

## DNA extraction from leaf sample

### Preparation of test samples

Cut  $\square$  2~5mm the test sample and put it in the micro test tube.  
(usually use 0.2ml or 0.5ml tubes for PCR)



### Reagents preparation

Mix the CellEase A and B (15 $\mu$ l CellEase A, 15 $\mu$ l CellEase B).



Add 30  $\mu$ l of the mixture to the samples.



Incubate at 72°C for 6 minutes. Then incubate at 94°C for 3 minutes.



Add 10 $\mu$ l of CellEase C to the test sample and stir them gently.



Transfer 5-7 $\mu$ l of extracts to PCR reaction mixture and amplify the target DNA fragment.

### PCR

5~7 $\mu$ l	Test sample
5.0 $\mu$ l	$\times 10$ buffer (+Mg <sup>2+</sup> )
5.0 $\mu$ l	dNTPs
1.0 $\mu$ l	Forward Primer (10pmol/ $\mu$ l)
1.0 $\mu$ l	Reverse Primer (10pmol/ $\mu$ l)
0.5 $\mu$ l	Ex Taq (5 U/ $\mu$ l)

Fill up to 50 $\mu$ l by distilled water

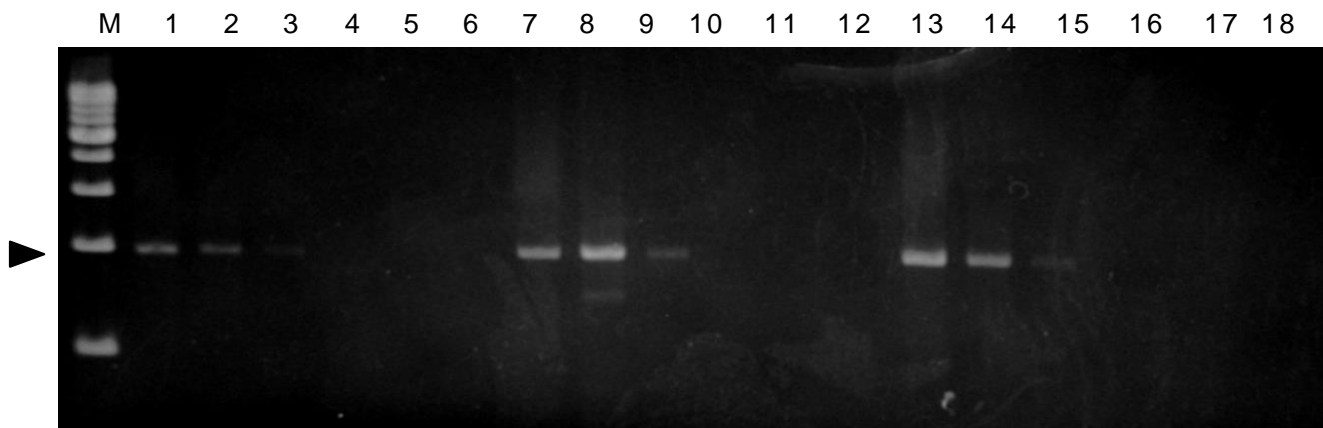
### PCR Cycle

94°C	1min
94°C	30sec
55°C	30sec
72°C	60sec
72°C	4min

35 Cycles

## <Results>

### ① DNA extraction from tomato leaf



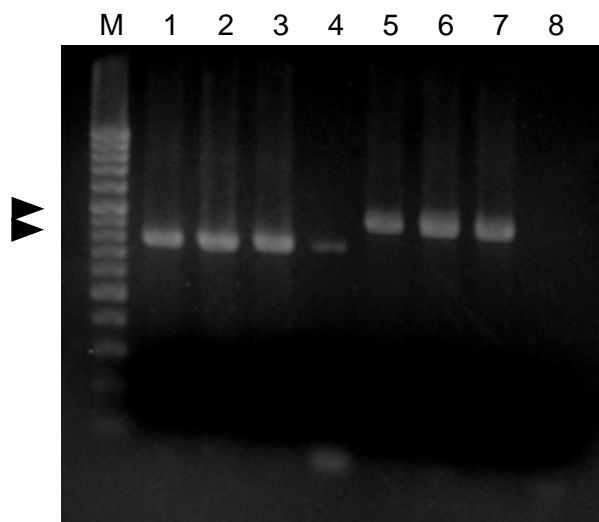
M Marker (500bp ladder)

1 + CellEase, Without dilution	Add 5 $\mu$ l to PCR	7 + CellEase, Without dilution	Add 6 $\mu$ l to PCR	13 + CellEase, Without dilution	Add 7 $\mu$ l to PCR
2 + CellEase, $\times$ 10 dilution	Add 5 $\mu$ l to PCR	8 + CellEase, $\times$ 10 dilution	Add 6 $\mu$ l to PCR	14 + CellEase, $\times$ 10 dilution	Add 7 $\mu$ l to PCR
3 + CellEase, $\times$ 100 dilution	Add 5 $\mu$ l to PCR	9 + CellEase, $\times$ 100 dilution	Add 6 $\mu$ l to PCR	15 + CellEase, $\times$ 100 dilution	Add 7 $\mu$ l to PCR
4 - CellEase, Without dilution	Add 5 $\mu$ l to PCR	10 - CellEase, Without dilution	Add 6 $\mu$ l to PCR	16 - CellEase, Without dilution	Add 7 $\mu$ l to PCR
5 - CellEase, $\times$ 10 dilution	Add 5 $\mu$ l to PCR	11 - CellEase, $\times$ 10 dilution	Add 6 $\mu$ l to PCR	17 - CellEase, $\times$ 10 dilution	Add 7 $\mu$ l to PCR
6 - CellEase, $\times$ 100 dilution	Add 5 $\mu$ l to PCR	12 - CellEase, $\times$ 100 dilution	Add 6 $\mu$ l to PCR	18 - CellEase, $\times$ 100 dilution	Add 7 $\mu$ l to PCR

※ The DNA extract was diluted by distilled water respectively and apply to PCR.

Primer: A part of heat shock protein gene (Hsc 70, 1kbp length) from Tomato (*Lycopersicon esculentum*)

### ② DNA extraction from rice leaf



M Marker (100bp ladder)

1~3	+CellEase add 6 $\mu$ l to PCR
4	-CellEase add 6 $\mu$ l to PCR
5~6	+CellEase add 6 $\mu$ l to PCR
8	-CellEase add 6 $\mu$ l to PCR

 **Biocosm Inc.**

Primer : IRF170

ORF100-PSPD

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