Foregene DNA Identification System 27Y

Product Manual

I. Product introduction

Foregene DNA Identification System 27Y uses six-color fluorescent labeling technology to amplify 27 Y-STR loci at one time. Including: DYS456, YGATAH4, DYS439, DYS19, DYS392, DYS576, DYS627, DYS391, DYS437, DYS570, DYS635, DYS448, DYS533, DYF387S1, DYS393, DYS389I, DYS390 , DYS389II, DYS438, DYS518, DYS460, DYS458, DYS481, DYS385, DYS449.

The kit has good anti-inhibition ability, and can directly amplify filter paper, FTA card, cotton swab, saliva card, oral swab, and can also be used to extract and purify DNA templates from case materials.

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 store the kit at 4°C before the reaction to avoid repeated freezing and thawing, after the kit is taken out for use, ; the enzyme should be stored at - 20°C;
3. After the reaction, the kit (including molecular weight internal standard and ladder) should be stored in the "electrophoresis detection room" at 4°C, avoid repeated freezing and thawing, and avoid contact with the pre-reaction kit to avoid contamination.

III. Preparations before amplification

1. Protocol settings

Table 1: PCR reaction program for ABI 9700 thermal cycler

Amplification procedure	ABI 9700 Thermal Cycler
Denaturation	9 6 °C, 5 minutes
28 cycles	94°C, 5 seconds
	60°C, 1 minute 10 seconds
Extension	60°C, 15 minutes
Cryopreservation	15°C
Total amplification time: 60 minutes	

2. Shaking and mixing of reagents

Vortex the 2 × PCR reaction master mix V and 5 × 27Y primer mixture for 10 seconds before use , and then centrifuge briefly to eliminate the uneven concentration of the solution caused by possible tube wall adsorption.

IV. PCR amplification system

Table 2: Standard hands-free amplification reaction system preparation composition

	Components	10 μl system (μl)
Master Mix	Deionized water	3.0
	2×PCR Reaction Master Mix V	5.0
	5 x 27Y Primer Mix	2.0

	Blood stain	Diameter 1.2mm
Total reaction volume		10.0

V. Spectral correction (Matrix correction, take 3100 genetic analyzer as an example)

1. Replace the old POP4 gel and electrophoresis buffer on the sequencer;
2. Add 8 μl of 6-color system spectral calibration reagent (6-color Matrix J6C4) to 200 μl of deionized formamide, vortex and mix well, and distribute 10 μl in each well of two rows of 16 wells in a 96-well plate;
3. Denature at 95°C for 3 minutes, and immediately put it on ice to cool for 3 minutes (this step is very important and cannot be omitted);
4. For spectrum correction electrophoresis, select " J6 " for Dye Set, select "Spect36_POP4_1" for Run Module, and fill in "2.0" for Lower of Matrix Condition Number Bounds in Edit Parameter, fill in "0.4" for Sensitivity, and fill in "0.95" for Minimum Quality Score ", the specific parameters can be adjusted according to the sensitivity of the instrument, so that the final detection peak height is controlled between 750rfu-4000rfu;
5. In order to obtain the best correction effect, it is recommended that the Q value be >0.95 , and more than 13 of the 16 capillaries pass through. If this standard cannot be reached, it is recommended to adjust the electrophoresis parameters and re-electrophoresis.

VI. Electrophoresis detection

1. Standard loading system: 8.5 μl formamide + 0.5 μl molecular weight internal standard ORG500 + 1 μl PCR product;
2. ORG500 to 1ml formamide , mix well, and add 9 μl to each sample well of a 96-well plate;
3. Allelic Ladder dosage: 1μl;
4. Establishment of the electrophoresis protocol: Click Protocol Manager, click New on the page, create a new protocol, and name it GoldenEye_J6. Select REGULAR for Type, select HID Fragment Analysis36_POP4 for Run Module, select J6 for dye set, the parameters in HID Fragment Analysis36_POP4 are set to default values, no need to be changed, the injection voltage is 3k Volts, and the injection time is 10 sec.

VII. Data analysis

1. Open the Gene Mapper ID software. When using this kit for the first time, you need to import panels, bin set, corresponding analysis method, and size standard (ORG500 : 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, and change the sample type of Ladder to "Allelic Ladder" in the "Sample Type" column; start analyzing the data.

Attached kit component list:

Table 3: Foregene DNA Identification System 27Y Component List

Reagent test kit	Component name	Volume (μl/tube)	Quantity (tube)
PCR pre-reaction kit	2×PCR Reaction Master Mix	1250	2
	5 x 27Y Primer Mix	500	2
	DNA Typing Standard 9948	20	2

	Deionized water	1700	2
Post-PCR Reaction Kit	27Y allele mixture Ladder	20	2
	Orange molecular weight internal standard ORG500	150	2