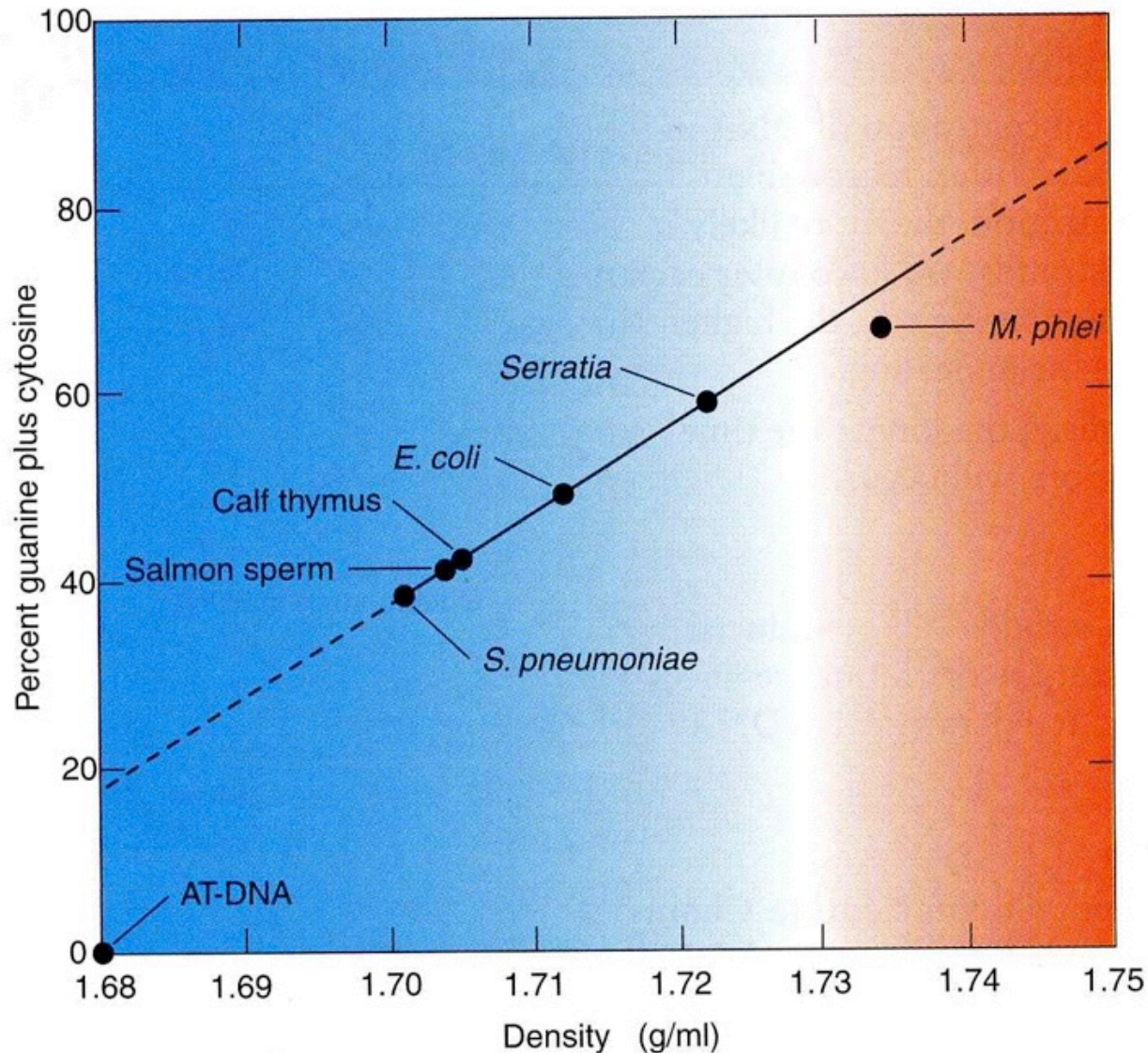


# Effects of variation in base composition

# G+C

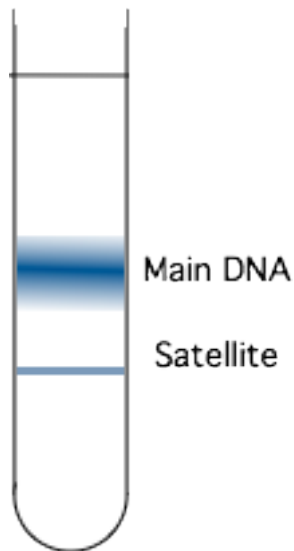
- Since any order of bases is possible along one of the two strands, it is possible to have different percentages of GC pairs and AT pairs in DNA from various species. The highest GC values are close to 80%, while the lowest are about 20%. These variations lead to changes in the physical biochemistry of the respective DNA molecules.

# Change in density



- The density of DNA rises almost linearly with increasing GC content

# Satellite DNA

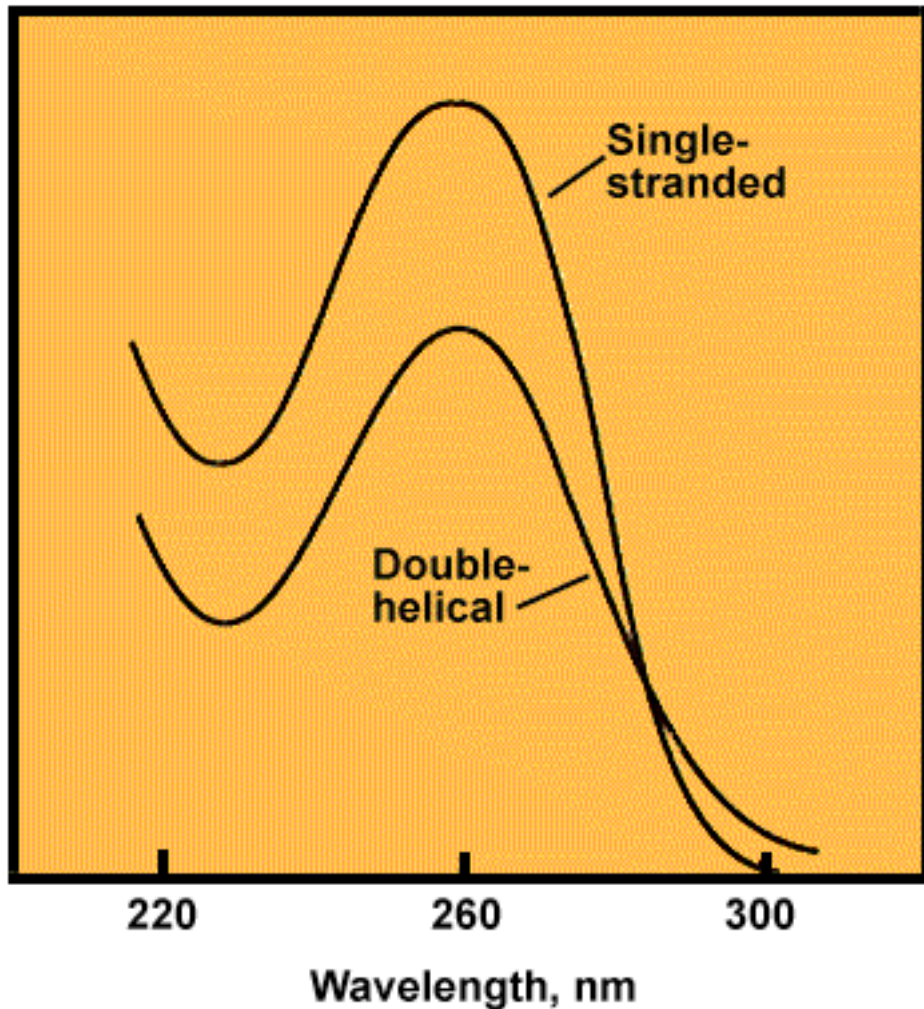


If the total DNA from many organisms is spun in a density gradient there will often be one or two outlying bands which are significantly higher or lower density than the rest. These are repeated sequences which are particularly GC or AT rich. These are termed 'satellite DNA'.

# DNA strands can separate

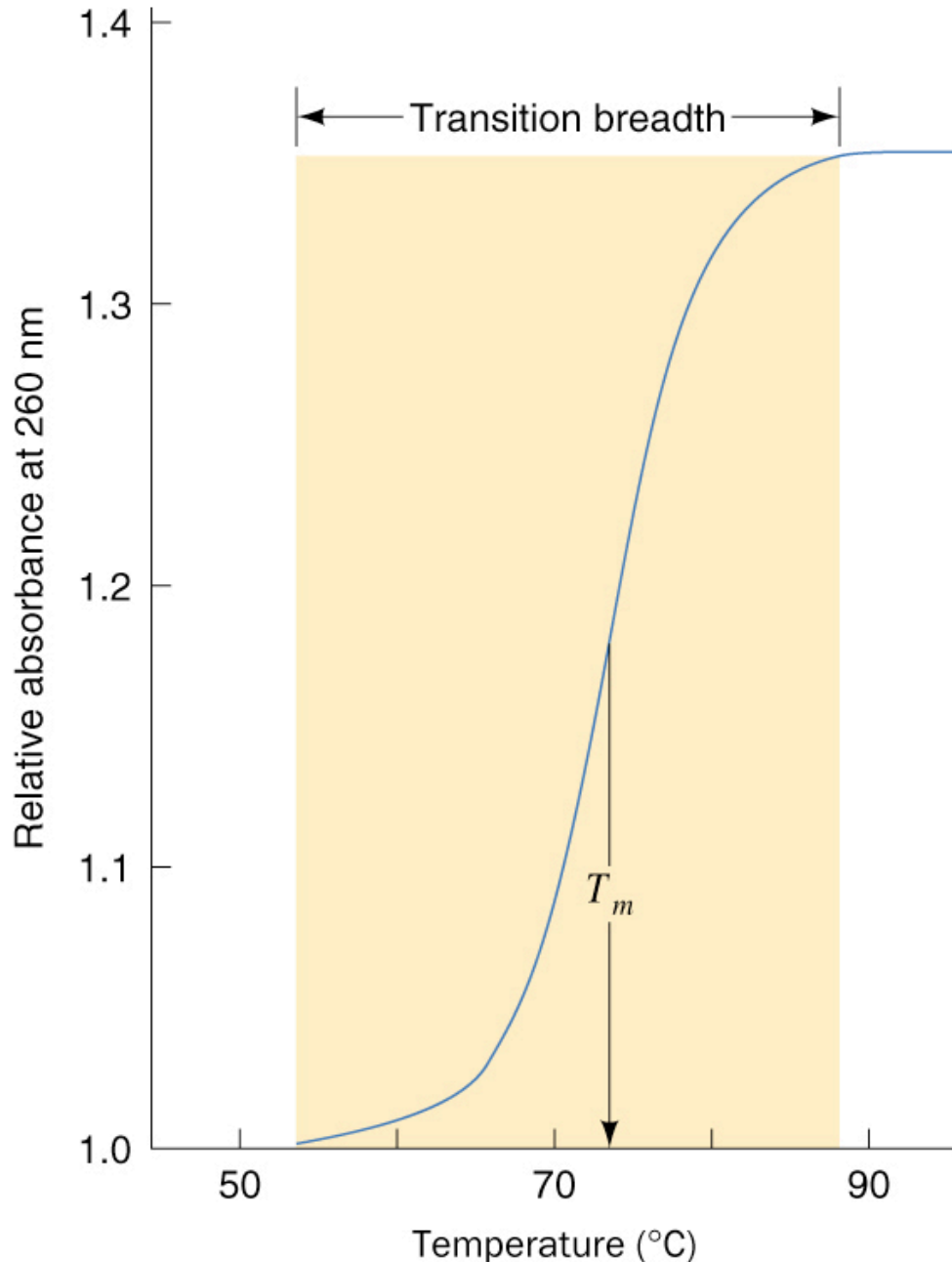
- Because the two strands are held together by hydrogen bonds, it is possible to overcome the attraction by several methods, low ionic strength, high pH, reagents such as formamide and heating. The simplest is heating.
- As the temperature goes up, so does the speed of the molecules and, at some point the random movement is so violent that the two strands are separated

# Hyperchromic shift



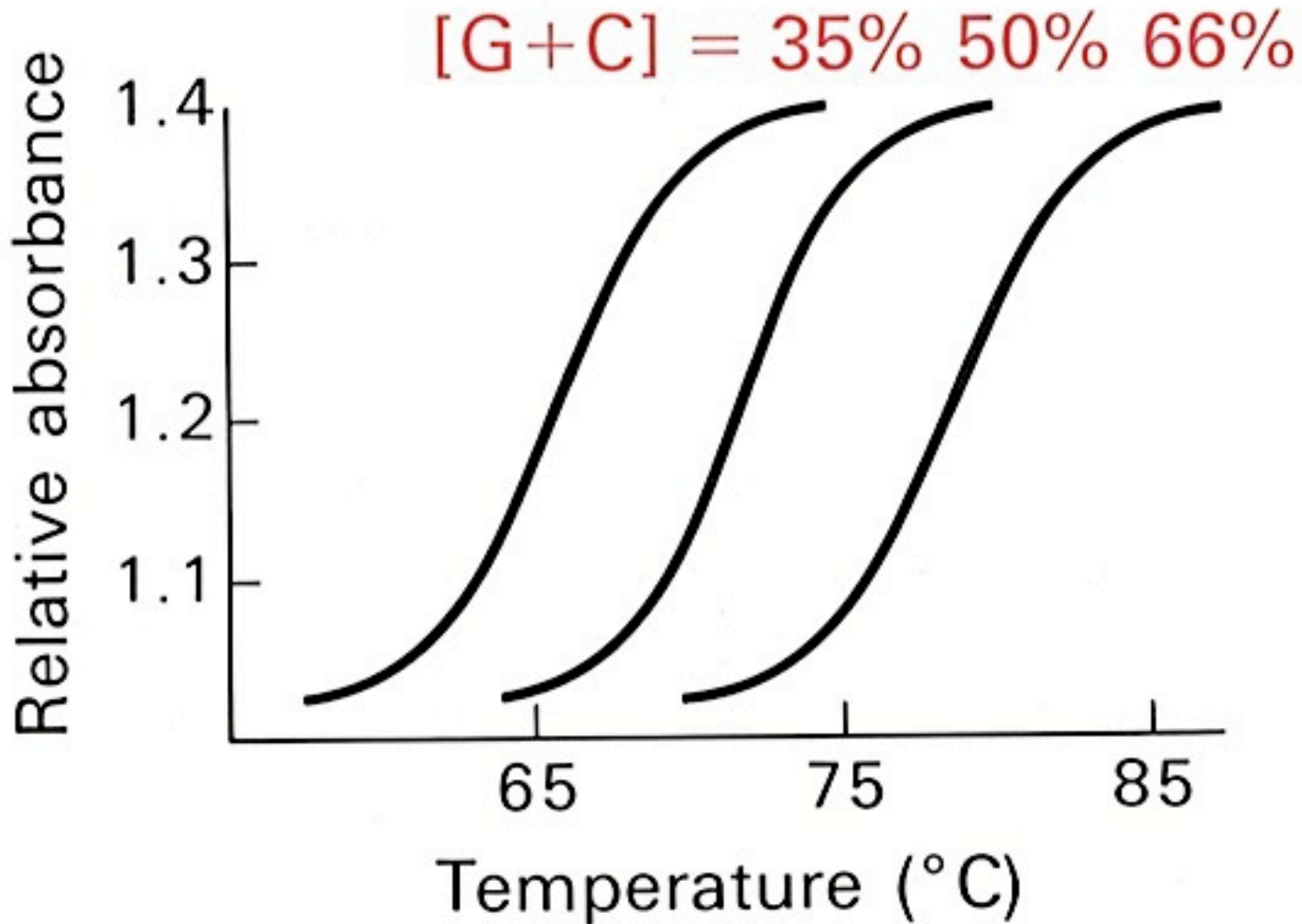
- Normally DNA absorbs UV light in a very characteristic fashion, with a peak absorbance at 260nm. Note that single-stranded DNA absorbs more strongly than double-stranded. This is termed the hyperchromic shift.

# Effect of heating

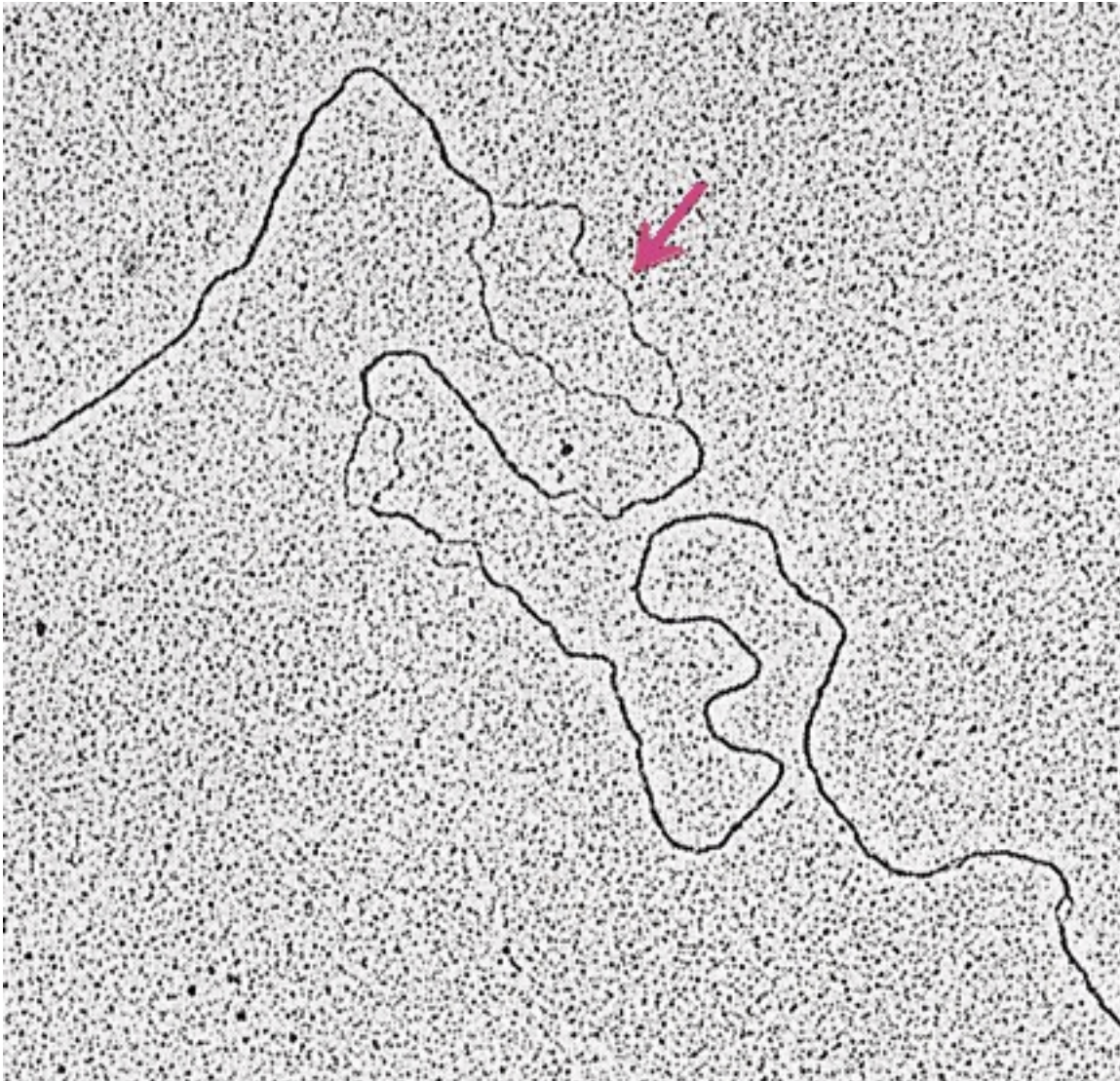


As the temperature rises, and the molecules separate into single strands, so the absorbance goes up. The temperature at which the halfway point is reached is termed  $T_m$

# Effect of %GC on Tm



# Mapping DNA using $T_m$



If a DNA molecule is heated, and then studied by EM, the AT-rich regions can be identified since they are the first to melt.

# Renaturation

The separation of the two strands when heated is not permanent. If the solution is cooled, strands will tend to reform double-stranded molecules. This depends on strands with complementary sequences coming close enough for hydrogen bonds to form.

Once any part of two strands form H bonds, the remaining base pairs form very quickly (zip up). This assumes that the initial match is correct.

AATTCAGTCAATTACGATC

TTAA

GTCACTTAATGCTAG will zip correctly

AATTCAGTCAATTACGATC

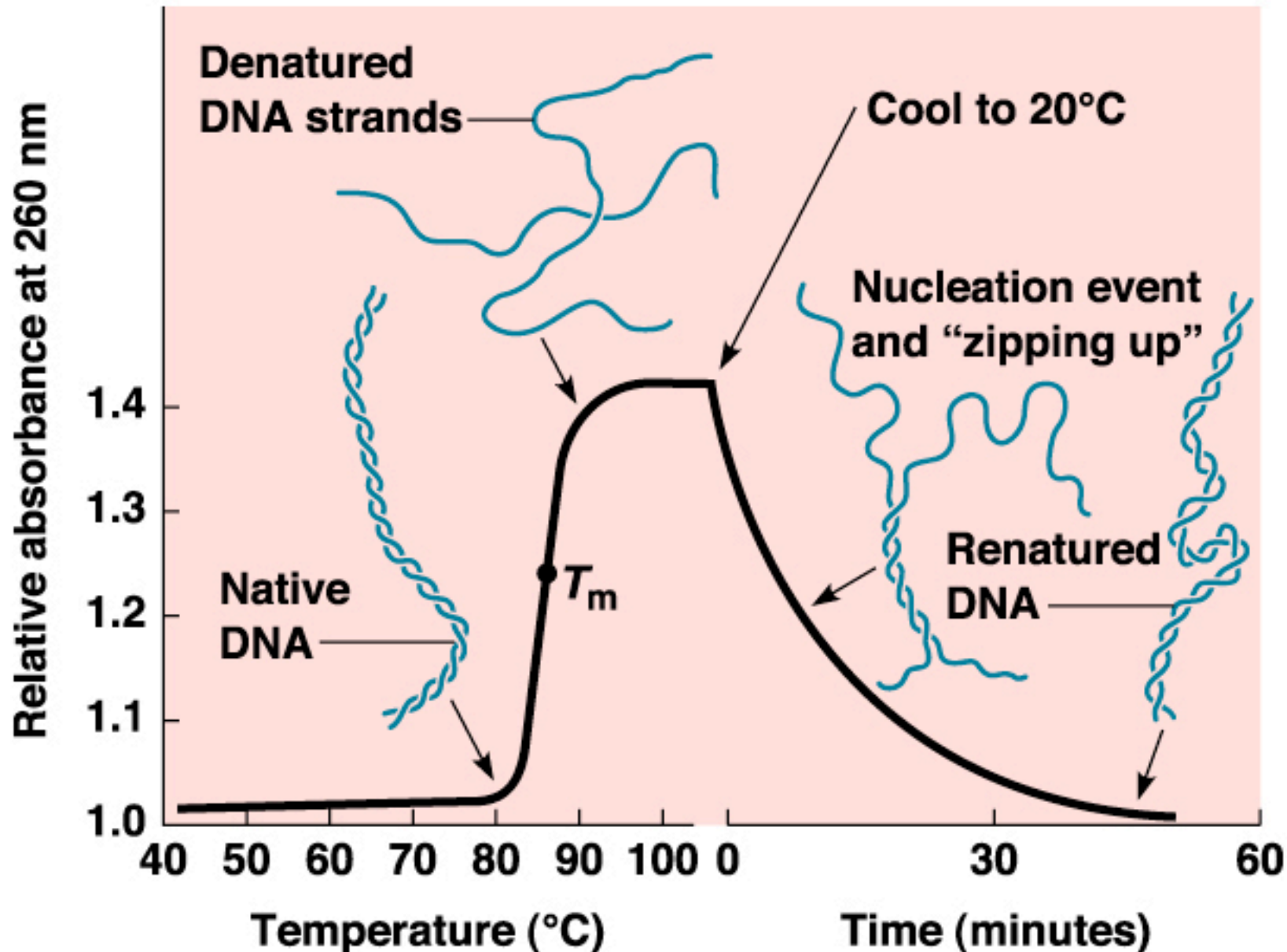
TTAA

GTCACTTAATGCTAG won't

# Partial renaturation

Since there are many places along a strand where the other strand might find a partial match, many of the double strands that form are not a perfect match. As a result, if you follow the loss of hyperchromicity as the solution cools, it will never come back to its starting point. The difference is a reflection of how many single-stranded regions are left.

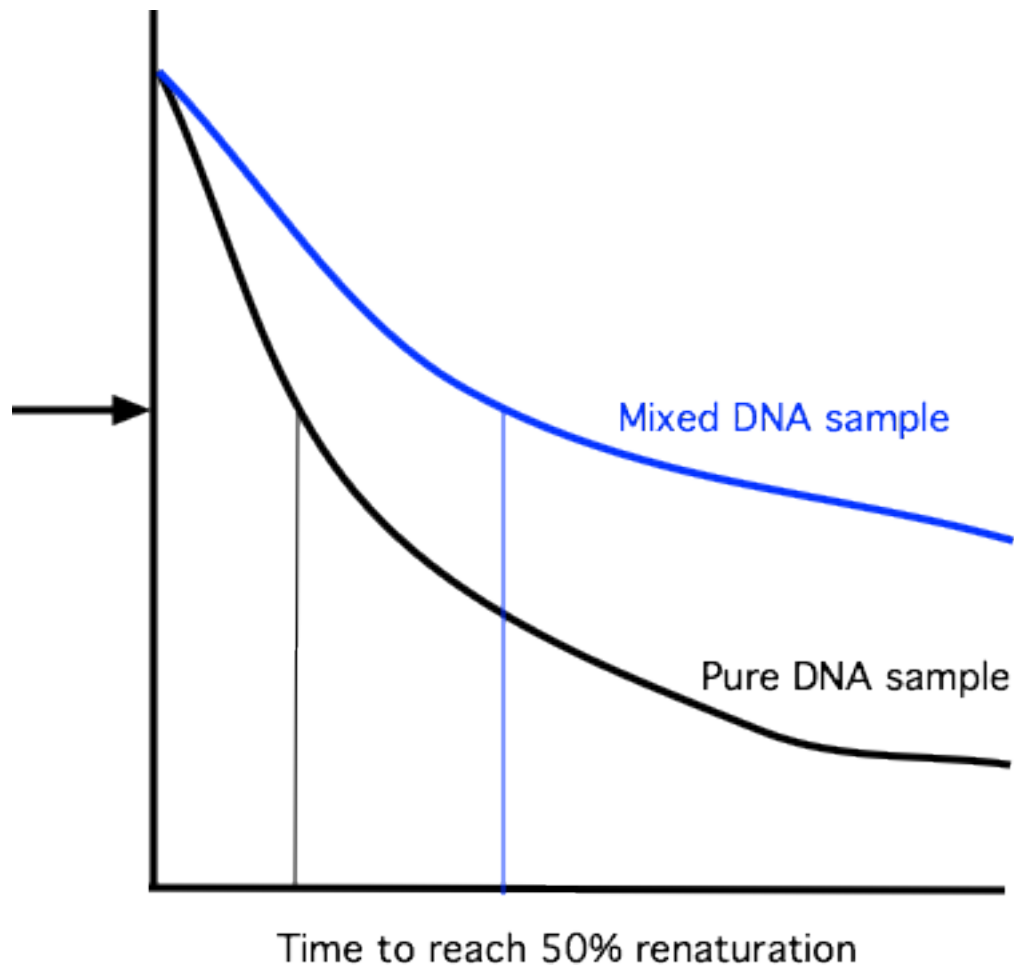
# Renaturation kinetics



# Testing for similarity

If two **different** DNAs are melted together, and then allowed to renature, **hybrid** molecules may form. Here, one of the strands pairs up with a strand from the other DNA molecule, rather than with its original partner. The chances of this happening mainly depend on how similar the two DNAs are in terms of sequence. If the second DNA was in fact the same as the first (e.g. two samples from the same organism) then the renaturation would look exactly the same as when either was allowed to renature on its own. In addition, the **rate** of renaturation will be the same in both cases. This will not be true if the two DNAs came from different sources.

# Renaturation kinetics in hybrids



There are two factors at work here. First, because half of the collisions are between dissimilar molecules, the rate of renaturation is slower. Second, because some of the molecules that form are hybrid, there will be more single-stranded regions in the resultant molecules. The difference in time to reach 50% renaturation is a measure of how dissimilar the two samples are.

# Cot<sub>1/2</sub>

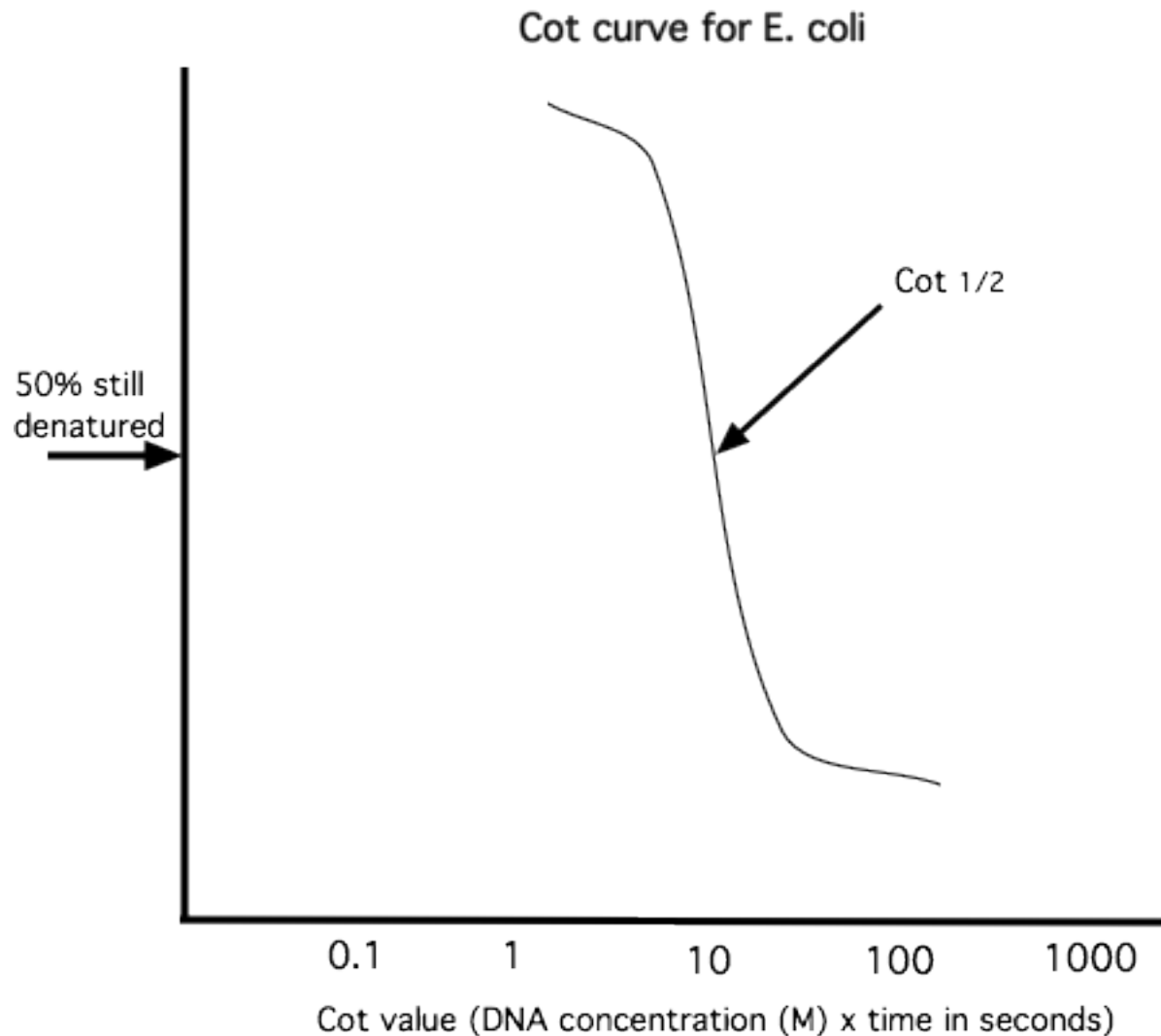
In the case of a single type of DNA being allowed to renature, the rate depends on three variables: the temperature at which the renaturation is being conducted, the ionic composition of the medium, and the concentration of DNA molecules. The first two are usually fixed.

The concentration of DNA will affect the number of molecules per unit volume, and therefore the number of collisions. In general the time needed for half the molecules to rejoin is inversely proportional to concentration. Thus, for any DNA sample, there is a unique number termed Cot<sub>1/2</sub> (Concentration of DNA x time to reach 50% renaturation)

What this says is that if you double the DNA concentration, it will reanneal in half the time and *vice versa*

# *E. coli* $Cot_{1/2}$ curve

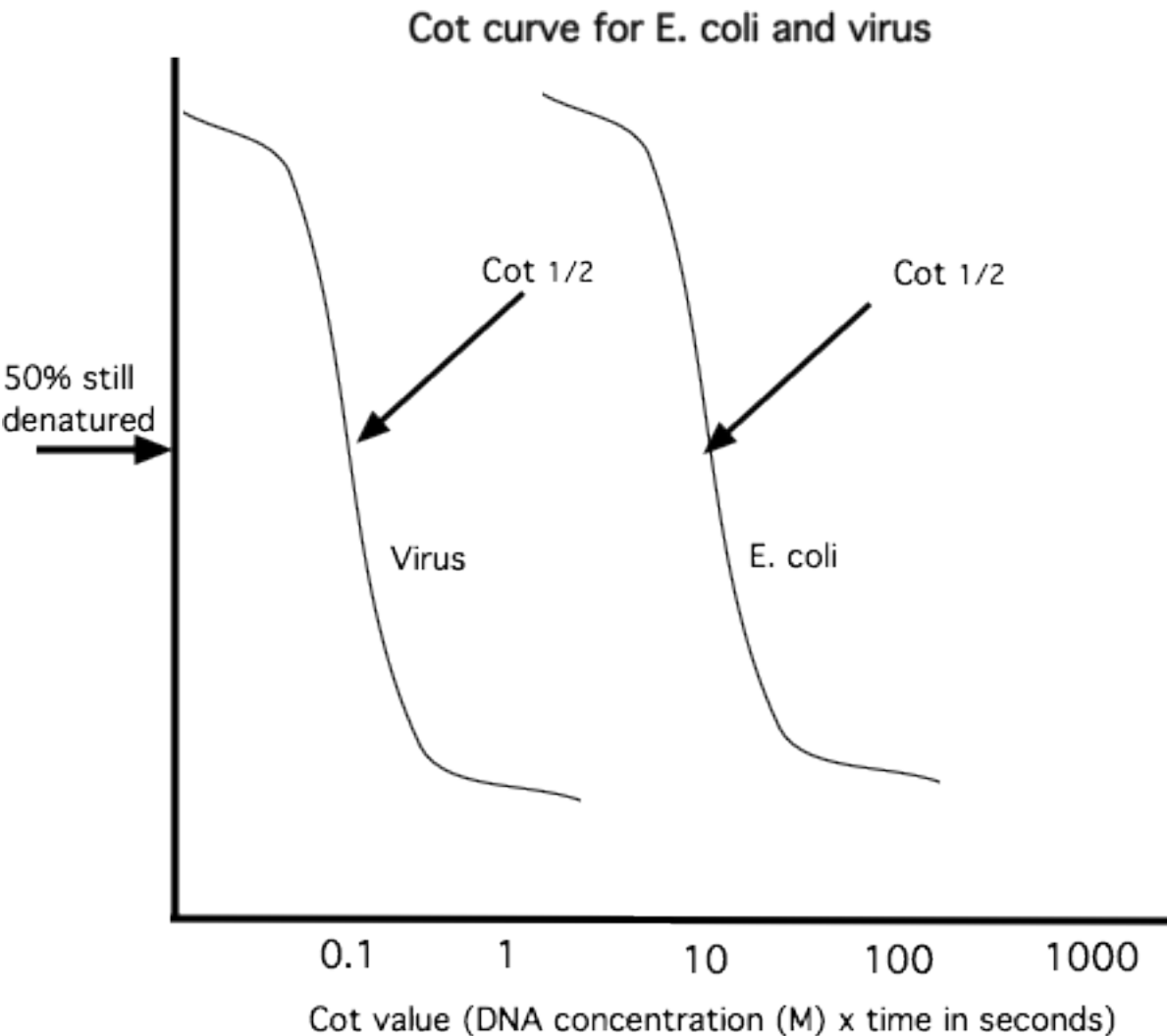
For *E. coli*, which contains a single piece of DNA about 4 million base-pairs long, the  $Cot_{1/2}$  value is approximately 10.



What will happen if the size of the DNA molecule is much smaller, such as a viral DNA of just 40,000 base pairs?

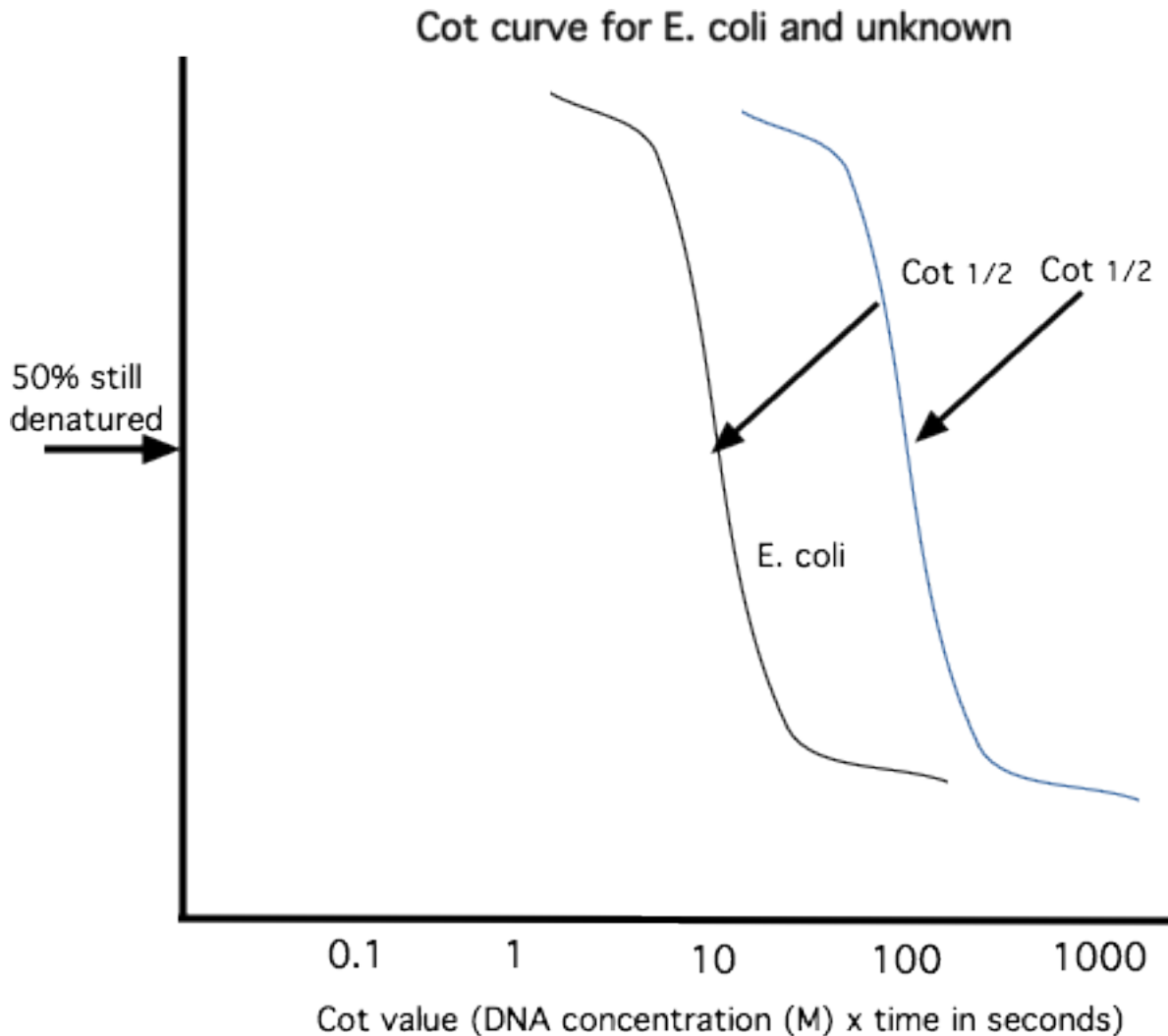
For the same concentration of DNA, there will be more copies

# Other curves



With a genome 100x smaller, there are 100x more copies/ml for the same concentration of DNA. Therefore they will find each other and reanneal at a lower Cot value: in fact 100 times lower. In general  $Cot_{1/2}$  is directly proportional to genome size, If you compare the value to that of an organism where you know the genome size, you can estimate the unknown quite accurately

# Estimating genome size

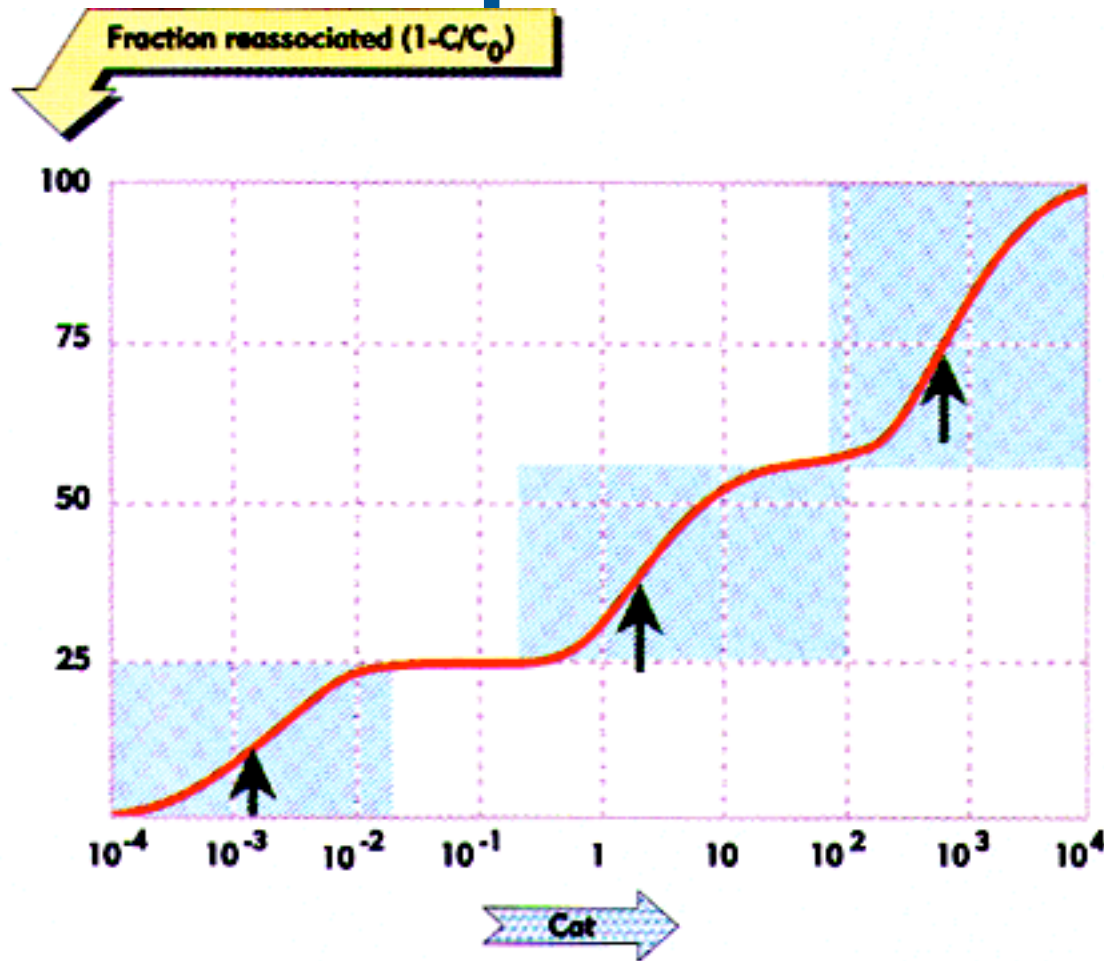


Since this organism has a Cot value ten times that of *E. coli*, we can estimate the size of its genome to be about 40 million base pairs

# Copy number

Generally the amount of DNA in a cell matches the size of the DNA molecule as estimated by its Cot value. This means that there is a single copy of the DNA in the cell. In some cases the two do not match. For example, the size of the chloroplast genome, as determined by its Cot value, is only 1/50th of the total amount of cpDNA in a cell. What this tells us is that there are 50 copies of the cpDNA in each cell. The same is true for mitochondrial DNA, and some other cases. A high copy number is a reflection of the presence of a large number of small molecules.

# Complex Cot curves



When Cot curves are generated for eukaryotes, a more complex result is seen. There are several rounds of reannealing. Some of these occur very early (fast), suggesting a small genome, others are intermediate or slow. The fast component consists of many short repeated sequences, the intermediate of a moderate number of fairly large pieces, and the slow of a single (unique) copy of a very large sequence.

	Fast Component	Intermediate Component	Slow Component
Percent of genome	25	30	45
Cot $_{1/2}$	0.0013	1.9	630
Complexity, bp	340	$6.0 \times 10^5$	$3.0 \times 10^8$
Repetition frequency	500,000	350	1

# Function of repeated sequences

- If these repeated sequences make up so much of the genome, what do they do?
- They are too small to code for proteins, and most do not have an open reading frame.
- Possibilities:
  - Junk, left over transposon sites
  - sRNA genes
  - other, so far undiscovered function

# Uses of renaturation and hybridization

- Because similar sequences will bind to each other, this can be used as a way of identifying the presence of a particular sequence using a 'probe'
- Probes may be obtained from existing DNA or RNA sequences, or can be synthesized to order. Generally a relatively short sequence is enough to provide a unique match. For example, a sequence of 10 bases (ten-mer) is only likely to show up once in a million base-pair sequence

# Methods using hybridization

- Liquid- already discussed
- *In situ*
- Filters
- Membranes

# *In situ* hybridization (ISH)

- Mary-Lou Pardue developed the basic methods in the 60's. Cells or chromosomes are spread onto a slide and heated, to denature the DNA. The slide is then incubated with a labeled probe that will hybridize to a complementary sequence.
- Most of the early work used radioactive labels that could be identified with autoradiography

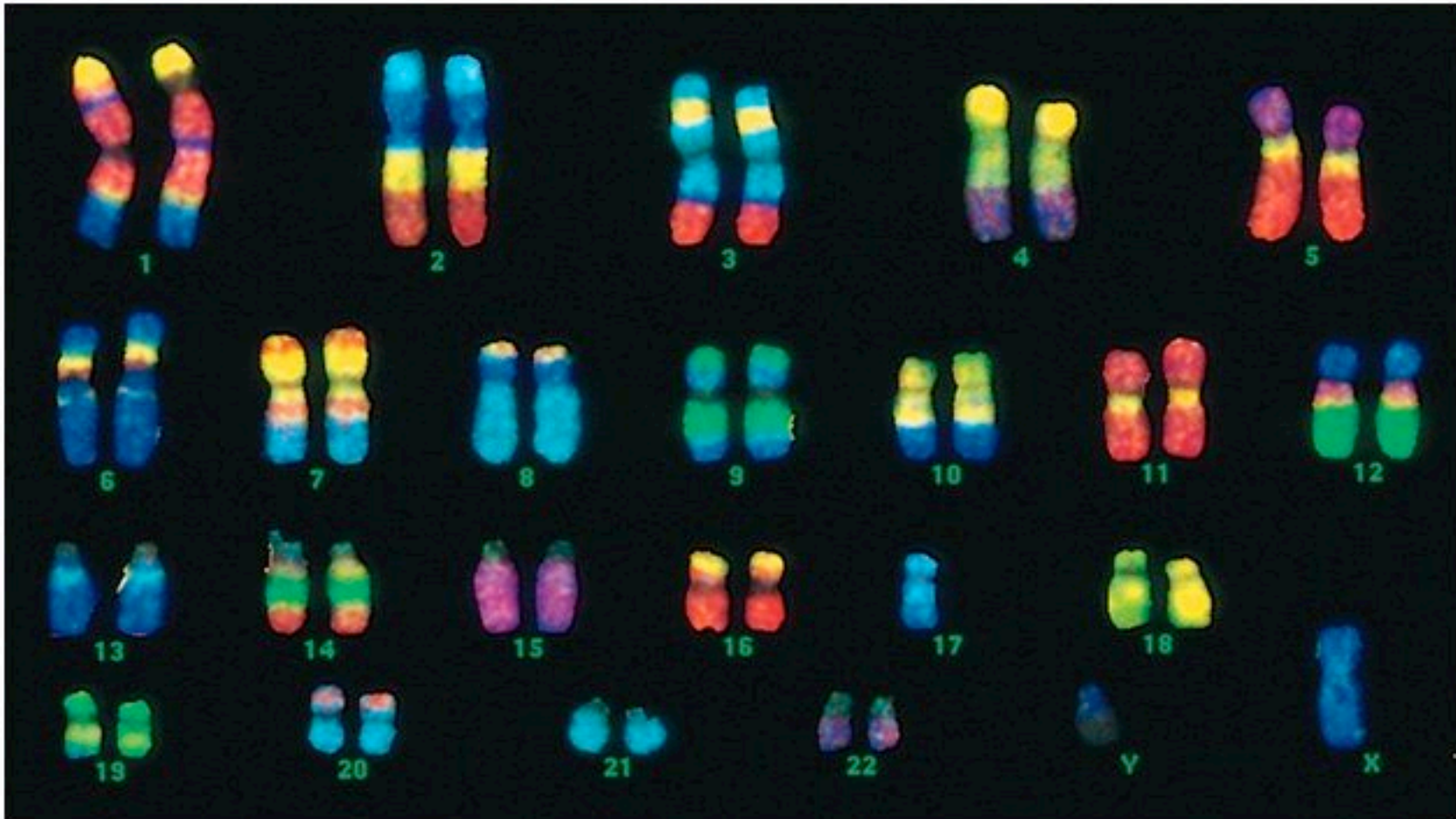


Telomere labeling



# FISH

- Most of the work now is done using fluorescent labeling of the probe



# Filter binding

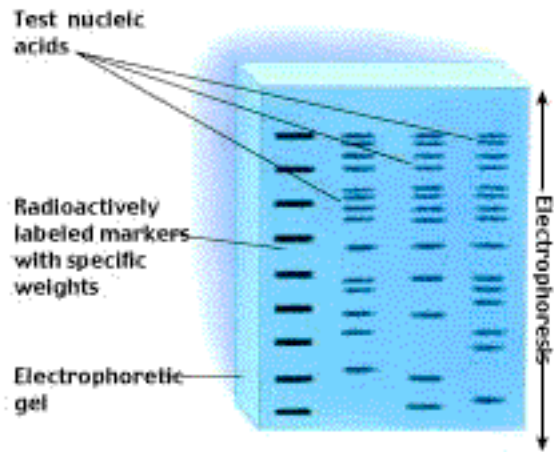
- To estimate the degree of hybridization, filter-binding is a convenient approach. The denatured target DNA is bound to a filter and then incubated with an excess of labeled probe. The more targets there are, the greater the amount of probe that will stay on the filter after washing.
- By making conditions harder for hybridization (stringent), it is possible to detect only very close fits and vv.

# Membrane binding

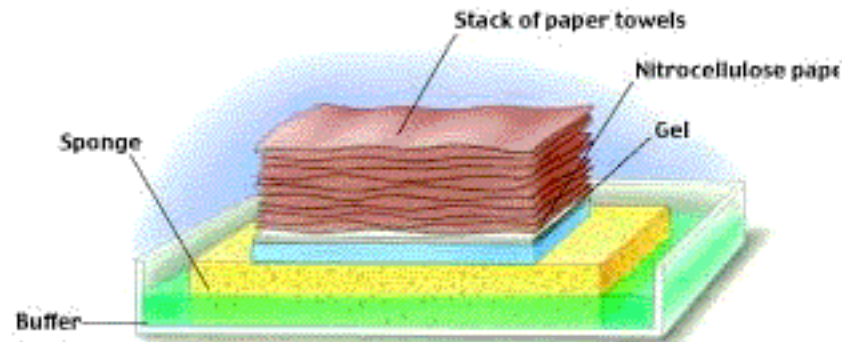
- DNA will stick very strongly to some materials, such as nitrocellulose. Once bound, it is virtually impossible to get it off.
- Membrane binding works in a fashion similar to filter binding, but you can arrange for different DNA molecules to be kept separate. The simplest way to do this is to transfer (blot) the DNA from a gel.

# Southern blot

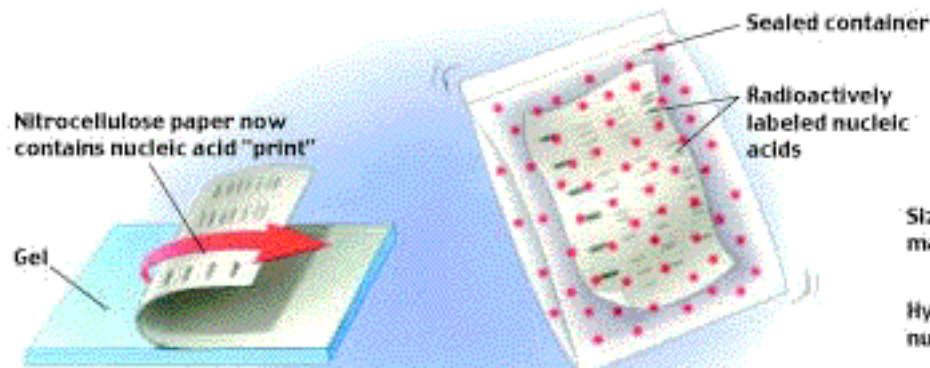
- The first, and commonest, use of blotting is the Southern blot. Here DNA fragments from a gel are transferred and then probed with labeled DNA fragments. If there is a match they will stick to the appropriate band(s)
- A Northern blot probes with labeled RNA rather than DNA



1. Electrophoresis is performed, using radioactively labeled markers as a size guide in the first lane.

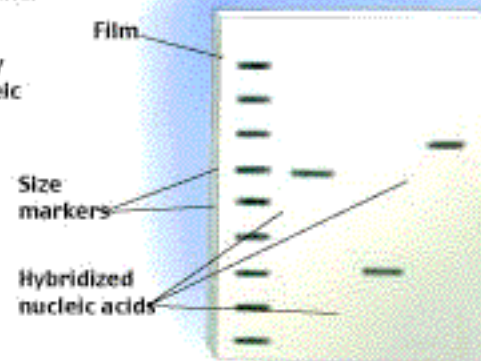


2. The gel is covered with a sheet of nitrocellulose and placed in a tray of buffer on top of a sponge. Alkaline chemicals in the buffer denature the DNA into single strands. The buffer wicks its way up through the gel and nitrocellulose into a stack of paper towels placed on top of the nitrocellulose.



3. Pattern on gel is copied faithfully, or "blotted", onto the nitrocellulose.

4. Blotted nitrocellulose is incubated with radioactively labeled nucleic acids, and then rinsed.



5. Photographic film is laid over the paper and is exposed only in areas that contain radioactivity (autoradiography). Nitrocellulose is examined for radioactive bands, indicating hybridization of the original nucleic acids to the radioactively labeled ones.