KURABO

Ver.17-08 E

Isolation of genomic DNA Quick Guide

Cheek Swab

QuickGene DNA tissue kit S (DT-S)

In this Quick Guide, the protocol for isolation of genomic DNA from animal tissue is a digest from the Handbook of QuickGene tissue kit L (DT-S) and the Operation Manual of QuickGene-Mini480. *** Before using, please read the Operation Manual.**

Wear protective gloves and safety goggles during the experiments.

step1 Preparations

In order to isolate the target genomic DNA, please prepare the following items.

1 Preparations

QuickGene-Mini480

DNA Tissue Kit S (DT-S)

Microtube (2 ml)

High grade ethanol (>99%)

Benchtop microcentrifuge

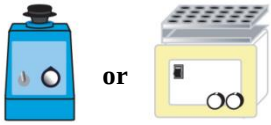
Heat block incubator (56℃)

Protective gloves

Safety goggles

Micropipettes
(P-200, P-1000 or other types)

Tube mixer



or

2 Preparations of Reagents

◆Proteinase K (EDT)

Store at 2-8℃.

◆ Tissue Lysis Buffer (MDT)

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubating at 55℃.

◆ Lysis Buffer (LDT)

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubating at 37℃.

◆ Wash Buffer (WDT)

Add 160 ml ethanol (>99%) into the bottle and mix well.

After adding the ethanol, close the cap and store at room temperature.

◆ Elution Buffer (CDT)

Use CDT for elution of genomic DNA.

Continue to step.2

step2 Protocol

In order to gain the target yield of DNA, please follow the protocol below.

When putting the swab into the tube and dissolving cells by a long time incubation, please use non-organic derived swabs (e.g. polypropylene shaft with polyester cotton swab etc.).
Regarding the collection of cheek cells, please follow the manual of cheek swab supplied by its producer.

1 Suspend cheek cells in PBS from cheek swab

1) Suspend cheek cells in 200-400 µl of 1x PBS buffer with swab cotton
2) Remove swab cotton from the buffer
3) Use 200 µl of solution for a sample

For the maximum yield, the following protocol is available.
1) Cut down swab cotton from its shaft, and put into 2ml microtube with 400 µl of 1x PBS buffer in
2) Set the heat block incubator at 56℃
3) Continue to "2) of 3. Prepare Lysate" below
*When transfer whole lysate into the cartridge at step 3, remove the swab cotton from the lysate.

2 Set the heat block incubator at 56℃

3 Prepare Lysate

1) Add 200 µl of the cheek cells (in PBS) into the bottom of 2 ml empty microtube
2) Add 10 µl of EDT and subsequently 200 µl of LDT
3) Mix with vortex mixer at the maximum speed for 15 sec
4) Flash spin down for several seconds to remove drops from the inside of the lid.
5) Incubate with the heat block incubator at 56℃ for 10 min

For the maximum yield, the extension of incubation time (up to 60 min) is available.
During the long incubation, please mix with vortex mixer sometimes.

6) Flash spin down for several seconds to remove drops from the inside of the lid.
7) Add 200 µl of ethanol (>99%), then mix with vortex mixer at the maximum speed for 15 sec
8) Flash spin down for several seconds to remove drops from the inside of the lid.

4 Complete the lysis

Continue to step.3

step3 Isolation protocol with QuickGene-Mini480

Use QuickGene-Mini480 to isolate genomic DNA.

QuickGene-Mini480 Workflow
The Pressurization mark 

in the workflow indicates the following operations.

1. Set holder into system. ※**Please read the User's Manual to know how to set the holder.**
2. Rotate pressurizing switch toward the front side to start pressurizing.
3. Make sure that there is no residual liquid in the cartridge and return the pressurizing switch to original position.
4. Move the holder to pressurize the next row. Repeat 2. and 3.
5. Pull out holder from system.