FOREGENE DNA Identification System 20A （Free DNA Extraction）

Concise Operation Guide v1.71

Storage

1. After receiving the kit frozen in dry ice or plastic ice packs, if it is not used temporarily, please store it below -20°C for a long time；
2. After the kit is taken out and used, the kit should be stored at 4°C before the reaction to avoid repeated freezing and thawing; Taq 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

Preparations before amplification

1. Amplication program settings

Table 1 PCR reaction program of ABI 9700 thermal cycler

	ABI 9700 Thermal Cycler
Denaturation	96°C, 2 Min
Amplication：30±1 Cycles	94°C, 5 Sec
	60°C, 1 Min 10 Sec
Extension	60°C, 30 Min
Low temperature preservation	15°C
[Note]：Please use ABI 9700 (Gold Base) thermal cycler MAX mode amplification.	

2. Shaking and mixing of reagents

In order to obtain the best amplification effect, it is recommended to vortex the reaction buffer (Reaction Buffer) and primer mix (Primer Set) for 10 seconds before use,then briefly centrifuge to rule out the uneven concentration of the solution caused by the possible tube wall adsorption.

PCR amplication system

Table II: Standard Amplification Reaction System Preparation Composition

	Component	**25 μl reaction system(μl)**
Master Mix	**Deionized water**	**9.6**
	2.5×PCR reaction bufferIII	**10.0**
	5×20A primer mix	**5.0**
	Taq polymerase III	**0.16**
Bloodstain		1.2mm DM

Total reaction system	25.0

Spectral correction (Matrix correction, taking Model 3100 Genetic Analyzer as an example)

1. Replace the old POP4 gel and electrophoresis buffer on the sequencer;
2. Add 8 μl of 5-color system spectral calibration reagent to 200 μl deionized formamide, shake and mix well, and distribute 10 μl in each well of 16 wells in two rows of 96-well platel;
3. Denature at 95°C for 3 minutes, immediately place on ice to cool for 3 minutes (this step is very important and cannot be omitted)9
4. For spectrum correction electrophoresis, select "E5" 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 "0.1" for Sensitivity, fill in "0.8" for Minimum Quality Score, and specify parameters It 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 >0.90, and more than 13 capillaries pass through 16 capillaries. If this standard cannot be reached, it is recommended to adjust the electrophoresis parameters and perform electrophoresis again.

Electrophoresis detection

1. Standard loading system: 8.5 μl formamide + 0.5 μl molecular weight internal standard ORG500 + 1 μl PCR product;
2. Batch sample loading system: add 50 μl molecular weight internal standard ORG500 to 1ml formamide, mix well, add 9 μl to each sample well of 96-well plate;
3. Establishment of the electrophoresis protocol: Click Protocol Manager, click New on the page, create a new protocol, and name it GoldenEye_E5. Select REGULAR for Type, select HID Fragment Analysis36_POP4 for Run Module, select E5 for dye set, set the parameters in HID Fragment Analysis36_POP4 to default values, no need to change, the injection voltage is 3k Volts, and the injection time is 4 sec.

Data analysis

1. Open the Genemapper ID software. When using this kit for the first time, you need to import the 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 data.

Attached kit component list:

200 Preps FOREGENE DNA Identification System 20A（Free DNA Extraction）

Kit Componet	Componet Name	Volume（μl /tube）	Quantity（tube）
PCR Pre-reaction reagent	2.5×PCR reaction buffer III	1000	2
	5×20A primer mix	500	2
	Control DNA 9947A（1ng/μl）	25	1
	Deionized water	1700	2
Taq Polymerase	Taq polymerase III	40	2
PCR Rear-reaction reagent	Allelic ladder20A	40	1
	Molecular weight internal standardORG 500	150	2
Note			

Note：The trial pack of this product is for 50 people!