GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits—PCR Amplification and CE

Catalog Numbers 4476135, 4482815, A43565

Pub. No. 4477593 **Rev.** D

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide* (Pub. No. 4477604 Rev. F or later). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The GlobalFiler™ PCR Amplification Kit (200-reaction Cat. No. 4476135 or 1,000-reaction Cat. No. 4482815) and the GlobalFiler™ IQC PCR Amplification Kit (200-reaction Cat. No. A43565) are 6-dye, short tandem repeat (STR) multiplex assays that amplify 21 autosomal STR loci, Amelogenin, 1 Y STR locus, and 1 Y insertion/deletion (Y indel) locus.

In addition to the loci listed above, the GlobalFiler™ IQC PCR Amplification Kit amplifies 2 Internal Quality Control (IQC) markers.

Note: The primer set and the allelic ladder in the GlobalFiler™ kit are different than the primer set and the allelic ladder in the GlobalFiler™ IQC kit. Do not interchange.

Before you begin

- Place this guide in the area of the lab where you perform post-PCR procedures.
- Set up samples (described in Quick Reference Pub. No. 4477594 Rev. E or later).

Perform PCR

1. Program the thermal cycling conditions.

IMPORTANT! Set the thermal cycler you are using as indicated: ProFlex™ PCR System: 97 Simulation ramping mode; Veriti™ Thermal Cycler: 100% ramping rate, *do not* use 9600 emulation mode; GeneAmp™ PCR System 9700: Max ramping mode.

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 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, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The ProFlex™ PCR System and the Veriti™ Thermal Cycler do not require a compression pad.

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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.

Genetic analyzer	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)[1]
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

[1] The allelic ladder in the GlobalFiler™ kit is different than the allelic ladder in the GlobalFiler™ IQC kit. Do not interchange.

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	3500 Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions
3500 3500xL	Windows™ 10	v3.3, v4	None	Plate templates: 6dye_36_POP4 (and _xl) Assay (DC4): AB_J6_LS_POP4 (and _xl), which contains instrument protocol AB_HID36_POP4_J6_NT3200. Assays (DCv3.3 and earlier): GF+Norm_POP4 (and _xl) and GF_POP4 (and _xl), which contain instrument protocol HID36_POP4 (and_xl)_J6_NT3200. All assays use the following conditions: • Run module: HID36_POP4 • Injection conditions: 1.2 kV/15 sec (24 sec for xl) • Run conditions: 13 kV/1550 sec • Dye Set J6
3500 3500xL	Windows™ 7	v3, v3.1	None	
3500 3500xL	Windows™ 7	v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	
3500 3500xL	Windows™ Vista	v1	HID Updater 3500 DC v2 (Cat. No. 4480670)	

Table 2 3130 Series instrument requirements

Genetic analyzer	Operating system	Data Collection Software	Additional software	Run modules and conditions
3130	Windows™7	v4	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*[1]				• HIDFragmentAnalysis36_POP4_1 • Injection conditions: 3 kV/5–10 sec • Run conditions: 15 kV/1500 sec • Dye Set J6

[1] We conducted validation studies using the 3130*xl*, 3500, and 3500xL configurations.

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

Prepare the samples for electrophoresis immediately before loading samples in the plate.

1. Pipet the required volumes of components into an appropriately sized, clear (non-colored), 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 reaction plate in the genetic analyzer, then start the electrophoresis run.

Data analysis

To set up the GeneMapper™ *ID-X* Software for data analysis, see the *GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide* (Pub. No. 4477604 Rev. F or later).

Limited product warranty

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Revision history: Pub. No. 4477593

Revision	Date	Description
D	21 August 2019	Add information for GlobalFiler™ IQC PCR Amplification Kit. Add information for 3500 Series Data Collection Software v3.1, v3.3, v4. For 3130xl, changed HIDFragmentAnalysis36_POP4_1 Injection conditions to 5–10 sec.
C	23 October 2018	Updated branding and trademarks, no technical changes.
B	07 July 2016	Add information for 3500 Series Data Collection 3 Software.
A	June 2013	New document.