Foregene DNA Identification System 38Y-C (Case)

Product Manual beta 1.0 (Beta version)

I. Product Introduction

Foregene DNA identification system 38Y-C uses six-color fluorescent labeling technology to amplify 38 Y chromosome STR loci , 3 Y- Indel loci and two internal quality control loci at one time for amplification Case samples extracted from purified DNA templates.

II. Reagent Storage

1. Please store them below -20°C (if not used temporarily) ,after receiving the kits frozen in dry ice or gel ice packs;

2. Please sotre the pre-reaction component PCR master mix is at -20°C, after the kit is taken out for useand 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

	27 cycles	28 cycles	29 cycles
Amount of template	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 properly adjust the amount of template added in the PCR system and the number of cycles in the PCR program according to their 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

In order to obtain the best amplification effect, it is recommended to vortex 2.5×PCR master mix C I and 5 × 38Y-C primer mixture for 10 seconds before use and then centrifuge at low speed to eliminate 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	10μl system (μl)
Master Mix	2.5×PCR Master Mix C I	4.0
	5 x 38Y-C Primer Mix	2.0
	DNA template	1- 4
	Deionized water	Fill up to a reaction volume of 10 μl

4. Protocol settings

Table 3: PCR amplification procedure

Pre- denaturation	95°C, 2 minutes
28 loops	94°C, 5 seconds
	60 °C, 2 minutes
Final extension	60°C, 10 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 per well;

Instrument type	3130 x 1	3500	3 500 xl
6-color Matrix	3μl	1.5μl	4μl
Deionized formamide	200μl	100 μl	250μl

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

Create a Run Module: Name it Goldeneye J6 Matrix, select SPECTRAL for Type, and Spect36_POP4 for Template ; in Run Module Settings: fill in 5 for Data_Delay_Time, fill in 1 000 for Run_Time , and fill in other parameters with default values ;

Create a Protocol: Name as Golden Eye J6, select J6 for Dye Set, and select Golden Eye J6 Matrix for Run Module .

4.2. 3500 Series

Create Dye Set : Dye Set Name is named GoldenEye J6, Chemistry is selected to Matrix Standard, Dye Set Template is selected to 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 (3500 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 = 0.3μl : 9.7μl ratio to prepare the loading system , 10μl/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 6 00: 65, 70, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 330, 360, 390, 420 , 450, 490, 500, 527 , 557 , 585 , 599);
2. Import the electrophoresis data, and select the corresponding analysis parameters such as panel, analysis method and size standard to analyze the data.

Annexed Table 1: Kit components (100 Tests)

Reagent test kit	Component name	Volume (μl/tube)	Quantity (tube)
Pre-reaction Kit	2.5×PCR Master Mix C I	1000	1
	5 x 38Y-C Primer Mix	500	1
	DNA Typing Standards 9948 (2ng/ μl)	25	1
	Deionized water	1700	1
Post-reaction kit	38Y-C Ladder	10	1
	Orange Molecular Weight Internal Standard J 600	160	1

Annexed Table 2: Kit locus information

Loci	Group	9948	Ladder allele
rs759551978	B	2	1, 2,
rs771783753	B	2	1, 2,
rs199815934	B	2	1, 2,
DYS437	B	15	13, 14, 15, 16, 17,
DYS393	B	13	8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS385	B	11,14	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,
DYS627	B	22	16, 17, 18, 19, 20, 21, 22, 23, 24, 25,
DYS444	B	12	10, 11, 12, 13, 14, 15, 16,
DYS596	B	16	14, 15, 16, 17,
DYS645	B	8	7, 8, 9,
DYS439	G	12	8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS391	G	10	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS481	G	24	20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32,
DYS19	G	14	10, 11, 12, 13, 14, 15, 16, 17, 18, 19,
DYF387S1	G	35,38	33, 34, 35, 36, 37, 38, 39, 40, 41,
DYS447	G	25	21, 22, 23, 24, 25, 26, 27, 28, 29,
DYS557	G	16	13, 14, 15, 16, 17, 18, 19, 20,
DYS593	G	15	13, 14, 15, 16, 17, 18,
Y_GATA_H4	Y	12	7, 8, 9, 10, 11, 12, 13, 14, 15,
DYS458	Y	18	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,
DYS533	Y	12	7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS448	Y	19	16, 17, 18, 19, 20, 21, 22, 23, 24,
DYS460	Y	11	8, 9, 10, 11, 12, 13, 14,
DYS527	Y	21,22	14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
DYS643	Y	11	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,
DYS456	R	17	13, 14, 15, 16, 17, 18,
DYS389I	R	13	10, 11, 12, 13, 14, 15, 16, 17,
DYS438	R	11	8, 9, 10, 11, 12, 13, 14,
DYS389II	R	31	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35,
DYS449	R	30	22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41,
DYS549	R	13	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,
DYS522	R	10	9, 10, 11, 12, 13, 14,
DYS392	P	13	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
DYS576	P	16	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,
DYS635	P	twenty three	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,
DYS390	P	twenty four	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
DYS518	P	38	32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42,
DYS570	P	18	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,

Annexed Graph 1: Allelic Ladder Typing Map

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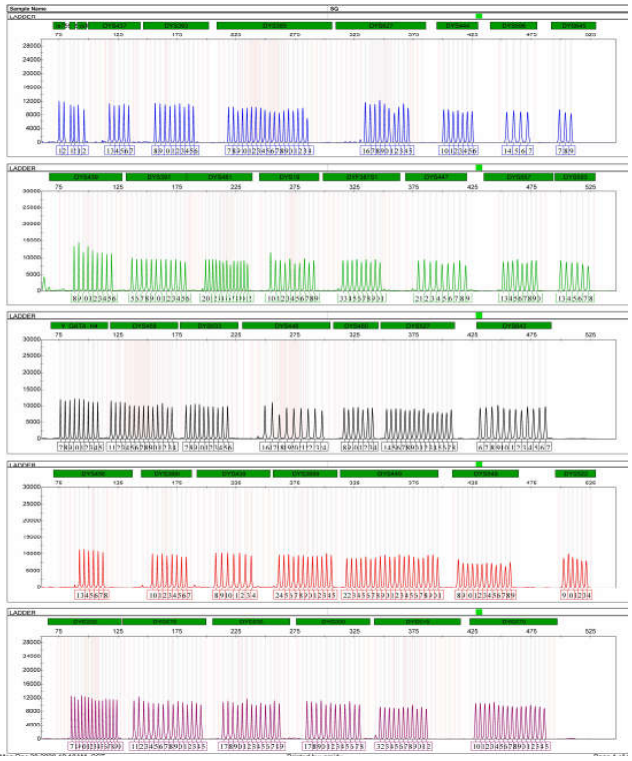
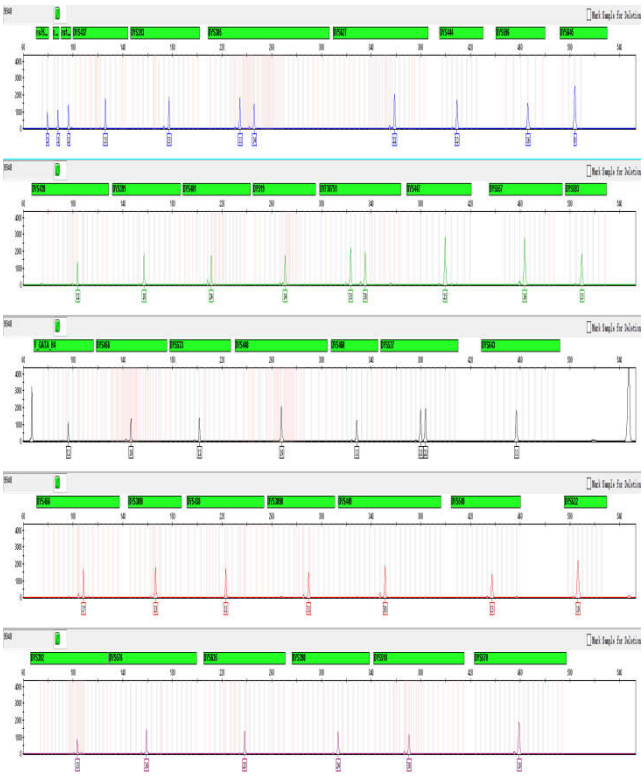


Annexed Graph 2: DNA typing standard 9948 typing map



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.

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