Analysis of Allelic Drop-out

I. Estimation of drop-out probabilities

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Low-template samples

- LT profiles present interpretational challenges
- Can be main profile or minor profile

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Allelic Drop-out

- Alleles present in biological sample may not be detected in resulting DNA profile
- This introduces ambiguity
- Ambiguity complicates assessment of
  - number of contributors
  - estimation of weight of evidence

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LR with *P*(*Do*)

- Likelihood ratios (LR) are the only solution that can allow for drop-out (DO)
- Methods exist to incorporate a factor for DO
- An empirical estimate of the Probability of Drop-out *P*(*Do*) improves the utility of a factor for DO
- An empirical estimate of DO is also necessary to validate expert systems that model DO

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Where's the data?

- A vast repository of data exists from which to estimate *P*(*Do*)
- Few empirical studies have been performed

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The Data

- NIST data (Butler and Hill, 2010)
  www.cstl.nist.gov/strbase/LTDNA.htm
  - Two single source samples
  - Dilution series (10 pg ,20 pg ,100 pg)
  - Identifiler, PowerPlex 16
  - Standard # PCR cycles
  - Each sample amplified 10X

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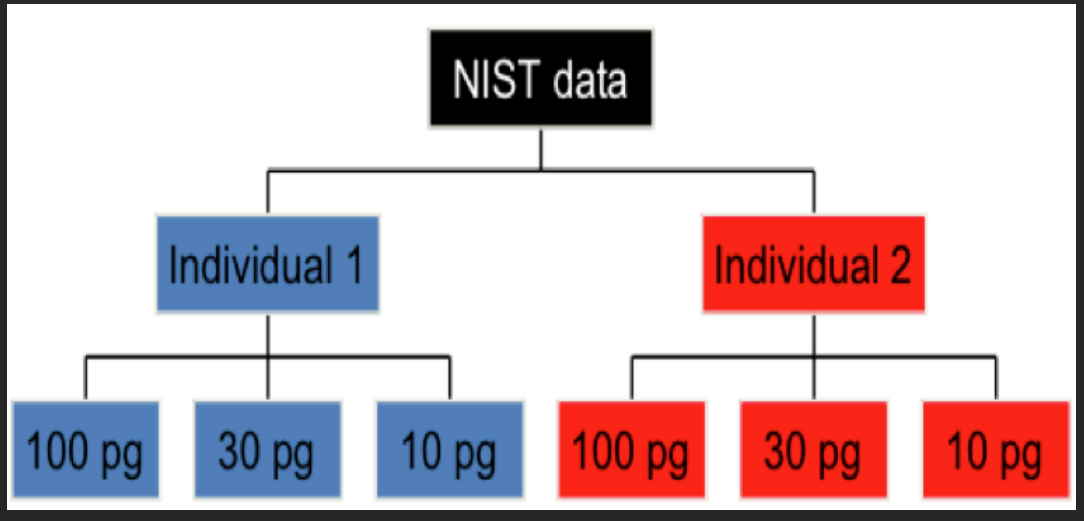
The Data



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Importance of a Threshold

- Determination of DO depends directly on the analytical threshold (AT) employed
- Therefore, the AT must also be empirically determined
- The purpose of an AT:
  - Maximize detection of true peaks
  - Minimize detection of artifacts

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The Question has Changed

- A standard meant for high quantity, high quality samples is no longer appropriate or useful.
- The technology has exceeded our ability to interpret and weight the results
- Low quantity, poor quality samples are routinely processed
- Complex, challenging results are routinely generated

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The Approach must change

- Arbitrary or overly conservative thresholds eliminate noise, *but at the cost of eliminating legitimate signal*
- Throwing away data is NOT conservative
  - human comfort levels leading to policy also lead to inaccuracy
- Eliminating true signal risks
  - false inclusions
  - false exclusions
  - under- or over-estimating the weight of the evidence
- The true value of the evidence is not realized
- Especially for low level, complex, compromised samples

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Empirical determination of AT

- Limit of Quantitation (LOQ) is one standard method to differentiate signal from noise
  - LOQ defined as [mean + 10 SD]
- The signal produced by current genetic analyzers has now become impenetrable to analysis of baseline noise
  - So we need a proxy ...

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SWGDAM 2010

1.1. Analytical threshold: The Laboratory should establish an analytical threshold based on signal-to-noise analyses of internally derived empirical data. As an example, an analytical threshold may be based on two times the intensity difference between the highest peak and lowest trough within the instrumental noise data. .....

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Calculated Thresholds

2X Max is a pretty good proxy for mean + 10 SD!

	2X Max Noise	LOQ
NIST	28	21

- Round up to 30 rfu for this study
- 30 rfu is pretty typical with current systems

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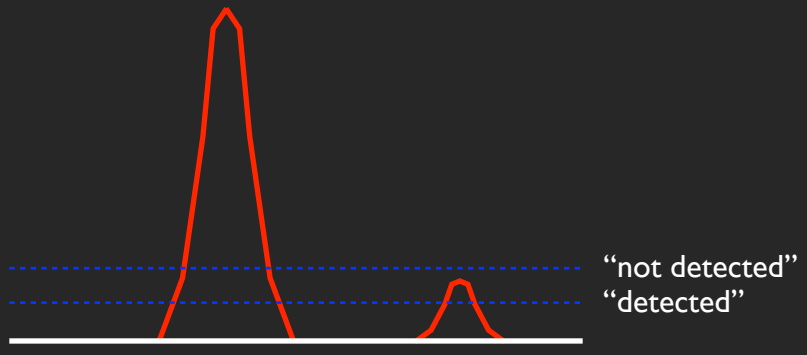
Effect of AT on DO

- If DO defined as:
  - the situation in which a particular peak known to exist in the sample fails to rise above the allelic detection threshold
- An allele that is actually present might not be "detected" in the profile



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Effect of AT on DO

- We compared
  - Empirically determined 30 RFU AT
  - Commonly used 50 RFU AT
- Not surprisingly, fewer instances of apparent DO occur using a lower detection threshold!

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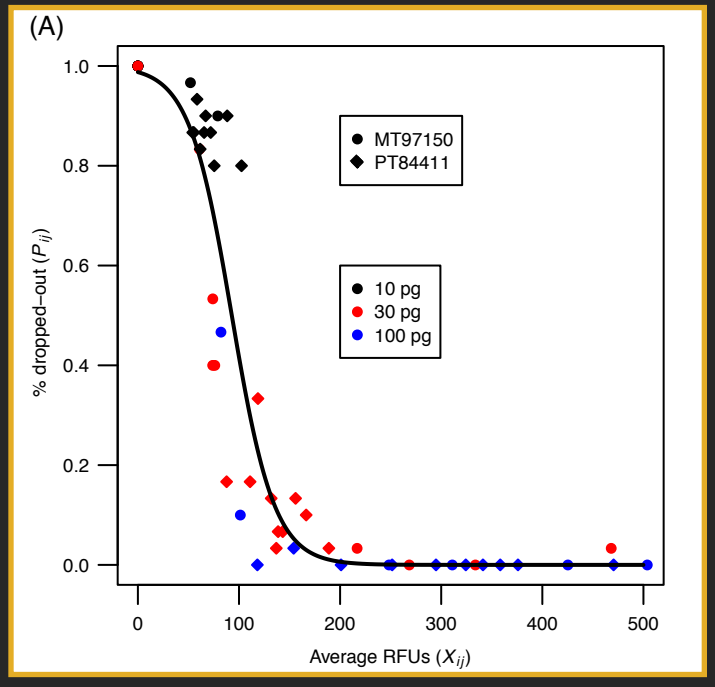
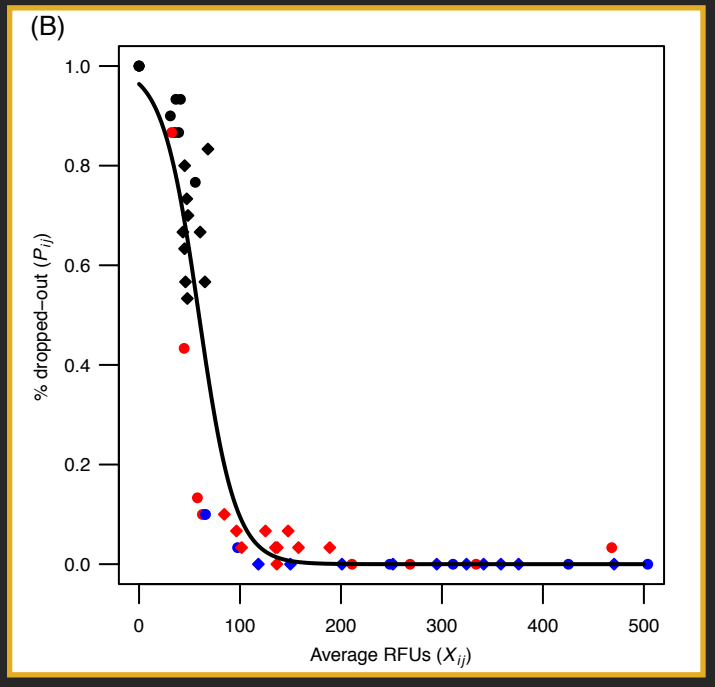
The Model

Identifiler data



50 rfu



30 rfu

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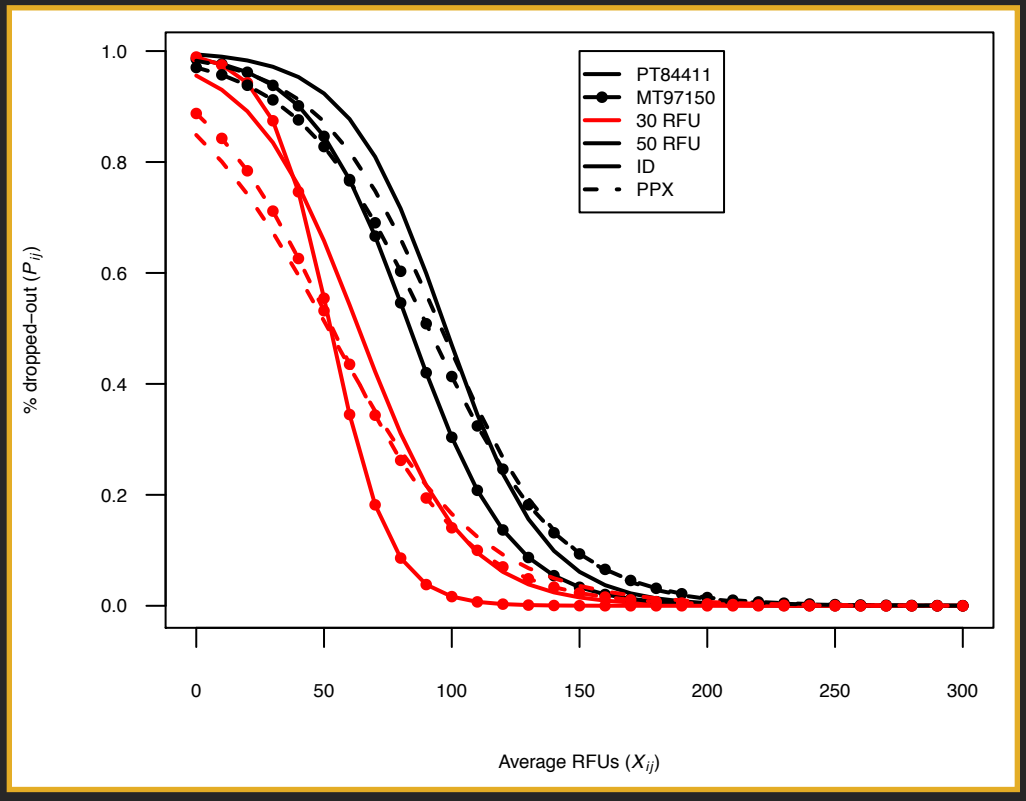
The Model



Logistic Curves
for 2 samples, IDfiler, PPX

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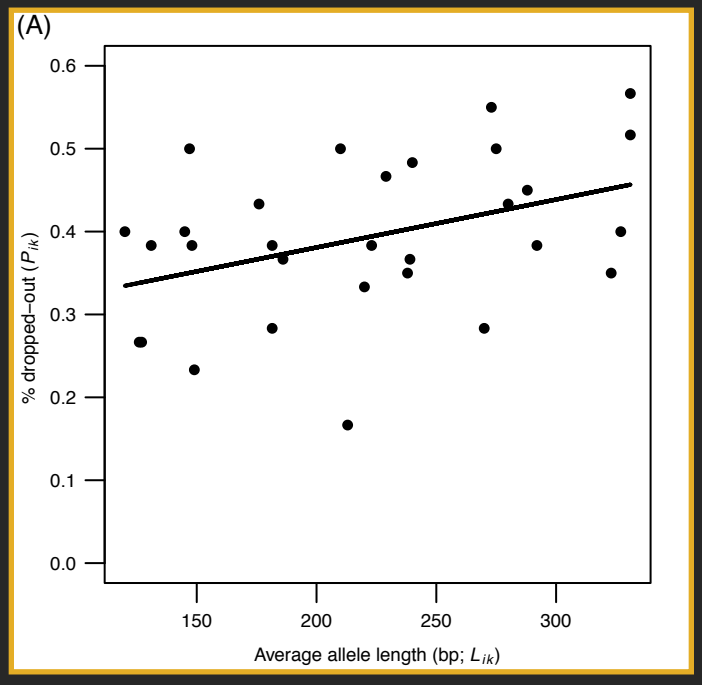
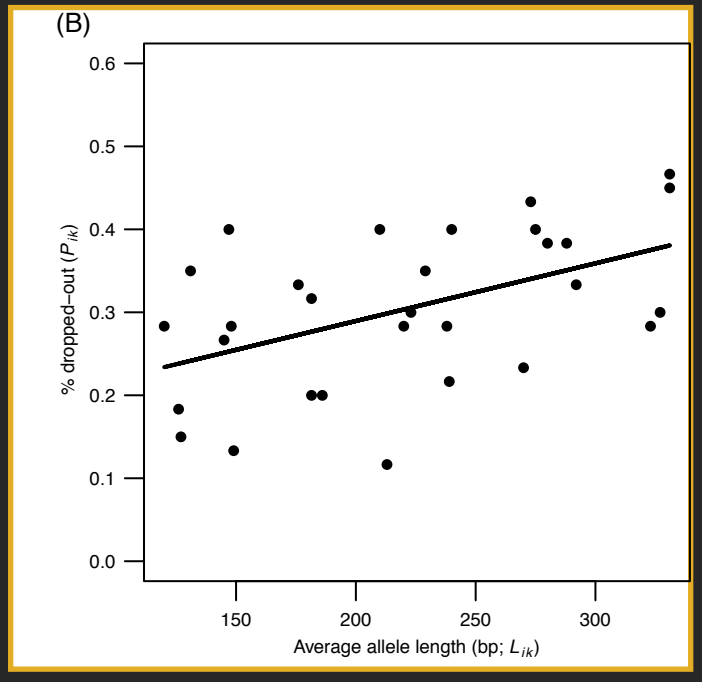
The Model

Identifiler data





50 rfu

30 rfu

allele length

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LR with DO

- Finally, the empirically determined *P*(*Do*) can be incorporated into a LR to estimate the weight of evidence for or against specific propositions
- Use sensitivity testing to test limits
- Test against known non-contributors to test information content of profile, results

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Next up ...

II. Evaluation of drop-out probabilities

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