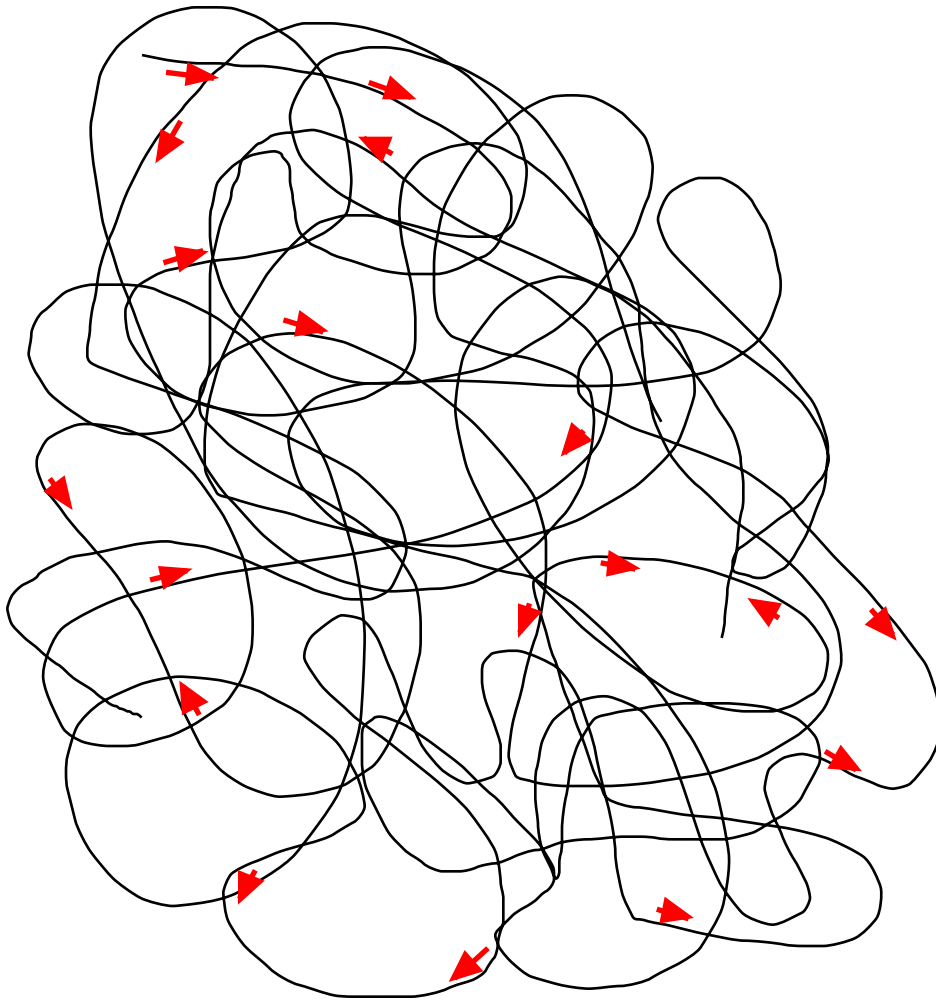


**Randomly Amplified
Polymorphic DNA
(RAPDs)**

RAPDs



Short "random" oligo



RAPDs Requirements

A Single Short Primer:

5' – CTATGGCTCT – 3'

OR

5' – ATCATATTCC – 3'

OR

5' – CACTCAGGTA – 3' ...

Very clean, quantified chromosomal DNA

PCR reaction components:

Low annealing temperature (i.e., 30 – 50° C)

High Salt concentration (i.e., ≥ 2.5 mM)

Probability of Priming

$$\begin{aligned} P(\text{dATP}) &= P(\text{dTTP}) = P(\text{dGTP}) = P(\text{dCTP}) \\ &= 0.25 \end{aligned}$$

$$P(\text{CT}) = 0.25 * 0.25 = 0.625$$

$$P(\text{CTC}) = 0.25 * 0.25 * 0.25 = 0.0156$$

$$P(\text{dNTP}_n) = (0.25)^n$$

$$\begin{aligned} P(\text{any 10 nucleotide sequence}) &= 0.25^{10} \\ &= 9.54 \times 10^{-7} \end{aligned}$$

Exceptions:

- GC content is usually higher than 50% (i.e., not 0.25 probability for each nucleotide)**
- Sequence is not random (i.e., sequence clusters or islands)**
- Primer is not really 10 nucleotides long**

Probability of Priming II

$$P(\text{any 10 nucleotides}) = 9.54 \times 10^{-7}$$

Or

Once in every 95,400,000 bp

The brown pelican (*Pelecanus occidentalis*) genome is approximately 1.9×10^9 bp. A specific 10 nt primers should find about 20 matching sites in the pelican genome. How many would be opposing and at proper distance?

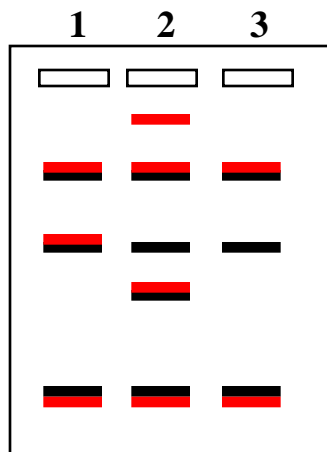
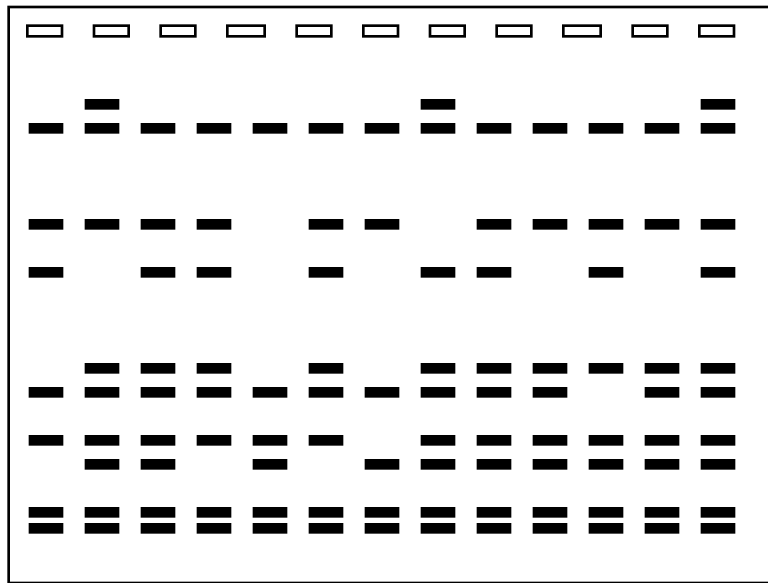
Or

A bacterial genome is about 100x smaller therefore the same random primers should find about 0.2 matching sites. (see figure in NAR 18:6532).

RAPD Loci

- **All kinds of regions:**
 - **Single copy**
 - **Low copy**
 - **Medium copy**
 - **High copy**
 - **Coding**
 - **Noncoding**
 - **Functional**
 - **Nonfunctional**
- **All that is required is that primers anneal opposing and the distance between them not be greater than about 3,000 bp.**
- **Scattered throughout the genome**
- **Moderately variable**
- **Can be used from individual to species (and higher) levels.**
- **Dominant Markers**
- **Allele is presence or absence of band**

RAPD Gel



Observed # of differences	2	0
Real # of differences	?	1

RAPD Strengths and Weaknesses

Advantage:

- **Easy to do**
 - **i.e., no cloning**
- **Moderately variable loci**
- **Mixture of regions**
 - **Some neutral some selected**
- **Very high throughput of samples**

Disadvantages:

- **Often unreliable (sample to samples and within the same sample)**
- **Dominant not co-dominant alleles**
- **Black box voodoo technology**