

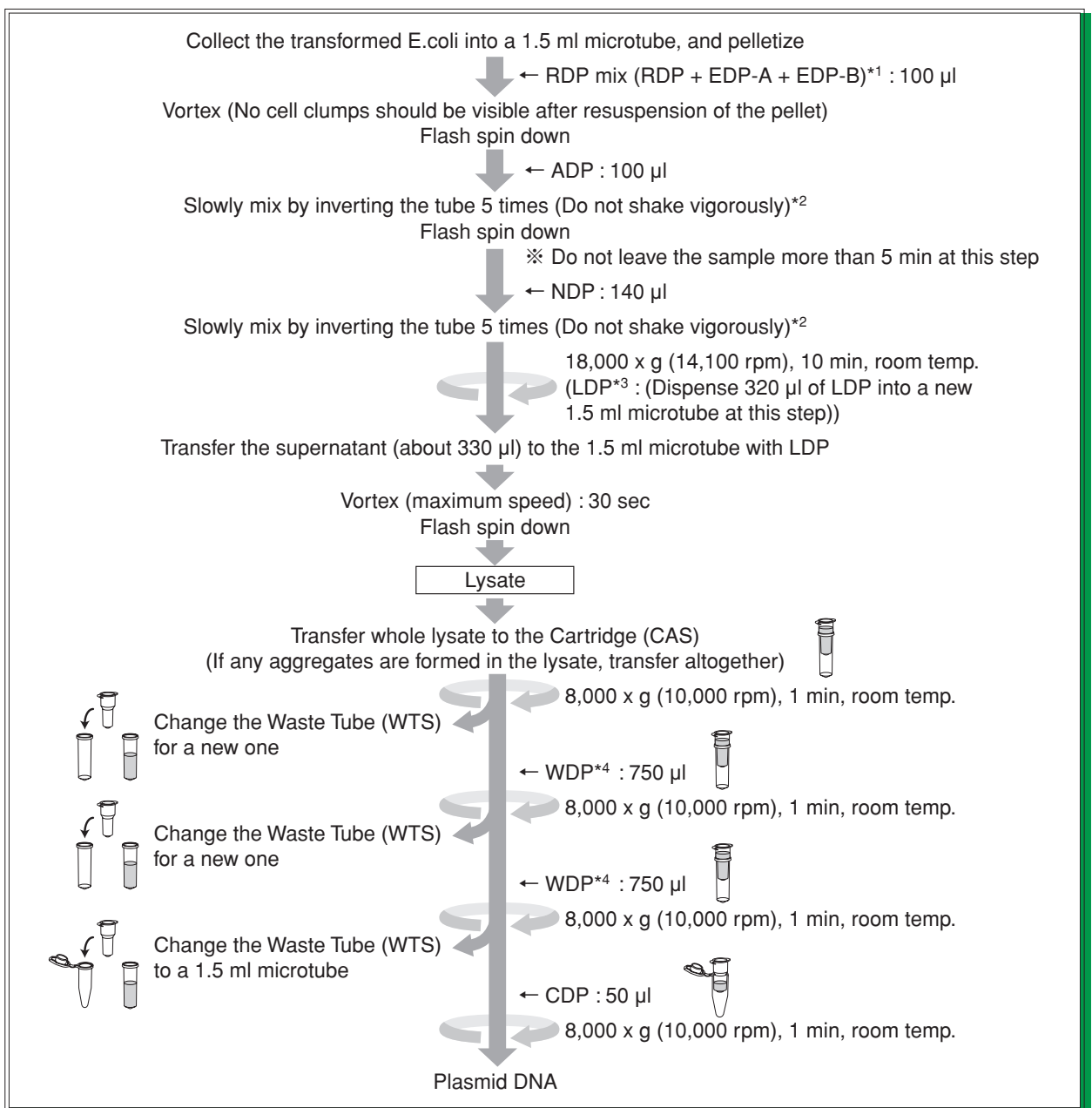


QuickGene Series Application Guide

Plasmid Extraction from E. coli

Kit : QuickGene SP kit Plasmid (Spin method)

Protocol



*1 : Before starting an extraction experiment, add 3 μ l of EDP-A and 1 μ l of EDP-B per every 100 μ l of RDP, and mix well.

*2 : After addition of ADP or NDP, immediately mix by inverting the tube 5 times.

Vigorous mixing results in the copurification 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 : Add 200 ml of >99% ethanol into the bottle and mix well by gently inverting the bottle before use.

Results : Extraction of Plasmid DNA from transformed E. coli

Plasmid DNA was extracted from 1 ml over-night culture of transformed E. coli in LB medium using QuickGene SP kit Plasmid and Spin column method (A Company).

E. coli : DH5 α (1×10^9)

Vector : pBlueScript II

Insert : GAPDH about 1 kb

● The yield and purity of plasmid DNA

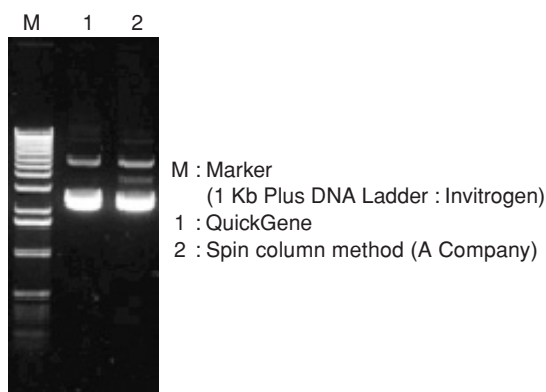
kit	Yield	A _{260/280}	A _{260/230}
QuickGene	23.4 μ g	1.97	2.32
Spin column method (A Company)	18.2 μ g	1.96	2.32

A_{260/280} : The ratio indicates the purity of nucleic acid from protein contamination (A_{260/280} >1.7).
(Protein contamination decreases the ratio.)

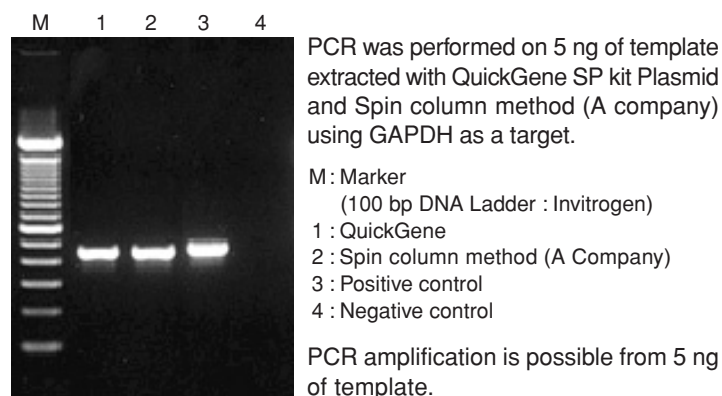
A_{260/230} : The ratio indicates the purity of nucleic acid from chaotropic salt (guanidium salt) contamination.
(Guanidium salt contamination decreases the ratio.)

The use of QuickGene SP kit Plasmid enables the high-yield and high-purity extraction of plasmid DNA from transformed E. coli.

● Electrophoresis of plasmid DNA

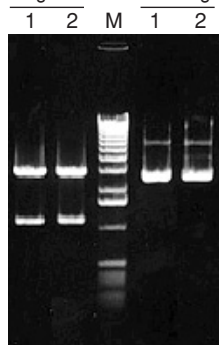


● PCR



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

After digestion Before digestion



Restriction enzyme digestion was performed for plasmid DNA extracted from transformed E. coli using QuickGene SP kit Plasmid and Spin column method (A company).

Restriction endonuclease (0.5 μ l each of *Not* I and *Xho* I) were added to 10 μ l of a reaction solution (including 1 μ l of the extracted plasmid). Then it was incubated for 2 hours at 37°C.

M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
1 : QuickGene
2 : Spin column method (A Company)

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

* Trademark and exclusion item

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