



QuickGene Series Application Guide

Bacterial Genomic DNA Extraction from Stool

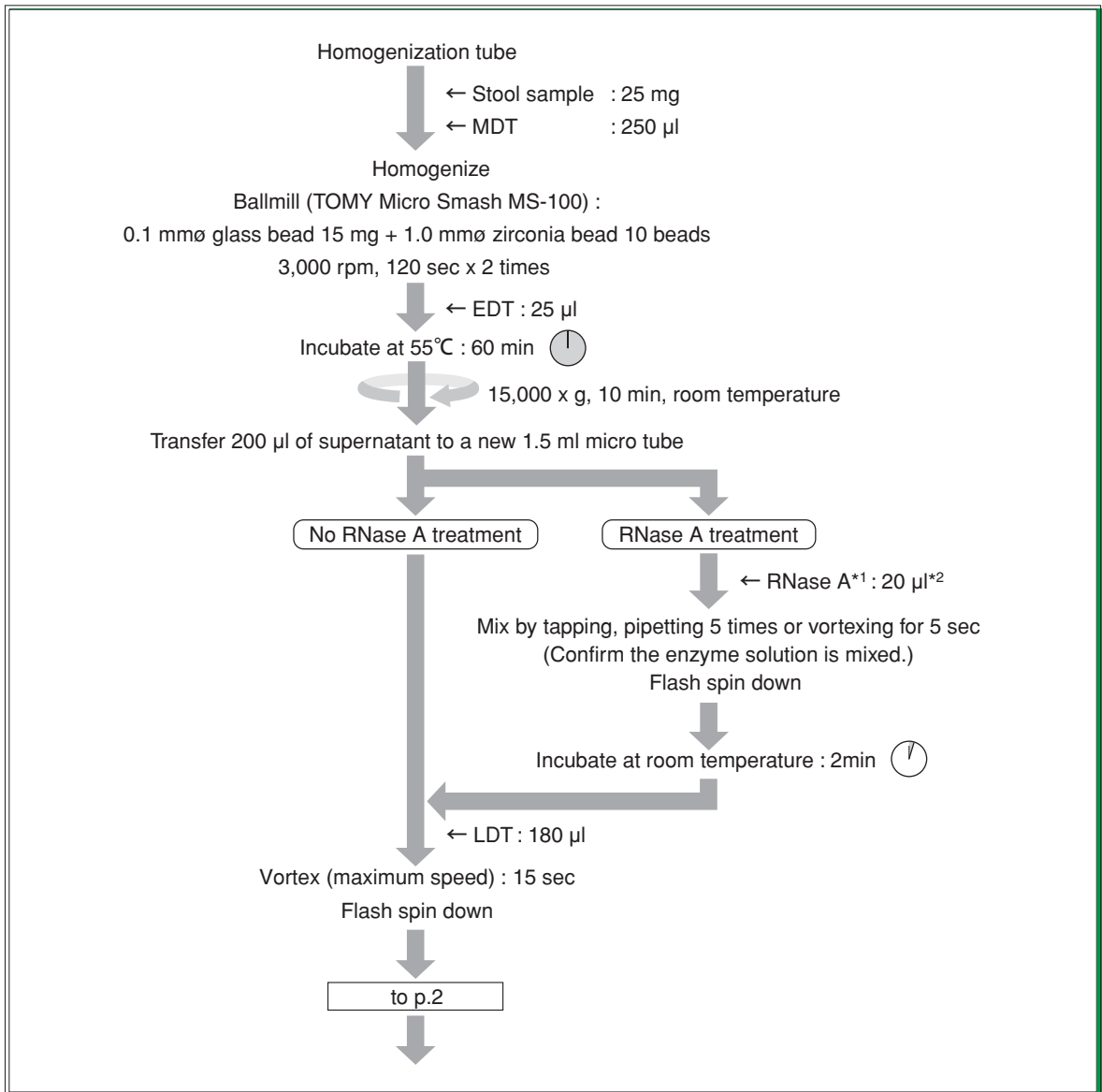
Kit : QuickGene DNA tissue kit S

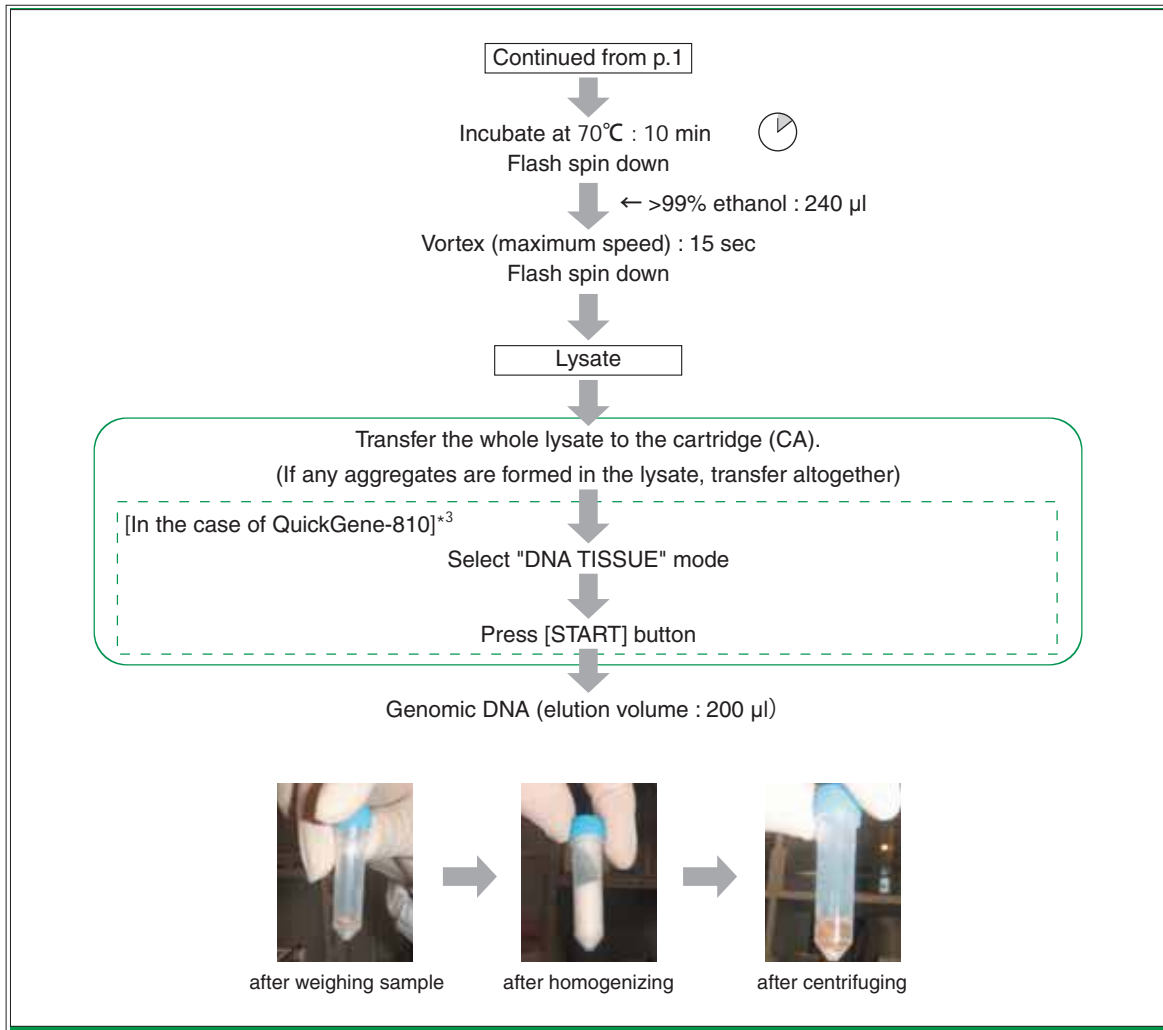
Model : QuickGene-810 / QuickGene-Mini80

Summary

Enables easy and rapid extraction of bacterial genomic DNA from stool.

● Protocol





*1 : RNase A is not contained in the kit. Please, prepare recommended RNase (refer to the following).

*2 : 60µl for RNase A (invitrogen Cat. No.12091).

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

* Perform extraction within 30 min after lysate preparation.

Recommended RNase

- Ribonuclease A (Sigma : Cat. No.R5125*^{1,*2} , :2 , R5500*^{1,*2} , R6513*¹ , R4642)
- Ribonuclease A (MP Biomedicals Cat. No. 101076*^{1,*2})
- RNase A (AMRESCO Cat. No. 0675*^{1,*2})
- RNase A (QIAGEN Cat. No. 19101)
- RNase A (invitrogen Cat. No. 12091)

*1 : Prepare 100 mg/ml solution with 10 mM Tris-HCl (pH 7.5) and 15 mM NaCl.

*2 : Incubate at 100°C for 15 min to deactivate DNase.

Results : Bacterial genomic DNA extraction from stool

Bacterial genomic DNA was extracted from stool using QuickGene system (QuickGene-810 and QuickGene DNA tissue kit S) and Spin column method (A company).

Stool samples No.1 : Adult 1 No.2 : Adult 2
No.3 : Infant 1 No.4 : Rat 1

* Bacterial genomic DNA was extracted from 25 mg of stool for QuickGene system, and from 100 mg of stool for Spin column method (A company).

● The yield and purity of genomic DNA

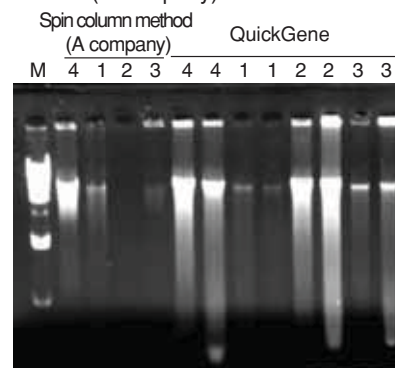
Sample	Yield				Purity(A260/280)			
	No.1	No.2	No.3	No.4	No.1	No.2	No.3	No.4
QuickGene	8.4 µg	23.7 µg	15.8 µg	34.4 µg	2.14	1.92	2.08	2.13
Spin column method (A company)	2.3 µg	0.6 µg	N.D	6.7 µg	2.08	1.36	N.D	1.70

No RNase A treatment.

The use of QuickGene system enables high-purity genomic DNA extraction from stool with little contamination of protein in higher yield than Spin column method (A company).

● Electrophoresis of genomic DNA

Electrophoresis was performed with genomic DNA extracted from stool using QuickGene system and Spin column method (A company).



Electrophoresis condition : 0.8% agarose

M : λ -Hind III
1 : No.1 Adult 1
2 : No.2 Adult 2
3 : No.3 Infant 1
4 : No.4 Rat 1

(-) (-) (-) (-) (+) (-) (+) (-) (+) (-) (+) (-)

(+) : RNase treatment, (-) : No RNase treatment

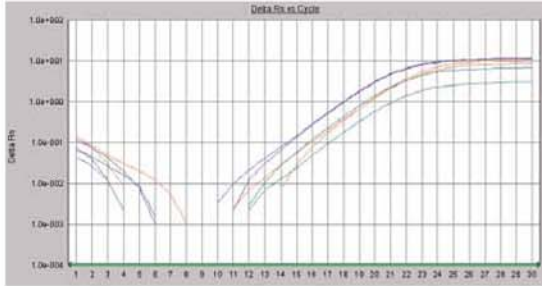
No decomposition was detected for extracted genomic DNA.

● Real Time PCR

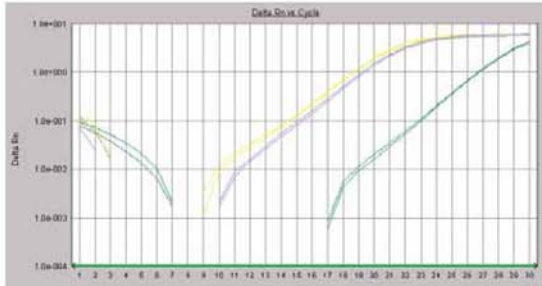
Real time PCR was performed with *Escherichia coli* specific primer for genomic DNA extracted from stool using QuickGene system and Spin column method (A company).

1 µl of eluate was used as a template (total reaction capacity, 10 µl : duplicate).

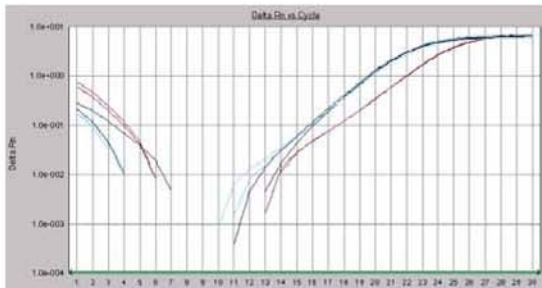
Applied Biosystem 7300 was used for Real Time PCR.



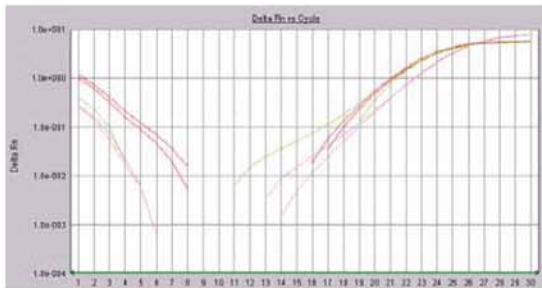
Azure : Adult 1 (QuickGene-810, RNase treatment)
 Green : Adult 1 (QuickGene-810, No RNase treatment)
 Orange: Adult 1 (Spin column method (A company), No RNase treatment)



Yellow : Adult 2 (QuickGene-810, DNase treatment)
 Azure : Adult 2 (QuickGene-810, No DNase treatment)
 Green : Adult 2 (Spin column method (A company), No RNase treatment)



Blue : Infant 1 (QuickGene-810, RNase treatment)
 Azure : Infant 1 (QuickGene-810, No RNase treatment)
 Brown : Infant 1 (Spin column method (A company), No RNase treatment)



Green : Rat 1 (QuickGene-810, RNase treatment)
 Pink : Rat 1 (QuickGene-810, No RNase treatment)
 Red : Rat 1 (Spin column method (A company), No RNase treatment)

Expression analysis was carried out in real time PCR for each genomic DNA.
 In addition, expression analyses were carried out in a similar way for *Lactobacillus* specific primer and *Clostridium coccooides-Eubacterium rectale* group specific primer.

Contributed by Creative Research Initiative "Sousei" of Hokkaido University and Endowed Research Department, Meiji Dairies Corporation.

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