36.1 Purpose:

To amplify DNA samples using the Globalfiler Express System (Thermo Fisher) using direct amplification.

36.2 Responsibility:

DNA Unit Personnel.

36.3 GlobalFiler Express Background:

36.3.1 The Thermo Fisher GlobalFiler Express System is a 24-locus STR multiplex PCR System. The six-color system allows for co-amplification and fluorescent detection of 21 STR loci: D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338; Amelogenin, 1 Y-STR loci: DYS391, and 1 insertion/deletion polymorphic marker on the Y chromosome: Y-indel.

36.3.2 These procedures generally follow those outlined in the "GlobalFiler Express PCR Amplification User Guide" for direct amplification with some changes in order to achieve one universal GlobalFiler Express amplification protocol for all substrate types. This protocol may be used for database and known sample processing only. Please refer to the manual for guidance and troubleshooting.

36.4 Preparation of samples:

36.4.1 <u>All FTA and non-FTA (blood and buccal) cards and blood on cloth samples</u>: See the GlobalFiler Express PCR Amplification Kit User Guide, Chapter 2, for general guidance and troubleshooting. See below for details.

36.4.2 <u>Punching a plate using the BSD Duet:</u>

NOTE: Detailed instructions can be found in Appendix I this SOP and DNA QR-326.

36.4.2.1 Turn on the BSD Duet.

36.4.2.2 Design the program to accommodate all the samples and controls.

36.4.2.3 Use the barcode reader when applicable.

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36.4.2.4 Punch all the samples and controls, ensuring samples were placed in the correct wells.

36.4.2.5 A 1.2mm punch is placed into a well of a 96-well PCR plate for each sample.

36.4.2.6 A 1.2 mm punch of a blank FTA card is used for the Reagent Blank (RB).

36.4.2.7 A 1.2 mm punch of an EP1 blood stained on an FTA card is used for the extraction positive control.

36.4.2.8 Turn off the BSD Duet when done.

36.4.2.9 When necessary, clean and perform maintenance on the BSD Duet as detailed in Appendix II of this SOP.

36.4.3 Manual Punching of FTA Cards:

36.4.3.1 FTA cards may be punched manually. This is generally only appropriate if the number of samples to be tested is small.

36.4.3.2 Ensure that the "DSS", "ID", or "DB" number on the laboratory barcode or on the foil envelope matches the sample number to be tested.

36.4.3.3 A 1.2mm punch is placed into 0.2 mL PCR tubes or 0.2 mL PCR strip tubes for each sample.

36.4.3.4 A 1.2 mm punch of a blank FTA card is used for the Reagent Blank (RB).

36.4.3.5 A 1.2 mm punch of an EP1 blood stained on an FTA card is used for the extraction positive control.

36.4.3.6 After punching each sample or control, clean the tip of the puncher by punching a disc out of clean filter paper or a clean FTA card.

36.4.3.7 After daily operation, follow the instructions below to clean the tip of the puncher:

a. Dip the tip into the 100% ethanol solution and swirl for 3-5 seconds.
b. Remove from the liquid and let air dry for 3-5 seconds.
c. Repeat steps a and b.
d. Tap the end of the tip on a clean kimwipe to remove any excess ethanol and wipe the tip with a clean/dry tissue.
e. Let the components dry thoroughly for at least 10 minutes before next use.

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36.4.4 All Swab Samples: See the GlobalFiler Express PCR Amplification Kit User Guide, Chapter 2, for general guidance and troubleshooting. See below for details.

36.4.4.1 To 1.5ml or 2.0ml tube, add swab sample. Sample size will vary according to type and quality of sample.

36.4.4.2 Add 400µl of Prep-N-Go Buffer™ to the tube. Be sure to cover the swab with liquid.

36.4.4.3 Add 400µl of Prep-N-Go Buffer™ to a blank tube to be used as a reagent blank for the swab samples, i.e. "RB Swab".

36.4.4.4 Incubate tube at 90°C for approximately 20 minutes. A thermomixer (preferred method) or oven may be used.

36.4.4.5 After the 90°C incubation, remove the tube from heat and allow to stand at room temperature for approximately 15 minutes.

36.4.4.6 Spin down tube and remove swab from the tube. Try to squeeze excess liquid from swab into tube. Discard the swab.

36.4.4.7 Extract may be stored at 4°C for up to 2 weeks. Long term storage may be below 4°C.

Note: For BODE Collector samples, the GlobalFiler Express procedure cannot be used. Refer to the procedure outlined in DNA SOP-19 Processing of Single Source Samples on EZ1 Advanced XL for these sample types.

36.5 Direct Amplification:

Notes: Although the GlobalFiler Express PCR Amplification Kit User Guide directs the user to treat punch samples and swab samples differently, this procedure will treat all samples similar to the FTA sample amplification.

If this is the first time opening the kit, add 80 µl of Master Mix Additive to the Master Mix tube and mix. Mark the cap of the Master Mix with a "+" to indicate that the Master Mix Additive has been added.

36.5.1 The master mix is prepared per manufacturer's instructions (GlobalFiler Express PCR Amplification Kit User Guide): Make enough master mix for the number of reactions needed plus additional reactions to compensate for pipetting volume variations. Use Worksheet DNA QR-29.

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Master Mix	6µl/reaction
Primer Mix	6µl/reaction
TE Buffer	3µl/reaction

Total Master Mix Volume (MM) 15 µl/reaction

36.5.2 A 15µl aliquot of the master mix (MM) is dispensed into 0.2mL amplification tubes, strip tubes, or into the wells of a 96-well plate, containing the FTA, non-FTA, and blood on cloth punch samples. Additionally, an aliquot is dispensed into the corresponding well or tube for positive and negative controls, as well as swab samples.

36.5.3 For each swab sample and the corresponding reagent blank for swabs, add 3µl of sample to the corresponding well or tube.

36.5.4 The RB and NEG control volume must be the maximum volume used for swab samples in that set. If swab samples are in the set, add an additional 3µl of TE Buffer to the NEG well/tube to achieve this increased volume. If the set does not contain any swabs, no additional volume of TE Buffer needs to be added to the NEG well. Note: Quality and quantity of sample may be taken into consideration when determining the volume of input DNA used for amplification.

36.5.5 Add 3µl (6ng) of undiluted POS control (007) to its corresponding well or tube. Cover plate with a plate seal.

36.5.6 The samples are amplified in a Veriti™ 96 well thermal cycler using the 100% ramp speed or a GeneAmp PCR System 9700 thermal cycler using the Max Mode as the ramp speed. The amplification parameters are 95°C for 1 min, followed by 25 cycles of (94°C for 3 sec, 60°C for 30 seconds), then 60°C for 8 min, and finally 4°C until the samples are removed from the thermal cycler. Note: When the Select Method Option screen appears on the 9700 thermal cyclers, select “Max” for the ramp speed and enter the reaction volume (15µl).

36.5.7 Alternatively, the samples (tubes only) are amplified in a BioRad C-1000 thermal cycler using the ramp speed of 2.2°C/sec. The amplification parameters are 95°C for 1 min, followed by 25 cycles of (94°C for 3 sec, 60°C for 30 seconds), then 60°C for 8 min, and finally 4°C until the samples are removed from the thermal cycler.

36.5.8 For detection of the amplification products, see DNA SOP-38 GlobalFiler, GlobalFiler Express, and Yfiler Plus Detection.

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APPENDIX I: Punching samples on the BSD Duet

Turning on the BSD Duet

1. In any order, start the computer and log on, turn on the BSD Duet using the switch on the right side, and turn on the air pump by plugging it in.

2. Double click the BSD 600 Menu icon to open BSD Duet software. Log on.

3. Click on Configure System and the Files tab. Select Input File Name by browsing to the file that was previously saved to a removable disk or shared drive. Set Sample Number Mode field to File Input.

4. Go to the Barcode tab. Check the box next to Samples (unless you are not using barcodes); the rest of the boxes should be unchecked. Click Save and Exit. If you are punching a full database plate and working from the existing full plate test, proceed to the section of this appendix titled *Punching a Full Plate*. If you are punching a partial database plate or known plate, proceed to the section of this appendix titled *Creating a Test for a Partial Plate*.

Creating a New Test

1. Before beginning, it is helpful to make a plate map on a worksheet if you have not yet done so.

2. Click the Edit Test Sequences box in the BSD Main Menu to open the Test Editor program.

3. Choose Create a new test.

4. Choose Microtitre.try as the type of tray.

5. From the Test pull down menu, select Test Configuration. Click the Automatic filling tab and change the Fill Direction to vertical. Click the Spot per cell tab and choose the number (up to 6) and size (1.2mm) of spots to be placed in cells. Click OK.

6. Choose a cell type for each cell of the tray. Double-click on a single cell to do this, or make a box around a group of cells and right-click to change them all to the same type.

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a. Samples (including QCs) = Sample
b. RB and EP1 = Control
c. Ladder, Neg, and Pos = Liquid Control, or Unused Cell
d. Blank = Unused Cell

7. Control Usage for Single Source Samples:

   a. For Database known samples, 1 RB, 1 Neg, and 1 Pos will run on a plate. 3 EP1s will run on a full plate, and at least 1 EP1 will run on a partial plate.

   b. For casework known samples, 1RB, 1 Neg, 1 Pos and at least 1 EP1 will run on a plate. Additional EP1 samples can be added for a plate containing a large number of known samples upon analyst discretion.

8. Change the Filling Sequence Numbers to differentiate between Controls (i.e. RB vs. EP1) or Liquid Controls. (By default, automatic filling is enabled for Samples only. Automatic filling can be disabled by un-checking the box in the Automatic Filling tab of the Test Configuration box).

9. Save your test in the BSD600 folder on the local disk C: drive by clicking the icon or using the pull-down File menu. Give your test a name when asked. There are no naming restrictions. Delete this test after punching, unless you plan to re-use it often.

10. Close the Test Editor program using the Exit pull-down or clicking the "x" box in the upper right corner. Proceed to section: *Punching a Plate*.

Creating a Test for a Partial Plate

Open the Edit Test Sequences program. Open test DB Plate. Mark unused sample and control wells as unused by double-clicking single cells or selecting a range and right-clicking. Go to the File menu, select Save As, rename the test, and save. Delete this test after punching, unless you plan to re-use it often.

Punching a Plate

1. Open the Distribute Spots program and follow the prompts on the program. For a full plate, choose "DB plate" as the test to punch. For a partial plate, choose the program created in *Creating a Partial Plate*, step 1 (see above). Make sure only one test is checked off. Check the boxes for Samples, Controls, and Cleaning. Remember when

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loading Slip-Prep plate to remove the white collar from the plate. Make sure well A01 is in the upper-left corner.

2. Before punching, intentionally scan an incorrect barcode to ensure reader is functioning properly. Document this on QR-326. Punch the plate, scanning LIMS barcodes for each sample before punching. If a barcode is incorrect, the program will pause and notify you.

3. To punch a card, slide the FTA card under the metal clamps and align the red laser dot with the spot to punch. The area punched should be stained, but isn't always. Press the pad to punch or use the automatic trigger. Between samples, use a blank card to do a cleaning punch.

4. The sensor only detects the spot in the chute, not whether it actually made it into the well. For this reason click Inspect Trays and check wells as necessary. It is easier to re-punch samples before the test is finished than at the end. If spots are not falling into the center of the well or there is too much static, correct the problem by increasing the air flow in the air pump and/or add more water to the bottle attached to the Duet and air pump.

5. After punching all samples and the input file is complete, click Continue Punching to punch controls. If multiple punches of the same control are being made, multiple laser spots will appear. The Duet will punch and distribute all spots from this card at once. If you want to punch spots individually, click Shrink Pattern.

6. When controls are punched, check to see that all spots are present, and click All Spots Present and End Run.

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Appendix II: Maintaining the BSD Duet

Cleaning the BSD Duet

1. The chute should be cleaned at least once every 400 punches, or 2 full plates. The cleaning should be logged on DNA QR-12. Be careful not to inhale dust from the Duet when cleaning.

2. Unplug the air pump and turn the Duet off.

3. Open the cover and lift the card platforms. Use the black handle to rotate the punching apparatus 90 degrees to the left.

4. Unscrew the inner chute (top part of the punching apparatus) and remove. Leave the outer chute attached by the wire.

5. Clean only the inner chute using 100% ethanol and the Duet cleaning tool. Remove debris from both chutes using a can of compressed air. Reattach the inner chute to the outer chute and to the BSD Duet.

6. Move the punching apparatus back into the upright position. Direct a strong flow of compressed air under the punch guide to remove debris collected on the clamps and card holding area.

7. Check for any stray spots inside the BSD Duet and remove. If necessary, clean any dusty areas.

8. Return the card platforms and cover to their original positions. The BSD Duet and air pump can be left off or on when not in use.

BSD Duet Punchbot Performance Checking

1. Design a program (refer to section: *Creating a New Test*) which will use the barcode reader. Punch the following size: 1.2mm punch, and punch the following types of punches: samples, controls, standards, and cleaning.

2. Run the program.

3. Confirm the following: The barcode reader functions properly, the 1.2mm puncher functions properly, and that all the punches were placed in the proper locations.

4. Fill out DNA QR-265 BSD Duet Performance Check.

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