

Biotechnology - a background guide

Table of contents

Preface

Background to the Guide

Biotechnology and translators/interpreters

- Biotechnology-related material requiring translation
- A note on spelling
- Online glossaries

About the author

Acknowledgements

Introduction - what is biotechnology?

Chapter 1 - Organisms, cells and molecules

Taxonomy

- Kingdoms of life
- Viruses
- Microbacteria and megaviruses

The Cellular Basis of Life

- Cell structure
- The anatomy of a cell
 - Nucleus
 - Ribosomes
 - Mitochondria (singular, mitochondrion)
 - Cytoskeleton
 - Golgi apparatus (complex)
 - Vesicles
 - Chloroplasts

Tools and techniques for cell biology

- Microscopy
 - Fluorescence microscopy
 - Confocal microscopy
 - Electron microscopy
- Differential centrifugation
 - Units used in centrifugation
- Isolating intracellular components

What is the difference between a living cell and an inert bag of chemicals?

The molecules of life

Getting down to basics: chemistry

- Elements

- Molecules
 - How molecular structures are displayed
 - Sizes of molecules
 - Macromolecules
- Chemical nomenclature
- Types of chemical bond
- Chemical reactions

Biochemistry

- Enzymes
 - Enzyme action
 - Nomenclature
- Metabolism and metabolic pathways
 - Coenzymes, vitamins and cofactors
 - Primary metabolism
- Proteins
 - Protein structure and function
 - Protein domains
- Nucleic acids
- Carbohydrates
 - Nomenclature
 - Carbohydrate-containing molecules
- Lipids

Chapter 2 - Microbial biotechnology

Introduction

Protein production by microorganisms

Secondary metabolism

- Biosynthetic pathways for polyketides

Applied microbiology

- Microbial cell growth
 - Growth media
 - Containment facilities
- Laboratory scale cell culture
 - Growth phases
- Production-scale fermentations
- A bioprocess example: penicillin production
 - Media preparation
 - Fermentation
 - Biomass removal and solvent extraction
- The search for novel microbes and products
 - The unculturables

Chapter 3 - Molecular genetics primer

Introduction

Genetics and the nature of the gene

- Mendelian genetics
- Chromosomes and heredity
- Genetics terminology
- Fruit flies and mutations
- Non-Mendelian inheritance
- Microbial genetics
- *E. coli* genetics and bacteriophages
- Biochemical genetics - one gene, one enzyme

Molecular biology

- The chemical nature of the gene
- The 'central dogma' of molecular biology

The DNA double helix and base pairs

- Different forms of DNA
- How DNA is copied and its information content extracted
- DNA and chromosomes
- Gene replication in viruses

Extracting information from genes

- Messenger RNA
- mRNA transcription
- Turning mRNA sequences into proteins
- Translating the code

Split genes and mRNA processing

- Other features of eukaryotic mRNA

Regulation of gene expression

- Transcriptional regulation

Translational regulation and protein modification

- Proteolysis
- Glycosylation
- Ubiquitylation
- Lipidation
- Phosphorylation
- Other modifications

Chapter 4 - Genetic engineering part 1

Introduction

The birth of recombinant DNA technology

- Restriction enzymes

Genetic engineering in practice

- Outline of workflow

Basic toolkit

- Agarose gel electrophoresis
- Radiolabelled molecules
- Detecting and measuring radioactivity
- Synthetic oligonucleotides
- Spectrophotometry- quantifying nucleic acids and other molecules
- Hybridisation probes
- Southern and northern blotting
- In situ hybridisation
- DNA modifying enzymes
- Buffers and reaction mixes
- Protein additives
- Metal ions and chelating agents
- Detergents

Isolating the DNA sequence to be cloned

- Obtaining protein sequence
- Complementary DNA - cDNA
- cDNA libraries
 - Screening libraries to find gene of interest

Amplifying DNA using the polymerase chain reaction - PCR

- Using PCR to screen cDNA libraries
- PCR and mRNA expression

Vectors

- Plasmids
 - Multiple cloning site
 - Antibiotic selection markers
 - lacZ enzyme
 - Ori and f1 ori
- Viral vectors
- Modifying the recombinant vector to optimise protein production
 - Codon usage bias
 - Sequences for ribosome binding
- Introducing vector DNA into bacteria
 - Vector purification
 - Bacterial transformation
- Analysing recombinant bacterial clones
 - Sequencing the DNA inserts

Using recombinant bacteria to produce protein

- Analysis of protein expression
 - SDS-polyacrylamide gel electrophoresis: SDS-PAGE
 - Staining the gel
 - Western blotting

- Purification of recombinant proteins
- Background to chromatography
 - Affinity chromatography
 - His-tags
 - Other purification tags
- Reporter genes

Bioinformatics

- Sequence analysis

Chapter 5 - Genetic engineering part 2

Protein expression in eukaryotes

- Why eukaryotes?
- Mammalian cell culture
 - Growth media
- Eukaryotic expression vectors
 - Introducing vectors into cells: transfection
 - Introducing genes into algae
 - Viral vectors and transduction
 - Baculovirus
 - Mammalian cells
 - Cell-free protein synthesis
- Industrial production of therapeutic proteins

Therapeutic antibodies

- Antibody isotypes
- Polyclonal antibodies
- Monoclonal antibodies
- Antibody engineering
 - CDR grafting
 - Phage display
 - Humanised mice
- Aptamers

Immunoassays

- Primary and secondary antibodies
- Biotin-streptavidin
- ELISA assays
- Immunoprecipitation
 - Chromatin immunoprecipitation (ChIP assays)
- Assays for cell surface proteins
 - Immunohistology
 - Flow cytometry
 - Quantum dots
 - Mass cytometry

Binding assays

- Affinity
- Laboratory techniques
 - Fluorescence resonance energy transfer - FRET
 - Surface plasmon resonance - SPR

Genetic engineering *in vivo*

- Model organisms
- Transgenic mice
- Transgenic ruminants - pharming
- Transgenic plants
 - First and second-generation crops
 - Plant-made pharmaceuticals
 - Gene transfer into plants
- Tracking gene expression *in vivo*
- Transgenic knockouts
- Fertilisation

Stem cells

- Creating stem cells for research and therapy
 - Somatic cell cloning
 - iPS stem cells

Genome editing

- Zinc finger nucleases (ZFNs)
- TALENS
- CRISPR-Cas9
- Conditional gene expression
- Gene therapy

Controlling mRNA and protein expression

- Small interfering RNAs (siRNAs)
- Short hairpin RNAs (shRNAs)
- Antisense oligonucleotides

Chapter 6 - The world of 'omics'

Introduction

Genomics

- The Human Genome Project
 - Human genome nomenclature
 - Genome sequencing and analysis
 - Next Generation Sequencing (NGS)
 - What is in the human genome?
- Transcriptomics
- DNA Sequence and disease
 - Single nucleotide polymorphisms-SNPs
 - Pharmacogenomics

DNA microarrays

- Microarray applications
 - Gene expression
 - SNP detection

Metagenomics

Epigenomics and epigenetics

- DNA methylation - the methylome

Proteomics

- Two-dimensional gel electrophoresis
- Identifying proteins
- Mass spectrometry
 - Protein fingerprints
- Protein arrays
- Protein complexes
 - Yeast two-hybrid screens
 - Tandem affinity purification (TAP)
- Displaying protein networks

Structural proteomics

- X-ray crystallography
- Nuclear magnetic resonance spectroscopy (NMR)
- Homology modelling
- Cryoelectron microscopy (CryoEM)

Chemical genomics and proteomics

Systems biology

Synthetic biology

- Synthetic gene circuits and DNA computers
- Synthetic life
- Human genome-write

Concluding remarks

Chapter 7 - Patent examples

Introduction

Some statistics

Example 1 - Anti-DLL3 antibodies and drug conjugates for use in melanoma

- Background
- General comments
- Main topics
- Biochemical nature and function of DLL3 protein
- DLL3 and melanomas
 - Signalling pathways in cancer
 - Determining expression levels of DLL3 mRNA
- 3) Generation and characterisation of antibodies to DLL3

- Creation of mouse monoclonal antibodies
- Characterising the mouse monoclonal antibodies
- Creation of humanised and chimaeric anti DLL3 antibodies

Example 2 - New Means and Methods for Producing Propanediol

- Background
- General comments
- Main topics
 - 1) Metabolic pathways
 - 2) Genetic engineering of relevant enzymes

Example 3 - Non-human animals having humanized Fc-gamma receptors

- Background
- General comments
- Main topics
 - 1) Background to FcγRI receptor
 - 2) Generation of FcγRI humanised animals

Example 4 - Assay for detection of JC virus DNA

- Background
- General comments
- Main topics
 - 1) DNA isolation from CSF
 - 2) Nucleic acid amplification

Example 5 - Production of DHA and other LC-PUFAs in plants

- Background
- General comments
- Main topics
 - 1) DNA constructs
 - 2) Plant cell transformation
 - 3) Analysis of transgenic seeds

Example 6 - Methods and Genetic Systems for Cell Engineering

- Background
- General comments
- Main topics

1) Background material

2) Examples of genetic constructs