DNA Fingerprint ing & Barcoding: diagnostic tool for rapid species recognition, unique identification, and discovery

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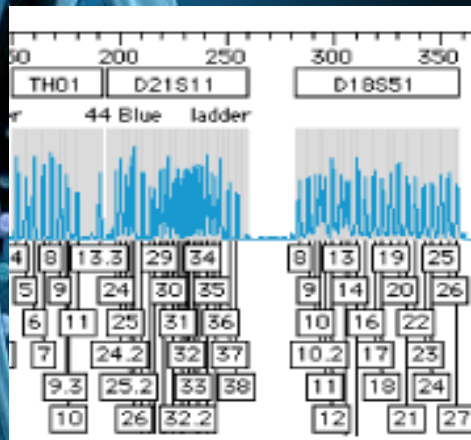
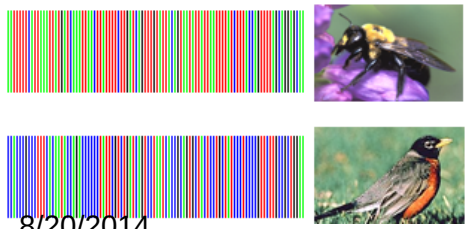
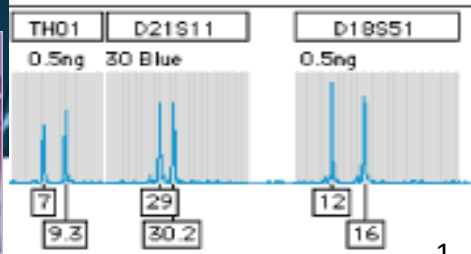


Attending as keynote speaker on “IGN 3rd student Conference, August 24-29, 2014, UNSRAT Menado





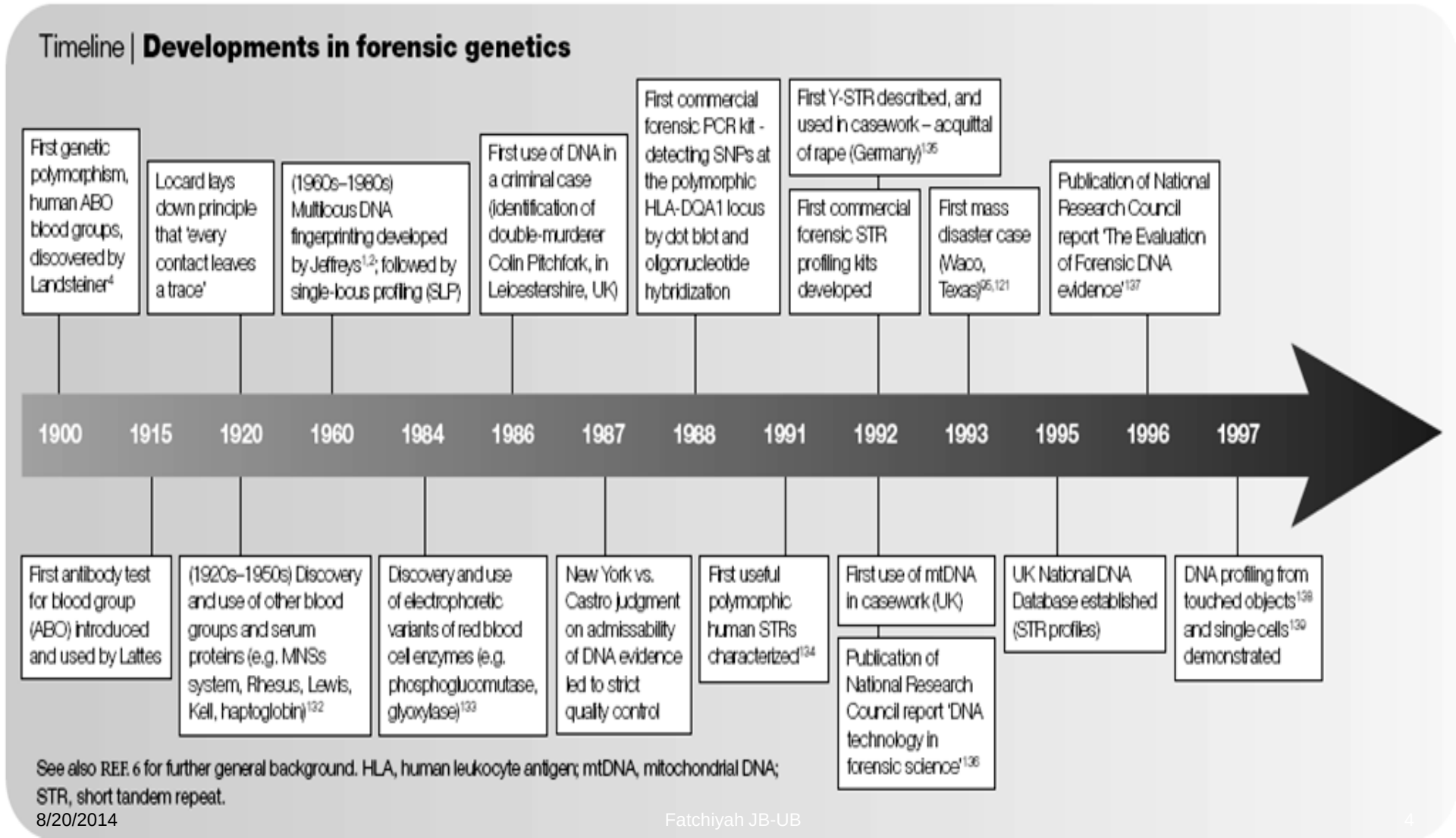
DNA fingerprinting

- The term **DNA fingerprinting** - or **genetic fingerprinting** - is applied to the scientific process whereby samples of DNA are collected, collated and used to match other samples of DNA, which may have been found at the scene of a crime
- DNA - or genetic - fingerprinting relies heavily on the principle that no two individuals share the same genetic code - except for identical twins and statistically those elements of DNA that are examined and used to obtain a match will be unique

Brief History of DNA Fingerprinting

- 1980 - Ray White describes first polymorphic RFLP marker
- 1985 - Alec Jeffreys discovers multi-locus VNTR probes
- 1985 - first paper on PCR
- 1988 - FBI starts DNA casework
- 1991 - first STR paper
- 1995 - FSS starts UK DNA database
- 1998 - FBI launches CODIS database

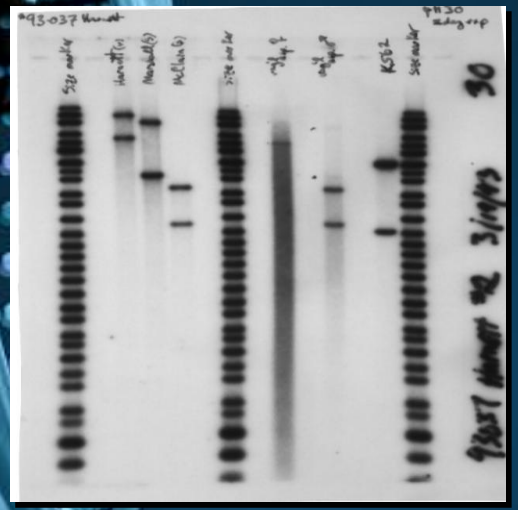
Development in Forensic Genetics

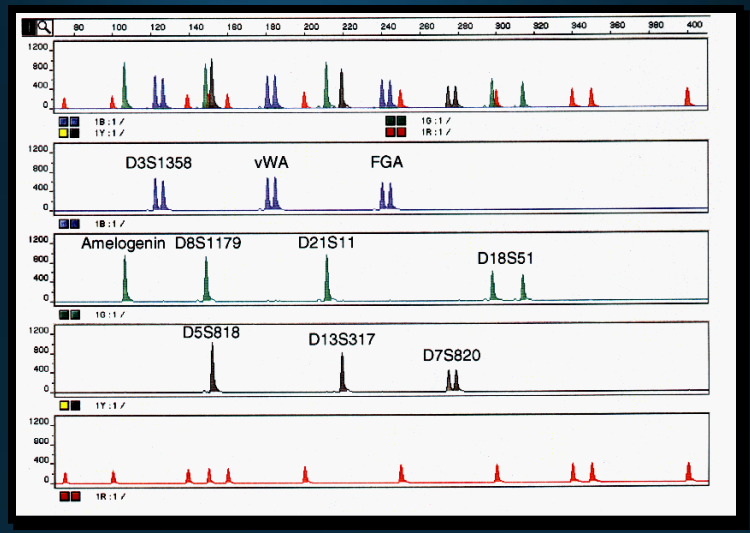


DNA content of biological samples:

Type of sample	Amount of DNA
Blood	30,000 ng/mL
stain 1 cm^2 in area	200 ng
stain 1 mm^2 in area	2 ng
Semen	250,000 ng/mL
Postcoital vaginal swab	0 - 3,000 ng
Hair	
plucked	1 - 750 ng/hair
shed	1 - 12 ng/hair
Saliva	5,000 ng/mL
Urine	1 - 20 ng/mL

Three generations of DNA testing



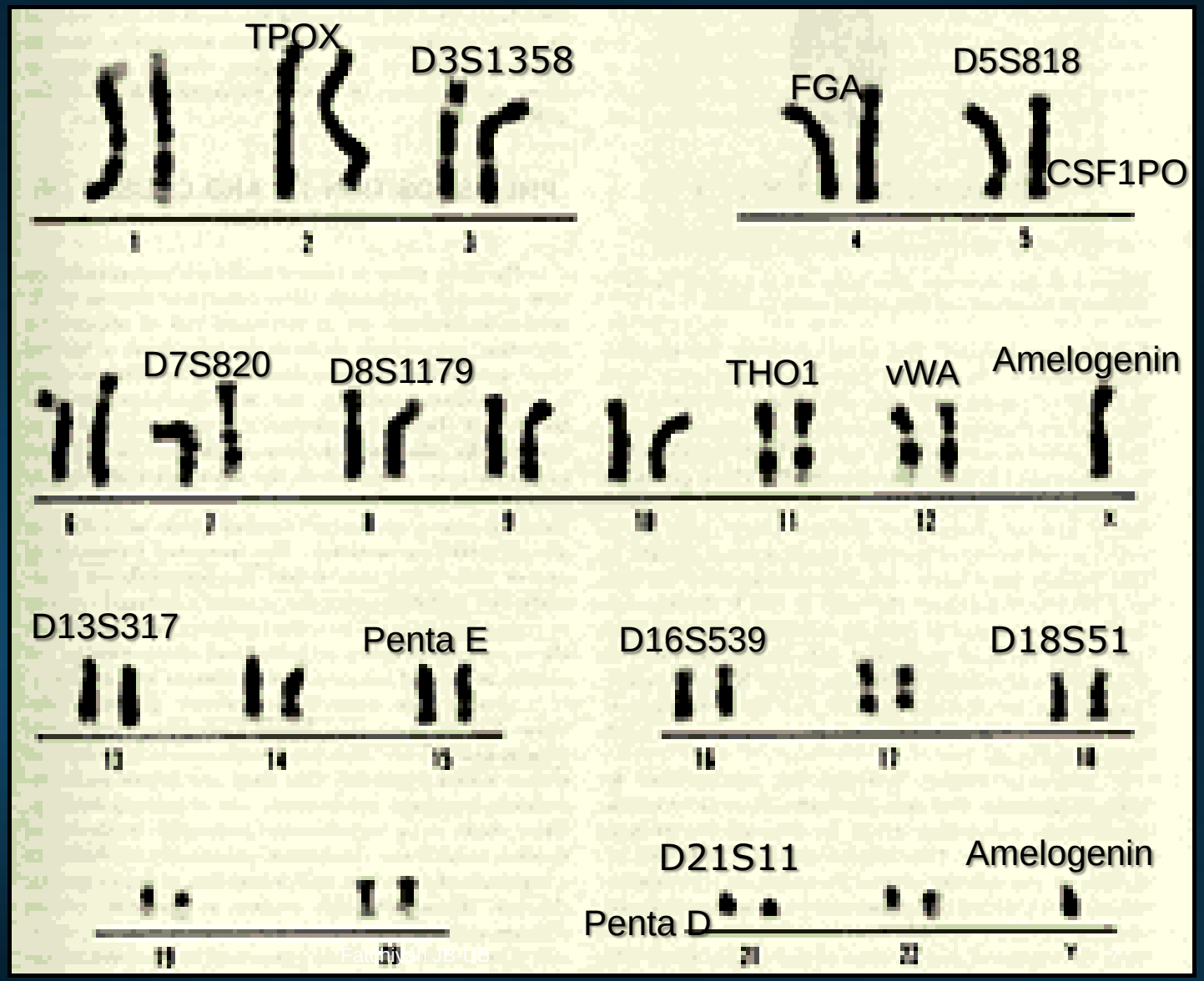


RFLP
AUTORAD
Allele = BAND

DQ-alpha
TEST STRIP
Allele = BLUE DOT

Automated STR
ELECTROPHEROGRAM
Allele = PEAK

Chromosome Spread showing the positions of the amplified loci of CODIS



The PowerPlex® 16 System amplifies 16 loci.

DNA profiling can give you a permanent record of your individual DNA profile.

- DNA profiling is used in forensic science to accurately identify individuals.
- This profile can be used to support any claims you have about genetic connections to grandparents, parents, siblings, or children now and in the future.

Sample Child Identification DNA Profile

Height	46 inches
Weight	47 pounds
Hair Color	Blonde
Eye Color	Brown
Ethnicity/Race	Caucasian
Gender	Female
Birthdate	5/20/1998

Genetic Marker	Allele A	Allele B
D8S1179	10	17
D21S11	28	29
D7S820	10	13
CSF1PO	11	12
D3S1358	16	
TH01	9.3	
D13S317	8	11
D16S539	9	11
D2S1338	19	21
D19S433	14	
vWA	14	16
TPOX	9	
D18S51	17	
AMEL	X	
D5S818	11	13
FGA	22	25

Case No. DP08-05476 Profile Date 8/27/2008

Statistical estimates: the product rule

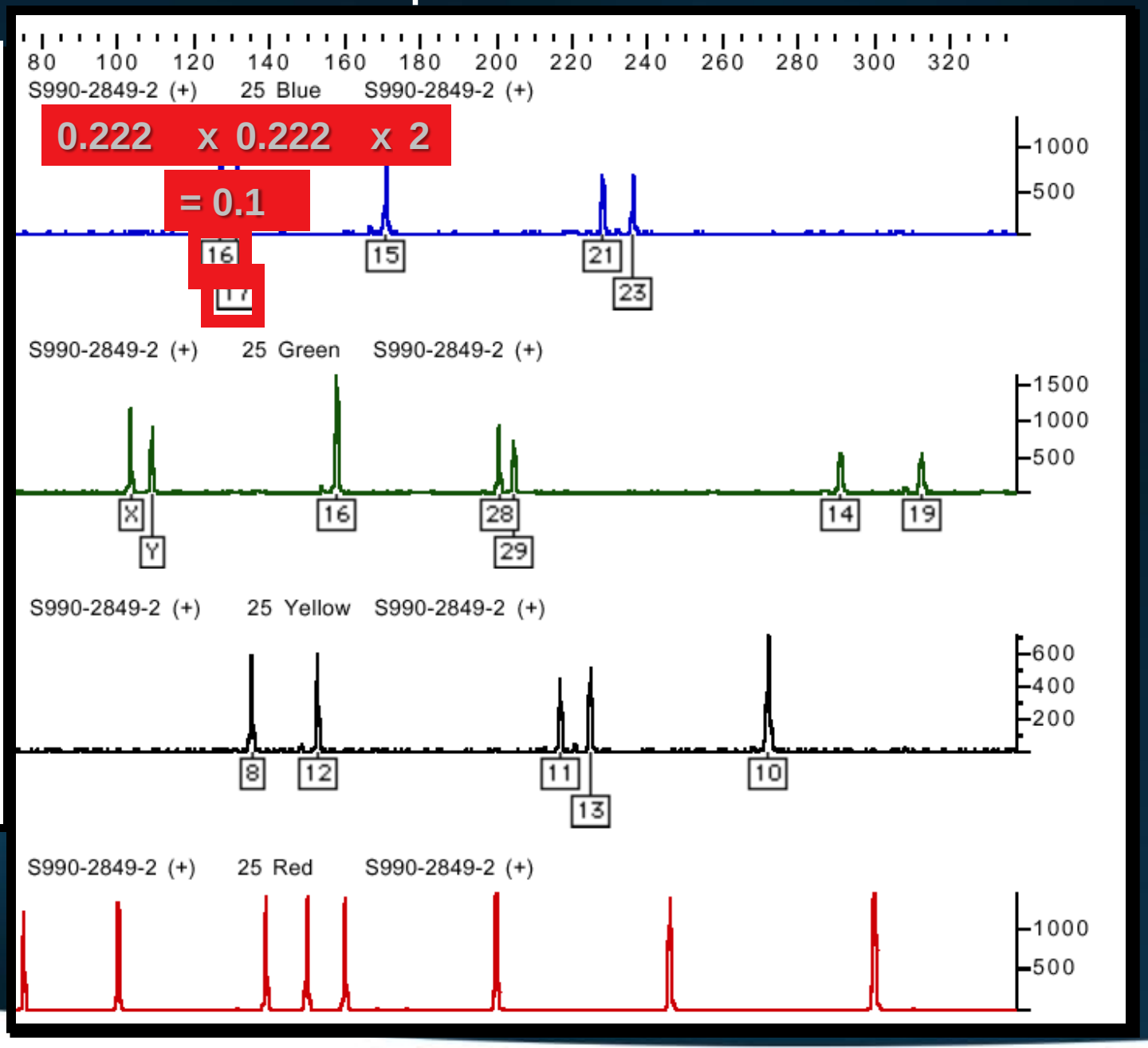
Allele Frequencies

Locus D3S1358
Race Caucasian
(N = 203)

Allele	Frequency
12	0.012
13	0.012
14	0.140
[redacted]	
19	0.012

Locus vWA
Race Caucasian
(N = 196)

Allele	Frequency
11	0.012
12	0.012
13	0.012
14	0.102
15	0.082



Statistical estimates: the product rule

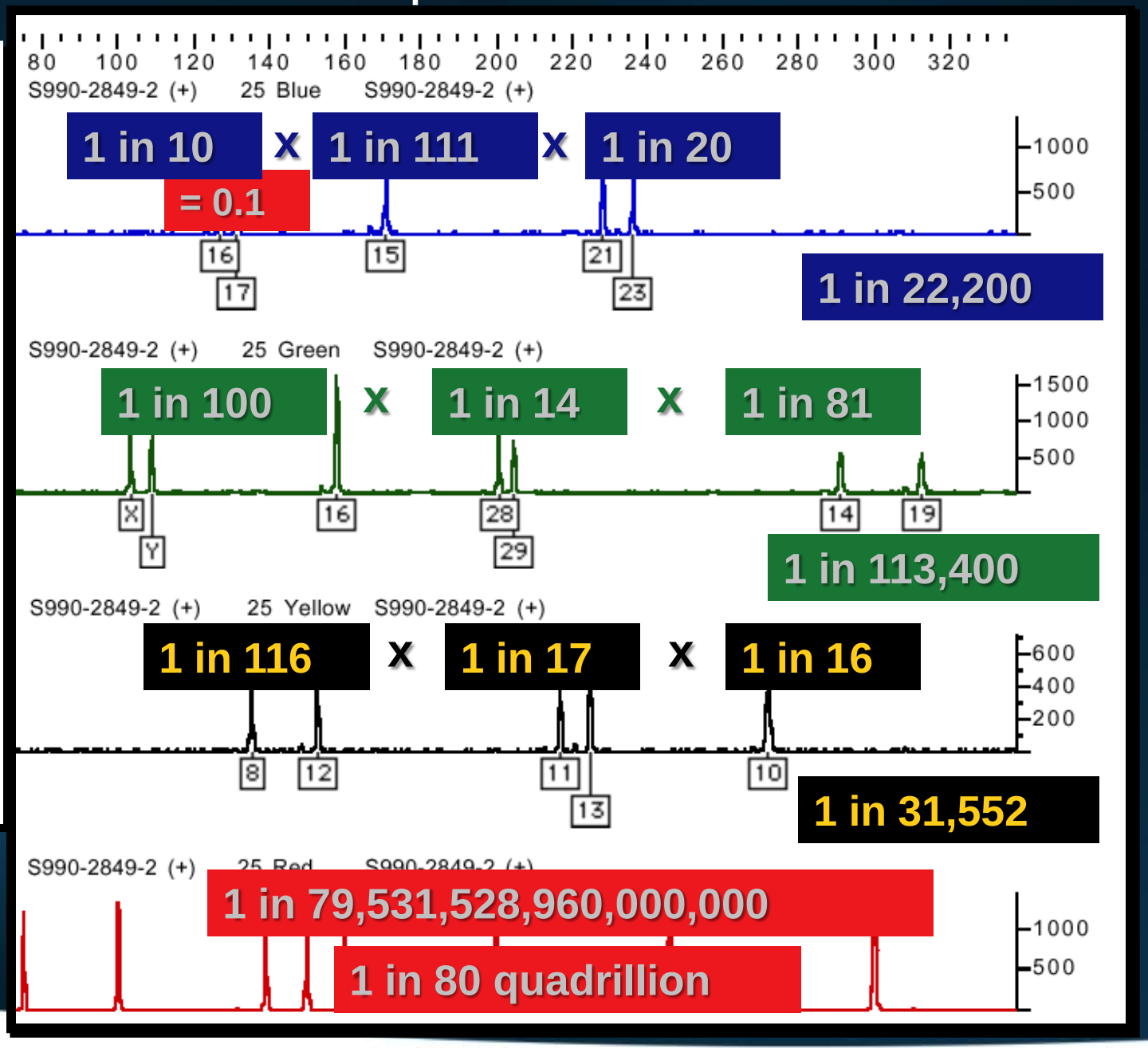
Allele Frequencies

Locus D3S1358
Race Caucasian
(N = 203)

Allele	Frequency
12	0.012
13	0.012
14	0.140
15	0.246
16	0.222
17	0.222
18	0.163
19	0.012

Locus vWA
Race Caucasian
(N = 196)

Allele	Frequency
11	0.012
12	0.012
13	0.012
14	0.102
15	0.082

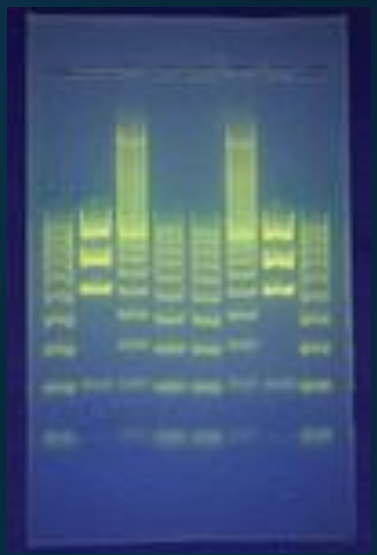


What are some of the DNA technologies used in forensic investigations?

- Restriction Fragment Length Polymorphism (RFLP)
- PCR Analysis
- STR Analysis
- Mitochondrial DNA Analysis
- Y-Chromosome Analysis

DNA fingerprinting procedures

- RFLP: Restriction Fragment Length Polymorphism. Special enzymes are used to cut segments of a sample from which DNA is extracted



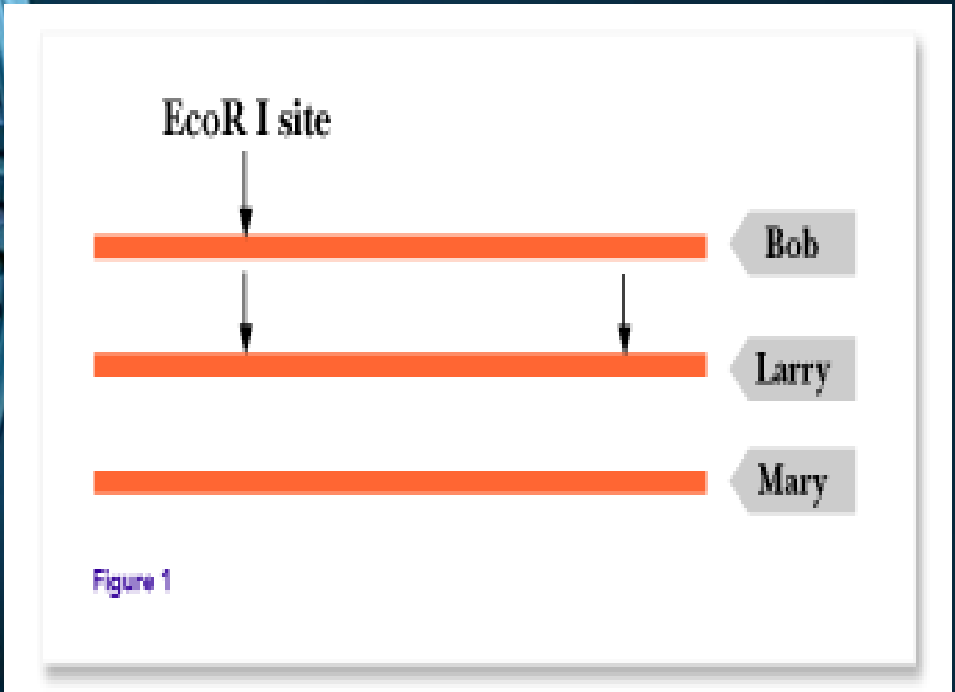
- Polymerase Chain Reaction can create multiple copies of the DNA sequence using as little as fifty molecules; this procedure can help to produce a usable DNA sample from a single human hair

Restriction Fragment Length Polymorphisms (RFLP)

- RFLPs are different fragment lengths of base pairs that result from cutting a DNA molecule with a restriction enzyme
- It is the length differences associated with DNA strands or RFLPs that allow one to distinguish one person from another.

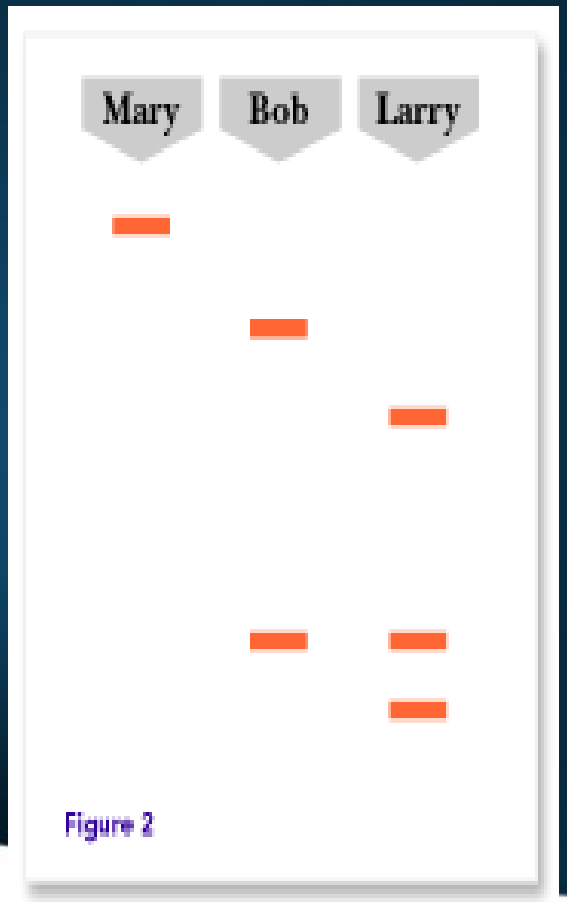
An Example Using EcoR I for a Question of Paternity

Recall: EcoR I cuts only at GAATTC



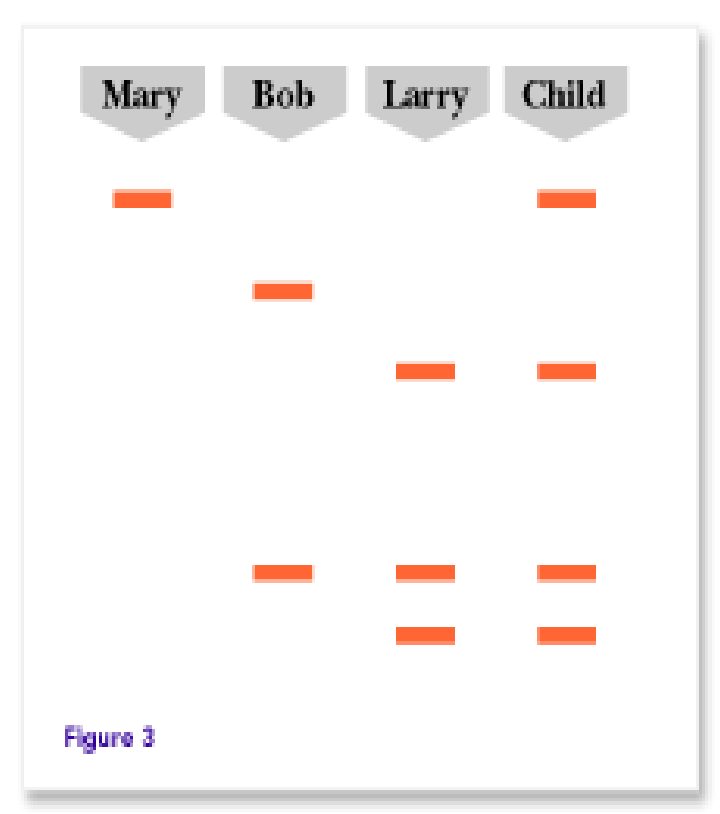
- EcoR I cuts a similar section of DNA on Bob, Larry, and Mary
- After the cut how many fragments Bob, Larry, and Mary have?
- Answer: 2, 3, and none

Resulting Picture after Electrophoresis



- The bigger fragments are near the top

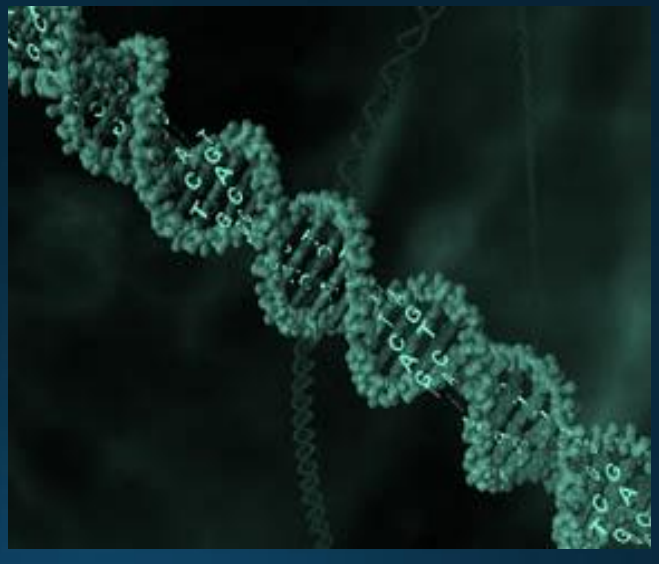
Paternity Test

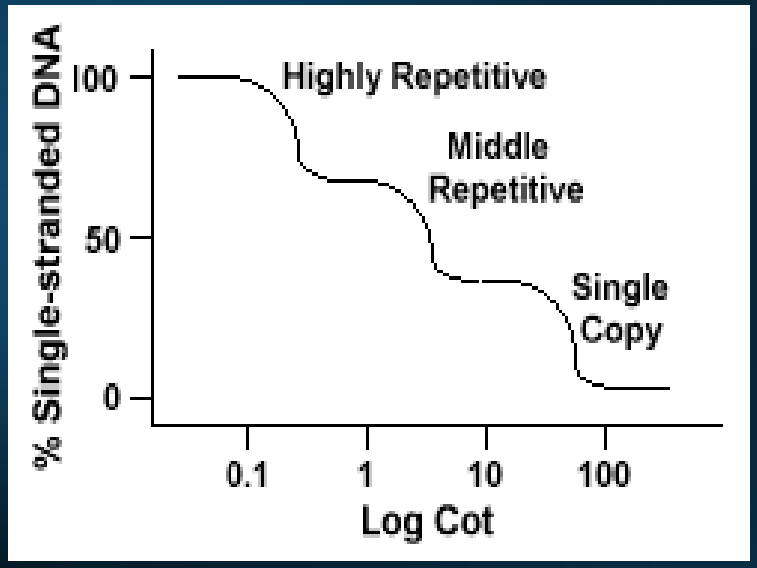


- In general the child's DNA must be a combination of Mary's DNA and one of the men. Which man is the father?
- Answer: Larry


FRANKLY I'M A BIT CONFUSED. ACCORDING TO THE GENETIC PRINTOUT THIS GENTLEMAN IS, IN FACT, A GOAT!
GENETIC RESEARCH
FRAN

Repetitive DNA in the Human Genome





- Less than 2% codes for Proteins
- 50% of the genome contains repeated sequences
  - No apparent function
  - Recombination?
  - For`mation of new genes?

- Types of repeated DNA
  - Tandomly repeated
    - Telomeres
    - Satellite (VNTRs)
    - Minisatellite (STRs)
  - Interspersed repetitive DNA
    - SINES (Alu sequences)
    - LINES
    - Transposable elements

Applications of DNA Forensics

- As the field of DNA forensics is ever increasing, the uses of DNA forensics are also increasing, and the current techniques are being refined.
- The ability of scientists to use DNA fingerprinting to identify sequencing patterns in many different organisms is creating new possibilities every day, the extent of which is still not fully known

- Diagnosis of/ Development of cures for Inherited Diseases
- ~ diagnosis of cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia, and many others
- ~ education of prospective parents as to the risks of having an affected child
- ~ identification of DNA patterns associated with diseases to help establish treatment
- Biological Evidence
- ~ paternity/maternity testing
- ~ linkage of suspects to crime scenes
- Identification of Individuals
- ~ missing persons and casualties

Other organisme

- Veterinary Applications
- ~ parentage testing of purebred animals
- ~ wildlife studies
- ~ identification of inheritance patterns of genetic diseases
- ~ identification of genetic patterns in populations
- ~ investigate genetic susceptibility of populations to diseases
- Agricultural Applications
- ~ breeding of dairy animals
- ~ cultivation of various crops

What is DNA barcoding?

- The term of **DNA Barcoding**: Derivation of a short DNA sequence(s) that enables species identification or recognition in a particular domain of life (eukaryotes).
- Focus to date—in animals—has been on a 658 base-pair (bp) fragment of the mitochondrial gene, *cytochrome oxidase subunit I* (COI).
- The Barcode of Life Initiative (BOLI) would resolve barcodes for named species and use a barcoding approach to assess undescribed biological diversity.
- Very controversial!

CENSUS OF MARINE LIFE

What *is* DNA Barcoding?

CMarZ




- ***Definition***: Derivation of short DNA sequence(s) that enables species identification or recognition in a particular domain of life (e.g., eucaryotes).
- ***Focus to date***: For animals, a 658 base-pair fragment of the mitochondrial gene, cytochrome oxidase subunit I (mtCOI).
- ***Barcode of Life Initiative (BOLI)*** will resolve barcodes for named species and use a barcoding approach to assess undescribed biological diversity.

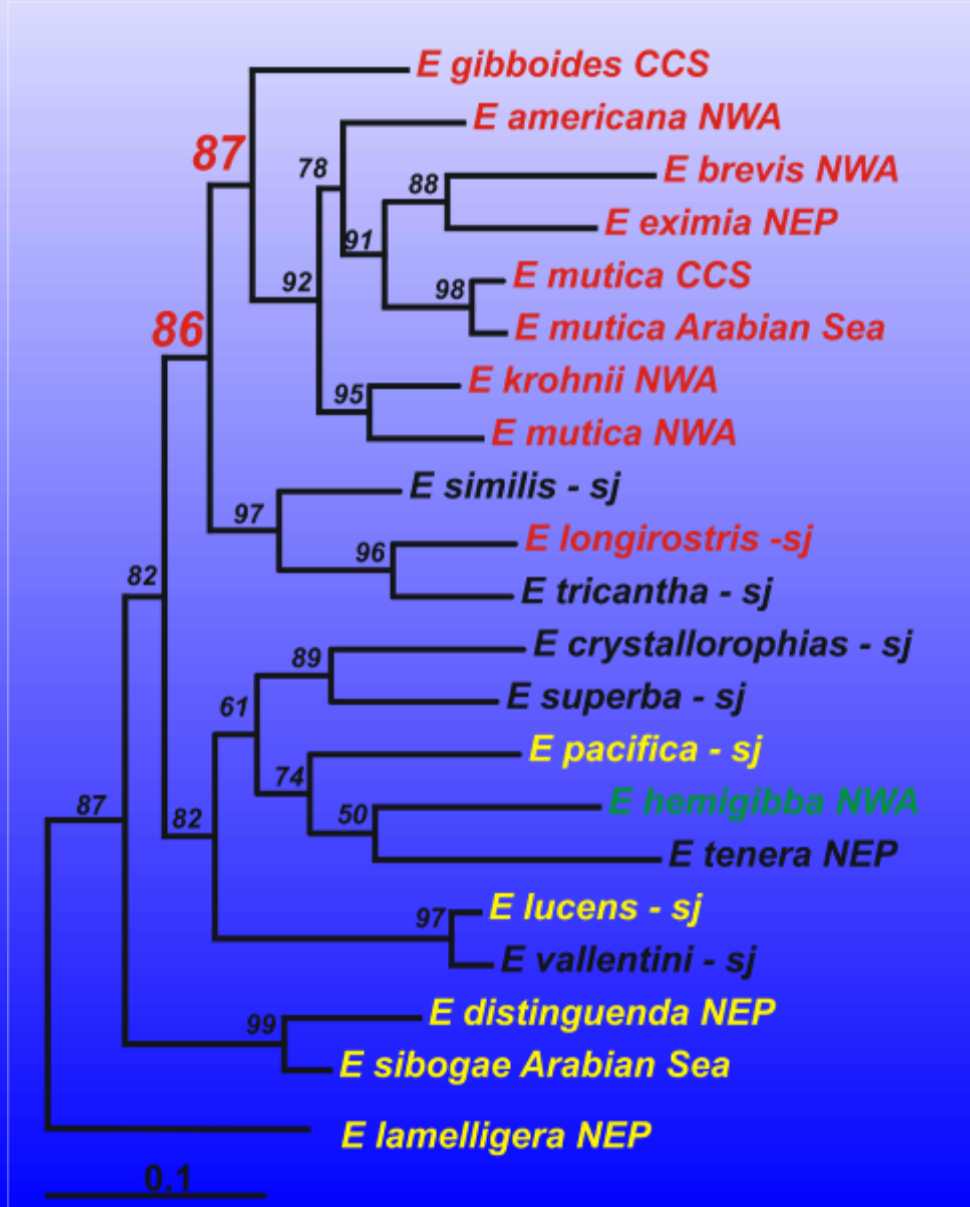
Barcoding Euphausiids (Crustacea)

Fourteen of 86 euphausiid species were identified by Peter Wiebe.

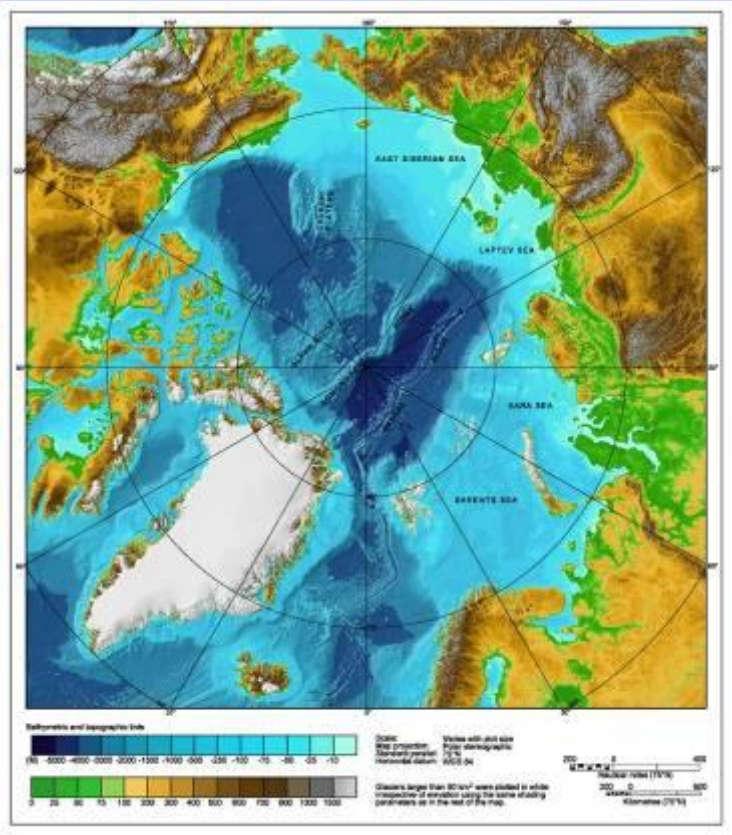
50 euphausiids — including 19 species of *Euphausia* — have been barcoded to date.

<u>Barcoding for Euphausiids</u>:

- Good at species identification
- Can reveal cryptic species



Barcoding Arctic Zooplankton



- ArcOD (Arctic Ocean Diversity) sends identified specimens of Arctic zooplankton for barcoding by CMarZ.
- Comprehensive DNA database of ~210 species of the Central Arctic assemblage is underway.
- DNA obtained from ~50 species already, comprising several dates and locations; work in progress at UConn

Update TRENDS in Parasitology Vol.19 No.12 December 2003 545

Research Focus

DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you

Nora J. Besansky, David W. Severson and Michael T. Ferdig

Center for Tropical Disease Research and Training, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA

a."the role of any molecular diagnostic is to aid research, not to serve as an end in itself.

b.Barcoding … is independent of questions as to whether individual taxa are species, what species are (or should be), and where they fit in a unified tree of life….

c.Barcoding is not an end in itself, but will boost the rate of discovery. The unique contribution of DNA barcoding to … taxonomy and systematic is a compressed timeline for the exploration and analysis of biodiversity."

Potential applications

- Facilitating identification and recognition of named (described) species:
  - linking life history stages, genders;
  - differentiating cryptic species;
  - identifying gut contents;
  - human disease vectors;
  - agricultural pests;
  - biosecurity (?).
- Surveying and inventorying biodiversity; e.g., flagging potentially new (undescribed) species.

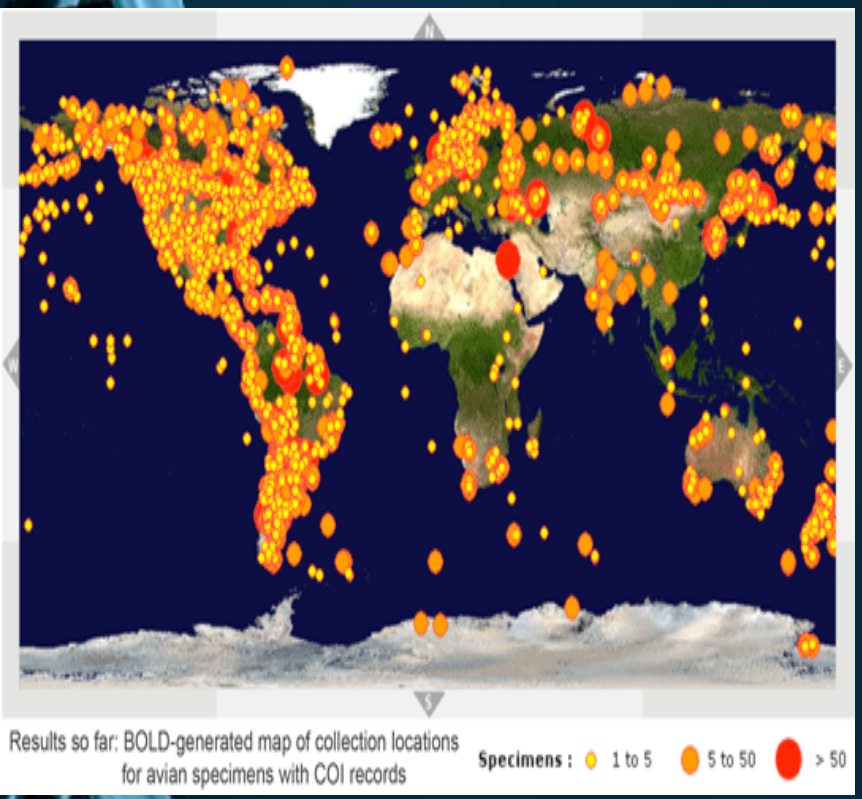
Strengths

- Offers alternative taxonomic identification tool for situations in which morphology is inconclusive.
- Focus on one or a small number of genes provides greater efficiency of effort.
- Cost of DNA sequencing is dropping rapidly due to technical advances.
- Potential capacity for high throughput and processing large numbers of samples.
- Once reference database is established, can be applied by non-specialist.

Weaknesses

- Assumes intra-specific variation is negligible, or at least lower than inter-specific values.
- No single gene will work for all taxa (e.g., COI is not appropriate for vascular plants, or even for some animals).
- Single-gene approach is less precise than using multiple genes; may introduce unacceptable error.
- Some of the most attractive aspects rely on future technology, e.g., handheld sequencer.

allbirdsbarcoding initiative



- The diversity of birds opens a window into the living world.
- The All Birds Barcoding Initiative (ABBI), launched in September 2005, aims to collect standardized genetic data in the form of DNA barcodes from the approximately 10,000 known species of world birds
- Avian COI barcode primers and protocols, with links to original papers, compiled August 2009, available at avian COI barcode primers.doc

16S ribosomal RNA

- The genes for ribosomal RNA have changed little over millions of years as organisms evolved. The slight changes that have occurred provide clues as to how closely or distantly various organisms are related.
- 16S rRNA gene is very short, just 1,542 nucleotide bases, it can be quickly and cheaply copied and sequenced

Methods Mol Biol. 2008;410:335-49.

16S rRNA targeted DGGE fingerprinting of microbial communities.

Tzeneva VA[1], Heilig HG, van Vliet WA, Akkermans AD, de Vos WM, Smidt H.

- biomarkers to monitor microbial communities in environment samples is small subunit ribosomal RNA (rRNA) and the corresponding genes have proven invaluable for advances in microbial ecology.
- the most commonly used approaches is denaturing gradient gel electrophoresis (DGGE) of PCR-amplified fragments. DGGE allows separation of DNA fragment mixtures of equal length depending on their sequence. The separation is based on their sequence-specific melting point in a polyacrylamide gel with a gradient of a denaturant chemical (generally a combination of urea and formamide).
- DGGE allows for a rapid analysis and comparison of microbial communities. Compositional diversity can be visualized using DGGE where each band in principle represents a bacterial phylotype. After staining bands are visualized at each position in the gel where DNA molecules stopped migration. In principle, DGGE fingerprinting can resolve single base pair differences.

The use of stable isotope probing techniques in bioreactor and field studies on bioremediation

- Stable isotope probing (SIP) is a molecular technique that allows investigators to follow the flow of atoms in isotopically enriched molecules through complex microbial communities into metabolically active microorganisms.
- Applications of SIP to biodegradation and bioremediation processes are still in their infancy. Biomarkers (especially sequences of 16S ribosomal RNA and functional genes) to biodegradation reactions in naturally occurring microbial communities.
- As extensive compilations of ecologically important genotypes and phenotypes accrue, predictive abilities for contaminant metabolism in particular habitats may be achieved

The science of DNA profiling is sound.

But, not all of DNA profiling is science.

This is especially true in situations involving: small amounts of starting material, mixtures, relatives, and analyst judgment calls.

Danke schön
Thank you
Terima kasih