DNA structure

Single strand : sugar-phosphate backbone (→ negatively charged)

4 different bases A G T C ; there is a 5' end and a 3' end.

Double helix : B-DNA : helix diameter = 18 Å

1 helix turn → 34 Å → 10 base pairs

(This is the <u>average</u> structure ; individual twist angles etc. depend on the sequence → recognition by proteins).

There is a major groove and a minor groove. In the helix, bases are towards the inside and the sugar-phosphate backbone towards the outside. Bases are exposed within the grooves : the edges of the bp form the floor of the grooves.

B-DNA : bases planes perpendicular to the axis of the helix (this is the normal, hydrated form, i.e. in vivo form)

A-DNA : bases planes are tilted ~ 15° ; (dehydrated, non-physiological conditions)

The sides of the bases exposed in the grooves contain N and O atoms which can form hydrogen bonds with proteins' side chains ;
each base pair presents a specific ~~sequence~~ pattern of hydrogen bond donor - acceptor atoms → recognition .

Major groove recognition patterns are more discriminatory than minor groove ones .

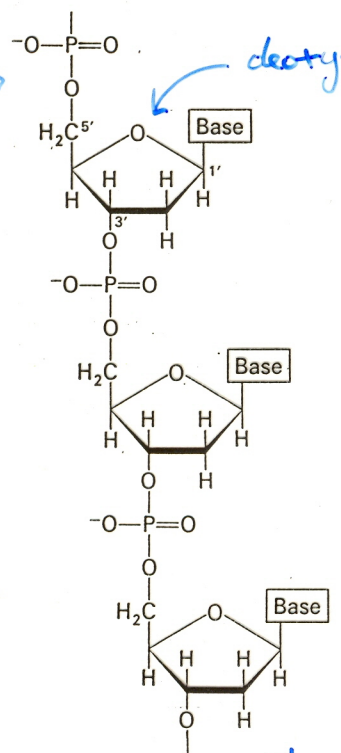
Persistence length of DNA : single stranded ~ 1 nm
double stranded ~ 50 nm

DNA structure

5' end

phosphate

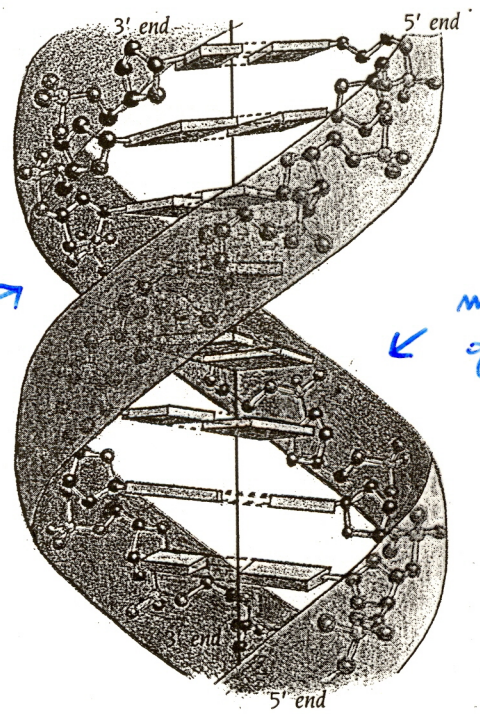
deoxyribose



3' end

Figure 23-2
Structure of part of a DNA chain.

Figure 6.1 Schematic drawing of B-DNA. Each atom of the sugar-phosphate backbones of the double helix is represented as connected circles within ribbons. The two sugar-phosphate backbones are highlighted by orange ribbons. The base pairs that are connected to the backbone are represented as blue planks. Notice that in B-DNA the central axis of this double helix goes through the middle of the base pairs and that they are perpendicular to the axis.



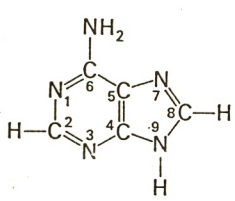
minor groove

major groove

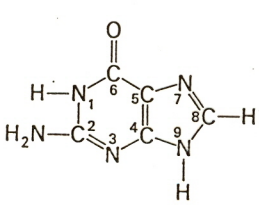
18 Å

1 helix turn → 34 Å → 10 base pairs

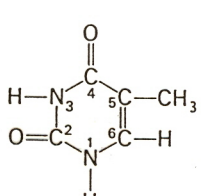
The four bases of DNA



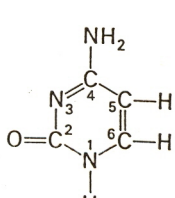
Adenine (A)



Guanine (G)

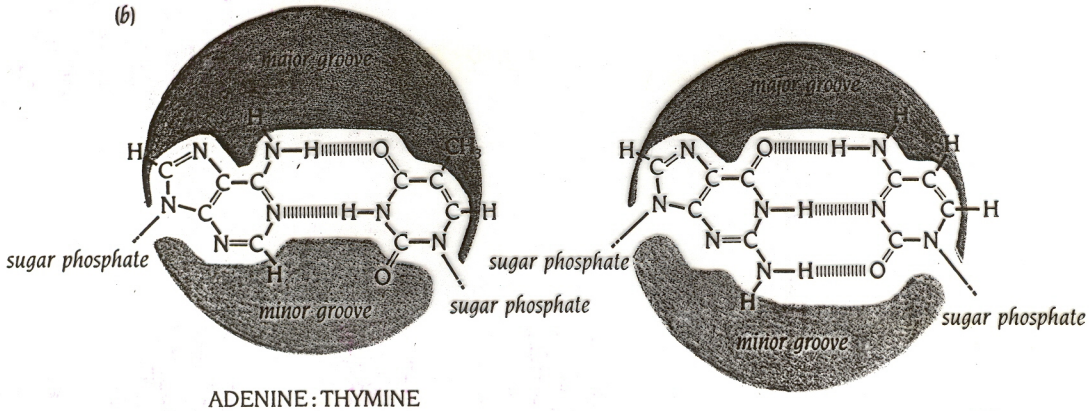


Thymine (T)



Cytosine (C)

Hydrogen bonding between the bases



2 hydrogen bonds

three hyd. bonds

B-DNA

minor groove

major groove

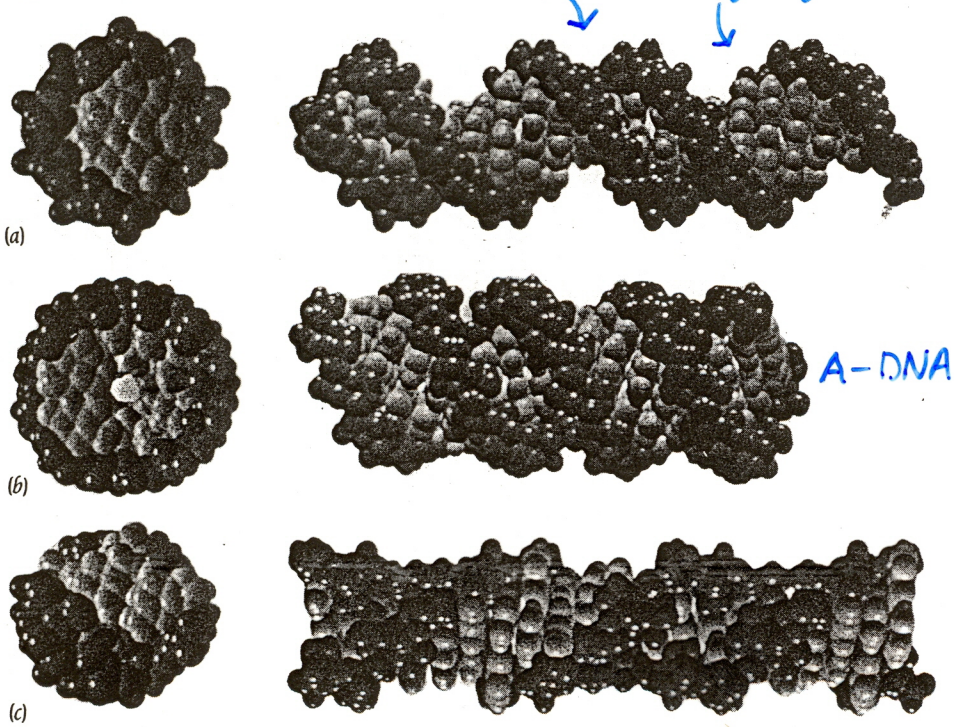
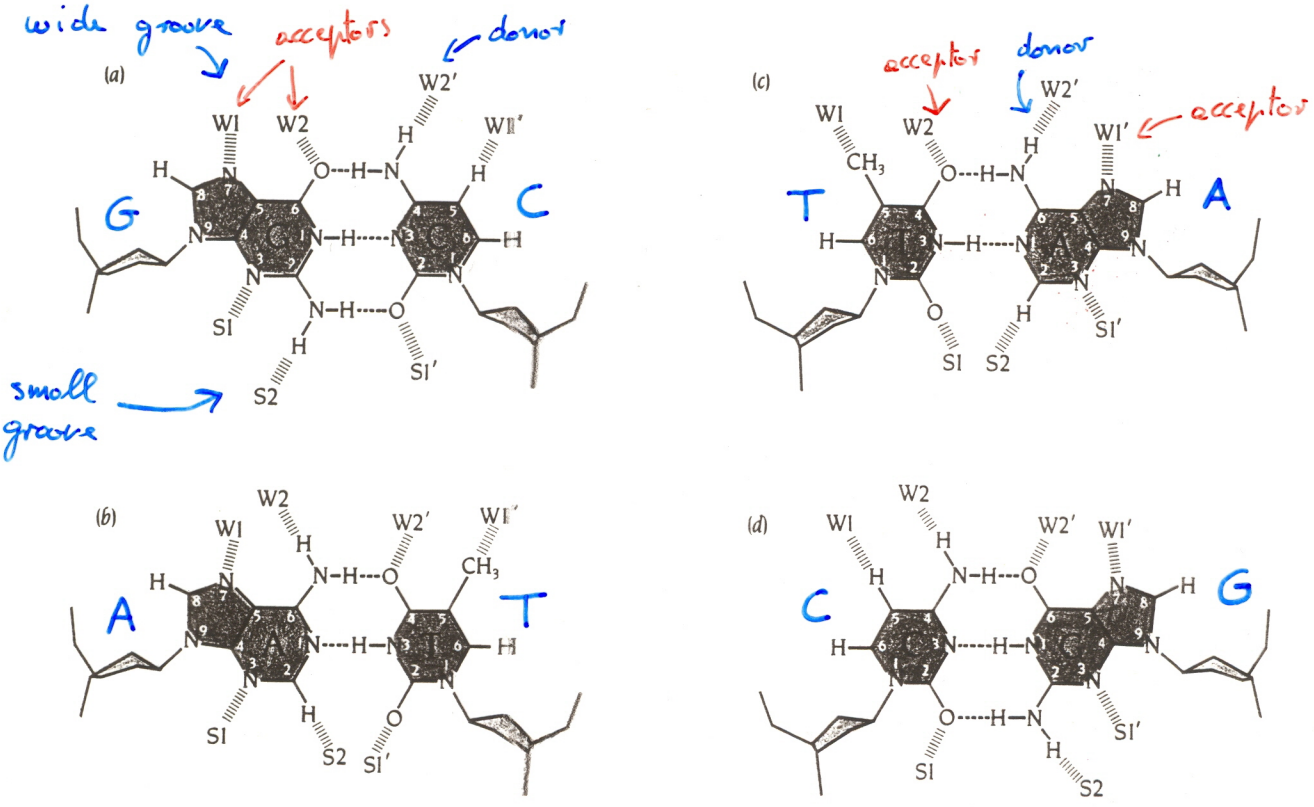


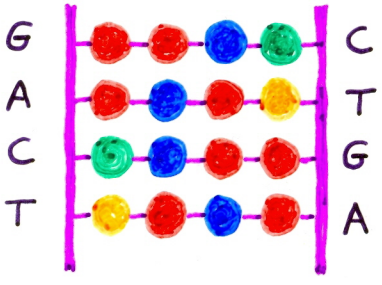
Figure 6.2 Three helical forms of DNA, each containing 22 nucleotide pairs, shown in both side and top views. The sugar-phosphate backbone is dark; the paired nucleotide bases are light. (a) B-DNA, which is the most common form in cells. (b) A-DNA, which is obtained under dehydrated nonphysiological conditions. Notice the hole along the helical axis in this form. (c) Z-DNA, which can form for selected DNA sequences under special circumstances. (Courtesy of Richard Feldmann.)

How base pairs can be recognized by DNA binding proteins

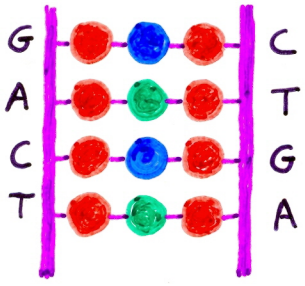


Recognition patterns :

major groove

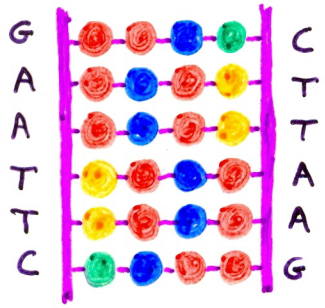


minor groove



- H-bond acceptor
- H-bond donor
- H atom
- methyl group (CH_3)

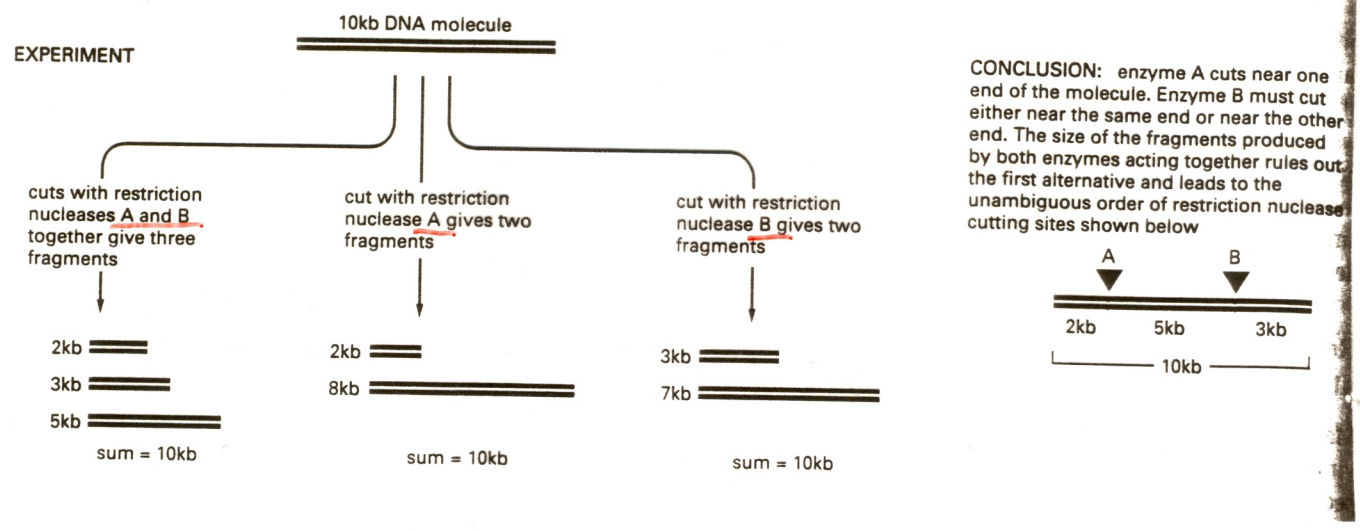
→ major groove patterns are more discriminatory



Recognition site for EcoRI (restriction enzyme)

Restriction mapping :

determining the relative position of cutting sites along the DNA molecule



Restriction map :

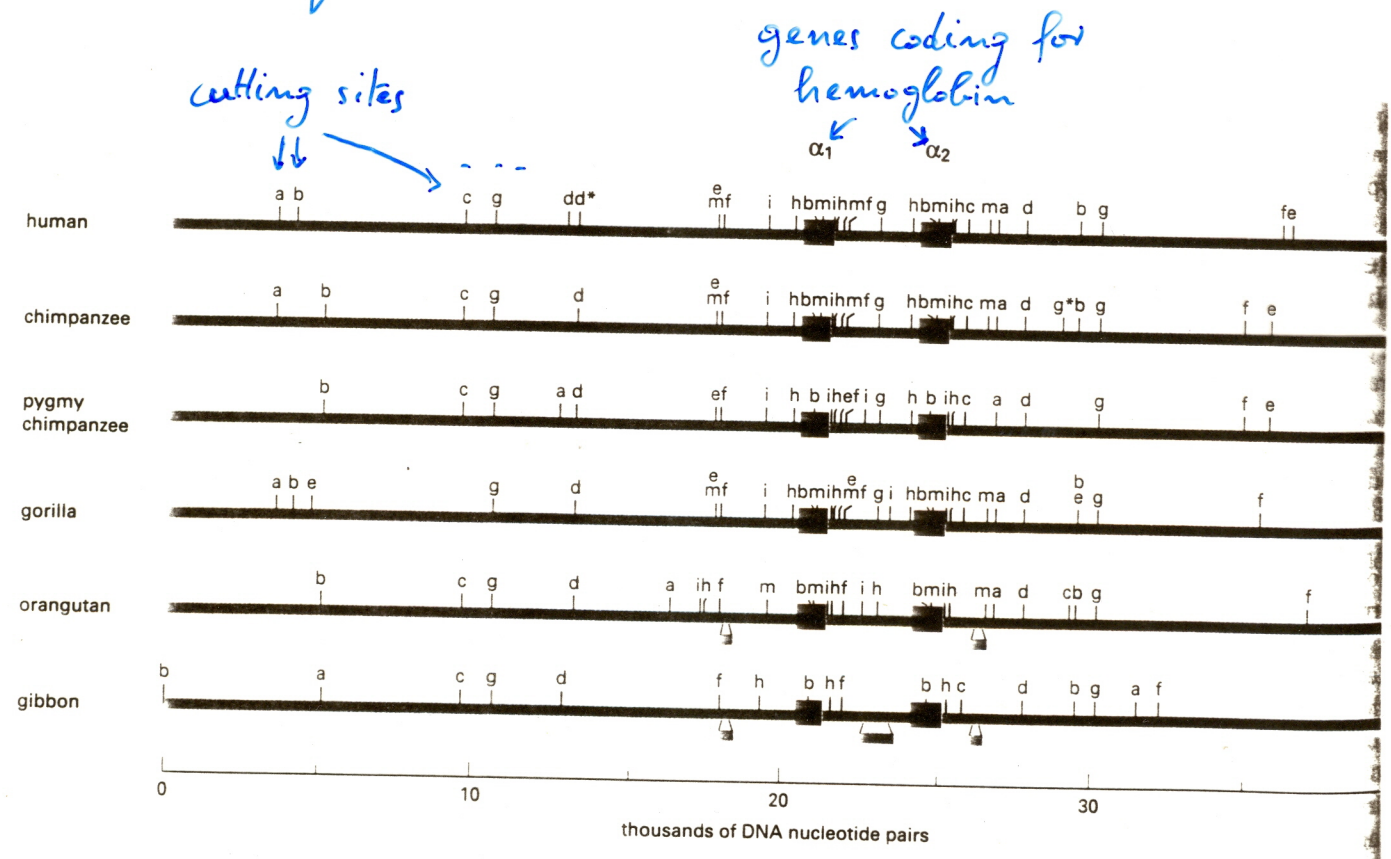
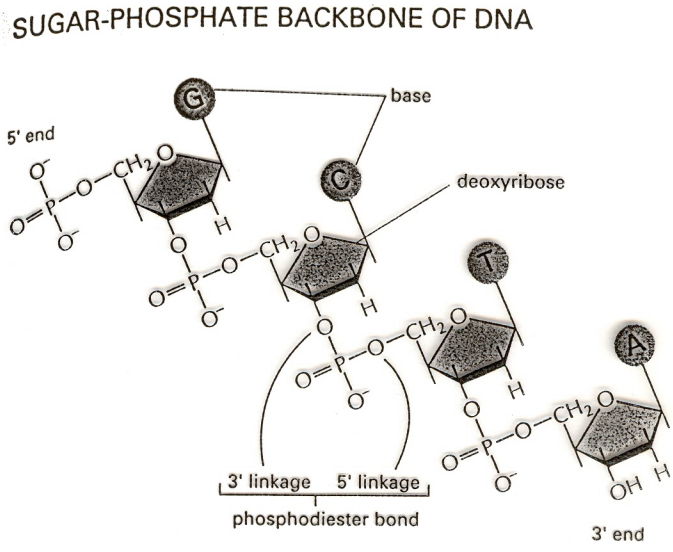
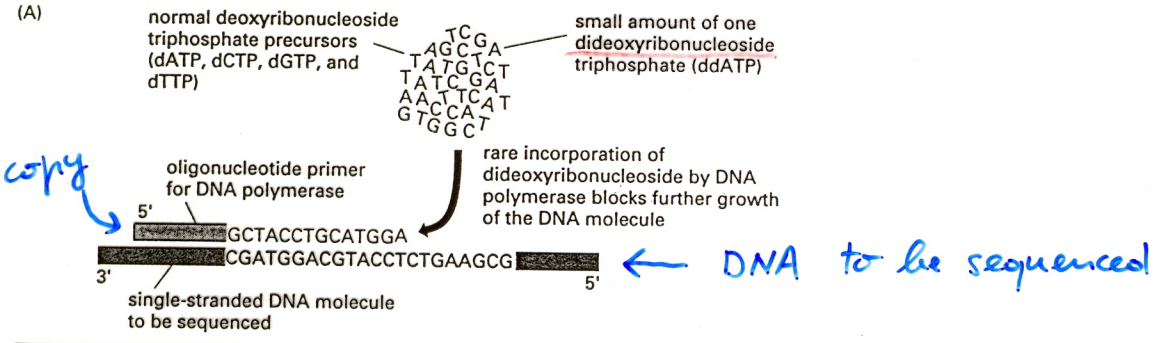
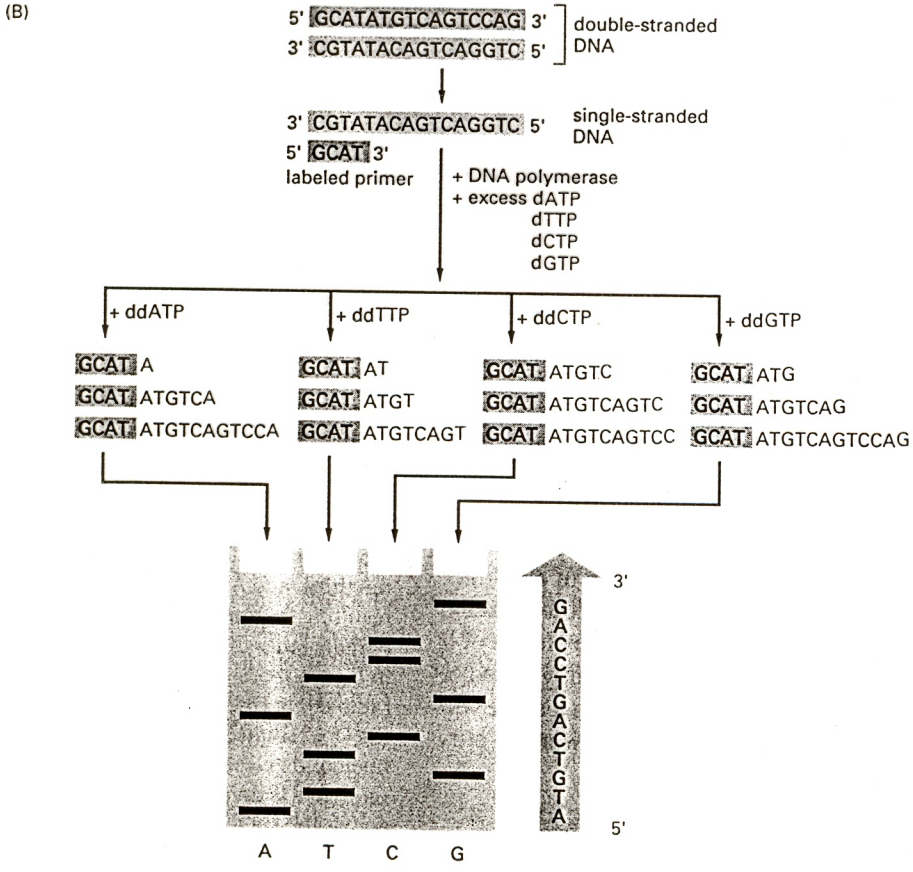


Figure 7–4 Restriction maps of human and various primate DNAs in a cluster of genes coding for hemoglobin. The two *red squares* in each map indicate the positions of the DNA corresponding to the two α-globin genes. Each letter stands for a site cut by a different restriction nuclease. As in Figure 7–3, the location of each cut was determined by comparing the sizes of the DNA fragments generated by treating the DNAs with the various restriction nucleases, individually and in combinations. Note that the chimpanzee, which is most closely related to humans, has the most similar restriction map, whereas the gibbon is more distantly related and has the most diverged map—including three DNA insertions. (Courtesy of Elizabeth Zimmer and Allan Wilson.)

DNA sequencing



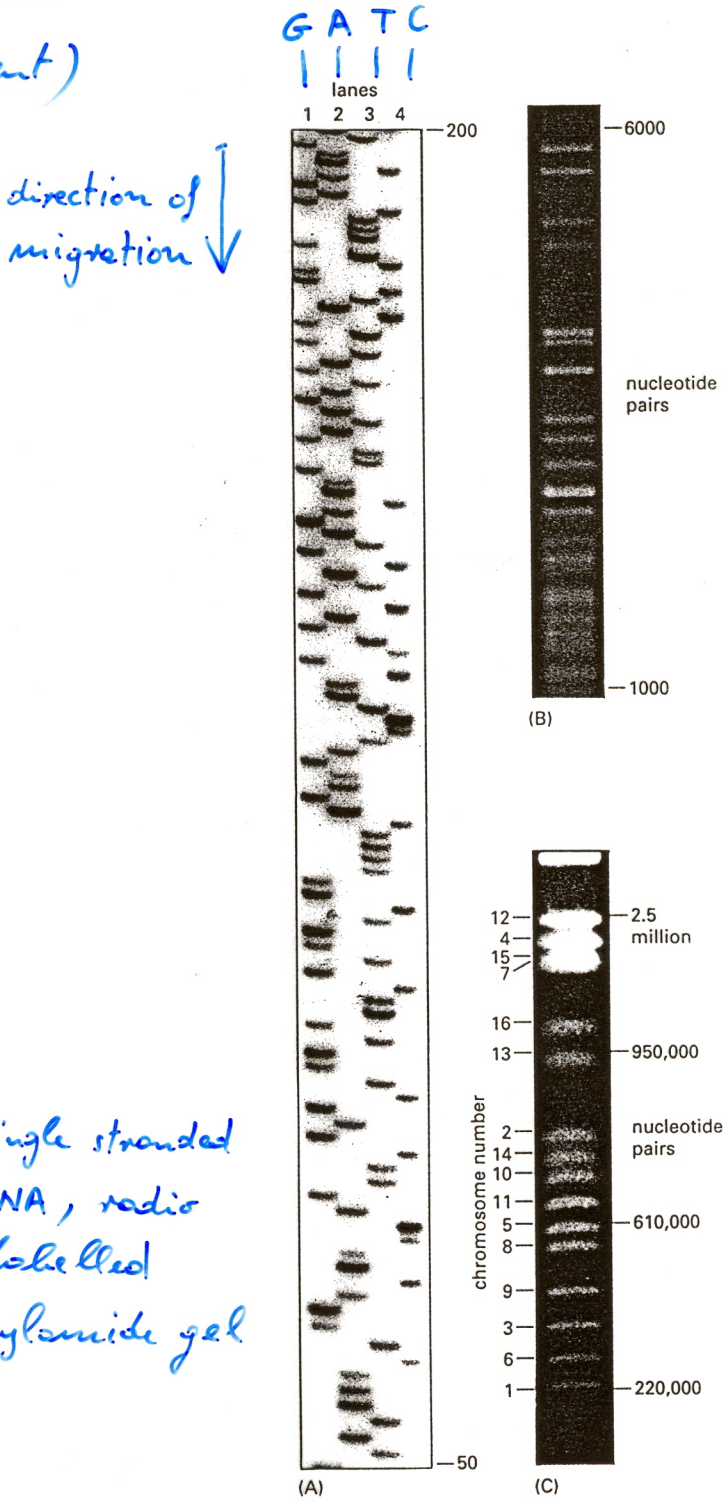




DNA gel electrophoresis

To label DNA :

- make a copy (DNA polymerase) in the presence of radioactive (labelled) nucleotides (^{32}P label)
- stain with Ethidium Bromide (fluorescent)



DNA (double stranded) labelled with Ethidium Bromide

pulsed field separation (DNA + EB)

single stranded DNA, radio labelled on polyacrylamide gel