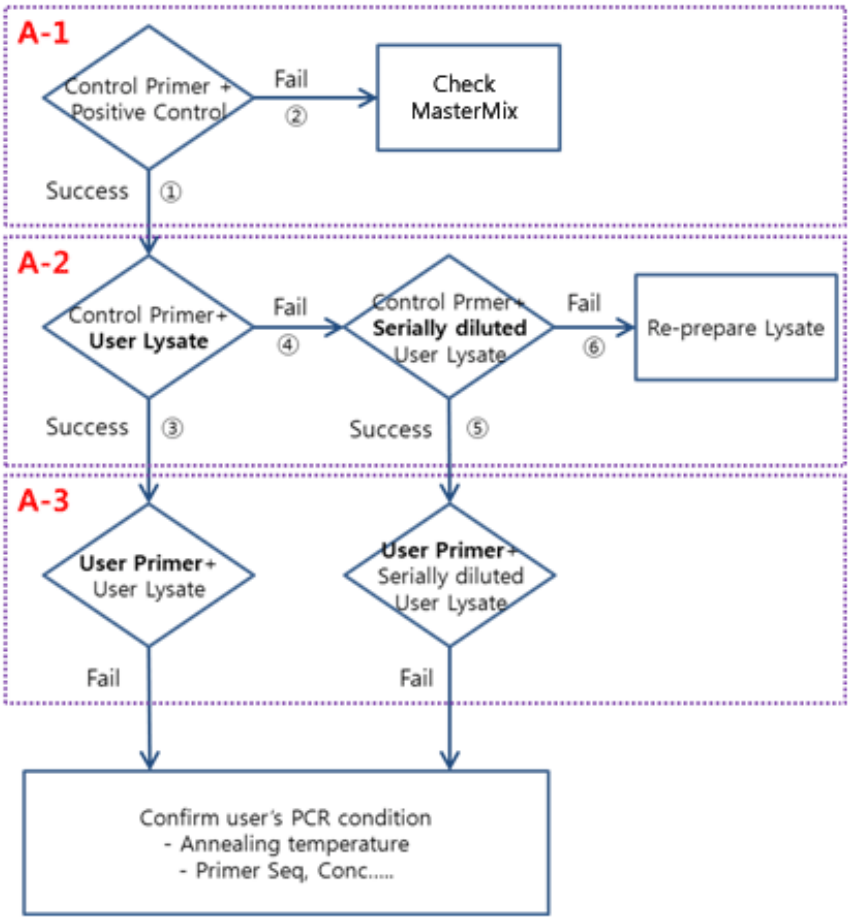


PCR was performed with 1 ul *Nicotiana* and *Capsicum* leaf lysate using the 1X Plant Direct Lysis Buffer solution in 20 ul PCR. And, pieces of *Nicotiana* and *Capsicum* leaf with 2 mm Harris Punch were placed directly into 20 ul PCR.
Lane 1 and 5: 1uL lysate
Lane 2 and 6: one piece,
Lane 3 and 7: two pieces
Lane 4 and 8: three pieces

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

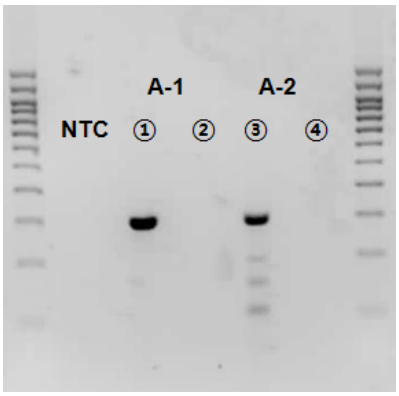


1. Perform the following experiment to confirm the performance of **Direct Taq PCR MasterMix** and/or **user's mouse tail lysate**.

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

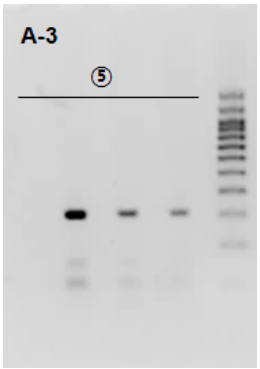
Step		Temp.	Time	Cycles
Initial Denaturation		95℃	5 min	1
Cycling	Denaturation	95℃	30 sec	35
	Annealing	50℃	30 sec	
	Extension	72℃	30 sec	
Final Extension		72℃	7 min	1

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.



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.

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
K0568600	EzWay™ Mouse Tail Direct PCR Kit	1 Kit