CHAPTER 15 - AUTOSOMAL STR AMPLIFICATION – AmpF*l*STR® Identifiler® Kits

AmpF*l*STR® Identifiler® Kit Storage:

Upon receipt of AmpF*l*STR® Identifiler® kits, use the PCR Amplification Kit Log to record date received and all lot numbers of each item in the kit. Included in the kit are the PCR reaction mix, primer set, Amp*l*iTaq Gold® DNA polymerase, AmpF*l*STR® Control DNA 9947A, mineral oil, and allelic ladder. After kit inventory, the AmpliTaq® Gold DNA polymerase is removed from the kit box and placed in the freezer in the DNA reagent preparation room. The allelic ladder is stored in the freezer located in the PCR amplification room. Proper precautions should be exercised when handling and transporting the allelic ladder since it contains amplified product. Allelic ladder will be thawed as needed. Following the initial use, the ladder will be stored in the refrigerator. On the date that the ladder is thawed and transferred to the refrigerator, an expiration date will be written on the box that is six months from the transfer date or the expiration date printed on the box by the manufacturer, whichever comes first. The remaining kit reagents should be stored in the kit box in the refrigerator located in the DNA reagent preparation room.

Amplified DNA Samples:

When STR typing with an AmpF*l*STR® Identifiler® kit is completed, the amplified DNA samples will be handled in the following manner:

1. Discard amplified DNA samples in the red "biohazard" bags if sufficient stain material and/or DNA extract remains for retesting (e.g., reference blood samples, large bloodstains, etc.).

2. Retain amplified DNA samples for those samples where the DNA extract and/or stain material was consumed in analysis. These samples are labeled and placed in the designated racks in the PCR amplification room freezer.

PCR Setup and Amplification:

Adding DNA to PCR reaction tubes should be done in the biosafety hood. Wear clean gloves and a lab coat. Masks may be worn at the analyst's discretion.

1. Check to make sure that the thermal cycler has been calibrated. Turn on the PE 9700 thermal cycler and select the "identifiler" file which has the following parameters:

    a. Initial incubation at 95^{o}C for 11 minutes.

    b. Step cycle (28 cycles): denature at 94^{o}C for 1 minute
    anneal at 59^{o}C for 1 minute

extend at 72°C for 1 minute

c. Final extension at 60°C for 90 minutes

d. Hold temperature: 4°C for overnight or weekend soak

(Note: another thermal cycler file named "identifiler(54)" was validated and is available for the amplification of samples suspected of having primer binding site mutations. This program utilizes a lower annealing temperature to reduce the stringency of the reaction.)

2. Determine the number of samples to be amplified, including a positive and negative control. Place the required number of PCR tubes into a rack and label them. Use 0.5 mL thin-walled PCR reaction tubes. Alternatively, amplification may be performed in an Optical 96-well Reaction Plate with strip caps, specifically using the PE 9700G silver block thermal cycler.

3. Mix/vortex the AmpF*l*STR® PCR Reaction Mix, AmpF*l*STR® Primer Set, and AmpliTaq Gold® DNA Polymerase tubes. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

4. Prepare the Identifiler® Master Mix as follows:

Number of samples x 10.5 µL	AmpF*l*STR® PCR Reaction Mix
Number of samples x 0.5 µL	Amp*li*Taq Gold® DNA Polymerase
Number of samples x 5.5 µL	AmpF*l*STR® Identifiler® Primer Set

5. Thoroughly mix the Identifiler® Master Mix tube and spin briefly in a microcentrifuge to remove any liquid from the cap.

6. Add TE buffer, PCR master mix, and DNA sample to the appropriate sample and control tubes, or wells of a 96-well reaction plate. This can be done several ways as described below. The DNA sample should always be added last. For Identifiler® reactions, the combined volume of TE buffer and DNA sample should equal 10 µL and the volume of PCR master mix added should equal 15 µL. The target DNA quantity is ~1 ng.

Sample Type	Concentration	Preparation Method
DNA sample	≤0.125 ng/µL	Add 10µL sample to PCR tube or well
DNA sample	>0.125 ng/µL	• Dilute a portion of the sample with TE buffer so that only ~1 ng of DNA is in a volume of 10µL. Add diluted sample to PCR tube or well. OR •Add a precalculated amount of TE buffer followed by a precalculated volume of DNA so that ~1ng of DNA is in a volume of 10µL. Always add the DNA sample last. NOTE: if using the latter method, the PCR master mix can be added either before or after the addition of TE buffer.
Extraction Control	Unknown, low concentration	Add the same amount of extract volume to the PCR tube that is equal to the least concentrated sample within the extraction set. Add TE buffer, if necessary, to bring the total combined volume up to 10µL.
Positive Control (AmpFℓSTR® Control DNA 9947A)	0.10 ng/µL	•Mix the AmpFℓSTR® Control DNA 9947A tube. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap. •Add 8-10µL (0.8-1 ng) of AmpFℓSTR® Control DNA 9947A to the positive control PCR reaction tube or well.
Negative Control	0 ng/µL	Add 10µL TE buffer to the negative control reaction tube or well.

8. If amplifying in tubes, using a rack, carry the loaded PCR reaction tubes into the PCR amplification/typing room. Do not place this rack down at any time in this room. If necessary, the rack can be placed in the analyst's laboratory coat pocket. Transfer the PCR reaction tubes into the designated wells of the thermal cycler and record the heat block position of each tube on the Amplification Data Sheet. Push the tubes down completely into the heat block and slide the lid forward to lock them into position. If using a 96-well reaction plate, cover the loaded wells with strip caps, and carry the plate into the PCR amplification/typing room, and place the plate into thermal cycler 9700G or 9700H. Push the plate down completely into the heat block and slide the lid forward to lock them into position.

9. Start the AmpF*l*STR® thermal cycler program.

10. After the amplification process, remove the tubes or plate from the thermal cycler. The amplified products are now ready for analysis by capillary electrophoresis. The amplified

products can be stored refrigerated (2 to 8°C) for short periods of time (less than two weeks). For longer periods, store the amplified products in the freezer (-15 to -25°C). The amplified products must be stored so that they are protected from light.

11. If a sample does not initially amplify, one of the following strategies can be used to overcome possible inhibitory effects:

- 2 µL of a 4 mg/mL solution of bovine serum albumin (BSA) can be added to the amplification reaction mix.
- The DNA extract may be diluted with TE buffer.
- The DNA extract can be subjected to additional washes with TE buffer in a Microcon® unit.

END

CHAPTER 16 - Y-STR AMPLIFICATION - AmpF*l*STR® Yfiler® Kit

AmpF*l*STR® Yfiler® Kit Storage and Quality Control Testing:

Upon receipt of AmpF*l*STR® Yfiler™ kit, use the PCR Amplification Kit Log to record date received and all lot numbers of each item in the kit. The kit includes the Yfiler® PCR Reaction Mix, Yfiler® Primer Set, Amp*l*iTaq Gold® DNA Polymerase, AmpF*l*STR® Control DNA 007 (male DNA), AmpF*l*STR® Control DNA 9947A (female DNA), and Yfiler® Allelic Ladder. After kit inventory, the Amp*l*iTaq® Gold DNA polymerase is removed from the kit box and placed in the freezer in the DNA reagent preparation room. The allelic ladder is stored in the refrigerator located in the PCR amplification room. Proper precautions should be exercised when handling and transporting the allelic ladder since it contains amplified product. Allelic ladder will be thawed as needed. Following the initial use, the ladder will be stored in the refrigerator. On the date that the ladder is thawed and transferred to the refrigerator, an expiration date will be written on the box that is six months from the transfer date or the expiration date printed on the box by the manufacturer, whichever comes first. The remaining AmpF*l*STR® kit reagents should be stored in the kit box in the refrigerator located in the DNA reagent preparation room.

The female control DNA (9947A) will only be used for quality control testing when the laboratory receives a new lot number of a Y-filer® amplification kit and is not required for routine casework amplification. For the quality control testing amplification, 1 µL (10 ng) of the female control DNA will be added to the PCR reaction.

Amplified DNA Samples:

When Y-STR typing is completed, the amplified DNA samples will be handled in the following manner:

1. Discard amplified DNA samples in the red "biohazard" bags if sufficient stain material and/or DNA extract remains for retesting (e.g., reference blood samples, large bloodstains, etc.).

2. Retain amplified DNA samples for those samples where the DNA extract and/or stain material was consumed in analysis. These samples are labeled and placed in the designated racks in the PCR amplification room freezer.

PCR Setup and Amplification:

Adding DNA to PCR reaction tubes should be done in the biosafety hood. Wear clean gloves and a lab coat. Masks may be worn at the analyst's discretion.

1. Check to make sure that the thermal cycler has been calibrated. Turn on either the 9700G or 9700H silver block thermal cycler and select the amplification program "y-filer". The Yfiler® thermal cycle program has the following parameters: