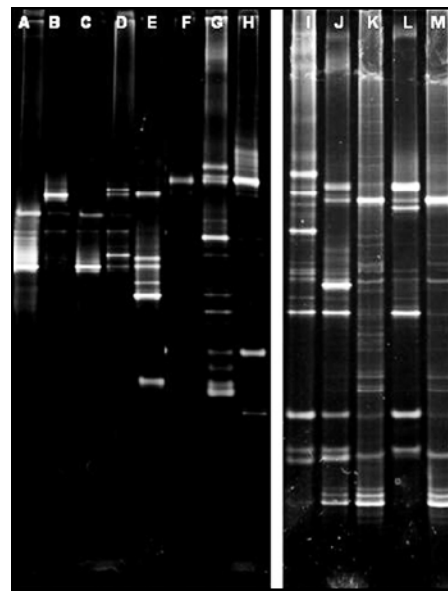
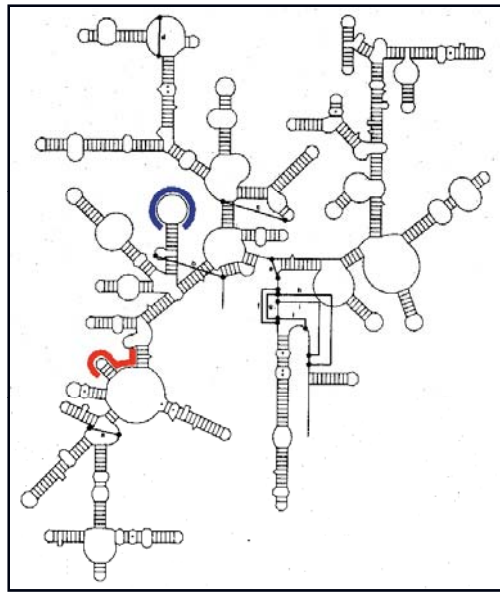


# Methods: Molecular Techniques

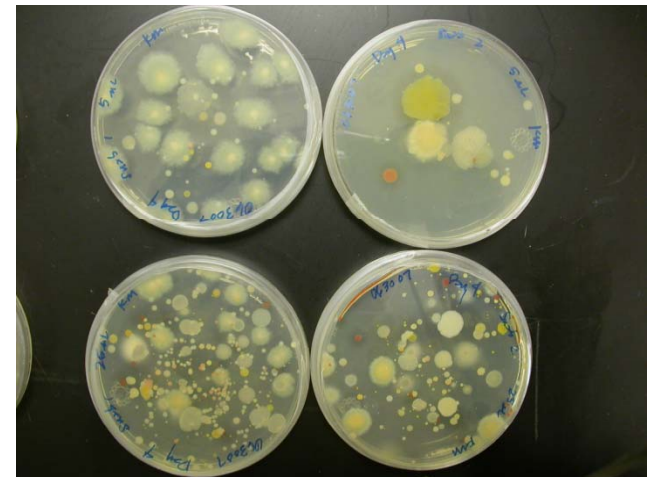


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# Introduction

- **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**

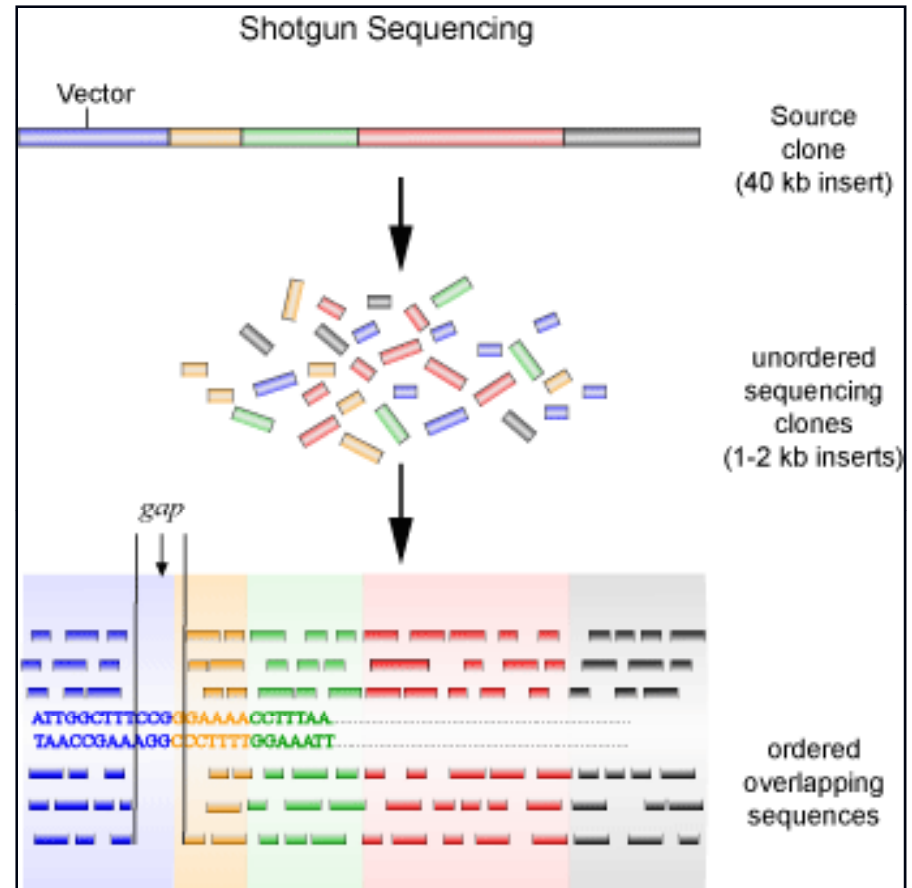


# Introduction

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

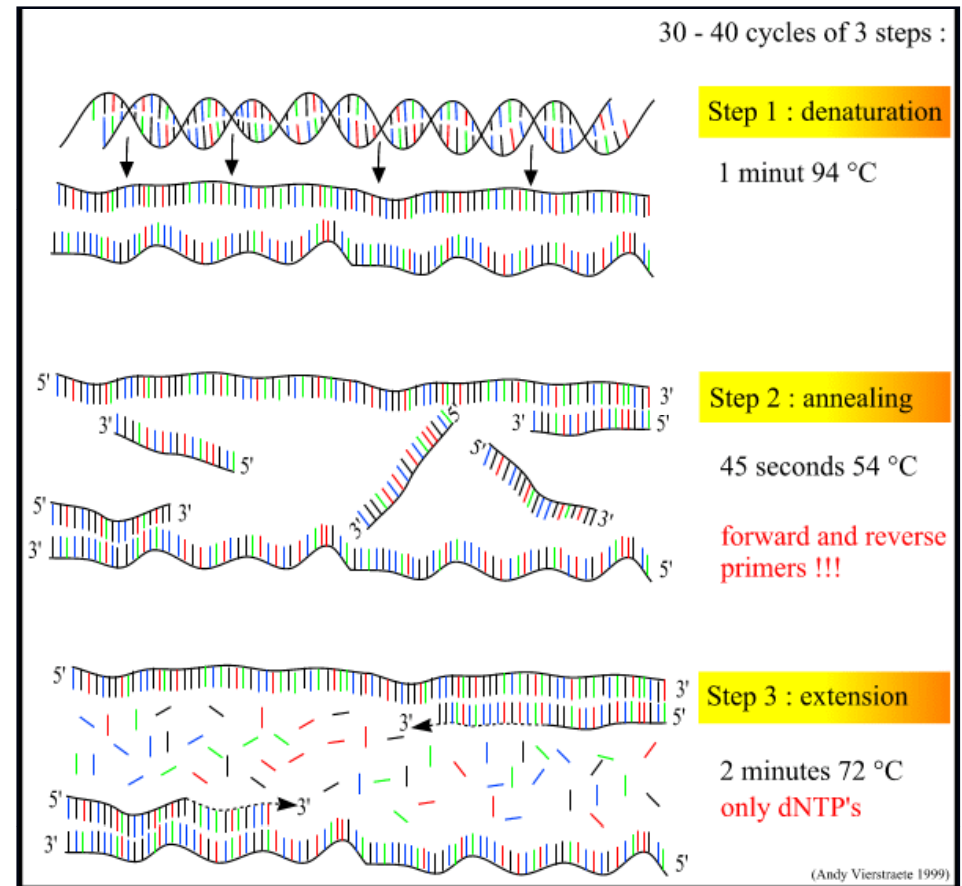
# Shotgun Method

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

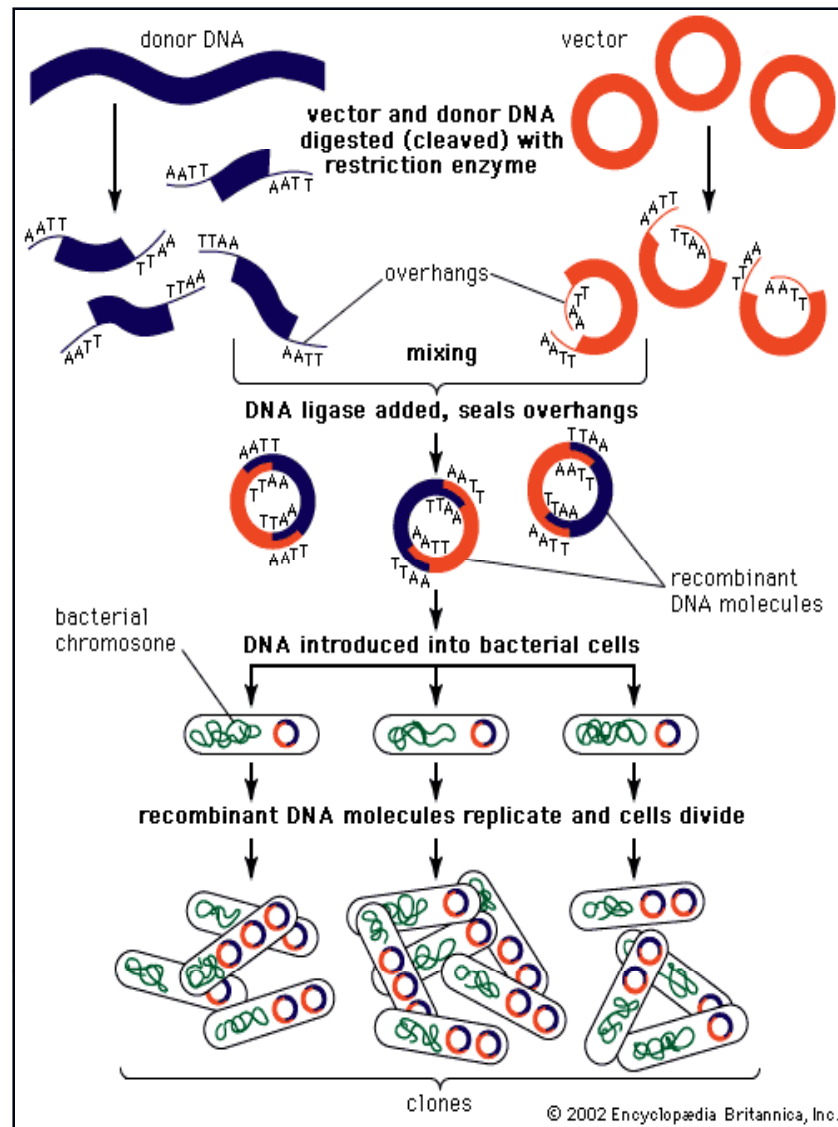


# PCR

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

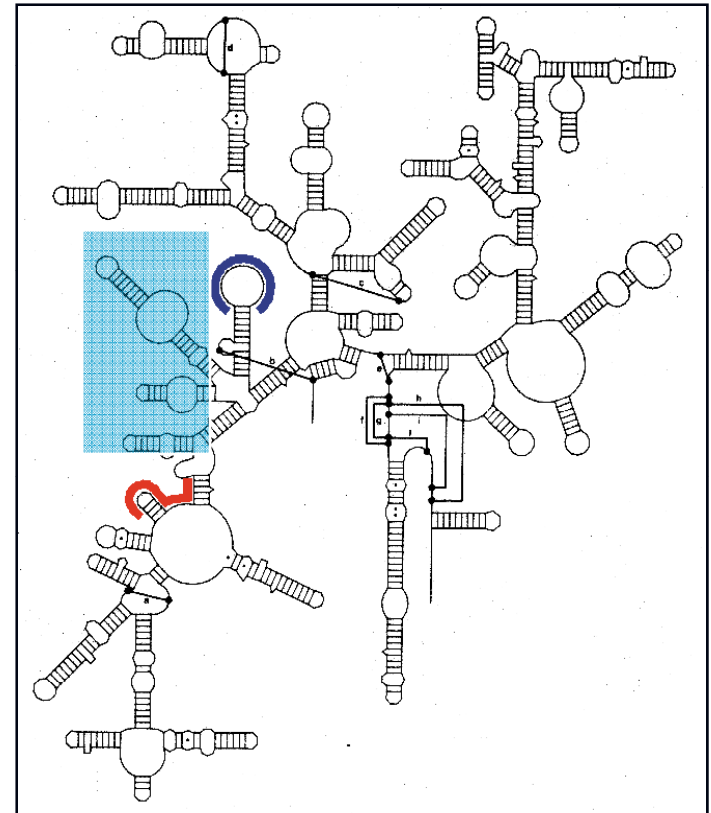


# Recombinant DNA Clones



# 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**

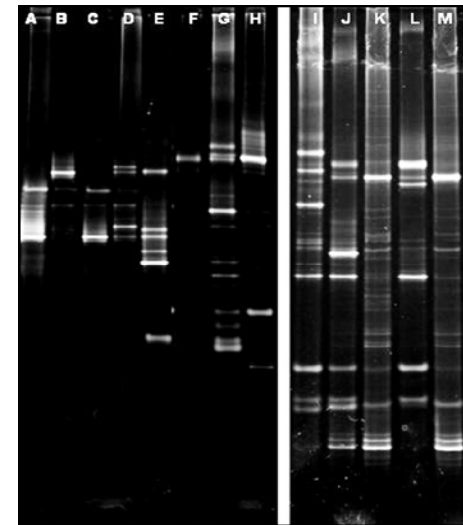
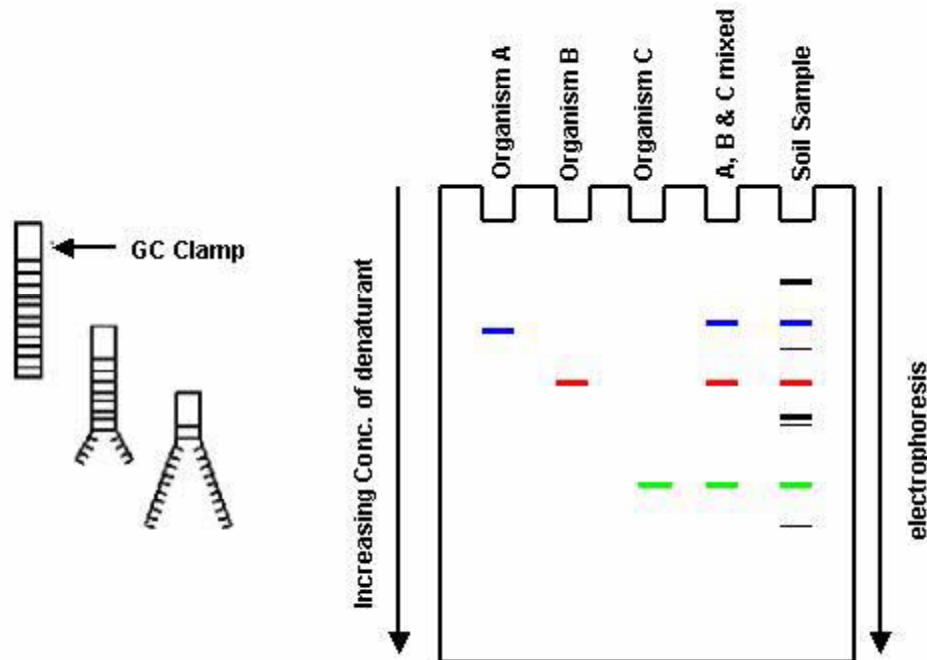


# Mutant vs. Wild Type

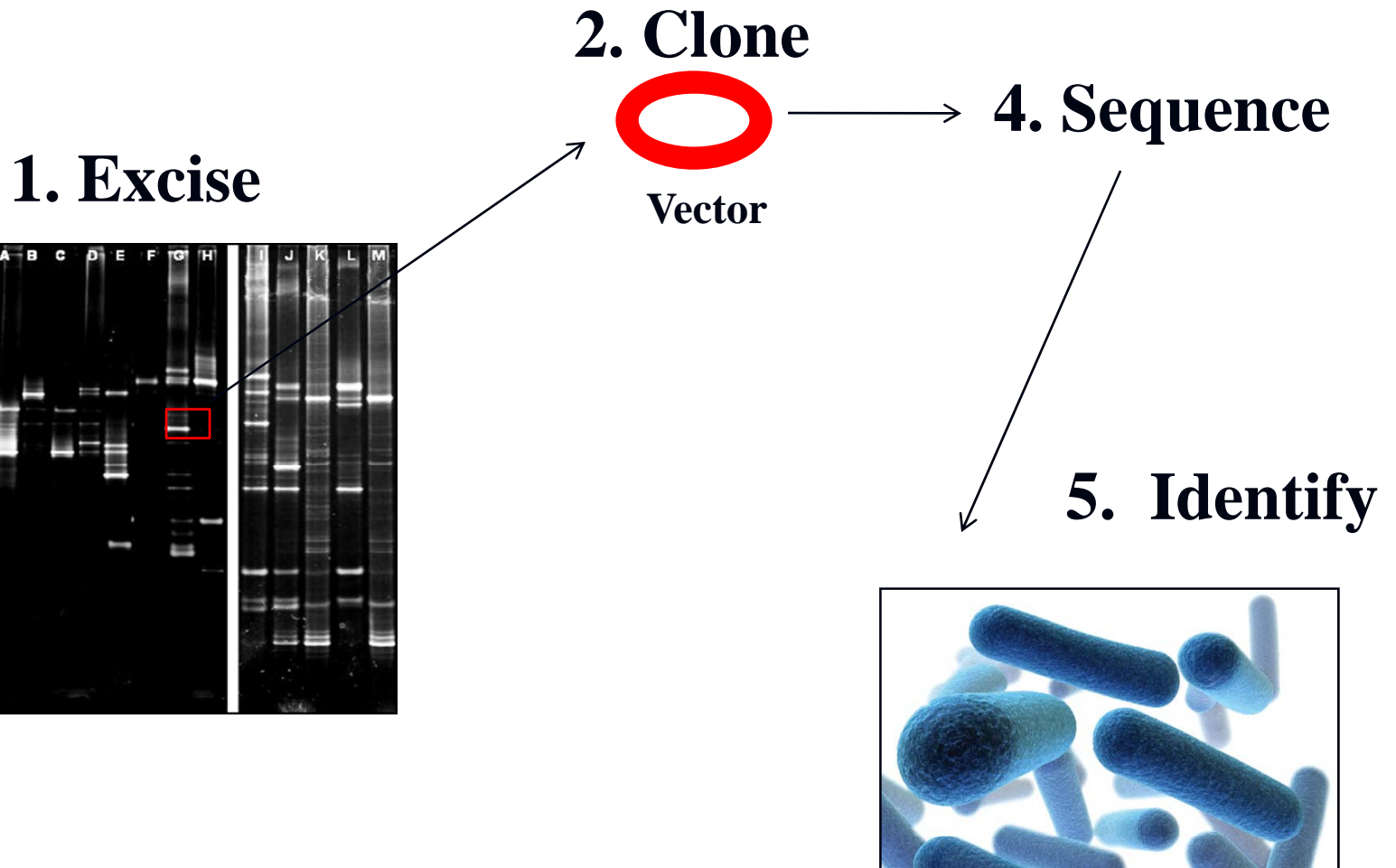
- **Loss of Function experiments**
  - **How: gene knockout**
  - **Why: pinpoint phenotypes controlled by genes**
- **Gain of functions – increased function**
- **Tracking –**
  - **How: WT gene replaced with ‘fusion’ gene**
  - **Why: Visualize gene modifications**
- **Gene Expression**
  - **How: 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.

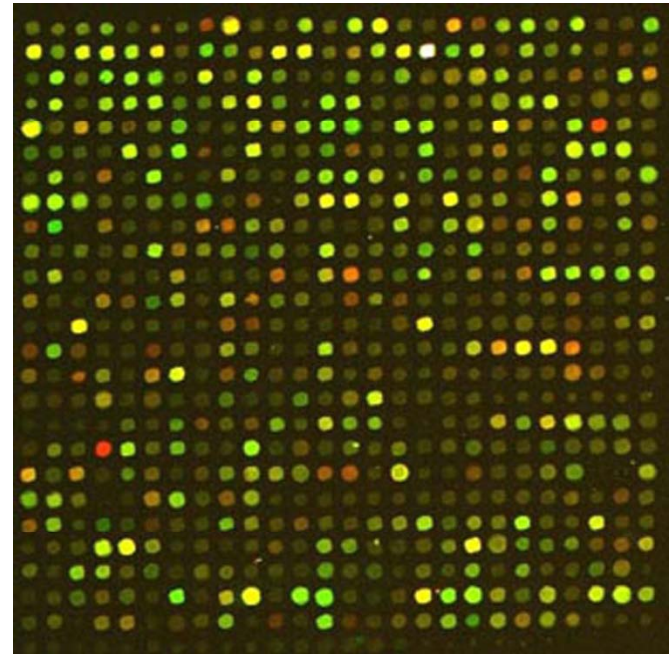


# DGGE



# DNA Micro-array

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

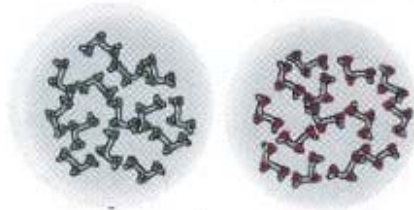


# DNA Micro-array

Make cDNA reverse transcript  
Label cDNAs with fluorescent dyes

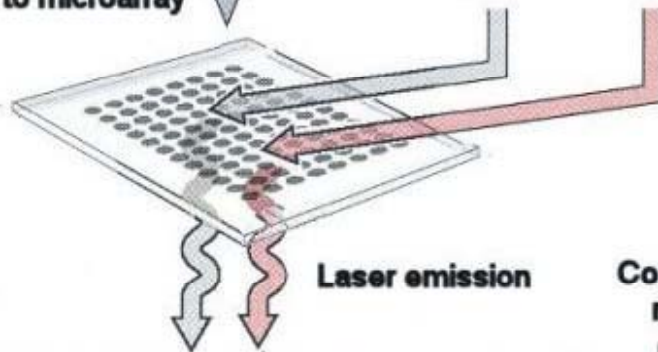
Control

Experimental



Hybridization to microarray

Laser excitation at dye-specific Hz

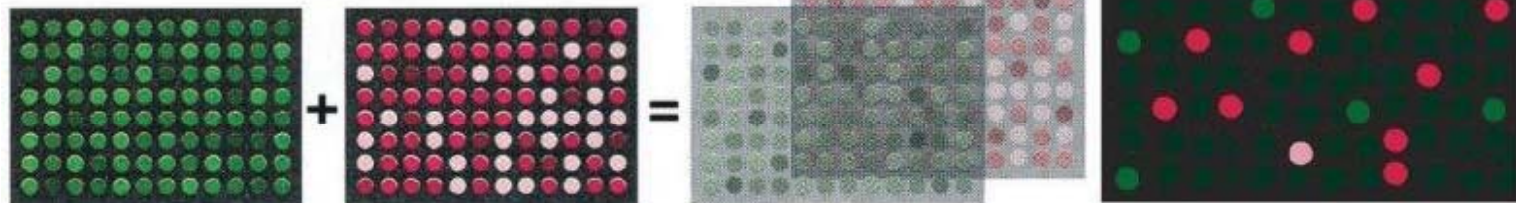


Principle of cDNA microarray assay for gene expression  
(after Gibson & Muse 2002)

Red = "up-regulation"

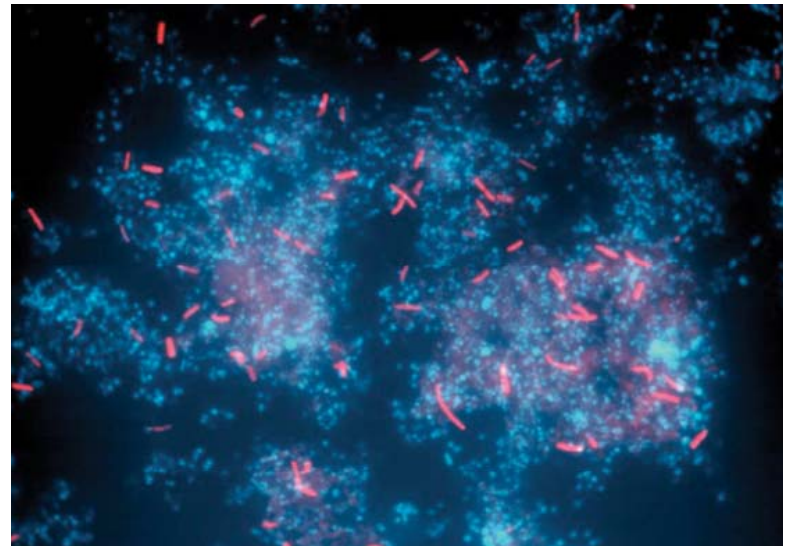
Green = "down-regulation"

Black = constitutive expression

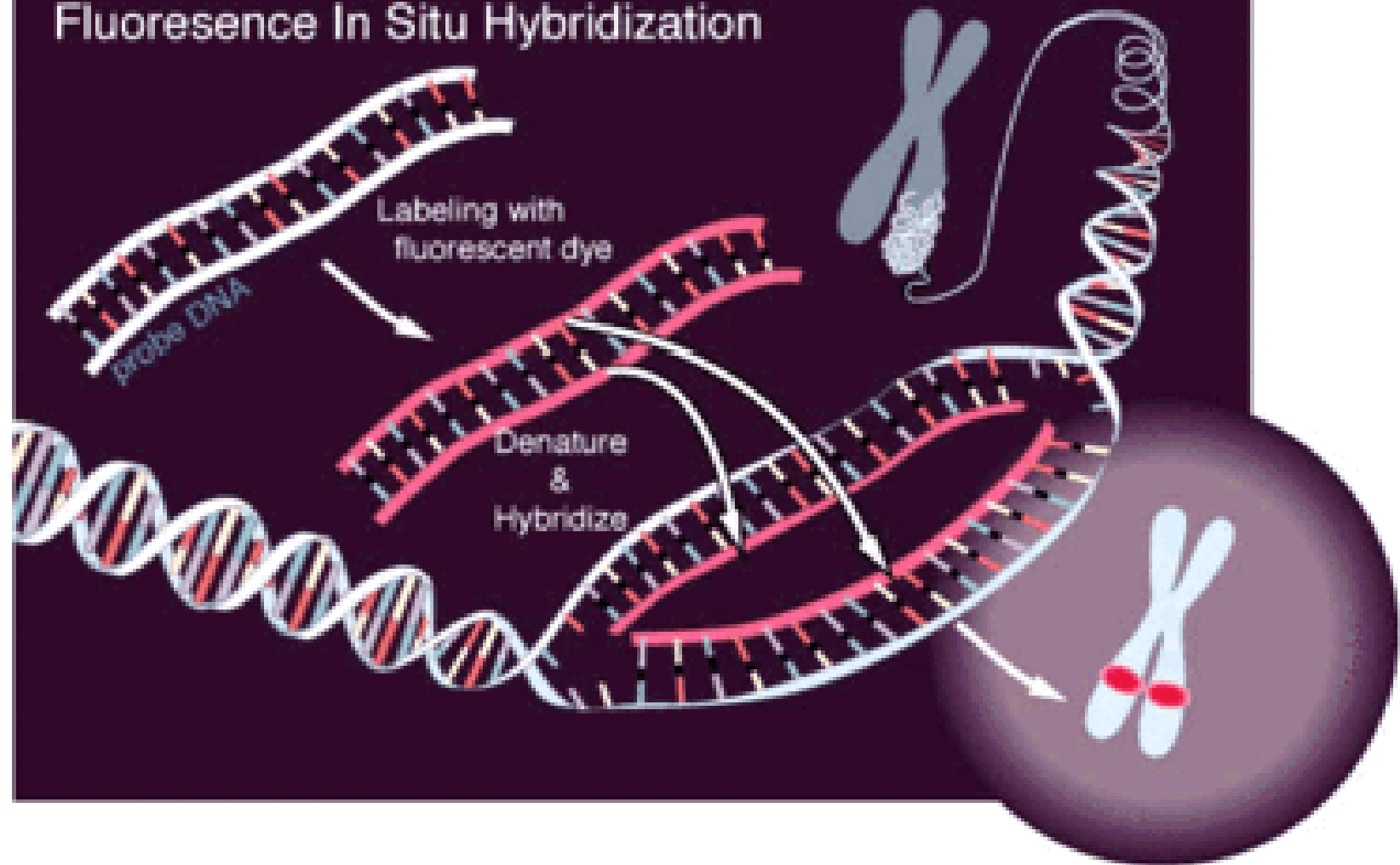


# Confocal Scanning Laser Microscopy

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



# Fluorescence In Situ Hybridization



# 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.**