Genomic DNA Extraction from Diverse Forensic Samples using GENTi™ Advanced Blood DNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System

Experimental Conditions

Materials Required

- GENTi™ 32 Advanced Automatic Extraction System (GTI032A)
- GENTi™ Advanced Blood DNA Extraction Kit (903-096A)
- Microcentrifuge & vortex mixer
- Thermomixer (e.g. HCM100-Pro, 5032103100, Supplier : D)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Buffer CL (Lysis for cigarette and fingerprint, 106-921)
- Proteinase K, 120 mg (392-103)
- PK Storage Buffer, 7 ml (392-903)

Sample Information

- Sample type and appropriate starting amount

Sample	Amount
Dried blood spot (DBS)	2 dots
Blood swab	1 ea
Cigarette butt	12 pieces of 2 cm^2
Fingerprint	12 pieces of 2 cm^2

- Extraction conditions
  - Extraction protocol : Blood_High Protocol (operation time : 37′ 07″)
  - Elution volume : 70 µl

Sample Preparation

- **Dried blood spot (DBS)**

1. Prepare human blood on the indicating FTA cards (DBS) collected within 3 days.
2. Collect the 5 mm diameter DBS samples using sterilized punching machine.
3. Transfer the two dots into 1st/7th well of the cartridge of GENTi™ Advanced Blood DNA Extraction Kit.

- **Blood swab**

1. Prepare human blood swab samples taken within 1 day.
2. Cut only the cotton part of swab and transfer it into 1st/7th well of the cartridge of GENTi™ Advanced Blood DNA Extraction Kit.

- **Cigarette butt**

1. Cut the outer paper of cigarette butt filter part into 12 equal parts of 2 cm^2 in size.
2. Transfer 12 pieces to a 2 ml microcentrifuge tube and add 300 µl of Buffer CL and 20 µl of Proteinase K solution (20 mg/ml).
3. Transfer the tube to a thermomixer and incubate at 1,500 rpm for 20 min at 56˚C.
4. Centrifuge at full speed for 1 min at room temperature.
5. Carefully collect 300 µl of the supernatant and transfer it into 1st/7th well of the cartridge of GENTi™ Advanced Blood DNA Extraction Kit.

- **Fingerprint**

1. Cut the fingerprinting into 12 equal parts of 2 cm^2 in size.
2. Transfer 12 pieces to a 2 ml microcentrifuge tube and add 300 µl of Buffer CL and 20 µl of Proteinase K solution (20 mg/ml).
3. Transfer the tube to a thermomixer and incubate at 1,500 rpm for 20 min at 56˚C.
4. Centrifuge at full speed for 1 min at room temperature.
5. Carefully collect 300 µl of the supernatant and transfer it into 1st/7th well of the cartridge of GENTi™ Advanced Blood DNA Extraction Kit.

*** All prepared samples are according to GENTi™ Advanced Blood DNA Extraction Kit protocol.**

Protocol

GENTi™ Advanced Blood DNA Extraction Kit protocol

*** For more details and methods, please refer to the handbook of GENTi™ Advanced Blood DNA Extraction Kit.**

1. Add each of samples to 1st/7th well of the cartridge of GENTi™ Advanced Blood DNA Extraction Kit.
2. Add 15 µl of dissolved Proteinase K solution (2 µg/µl, not provided) to 1st/7th well.
3. Load the cartridge on the tray of GENTi™ 32 Advanced Automatic Extraction System.
4. Insert the magnetic rod cover to the end to strip bracket.
5. Select the correct extraction protocol (Blood_High Protocol) and operate the extraction system.

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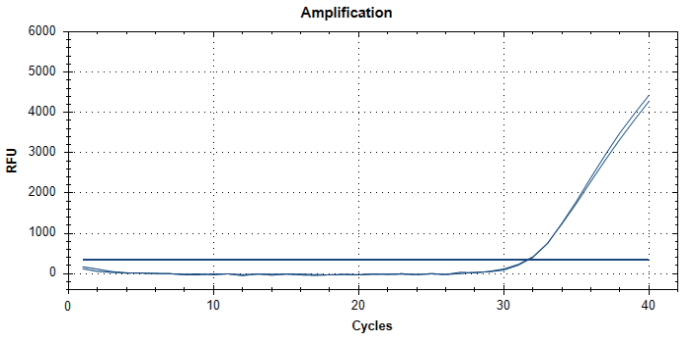
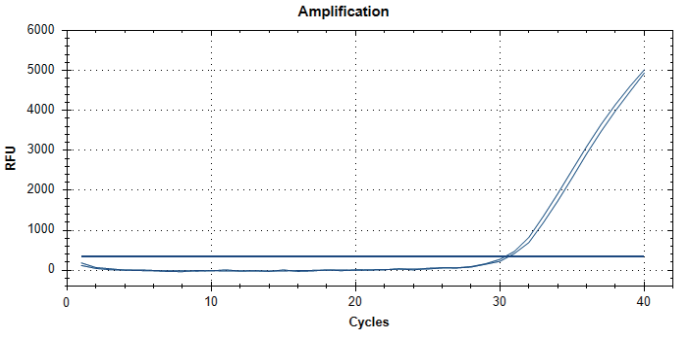
Result



DBS	Yield (ng/µl)	$A_{260/280}$	$A_{260/230}$	C_q Value
#1	3.8	1.81	1.13	31.72
#2	2.9	1.81	0.43	32.62

Figure 1. Result of the yield and purity of genomic DNA extracted from dried blood spots and real-time PCR analysis using it as a template.
Blood genomic DNA were extracted from dried blood spot with GENTi™ Advanced Blood DNA Extraction Kit on GTENTi™ 32 Automatic Extraction System. The quality and yield of eluted DNA were checked by measuring the absorbance and determining the $A_{260/280}$ and $A_{260/230}$ ratio using a spectrophotometer. Subsequently, DNA quantitation was performed in duplicate using a TaqMan-based real-time PCR assay (on CFX-96).
- Spectrophotometer : NanoDrop™ 2000/2000c (ND-2000)
- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
- Primer and probe : Human GAPDH Primer/Probe



Blood swab	Yield (ng/µl)	$A_{260/280}$	$A_{260/230}$	C_q Value
#1	5.5	2.24	0.89	30.65
#2	5.6	2.18	1.10	30.38

Figure 2. Result of the yield and purity of genomic DNA extracted from blood swab and real-time PCR analysis using it as a template.
Blood genomic DNA were extracted from blood swab with GENTi™ Advanced Blood DNA Extraction Kit on GTENTi™ 32 Automatic Extraction System. The quality and yield of eluted DNA were checked by measuring the absorbance and determining the $A_{260/280}$ and $A_{260/230}$ ratio using a spectrophotometer. Subsequently, DNA quantitation was performed in duplicate using a TaqMan-based real-time PCR assay (on CFX-96).
- Spectrophotometer : NanoDrop™ 2000/2000c (ND-2000)
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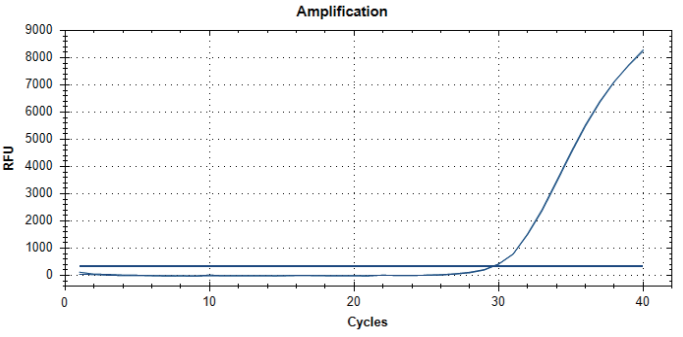

Cigarette butt	Yield (ng/µl)	$A_{260/280}$	$A_{260/230}$	C_q Value
#1	5.3	2.05	0.92	29.67
#2	5.6	2.16	0.96	29.70

Figure 3. Result of the yield and purity of genomic DNA extracted from cigarette butt and real-time PCR analysis using it as a template.
Genomic DNA were extracted from lip epidermal cells present in cigarette butts with GENTi™ Advanced Blood DNA Extraction Kit on GTENTi™ 32 Automatic Extraction System. Buffer CL and Proteinase K solution were added to the samples prior to DNA extraction for optimal performance The quality and yield of eluted DNA were checked by measuring the absorbance and determining the $A_{260/280}$ and $A_{260/230}$ ratio using a spectrophotometer. Subsequently, DNA quantitation was performed in duplicate using a TaqMan-based real-time PCR assay (on CFX-96).
- Spectrophotometer : NanoDrop™ 2000/2000c (ND-2000)
- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
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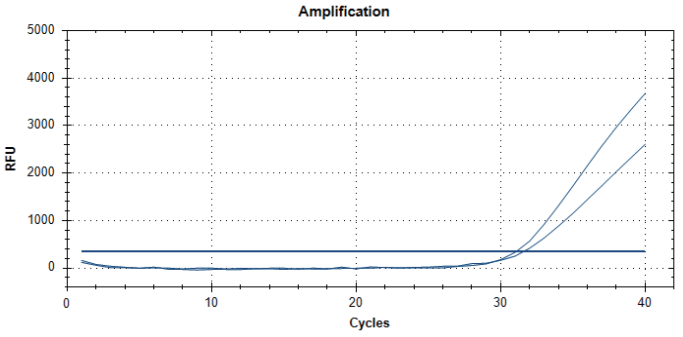

Fingerprint	Yield (ng/µl)	$A_{260/280}$	$A_{260/230}$	C_q Value
#1	2.9	2.14	0.94	31.07
#2	2.5	1.84	0.86	31.59

Figure 4. Result of the yield and purity of genomic DNA extracted from fingerprint and real-time PCR analysis using it as a template.
Genomic DNA were extracted from epidermal cells present in fingerprint with GENTi™ Advanced Blood DNA Extraction Kit on GENTi™ 32 Automatic Extraction System. Buffer CL and Proteinase K solution were added to the samples prior to DNA extraction for optimal performance. The quality and yield of eluted DNA were checked by measuring the absorbance and determining the $A_{260/280}$ and $A_{260/230}$ ratio using a spectrophotometer. Subsequently, DNA quantitation was performed in duplicate using a TaqMan-based real-time PCR assay (on CFX-96).
- Spectrophotometer : NanoDrop™ 2000/2000c (ND-2000)
- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
- Primer and probe : Human GAPDH Primer/Probe