8. Genomic DNA Extraction from Bacteria

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-1

Bacterial Genomic DNA Extraction from Stool

Protocol

Homogenization tube

← Stool sample : 25 mg
← MDT : 250 µl

Homogenize
Ballmill (TOMY Micro Smash MS-100) :0.1 mmφ glass bead 15 mg + 1.0 mmφ zirconia bead 10 beads, 3,000 rpm, 120 sec x 2 times

← EDT：25 µl

Incubate at 55°C : 60 min

15,000 x g, 10 min, RT

Transfer 200 µl of supernatant to a new 1.5 ml micro tube

No RNase A treatment

RNase A treatment

← RNase A*1 : 20 µl*2

Mix by tapping, pipetting 5 times or vortexing for 5 sec (Confirm the enzyme solution is mixed.)
Flash spin down

Incubate at RT : 2 min

← LDT：180 µl

Vortex (maximum speed) : 15 sec
Flash spin down

Incubate at 70°C for 10 min
Flash spin down

← >99% ethanol：240 µl

Vortex (maximum speed) : 15 sec
Flash spin down

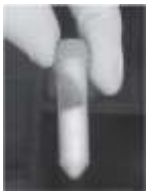
Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA
(Elution volume : 200 µl)


after weighing sample


after homogenizing


after centrifuging

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

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

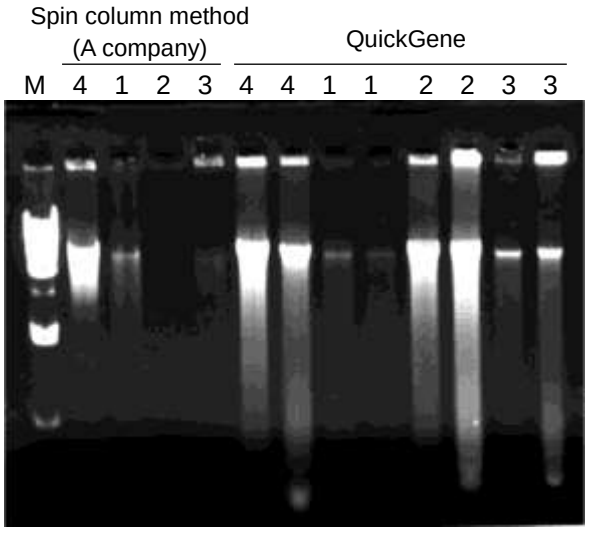
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

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

Electropherogram



Electrophoresis condition : 0.8% agarose
M : λ-Hin d 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.

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4
QuickGene	8.4 µg	23.7 µg	15.8 µg	34.4 µg
Spin column method (A company)	2.3 µg	0.6 µg	N.D	6.7 µg

Protein contamination：A260/280

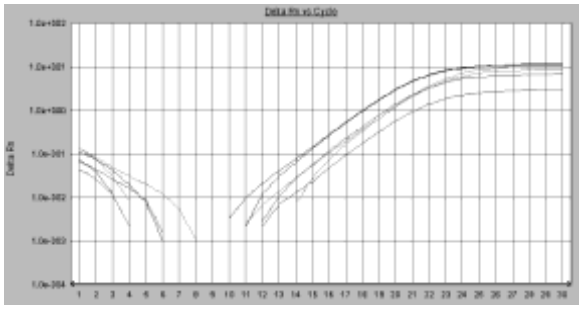
Sample	No.1	No.2	No.3	No.4
QuickGene	2.14	1.92	2.08	2.13
Spin column method (A company)	2.08	1.36	N.D	1.70

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).

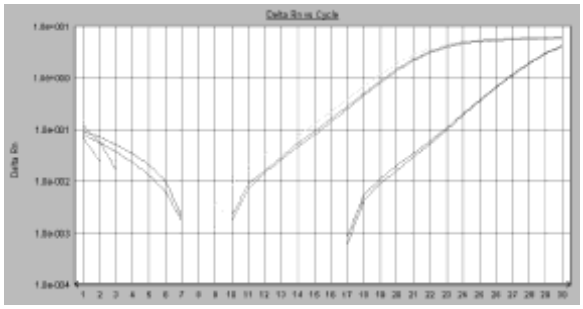
Other

・Real Time PCR

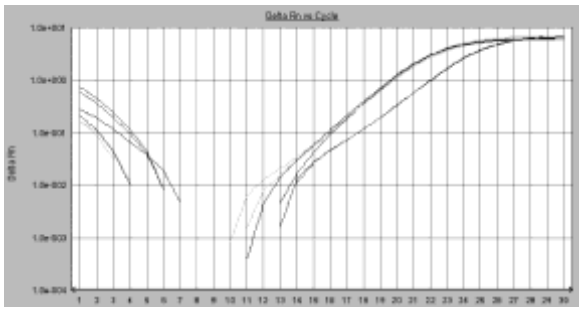
Real time PCR was performed with Escherichia coli specific primer for genomic DNA extracted from stool using QuickGene system and Spin column method (A company).
1µl of eluate was used as a template (total reaction capacity, 10 µl : duplicate). Applied Biosystem 7300 was used for Real Time PCR.



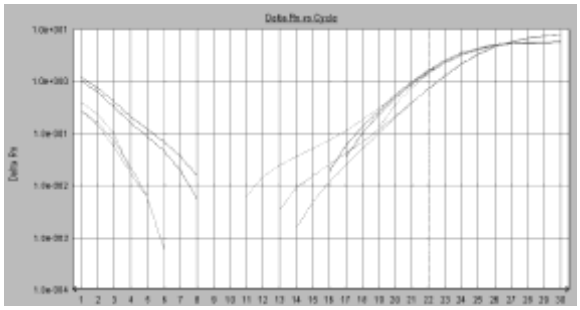
Azure : Adult 1(QuickGene-810, RNase treatment)
Green : Adult 1(QuickGene-810, No RNase treatment)
Orange: Adult 1(Spin column method (A company), No RNase treatment)



Yellow : Adult 2 (QuickGene-810, DNase treatment)
Azure : Adult 2 (QuickGene-810, No DNase treament)
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 coccoides- Eubacterium rectale group specific primer.

Common protocol is usable for the following

No Data

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).

DF-2

Fosmid DNA Extraction from *E.coli*

Protocol

Pellet of transformed *E.coli* in 2.0 ml micro centrifuge tube

↓ ← RDP mix (RDP + EDP01 *1) : 100 µl

Mix thoroughly by vortexing (Maximum speed)

Flash spin down

↓ ← ADP : 100 µl

Mix with inversion 5 times *2

Flash spin down

↓ ← NDP : 140 µl

Mix with inversion 5 times *3

↓ ⟲ 18,000 x g, 10 min, RT

Transfer the supernatant (about 330 µl) to new micro tube dispensed 320 µl of LDP

↓

Mix thoroughly by vortexing (Maximum speed) : 30 sec.

Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.

(from the step after transferring the lysate into the cartridge)

↓

[Fosmid DNA
(Elution volume : 50 µl)]

*1 Add total amounts of EDP-01 to RDP bottle, and mix well

*2 Don't leave more than 5 min. at this stage

*3 Mix with inversion the tube immediately after addition of ADP or NDP. The solution should be mixed by inverting the tube 5 times gently. Chromosomal DNA will be extracted if the solution is vortexed.
If you shake the tubes, a lot of genomic DNA will be extracted with plasmid DNA, however incomplete mixing at this time, yield may decline.

Results

No Data

Common protocol is usable for the following

Plasmid

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-3

Genomic DNA Extraction from Actinomyces

Protocol

Homogenization tube

← Fungus body : about 10 mg

← 5 mmφ zirconia ball

Freeze in liquid nitrogen for 30 sec

Homogenize：TissueLyser Adaptor Set [30Hz, 1 min]

← MDT : 180 µl

Make tapping to disperse well

← EDT : 20 µl

← RNase A (100 mg/ml) : 4 µl

Tapping 5 times *1 & Flash spin down

Reaction at RT for 2 min

← LDT：180 µl

Vortex for 15 sec (maximum speed) & Flash spin down

Incubation at 70°C：10 min & Flash spin down

← >99% ethanol：240 µl

Vortex for 15 sec (maximum speed) & Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA

*1 Confirm enzyme solution is mixed.

Results

No Data

Common protocol is usable for the following

No Data

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).

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QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-4

Genomic DNA Extraction from Filamentous Bacterium

Protocol

1.5 ml micro tubes

← Disrupted fungus body : 5 mg - 100 mg *1

Freeze at -80°C for more than 1 hour (in use of liquid nitrogen, 30 sec)

← MDT : 180 µl

← EDT *2 : 20 µl

Make tapping to disperse well

Flash spin down

Incubation at 55°C : 10 min (mixing sometimes)

16,000 x g, 10 min, RT

Transfer supernatant to a new 1.5 ml micro tube *3

← <Option> RNase A treatment *4

← LDT *5 : 180 µl

← >99% ethanol : 240 µl

Vortex for 15 sec (maximum speed)

Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA
(Elution volume : 200 µl)

*1 Usable amount varies depending on kind of fungus etc.
In the case of sample extracted for the first time with this kit, please carry out preliminary experiment.

*2 This process can be omitted in case of no EDT effect

*3 In case debris is not precipitated perfectly, centrifuge again.

*4 RNase A (100 mg/ml) : 20 µl Tapping (Confirm enzyme solution is mixed)
Flash spin down (several seconds)
↓
Incubate at RT for 2 min

*5 In case precipitate is generated after LDT addition, incubate at 70°C for several minutes and add >99% ethanol after dissolving precipitate.

Results

No Data

Common protocol is usable for the following

No Data

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).

KURABO

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-5

Genomic DNA Extraction from Gonococcal Bacteria (*Neisseria gonorrhoeae*)

Protocol

Suspension of bacteria harvested from liquid medium after culture or agar medium

↓ 5,000 x g, 5 min → Remove supernatant

Pelleted bacteria (about 5 mg of wet bacterial cell)

↓ ← MDT：180 µl

↓ ← EDT：20 µl

Suspend bacteria well by pipetting so as not to leave bacteria agglomerates

Incubate at 55°C : 15 min*1

↓ ← LDT：180 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Incubation at 70°C：10 min

Flash spin down

↓ ← >99% ethanol：240 µl

Vortex (maximum speed)：15 sec & Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Genomic DNA
(Elution volume : 200 µl)

*1 If bacteria agglomerates remain at this time, break them by pipetting and incubate again.

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).

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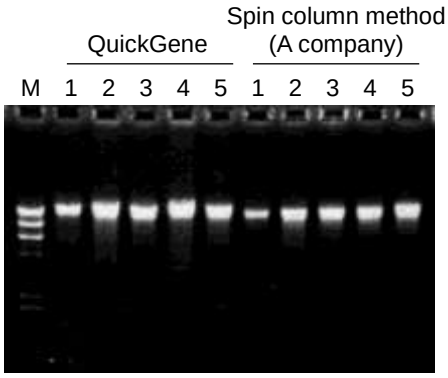
Results

Bacterial strain : Clinical isolates No.1 ~ 5 extracted from about 4.5 ~ 6 mg of each wet fungi

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE
M : λ-*Hin* d III
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4
5 : Bacterial strain No.5

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4	No.5
QuickGene	8.5 µg	7.1 µg	11.2 µg	11.0 µg	7.3 µg
Spin column method (A company)	3.2 µg	6.6 µg	5.8 µg	6.5 µg	4.6 µg

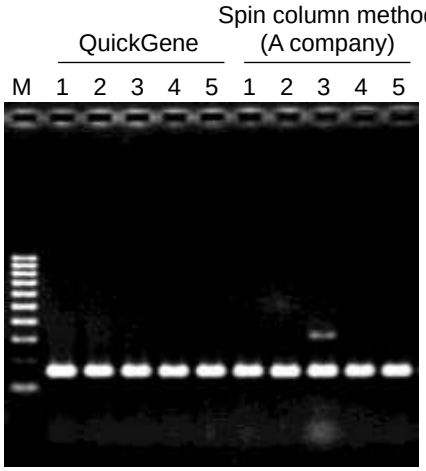
Protein contamination：A260/280

Sample	No.1	No.2	No.3	No.4	No.5
QuickGene	1.97	2.06	2.39	2.03	2.04
Spin column method (A company)	2.11	2.05	2.46	2.00	2.05

Other

- PCR

ParC gene in subunit of topoisomerase IV as target of fluoroquinolone antibacterial agent was detected by PCR for genomic DNA extracted using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4
5 : Bacterial strain No.5

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

Helicobacter pylori, *Pseudomonas aeruginosa*

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-6

Genomic DNA Extraction from Hay Bacillus

Protocol

Harvest hay bacillus and pelletize (5,000 g×10 min) Hay bacillus pellet *1

← STET : 180 µl *2

Vortex (Disperse cells well)

Incubate at 37°C for 30 min

← EDT：20 µl

Tapping 5 times (Confirm enzyme solution is mixed)

← RNase A : 20 µl (optional) *3

Tapping 5 times (Confirm enzyme solution is mixed)

Flash spin down

Incubation at 25°C：2 min

← LDT：180 µl

Vortex for 15 sec (maximum speed) *4 & Flash spin down

Incubation at 70°C：10 min *5

Flash spin down

← >99% ethanol : 240 µl

Vortex for 15 sec (maximum speed) *6 & Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA

*1 Less than 1×10^9 bacillus (OD : 0.8　1.2 ml)

*2 STET : 20mM Tris-HCl (pH8), 2 mM EDTA (pH8), 1.2%
Triton×100, 20 mg/ml Add lysozyme before use.

*3 Amount decreasing is possible depending on RNA amount being expressed in cell

*4 Make homogeneous solution. (When needed, make pipetting.)

*5 When needed, apply agent deactivation (boiling) treatment.

*6 Make homogeneous solution.

Results

No Data

Common protocol is usable for the following

No Data

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).

KURABO

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-7

Genomic DNA Extraction from *Helicobacter pylori*

Protocol

Suspension of bacteria harvested from liquid medium after culture or agar medium

← 5,000 x g, 5 min → Remove supernatant

Pelleted bacteria (about 4 mg of wet bacterial cell)

← MDT : 180 µl

← EDT：20 µl

Suspend bacteria well by pipetting so as not to leave bacteria agglomerates

Incubation at 55°C：15 min *1

← LDT：180 µl

Vortex (maximum speed) : 15 sec

Flash spin down

Incubation at 70°C：10 min

Flash spin down

← >99% ethanol：240 µl

Vortex (maximum speed)：15 sec

Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA
(Elution volume : 200 µl)

*1 If bacteria agglomerates remain at this time, break them by pipetting and incubate again.

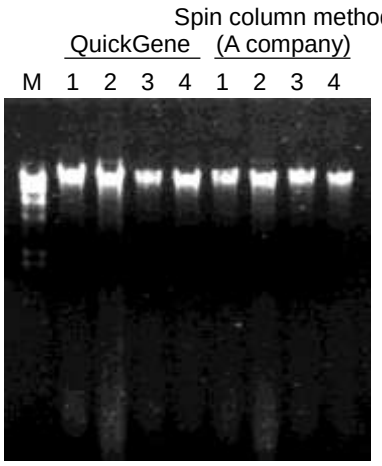
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Bacterial strain : Clinical isolates No. 1 ~ 4 extracted from about 4 mg of each wet bacterial cell

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ –*Hin* d III
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

Sample	No.1	No.2	No.3	No.4
QuickGene	3.0 µg	4.2 µg	2.0 µg	3.2 µg
Spin column method (A company)	2.9 µg	4.7 µg	1.0 µg	2.9 µg

Protein contamination：A260/280

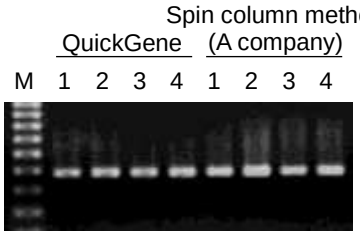
Sample	No.1	No.2	No.3	No.4
QuickGene	2.01	1.91	1.88	1.93
Spin column method (A company)	1.92	1.88	1.78	1.82

Other

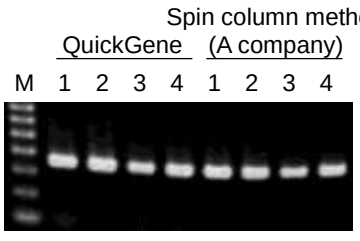
- PCR

16s ribosomal RNA(A) gene and neutrophil-activating protein (NAP)(B) gene of *Helicobacter pylori* were detected by PCR for genomic DNA extracted using QuickGene system and Spin column method (A company).

HprRNA



HpNAP



Electrophoresis condition :
2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1 : Bacterial strain No.1
2 : Bacterial strain No.2
3 : Bacterial strain No.3
4 : Bacterial strain No.4

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

Gonococcal Bacteria (*Neisseria gonorrhoeae*), *Pseudomonas aeruginosa*

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-8

Genomic DNA Extraction from Methicillin-resistant *Staphylococcus aureus* (MRSA)

Protocol

Suspension of bacteria harvested from liquid medium after culture or agar medium

← 5,000 x g, 5 min → Remove supernatant

Bacteria pellet (about 4 mg of wet fungi)

← 20 mM Tris-HCl (pH7.5), 20 µg/ml lysostaphin (Sigma-Aldrich) *1 : 160 µl

Suspend bacteria well

Incubation at 37°C：30 min (Vortex occasionally)

← EDT：20 µl
← 10% Triton X-100 : 20 µl
← LDT：180 µl

Vortex (maximum speed) : 15 sec & Flash spin down

Incubation at 70°C：30 min

Flash spin down

← >99% ethanol：240 µl

Vortex (maximum speed)：15 sec & Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA
(Elution volume : 200 µl)

*1 "20 mM Tris-HCl (pH7.5), 20 µg/ml lysostaphin (Sigma-Aldrich)" is not contained in the kit. Add lysostaphin immediately before use.

Results

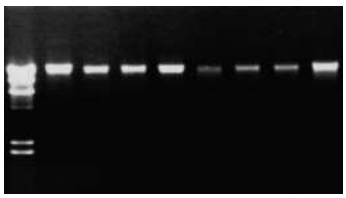
Bacterial : Standard strain (ATCC25923) of Methicillin-sensitive *Staphylococcus aureus* (MSSA)
Strain : Clinical isolates, No.1 ~ 3, of Methicillin-resistant Staphylococcus aureus (MRSA) isolated from about 4 mg of each wet bacterial cell

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).

KURABO

Electropherogram

Spin column method
QuickGene (A company)
M 1 2 3 4 1 2 3 4



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ-*Hin* d III
1 : MSSA ATTC strain
2 : MRSA No.1
3: MRSA No.2
4: MRSA No.3

The yield of genomic DNA

Sample	MSSA	MRSA No.1	MRSA No.2	MRSA No.3
QuickGene	16.0 µg	14.4 µg	10.2 µg	10.3 µg
Spin column method (A company)	2.7 µg	4.6 µg	9.1 µg	12.5 µg

Protein contamination：A260/280

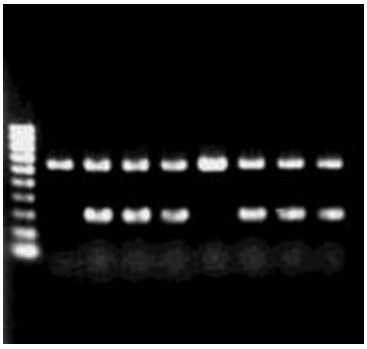
Sample	MSSA	MRSA No.1	MRSA No.2	MRSA No.3
QuickGene	1.76	1.70	1.70	1.76
Spin column method (A company)	1.80	1.76	1.73	1.95

Other

- PCR

For genomic DNA extracted using QuickGene system and Spin column method (A company), *FemA* gene of *Staphylococcus aureus* and *mecA* gene of MRSA were detected by PCR method [Jonas, D. et al.「Rapid PCR based Identification of Methicillin resistant *Staphylococcus aureus* from Screening Swabs.」J. Clin. Microbiol. 2002 ; 40, 1821-1823.].

Spin column method
QuickGene (A company)
M 1 2 3 4 1 2 3 4



Electrophoresis condition : 2% agarose / 1 x TAE

M : 100 bp DNA Ladder
1: MSSA ATT strain
2: MRSA No.1
3: MRSA No.2
4: MRSA No.3

Only *femB* for MSSA (ATCC standard strain) and both of *femB* and *mecA* for MRSA were detected.

Common protocol is usable for the following

No Data

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-9

Genomic DNA Extraction from Penicillin-resistant *Streptococcus Pneumoniae* (PRSP)

Protocol

Bacterial cell pellet *1 : about 4 mg

← A solution *2 : 160 µl

Vortex (maximum speed) : 15 sec & Flash spin down

Incubate at 37°C mixing occasionally : 30 min *3

← LDT : 180 µl

← EDT : 20 µl

Vortex (maximum speed) : 15 sec & Flash spin down

Incubation at 70°C : 30 min

← >99% ethanol : 240 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Genomic DNA
(Elution volume : 200 µl)

*1 Condition of centrifuging for harvest (5,000 x g, 5 min)

*2 A solution : 20 mM Tris-HCl (pH 7.5)
2 mM EDTA
1.2% Triton X-100
20 mg/ml lysozyme
* lysozyme is added when needed

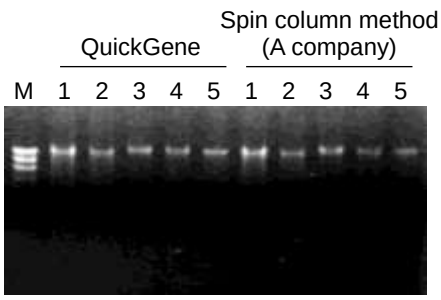
*3 The solution may become milk-white and turbid, or precipitate may be generated. However, dissolution takes place in the next step.

Results

Fungal strain

No.1: R6 (*Streptococcus pneumoniae* standard strain)
No.2: PISP clinical isolate (penicillin-moderately resistant *Streptococcus pneumoniae*)
No.3: PISP clinical isolate (penicillin-moderately resistant *Streptococcus pneumoniae*)
No.4: PRSP clinical isolate (penicillin-highly resistant *Streptococcus pneumoniae*)
No.5: PRSP clinical isolate (penicillin-highly resistant *Streptococcus pneumoniae*)

Electropherogram



M : λ-*Hin* d III
1 : No.1
2 : No.2
3 : No.3
4 : No.4
5 : No.5

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).

KURABO

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4	No.5
QuickGene	12.6 µg	4.8 µg	8.6 µg	9.1 µg	8.3 µg
Spin column method (A company)	10.6 µg	5.8 µg	10.0 µg	8.0 µg	5.4 µg

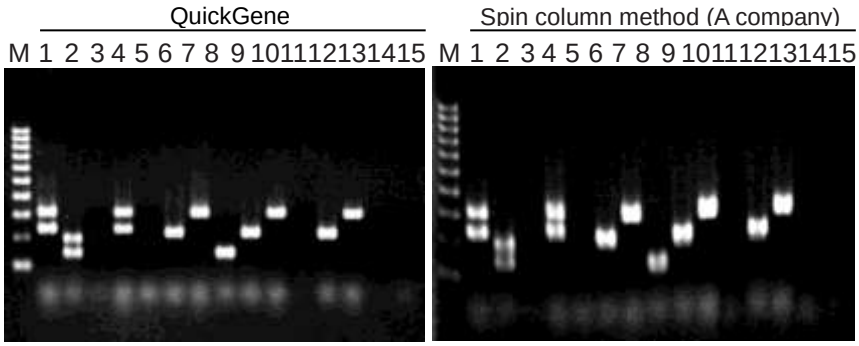
Protein contamination：A260/280

sample	No.1	No.2	No.3	No.4	No.5
QuickGene	1.88	2.14	1.74	2.00	1.96
Spin column method (A company)	2.11	1.75	1.96	1.70	2.05

Other

- PCR

LytA gene [*4], penicillin binding protein gene [*5] (*pbpla, pbp2x, pbp2b*) and macrolide-resistant gene (*mef* (A), *erm* (B)) were detected by PCR for genomic DNA extracted from *Streptococcus pneumoniae* using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose / 1 x TAE

M: 100bp ladder
1: No.1 R6/*lytA*, *pbpla*
2: No.1 R6/*pbp2x*,*pbp2b*
3: No.1 R6/*mef* (A),*erm* (B)
4: No.2 PISP/*lytA*, *pbpla*
5: No.2 PISP/*pbp2x*,*pbp2b*
6: No.2 PISP/*mef* (A),*erm* (B)
7: No.3 PISP/*lytA*,*pbpla*
8 : No.3 PISP/*pbp2x*,*pbp2b*
9 : No.3 PISP/*mef* (A),*erm* (B)
10 : No.4 PRSP/*lytA*,*pbpla*
11 : No.4 PRSP/*pbp2x*,*pbp2b*
12 : No.4 PRSP/*mef* (A),*erm* (B)
13 : No.5 PRSP/*lytA*,*pbpla*
14 : No.5 PRSP/*pbp2x*,*pbp2b*
15 : No.5 PRSP/*mef* (A),*erm* (B)

*4 : Lytic enzyme gene and positive control for *Streptococcus pneumoniae*.
*5 : Primer is designed so that gene is not amplified in case that resisitance mutation takes place.

		lytA	*pbp1a*	*pbp2x*	*pbp2b*	*mef* (A)	*erm* (B)
No.1	R6	+	+	+	+	-	-
No.2	PISP	+	+	-	-	-	+
No.3	PISP	+	-	-	+	-	+
No.4	PRSP	+	-	-	-	-	+
No.5	PRSP	+	-	-	-	-	-

For No.1 R6, neither resistance mutation of penicillin binding protein gene nor macrolide resistant gene was detected.
For No.2 PISP, resistance mutation of *pbp2x, pbp2b* and existence of *erm* (B) were recognized.
For No.3 PISP, resistance mutation of *pbpla, pbp2x* and existence of *erm* (B) were recognized.
For No.4 PRSP, resistance mutation of *pbpla, pbp2x, pbp2b* and existence of *erm* (B) were recognized.
For No.5 PRSP, resistance mutation of *pbpla, pbp2x, pbp2b* was recognized, while existence of macrolide resistant gene was not recognized.
As described above, excellent results of PCR analyses of medical agent resistant gene were obtained.

Common protocol is usable for the following

Vancomycin-resistant Enterococcus (VRE)

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).

KURABO

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-10

Genomic DNA Extraction from *Pseudomonas aeruginosa*

Protocol

Susupension of bacteria harvested from liquid medium after culture or agar medium

← 5,000 x g, 5 min → Remove supernatant

Pelleted bacteria (about 5 mg of wet bacterial cell)

← MDT : 180 µl

← EDT：20 µl

Suspend bacteria well by pipetting so as not to leave bacteria agglomerates as possible

Incubation at 55°C：15 min *1

← LDT：180 µl

Vortex (maximum speed) : 15 sec & Flash spin down

Incubation at 70°C：10 min

Flash spin down

← >99% ethanol：240 µl

Vortex (maximum speed)：15 sec & Flash spin down

Lysate

Transfer all contents of the micro tube into the cartridge of QuickGene

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

Genomic DNA
(Elution volume : 200 µl)

*1 If bacteria agglomerates remain at this time, break them by pipetting and incubate again.

Results

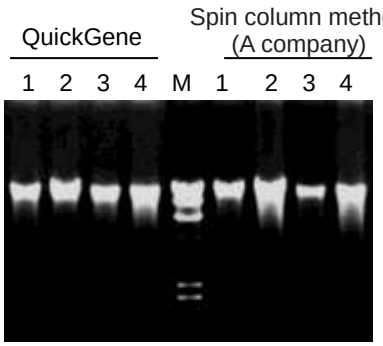
Bacterial strain
No.1 : S792 (serotype G)
No.2 : S728 (serotype G, mucoid strain)
No.3 : S715 (serotype E)
No.4 : S1067 (rough strain)

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).

KURABO

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

Electropherogram



Electrophoresis condition : 1.5% agarose / 1 x TAE

M: λ –*Hin* d III
1: No.1 S792 (serotype G)
2: No.2 S728 (serotype G, mucoid strain)
3: No.3 S715 (serotype E)
4: No.4 S1067 (rough strain)

No decomposition was detected for extracted genomic DNA.

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4
QuickGene	11.4 µg	12.4 µg	10.0 µg	14.0 µg
Spin column method (A company)	10.8 µg	14.0 µg	7.4 µg	13.0 µg

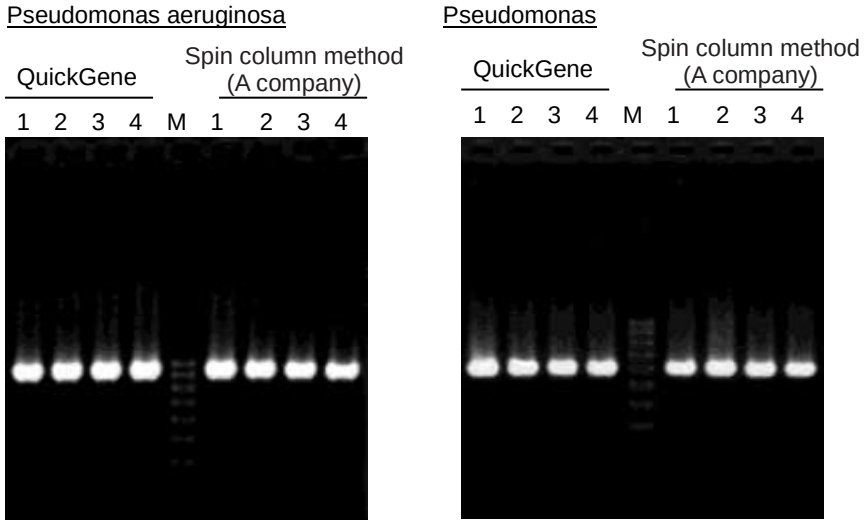
Protein contamination：A260/280

sample	No.1	No.2	No.3	No.4
QuickGene	2.23	1.90	2.31	2.18
Spin column method (A company)	1.96	1.78	1.93	2.12

Other

- PCR

16s rRNA gene was detected by PCR with primer characteristic to *Pseudomonas aeruginosa* and that characteristic to *Pseudomonas* genus, for genomic DNA extracted from *Pseudomonas aeruginosa* using QuickGene system and Spin column method (A company).



Electrophoresis condition :
2% agarose / 1 x TAE

M: 100 bp DNA Ladder
1 : No.1 S792
2 : No.2 S728
3 : No.3 S715
4 : No.4 S1067

PCR products were detected for each genomic DNA.

Common protocol is usable for the following

Gonococcal Bacteria (*Neisseria gonorrhoeae*), *Helicobacter pylori*

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-11

Genomic DNA Extraction from Vancomycin-resistant *Enterococcus* (VRE)

Protocol

Bacterial cell pellet *1 : about 4 mg

← A solution *2 : 160 µl

Vortex (maximum speed) : 15 sec & Flash spin down

Incubate at 37°C mixing occasionally : 30 min *3

← LDT : 180 µl

← EDT : 20 µl

Vortex (maximum speed) : 15 sec & Flash spin down

Incubation at 70°C : 30 min

← >99% ethanol : 240 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Genomic DNA
(Elution volume : 200 µl)

*1 Condition of centrifuging for harvest (5,000 x g, 5 min)

*2 A solution :
20 mM Tris-HCl (pH 7.5)
2 mM EDTA
1.2% Triton X-100
20 mg/ml lysozyme
* lysozyme is added when needed

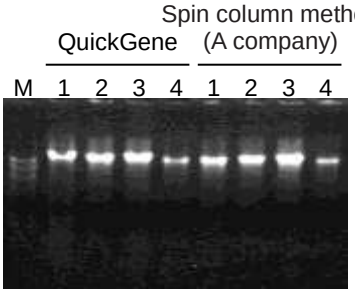
*3 The solution may become milk-white and turbid, or precipitate may be generated. However, dissolution takes place in the next step.

Results

Fungal strain

No.1 : Vancomycin sensitive *E.faecium* (Vancomycin sensitive *Enterococcus* clinical isolate)
No.2 : Vancomycin sensitive *E.faecalis* (Vancomycin sensitive *Enterococcus* clinical isolate)
No.3 : Vancomycin resistant *E.faecalis* (Vancomycin resistant *Enterococcus* clinical isolate)
No.4 : Vancomycin resistant *E.faecalis* (Vancomycin resistant *Enterococcus* clinical isolate)

Electropherogram



M : λ-*Hin* d III
1: No.1
2: No.2
3: No.3
4: No.4

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).

KURABO

The yield of genomic DNA

sample	No.1	No.2	No.3	No.4
QuickGene	11.1 µg	7.4 µg	9.6 µg	3.0 µg
Spin column method (A company)	4.2 µg	7.0 µg	11.1 µg	1.8 µg

Protein contamination：A260/280

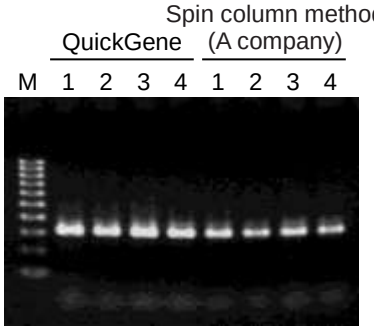
sample	No.1	No.2	No.3	No.4
QuickGene	2.03	1.75	1.94	1.78
Spin column method (A company)	1.73	1.70	1.96	1.70

Other

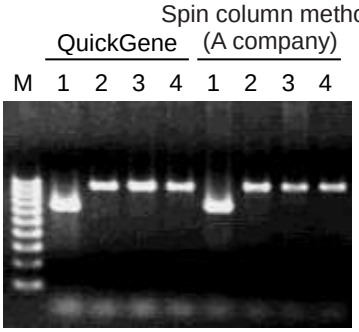
・ PCR

Enterococcus 16S rRNA, E.faecium [*4], *E.faecalis* [*5] and medical agent resistant gene (*vanA* [*6], *vanB* [*7]) were detected by PCR for genomic DNA extracted from *Enterococcus* using QuickGene system and Spin column method (A company).

Enterococcus 16S rRNA



E. faecium, E. faecalis

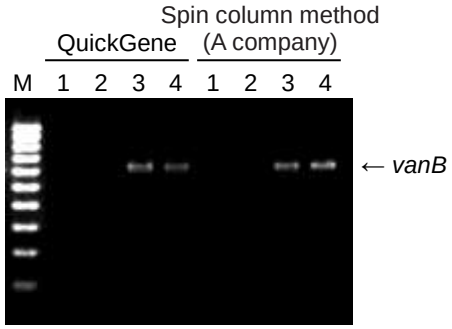


Electrophoresis condition :

2% agarose / 1 x TAE

M: 100 bp DNA Ladder
1 : No.1 Vancomycin sensitive *E.faecium* (Vancomycin sensitive *Enterococcus* clinical isolate)
2 : No.2 Vancomycin sensitive *E.faecalis* (Vancomycin sensitive *Enterococcus* clinical isolate)
3 : No.3 Vancomycin resistant *E.faecalis* (Vancomycin resistant *Enterococcus* clinical isolate)
4 : No.4 Vancomycin resistant *E.faecalis* (Vancomycin resistant *Enterococcus* clinical isolate)

vanA , vanB



No.1 Vancomycin sensitive *E.faecium* was identified to be *vanA*, *vanB* negative *E.faecium*.
No.2 Vancomycin sensitive *E.faecalis* was identified to be *vanA*, *vanB* negative *E.faecalis*.
No.3, 4 Vancomycin sensitive *E.faecalis* were identified to be *vanA* negative, *vanB* positive *E.faecalis*.
For each primer use, good results were obtained , which were consistent with those of biochemical examinations.

*4 : *E.faecium* -specific primers (658 bp)
*5 : *E.faecalis* -specific primers (941 bp)
*6 : Medical agent-resistant gene *vanA* (732 bp)
*7 : Medical agent-resistant gene *vanB* (635 bp)

Common protocol is usable for the following

Penicillin-resistant *Streptococcus Pneumoniae* (PRSP)

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-12

Genomic DNA Extraction from Yeast

Protocol

Yeast pellet (5×10^{7}cells) *1
↓ ← Sorbitol Buffer *2 : 600 µl
Mix by Pipetting
↓ ← Lyticase : 200 U
Vortex (maximum speed) : 15 sec
Incubation at 37°C：30 min
↓ 300 x g, 10 min, RT
Discard supernatant
↓ ← MDT：180 µl
↓ ← EDT：20 µl
Vortex (maximum speed) : 15 sec & Flash spin down
Incubation at 55°C：15 min
↓ ← <Optional> RNase treatment *3
↓ ← LDT : 180 µl
Vortex 15 sec (maximum speed) & Flash spin down
Incubation at 70°C：10 min
Flash spin down
↓ ← >99% ethanol：240 µl
Vortex (maximum speed) : 15 sec & Flash spin down
↓
[Lysate]
↓
Transfer all contents of the micro tube into the cartridge of QuickGene
↓
Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)
↓
[Genomic DNA]

*1 Yeast culture
↻ 5,000 x g, 10 min, RT
Yeast pellet

*2 <Sorbitol Buffer>
1M Sorbitol
100mM EDTA
0.1% β-mercaptoethanol

*3 (Optional process)
RNase A : 20 µl
Mix by tapping 5 times
↓
Flash spin down
Incubate (25°C, 2 min)

Results

No Data

Common protocol is usable for the following

No Data

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).

KURABO

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-13

Genomic DNA Extraction from Yeast (Bead homogenization method)

Protocol

2 ml micro tube

↓ ← Yeast cell pellet *1

Freeze the pellet rapidly *2

↓ ← 4.8 mmφ stainless-steel beads : 2 beads

Homogenize：MS-100R (TOMY SEIKO Co.Ltd.) [3,000 rpm, 30 sec]

↓

Refreeze for 3 min.

↓

Homogenize：MS-100R (TOMY SEIKO Co.Ltd.) [3,000 rpm, 30 sec]

Flash spin down

↓ ← MDT : 180 µl + EDT : 20 µl

Incubation at 55 °C：30 - 60 min

↓ ← 15,000 rpm, 10 sec, RT

Transfer the supernatant (200 µl) to a new 1.5 ml micro tube

↓ ← LDT : 180 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Incubation at 70°C：10 min

Flash spin down

↓ ← >99% ethanol：240 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Lysate

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

Genomic DNA
(Elution volume : 50 µl)

*1 Harvest whole yeast cells by centrifuging after shaking culture in 5 ml YPAD at 30°C for 16 hours [OD600 = app. 3]

*2 Immerse the tube in dry ice-ethanol for more than 10 min.

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).

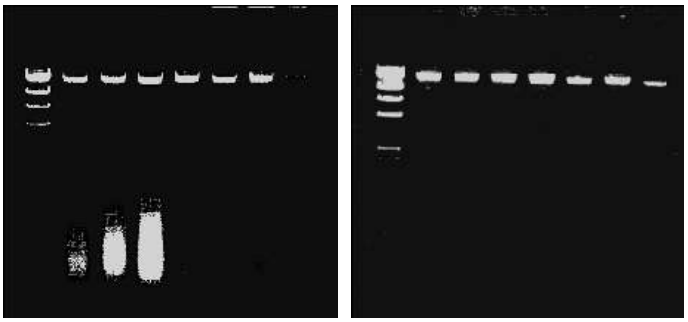
KURABO

Results

Electropherogram

Without RNase treatment
M 1 2 3 4 5 6 7

With RNase treatment
M 1 2 3 4 5 6 7



M : λ-*Hin* d III digest
1 : Parental strain
2 ~ 7 : Candidate null mutants

The yield of genomic DNA / Protein contamination：A260/280

Sample (without RNase treatment)	Yield (μg)	Purity (A260/280)
1	79.4	2.12
2	111.1	2.13
3	127.8	2.12
4	35.0	2.01
5	30.2	1.85
6	53.3	1.99
7	10.7	1.67

Common protocol is usable for the following

No Data

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).

QuickGene

QuickGene DNA tissue kit S (DT-S)
QuickGene SP kit DNA tissue (SP-DT)

DF-14

Helicobacter Pylori Genomic DNA from Human Stool

Protocol

Suspend human stool into PBS (-) so as to be about 10% (w/v),
and disrupt undissolved agglomerates as possible by pipetting.

↓ 3,000 rpm, 5 min, RT

Remove insoluble matter

Collect supernatant (stool emulsion)

↓ ← EDT：20 µl
← MDT：180 µl
← Stool emulsion：100 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

incubation at 55°C：1 hour

↓ 5,000 rpm, 5 min, RT
← LDT：180 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

Incubation at 70°C：10 min

Flash spin down

↓ ← >99% ethanol：240 µl

Vortex (maximum speed) : 15 sec & Flash spin down

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

[Genomic DNA
(Elution volume : 200 µl)]

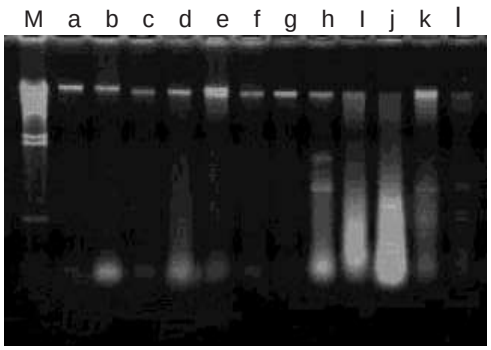
Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

KURABO

Results

Electropherogram

Agarose electrophoresis profile of DNA derived from human stool (1.5% agarose gel)



M : marker (λ-*Hin* d III)
a, g : No.1 (stool, *pylori* positive)
b, h : No.2 (stool, *pylori* positive)
c, i : No.3 (stool, *pylori* positive)
d, j : No.4 (stool, *pylori* positive)
e, k : No.5 (stool, *pylori* negative)
f, l : No.6 (stool, *pylori* negative)

a ~ f : QuickGene
g ~ l : A company

The yield of genomic DNA (μg)

	No.1	No.2	No.3	No.4	No.5	No.6
QuickGene	0.48	1.92	0.40	1.48	3.28	1.32
A company Spin column method	2.48	0.76	1.36	4.8	5.68	0.48

Low yield analytes were found for QuickGene system, while many low molecular weight substances which are considered to be due to decomposition were found by agarose electrophoresis profile for samples refined with A company kit. It was considered that yield becomes high values as calculation based on ultraviolet absorption includes absorption of low molecular weight substances.
From above results, it is considered that in QuickGene system genomic DNA which is less decomposed is refined effectively.

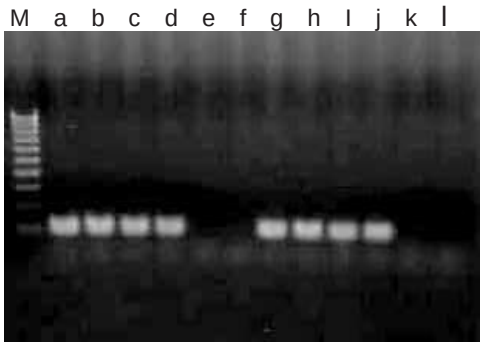
Protein contamination：A260/280

	No.1	No.2	No.3	No.4	No.5	No.6
QuickGene	1.73	2.10	1.74	1.90	2.03	1.96
A company Spin column method	1.83	1.76	1.72	1.70	1.65	1.73

Other

・ PCR

Detection of genomic DNA coding Pylori bacterium 16S rRNA by nested PCR



M : marker (100 bp ladder)
a, g : No.1 (stool, *pylori* positive)
b, h : No.2 (stool, *pylori* positive)
c, i : No.3 (stool, *pylori* positive)
d, j : No.4 (stool, *pylori* positive)
e, k : No.5 (stool, *pylori* negative)
f, l : No.6 (stool, *pylori* negative)

a ~ f : QuickGene
g ~ l : A company

Using DNA prepared from human stool with QuickGene, DNA of Pylori bacterium could be detected from stool of patient who was diagnosed to be positive by nested PCR with testmate rapid Pylori anti body kit

Common protocol is usable for the following

No Data

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).

DF-15

Plasmid DNA Extraction from *E. coli*

Protocol

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

Transfer the supernatant (about 330 µl) to the 1.5 ml microtube with LDP

↓

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

↓

[Lysate]

↓

Transfer all contents of the micro tube into the cartridge of QuickGene

↓

Refer to the extraction protocol for each device written in the kit handbook.
(from the step after transferring the lysate into the cartridge)

↓

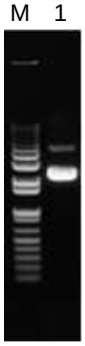
[Plasmid DNA
(Elution volume : 50 µl)]

*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.

Results

Electropherogram



M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
1 : QuickGene

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).

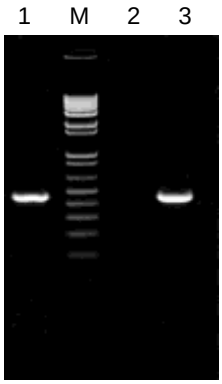
The yield of plasmid DNA / Protein contamination：A260/280 / Chaotropic salt contamination：A260/230

Kit	Yield	A260/280	A260/230
QuickGene	21.4 µg	1.99	2.49

Other

- PCR

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

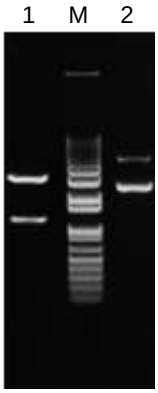


M : Marker (100 bp DNA Ladder : Invitrogen)
1 : QuickGene
2 : Negative control
3 : Positive control

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



M : Marker (1 Kb Plus DNA Ladder : Invitrogen)
1 : QuickGene (*Not* I + *Xho* I)
2 : None

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

Common protocol is usable for the following

Fosmid

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).