1. GLOBALFILER® EXPRESS AND YFILER® PLUS DIRECT AMPLIFICATION OF STANDARDS

A. Introduction

(1) STR (short tandem repeat) loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length. These abundant repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers which may be detected using the polymerase chain reaction (PCR).

(2) The Globalfiler® Express PCR Amplification Kit (Globalfiler Express or GFE) is a short tandem repeat (STR) 6-dye multiplex assay that amplifies 21 autosomal STR loci, amelogenin, 1 YSTR locus and 1 Y indel locus in a single PCR (total of 24 loci). GFE is a "megaplex" kit, designed to amplify the expanded loci required for NDIS participation. The expanded multiplex allows for an increased overlap with European kits and thus, allows for more loci to be searched in international databases.

(3) The Yfiler® Plus PCR Amplification Kit (Yfiler Plus or YFP) is a male specific PCR amplification kit that amplifies 27 loci in a single PCR reaction using 6-dye chemistry. YFP provides specific and sensitive detection of male haplotype DNA from the Y-chromosome even in the presence of an overwhelming amount of female DNA. The same amplification kit can be used for amplification of normalized, extracted DNA or for direct amplification of reference standards.

(4) Both GFE and YFP (direct amplification protocol) are optimized for direct amplification of known standards, eliminating the need for extraction and quantitation. The kits may be used on known reference standards on the following substrates: buccal swabs, buccal filters, buccal combs, bloodstain cards prepared from blood tubes, blood blots, and blood patches.

B. General Procedure

(1) Prior to processing, the following known reference samples will be portioned into a microcentrifuge tube: one half of one buccal swab, a 3x3mm sample from a buccal filter paper, one "tooth" from a buccal comb, a 3mm punch or 3x3mm sample from a bloodstain card, approximately 5x5mm cutting from a blood blot or, approximately 1x1cm cutting from a blood patch.

(a) NOTE: If analyses using these portion sizes and the approved lysate amounts/injection conditions fail to obtain an interpretable DNA profile (either too much or too little DNA), the portion size may be decreased or increased accordingly. Modifications to the portion size may only be attempted after the most or least sensitive conditions have been performed. For instance, if one half of one buccal swab amplified using 1µl of lysate and injected for 12 seconds results in overblown data, it is acceptable to use one quarter or one eighth of a swab. Or, if a complete profile was not obtained using a 3x3mm portion from a bloodstain card using 3µl of lysate and a 24-second injection, it is acceptable to try a 5x5mm or larger cutting.

(2) A batch is a set of samples lysed using the same preprocessing steps during one session.

(a) If none of the known reference samples are consumptive, only one reagent blank is required per batch of samples. The reagent blank should be placed at the end of its associated batch and it should remain at the end of its samples throughout all DNA processing steps.

(3) The samples may be processed in any PCR hood that is decontaminated with 10% bleach followed by a water or alcohol rinse prior to and after use.

(a) Use the reaction mix, primer set, and control DNA for the correct amplification kit. Do not mix components from different lot numbers of kits together.

(4) Amplification occurs in the designated amplified DNA work area after setting up the samples.

C. Receipt and Storage of Kits

(1) Upon initial receipt, the GFE kit components should be stored as follows:

(a) GFE Master Mix: -15 to -25°C upon receipt; 2 to 8°C after addition of Additive for up to 6 months or the expiration date on kit (whichever comes first).

(b) Master Mix Additive: -15 to -25°C upon receipt; discard tube after addition to the Master Mix tube.

(c) GFE Primer Set: -15 to -25°C upon receipt; 2 to 8°C after initial use for up to 6 months or the expiration date on kit (whichever comes first).

(d) DNA Control 007: -15 to -25°C upon receipt; 2 to 8°C after initial use up to the expiration date on kit.

(e) GFE Allelic Ladder: -15 to -25°C upon receipt; 2 to 8°C after initial use up to the expiration date on kit. NOTE: should be stored in post-PCR room.

(2) Upon initial receipt, the YFP components should be stored as follows:

(a) YFP Master Mix: -15 to -25°C upon receipt; 2 to 8°C after initial use up to the expiration date on kit.

(b) YFP Primer Set: -15 to -25°C upon receipt; 2 to 8°C after initial use up to the expiration date on kit.

(c) DNA Control 007: -15 to -25°C upon receipt; 2 to 8°C after initial use up to the expiration date on kit.

(d) YFP Allelic Ladder: -15 to -25°C upon receipt; 2 to 8°C after initial use up to the expiration date on kit. NOTE: should be stored in post-PCR room.

(3) The fluorescent dyes attached to the primers are light sensitive. Store the primer sets, allelic ladders, and amplified DNA protected from light.

D. Pre-processing

(1) Add 200µl of Prep-n-Go Buffer™ (PGB) to each MCT containing a portion of known standard and the MCT(s) containing the reagent blank(s).

(2) Vortex for approximately 5 seconds.

(3) Incubate at 70°C for a minimum of 20 minutes. Allow the samples to rest at room temperature for a minimum of 15 minutes to cool the lysate (for accurate pipetting) before proceeding to amplification.

(4) Vortex for approximately 5 seconds.

(5) If necessary, briefly centrifuge at full speed to remove liquid from cap.

(6) For general processing, the PGB can be added to the samples and then while they incubate, the amplification plates or tubes can be prepared. After the plates/tubes are prepared with the amplification reagents and PGB, vortex the samples and proceed with adding the necessary volume from lysates.

E. Amplification

(1) Upon first use of either kit, thaw the necessary kit components. Vortex for approximately 3 seconds and centrifuge briefly before opening tubes.

(a) NOTE: upon first use of GFE master mix, add 80µl of Master Mix Additive to the Master Mix tube (if using a kit with 1000 reactions, add 390µl). Gently invert the Master Mix tube 10 times and centrifuge briefly. The tube should be marked with a (+) to indicate the Additive has been added. Discard the Master Mix Additive.

(2) After first use, remove all necessary kit components from the refrigerator. Vortex for approximately 3 seconds and centrifuge briefly before opening tubes.

(3) For GFE, prepare a PCR master mix by adding the following volumes of reagents to an appropriately-sized MCT (the additional samples are to provide sufficient volume to account for potential reagent loss; more or fewer additional samples may be added depending upon the size of the batch):

(a) (Number of samples+2) x 6 µl GFE Master Mix

(b) (Number of samples+2) x 6 µl GFE Primer Set

(c) Vortex for 3 seconds, then centrifuge briefly.

(4) For YFP, prepare a PCR master mix by adding the following volumes of reagents to an appropriately-sized MCT (the additional samples are to provide sufficient volume to account for potential reagent loss; more or fewer additional samples may be added depending upon the size of the batch):

(a) (Number of samples + 2) x 10 µl YFP Master Mix

(b) (Number of samples + 2) x 5 µl YFP Primer Set

(c) (Number of samples + 2) x 10 µl TE Buffer

(d) Vortex for 3 seconds, then centrifuge briefly.

(5) Label 0.2 ml reaction tubes with the unique identifier from the Amplification Worksheet (example: initial, Julian date, tube number-K213/1) or use 0.2 ml strip tubes or a 96 well plate.

(6) Add 1µl of PGB to each tube or well that will contain a lysate from a known reference sample or reagent blank. NOTE: this volume may be adjusted if the amount of lysate is adjusted. The total lysate plus PGB volume must be 3µl.

(7) Add 3µl of PGB to each tube or well for a negative amplification control.

(8) Add the appropriate amount of PGB to the tube/well that will contain the positive amplification control.

(a) For GFE: add 2.25µl

(b) For YFP: add 2.0µl.

(9) Dispense the appropriate amount of amplification master mix into each tube or well.

(a) For GFE: add 12µl of master mix.

(b) For YFP: add 25µl of master mix.

(10) Transfer 2µl of each reference sample lysate and the reagent blank(s) into the appropriate wells. During the transfer, it is preferable to pipette the sample up and down a few times before removing the sample for the amplification tubes. NOTE: The lysate volume for all samples may be adjusted if necessary to develop a full profile that is on scale for ease of interpretation. Volumes of both 1µl and 3µl are also acceptable for use, and the volume of PGB (to total an input volume of 3µl) should be adjusted accordingly.

(11) Add the appropriate amount of DNA Control 007 to the appropriate well for the positive amplification control. NOTE: the volume of DNA Control 007 may be adjusted as necessary to ensure all peaks are present and on-scale. If adjusted, the volume of PGB must be adjusted accordingly to reach to a total input volume of 3µl.

(a) For GFE: add 0.75µl of DNA Control 007.

(b) For YFP: add 1.0µl of DNA Control 007.

(12) The final volume is 15 µl for GFE samples and 28 µl for YFP samples.

(13) Seal the tubes or plate as appropriate, using strip caps or adhesive sealing foil.

(14) Transport the samples to the post-amplification room.

(15) Centrifuge the tubes or plate for approximately 20 seconds (may be done prior to transport to post-amp room).

(16) Turn on the ProFlex™ PCR System. Place the tubes or plate into the sample block. Be sure to push the tubes down completely into the rack. If using a plate, it is recommended to use a compression pad to additionally prevent evaporation during thermal cycling. Close the lid and lock.

(17) Select the appropriate amplification cycle program.

(a) The GFE thermal cycling condition starts with an initial 95°C hold for 1 minute, followed by 94°C for 3 seconds and 60°C for 30 seconds, repeated 28 times. This is followed by a 60°C hold for 16 minutes. The samples are then brought to 4°C and may be held there for up to 24 hours.

- ProFlex™ – Select “Set up Run”. Select “Open Method”. Select “GFE”. Select “Verify Block”. Select “Start Run”. This will start the cycling. The ProFlex™ is set to a reaction volume of 25µL and runs using the 9700 simulation mode.

(b) The YFP thermal cycling condition starts with an initial 95°C hold for 1 minute, followed by 94°C for 4 seconds, and 61.5°C for 1 minute, repeated 28 times. This is followed by a 60°C hold for 22 minutes. The samples are then brought to 4°C and may be held there for up to 24 hours.

- ProFlex™ – Select “Set up Run”. Select “Open Method”. Select “YFP DIR”. Select “Verify Block”. Select “Start Run”. This will start the cycling. The ProFlex™ is set to a reaction volume of 28µL and runs using the 9600 simulation mode.

(18) After the amplification is complete, remove the tubes from the instrument block and store

the amplified products protected from light. The amplified products can be stored in a refrigerator for short periods of time, or in a freezer for long-term storage.

(19) Soak the rack used to transport the samples from the designated PCR amplification set-up area into the designated amplified DNA work area in a 10% bleach solution prior to removing from the designated amplified DNA work area.

(20) After typing is completed, discard the amplified DNA tubes by double bagging and disposing as hazardous waste.