8. Genomic DNA Extraction from Bacteria
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

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
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)

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

*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).
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-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.

The yield of genomic DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
</tr>
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<tbody>
<tr>
<td>QuickGene</td>
<td>8.4 µg</td>
<td>23.7 µg</td>
<td>15.8 µg</td>
<td>34.4 µg</td>
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<tr>
<td>Spin column method (A company)</td>
<td>2.3 µg</td>
<td>0.6 µg</td>
<td>N.D</td>
<td>6.7 µg</td>
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Protein contamination: A260/280

<table>
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<th>No.1</th>
<th>No.2</th>
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<tr>
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<td>2.08</td>
<td>1.36</td>
<td>N.D</td>
<td>1.70</td>
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</table>

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 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 cocoides- 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).
**Fosmid DNA Extraction from *E.coli***

**Protocol**

1. **Pellet of transformed *E.coli* in 2.0 ml micro centrifuge tube**
   - RDP mix (RDP + EDP01 *) : 100 µl
   - Mix thoroughly by vortexing (Maximum speed)
   - Flash spin down
   - ADP : 100 µl
   - Mix with inversion 5 times 
   - Flash spin down
   - NDP : 140 µl
   - Mix with inversion 5 times
   - 18,000 x g, 10 min, RT

2. **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

3. **Transfer all contents of the micro tube into the cartridge of QuickGene**

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

5. **Fosmid DNA**
   (Elution volume : 50 µl )

**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).*
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

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).
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)

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).
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

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).

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

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

Electropherogram

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
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<td>4.6 μg</td>
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The yield of genomic DNA

<table>
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<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
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<td>2.05</td>
<td>2.46</td>
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Protein contamination: A260/280

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<td>2.00</td>
<td>2.05</td>
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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).

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<th>Sample</th>
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<th>No.5</th>
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<td>2.05</td>
<td>2.46</td>
<td>2.00</td>
<td>2.05</td>
</tr>
</tbody>
</table>

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).
Genomic DNA Extraction from Hay Bacillus

**Protocol**

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

- **STET**: 180 µl

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)

- 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) & 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

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**

**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).
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

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).
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

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
</tr>
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<td>2.9 µg</td>
<td>4.7 µg</td>
<td>1.0 µg</td>
<td>2.9 µg</td>
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Protein contamination: A260/280

<table>
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<tr>
<th>Sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
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<tbody>
<tr>
<td>QuickGene</td>
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<td>1.91</td>
<td>1.88</td>
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<tr>
<td>Spin column method (A company)</td>
<td>1.92</td>
<td>1.88</td>
<td>1.78</td>
<td>1.82</td>
</tr>
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</table>

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

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).
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)

**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).


### Electropherogram

- **Spin column method**
  - QuickGene
  - (A company)

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>MSSA (µg)</th>
<th>MRSA No.1 (µg)</th>
<th>MRSA No.2 (µg)</th>
<th>MRSA No.3 (µg)</th>
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<tr>
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<td>10.2</td>
<td>10.3</td>
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<td>2.7</td>
<td>4.6</td>
<td>9.1</td>
<td>12.5</td>
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### Protein contamination: A260/280

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<tr>
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<th>MRSA No.2</th>
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<td>Spin column method (A company)</td>
<td>1.80</td>
<td>1.76</td>
<td>1.73</td>
<td>1.95</td>
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</table>

### Other

- **PCR**


Spin column method

- QuickGene
- (A company)

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).
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)

### 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

<table>
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<tr>
<th>QuickGene</th>
<th>Spin column method</th>
</tr>
</thead>
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<tr>
<td>M 1 2 3 4 5</td>
<td>1 2 3 4 5</td>
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<table>
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<td>4</td>
<td>No.4</td>
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<tr>
<td>5</td>
<td>No.5</td>
</tr>
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</table>

\(\lambda\)-Hin d III

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 genomic DNA

<table>
<thead>
<tr>
<th>sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
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<td>5.4 µg</td>
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</table>

Protein contamination : A260/280

<table>
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<tr>
<th>sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
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<td>1.75</td>
<td>1.96</td>
<td>1.70</td>
<td>2.05</td>
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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).

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

  Electrophoresis condition : 2% agarose / 1 x TAE

  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 (e.g. when extracting DNA, RNA is also extracted).
Genomic DNA Extraction from *Pseudomonas aeruginosa*

**Protocol**

**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 as possible

- Incubation at 55°C: 15 min

- 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)

**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).
**Electropherogram**

<table>
<thead>
<tr>
<th></th>
<th>QuickGene</th>
<th>Spin column method (A company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis condition</td>
<td>1.5% agarose / 1 x TAE</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>λ – <em>Hin</em> III</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No.1 S792 (serotype G)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No.2 S728 (serotype G, mucoid strain)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No.3 S715 (serotype E)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No.4 S1067 (rough strain)</td>
<td></td>
</tr>
</tbody>
</table>

No decomposition was detected for extracted genomic DNA.

**The yield of genomic DNA**

<table>
<thead>
<tr>
<th>sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>11.4 µg</td>
<td>12.4 µg</td>
<td>10.0 µg</td>
<td>14.0 µg</td>
</tr>
<tr>
<td>Spin column method (A company)</td>
<td>10.8 µg</td>
<td>14.0 µg</td>
<td>7.4 µg</td>
<td>13.0 µg</td>
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</table>

**Protein contamination : A260/280**

<table>
<thead>
<tr>
<th>sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>2.23</td>
<td>1.90</td>
<td>2.31</td>
<td>2.18</td>
</tr>
<tr>
<td>Spin column method (A company)</td>
<td>1.96</td>
<td>1.78</td>
<td>1.93</td>
<td>2.12</td>
</tr>
</tbody>
</table>

**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).

**Pseudomonas aeruginosa**

<table>
<thead>
<tr>
<th></th>
<th>QuickGene</th>
<th>Spin column method (A company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis condition</td>
<td>2% agarose / 1 x TAE</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>100 bp DNA Ladder</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No.1 S792</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No.2 S728</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No.3 S715</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No.4 S1067</td>
<td></td>
</tr>
</tbody>
</table>

**Pseudomonas**

<table>
<thead>
<tr>
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<th>QuickGene</th>
<th>Spin column method (A company)</th>
</tr>
</thead>
<tbody>
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<td>Electrophoresis condition</td>
<td>2% agarose / 1 x TAE</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>100 bp DNA Ladder</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No.1 S792</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No.2 S728</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No.3 S715</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No.4 S1067</td>
<td></td>
</tr>
</tbody>
</table>

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

Protocol

Bacterial cell pellet: about 4 mg

A solution: 160 µl

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

Incubate at 37°C mixing occasionally: 30 min

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)

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

Spin column method

QuickGene (A company)

M : λ-Hind 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).
The yield of genomic DNA

<table>
<thead>
<tr>
<th>sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>11.1 µg</td>
<td>7.4 µg</td>
<td>9.6 µg</td>
<td>3.0 µg</td>
</tr>
<tr>
<td>Spin column method (A company)</td>
<td>4.2 µg</td>
<td>7.0 µg</td>
<td>11.1 µg</td>
<td>1.8 µg</td>
</tr>
</tbody>
</table>

Protein contamination : A260/280

<table>
<thead>
<tr>
<th>sample</th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>2.03</td>
<td>1.75</td>
<td>1.94</td>
<td>1.78</td>
</tr>
<tr>
<td>Spin column method (A company)</td>
<td>1.73</td>
<td>1.70</td>
<td>1.96</td>
<td>1.70</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>QuickGene</th>
<th>Spin column method (A company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
</tbody>
</table>

| 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) |

*E. faecium, E. faecalis*

<table>
<thead>
<tr>
<th>Sample</th>
<th>QuickGene</th>
<th>Spin column method (A company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
</tbody>
</table>

Electrophoresis condition :
2% agarose / 1 x TAE

vanA, vanB

<table>
<thead>
<tr>
<th>Sample</th>
<th>QuickGene</th>
<th>Spin column method (A company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
</tbody>
</table>

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.

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).
Genomic DNA Extraction from Yeast

Protocol

Yeast pellet (5×10^7 cells) \(^*1\)
Mix by Pipetting
Vortex (maximum speed) : 15 sec
Incubation at 37°C : 30 min
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

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).
Genomic DNA Extraction from Yeast (Bead homogenization method)

Protocol

2 ml micro tube

Yeast cell pellet

Freeze the pellet rapidly

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).
Results

Electropherogram

Without RNase treatment

With RNase treatment

\[ \lambda - H\text{in} \text{ III digest} \]
\[ 1 : \text{Parental strain} \]
\[ 2 \sim 7 : \text{Candidate null mutants} \]

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

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

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).
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).
Results

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

<table>
<thead>
<tr>
<th>M</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
<th>k</th>
<th>l</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>g</td>
<td>No.1 (stool, pylori positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>h</td>
<td>No.2 (stool, pylori positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>i</td>
<td>No.3 (stool, pylori positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>j</td>
<td>No.4 (stool, pylori positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>k</td>
<td>No.5 (stool, pylori negative)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>l</td>
<td>No.6 (stool, pylori negative)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a ~ f : QuickGene
g ~ l : A company

The yield of genomic DNA (μg)

<table>
<thead>
<tr>
<th></th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
<th>No.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>0.48</td>
<td>1.92</td>
<td>0.40</td>
<td>1.48</td>
<td>3.28</td>
<td>1.32</td>
</tr>
<tr>
<td>A company Spin column method</td>
<td>2.48</td>
<td>0.76</td>
<td>1.36</td>
<td>4.8</td>
<td>5.68</td>
<td>0.48</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
<th>No.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickGene</td>
<td>1.73</td>
<td>2.10</td>
<td>1.74</td>
<td>1.90</td>
<td>2.03</td>
<td>1.96</td>
</tr>
<tr>
<td>A company Spin column method</td>
<td>1.83</td>
<td>1.76</td>
<td>1.72</td>
<td>1.70</td>
<td>1.65</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Other

• PCR

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

<table>
<thead>
<tr>
<th>M</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>g</td>
<td>No.1 (stool, pylori positive)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>h</td>
<td>No.2 (stool, pylori positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>i</td>
<td>No.3 (stool, pylori positive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>j</td>
<td>No.4 (stool, pylori positive)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>k</td>
<td>No.5 (stool, pylori negative)</td>
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<tr>
<td>f</td>
<td>l</td>
<td>No.6 (stool, pylori negative)</td>
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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).
**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) \(^2\)

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

<table>
<thead>
<tr>
<th>Kit</th>
<th>Yield</th>
<th>A260/280</th>
<th>A260/230</th>
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<tbody>
<tr>
<td>QuickGene</td>
<td>21.4 µg</td>
<td>1.99</td>
<td>2.49</td>
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</table>

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