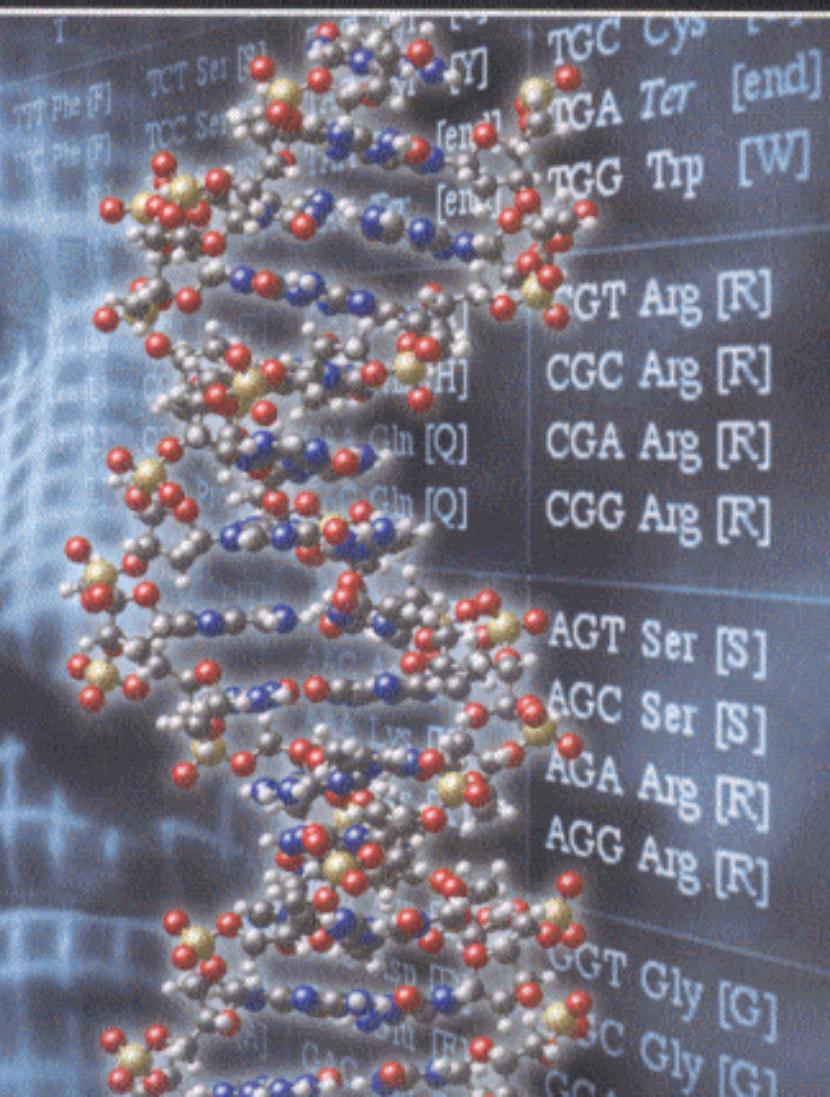


Calculations for Molecular Biology and Biotechnology

A Guide to Mathematics in the Laboratory

Frank H. Stephenson



Contents

Foreword	xiv
Chapter 1: Scientific Notation and Metric Prefixes ..	1
Introduction.....	1
Significant Digits.....	1
Rounding Off Significant Digits in Calculations.....	2
Exponents and Scientific Notation.....	4
Expressing Numbers in Scientific Notation.....	4
Converting Numbers from Scientific Notation to Decimal Notation.....	6
Adding and Subtracting Numbers Written in Scientific Notation.....	8
Multiplying and Dividing Numbers Written in Scientific Notation.....	9
Metric Prefixes.....	13
Conversion Factors and Canceling Terms.....	14
 Chapter 2: Solutions, Mixtures, and Media.....	 18
Introduction.....	18
Calculating Dilutions: A General Approach.....	18
Concentrations by a Factor of X.....	20
Preparing Percent Solutions.....	22
Diluting Percent Solutions.....	23
Moles and Molecular Weight: Definitions.....	27
Molarity.....	28
Diluting Molar Solutions.....	31
Converting Molarity to Percent.....	32
Converting Percent to Molarity.....	33
Normality.....	34
PH.....	35
pK_a and the Henderson–Hasselbalch Equation.....	39

Chapter 3: Cell Growth.....	42
The Bacterial Growth Curve.....	42
Sample Data.....	45
Manipulating Cell Concentration.....	46
Plotting OD ₅₅₀ vs. Time on a Linear Graph	48
Plotting the Logarithm of OD ₅₅₀ vs. Time on a Linear Graph.....	49
Logarithms	49
Sample OD ₅₅₀ Data Converted to Log Values.....	50
Plotting Log OD ₅₅₀ vs. Time.....	50
Plotting the Log of Cell Concentration vs. Time.....	51
Determining Log Values.....	51
Calculating Generation Time.....	52
Slope and Growth Constant.....	52
Generation Time.....	53
Plotting Cell Growth Data on a Semilog Graph.....	55
Plotting OD ₅₅₀ vs. Time on a Semilog Graph.....	55
Estimating Generation Time from a Semilog Plot of OD ₅₅₀ vs. Time.....	56
Plotting Cell Concentration vs. Time on a Semilog Graph.....	57
Determining Generation Time Directly from a Semilog Plot of Cell Concentration vs. Time.....	59
Plotting Cell Density versus OD ₅₅₀ on a Semilog Graph.....	60
The Fluctuation Test.....	61
Fluctuation Test Example.....	63
Variance.....	64
Measuring Mutation Rate.....	66
The Poisson Distribution.....	67
Calculating Mutation Rate by Using the Poisson Distribution.....	68
Using a Graphical Approach to Calculate Mutation Rate from Fluctuation Test Data.....	69
Mutation Rate Determined by Plate Spreading.....	74
Measuring Cell Concentration on a Hemocytometer.....	75

Chapter 4: Working with Bacteriophage.....	77
Introduction.....	77
Multiplicity of Infection.....	77
Probabilities and Multiplicity of Infection	79
Measuring Phage Titer	85
Diluting Bacteriophage	86
Measuring Burst Size.....	87
Chapter 5: Quantitation of Nucleic Acids.....	90
Quantitation of Nucleic Acids by Ultraviolet Spectroscopy..	90
Determining the Concentration of Double-Stranded DNA...	91
Using Absorbance and an Extinction Coefficient to Calculate Double-Stranded DNA Concentration.....	94
Calculating DNA Concentration as a Millimolar (mM) Amount.....	96
Determining the Concentration of Single-Stranded DNA Molecules.....	97
Single-Stranded DNA Concentration Expressed in $\mu\text{g/mL}$	97
Determining the Concentration of High-Molecular-Weight Single-Stranded DNA in pmol/ μL	98
Expressing ssDNA Concentration as a Millimolarity Amount....	98
Oligonucleotide Quantitation.....	99
Optical Density (OD) Units.....	99
Expressing an Oligonucleotide's Concentration in $\mu\text{g/mL}$	100
Oligonucleotide Concentration Expressed in pmol/ μL	100
Measuring RNA Concentration.....	103
Molecular Weight, Molarity, and Nucleic Acid Length	104
Estimating DNA Concentration on an Ethidium Bromide– Stained Gel.....	108

Chapter 6: Labeling Nucleic Acids with Radioisotopes.....	109
Introduction.....	109
Using Radioactivity: The Curie	109
Estimating Plasmid Copy Number.....	110
Labeling DNA by Nick Translation.....	112
Determining Percent Incorporation of Radioactive Label from Nick Translation.....	113
Calculating Specific Radioactivity of a Nick Translation Product..	114
Random Primer Labeling of DNA.....	114
Random Primer Labeling – Percent Incorporation.....	115
Random Primer Labeling – Calculating Theoretical Yield.....	116
Random Primer Labeling – Calculating Actual Yield.....	117
Random Primer Labeling – Calculating Specific Activity of the Product.....	118
Labeling 3' Termini with Terminal Transferase.....	119
3'-End Labeling with Terminal Transferase – Percent Incorporation.....	119
3'-End Labeling with Terminal Transferase – Specific Activity of the Product.....	120
cDNA Synthesis.....	121
First Strand cDNA Synthesis.....	121
Second Strand cDNA Synthesis.....	125
Homopolymeric Tailing.....	128
<i>In Vitro</i> Transcription.....	133
Chapter 7: Oligonucleotide Synthesis.....	136
Introduction.....	136
Synthesis Yield.....	136
Measuring Stepwise and Overall Yield by the DMT Cation Assay.....	139
Overall Yield.....	139

Stepwise Yield.....	140
Calculating Micromoles of Nucleoside Added at Each Base Addition Step.....	142
Chapter 8: The Polymerase Chain Reaction.....	143
Introduction.....	143
Template and Amplification.....	143
Exponential Amplification.....	145
PCR Efficiency.....	147
Calculating the T_m of the Target Sequence.....	151
Primers.....	153
Primer T_m	158
Calculating T_m Based on Salt Concentration, G/C Content, and DNA Length.....	159
Calculating T_m Based on Nearest-Neighbor Interactions.....	160
dNTPs.....	165
DNA Polymerase.....	168
Calculating DNA Polymerase's Error Rate.....	169
Quantitative PCR.....	171
Chapter 9: Recombinant DNA.....	186
Introduction.....	186
Restriction Endonucleases.....	186
The Frequency of Restriction Endonuclease Cut Sites.....	188
Calculating the Amount of Fragment Ends.....	189
The Amount of Ends Generated by Multiple Cuts.....	190
Ligation.....	192
Ligation Using Lambda-Derived Vectors.....	194
Packaging of Recombinant Lambda Genomes.....	200
Ligation Using Plasmid Vectors.....	203
Transformation Efficiency.....	207
Genomic Libraries: How Many Clones Do You Need?.....	208

cDNA Libraries: How Many Clones Are Enough?.....	210
Expression Libraries.....	211
Screening Recombinant Libraries by Hybridization to DNA Probes.....	212
Oligonucleotide Probes.....	214
Hybridization Conditions.....	216
Hybridization Using Double-Stranded DNA Probes.....	223
Sizing DNA Fragments by Gel Electrophoresis.....	224
Generating Nested Deletions Using Nuclease BAL 31.....	237
 Chapter 10: Protein.....	 242
Introduction.....	242
Protein Quantitation by Measuring Absorbance at 280 nm... Using Absorbance Coefficients and Extinction Coefficients to Estimate Protein Concentration.....	242 243
Relating Absorbance Coefficient to Molar Extinction Coefficient.....	245
Determining a Protein's Extinction Coefficient.....	246
Relating Concentration in Milligrams per Milliliter to Molarity.....	248
Protein Quantitation Using A_{280} When Contaminating Nucleic Acids Are Present.....	249
Protein Quantitation at 205 nm.....	250
Protein Quantitation at 205 nm When Contaminating Nucleic Acids Are Present.....	251
Measuring Protein Concentration by Colorimetric Assay – The Bradford Assay.....	252
Using β -Galactosidase to Monitor Promoter Activity and Gene Expression.....	254
Assaying β -Galactosidase in Cell Culture.....	255
Specific Activity.....	257

Assaying β -Galactosidase from Purified Extracts.....	258
The CAT Assay.....	260
Calculating Molecules of CAT.....	263
Use of Luciferase in a Reporter Assay.....	265
<i>In Vitro</i> Translation – Determining Amino Acid Incorporation.....	266
Chapter 11: Centrifugation.....	270
Introduction.....	270
Relative Centrifugal Force (<i>g</i> Force).....	270
Converting <i>g</i> Force to Revolutions per Minute.....	272
Determining <i>g</i> Force and Revolutions per Minute by Use of a Nomogram.....	273
Calculating Sedimentation Times.....	275
Chapter 12: Forensic Science.....	278
Introduction.....	278
Alleles and Genotypes.....	278
Calculating Genotype Frequencies.....	280
Calculating Allele Frequencies.....	281
The Hardy–Weinberg Equation and Calculating Expected Genotype Frequencies.....	282
The Chi-Square Test: Comparing Observed to Expected Values.....	286
Sample Variance.....	290
Sample Standard Deviation.....	291
P_i : The Power of Inclusion.....	292
P_d : The Power of Discrimination.....	293
DNA Typing and a Weighted Average.....	294
The Multiplication Rule.....	295
Index.....	297