DF-16

Automated_Plasmid DNA Extraction from *E. coli*

Protocol

Collect the transformed *E. coli* into a 1.5 ml microtube, 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 microtube

Transfer the supernatant (about 330 µl) to the 2 ml microtube*4 with LDP

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

Set into the device

Protocol: PLASMID

(Elution volume : 50 µl *5)

*Please refer to Quick Start Guide or operation manual to know how to set sample tube.

1. Apply the lysate into the cartridge
2. Pressurizing
3. Wash 2 times by Wash Buffer (WRT)
4. Add selected volume of Elution buffer and elute plasmid DNA into collection tube.

Plasmid DNA

*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.

*3 Add 44 ml of >99% ethanol into the bottle and mix well by gently inverting the bottle before use.

*4 Following microtube are recommended.
#BM4020
(BM instrument co., ltd)
#72.695.700,
#72.695.500S
(SARSTEDT)

*5 The volume of the eluate from each cartridge is 100 µl.
The volume of CRT can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

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).

Results

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

Kit	A260/280	A260/230	Yield	S.D.
QuickGene	1.98	2.23	29.5 µg	4.07

N=4

Common protocol is usable for the following

Fosmid

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