Development Areas

- Automate the STR typing process

    - Implement robotic liquid handlers in the performance of STR typing

    - Replace the slot blot hybridization procedure with a real-time PCR based assay for quantification of human DNA

- Develop a software tool for the analysis and interpretation of forensic STR data

Automation Project Aims

Provide an integrated package to:

1. Extract / purify DNA from reference samples:

   a. Blood on FTA (without releasing from paper)

   b. Saliva on FTA (without releasing from paper)

   c. Buccal swabs

   d. Blood on non-FTA paper

2. Set-up real-time PCR quantification reactions

3. Dilute DNA extracts to working concentrations

4. Set-up PCRs to amplify the 13 CODIS STR loci

5. Prepare PCR products for analysis by capillary electrophoresis

Implementation of Robotic Liquid Handlers

- Two robotic workstations were planned:

    - Pre-amplification robot

    - Post-amplification robot

- The robotic workstations will reside in separate rooms.

- TECAN robots with fixed tips were selected.

The project was outsourced to

Pre-Amplification Robot:
TECAN Freedom EVO 200

Automated Procedure	Human Intervention
Extract / purify DNA	
Set up real time-PCR quantification assays (if needed)	
Store extract plates in carousel	
	Human moves plate to ABI 7000 (real time PCR instrument)
	Human transfers quantification data to pre-amp robot
Remove plate with extracts or FTA punches from storage carousel	
Normalize DNA extracts by transfer and dilution (if needed)	
Set up amplification plates	
	Human moves plates to thermal cycler for STR amplification

Pre-Amplification Robot:
TECAN Freedom EVO 200
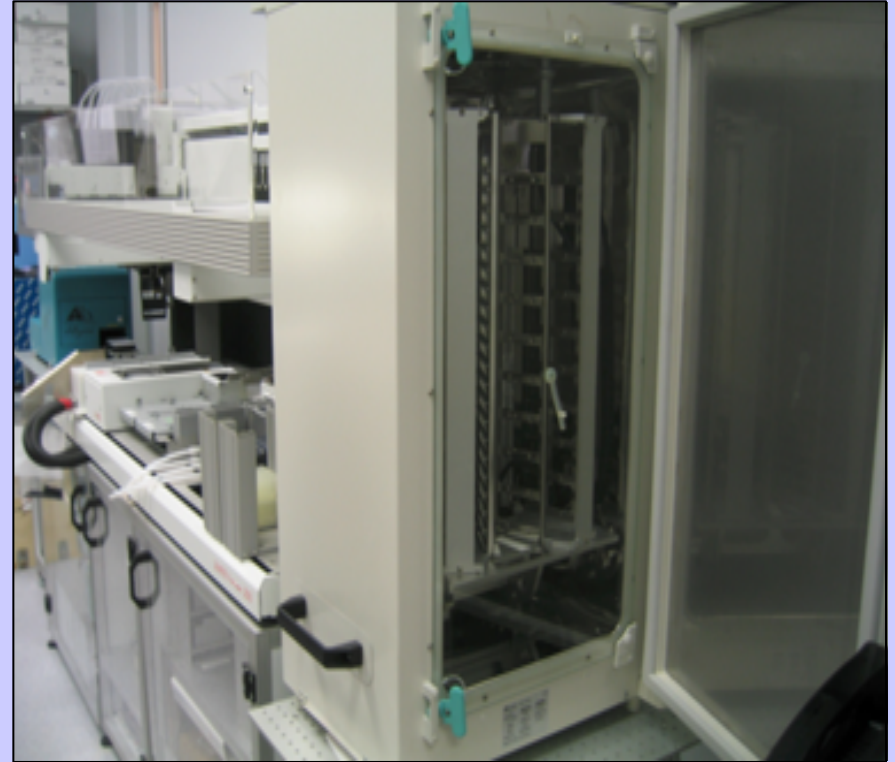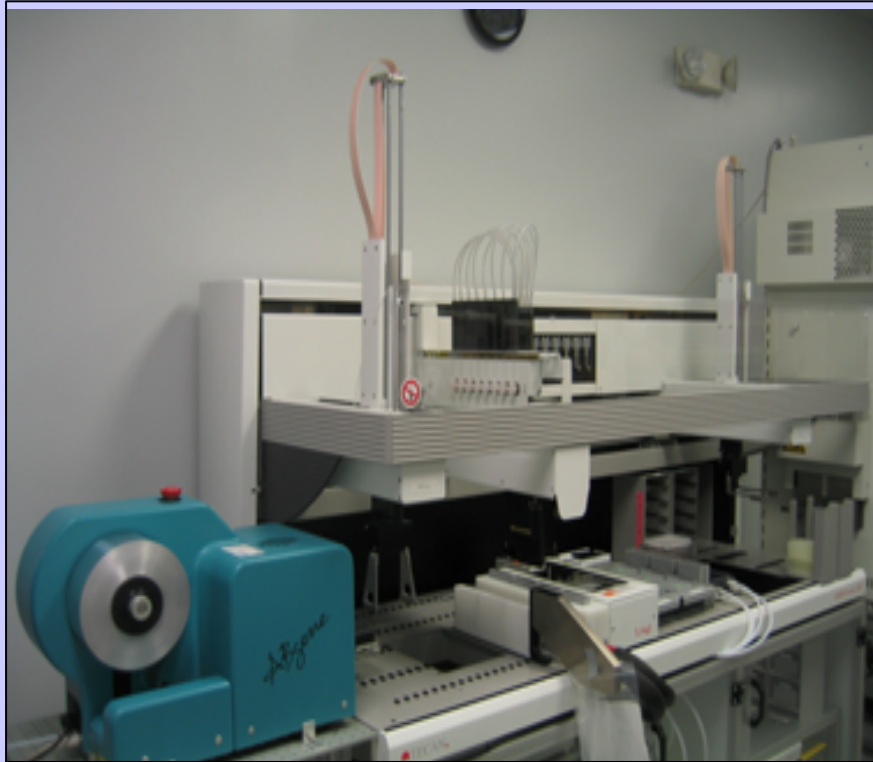
TECAN Genesis Logistic Workstation 200

Post-Amplification Robot:
TECAN Freedom EVO 150

Automated Procedure	Human Intervention
	Human moves plates to post-amp robot
Prepare samples for capillary electrophoresis	
	Human moves plate to 3100 CE instrument

Automated DNA Extraction

Three purification methods were evaluated for blood and saliva stains on FTA paper:

- Modified Whatman procedure (2x final drying time)

- Modified Whatman procedure (↓ buffer volumes)

- Modified Whatman procedure (↑ lysis time) - selected

Three commercially available kits amenable to high throughput extraction were evaluated for blood on non-FTA paper and buccal swabs:

- QIAamp^R 96 DNA blood kit (QIAGEN)

- DNA IQ^{TM} System (Promega Corp.) - selected

- Nucleospin^R 96 Blood Kit (Macherey-Nagel)

Selected processes were validated on the pre-amplification robot for each sample type

Automation of DNA Quantitation

- Developed a human-specific real-time PCR quantification assay:
  - Alu-based assay / TaqMan^R chemistry

- Evaluated three existing human-specific real-time PCR quantification assays:
  - Two non-commercial:
    - Alu-based / SYBR^R Green (Nicklas and Buel, JFS 2003)
    - THO1-based / TaqMan^R chemistry (Richard et al. JFS 2003)
  - One commercially available:
    - Quantifiler^{TM} Human DNA Quantification Kit (ABI) – selected

- Validated the robotic set-up of the Quantifiler^{TM} Human DNA Quantification Kit

Automated DNA Normalization and Set-Up for Amplification

- The pre-amplification robot was programmed to:

  - transfer and dilute DNA samples to a uniform working concentration based on output from the real-time PCR assay.

  - set-up amplification reactions with the following STR kits:
    - AmpFISTR^R Cofiler^{TM}
    - AmpFISTR^R Profiler Plus^{TM} ID
    - AmpFISTR^R Identifiler^{TM}
    - GenePrint^R PowerPlex^{TM} 16

- The two process were validated on the robot.

Automated Set-Up for Capillary Electrophoresis

- The post-amplification robot was programmed to set-up trays for CE:

  - Prepare loading solution mix
  - Heat denature
  - Centrifuge materials to bottom of wells
  - Store at 4°C until instrument loading.

- The process was validated on the robot.

Process Integration on the Pre-Amplification Robot

- Individual validated processes on the pre-amplification robot were integrated through software development

  FTA purification and AmpFISTR^R Profiler Plus^{TM} ID set-up
  FTA purification and AmpFISTR^R COfiler^{TM} set-up
  FTA purification and AmpFISTR^R Identifiler^{TM} set-up
  FTA purification and GenePrint^R PowerPlex^{TM} 16 set-up

  DNAIQ^{TM} extraction and Quantifiler^{TM} set-up

  Extract normalization and AmpFISTR^R Profiler Plus^{TM} ID set-up
  Extract normalization and AmpFISTR^R COfiler^{TM} set-up
  Extract normalization and AmpFISTR^R Identifiler^{TM} set-up
  Extract normalization and GenePrint^R PowerPlex^{TM} 16 set-up

- The integrated processes were validated

Project Status

- Robots have been delivered to the FBI

- Undergoing testing to confirm successful execution of the integrated STR typing process

Expert System for Forensic STR Applications

Goal: Develop a software tool for the analysis and
interpretation of single source <u>and</u> mixed samples

- Start with raw fluorescent STR data files
- Perform "Genescan" functions
- Perform "Genotyper" functions
- Apply artifact detection rules
- Perform quality control checks
- Accept, reject, or flag data for review
- Mimic the decision making review process of a DNA Examiner

The project was outsourced to   THE UNIVERSITY *of* TENNESSEE

STRESP Development to Date

- Applies to single source samples

- Accepts data files from the ABI PRISM^R 310 or 3100 Genetic Analyzer

- Accepts data generated with the following STR amplification kits:

  - AmpFISTR^R Cofiler^R

  - AmpFISTR^R Profiler Plus^{TM} ID

  - AmpFISTR^R Identifiler^{TM}

- User authentication is required to invoke the program

- All usage activities are kept in an audit log

STRESP Development to Date (Continued)

- Applies "rules" for artifact detection (e.g. –A, stutter, etc.)

- Performs quality control checks (e.g. confirms size standard peaks properly defined)

- Analysis parameters are adjustable, but all modifications are logged to audit log

- Analysis results can be edited, but all edits are logged into the audit log

- Results in human-readable reports and in a format suitable for uploading into CODIS (CMF)

- Stand alone

Future STRESP Developments

- **Address mixed source samples**

- **Results in a format suitable for input into Popstats**

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