PROBLEMS

14.1. Given the following information, calculate the probability of forming a plasmid-free cell due to random segregation for a cell with 50 plasmid monomer equivalents:

a. 40% of the total plasmid DNA is in dimers and 16% in tetramers.

b. The distribution of copy numbers per cell is as follows, assuming monomers only:

≤ 3 plasmids	~ 0%
4 to 8	4%
9 to 13	10%
14 to 18	25%
19 to 23	25%
24 to 28	20%
29 to 33	12%
34 to 38	4%
≥ 39	~ 0%

14.2. Assume that all plasmid-containing cells have eight plasmids; that an antibiotic is present in the medium, and the plasmid-containing cells are totally resistant; and that a newly born, plasmid-free cell has sufficient enzyme to protect a cell and its progeny for three generations. Estimate the fraction of plasmid-containing cells in the population in a batch reactor starting with only plasmid-containing cells after five generations.

14.3. Consider an industrial-scale batch fermentation. A 10,000 l fermenter with 5×10^{10} cells/ml is the desired scale-up operation. Inoculum for the large tank is brought through a series of seed tanks and flasks, beginning with a single pure colony growing on an agar slant. Assume that a colony (10^6 plasmid-containing cells) is picked and placed in a test tube with 1 ml of medium. Calculate how many generations will be required to achieve the required cell density in the 10,000 l fermenter. What fraction of the total population will be plasmid-free cells if $\mu_+ = 1.0\ h^{-1}$, $\mu_- = 1.2\ h^{-1}$, and $P = 0.0005$?

14.4. Assume that you have been assigned to a team to produce human epidermal growth factor (hEGF). A small peptide, hEGF speeds wound healing and may be useful in treating ulcers. A market size of 50 to 500 kg/yr has been estimated. Posttranslational processing is not essential to the value of the product. It is a secreted product in the natural host cell. Discuss what recommendations you would make to the molecular-biology team leader for the choice of host cell and the design of a reactor. Make your recommendations from the perspective of what is desirable to make an effective process. You should point out any potential problems with the solution you have proposed, as well as defend why your approach should be advantageous.

14.5. Develop a model to describe the stability of a chemostat culture for a plasmid-containing culture. For some cultures, plasmids make a protein product (e.g., colicin in *E. coli*) that kills plasmid-free cells but does not act on plasmid-containing cells. Assume that the rate of killing by colicin is $k_T C n_-$, where k_T is the rate constant for the killing and C is the colicin concentration. Assume that the colicin production is first order with respect to n_+.

14.6. Consider the following data for *E. coli* B/r-pDW17 grown in a minimal medium supplemented with amino acids. Estimate $\Delta\mu$ and R. Compare the stability of this system to one with a glucose-minimal medium (Example 14.2).