

DNA extraction from Bacteria

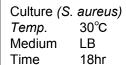
Reagents preparation

Mix the Cellease A and B (2 ul Cellease A, 2 ul CellEase B)



Preparation of test samples

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





Add 4ul of the Cellease mixture to the samples (5µl).



Incubate at 37°C for 6 minutes
Then incubate at 95°C for 3 minutes



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

PCR

5~8ul Test sample
5.0 ul ×10 buffer(+Mg²⁺)
5.0 ul dNTPs
1.0 ul Forward Primer (10pmol/ul)
1.0 ul Reverse Primer (10pmol/ul)
0.5 ul Ex Taq (5 U/ul)

Fill up to 50 ul by distilled water

PCR Cycle

 94°C
 1min

 94°C
 30sec

 55°C
 30sec

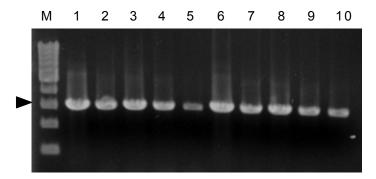
 72°C
 60sec

 72°C
 4min

35 Cycles

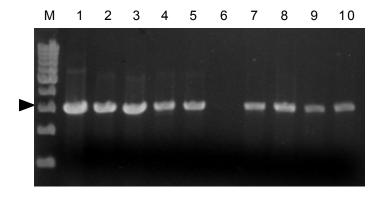
< Results >

·CellEase Bacteria II



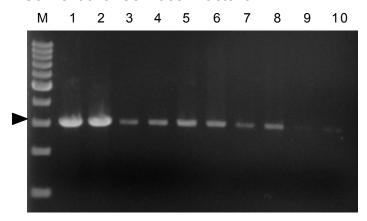
- M Marker (500bp ladder)
- 1 Undiluted Sample Add 5µl of DNA extract to PCR
- 2 × 10 dilution
- 3×10^2 dilution
- 4×10^3 dilution
- 5×10^4 dilution
- 6 Undiluted Sample Add 5µl of DNA extract to PCR
- 7 × 10 dilution
- 8×10^2 dilution
- 9×10^3 dilution
- 10×10^4 dilution

·CellEase Bacteria II



- M Marker (500bp ladder)
- 1 Undiluted Sample Add 7µl of DNA extract to PCR
- 2 × 10 dilution
- 3×10^2 dilution
- 4×10^3 dilution
- 5×10^4 dilution
- 6 Undiluted Sample Add 8μl of DNA extract to PCR
- × 10 dilution
- 8×10^2 dilution
- 9×10^3 dilution
- 10×10^4 dilution

·Conventional CellEase Bacteria



- M Marker (500bp ladder)
- 1,2 Undiluted Sample
- 3.4 × 10 dilution
- 5.6×10^2 dilution
- 7.8×10^3 dilution
- 9,10 \times 10⁴ dilution
- X The protocol of conventional CellEase kit was followed by the original instruction manual.

As a results, $6\sim7\mu l$ of DNA extract was thought to be best for PCR ($50\mu l$ total reaction volume). The clear DNA bands were detected from more than $\times\,10^4$ dilution of DNA extracts by using CellEase Tissue II



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