Yfiler™ Plus PCR Amplification Kit – PCR Amplification and CE

Catalog Numbers 4484678 and 4482730

Pub. No. 100030923 **Rev.** C

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Yfiler™ Plus PCR Amplification Kit User Guide* (Pub. No. 4485610). 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 for amplification from extracted DNA and/or direct amplification of single-source samples.

Before you begin

1. Set up samples:
   - Blood or buccal samples that are collected on treated paper substrates (described in Quick Reference Pub. No. 100030920)
   - Blood samples that are collected on untreated paper substrates (described in Quick Reference Pub. No. 100030920)
   - Buccal samples that are collected on swab substrates (described in Quick Reference Pub. No. 100030922)
   - DNA casework samples (described in Quick Reference Pub. No. 100030921)
2. Place this guide in the area of the lab where you perform post-PCR procedures.

Perform PCR

1. Program the thermal cycling conditions.

IMPORTANT!

- If you are using the ProFlex™ 96-well PCR System, then run in the GeneAmp™ **PCR System 9600 Simulation Mode**.
  For instructions on how to configure the ProFlex™ 96-well PCR System to run GeneAmp™ PCR System 9600 Simulation Mode, see the *ProFlex™ PCR System User Guide* (Cat. No. MAN0007697).
- If you are using the GeneAmp™ PCR System 9700 with a 96-well silver or gold-plated silver block, then select **9600 Emulation Mode**.
  For instructions on how to configure the GeneAmp™ PCR System 9700 to run 9600 Emulation Mode, see the *GeneAmp™ PCR System 9700 Base Module User Manual* (Cat. No. 4303481).
- If you are using the Veriti™ 96-Well Thermal Cycler, then select **9600 Emulation Mode**.
  For instructions on how to configure the Veriti™ 96-Well Thermal Cycler to run 9600 Emulation Mode, see the *Veriti™ 96-Well Thermal Cycler AmpFℓSTR™ Kit Validation User Bulletin* (Cat. No. 4440754).

Initial incubation step	Optimum cycle number		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE (Direct Amplification 26–29) (Extracted DNA 30)		HOLD	HOLD
95°C, 1 minute	94°C, 4 seconds	61.5°C, 1 minute	60°C, 22 minutes	4°C, up to 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.

IMPORTANT! If you are using adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti™ Thermal Cycler does not require a compression pad.

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

If you are storing the DNA...	Then place at...
<2 weeks	2°C to 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(s)
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500*xl*	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 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. Perform internal validation studies to verify the required allelic ladder injection frequency, to ensure accurate genotyping of all samples in your laboratory environment.

Electrophoresis instrument requirements

For more information, see the "Related documentation" section in the user guide.

Table 1 3500 Series instrument requirements

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions (installed with the HID Updater)
3500 3500xL	Windows™ Vista	3500 Data Collection Software v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	Plate templates: 6dye_36_POP4 (and _xl) Assays: GF+Norm_POP4 (and _xl) and GF_POP4 (and _xl), which contain instrument protocol HID36_POP4 (and_xl)_J6_NT3200 with the following conditions: • Run module: HID36_POP4 (and _xl) • Injection conditions: 1.2 kV/16 sec (24 sec for xl)[1] • Alternate injection conditions: 1.5 kV/16 sec (24 sec for xl)[2] • Run conditions: 13 kV/1550 sec • Dye Set J6
3500 3500xL	Windows™ 7	3500 Data Collection Software v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	Same as 3500 Data Collection Software v2 listed above
3500 3500xL	Windows™ 7	3500 Data Collection Software v3	None	Same as 3500 Data Collection Software v2 listed above

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

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

Table 2 3130 Series instrument requirements

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Run modules and conditions
3130	Windows™ 7	Data Collection Software v4[1]	3130/3730 DC v4 6-Dye Module v1	• HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/5 sec • Run conditions: 15 kV/1500 sec • Dye Set J6
3130 *xl*				• HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/10 sec • Alternate injection condition for the 3130*xl*: 3 kV/13 sec[2] • Run conditions: 15 kV/1500 sec • Dye Set J6

[1] Requires activation of 6-dye license.

[2] This kit was developed using two injection voltage conditions for the 3130*xl*; 3 kV/10 sec and 3 kV/13 sec. You are encouraged to explore both options during validation to determine which protocol provides the best results on your instrumentation.

Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)

This procedure applies to the 3500 Series and 3130 Series instruments.

Prepare the samples for electrophoresis immediately before loading.

1. Pipet the required volumes of components into an appropriately sized polypropylene tube:

Reagent	Volume per reaction
GeneScan™–600 LIZ™ Size Standard v2.0	0.4 µL
Hi-Di™ Formamide	9.6 µL

Note: Include volume for additional samples to provide excess volume for the loss that occurs during reagent transfers.

IMPORTANT! The volume 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. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide/size standard mixture
   - 1 µL of PCR product or Allelic Ladder

   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 at 95°C for 3 minutes.

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. 4485610).

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

Revision history: Pub. No. 100030923

Revision	Date	Description
C	10 January 2019	In "Perform PCR" on page 1, update the cycle number recommendation to "CYCLE (Direct Amplification 26–29) (Extracted DNA 30)".
B	27 December 2016	Non-technical changes: reorganized content
A	09 December 2014	New document