



QuickGene

QuickGene Series **Application Guide**

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

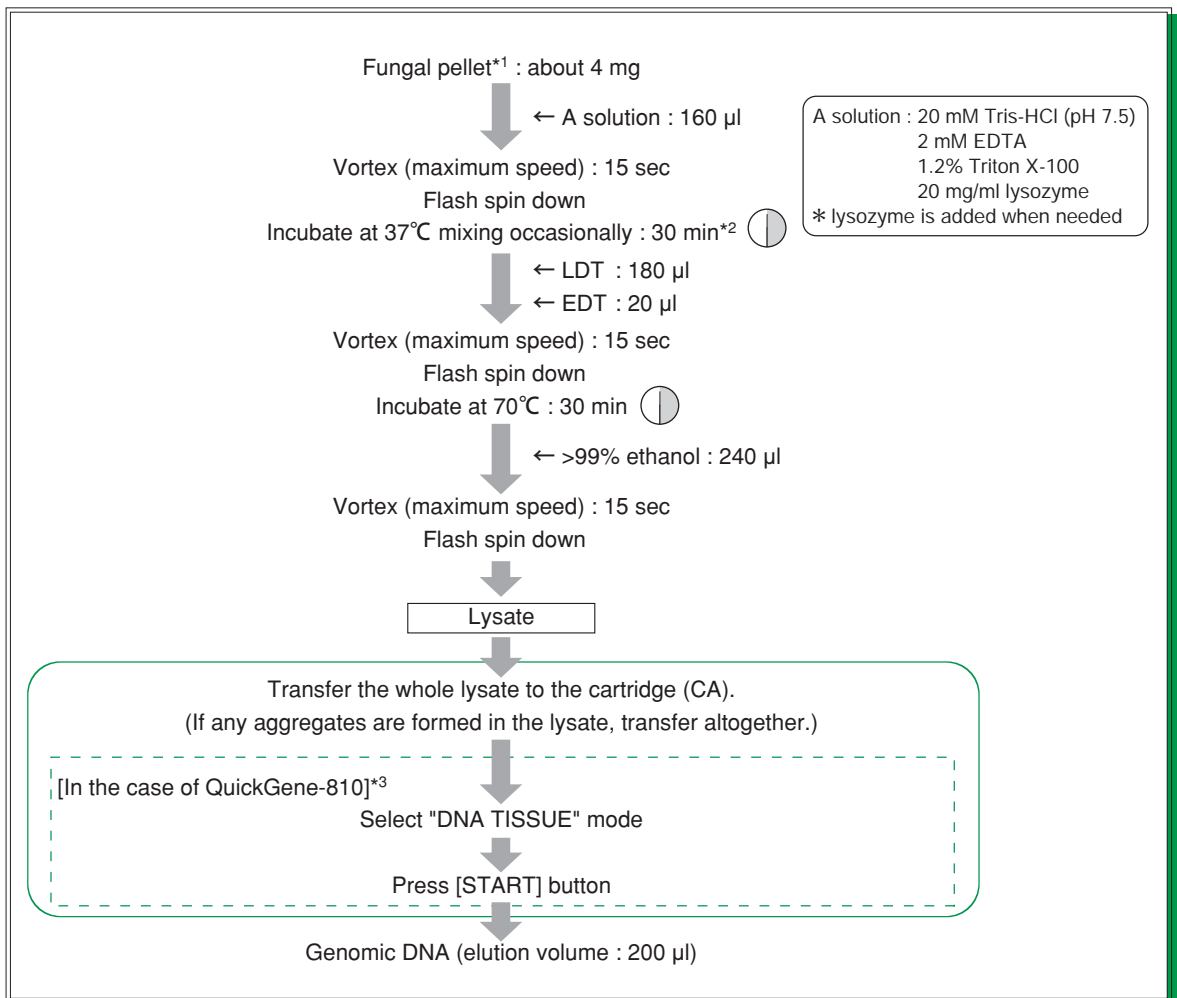
Kit : QuickGene DNA tissue kit S

Model : QuickGene-810 / QuickGene-Mini80

## Summary

Enables easy and rapid genomic DNA extraction from penicillin-resistant *Streptococcus pneumoniae* (PRSP)

## ● Protocol



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

\*2 : The solution may become milk-white and turbid, or precipitate may be generated. However, dissolution takes place in the next step.

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

\* Perform extraction within 30 min after lysate preparation.

## Results : Genomic DNA extraction from *Streptococcus pneumoniae*

Genomic DNA was extracted from each ~ 4 mg of the following wet fungi using QuickGene system (QuickGene -800 and QuickGene DNA tissue kit S) and Spin column method (A company).

- 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*)

### ● The yield and purity of genomic DNA

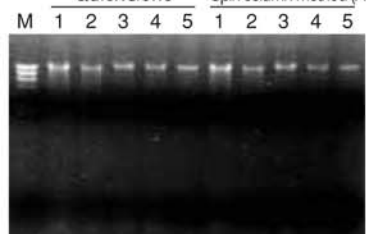
Sample	Yield					Purity(A260/280)				
	No.1	No.2	No.3	No.4	No.5	No.1	No.2	No.3	No.4	No.5
QuickGene	12.6 µg	4.8 µg	8.6 µg	9.1 µg	8.3 µg	1.88	2.14	1.74	2.00	1.96
Spin column method (A company)	10.6 µg	5.8 µg	10.0 µg	8.0 µg	5.4 µg	2.11	1.75	1.96	1.70	2.05

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

### ● Electrophoresis of genomic DNA

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

QuickGene Spin column method (A company)



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -Hind III

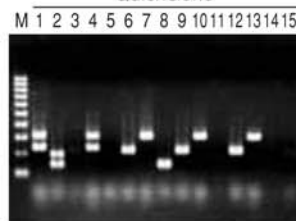
- 1 : No.1 R6(*Streptococcus pneumoniae* standard strain)
- 2 : No.2 PISP clinical isolate(penicillin-moderately resistant *Streptococcus pneumoniae*)
- 3 : No.3 PISP clinical isolate(penicillin-moderately resistant *Streptococcus pneumoniae*)
- 4 : No.4 PRSP clinical isolate(penicillin-highly resistant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

No decomposition was detected for extracted genomic DNA.

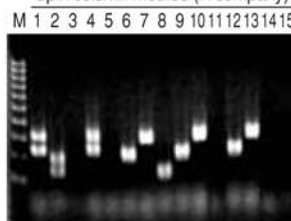
### ● PCR

*lytA* gene<sup>\*4</sup>, penicillin binding protein gene<sup>\*5</sup> (*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).

QuickGene



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 : *lytA* gene and positive control for *Streptococcus pneumoniae*.

\*5 : Primer is designed so that gene is not amplified in case that resistance mutation takes place.

		<i>lytA</i>	<i>pbp1a</i>	<i>pbp2x</i>	<i>pbp2b</i>	<i>mef(A)</i>	<i>erm(B)</i>
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 *pbp1a*, *pbp2x* and existence of *erm(B)* were recognized.

For No.4 PRSP, resistance mutation of *pbp1a*, *pbp2x*, *pbp2b* and existence of *erm(B)* were recognized.

For No.5 PRSP, resistance mutation of *pbp1a*, *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.

#### \* Trademark and exclusion item

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North American Distributor

www.autogen.com

AutoGen, Inc. 84 October Hill Road Holliston, MA 01746

Tel: 508.429.5965; Fax: 508.429.9765; E-mail: info@autogen.com