Today's lecture

- ΔE and ΔG: $\Delta G = \Delta E - T\Delta S$ (should have given this to you previously)
- DNA, RNA & Proteins: ΔG and stability of molecules
- It takes many proteins to open up DNA
- Energy Source: ATP
- Practical Applications of DNA: Forensics, Clinical, Caveman's DNA

Homework

Due by:
beginning of class on Monday, Feb 6th

1. Read Chpt 5 of Campbell
2. (A lot of reading, but…_

2. Do web-site homework on reading, Chpt 5
(will probably take over an hour)

Homework Set #2

(fair length)

On web-site under HW 2.
(PDF is there.)

Boltzmann factor & Degeneracy

E_1 Temp, T

E_0

The Boltzmann factor, $e^{-E_i/kT}$

The probability of finding a molecule with energy E_i is equal to

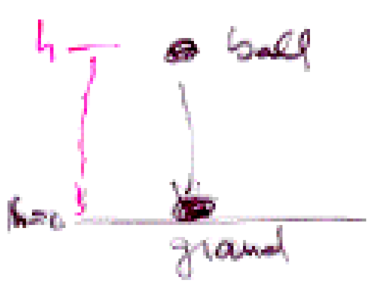
$$P(E_i) = \frac{1}{Z} \cdot e^{-E_i / k_B T}$$

where the constant Z is called the partition function to make sure the sum of all probabilities equals 1.

$$Z = \sum_i e^{-E_i / k_B T}$$

Simple case: Ball in gravitational field; DNA oligos....

Thermal fluctuations, finite probability of being at height, h.



$E = mgh$

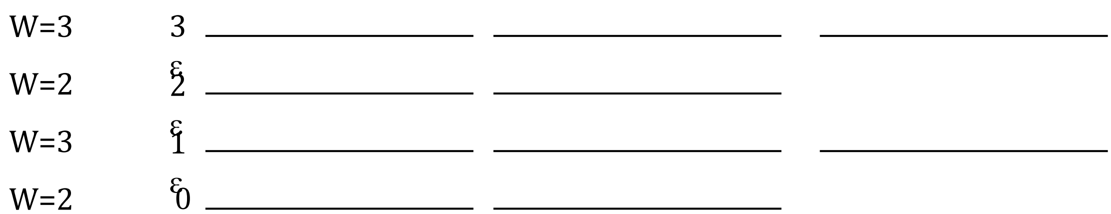
$E_o \; h = 0$

$E_1 \; h = (mg)(h \text{ meter})$

$$\frac{P(h)}{P(O)} = e^{-mgh/kT}$$

Boltzmann factor & Degeneracy

- Generalize the definition of the free energy to include degeneracy.
- Each energy level may be populated with several molecules, i.e. have many accessible states. We define the multiplicity W_i as the number of accessible states with energy E_i. For example:



Assume that a more general formula for the probability

$$P(E_i, W_i) = (W_i/Z)\, e^{-E_i/kT}$$

of finding a molecule with energy E_i, with the multiplicity factor W_i.

Using $W_i = \exp[\ln W_i]$; and later define $S = k\ln[W_i]$; $G = E - TS$

$$P(E_i, W_i) = (W_i/Z)\exp(-E_i/kT) = (1/Z)\,[\exp(\ln W_i)]\,\exp(-E_i/kT)$$

$$= (1/Z)\exp -(E_i - kT\ln W_i)/kT$$

Define $S = k\ln[W_i]$

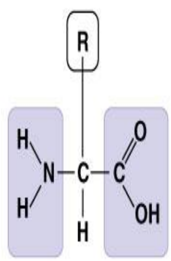
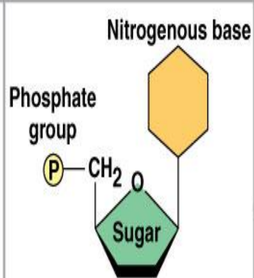
$$P(E_i, W_i) = (1/Z)\exp -(E_i - TS)/kT = = (1/Z)\exp -(F_i)/kT$$

where F = Helmholtz free energy which is same as Gibb's Free Energy for liquids (non-gasses).

Note: ΔG because always energy w.r.t. some zero (like E, ΔE); define E and S. Typically, 1M concentration.

Figure 5.UN02b

So far, we’ve learned about 2 (out of 4) macromolecules

Large Biological Molecules	Components	Examples	Functions
CONCEPT 5.4 Proteins include a diversity of structures, resulting in a wide range of functions	 Amino acid monomer (20 types)	• Enzymes • Structural proteins • Storage proteins • Transport proteins • Hormones • Receptor proteins • Motor proteins • Defensive proteins	• Catalyze chemical reactions • Provide structural support • Store amino acids • Transport substances • Coordinate organismal responses • Receive signals from outside cell • Function in cell movement • Protect against disease
CONCEPT 5.5 Nucleic acids store, transmit, and help express hereditary information	 Nucleotide monomer	**DNA:** • Sugar = deoxyribose • Nitrogenous bases = C, G, A, T • Usually double-stranded	Stores hereditary information
		RNA: • Sugar = ribose • Nitrogenous bases = C, G, A, U • Usually single-stranded	Various functions during gene expression, including carrying instructions from DNA to ribosomes

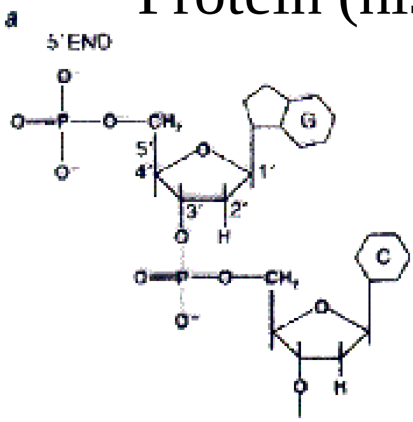
What we've learned so far: plus a little bit…

- DNA contains all the information that is us (with some modifications due to environment).
- Yet there are over 200 types of cells each of which has the exact same DNA. Obviously how the DNA is expressed…meaning proteins, vary from cell to cell.
- There are 3 billion bases pairs in DNA. They're divided into 46 pieces of DNA—called chromosomes. 23 from father, 23 from mother.

  (1 sex chromosome from mother, one from father.)

Size Scales of DNA (+ Protein)

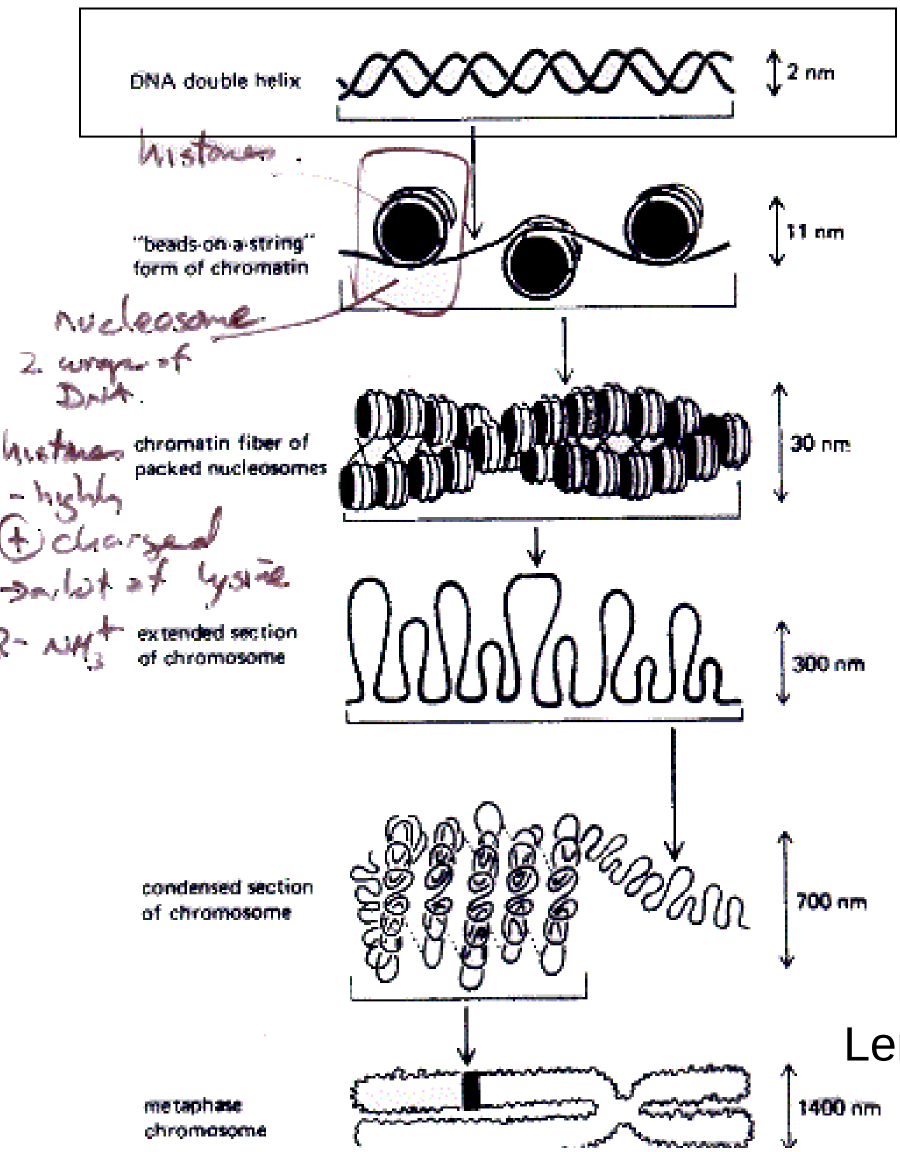
Chromatin = Complex of DNA + Protein (histones + non-histones)



Nucleotides

[4 Diff. types, A,T,C,G]

8/17/06



How many base pairs in human cell?

3 x 10^9 = 3 billion

How much length of DNA in a cell?

~ 1 meter

Flexibility of DNA?

~ 1 meter packed in 3-10 mm (size of nucleus)

chromosomes?

46

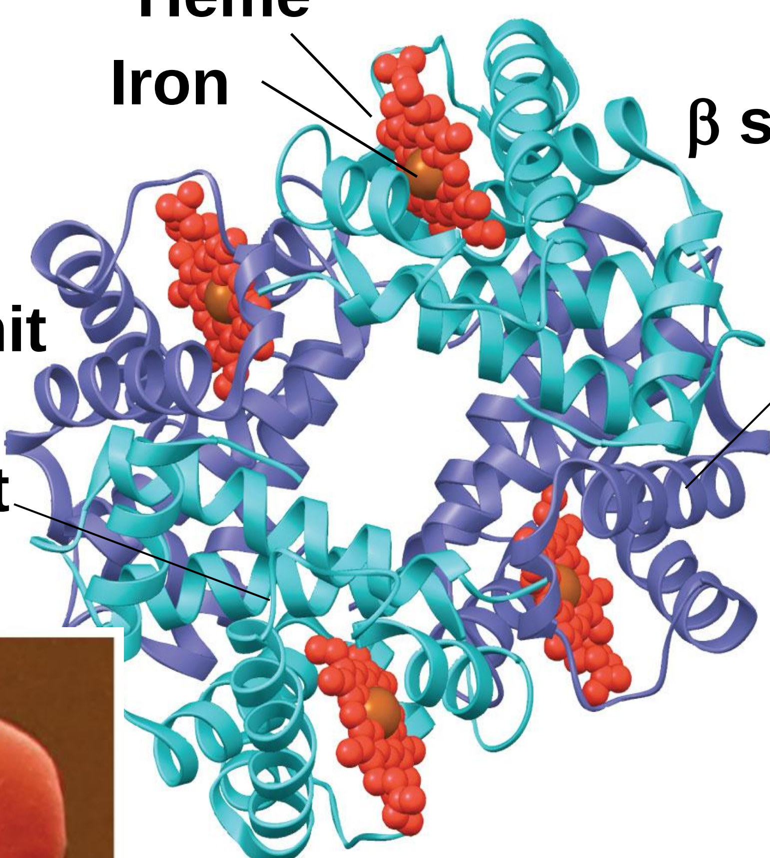
Length/chromosomes?

~ 1/50 meter = 2 cm!

What we've learned so far: plus a little bit…

- DNA is further divided into genes, where each gene is transcribed into RNA (a modified copy of DNA) which is translated into 1 polypeptide ("protein").
  - Polypeptide is linear arrangement of amino acids. If one strand makes a protein then 1 gene = 1 protein;
  - If quaternary structure, more than 1 gene = polypeptide; e.g. hemoglobin, 2 α, 2 β proteins; therefore made up of 2 genes

Figure 5.20i



Hemoglobin

What we've learned so far: plus a little bit…

- Can tell where on DNA a protein starts because every protein starts with ATG (Amino Acid = Methionine) and ends with 1 of 3 "stop codons" in DNA:
- TAG: "They Are Gone"
- TAA: "They Are Away"
- TGA: "They're Going Away"

A protein is typically about ≈1,500 bases of DNA (100-10,000 aa long).

There are ≈20,000 genes.

What percentage of DNA codes for proteins?

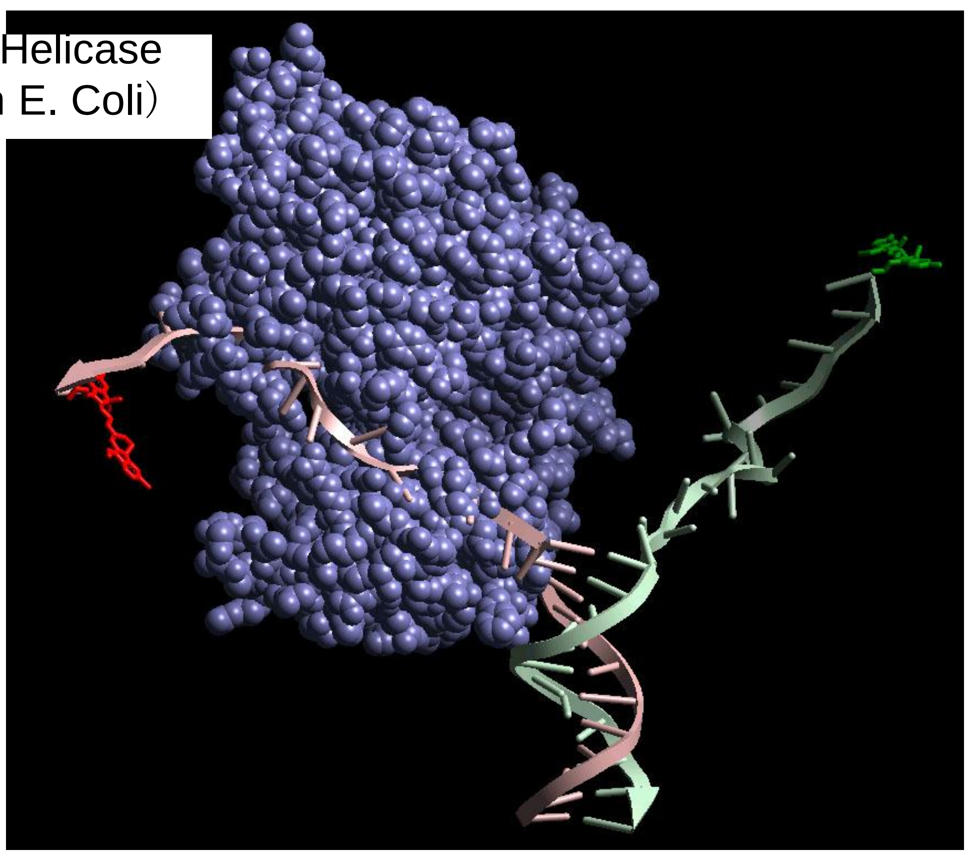
≈10% of DNA codes for proteins.

>90% of DNA is called "junk" DNA
Unclear what, if anything, it does!
May be evolutionary baggage.
May code for RNA which does not make proteins but controls protein expression.

What we've learned so far: plus a little bit…

- DNA is made up of weak bonds, held together by H-bonds, but together, many weak bonds are very strong.
- But in order to copy itself, need to split from double stranded to single stranded.
- Needs proteins which catalyze reaction. (Helicase…)
- Where does it get the energy to do this? ATP

Rep Helicase (from E. Coli)

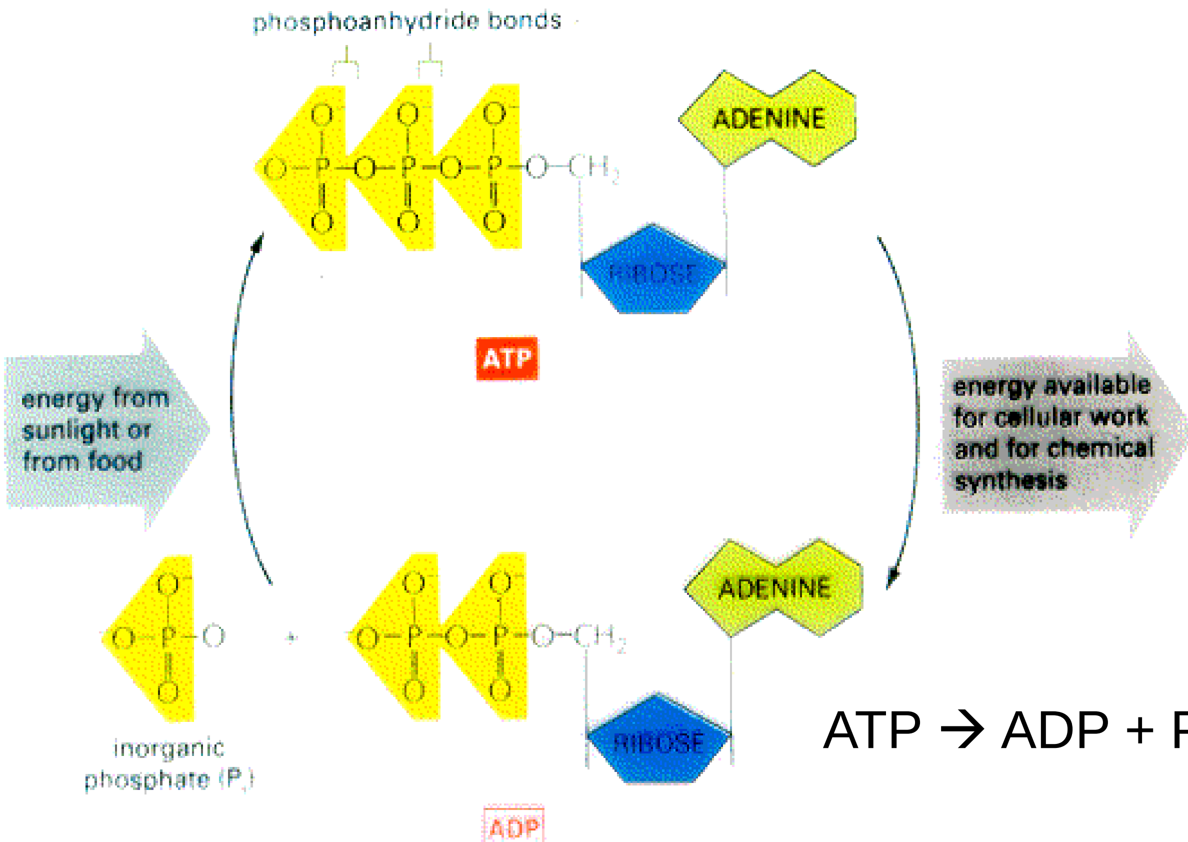


Taekjip Ha, UIUC, Nature, 2002

ATP (Adenosine triphosphate) is the universal food currency of all cells

Nucleic acids perform several roles

1. Immediate source of energy in cell (ATP)



ATP → ADP + P_i

ATP is high energy because of electrostatic repulsion of negatively charged oxygens.

(Entropy is also generally increased because 1 molecule → 2 molecules.)

Adenosine rings used as recognition/binding site by enzymes

Adenosine most commonly used, but dNTP also used.

Energetics of ATP

1 ATP= 80-100 pN-nm of energy at 37 ºC

= 20-25 kT of energy

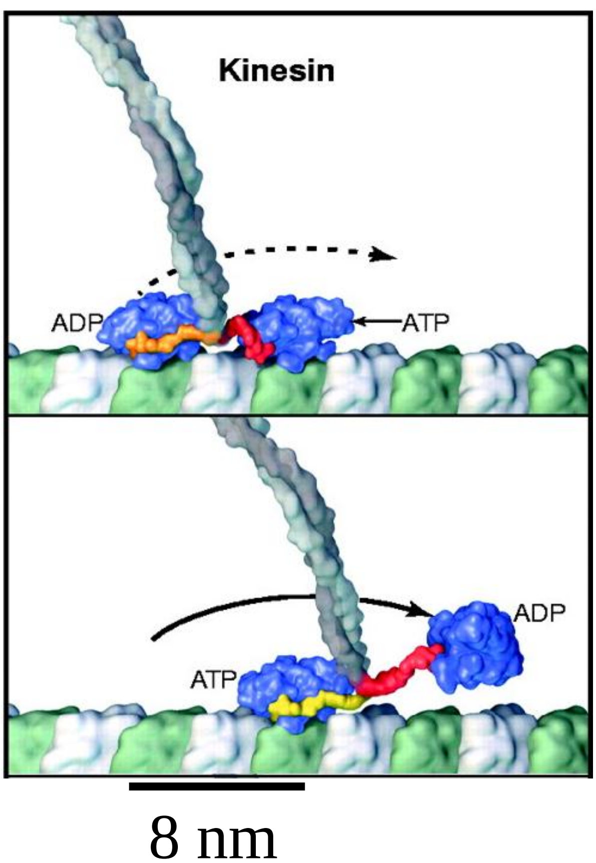
(much more than kT = 4 pN-nm)

A lot of energy

Why do I say 80 to 100 pN-nm? Why not an exact amount?

What counts is ΔG, not ΔE, where ΔG = ΔE -TΔS

ATP → ADP + P_i : depends on [ADP] & also [P_i] concentration



Kinesin– molecular motor

Uses ATP to move objects. Takes 8.3 nm steps and has a maximum force output of 7 pN.

How efficient?

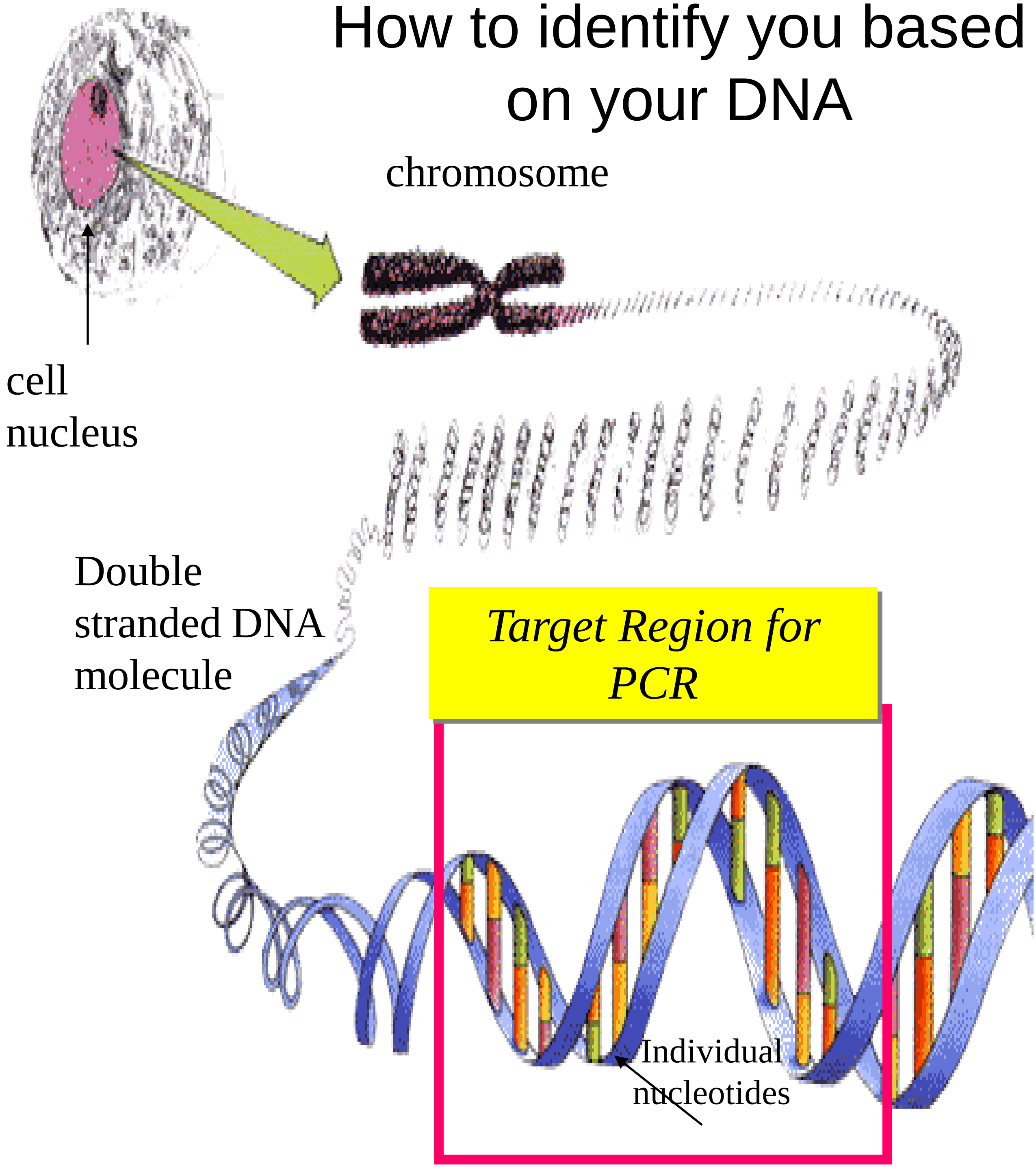
8.3 nm x 7 pN = 58 pN 80-100pN = 58-72%

Very efficient!

Car engine 20% efficient.

DNA in the Cell

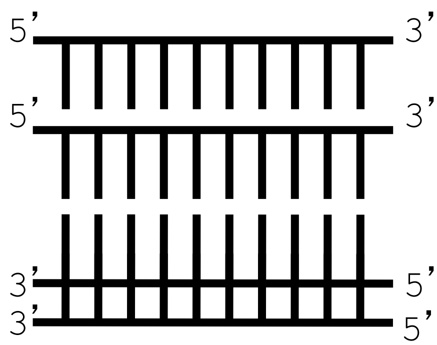
How to identify you based on your DNA



Polymerase Chain Reaction.

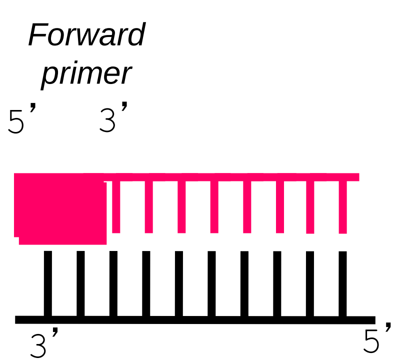
Invented 1990; Nobel Prize in 1993: Kary Mullis

DNA Amplification with the Polymerase Chain Reaction (PCR)



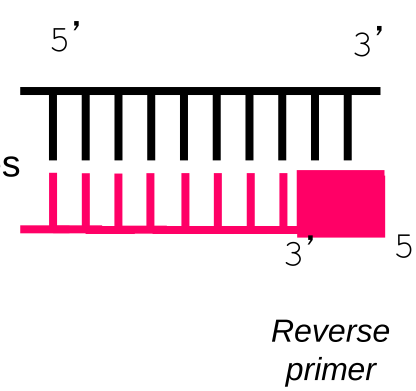
Starting DNA Template

Separate strands (**denature**)

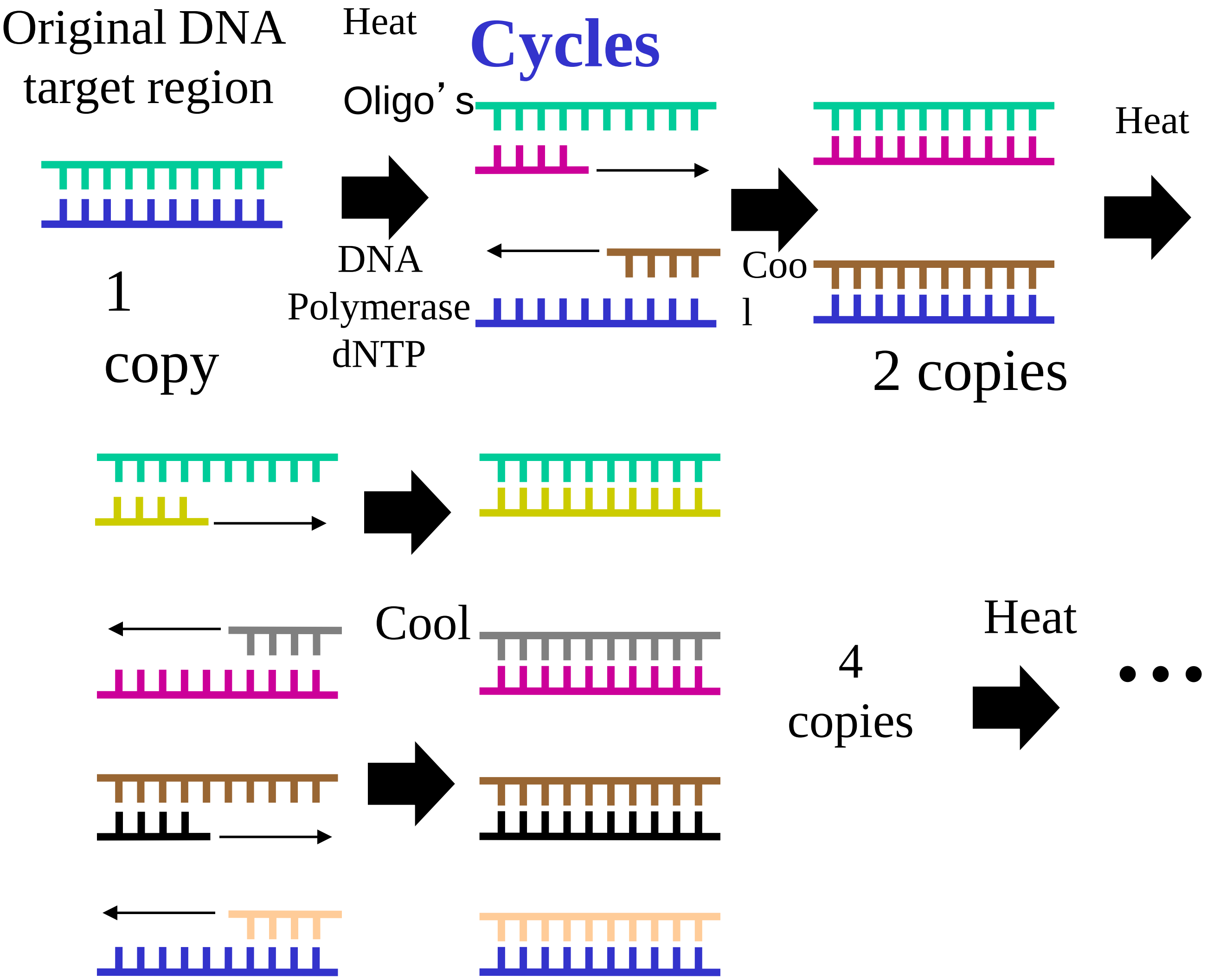


Make copies (**extend primers**)

Add primers (**anneal**)



PCR (Polymerase Chain Reaction) Copies DNA Exponentially through Multiple Thermal Cycles



In 32 cycles at 100% efficiency, 1.07 billion copies are created

To work, what property of DNA polymerase has to have?

New Scientists (1998)…Yellowstone's bugs land up in court **...** Microorganisms from **hot** springs are especially valuable because their enzymes are not easily destroyed by heat.

Applications of PCR

(see next set of slides)