



Saint Louis County Police Crime Laboratory

Serve a population of ~1 million

- \geq 12 employees in the Bio/DNA unit Currently (2015) we have 2.5 biologists, 1 technician (training in Biology), 5 full-time DNA analysts (one just signed off for DNA), 3 supervisory/DNA analysts (section supervisor, TL, and CODIS Administrator)
- \succ Biologists perform the sampling step for DNA = huge time savings for DNA
- > We use EZ1s for extraction (Erase on EZ1s for differentials, Tecan in validation), 7500s, 9700s, and 3500s for CE- We average ~ 1000 cases per year in DNA

>GlobalFiler online in June of 2014

Location of GlobalFiler Loci in the Genome:



Protocol Overview and Optimization

GlobalFiler Express Kit: <u>40 min. amp!</u>

	Prep-N-Go buffer volume	Incubation Time/Temp	DNA Input volume	Total Reaction Volume	Cycle #	Injection Time (3500)
Original Protocol	<u>FTA</u> = 2 punches in 20uL, <u>Oral swab</u> = 1/2 swab in 200uL, <u>Whatman buccal</u> <u>swabs</u> = ~3 teeth in 200uL	20 min, 37ºC	3uL	15uL	27	10,15,20 seconds
Optimized Protocol	SAME	SAME	1uL and 2uL input volumes added	SAME	SAME	5 second injection time added

GlobalFiler Casework Kit:

DNA Target Amount	DNA Input volume	Total Reaction Volume	Cycle #	Injection Time (3500)	GeneMapper ID-X v1.4 Settings
1.5-2ng	Up to 15uL	25uL	29	10,15,20 seconds	Local Southern Sizing Method, Peak Window Size=11

Analytical and Stochastic Thresholds for Casework Analysis:

	Analytical Threshold	Stochastic Threshold - 5*, 10, 15 second injection times	Stochastic Threshold - 20 second injection time
GlobalFiler Express Kit	80 RFU	300 RFU *5 second injection time only used with Express Kit	300 RFU (stochastic threshold validated using 20 second injection time samples)
GlobalFiler Casework Kit	100 RFU	400 RFU	550 RFU

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Casework Experience with the GlobalFiler STR Kits Saint Louis County Police Crime Laboratory

Kyra Groeblinghoff, Nicholas Homa, Shanna Saunders

Master Mix Additive (MMA) Update

- \succ Customers reported seeing elevated NEDTM peak heights as well as a reverse ski slope in the purple channel (SIDTM)
- \succ Suspected cause is the old MMA formulation which used a phosphate buffer - phosphate likely forming a precipitate $(Mg_3(PO_4)_2)$ which reduces the available Mg^{2+} in the reaction
- \succ Higher input DNA samples (\geq 4ng) have a more pronounced effect due to the balance of primers and the excess DNA using up even more of the Mg²⁺
- \succ Updated kit uses BSA in a Tris buffer and MMA is no longer in a separate tube
- \succ Only applies to the Casework kit, no changes to the Express kit

> Performance check needed!

Sensitivity Series:

Sample ID	Plexor Quant value (ng/ul)	Plexor factor	Adjusted Quant value (ng/ul)	ul added to reaction	Total DNA (ng) in Amp reaction
KJR-stock (average*)-3ul	5.38	0.651	3.50238	3	10.507
KJR-stock (average*)-2ul	5.38	0.651	3.50238	2	7.0047
KJR-Dil-1-15ul	0.538	0.651	0.350238	15	5.2535
KJR-Dil-1-10ul	0.538	0.651	0.350238	10	3.5023
KJR-Dil-2	0.0538	0.651	0.0350238	10	0.3502
KJR-Dil-3	0.00538	0.651	0.00350238	10	0.0350

Known/non-probative:

Sample ID	Source	Body Fluid/Substrate
F1/F2- SERI	SERI R675B lot# 1003001 + Saliva from 4024 (differential extraction performed)	Semen+saliva on fabric
NIST	Bloodstain card extract	Blood on FTA
KJR	Whole blood with EZ1 TTD extraction	Blood
S-MIX-A	CTS-14-575#3 (no differential performed)	Blood+semen on fabric

Performance Check Results – MMA Update NED CHANNEL AVG PEAK HEIGHTS, NEW (N) VS OLD (O)



- \succ The "old" kit had been thawed only 4 days prior to amplification
- \succ Data indicates the level of precipitate formation may be related to the length of time the kit sits refrigerated after MMA is added

Interesting Case #1 – 1990 Homicide				
Scenario:Female maintenance worker was found stabbed and strangled in the men's restroom of a local seminary. Original suspect was the victim's boss - a glove with a bloodstain (Q11) was located in a gym bag at the suspect's residence.Evidence:(Q14 not previously tested)Q11 - 2001 extract of RBS from glove located in suspect's residenceQ14 - swabs from restroom sink, back of fixture				
Globalfiler was just being brought online (waiting for NDIS approval) These samples were the first run with GlobalFiler once it came online				
70 110 150 190 230 271 2000 0 15 16 19 11 15 16 18 12 Filer_Panel_v1	QTT: QTT			
70 110 150 190 230 270 4000 0 2000 0 2 X 10 14 2831.2 Y 12 Filer_Panel_v1	GlobalFiler (purple channel not shown)			
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	 Full profile obtained! Minor noted that was not 			
Filer_Panel_v1 022S1045 05S818 013S317 07S8 70 110 150 190 230 270 $4000^{$	dotoctod			
Q14 - Identifiler Results: $ \begin{array}{c} \hline 021511 & D75820 & CSF1P0 \\ \hline 000 & 110 & 150 & 190 & 230 & 270 & 310 & 350 \\ \hline 1013 & 28 & 10 \\ \hline 12 & 29 & 10 \end{array} $	Q14 - GlobalFiler Results:			
$r_v 1.2X$ D3S1358 TH01 D13S317 D16S539 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1338 D2S1358 D2S1338 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S1358 D2S15 D18S51	I16 Filer_Panel_v1 III D21S11 D18S51 DYS391 70 110 150 190 230 270 310 350 33 8000 IIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII			
70 110 150 190 230 270 310 350 000 110 150 190 230 270 310 350 13 17 8 13 14 18 15 13 17 8 14 18 er_v1.2X 70 110 150 190 230 270 310 350	Filer_Panel_v1 D2S441 D19S433 TH01 FGA 70 110 150 190 230 270 310 350 39 4000 4000 4000 4000 4000 4000 4000 400			
$\mathbf{\hat{P}}_{0} = \mathbf{\hat{P}}_{0} + \mathbf{\hat{P}}_{11} + \mathbf{\hat{P}}_{12} + \mathbf{\hat{P}$	11 11 15 10 10 10 10 10 10 10 10 10 10			
 (or more) contributors, male detected, looks promising ➢ Q14 - Globalfiler results, Mix of at least 3 contributors, <u>at least 2 males</u> (Y indel), major contributor is the victim - Bummer! 	Filer_Panel_v1			
Although the new analysis did not "solve" the case, it was incredibly valuable to see a direct comparison of the Identifiler and GlobalFiler kits on a casework sample				
> The GlobalFiler kit is far more sensitive than Identifiler				
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DNA Technical Leader				
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Interesting Case #2 – "Yahoo"

Scenario:

Suspect broke into an unoccupied home (resident had recently passed away - relatives had been packing up belongings). Suspect steals a TV and other items, then steals the victim's car. He is found in the city passed out in the stolen car (high on heroin).

Evidence: Q1 - swabs of blood from bathroom wall Q3 - swabs from mouth of "Yahoo" bottle





Contamination?

shown)

Apparent minor

Known was run with GF Express, samples run with GF Casework kit –therefore can't be from EZ1 or extraction/amplification reagents

contributor...~11:1 mixture

ratio (no minor detected in

the Blue channel - not

The plot thickens... Apparent minor contributor from buccal swab matches the minor contributor from the "Yahoo" bottle

Possible Explanations:

> Officer collected swabs at same time (or used contaminated) swabs)? – no. Per officer, he collected the Yahoo bottle swabs but the buccal swab was collected at the city jail the following day... > Chimera?

CODIS hit to a multi-allelic offender solves the mystery. The suspect is a bone marrow recipient!

- Interesting that one saliva sample has a mixture ratio of 11:1 (buccal swab) and the other 2:1 (Yahoo)
- The MO Highway Patrol has 7 multi-allelic offenders (out of 263,755 offenders (0.002%) as of 3/5/15)

Summary

>Overall we are very pleased with the GlobalFiler kits.

- > The significantly reduced processing/amplification times has improved our throughput and allowed for expedited analysis.
- > The 15uL input volume allows for dramatically increased sensitivity.
- > The additional loci provide more definitive information with respect to the number of contributors and the gender of contributors.
- \succ The additional loci provide an amazing power of discrimination - Random Match Probabilities are routinely in the nonillion-decillion range (10³⁰-10³³)