Signature Science and 24plex — A Timeline

Dec 2013

Signature Science Became an Accredited Forensic DNA Laboratory

Dec 2017

24plex Kit Validation – Original Formulation

April 2020

24plex STRmix™ Validation – Original Formulation

June 2022

24plex Kit and STRmix™ Validations – New Formulation

July 2022

Casework using 24plex Halted

Oct 2022

24plex Performance Check – New Formulation Tighter Specification
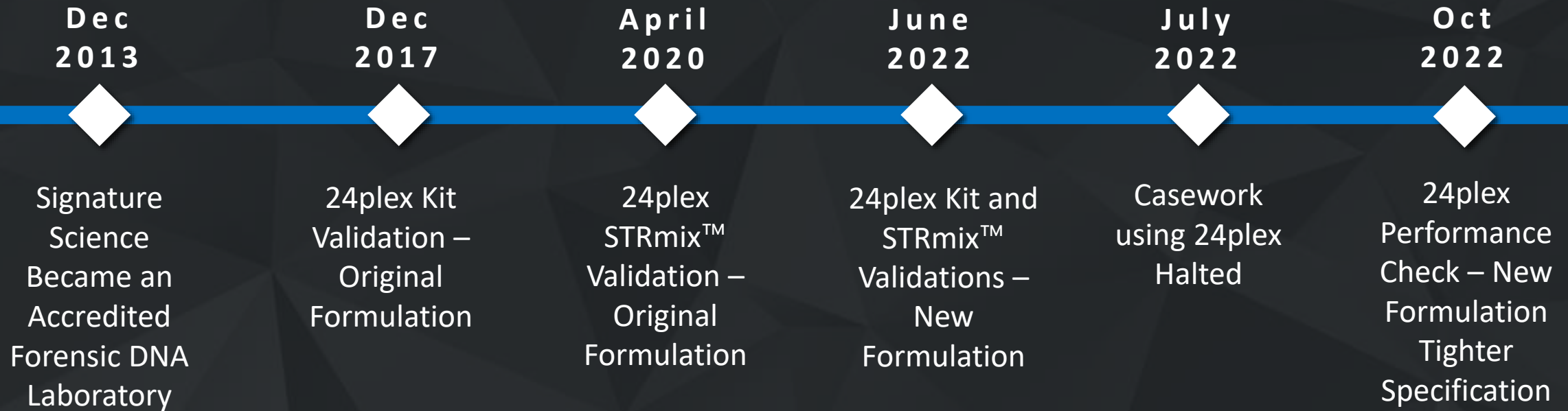
# QIAGEN Notification Memo — Investigator 24plex QS Kits, June 2021

Notification Memo - Investigator 24plex QS Kits

Dear valued QIAGEN customer,

We are writing to provide you with a final update regarding the changes to the Investigator 24plex QS Kit (100), Cat No 382415, and Investigator 24plex QS Kit (400), Cat No 382417, which was originally detailed in a QIAGEN Notification Memo dated December 2020. These changes were enacted to assist in alleviating customer reported -A at some loci:

- Final update regarding changes to 24plex kit to alleviate minus A
  - Increased final extension time
  - Increased concentration of dATP and Magnesium in reaction mix

- The Institute of Environmental Science & Research (ESR) recommended current 24plex QS/STRmix™ users to perform a performance check

Minus A in Original Formulation
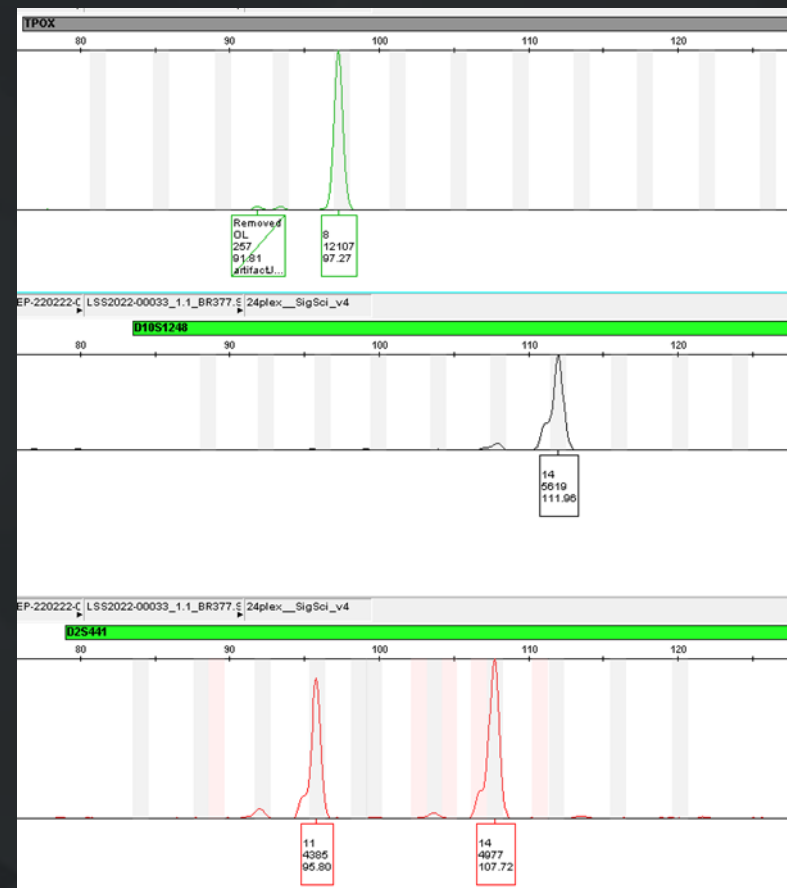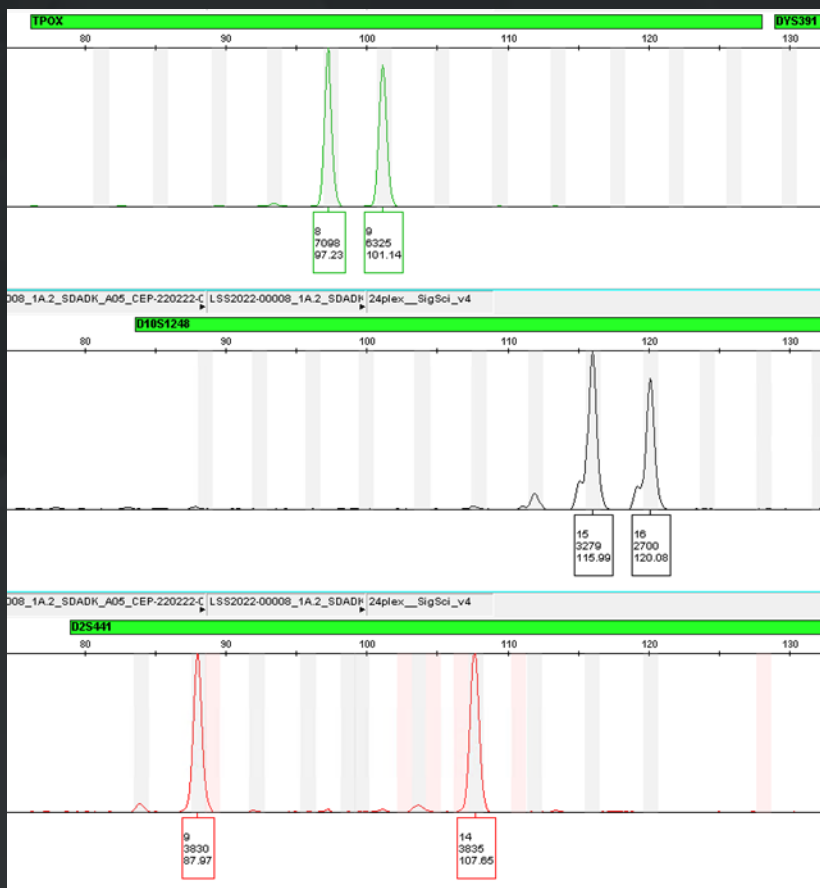
Performance Check Versus Validation

- Signature Science (SigSci) elected to perform additional validation efforts to assess the new formulation with an increase in PCR cycle as well as an additional CE injection assay

  - Increase from 29 to 30 cycles

  - Add an additional lower injection parameter (1.2kV/15 second)

Validation Efforts

- Kit Validation
  - Precision Study
  - Sensitivity and Stochastic Studies
  - Accuracy and NIST-traceability Study
  - Mixture Study
  - Concordance/Comparison to Original Procedures
  - Contamination Assessment

- Supplemental STRmix™ Validation
  - Two different model makers
    - 1.6kv/33 sec
    - 1.2kV/15 sec
 - Sensitivity and Specificity
    - Fifty mixtures
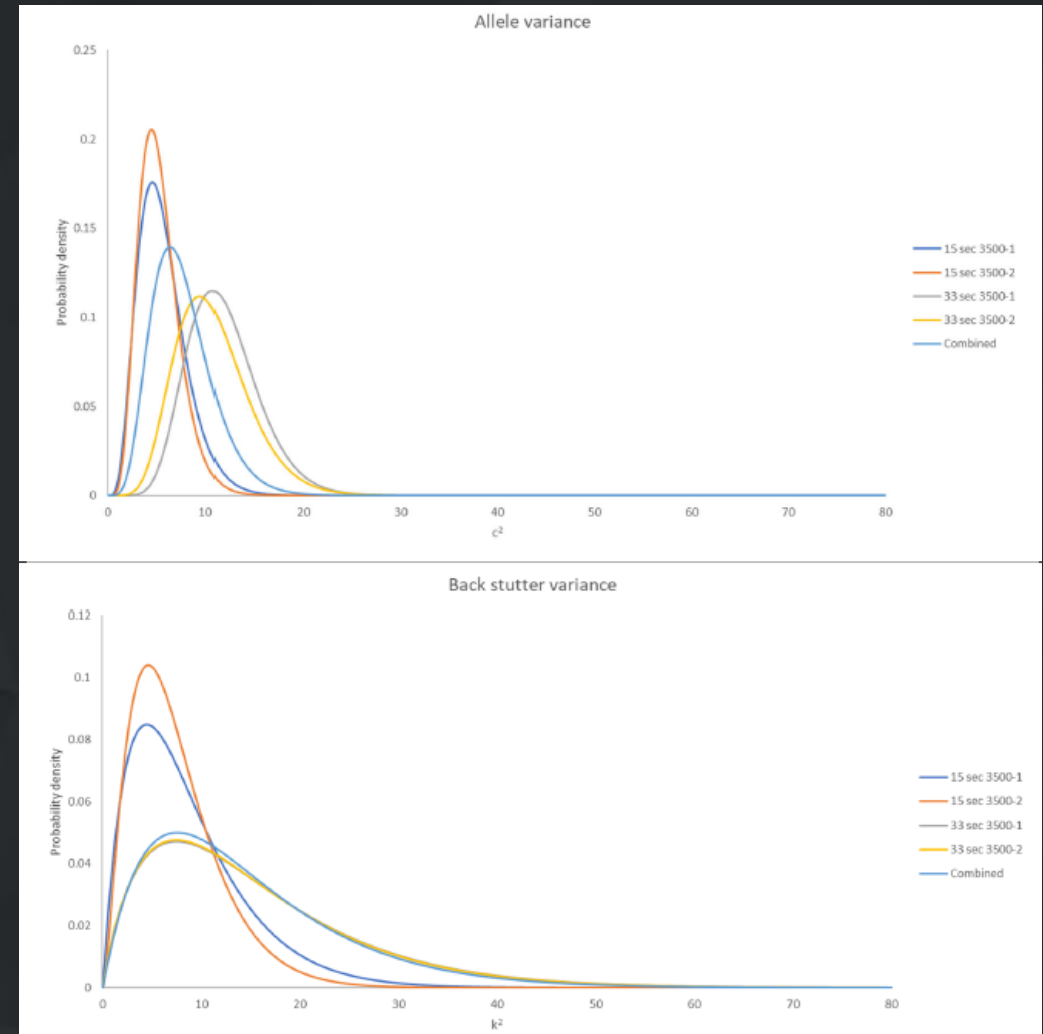
Unexpected Stutter Seen in Validation of New Formulation

- Three mixture samples had multiple locations where STRmix™ gave high weightings to what was known to be a stutter peak

- Increased iterations were utilized to attempt to resolve false exclusions/unintuitive likelihood ratios (LRs)
  - Aids in allowing other genotype weights to be explored

- Resolution occurred for two samples
  - LR falsely conservative due to low weightings as individuals did not align with elevated stutter peak(s)

- Remaining sample had locus with a per locus likelihood ratio of 0 ignored
  - Resolved false exclusion

Original 3-Person Validation Sample for New Formulation (10:5:1, K9:K4:K10)

D22 → K9=15,16/K4=15,16/K10=16,16
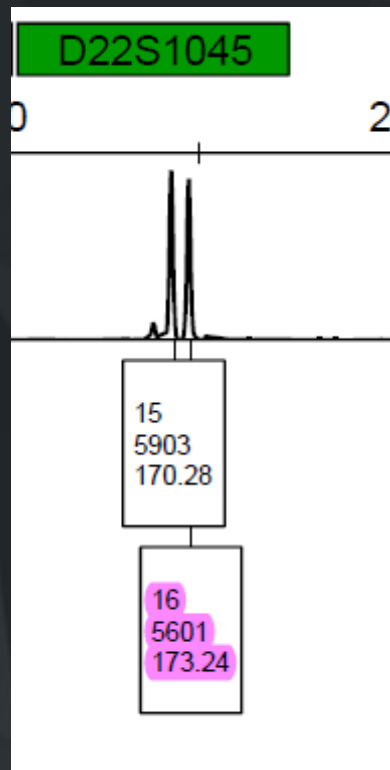
Contributor #3

Genotype Weights → 16,16 not there

D22S1045	14, 17	52.39%	14, F
	14, 16	17.97%	
	14, 15	17.37%	
	14, 14	10.44%	
	Q, 14	1.80%	
	17, 17	0.01%	
	16, 17	0.01%	
	15, 17	0.00%	
	15, 15	0.00%	
	15, 16	0.00%	

D22S1045 Back Stutter Example

- D22 uses allele regression as best explanatory variable for stutter

- For a 15 allele, STRmix™ is anticipating a 14 stutter peak to be around 6.5% of the 15, whereas in the actual sample it is 10.4%

D22S1045 Back Stutter Ratio ~ Allele

$y = 0.00988x - 0.08293$ $R^2 = 0.86200$ Locus Average = 0.06120
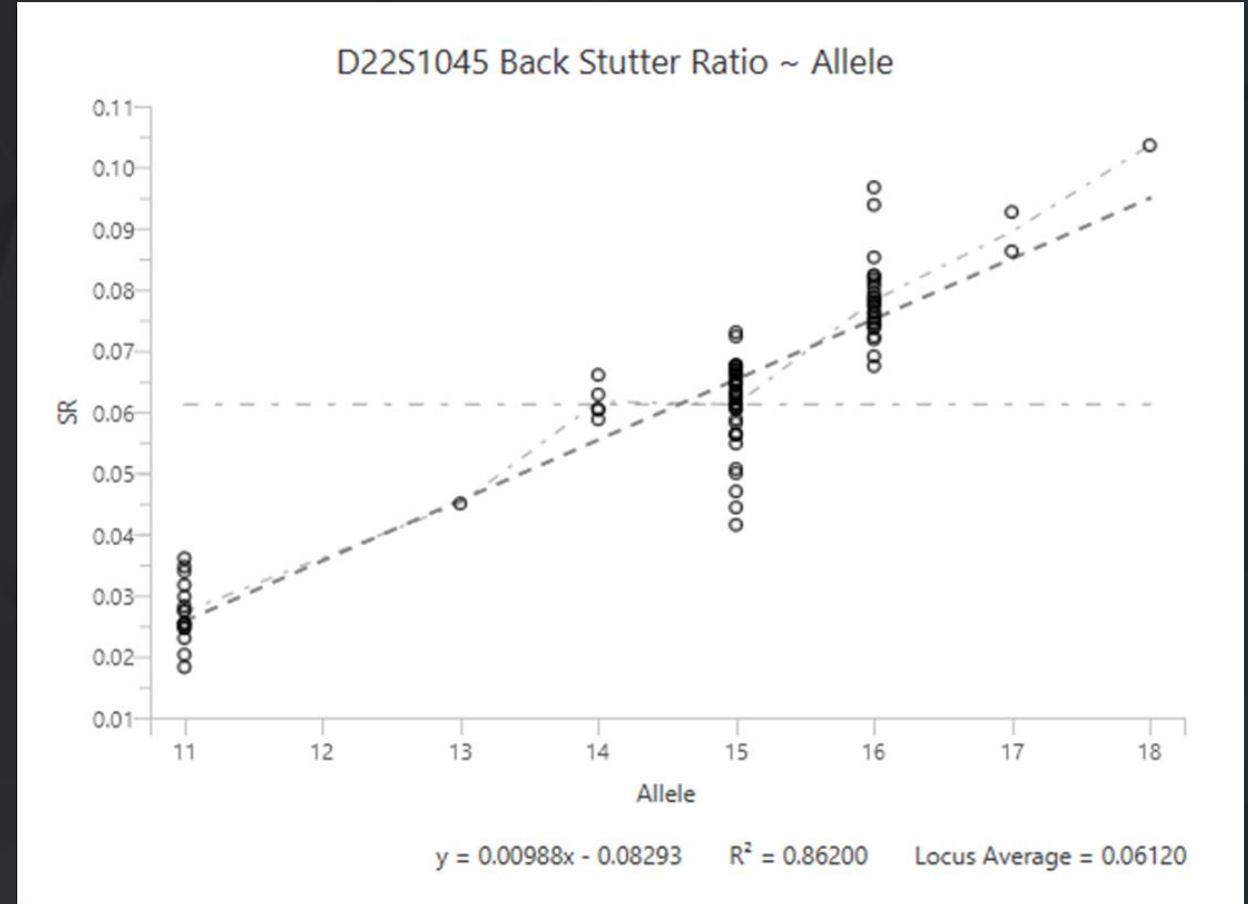
K10 Comparison Resulted in a LR=0

- D22S1045 was the only locus with a per locus LR of 0

- Most of the other loci resulted in a per locus LR greater than 1

- The sample was re-run using additional iterations
  - Low weighting for 16,16 (0.00%)
  - New LR=59.9 thousand
    - More intuitive given comparison of known to electropherogram

PER LOCUS LIKELIHOOD RATIOS

TABLE 1 OF 2

LOCUS	NIST1036_AFAM 0.01b(1.0, 1.0)			NIST1036_ASIAN 0.01b(1.0, 1.0)		
	Pr(E|Hp)	Pr(E|Hd)	LR	Pr(E|Hp)	Pr(E|Hd)	LR
TH01	8.10286E-4	3.32692E-4	2.43555E0	5.36031E-5	8.16211E-6	6.56731E0
D3S1358	7.93376E-5	5.35325E-5	1.48204E0	3.83493E-5	3.18594E-5	1.20371E0
vWA	1.21676E-3	1.13270E-4	1.07421E1	4.01955E-3	3.36179E-5	1.19565E2
D21S11	4.10716E-6	5.84535E-7	7.02637E0	2.48690E-6	7.42746E-7	3.34825E0
TPOX	1.17773E-6	6.27461E-5	1.87698E-2	5.21496E-6	7.24494E-6	7.19807E-1
DYS391						
D1S1656	7.43912E-5	1.83471E-4	4.05466E-1	4.04628E-6	1.02979E-5	3.92923E-1
D12S391	1.41478E-5	5.85424E-6	2.41667E0	1.86516E-5	1.02164E-5	1.82565E0
SE33	1.45182E-8	1.64432E-9	8.82930E0	9.66903E-8	3.39544E-8	2.84765E0
D10S1248	1.26764E-4	1.32804E-5	9.54523E0	2.66927E-4	3.67980E-5	7.25384E0
D22S1045	0	3.01067E-4	0	0	9.33329E-5	0
D19S433	1.31078E-4	5.91682E-5	2.21535E0	1.54007E-4	8.70968E-5	1.76823E0
D8S1179	4.73553E-6	2.26781E-6	2.08815E0	5.10914E-7	2.10920E-7	2.42232E0
D2S1338	2.88259E-5	1.26141E-5	2.28521E0	6.32028E-5	2.98943E-5	2.11421E0
D2S441	9.14904E-4	7.81417E-5	1.17083E1	8.41642E-4	4.93656E-5	1.70492E1
D18S51	6.08610E-5	7.92455E-6	7.68005E0	4.10023E-6	1.60588E-6	2.55326E0
FGA	1.98417E-5	8.76608E-6	2.26346E0	6.68358E-5	3.10959E-5	2.14934E0
D16S539	6.70678E-4	1.46410E-4	4.58082E0	3.59506E-4	6.05461E-5	5.93772E0
CSF1PO	7.85608E-4	2.01934E-4	3.89043E0	2.95981E-3	7.86501E-4	3.76327E0
D13S317	3.68283E-4	1.66841E-4	2.20739E0	8.67276E-7	2.27486E-7	3.81245E0
D5S818	3.88938E-4	1.87753E-4	2.07154E0	9.21456E-4	3.87433E-4	2.37836E0
D7S820	4.47459E-5	4.52766E-5	9.88278E-1	6.28627E-5	5.19351E-5	1.21041E0
LR TOTAL			0			0
FACTOR OF N! LR			0			0
99% 1-SIDED LOWER HPD INTERVAL			0			0

Evaluation of Problematic Samples

- Moved forward with completion of validation

- Advised by ESR to keep a close eye on casework and possible stutter remodeling may be needed in the future

- Started amplifying casework samples with the new formulation of 24plex in July 2022

- Prepared materials for analysts to go over in depth what was seen during the validation

QIAGEN Product Notification, July 2022

Product Notification Investigator 24plex QS Kit, cat nos 382415 and 382417

Dear valued customer,

In discussion with some of our customers, we have been made aware of varying levels of stutter formation between Investigator 24plex QS lots they received.

- Varying levels of stutter formation were discovered between different lots of 24plex

- Stutter levels observed were not in line with the stutter model established previously for STRmix™ analysis

- A raw material used for production of Fast Reaction Mix 2.0 was found to be the cause

- A tighter specification was being made

QIAGEN Update on Stutter Variation Observed with STRmix™ and Investigator® 24plex QS Kits, August 2022

Update on stutter variation observed with STRmix™ and Investigator® 24plex QS Kits

Affected products: Cat. Nos. 382415 and 382417

Dear valued customer,

We recently wrote to inform you of reports of some STRmix customers observing varying levels of stutter formation between Investigator 24plex QS lots they received. We would like to provide an update following a joint investigation by ESR and QIAGEN.

- Confirmed impurity found in raw material was root cause for issue

- ESR is working on providing guidance about whether laboratory data will be impacted

- The variability was only observed between lots and not within lots

ESR Impact of Investigator® 24plex QS Lot Variation on Stutter Rates and STRmix™ Performance, August 2022
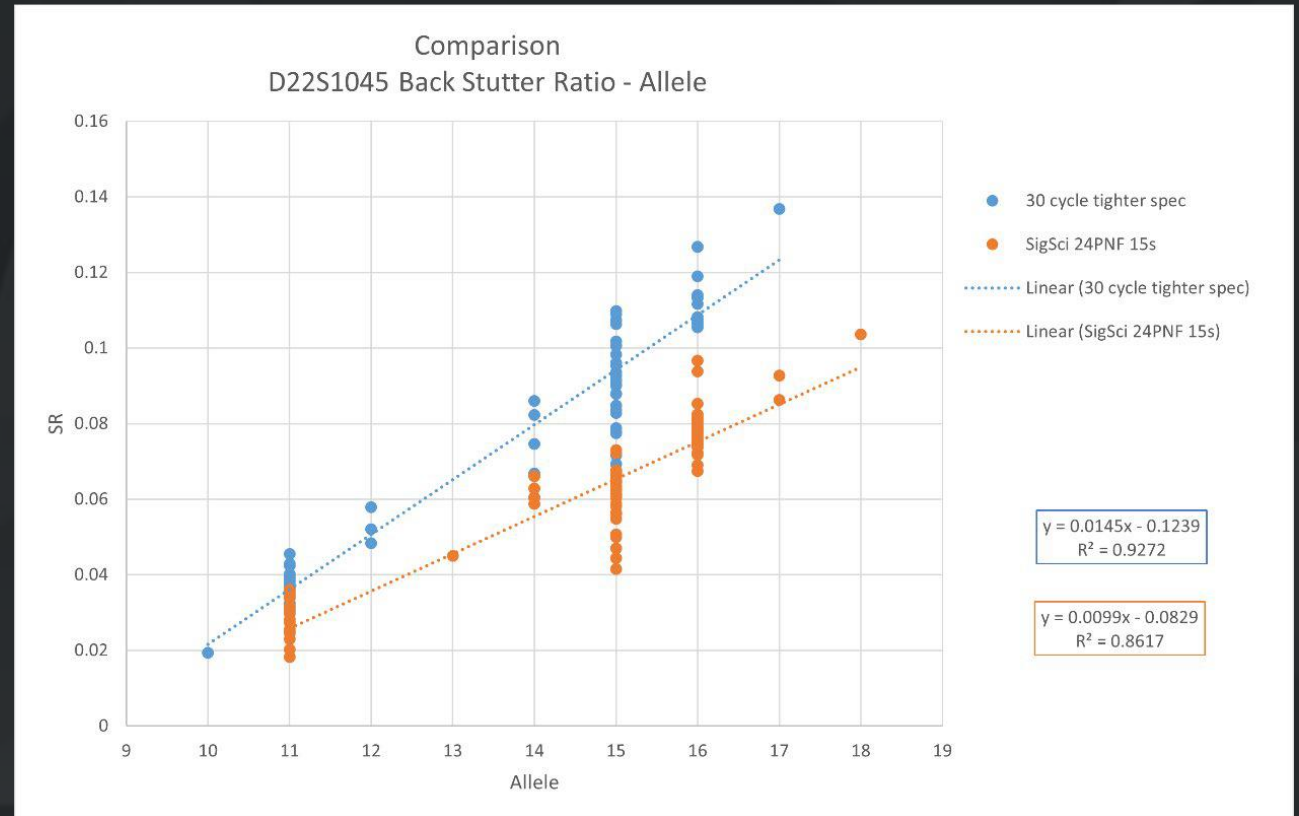
- Unexpected peak modeling found by Suffolk County Crime Lab in June 2022

- Counterintuitive minor component genotype weights were seen for samples with minor contributors who had peak heights similar in size to stutter products

- Recommended to compare current lots of 24plex with 24plex manufactured with tighter raw material specification, with special attention to the D22S1045 locus

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Impact of Investigator® 24plex QS lot variation on stutter rates and STRmix™ performance

# Comparison of Stutter Between 30 Cycle SigSci Validation Data and 30 Cycle Tighter Specification data

- Comparative data was provided by ESR as original 24plex validation done using 29 cycles

- While slight differences were observed at other loci, D22S1045 had the most dramatic difference

- The differences observed between the regression lines for D22S1045 could lead to the expected stutter height for some peaks being quite different to the observed height

Comparison
D22S1045 Back Stutter Ratio - Allele

- 30 cycle tighter spec
- SigSci 24PNF 15s
- Linear (30 cycle tighter spec)
- Linear (SigSci 24PNF 15s)

$y = 0.0145x - 0.1239$
$R^2 = 0.9272$

$y = 0.0099x - 0.0829$
$R^2 = 0.8617$

Results of Stutter Evaluation

- Address Minus A issue present in casework samples

- Create new stutter ratio modeling using samples generated with the tighter specification of 24plex

- Re-do Model Maker incorporating the new stutter files followed by a performance check of these changes
    - Re-evaluated stochastic threshold and stop-at-quant/male screening thresholds
    - Re-ran problematic samples from validation using new stutter model to see if resolution occurred
    - Created new mixtures to evaluate

# Minus A Noted in Casework Samples Using New Formulation
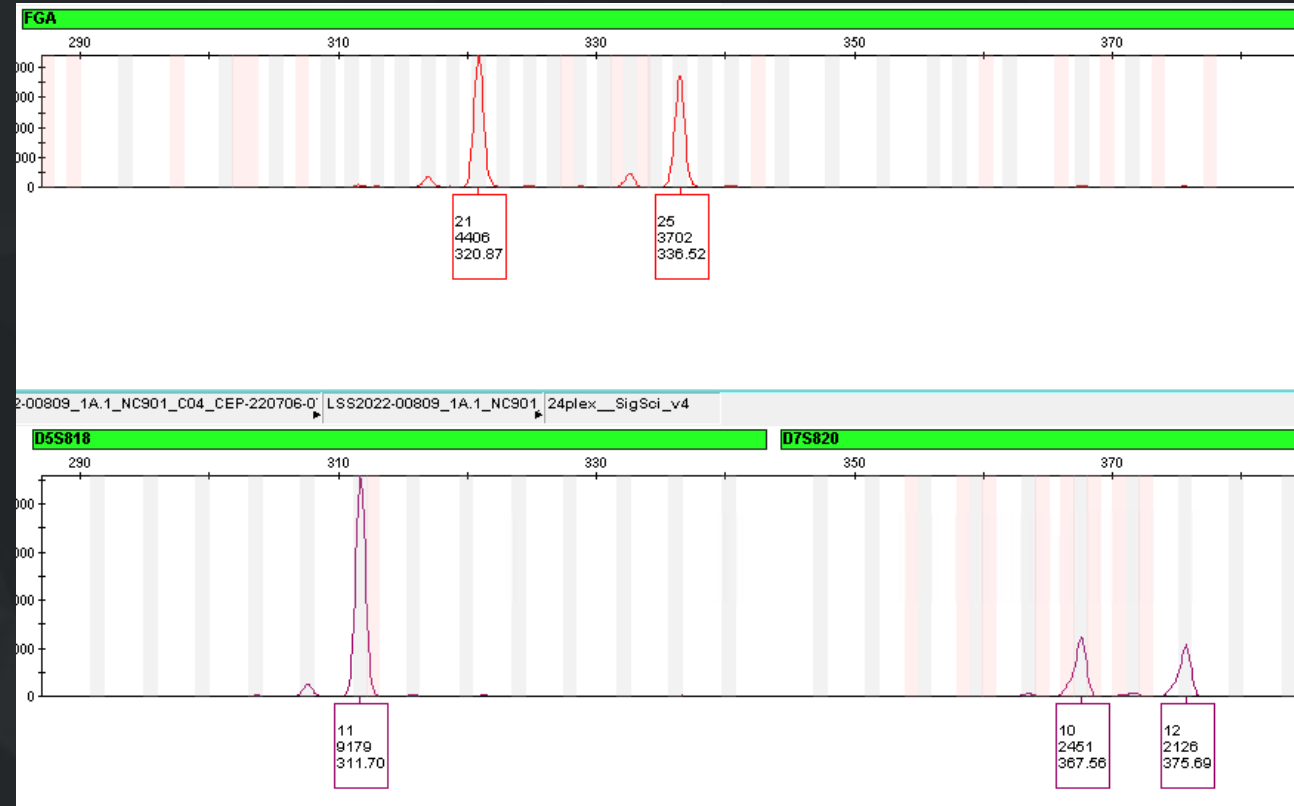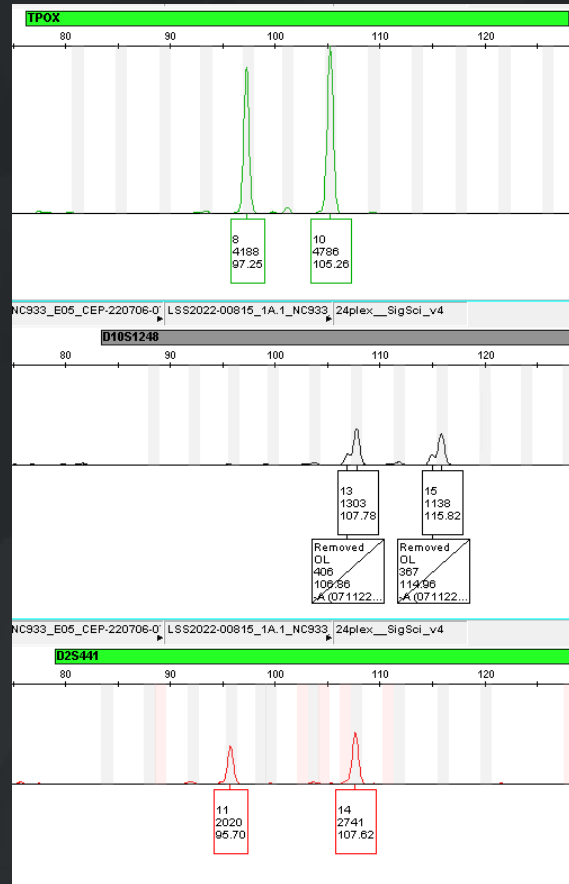
Resolution of Minus A

- Advised by QIAGEN to:
  - Remove Master Mix components from storage immediately before Master Mix is made and returned immediately thereafter
  - Utilize a frozen plate block from freezer and place 96-well plate onto it for amplification set-up
  - Lower than expected peak heights were sometimes still seen at D7
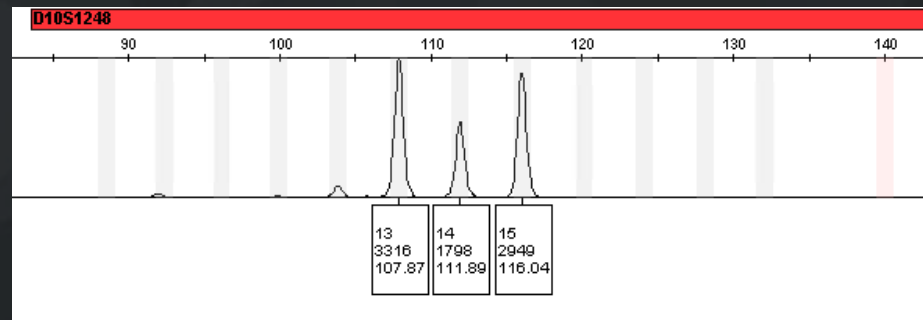
Comparison of Stutter Allele Regression at D22S1045
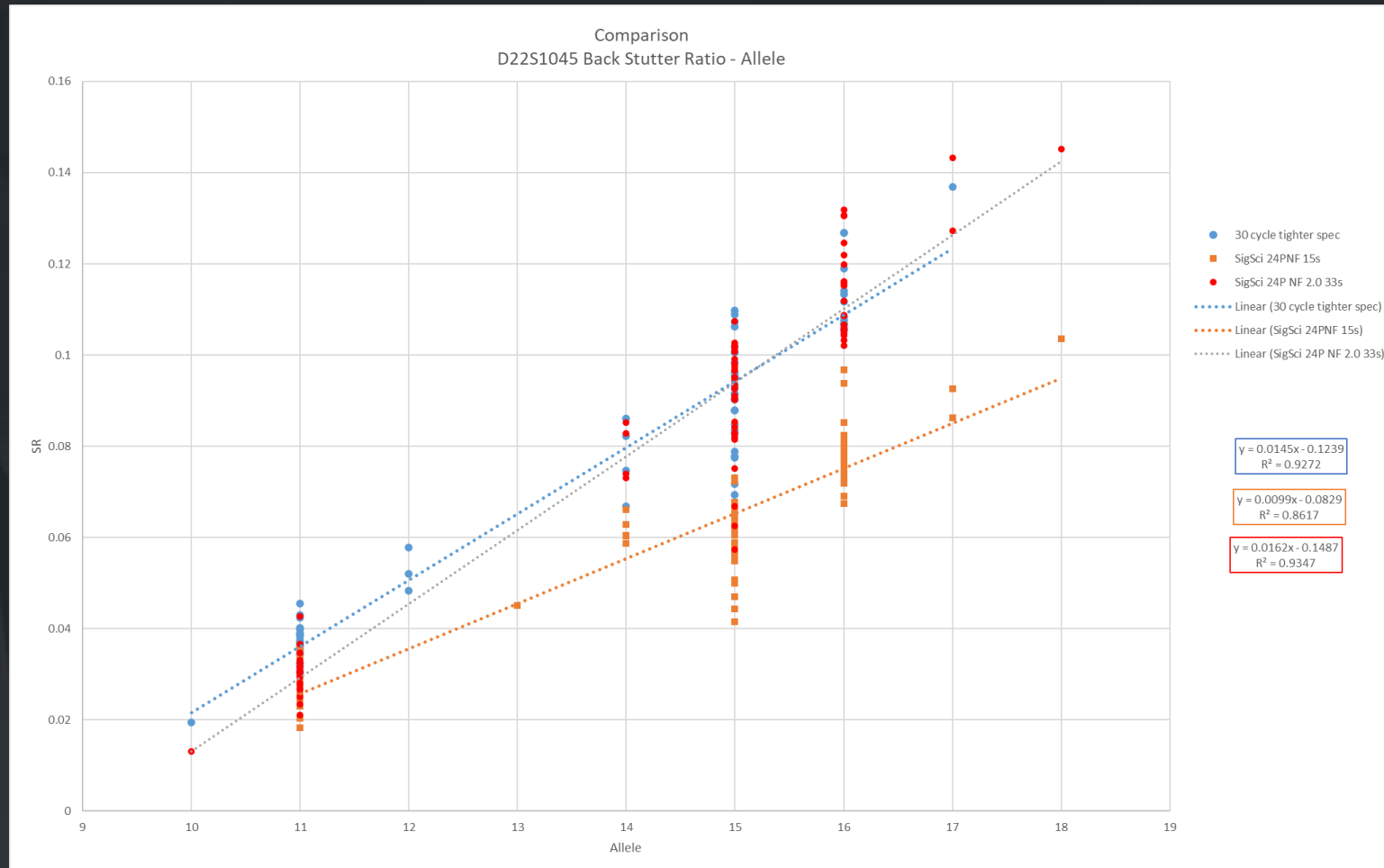
Original 3-Person Validation Sample (10:5:1, K9:K4:K10) Versus Same Sample Re-Run Using New STRmix™ Model

Contributor #3 Original
Genotype Weights → 16,16 not there

D22S1045	14, 17	52.39%	14, F
	14, 16	17.97%	
	14, 15	17.37%	
	14, 14	10.44%	
	Q, 14	1.80%	
	17, 17	0.01%	
	16, 17	0.01%	
	15, 17	0.00%	
	15, 15	0.00%	
	15, 16	0.00%	

Contributor #3 Re-run
Genotype Weights → 16,16 top choice

D22S1045	16, 16	20.38%	F, F
	15, 16	20.05%	
	15, 15	17.22%	
	16, 17	12.52%	
	15, 17	10.74%	
	14, 16	3.73%	
	14, 15	3.46%	
	17, 17	2.91%	
	14, 17	2.23%	
	Q, 16	2.05%	
	Q, 15	1.89%	
	Q, 17	1.49%	
	Q, 14	0.48%	
	Q, Q	0.43%	
	14, 14	0.42%	

Performance Check Completion

- Two batches of casework (~90 cases) had to be reamplified using the tighter specification of 24plex

- Analysts told to still pay close attention to intuitiveness of the LR generated for a person of interest
  - Thorough manual comparison of individual to EPG as well as contributor assignment/genotype weights
  - Close evaluation of all per locus LRs with special attention to any per locus LR that equals 0

Current status

- Casework utilizing 24plex has been successfully re-established using the tighter specification of the New Formulation of 24plex

- Minus A issue resolved at D10

- As lower than expected peak heights were sometimes still seen at D7 during the performance check, analysts were told to ignore the locus if issues arise during STRmix™ analysis or perform rework
  - This has not been needed to date

Questions?

Samantha Wandzek

swandzek@signaturescience.com

Special thanks to QIAGEN and ESR for continued support during the transition to the new formulation of 24plex