

DF-15

## Plasmid DNA Extraction from *E. coli*

### Protocol

Collect the transformed *E. coli* into a 1.5 ml micro tube, and pelletize

↓ ← RDP mix (RDP + EDP-01) \*1 : 100  $\mu$ l

Vortex (No cell clumps should be visible after resuspension of the pellet)

Flash spin down

↓ ← ADP : 100  $\mu$ l

Slowly mix by inverting the tube 5 times (Do not shake vigorously) \*2

Flash spin down (Do not leave the sample more than 5 min at this step)

↓ ← NDP : 140  $\mu$ l

Slowly mix by inverting the tube 5 times (Do not shake vigorously) \*2

↻ ↓ 18,000 x g (14,100 rpm), 10 min, RT

Dispense 320  $\mu$ l of LDP \*3 into a new 1.5 ml micro tube

Transfer the supernatant (about 330  $\mu$ l) to the 1.5 ml microtube with LDP

↓

Vortex (maximum speed) : 30 sec & Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.  
(from the step after transferring the lysate into the cartridge)

↓

Plasmid DNA  
(Elution volume : 50  $\mu$ l)

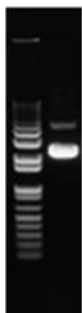
\*1 Before starting an extraction experiment, add total amounts of EDP-01 to RDP bottle, and mix well. In the case of storing RDP mix, it is recommended to preserve it under refrigeration (2-8°C) and use within 6 months.

\*2 After addition of ADP or NDP, immediately mix by inverting the tube 5 times. Vigorous mixing results in the co-purification of much of genomic DNA. Too slow mixing causes inadequate blending of liquids, resulting in deterioration in the yield of plasmid DNA.

### Results

#### Electropherogram

M 1



M : Marker (1 Kb Plus DNA Ladder : Invitrogen)  
1 : QuickGene

Depending on sample and storage conditions, nucleic acid may not be extractable.  
Therefore, we cannot guarantee accurate data.  
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

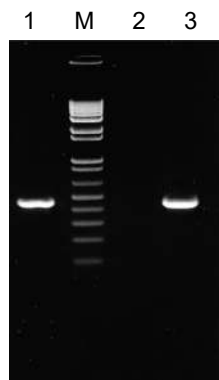
■ The yield of plasmid DNA / Protein contamination : A260/280  
/ Chaotropic salt contamination : A260/230

Kit	Yield	A260/280	A260/230
QuickGene	21.4 µg	1.99	2.49

■ Other

• PCR

PCR was performed on 5 ng of template extracted with QuickGene system using *GAPDH* as a target.

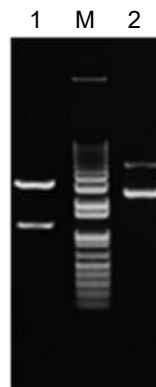


M : Marker (100 bp DNA Ladder : Invitrogen)  
1 : QuickGene  
2 : Negative control  
3 : Positive control

PCR amplification is possible from 5 ng of template.

• Restriction enzyme digestion with *Not* I and *Xho* I

Restriction enzyme digestion was performed for plasmid DNA extracted from transformed *E. coli* using QuickGene system. Restriction endonuclease (0.5 µl each of *Not* I and *Xho* I) were added to 10 µl of a reaction solution (including 1 µl of the extracted plasmid). Then it was incubated for 2 hours at 37° C.



M : Marker (1 Kb Plus DNA Ladder : Invitrogen)  
1 : QuickGene (*Not* I + *Xho* I)  
2 : None

From these results, it is understood that restriction endonuclease cleavage is practicable.

■ Common protocol is usable for the following

Fosmid