PCR Amplification Guide

Philisa® Thermal Cycler and Philisa*FAST*™ DNA Polymerase

For use with the Life Technologies™ AmpFℓSTR® Identifiler® Plus PCR Amplification Kit Primer Set

Pre-PCR Amplification

PCR Amplification will be performed on the Streck Philisa Thermal Cycler using the Philisa PCR Tubes. The Streck Philisa Thermal Cycler shall be placed in the amplified DNA work area.

All Pre-PCR setup steps (extraction, purification, quantification, etc.) should be consistent with the lab's standard methods and following the guidelines listed in the Identifiler® Plus User Guide, Chapter 2.

Additional materials required for this protocol include:

- ART Gel extended length Aerosol Barrier pipette tips (Fisher catalog number: 212369)
- Philisa*FAST* DNA Polymerase Kit (Streck Catalog number: 250024)
- Streck Philisa Thermal Cycler (Streck catalog number: 250000)
- Streck Philisa PCR Tubes (Streck catalog number: 250001)
- Streck Philisa PCR Tube Rack (Streck catalog number: 250022)

Prepare the amplification kit reactions

1. **Calculate the volume of each component needed to prepare the Master Mix, using the table below:**

Master Mix Component	Volume per Reaction (µl)
AmpFℓSTR Identifiler Plus Primer Set	6.0
Philisa*FAST* 10X Fast Buffer I or II	2.75
Philisa*FAST* dNTP mixture	2.0
Philisa*FAST* DNA Polymerase(5 U/µl)	0.5
PCR Grade Water*	3.75
Total Master Mix Volume per Reaction	**15.00**

* For low copy number DNA, water can be left out of the Master Mix and replaced with an equal volume of additional template DNA in the Philisa PCR Tube.

Guide continues on the reverse side of this document.

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2. **Prepare reagents (Initial use)**

   - Thaw AmpFℓSTR Identifiler Plus Primer Set, Philisa*FAST* 10X Fast Buffer I, Philisa*FAST* dNTP Mixture, and Philisa*FAST* DNA Polymerase.

     Note: Component thawing is only required for the initial use of the STR Kit. Philisa*FAST* components should then be stored between 2 and 8 °C and do not require subsequent thawing. DO NOT refreeze the components. Philisa*FAST* Enzyme should be stored at -20 °C.

   - Vortex the components at medium setting for 5 seconds and briefly pulse spin in the micro-centrifuge prior to opening the tubes.

3. **Pipette the required volumes of each component into an appropriately sized polypropylene tube (Example: 1.5 ml Eppendorf tube).**
4. **Vortex the reaction mix for 3 seconds then centrifuge briefly.**
5. **Place the Philisa PCR Tubes into the Philisa Tube Rack and pipette 15 µl of the reaction into each tube.**
6. **Prepare the DNA Samples:**

DNA Sample	Preparation
Negative Control	Add 10 µl of low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to the Philisa PCR Tube.
Test DNA	Dilute an aliquot of the test DNA with low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to a concentration of 0.1 ng/µl. Add 10 µl of this mixture to the Philisa PCR Tube.
Low Template Test DNA	If test DNA concentration is < 0.1 ng/µl, eliminate water from Master Mix and add 15 µl of test DNA to the Philisa PCR Tube.
Positive Control	Add 10 µl of 9947A Control DNA (0.1 ng/µl) to the Philisa PCR Tube.

Perform PCR

Note: Refer to the Philisa Thermal Cycler Operator's Manual for complete instructions.

1. Program the Philisa Thermal Cycler using the following cycling parameters:

PCR Step	Temperature	Time
Initial Incubation Step	96 °C	60 sec
Three-Step PCR (29 Cycles)	96 °C	5 sec
	61 °C	15 sec
	72 °C	2 sec
Final Extension	72 °C	15 sec

2. **Load the PCR Tubes into the Philisa Thermal Cycler wells and close the lid.**
3. **Press the Start Run button to begin the protocol.**
4. **Upon completion of the run, store the amplified DNA products protected from light and at the following temperature ranges:**

DNA Sample	Preparation
Less than 2 weeks	2 – 8 °C
Greater than 2 weeks	-15 – -25 °C

5. **Proceed to electrophoresis step following the guidelines listed in the Identifiler Plus Users Guide, Chapter 3.**