

JS 190- Population Genetics- Assessing the Strength of the Evidence

I. Pre class activities

- a. Quiz then Review Assignments and Schedule

II. Learning Objectives

- a. Overview of Validation
Developmental vs Internal: SWGDAM vs DAB
- b. Define Genetic Concordance or “Match”
- c. Understand the evaluation of Results- Where the rubber meets the road! Genetic concordance under 3 circumstances
- d. Frequency estimate calculations- The strength of the result of the inference of common source between the biological evidence and reference donor.
 - 1. Hardy-Weinberg Equilibrium-Definition
 - 2. HWE- Assumptions-
 - 3. In class “mating” under HWE /w selection and migration!
 - 4. Linkage equilibrium frequencies



Assignments

- Extra credit due weds 09 May
- Find an article from 2012 on forensic DNA and statistics/population genetics in the news and
- Write 500 word summary with 3Q and 3A

190 Remaining Schedule

- Mon 7 May: Validation and Population Genetics- Virtual Lab Troubleshooting
- Weds 9 May: Dr. Charles Brenner
- Mon 14 May:
 - Ethics of Genetic Testing and Privacy Issues
 - Future of Forensic DNA
 - Last class- Hand in lab books
 - SOTES

General validation considerations

from www.cstl.nist.gov/strbase/validation/SWGDAM_Validation.doc and
<http://www.cstl.nist.gov/strbase/dabqas.htm>

- Validation is the process by which the scientific community acquires the necessary information to
 - (a) Assess the ability of a procedure to obtain reliable results.
 - (b) Determine the conditions under which such results can be obtained.
 - (c) Define the limitations of the procedure.
- The validation process identifies aspects of a procedure that are critical and must be carefully controlled and monitored.

Two Types: Developmental vs. Internal Validation

- There are two types of validation required to implement or modify technologies for forensic DNA analysis—developmental and internal.
- The application of existing technology to the analysis of forensic samples does not necessarily create a new technology or methodology.

Developmental vs. Internal Validation

- Developmental validation is the demonstration of the accuracy, precision, and reproducibility of a procedure by the manufacturer, technical organization, academic institution, government laboratory, or other party. Developmental validation must precede the use of a novel methodology for forensic DNA analysis.
- Internal validation is conducted by each forensic DNA testing laboratory and is the in-house demonstration of the reliability and limitations of the procedure. Prior to using a procedure for forensic applications, a laboratory must conduct internal validation studies.

Developmental Validation

- **2.1 Characterization of genetic markers:**
 - **2.1.1 Inheritance:** The mode of inheritance of DNA markers demonstrated through family studies.
 - **2.1.2 Mapping:** The chromosomal location of the genetic marker
 - **2.1.3 Detection:** Technological basis for identifying the genetic marker.
 - **2.1.4 Polymorphism:** Type of variation analyzed.
- **2.2 Species specificity**
- **2.3 Sensitivity studies:** When appropriate, the range of DNA quantities able to produce reliable typing
- **2.4 Stability studies:** The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults has been extensively documented. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated using known samples
- **2.5 Reproducibility:** The technique should be evaluated in the laboratory and among different laboratories to ensure the consistency of results. Specimens obtained from donors of known types should be evaluated.
- **2.6 Case-type samples:** The ability to obtain reliable results should be evaluated using samples that are representative of those typically encountered by the testing laboratory. When possible, consistency of typing results should be demonstrated by comparing results from the previous procedures to those obtained using the new procedure.
- **2.7 Population studies:** The distribution of genetic markers in populations should be determined in relevant population groups. When appropriate, databases should be tested for independence expectations.
- **2.8 Mixture studies:** The ability to obtain reliable results from mixed source samples should be determined.
- **2.9 Precision and accuracy:** The extent to which a given set of measurements of the same sample agree with their mean and the extent to which these measurements match the actual values being measured should be determined.
- **2.10 PCR-based procedures:** Publication of the sequence of individual primers is not required in order to appropriately demonstrate the accuracy, precision, reproducibility, and limitations of PCR-based technologies.

Internal Validation

- **3.1 Known and nonprobative evidence samples:** The method must be evaluated and tested using known samples and, when possible, authentic case samples; otherwise, simulated case samples should be used. DNA profiles obtained from questioned items should be compared to those from reference samples. When previous typing results are available, consistency as to the inclusion or exclusion of suspects or victims within the limits of the respective assays should be assessed.
- **3.2 Reproducibility and precision:** The laboratory must document the reproducibility and precision of the procedure using an appropriate control(s).
- **3.3 Match criteria:** For procedures that entail separation of DNA molecules based on size, precision of sizing must be determined by repetitive analyses of appropriate samples to establish criteria for matching or allele designation.
- **3.4 Sensitivity and stochastic studies:** The laboratory must conduct studies that ensure the reliability and integrity of results. For PCR-based assays, studies must address stochastic effects and sensitivity levels.
- **3.5 Mixture studies:** When appropriate, forensic casework laboratories must define and mimic the range of detectable mixture ratios, including detection of major and minor components. Studies should be conducted using samples that mimic those typically encountered in casework (e.g., postcoital vaginal swabs).
- **3.6 Contamination:** The laboratory must demonstrate that its procedures minimize contamination that would compromise the integrity of the results. A laboratory should employ appropriate controls and implement quality practices to assess contamination and demonstrate that its procedure minimizes contamination.
- **3.7 Qualifying test:** The method must be tested using a qualifying test. This may be accomplished through the use of proficiency test samples or types of samples that mimic those that the laboratory routinely analyzes. This qualifying test may be administered internally, externally, or collaboratively.

DNA Advisory Board QA standards

<http://www.cstl.nist.gov/strbase/QAS/Final-FBI-Director-Forensic-Standards.pdf>

- **8.2.1 Developmental validation** studies shall include, where applicable, characterization of the genetic marker, species specificity, sensitivity studies, stability studies, reproducibility, case-type samples, population studies, mixture studies, precision and accuracy studies, and PCR-based studies. PCR-based studies include reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls, and product detection studies. All validation studies shall be documented.
- **8.3.1 Internal validation** studies conducted after the date of this revision shall include as applicable: known and non-probative evidence samples or mock evidence samples, reproducibility and precision, sensitivity and stochastic studies, mixture studies, and contamination assessment. Internal validation studies shall be documented and summarized. The technical leader shall approve the internal validation studies.
- 8.3.1.1 Internal validation data may be shared by all locations in a multi-laboratory system. Each laboratory in a multi-laboratory system shall complete, document and maintain applicable precision, sensitivity, and contamination assessment studies. The summary of the validation data shall be available at each site.
- 8.3.2 Internal validation shall define quality assurance parameters and interpretation guidelines, including as applicable, guidelines for mixture interpretation.
- 8.3.3 A complete change of detection platform or test kit (or laboratory assembled equivalent) shall require internal validation studies.



Can the Validation Process in Forensic DNA Typing Be Standardized?

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¹National Institute of Standards and Technology

²Pennsylvania State Police DNA Laboratory

15th International Symposium on Human Identification
Phoenix, AZ
October 6, 2004

Presentation Outline

- Summary of Findings (Community Consensus?)
 - Literature review
 - Interviews with labs
 - **Validation questionnaire**
- Steps Involved in Going “On-Line”
- Resources Under Development to Aid Future Validation Efforts

Statement of Project Purpose

- Review validation practices currently in use and available standards and guidelines
- Refine general philosophy of validation and steps involved with goal to see if these steps can be standardized
- Attempt to define a minimum number of samples that could be recommended for various validation scenarios
 - Is there a consensus in the community (or can there ever be)?

Validation Definitions

ISO 17025

5.4.5.1 Validation is the **confirmation by examination** and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled

DAB Quality Assurance Standards for Forensic DNA Testing Laboratories

2 (ff) Validation is a **process by which a procedure is evaluated** to determine its efficacy and reliability for forensic casework analysis and includes:

To demonstrate that a method is suitable for its intended purpose...

International Symposia on Human Identification and the Topic of Validation

#	Year	Validation in Title	Total Talks	Validation in Title	Total Posters	%
1	1989	1	10	--	--	10.0
2	1991	0	21	0	14	0
3	~10% out of 1,220 presentations have "validation" in the title					
4						
5						
6						
7	1996	2	30	1	77	2.8
8	1997	3	34	11	81	12.1
9	1998	3	25	14	80	16.2
10	1999	0	44	7	70	6.1
11	2000	8	33	11	107	13.6
12	2001	4	30	7	76	10.4
13	2002	2	27	8	78	9.5
14	2003	4	26	17	86	18.8
15	2004					
	TOTAL	34	384	91	836	10.2

Conventional forensic DNA typing methods are now widely used and accepted in courts of law. However, new technologies, software, or instrumentation will continue to be developed and therefore need to be validated in laboratories prior to use in casework.

Can we learn from the past as we move into the future?

DAB Quality Assurance Standards for Forensic DNA Testing Laboratories

Manufacturer

- (1) **Developmental validation** is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples.
- (2) **Internal validation** is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

Forensic Lab

SWGDM Revised Validation Guidelines

Section 1.1 Validation is the process by which the scientific community acquires the necessary information to

- (a) Assess the ability of a procedure to obtain reliable results.
- (b) Determine the conditions under which such results can be obtained.
- (c) Define the limitations of the procedure.

The validation process identifies aspects of a procedure that are critical and must be carefully controlled and monitored.

Reliability, Reproducibility, Robustness, Range

PubMed Literature Search

<http://www.ncbi.nlm.nih.gov/PubMed>

Search Results with term “validation” (9/8/04)

- *J. Forensic Sci.* - 71 references
- *Int. J. Legal Med.* - 21 references
- *Forensic Sci. Int.* - 47 references
- *Electrophoresis* – 62 references (12 on DNA)

- All of PubMed - **28,035 references**

Review of Promega conference proceedings:

125 with “validation” in title of talk or poster

Total number of papers examined: **64**

Representative Labs Interviewed

- **Montgomery County Crime Lab** – **small lab**, 3 analysts, ~180 cases/year; using PP16 and ABI 310
- **Orchid Cellmark** – **private contract lab**, 40 analysts and technicians, ~5,000 cases/year; Profiler Plus/COfiler and Identifiler with ABI 310 and ABI 3100; extensive court experience
- **AFDIL** – **large federal lab**, ~120 analysts/technicians, remains identification rather than strictly forensic cases, >1,000 cases/year (mtDNA & STRs); Profiler Plus/COfiler and PP16 with ABI 377 and ABI 3100

Information from interviews is included in the written report of this project...

Contacting the Community

- Validation Standardization Questionnaire handed out at NIJ DNA Grantees meeting (June 28-30, 2004)
- Emails sent to >200 scientists (July-Aug 2004)
 - Attendees from the NIJ DNA Grantees meeting
 - Participants in NIST interlaboratory studies
 - Contacts through STRBase website
- Responses from 52 scientists were compiled
 - Covering 27 states + Puerto Rico, 4 companies, 2 outside US
- Specific interviews were conducted to gain perspectives from a small lab, a large lab, a private lab, and court testimony experience

Review of Survey Questions

- What is validation?
- How do you know when you are finished validating a kit, instrument, software, or procedure?
- What steps are needed in internal validation and how many samples should be run at a minimum?
- **How many total samples do you think it takes to internally “validate” a new forensic kit?**
- How many different sets of samples are needed? Over what time period?
- Where do you look for guidance currently in terms of validation?
- **What are some kits, software, instruments that you are considering for validation in the next year?**
- How are validation, training, and proficiency testing related to one another?
- Do you think that the process of validation can be standardized?
- If a standard protocol or set of guidelines existed for validation, would you use it?
- If a standard set of samples existed for performing validation testing, would you use them?

Used to help define specific examples ...

How do you know when you are finished with a validation study? (2)

- “Once a reasonable body of data has been assembled and analyzed, quirks have been revealed, and the upper and lower limits of the system have been challenged using a range of samples that one could expect to encounter in the everyday operation of the system”
- “When you achieve accuracy and precision to the desired statistical level of certainty”
- “You can never know...but it is always nice to have more samples!”
- “Validation is never complete”

Survey Summary for Recommended Precision Studies

A few of the responses:

- “100 allelic ladder injections”
- “1 allelic ladder with 10 injections”
- “Depends upon the system being tested. For a databanking system, 50-100 runs of 50-100 specimens. Again, stats tell you when you’ve processed enough specimens to understand the system.”
- “Minimum: Run one sample at least 8 times.
Recommended: Run at least two samples plus allelic ladder at least 8 times.” (24 sample-runs)

How do you know when you are finished with a validation study? (1)

- “When you have demonstrated that it works as expected over a range of samples that is representative of what is seen in casework”
- “When repeat performance gave the same result”
- “When you pull the toothpick out and it is dry?... Meet at least minimum expectations and DAB guidelines”
- “You are very comfortable that you know how it works and your documentation will convince a reviewer you have put the kit thru a rigorous review/test.”

Validation Standardization Questionnaire (conducted June-August 2004)

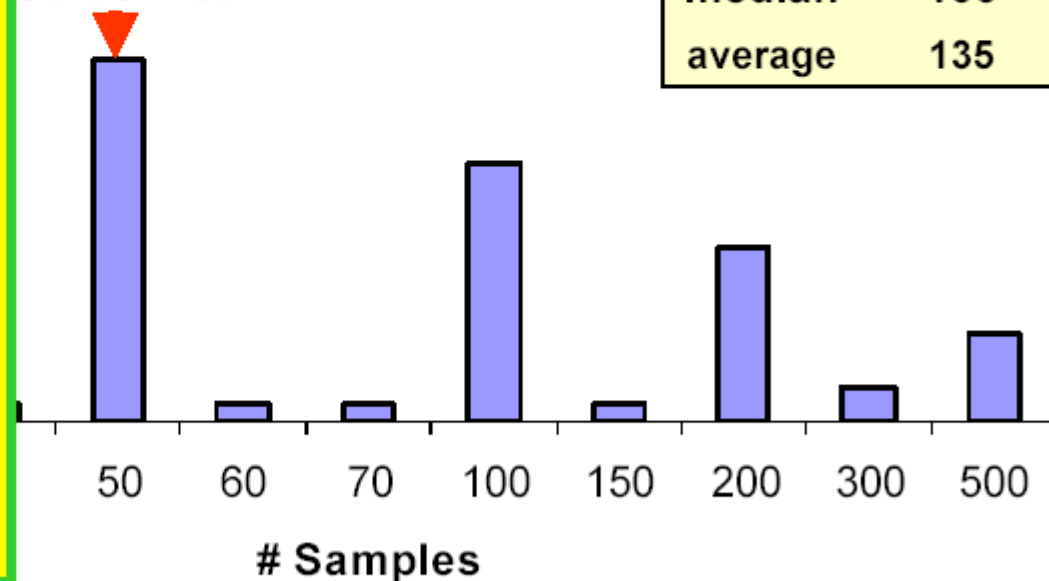
Survey Summary for Recommended Total Number of Samples to Internally Validate a New Forensic Kit

To Validate a "New" Kit

SWGDM
Guidelines

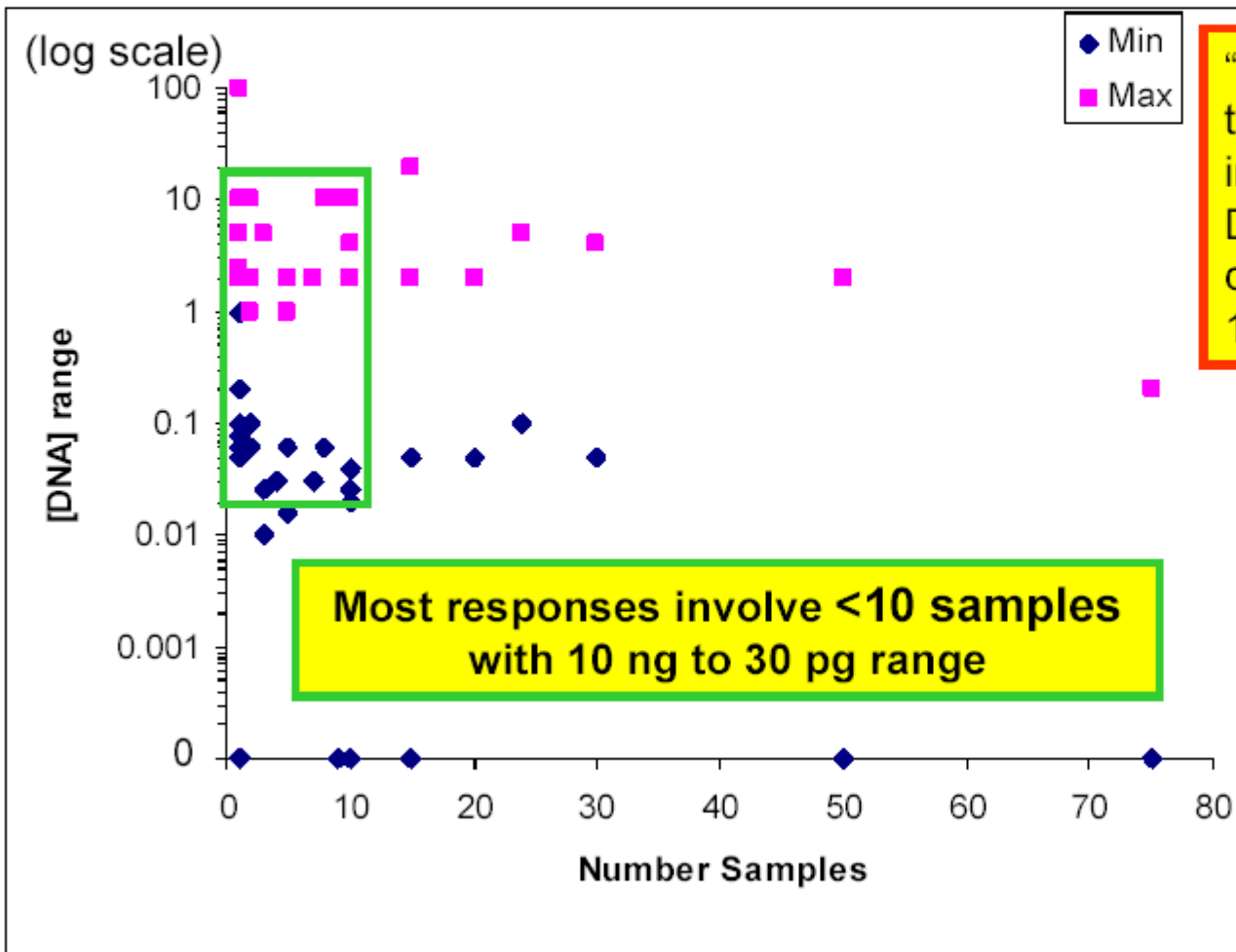
min	5
max	500
median	100
average	135

"As many as it takes to determine working parameters and appropriate interpretation guidelines of systems employed in a working environment. In most cases a minimum of 50 sample-runs is preferred. (One sample run once equals one sample-run.)"



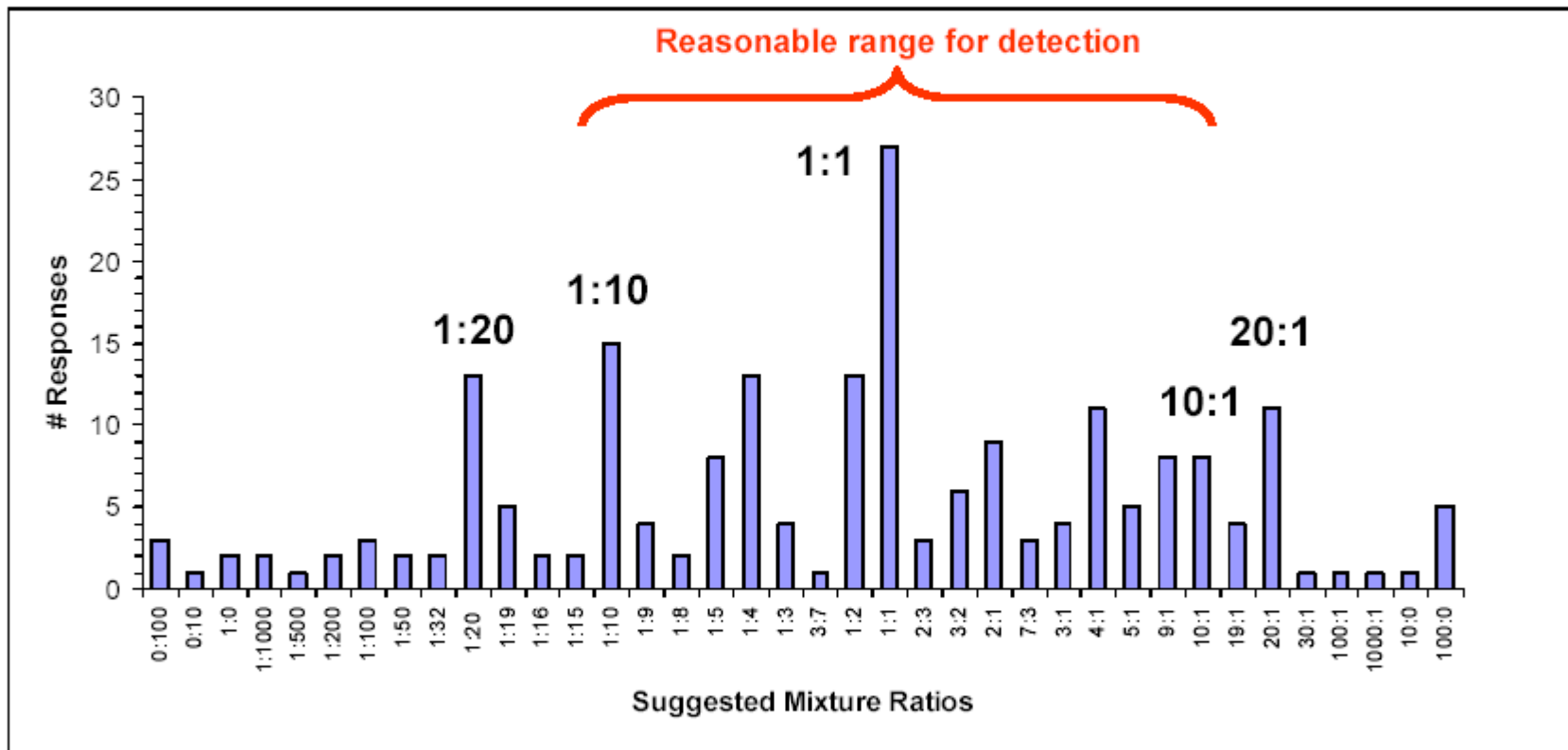
Choices in survey were: 10, 50, 500, or other _____

Survey Summary for Recommended Sensitivity Studies



“Need to run samples that challenge interpretation at high DNA and low DNA concentrations—e.g., 10 ng and <0.2 ng”

Survey Summary for Recommended Mixture Studies



Some Recommended Numbers of Samples:

- 5 different 2-person mixtures
- 50 amplifications from at least 10 different mixtures
- 1 set of samples (ranging from 1:10 to 10:1)

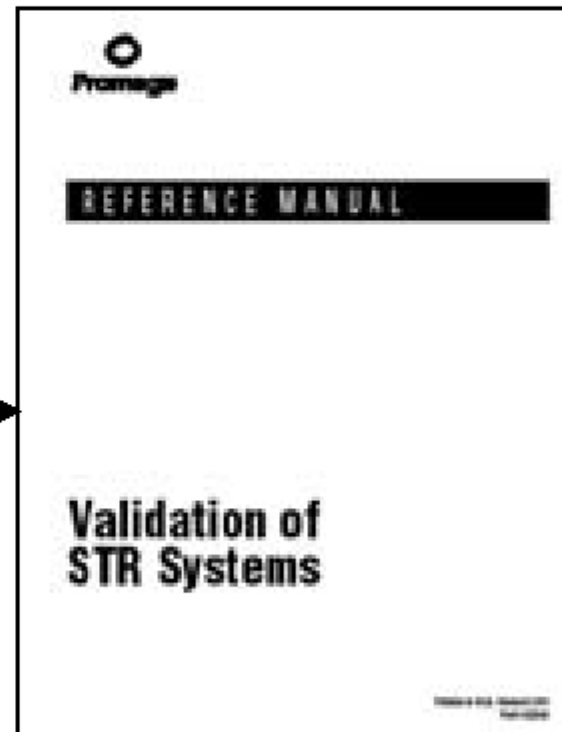
Survey Summary for Recommended **Non-Probative Cases**

A few of the responses:

- Most responses were between 5-10 cases (range 3-25)
- “More important that the number of cases is the range of forensic samples that are typed during validation.”
- “Complete cases are not required to test a system.
Recommended: **Run at least 8 mock non-probative samples.** Note: Non-probative samples are not guaranteed to provide complete profiles. They are needed only to show that false results are not generated. Lack of results or incomplete results do not affect the validity of a validation.”

Where do you look for guidance currently in validation?

- SWGDAM
- DAB standards and ISO 17025
- Other scientists
- Literature publications
- Presentations at meetings
- Promega's validation guide
- FBI studies and publications
- NIST studies and publications
- Previous scientific training
- Common sense



Published in March 2001

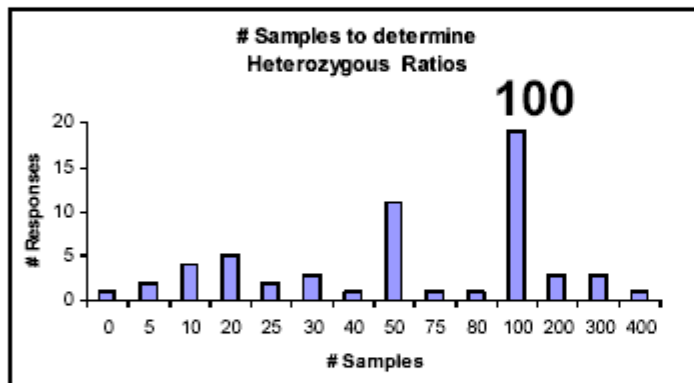
Survey Summary for Recommended **Non-Human Cases**

A few of the responses:

- “10-20 food animals, companion animals, local wildlife, ferrets”
- “I don’t believe this is necessary in internal validation if external results are published. This would not be expected to vary in different analysts’ hands.”
- “I’ve trusted system manufacturers to handle this. Should I have?”
- “Minimum: Include information from developmental studies. If performing developmental studies, include at least bacterial and yeast/fungal example, plus mammalian and non-mammalian examples.”

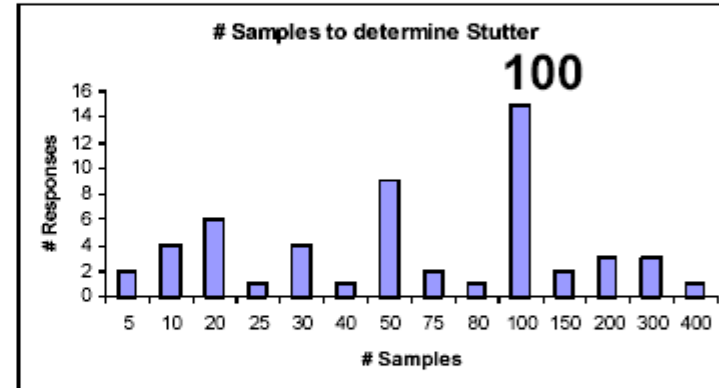
Survey Summary for Recommended Numbers of Samples

to Determine Heterozygote Peak Height Ratios and Stutter Values



min	0
max	400
median	50
average	85

Heterozygote Peak Height Ratios



min	5
max	400
median	63
average	88

Stutter Values

Can Validation be Standardized?

Statements from survey responders...

Over 86% (45/52) said yes

Those who responded “no” said

- “to some degree it can be, however, validation is specific to the platform, kits, ...”,
- “a start-up lab should do much more than an experienced lab...”,
- “validation builds on previous work by lab or published data”,
- “parts of it can be standardized; I don’t think the non-probative cases could be”, and
- “only in a general way, as with the SWGDAM guidelines. The uniqueness of each new procedure would make standardization difficult.”

Our Conclusion...

to a certain extent it can...but everyone will always have a different comfort level...and **inflexible, absolute numbers for defined studies will not likely be widely accepted**

If a Standard Protocol or Set of Guidelines Existed for Validation, Would You Use It?

90% (47/52) said yes

Some responses

- “No-I would reference them. I may not completely abide by them but I would certainly review them”,
- “No-but it would be taken into consideration”,
- “Yes-we would have to or there would be problems in court”,
- “Yes-as long as they remain updated, relevant and feasible guidelines and do not become dogma”,
- “Yes-if it would pass an audit for validation”, and
- “Yes-unless they were far less stringent than current practice.”

Validation Section of the DNA Advisory Board Standards
issued July 1998 (and April 1999); published in *Forensic Sci. Comm.* July 2000

STANDARD 8.1 The laboratory shall use validated methods and procedures for forensic casework analyses (*DNA analyses*).

8.1.1 Developmental validation that is conducted shall be appropriately documented.

8.1.3 Internal validation shall be performed and documented by the laboratory.

If a Standard Set of Samples Existed for Performing Validation Testing, Would You Use Them?

90% (47/52) said yes

Some responses

- “Yes-would love to have something like that available; we are always eager to have benchmarks for assessment”,
- “Yes-these types of samples would cut down on time for validation. It would be efficient if they were ready for the particular type of validation...”,
- “Yes-as long as they are readily available at a reasonable price”,
- “No-this approach is not recommended. It is most important that systems work with the materials available in individual laboratories. Laboratories should be allowed, even encouraged, to select their own preferred materials. Choices for such selection of standard materials for within laboratory analyses and cross-laboratory comparison already exist from a variety of government and commercial entities.”

There are Different Opinions...

in Who Should Perform Validation

Development of New STRs for Forensic Casework: Criteria for Selection, Sequencing & Population Data and Forensic Validation

Angel Carracedo and M.V. Lareu

Institute of Legal Medicine. University of Santiago de Compostela, Spain

<http://www.promega.com/geneticidproc/ussymp9proc/content/21.pdf>

Validation studies following similar parameters to those recommended by TWGDAM were carried out. These include robustness, stability, mixtures, non-human studies, mutation rate and checking for independence with other loci. In our opinion the final validation of a system cannot be carried out by individual groups and companies and should always be performed by an internationally established validation group. In Europe a final assessment and intercomparison exercises are usually performed by the EDNAP group, a working group of the ISFH.

Abstract from talk presented at Promega meeting in 1998

Revised SWGDAM Validation Guidelines (July 2004)

http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm



Forensic Science Communications July 2004 – Volume 6 – Number 3

Standards and Guidelines

Revised Validation Guidelines

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Scientific Working Group on DNA Analysis Methods
(SWGDM)

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3. Internal Validation

**... a total of at least 50 samples
(some studies may not be necessary...)**

Program for DNA Analysis by the Technical Working Group on DNA Analysis Methods (*Crime Laboratory Digest* 1995:22(2):21-43) has been revised due to increased laboratory experience, the advent of new technologies, and the issuance of the Quality Assurance Standards for Forensic DNA Testing Laboratories by the Director of the FBI (*Forensic Science Communications* available: www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm).

The document provides validation guidelines and definitions approved by SWGDAM July 10, 2003.

A Thoughtful Comment from One Interviewee

Before a set of validation experiments is performed...

- The question should be asked “Do we already know the answer to this question from the literature or a previous study performed in-house?”
- If the answer is “yes” and we document how we know this answer, **then there is no need to perform that set of validation experiments.**

A good example of this scenario is non-human DNA studies.

How an Assay Evolves

NIJ-funded project
or company efforts

Performed by
manufacturer

Performed by
forensic lab

Research

Development

Optimization

Pre-Validation

Validation

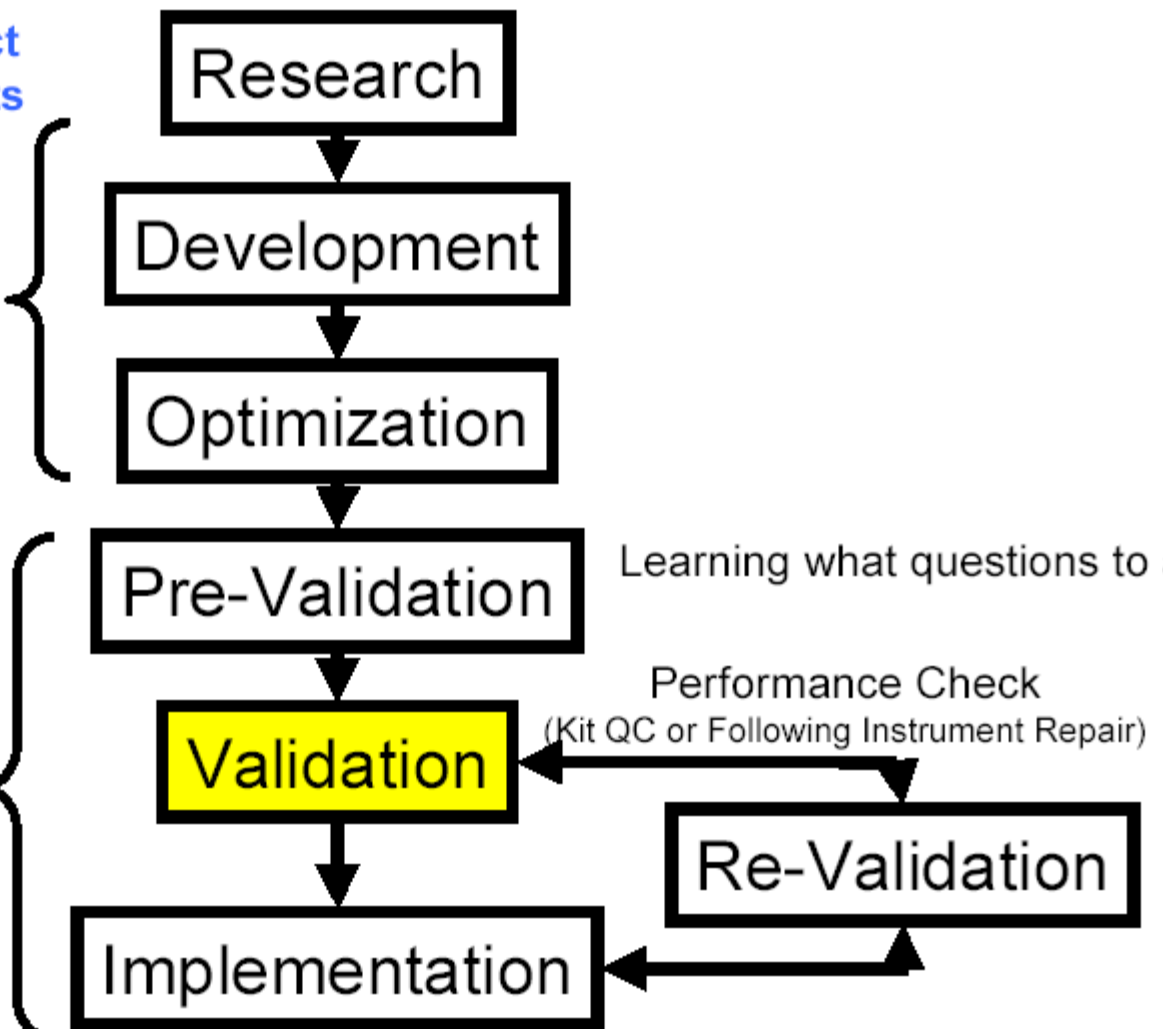
Implementation

Learning what questions to ask

Performance Check
(Kit QC or Following Instrument Repair)

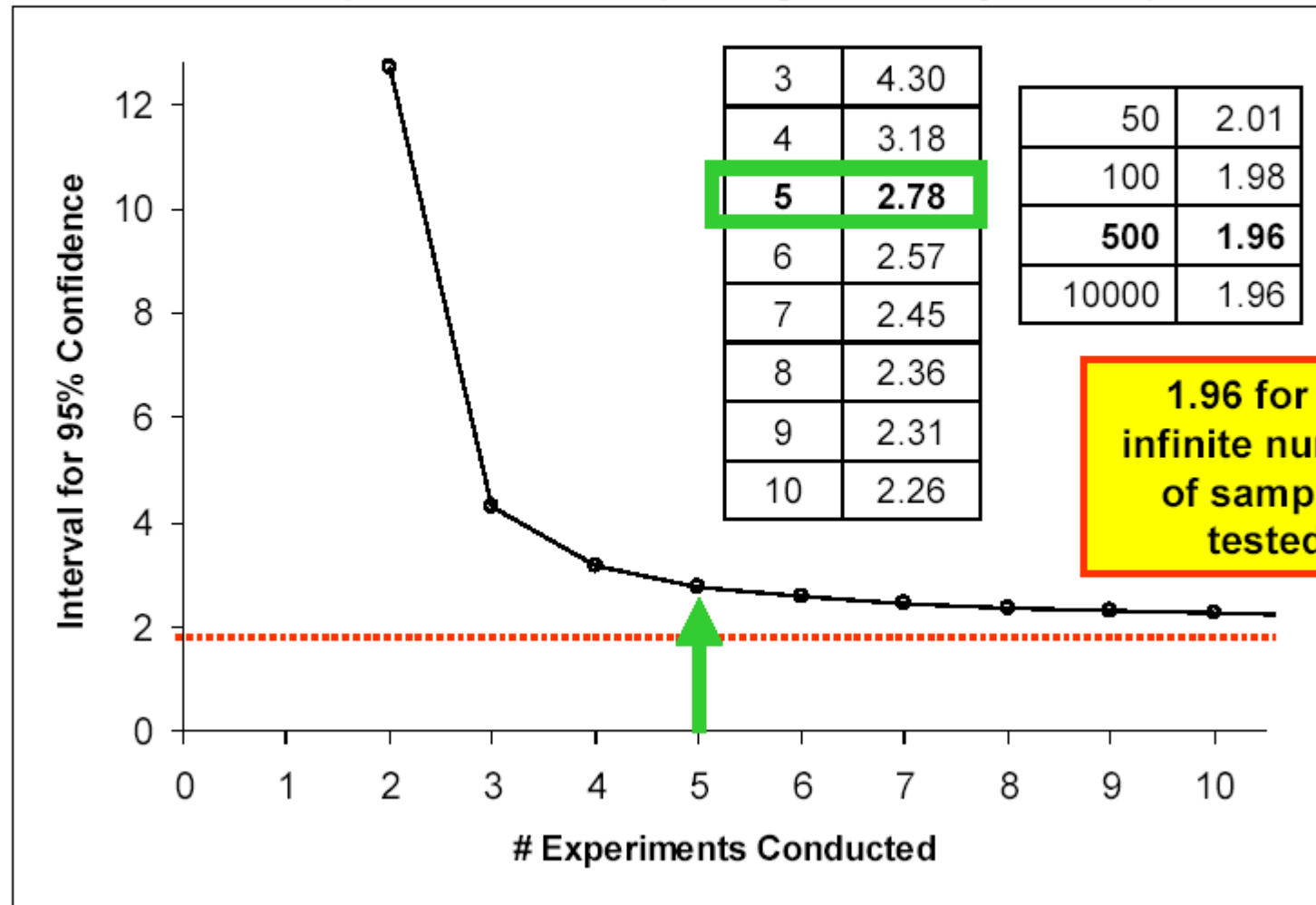
Re-Validation

Writing SOP, Training Others and Going "On-Line"



A Comment on Minimum Numbers of Samples for Validation Studies...

Impact of Number of Experiments on Capturing Variability in a Population of Data



Example: PowerPlex 16

- Switch from ProfilerPlus/COfiler kits to PowerPlex 16
- Retaining same instrument platform of ABI 310

Recommendations:

- Concordance study (somewhat, but better to review literature to see impact across a larger number of samples and which loci would be expected to exhibit allele dropout-e.g., D5S818)
- Stutter quantities, heterozygote peak height ratio
- Some sensitivity studies and mixture ratios
- **Do not need precision studies to evaluate instrument reproducibility**

Resources to Aid Future Validation Studies

- STRBase Validation Website
 - <http://www.cstl.nist.gov/biotech/strbase/validation.htm>
 - Examples with recommended minimum numbers
 - Validation summary sheets
- NIST Calibration Data Set
 - **set of ~200 sample data files** that can be used to evaluate common STR typing “artifacts” such as stutter, non-template addition, spikes, peak imbalance, tri-allelic patterns, variant alleles, single base resolution
 - [will help meet NDIS Appendix B requirements for Expert Systems evaluation](#)
- Quality Control Program (Dave Duewer, NIST)
 - Software to monitor STR electropherogram performance (resolution, sensitivity) over time

New Validation Homepage on STRBase

<http://www.cstl.nist.gov/biotech/strbase/validation.htm>

Validation Information to Aid Forensic DNA Laboratories

Validation Summary Sheets

We are initiating an effort to catalog... The purpose of this effort is to provide a number of samples run as part of the laboratories. These validation summary sheets are used in accordance with FBI Laboratory Guideline 1.2.2.1 that "internal validation..."

Below is listed a compilation of reference house assays, instrumentation, and... [biotechnology](#) is listed at the bottom of each **Summary Sheet** (note that not all valid...

NA, Assay, or Instrument	Reference
PowerPlex Y	Kerika
Profiler Plus	Frank et al (2002), Pawlowski
COfiler	Leibell et al (2002)
SQM Plus	Collins et al (2002)
AmpFISTR Blue	Walsh et al (2002)
AmpFISTR Green I	Holt et al (2002)
Profiler	Holt et al (2002)
Profiler Plus AD	Leibell et al (2002)
Identifiler	Collins et al (2002)

Address: http://www.cstl.nist.gov/biotech/strbase/validation/YSI_PowerPlexY.htm

PowerPlex Y Validation

Reference: Kerika et al (2004) *Forensic Sci. Int.*, in press

What validated? **Where published?**

Study Completed **Description of Samples Tested (performed in 7 labs and Promega)** **# Done**

Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 MF mixture series x 11 ratios (1:0.1, 1:1, 1:2, 1:3, 1:5, 1:10, 1:20, 1:30, 1:50, 1:100) (5:100, 10:100, 25:100, 50:100, 100:100) (5:100, 10:100, 25:100, 50:100, 100:100) (5:100, 10:100, 25:100, 50:100, 100:100)	132
Mixture Ratio (male:male)	6 labs x 2 MM mixtures series x 11 ratios (1:0.1, 1:1, 1:2, 1:3, 1:5, 1:10, 1:20, 1:30, 1:50, 1:100)	132
Sensitivity	7 labs x 2 series x 6 amounts (10, 50, 250, 1250, 6250, 31250)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2398	6
Precision (ABI 3100 and ABI 3130)	10 ladder replicates + 10 sample replicated + 10 ladders + 8 samples for 377	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS395 but no studies were noted)	
Cycling Parameters	5 cycles (95/95/95/95) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/56/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/75/125/250/500) x 5 amounts + 5 concentrations	60
Thermal cycler test	4 models (ABI3100/3130/3130XL) x 1 sample + 3 models x 3 sets x 12 samples	76
Male-specificity	2 females x 1 titration series (5000 ng female DNA) x 5 amounts	10
TagAid polymerase titration	5 amounts (1.38/0.69/0.34/0.17/0.085 ng) x 4 quantities (10, 50, 250, 13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1.5x/3x/6x) x 4 quantities (10, 50, 250, 13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (10, 50, 250, 13 ng DNA)	20
TOTAL SAMPLES EXAMINED		1269

How?

Comments: **Other information and conclusions**

Validation Summary Sheet for PowerPlex Y

<u>Study Completed</u> (17 studies done)	<u>Description of Samples Tested</u> (performed in 7 labs and Promega)	<u># Run</u>
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (1:0, 1:1, 1:10, 1:100, 1:300, 1:1000, 0.5:300, 0.25:300, 0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
Mixture Ratio (male:male)	6 labs x 2 M/M mixtures series x 11 ratios	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/15/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (480/2400/9600/9700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
TaqGold polymerase titration	5 amounts (1.38/2.06/2.75/3.44/4.13 U) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20

Krenke et al. (2004) *Forensic Sci. Int.*, in press

TOTAL SAMPLES EXAMINED

1269

Genetic Concordance or “Match”

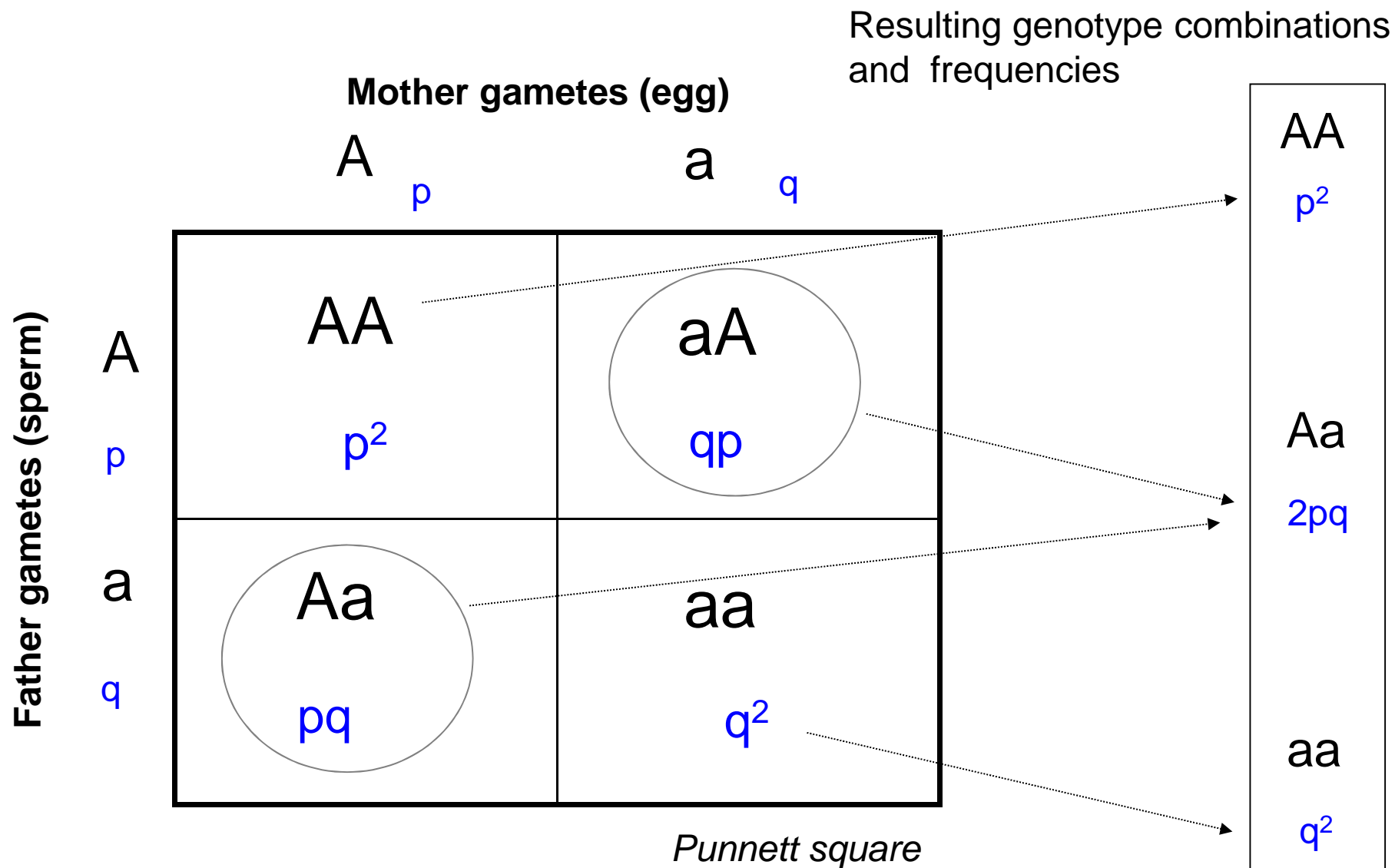
- Scientists: Match- reserved to no significant differences observed between 2 samples in the particular test(s) conducted. May be different but the test failed to reveal.
- DNA tests are limited to a very small % of the human genome
- Public- Match connotes absolute individualization. Therefore conclusion of “genetic concordance simply describes the fact that two samples show the same genotypes.

Continuous vs Discrete alleles

- Continuous alleles : RFLP: continuous alleles are not resolved- e.g. 99 vs 100 repeats are too similar
- Discrete alleles- PCR: method clearly differentiates the types- exact number of repeats can be determined
- Analogy: Continuous alleles- Too close to call the difference between the two runners
Discrete: Every runner has exact speed(size) detected.

Genetic concordance under 3 circumstances

- 1. Samples come from a common source- evidence comes from the same individual providing the reference sample
- 2. Concordance is a coincidence- someone other contributed the evidence
- 3. Concordance is an accident (erroneous)- collection, analytical or clerical error – the evidence and reference appear to have the same profile
- The strength of the concordance depends on which of the 3 scenarios produced the result. If 1, then the strength of the inference becomes the next critical question



Freq (A) = p

Freq (a) = q

$p + q = 1$

$(p + q)^2 = p^2 + 2pq + q^2$

Figure 19.3, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

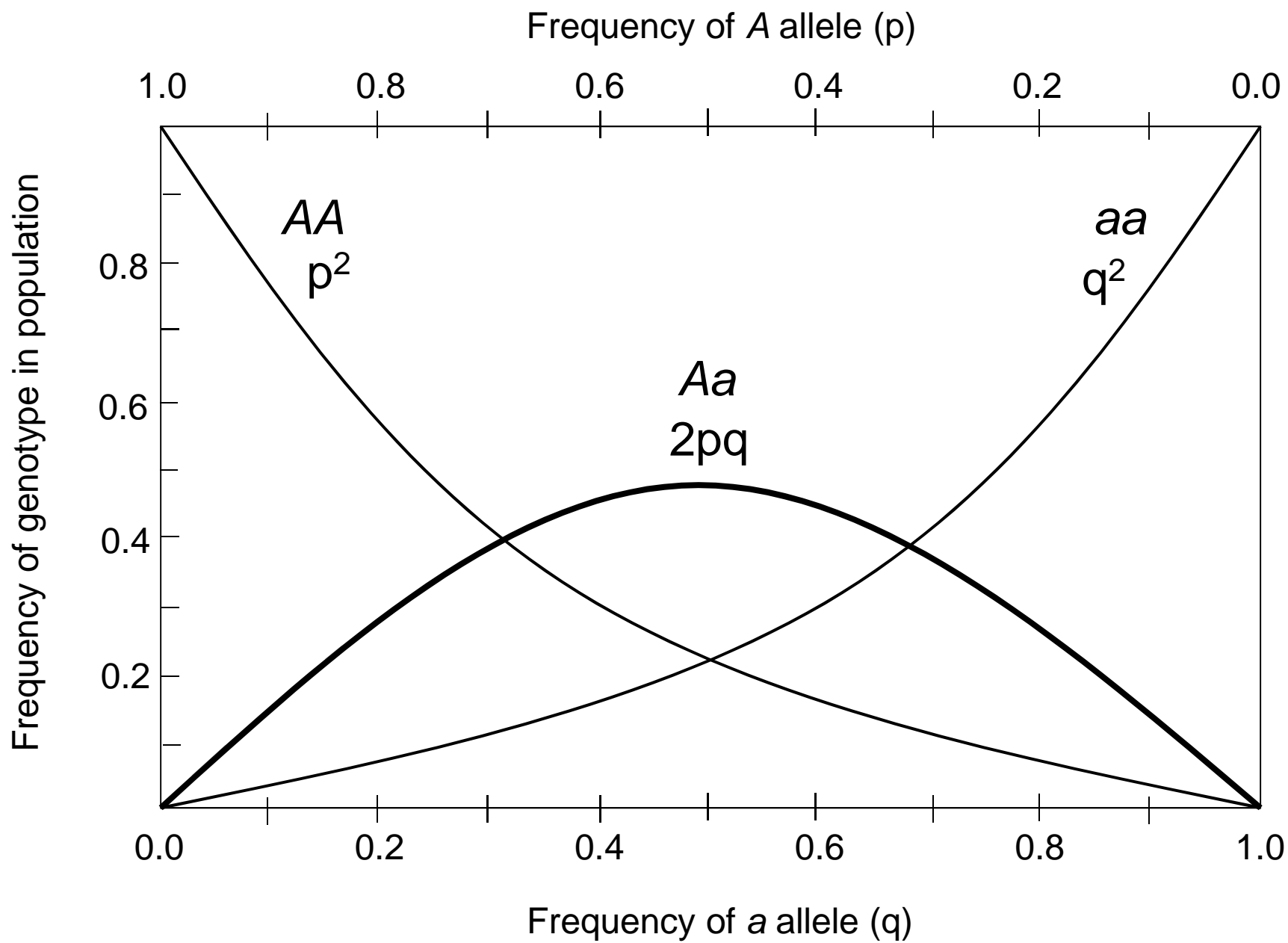


Figure 19.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

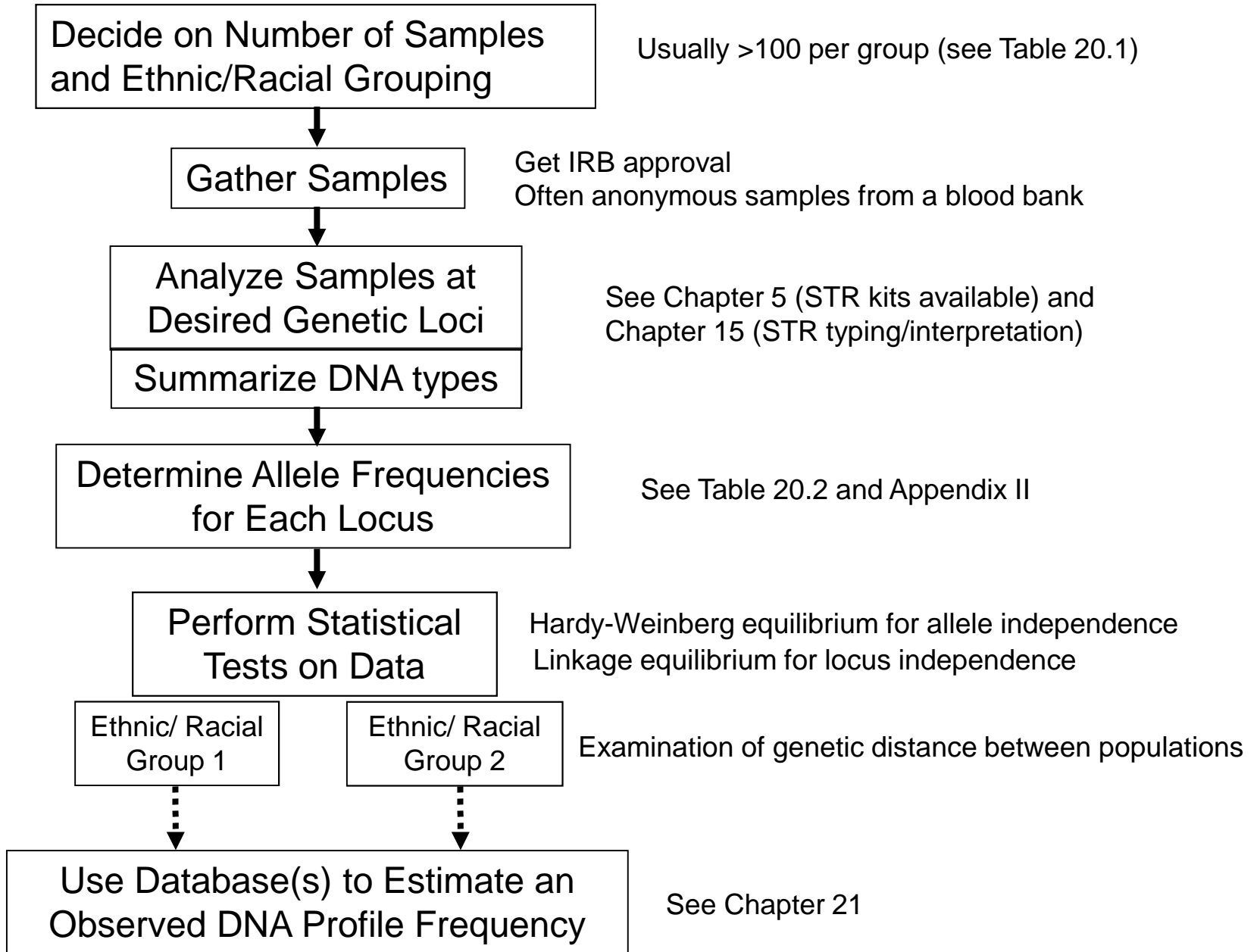


Figure 20.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

U.S. Population Samples

(Appendix II)

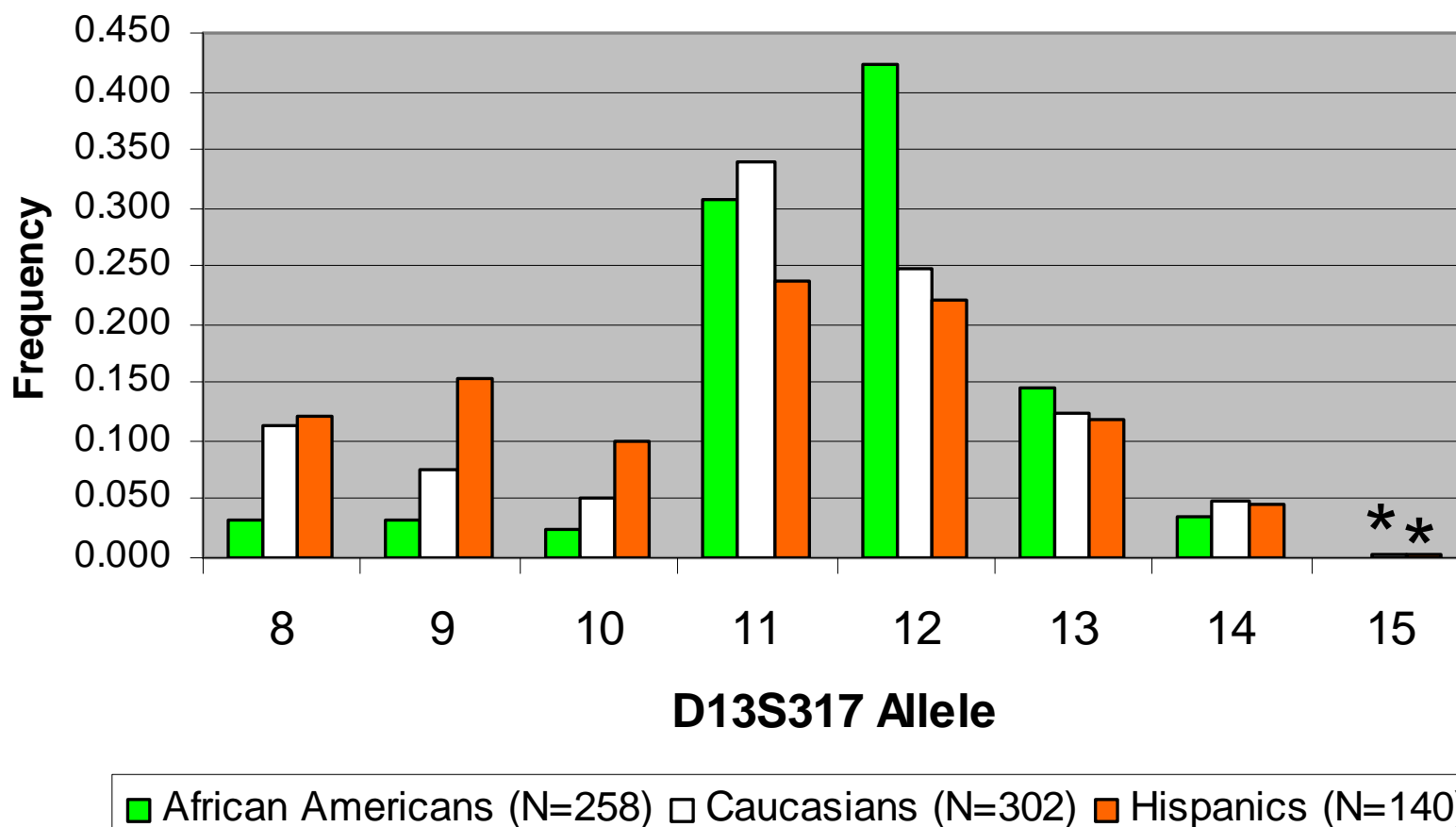


Figure 20.3, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

How Statistical Calculations are Made

- **Generate data** with set(s) of samples from desired population group(s)
 - Generally only 100-150 samples are needed to obtain reliable allele frequency estimates
- **Determine allele frequencies** at each locus
 - Count number of each allele seen
- Allele frequency information is used to **estimate the rarity of a particular DNA profile**
 - Homozygotes (p^2), Heterozygotes ($2pq$)
 - Product rule used (multiply locus frequency estimates)

For more information, see Chapters 20 and 21 in *Forensic DNA Typing, 2nd Edition*

Assumptions with Hardy-Weinberg Equilibrium

The Assumption	The Reason
Large population	Lots of possible allele combinations
No natural selection	No restriction on mating so all alleles have equal chance of becoming part of next generation
No mutation	No new alleles being introduced
No immigration/emigration	No new alleles being introduced or leaving
Random mating	Any allele combination is possible

None of these assumptions are really true...

Individual Genotypes Are Summarized and Converted into

Genotype Array	8	9	10	11	12	13	14	15		Allele Count	Observed Frequency
	8,8	8,9	8,10	8,11	8,12	8,13	8,14	8,15			
8	9	9	1	17	13	10	0	0	8	68	0.11258
		9,9	9,10	9,11	9,12	9,13	9,14	9,15			
9		1	2	15	10	4	3	0	9	45	0.07450
			10,10	10,11	10,12	10,13	10,14	10,15			
10			2	12	6	3	2	1	10	31	0.05132
				11,11	11,12	11,13	11,14	11,15			
11				37	54	21	12	0	11	205	0.33940
					12,12	12,13	12,14	12,15			
12					21	18	7	0	12	150	0.24834
						13,13	13,14	13,15			
13						7	5	0	13	75	0.12417
							14,14	14,15			
14							0	0	14	29	0.04801
								15,15			
15								0	15	1	0.00166
										604	

The 11,12 genotype was seen **54 times** in 302 samples (604 examined chromosomes)

Table 20.2, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Allele Frequency Tables

Butler *et al.* (2003)
JFS 48(4):908-911

Einum *et al.* (2004)
JFS 49(6)

Allele frequencies denoted with an asterisk (*) are below the 5/2N minimum allele threshold recommended by the National Research Council report (NRCII) *The Evaluation of Forensic DNA Evidence* published in 1996.

D3S1358

Most common allele

	<u>Caucasian</u> N= 302	<u>Caucasian</u> N= 7,636		<u>African American</u> N=258	<u>African American</u> N= 7,602
<u>Allele</u>			<u>Allele</u>		
11	0.0017*	0.0009	11	--	0.0003*
12	0.0017*	0.0007	12	--	0.0045
13	--	0.0031	13	0.0019*	0.0077
14	0.1027	0.1240	14	0.0892	0.0905
15	0.2616	0.2690	15	0.3023	0.2920
15.2	--	--	15.2	0.0019*	0.0010
16	0.2533	0.2430	16	0.3353	0.3300
17	0.2152	0.2000	17	0.2054	0.2070
18	0.15232	0.1460	18	0.0601	0.0630
19	0.01160	0.0125	19	0.0039*	0.0048
20	0.0017*	0.0001*	20		

Hardy Weinberg Equilibrium

- A predictable relationship exists between allele frequencies and genotype frequencies at a single locus. This mathematical relationship allows for the estimation of genotype frequencies in a population even if the genotype has not been seen in an actual population survey

Hardy Weinberg conditions

- Large population- several 100 or more
- Approximately Random mating
- Negligible to No mutation
- Negligible to No migration
- Negligible to No selection
- “Don’t be ridiculous” over long periods this will not hold
- Over short periods HWE may apply
- Maintenance of constant allele frequencies also means genetic variability within a population is not blended away over successive generations.

How a mathematician got into biology

- Story from Mange and Mange- 1999
- Hardy loved pure math- which he considered useless but beautiful! Disliked practical applications. This was one of the most significant practical applications of math in history
- Published in Science to avoid letting his pure math colleagues see it!

HWE

- The cards are your alleles. The alleles are Red= R and Black = B. Individual cards make up the "gene pool"
This gene pool contains 40 black cards and 60 red cards, so the frequency of the black allele is $40/100 = 0.4$, and the frequency of the red allele is $60/100 = 0.6$.
- p = frequency of R and q = frequency of B
- HWE law- Part 1- Under HWE conditions
 - Frequency of the genotype R/R = p^2
 - Frequency of the genotype B/B = q^2
 - Frequency of the genotype R/B= $2pq$
 - $P^2+2pq+q^2 = 1$
- HWE law- Part 2
 - As long as HW conditions prevail allele and genotype frequencies do not change.

HWE simulation

- See handout- We will be mating in class!
- Get a pair of cards from the instructor.
- In your teams calculate the allele frequencies of R = red cards and B = black cards
- Also tally the genotype frequencies
- E.g. number of RR, RB and BB
- Team captains provide a summary and write down the results on the black board

Random mating, no selection

- "Random mating" means that mating is without regard to the genotype of the individuals. It is important that individuals not know about alleles of potential mates.
- Get up and mingle in the room, carrying your cards and keep the cards in pairs so that the color cannot be seen by other students.
- Say "hello" when you encounter another student. The fourth time they you say "hello" to someone you will mate
- That means you randomly give one card each to form your first offspring. Then, take your card back and mate again with the same partner. Be sure to write down the genotypes of your offspring.
- When a pair has been mated, that pair should not be mated again in this round of mating. Students continue to mill around until all of their individuals have been mated.

Tally the results

- In small groups, add up the total number of red cards, black cards and genotypes
- Write down your results on the blackboard.
- Did the frequency of the R and B change?
- Do the genotypes of the offspring observed match the genotypes predicted?
- Now become your offspring and repeat the mating and tally.

Random mating- Selection

- Now we will mate with selection.
Whenever a RR genotype is formed, this is a lethal combination and results in death.
- Therefore, this combination results in no viable offspring.
- When you mate this time, eliminate any RR genotypes from your tallies.
- Repeat the tally as before

Random mating- Migration

- 5 students will migrate and become geographically isolated
- Mating occurs within two separate populations.
- We will tally results for these 2 populations
- Do you expect the results to be the same as before? Why or why not?