

Automated DNA Extraction and Amplification for Portable Forensic Analysis

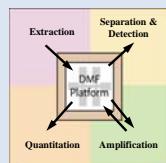
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Introduction

While genotyping or DNA fingerprinting has become a mainstay of criminal forensics, new DoD and DHS applications are motivating an increased focus on speed, portability, and ruggedization for operation outside traditional laboratory facilities.

- Kinship determination at border crossings & ports of entry
- Combatant identification at detention facilities and in the field
- Associating suspects with items or locations in criminal/terror cases

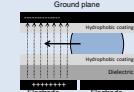


To address these emerging applications, Sandia has developed the Battlefield Automated DNA Analysis & Sampling System, a compact Short Tandem Repeat (STR) genotyping platform featuring

- Modular architecture with central digital microfluidic (DMF) hub for sample routing
- Individually optimized DNA extraction, quantitation, amplification, and separation modules
- Fully automated operation for push-button, sample-in-answer-out functionality



Transparent indium-tin oxide electrodes allow microliter droplets to move along stepping-stone-like electrode paths.

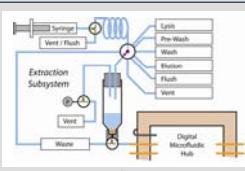


Cross section view illustrating DMF droplet actuation. Droplets move stepwise from one electrode to the next when voltage is applied to the destination electrode.

Materials & Methods



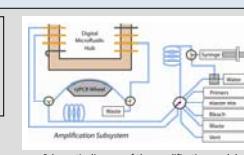
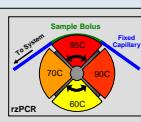
Overview of the extraction module prototype hardware.



Introducing a sample buccal swab into the system.

Extraction Module:

- Adapts off-the-shelf Zymo spin extraction assay using headspace pressure instead of centrifugation
- Buccal swab samples introduced into single-use, replaceable Zymo tube
- Buffers delivered to Zymo tube via multiport valve from fluid reservoirs to automate lysis, pre-wash, wash, and elution steps
- A series of ~20 samples tested comparing the extraction module vs. benchtop protocol
- Initial quantitation by fluorescence-based Qubit assay



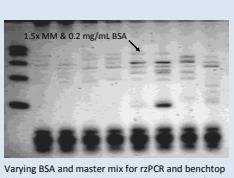
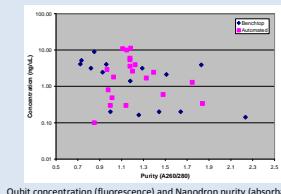
Amplification Module:

- Rotary Zone polymerase chain reaction (rzPCR) design for amplification of extracted DNA
- Sample in capillary tube held stationary against wheel with segments maintained at protocol temperatures
- Stepper motor rotates wheel relative to fixed sample tube for PCR cycling
- Much faster than conventional heated/cooling-block thermal cyclers (~1 sec ramp time)
- Capillary interface allows full automation of PCR sample prep and thermal cycling protocol
- Compact, portable, low power (<12W steady)

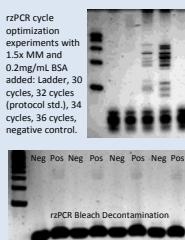
Results

Automated Extraction Module:

- Zymo protocol successfully automated using pressure-driven approach
- DNA purity and concentration of automated Zymo module comparable to centrifuge-based benchtop Zymo protocol
- Automated system extracted on average 100 +/- 108 ng DNA per swab vs. 60.9 +/- 70.8 ng for the benchtop Zymo protocol (30 uL elution volume)
- DNA extracted by automated system successfully analyzed with PowerPlex 16HS short tandem repeat genotyping kit



rzPCR cycle optimization experiments with 1.5x MM and 0.2 mg/mL BSA. Lanes L to R: Ladder, 1.5x MM + 0.2mg/mL BSA, 1.5x MM + 0.2mg/mL BSA, 20 cycles, 32 cycles (protocol std.), 34 cycles, 36 cycles, negative control.



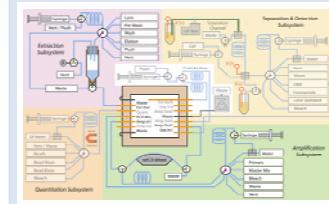
rzPCR Amplification Module:

- Successful rzPCR implementation of PowerPlex 16 HS
- Preliminary results indicate that additional amounts of bovine serum albumin (BSA) and Master mix (MM) appear to improve rzPCR amplification efficiency
- Optimization experiments show that rzPCR requires some additional cycles (~35 vs. 32) to compensate for slightly lower efficiency than benchtop PCR
- Bleach decontamination method proved effective in preventing carryover between rzPCR runs

Conclusions & Future Work

Conclusions:

- Automated Zymo extraction offers promising alternative to labor-intensive benchtop process
- Samples extracted from automated system suitable for benchtop PCR or rzPCR amplification
- rzPCR provides an effective automation and integration-ready method for DNA amplification
- Properly optimized, rzPCR can execute even multiplex PCR protocols such as 16-locus STR
- Decontamination protocols avoid the need for conventional disposable tube-based methods



Future work:

- Demonstrate integrated operation of extraction & amplification modules using DMF hub
- Complete testing & optimization of separation & detection module for genotype readout
- Finalize design & test quantitation module to enable robust operation despite highly variable buccal cell DNA extraction
- Complete full system integration of all four modules with central DMF hub and demonstrate end-to-end operation from buccal swab introduction to DNA fingerprint output
- Explore extraction & analysis of DNA from other sample types (blood, hair, tissue, bone, etc.)
- Show system utility in relevant applications



Acknowledgments

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