Rapid DNA Testing Approaches for Reference Samples

Erica L.R. Butts and Peter M. Vallone

National Institute of Standards and Technology (NIST)
100 Bureau Drive MS 8314, Gaithersburg, MD 20899-8314

Poster # 30 at:
23th ISHI, Nashville, TN
October 15-18, 2012

Email: erica.butts@nist.gov
Phone: 301-975-5107

Current ways your laboratory can perform Rapid DNA Typing:

What is Rapid DNA Typing? Recently, various claims have been made to the term 'Rapid DNA'. It is important to understand the distinctions.

Three main areas exist within Rapid DNA Typing: **Services**, **Techniques**, and **Instruments**

Rapid DNA (Services)

Such as Bode Rapid DNA Service™. This is a lab-based quick turnaround **(< 2 h)** approach using techniques/methods familiar to a forensic laboratory (PCR, CE separation, etc). **This is not a field portable instrument**. The profiles are for **investigative leads** (not for uploading into CODIS).

Rapid DNA (Instruments)

New fully integrated (swab in ➡ profile out) platforms developed by IntegenX, NetBio, ZyGem/Lockheed, and Univ of AZ intended for the STR typing of reference samples.

Throughput: 5-8 samples
Time: 90 minutes per 5-8 profiles
Cost: >$200 per sample

The eventual goal for these platforms is to develop STR profiles in a non-laboratory setting. This will rely on proper validation and NDIS approval paths if information will be intended for CODIS comparison.

Rapid DNA Techniques: genotype results generated in less than 2 hours with standard laboratory equipment and protocols are shown below

Acknowledgements:
This work was funded in part through an interagency agreement between NIST and the FBI Biometrics Center of Excellence. We would also like to thank Life Technologies and Promega for providing the STR kits used for testing and analysis.

References:
1. Promega Corporation. (2012). PowerPlex Fusion System Technical Manual. Madison, WI
2. Promega Corporation. (2012). PunchSolution Kit Technical Manual. Madison, WI
3. Promega Corporation. (2012). SwabsSolution Kit Technical Manual. Madison, WI
4. Applied Biosystems. (2012). GlobalFiler™ Express Kit Early Evaluation Study Guide. Foster City, CA
5. *ZyGEM. Quick-Start Guide*
6. P.M. Vallone, C.R. Hill, D. Podini, J.M. Butler. Rapid amplification of commercial STR typing kits. Forensic Sci. Int. Genet. Sup. Series 2 (2009) 111-112.
7. A. Foster, N. Laurin. Development of a fast PCR protocol enabling rapid generation of AmpFℓSTR Identifiler profiles for genotyping of human DNA. Investigative Genetics 2012 3:6.

Fast PCR

Fast PCR protocols can be adopted into a STR typing workflow

Buccal Swabs (Cotton)

Setup: 3 min 35 sec

Prep-N-Go Buffer

Incubate swab at room temperature in 400 µL Prep-n-Go Buffer

***forensic*GEM Saliva**

One buccal swab washed with 500 µL DNA-free water and incubated at room temperature for 15 minutes. 20 µL elute added to reaction mix.

75 °C for 15 minutes followed by 95 °C for 5 minutes

2 µL solution added to **Rapid Identifiler PCR Setup**

Setup: 6 min 15 sec
Incubation: 20 min

Fast cycling: The use of robust enzymes and faster thermal cyclers to reduce the amount of time required for PCR amplification.

Current Rapid Identifiler PCR Setup [6]:
2.0 µL Identifiler Primers
5.0 µL Takara Perfect Real Time Mix
0.25 µL Takara SpeedSTAR Polymerase
0.75 µL Water
2.0 µL DNA

Cycling Parameters (28 cycles) [7]:
95°C 1 min | 95°C 5 s | 61°C 15 s | 72°C 1 min

Thermal Cyclers: ABI 9700 and Streck Philisa

ABI 9700
Heating mechanism: Peltier block (Al)
Tube format: 0.2 mL tubes or plates
96 reactions per instrument

Streck Philisa
Heating mechanism: Peltier block (Al)
Tube format: proprietary 50 µL tubes
8 reactions per instrument

Require the use of gel loading tips to load PCR product into CE setup plate due to tube design

Prep-N-Go	Prep-N-Go
PCR Setup: 5 min 42 sec	**PCR Setup**: 2 min 7 sec
PCR: 36 Minutes	**PCR: 14 Minutes**
***forensic*GEM Saliva**	***forensic*GEM Saliva**
PCR Setup: 7 min 18 sec	**PCR Setup**: 6 min 48 sec
PCR: 36 Minutes	**PCR: 14 Minutes**

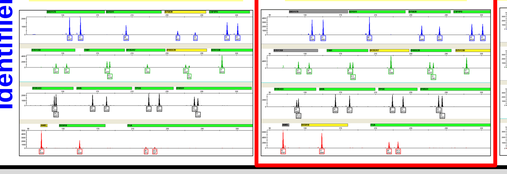
CE Conditions:
1.2 kV for 7 sec

CE Run Time: 38 minutes

Analysis:
GeneMapper *ID-X* v1.2 with an analytical threshold of 50 RFU

STR genotype results were generated in less than 2 hours with standard laboratory equipment and protocols. The validation of Direct PCR STR kits or Fast PCR protocols will allow a forensic lab to *have 'Rapid DNA' capabilities*.

Identifiler



Technique	Sample Type	Pre-PCR Techniques	PCR Amplification	CE & Analysis	Results & Final STR Typing Times
Rapid DNA Typing	8 samples for each set of parameters	Various techniques suggested to prepare multiple substrates for PCR amplification	**Fast PCR**: Identifiler **Direct PCR**: GlobalFiler Express and PowerPlex Fusion	Applied Biosystems 3500 Genetic Analyzer	The **total amount of time** from sampling to complete profile **for 8 samples**, to include all liquid handling steps, PCR, CE, sample transfer steps, and data analysis.

Direct PCR

Direct PCR kits speed up processing by **bypassing extraction** and can perform a direct amplification in **less than 90 minutes**. PCR products are separated and detected on a traditional CE system.

Blood FTA

Punching: 2 min 21 sec

Blood S&S 903

Punching: 2 min 26 sec

Buccal Swabs (Cotton)

None

Prep-N-Go Buffer
(for use with GlobalFiler)

3 µL added with PCR setup and one 1.2 mm punch

Setup: 58 sec
Incubation: 30 min

Promega PunchSolution
(for use with Fusion)

10 µL PunchSolution Reagent incubated at 70 °C for 30 minutes

***forensic*GEM Storage Card (Blood)**
(for use with GlobalFiler or Fusion)

One 1.2 mm punch washed with 100 µL DNA-free water and incubated at room temperature for 15 minutes. Remove water and add reaction mix to the 1.2 mm punch.

75 °C for 15 minutes followed by 95 °C for 5 minutes

Setup: 5 min 28 sec
Incubation: 35 min

2 µL solution added to GlobalFiler
3 µL solution added to Fusion

Setup: 3 min 35 sec

Prep-N-Go Buffer
(for use with GlobalFiler)

Incubate swab at room temperature in 400 µL Prep-n-Go Buffer

Setup: 1 min 40 sec
Incubation: 30 min

Promega SwabSolution
(for use with Fusion)

One buccal swab incubated at 70 °C for 30 minutes in 1 mL SwabSolution Reagent

***forensic*GEM Saliva**
(for use with GlobalFiler or Fusion)

One buccal swab washed with 500 µL DNA-free water and incubated at room temperature for 15 minutes. 20 µL elute added to the reaction mix.

75 °C for 15 minutes followed by 95 °C for 5 minutes

Setup: 6 min 15 sec
Incubation: 20 min

2 µL solution added to GlobalFiler
3 µL solution added to Fusion

GlobalFiler Express
6-dye Technology
24plex
3500 Instruments only

PCR Setup (Punches):
6 µL Master Mix
6 µL Primer Set
3 µL Prep-n-Go

Setup: 1 min 34 sec

Cycling Conditions:
ABI 9700
95 °C for 1 minute

94 °C for 3 seconds
60 °C for 30 seconds
Cycle for 26 cycles

60 °C for 8 minutes

GlobalFiler Cycling Time:
42 minutes
15 seconds

PCR Setup (Swabs):
6 µL Master Mix
6 µL Primer Set
3 µL Prep-n-Go or DNA

Setup: 2 min 7 sec

PowerPlex Fusion
5-dye Technology
24plex
3100 or 3500 Instruments

PCR Setup (Punches):
5 µL Master Mix
5 µL Primer Set
15 µL Water

Setup: 1 min 33 sec

Cycling Conditions:
ABI 9700
96 °C for 1 minute

94 °C for 10 seconds
59 °C for 60 seconds
72 °C for 30 seconds
Cycle for 26 cycles

60 °C for 20 minutes

Fusion Cycling Time:
1 hour
35 minutes
45 seconds

PCR Setup (Swabs):
5 µL Master Mix
5 µL Primer Set
13 µL Water
2 µL Swab Extract/DNA

Setup: 2 min 19 sec

CE Conditions:

GlobalFiler:
1.2 kV for 16 sec
1550 sec Run Time
13.0 kV Run Voltage
15.0 kV PreRun Voltage

Fusion:
1.2 kV for 24 sec
1300 sec Run Time
15.0 kV Run Voltage

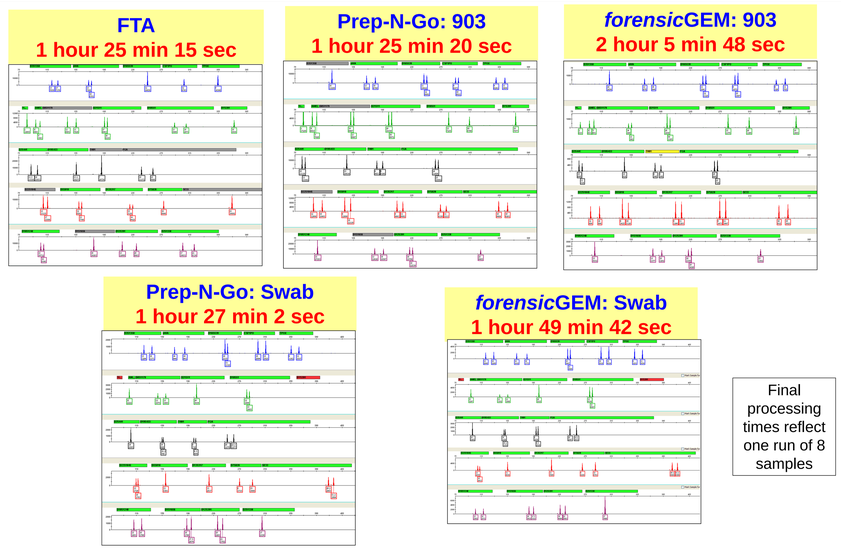
GlobalFiler CE Run Time:
39 minutes
5 seconds

Fusion CE Run Time:
37 minutes
30 seconds

Analysis:
GeneMapper *ID-X* v1.2 with an analytical threshold of 200 RFU for both GlobalFiler and Fusion

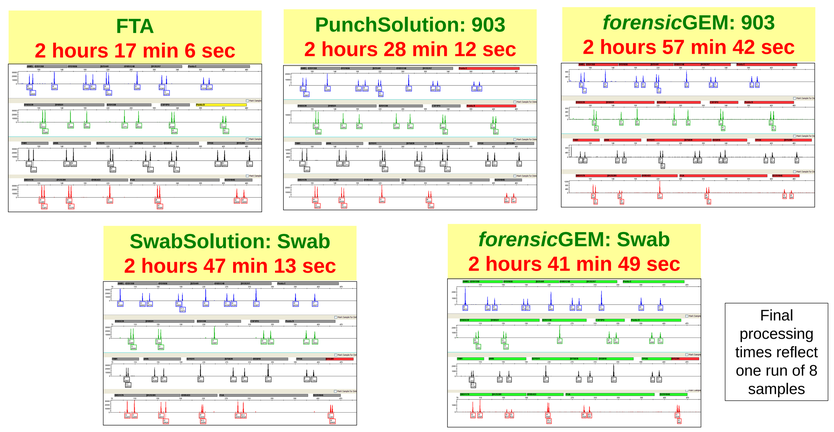
Analysis of quality single source reference samples: 45 sec

GlobalFiler Express



Final processing times reflect one run of 8 samples

PowerPlex Fusion



Final processing times reflect one run of 8 samples

Poster available for download from STRBase: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm