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 µl

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

Flash spin down

↓ ← ADP : 100 µl

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 µl

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 µl of LDP *3 into a new 1.5 ml micro tube

Transfer the supernatant (about 330 µl) 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)

↓

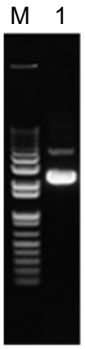
[Plasmid DNA
(Elution volume : 50 µl)]

*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 : 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).

KURABO

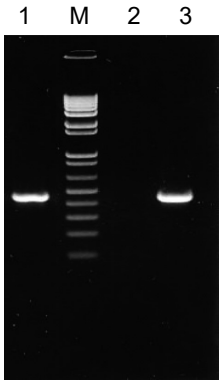
The yield of plasmid DNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

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.

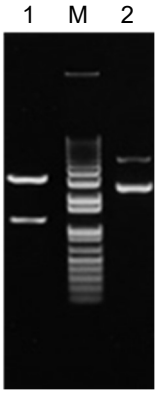


M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Negative control
3 : Positive control

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

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).