Foregene DNA Identification System Y Plus (Non-Extraction)

Product Manual beta 3.2

I. Product introduction

The Foregene DNA identification system Y Plus uses six-color fluorescent labeling technology to amplify 20 Y chromosome core loci, 15 preferred loci, 6 alternative loci and 3 Y indel loci at one time .

II. Reagent Storage

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

2. Please store the pre-reaction component 4×premix VII at -20°C,after the kit is taken out for use, and the other 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

The concentration of DNA typing standard 9948 is 2ng/μl 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

In order to obtain the best amplification effect, it is recommended to vortex the previous 4×master mix VII component and 5×Y Plus primer mixture for 10 seconds 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 1:The preparation composition of Standard (extraction-free) amplification system

	Components	25 μl system (μl)	10μl system (μl)
Master Mix	4 × Master Mix VII	6.25	2.5
	5×Y Plus Primer Mix	5.0	2.0
	Deionized water	13.75	5.5
Blood stain		Diameter 1.2mm	Diameter 1.2mm
Total reaction volume		25 μl	10 μl

4. Protocol settings

Table 2: PCR amplification procedure

Denaturation	95°C, 2 minutes
27 ±1 cycle	94°C, 5 seconds
	60 °C, 1 minute 30 seconds
	62 °C, 1 minute
Extension	60°C, 5 minutes
Cryopreservation	15°C

NOTE: Use an ABI 9700 (Gold Block) cycler and 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 l	3500	3500 xl
6-color Matrix	8 μl	2.7 μl	5 μl
Deionized formamide	200 μl	100 μl	250 μl

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 as Goldeneye J6 Matrix, choose SPECTRAL for Type, and Spect36_POP4 for Template; **Run Module Settings**: fill in 3 for Injection_Voltage, for **Injection_Time** ： fill in 10, **Run_Time**：fill in1500, and fill in other parameters according to the default values. ②Create Protocol: Name as Golden Eye J6, select J6 for Dye Set, and select Golden Eye J6 Matrix for Run Module ; in Edit Parameter, fill in 2.0 for Lower of Matrix Condition Number Bounds, and fill in 20 for Upper.

4.2. 3500 series: Create Dye Set: Dye Set Name is named as GoldenEye J6, Chemistry is selected to Matrix Standard, Dye Set Template is selected to J6 Template ; in the Parameters option, Matrix Condition Number Upper Limit is set to 8.0 (adjustable according to actual conditions), and After Scan is set to Subtract 200 from the Scan point value of the first fragment peak of the matrix, add 200 to the Scan point value of the largest fragment peak of the matrix in Before Scan, and fill in other parameters with default values.

Foregene Co., Ltd.
Add.: D2,block A, Tri-medical innovation center, No.18, North Bayi Road, Yongning Street, 611130, Chengdu, China
Tel: +86-28-86050301 www.foreivd.com

5. In order to obtain the best correction effect, it is recommended to set the Sensitivity to 0.4 and 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. According to the molecular weight internal standard : deionized formamide=0.5 μl : 9.5 μl ratio to prepare the sample loading system, 10 μl / well for packing ;
2. Amount of PCR product used: 1 μl , amount of allele 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. Open the Genemapper ID software, import the panels and bin files , and establish Analysis Method and Size Standard (T500: 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, select the corresponding analysis parameters such as Panel, Analysis Method and Size Standard to analyze the data.

Annexed Table 1: Kit Components (100 Tests)

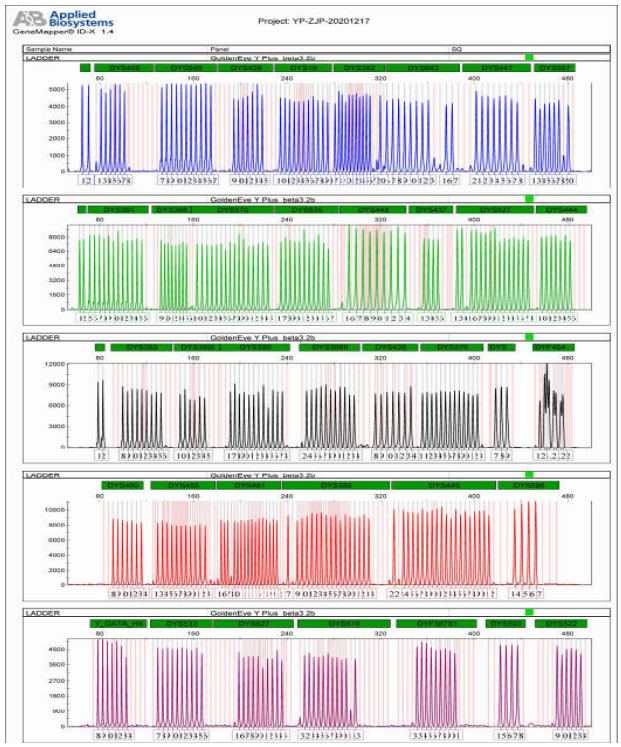
Reagent test kit	Component Name	Volume (μl/tube)	Quantity (tube)
Pre-reaction Kit	4 × Master Mix VII	625	1
	5×Y Plus Primer Mix	500	1
	DNA Typing Standards 9948 (2.0 ng/μl)	25	1
	Deionized water	1700	1
Post-reaction kit	Y Plus Ladder Molecular weight internal standard T 500	40	1
		150	1

Annexed Table 2: Kit locus information

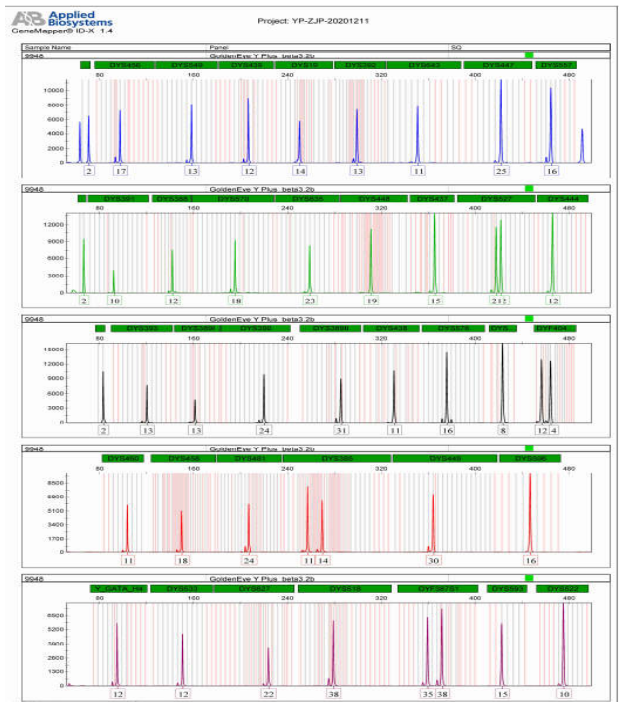
Loci	Group	Control DNA	Allele
rs199815934	B	2	1, 2
DYS456	B	17	13, 14, 15, 16, 17, 18,
DYS549	B	13	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,
DYS439	B	12	9, 10, 11, 12, 13, 14, 15,
DYS19	B	14	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
DYS392	B	13	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20,
DYS643	B	11	6, 7, 8, 9, 10, 11, 12, 13, 16, 17,
DYS447	B	25	21, 22, 23, 24, 25, 26, 27, 28,
DYS557	B	16	13, 14, 15, 16, 17, 18, 20
rs771783753	G	2	1, 2,
DYS391	G	10	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS388	G	12	9, 10, 11, 12, 13, 14, 15, 16,
DYS570	G	18	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,
DYS635	G	23	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27
DYS448	G	19	16, 17, 18, 19, 20, 21, 22, 23, 24,
DYS437	G	15	13, 14, 15, 16
DYS527	G	21,22	13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
DYS444	G	12	10, 11, 12, 13, 14, 15, 16,
rs759551978	Y	2	1, 2,
DYS393	Y	13	8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS389I	Y	13	10, 11, 12, 13, 14, 15,
DYS390	Y	24	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
DYS389II	Y	31	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34,
DYS438	Y	11	8, 9, 10, 11, 12, 13, 14,
DYS576	Y	16	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,
DYS645	Y	8	7, 8, 9,
DYF404S1	Y	12,14	12, 13, 13.2, 14, 15, 15.2, 16.2, 17,
DYS460	R	11	8, 9, 10, 11, 12, 13, 14,
DYS458	R	18	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,
DYS481	R	24	16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32,
DYS385	R	11,14	7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,
DYS449	R	30	22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42,
DYS596	R	16	14, 15, 16, 17,
Y_GATA_H4	P	12	8, 9, 10, 11, 12, 13, 14,
DYS533	P	12	7, 8, 9, 10, 11, 12, 13, 14, 15, 16,
DYS627	P	22	16, 17, 18, 19, 20, 21, 22, 23, 24, 25,
DYS518	P	38	32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 43,
DYF387S1	P	35,38	33, 34, 35, 36, 37, 38, 39, 40, 41,
DYS593	P	15	15, 16, 17, 18,
DYS522	P	10	9, 10, 11, 12, 13, 14,

Annexed Graph 1: Allelic Ladder Typing Map

Foregene Co., Ltd.
Add.: D2,block A, Tri-medical innovation center, No.18, North Bayi Road, Yongning Street, 611130, Chengdu, China
Tel: +86-28-86050301 www.foreivd.com



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.
3. The trial pack is for 25 people, and the attached table 1 is the composition list for 100 people, and the trial pack comes with the Spectral Calibration.

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