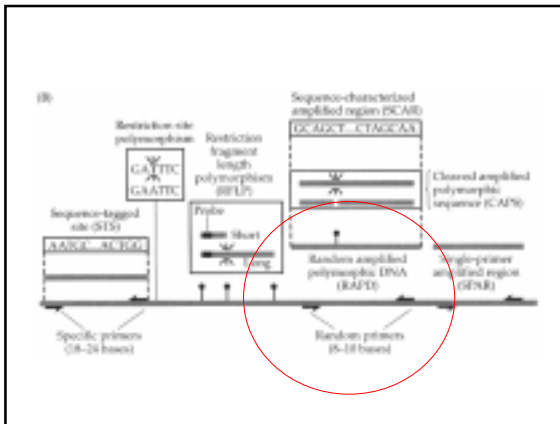


nucleic acid variation

- indirect
 - DNA/DNA hybridization
 - enzymatic degradation
 - restriction enzyme digestion (RFLP)
 - amplification polymorphism (RAPD)
 - repeat sequence variation (VNTR / STR)
- direct
 - sequencing

DNA POLYMORPHISMS

- AMPLIFICATION INDIRECT ESTIMATES OF SEQUENCE DIVERGENCE
 - PCR BASED MEASURES
 - RAPD
 - VARIATION IN PRIMER SITES
 - STS
 - SEQUENCE TAGGED SITES with RFLP



RAPD randomly amplified polymorphic DNA

- PCR based technique
 - use of single short oligonucleotide primer - often 10 base



RAPD randomly amplified polymorphic DNA

- PCR based technique
 - use of single short oligonucleotide primer - often 10 base



RAPD randomly amplified polymorphic DNA

- Multiple "loci" amplify
 - many different locations of 10 bp primer site
 - Any location with inverted repeat can amplify




RAPD

randomly amplified polymorphic DNA

- Multiple “loci” amplify
 - nucleotide changes in one or both sites can “destroy” a band and produce a polymorphism


PRIMER 1



RAPD

randomly amplified polymorphic DNA

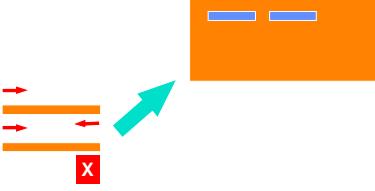
- “+” alleles show dominance



RAPD

randomly amplified polymorphic DNA

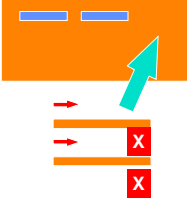
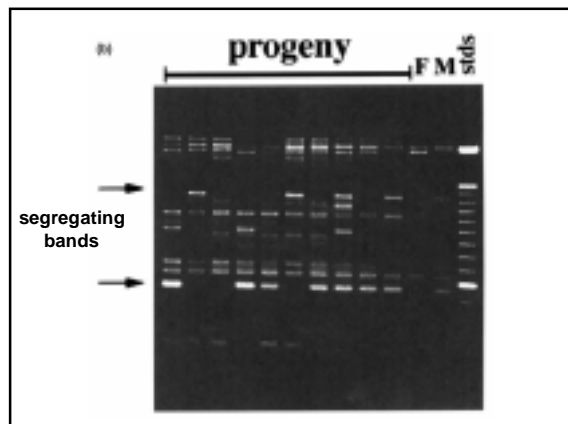
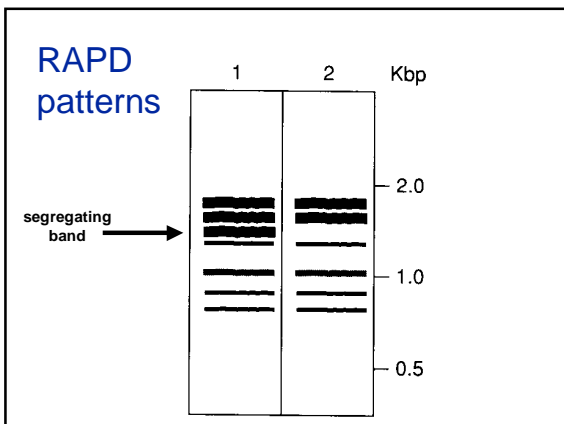
- “+” alleles show dominance
- “-” allele is recessive

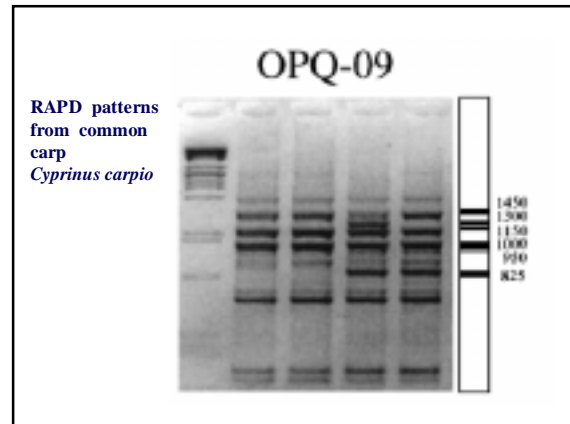
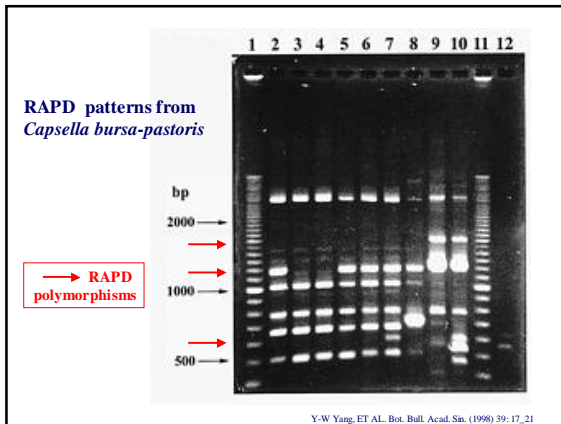


RAPD

randomly amplified polymorphic DNA

- “+” alleles show dominance
- “-” allele is recessive



RAPD

randomly amplified polymorphic DNA

- “+” alleles show dominance
- “-” allele is recessive

Primer			OPQ-09				
Band (bp)	1476	1306	1176	1004	976	827	
Atsuka	100	12.5	100	100	6.25	53.8	
Disneyes	100	10.2	100	160	6.12	29.1	

- Frequencies estimated from frequency of recessives (-), assuming HWE

RAPD

randomly amplified polymorphic DNA

- Can be used to screen a genome quickly
- Many highly variable bands often seen
 - dominance limits ability to calculate allele frequency
 - conditions must be carefully controlled
 - RAPD can produce spurious amplification
 - number of nucleotide differences between “alleles” unknown

DNA POLYMORPHISMS

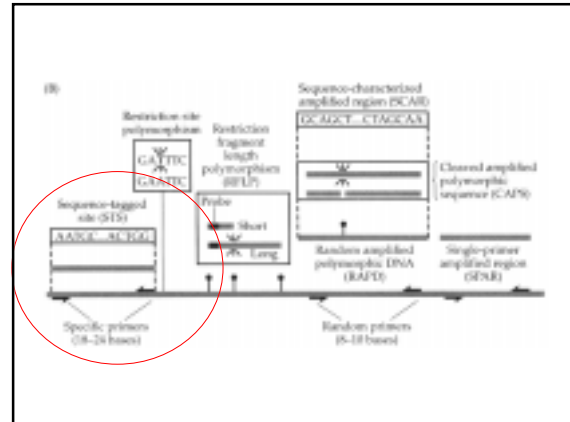
- AMPLIFICATION INDIRECT ESTIMATES OF SEQUENCE DIVERGENCE
 - PCR BASED MEASURES
 - **RAPD**
 - VARIATION IN PRIMER SITES
 - **STS**
 - SEQUENCE TAGGED SITES with RFLP

HOW DO WE VISUALIZE RFLP?

- Use sequence with large amounts of DNA
 - mtDNA, cpDNA or rRNA
 - (limited # sequences)
- use Southern blotting with cloned probes
- **use PCR**

STS

- **SEQUENCE TAGGED SITE**
 - site with unique location in the genome which can be identified by amplification using a specific pair of PCR primers
 - Primers developed to be STS locus specific

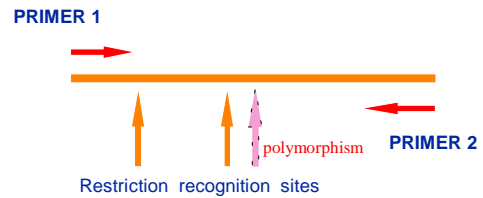


STS

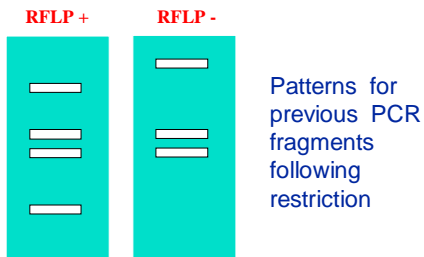
COMBINATION OF PRIMER 1 + PRIMER 2
REPRESENTS UNIQUE SITE



Combine STS with RFLP



Combine STS with RFLP



STS-RFLP PROBLEM:

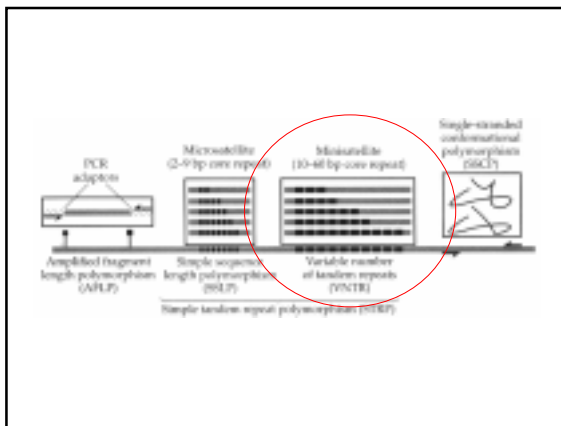
- STS products are usually small (<500 bp)
- Limited degree of nucleotide polymorphism
 - (< 1/100? nucleotides variable)

nucleic acid variation

- indirect
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- direct
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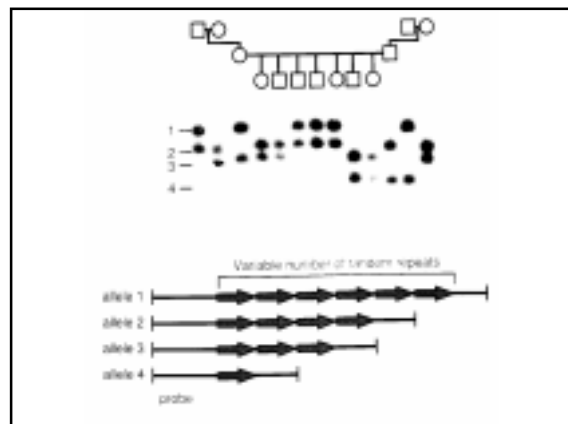
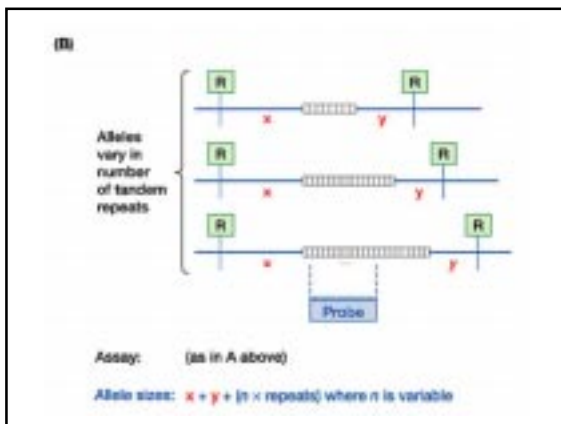
DNA VARIATION

- RESTRICTION SITE VARIATION
 - "RANDOM SITES" - RFLP
 - TARGETED SEQUENCES
 - PCR - STS
 - **SPECIAL SIZE VARIATION**
 - VNTR
 - STR



VNTR LOCI

- Variable Number of Tandem Repeats
 - hypervariable loci or **minisatellite** sequences
 - Special kind of RFLP sequence
 - variation in number of repeats of short (30-300 bp) core segment between constant restriction sites.

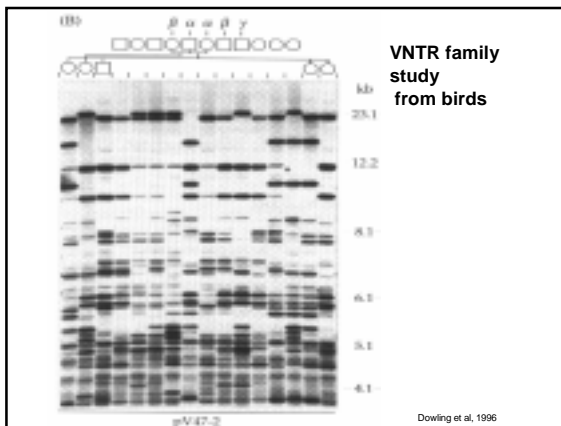


VNTR LOCI

- "mutational" process probably involves unequal cross-over or replication slippage
- High levels of polymorphism
 - MANY alleles
- usually visualized by Southern blotting

VNTR LOCI

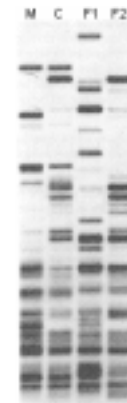
- VNTR probes:
 - **Multilocus probe:** Jeffreys' probes
 - core sequence hybridizes to several (many) different locations
 - not utilized widely in human genetics
 - gene frequency estimation difficult



DNA Multilocus VNTR Patterns DNA Forensics: Paternity

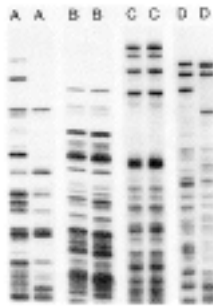
Shown are DNA "fingerprints" from a new mother (M), her recently born child (C), and two possible fathers (F1, and F2). The mother and the child share some DNA bands since they also share 50% of their genes but they each have bands that are not represented among the bands of the other.

The two possible fathers have dissimilar DNA banding patterns. The question is does one of the possible fathers have several bands similar to the child which are not shared with either the mother or the other possible father.



DNA Multilocus VNTR Patterns from four sets of twins

Shown are DNA banding patterns from four sets of twins. If the band patterns are identical, then the twins should be identical (maternal) but if the band patterns of two twins are different, then the twins are non-identical (fraternal). Non-identical twins should share some of their DNA bands since they share 50% of the genes.



VNTR LOCI

- VNTR probes:
 - **Single Locus probes**
 - core sequence hybridizes to one unique locus in genome
 - preferred VNTR sequences because variation can be assigned to locus

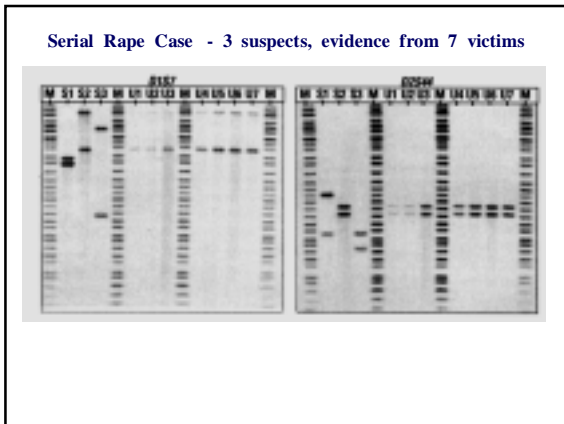
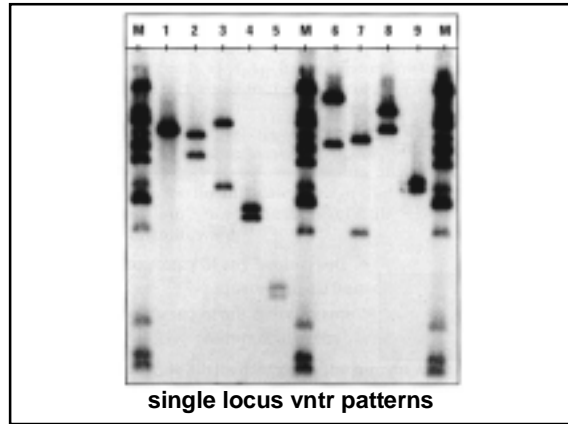
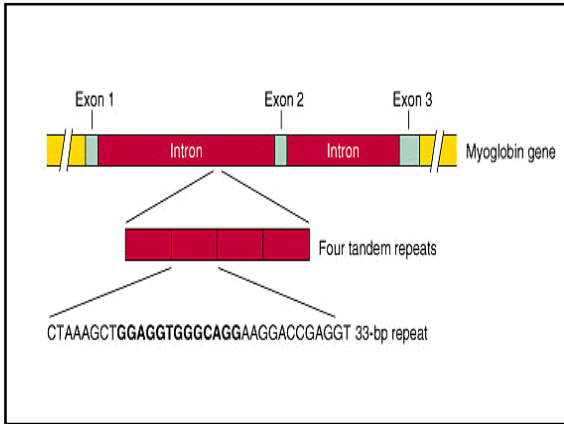


TABLE 6.2 Allele frequencies for LCR Conversions at four tetranucleotide loci.
Source: Stone et al. (1992), based on 10,000 alleles.

Allele Designation	Locus D1S1	Locus D2S13	Locus D3S13	Locus D4S13
1	0.001	0.007	0.003	0.004
2	0.006	0.003	0.003	0.004
3	0.006	0.004	0.007	0.006
4	0.013	0.004	0.004	0.014
5	0.012	0.004	0.005	0.014
6	0.014	0.004	0.013	0.014
7	0.010	0.013	0.010	0.010
8	0.020	0.016	0.010	0.017
9	0.011	0.006	0.008	0.014
10	0.014	0.007	0.009	0.017
11	0.008	0.003	0.002	0.008
12	0.011	0.007	0.006	0.010
13	0.006	0.004	0.006	0.009
14	0.007	0.007	0.007	0.010
15	0.017	0.007	0.007	0.016
16	0.011	0.007	0.006	0.016
17	0.008	0.017	0.007	0.010
18	0.010	0.012	0.007	0.010
19	0.010	0.014	0.007	0.010
20	0.010	0.008	0.008	0.010
21	0.017	0.008	0.008	0.010
22	0.017	0.008	0.008	0.010
23	0.017	0.008	0.008	0.010
24	0.010	0.008	0.008	0.010
25	0.010	0.008	0.008	0.010
26	0.010	0.008	0.008	0.010

- VNTR limitations**
- may be difficult to score size of "allele" accurately
 - limited number of VNTR loci
 - non-random distribution in genome

- VNTR applications**
- DNA fingerprinting - forensic
 - paternity analysis
 - analysis of linkage to other loci
 - (limited because of nonrandom locus distribution in genome)