



Detection and Analysis of DNA Mixtures With the MiSeq[®] FGx[™]

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Disclaimer

- There is no real or apparent conflicts of interest related to the content of this presentation.

Overview

- **Goal** – Use next generation sequencing (NGS) to detect challenging mixtures
- **Methods** – Produce data sets on the MiSeq FGx[®] testing sensitivity, concordance, and mixture detection using ForenSeq[™] DNA Signature Prep

Background

- Detection and deconvolution of mixtures is an ongoing focus of research and training in forensic laboratories
- Mixtures represent a crucial portion of casework
 - Sexual assault kits
 - Touch DNA samples
 - Property crime
- Many NGS chemistries commercially available
 - Potential benefits over capillary electrophoresis (CE)

NGS and Mixtures

NGS has many potential advantages

- Enhanced sensitivity
- Detection of sequence based Isoalleles
- More markers

Disadvantage

- No probabilistic genotyping software available

Binary Mixture Deconvolution

Capillary Electrophoresis



The screenshot shows the ARMEDXPert software interface for a binary mixture deconvolution analysis. The main window displays the following information:

- File:** 2800M-NIST A, 1-3_R702-A504_S12_L001_R1_001.fastq
- Locus:** D4S2408 (2)
- Alleles:** 8 UO, 9 ZI
- RFUs:** 496, 746
- BPs:** 46, 50
- Contributor #:** 2
- Operations:** Highest to lowest #, Ignore alleles below mPH, Lock locus on report
- Parameters:** PHr 0.50, mPH 50, mP 0.00, Multi PHr, HT 300
- Buttons:** Popout calls, View call report, Add Comment
- Peaks:** 8 UO (496), 9 ZI (746)
- Mixture Information:** All combinations have: PHr >= 0.5, MPh >= 50, mP >= 0
- Deconvolution Results:**
 - For a 2-contributor 2-allele mixture of types AA & AB: 2/2-combination(s):
8 UO, 9 ZI(phr = 0.60; p = 0.96) • 8 UO(p = 0.04) [24 : 1]
8 UO, 9 ZI(phr = 1.00; p = 0.80) • 9 ZI(p = 0.20) [4 : 1]
 - For a 2-contributor 2-allele mixture of types AA & BB: 1/1-combination(s):
9 ZI(p = 0.60) • 8 UO(p = 0.40) [1.5 : 1]
 - For a 2-contributor 2-allele mixture of types AB & AB: 1/1-combination(s):
8 UO, 9 ZI(phr = 0.66) • 8 UO, 9 ZI(phr = 0.66)

Next Generation Sequencing

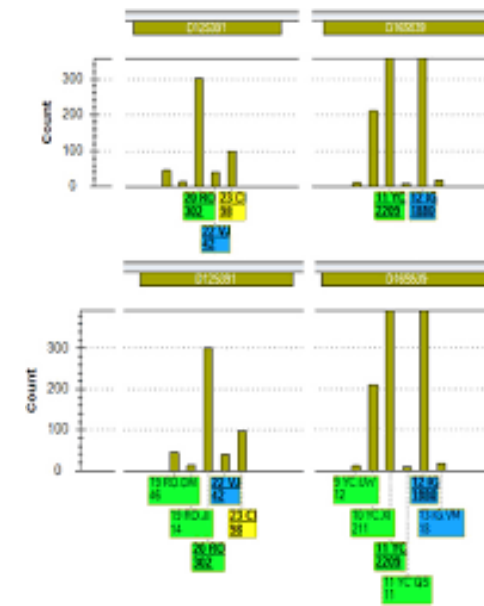
The image shows the cover of the journal "Forensic Science International: Genetics". It features the Elsevier logo on the left, the journal title in the center, and the ScienceDirect logo on the right. The journal homepage is listed as www.elsevier.com/locate/bscgen.

Research paper

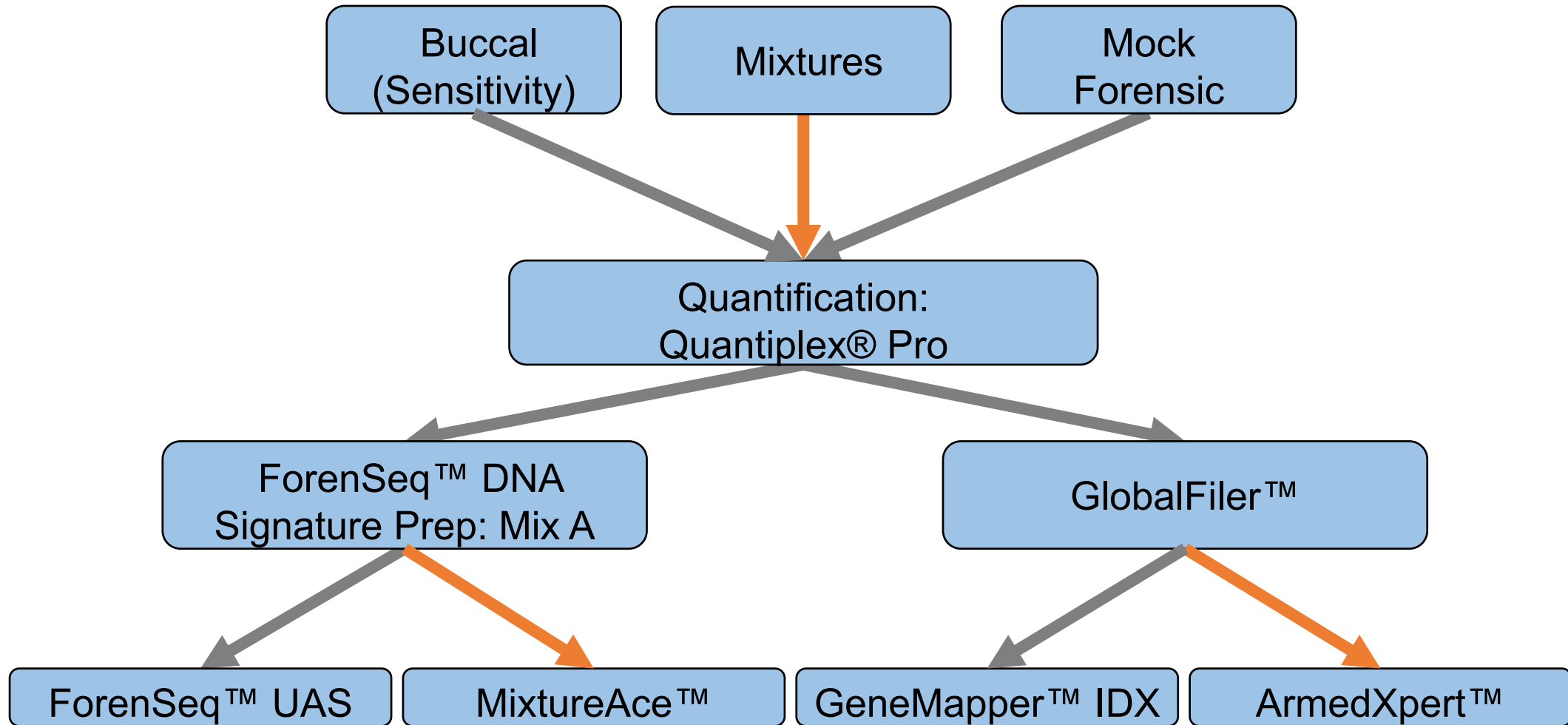
Evaluation of ArmedXpert software tools, MixtureAce and Mixture Interpretation, to analyze MPS-STR data

Vishakha Sharma^a, Brian Young^b, Luigi Armogida^b, Amber Khan^c, Elisa Wumbach^{a,*}

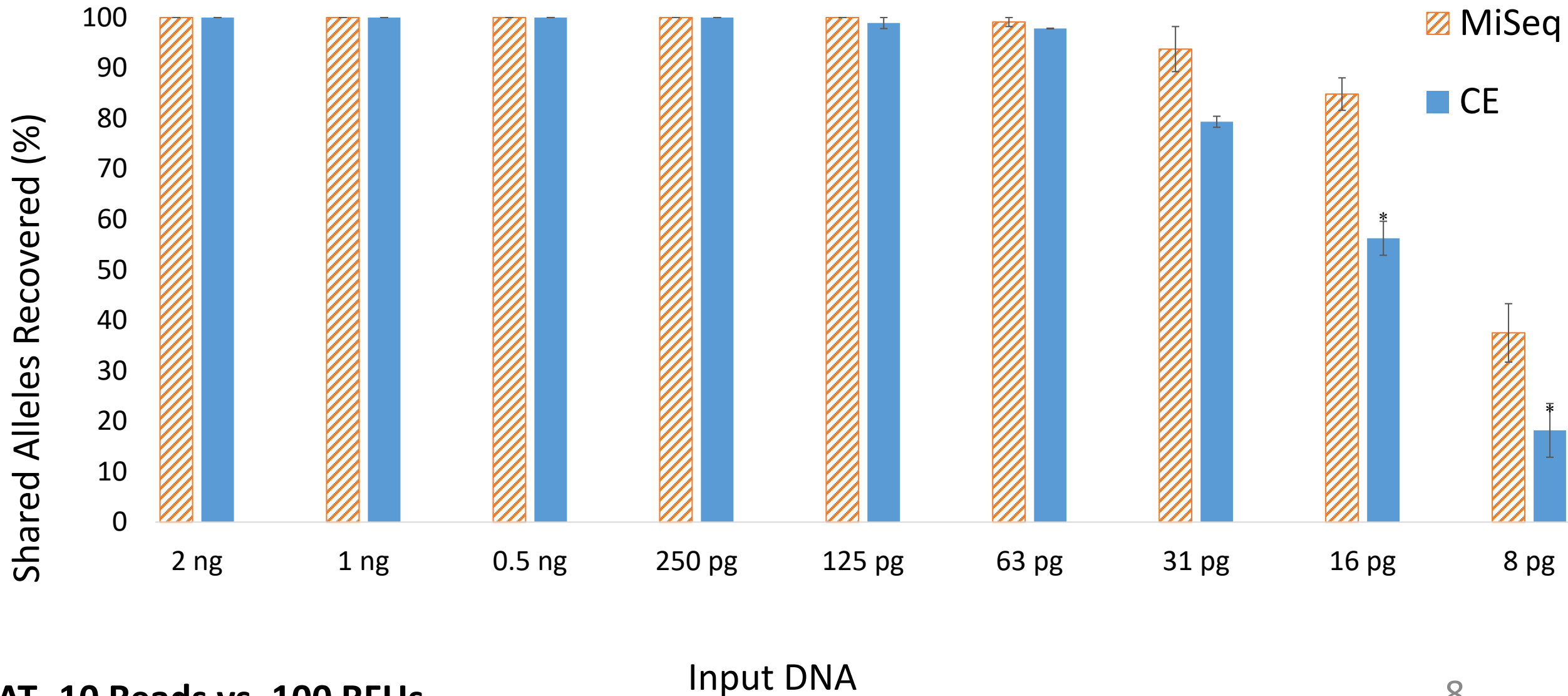
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^c New York City Public Health Laboratory, Department of Health and Mental Hygiene, 455 East 26th Street, New York, NY 10016, USA



Experimental Design



Sensitivity: ForenSeq™ and GlobalFiler™

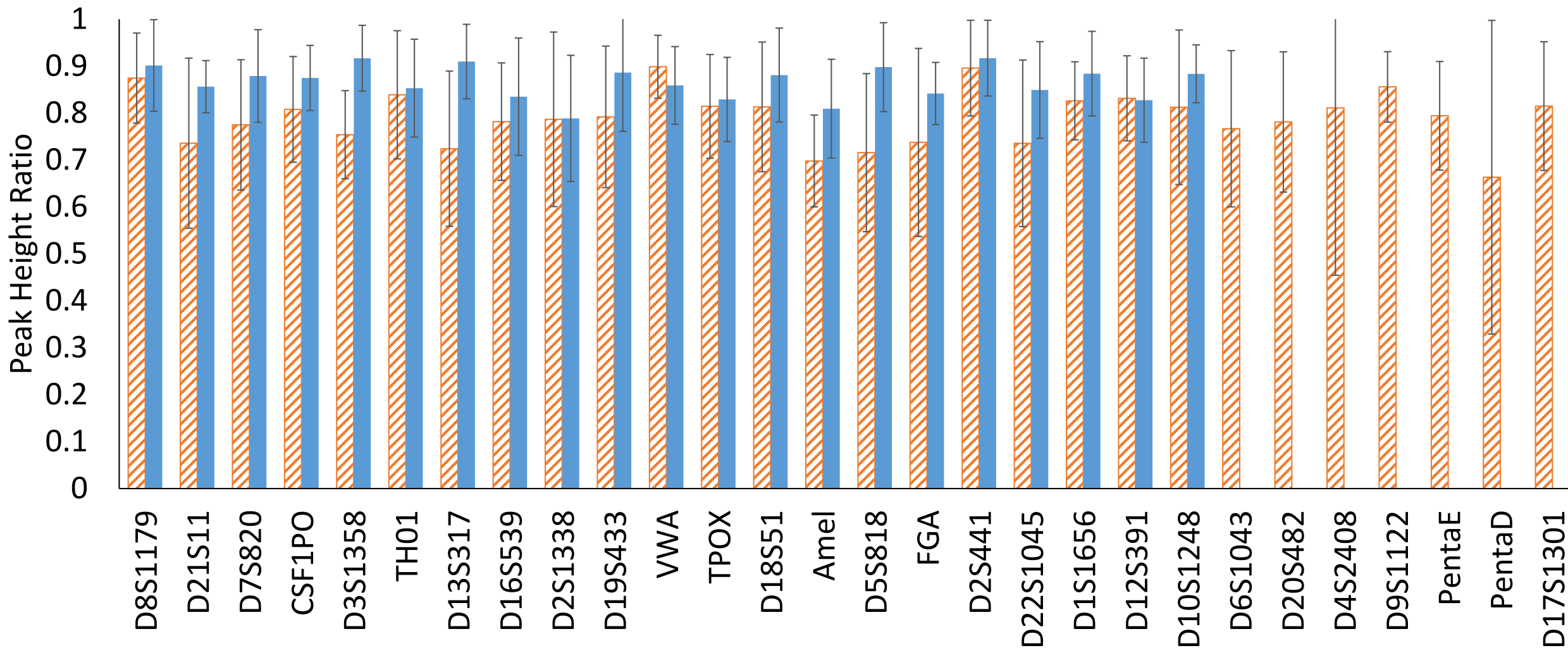


Concordance: ForenSeq™ and GlobalFiler™

Sample	D3	vWA	D16	CSFIPO	TPOX	Amel	D8	D21	D18	DYS	D2S4	D19	TH01	FGA	D22	D5	D13	D7	D10	D1	D12	D2S13
Buccal 1	16,18	16,18	10,11	11,12	8,8	X,X	11,13	29,30	12,16		14,14	13,14	6,9,3	21,26	15,17	11,11	12,13	8,12	13,14	12,15,3	17,19	17,19
Buccal 2	15,17	17,19	11,12	12,12	8,8	X,Y	13,15	32,2,33,2	17,18	10	10,11	14,15	9,9	23,23	16,16	11,12	11,11	11,11	14,15	15,15	18,19	19,20
Buccal 3	16,17	15,17	10,14	12,12	8,11	X,X	12,13	30,30	13,16		10,11	13,14	6,7	22,23	14,15	11,11	8,10	12,12	13,13	13,14	18,25	18,20
Buccal 4	14,18	16,17	12,12	10,13	8,8	X,Y	11,12	27,31,2	14,15	11	10,10	13,15	6,9,3	19,24	11,16	12,12	10,11	9,11	14,15	17,3,18,3	17,18	23,25
Buccal 5	14,18	17,19	11,13	10,12	9,11	X,X	13,15	30,31,2	15,16		10,11	12,13	6,8	22,29	15,15	11,12	8,8	11,13	12,14	17,3,17,3	20,21	20,24
Buccal 6	16,18	17,19	9,12	7,9	8,11	X,X	12,12	28,28	14,15		10,14	13,14	7,9,3	22,23	15,16	12,13	11,14	11,12	11,17	13,13	18,21	17,18
Buccal 7	17,19	15,16	11,12	10,11	8,11	X,X	11,13	27,30	12,14		11,14	13,2,14	8,9,3	20,22	15,15	11,12	9,11	10,11	15,16	16,16	20,22	17,24
Buccal 8	15,15	16,18	9,11	10,10	8,10	X,Y	11,13	28,35	16,20	11	10,14	14,15,2	6,9	20,23	16,16	12,12	11,13	11,11	11,14	12,14	18,19	19,22
Bone 1	16,16	18,18	12,13	10,11	8,8	X,X	13,14	29,31	15,17		10,15	13,15	6,8	22,25	15,15	11,11	8,12	10,11	15,18	12,17	17,3,18	20,20
Bone 2	15,17	16,16	12,14	10,12	8,12	X,Y	12,13	29,29	15,15	12	10,14	12,14	8,9	22,23	15,15	11,12	8,11	9,10	14,15	11,16	21, Q	19,20
Bone 3	16,17	17,18	11,13	10,12	8,9	X,X	12,14	28,31,2	13,16		14,15	14,15	7,8	20,25	15,16	12,13	8,10	8,12	13,13	13,17,3	16,16	17,20
Bone 4	16,17	17,18	11,13	10,12	8,9	X,X	12,14	28,31,2	13,16		14,15	14,15	7,8	20,25	15,16	12,13	8,10	8,12	13,13	13,17,3	16,16	17,20
Muscle 1	15,17	16,18	11,13	11,12	8,8	X,Y	12,13	31,2,31,2	12,18	11	10,14	14,14	9,9,3	20,24	15,16	11,12	10,12	8,11	14,15	12,13	15,21	23,25
Muscle 2	15,15	14,16	10,11	11,12	8,11	X,Y	13,13	29,32,2	14,16	11	10,14	12,15	7,9,3	19,20	16,16	12,12	9,14	8,12	14,15	15,16	21,24	17,20
Muscle 3	16,17	16,20	12,13	10,12	8,8	X,Y	13,14	30,33,2	12,16	11	11,3,14	11,15	9,9	21,22	15,16	9,9	12,14	11,11	16,16	11,15	18,19	17,18
Muscle 4	16,18	18,18	11,11	11,12	8,8	X,X	12,13	32,33,2	14,19		10,14	13,16	9,9,3	20,25	15,16	12,12	12,14	8,11	14,16	14,17,3	19,3,21	19,23
Blood 1	15,16	14,16	11,12	11,12	8,8	X,X	13,14	27,29	12,18		14,14	13,14	7,9,3	19,21	15,15	11,13	11,12	9,11	14,14	16,3,17,3	18,20	22,24
Blood 2	14,14	16,16	11,11	10,10	8,11	X,Y	13,15	30,32	12,16	10	10,14	12,13	6,9,3	22,27	15,16	9,13	8,12	10,11	13,14	12,12	21,23	20,22
Blood 3	18,18	15,15	9,11	11,12	9,11	X,Y	13,14	28,31,2	13,19	10	10,11	15,16	7,9,3	19,21	15,16	11,12	11,13	11,11	14,15	13,15	15,18,3	17,20
Hair 1	15,17	17,18	11,14	10,11	9,11	X,Y	10,13	27,29	15,18	10	11,11	13,13	6,6	19,24	12,15	11,12	9,12	11,11	13,17	13,16,3	17,19	20,23
Hair 2	14,14	16,16	11,11	10,10	8,11	X,Y	13,15	30,32	12,16	10	10,14	12,13	6,9,3	22,27	15,16	9,13	8,12	10,11	13,14	12,12	21,23	20,22
Hair 3	15,16	14,16	11,12	11,12	8,8	X,X	13,14	27,29	12,18		14,14	13,14	7,9,3	19,21	15,15	11,13	11,12	9,11	14,14	16,3,17,3	18,20	22,24

- All samples concordant
- **Green:** matching genotype
- **Yellow:** additional recovery in NGS results
 - Bone samples
- **Red:** additional recovery in CE results

Heterozygote Balance and Stutter

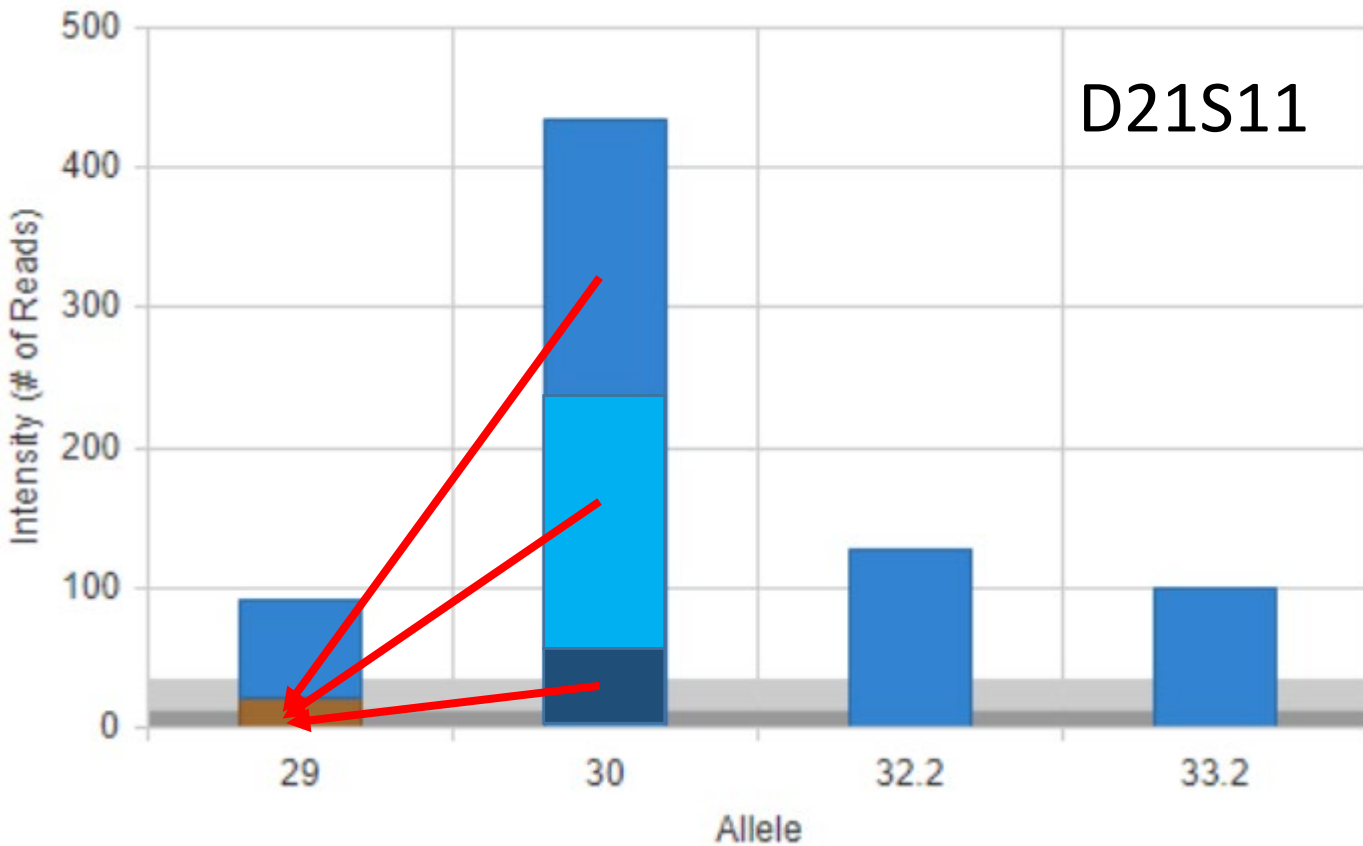


▨ DNA Sig Prep
 PHR: 79.1%±16.1%
 Loci specific stutter: 7.5%-33%

■ GlobalFiler
 PHR: 86.6%±9.3%
 Allele specific stutter

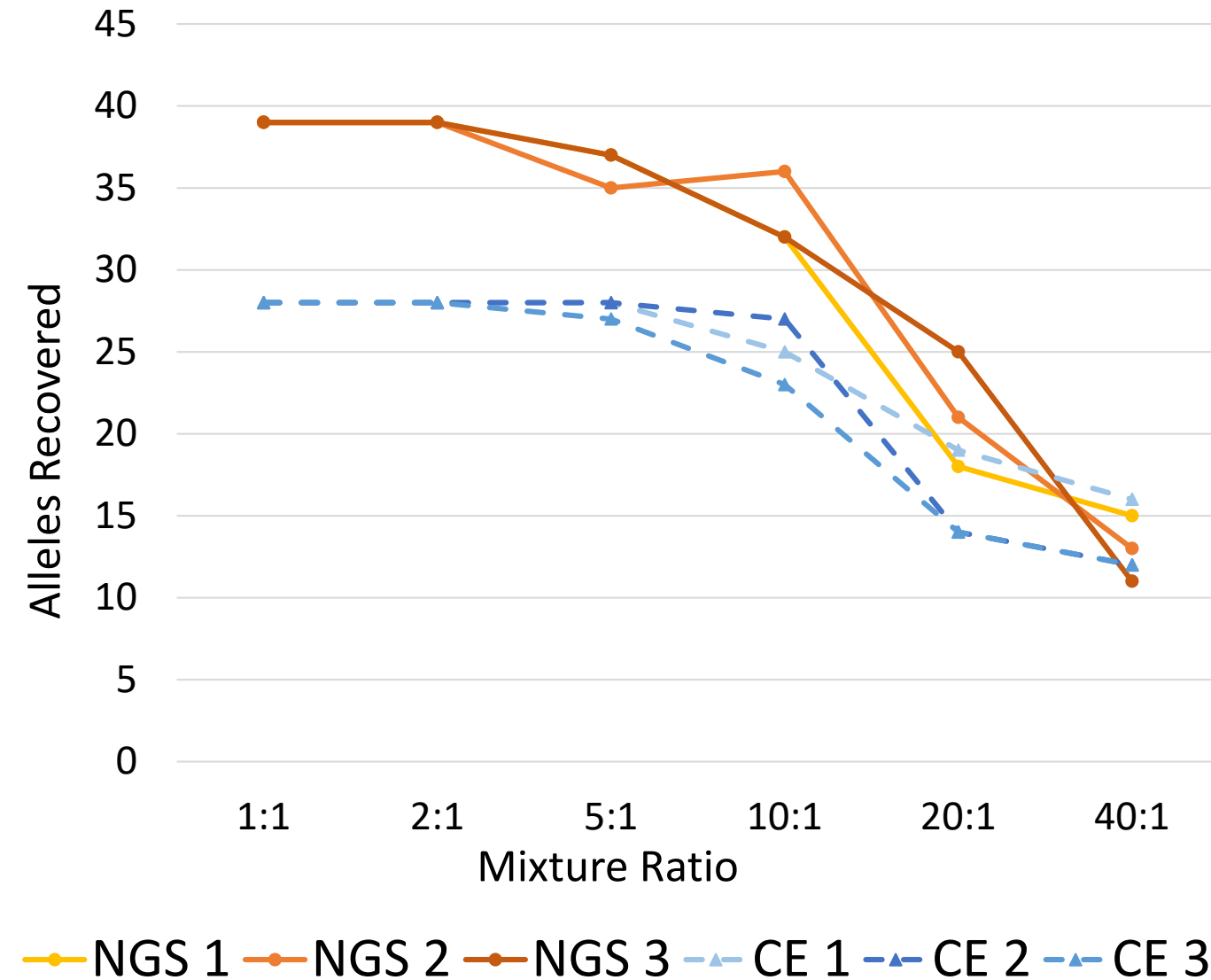
Sequenced Based Allele Calls

```
TATCTATCTAATCTATCTGTCTGTCTGTCTGTCTGTCTGTCTGTCTATCTATCTATATCTATCTATCTATCATCTATCTATCTATCCATATCTATCTATC
TATCTATCTATCTGTCTGTCTGTCTGTCTGTCTGTCTGTCTGTCTATCTATCTATATCTATCTATCATCTATCTATCCATATCTATCTATCTATCTATCTATC
TATCTATCTGTCTGTCTGTCTGTCTGTCTGTCTGTCTATCTATCTATATCTATCTATCATCTATCTATCCATATCTATCTATCTATCTATCTATC
```



3 Person Mixture

2 Person Mixtures



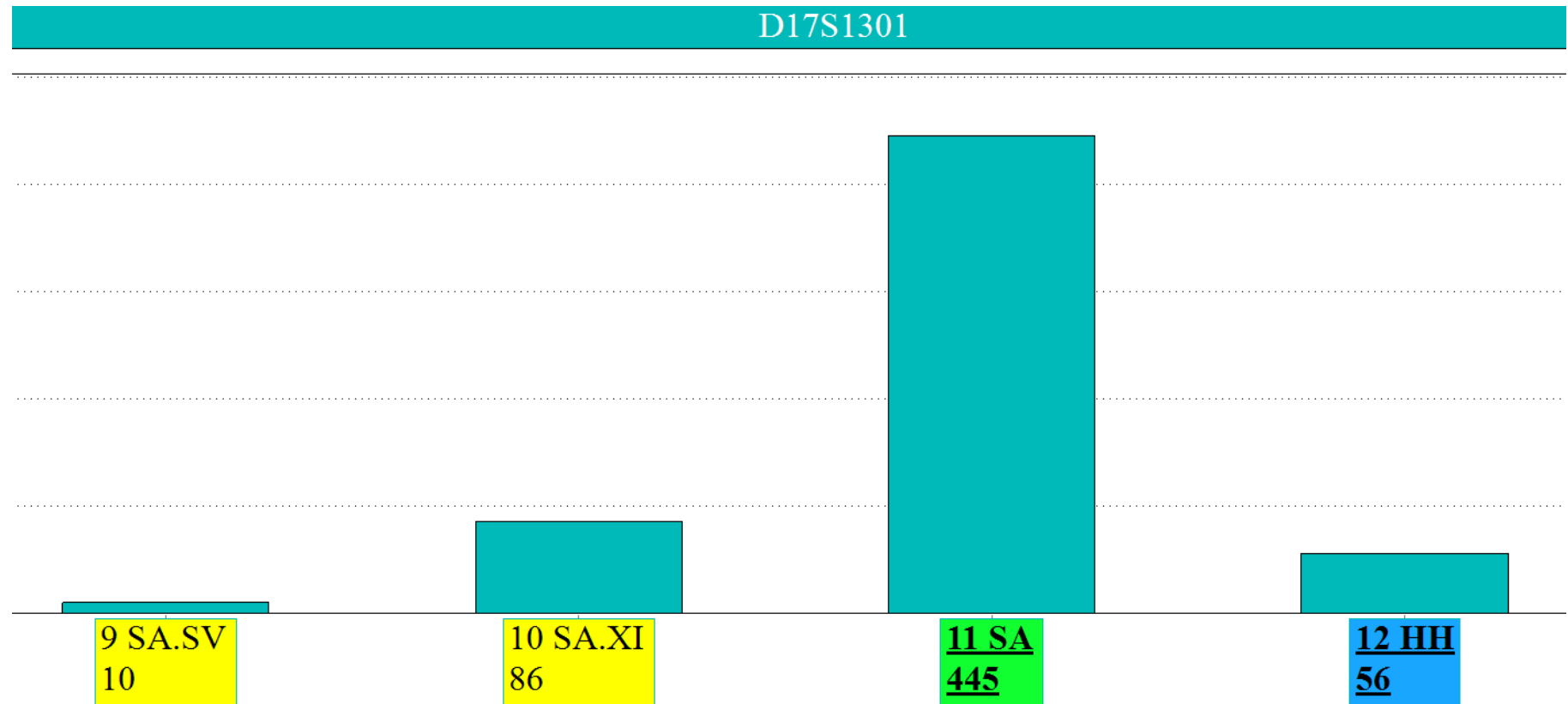
Random Match Probability

Mixture	NGS	NGS CODIS	CE
20:1	3.52E+12	1.82E+09	6.9125E+6
20:1	2.83E+18	1.25E+10	1.4632E+11
20:1	3.32E+18	8.94E+12	8.8174E+7
40:1	1.33E+10	1.40E+07	7.206E+5
40:1	1.11E+10	6.31E+07	2.8934E+9
40:1	3.27E+08	3.95E+05	7.206E+5

NGS: 38 unique minor alleles

CE: 27 unique minor alleles

Allele Masking



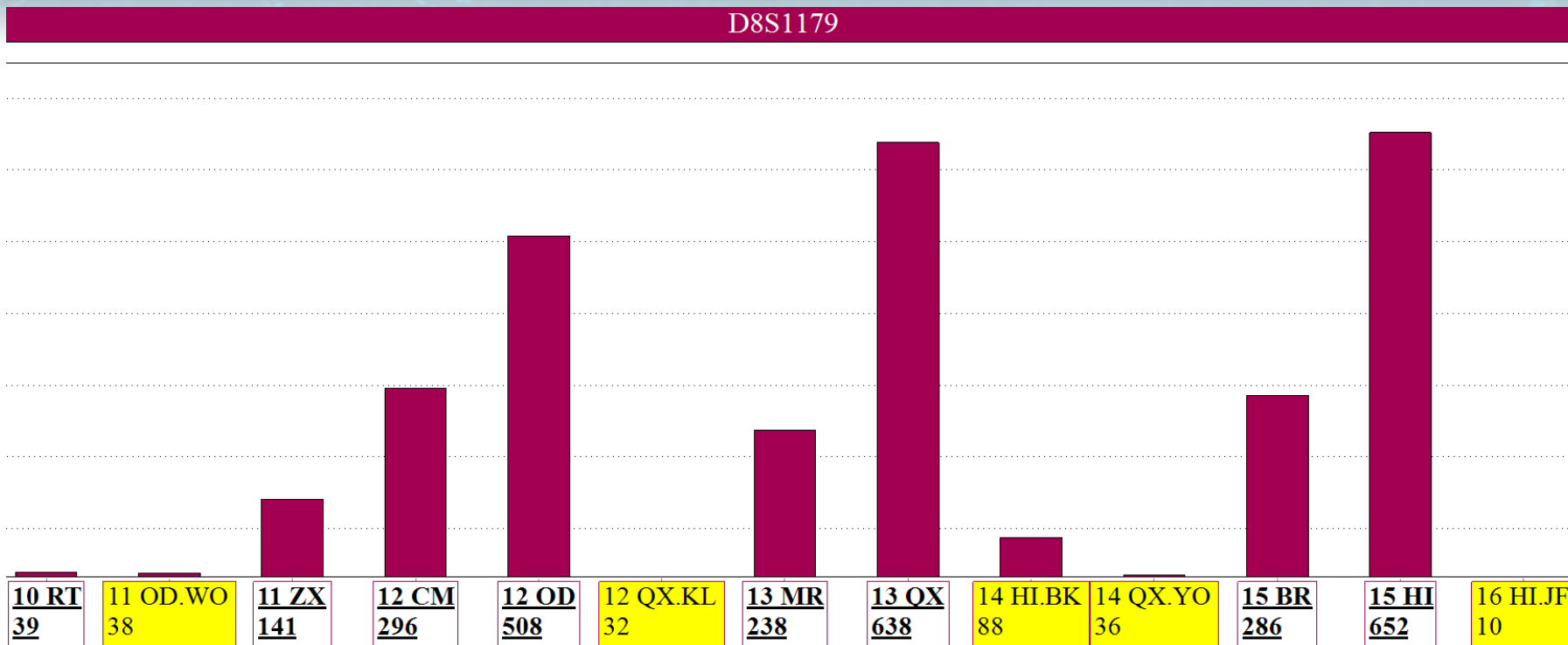
Major: 11 SA, 11 SA
Minor: 10 XI, 12HH

D17 Stutter Threshold:
20%

- Masking started at 5:1 ratio, N-1 position
- Allele masking in stutter position more prevalent in NGS chemistry
- Inherently higher stutter observed in NGS compared to CE

4 Person: Isoalleles

D8S1179



7 donor alleles

- 3 isoalleles

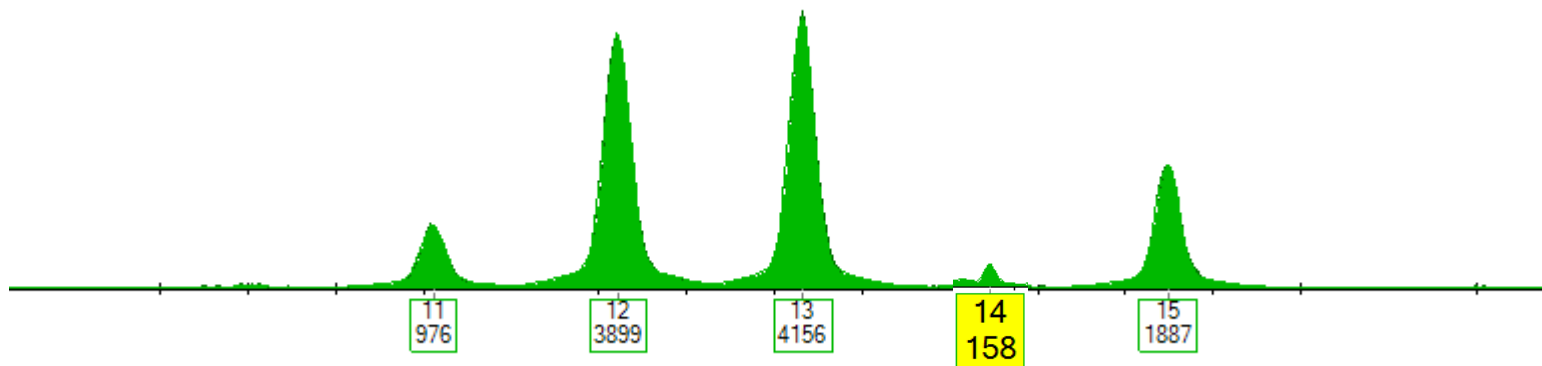
6 stutter peaks

Peak Names= ##.XX.YY

is peak length

XX is peak sequence

YY is stutter sequence



Donor 1: 11ZX, 13MR

Donor 2: 13MR, 15BR

Donor 3: 12OD, 12CM

Donor 4: 13QX, 15HI

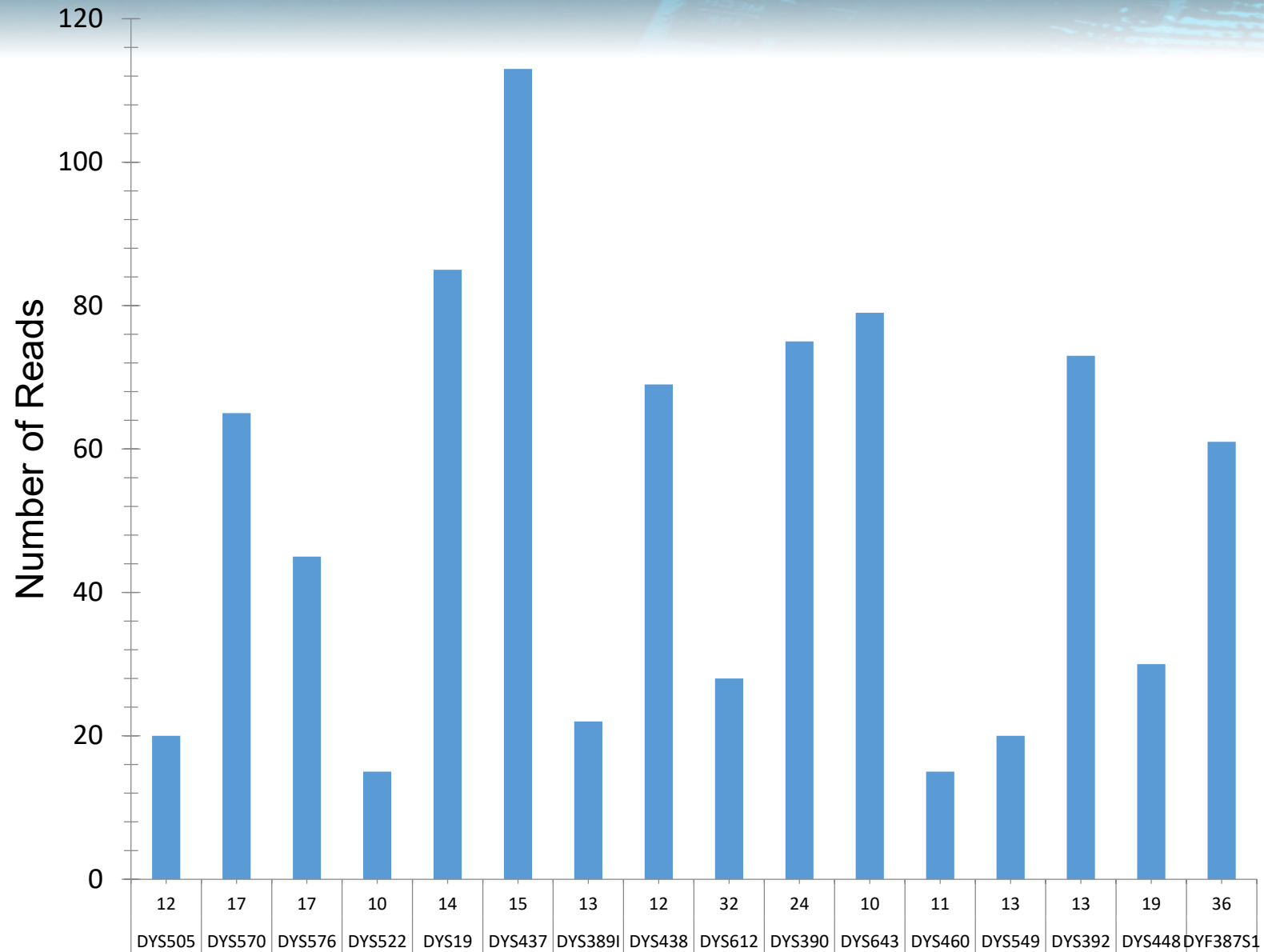
3-4 Person Mixtures

- NGS = additional unique alleles
- 2 person: 3 additional alleles
- 3 person: 7 – 12 additional alleles
- 4 person: 13 – 18 additional alleles

Mixture	Major	Medium	Medium 2	Minor
2 Person	Female 1	Male 1		
3 Person 1	Female 2	Male 1	Female 1	
3 Person 2	Male 2	Male 1	Female 1	
3 Person 3	Male 2	Female 3	Female 1	
3 Person 4	Male 2	Female 3	Female 4	
3 Person 5	Female 5	Female 3	Female 4	
4 Person 1	Male 2	Female 2	Male 1	Female 1
4 Person 2	Female 3	Female 2	Male 1	Female 1
4 Person 3	Female 3	Female 4	Male 1	Female 1
4 Person 4	Female 3	Female 4	Female 5	Female 1
4 Person 5	Female 3	Female 4	Female 5	Male 3

4 Person Mixture 5

- 3 Female: 1 Male in ratio of 8:4:2:1
 - Male is minor contributor 1/15 of total input
- 16 of 24 Y-STRs sequenced



Conclusions

Advantages

- More Autosomal STRs
- Includes Y-STRs and X-STRs
- Allele sequence (Isoallele)
 - Better match statistic
 - Less allele stacking
 - Less allele masking

Disadvantages

- Elevated stutter (thresholds)
- Peak height ratio variability
- No probabilistic genotyping systems

Recommendation 1: Implement probabilistic genotyping

Recommendation 2: Simplified assay design (ForenSeq™ MainstAY)

Acknowledgements



Questions

Rachel Houston, PhD

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