Foregene DNA Identification System 30 A-C (Case)

Product Manual beta 1.0

I. Product Introduction

Foregene DNA identification system 30A-C uses six-color fluorescent labeling technology to amplify 29 autosomal STR loci , 1 Amel locus, 1 Yindel locus, and 1 human species-specific Sexual sites, 2 internal quality control sites, used to amplify the DNA template extracted and purified from Case materials.

II. Reagent Storage

1. Please store below -20°C,,after receiving the kits frozen in dry ice or gel ice packs.
2. Please store the pre-reaction component 2.5× premix CII at -20°C after the kit is taken out for use,, and the remaining pre-reaction reagents are stored at 4°C to avoid repeated freezing and thawing. **If the single dosage is small, it is recommended to store at -20°C after aliquoting** ;
3. Store the components after the reaction at 4°C, avoid repeated freezing and thawing, and do not touch the reagents before the reaction to avoid contamination.

III. PCR Amplification

1. DNA template Amount

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

	28 cycles	29 cycles
Template amount	0. 06ng - 4ng _	0.06ng - 2ng _ _

The concentration of DNA typing standard 9948 is 1 ng/ μ l in this kit. Users can adjust properly the amount of template added in the PCR system and the number of cycles in the PCR program according to own DNA template concentration. If the amount of template is too low, some alleles may be missing, and if the amount of template is too high, it may lead to results beyond the detection range of the genetic analyzer.

2. Shaking and Mixing Reagents

Vortex the 2.5× premix C Ⅱ and 5 × 30 AC primer mixture for 10 seconds before using, and then centrifuge at low speed to rule out the uneven concentration of the solution caused by possible tube wall adsorption.

3. Amplification system preparation

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

	Components	25ul system	10μl system
Master Mix	2.5× Premix C Ⅱ	10.0	4.0
	5 x 30 AC Primer Mix	5.0	2.0
	DNA template	2.5— 10	1— 4
	Deionized water	Make up to a reaction volume of 25 μl	Make up to a reaction volume of 10 μl

4. Protocol settings

Table 2: PCR amplification procedure

Denaturation	95°C, 2 minutes
2 8-29 cycles	94°C, 5 seconds
	60 °C, 2 minutes
Extension	60°C, 2 minutes
Cryopreservation	15°C

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

IV. Spectral Correction

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	3130 x l	3500	3 500 xl
6-color Matrix	8 μl	1.5 μl	5 μl
Deionized formamide	200μl	100 μl	250 μ l _

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: ① Create a Run Module: **Name it GoldeneyeJ6Matrix,** choose SPECTRAL for Type, and Spect36_POP4 for Template; **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 ; ② Create Protocol: Name it **Golden EyeJ6** , choose J6 for Dye Set, choose Golden EyeJ6Matrix 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 (3 130 series) or 3000rfu-20000rfu (3 500 series) . If this standard cannot be reached, it can be adjusted according to the actual electrophoresis situation.

V. Electrophoresis Detection

1. According to the molecular weight internal standard : deionized formamide = 0.5 μl : 9.5 μl ratio to prepare the sample loading system , 10 μ l / well for packing ;
2. The amount of PCR product used: 1 μl , the amount of Allelic Ladder used: 1 μl ;
3. After denaturation at 95°C for 3 minutes , quickly cool at 0°C for 3 minutes;
4. Electrophoresis detection: The recommended injection voltage of 3130 series is 3k Volts, and the injection time is 10 sec; the injection voltage of 3500 series is 1.2k Volts, and the injection time is 24 seconds.

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× Premix C Ⅱ	10 00	2
	5 x 30 AC Primer Mix	500	2
	DNA Typing Standards 9948 (1 ng/ μl)	40	1
	Deionized water	1700	2
Post-reaction kit	30 AC Ladder	5 0	1
	Molecular weight internal standard JR 500	150	2

able 2 Kit locus information

Loci	Group	Control DNA	Allele
D3S1358	B	15,17	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
CSF1PO	B	10,11	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
D2S441	B	11,12	8.1, 9, 10, 11, 11.3, 13, 14, 15,
D21S11	B	29,30	24, 24.2, 25, 26, 27, 27.2, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36.2, 37, 38, ,
Penta E	B	11	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26
D15S659	B	16,18	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,
D8S1179	G	12,13	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,
D5S818	G	11,13	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
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	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
Penta D	G.	8.12	2.2, 3.2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
D8S1132 _	G.	2 0.24	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,25, 26,
yindel	AND	2	1, 2,
Amel	AND	X,Y	X,Y,
vWA	AND	17	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22.1, 23.1, 24.1,
D2S1338	AND	23	11, 12, 13, 14, 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	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,
D6S477	Y	11,16	10, 10.2, 11, 11.2, 12, 13, 14, 15, 16, 17, 18, 19,
TH01	R	6,9.3	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3,
D12S391	R	18,24	14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27,
TPOX	R	8,9	7, 8, 9, 10, 11, 12, 13, 14, 15,
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,
D19S253	R	11,12	7, 8, 9, 10, 11, 12, 13, 14, 15,
D13S317	P	11	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
D1S1656	P	14,17	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3,
D10S1248	P	12,15	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,
D6S1043	P	12	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 21.3, 23, 24, 25,
D7S820	P	11	6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
D 10S1435	P	1 2,13	9, 10, 11, 12, 13, 14, 15,
D 3S3045	P	1 2,14	8, 9, 10, 11, 12, 13, 14, 15, 16,

Table1 :Allelic Ladder Typing Map

Foregene Co., Ltd
Address: D2,block A, Tri-medical innovation center, No.18, North Bayi Road, Yongning Street, 611130, Chengdu, China
Tel:+86-28-86050301 E-mail:overseas@foregene.com

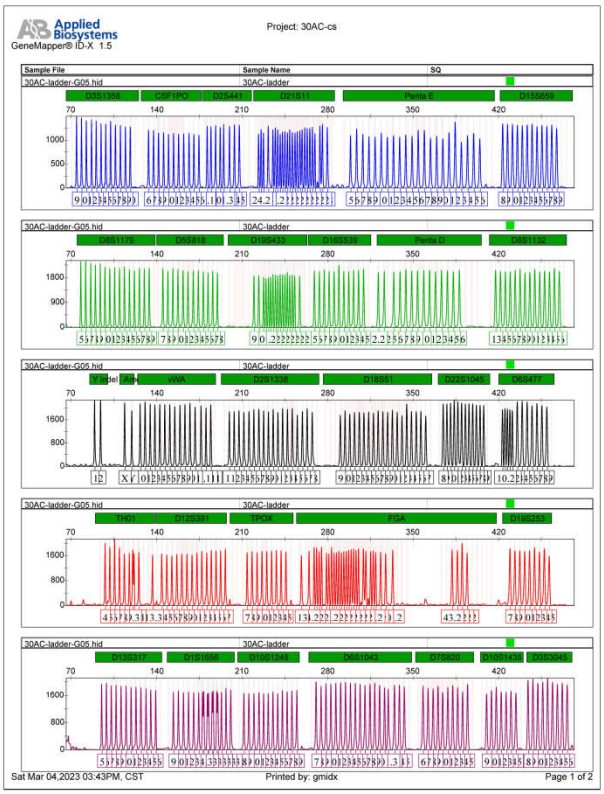
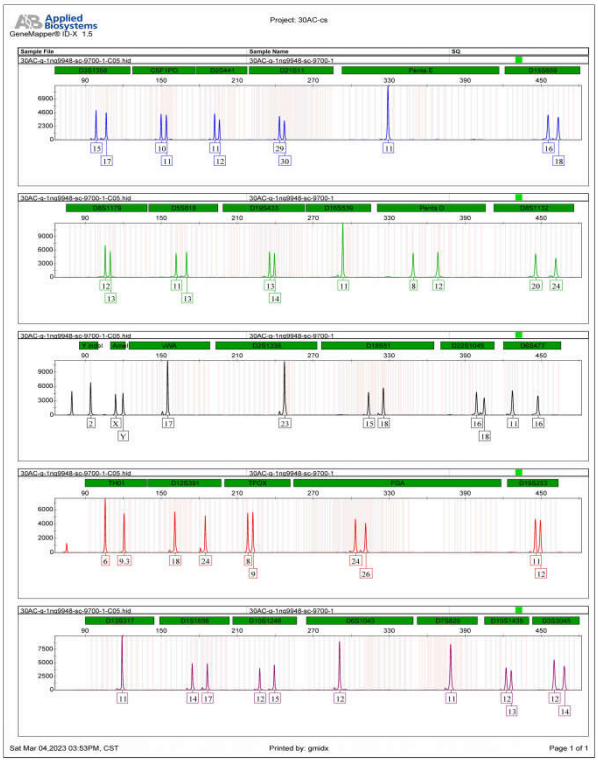


Table 2:DNA typing standard 9948 typing map



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.