

**Development of Protocols for Rapid Amplification of STR Typing Kits:
The Use of 'Non-Standard' Thermal Cyclers**


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final version can be found at
<http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#Presentations>

Rapid PCR Applications

- Faster sample-to-answer
 - Successful rapid PCR cycling reduces STR workflow times (**less than 2 hours in the laboratory**)
 - Single source reference and databasing samples
- Increased throughput (more runs per day)
- Integrated platforms for forensics and biometrics
 - Rapid DNA instruments (swab in → answer out)

Thermal Cycling Times for Commercial STR Kits

Year	Run on a 9700 thermal cycler	Hot start	Time per cycle	Cycles	Post soak	Total time
1997/98	Profiler Plus/Cofiler	11 min	3 min	28	60 min	2:52
1999	SGM Plus	11 min	3 min	28	45 min	2:53
2000	PowerPlex 16	12 min	1 min 45 s	32	30 min	3:00
2001	Identifiler	11 min	3 min	28	60 min	2:58
2003	PowerPlex Y	12 min	1 min 45 s	32	30 min	3:18
2004	Yfiler	11 min	3 min	30	80 min	2:45
2007	PowerPlex S5	2 min	4 min	30	45 min	3:21
2007	minifiler	11 min	3 min 20 s	30	45 min	3:16
2009	ESI 16, 17 ESX 16,17	2 min	4 min	30	45 min	3:22
2009	PowerPlex 16 HS	2 min	1 min 45 s	32	30 min	2:42
2009	NGM	11 min	3 min 20 s	29	10 min	2:33
2009	Identifiler Direct	11 min	3 min	26	25 min	2:34
2010	Identifiler Plus	11 min	3 min 20 s	28	10 min	2:18
2011	PowerPlex 18D	2 min	1 min 10s	27	20 min	1:25
2012	NGM Express (direct)	1 min	48 s	26	5 min	0:45
2012	PowerPlex 21	1 min	1 min 40 s	30	10 min	1:23
2012	PowerPlex Y23	2 min	1 min 40 s	30	20 min	1:33
2012	PowerPlex Fusion	1 min	1 min 40 s	30	10 min	1:24
2012	GlobalFiler Express (direct)	1 min	33 s	26	8 min	0:40

DNA Polymerases

- AmpliTaq Gold® is typically used
 - Heat activated (avoid non-specific PCR products)
- Takara SpeedSTAR™ HS DNA Polymerase
 - Extension times of 100 bp/s are possible (compared to 20 bp/s for other polymerases)
 - Hot-start formulation is antibody mediated
- Qiagen
 - QIAGEN Fast Cycling PCR Kit
- New England Biolabs/Finnzymes
 - Phusion and Phire DNA Polymerases
 - Q5
- KAPA Biosystems
 - KAPA2G Fast PCR Kits
- Biotium
 - Cheetah™ Taq
- Fermentas
 - PyroStart Master Mix
- EMD Millipore
 - KOD DNA Polymerase

DNA Polymerase Characteristics

Why are cycling times decreasing?

- Accuracy (geometric selection)
- Proofreading (3' – 5' exonuclease activity)
- **Processivity**: the number of nucleotides incorporated before dissociation

E.Coli polymerase III

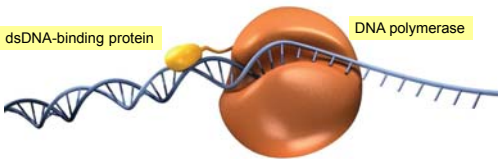
- Example: E.Coli polymerase III subunit (alone)
 - Processivity = 10 nt
 - Speed = 20 nt/s
- If associated with **sliding clamp** (and replisome subunits)
 - Processivity = 50 kb
 - Speed = 1000 nt/s

Taq Polymerase: P = 50 nt, S = 20 nt/s

Pomerantz, R. T. & O'Donnell, M. (2007) *Trends Microbiol.* 15, 156–164



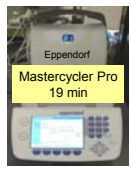
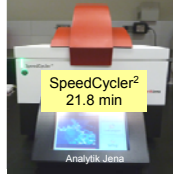


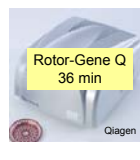

Chimeric Polymerases

- Based on work Wang et al 2004 NAR 32: 1197-1207
 - Enhance polymerase **processivity** by covalently linking a non-specific ds binding protein to the polymerase domain
 - 16 to 32 fold increase in polymerase efficacy



http://www.thermo.com/pcr/enzymes-master-mixes-and-reagents/prime-hot-start-ii-dna-polymerase/
https://www.nsb.com/tools-and-resources/feature-articles/anatomy-of-a-polymerase-how-structure-affects-function

Developing Protocols for 8 Thermal Cyclers

 9700 36 min Life Technologies	 Piko 30.5 min Thermo Scientific	 Mastercycler Pro 19 min Eppendorf	 SpeedCycler ² 21.8 min Analytik Jena
 Palm 17 min Atrium	 SmartCycler 21.8 min Cepheid	 Rotor-Gene Q 36 min Qiagen	 Philisa 17 min Streck

Cycling times given for a 3-step 28 cycle protocol

PCR Thermal Cycling Profile

Standard Identifier cycling parameters					3-step ≈3 hours
95°C 10 min	95°C 1 min	59°C 1 min	72°C 1 min	60°C 60 min	
Reduced PCR artifacts					2-step 31 min
95°C 1 min	95°C 5 s	61°C 15 s	72°C 1 min	72°C	

Demonstration of rapid multiplex PCR amplification involving 16 genetic loci Vallone et al. Forensic Sci Int Genet. 2008 3:42-45

Development of a fast PCR protocol enabling rapid generation of AmpFSTR® Identifier® profiles for genotyping of human DNA Foster and Laurin Investig Genet. (2012) 3:6

28 cycles on GeneAmp 9700

Comparative Throughput (Cycling)

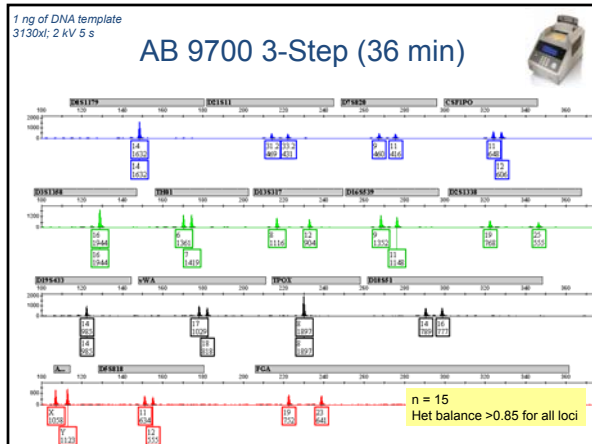
Cycler	# samples	3 step		2 step	
		Fastest Cycling Time (min)	2 step	Fastest Cycling Time (min)	2 step
GeneAmp PCR System 9700	96	36	31	1	36
Mastercycler Pro S	96	19	17	1	19
Rotor-Gene Q	72	36	32	2	72
SmartCycler	16	22	18	6	132
Philisa	8	17	14	12	204
Piko	96	30	26	1	30
SpeedCycler ²	96	22	19	1	22
Palm PCR	12	17	17	8	136

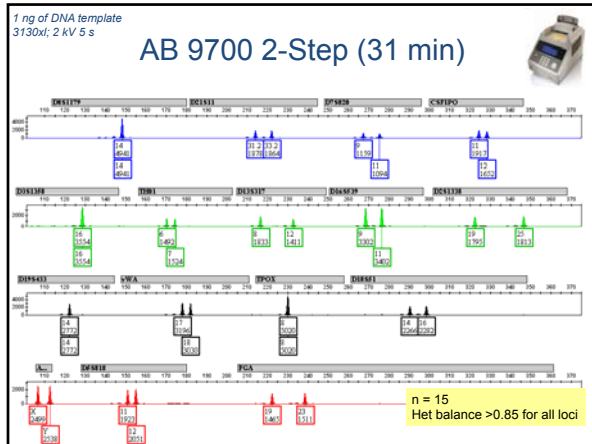
- Varying characteristics of heating/cooling and tube (reaction vessel)
- Rotor-Gene Q and SmartCycler are real-time PCR instruments

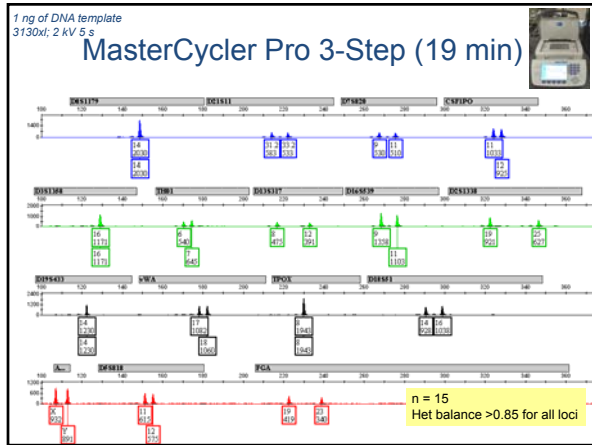
While cycling times may be rapid, the throughput in some cases is reduced from the standard 96-well format

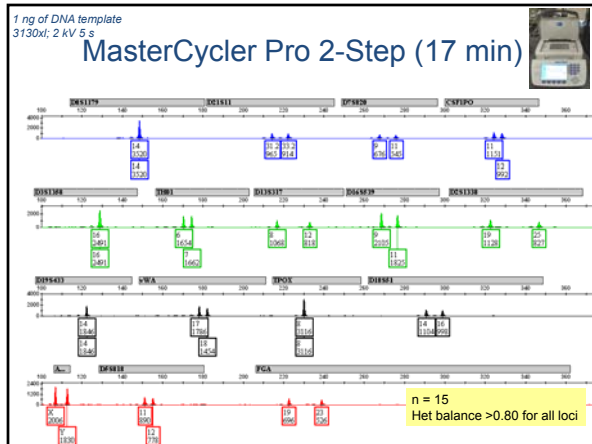
Experiments and PCR Conditions

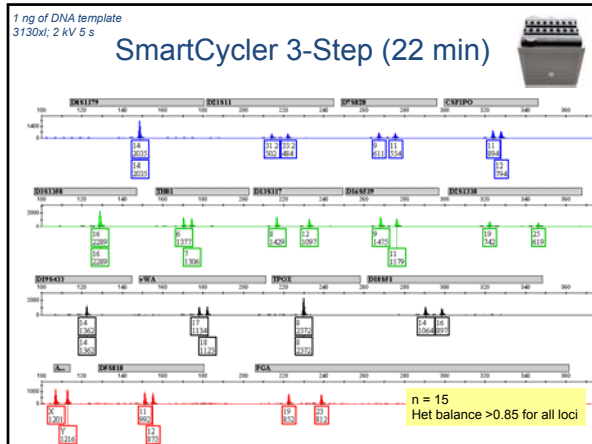
- Develop a successful PCR protocols for each cycler with 2- and 3-step cycling conditions
- Sensitivity study: 1 sample, 7 concentrations in duplicate; compare 2- and 3-step PCR protocols
- Rapid STR typing workflow example (less than 2 hours)
 - 1 X Takara PCR mastermix, 1 U SpeedStar polymerase *Premix Ex Taq™* (Perfect Real Time)
 - 10 μ L total reaction volume in a thin walled tube (8-strip) or proprietary tube
 - 2 μ L of Identifiler PCR primer mix
 - ~1 ng of template DNA
 - 2- and 3-step cycling conditions
 - Separation and detection on a 3130xl or 3500/3500xl

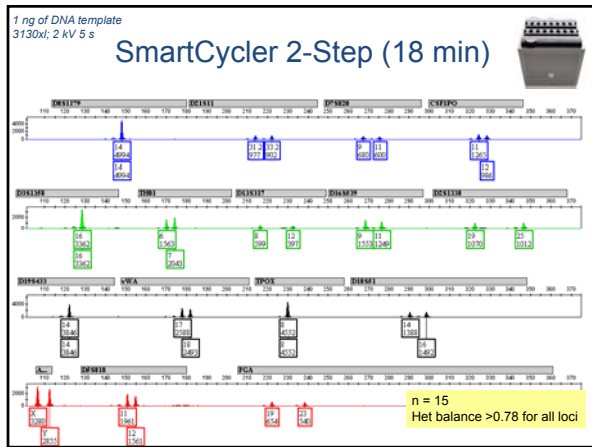


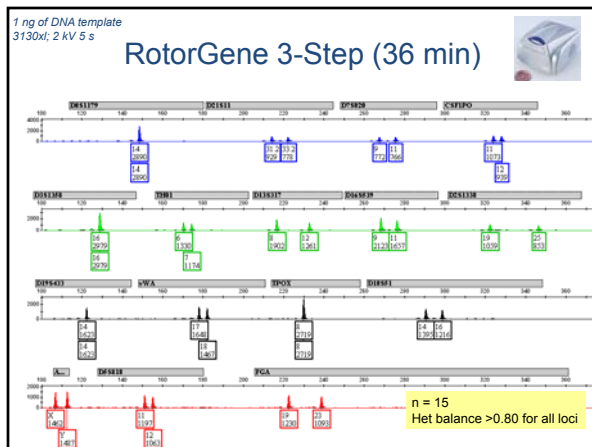


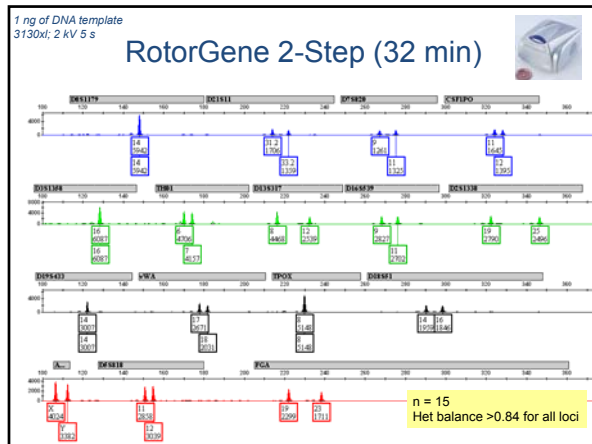


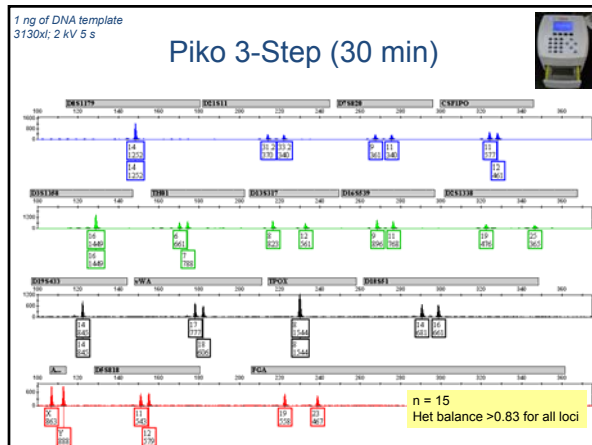


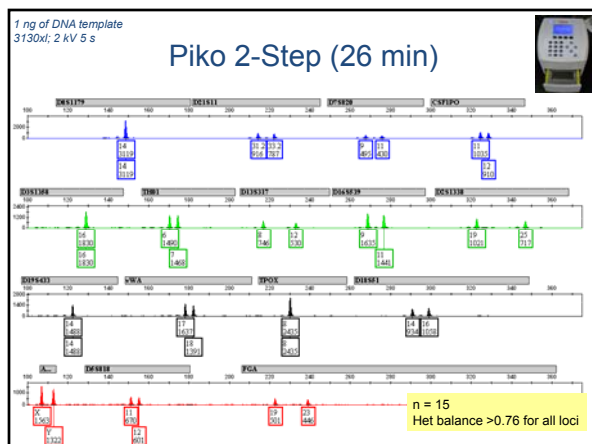


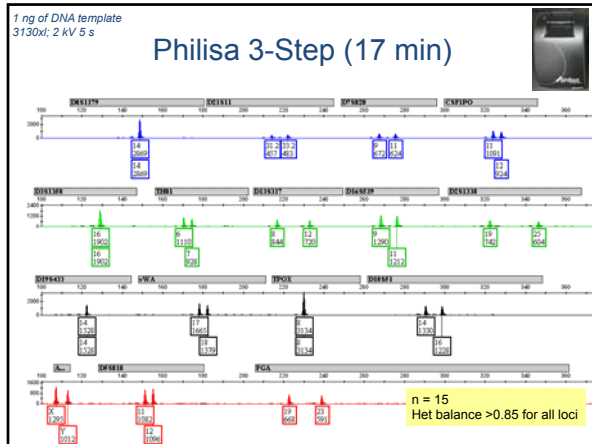


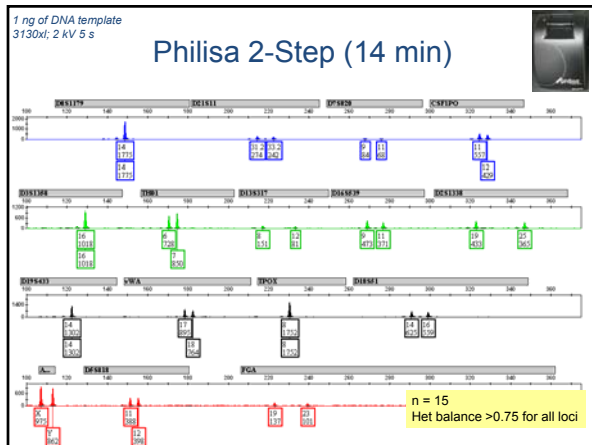


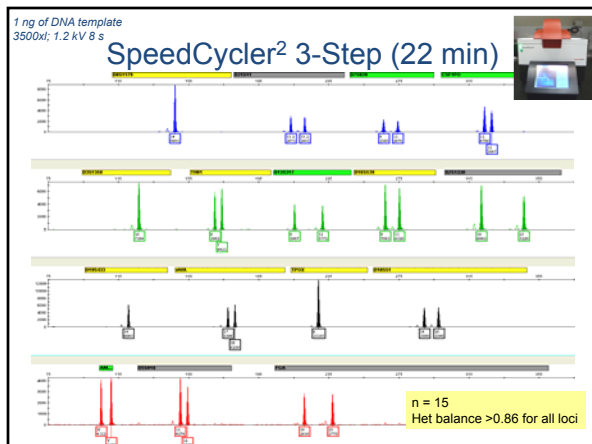


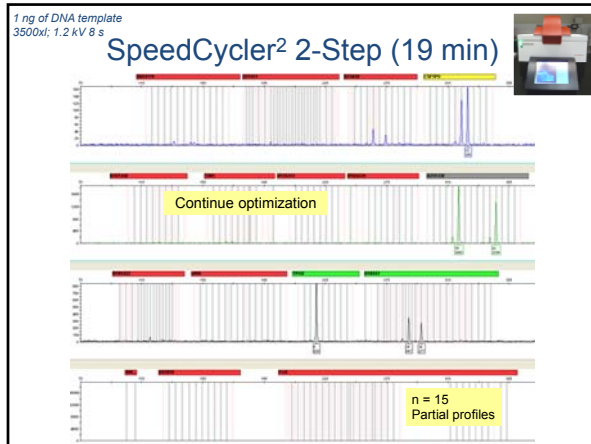












Sensitivity

	2-Step Cycling						3-Step Cycling								
	9700	SmartCycler	MasterCycler Pro	Rotor-Gene	Piko	SpeedCycler2	9700	SmartCycler	MasterCycler Pro	Rotor-Gene	Piko	SpeedCycler2			
1000 pg	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green			
750 pg	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green			
500 pg	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green			
250 pg	Yellow	Yellow	Green	Green	Green	Green	Yellow	Yellow	Green	Green	Green	Green			
125 pg	Red	Red	Yellow	Green	Green	Green	Red	Red	Yellow	Green	Green	Green			
62.5 pg	Red	Red	Red	Yellow	Green	Green	Red	Red	Red	Yellow	Green	Green			
31.3 pg	Red	Red	Red	Red	Yellow	Green	Red	Red	Red	Red	Yellow	Green			
min	31	18	17	32	26	14	19	min	36	22	19	36	30	17	22

1 sample; each concentration point in duplicate

■ Both Alleles Observed
■ Allele Dropout
■ Locus Dropout

Rapid STR profiling in a lab setting

Bode Technology

Allow DNA to Drive Your Investigation
Introducing Same-Day DNA Service

Court-Ready DNA Analysis in One Day

Utilize DNA for Immediate Response to Critical Investigations

Same-Day DNA Service™ - Court-Ready Report in One Day

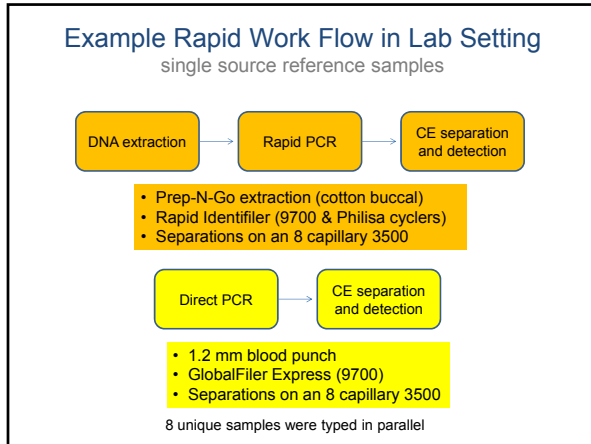
Rapid DNA Service™ - Forensic Sample (evidence or reference items) to DNA Profile in < 90 mins.

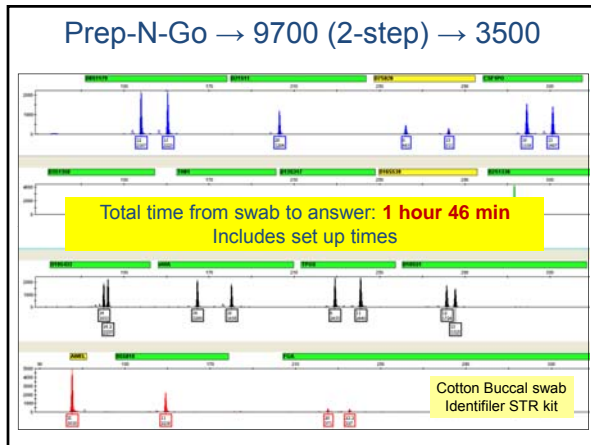
www.Bodetech.com/SameDayDNA

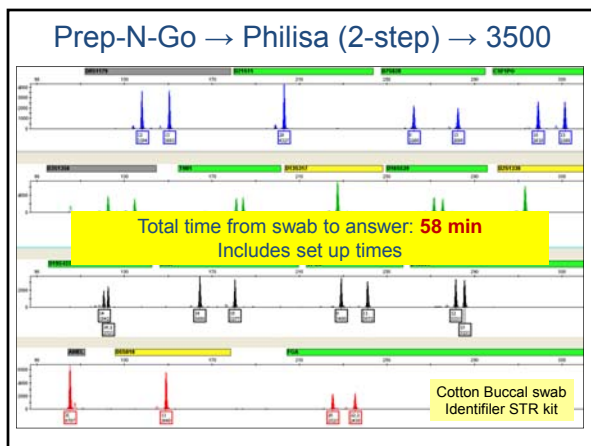
NIST presentations on Rapid STR Typing

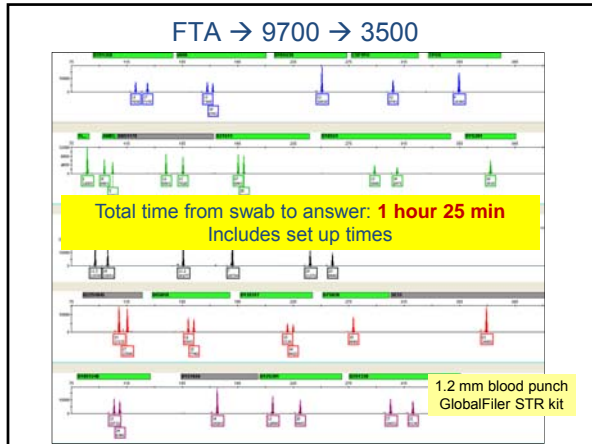
MAAFS 2012
"Rapid DNA Testing Approaches for Reference Samples"
http://www.cstl.nist.gov/strbase/pub_pres/Butts-MAAFS2012-rapid-DNA-testing.pdf

Promega 2012
"Rapid DNA Testing Approaches for Reference Samples"
http://www.cstl.nist.gov/strbase/pub_pres/Butts-ISH2012-Rapid-DNA.pdf









Conclusions

- Successful protocols developed for 7/8 cyclers tested
 - 14 min PCR on Philisa cycler
- Continue work on Palm PCR and SpeedCycler²
- Under the stated conditions sensitivity is around 250-500 pg of template DNA
- 2-step PCR protocol:
 - Faster
 - Similar sensitivity compared to 3-step
 - Comparable RFUs; peak height balance and stutter
 - Fewer PCR artifacts
- Complete STR profiling in < 2 h (swab-to-answer)

Thank you for your attention!

Questions?

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NIJ – Interagency Agreement with the Office of Law Enforcement Standards
