



QuickGene Series Application Guide

Plasmid DNA isolation from E. coli

Kit : QuickGene Plasmid kit S II

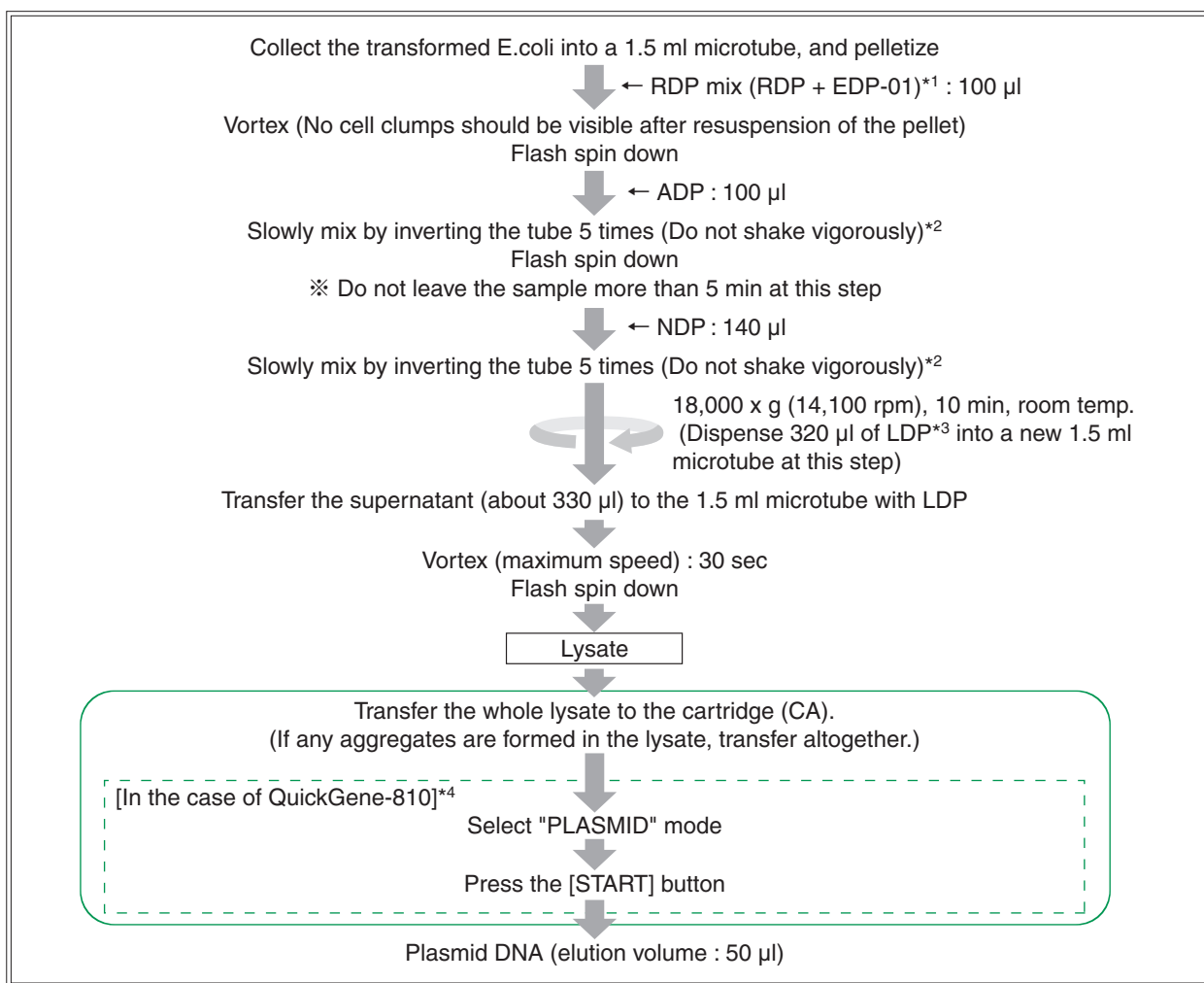
Model : QuickGene-810/QuickGene-Mini80

Summary

Enables easy and rapid isolation of high purity plasmid DNA from E. coli

● Protocol

This protocol is used with QuickGene Plasmid kit S II and 1-2 ml over night cultures (37°C, 12-16 hr) of E. coli grown in LB medium.



*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 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 : In the case of QuickGene-Mini80, please refer to the Kit Handbook for details.

* Perform extraction within 30 min after lysate preparation.

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 system (QuickGene and QuickGene Plasmid kit S II).

E. coli : DH5 α (1×10^9)

Vector : pBlueScript II

Insert : GAPDH about 1 Kb

● The yield and purity of plasmid DNA

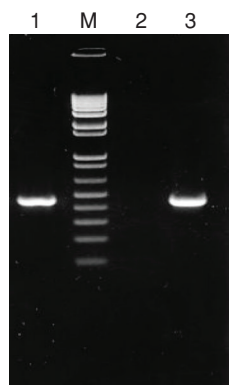
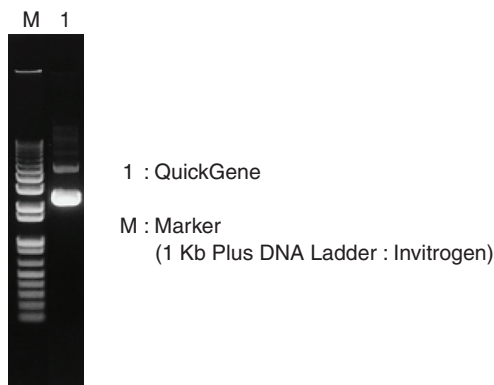
Kit	Yield	A _{260/280}	A _{260/230}
QuickGene	21.4 μ g	1.99	2.49

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 system enables the high-yield and high-purity extraction of plasmid DNA from transformed E. coli.

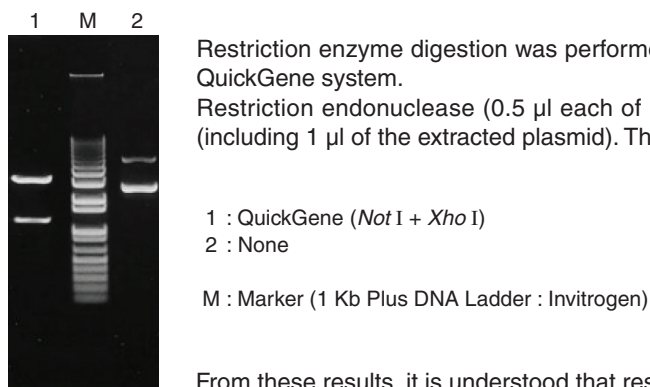
● Electrophoresis of plasmid DNA ● PCR



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

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 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.

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

* Trademark and exclusion item

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