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Two person mixture interpretation LR binary method

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Definition of heterozygote balance

- Two definitions of heterozygote balance or peak height ratio:

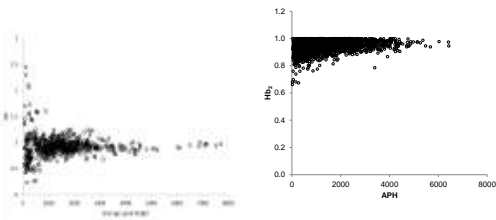
$$Hb_1 = \frac{O_{HMW}}{O_{LMW}} \quad Hb_2 = \frac{O_{smaller}}{O_{larger}}$$

- Where O is observed peak height.
- Hb_1 has the highest information content because it maintains peak order.
- Hb_2 may be obtained from Hb_1 but not vice versa.

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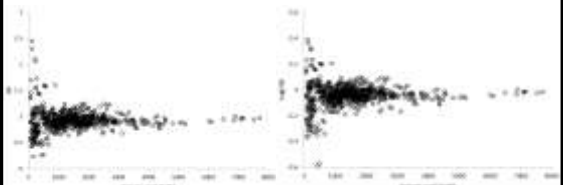
Hb versus average peak height



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Hb versus average peak height

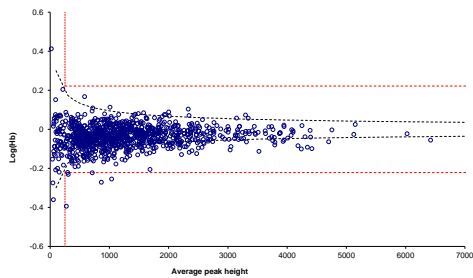


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Variability of Hb

Conventional thresholds
95% intervals



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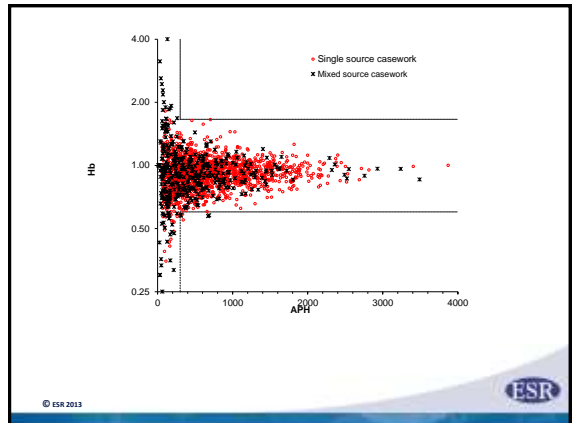
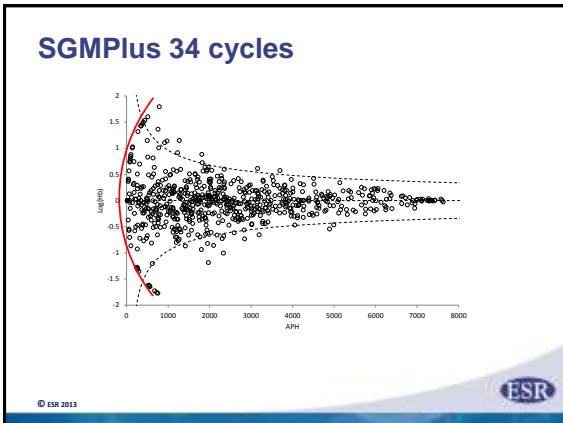
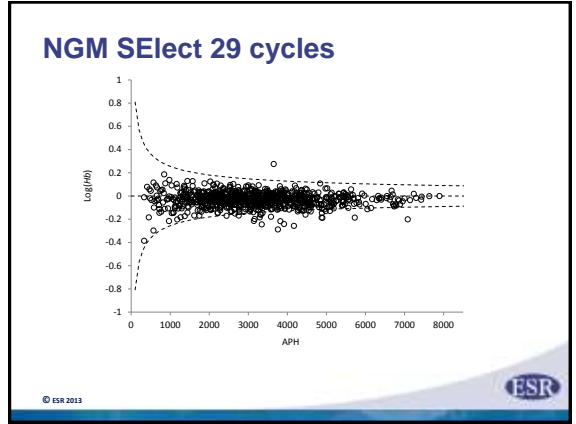
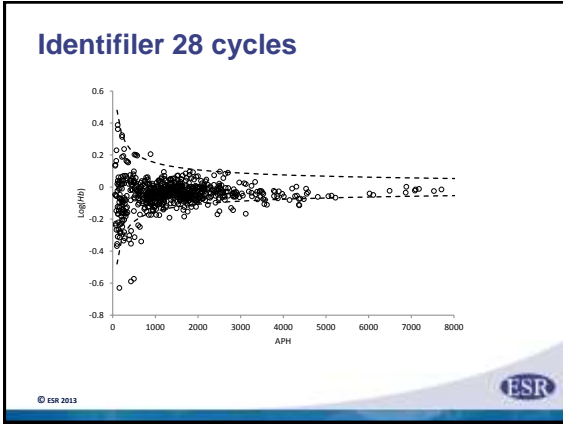
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Conclusion

- The mean of heterozygote balance is unaffected by average peak height.
- The variance about this mean is much lower at high average peak heights.
- This is true over multiple kits and PCR cycle numbers.

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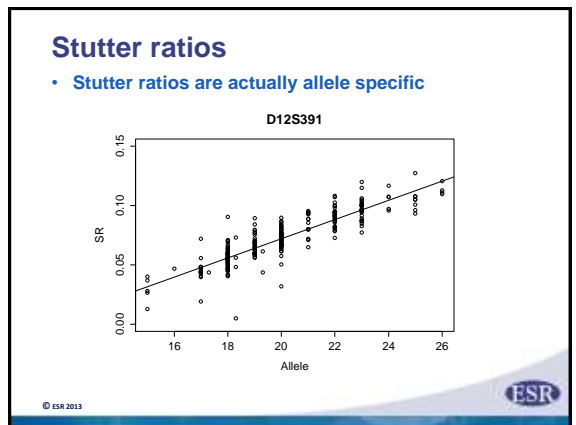
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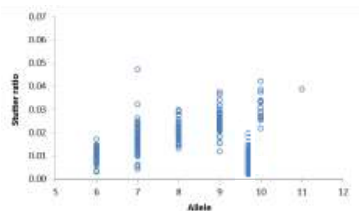
2. Stutter ratios

- Traditionally we apply a threshold at analysis to remove stutter
 - Locus specific
 - Kit specific
- What if your minor POI was approximately same RFU as stutter?
- Is removing stutter peaks conservative?
- What if a stutter peak was actually allelic and excluded your POI?

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TH01 stutter



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TH01 repeat structure

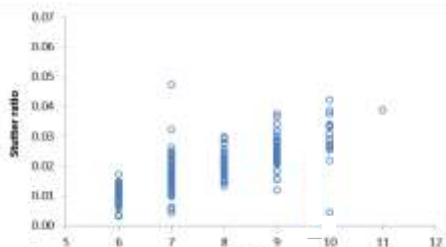
Common TH01 allele sequences		
Repeat structure	Allele	LUS
[AATG] ₆	6	6
[AATG] ₇	7	7
[AATG] ₈	8	8
[AATG] ₉	9	9
[AATG] ₆ ATG[AATG] ₃	9.3	6

Longest uninterrupted stretch of basic repeat motifs is a good predictor of stutter ratio.

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TH01 Stutter ratio versus LUS



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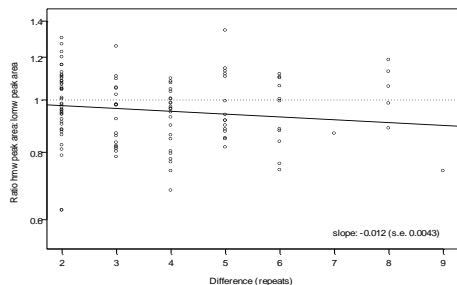
Stutter

- It is not possible to interpret mixtures well without a thorough consideration of stutter

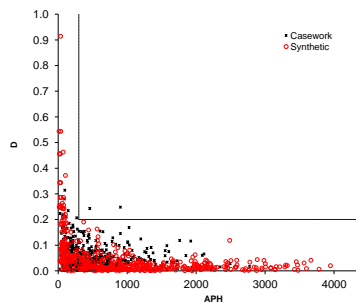
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D18



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	Number of alleles showing					
	1	2	3	4	5	6
D3	0.00	0.05	0.37	0.46	0.12	0.00
VWA	0.00	0.04	0.29	0.47	0.19	0.02

This has been
a major issue in some Australian cases

D18	0.00	0.01	0.11	0.39	0.40	0.10
D19	0.00	0.08	0.35	0.40	0.15	0.01
THO	0.00	0.07	0.40	0.44	0.09	0.00
FGA	0.00	0.01	0.14	0.42	0.35	0.07

The proportion of three person mixtures that would present four or fewer alleles for the SGM+™ is 0.033.
The result for the Profiler Plus™ loci was 0.062

Combinations without area

- Four peak locus
- ABCD
- 6 combinations
- 3 pairs

RM1	RM2
AB	CD
AC	BD
AD	BC
BC	AD
BD	AC
CD	AB

Combinations without area

- Three peak locus ABC
- 12 combinations

RM1	RM2
AA	BC
BB	AC
CC	AB
AB	AC
AB	BC
AC	BC

RM1	RM2
BC	AA
AC	BB
AB	CC
AC	AB
BC	AB
BC	AC

Combinations without area

- Two peak locus AB
- 7 combinations

RM1	RM2
AA	BB
AB	BB
AB	AA
AB	AB

RM1	RM2
BB	AA
BB	AB
AA	AB

Combinations without area

RM1	RM2
AA	AA

- One peak locus A
- 1 combination

Eliminating Combinations with area

- Guidelines
- Four peak locus
- ABCD
- For each het
- Simple het guideline

$$0.6 \leq \frac{\phi_1}{\phi_2} \leq 1.66$$

RM1	RM2
AB	CD
AC	BD
AD	BC
BC	AD
BD	AC
CD	AB

Eliminating Combinations with area

- Three peak locus
- ABC
- For each het

RM1	RM2
AA	BC
BB	AC
CC	AB
AB	AC
AB	BC
AC	BC

$0.6 \leq \frac{\phi_1}{\phi_2} \leq 1.66$
 Shared het guideline
 $0.6 \leq \frac{\phi_3}{\phi_2 + \phi_3} \leq 1.66$ Shared allele

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Eliminating Combinations with area

- Two peak locus AB
- 7 combinations

RM1	RM2
AA	BB
AB	BB
AB	AA
AB	AB

Het Hom guideline
 $0.6 * \phi_1 \leq \phi_s$
 Simple het guideline
 $0.6 \leq \frac{\phi_1}{\phi_2} \leq 1.66$

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Eliminating combinations

- What is left?
- Calculate Mx for each combination

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Eliminating Combinations with area 4 peak loci

RM1	RM2
AB	CD
AC	BD
AD	BC
BC	AD
BD	AC
CD	AB

$$\hat{M}_X = \frac{\phi_1 + \phi_2}{\phi_1 + \phi_2 + \phi_3 + \phi_4}$$

$$1 - \hat{M}_X = \frac{\phi_3 + \phi_4}{\phi_1 + \phi_2 + \phi_3 + \phi_4}$$

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Eliminating Combinations with area 3 peak loci

RM1	RM2
AA	BC
BB	AC
CC	AB
BC	AA
AC	BB
AB	CC
AB	BC

$$\hat{M}_X = \frac{\phi_{Hom}}{\phi_{Hom} + \phi_2 + \phi_3}$$

$$\hat{M}_X = \frac{\phi_1 + \phi_2}{\phi_1 + \phi_2 + \phi_{Hom}}$$

$$\hat{M}_X = \frac{\phi_{UL}}{\phi_{UL} + \phi_{UR}}$$

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Eliminating Combinations with area 2 peak loci

RM1	RM2
AA	BB
AB	BB
AB	AA
AB	AB

$$\hat{M}_X = \frac{\phi_L}{\phi_L + \phi_R}$$

$$\hat{M}_X = \frac{2\phi_U}{\phi_U + \phi_S}$$

No info

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Eliminating combinations

- What is left?
- Calculate Mx for each combination
- Are any combinations inconsistent on the basis of Mx?

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	Hb	Hb	Mx
7	71		
8	1188	8,9	12,13
9	884	8,12	9,13
10		8,13	9,12
11	121	12,13	8,9
12	2020	9,13	8,12
13	1920	9,12	8,13

$M_x \approx 0.75$

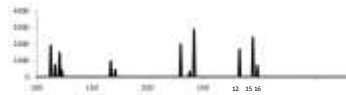
POI = 12,13 V = 8,9

H₁: POI+V

H₂: V + U

LR=

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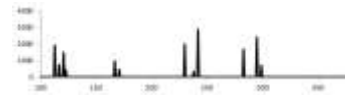


	Hb	Hb	Mx
11	120		
12	1680	12,12	15,16
13		15,15	12,16
14	145	16,16	12,15
15	2416	12,15	12,16
16	712	12,15	15,16
		12,15	16,16
		12,16	12,15
		12,16	15,15
		12,16	15,16
		15,16	12,12
		15,16	12,12
		15,16	12,15
		15,16	12,16

$M_x \approx 0.75$

POI = 12,15 V = 15,16

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	Hb	Hb	Mx
11	120		
12	1680		
13			
14	145		
15	2416		
16	712	12,15	15,16
		12,15	16,16

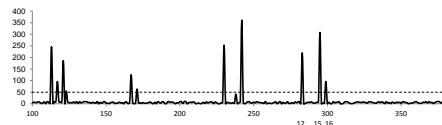
$M_x \approx 0.75$

POI = 12,15 V = 15,16

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Using F designations



12,15,16, F

POI = 12,15 V = 15,16

If we allow drop out at 300RFU then we would pair

12,F or 15,F or 16,F

We can interpret this because there are no non-concordances (see Jo-Anne Bright's lecture)

These models cannot reliably interpret all non-concordances

Non-concordance: When an allele in the POI genotype is not present in the crime profile

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