



QuickGene Series Application Guide

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

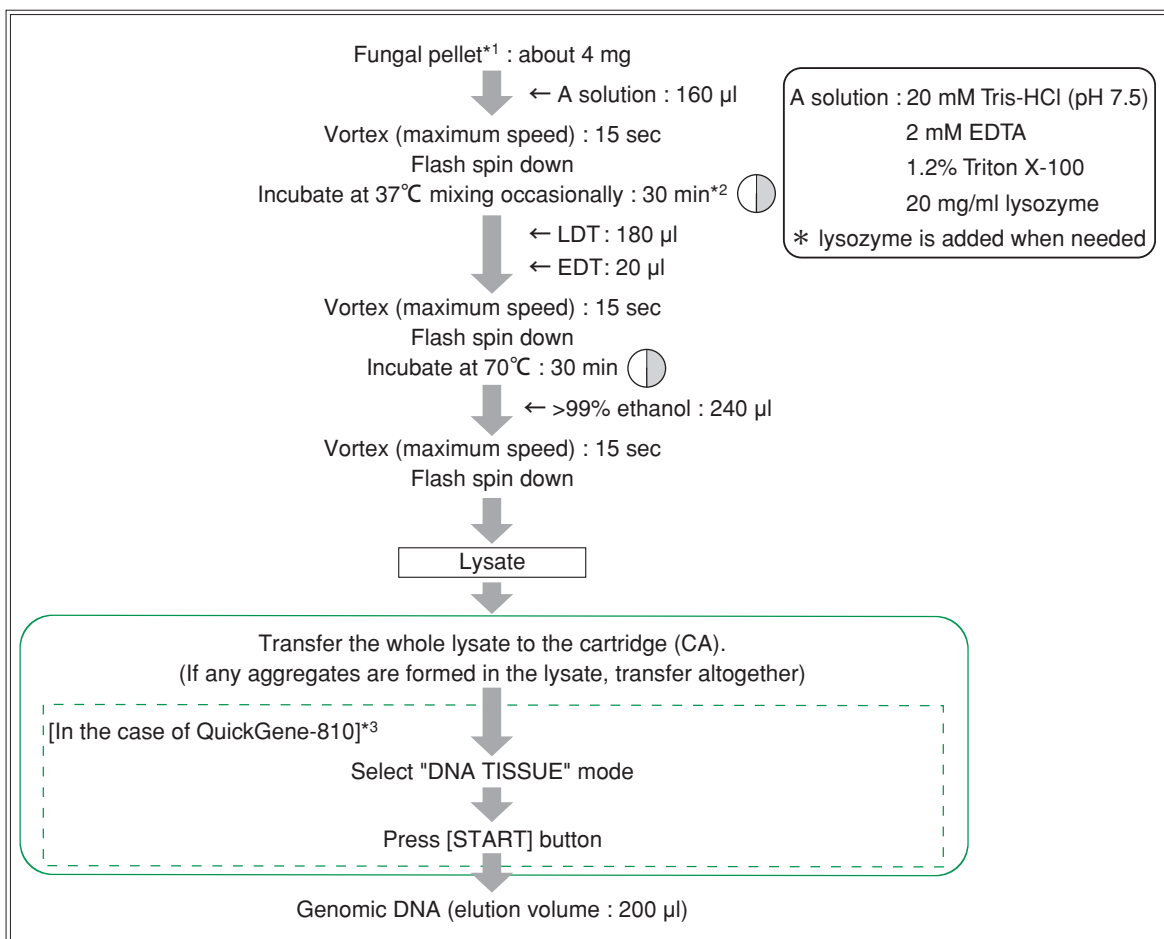
Kit : QuickGene DNA tissue kit S

Model : QuickGene-810 / QuickGene-Mini80

Summary

Enables easy and rapid genomic DNA extraction from vancomycin-resistant *Enterococcus* (VRE)

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 detail.

* Perform extraction within 30 min after lysate preparation.

Results : Genomic DNA extraction from *Enterococcus*

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 : Vancomycin sensitive <i>E.faecium</i>	(Vancomycin sensitive <i>Enterococcus</i> clinical isolate)
	No.2 : Vancomycin sensitive <i>E.faecalis</i>	(Vancomycin sensitive <i>Enterococcus</i> clinical isolate)
	No.3 : Vancomycin resistant <i>E.faecalis</i>	(Vancomycin resistant <i>Enterococcus</i> clinical isolate)
	No.4 : Vancomycin resistant <i>E.faecalis</i>	(Vancomycin resistant <i>Enterococcus</i> clinical isolate)

● The yield and purity of genomic DNA

Sample	Yield				Purity(A260/280)			
	No.1	No.2	No.3	No.4	No.1	No.2	No.3	No.4
QuickGene	11.1 µg	7.4 µg	9.6 µg	3.0 µg	2.03	1.75	1.94	1.78
Spin column method (A company)	4.2 µg	7.0 µg	11.1 µg	1.8 µg	1.73	1.70	1.96	1.70

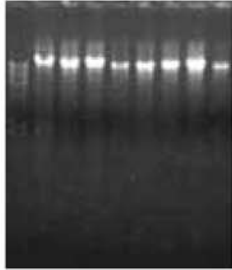
The use of QuickGene system enables high-purity genomic DNA extraction with little contamination of protein in high yield and in better reproducibility than spin column method.

● Electrophoresis of genomic DNA

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

QuickGene Spin column method (A company)

M 1 2 3 4 1 2 3 4



Electrophoresis condition : 1.5% agarose / 1 x TAE

M : λ -Hind III

- 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)

No decomposition was detected for extracted genomic DNA.

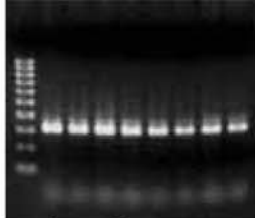
● PCR

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

QuickGene Spin column method (A company)

M 1 2 3 4 1 2 3 4



vanA, *vanB*

QuickGene Spin column method (A company)

M 1 2 3 4 1 2 3 4

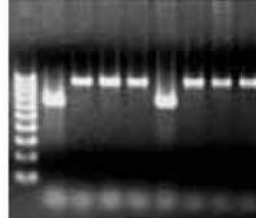


← *vanB*

E. faecium, *E. faecalis*

QuickGene Spin column method (A company)

M 1 2 3 4 1 2 3 4



← *E. faecalis*
← *E. faecium*

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)

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)

* Trademark and exclusion item

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