

# NGMSE the Irish Experience

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23<sup>rd</sup> May 2014



# Multiplex History

- SGM (1999)
- SGM+ (2006)
- Moved to NGMSE (2013)

# Preparing for Database

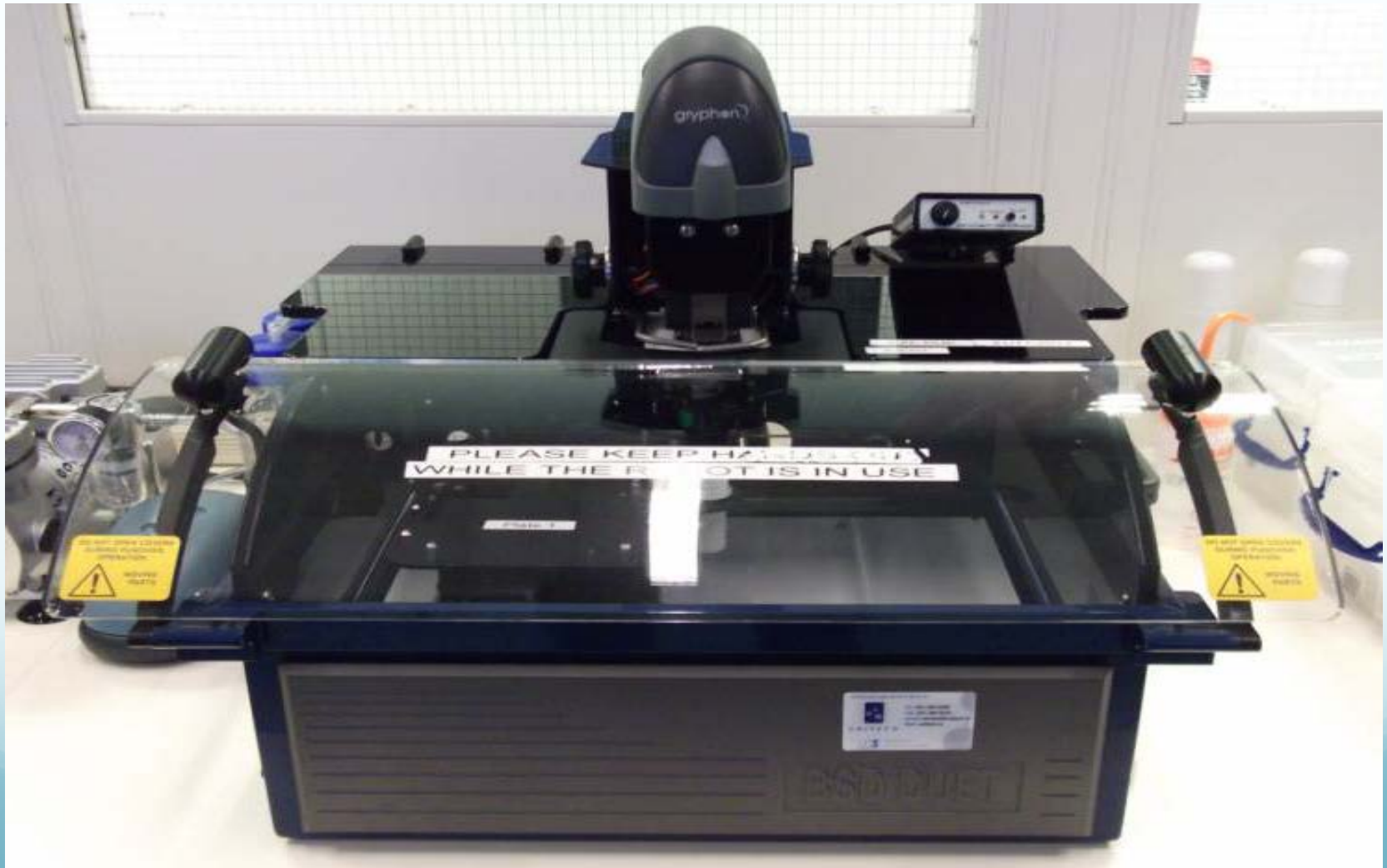
- NGMSE part of major changes
- FTA, Robotics (Tecan, Hamilton, EZ1)
- 3500



# FTA Card after transfer of Buccal Cells



# Puncher to punch a 1.2mm disc



# Robotics for Buccal Cell DNA profiling



# Manual DNA extraction with EZ1



# Fully automated Crimestain DNA profiling



# Fully automated Capillary Electrophoresis set up



## **Hamilton Star:**

- Lysate purification with magnetic beads; Qiasymphony
- Quantification set up; Quantifiler Duo
- Amplification set up; NGMSE

## **Hamilton Starlet:**

- CE set up for 3130 XL and 3500XL

# 17plex

- Tender for the supply of STR kits
- Life Technologies/Promega
- Life Technologies won tender on price and the possibility of direct amplification

# Validation

- SWGDAM and ENSFI Guidelines
- NGMSE vs SGM+
- 3500 vs 3130

# Validation results

- 1ng input, 29 cycles (FTA refs, 26 cycles)
- Amp volume 25 $\mu$ l
- Dilution sensitivity done to 10pg
- EZ1 was more sensitive than the Hamilton at low DNA samples.

# Dilution results

- 11/12 full profiles with 250pg (1=31/32)
- 8/12 full profiles with 125pg (3= 31/32)
- Average 27/32 with 100pg
- Big reduction at 50pg

# Validation Observations

- Stutter comparable to Life Technologies
- Upper stutter more apparent than Life Technologies
- Pull up levels deemed acceptable
- Average intra- and inter-loci balance is  $>60\%$
- However intra-loci balance values of  $< 60\%$  observed approx. 3% of the time
- Analysis thresholds

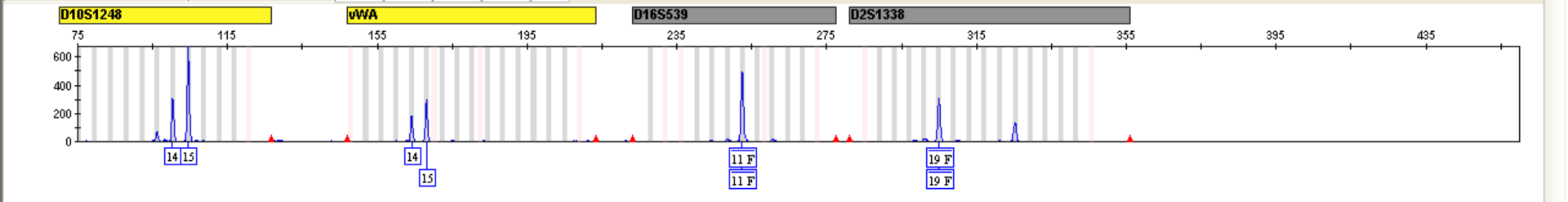
# Analysis Thresholds

3130		3500
Homozygous	270rfu	560rfu
Heterozygous	50rfu	175rfu

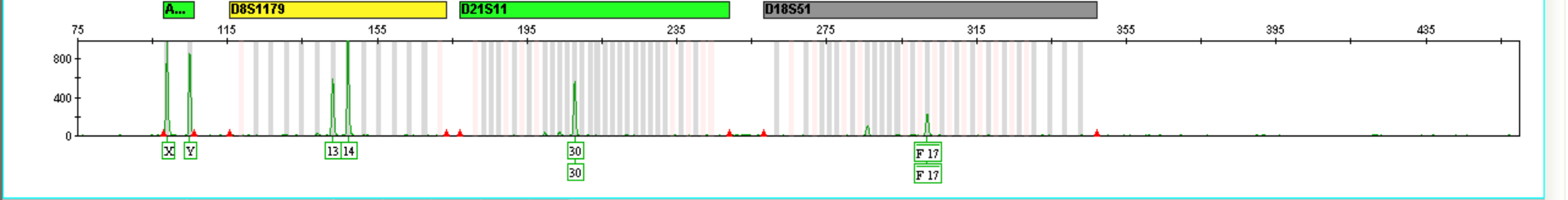
Plot Setting: Trad. Genotype Plot\_New Kits Panes: 4

Sample Name Panel SQ SSPK MIX OMR CGQ

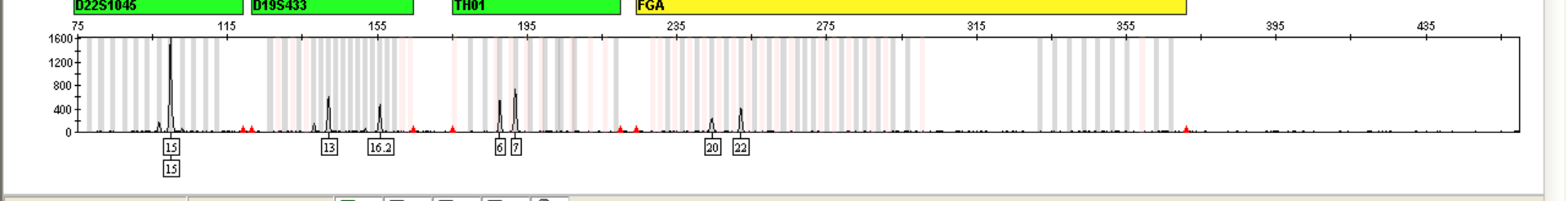
S14200631 NGMSElect\_panel\_v2X  Mark Sample for Deletion



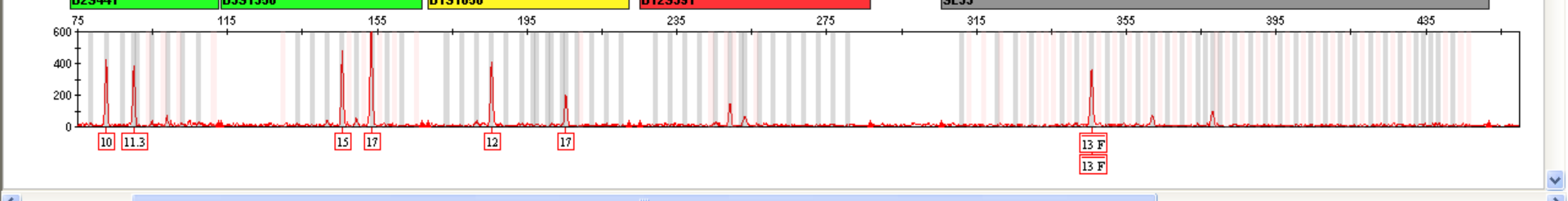
S14200631 NGMSElect\_panel\_v2X  Mark Sample for Deletion



S14200631 NGMSElect\_panel\_v2X  Mark Sample for Deletion



S14200631 NGMSElect\_panel\_v2X  Mark Sample for Deletion

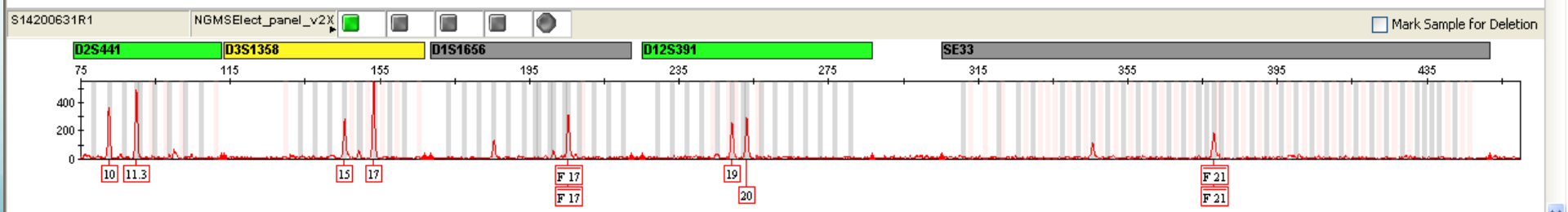
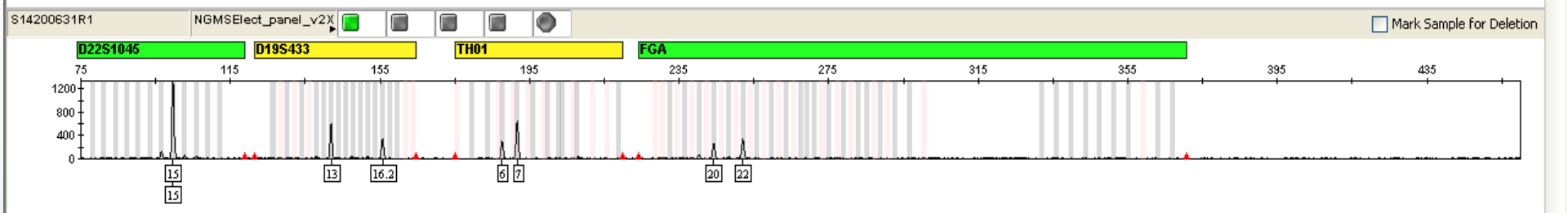
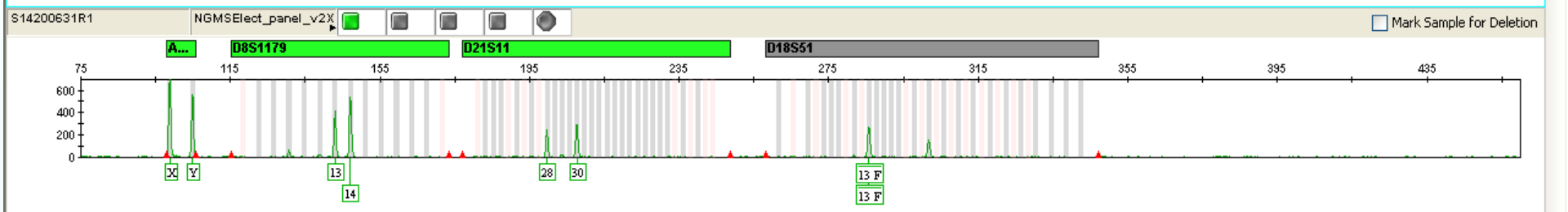
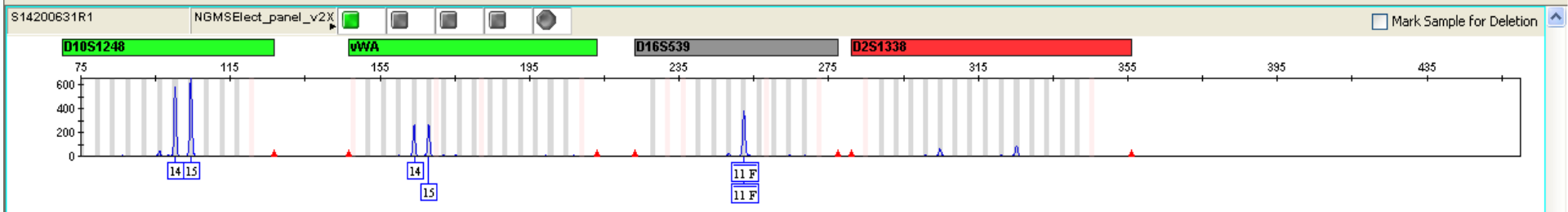


Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
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B-1	S14200631_05032			35.44	1601	14908	2513
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Plot Setting: Trad. Genotype Plot\_New Kits Panes: 4

Sample Name	Panel	SQ	SSPK	MIX	OMR	CGQ
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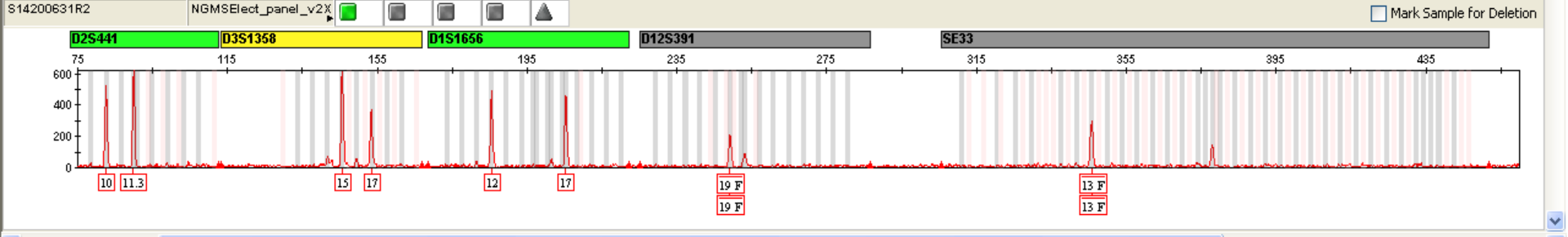
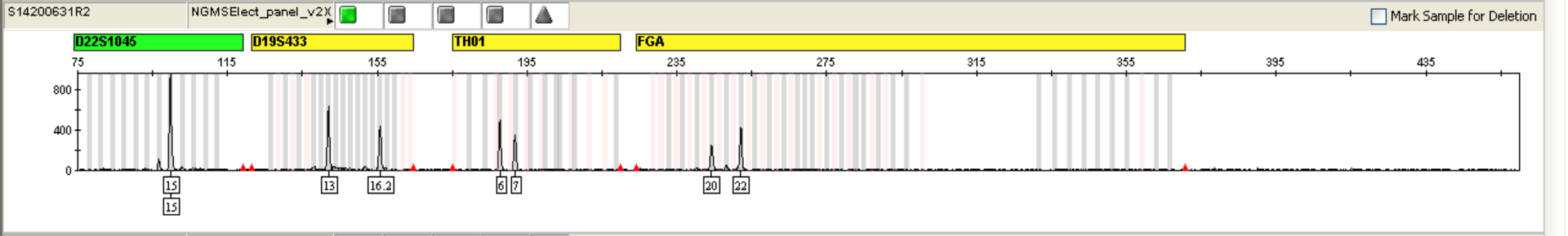
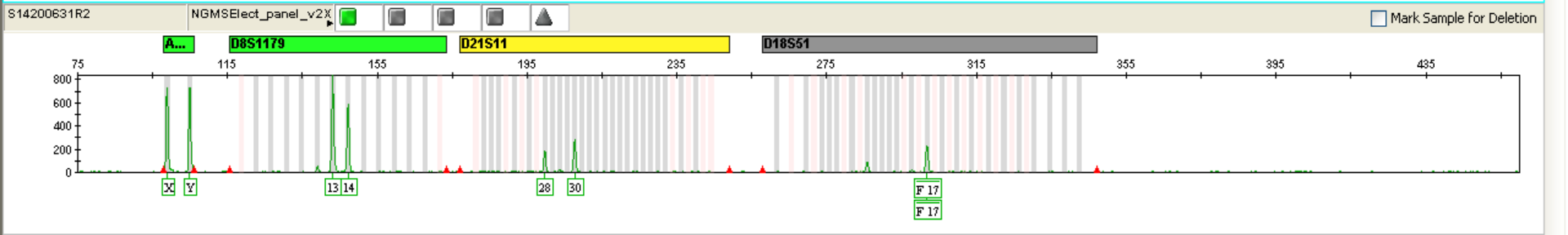
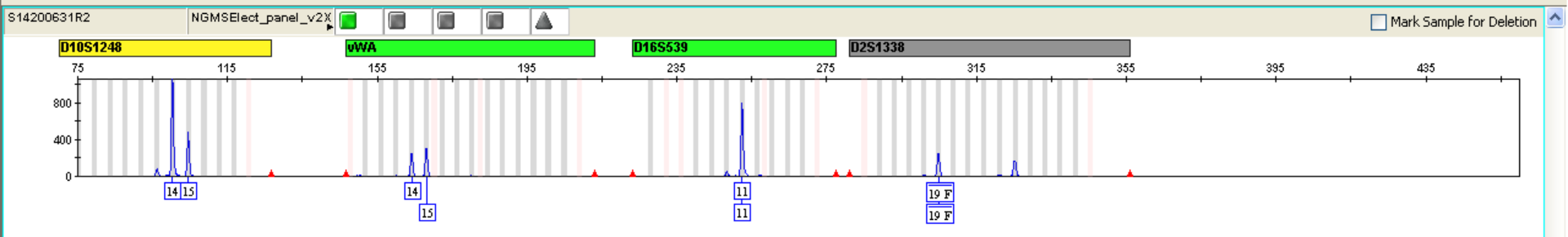


Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
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B,1	S14200631R110C			30.96	499	6448	2512
B,2	S14200631R110C			35.34	368	2900	2560
B,3	S14200631R110C			36.44	498	2479	2572

Plot Setting: Trad. Genotype Plot\_New Kits | Panes: 4

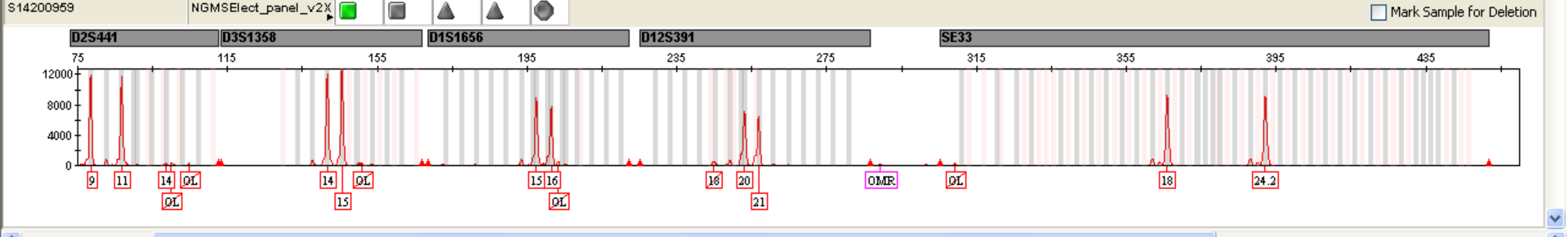
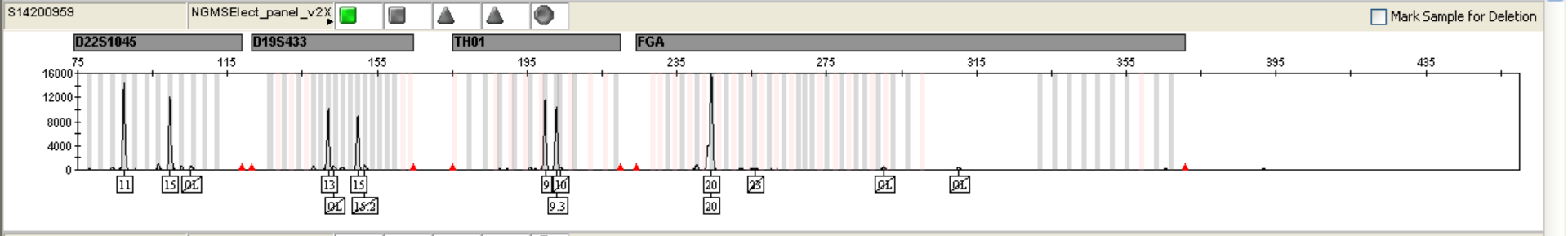
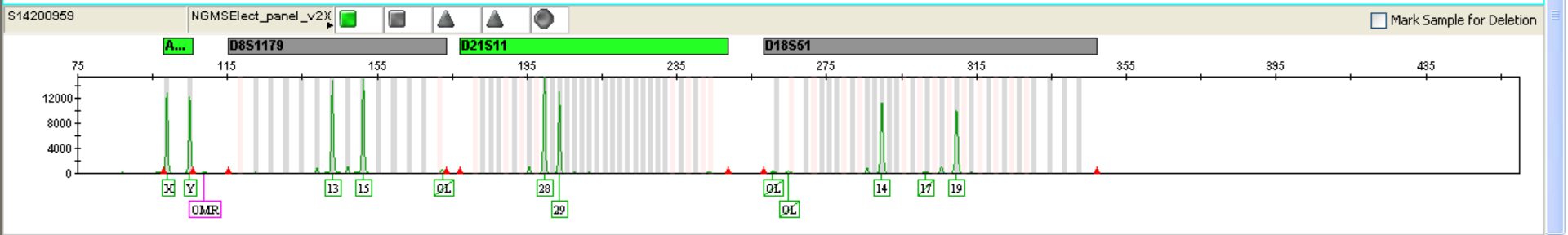
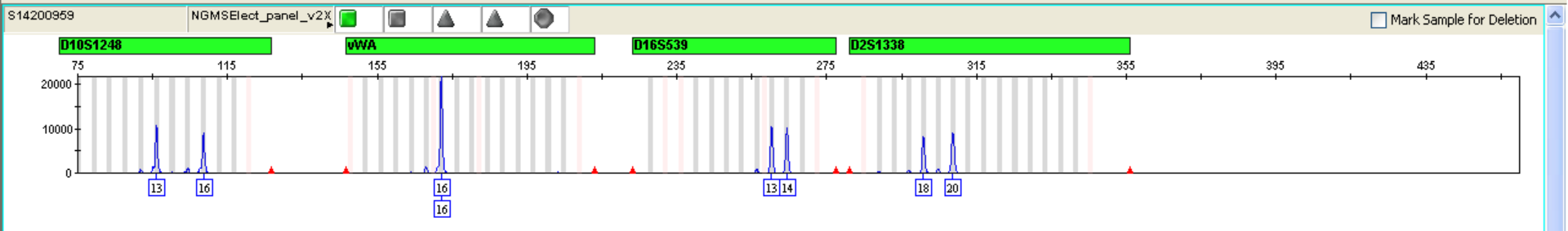
Sample Name | Panel | SQ | SSPK | MIX | OMR | CGQ



Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
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# Casework Observations

- Stutter and pull up more apparent
- Peak imbalance a problem when looking at mixtures



Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
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# Environmental DNA

- Increased cleaning
  - Microsol to Virkon
- Increased awareness of ‘non-lab’ DNA
  - Initial submission site, outside surface of bags

# Results for 'touch' profiles

	<b>Full/Partial (Single)</b>	<b>Mixture (major/minor)</b>	<b>Not Interpreted</b>	<b>Insufficient</b>
<b>Sample Num.</b>	21	16	95	133
<b>%</b>	8	6	36	50

# Minitapes of worn items (hats, gloves, balaclavas)

<b>%</b>	<b>Single</b>	<b>Major/minor</b>	<b>Not interpreted</b>	<b>Insufficient/negative</b>
<b>SGM+</b>	30	5	10	55
<b>NGM SE</b>	10	50	40	0

# Negative Consequences

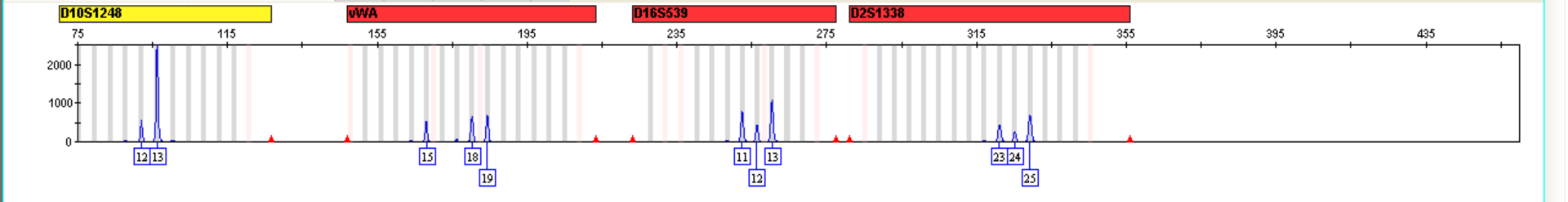
Big increase in time needed for analysis and interpretation  
...due to

- Increased detection of low level contributors
- General increase in mixture detection
- Peak imbalance, stutter, pull up

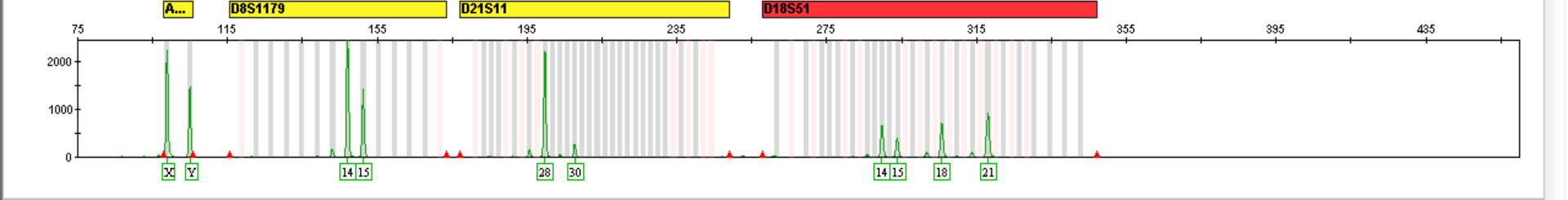
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Sample Name Panel SQ SSPK MIX OMR CGQ

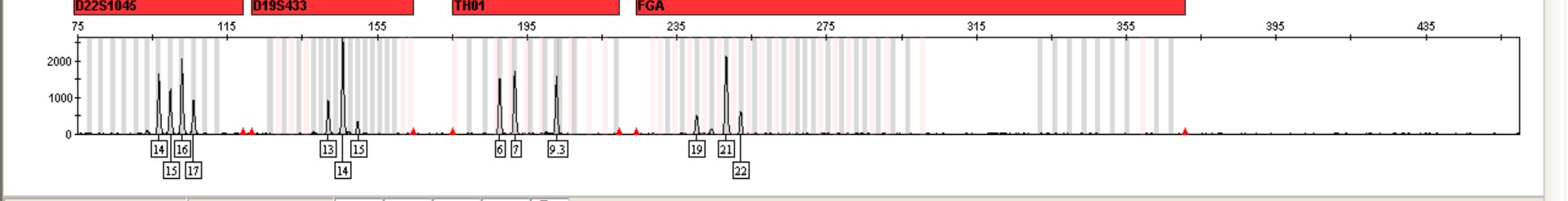
75(B3627R1) NGMSElect\_panel\_v2X  Mark Sample for Deletion



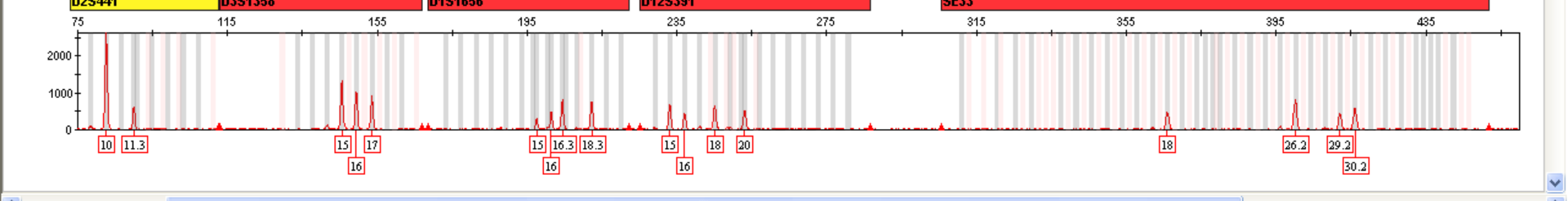
75(B3627R1) NGMSElect\_panel\_v2X  Mark Sample for Deletion



75(B3627R1) NGMSElect\_panel\_v2X  Mark Sample for Deletion



75(B3627R1) NGMSElect\_panel\_v2X  Mark Sample for Deletion



Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
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# Future Needs

Tools for interpretation of more complex mixtures

- RMP
- Classical LR
- RMNE
- LRmix/STRmix ?

For Body Identification

- POPSTATS

# Positive Consequences

**More profiles!**

# LIMS: Tender awarded in 2012



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