

Ingenomics™ Mini AutoProfiler STR Kit

1. Product introduction

Ingenomics™ Mini AutoProfiler STR Kit Uses 5-color fluorescence markers to detect 20 STR loci and 1 gender loci by multiple amplification, which is used for forensic analysis, genetic relationship testing, scientific research and other human genetic identification. The detected sites contained 13 CODIS loci (CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11) (Budowle et al., 1998). In addition, the expanded CODIS and ESS locus (Penta D, Penta E, D2S441, D2S1338, D12S391 and D19S433) highly polymorphic D6S1043 were also included.

This product is ISO18385 certified forensic grade product. According to the scope of the standard, the certification only includes the amplification reagents, while the Size Standard, Matrix Standards, Control DNA and Allelic Ladder post-amplification analysis reagents are not within the scope of this standard.

2. Reagent storage

- After receiving the kit, if it is not used temporarily, please store it at -20°C for a long time.
- After the kit is taken out for use, please store it at 4°C and avoid repeated freezing and thawing; if it will not be used for a long time, please store the Master Mix in the enzyme kit at -20°C.
- Please store the detection component kit in the "electrophoresis detection area" at 4°C, avoid repeated freezing and thawing, and avoid contact with the amplification component kit to avoid contamination.

3. Genetic Analyzer

For applications with the Applied Biosystems® 3500/3500XL Genetic Analyzers, we recommend preheating the oven to 60°C before use. Use the following parameters when setting up the instrument program. Please refer to the instrument user manual for more details.

Table 1 Genetic Analyzer Parameter settings

Genetic Analyzer	Run module	Dye set	Injection voltage, time	
ABI®3500/3500XL	HID36_POP4	ING-5	3 kV	10sec*
ABI®3130/3130XL	HIDFragmentAnalysis36_POP4	ING-5	3 kV	10sec*
ABI®3730/3730XL	Fragment Analysis	ING-5	3 kV	10sec*

*The injection time can be modified according to the height of the peak. The proposed modification range is (2-24sec), to increase or decrease the height of the observed peak.

4. PCR amplification system

Please always vortex the fully thawed Master Mix and Primer Mix for 10 seconds before preparing the system, then centrifuge briefly.

Table 2 Ingenomics™ Mini AutoProfiler STR Kit amplification system

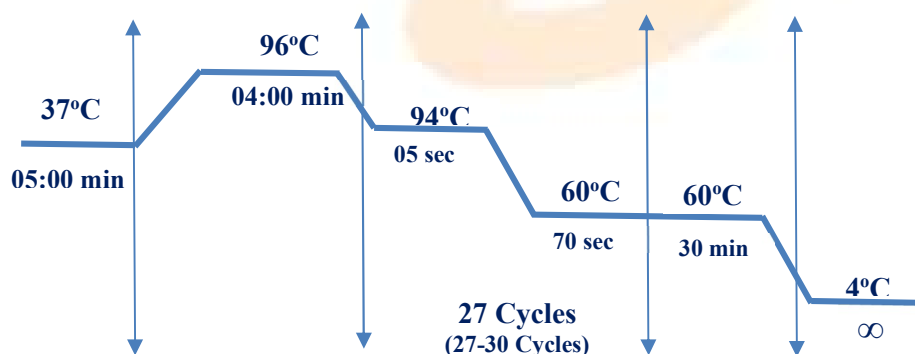
Reactive components	25 µL System addition amount
Ingenomics™ Mini AutoProfiler 2.5× Buffer D	10 µL
Ingenomics™ Mini AutoProfiler 5× Primer Mix	5 µL
Ingenomics™ Mini AutoProfiler Polymerase Mix II	0.625 µL
Template DNA	1 ng
Add dH ₂ O to the final volume of the reaction	add to 24µL

NOTE: (1) If the PCR amplification mixture is not thoroughly mixed, it may result in a decrease in amplification yield or an imbalance between loci.

(2) When determining the number of amplification reactions, positive and negative controls should be included. Add 1-2 reactions to eliminate pipetting errors. This step wastes a small amount of reagents but ensures that all samples have sufficient PCR reaction system, and also ensures that each reaction tube contains the same PCR reaction system.

5. Amplification procedure

Figure 1 ABI 9700 Thermal Cycler PCR Reaction Program Setup



Mini AutoProfiler PCR Conditions

Note: (1) When using the GeneAmp® PCR System 9700 Thermal Cycler, the program must be run at the maximum rate of temperature change (silver- or gold-plated thermal block plate). The rate of temperature change can be set after the thermal cycler has started running. Select "Method" to enter the setting options interface, select "9700" as the rate setting mode, and then enter the final reaction volume.

(2) Prolonged storage of amplified samples at 4 °C or higher may degrade the product.

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- (3) The number of amplification cycles and the final extension time depends on the specific sample. It is recommended to use 28 cycles and the final extension for 60min.

6. Electrophoresis Detection

- (1) Standard sample loading system:

Component	Volume (μL)
Formamide	8.8~8.5
Size standard ING550	0.2~0.5
PCR product	0.5~1.5*

*Appropriately increase or decrease the amount of PCR product according to the product concentration and the sensitivity of the sequencer, the recommended amount is 1 μL.

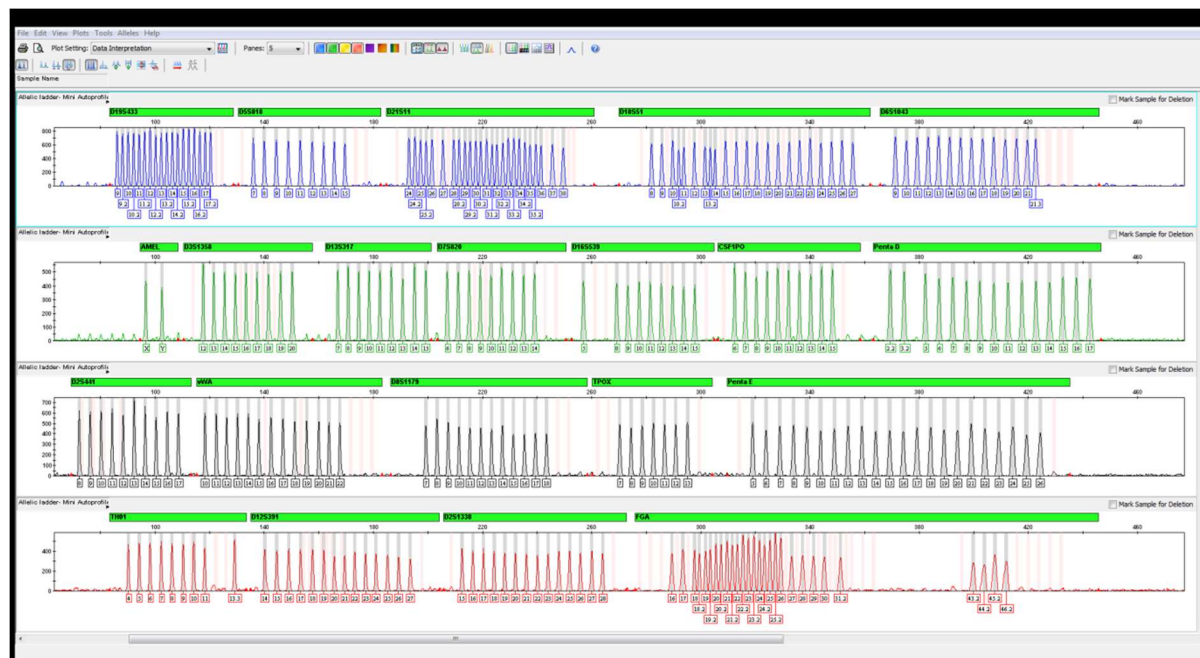
1. Vortex formamide and Ingenomics™ Size Standard QD5500 mixture for 10–15 seconds.
2. Pipet 10μl of mixture into each well.
3. Add 1μl of amplified sample (or 1μl of Allelic Ladder) to each well. Cover wells with appropriate septa.

Supplementary Table 1: Kit Components Table

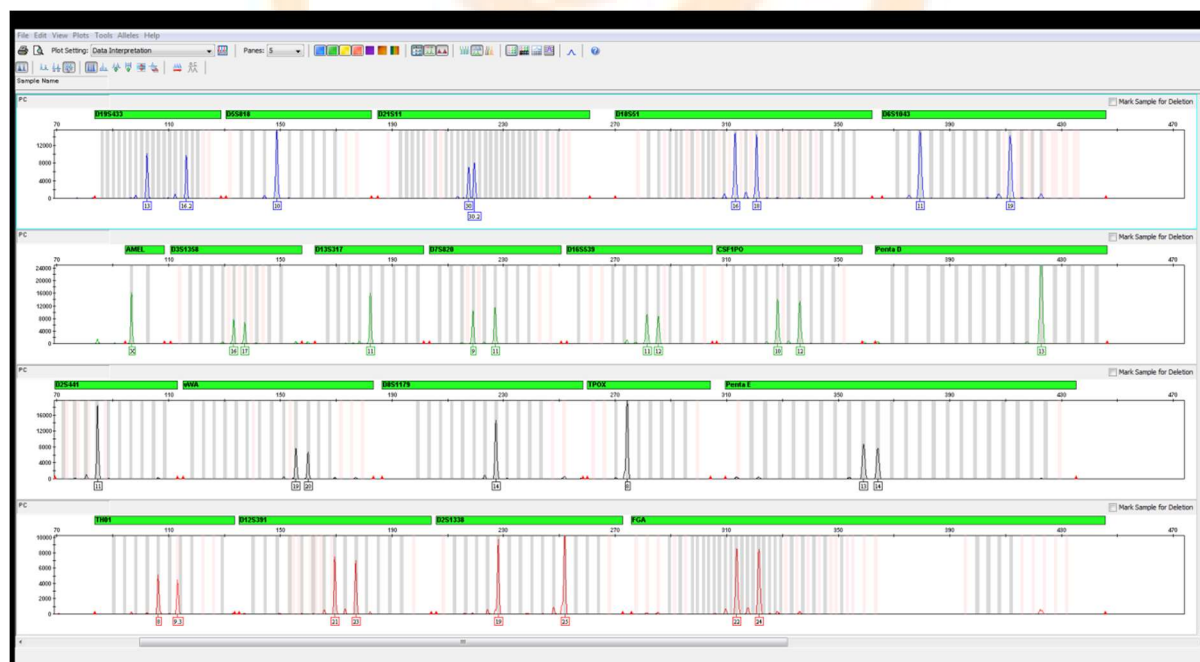
Reagent test kit	Component name	200 reactions
Amplification Component Kit	Ingenomics™ Mini AutoProfiler 5× Primer Mix	250μL *4
	Control DNA (2ng/μL)	25μL *1
	Nuclease-Free Water	1800μL * 2
Taq enzyme kit	Ingenomics™ Mini AutoProfiler 2.5 × Buffer D	500μL *4
	Ingenomics™ Mini AutoProfiler Polymerase Mix II	32μL *4
Detection component kit	Ingenomics™ Allelic Ladder	40μL *2
	Ingenomics™ Size Standard ING550	75μL * 2
	5-Dye Matrix Standards	25μL *1

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GeneMapper® ID-X Software plot of the Ingenomics™ Mini AutoProfiler Allelic Ladder.



9948A DNA Control (1 ng) amplified with the Ingenomics™ Mini AutoProfiler STR Kit and analyzed on an Applied Biosystems® 3500 Genetic Analyzer.

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Supplementary Table 2: Ingenomics™ Mini AutoProfiler Genetic Information

Locus	Fluorescent Label	DNA CONTROL
D19S433	FAM	13/16.2
D18S51	FAM	10
D5S818	FAM	30/30.2
D21S11	FAM	16/18
D6S1043	FAM	11/19
AMEL	HEX	X
D3S1358	HEX	16/17
D13S317	HEX	11
D7S820	HEX	9/11
D16S539	HEX	11/12
CSF1PO	HEX	10/12
Penta D	HEX	13
D2S441	1.552	11
Vwa	1.552	19/20
D8S1179	1.552	14
TPOX	1.552	8
Penta E	1.552	13/14
THO1	I.R600	8/9.3
D12S391	I.R600	21/23
D2S1338	I.R600	19/25
FGA	I.R600	22/24

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