

# 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 (903-048A/903-096A)
- ♦ Buffer CL (lysis for cigarette butt and fingerprint, 106-921)
- ♦ Proteinase K, 120 mg (392-103)
- ♦ PK Storage Buffer, 7 ml (392-903)
- ♦ 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.)

### 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 <sup>2</sup> |
| Fingerprint            | 12 pieces of 2 cm <sup>2</sup> |

- ♦ 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<sup>2</sup> 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<sup>2</sup> 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

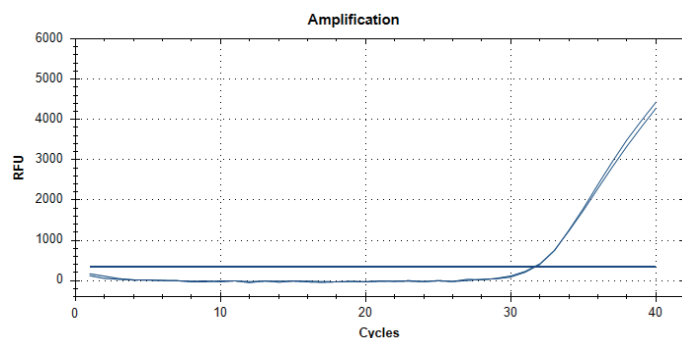
### GENTi™ Advanced Blood DNA Extraction Kit Protocol

\* 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

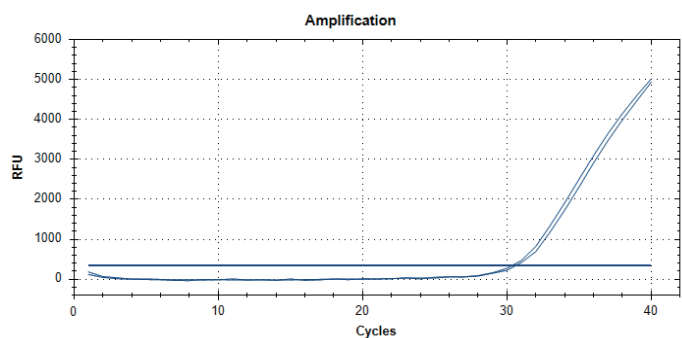


| DBS | Yield (ng/μl) | A <sub>260/280</sub> | A <sub>260/230</sub> | C <sub>q</sub> Value |
|-----|---------------|----------------------|----------------------|----------------------|
| #1  | 3.8           | 1.81                 | 1.13                 | 31.72                |
| #2  | 2.9           | 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 GENTi™ 32 Automatic Extraction System. The quality and yield of eluted DNA were checked by measuring the absorbance and determining the A<sub>260/280</sub> and A<sub>260/230</sub> 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)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391A)
- Primer and probe: human GAPDH primer/probe

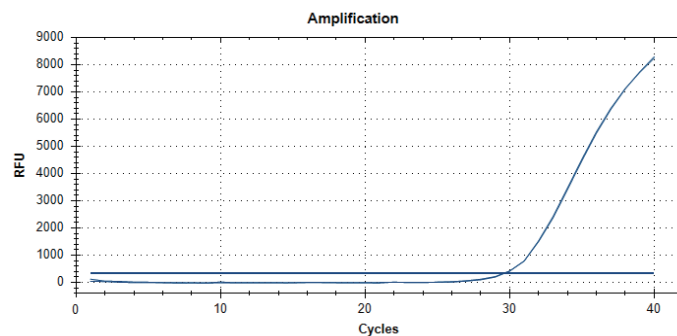


| Blood swab | Yield (ng/μl) | A <sub>260/280</sub> | A <sub>260/230</sub> | C <sub>q</sub> Value |
|------------|---------------|----------------------|----------------------|----------------------|
| #1         | 5.5           | 2.24                 | 0.89                 | 30.65                |
| #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 GENTi™ 32 Automatic Extraction System. The quality and yield of eluted DNA were checked by measuring the absorbance and determining the A<sub>260/280</sub> and A<sub>260/230</sub> 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)
- Real-time PCR system: CFX-96™ System (1855201)
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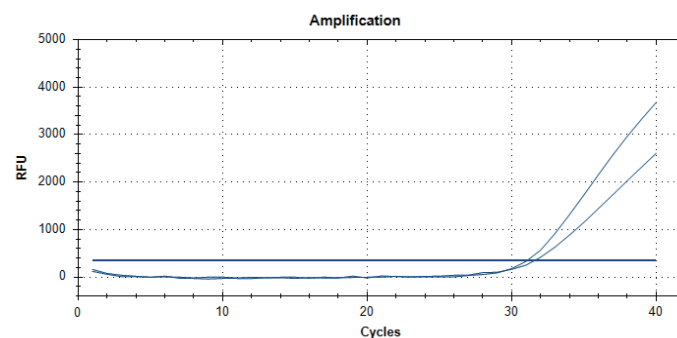


| Cigarette butt | Yield (ng/μl) | A <sub>260/280</sub> | A <sub>260/230</sub> | C <sub>q</sub> Value |
|----------------|---------------|----------------------|----------------------|----------------------|
| #1             | 5.3           | 2.05                 | 0.92                 | 29.67                |
| #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 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<sub>260/280</sub> and A<sub>260/230</sub> 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)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391A)
- Primer and probe: human GAPDH primer/probe



| Fingerprint | Yield (ng/μl) | A <sub>260/280</sub> | A <sub>260/230</sub> | C <sub>q</sub> 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<sub>260/280</sub> and A<sub>260/230</sub> 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)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391A)
- Primer and probe: human GAPDH primer/probe