KURABO

HANDBOOK

QuickGene SP kit DNA whole blood (SP-DB)

For extraction of genomic DNA from whole blood (Spin method)

Ver.2.0

Contents

Warning For research use only.
Not recommended or intended for diagnostic or clinical application for humans or animals.

1. Introduction

QuickGene porous membrane to immobilize nucleic acid has large specific surface area and uniform & fine porousness. So you can successfully extract genomic DNA with high yield; moreover, with its patented thin membrane, most contaminants are eliminated.

When using this kit, high quality and high yield genomic DNA can be extracted and also purified from whole blood. No hazardous organic solvents such as phenol and chloroform are used.
The extraction can be completed in about 35 min from the preparation of lysate (in the case of 8 samples). The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, Southern blotting and other applications.

Please be sure to read this handbook carefully before using this kit.

2. Kit Components and Storage Conditions

2-1 Kit Components (96 Preps)

☐ Protease	EDB	1 vial
☐ Lysis Buffer	LDB	30 ml
☐ Wash Buffer	WDB	125 ml
☐ Elution Buffer	CDB	100 ml
☐ Cartridges	CAS	96
☐ Waste Tubes	WTS	192

2-2 Storage Conditions

All reagents are stable for one year after purchase at room temperature (15-28°C). Reconstituted EDB is stable for 2 months when stored at 4°C. Storage at –20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20°C is recommended.

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents
- >99% Ethanol (for preparation of lysate and WDB working solution)
- Nuclease-free water (for dissolving EDB)

[2] Equipments
- Micropipettes and tips
- 1.5 ml microtubes (for preparation of lysate and collection of DNA)
- Tube stand
- Vortex mixer (maximum speed at 2,500 rpm or more)
- Microcentrifuge (c.a. 6,000 × g (8,000 rpm)) *

  * Some centrifuges may be unsuitable for use. Please check the specifications before use.
- Heat block or water bath (at 56°C)

4. Safety Warnings

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- All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, disposable gloves and safety goggles during the experiments are highly recommended. In case of contact between the reagents and the eyes, skin, or clothing, wash immediately with water.
  (See the Material Safety Data Sheet for specific recommendations, http://www.kurabo.co.jp/bio/English/)

◆ Protease EDB
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ LDB (Lysis Buffer)
- **Harmful if ingested.**
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ WDB (Wash Buffer)
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ CDB (Elution Buffer)
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ Use or storage of LDB at high temperature should be avoided.

◆ Any solution and flow-through containing LDB should not be mixed with bleach.

◆ **In the case of using potentially infectious samples :**
Wear a suitable laboratory coat, disposable gloves and safety goggles during the experiments.

◆ **Disposal of waste fluid and consumables when using potentially infectious samples :**
After use, dispose of potentially infectious samples and consumables by incineration, high-temperature decontamination, sterilization, or disinfection in accordance with applicable laws. When entrusting waste disposal to licensed hazardous waste disposal contractors, use specially controlled waste management forms (manifest), if applicable.

5. Precautions

◆ Handling of Starting Material

- The yield of DNA might decrease when the number of leucocytes exceeds 2×106cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×106cells/200 µl.
  The Cartridge (CAS) might clog when the number of leucocytes exceeds 5×10^6cells/200 µl. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.
- Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
- Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Use a whole blood sample within 3 days after collection. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

◆ Use of Reagent

- After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CAS) might clog when dissolution of EDB is insufficient.
- Use or storage of LDB at high temperature should be avoided.
- Any solution and flow-through containing LDB should not be mixed with bleach.

◆ Procedure of Extraction

- Use a vortex mixer able to stir at 2,500 rpm or more. Weak vortex may cause insufficient dissolution, lead to decrease of the yield of DNA or clogging of the Cartridge (CAS).
- When setting a Cartridge (CAS) to a Waste Tube (WTS), set it tightly.
- Close the cap of Cartridge (CAS) tightly before placing it in the microcentrifuge.
- During the procedure, work quickly without interruption.
- The yield of DNA varies depending upon sample conditions. The standard yield is 4 to 8 µg from 200 µl of whole blood samples.
- Use QuickGene SP kit DNA whole blood (SP-DB) at room temperature (15-30°C). In case of using at lower or higher temperature, it may affect the extraction performance.

- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Centrifuge as described. (speed, time, etc.)

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene SP kit DNA whole blood (SP-DB) is evaluated routinely on a lot-to-lot uniformity.
- Yield and quality of extracted genomic DNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

7. Product Description

DNA and RNA are included in eluate extracted with this kit. Table 1 shows the average of yield and purity (A260/280) of genomic DNA extracted from 200 µl of whole blood samples.
The yield varies depending upon sample conditions.

Table 1

Sample	Amount of genomic DNA (µg)	A260/280
Whole blood (200 µl)	4 - 8	1.97

8. Protocol

8-1 Preparations of Reagents

◆EDB (Lyophilized)
When using EDB, pipette 3.3 ml of nuclease-free water into the vial containing lyophilized Protease. Dissolve it completely. Reconstituted EDB is stable for 2 months when stored at 4°C. Storage at –20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20°C is recommended.

Notices Dissolve EDB completely by the following method, and then use the solution.
Add 3.3 ml of nuclease-free water, close cap and mix with inversion the bottle.
Leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CAS) might clog when dissolution of EDB is insufficient.

◆ LDB (30 ml)
Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.

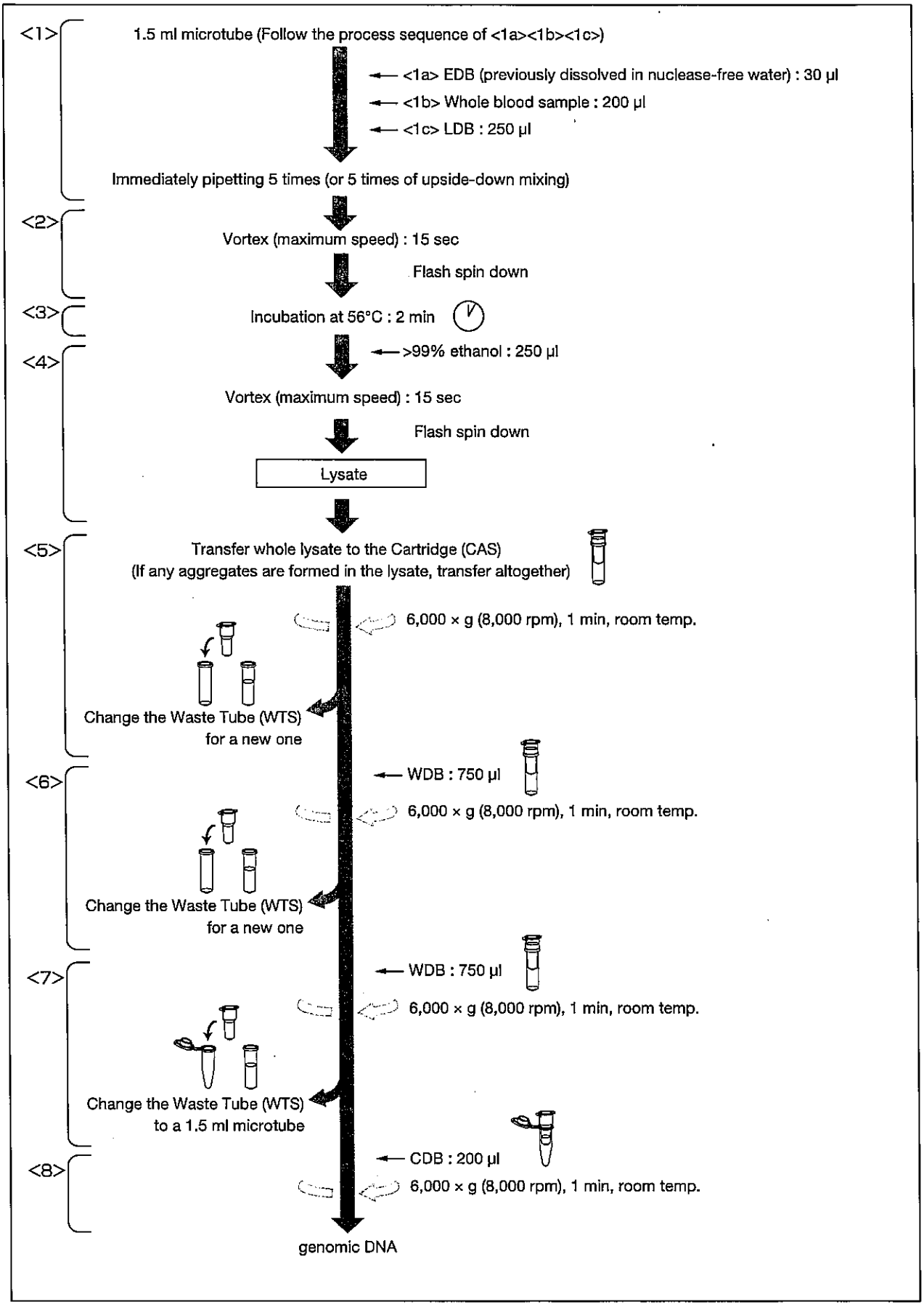
◆ WDB (125 ml)
WDB is supplied as a concentrate.
Add 125 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ CDB (100 ml)
Use CDB for elution of DNA.

8-2 Workflow and Details of Protocol

- Cool down all reagents to room temperature before use.
- Set the temperature of a heat block or a water bath to 56°C (it is used in step <3>).
- Check that 125 ml of >99% ethanol is added to WDB before starting an experiment.
- This kit is designed for extraction of genomic DNA from 200 µl of whole human blood. Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Follow the volume of sample and buffers described in the workflow at p.8.
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- Avoid touching the filter in the Cartridge (CAS) with the pipette tip.
- All steps of the protocol should be performed at room temperature (15-30°C).
- During the procedure, work quickly without interruption.
- Please avoid a continuous driving of the microcentrifuge to prevent the temperature in the centrifuge chamber from rising. It might adversely affect the extraction performance.
- Any solution and flow-through containing LDB should not be mixed with bleach.
- Centrifuge as described. (speed, time, etc.)
- When using potentially infectious samples for experiments, dispose of them according to applicable regulations.
- When taking the Cartridge (CAS) and the Waste Tube (WTS) out of the centrifuge, take carefully not to allow contact between the flow-through and the Cartridge. If flow-through is splashed, perform flash spin down for several seconds.
- There is no influence on the yield or purity of DNA even if centrifugation is performed at speed beyond 6,000 × g (8,000 rpm), but be cautious not to exceed 8,000 × g (10,000 rpm).

Workflow


<1>
1.5 ml microtube (Follow the process sequence of <1a><1b><1c>)
<1a> EDB (previously dissolved in nuclease-free water) : 30 µl
<1b> Whole blood sample : 200 µl
<1c> LDB : 250 µl
Immediately pipetting 5 times (or 5 times of upside-down mixing)
<2>
Vortex (maximum speed) : 15 sec
Flash spin down
<3>
Incubation at 56°C : 2 min
<4>
>99% ethanol : 250 µl
Vortex (maximum speed) : 15 sec
Flash spin down
Lysate
<5>
Transfer whole lysate to the Cartridge (CAS)
(If any aggregates are formed in the lysate, transfer altogether)
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
for a new one
<6>
WDB : 750 µl
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
for a new one
<7>
WDB : 750 µl
6,000 × g (8,000 rpm), 1 min, room temp.
Change the Waste Tube (WTS)
to a 1.5 ml microtube
<8>
CDB : 200 µl
6,000 × g (8,000 rpm), 1 min, room temp.
genomic DNA

Details of Protocol

<1> Follow the protocol of <1a> to <1c> exactly. Do not add LDB directly after addition of EDB to a 1.5 ml microtube. In case the procedure is changed, the yield of DNA may not be obtained.

<1a> Add 30 µl of EDB (previously dissolved in nuclease-free water) to the bottom of a 1.5 ml microtube.

<1b> Add 200 µl of a whole blood sample.

After adding the whole blood, immediately proceed to step <1c>.
Leaving the samples for a long time before addition of LDB might decrease the yield of DNA.

<1c> Add 250 µl of LDB, then immediately pipette 5 times.

Instead of pipetting, mixing upside-down 5 times can be performed.
In order to ensure efficient lysis, it is essential to mix thoroughly the sample and LDB. Pipette (or mix upside-down) surely in order to mix EDB, whole blood and LDB efficiently.

<2> Vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

Surely vortex for 15 sec at the maximum speed. The speed of 2,500 rpm or more is recommended. If you do not have such a vortex mixer, pipette (or mix upside-down) completely at step <1c>.
In case mixing is insufficient, the yield of DNA might decrease or the Cartridge (CAS) might clog.

<3> Incubate at 56°C for 2 min.

Prolongation of the incubation time up to 5 min does not affect the yield.

<4> Add 250 µl of >99% ethanol, and vortex at the maximum speed for 15 sec. Flash spin down for several seconds to remove drops from the inside of the lid.

Mix the sample and the ethanol enough. Vortex at the same speed as in step <2>.

<5> <Applying lysate> Carefully transfer the whole lysate from steps <1> - <4> to the Cartridge (CAS). Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and the Waste Tube out of the microcentrifuge. Attach the Cartridge onto a new Waste Tube (WTS, provided), and discard the Waste Tube and flow-through (filtrate).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Perform the extraction operation quickly after completion of lysate. It is possible to leave it until 30 min if necessary.
If any lysate still remains in the Cartridge, centrifuge again.

<6> <First wash> Open the cap of the Cartridge (CAS) carefully, and add 750 µl of WDB. Close the cap tightly, and centrifuge at 6,000 × g (8,000rpm) for 1 min at room temperature. Carefully take the Cartridge and the Waste Tube out of the microcentrifuge. Attach the Cartridge onto a new Waste Tube (WTS, provided), and discard the Waste Tube and flow-through.

If any WDB still remains in the Cartridge, centrifuge again.

<7> <Second wash> Open the cap of the Cartridge (CAS) carefully, and add 750 µl of WDB. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1min at room temperature. Carefully take the Cartridge and the Waste Tube out of the microcentrifuge. Attach the Cartridge onto a 1.5 ml microtube (not provided), and discard the Waste Tube and flow-through.

If any WDB still remains in the Cartridge, centrifuge again.

<8> <Elution> Open the cap of the Cartridge (CAS) carefully, and add 200 µl of CDB. Close the cap tightly, and centrifuge at 6,000 × g (8,000 rpm) for 1 min at room temperature. Carefully take the Cartridge and the 1.5 ml microtube out of the microcentrifuge, and discard the Cartridge.

The volume of CDB can be reduced to 50 µl, but in that case, elution efficiency might decrease by about 20%.

In case the genomic DNA recovered is not used immediately, surely close the cap of the 1.5 ml microtube, and then preserve at 4°C or –20°C.

In case of storing genomic DNA for a long time, it is recommended to preserve at –20°C.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene SP kit DNA whole blood (SP-DB).

(1) Low yield or no DNA obtained :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Inadequate dissolution of EDB	After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4°C. Do not use EDB preserved for a longer period than 2 months. Storage at –20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20°C is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Inappropriate volume of whole blood sample	If the volume of a whole blood sample is too much, reduce it to the prescribed volume (200 µl). Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
Use of too much amount of leucocytes	The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.6.)
Incomplete addition of whole lysate to the Cartridge (CAS)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Rupture of filter	Be careful not to allow pipette tip to contact with a filter in the Cartridge (CAS).
Use of reagents other than CDB to elute DNA	Use CDB to elute DNA.
DNA degradation	Refer to (3) "DNA degradation".
Elevated centrifuge chamber temperature	In order to prevent elevation of centrifuge chamber temperature, avoid continuous operation of centrifuge. It may adversely affect the extraction performance.

(2) Clogging of Cartridge (CAS) occurs :

Cause	Action
Use of too much amount of a whole blood sample	Reduce it to the prescribed volume (200 µl).
Use of too much amount of leucocytes	The Cartridge (CAS) might clog when the number of leucocytes exceeds 5×10^6cells/200 µl. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).

(3) DNA degradation :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

(4) Purity of DNA is low :

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4°C. Do not use EDB preserved for a longer period than 2 months. Storage at –20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20°C is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 1.5 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) → Whole blood sample → LDB.
Insufficient homogenization after addition of LDB	Immediately after addition of LDB, pipette (or mix upside-down), and then vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
Inappropriate volume of ethanol in lysate	Add the prescribed volume of >99% ethanol.
Insufficient homogenization after addition of ethanol	After addition of ethanol, vortex sufficiently (for 15 sec). Perform vortex at the maximum speed (2,500 rpm or more is recommended).
No addition of the prescribed volume of ethanol to WDB	Before using WDB for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.6.)
Improper washing procedure	Wash twice with 750 µl of WDB.
Inappropriate centrifugal speed	When centrifuging Cartridge (CAS), centrifuge at 6,000 × g (8,000 rpm).
Contact Cartridge (CAS) with flow-through	When taking the Cartridge and the Waste Tube (WTS) out of the microcentrifuge, take them out carefully. If flow-through is splashed, perform flash spin down for several seconds.
Use of reagents other than CDB to elute DNA	Use CDB to elute DNA.

(5) Subsequent experiments such as PCR etc. do not proceed well :

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Low purity of DNA	Refer to (4) "Purity of DNA is low".
DNA degradation	Refer to (3) "DNA degradation".

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at room temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 37°C. Cool down it to room temperature before use.

(7) Waste Tube (WTS) is ruptured :

Cause	Action
Centrifugation exceeding a specified speed (6,000 × g (8,000 rpm))	Centrifuge at the specified speed (6,000 × g (8,000 rpm)).

10. Ordering Information

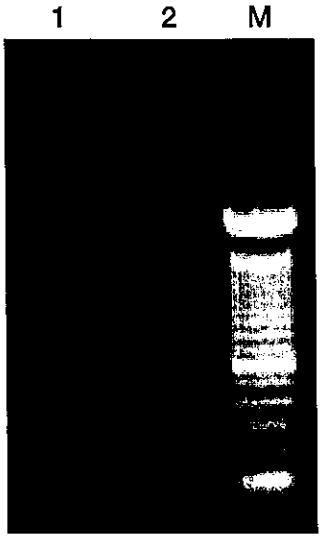
Product	Cat #
QuickGene SP kit DNA tissue	SP-DT
For extraction of genomic DNA from tissues	
QuickGene SP kit DNA whole blood	SP-DB
For extraction of genomic DNA from whole blood	

Appendix 1 Examples of the Data with QuickGene SP kit DNA whole blood (SP-DB)

● PCR

An example of PCR of genomic DNA extracted with this kit.
PCR was performed with 0.1 ng of genomic DNA extracted from 200 µl of a whole blood sample with this kit using G3PDH as a target.

Figure 1



2% Agarose gel/1 × TAE

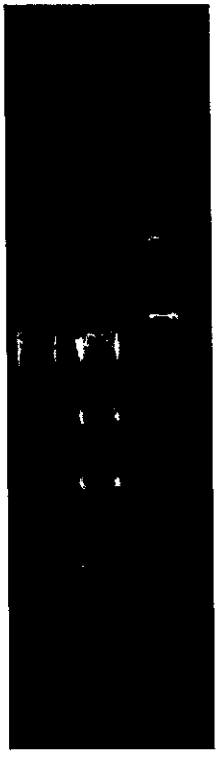
Lane	Sample
1	200 µl of a whole blood sample
2	Negative control
M	100 bp Ladder (Invitrogen)

As a result of this PCR, the band of the amplification product from 0.1 ng of genomic DNA template was detected.

● Results of pulse field electrophoresis

The length of genomic DNA extracted with this kit.

Figure 2



1% Agarose gel/0.5×TBE

Lane	Sample
1	DNA extracted from 200 µl of a whole blood sample with this kit
M1	λ Hind Ⅲ digest
M2	Midrange PFG Marker Ⅱ (NEB)

From the result, genomic DNA extracted with this kit has a length of less than 140 kb.

KURABO

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SP-DB_HB-E_V20