

**QuickGene** QuickGene Series **Application Guide**

## Genomic DNA Isolation from Mouse Sperm

### QuickGene DNA Tissue Kit S

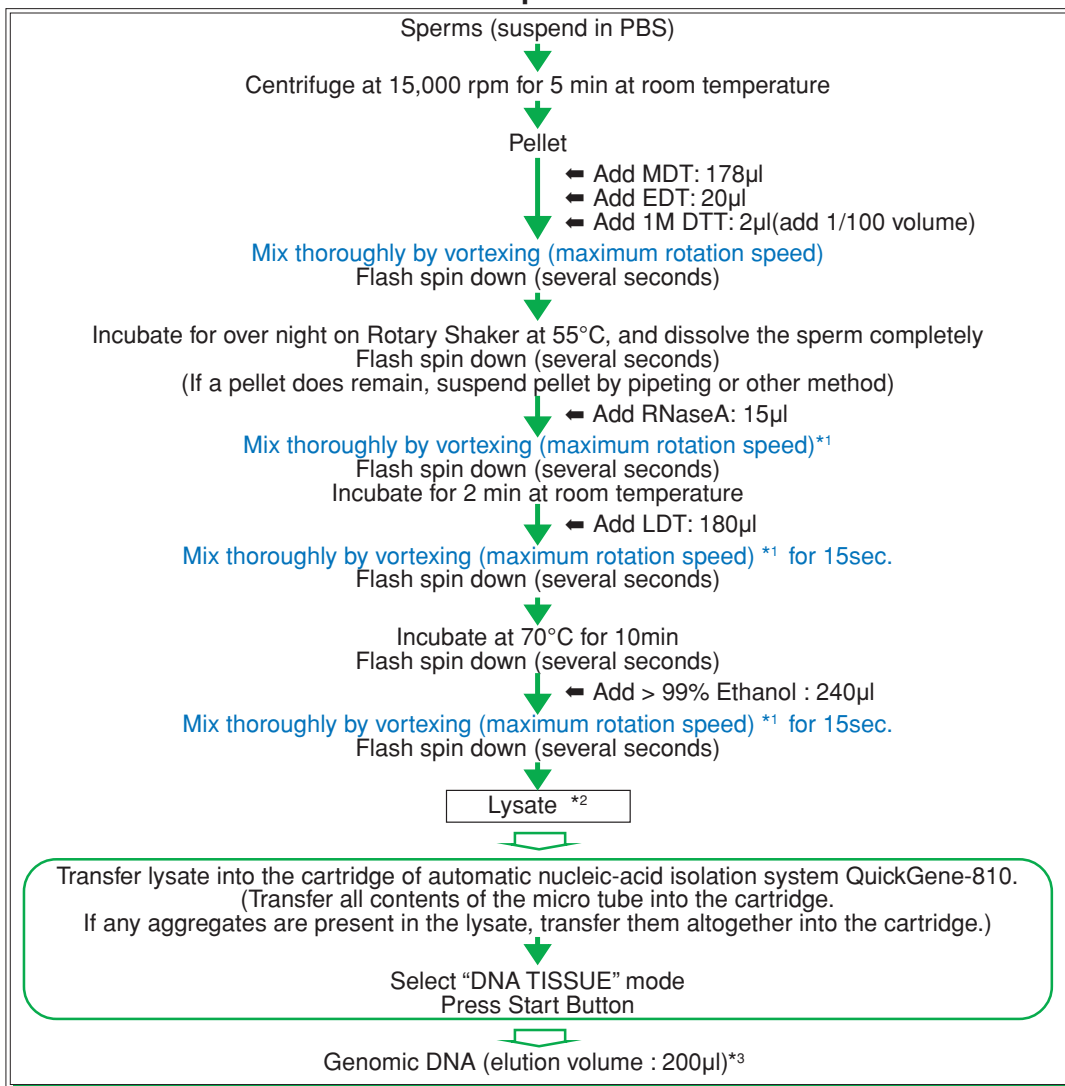
Enables easy and rapid isolation of genomic DNA from mouse sperm.  
Furthermore, the isolated genomic DNA is highly pure and methylation is retained.

#### Features

- Simultaneously extracts genomic DNA from 8 sets of Lysate in only 13 minutes
- Sophisticated genomic DNA isolation system without centrifugation
- Safety operation without using hazardous solvent such as phenol
- Isolated genomic DNA should be sufficiently pure and the yield is enough for PCR, restriction enzyme digestion, Southern Blotting and other applications because of uncontaminated protein and chaotropic salt.

#### Protocol

#### Genomic DNA isolation from Mouse Sperm



\*1 : Mix completely by vortexing at the maximum speed.  
If the mixing is not enough by vortexing, use tapping, pipetting or inverting.

\*2 : Transfer the lysate into the cartridge within 30min.

\*3 : This elution volume is initial value of "DNA TISSUE" mode.

## Components of the Kit

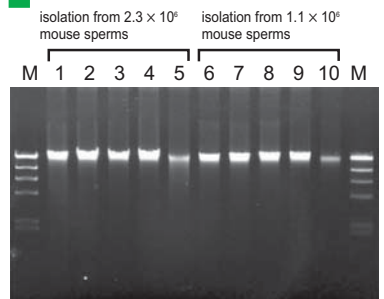
- Proteinase K (EDT)
- Tissue lysis buffer (MDT)
- Lysis buffer (LDT)
- Wash buffer (WDT)
- Collection buffer (CDT)
- Cartridges (CA)
- Collection tubes (CT)
- Caps (CAP)
- Waste tubes (WT)

## Preparation of reagents

- Wash buffer (WDT)  
Add 160ml of > 99% Ethanol into the bottle and mix with inversion the bottle gently before using.

## Results : Genomic DNA isolation from mouse sperm

### 1) AGE of isolated genomic DNA (Sample: $2.3 \times 10^6$ and $1.1 \times 10^6$ mouse sperms)



Genomic DNA isolated from mouse sperm (100ng)

Genomic DNA was isolated from  $2.3 \times 10^6$  and  $1.1 \times 10^6$  mouse sperms by using the QuickGene-810 system (QuickGene-810 and QuickGene DNA tissue kit S) and phenol/chloroform method. Agarose gel electrophoresis was performed with isolated genomic DNA.

Lane M:  $\lambda$ -Hind III marker  
Lanes 1-4, 6-9: QuickGene-810  
Lanes 5, 10: Phenol/chloroform

The DNA isolated with phenol/chloroform showed smear of DNA (Lanes 5 and 10), not shown in QuickGene-810 (Lanes 1-4, 6-9) isolated DNA.

The DNA isolated with QuickGene-810 has higher yield and higher purity than the isolated DNA with phenol/chloroform.

### 2) The yield of genomic DNA ( $\mu\text{g}$ ) (calculated from $A_{260 \text{ nm}}$ )

Number of sperm	$2.3 \times 10^6$	$1.1 \times 10^6$
QuickGene-810	3.99	3.99
Phenol/chloroform method	5.48	2.20

The yield with phenol/chloroform method seems higher than the yield with QuickGene-810 by calculation from  $A_{260}$  however it is due to the degradation of DNA.

The yield of QuickGene-810 system is higher than the yield of phenol/chloroform method as the electrophoresis data shows.

### 3) The purity of genomic DNA (determination of protein contamination): $A_{260/280}$

Number of sperm	$2.3 \times 10^6$	$1.1 \times 10^6$
QuickGene-810	1.75	1.73
Phenol/chloroform method	1.6	1.93

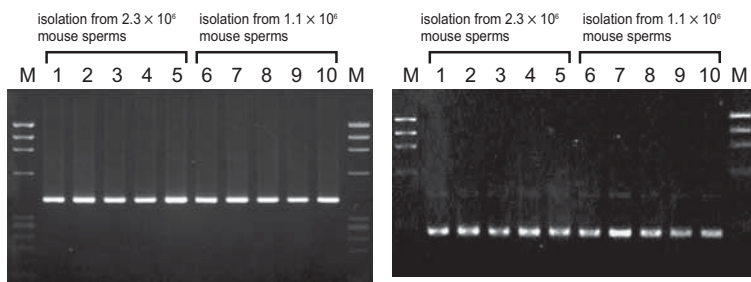
$A_{260/280}$ : Indicator of protein contamination ( $A_{260/280} > 1.7$ ). (Protein contamination lowers the absorbance ratio.)

QuickGene-810 system enables the isolation of high-purity genomic DNA with little protein contamination.

#### 4) Bisulfite treatment \* and PCR

1 µg of mouse sperm genomic DNA isolated using QuickGene-810 system or the phenol/chloroform method, was treated with bisulfite and used for PCR template.

PCR amplification targeting the differentially methylated regions (DMR) of H19 and Igf2r was performed successfully by using 250 ng genomic DNA treated with bisulfite.



H19 Bisulfite PCR electropherogram

Igf2r Bisulfite PCR electropherogram

Lane M:  $\phi$  × 174/Hae III marker  
Lanes 1-4, 6-9: QuickGene-810  
Lanes 5, 10: Phenol/chloroform

No difference in DNA after bisulfite treatment was seen between the QuickGene-810 isolation method and phenol/chloroform isolation method.

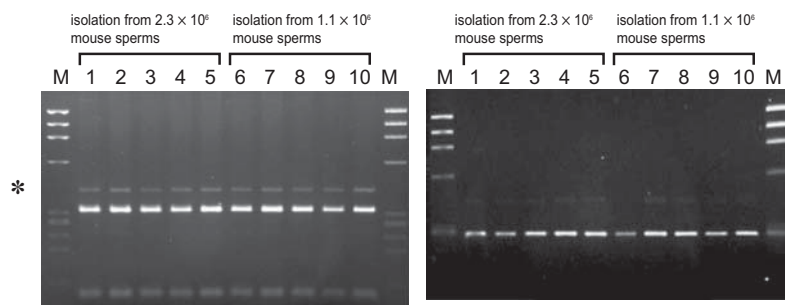
#### \* Bisulfite treatment

Bisulfite treatment converts nonmethylated cytosine (C) in the genomic DNA to uracil (U). Methylated cytosine is structurally stable so it is not converted to uracil.

(<http://www.mbcl.co.jp/data/gene/20.html>)

#### 5) DNA methylation analysis by using combined bisulfite restriction assay (COBRA)

The PCR products H19 DMR and Igf2r DMR obtained in 3) were digested by restriction enzymes HpyCH4IV And Csp45I, respectively. Agarose gel electrophoresis photos of the results are shown.



H19 COBRA electropherogram

Igf2r COBRA electropherogram

Lane M:  $\phi$  × 174/Hae III marker  
Lanes 1-4, 6-9: QuickGene-810  
Lanes 5, 10: Phenol/chloroform

H19 DMR is almost completely methylated and Igf2r DMR is demethylated.

\* Band indicates nonmethylated band

Therefore, it is confirmed that the methylated portion of sperm DNA isolated QuickGene-810, like the phenol/chloroform isolation method, is conserved.

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