With QuickGene-Mini8L

Research use only

KURABO

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Isolation of genomic DNA Quick Guide

Buffy Coat

QuickGene DNA whole blood kit L (DB-L)

In this Quick Guide, the protocol for isolation of genomic DNA from buffy coat is modified based on the Handbook of QuickGene whole blood kit L (DB-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	10 ml pipette or pipette aid	Tube mixer
DNA whole blood kit L (DB-L) 		
High grade ethanol (>99%)		
1.5 ml microtube (for DNA collection)		
15 or 50 ml centrifuge tube (for lysate preparation)		
Micropipettes (P-1000)		
Water bath (56℃)		
Protective gloves		
Safety goggles		

2 Preparations of Reagents

◆Protease (EDB)

Add 3.3 ml nuclease-free water into the vial containing lyophilized Protease, leave it for 30 minutes or more at room temperature with occasionally stirring it. Dissolve it completely.

(Reconstituted EDB is stable for 2 months when stored at 4℃. More than 2 months, Dividing the solution into aliquots and storage at -80℃ is recommended.)

◆ Lysis Buffer (LDB)

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

◆ Wash Buffer (WDB)

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 (CDB)

Use CDB 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.

1 Prepare buffy coat sample

Prepare 500 µl of buffy coat samples in each procedures using a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin. In case of cartridge clogging, use less starting material in the procedure of "4 Prepare Lysate".
Buffy coat samples may be used immediately or stored at –20° C or –70° C. Frozen samples should be thawed quickly in a 37° C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25° C) before beginning the procedure.

2 Set the temperature of the water bath at 56℃

3 Set the consumables to QuickGene-Mini8L

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

4 Prepare Lysate

1) Add 300 µl of EDB (previous dissolved in nuclease-free water) into bottom of a 15 ml centrifuge tubes.
2) Add 500 µl of buffy coat sample. (After adding the buffy coat sample, immediately process to 3))
3) Add 2.5 ml LDB, and mix the sample with shaking upside-down intensely 10 times immediately.

Mix the sample with shaking upside-down thoroughly, and mix EDB, whole blood sample and LDB well.
Next step is mixing the solutions by vortex mixer.
If you don't have a vortex mixer at the speed of 2,500 rpm or more, please mix upside-down completely in this step.

4) Mix with vortex mixer at the maximum speed (2,500 rpm or more) for 15 seconds.

In case mixing is insufficient, the yield of DNA might decrease or the cartridge (CA) might clog.

5) Incubate with water bath at 56℃ for 5 minutes.

The maximum incubation time is 10 minutes.
When you use the heating block, you have to incubate at 56℃ for 30 minutes.

6) Add 2.5 ml of ethanol (>99%), and mix the sample with shaking upside-down intensely 10 times immediately.
7) Mix with vortex mixer at the maximum speed (2,500 rpm or more) for 15 seconds.

In case mixing is insufficient, the yield of DNA might decrease or the cartridge (CA) might clog.

5 Complete the lysis

Perform the isolation operation within 30 minutes, after completing the lysis.

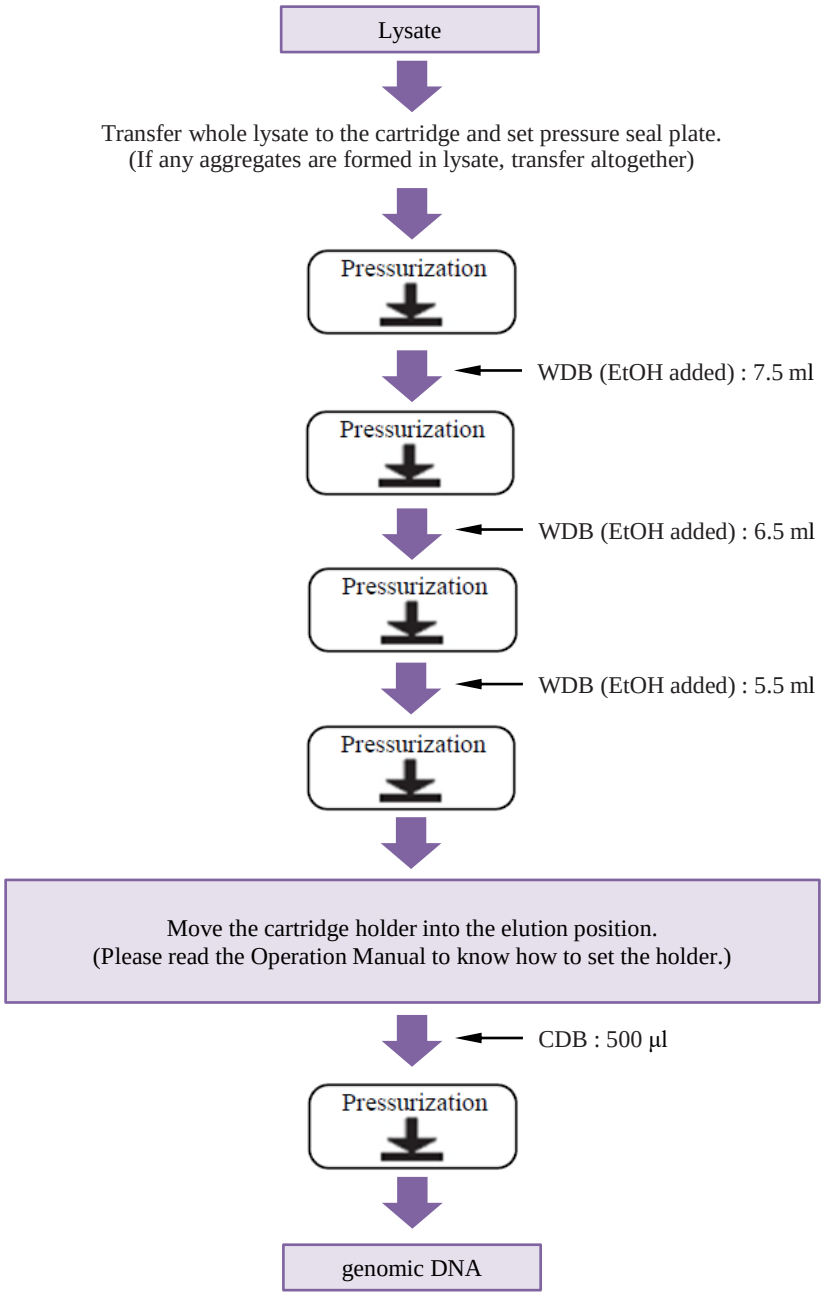
Continue to step.3

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.



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