Forensic DNA Analysis

Forensics, pertaining to the courts either criminal or civil

Forensics DNA analysis is the use of DNA evidence

Used in:

- paternity suites
- victim identification
- identifying suspects

Originally identification was limited to:

Physical attributes such as; ethnicity, gender, height, weight, hair color, etc.

Friction-ridge identification or fingerprinting

Blood-antigen & serum proteins, ABO blood groups

Even though two unrelated humans differ in their DNA only by 0.1 to 0.2% there are still up to 6 million basepair differences

It is these differences that are used to create a unique DNA "fingerprint" also known as DNA profile

Restriction Fragment Length Polymorphism (RFLP)

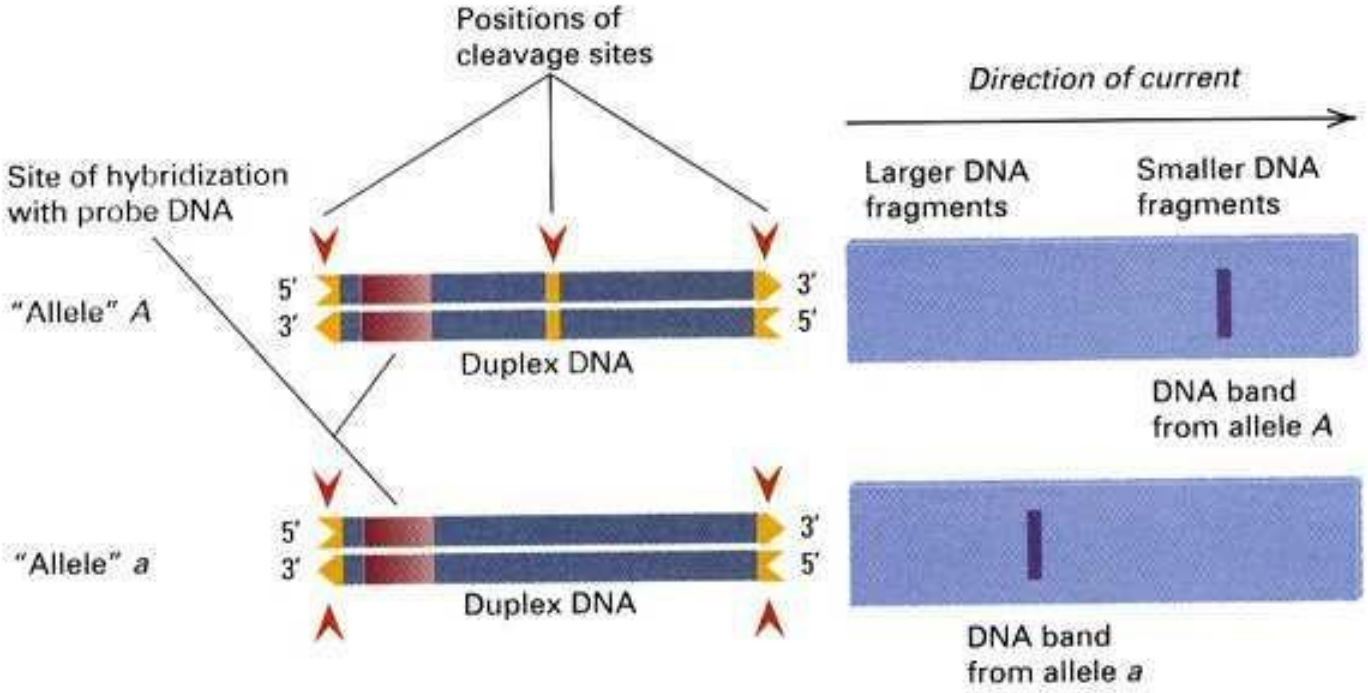
Detects a single basepair change in DNA

Must occur within a restriction enzyme cleavage sequence to be visible

Often used in disease screening such as in the detection of sickle cell anemia

DNA fragments are often visualized by Southern Blot

RFLP



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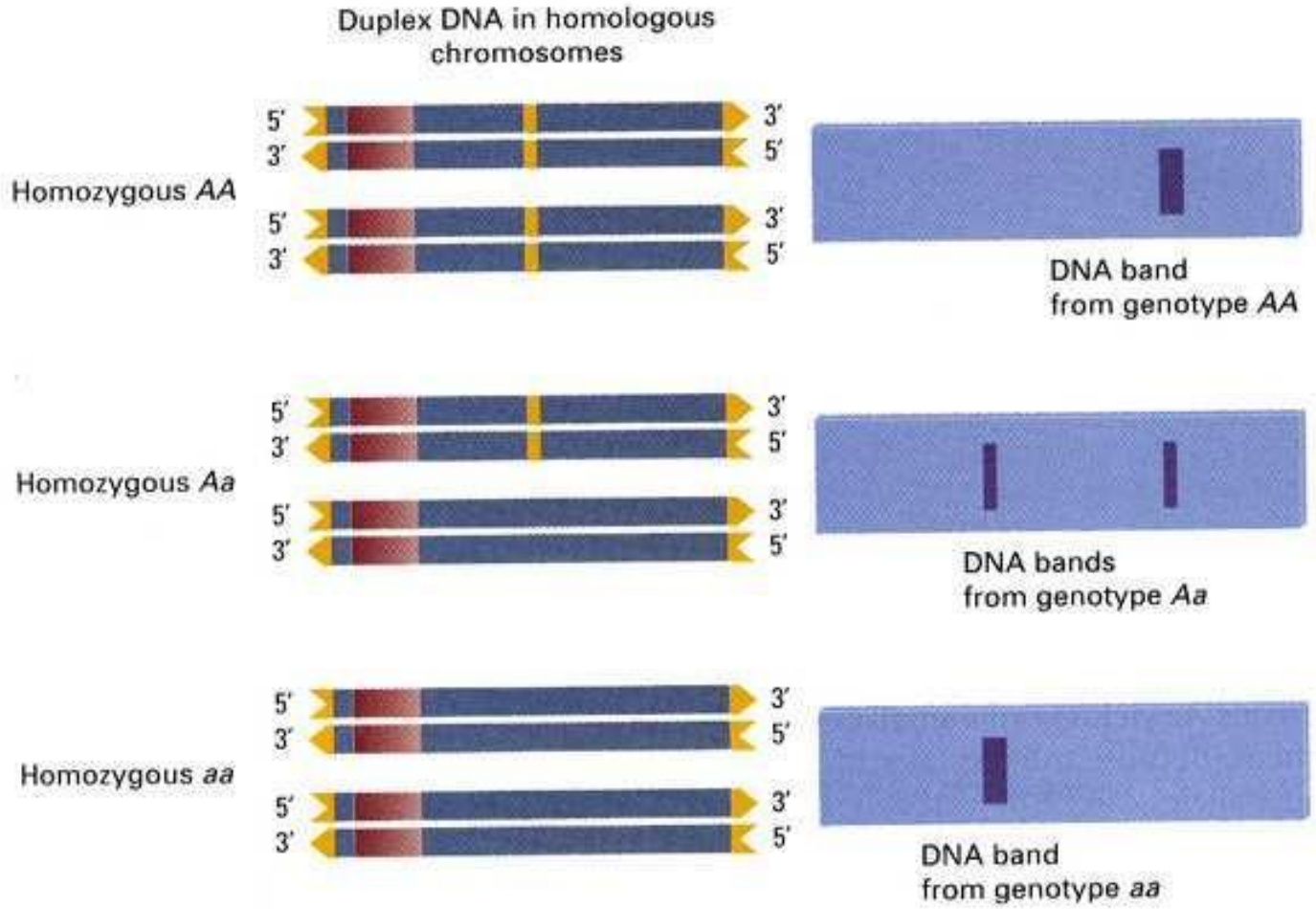

Duplex DNA in homologous chromosomes
Homozygous AA
5′
3′
3′
5′
DNA band from genotype AA
Homozygous Aa
DNA bands from genotype Aa
Homozygous aa
DNA band from genotype aa

DNA Fingerprinting

First described in 1985 by Alec Jeffreys as a method for identifying individuals by their unique pattern of DNA banding

First use of DNA fingerprinting was in a 1985 immigration case in the UK. It identified a child as being the offspring of a British citizen

It was then used to rule out a suspect in a rape/murder case in England in 1986

During the late 80s/early 90s US courts questioned the validity of DNA profiling

The debates centered on evidence collection procedures, training of technicians, & the statistics used to establish a match

By the mid 1990s DNA profiling was shown to be scientifically valid and DNA evidence became admissible

What creates this unique pattern?

Satellite DNA: repetitive DNA sequence.

- Macrosatellite: core sequence 100 to 6500bp
- Minisatellite: core sequence of 10-20bp repeated multiple times
- Microsatellite: small arrays of tandem repeats of 2 to 4bp in length
- (AT)n account for 0.3% of the human genome
- (CATG)n accounts for 0.5% of the human genome

Repeats of Satellite DNA

Repeat units vary in length from 2bp to long stretches of 6000bp or more

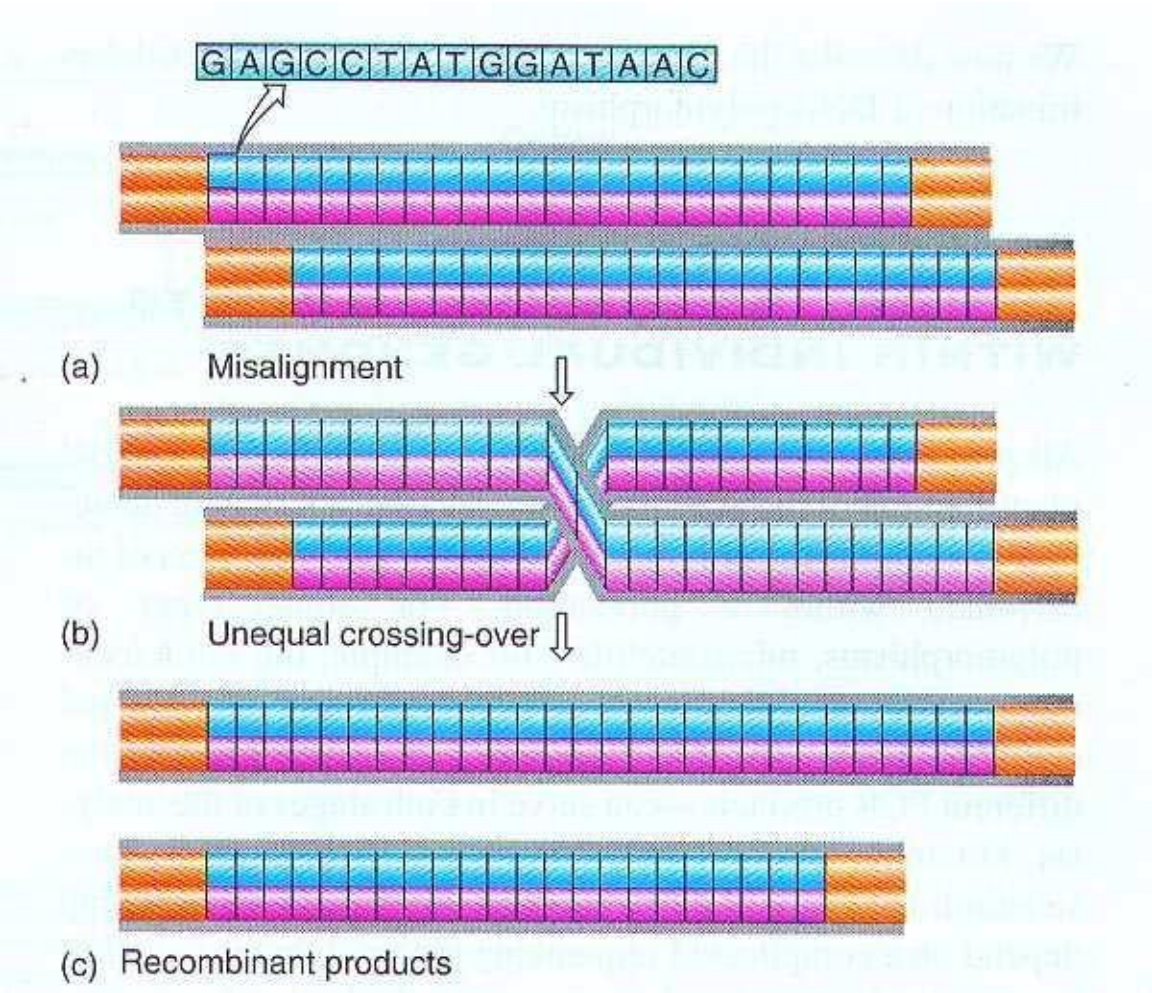
These repeat units are lined up head to tail and compose satellite DNA and are interspersed throughout the genome

The number of units varies person to person

Thus these sequences are called VNTRs (variable number of tandem repeats)

A VNTR is a locus that is hypervariable due to a large number of alleles each characterized by a different number of repeat units

One Mechanism of VNTR Creation



http://www.usask.ca/biology/rank/316/genomics/genomics.htm

Southern blotting can be used to visualize the variation

Probes specific to the repeat unit are hybridized to DNA cut with a restriction enzyme that cuts just outside the VNTR

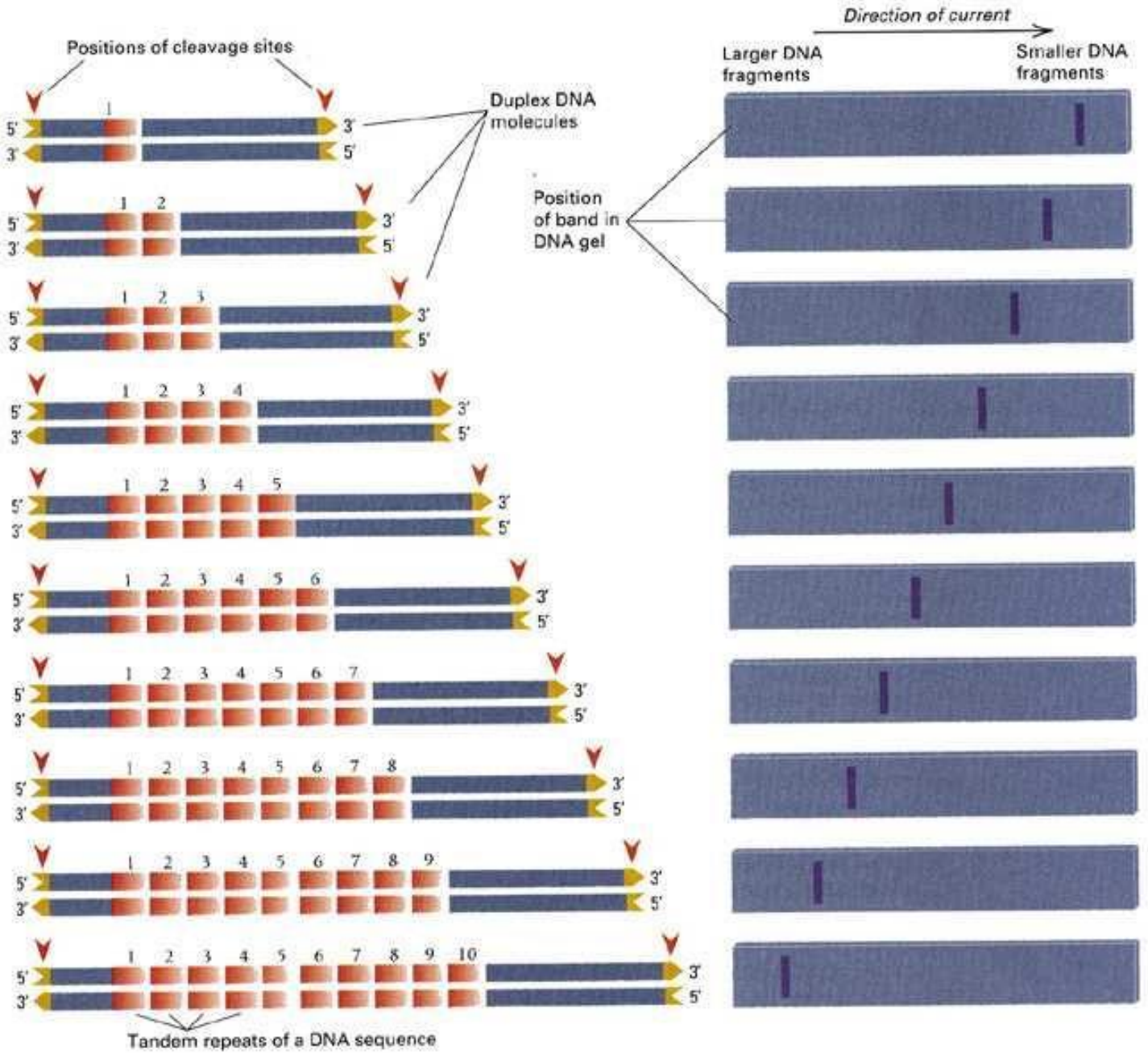
This allows for the difference in VNTR length to be detected

Two common probes are known are:

33.6 $(AGGGCTGGAGG)_{18}$

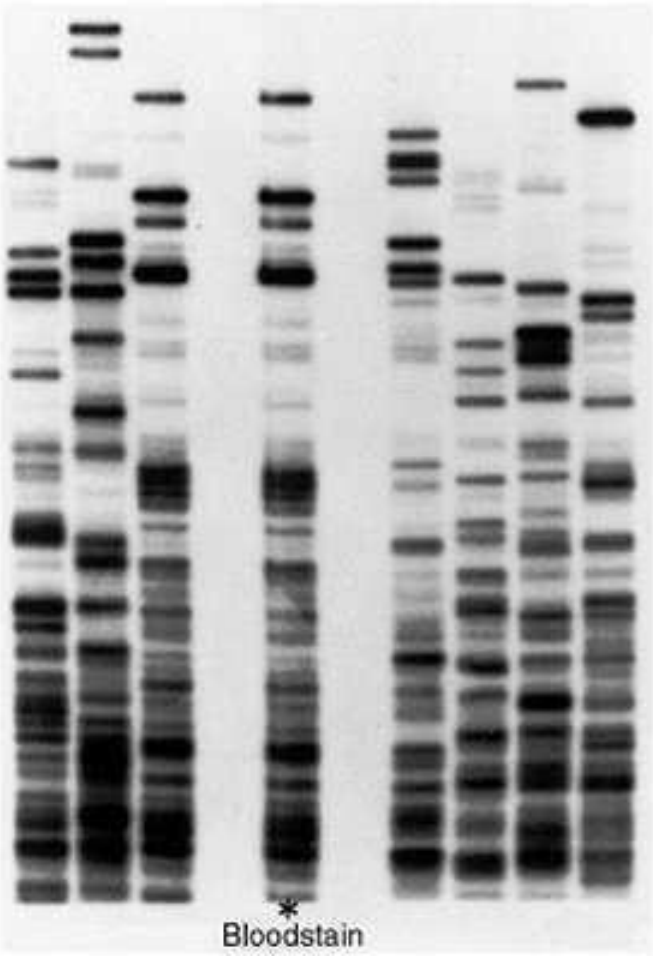
31.5 $(AGAGGTGGGCAGGTGG)_{29}$

These are multi-locus minisatellite probes and show about 17 different DNA bands for each individual



http://bioweb.uwlax.edu/GenWeb/Molecular/Bioinformatics/Unit_3/Lec_3-1/figs3-1/figs3-1.htm

Multi-loci DNA Fingerprint



http://www.mun.ca/biology/scarr/DNA_fingerprinting.htm

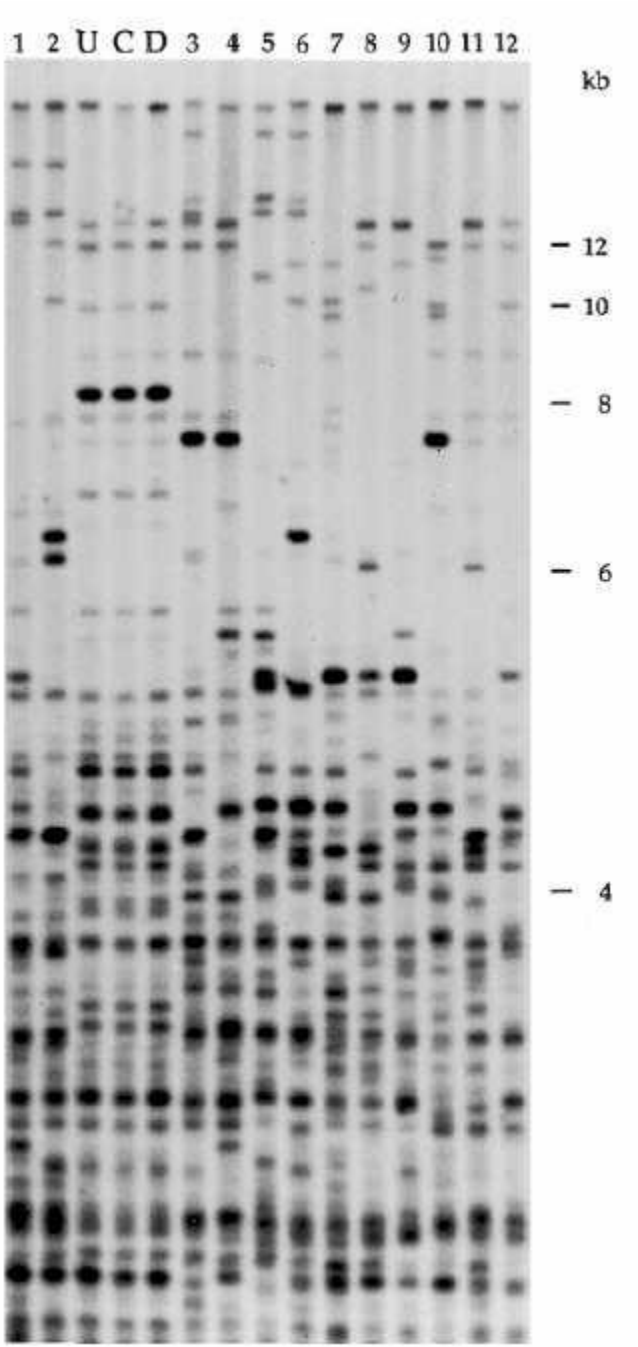
Multi-locus analysis of Dolly used to prove she was a clone

1 –12 are control sheep

U is original udder cells

C is cells from culture

D is Dolly blood cells

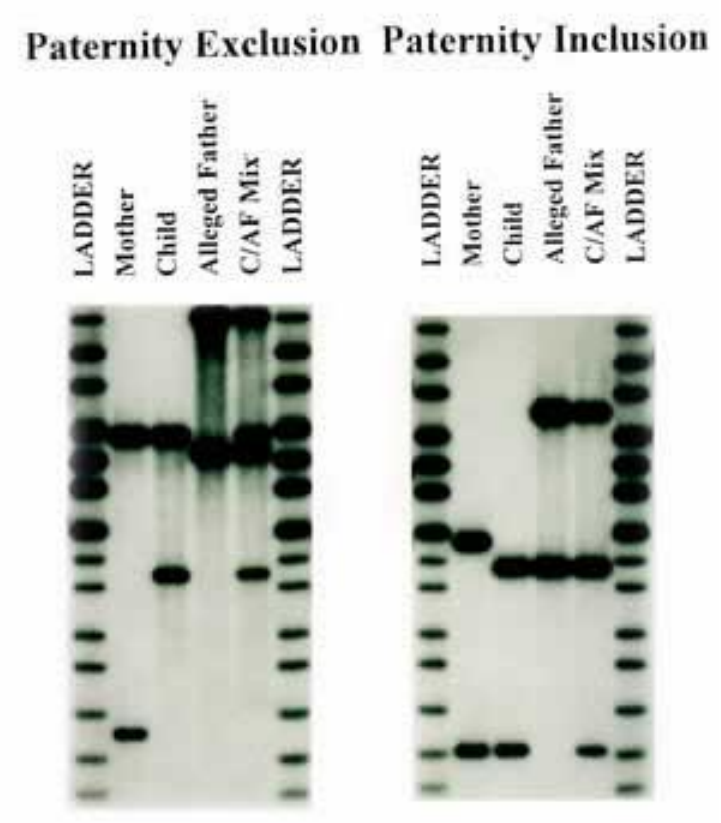


Single-Locus VNTR

Single-locus mini/microsatellite VNTRs generates at most two bands

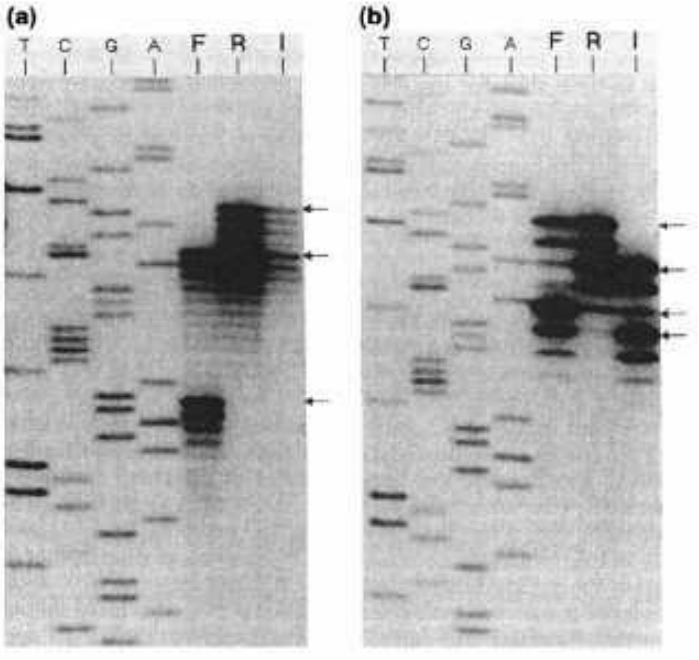
Though not as unique as multi-locus VNTRs they are simple to use

Multiple single-locus VNTRs are used to give a DNA fingerprint



http://www.genelex.com/paternitytesting/paternityslide2.html

Actin Mfd49



Skeletal remains exhumed from a site in Brazil in 1985 that were thought to be those of the Nazi, Josef Mengele

The profile of DNA extracted from a femur (F) was compared with those of his son (R) and wife (I) at 10 different loci, & found to be fully compatible with paternity of Mengele's son

PCR amplification of VNTR

PCR is particularly useful in forensic analysis as it allows minute amounts of DNA to be analyzed

DNA can be obtained from blood stains, semen, saliva, or hair roots

Instead of digesting the DNA PCR is used to amplify the VNTRs and the products are run on a gel and visualized by staining

This process requires primers that anneal just outside the VNTR

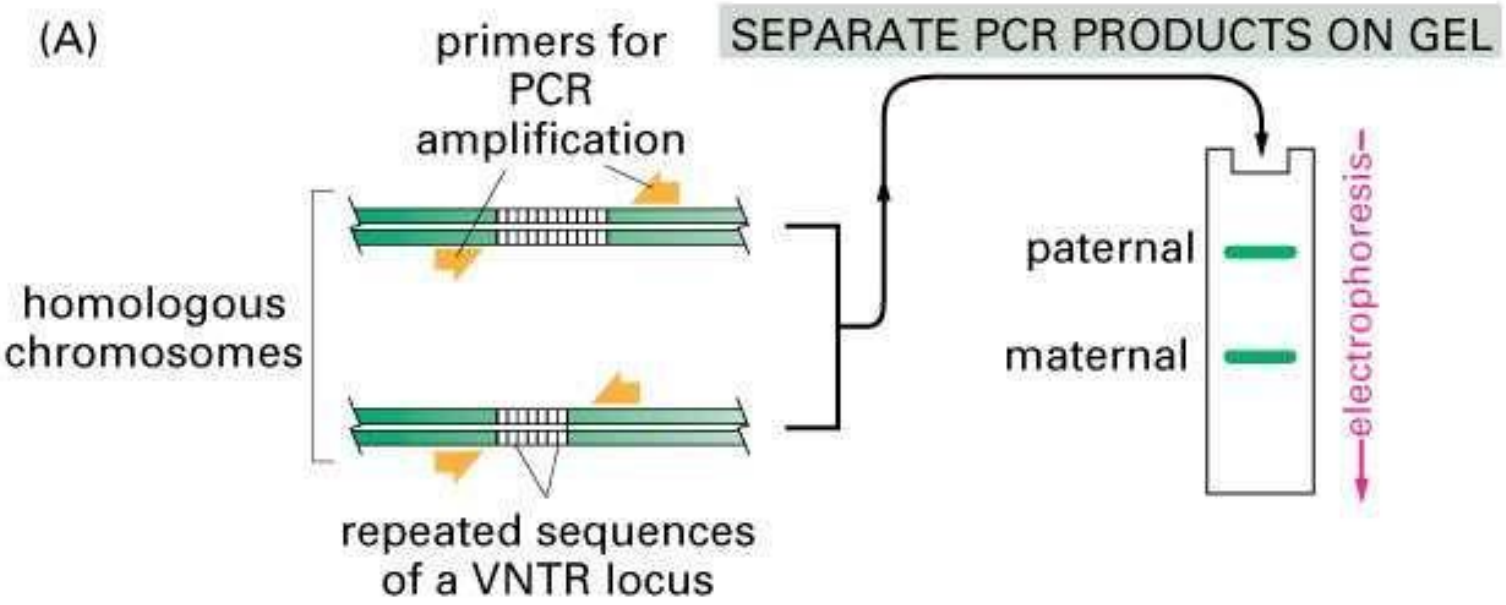


Figure 10-30 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

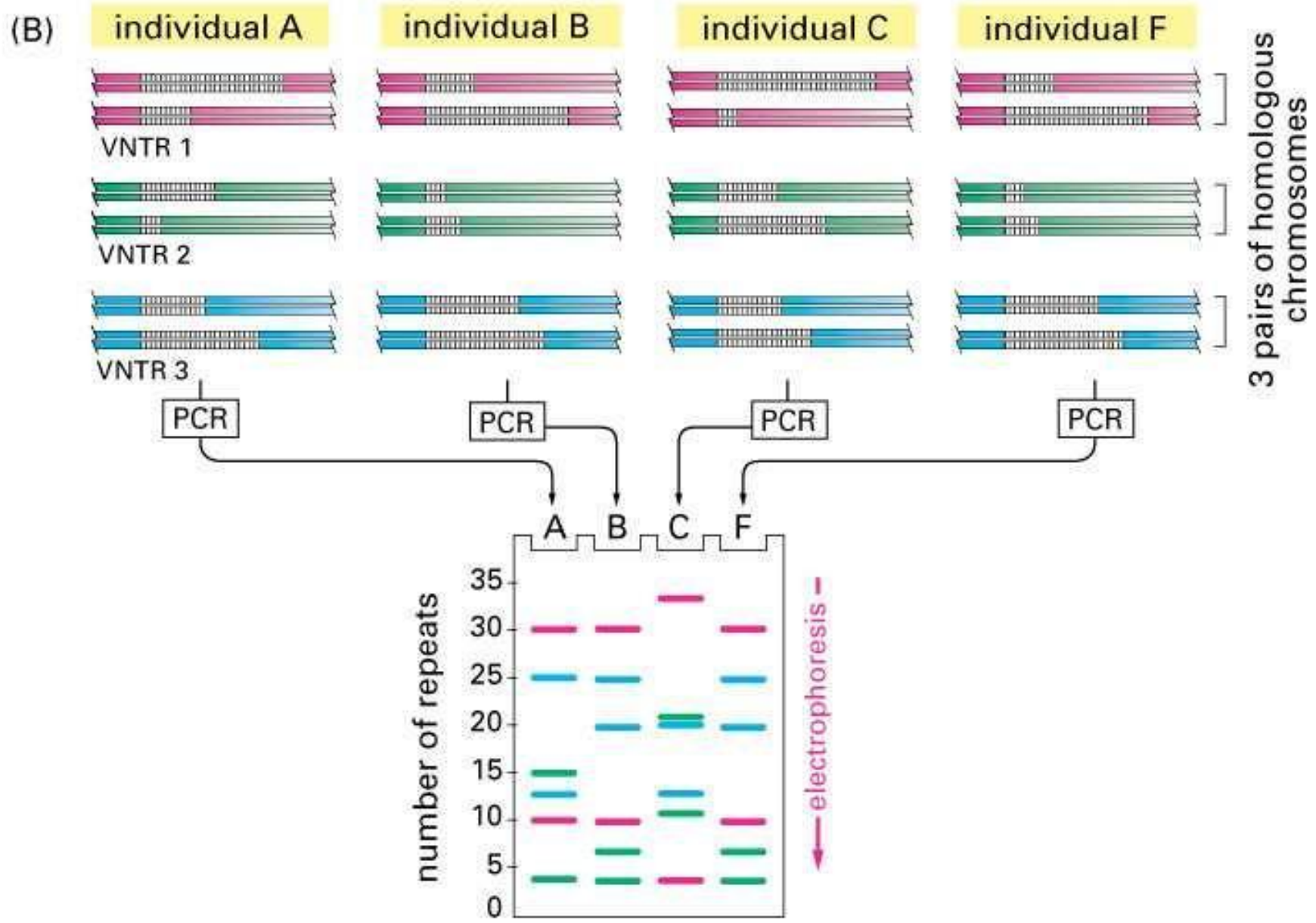


Figure 10-30 part 2 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Short Tandem Repeats (STR)

Are a variation on VNTRs, but use the smallest repeats units often only 2 to 4 bp in length

aatttttgtatttttttttagagacggggtttcaccatgttggtcaggctgactatggagt

tattttaaggttaatatatataaagggtatgatagaacacttgtcatagtttagaacgaa

ctaacgatagatagatagatagatagatagatagatagatagatagatagatagacagat

tgatagttttttttttatctcactaaatagtctatagtaaacatttaattaccaatatttg

13 core loci of tetrameric repeats are tested together to make a DNA profile

The sequence above is locus D7S280 which is located on

STRs are isolated using PCR

Primers have been developed to allow amplification of multiple STR loci in a single reaction mixture

Each primer set has been optimized such that its product, no matter the number of STRs, is not the same size as any of the other products

Each primer set has unique fluorescent molecules covalently linked to them so that they may be visualized immediately by a computer

Following the PCR reaction, internal DNA length standards are added to the reaction mixture

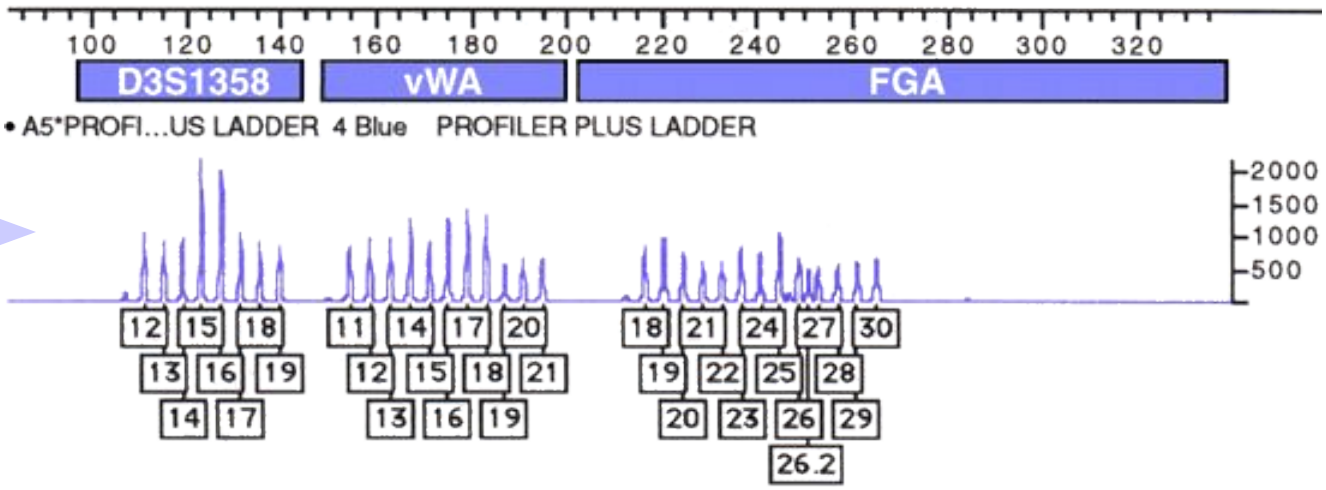
The DNAs are separated by length in a capillary gel electrophoresis machine

As DNA peaks elute from the gel they are detected with laser activation

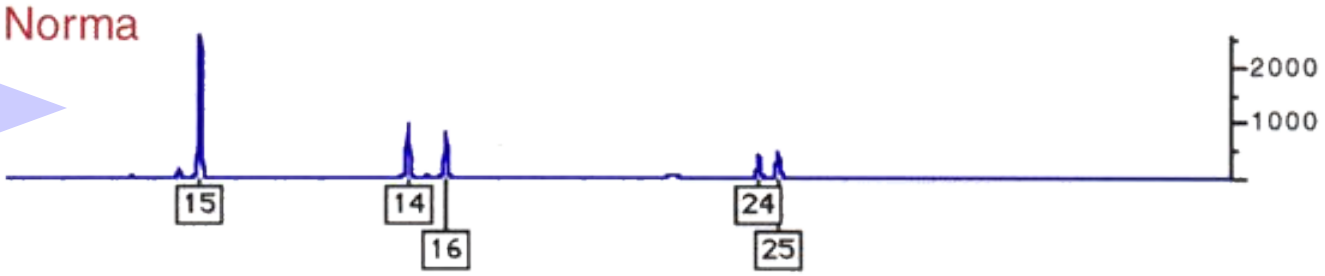
The results are then graphed by a computer which compares them to a standard

Analysis of 3 STRs, D3S1358, vWA, & FGA

Reference standards with the known alleles for each STR locus



Profile of test subject



Genotype is 15, 15 @ D3S1358, 14, 16 @ vWA, & 24, 25 @ FGA

http://www.biology.arizona.edu/human_bio/activities/blackett2/str_analysis.html

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

Example of a DNA profile using the 13 CODIS STR

The odds of another person having this profile 1 in 7.7×10^{15}

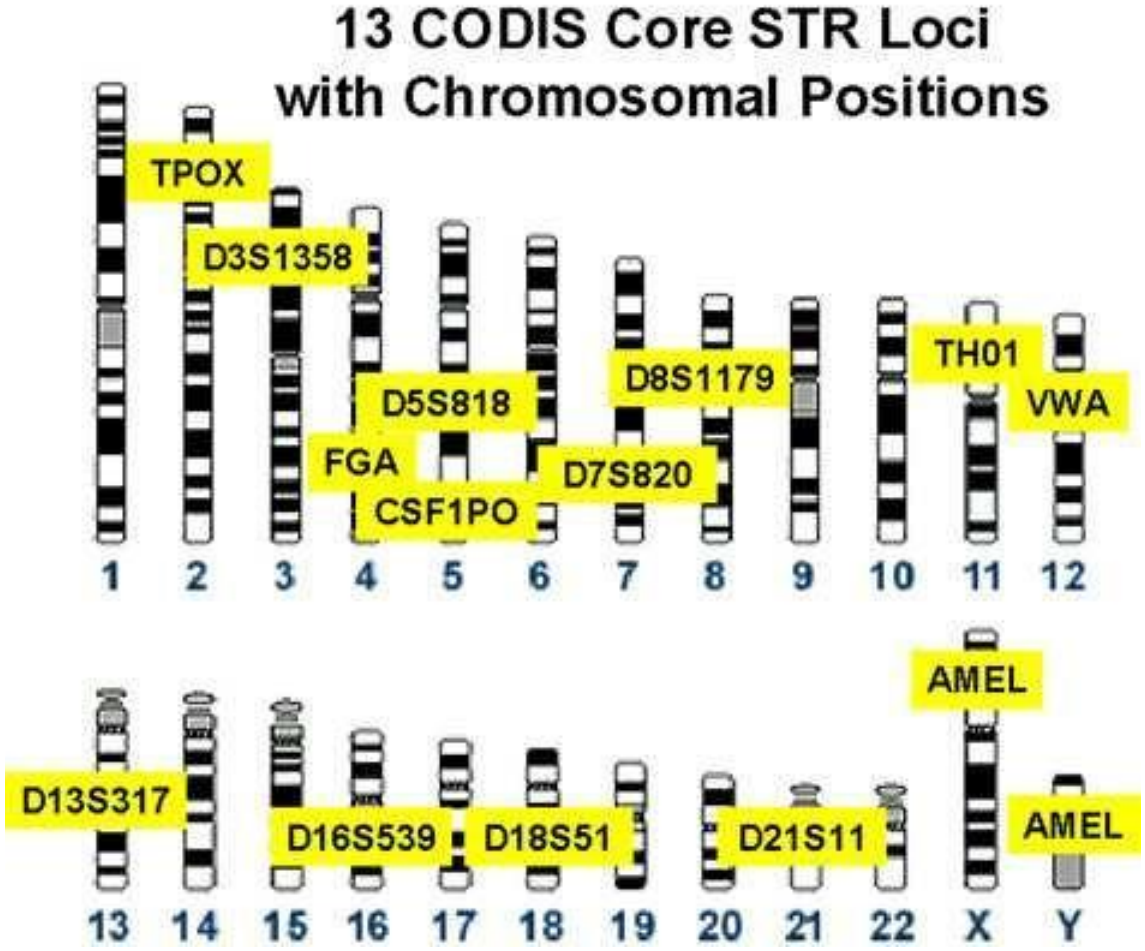
CODIS (Combined DNA Index System)

In 1997, the FBI announced the selection of 13 STR loci to constitute the core of the United States national database, CODIS

All forensic laboratories that use the CODIS system can contribute to the national database

The STRs alleles are easily genotyped using commercial kits

All data from these analyses are digital thus easily placed in the database



http://www.cstl.nist.gov/div831/strbase/images/codis.jpg

Newer Typing Techniques

MiniSTR uses shorter PCR primers giving shorter pieces of DNA to analyze. Developed for WTC (World Trade Center) recovery since the DNA recovered from the site was degraded significantly

Single Nucleotide Polymorphisms (SNPs) single basepair mutations mainly used in medical analysis, but being modified for forensics.

Mitochondrial DNA (mtDNA) analysis of this DNA which is more abundant, hardier, but not unique provides supplemental information increasing the ability to make a statistical match - maternal inheritance