

DNA extraction from Bacteria

Reagents preparation

Mix the CellEase A and B (2 μ l CellEase A, 2 μ l CellEaseB)



Preparation of test samples

Directly or stepwise diluted Bacterial cells (5 μ l) were transferred to the tube (usually use 0.2ml or 0.5ml tubes for PCR)

Culture (*Staphylococcus. aureus*)

Temp. 30°C

Medium LB

Time 18hr



Add 4 μ l of the CellEase mixture to the samples (5 μ l).



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



Transfer 5-8 μ l of extracts to PCR reaction mixture and amplify the target DNA fragment

PCR

5~8 μ l	Test sample
5.0 μ l	\times 10 buffer (+Mg ²⁺)
5.0 μ l	dNTPs
1.0 μ l	Forward Primer (10pmol/ μ l)
1.0 μ l	Reverse Primer (10pmol/ μ l)
0.5 μ l	Ex Taq (5 U/ μ l)

Fill up to 50 μ l by distilled water

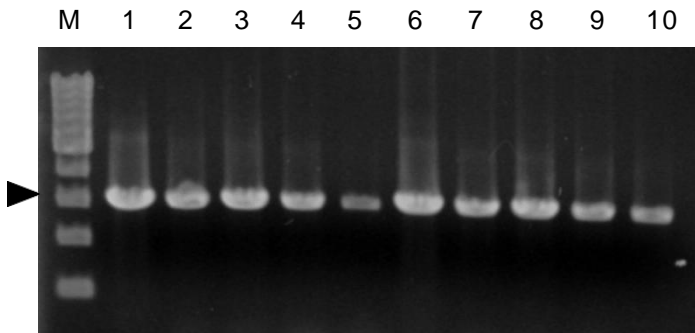
PCR Cycle

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

35 Cycles

<Results >

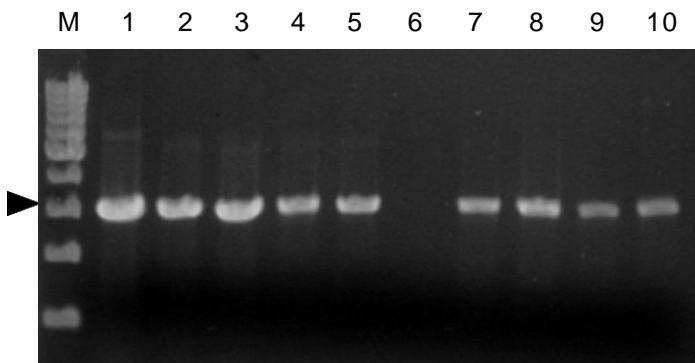
CellEase Bacteria II / *Staphylococcus aureus*



M Marker (500bp ladder)

- 1 Undiluted Sample Add 5 μ l of DNA extract to PCR
- 2 $\times 10^1$ dilution
- 3 $\times 10^2$ dilution
- 4 $\times 10^3$ dilution
- 5 $\times 10^4$ dilution
- 6 Undiluted Sample Add 6 μ l of DNA extract to PCR
- 7 $\times 10^1$ dilution
- 8 $\times 10^2$ dilution
- 9 $\times 10^3$ dilution
- 10 $\times 10^4$ dilution

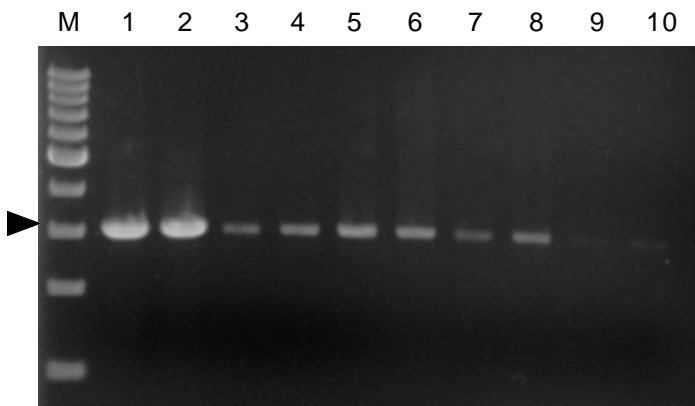
CellEase Bacteria II



M Marker (500bp ladder)

- 1 Undiluted Sample Add 7 μ l of DNA extract to PCR
- 2 $\times 10^1$ dilution
- 3 $\times 10^2$ dilution
- 4 $\times 10^3$ dilution
- 5 $\times 10^4$ dilution
- 6 Undiluted Sample Add 8 μ l of DNA extract to PCR
- 7 $\times 10^1$ dilution
- 8 $\times 10^2$ dilution
- 9 $\times 10^3$ dilution
- 10 $\times 10^4$ dilution

Conventional CellEase Bacteria



M Marker (500bp ladder)

- 1,2 Undiluted Sample
- 3,4 $\times 10^1$ dilution
- 5,6 $\times 10^2$ dilution
- 7,8 $\times 10^3$ dilution
- 9,10 $\times 10^4$ dilution

※The protocol of conventional CellEase kit was followed by the original instruction manual.

As a results, 6~7 μ l of DNA extract was thought to be best for PCR (50 μ l total reaction volume).
The clear DNA bands were detected from more than $\times 10^4$ dilution of DNA extracts by using CellEase Bacteria II