

# NIST Experience with FSS-i<sup>3</sup> v4.1.3 Software Upgrade

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## Disclaimers

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The Forensic Science Service **FSS-i<sup>3</sup> v4.1.3** integrity interpretation innovation

Forensic systems designed by forensic scientists for forensic scientists

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## Packaging for FSS i-Cubed Software



## Cost to the End User

- **Software**
  - Receive the v4.1.3 upgrade software
  - single copy, single computer **\$20,000**
- **Maintenance agreement**
  - **\$4,000** per year (20% of total software cost per year, max \$15,000)
  - Software upgrades and patches are included
- **Training**
  - **\$2,000** if at Promega (plus your travel expenses)
  - \$12,000 for up to 5 people if performed in your lab
- Requires GeneMapper ID or GeneScan/Genotyper software to already be in place in your lab

**Minimum starting cost of \$26,000**

## NIST Experience with Software Purchase

- Attempted to purchase directly from FSS
  - No quote provided by Chris Macquire despite multiple attempts and email agreements to do so (Dec 2004, Jan, Feb, May, June 2005)
- Quote for software from Promega on Oct 18, 2005
  - Told that we had to purchase \$4,000 maintenance agreement along with at least \$2,000 training (plus travel expense to Madison, WI)
  - NIST contract officer signed off Dec 19, 2005
- Promega installed **v4.0.1** software January 3, 2006
- Becky Hill went to Madison, WI Jan 9-13, 2006 for first training class held at Promega
- Received **v4.1.2d** August 3, 2006, installed by Bob McLaren
- Received and installed **v4.1.3** patch September 9, 2006

### Overview of Software Components



**i-STress** quickly and accurately calls your allele types and objectively assesses the quality of your data. This allows analysts to reduce the time spent manually reviewing data and focus on "problem" samples.

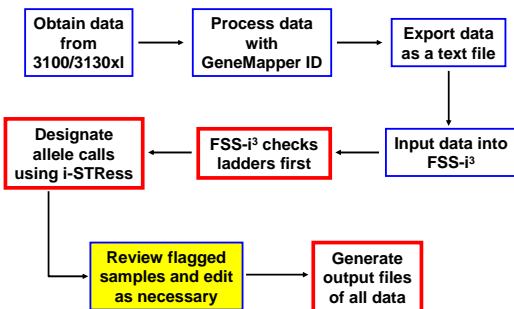
**i-STReam** module evaluates two-person DNA mixtures and produces a best-fit major profile. This aids the reporting analyst in mixture deconvolution and unbiased interpretation.

**i-integrity** checks for potential sample-to-sample contamination within a batch by comparing all alleles called in a sample to the alleles in every other sample in the batch.

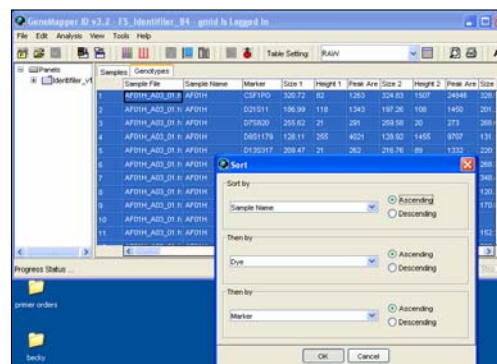
<http://www.promeqa.com/applications/profile.asp?appname=Genetic+Identity&sku=FSSi3&spl=off>

## Introduction to FSS-i<sup>3</sup> Software

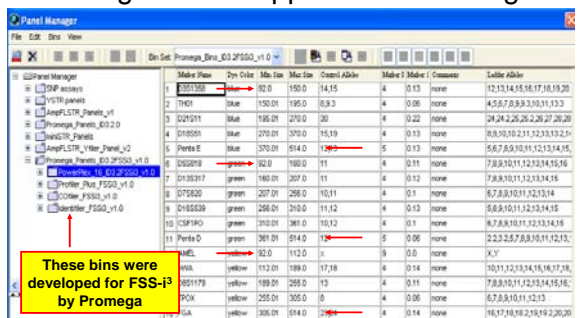
### FSS-i<sup>3</sup> Flow Chart



### GeneMapper ID Raw Data Sorted



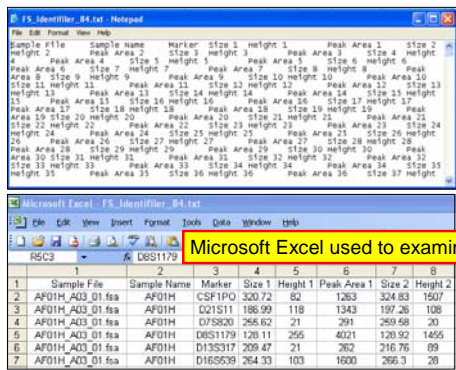
### Change GeneMapper ID Bin Settings



These bins were developed for FSS-i<sup>3</sup> by Promega

Min/Max Sizes were adjusted to be the same for all dye channels, and marker bin settings were lined up so there were no gaps within a dye channel

### Text File Saved from GeneMapper ID "Raw Data"



Microsoft Excel used to examine data

### FSS-i3 Input Template

Controls what and how information will be brought into the FSS-i3 program

### Import data as text file into FSS-i3

### PowerPlex16 D3S1358 ladder

At least one ladder must pass for rules to be applied and alleles designated for each sample

### FSS-i3 Rule Sets and Thresholds

Each multiplex kit can have multiple rulesets

### Images from FSS-i3 i-STress

### Editing samples

### FSS-i3 Output Template

Controls what and how information will be exported from the FSS-i3 program

### Output Files Created by FSS-i3 i-STress

C:\Program Files\FSSi3 v412d\i-STress\Files\PP16 Combo2 Output Files

Batch ID	Sample ID	Locus ID	Major Designation 1	Major Designation 2	Minor Designation 1	Minor Designation 2	i-STress Major	i-STress Minor	Rules Fired	Change Log
PP16 Combo2	MT9492	D3S1358	14	15	F	F	14,15	-	no rules fired	no change
PP16 Combo2	MT9492	TH01	6	9,3	F	F	6,9,3	-	no rules fired	no change
PP16 Combo2	MT9492	D1S11	29	32,2	F	F	29,32,2	-	no rules fired	no change
PP16 Combo2	MT9492	D1S51	15	15	F	F	15,15	-	no rules fired	no change
PP16 Combo2	MT9492	Para E	11	15	F	F	11,15	-	no rules fired	no change
PP16 Combo2	MT9492	D5S818	9	12	F	F	9,12	-	no rules fired	no change
PP16 Combo2	MT9492	D1Z317	9	16	F	F	9,16	-	no rules fired	no change
PP16 Combo2	MT9492	D7S820	9	9	F	F	9,9	-	no rules fired	no change
PP16 Combo2	MT9492	D1S639	9	12	F	F	9,12	-	no rules fired	no change
PP16 Combo2	MT9492	CSF1PD	11	12	F	F	11,12	-	no rules fired	no change
PP16 Combo2	MT9492	Para D	12	13	F	F	12,13	-	no rules fired	no change

Files are created in html but can easily be imported into Excel for sorting and review

### Audit Files Created by FSS-i3 i-STress

C:\Program Files\FSSi3 v412d\i-STress\Files\PP16 Combo2 Audit Files

Name	Size	Type	Date Modified
PP16 Combo2 samples full data		File Folder	10/4/2006 11:27 AM
PP16 Combo2 samples summary		File Folder	10/4/2006 11:26 AM
PP16 Combo2_Edits Check.html	7 KB	HTML Document	10/4/2006 11:27 AM
PP16 Combo2 @BPM.csv	2 KB	Microsoft Office Exc...	10/5/2006 3:04 PM
PP16 Combo2 Ladder Compos...	63 KB	HTML Document	10/5/2006 2:57 PM
PP16 Combo2 Ladder Summar...	113 KB	HTML Document	10/5/2006 2:57 PM
PP16 Combo2 Ladder Virtual ...	97 KB	HTML Document	10/5/2006 2:57 PM
PP16 Combo2 RAW Designat...	230 KB	Microsoft Excel Wor...	10/5/2006 3:04 PM
PP16 Combo2 SubSetUsed.html	27 KB	HTML Document	10/5/2006 3:04 PM
PP16 Combo2 Sample Audit.html	4,054 KB	HTML Document	10/4/2006 11:26 AM

Lane: 13  
Barcode: MT94877  
Result For Lane: FF

Locus	Final Result	Designation	Size (bp)	Height (a.u)	Area (a.u)	Rules Fired	Inspected	Change Log
D18S51		04.2	207.53	59	540	Ref: Jap AB	3/6/04	Operator: Don Time: 10/05/2006 11:34:13 AM Machine: p41509
		04.2	201.00	53	786	-	-	-
		14	300.30	65	1009	-	-	-
		15	312.20	1308	14045	-	-	-
		16	318.10	740	8224	-	-	-
		-	338.20	76	5074	-	-	-
		-	342.20	77	802	-	-	-

## New Features of v4.1.3 FSS-i3 Upgrade

### Creating new multiplexes with Multiplex Manager

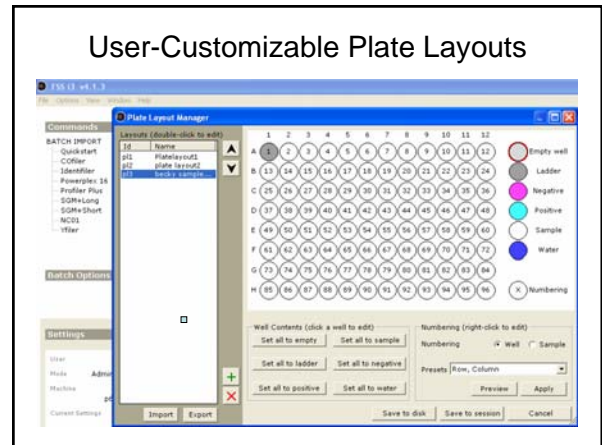
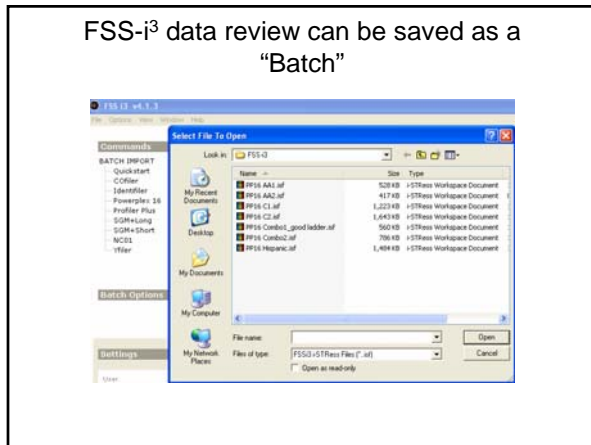
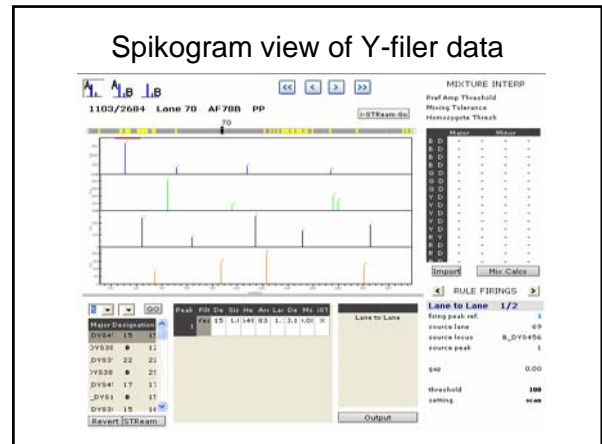
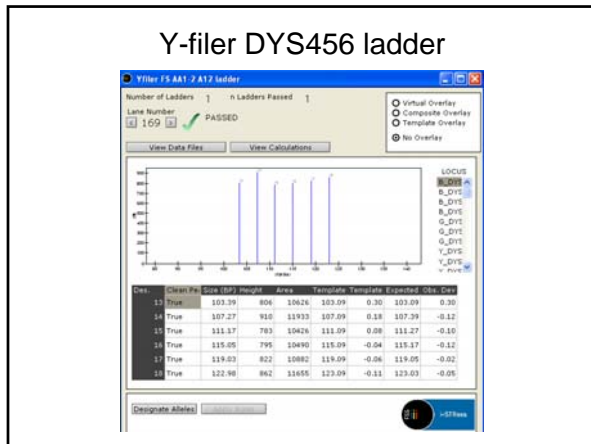
Y-filer loci were added manually

### Ladder editor for new multiplexes (Y-filer)

Size	Designation	Deviation	Position from anchor
243.67	7	0	0
247.67	9	4	4
251.67	9	8	8
255.67	10	4	12
259.67	15	4	16
263.67	12	4	24
267.67	15	4	28
271.67	16	4	32
275.67	15	4	36
279.67	17	4	40
283.67	18	4	44
287.67	13	4	48

The allelic peaks shown are graphical representations and do not represent true allelic morphology or intensity





# Work Performed at NIST

- ### Allele Concordance Studies at NIST
- Manual calls
    - with GeneScan/Genotyper v3.7
    - with GeneMapper ID v3.2
  - Automated calls with GM/FSS-i<sup>3</sup>
  - Comparison of output with Excel spreadsheets written by Dave Duewer (NIST)

Single Source Samples Examined with i-STRess



- We have previously examined **262 Identifier** samples with v4.0.1. Excellent concordance was found and the results can be found in past presentations:  
[http://www.cstl.nist.gov/biotech/strbase/pub\\_pres/NIST\\_FSSi3\\_Mar2006.pdf](http://www.cstl.nist.gov/biotech/strbase/pub_pres/NIST_FSSi3_Mar2006.pdf)  
[http://www.cstl.nist.gov/biotech/strbase/pub\\_pres/PromegaTechTour\\_NIST\\_FSSi3.pdf](http://www.cstl.nist.gov/biotech/strbase/pub_pres/PromegaTechTour_NIST_FSSi3.pdf)
- In this presentation I will focus on results from **656 PowerPlex16** samples run with v4.1.3.

Data Comparison Between Methods

- Dave Duewer (NIST Analytical Chemistry Division) has written several computer programs to convert and compare FSS-i3 data that utilize Excel macros
  - **DNA\_FSSi3\_Convert.xls** (converts data format)
  - **STR\_MatchSamples.xls** (compares samples)
- These programs will be made available to the community after additional testing and refinement
  - <http://www.cstl.nist.gov/biotech/strbase/software.htm>

DNA\_FSSi3\_Convert.xls

First five columns in FSS-i3 output are converted to be like GeneMapper ID allele designation table

Batch ID	Sample ID	Locus ID	Major Designation 1	Major Designation 2	
PP16	Hispanic	GT37463	D3S1358	15	17
PP16	Hispanic	GT37463	TH01	6	9,3
PP16	Hispanic	GT37463	D21S11	28	
PP16	Hispanic	GT37463	D18S51	15	
PP16	Hispanic	GT37463	Penta E	7	19
PP16	Hispanic	GT37463	D5S818	9	13

Each row is an individual locus

Data Transformation

Sample	D3S1358	D3S1358	TH01	TH01	D21S11	D21S11	D18S51	D18S51	Penta E	Penta E
MT94892	14	15	6	9,3	29	32,2	15	15		
TT50701	14	16	6	9,3	29	30	14	19		
UT57287	16	16	6	6	28	30	12	15		
PT84244	14	15	7	9	28	30	15	18		

Each row is an individual sample

STR\_MatchSamples.xls

Summary Table:

#Samples	#ExactMatch	#Unmatched
95	67	28

Two or more data sets can be compared to one another

Creates a list of all samples that are fully concordant at all loci between the samples being compared

Similar to i-nTEGRITY in looking for samples with closest genotypes through comparing each sample to all others

Exact Matches (Full Concordance) Observed with STR\_MatchSamples.xls Program

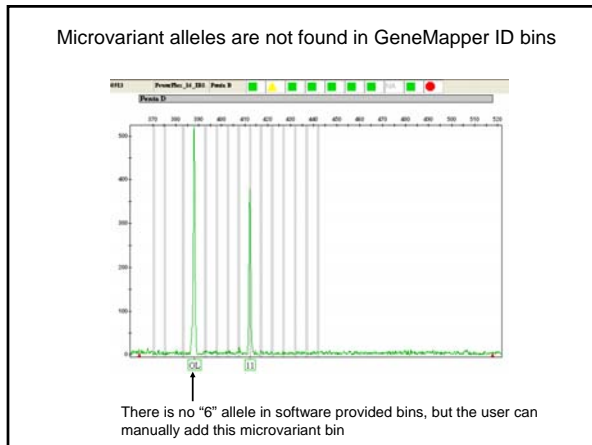
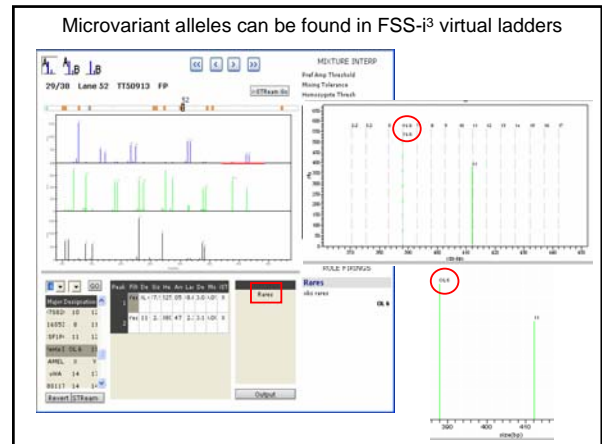
Type	Sample Description	Penta_D	Penta_E	TH01	TPOX	vWA
Unmatched	GT37019:all data	2,2,11	5,13	6,7	8,9	17
Unmatched	GT37019:all PP16 GM samples	2,2,11	5,13	6,7	8,9	14,17
ExactMatch	BC11352:all data, BC11352:all PP16 GM samples	10,11	7,12	6,9,3	8	14,17
ExactMatch	GA05070:all data, GA05070:all PP16 GM samples	13,14	7,17	7,9	8,12	14,19
ExactMatch	GA05071:all data, GA05071:all PP16 GM samples	10,11	11,12	7,9,3	8,11	16,17
ExactMatch	GC03394:all data, GC03394:all PP16 GM samples	10,11	12,15	6,7	8	17,18

- Unmatched sample type flags discordant calls
- ExactMatch sample type indicates full concordance between FSS-i3 and GeneMapper ID samples

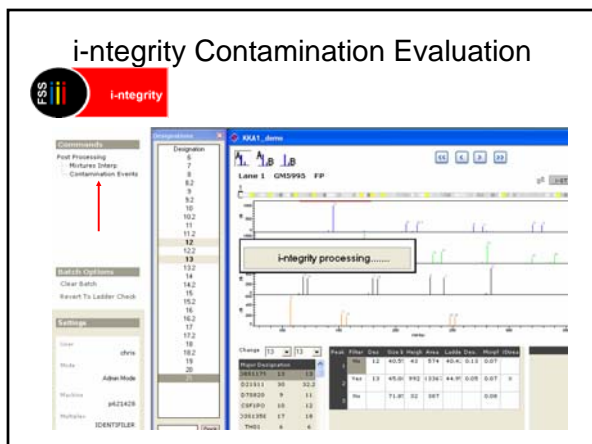
Concordance Evaluation

- PowerPlex16 data collected on ABI 3130xl; 656 samples processed in GeneMapper ID and FSS-i3
- Typed manually with GeneMapper ID
- Same data processed through GeneMapper ID/FSS-i3
- Results from **656 samples compared**
  - 613 samples matched with no data review
  - 43 pairs exhibited a mismatch with **unedited** FSS-i3 results
- Examination of mismatches to determine which rules were fired and if user would be able to make correct calls following editing: **All calls were concordant after careful review**

- If No Rules Fired, Were There Any Mistakes?
- Each genotype was carefully re-reviewed manually
  - **No discrepancies (discordance) were noted in calls based on rules set**
  - **This observation provides confidence that when no rules are fired, data quality is acceptable in the data sets reviewed thus far...**



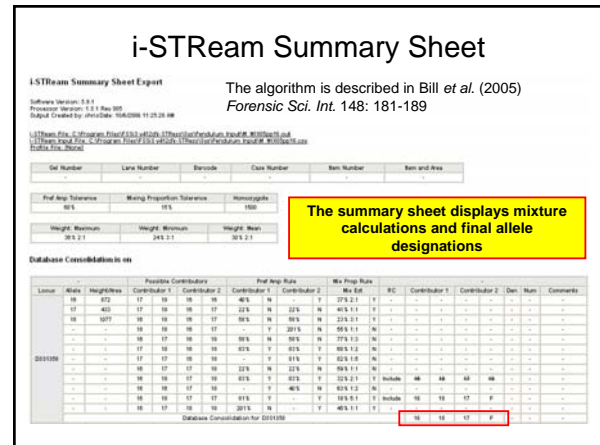
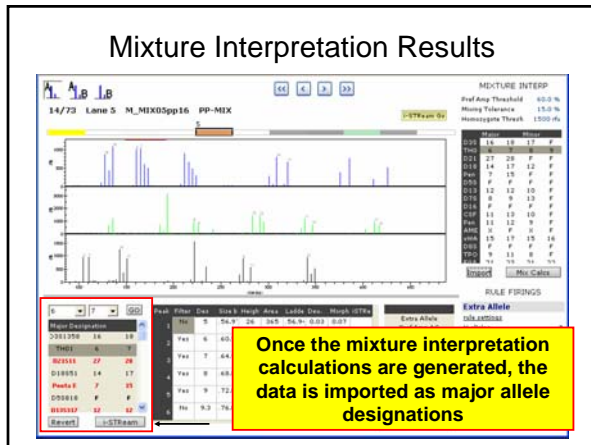
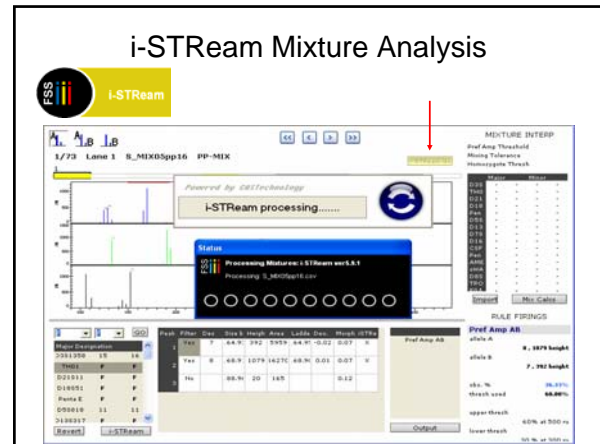
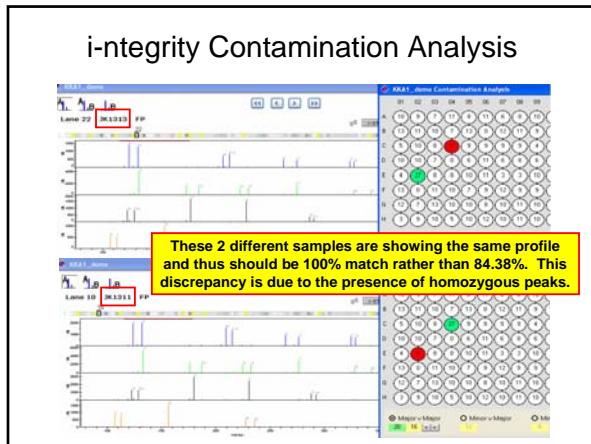
- ### Reviewing a Large Data Set
- Nice Features
- Rapid check of all allelic ladders and generation of composite allelic ladders
  - Rapid processing of data
- Cumbersome Features
- Having to click through every sample in order to review rule firings



### i-integrity Contamination Analysis

Lane 1	Barcode	Lane 2	Barcode	Match Count	Match %
13	OM6013	1	OM5995	18 / 32	56.25
22	JK1313	10	JK1311	27 / 32	84.38
50	GU8293	43	JK1021	16 / 32	50.00
51	JK107	44	JK1132	16 / 32	50.00
51	JK107	50	GU8293	17 / 32	53.13
62	GU8900	59	JK1464	16 / 32	50.00
69	JK1299	33	JK1290	16 / 32	50.00
73	OM8991	68	JK1138	17 / 32	53.13
75	JK116	63	JK108	16 / 32	50.00
85	OM871	29	JK842	16 / 32	50.00

These 2 samples are showing a high match % of 84.38, so we want to examine these samples further



- ### In Summary
- The FSS-i3 v4.1.3 upgrade is a vast improvement over v4.0.1
- Opens up capabilities for creating new multiplex kits (Y-filer, PowerPlex Y and custom assays)
  - Projects can be saved during data analysis sessions
  - i-STReam provides mixture results much more quickly
  - It is now possible to alter the plate layouts
  - Can edit the input and output templates

- ### Future Plans
- We plan to explore i-STReam capabilities further
  - We will run more data sets that are available at NIST
  - Release additional software tools on STRBase (Dave Duewer programs) soon
  - Publish recommendations on approaches for validation of expert system software



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Bob McLaren



**Training at Promega:**  
Bob McLaren  
Kim Huston  
Curtis Knox

[http://www.promega.com/profiles/901/Profiles/DNA\\_901\\_16.pdf](http://www.promega.com/profiles/901/Profiles/DNA_901_16.pdf)