Ingenomics™ Y Profiler STR Kit

1. Product Introduction

The Ingenomics™ Y Profiler STR Kit uses 6-color dye chemistry and multiplex PCR amplification to detect 38 Y chromosome loci. It can be used for human identification, such as forensic analysis, kinship detection and scientific research. The extracted DNA template can be amplified directly and the direct amplification of blood spots or saliva spots using filter paper or FTA card as the sample source can also be carried out using this kit.

The detected genetic loci contain DYS393, DYS570, DYS19, DYS392, DYS549, Y GATA H4, DYS460, DYS458, DYS481, DYS635, DYS448, DYS533, DYS456, DYS389I, DYS390, DYS389II, DYS438, DYS576, DYS391, DYS439, DYS437, DYS385a/b, DYS643, DYF387S1, DYS627, DYS449, DYS518, Y- Indel, DYS447, DYS444, DYS557, DYF404S1, DYS527a/b, and DYS596.

This product is ISO18385 certified and is of forensic grade standard. According to the scope of the standard, the certification 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 immediately, please store it at -20°C for a long-time storage.
- After the kit is opened for first use, store the kit components at 4°C and avoid repeated freezing-thawing; if the kit, post first use, is not further intended to be used for a long period, please store the Master Mix and the enzyme components at -20°C.
- Please store the post-amplification components of the kit at 4°C, avoid repeated freezing and thawing, and avoid contact with the pre-amplification kit components to prevent contamination.

3. Genetic Analyzer

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

Table 1: Ingenomics™ Y Profiler STR Kit genetic analyzer parameter settings

Genetic Analyzer	run module	dye group	Injection voltage, time
ABI® Model 3500	HID36_POP4	ING6	3KV 10S*
ABI® Model 3130xL	HIDFragmentAnalysis36_POP4	ING6	3KV 10S*

The injection time can be modified according to the height of the peak, and the recommended modification range is (2-24 seconds) to increase or decrease the observed signal value.

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™ Y Profiler STR Kit amplification system

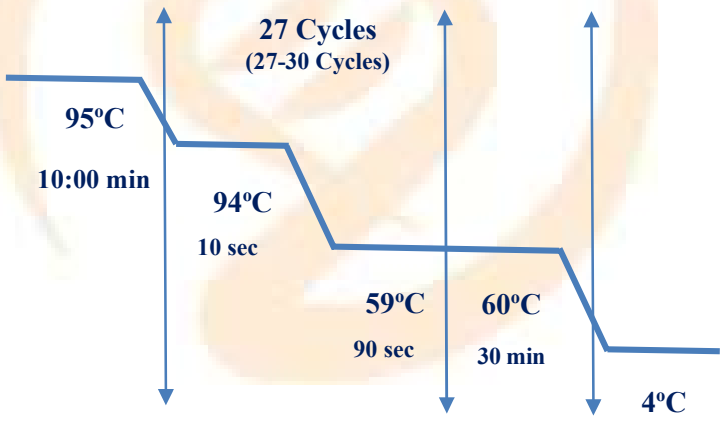
Reactive components	25μL system addition amount
Ingenomics™ Y Profiler 2 × Master Mix V	12.5μL
Ingenomics™ Y Profiler 5× Primer Mix	5μL
Template DNA	1.2mm diameter* (blood card or blood filter paper) DNA template (0.5ng-2ng)
Add dd H 2 O to the final volume of the reaction	25μL

* Please ensure immersion complete the filter paper or FTA card in the reaction solution system, otherwise it may cause amplification failure; determine the number of amplification reactions, including positive and negative controls. Add 1-2 amplification reaction systems to eliminate pipetting errors.

* For newly collected blood cards, please dry the samples at 95℃ for 10 min for better results.

5. Amplification Procedure

Figure 1: Thermal Cycler PCR Reaction Program Setup (ABI 9700)



Y Profiler PCR Conditions

Note:

1) Prolonged storage of amplified samples at 4 °C or higher may degrade the product, and diminish data quality obtained.

2) The number of amplification cycles and the final extension time depends on the specific sample. It is recommended to run samples for 28 cycles and the final extension for 60 min. (Users are however suggested to standardize the protocols as needed)

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.

2. Vortex formamide and Ingenomics™ Size Standard ING550 mixture for 10–15 seconds.
3. Pipet 10μl of mixture into each well.
4. Add 1μl of amplified sample (or 1μl of Allelic Ladder) to each well. Cover wells with appropriate septa.

Supplementary Table 1: Kit Components

Reagent test kit	Component name	100 reactions
Amplification Component Kit	Ingenomics™ Y Profiler 5× Primer Mix	500μL*1
	Control DNA (2ng/μL)	25μL*1
	Nuclease-Free Water	1800μL * 1
Taq enzyme kit	Ingenomics™ Y Profiler 2 × Master Mix V	1250μL*1
Detection component kit	Ingenomics™ Y Profiler Allelic Ladder	40μL*1
	Ingenomics™ Size Standard ING550	150μL * 1
	6-Dye Matrix Standards	25μL*1

Supplementary Table 2: Ingenomics™ Y Profiler STR Kit Genotyping Information

Locus	Fluorescent label	9948 Genotypes
rs2032678	FAM	2
DYS393	FAM	13
DYS570	FAM	18
DYS19	FAM	14
DYS392	FAM	13
DYS549	FAM	13
Y GATA H4	FAM	12
DYS444	FAM	12
DYS460	HEX	11
DYS458	HEX	18
DYS481	HEX	24
DYS635	HEX	23
DYS438	HEX	11
DYS447	HEX	25
DYS596	HEX	16
DYS456	TAINGA	17
DYS389I	TAINGA	13
DYS390	TAINGA	24
DYS389II	TAINGA	31
DYS448	TAINGA	19
DYS533	TAINGA	12
DYS449	TAINGA	30
DYS391	ROX	10
DYS439	ROX	12
DYS437	ROX	15
DYS385a/b	ROX	11/14
DYS643	ROX	11
DYS518	ROX	38
DYS576	PURP	16
DYF404S1	PURP	12/14
DYF387S1	PURP	35/38
DYS627	PURP	22
DYS527a/b	PURP	21/22
DYS557	PURP	16