Jeffreys et al. 1985

Individual-specific "fingerprints" of human DNA

Background and Introduction

Paper title: Individual-specific "fingerprints" of human DNA

Alec Jeffreys, Victoria Wilson, and Swee Lay Thein

Some useful definitions for understanding this paper...

Minisatellite:

- Simple tandem-repetitive regions of DNA
- Also known as a variable number tandem repeats (VNTR)
- Section of DNA containing 10-60bp
- Dispersed in human genome
- Show substantial length polymorphism from unequal exchanges
    - Alters number of short tandem repeats in satellite
- Unique 10-15bp core in minisatellite
    - Almost invariant sequence “GGGCAGGA*X*G” where ‘*X*’ is any base
    - Acts as recombination signal in generating hypervariable regions

Microsatellite:

- Short-tandem repeats (STR)
- Section of DNA containing 2-6bp
- Used as genetic marker (tagging) in kinship and population studies
- Used for studies in gene deletion and duplication
- Can cause diseases; cancer, neurodegenerative disorder

Probe:

- Hybridizing probe
- Core sequence repeated in tandem
- Detects highly polymorphic minisatellites simultaneously
- Provides set of genetic markers for each individual
- Used in human-linkage analysis

DNA fingerprinting:

- Many variant $(core)_n$ probes used to find specific individual DNA fingerprint set
- Detecting many hypervariable satellites produces somatically stable fingerprint
- Fingerprint is specific to individual or individual and identical twin
- Many applications; paternity, forensics, etc.

"The obtaining or comparing of genetic fingerprints for identification; *spec.* the comparison of DNA in a person's blood with that identified in matter found at the scene of crime etc."

-Oxford English Dictionary

Southern blot:

Comparison of Gel Electrophoresis and Southern Blot images

Mini Case Study:
Often there are families that try to cover up an "underage" or out of "wedlock" pregnancy by portraying the baby as the daughter's sister. Would you be able to tell, with the use of DNA fingerprinting, if the baby was indeed the daughter's sister or the daughter's daughter? (Refer to diagram on board)

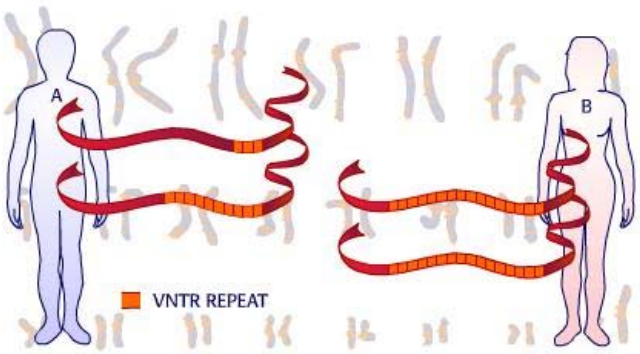
Jeffreys' Breakthrough

- Jeffreys helped show that it is possible to design probes for the cloning of individual polymorphic minisatellite regions from human DNA and that these can analyze multiple hypervariable regions.
- Single-copy human DNA probes are used to detect restriction fragment length polymorphisms.
- Cut DNA into fragments based on the restriction sites.
- Problems?
- Single nucleotide polymorphisms only detect changes in nucleotides for a specific sequence.

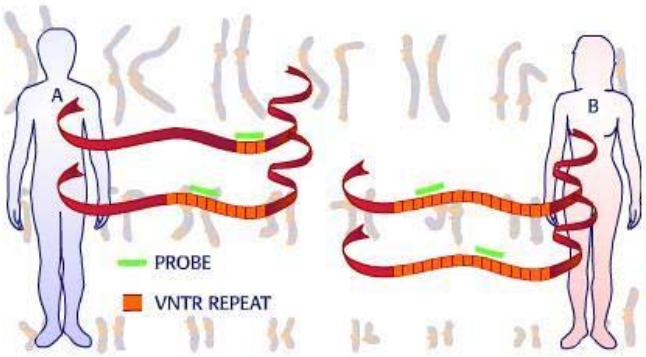
- If the restriction sites cut before the nucleotide change/substitution, you may completely miss the polymorphism!
- Since the mean heterozygosity of human DNA is low, not many of the restriction endonucleases will detect a RFLP at a given locus.
- The new method can detect DNA polymorphisms based on the number and size of the repeats determined by the probe, so it is a much more accurate way of detecting RFLPs.

Probe for Variable Human DNA:

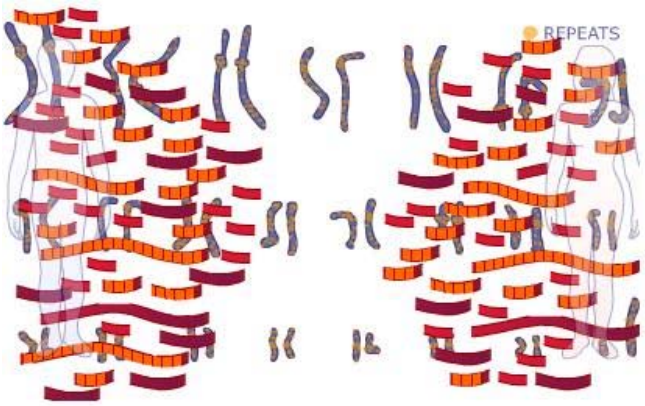
In 1984, Alec Jeffreys first developed his groundbreaking technique using DNA variations to establish unique human identities.



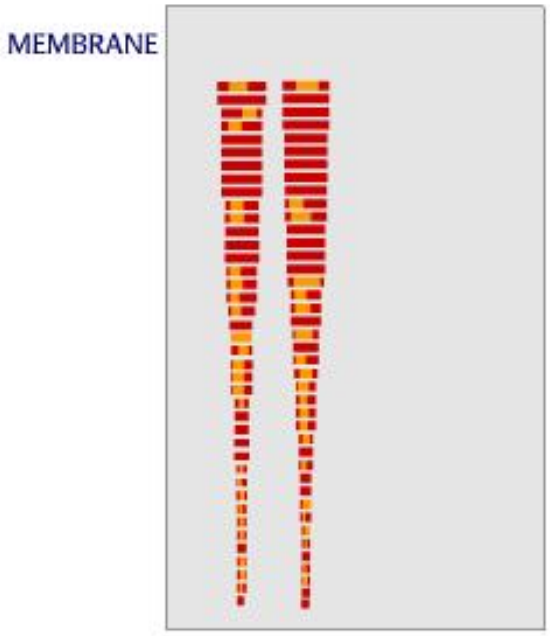
Jeffreys looked at differences in DNA sequence, VNTRs. Differences in the number of repeats created different alleles at a locus on a chromosome. Person A is heterozygous at the locus, person B is homozygous.



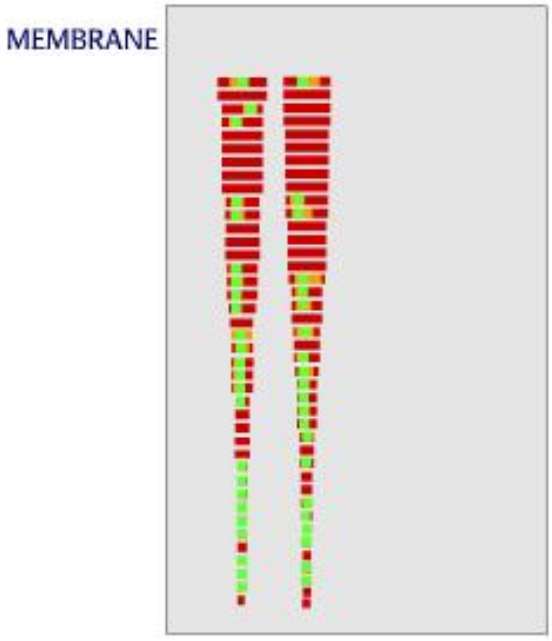
Jeffreys identified DNA sequences that matched the VNTR repeats, he then used these sequences as probes to detect additional VNTR regions.



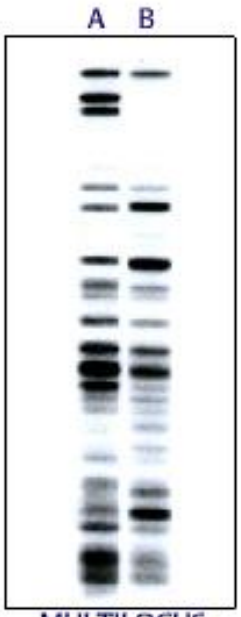
A restriction digest of a person's entire genome produces millions of DNA fragments of different sizes. Jeffreys realized that he could use the probe to identity the subset of restriction fragments containing the VNTR repeat.



The restriction fragments were loaded into an agarose gel and separated by electrophoresis smaller fragments migrate faster than larger ones. Next, the DNA fragments were transferred to a solid membrane, in a process called Southern Blotting.



Jeffreys labeled his probes with ^{32}P, and these probes bond to any fragment containing a VNTR (or minisatellite).



When Jeffreys exposed the membrane to X-ray film, radioactivity from the probes produced dark bands that corresponded to the positioning of the VNTRs. Now, the original method was known as multi-locus fingerprinting.

Experiments

Table 1 Similarities of DNA fingerprints between random pairs of individuals

Probe	DNA fragment size (kb)	No. of fragments per individual ±s.d.	Probability x that fragment in A is present in B	Maximum mean allelic frequency/ homozygosity
33.6	10–20	2.8 ± 1.0	0.11	0.06
	6–10	5.1 ± 1.3	0.18	0.09
	4–6	5.9 ± 1.6	0.28	0.14
33.15	10–20	2.9 ± 1.0	0.08	0.04
	6–10	5.1 ± 1.1	0.20	0.10
	4–6	6.7 ± 1.2	0.27	0.14

- 8 µg of blood DNA from 20 unrelated British people
- Jeffreys compared similarities of DNA fingerprints between random pairs of individuals
- Three fragment sizes were used for probes 33.6 and 33.15
- Averaged number of fragments from Hinf*I* digest (of minisatellite) for each fragment size
- Calculated probability that bands in person A are present in person B
- Smaller fragment size and larger number of bands allows for higher probability of bands being present in A and B
- Unknown proportion of co-migrating bands in A and B derived by chance from different loci
- Estimates for frequency and homozygosity are maximal in this case
- Accuracy depends on resolution of fragments

Some useful terms:

- x probability that an allele A is present in B ($x=2q-q^2$)
- Allele frequencey is low. $q^2 \ll q$; therefore $x=2q$ (mean probability)
- Little variance among alleles.
- Mean homozygosity given by mean q
- Mean probability that all fragments detected by probe 33.15 are in A and B is; $0.08^{2.9} \times 0.20^{5.1} \times 0.27^{6.7} = 3 \times 10^{-11}$ (Maximum)

Hypervariable fingerprints of human DNA

Individual identification using DNA fingerprints

DNA fingerprints and their use in studying family relationships

Practical Applications

- Jeffreys' discovery of the polymorphic minisatellite region and the experiments in designing probes will lead to a better understanding of unequal exchange within chromosomes and gene homogenization
- His experiments were able to show the importance of the shared core sequence as a recombination signal
- He hoped it could be used in general human segregation analysis

In particular:

- Detecting specific bands with disease loci
- Studying marker loss in tumors
- Paternity and maternity testing
- Forensic applications
- Detecting inbreeding

Sarbah vs Home Office

- The earliest cases using DNA finger printing
- Is Andrew Sarbah Christiana Sarbah's son?
- Blood samples from Christiana, Andrew, and Christiana's three confirmed children
- Compare the banding pattern of each individual
- Create a paternal band pattern
- About half of Andrew's bands matched the paternal copy and all the other bands were present in Christiana's banding pattern.
- The chances of this happening if **Christiana was Andrew's aunt are 1 in 1 trillion**

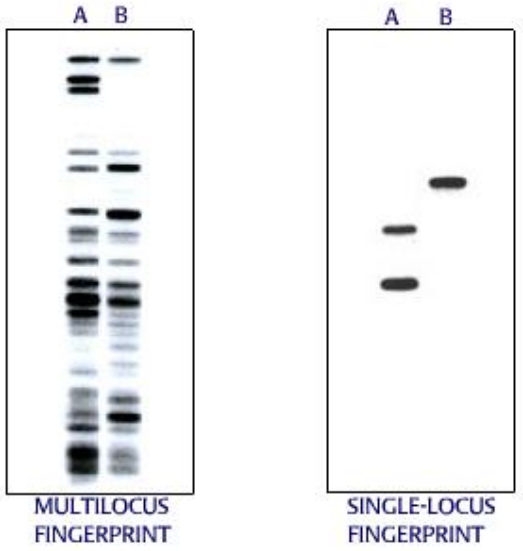
Richard Buckland Trial

- Between 1983 and 1986 Lynda Mann and Dawn Ashworth were raped and murdered
- Richard Buckland was the prime suspect
- Semen samples found on the girls and taken from Pitchfork, proved Richard Buckland's innocence.
- Colin Pitchfork (a baker) was sentenced to life in prison and Richard Buckland was set free

Click here for further information

Since 1985

- In 1988 Nakamura et al from the University of Utah created single locus probes for the D1S80 locus on chromosome 1.
- The result was much clearer, more concise but still offered the same result as Jeffreys experiments two years previous.



- In 1991 Jeffreys developed a new DNA typing system which took two days instead of several weeks.
- This method used PCR
- Used microsatellites (single tandem repeat) instead of minisatellites
- Useful for trace DNA or degraded DNA typing

Problems with DNA fingerprinting

- There are many crime cases where DNA is found but they do not have a match for that sample.
- DNA fingerprinting operates under probabilities
- The sample found and the sample of an individual are compared based on minisatellites
- 1 in 20 billion vs 1 in 20

Difficulties can arise with

- Minisatellite regions in populations. Some populations will have specific mini satellite regions that other populations around the world do not have.

- The probabilities used to identify DNA matches will change based on genetic background (eg, racial group, isolated populations)
- To combat this issue governments have set up several databases containing DNA of individuals classified in groups.

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