

STR Amplification of DNA DB Samples

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STR Amplification of DNA DB Samples

1 INTRODUCTION

These procedures are for performing direct amplification of nuclear DNA with the polymerase chain reaction (PCR) using the GlobalFiler[®] Express (GFE) PCR Amplification Kit on DNA databasing samples received by the Federal DNA Database Unit (FDDU).

2 SCOPE

These procedures apply to DNA personnel who perform direct amplification using the GFE Kit on DNA databasing samples.

3 EQUIPMENT

3.1 Equipment/Materials

- General Laboratory Supplies
- STACS[™] Database (STACS-DB) Software (Sample Tracking and Control Solutions [STACS DNA Inc.] part of InVita Healthcare Technologies), version 6.9 or above
- Barcode printer with appropriately sized labels (2.0" x 0.5" or equivalent)
- Barcode Scanner, Hand-held (Symbol LS4000i, 4008i, LS4071 or equivalent)
- Plate Sealer, microplate (Agilent PlateLoc or equivalent) with Heat Seal
- Optical Adhesive Covers (Applied BioSystems or equivalent)
- Tecan Robotic Workstation (Amplification Setup)
 - Tecan EVOware Software, version 2.8 or higher (Tecan)
- Fluent Workstation (Tecan LabWerx Customized Workstation)
 - FluentControl Software, version 2.3 or higher (Tecan)
- Optical Cover Compression Pads (Applied BioSystems or equivalent)
- Thermal Cycler (Applied BioSystems GeneAmp[®] PCR System 9700 or ProFlex[™] PCR System)
- Plate centrifuge (Eppendorf Model 5804 or equivalent)
- Laboratory incubator (Isotemp or equivalent)

3.2 Reagents

- Liquinox[™] Critical Cleaning Liquid Detergent (Alconox or equivalent)
- Water, reagent grade (VWR or equivalent)
- Bleach, 3% (molecular grade or equivalent)
- GlobalFiler[®] Express PCR Amplification Kit (Applied BioSystems)
 - Contains GlobalFiler[®] Express Master Mix, Master Mix Additive, GlobalFiler[®] Express Primer Set, GlobalFiler[®] Express Allelic Ladder, and DNA Control 007

4 STANDARDS AND CONTROLS

Two Combo controls (aka negative) and two Blood/Buccal Internal Standard (BIS) controls are included on each amplification plate.

The BIS is prepared as described in the DNA procedure for reagents (i.e., BIO-103) and is added to the plate during the sample punch procedure. These controls will be interpreted according to the criteria in the applicable FDDU interpretation procedure (i.e., BIO-315).

5 PROCEDURE

Refer to the DNA Procedure Introduction (i.e., BIO-100) for applicable laboratory quality assurance and cleaning instructions.

When using a Robotic Workstation, ensure general instrument cleaning and maintenance is done prior to use, as needed. See Appendix A for additional guidance.

5.1 SwabSolution Incubation

For plates prepared using SwabSolution, perform the following steps any time prior to loading the plate onto the Tecan instrument or before manually adding master mix to the plate.

- A. Ensure that the plate is adequately sealed after punching, then quick-spin the 96-Well plate(s) in a centrifuge (approximately 30 seconds).
- B. Incubate the plate(s) at 70°C for 10 minutes.
- C. After incubation, quick-spin the 96-Well plate(s) again in a centrifuge (approximately 30 seconds) before proceeding to Master Mix addition.

5.2 Amplification Setup

The Amplification Setup procedure may be performed manually or automated.

- A. Prior to the first use of a GFE kit, prepare the GFE Master Mix tube by adding Master Mix Additive to each GFE Master Mix tube.
 - o For a 200 reaction kit tube: Add 80µL of Additive
 - o For a 1000 reaction kit tube: Add 390µL of Additive

NOTE: Amplification WILL FAIL without the Master Mix Additive in the GFE Master Mix.

- B. Prepare the amplification master mix for the required number of samples. Include extra samples in the calculation for overage. Ensure the preparations of the amplification master mix have been recorded in the *Chemical Preparation* module of STACS-DB.

GlobalFiler Express MM	
	Per Sample (µl)
GFE Primer Set	6
GFE Master Mix w/Additive	6

- C. Within STACS-DB, select plate(s) to be processed and select the appropriate scenario.
1. Additionally, for automated processing only:
 - Scan the instrument barcode on the Robotic Workstation.
 - Ensure the Robot Maintenance Checks have been performed.
 - Indicate whether each check passed.
- D. If not previously performed during the selection of the plate(s), scan the barcode on each of the 96-Well plate(s). Scan the barcode of each reagent required for the selected scenario. Select "Process" and proceed with the Amplification Setup procedure.
1. Additionally, for automated processing only:
 - STACS-DB launches the robotic software
 - If necessary, enter the appropriate user name and password at the robotic software log-in screen
 - Verify that the appropriate script has been opened.
 - Ensure the required reagents and the selected 96-Well plate(s) have been loaded on to the instrument.
 - On the Tecan EVO, the plate(s) is loaded onto the instrument deck.
 - If utilizing a robotic carrier, ensure plate(s) is loaded into the carrier with A1 in the back left corner
 - Ensure the instrument has been properly flushed and no air bubbles are visible in the tubing or syringes
 - Initiate the robotic software process.
 - Indicate the number of plates to be processed.
 - Start the script.

The following Amplification Setup procedure will be performed manually or by the Robotic Workstation:

- A. Add 12 μ L of GlobalFiler Express Amplification master mix to each allocated well.
 - GFE Ladder wells do not receive master mix.
- B. The Robotic Workstation can heat-seal the 96-Well plate(s) with a foil cover and centrifuge each plate(s) at 1000 RPM for approximately 1 minute. Alternatively, the plate(s) may be manually sealed with a foil cover or an optical cover and centrifuged.
- C. Visually inspect the plate(s) and indicate the result in STACS-DB as successful, failed or aborted. Comments and observations must be entered for plates with process failed results. If the plate(s) were processed on a Robotic Workstation, indicate in STACS-DB whether the bleach process was performed.

5.3 GlobalFiler® Express PCR Amplification

- A. If necessary, quick-spin the sealed 96-Well plate(s) in a centrifuge.
- B. Load each sealed and centrifuged plate into a thermal cycler and place an ABI Optical Compression Pad on each plate and close the thermal cycler(s) lid(s).
- C. Select the appropriate method on the thermal cycler.

GlobalFiler Express PCR Amplification Method (on 9700 or ProFlex)			
	Temperature (C)	Time	Sample Type and Cycles
Hold	95	1 minute	
Cycle	94	3 seconds	Buccal or Blood (26 or 28 cycles)
	60	30 seconds	
Hold	60	8 minutes	
Hold	4	Forever (∞)	

- D. Start the thermal cycler. Ensure the reaction volume is 15 μ L and the ramp speed is MAX on a 9700 or GeneAmp™PCR System 9700 simulation mode on a ProFlex.
- E. Ensure the *Thermal Cycler Bar Code* and the *Plate Bar Code* for each plate to be amplified has been scanned into STACS-DB.
- F. Indicate the result of the process in STACS-DB as successful, failed or aborted. Comments and observations must be entered for plates with process failed results.
- G. After amplification, store plate(s) refrigerated ($4^{\circ}\text{C} \pm 3^{\circ}\text{C}$) in the post-amplification laboratory .

NOTE: The MicroAmp support base used to transport the 96-Well plate from the pre-amplification laboratory space to the post-amplification laboratory space must be cleaned with a 10% bleach solution and/or with 70% isopropyl alcohol prior to or immediately upon return to pre-amplification laboratory space. This practice will minimize the transport of amplified DNA product from post-amplification laboratory space to pre-amplification space.

6 LIMITATIONS

The appropriate processing methods are selected for a plate based on the sample type added to the plate and the amplification kit to be used. Based on internal studies, only the combinations of processes listed below are approved for use.

Sample Type	Amp Kit	Amp Setup	Punch Size	Thermal Cycler	Cycles
Buccal (FTA or Non-FTA) or Blood (FTA)	GlobalFiler Express	Manual or Automated using a Tecan EVO Robotic Workstation	1.2mm	9700 or ProFlex	26 or 28

7 SAFETY

All FDDU samples that contain blood are considered potentially infectious regardless of the perceived status of the source individual or the age of the material. All FDDU personnel who work with such material will refer to the [FBI Laboratory Safety Manual](#) for personal safety information and proper disposal of the chemical and biohazardous waste.

8 REFERENCES

The procedures described here are derived from a variety of sources. Portions of the protocol come directly from some of the references cited below.

Federal Bureau of Investigation. Quality Assurance Standards for DNA Databasing Laboratories, current version.

Sample Tracking and Control Solutions [STACS DNA Inc.] part of InVita Healthcare Technologies. *STACS™ Database (STACS-DB) User's Guide*.

Applied Biosystems. *GeneAmp® PCR System 9700 User's Manual Set*. Applied Biosystems, Foster City, CA. 1997.

Applied Biosystems. *Proflex™ PCR System User Guide*. Life Technologies Corporation | Carlsbad, CA. 2016.

Applied Biosystems. *GlobalFiler® Express PCR Amplification Kit User's Manual*. Applied Biosystems, Foster City, CA.

Applied Biosystems. *GlobalFiler® Express PCR Amplification Kit User Guide*. Applied Biosystems, Foster City, CA.

9 REVISION HISTORY

Revision	Issued	Changes
00	02/04/2022	Reformatted DNA 305-11 into new template and assigned new Doc ID. Revisions to remove Identifiler Direct.

10 APPENDIX A: INSTRUMENT MAINTENANCE

Refer to the DNA procedure for equipment calibration and maintenance (i.e., BIO-104) for additional information and applicable frequency requirements.

10.1 Robotic Workstations

10.1.1 Tecan Instrument Cleaning and General Maintenance Guidance

- **Seal Integrity Check** - Verify that the tubing and syringes (plunger lock screws) are tight and no air bubbles are being introduced into the system.
- **Tip Check** - Verify that the LIHA tips (as appropriate) are tight, free of clogs and not dripping system liquid (RO purified water).
- **Robotic Arms Check** - Verify that the Tecan robotic arms (as appropriate) are moving and working properly.
- **System liquid level check** - Check and refill the system liquid (i.e., water), if necessary. When refilled, the system liquid should be allowed to de-gas overnight.
- **Clean tips with alcohol** - Clean the outside of the fixed Tecan tips with 70% isopropyl alcohol each workday before use and if they become visually soiled.
- **Bleach deck** - Decontaminate the Tecan workdeck with 10% Bleach each workday before use and if it becomes visually soiled. The barcode scanners should be cleaned with a lint-free cloth only, as needed.
- **System liquid flush** - Flush the instrument thoroughly until no air bubbles are visible in the tubing or syringes.
- **RoboScrub** - Weekly, as used, The Tecan Robotic workstations that utilize the Liquid Handling Arm (LIHA) should be flushed with a mild detergent (e.g., Liquinox) to clean the inside of the Tecan tips, tubing and syringes to maintain the precision and accuracy of liquid handling by the Tecan Robotic Workstation. The RoboScrub solution should remain in the Tecan for a minimum of 15 minutes. Following the elapsed time, flush the Tecan with water until there are no visible air bubbles in the tubing or syringes
- **Bleach process** - Following each run on the Tecan EVO workstations, decontaminate the inside and outside of the Tecan tips with 3% Bleach and flush with water. There is a Bleach Wash script that will perform this process.

10.1.2 Performance Verification (aka Artel)

An Artel MVS (Multichannel Verification System) and NIST traceable standards are used to test the accuracy and precision of the liquid handling by a Tecan Robotic Workstation. Refer to the Artel MVS Multichannel Verification System User Guide for operation of the Artel MVS.

For the LIHA (typically configured with eight (8) fixed tips), perform a minimum of 6 repetitions with each tip for each volume.

The accuracy and precision results must be within the tolerance limits set by the FDDU for each volume.

- At times, it may be necessary to modify/optimize the Tecan liquid class parameters (e.g., offset and factor) to ensure that the accuracy and precision of the Tecan continually meets the FDDU tolerance limits.
- The tolerance limits, Artel data, and any modifications to Tecan liquid classes must be maintained in a notebook or an electronically accessible file/folder for reference.

10.2 Thermal Cycler General Maintenance and Performance Verification

10.2.1 9700

Refer to the *GeneAmp*® PCR System 9700 User's Manual Set 96-Well Sample Block Module User's Manual for instructions on how to perform the following procedures.

- A. **Cleaning** - Refer to *Cleaning the sample wells* and *Cleaning the sample block cover* sections.
- B. **Temperature Verification Test** - This test procedure requires the use of a Temperature Verification System (Applied Biosystems) and verifies that the thermal cycler remains within the temperature accuracy specification. Refer to *Running the Calibration Verification Test* section.
- C. **Temperature Non-uniformity Test** - This test procedure requires the use of a Temperature Verification System and verifies the temperature uniformity of the sample wells in the thermal cycler. Refer to *Running the Temperature Non-uniformity Test* section.
- D. **Rate Test and Cycle Test** - These procedures verify the integrity of the cooling and heating system of a thermal cycler. Refer to *Running System Performance Diagnostics* section.

10.2.2 ProFlex™

Refer to the ProFlex™ PCR System User Guide for instructions on how to perform the following procedures.

- A. **Cleaning** – Refer to the chapter for maintaining the instrument for instructions on how to clean the sample wells and heated cover.
- B. **Verify Block Temperature** – These tests are found in the *Block Verification Test* screen and requires the use of a Temperature Verification System for the following test types:
- C. **Heated Cover Test** - This test verifies the proper functioning of the heated cover.

- D. **Temperature Verification Test** - This procedure verifies that the thermal cycler remains within the temperature accuracy specification.
- E. **Temperature Non-Uniformity Test** - This procedure verifies the temperature uniformity of the sample wells in the thermal cycler.

11 APPENDIX B: QC OF CRITICAL REAGENTS

11.1 GlobalFiler Express Kit

- A. Each new lot of GlobalFiler Express Kits will be evaluated by testing it jointly with the current lot using the following samples per lot, at minimum:
 - 5 - punches (1.2mm) of a BIS (buccal) control
 - 1 - Combo control
 - 4 - 007 controls with a template quantity of 5 ng
- B. Amplify the plate at 26 cycles using the GlobalFiler Express protocol. Inject the plate, with at least 2 allelic ladders per lot, 3x at the current CE settings.
- C. Acceptance Criteria:

The allelic ladders from both the new lot and current lot of kits must be used separately to analyze the QC data with GeneMapper ID-X. Data will be analyzed using normalization. Criteria for acceptance of kits for use with FDDU samples are:

1. BIS and 007 samples:
 - i. Acceptable peak morphology and balance at each locus.
 - ii. Average peak heights of all alleles are greater than 175 RFU.
 - iii. Correct typing results obtained.
 - iv. No allelic peaks, other than those attributable to the BIS and 007 controls, are detected.
2. Each ladder allele is greater than 175 RFU.
3. Acceptable results for the negative controls.

If all the above criteria are met then the new lot is approved for use and the allelic ladders included in both the new lot and current lot of kits may be used for the analysis of samples both within and between lots of GlobalFiler Express kits. Enter the results of the QC procedures in STACS-DB using the *Receiving* modules.