Yfiler™ Plus PCR Amplification Kit

PCR Amplification and CE

Catalog Numbers 4484678 and 4482730

Pub. No. MAN1001551 Rev. A

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Yfiler™ Plus PCR Amplification Kit User Guide* (Pub. No. MAN0030230). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Yfiler™ Plus PCR Amplification Kit (100-reaction Cat. No. 4484678 or 500-reaction Cat. No. 4482730) is a 6-dye, short tandem repeat (STR) multiplex assay that amplifies 27 Y-STR loci in a single reaction.

The kit is optimized to allow amplification from extracted DNA or direct amplification from some types of single-source samples.

Before you begin

1. Set up samples:
   - Blood or buccal samples that are collected on treated paper substrates; see *Yfiler™ Plus PCR Amplification Kit—PCR Setup: Treated and Untreated Paper Substrates Quick Reference* (Pub. No. MAN1001550)
   - Blood samples that are collected on untreated paper substrates; see *Yfiler™ Plus PCR Amplification Kit—PCR Setup: Treated and Untreated Paper Substrates Quick Reference* (Pub. No. MAN1001550)
   - Buccal samples that are collected on swab substrates; see *Yfiler™ Plus PCR Amplification Kit—PCR Setup: Swab Substrate Quick Reference* (Pub. No. MAN1001549)
   - DNA casework samples; see *Yfiler™ Plus PCR Amplification Kit—PCR Setup: Extracted DNA Quick Reference* (Pub. No.MAN1001548)
2. Place this guide in the laboratory area where you perform post-PCR procedures.

Extracted DNA: Perform PCR

1. Program the thermal cycler.
   a. Set the ramping mode to **9600 Simulation**.
   b. Set the thermal cycling conditions as shown in the following table.

Initial incubation step	Cycle (30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C 1 minute	94°C 4 seconds	61.5°C 1 minute	60°C 22 minutes	4°C ≤24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

2. Load the plate or tubes into the thermal cycler, close the heated cover, then start the run.

3. When the run is complete, store the amplified DNA.

Storage time	Temperature
<2 weeks	2–8°C
>2 weeks	–25°C to –15°C

IMPORTANT! Protect the amplified DNA from light.

Direct amplification: Perform PCR

1. Program the thermal cycler.
   a. Set the ramping mode to **9600 Simulation**.
   b. Set the thermal cycling conditions as shown in the following table.

Initial incubation step	Optimum cycle number (26–29)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C 1 minute	94°C 4 seconds	61.5°C 1 minute	60°C 22 minutes	4°C ≤24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

2. Load the plate into the thermal cycler, close the heated cover, then start the run.

3. When the run is complete, store the amplified DNA.

Storage time	Temperature
<2 weeks	2–8°C
>2 weeks	–25°C to –15°C

IMPORTANT! Protect the amplified DNA from light.

Allelic ladder requirements for electrophoresis

To accurately genotype samples, you must run an allelic ladder with the samples.

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder
SeqStudio™ 24 Flex Genetic Analyzer	1 per injection	24 samples	23 samples + 1 allelic ladder
SeqStudio™ 8 Flex Genetic Analyzer	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
SeqStudio™ Genetic Analyzer	1 per 6 injections	4 samples	23 samples + 1 allelic ladder
3500xL Genetic Analyzer	1 per injection	24 samples	23 samples + 1 allelic ladder
3500 Genetic Analyzer	1 per 3 injections	8 samples	23 samples + 1 allelic ladder

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between runs. Follow the guidelines in the preceding table, which should account for normal variation in run speed. To facilitate accurate genotyping of all samples in your laboratory environment, perform internal validation studies to verify the required allelic ladder injection frequency.

Data collection software setup

Note: For detailed procedures, see the appropriate user documentation for your instrument.

Table 1 Software setup: SeqStudio™ Flex Series Genetic Analyzer for Human Identification

SeqStudio™ Flex Data Collection Software	*(Optional)* Additional software	Run parameters
v1.1.1	• SAE Administrator Console v2.1 • SeqStudio™ Plate Manager Software v2.1, v2.1.1 • SeqStudio™ Flex Remote Monitoring Software	**Injection protocol:** HID_Protocol_J6_36_POP4(xl)
		Size standard: GS600 LIZ (60–460)
		Dye set: J6 (DS-36)
		Run module: HID_J6_36_POP4 (xl)
		Injection conditions: 1.2 kV/15 seconds (xl: 24 seconds)
		Run conditions: 13 kV/1,550 seconds

Table 2 Software setup: SeqStudio™ Genetic Analyzer for HID

SeqStudio™ Data Collection Software	*(Optional)* Additional software	Run parameters	Plate setup
v1.2.1, v1.2.4, v1.2.5	• SAE Administrator Console v2.0, v2.1 • SeqStudio™ Plate Manager Software v1.2, v1.3	**Run Module:** HID Analysis	**Kit:** Yfiler™ Plus kit
		Injection conditions: 1.2 kV/10 seconds	**Dye set:** J6 (DS-36)
		Run conditions: 11 kV/1,120 seconds	**Size standard:** GS600 LIZ (60–460)

Table 3 Software setup: 3500 Series Genetic Analyzer for Human Identification

Operating system	3500 Data Collection Software	Run parameters
Windows™ 10	v4, v4.0.1	**Assay:** AB_J6_LS_POP4(xl)
		Instrument protocol: AB_HID36_POP4(xl)_J6_NT3200
		Run module: HID36_POP4(xl)
		Injection conditions: 1.2 kV/16 seconds (xl: 24 seconds)[1]
		Alternate injection conditions: 1.5 kV/16 seconds (xl: 24 seconds)[2]
		Run conditions: 13 kV/1,550 seconds
		Dye set: J6

[1] This kit was developed using an injection time of 16 seconds on the 3500 instrument. This is different from the default injection time of 15 seconds. You will need to change the instrument protocol accordingly.

[2] This kit was developed using two injection voltage conditions for the 3500 instrument: 1.2 kV/16 seconds and 1.5 kV/16 seconds. You are encouraged to explore both options during validation to determine which protocol provides the best results on your instrument.

Prepare samples for electrophoresis and start the run

Prepare the samples for electrophoresis immediately before loading.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set, amplified DNA, allelic ladder, and size standard from light when not in use.

1. Pipet the required component amounts into an appropriately sized polypropylene tube.

Component	Amount per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.4 µL
Hi-Di™ Formamide	9.6 µL

Note: Include additional samples in your calculations to account for the loss that occurs during reagent transfers.

IMPORTANT! The amount of size standard indicated in the table is a suggested amount. Determine the appropriate amount of size standard based on your experiments and results.

2. Vortex the tube, then briefly centrifuge.

3. Pipet the required component amounts into each well of a MicroAmp™ Optical 96-Well Reaction Plate.

Component	Amount per reaction
Formamide/size standard mixture	10 µL
PCR product or allelic ladder	1 µL

Note: For blank wells, add 10 µL of Hi-Di™ Formamide.

4. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.

5. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.

6. Immediately place the plate on ice for 3 minutes.

7. Place the sample tray on the autosampler, then start the electrophoresis run.

Data analysis

To set up the GeneMapper™ *ID-X* Software for data analysis, see the *Yfiler™ Plus PCR Amplification Kit User Guide* (Pub. No. MAN0030230).

Limited product warranty

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Revision history: Pub. No. MAN1001551 A

Revision	Date	Description
A	22 April 2025	New document for the Yfiler™ Plus PCR Amplification Kit; replaces Pub. No. 100030923. The following changes were made: • Compatible instruments, compatible software, and materials required were updated (throughout the document). • Copy edits and formatting changes were made to align with current documentation style (throughout the document).
C	10 January 2019	The cycle number recommendation was updated to "CYCLE (Direct Amplification 26–29) (Extracted DNA 30)".
B	27 December 2016	Content was reorganized. Non-technical changes only.
A	9 December 2014	New document for the Yfiler™ Plus PCR Amplification Kit.