



# Microarray-based Functional Analysis

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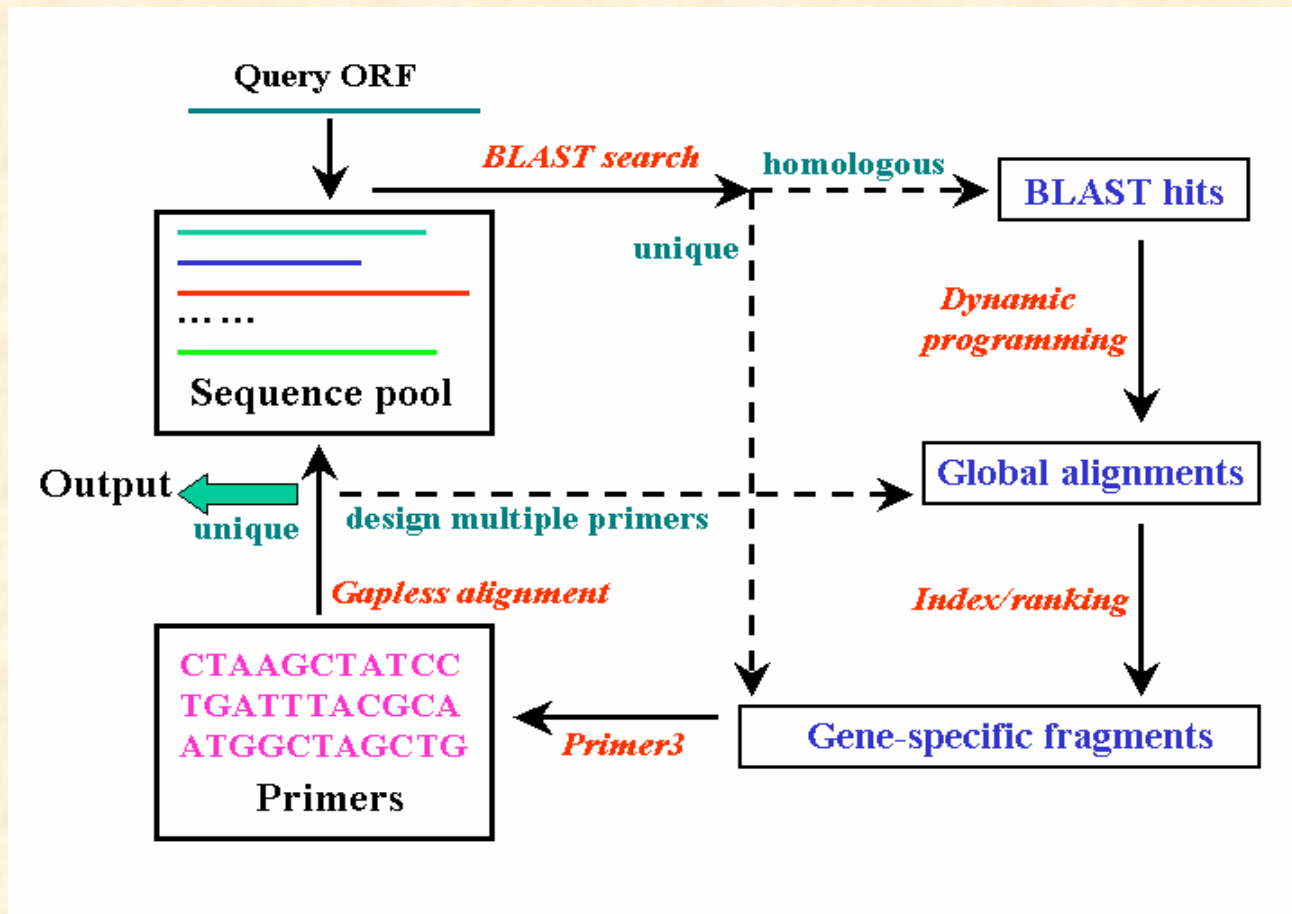
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# Outline

- **Construction and evaluation of whole genome microarrays**
- **Gene expression analysis using microarrays**
- **Mutagenesis and phage display**

# Designing primers for amplifying unique probes --- PRIMERGEN



DNA fragments less than 75% homology are used as probes.

Xu, D., G. Li, L. Wu, J.-Z. Zhou, and Y. Xu. 2002. *Bioinformatics*, 18(11):1432-1437, 2002.

# Preparation of PCR products for printing



20 ul PCR reaction using genomic DNA as template, gel check

100 ul PCR reaction in 96 well plates using PCR products as template, 8-32 X

Pool them together, purification with robots



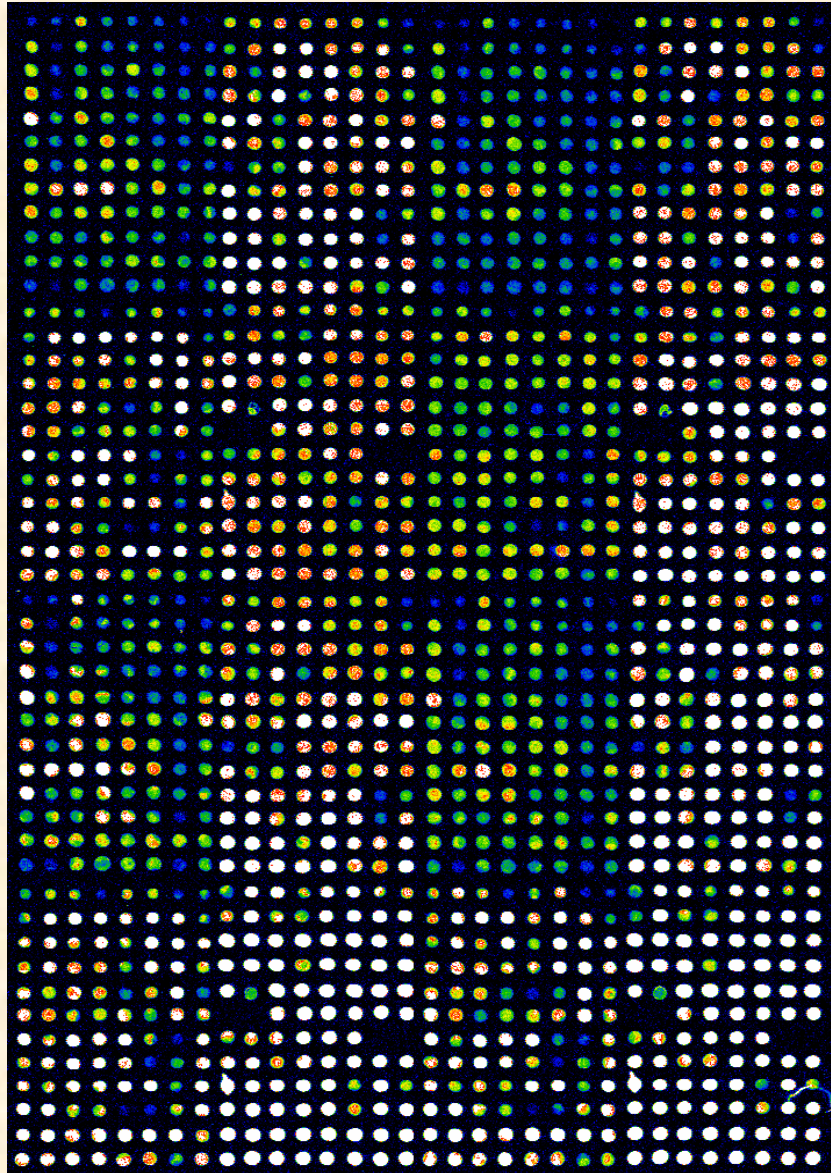
Dissolved the pellet in 50 ul, gel check for correct size, DNA concentration



Addition 50 ul of DMSO for printing



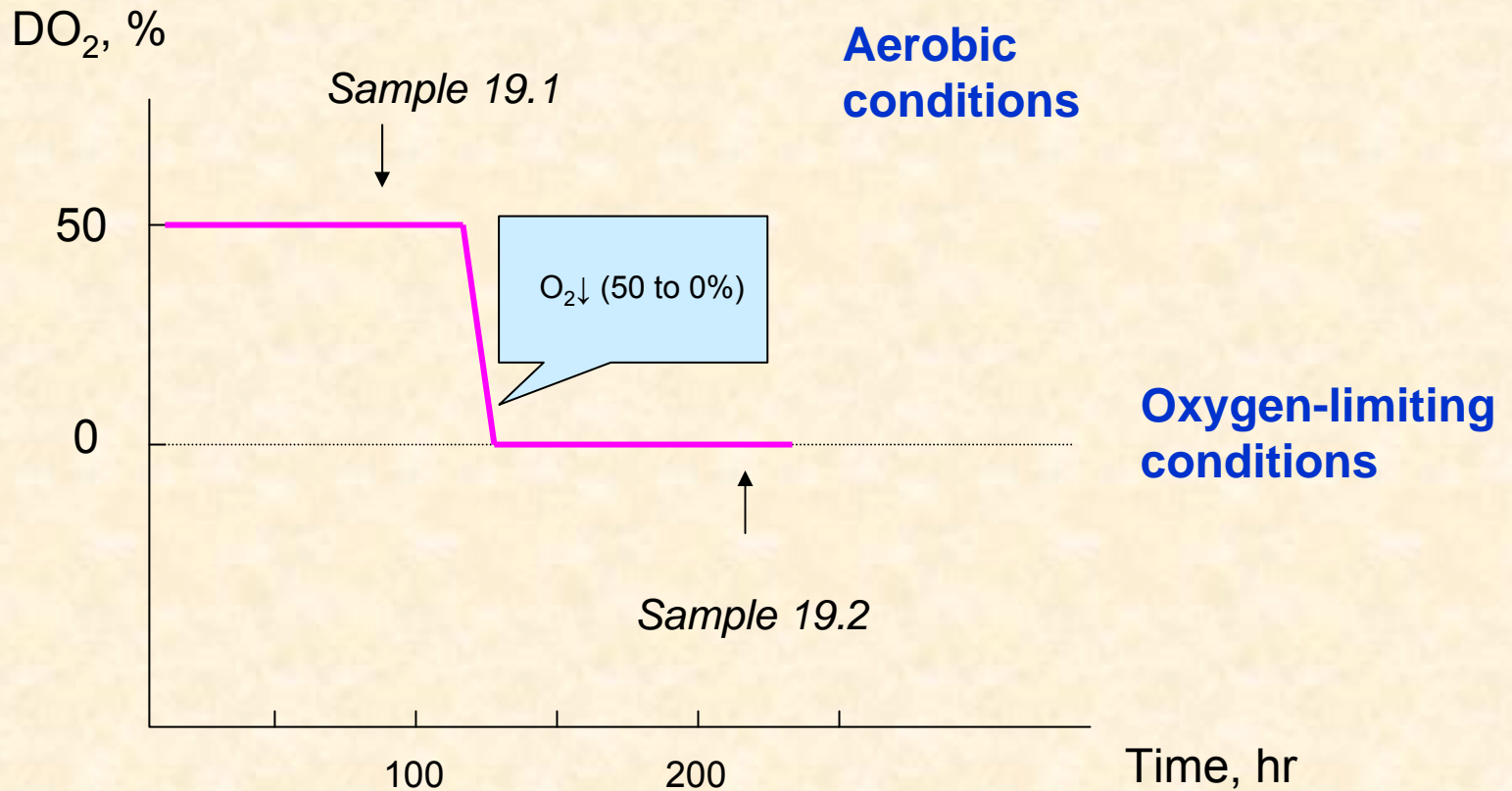
# Printing Quality Evaluation by POPO™-1 Dye Staining



- One slide of each batch was stained with POPO™-1 after CCD camera scanning and post processing.



# Aerobic-anaerobic experiment



M1 defined medium (3.0 L liquid volume)  
6 mM Na lactate, no  $\text{Ca}^{2+}$ , specific growth rate  $\mu=0.12$







# Microarray comparative profiling of gene expression patterns displayed under steady-state aerobic to oxygen-limiting growth conditions (CR-19)

 Genes induced under O<sub>2</sub>-limiting conditions     Genes repressed under O<sub>2</sub>-limiting conditions

Protein/amino acid biosynthesis:  **ribosomal proteins S, L  
glycyl-, phenylalanyl-, tyrosyl-tRNA synthetases  
Ala, Arg, Asp, Cys, Orn, Tyr biosynthetic genes**  
 **thiamine biosynthesis**

Intermediary carbon metabolism:  **acetyl-CoA metabolism, TCA cycle genes  
acetolactate synthetases II, III**  
 **isopropylmalate metabolism**

Energy metabolism / e<sup>-</sup>-transfer:  **cytochromes *cbb*<sub>3</sub>-, *d*- oxidases  
*omcA*, *fdh*, Ni/Fe hydrogenase  
NADH:ubiquinone reductase, ATP synthase**  
 **cytochrome *c*-oxidase, NADH dehydrogenase I**

Regulatory genes:  ***arcA*, *cheV*, *rseA*, sigma-70  
TetR-, RstA-family regulators**  
 **sigma-70 ECF family**

# Shewanella Federation

An Integrated Approach to the Study of Anaerobic Energy Metabolism

## Defining Gene Function through Deletion Mutagenesis

**GLOBAL REGULATORS:** *etrA*, *narQ*, *fur*, *crp*, *arcA*, *envZ*

**cAMP-BINDING REGULATORS:** *cAMP1*, *cAMP2*, *cAMP3*

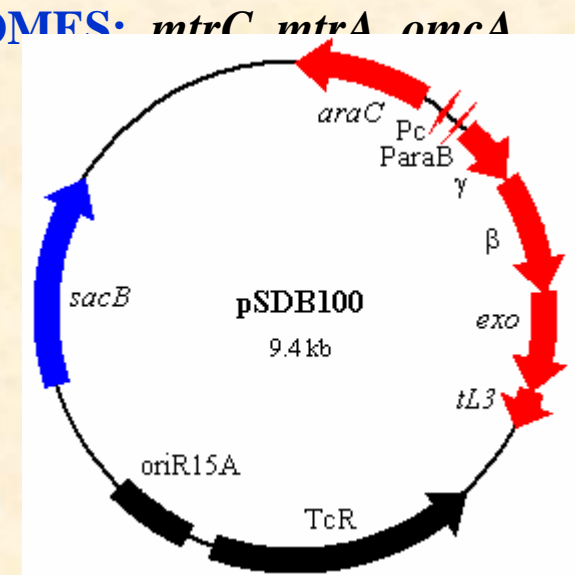
**ADENYLATE CYCLASES:** *cya1*, *cya2*, *cya3*

**OUTER MEMBRANE PROTEINS AND CYTOCHROMES:** *mtrC*, *mtrA*, *omcA*

**SIGMA FACTORS:** *rpoH*, *rpoE*

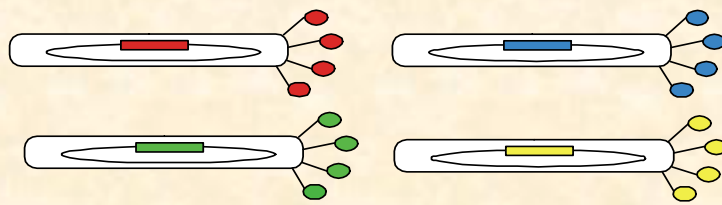
**STRESS RESPONSE:** *oxyR*, *bolA*, *dps*

**DOUBLE MUTANTS:** *etrA-fur*, *etrA-crp*, *cpxR-cpxA*

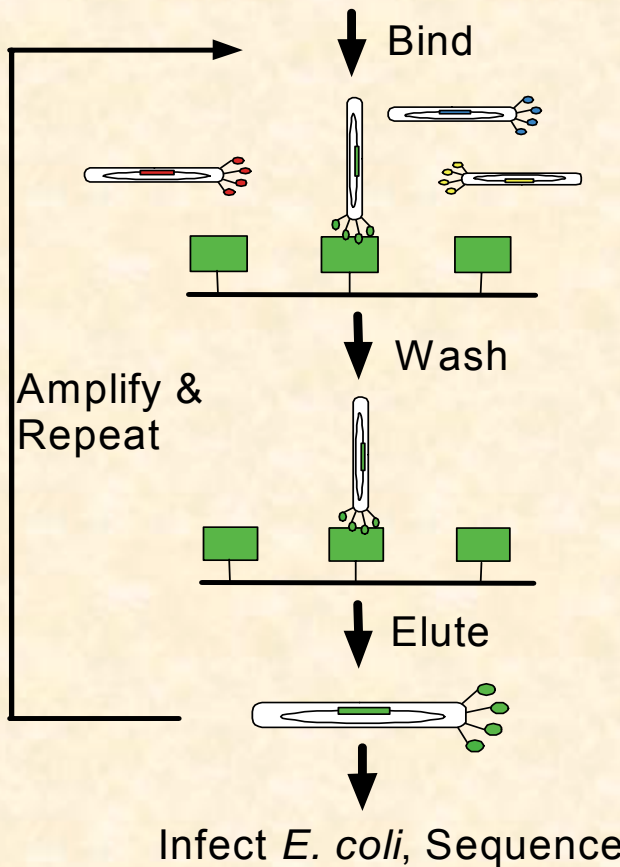




# Phage display for protein-ligand interaction



## Random phage library



Phage Display Libraries Constructed with Randomly Sheared Genomic DNA

Organism

Library size

*Escherichia coli* MG1655

$5.4 \times 10^6$

*Shewanella oneidensis* MR-1

$1.0 \times 10^7$

\*Insert size: 350-1000 base pairs

Cloning all *Shewanella* genes into universal vectors is in progress



# **The 11<sup>th</sup> International Conference on Microbial Genomes**

- **September 28 – October 2, 2003**
- **Durham, North Carolina**
- **Zhouj@ornl.gov**