Methods: Molecular Techniques

OEST 740
043008
Introduction

Molecular Biology – investigates the structure and function of biological molecules

- Fundamental Data
- Not limited by cultivation
- Rapid diagnostics
- Multi-level Analysis
  - From community to single cell
Introduction

Levels of Analysis
- Detection of gene or gene product
  - Functional capacity
- Compare gene sequences
  - Identity
  - Phylogeny and Evolution
  - Adaptation
- Monitor Gene expression
  - Responses to environment or mode of growth
  - (i.e. biofilm phenotype)
Shotgun Method

- Crude method of sequencing or cloning
- Primary step does not involve physical map of source clone
  - unguided
- Sequence contig multiple times
PCR

- Polymerase Chain Reaction
- Applications
  - Directed cloning/sequencing
  - Community Fingerprint
  - Number of Gene Copies

30 - 40 cycles of 3 steps:

Step 1: denaturation
1 minute 94 °C

Step 2: annealing
45 seconds 54 °C
forward and reverse primers !!!

Step 3: extension
2 minutes 72 °C
only dNTPs

(Andrzej Verinckx 1999)
Recombinant DNA Clones

1. **Vector and Donor DNA**
   - Vector and donor DNA are digested (cleaved) with restriction enzyme.
   - Overhangs are produced.

2. **Mixing and DNA Ligase**
   - DNA ligase is added, sealing the overhangs.
   - Recombinant DNA molecules are formed.

3. **Introduction into Bacterial Cells**
   - Recombinant DNA molecules are introduced into bacterial cells.
   - Bacterial chromosome expands.

4. **Replication and Cloning**
   - Recombinant DNA molecules replicate, and cells divide.
   - Clones are produced.

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Genetic Sequencing

- 16s rRNA
  - Phylogenetic analysis
  - Conserved Regions
    - Cloning and sequencing
  - Variable regions
    - Information
- Functional gene sequencing
Mutant Type vs. Wild Type

- Direct manipulation of genes using molecular cloning and transformation to alter structure and characteristic of genes
  - Isolation of genes
  - Insertion of genes into transfer vector
  - Transformation
- Recombinant DNA technology, gene splicing, gene modification, etc.
- Attachment, structural development, QSS
Mutant vs. Wild Type

- **Loss of Function experiments**
  - How: gene knockout
  - Why: pinpoint phenotypes controlled by genes

- **Gain of functions – increased function**

- **Tracking –**
  - How: WT gene replaced with ‘fusion’ gene
  - Why: Visualize gene modifications

- **Gene Expression**
  - How: Reintroduce gene promoter with protein coding region replaced by reporter gene
  - Why: Where and When data
DGGE

- Denaturing Gradient Gel Electrophoresis
  - Large population analysis
  - Fingerprint of genetic diversity
  - Comparison allows the visualization of presence or absence of particular species.

[Diagram showing DGGE gel with bands for different samples and a legend indicating GC Clamp.]
DGGE

1. Excise

2. Clone
   Vector

4. Sequence

5. Identify
DNA Micro-array

- Snap-shot of gene expression
- Differential Gene Express – Biofilm phenotype
  - Resistance
DNA Micro-array

Make cDNA reverse transcript
Label cDNAs with fluorescent dyes

Control

Experimental

Hybridization to microarray
Laser excitation at dye-specific Hz

Laser emission

Computer calculates ratio of intensity

Principle of cDNA microarray assay for gene expression
(after Gibson & Muse 2002)

Red = "up-regulation"
Green = "down-regulation"
Black = constitutive expression
Confocal Scanning Laser Microscopy

- In-situ analysis
  - Green Fluorescent Protein (GFP)
    - Pro-spatial and temporal resolution
    - Con-Somewhat limited
  - In-situ gene expression
    - FISH
    - Require molecular probes
Summary

- Molecular techniques are invaluable to understanding the structure of biofilms on a biogeochemical level.

- Providing useful information to the questions posed in biofilm research as to who, where, when and how.

- As well as, identifying how biofilm microbial populations respond to various environmental variability and stress.