

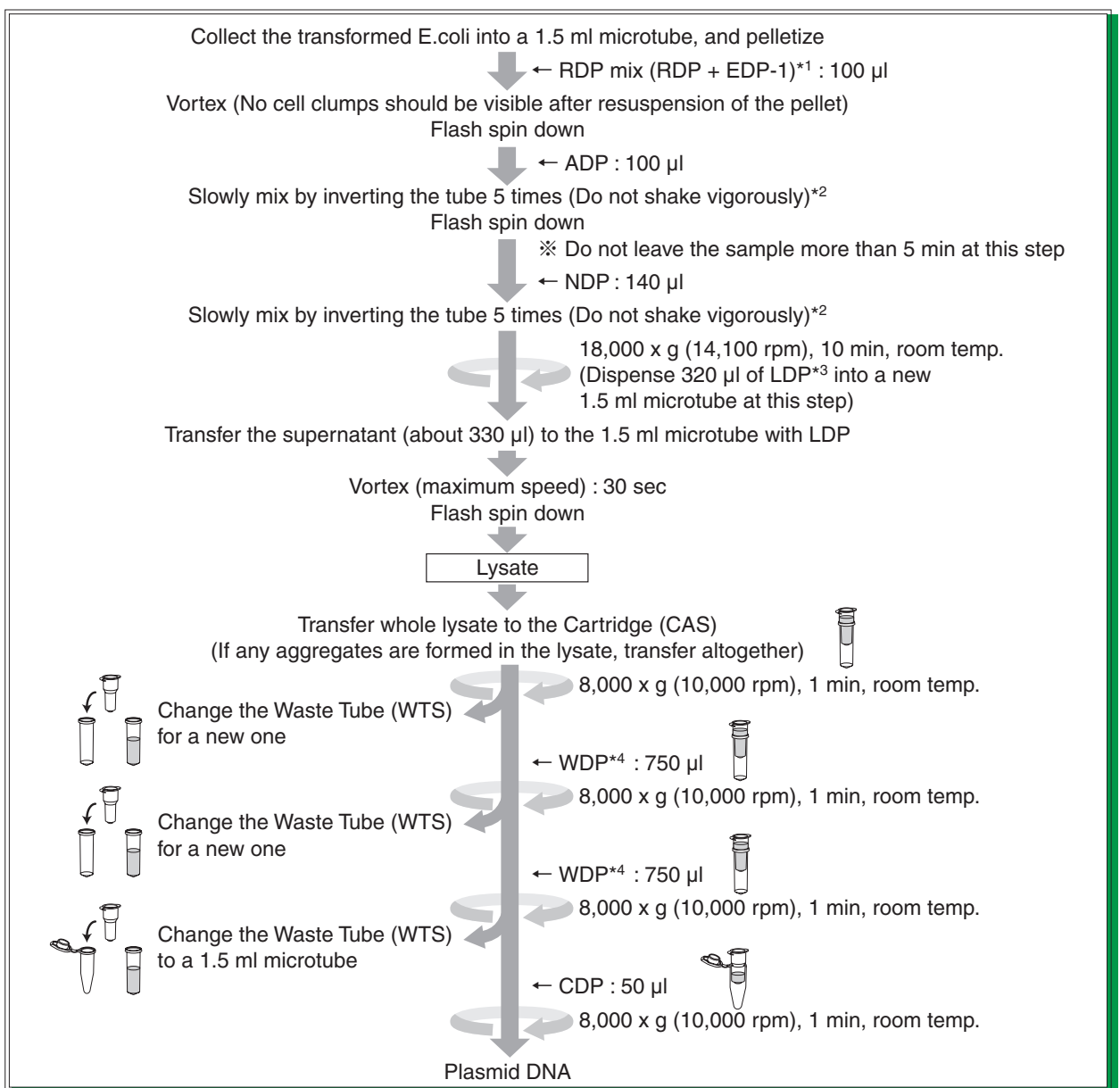


QuickGene Series Application Guide

Plasmid DNA Extraction from E. coli

Kit : QuickGene SP kit Plasmid II (Spin method)

Protocol



*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 : Add 200 ml of >99% ethanol into the bottle and mix well by gently inverting the bottle before use.

* 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 SP kit Plasmid II .

E. coli : DH5 α (1×10^9)

Vector : pBlueScript II

Insert : GAPDH about 1 Kb

● The yield and purity of plasmid DNA

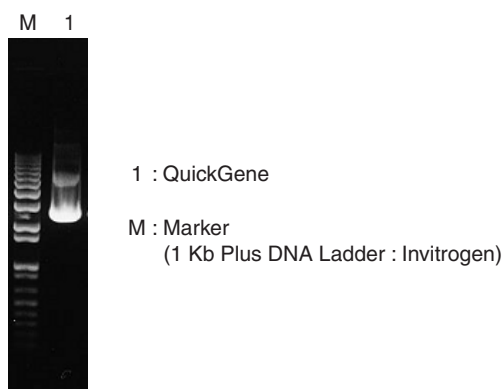
Kit	Yield	A _{260/280}	A _{260/230}
QuickGene	30.9 μ g	2.00	2.43

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

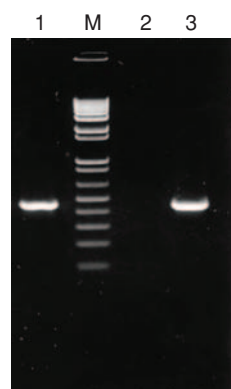
A_{260/230} : The ratio indicates the purity of nucleic acid from chaotropic salt (guanidium salt) contamination.
(Guanidium salt contamination decreases the ratio.)

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



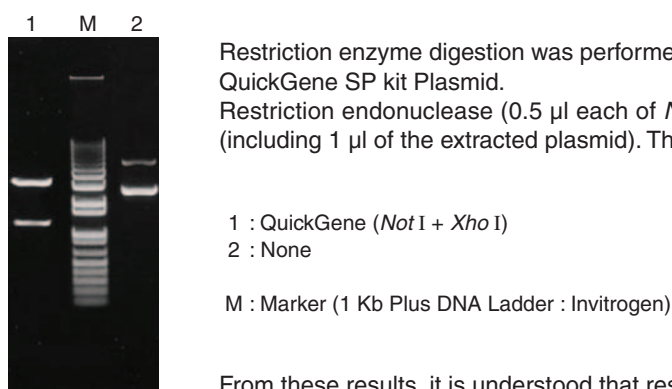
PCR was performed on 5 ng of template extracted with QuickGene SP kit Plasmid using GAPDH as a target.

1 : QuickGene
2 : Negative control
3 : Positive control

M : Marker
(100 bp DNA Ladder : Invitrogen)

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 SP kit Plasmid.

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

Right to registered names etc. used in this Application Guide is protected by law especially even in the case of no denotation.

FUJIFILM

FUJIFILM Corporation 7-3, Akasaka 9-Chome, Minato-ku, Tokyo 107-0052, Japan, Tel : +81-3-6271-2158, Fax : +81-3-6271-3136 • E-mail : sginfo@fujifilm.co.jp

FUJIFILM Europe GmbH Heesenstr.31, 40549 Dusseldorf, Germany, Tel:+49-211-5089-174, Fax:+49-211-5089-9144 • E-mail:lifescience@fujifilm-europe.de

FUJIFILM UK Ltd. Unit 12 St Martins Way, St Martins Business Centre, Bedford, MK42 0LF, U.K., Tel:+44-1234-245291, Fax:+44-1234-245293 • E-mail:lifesciences@fujifilm.co.uk

富士胶片(中国)投资有限公司 31st floor, Hong Kong New World Tower, No.300 Huai Hai Zhong Road, Shanghai, P.R China, Tel:+86-21-3302-4655 ext.363, Fax:+86-21-6384-3322 • E-mail:wqxiang@fujifilm.com.cn

FUJIFILM Medical Systems U.S.A., Inc. Tel:+1-866-902-3854 Fax:+1-203-327-6485 • E-mail:don.wilke@fujimed.com

<http://lifescience.fujifilm.com/>