

DNA FINGERPRINTING, POLYMERASE CHAIN REACTION

3/2/94, rvsd 6 March 1995, 5 March 1997, 26 Feb 01, 1 March 02, 16 Feb 04, 3 March08, 2 March09, 3Mar10
gmlsl 6th p. 509-511 , Russel, p. 495-496, Weaver&Hedrick, p. 458-460, gmlsl 7th: RFLP: p 404,
9th: 16, 151, 611, 719,

Polymerase Chain Reaction: (p 731)

Uses DNA polymerase from *Thermus aquaticus*, temp resistant polymerase = *Taq* polymerase

- 1) select 2x DNA to be amplified
- 2) **Add primer:** select or create primer to bind at each end of target sequence, **flanking** it
- 3) **heat 2x DNA**, allow primer to anneal, cool
- 4) **Add *Taq*** and 4 dNTPs to synthesize complimentary strands
- 5) **heat denature again** to produce ssDNA (*Taq* not affected)
- 6) **synthesis cycle again**, this time, newly synthesized strand = length between primers
- 7) **geometrically increase amt of DNA** by cycling heating and cooling

Because of large number of variable number tandem repeats, multiple alleles allow genetic differentiation (see p 152: parents each have two different alleles, children have assorted alleles)

Restriction Fragment Length Polymorphism (RFLP)

Cutting DNA from any two individuals can result in fragments of different lengths. (P 148)

Special class of RFLP: Variable Number Tandem Repeats: p 151, 611,

short sequence (15-100 bp long) repeated in variable numbers to yield 1 kb to 5 kb sequences at different loci. (20% of human genome consists of non-functional repeats, many introns.)

FORENSIC APPLICATIONS (overview, p 16)

Identify individuals from DNA from blood, semen, etc (paternity, crime scene analysis, etc)

- 1) **Isolate and purify DNA** (if inadequate amts, polymerase chain reaction used). (Hair samples must be digested with protease K to release cells.) Check for integrity by preliminary electrophoresis, should form major single 15 Kb band, not smear indicating degradation
- 2) **Digest with restriction enzyme** which **does not cut within the tandem repeat sequence** (Commonly Pst I, Hae III or Hin fl used). Run on gel to ensure digestion complete.
- 3) Transfer by **Southern Blot** to nylon membrane, fix by baking. (P 728)
- 4) **Hybridize the DNA on the membrane** with end labeled probe (D2S44 or DXYS14 commonly used) for hypervariable regions of **VNTR (variable number tandem repeats)** often have minor differences in sequence, but consensus sequence is conserved. With 7 or 8 such loci, it is nearly impossible for two people to randomly have identical pattern.
To prepare the **end labeled** probe ("end labeling"): p 725,
A) remove 5' PO₄ on cleaved end with **alkaline phosphatase**
B) use **polynucleotide kinase** and AT³²P to label 5' end with ³²P
Rinse and dry the membrane filter with the hybridized, annealed probe
- 5) Identify the sizes of the restriction fragments with **autoradiography** (p 16, 151 for an example gel)

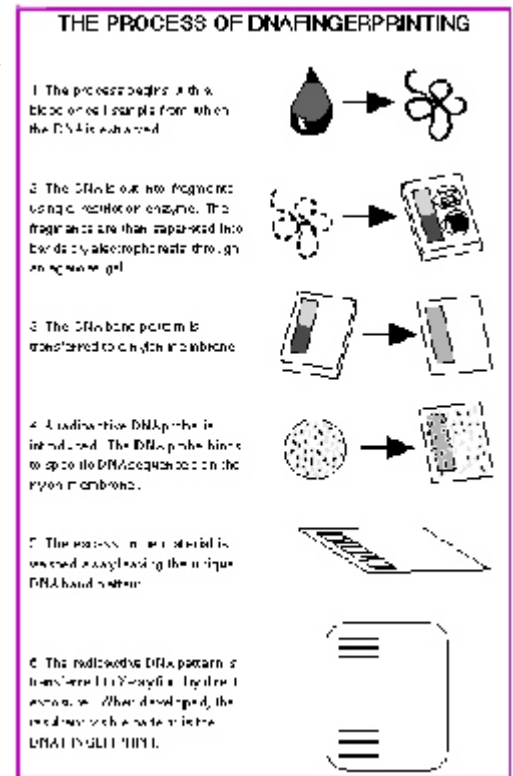
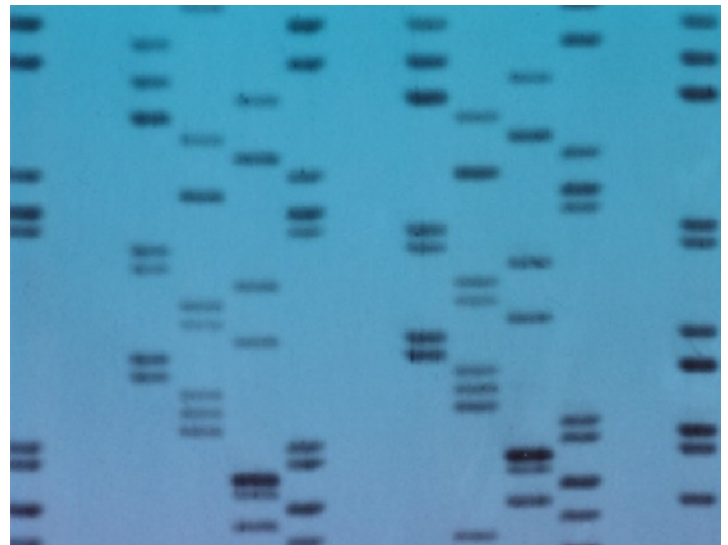


Figure 3.



Fingerprint of crime scene DNA is on the L, then that of nine suspects.

