



Towards threshold determination

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1. Threshold purpose
2. Experimental data
3. Heterozygote balance
4. Dropout & homozygote
5. Stutters
6. Conclusions & future work

1. *Threshold purpose*

- ◆ Profile designation
- ◆ Separation of mixed profiles
- ◆ Weight of evidence calculations: likelihood ratios

2. *Experimental data*

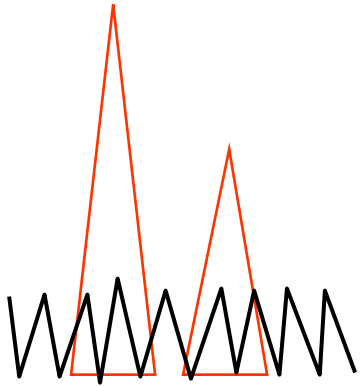
- ◆ Experimental data that allows the derivation of a methodology for setting thresholds
- ◆ DNA quantity
 - Dilution series from 50 to 500 in increment of 50 pg/μl
 - Quantifiler
- ◆ 51 donors (FSS staff)
- ◆ PCR blocks: 9700, Tetrads
- ◆ CEs: 3100, 3130 Excel

3. *Heterozygote balance*

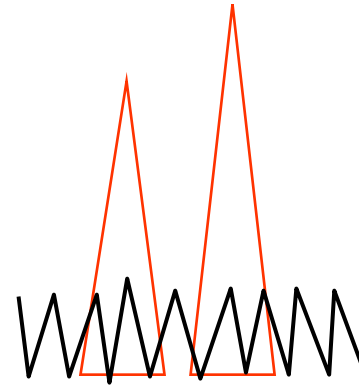


Heterozygote balance

3. Heterozygote balance

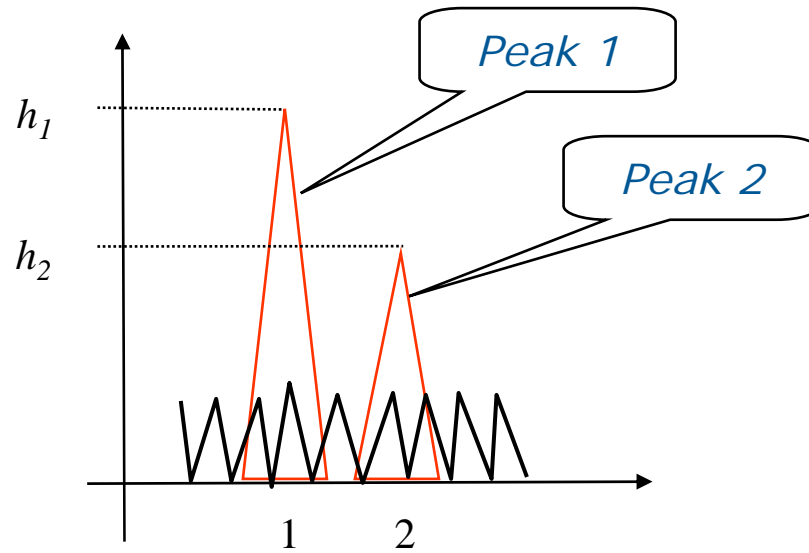


The high-molecular-weight peak is smaller



The low-molecular-weight peak is smaller

3. Heterozygote balance



$$\text{Heterozygote Balance} = \frac{h_1}{h_2}$$

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$$\text{Heterozygote Balance} = \frac{h_1}{h_2}$$

threshold

threshold

lower limit < heterozygote balance < *upper limit*

These limits may change with mean height

$$\text{mean height} = \frac{h_1 + h_2}{2}$$

3. Heterozygote balance

For each value of mean height
we can compute a lower limit and an upper limit

How

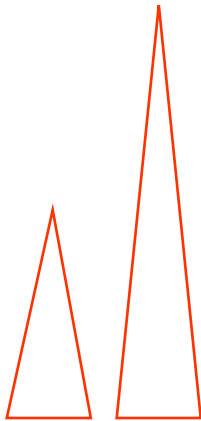
- estimate the joint probability distribution of mean height and heterozygote balance
- compute the conditional distribution of heterozygote balance given mean height
- compute 95% probability intervals

3. Heterozygote balance

Mean-height	Heterozygote balance threshold	
	lower limit	upper limit
m1	0.45	2.4
m2	0.50	2.0
m3	0.60	1.8
⋮	⋮	⋮
⋮	⋮	⋮

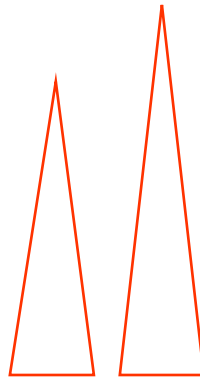
3. Heterozygote balance

Mean-height	Heterozygote balance threshold	
	lower limit	upper limit
m	0.60	1.8



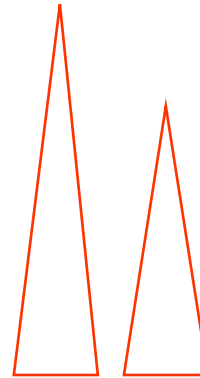
$$\frac{h_1 + h_2}{2} = 600$$

$$\frac{h_1}{h_2} = 0.60$$



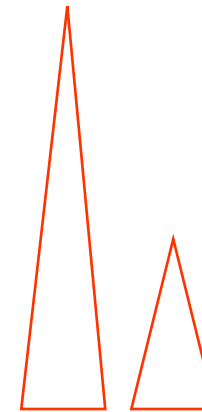
$$\frac{h_1 + h_2}{2} = 600$$

$$\frac{h_1}{h_2} = 0.80$$



$$\frac{h_1 + h_2}{2} = 600$$

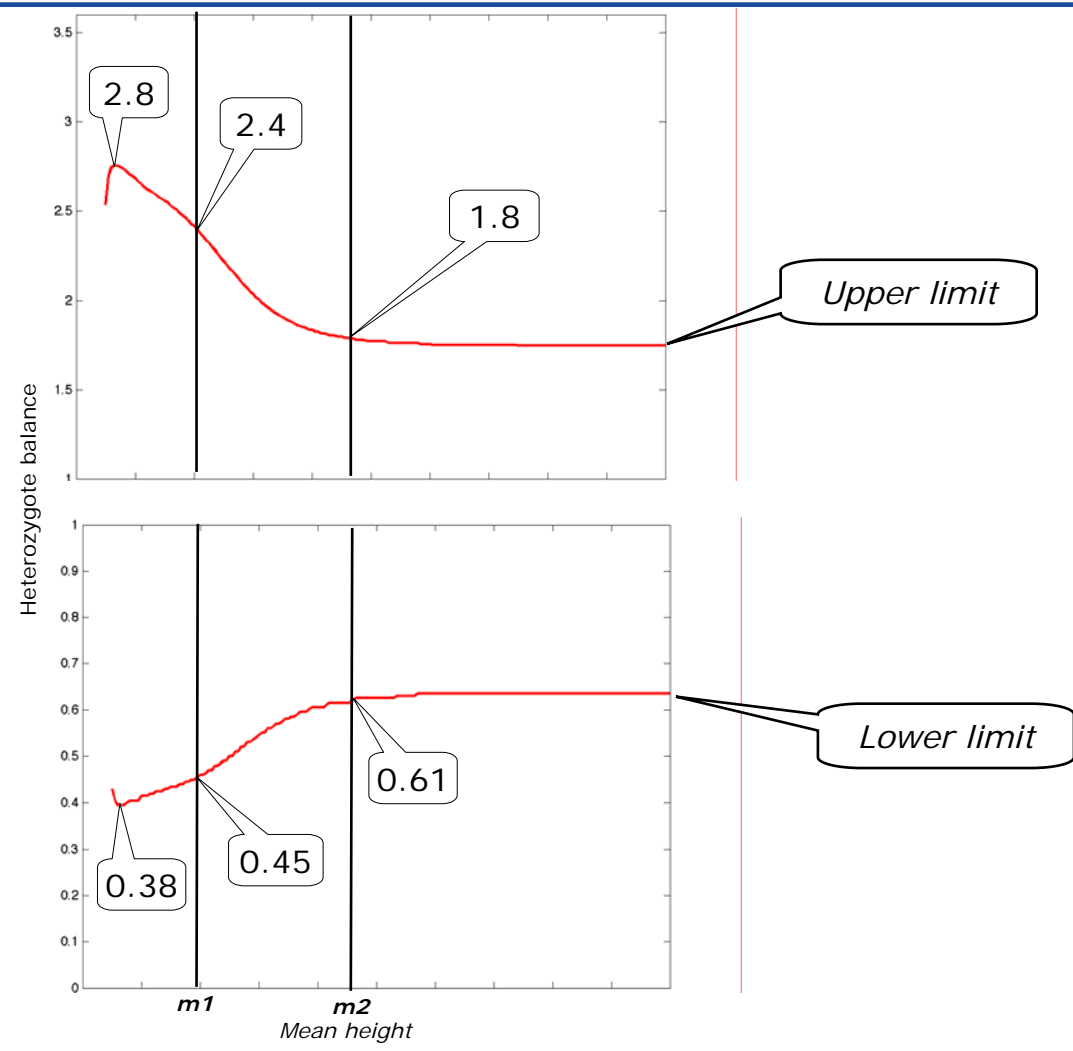
$$\frac{h_1}{h_2} = 1.4$$



$$\frac{h_1 + h_2}{2} = 600$$

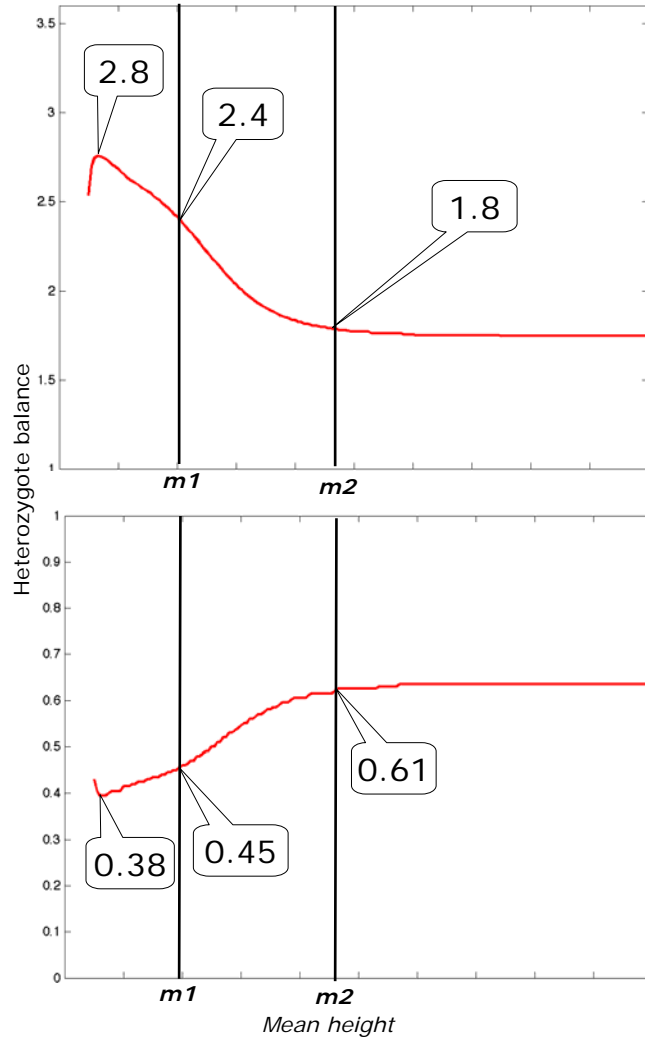
$$\frac{h_1}{h_2} = 1.8$$

3. Heterozygote balance



Gill, Curran & Elliot (2005). A graphical simulation model of the entire DNA process associated with the analysis of STR loci. *Nucleic acid research* 33, 632-643

3. Heterozygote balance

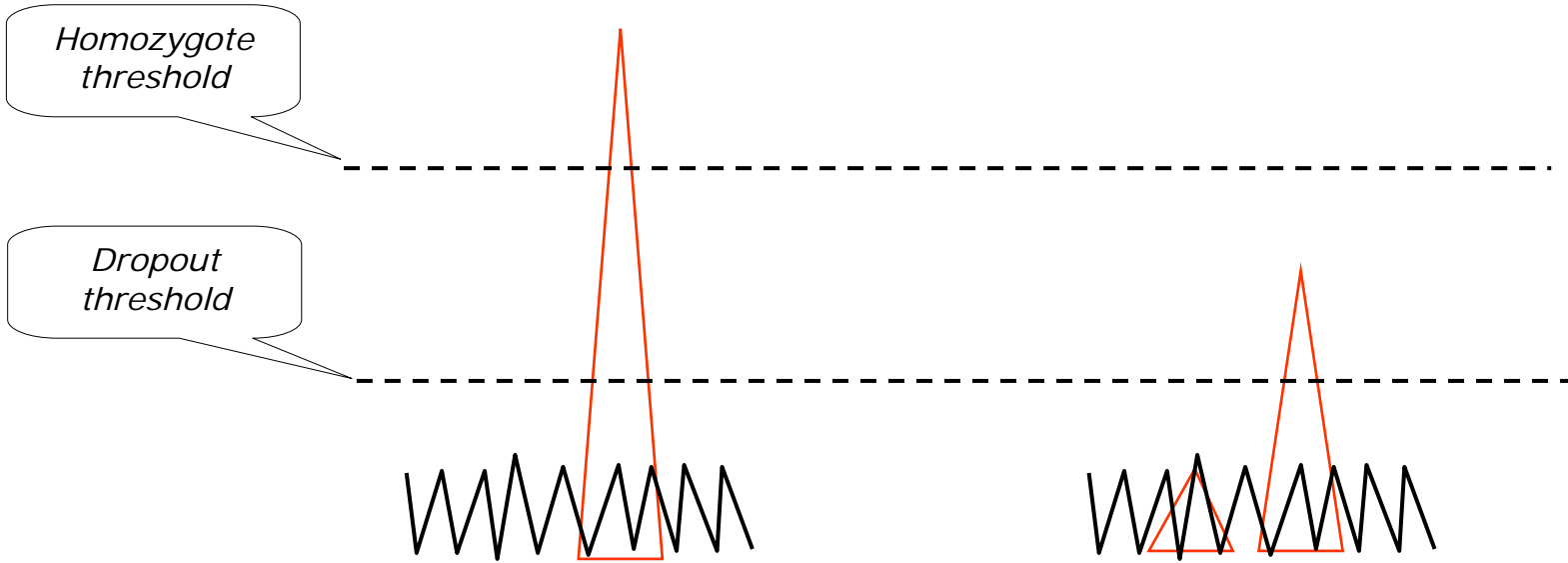


Mean-height region	Heterozygote balance threshold	
	Lower limit	Upper limit
Less than m_1	0.38	2.8
Between m_1 and m_2	0.45	2.4
More than m_2	0.61	1.8

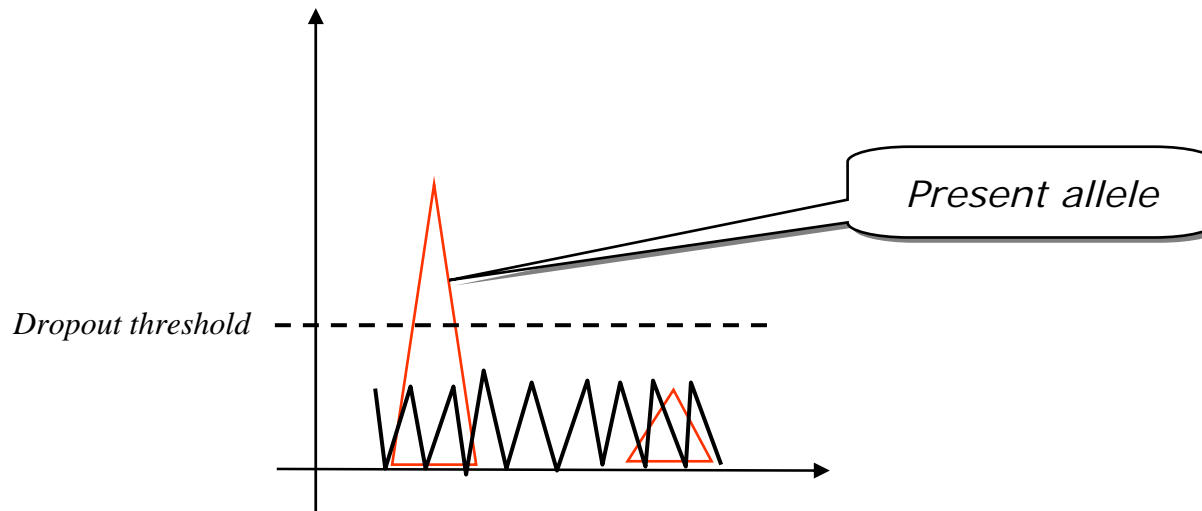
4. *Homozygote threshold*

Homozygote threshold

4. Homozygote threshold



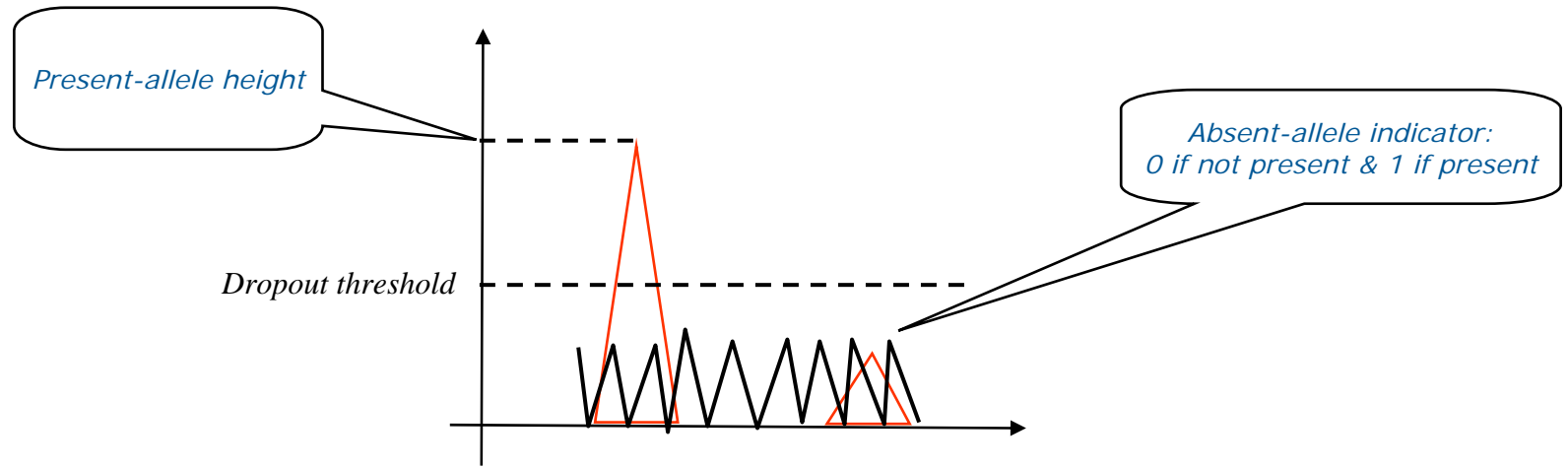
4. Homozygote threshold



Probability of dropout as a function of present-allele height

We expect to quantify what we already know: the higher the present allele is, the lower the dropout probability

4. Homozygote threshold

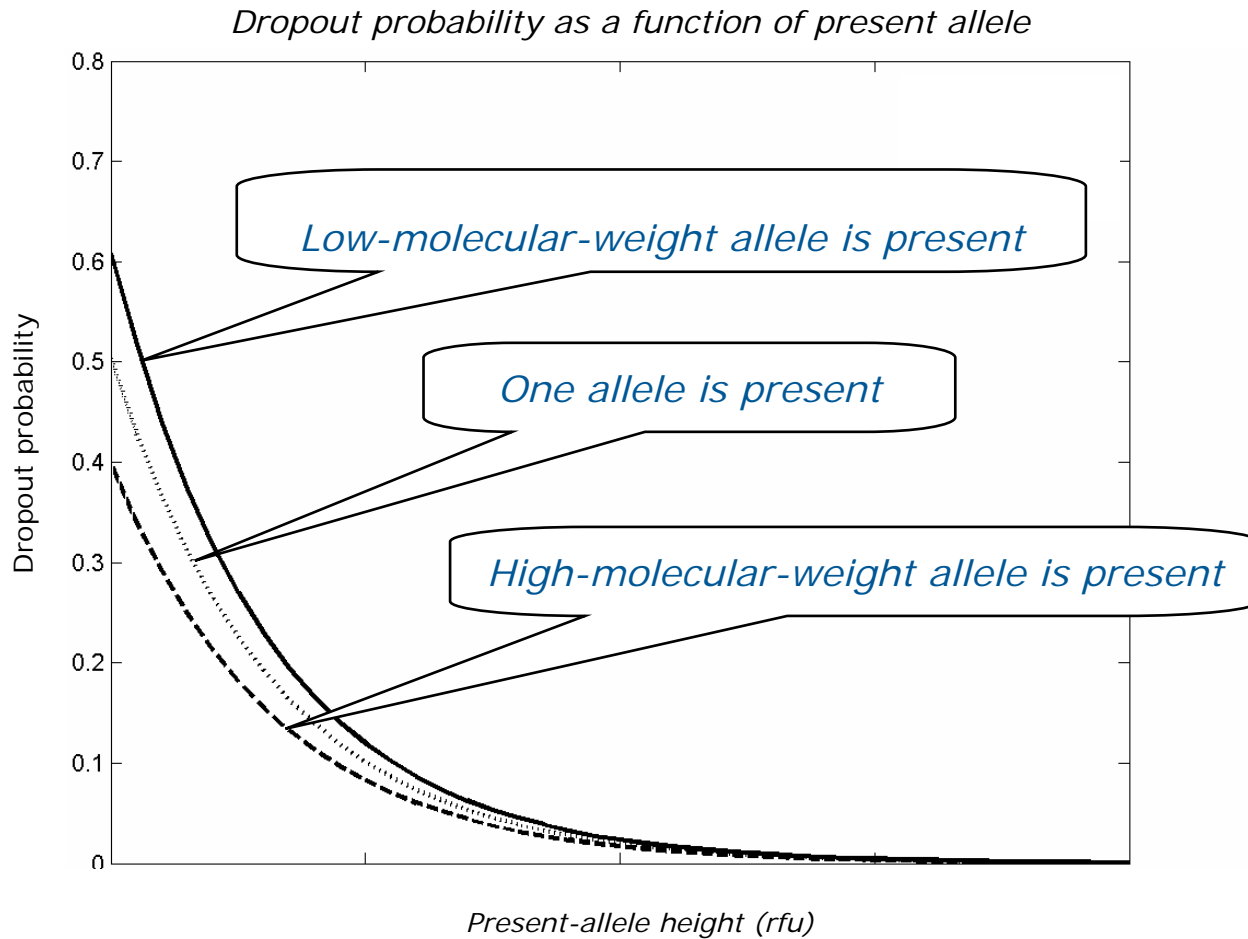


Data

Absent-allele indicator	Present-allele height
0	120
1	60
⋮	⋮

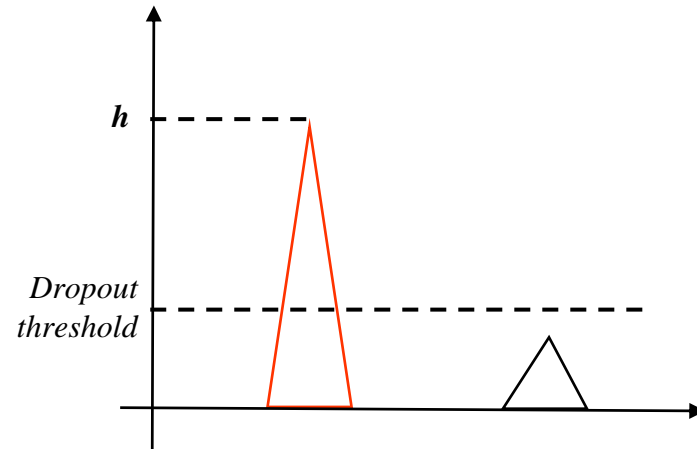
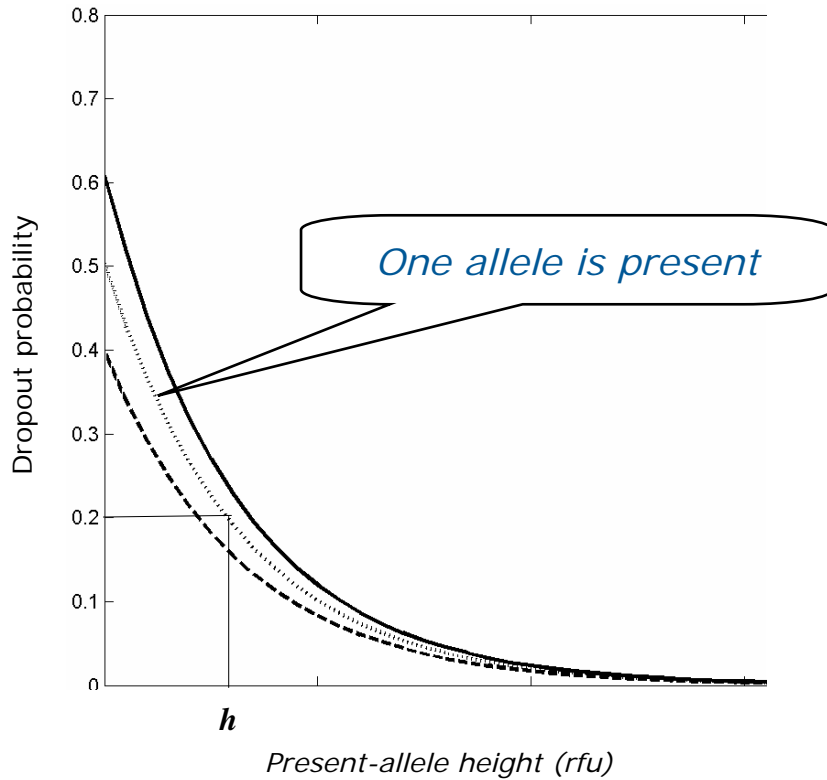
Logistic regression calculated from allele indicator & present-allele height

4. Homozygote threshold



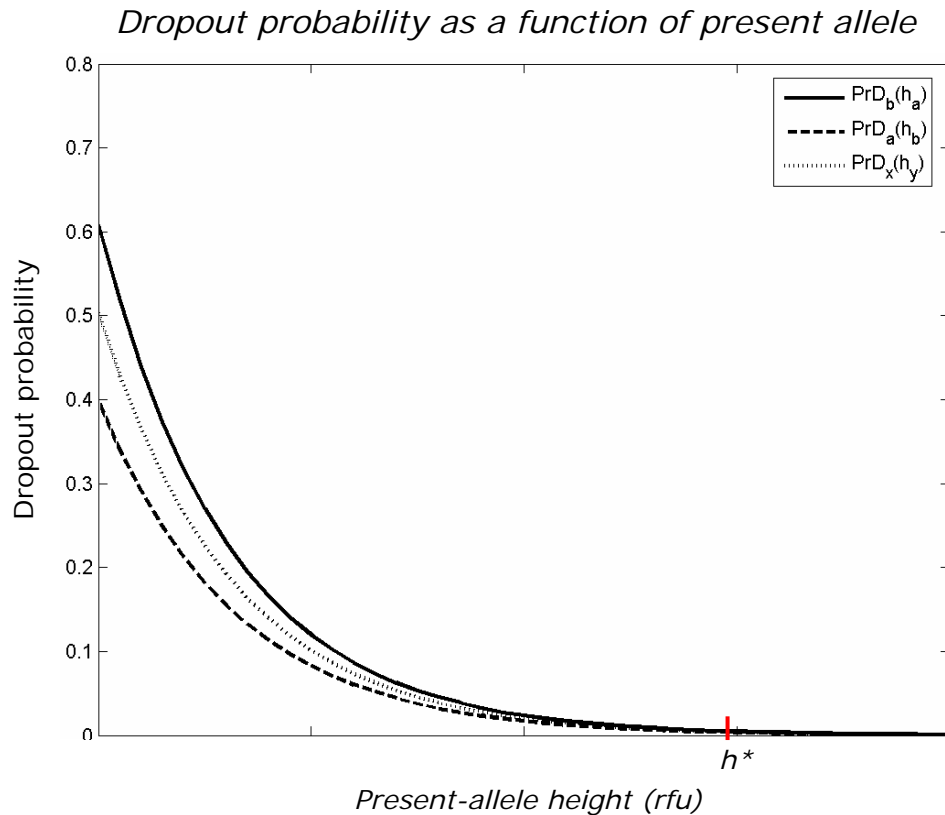
4. Homozygote threshold

Dropout probability as a function of present allele



- If
- donor is heterozygous and
 - the height of present allele is h ,
- then
- 20% of times partner-allele height is below the dropout threshold

4. Homozygote threshold

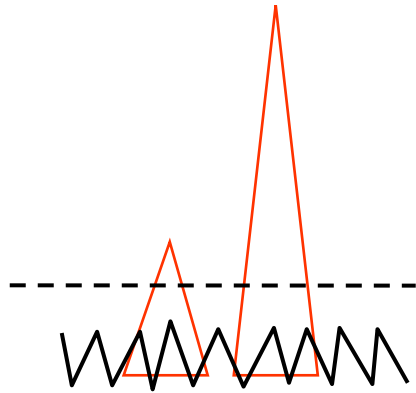


Homozygote threshold h^ can be the height of present allele that gives you a low probability that, if the donor is heterozygote, the partner allele is below the dropout threshold*

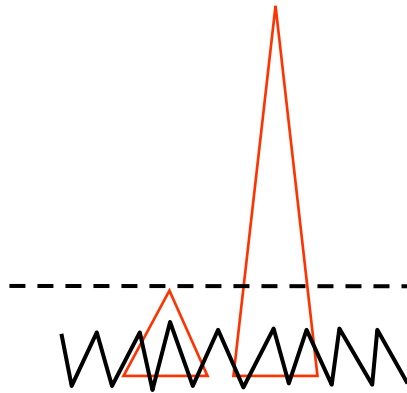
5. Stutters

Stutters

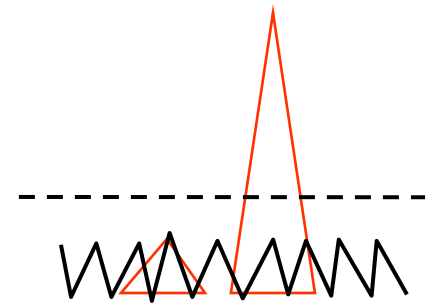
5. Stutters



Visible above the dropout threshold

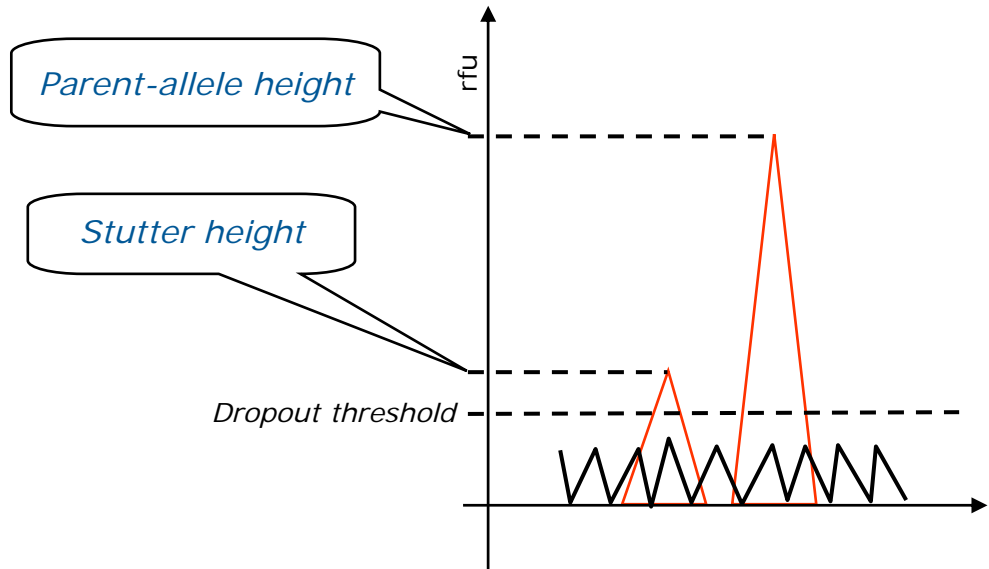


Visible but below the dropout threshold



Embedded in the baseline

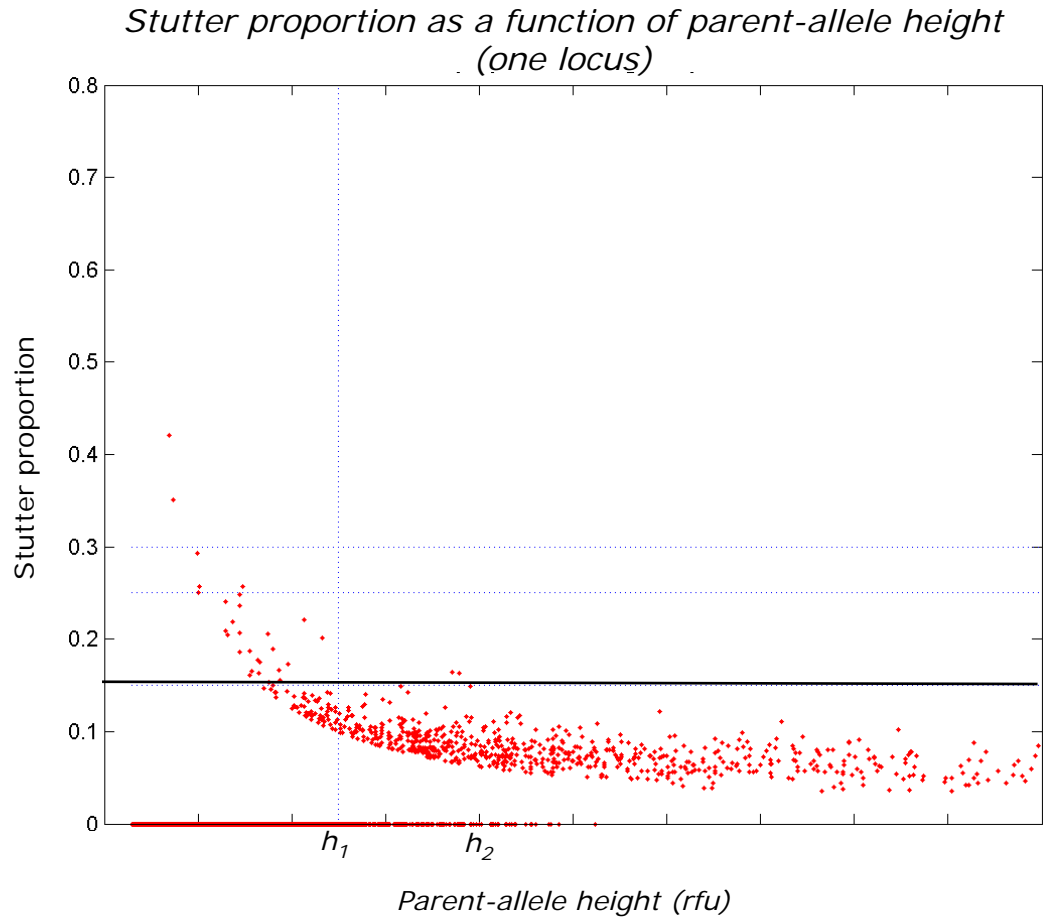
5. Stutters



$$\text{Stutter proportion} = \frac{\text{Stutter height}}{\text{Parent-allele height}}$$

We are interested in changes of stutter proportion as parent-allele height changes

5. Stutters



6. *Conclusions & future work*

- ◆ We have a methodology for selecting thresholds
- ◆ Data used should be representative of casework
- ◆ Currently working on the determination of minimum number of profiles to inform this methodology
- ◆ The methods that use thresholds should be validated

Thank you for your attention