FOREGENE DNA Identification System 30A (Non-Extraction)

Product Manual beta 2. 2

I. Product Introduction

FOREGENE DNA identification system 30A uses six-color fluorescent labeling technology to amplify 29 autosomal STR loci , one Amel locus and one Yindel locus at one time, and can directly amplify filter paper, FTA cards, cotton swabs, saliva cards, and mouth swab.

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 4×premix VII at -20°C, after the kit taken out for using , and store the other remaining pre-reaction reagents 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 number of cycles in the PCR program according to the amount of DNA template. 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 4× master mix VII and 5×30A primer mixture for 10 seconds before use , 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 1: The prepartion composition of Standard (Extraction-free) amplification reaction

	Components	25 μl system (μl)	10 μl system (μl)
Master Mix	4 × Master Mix VII	6.25	2.5
	5 x 30A Primer Mix	5.0	2
	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
28±2 cycles	94°C, 5 seconds
	59°C, 2 minutes
Extension	60°C, 5 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 , 10μl per well;

Instrument type	3130 x 1	3500	3 500 xl
6-color Matrix	8 μl	2.7 μl	5 μl
Deionized formamide	200μl	100 μl	250 μ 1 _

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: Named GoldeneyeJ6Matrix, select SPECTRAL for Type, and Spect36_POP4 for Template ; Run Module Settings: **Injection_Voltage:fill in 3** , **Injection_Time:fill in 10** , **Run_Time** :fill in 1500 , and fill in other parameters according to default values. ② Create Protocol: Name as **Golden Eye yeJ6, select J6 for Dye Set, and select Golden Eye yeJ6Matrix** 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 GoldenEye J6**, Chemistry selects Matrix Standard, Dye Set Template selects 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 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.

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. Configure the sample loading system according to the ratio of molecular weight internal standard : deionized formamide = 0.5μl : 9.5μl , and distribute 10μl/ well ;
2. Amount of PCR product used: 1 μl , 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 (JR-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	4× Master Mix VII	625	2
	5 x 30 A primer mix	500	2
	DNA Typing Standards 9948 (2ng/ μl)	2 5	1
	Deionized water	1700	2
Post-reaction kit	30A Ladder _ Molecular weight internal standard JR 500	40 150	1 2

Tale 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	AND	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,

Annexed graph 1: Allelic Ladder Typing Map

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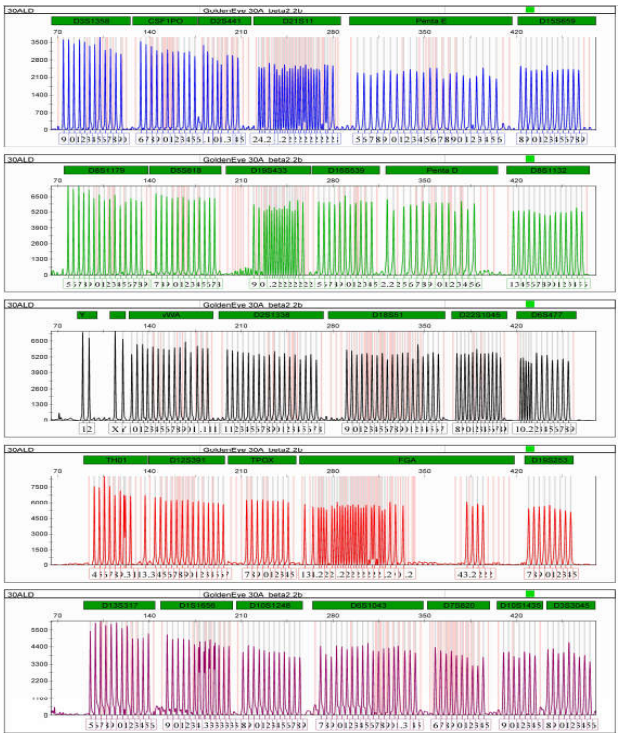
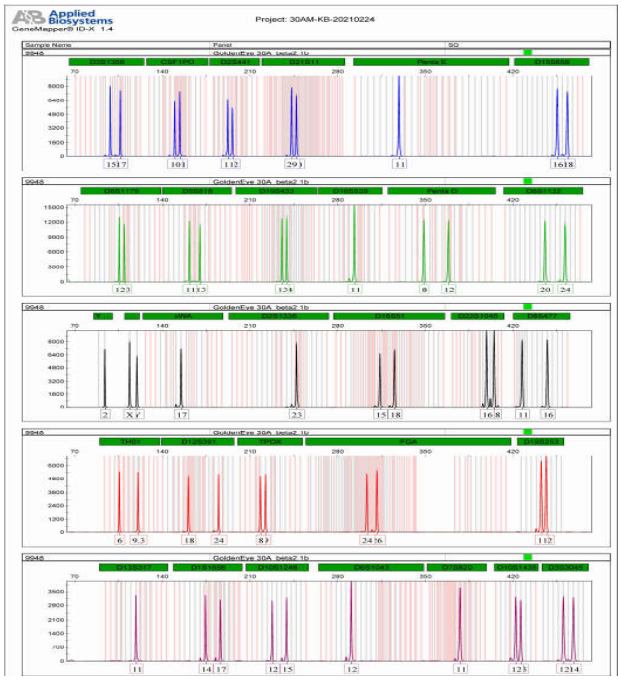


Annexed graph 2: DNA typing standard 9948 typing map



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.
3. The trial pack is for 25 people, the attached table is the composition list for 200 people, and the trial pack comes with Spectral Calibration!