2012 Mixture Interpretation Workshop:

Mixtures **U**sing *SOUND* **S**tatistics, **I**nterpretation, & **C**onclusions

Introduction to Interpretation: Statistical Approaches and Assumptions

John M. Butler

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Nashville, TN

BOSTON UNIVERSITY

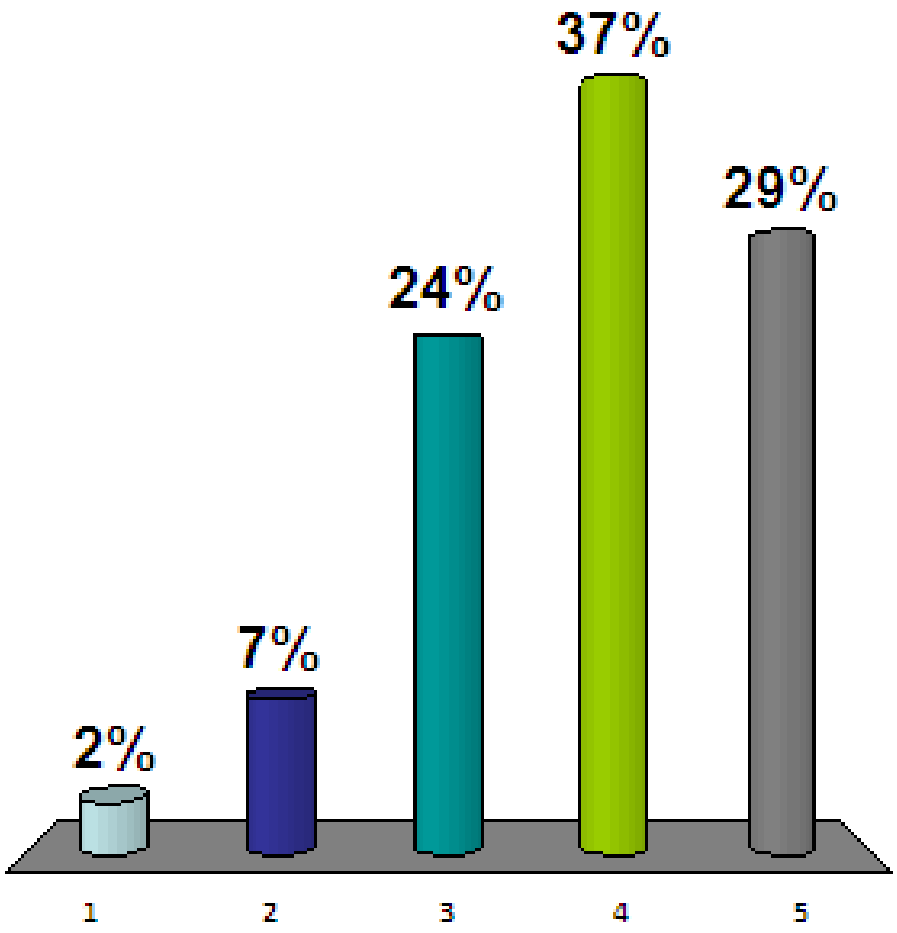
NIST

Which of the topics below would be your first choice for additional training?

From one of the regional mixture workshops (Apr – June 2011)

1. Relevant literature
2. How to validate thresholds
3. How to develop relevant SOPs
4. **Interpretation of low level mixtures**
5. **Statistics**

2/3 want more information on these topics



Planned Presentation Outline

- Overview/thoughts on interpretation & statistics
- SWGDAM 2010 interpretation guidelines
- Thoughts on setting thresholds
- Problems with CPI/CPE statistics
- Take home messages

- ***Workshop goals***: we are not spending a lot of time on good 2-person mixtures; we are trying to help define challenges and uncertainty with more complex mixtures and ways to address them

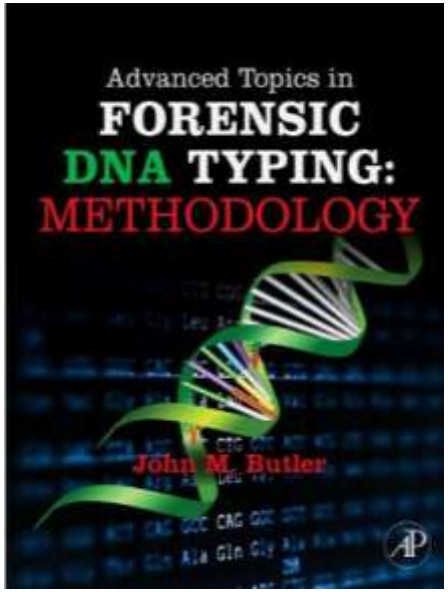
Steps Involved in Process
of Forensic DNA Typing

1) Data Interpretation
2) Statistical Interpretation

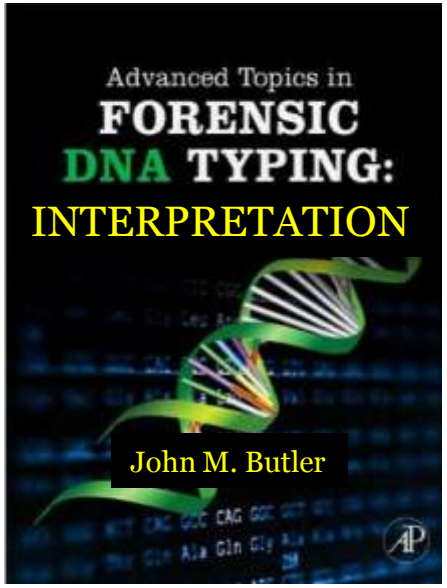
Gathering the Data **Understanding the Data**

Collection/Storage/ Characterization → **Extraction/ Quantitation** → **Amplification/ Marker Sets** → **Separation/ Detection** → **Interpretation** → **Report**

Advanced Topics: Methodology



Advanced Topics: Interpretation

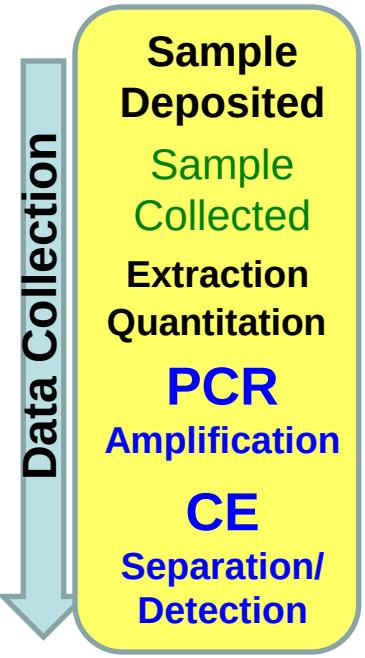


Purpose in Writing a Book on Interpretation

- Each of us thinks our own way is correct – but misinterpretations have given rise to a variety of approaches being undertaken today, some of which are not correct…

- I believe that **a better understanding of general principles will aid consistency and quality of work being performed**

Steps in DNA Interpretation



Signal observed

Peak (vs. noise) → **Allele** (vs. artifact) → **Genotype** (allele pairing) → **Profile** (genotype combining)

A threshold is a value used **to reflect reliability of information** (generally you are more confident of data above a threshold than below)

Data Interpretation

Analytical Threshold — Peak

Stutter Threshold — Allele

Stochastic Threshold — ***All Alleles Detected?***

Peak Height Ratio — Genotype(s)

Mixture Ratio — Contributor profile(s)

Comparison to Known(s)
Weight of Evidence (Stats)

Overview of the SWGDAM 2010 Interp Guidelines

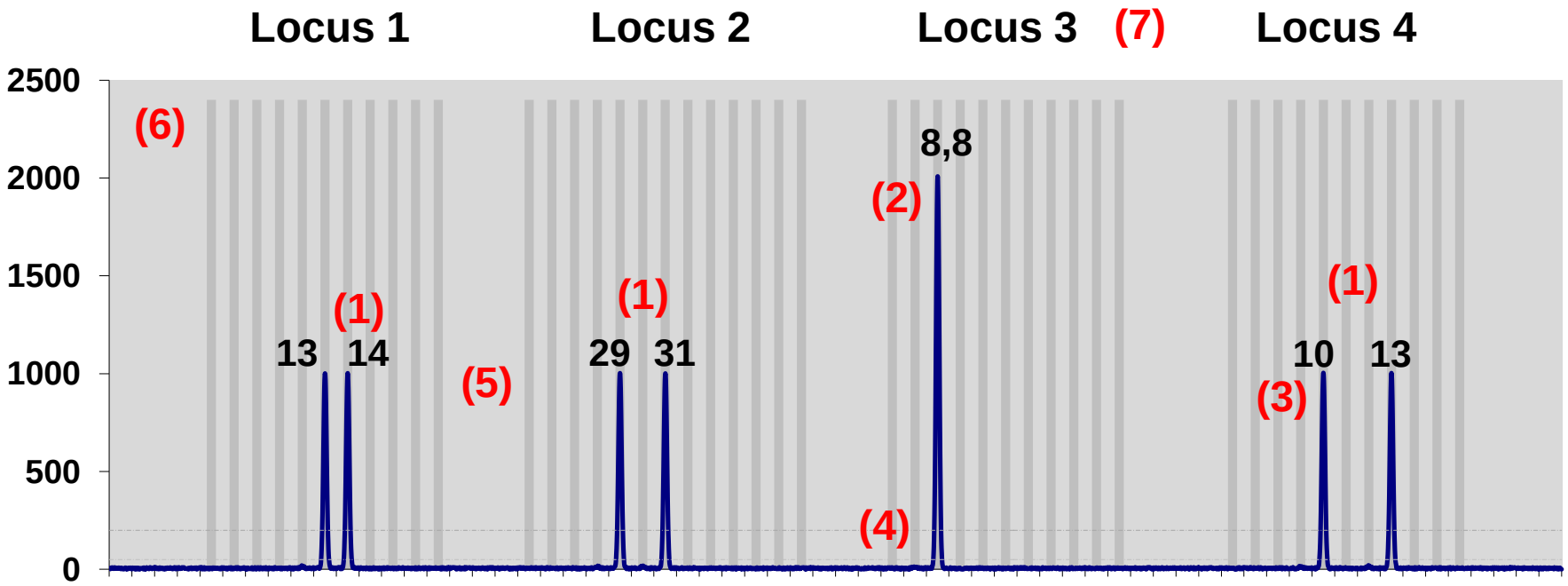
1. Preliminary evaluation of data – **is something a peak and is the analysis method working properly?**
2. Allele designation – **calling peaks as alleles**
3. Interpretation of DNA typing results – **using the allele information to make a determination about the sample**
    1. Non-allelic peaks
    2. Application of peak height thresholds to allelic peaks
    3. Peak height ratio
    4. Number of contributors to a DNA profile
    5. Interpretation of DNA typing results for mixed samples
    6. Comparison of DNA typing results
4. Statistical analysis of DNA typing results – **assessing the meaning (rarity) of a match**

Other supportive material: statistical formulae, references, and glossary

D.N.A. Approach to Understanding

- **D**octrine or Dogma (why?)
  - A fundamental law of genetics, physics, or chemistry
    - Offspring receive one allele from each parent
    - Stochastic variation leads to uneven selection of alleles during PCR amplification from low amounts of DNA templates
    - Signal from fluorescent dyes is based on …
- **N**otable Principles (what?)
  - The amount of signal from heterozygous alleles should be similar
- **A**pplications (how?)
  - Peak height ratio measurements

Using **Ideal Data** to Discuss Principles



(1) 100% PHR between heterozygous alleles
(2) Homozygotes are exactly twice heterozygotes due to allele sharing
(3) No peak height differences exist due to size spread in alleles (any combination of resolvable alleles produces 100% PHR)
(4) No stutter artifacts enabling mixture detection at low contributor amounts
(5) Perfect inter-locus balance
(6) Completely repeatable peak heights from injection to injection on the same or other CE instruments in the lab or other labs
(7) Genetic markers that are so polymorphic all profiles are fully heterozygous with distinguishable alleles enabling better mixture detection and interpretation

Challenges in real-world data

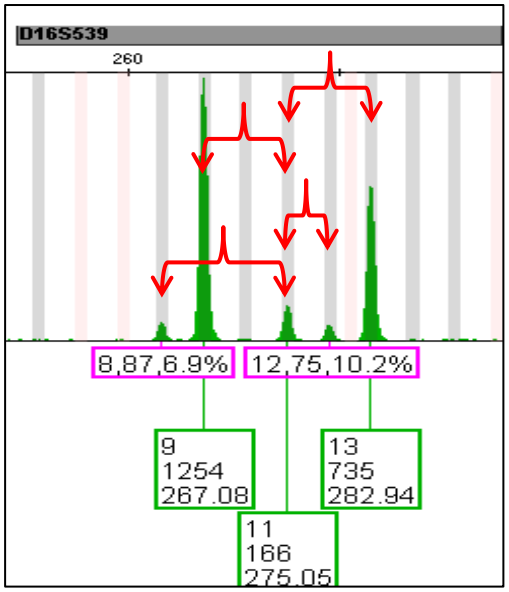
- **Stochastic (random) variation** in sampling each allele during the PCR amplification process
  - This is highly affected by DNA quantity and quality
  - Imbalance in allele sampling gets worse with low amounts of DNA template and higher numbers of contributors
- **Degraded DNA** template may make some allele targets unavailable
- **PCR inhibitors** present in the sample may reduce PCR amplification efficiency for some alleles and/or loci
- **Overlap of alleles** from contributors **in DNA mixtures**
  - Stutter products can mask true alleles from a minor contributor
  - Allele stacking may not be fully proportional to contributor contribution

Do You Have Uncertainty in Your Data?

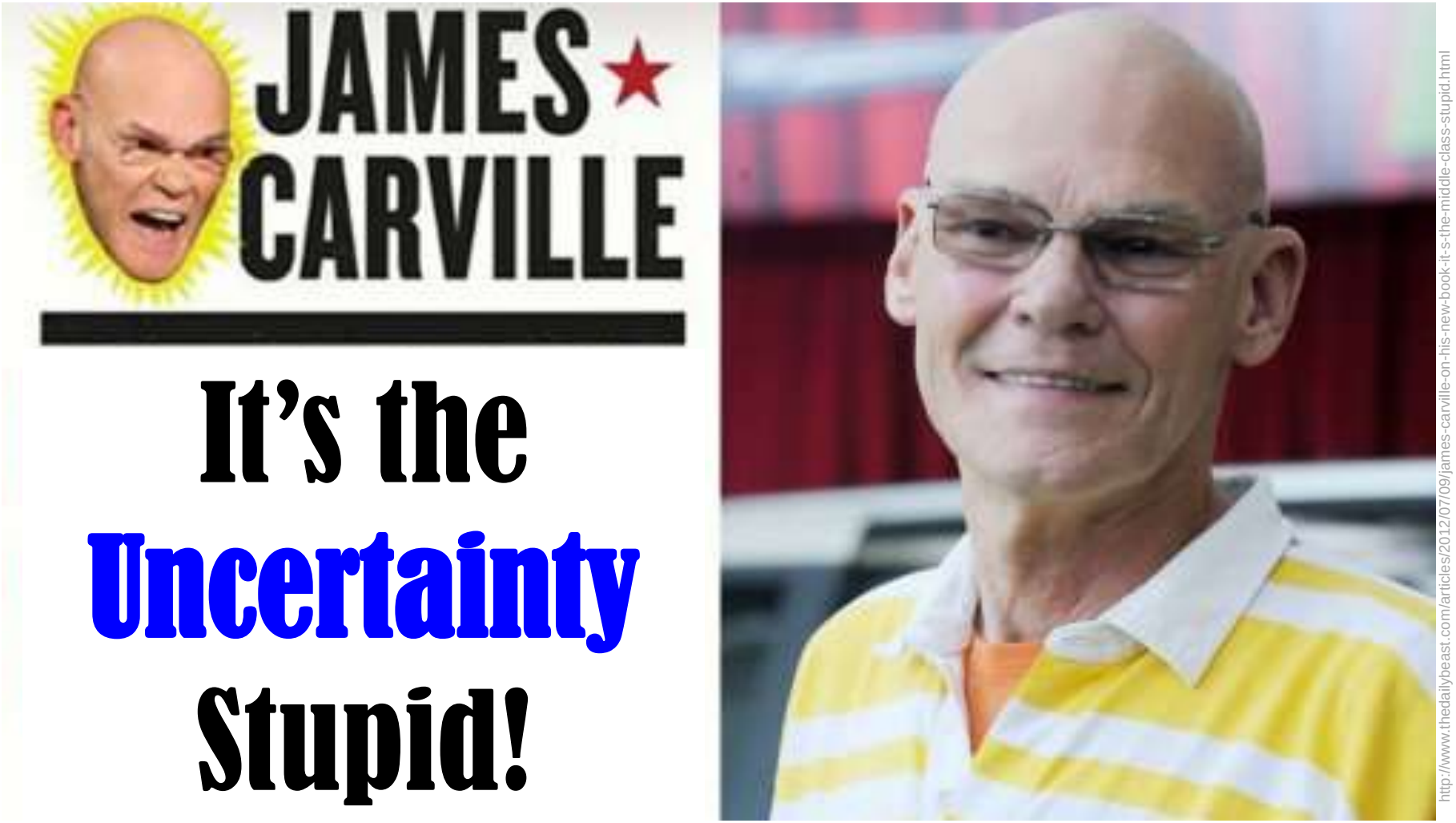
- **If allele dropout is a possibility** (e.g., in a partial profile), then there is uncertainty in whether or not an allele is present in the sample…and therefore what genotype combinations are possible

- **If different allele combinations are possible** in a mixture, then there is uncertainty in the genotype combinations that are possible…

Possible allele pairing with the 11



It is the Uncertainty that Matters…



Uncertainty and Probability

- "Contrary to what many people think, **uncertainty is present throughout any scientific procedure**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

- "It is now recognized that **the only tool for handling uncertainty is probability**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

Results Depend on Assumptions

- "Although courts expect one simple answer, statisticians know that **the result depends on how questions are framed and on assumptions tucked into the analysis**."
  - Mark Buchanan, Conviction by numbers. *Nature* (18 Jan 2007) 445: 254-255

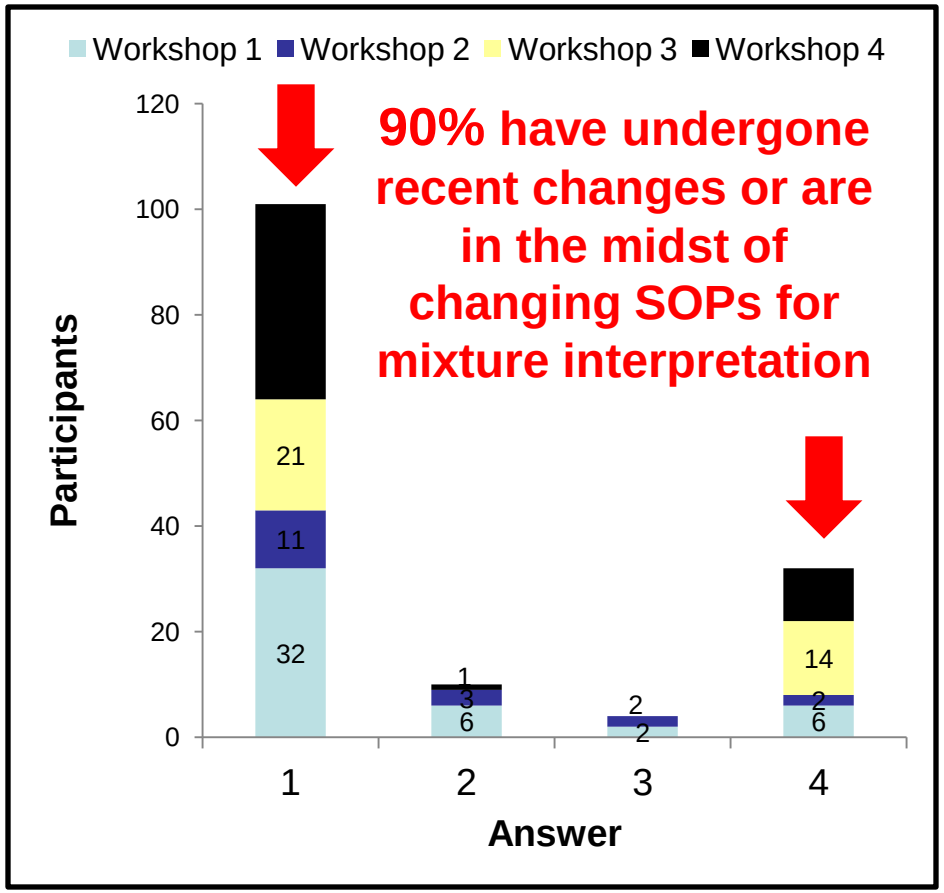
Is your lab in the process of changing your protocols?

Perhaps lowering your expected PHR 70% down to 55%?

Has your lab implemented changes to your SOPs based on the new guidelines?

1. Yes
2. No
3. Reviewed SOPs but no changes needed
4. Working on it

N=147
Regional mixture workshops
(Apr – June 2011)



From ISHI 2011 poster "Impact of the SWGDAM Mixture Interpretation Guidelines: Successes, Issues and Suggested Future Directions"

Stats Required for Inclusions

SWGDAM Interpretation Guideline 4.1:

> "**The laboratory must perform statistical analysis in support of any inclusion** that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis."

Buckleton & Curran (2008): "There is a considerable aura to DNA evidence. Because of this aura **it is vital that weak evidence is correctly represented as weak or not presented at all**."

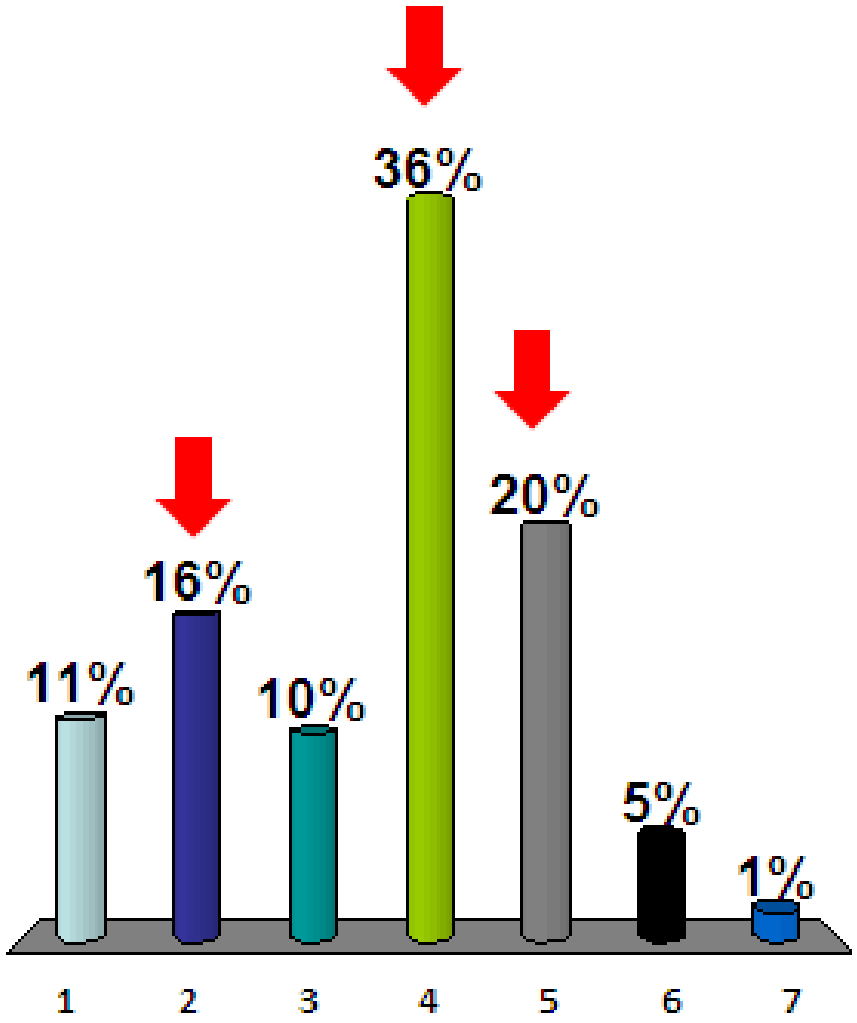
Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

Last Year's Response

What kind of mixture statistic does your lab use?

72% using CPI

1. LR
2. CPE (RMNE, CPI)
3. RMP
4. CPE or RMP
5. Other combinations
6. Probabilistic modeling (e.g., TrueAllele)
7. We don't use stats (contradicting the new guidelines – section 4.1)



Data from 138 responses
ISHI Mixture Workshop (Oct 2011)

CPE/CPI (RMNE) Limitations

- A CPE/CPI approach assumes that ***all alleles*** **are present** (i.e., cannot handle allele drop-out)
- Thus, statistical analysis of low-level DNA CANNOT be correctly performed with a CPE/CPI approach because some alleles may be missing
- Charles Brenner in his AAFS 2011 talk addressed this issue
- Research is on-going to develop allele drop-out models and software to enable appropriate calculations

Notes from Charles Brenner's AAFS 2011 talk

The Mythical "Exclusion" Method for Analyzing DNA Mixtures – Does it Make Any Sense at All?

1. The claim that it requires **no assumption about number of contributors** is mostly wrong.
2. The supposed **ease of understanding** by judge or jury is really an illusion.
3. **Ease of use** is claimed to be an advantage particularly for complicated mixture profiles, those with many peaks of varying heights. The truth is the exact opposite. **The exclusion method is completely invalid for complicated mixtures.**
4. The exclusion method is only **conservative** for guilty suspects.

Conclusion: "Certainly no one has laid out an explicit and rigorous chain of reasoning from first principles to support the exclusion method. It is at best guesswork."

Brenner, C.H. (2011). The mythical "exclusion" method for analyzing DNA mixtures – does it make any sense at all? *Proceedings of the American Academy of Forensic Sciences*, Feb 2011, Volume 17, p. 79

DAB Recommendations on Statistics

February 23, 2000

Forensic Sci. Comm. 2(3); available on-line at
http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
  - Devlin, B. (1993) Forensic inference from genetic markers. *Statistical Methods in Medical Research* 2: 241–262.
- Likelihood ratios (LR)
  - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

- Discussion about exclusion probabilities in Paternity cases.

Two types:

(1) Conditional Exclusion Probability - excluding a random man as a possible father, given the mother-child genotypes for a particular case.

(2) Average Exclusion Probability – excluding a random man as a possible father, given a randomly chosen mother-child pair.

Statistical Methods in Medical Research 1993; **2**: 241–262

Forensic inference from genetic markers

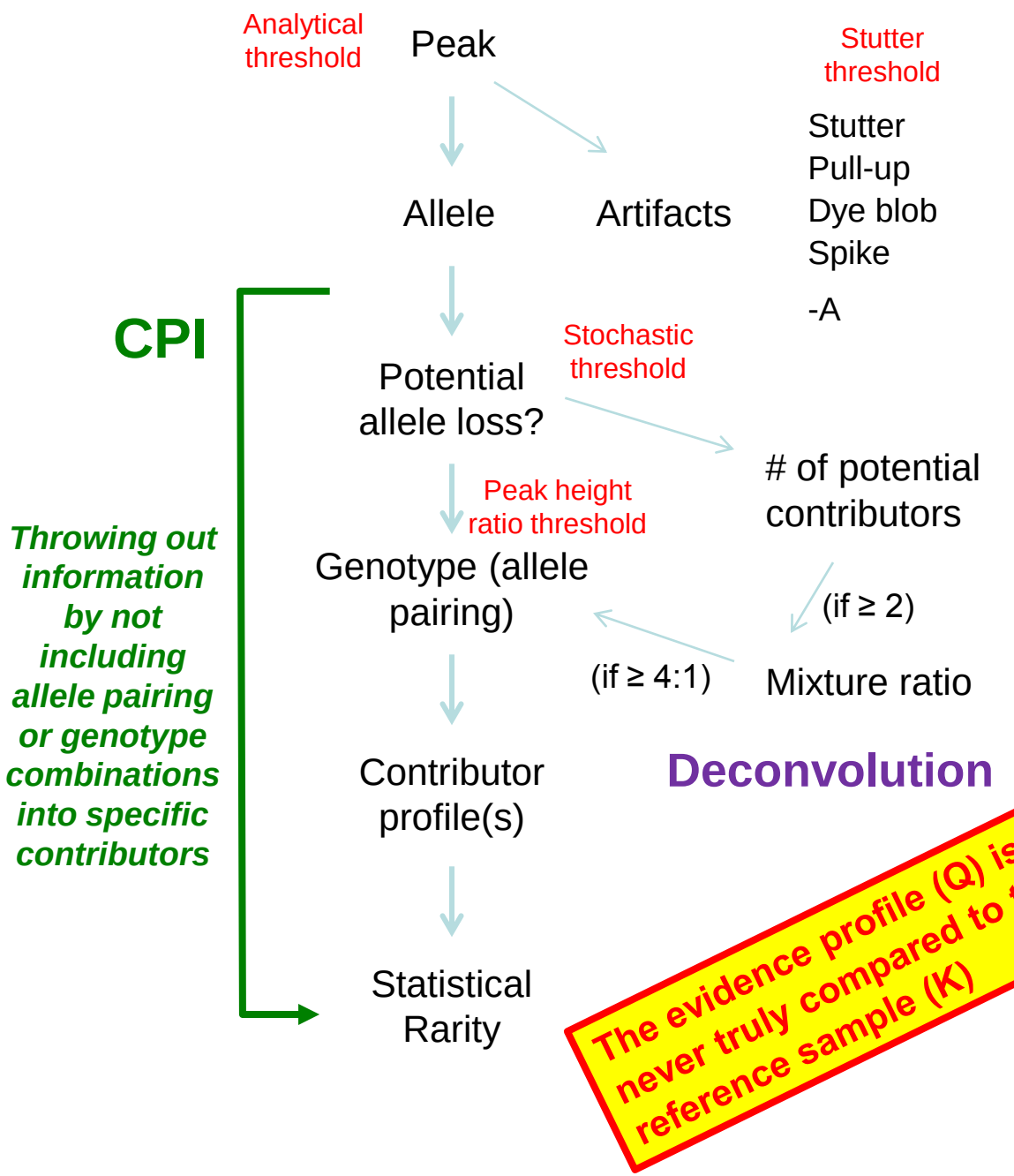
B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

“The theoretical concept of exclusion probabilities, however, makes no sense within the framework of normal mixture models.”

“The interpretation of conditional exclusion probability is obvious, which accounts for its value in the legal arena. Unlike [LR], however, it is not fully efficient.”

Problem with CPI Approach

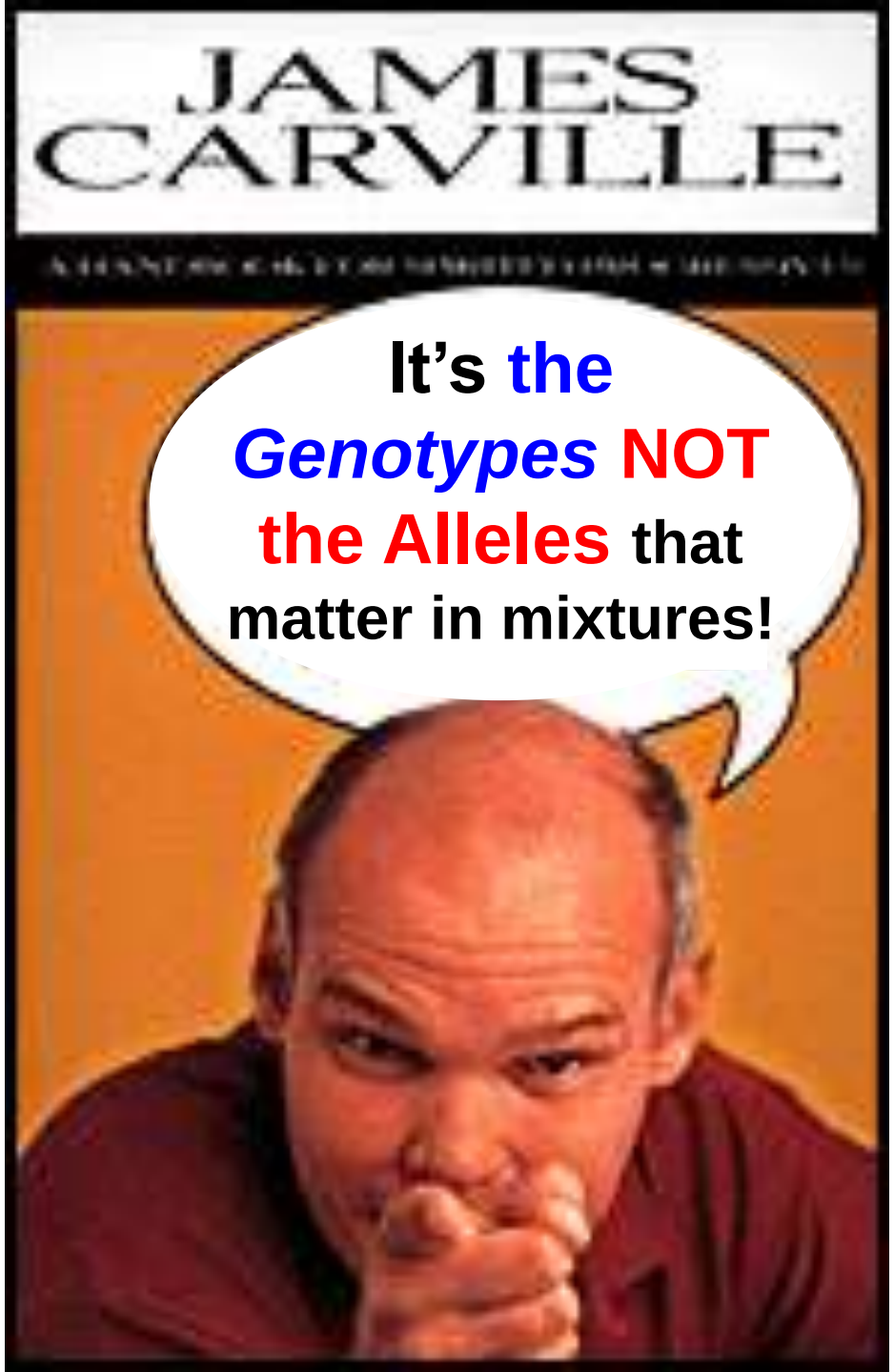


CPI deals with alleles NOT specific genotype combinations

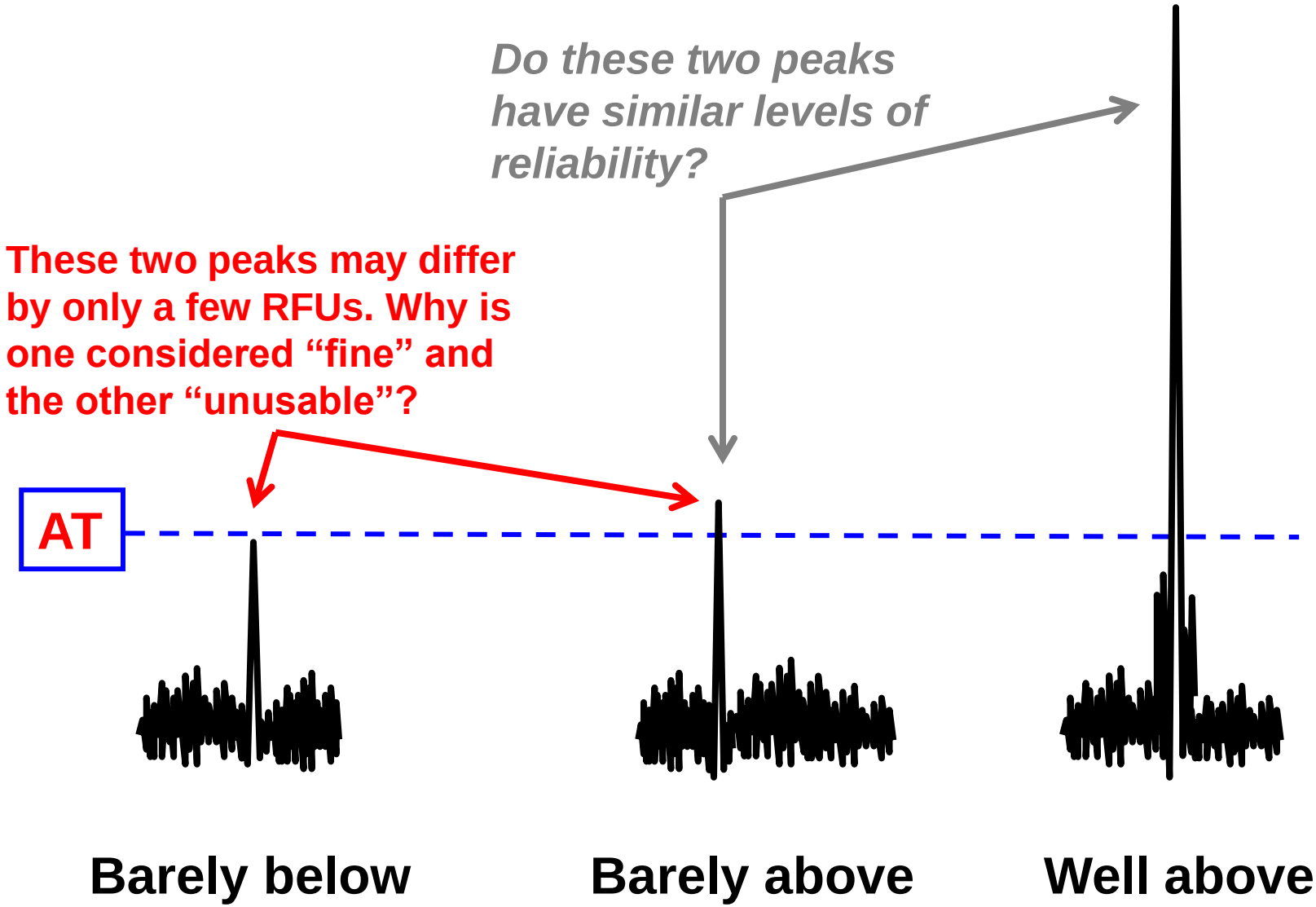
Q → K Comparison

Report Issued with conclusions (inclusion, exclusion, inconclusive)

Just Remember



What is the meaning of a threshold?

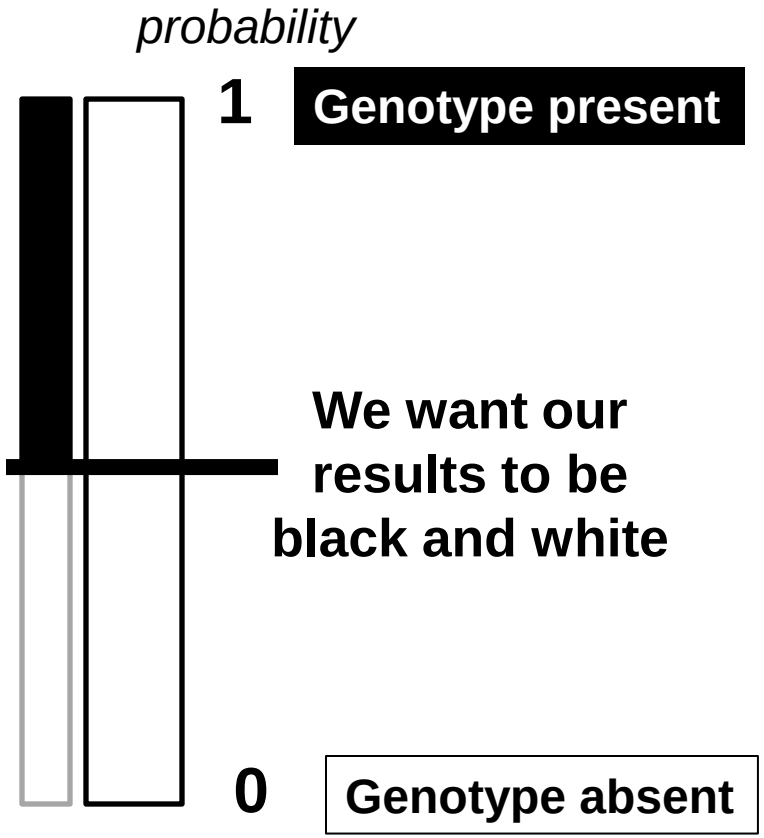


Keep in Mind…

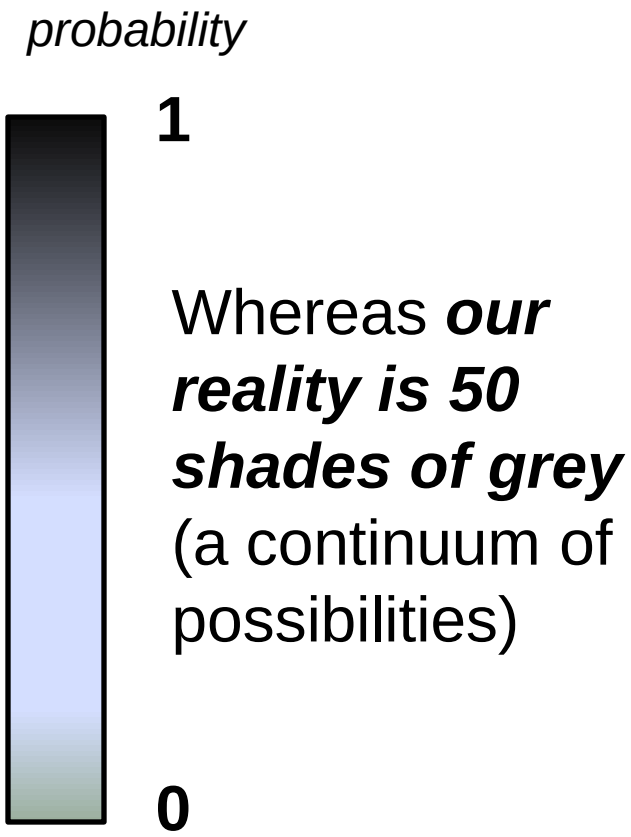
"The use of bounds **applied to data that show continuous variation** is common in forensic science and is often a pragmatic decision. However it should be borne in mind that applying such bounds has arbitrary elements to it and that **there will be cases where the data lie outside these bounds**."

Bright, J.A., et al. (2010). Examination of the variability in mixed DNA profile parameters for the Identifiler multiplex. *Forensic Science International: Genetics, 4,* 111-114.

Approaches to Data Interpretation:
Binary vs Probabilistic



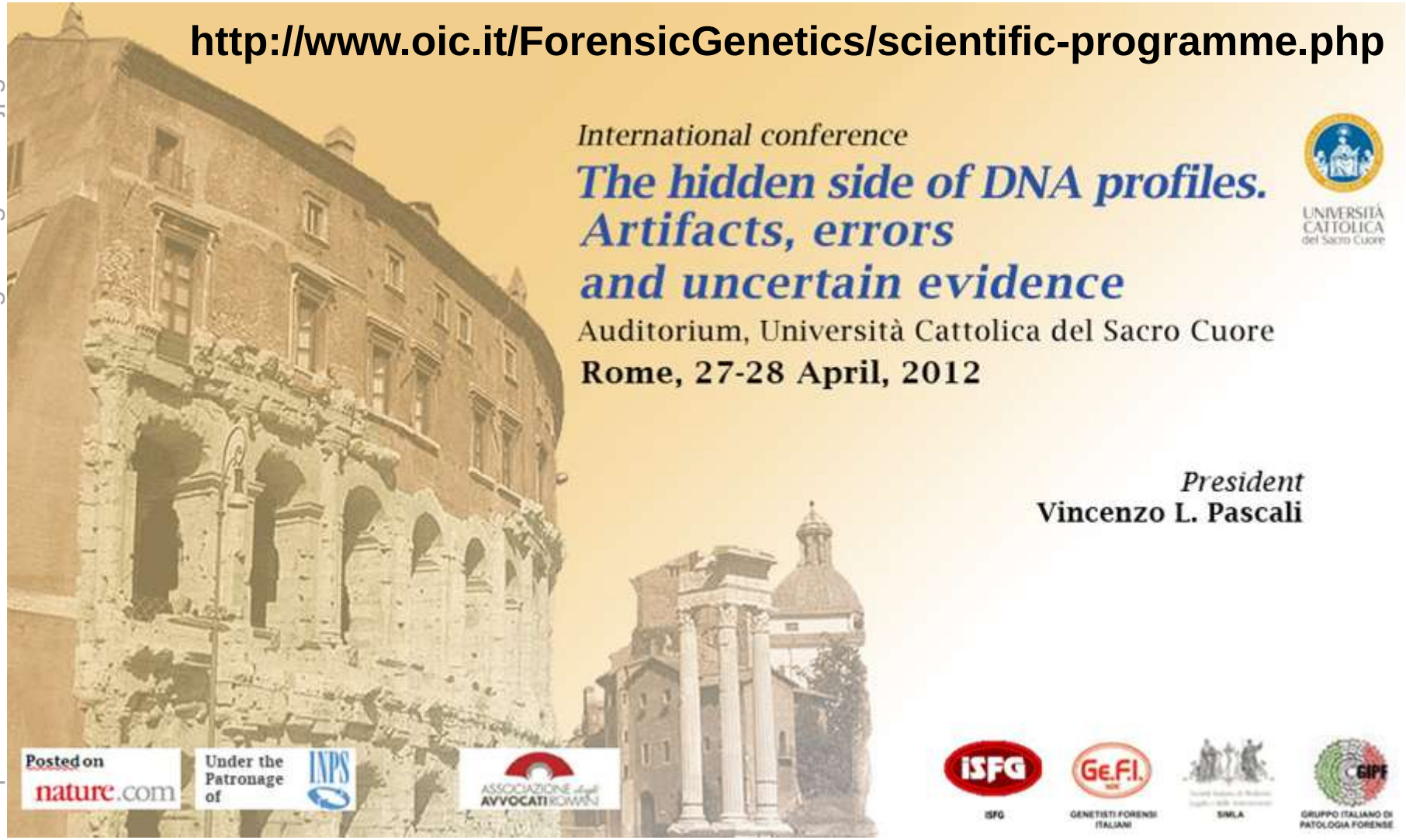
Binary Approach



Whereas ***our reality is 50 shades of grey*** (a continuum of possibilities)

Probabilistic Approach

Conference Held in Rome Earlier This Year



http://www.oic.it/ForensicGenetics/immagini/img-home.jpg

Bruce Budowle

University of North Texas Health Science Center

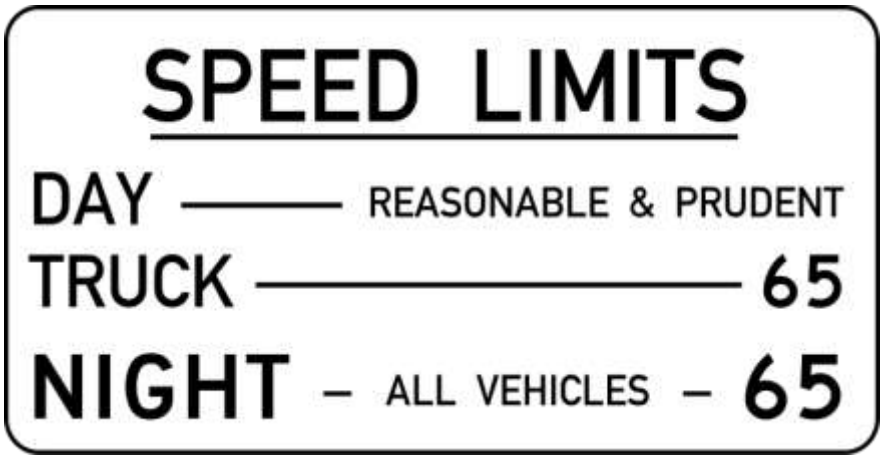
- "**We put thresholds in place to help protect us from risk of making wrong decisions**. They have value."

- **Compares thresholds to speed limits**, which are set for safety reasons

Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Do you leave thresholds and protocols up to “analysts’ discretion”?

http://korsgaardscommentary.blogspot.com/2011/10/its-time-to-put-brakes-on-speed-limit.html



http://en.wikipedia.org/wiki/File:MONTANA-PR.svg

Typical speed limit sign that one would see at the Montana state line from December 1995 to June 1999

http://1.bp.blogspot.com/-5gagI4xZbT0/TdvMBGODBZI/AAAAAAAAJYo/Pj9MRqANvvs/s400/speed-limit-change-sign-537.jpg

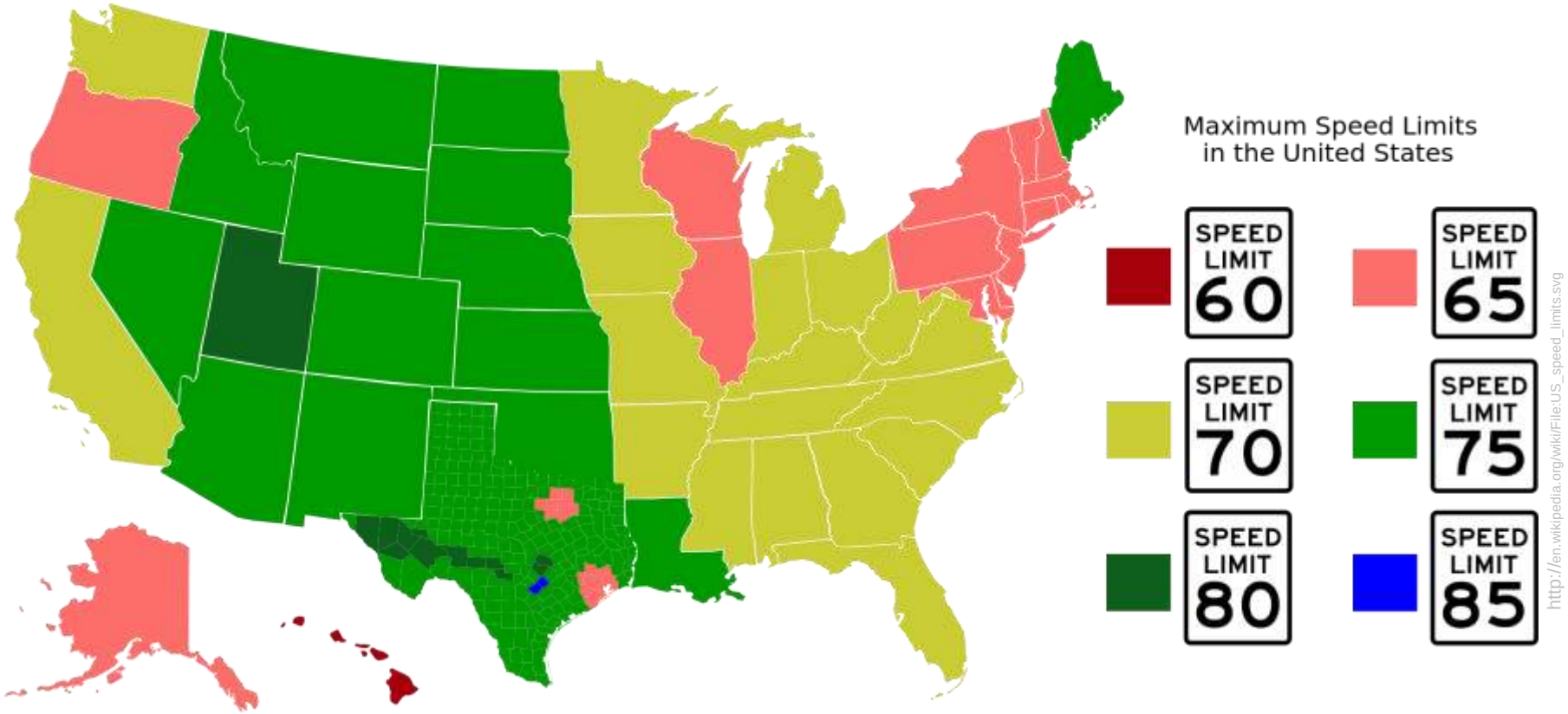
A Potential Outcome!

Do you carefully try to regulate everything with specific protocols?

http://www.lostrepublic.us/archives/category/monetary-policy/privatized-roads

Truly **a protocol with specificity**…. we even have **an auditor**, the local chief of police!

A variety of approaches exist for how protocols and thresholds are set…



http://en.wikipedia.org/wiki/Speed_limits_in_the_United_States

How Speed Limits Are Set?

http://www.crab.wa.gov/LibraryData/REPORTS/EngineerAnswers/Article03-04SpeedLimits.pdf

The posted speed limit for a road is set in slightly different ways in different counties. The most common way though, is to use **the "85th percentile" speed**. 85 out of 100 drivers will choose this speed no matter what the signs say. Many studies have shown this method to be safe, practical and enforceable. It also doesn't depend on the opinion of one person.

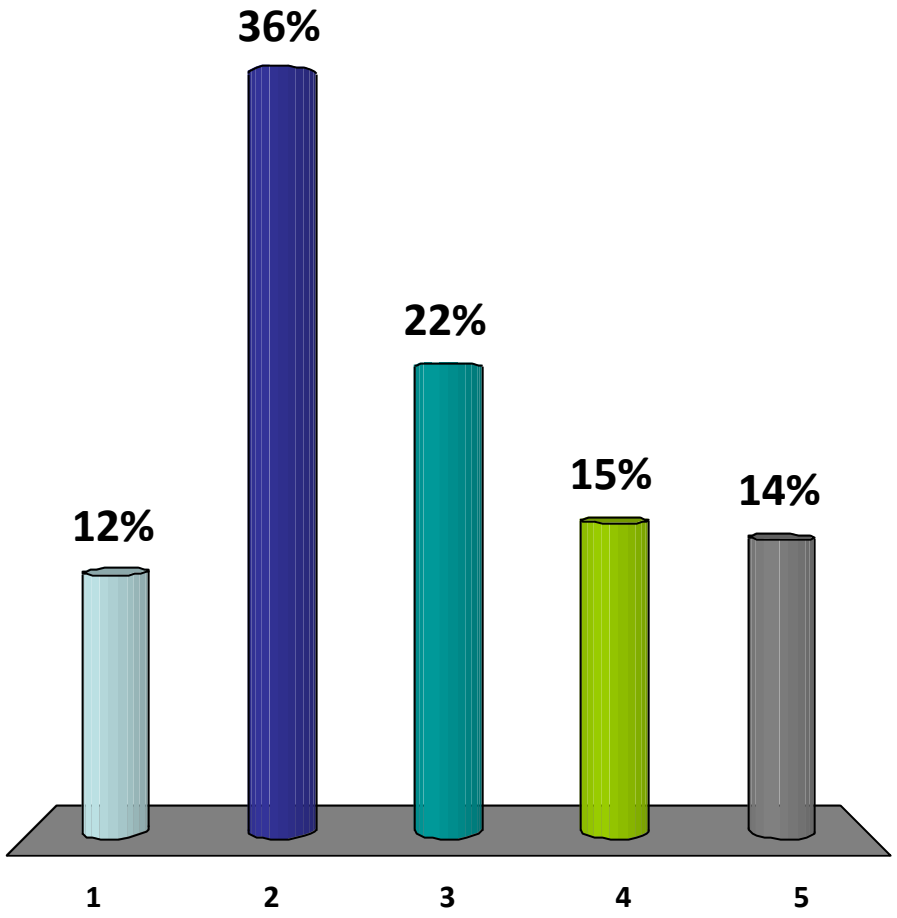
The 85th percentile speed is easily determined with special traffic counters that check the traffic on the roadway. The speed limit can then be set at the next lower 5 miles per hour. For example, if the traffic counters show 38 mph, the limit would be set at 35 mph. The speed limit may be set another 5 mph lower if there are features not obvious to the driver. These may include unusual roadside or traffic conditions including a high number of accidents.

How were the RFU levels set for your laboratory stochastic threshold?

(select only one)

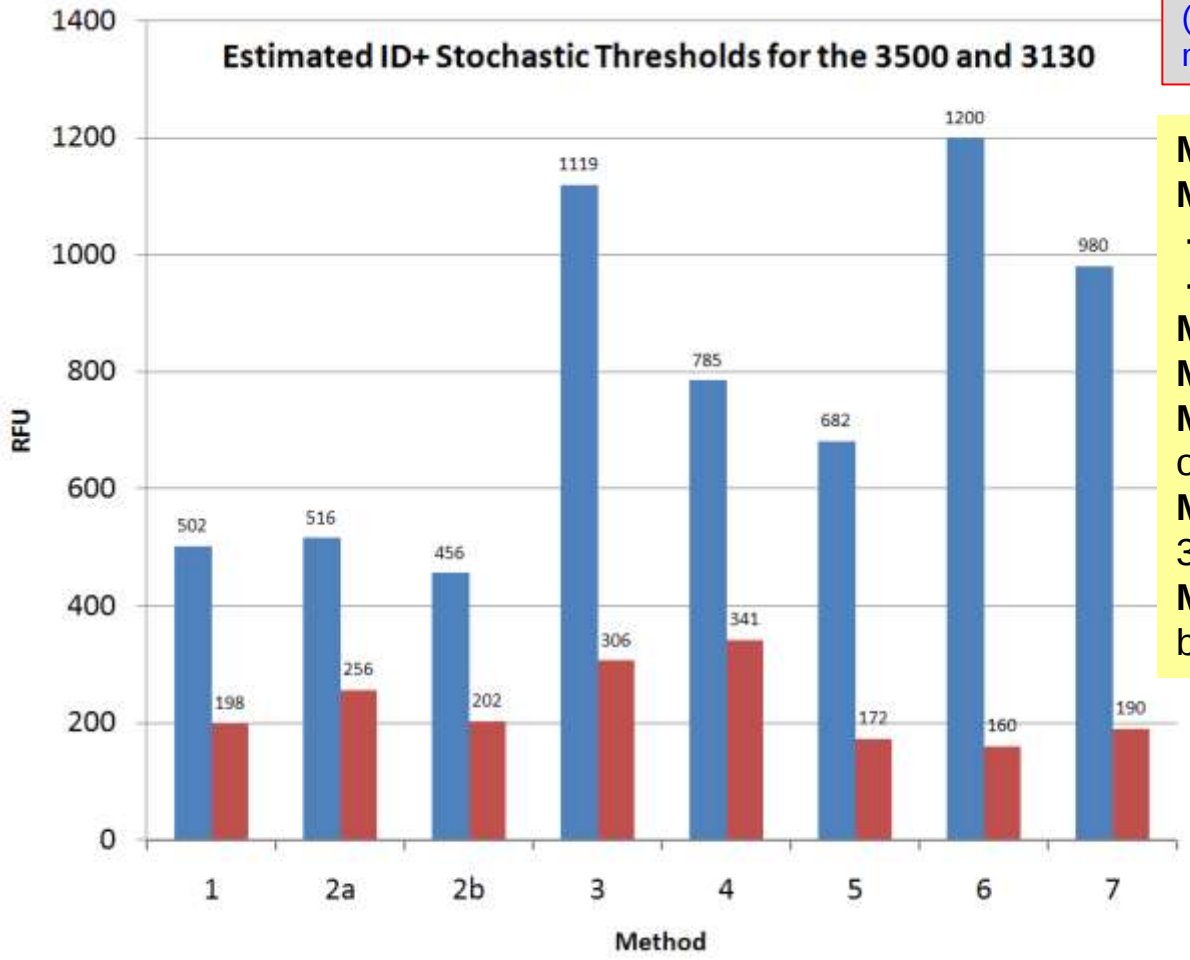
Data from 121 responses
ISHI Mixture Workshop (Oct 2012)

1. +2 SD
2. +3 SD
3. Above all dropout data
4. My TL established; I have no idea how
5. We do not use a stochastic threshold



Different approaches to determining a stochastic threshold

Results from CA DOJ Identifiler Plus validation experiments



Studied 3 DNA samples with serial dilutions (1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.016 ng), multiple amps of each template quantity

Method 1: tallest false homozygote
Method 2: false homo. ave. +3SD
- **2a:** using most relevant input amount
- **2b:** using all observed false homo.

Method 3: average PH het. +3 SD
Method 4: ave. PHR -3 SD vs. signal
Method 5: AT divided by minimum observed PHR
Method 6: partial profile at ~150 pg and 3x AT
Method 7: where majority of PHRs fall below 60%

Blue bars: 3500 ST
Red bars: 3130 ST

Sonja Klein (CA DOJ) presentation at the CAC meeting (Sacramento, CA), October 25, 2011: "Approaches to estimating a stochastic threshold"

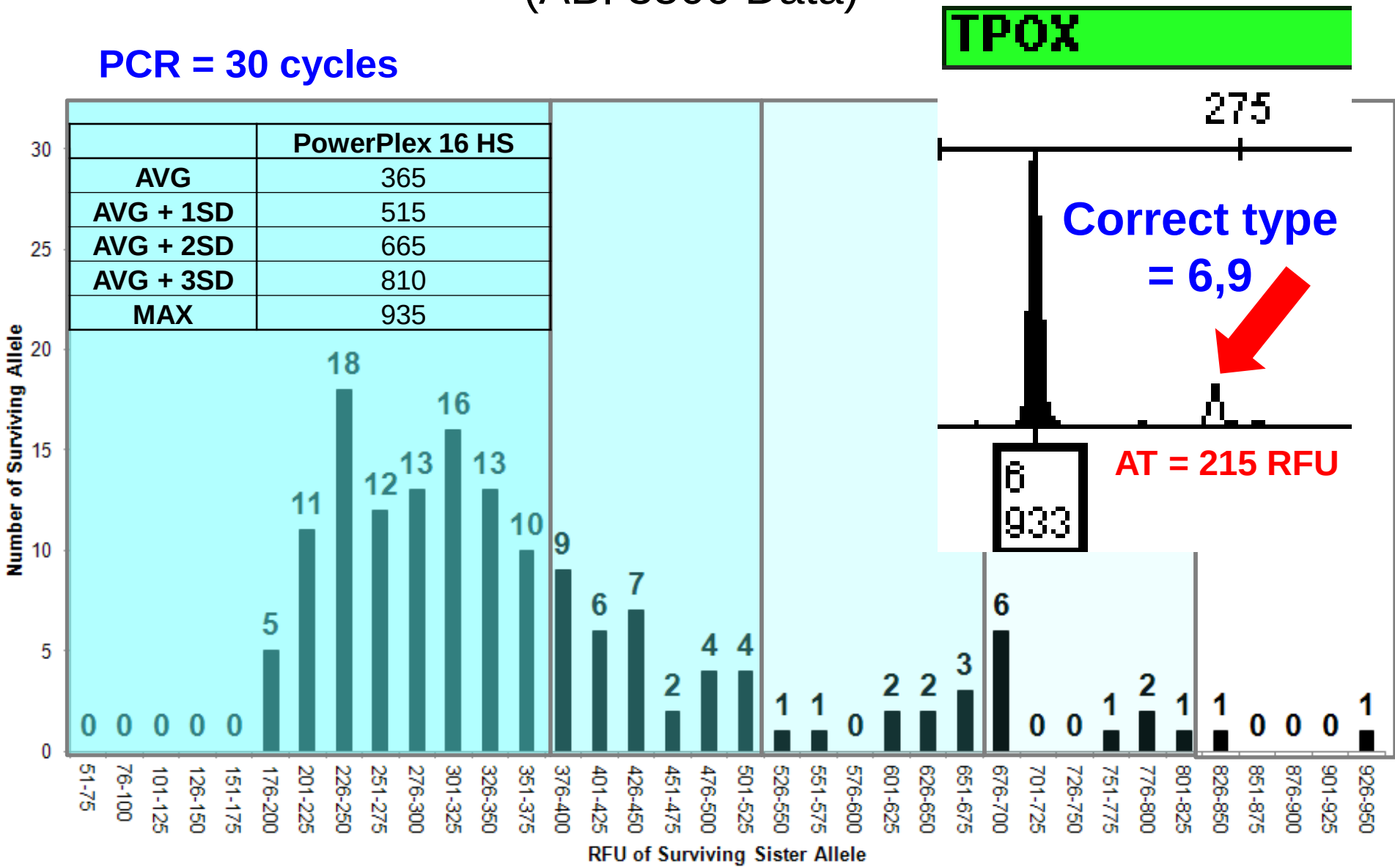
Data from Erica Butts (NIST), ISHI 2011 poster

PowerPlex 16 HS Stochastic Threshold

(ABI 3500 Data)

PCR = 30 cycles

	PowerPlex 16 HS
AVG	365
AVG + 1SD	515
AVG + 2SD	665
AVG + 3SD	810
MAX	935



Limitations of Stochastic Thresholds

- The possibility of allele sharing with a complex mixture containing many contributors may make a stochastic threshold meaningless

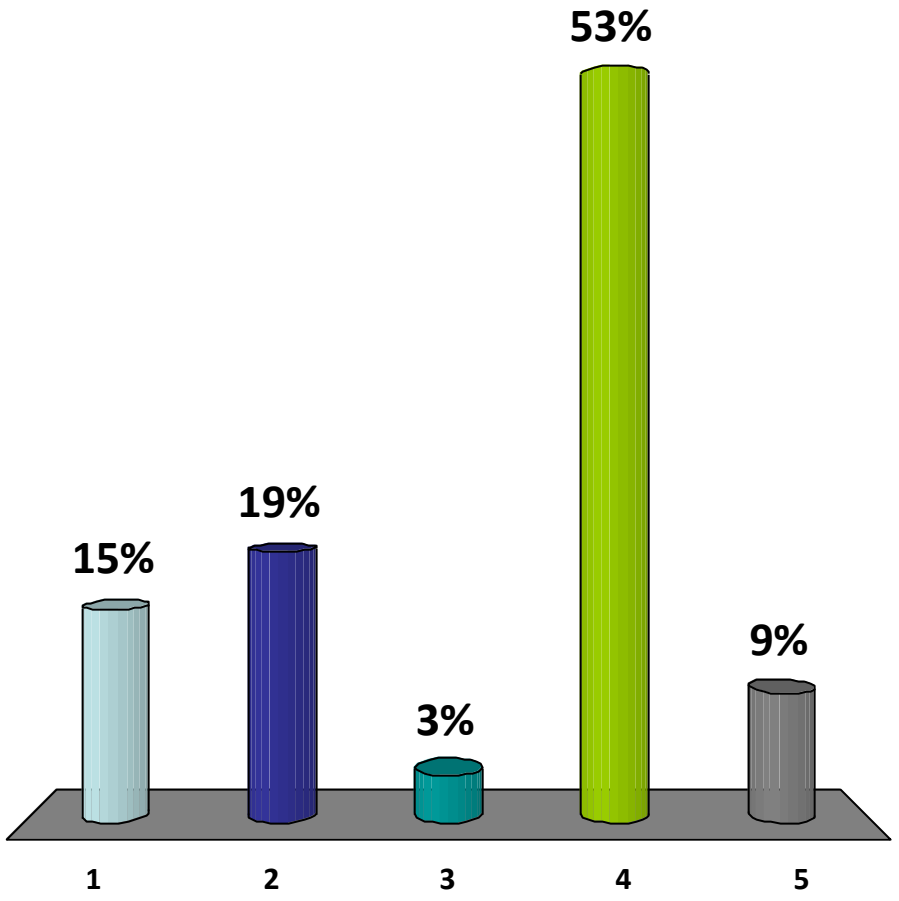
- “Enhanced interrogation techniques” to increase sensitivity (e.g., increased PCR cycles) may yield false homozygotes with >1000 RFU

- **New turbo-charged kits with higher sensitivity will need to be carefully evaluated to avoid allele drop-out and false homozygotes**

If your laboratory uses a stochastic threshold (ST), it is:

1. Same value as our analytical threshold (**we don't use a ST**)
2. About twice as high as our AT (e.g., AT = 50 and ST = 100 RFU)
3. Less than twice as high as our AT
4. Greater than twice as high as our AT
5. I don't know!

Data from 120 responses
ISHI Mixture Workshop (Oct 2012)



Last Year's Response

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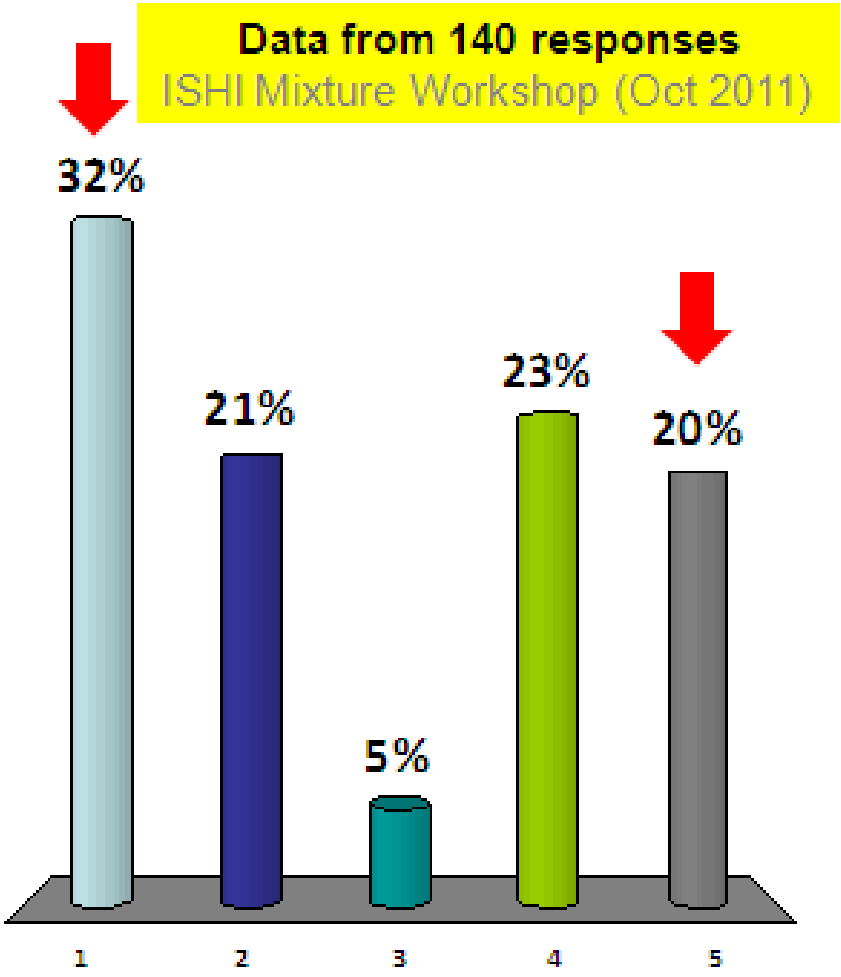
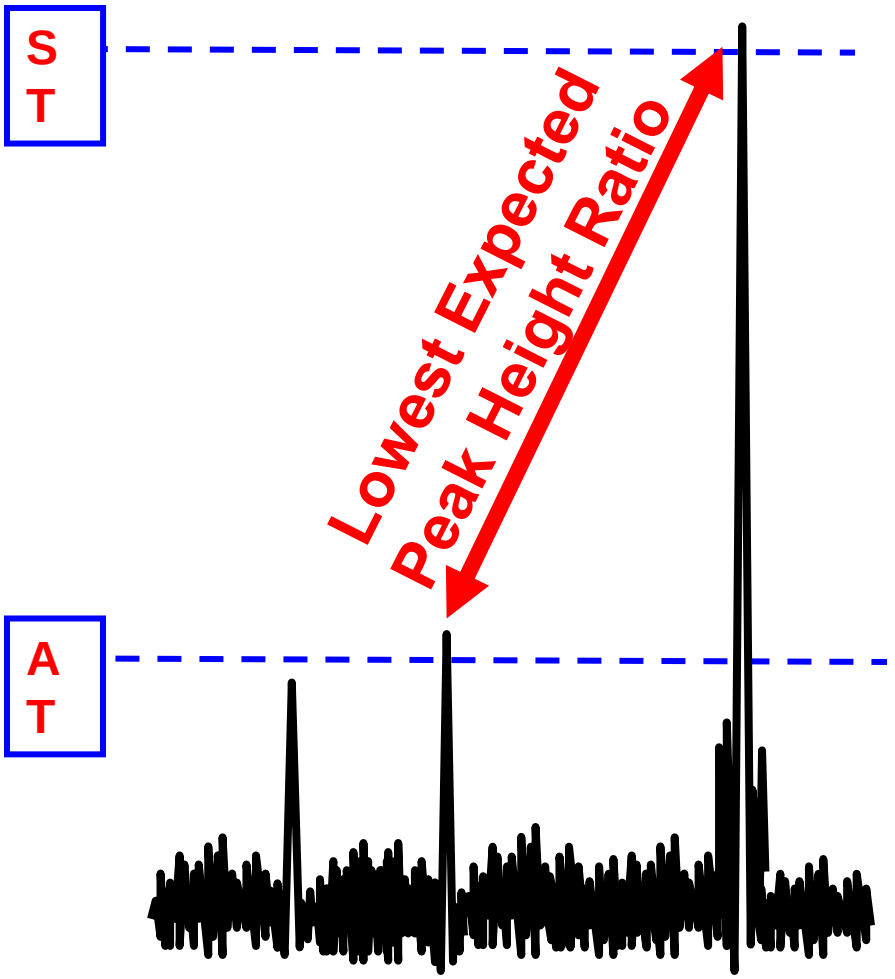


Stochastic and Analytical Thresholds

Impact Lowest Expected Peak Height Ratio



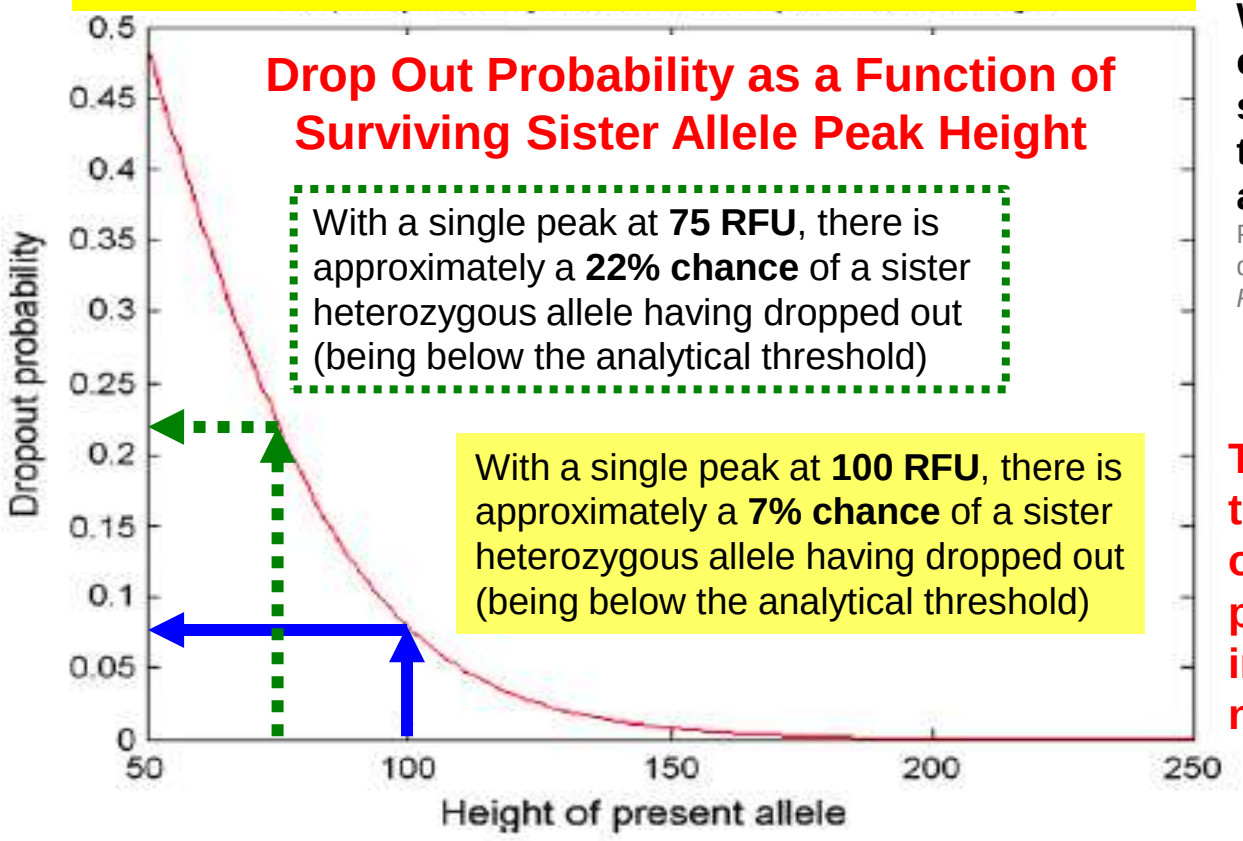
The lower you go trying to analyze low-level data… (i.e., more sensitive STR kits)

the worse your expected peak height ratios for single-source samples

Therefore, there is greater uncertainty with associating genotypes of contributors in mixtures (or even determining that you have a mixture)

Setting a Stochastic Threshold is Essentially Establishing a Risk Assessment

How much error are you willing to accept?



"Currently, most laboratories use an arbitrary stochastic threshold. **When a protocol is changed, especially if it is made more sensitive to low-level DNA, then the stochastic threshold must also change**."

Puch-Solis R, et al. (2011). Practical determination of the low template DNA threshold. *Forensic Sci. Int. Genet.* 5(5): 422-427.

The position and shape of this curve may change based on anything that can impact peak detection (e.g., CE injection time, PCR cycle number, post-PCR cleanup).

Gill, P., et al. (2009). The *low-template* (stochastic) threshold-Its determination relative to risk analysis for national DNA databases. *FSI Genetics*, 3, 104-111.

Stochastic Threshold Summary

- A stochastic threshold (ST) may be established for a specific set of conditions to reflect possibility of allele drop-out, which is essential for a CPE/CPI stats approach
- ST should be re-examined with different conditions (e.g., higher injection, sample desalting, increase in PCR cycles)
- ST will be dependent on the analytical threshold set with a method and impacts the lowest expected peak height ratio
- Assumptions of the number of contributors is key to correct application of ST

Coupling of Statistics and Interpretation

- **The CPE/CPI approach** for reporting an inclusionary statistic **requires that all alleles be observed** in the evidence sample

- If allele drop-out is suspected at a locus, then any allele is possible and the probability of inclusion goes to 100% -- in other words, the locus is effectively dropped from consideration for statistical purposes

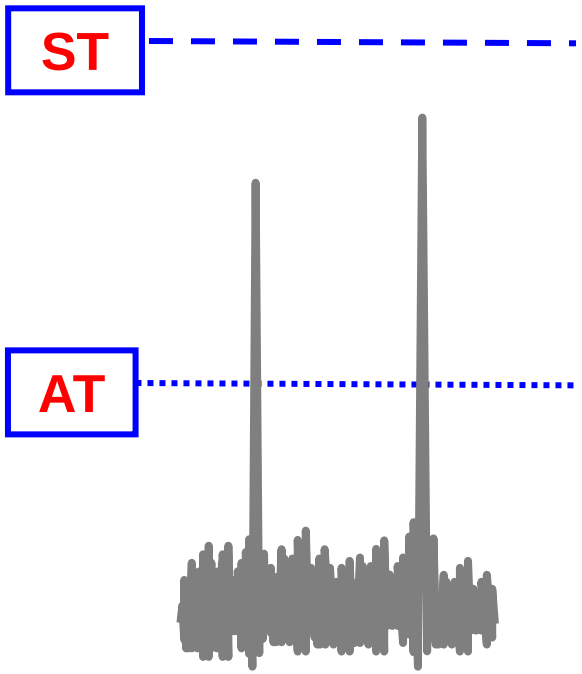
- If alleles are seen below the established stochastic threshold, then the locus is typically eliminated (“INC” – declared inconclusive) in many current lab SOPs

Impact of Dropping Loci

- The less data available for comparison purposes, the greater the chance of falsely including someone who is truly innocent

- Are you then being “conservative” (i.e., erring in favor of the defendant)?

Can This Locus Be Used for Statistical Calculations?



It depends on your assumption as to the number of contributors!

If you assume <u>a single-source sample</u>, then you can assume that the detection of two alleles fully represents the heterozygous genotype present at this locus.

If you assume (from examining other loci in the profile as a whole) that the sample is a mixture of two or more contributors, then there may be allele drop-out and all alleles may not be fully represented.

Likelihood Ratio (LR)

- Provides ability to express and evaluate both the prosecution hypothesis, H_p (the suspect is the perpetrator) and the defense hypothesis, H_d (an unknown individual with a matching profile is the perpetrator)

$$LR = \frac{\Pr(E \mid H_p)}{\Pr(E \mid H_d)}$$

- **The numerator, H_p, is usually 1** – since in theory the prosecution would only prosecute the suspect if they are 100% certain he/she is the perpetrator

- The denominator, **H_d**, is typically the profile frequency in a particular population (based on individual allele frequencies and assuming HWE) – i.e., **the random match probability**

Take Home Messages

- Inclusionary statements (including “cannot exclude”) need statistical support to reflect the relevant weight-of-evidence
- Stochastic thresholds are necessary if using CPI statistics to help identify possible allele dropout
- CPI is only conservative for guilty suspects as this approach does a poor job of excluding the innocent
- Uncertainty exists in scientific measurements and increases with complex mixtures (low level DNA and/or >2 contributors)
- An increasing number of poor samples are being submitted to labs – labs may benefit from developing a complexity threshold

President John F. Kennedy

Yale University commencement address (June 11, 1962)

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. **We enjoy the comfort of opinion without the discomfort of thought**."

http://www.jfklibrary.org/Research/Ready-Reference/Kennedy-Library-Miscellaneous-Information/Yale-University-Commencement-Address.aspx

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