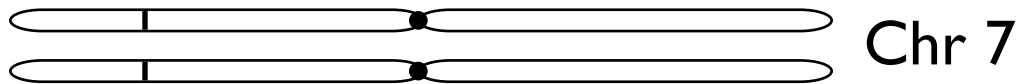


**What's polymorphic** about microsatellite repeats?



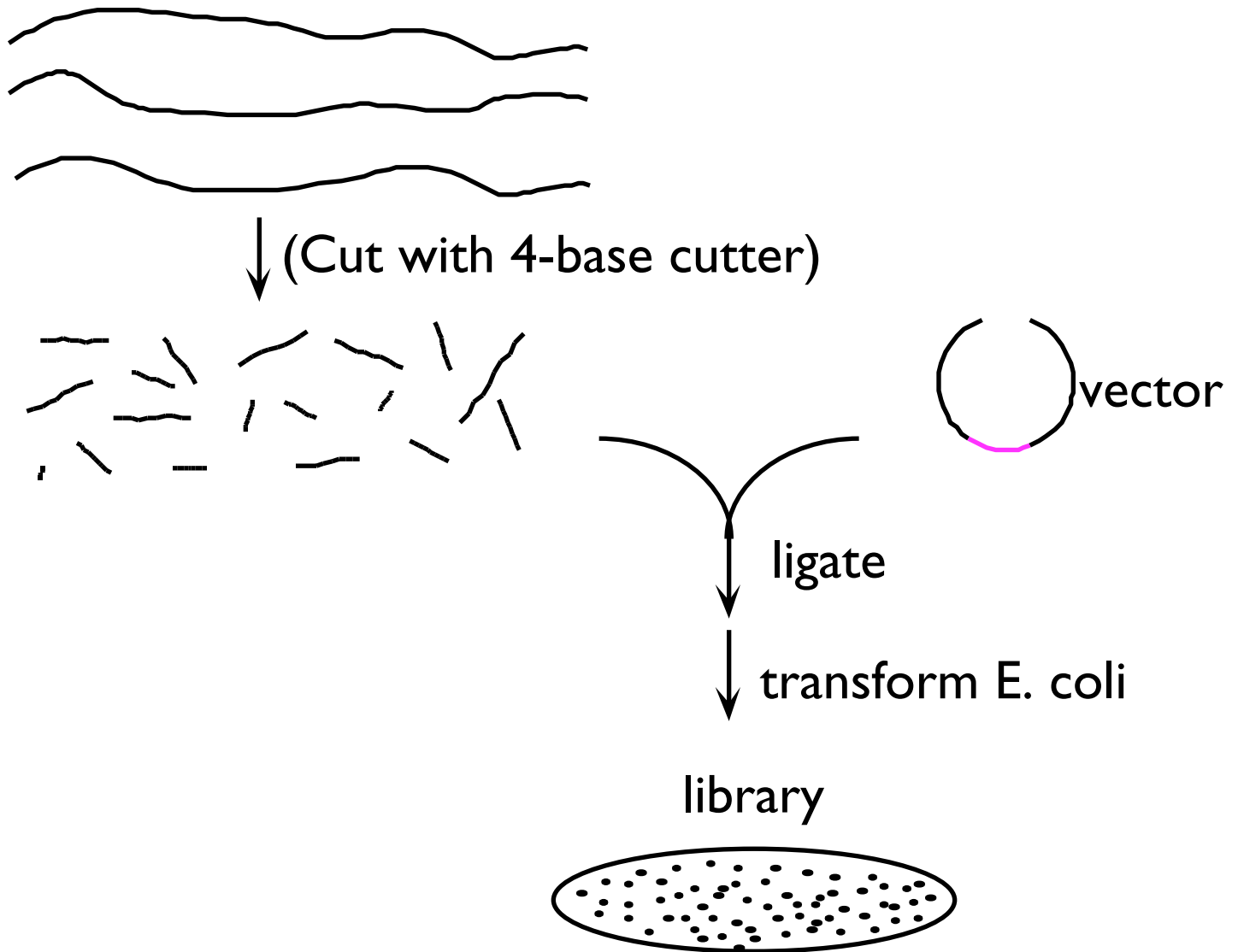
Person 1  $\left\{ \begin{array}{l} 21 \text{ repeats on chromosome 7 homolog 1} \\ 33 \text{ repeats on chromosome 7 homolog 2} \end{array} \right.$

Person 2  $\left\{ \begin{array}{l} 30 \text{ repeats on chromosome 7 homolog 1} \\ 18 \text{ repeats on chromosome 7 homolog 2} \end{array} \right.$

**The advantage of microsatellite repeats:**

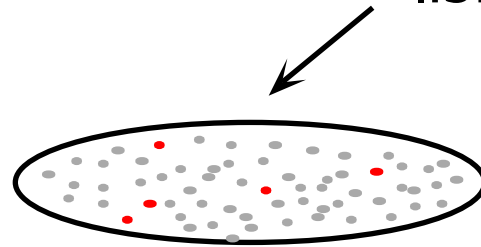
**Map construction:** Identifying repeats and their genomic locations

**Step I.** Make genomic **library** of short inserts



## Step 2. Identify repeat-containing clones

Synthesize DNA for probe:  $\longrightarrow$  probe the library  
e.g.,  $(CA)_{20}$



identify positives

$\downarrow$   
sequence the inserts

### Clone 1

...TTACACCGAACACGCCAAGAGAAACACACACA  
CACACACACACACACAATACGGTTTCGGTGGTTA  
ATTAGCT...

### Clone 2

...TAATTTAATTTTAATTGGGTTTCACACACA  
CACACACACACACACACACACACACACACA  
CACAGTTTGATTATTGCTACTTAC...

etc.

**Step 3.** Identify chromosomal locations of the repeat sequences

e.g., by hybridization to metaphase chromosomes  
(somatic cell hybrids come in handy!)

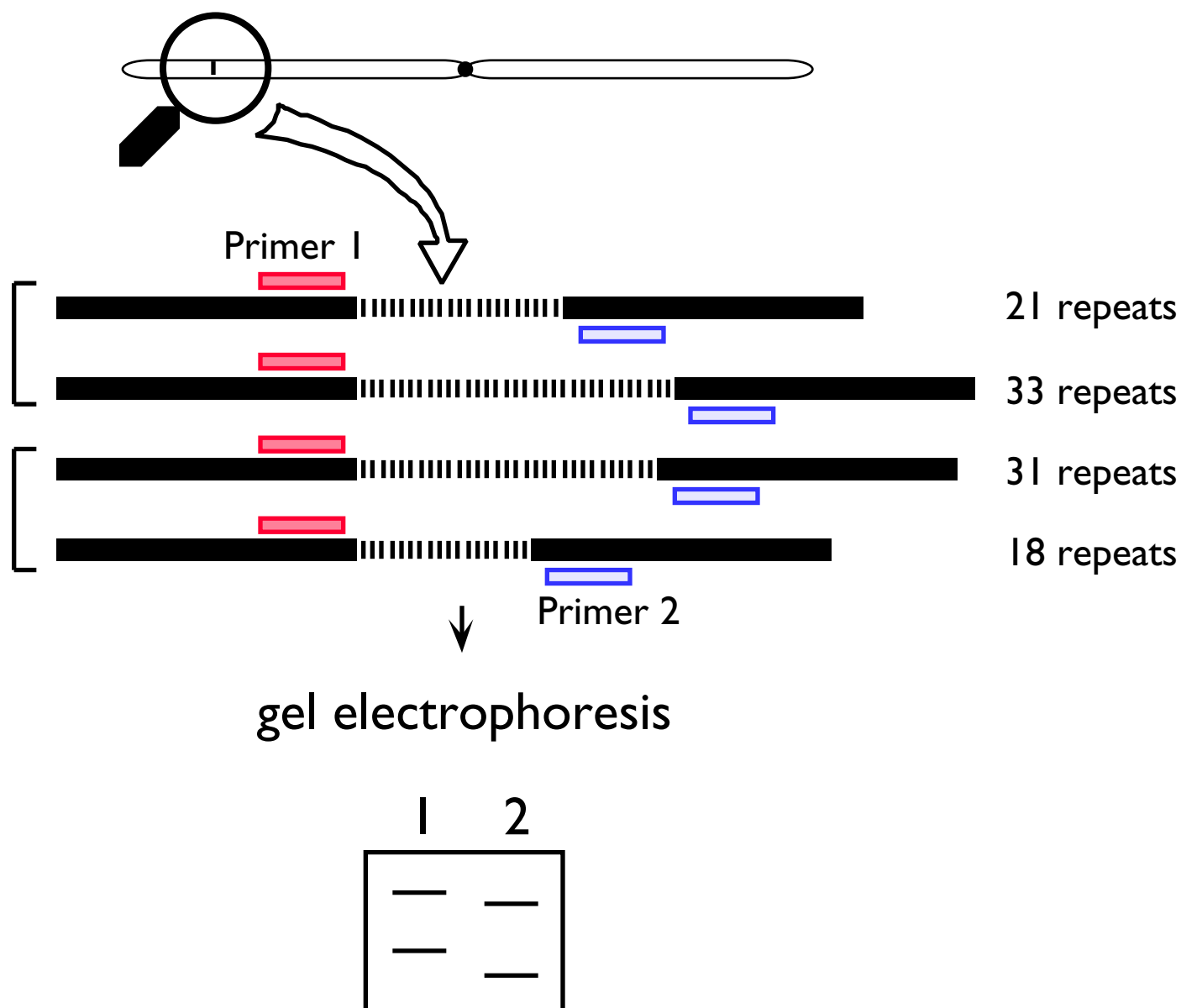
**Step 4.** Constructing a profile: How many alleles in the population? How frequent?

Usually done by **Polymerase Chain Reaction (PCR)**

# Determining repeat number at a polymorphic locus...

- ◆ PCR using unique sequence (flanking the repeat) as primers

Using our chromosome 7 example again:



# Using polymorphisms to map disease genes

- ◆ Score disease gene allele based on overt phenotype
- ◆ Score polymorphic alleles based on PCR analysis
- ◆ Ask: can recombinants be detected?

## In practice:

- ◆ Obtain DNA sample from all family members (blood ⇨ tissue culture)
- ◆ For each individual:
  - ◇ score disease phenotype, determine genotype
  - ◇ score polymorphism on each homolog (e.g., 21,33) for each of many polymorphisms
- ◆ For each polymorphism, calculate **Lod score** for various map distances

**Lod score = log of odds of linkage**

$$= \log_{10} \left[ \frac{\text{likelihood of linkage}}{\text{likelihood of not being linked}} \right]$$