

BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 21 – Methods

Restriction enzymes

- palindromic recognition sequences
- overhanging ends (5' or 3') vs. blunt ends after cutting
- probability of occurrence of cutting depending on length of rec. seq.
- role of methylation

Electrophoresis of nucleic acids

- agarose vs. polyacrylamide gels
- charge on nucleic acid and direction of electrophoresis

Blots – general idea; Southern blot (DNA); northern blot (RNA)

Recombinant DNA; DNA cloning – what do they mean?

Types of cloning vectors used in E. coli and rationale for using various types

Basic features of a plasmid

DNA libraries – genomic vs. cDNA; what is difference?

How is cDNA made?

What is difference between a selection and a screen?

How to screen a library?

PCR

- inventor Kary Mullis
- some uses
- describe steps and how it results in exponential amplification of DNA

Describe site-directed mutagenesis of DNA – Quickchange method

Dideoxy sequencing method

- inventor Fred Sanger
- steps; how does it work?
- role of dideoxy nucleoside triphosphates as chain terminators

Next generation DNA sequencing

- names of four different technologies
- general idea in comparison to dideoxy sequencing
- understand basics of 454 (pyro sequencing)
- understand basics of Solexa sequencing
- what are benefits and limitations?

Basic strategy behind genome sequencing

- contigs, scaffolds, “personal genomes”

DNA microarrays

- understand basic technique and general usage

Protein gels

- SDS-PAGE: what is SDS and what does it do?
- Role of beta-mercaptoethanol or dithiothreitol to reduce disulfide bonds
- Immunoblots (western blots) – what are they and basic technique

What is the proteome? What are some goals of proteomics research?

General ideas behind protein identification and sequencing by mass spectrometry

Electrophoretic mobility shift assay (EMSA) – understand basic technique and use

Footprinting – understand basic technique and use

Compare some aspects of EMSA and footprinting

Chromatin Immunoprecipitation (ChIP) – understand basic technique and use

SELEX or in vitro selection – understand basic technique and use