



QuickGene Series Application Guide

DNA Isolation from Branchia of Koi Herpes Virus (KHV) Infected Fish

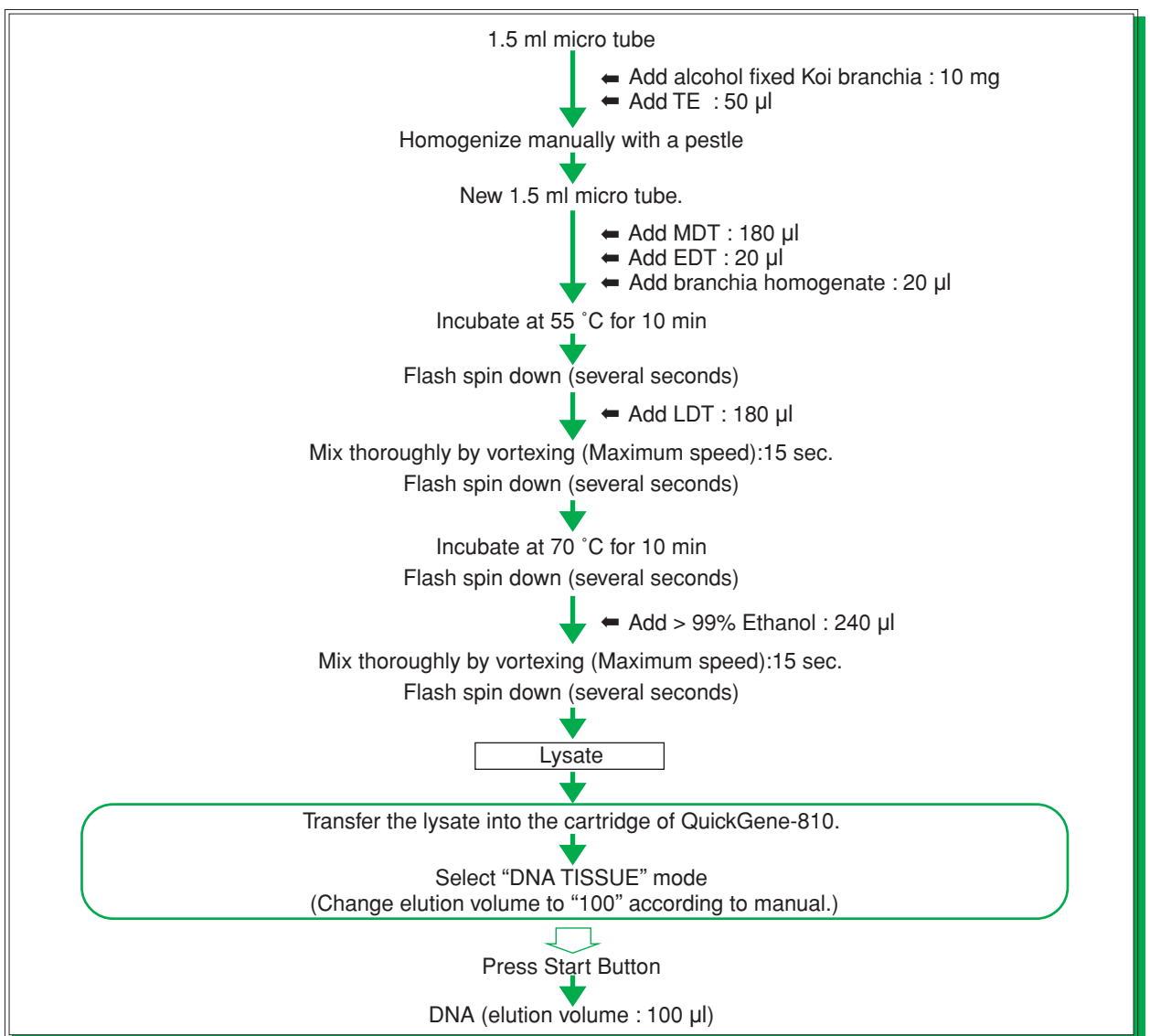
QuickGene tissue Kit S / QuickGene-810

Enables easy and rapid isolation of high-purity DNA from branchia of Koi Herpes Virus infected fish

Summary

- Total DNA of branchia of Koi Herpes Virus infected fish was extracted, and only Koi Herpes Virus DNA was detected by PCR.

Protocol



* Perform isolation within 30 min after lysate preparation.

Results : DNA isolation from branchia of Koi Herpes Virus infected fish

DNA was isolated from branchiae of normal fish (2 fish) and Koi Herpes Virus infected fish (4 fish) by using QuickGene-810 system(QuickGene-810 and QuickGene DNA tissue kit S).

● The yield and purity of DNA

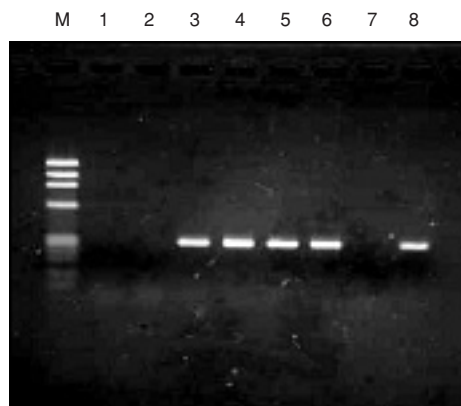
	No.	Yield(μg)	A _{260/280}
Normal fish	1	4.24	2.19
	2	4.07	2.27
Infected fish	1	0.67	2.04
	2	1.28	2.39
	3	2.41	2.10
	4	2.35	1.99

A_{260/280} ratio: an indicator of protein contamination (A_{260/280} >1.7).
(The ratio decreases with increasing the level of protein contamination.)

● PCR

DNA isolated by using QuickGene-810 system was used for PCR template.
PCR was performed according to the method by Yuasa et al, Improvement of a PCR method with the *Sph* 1-5 primer set for the detection of Koi herpesvirus (KHV), Fish Pathology, 40, 37-39(2005).

Primer : *Sph* I-5F, *Sph* I-5R



M : λ × 174-*Hae* III digest

- 1 : Normal fish No.1
- 2 : Normal fish No.2
- 3 : Infected fish No.1
- 4 : Infected fish No.2
- 5 : Infected fish No.3
- 6 : Infected fish No.4
- 7 : Negative control
- 8 : Positive control

PCR amplification similar to that for positive control was confirmed for infected fish, No.1-4.

Contributed by Hiroshima Pref. Fisheries & Marine Technology Center