FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

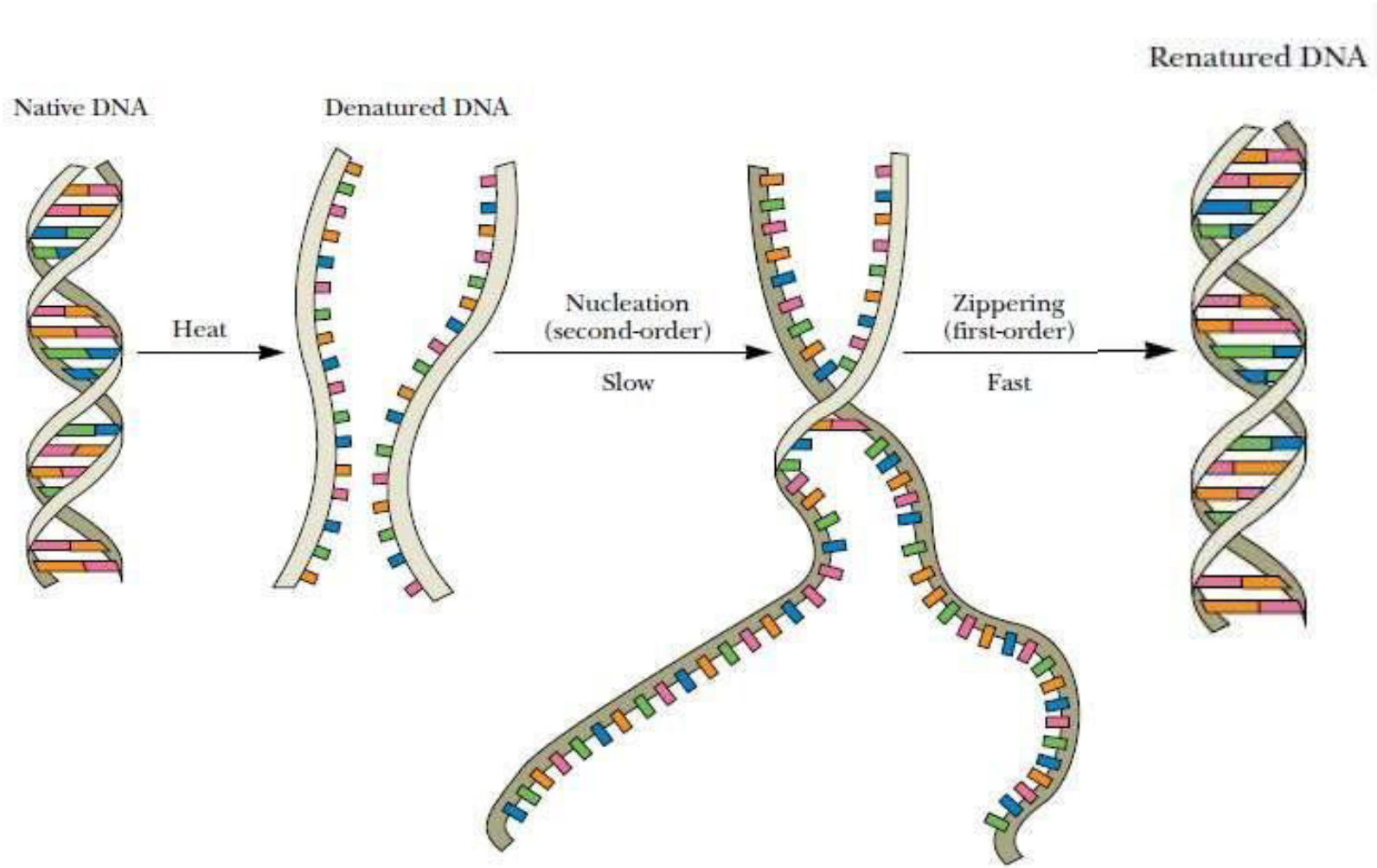
DNA Renaturation

- Denatured DNA will **renature** to re-form the duplex structure if the denaturing conditions are removed (that is, if the solution is cooled, the pH is returned to neutrality, or the denaturants are diluted out).
- Renaturation requires re-association of the DNA strands into a double helix, a process termed **reannealing.**
- For this to occur:

  (1)Strands must realign themselves so that their complementary bases are once again in register (NUCLEATION PROCESS)

  (2)Helix can be zippered up (Figure 12.19).

- Renaturation is dependent on DNA concentration and time. Many of the realignments are imperfect, and thus the strands must dissociate again to allow for proper pairings to be formed.
- The process occurs more quickly if the temperature is warm enough to promote diffusion of the large DNA molecules but not so warm as to cause melting.


Native DNA
Denatured DNA
Renatured DNA
Heat
Nucleation
(second-order)
Slow
Zippering
(first-order)
Fast

Renaturation Rate and DNA Sequence Complexity—$C0t$ Curves

- The renaturation rate of DNA is an excellent indicator of the sequence complexity and the size of the DNA.

- For example, bacteriophage T4 DNA contains about $2x10^5$ nucleotide pairs, whereas *Escherichia coli* DNA possesses $4.64x10^6$. *E. coli* DNA is considerably more complex in that it encodes more information. Or we may say that for any given amount of DNA (in grams), the sequences represented in an *E. coli* sample are more heterogeneous, that is, more dissimilar from one another, than those in an equal weight of phage T4 DNA. Therefore, it will take the *E. coli* DNA strands longer to find their complementary partners and reanneal. This situation can be analyzed quantitatively.

DNA Sequence Complexity and C_0t Curve

- If c is the concentration of single-stranded DNA at time t, then the second-order rate equation for two complementary strands coming together is given by the rate of decrease in c:

-- $dc/dt = k_2c^2$

where k_2 is the second-order rate constant.

- Starting with a concentration, C_0, of completely denatured DNA at $t0$, the amount of single-stranded DNA remaining at some time t is

$C/C_0 = 1/(1 + k_2C_0t)$

where the units of C are moles of ntd per L and t is in seconds.

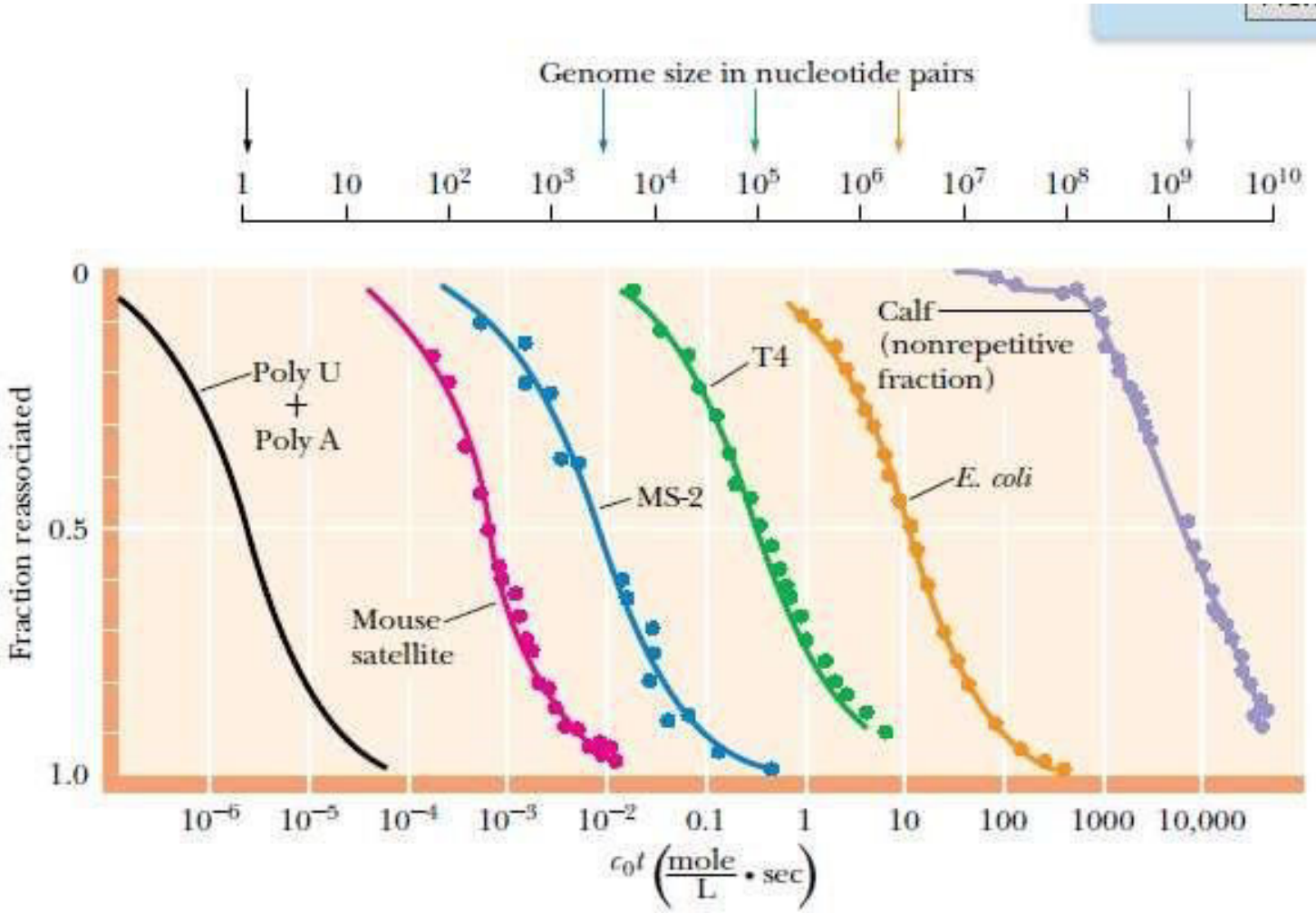
- Then the time for half of the DNA to renature (when $C/C_0 = 0.5$), according to the second order rate equation, is defined as $t = t_{1/2}$. Then,

$0.5 = 1/(1 + k_2C_0t_{1/2})$ and thus $1 + k_2C_0t_{1/2}$ comes out to be 2 yielding $C_0t_{1/2} = 1/k_2$

- A graph of the fraction of single-stranded DNA reannealed (C/C_0) as a function of C_0t on a semilogarithmic plot is referred to as a C_0t (pronounced "cot") curve (Figure).

*C*0*t* Curves

rates of reassociation of denatured DNA from various sources and illustrate how the rate of reassociation is inversely proportional to genome complexity. The DNA sources are as follows: poly A+poly U, a synthetic DNA duplex of poly A and poly U polynucleotide chains; mouse satellite DNA, a fraction of mouse DNA in which the same sequence is repeated many thousands of times; MS-2 dsRNA, the double-stranded form of RNA found during replication of MS-2, a simple bacteriophage; T4 DNA, the DNA of a more complex bacteriophage; *E. coli* DNA, bacterial DNA; calf DNA (nonrepetitive fraction), mammalian DNA (calf) from which the highly repetitive DNA fraction (satellite DNA) has been removed. Arrows indicate the genome size (in bp) of the various DNAs. *(From Britten, R. J., and Kohne, D. E., 1968.* Science ***161****:529–540.)*


Genome size in nucleotide pairs
1
10
10^2
10^3
10^4
10^5
10^6
10^7
10^8
10^9
10^{10}
Fraction reassociated
0
0.5
1.0
Poly U
+
Poly A
Mouse
satellite
MS-2
T4
Calf
(nonrepetitive
fraction)
E. coli
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
0.1
1
10
100
1000
10,000
$c_0 t \left(\frac{\text{mole}}{\text{L}} \cdot \text{sec}\right)$