Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

AMPFℓSTR® YFILER™ AMPLIFICATION AND LONG TERM STORAGE

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1 PREPARATION OF EXTRACTS FOR Y-STR ANALYSIS

The optimal target concentration range for amplification using the AmpFℓSTR® Yfiler™ System Kit has been determined through internal validation to be 0.5 to 1.0 ng/10 µL.

1.1 Wet extracts may be concentrated prior to amplification in an effort to meet the target concentration range.

1.1.1 The extracts may be concentrated using either a Microcon® concentrator assembly or by drying/evaporating and reconstitution. Refer to Appendix A for these procedures.

NOTE: If a STRONG signal was observed using the Plexor® HY System, but little or no amplification product observed, a higher concentration (i.e., 2 ng) of the isolated male DNA may be used to enhance the possibility of obtaining amplified product from a potentially degraded sample.

NOTE: If the female/male DNA ratio is very high, it may be necessary to target less than 0.5 ng male DNA to decrease the amount of female DNA added to the amplification reaction to reduce the likelihood of inhibitory effects caused by the large amount of female DNA in the reaction.

NOTE: If a sample is suspected to be inhibited, the sample may be further diluted or purified using a Microcon® concentrator assembly.

1.2 Dry extracts will be resolubilized following the procedure detailed in Appendix A.

1.3 After testing the remaining extracts/extract tubes will be dried using one of the procedures in Appendix A and returned to the originating examiner (if applicable) for return to the agency with the evidence. Alternatively, the wet extracts may be returned to the originating examiner for drying and return to the agency. If the examiner conducting the Y-STR testing is the original examiner, the extracts/tubes will be dried and returned with the evidence.

1.3.1 Drying and returning extracts to the submitting agency from known reference samples is optional; however, all extracts provided by an originating examiner will be returned to that originating examiner.

2 MANUAL PCR SET UP AND AMPLIFICATION

A dedicated area, such as a biological hood or a separate room, should be used for preparing PCR amplification reactions. All equipment and supplies used to prepare amplification reactions should be kept in this dedicated "clean" area at all times. These items will not be used to handle amplified DNA or other potential sources of contaminating DNA. Trace amounts of amplified DNA, if carried over into other samples before amplification, can lead to results that can be misinterpreted. Amplified DNA or equipment and supplies used to handle amplified DNA will NOT be brought into the designated "clean" area. Clean disposable laboratory gloves will be worn while preparing samples for PCR amplification and will be changed frequently or whenever there is a chance they have been contaminated with DNA. Touching the inside surface of the tube caps will be avoided.

DNA samples will be added to the amplification tubes or amplification plate AFTER the AmpFℓSTR® Yfiler™ master mix has been added to all tubes or all wells used in the plate. Each tube will be capped after sample DNA is added before proceeding to the next sample tube. Pipette tips will be changed after the addition of each DNA sample before proceeding to the next sample tube. The negative control should be processed and/or capped last, as this provides a control check for contamination during PCR set up.

2.1 Equipment

- Thermal Cycler - 9700
- Pipettes - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL, 1000 µL
- 8- Channel Pipette – Range 0.5 µL to 10 µL
- Repeater pipette
- Biological Safety Hood
- Microcentrifuge tube racks

2.2 Materials

- Sterile ART pipette tips - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL, 1000 µL
- Microcentfuge tubes
- Kimwipes
- Gloves
- Sterile 0.2 mL tubes (amp tubes)
- 96 well amplification plates (TempPlate(R) III P/N 1402-9700 or similar)
- Strip caps
- Adhesive sealing foil – VWR P/N 60941-112
- Compression pad (ABI P/N 4312639 or similar)

2.3 Reagents

- Sterile Type I Water
- AmpFℓSTR® Yfiler™ Amplification Kit (Applied Biosystems, Inc. P/N 4359513)

NOTE: The AmpFℓSTR® Yfiler™ Allelic Ladder supplied in the AmpFℓSTR® Yfiler™ System kit does not require amplification and should not be stored in the sample preparation area.

2.4 AmpFℓSTR® Yfiler™ Amplification Procedure for the 9700 Thermal Cycler

2.4.1 Amplification Parameters for the 9700 Thermal Cyclers:

2.4.1.1 Turn on the 9700 thermal cycler. If a program for AmpFℓSTR® Yfiler™ amplification has not already been stored in the thermal cycler, program as follows using the step cycle file, change the default setting to:

Initial incubation for 11 minutes at 95ºC.

Denature at 94ºC for 60 seconds
Anneal at 61ºC for 60 seconds
Extend at 72ºC for 60 seconds

Program for 30 cycles

Final extension at 60ºC for 80 minutes

Final hold at 4ºC.

This program may be saved as a “User File” for later use.

2.4.2 Transfer the AmpFℓSTR® Yfiler™ System amplification reagents to the designated "clean" area.

2.4.3 Place the required number of labeled sterile amp tubes (or 96-well plate, including one label for entire plate indicating the plate name) in a rack.

2.4.4 Calculate the required volume of each PCR amplification component to prepare a master mix by multiplying the volume by the number of samples. This should include reagent blanks, positive and negative amplification controls and additional reaction volumes (depending on the number of samples being amplified) to compensate for any pipetting variation. The PCR master mix should be prepared in a clean microcentrifuge tube.

Reaction Master Mix:

9.2 µL Yfiler™ PCR Reaction Mix
5.0 µL Yfiler™ Primer Set
0.8 µL AmpliTaq™ Gold Enzyme (4 Units)

2.4.5 Add 15 µL of PCR master mix to each labeled sterile amp tube or well of the 96 well plate using sterile ART tips. Cap each tube and ensure that the solution is at the bottom of the tube by gently tapping each tube on the counter top.

NOTE: Each AmpFℓSTR® Yfiler™ System amplification is performed in a final volume of 25 µL. Exactly 10 µL has been allocated for the sample DNA.

2.4.6 Prepare the sample DNA and controls as follows:

2.4.6.1 Open the tube containing the DNA extract or dilution of the DNA extract and carefully add 10 µL of sample DNA (0.5 to 1.0 ng/10 µL is, in general, the optimal target concentration, however the full 10 µL of undiluted DNA may be added when the target concentration cannot be reached) to the appropriate labeled amp tube or well in 96-well amplification plate.

2.4.6.2 Positive Amplification Control: Gently mix by hand the Control DNA followed by a light tapping of the tube on the counter top to remove any liquid from the cap. Carefully add 10 µL of the Control DNA (may be diluted with Type I water to a concentration of .5ng/10uL, if desired) to the appropriate labeled amp tube or well in the 96 well amplification plate.

NOTE: A stock dilution of Control DNA may be prepared using sterile Type I Water and stored in the refrigerator.

2.4.6.3 Negative Amplification Control: Carefully add 10 µL of the same sterile Type I water used to create any dilutions of associated DNA extract samples to the appropriate labeled amp tube or well in the 96 well amplification plate.

2.4.6.4 Reagent Blank(s): Carefully add 10 µL of the reagent blank to the appropriate labeled amp tube or well in the 96 well amplification plate.

2.4.7 Place the capped amp tubes or the sealed 96 well amplification plate into the thermal cycler. Push the tubes or plate down completely into the block. If a 96 well amplification plate is used, a compression pad (ABI P/N 4312639 or similar) must be placed over the sealed plate. Place the thermal cycler lid over the samples, and tighten.

2.4.8 When the thermal cycler prompts the user for the sample volume, choose 25 µL.

2.4.9 Choose the appropriate amplification file and start the program.

NOTE: The PCR amplification program will run for approximately 4 hours.

2.4.10 After the amplification process is complete remove the tubes or 96 well amplification plate from the thermal cycler.

2.4.11 The samples should be stored only in the post-amplification area of the laboratory and in the dark at 2 to 8 °C for a few days or at -20 °C for extended periods.

APPENDIX A - PROCEDURES FOR CONCENTRATING OR DRYING EXTRACTS AND RESOLUBILIZATION FOR Y-STR ANALYSIS

1 Equipment

- DNA concentrator/evaporator
- Microcentrifuge
- Pipettes - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL, 1000 µL

2 Materials

- Plastic zip closure bags or similar storage medium
- Sterile ART pipette tips - 2 µL, 10 µL, 20 µL, 100 µL and/or 200 µL, 1000 µL
- Microcentrifuge tubes and lids
  Kimwipes

3 Reagents

Type I water

4 Concentrating Extracts

4.1 Pulse spin the microcentrifuge tubes containing the extracts to force the liquid extract to the bottom of the tubes.

4.2 Insert a Microcon® 100 concentrator or Microcon® 50 concentrator into a labeled filtrate vial (microcentrifuge tube provided with the Microcon® assembly).

OPTION: 100 µL Type I water may be added to wet the filter, if desired.

4.3 Transfer the extract to the Microcon® concentrator and place the cap from the filtrate vial on the concentrator.

4.4 Spin the Microcon® assembly in a microcentrifuge for 10-40 minutes at approximately 5,000 rpm until the volume is reduced.

4.5 Carefully remove the concentrator unit from the Microcon® assembly and discard the fluid from the filtrate vial. Return the concentrator to the top of the filtrate vial.

4.6 Remove the cap from the concentrator and add a minimum of 10 µL Type I water.

4.7 Remove the concentrator from the filtrate vial and discard the vial. Carefully invert the concentrator and place into a new labeled retentate vial (same type of tube as the filtrate vial).

4.8 Spin the Microcon® assembly with the inverted concentrator in a microcentrifuge for 5 minutes at 5,000 rpm.

4.9 Discard the concentrator unit and place the cap on the retentate cup.

5 Drying Extracts

Evidence samples and known reference samples will be dried down at a separate time and/or in a separate place. If an evaporator is used, either evidence or known references may be placed in the evaporator at one time, but not both. Two evaporators may be used simultaneously – one for evidence and one for knowns, if desired.

5.1 Using an evaporator to dry the extracts

5.1.1 Pulse spin the microcentrifuge tubes containing the extracts to force the liquid extract to the bottom of the tubes.

5.1.2 Open the tubes.

5.1.3 Place the tubes into the evaporator and turn on at room temperature.

5.1.4 Once the extracts are dry, close the cap or place a new cap on each tube. If the extracts are dried for return to the agency, place the capped tubes into a plastic zip closure bag or similar storage medium.

5.2 Air drying the extracts

5.2.1 Pulse spin the microcentrifuge containing the extracts to force the liquid extract to the bottom of the tubes.

5.2.2 Open the tubes.

5.2.3 Place the tubes in an appropriate location such as a drying cabinet or hood to allow the samples to air dry. The rack containing the tubes may be covered with a Kimwipe, if desired.

5.2.4 Once the extracts are dry, close the cap or place a new cap on each tube. If the extracts are dried for return to the agency, place the capped tubes into a plastic zip closure bag or similar storage medium.

6 Resolubilizing Extracts

6.1 Add a volume of Type I water to the microcentrifuge tube(s) that is consistent with the sample volume prior to drying.

6.1.1 If the sample is being reconstituted for further testing and the optimal target amplification target will not be met by reconstituting in this amount, a smaller volume may be used as long as the associated reagent blank is treated in the same manner.

6.2 Place the tube(s) in a 56^0C incubator or heat block for a minimum of 2 hours, but no more than 3 hours.

6.3 If no associated reagent blank is available (only allowable for extracts created prior to July 1, 2009), a reconstitution blank must be created with the same amount of Type I water as used for the sample(s) and this amount must be consistent with the sample volume prior to drying.

APPENDIX B – REFERENCES

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