KURABO

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Isolation of genomic DNA Quick Guide

Tissue DNA

QuickGene DNA tissue kit L (DT-L)

In this Quick Guide, the protocol for isolation of genomic DNA from tissue is a digest from the Handbook of QuickGene tissue kit L (DT-L) and the Operation Manual of QuickGene-Mini8L. * 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-Mini8L
- DNA tissue kit L (DT-L)

- High grade ethanol (>99%)
- 1.5 ml microtube (for DNA collection)
- 15 or 50 ml centrifuge tube (for lysate preparation)
- Micropipettes (P-1000)
- Centrifuge (2,500x g (3,500 rpm))
- Water bath (70℃)
- Shaker incubator (55℃)
- Protective gloves
- Safety goggles

10 ml pipette or pipette aid

Tube mixer

RNase (option)

Ribonuclease A / Sigma-Aldrich
*Cat. No. R5125 *1, *2*
*Cat. No. R5500 *1, *2*
*Cat. No. R6513 *1*
Cat. No. R4642

Ribonuclease A / MP Biomedicals
*Cat. No. 101076 *1, *2*

RNase A / AMRESCO
*Cat. No. 0675 *1, *2*

RNase A / QIAGEN
Cat. No. 19101

RNase A / Thermo Fisher Scientific
Cat. No. 12091

**1 : Prepare 100 mg/ml solution with 10 mM Tris-HCl (pH 7.5), 15 mM NaCl.*
**2 : Incubate at 100℃ for 15 minutes to deactivate DNase.*

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.

QuickGene DNA tissue kit L (DT-L) basically corresponds to genomic DNA isolation from 5 mg to 100 mg of mouse liver.
For example, from 100 mg liver tissue of Balb/c mouse (7 weeks, ♀), 80 µg genomic DNA can be gained.
The volume of the elute from each cartridge is 500 µl.
The volume of CDT can be reduced to 100 µl, but in that case, elution efficiency might be decreased.

* Following collection of tissue from animal, the described volume should be immediately immersed in MDT.
If the tissue sample is not used immediately, the tissue should be flash frozen with liquid nitrogen and stored at -20°C or -80°C.
Do not leave tissue at room temperature. Repeatedly freezing or thawing should be avoided. Genomic DNA may degrade.

1 Lyse tissue

1) Set the temperature of shaker incubator at 55℃
2) Cut tissue into small blocks 5 mm square or less, and put the tissue into 15 ml (or 50 ml) centrifuge tube.
3) Add 1.8 ml of MDT and subsequently 200 µl of EDT.
4) Lyse the tissue completely with stirring by shaker at 55℃ for several hours till over night.
5) Centrifuge at 2,500x g (3,500 rpm) at room temperature for 3 minutes.
6) Transfer the supernatant to a new centrifuge tube without sucking in the unlysed portion of tissue (unlysed residue, gelatinous substance, etc.).

2 Set the temperature of the water bath at 70℃

3 Set the consumables to QuickGene-Mini8L

Regarding setting of the consumables, please refer to the Operation Manual of QuickGene-Mini8L.

4 RNase treatment (Option)

1) Add 100 µl of 100 mg/ml RNase (in the case of R4642 (Sigma-Aldrich)) to the sample in the centrifuge tube.

Please change the adding volume of RNase depending on the sample or RNase type.

2) Mix RNase well with the sample by mixing with vortex mixer for 5 seconds.
3) Incubate at room temperature for 2 minutes.

5 Prepare Lysate

1) Add 1.8 ml of LDT to the sample in the centrifuge tube.
2) Mix with vortex mixer at the maximum speed (2,500 rpm or more) for 15 seconds.

A white precipitate may form by addition of LDT, which in most cases will dissolve during the incubation at 70℃.

3) Incubate with water bath at 70℃ for 10 minutes.
4) Add 2.4 ml of ethanol (>99%).
5) Mix with vortex mixer at the maximum speed (2,500 rpm or more) for 15 seconds.

If the room temperature is low, a white precipitate may form by addition of ethanol.
After dissolving the precipitate at 55℃, please proceed to the next step after returning to room temperature.

6 Complete the lysis

Perform the isolation operation quickly after completing the lysis.

Continue to step.3

step3 Isolation protocol with QuickGene-Mini8L

Use QuickGene-Mini8L to isolate genomic DNA.

QuickGene-Mini8L Workflow
The Pressurization mark in the workflow indicates the following operations.

1. Set holder into system. ※Please read the Operation 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.