# Foregene DNA Identification System 25A-C（Case）

Product Manual beta 3.0 (beta version)

## I. Product Introduction

Foregene DNA Identification System 25A-C uses six-color fluorescence labeling technology to amplify 23 autosomal STR loci, one Amel loci, one Yindel loci and two internal quality control loci at a time for the amplification of DNA templates extracted and purified from case samples.

## II. Reagent Storage

1. After receiving the kit, please store it below -20℃ if you do not use it for the time being.

2. After the kit is taken out and used, the pre-reaction component PCR premixed solution is stored at -20℃, and the remaining pre-reaction reagents are stored at 4℃ to avoid repeated freeze-thaw. **If the single dosage is small, it is recommended to store at -20℃ after packaging.**

3. After the reaction, the components are kept at 4℃ to avoid repeated freeze-thaw. Do not touch the pre-reaction reagents to avoid pollution.

## III. PCR Amplification

1. DNA template Amount

Table 1：Optimum range of template amount under different cycle numbers

| | 27 cycles | 28 cycles | 29 cycles |
|---|---|---|---|
| Template amount | 0.06ng-3ng | 0.03ng-2ng | 0.03ng-1ng |

It is recommended to dilute the positive standard 9948 of this kit to 0.5ng/μl before use. Users can adjust the amount of templates added into the PCR system and the number of cycles in the PCR program according to their own DNA template concentration. Too low a template may result in the deletion of some alleles, and too high a template may result in results beyond the detection range of genetic analyzer.

2. Shock mixing of reagents

In order to obtain the optimal amplification effect, it is recommended to swirl 2.5×PCR premixed solution C I and 10×25A-C primer mixture for 10 seconds before use and centrifuge at low speed to eliminate the uneven concentration of solution caused by possible tube wall adsorption.

2. Amplification system preparation

Table 2: The Preparation Composition of Standard extraction and amplification system

| | Components | 10μl system(μl) |
|---|---|---|
| Master Mix | 2.5×PCR Premix C I | 4.0 |
| | 10×25A-CPrimer Mix | 1.0 |
| | DNA template | 1—5 |
| | Deionized water | Make up to a reaction volume of 10μl |

3. Amplification program setup

Table 3: PCR amplification procedure

| | |
|---|---|
| Predegeneration | 95°C, 2minutes |
| 28±1 cycle | 94°C, 5seconds |
| | 60°C, 2minutes |
| Extension | 60°C, 10minutes |
| Cryopreservation | 15°C |

Note: If using ABI 9700 (Gold Block) or Veriti thermal cycler, please perform amplification in MAX mode.

## IV. Spectral Calibration

1. Make sure that the POP4 gel and Buffer of the sequencer are within the validity period;

2. Mix the 6-color Matrix spectral calibration reagent and deionized formamide according to the following ratio, vortex and mix, and distribute to a 96-well plate , 10 μl PCR well;

| Instrument type | 3130xl | 3500 | 3500xl |
|---|---|---|---|
| 6-color Matrix | 3μl | 1.5μl | 4μl |
| Deionized formamide | 200μl | 100μl | 300μl |

3. After denaturation at 95°C for 3 minutes , quickly cool at 0°C for 3 minutes;

4. Spectral Calibration Recommended Parameters

4.1. 3130 Series: ①Create a Run Module: Name it Goldeneye J6 Matrix, choose Type SPECTRAL, and Template Spect36_POP4; Run Module Settings: fill in 5 for Data_Delay_Time, Run_Time: fill in 1 000 , and fill in other parameters according to the default values ; ②Create Protocol: Name it Golden Eye J6 , choose Dye Set J6, choose Golden Eye J6 Matrix for Run Module

4.2. 3500 Series: Create Dye Set : Dye Set Name is named GoldenEye J6, Chemistry is Matrix Standard, Dye Set Template is J6 Template.

5. In order to obtain the best correction effect, it is recommended to set the Sensitivity to 0.4, the Minimum Quality Score to 0.95 , and at the same time ensure that the spectral correction peak height is between 750rfu-4000rfu (3130 series) or 3000rfu-20000rfu ( 3500 series) . If this

standard cannot be reached, it can be adjusted according to the actual electrophoresis situation.

## V. Electrophoresis Detection

1. The loading system was prepared according to the internal standard of molecular weight: deionized formamide =0.3μl:9.7μl, and 10μl/ well was divided.

2. PCR product usage: 1μl, Allelic Ladder usage: 1μl;

3. 95℃ denaturation for 3 minutes, quickly put to 0℃ cooling for 3 minutes;

4. Electrophoretic detection: 3130 series injection voltage 3k Volts, injection time 10 sec; The 3500 series injection voltage is 1.2k Volts, injection time is 24 sec.

## VI. Data Analysis

1. Import panels and bin files , establish Analysis Method and Size Standard (J R 500: 65, 70, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 330, 360, 390, 420 , 450, 490, 500);

2. Import the electrophoresis data, and select the corresponding analysis parameters such as panel, analysis method and size standard to analyze the data.

Table 1: Kit components (200 Tests)

| Reagent test kit | Component name | Volume (μl/tube) | Quantity (tube) |
|---|---|---|---|
| Pre-reaction Kit | 2.5×PCR Premix C I | 1000 | 1 |
| | 10×25A-CPrimer Mix | 250 | 1 |
| | DNATyping Standards 9948（2ng/μl） | 25 | 1 |
| | Deionized water | 1700 | 1 |
| Post-reaction kit | 25A-C Ladder | 40 | 1 |
| | Orange molecular weight internal standard JR 500 | 150 | 1 |

Table 2: Kit locus information

| loci | Group | 9948 | Ladder allele |
|---|---|---|---|
| D3S1358 | B | 15,17 | 12, 13, 14, 15, 16, 17, 18, 19, 20, |
| CSF1PO | B | 10,11 | 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, |
| D2S441 | B | 11,12 | 8.1, 9, 10, 11, 11.3, 13, 14, 15, |
| D21S11 | B | 29,30 | 26, 27, 27.2, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 35.2, 36.2, |
| Penta E | B | 11,11 | 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 |
| D8S1179 | G | 12,13 | 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, |
| D5S818 | G | 11,13 | 7, 8, 9, 10, 11, 12, 13, 14, 15, |
| D19S433 | G | 13,14 | 9, 10, 11, 11.2, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, |
| D16S539 | G | 11,11 | 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, |
| Penta D | G | 8,12 | 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, |
| Yindel | Y | 2 | 1, 2, |
| Amel | Y | X,Y | X, Y, |
| vWA | Y | 17,17 | 13, 14, 15, 16, 17, 18, 19, 20, 21, 22.1, |
| D2S1338 | Y | 23,23 | 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, |
| D18S51 | Y | 15,18 | 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, |
| D22S1045 | Y | 16,18 | 11, 12, 14, 15, 16, 17, 18, |
| TH01 | R | 6,9.3 | 5, 8, 9, 9.3, 10, |
| D12S391 | R | 18,24 | 14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27, |
| TPOX | R | 8,9 | 7, 8, 9, 10, 11, 12, 13, |
| FGA | R | 24,26 | 13, 14.2, 15.2, 16, 16.2, 17, 18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25.2, 26, 27, 27.2, 28, 29, 30, 31.2, 43.2, 44.2, 45.2, 46.2, |
| D13S317 | P | 11,11 | 7, 8, 9, 10, 11, 12, 13, 14, |
| D1S1656 | P | 14,17 | 10, 11, 12, 13, 14, 15, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, |
| D10S1248 | P | 12,15 | 10, 11, 12, 13, 14, 15, 16, 17, 18, |
| D6S1043 | P | 12,12 | 9, 10, 11, 12, 13, 14, 15,16, 17, 18, 19, 20, 21, 21.3, |
| D7S820 | P | 11,11 | 6, 7, 8, 10, 11, 12, 13, 14, |

Figure 1: Ladder genotyping of alleles

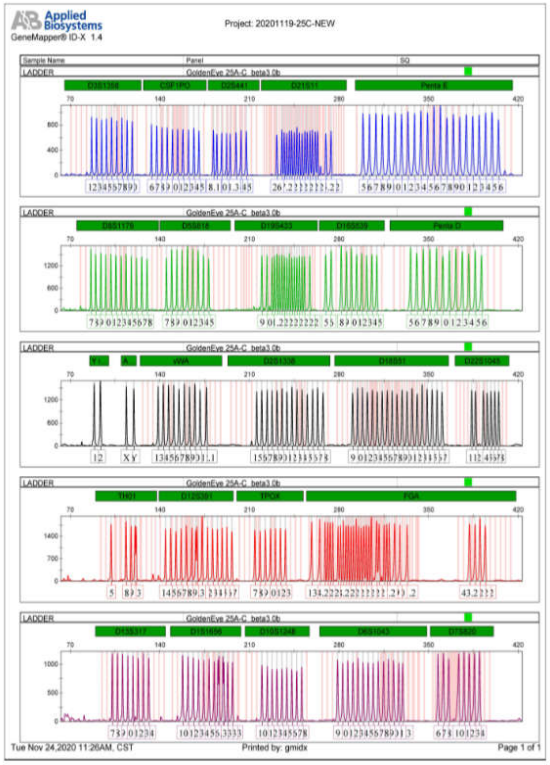


Figure 2: DNA typing standard 9948 typing map

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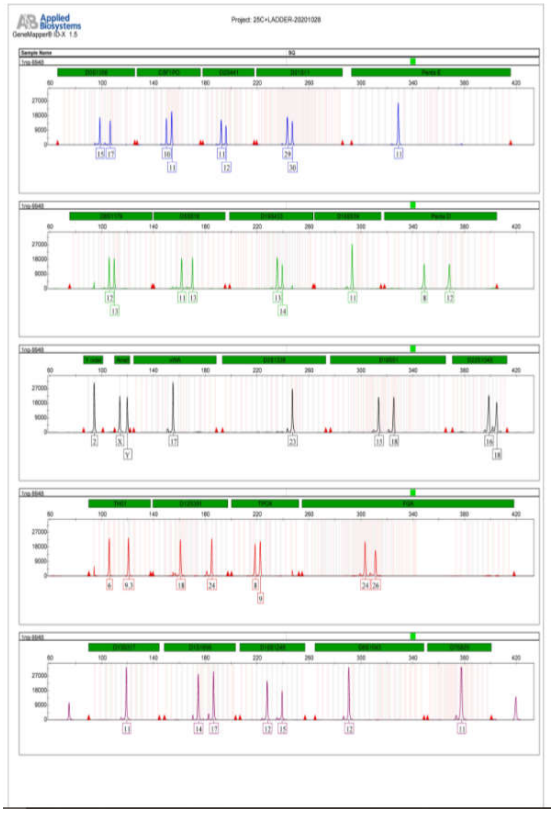

**Notice:**

1. This kit is valid for one year, see the product packaging for the production date and batch number.
2. For Research Use Only.

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