

Microarray-based Functional Analysis

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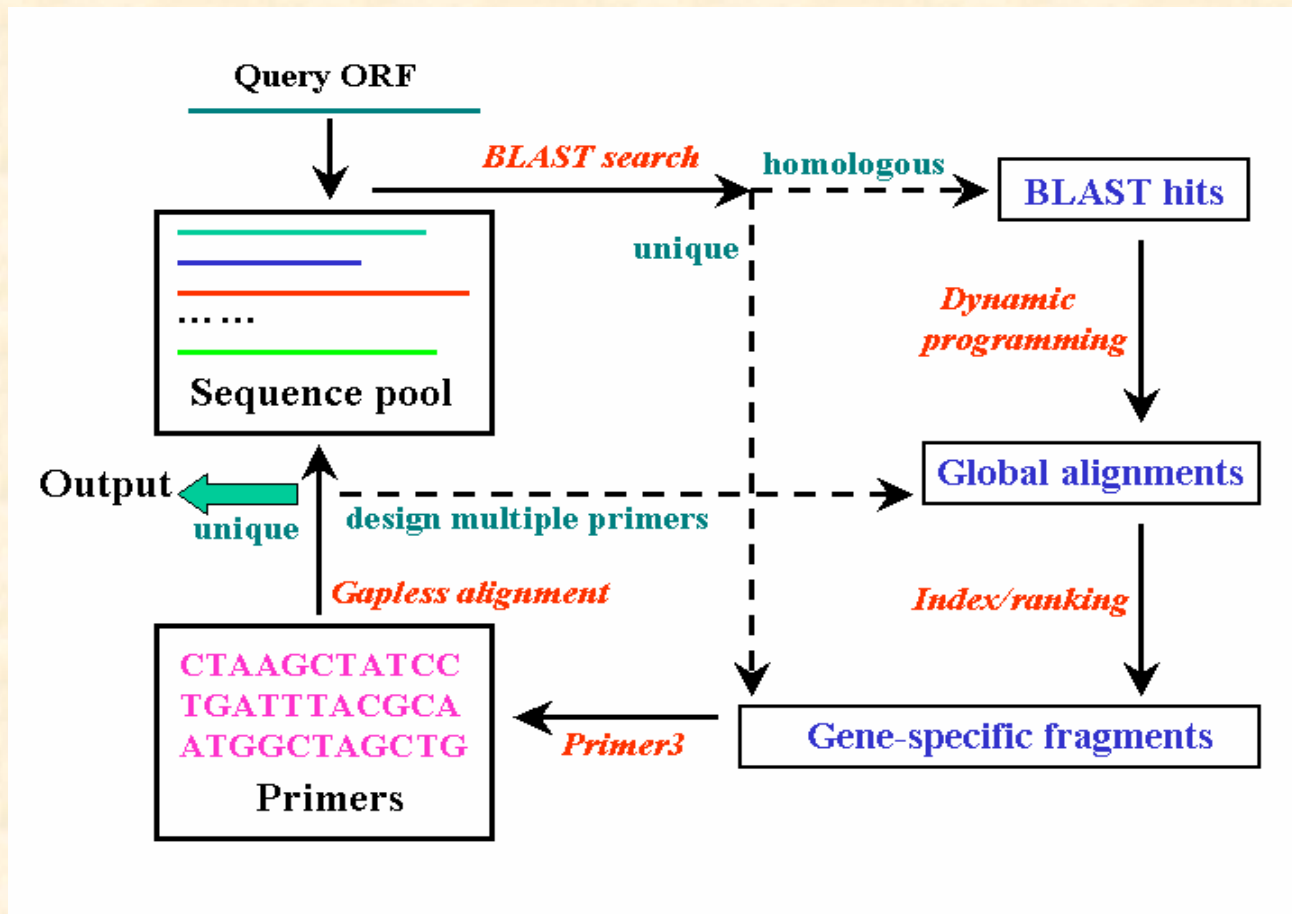
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Outline

- **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



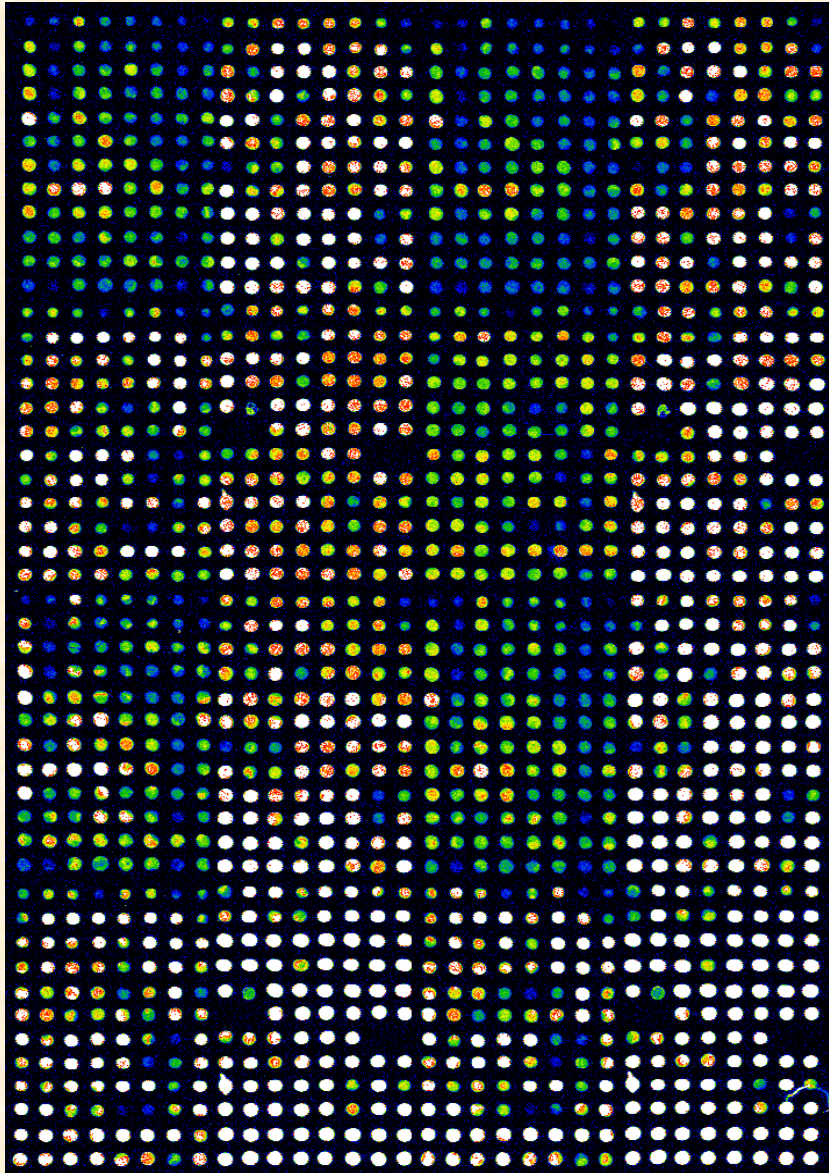
Dissolved the pellet in 50 ul, gel check for correct size, DNA concentration



Addition 50 ul of DMSO for printing



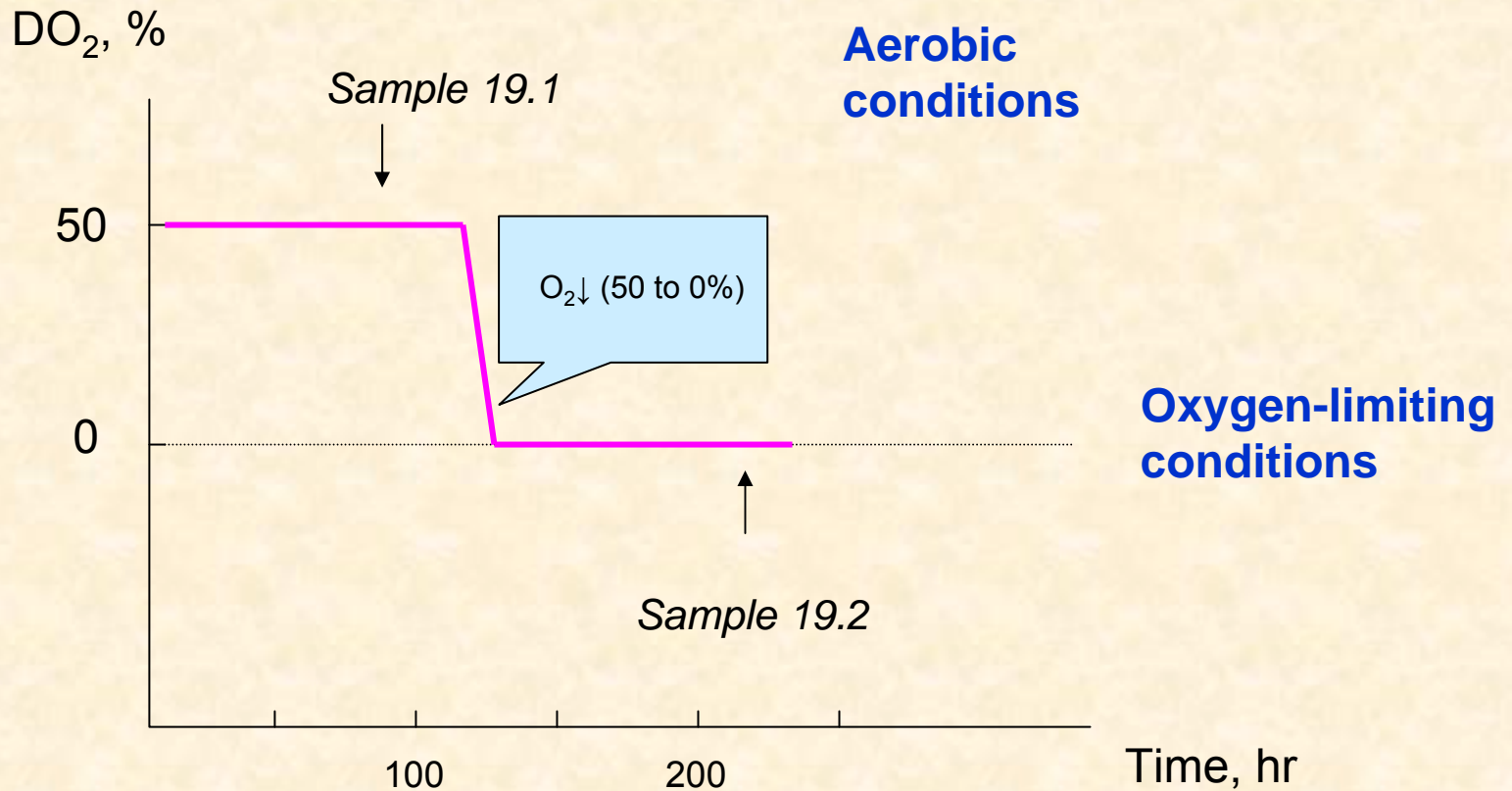
Printing Quality Evaluation by POPO™-1 Dye Staining



- 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 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 O₂-limiting conditions  Genes repressed under O₂-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⁻-transfer:  **cytochromes *cbb*₃-, *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*

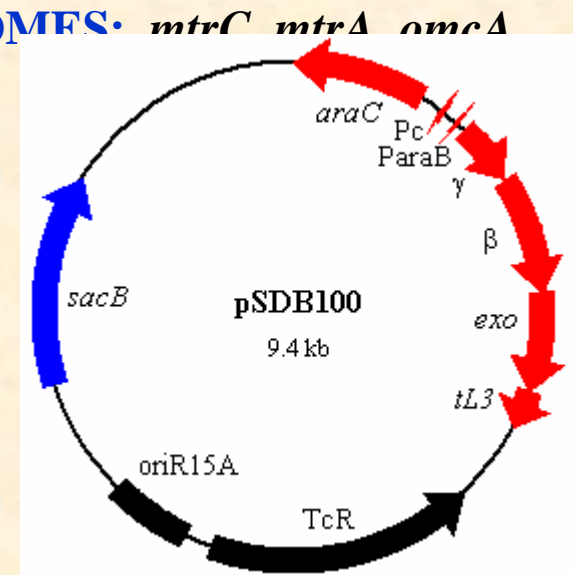
ADENYLATE CYCLASES: *cya1*, *cya2*, *cya3*

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

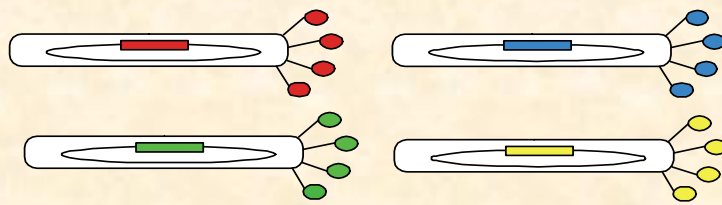
SIGMA FACTORS: *rpoH*, *rpoE*

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

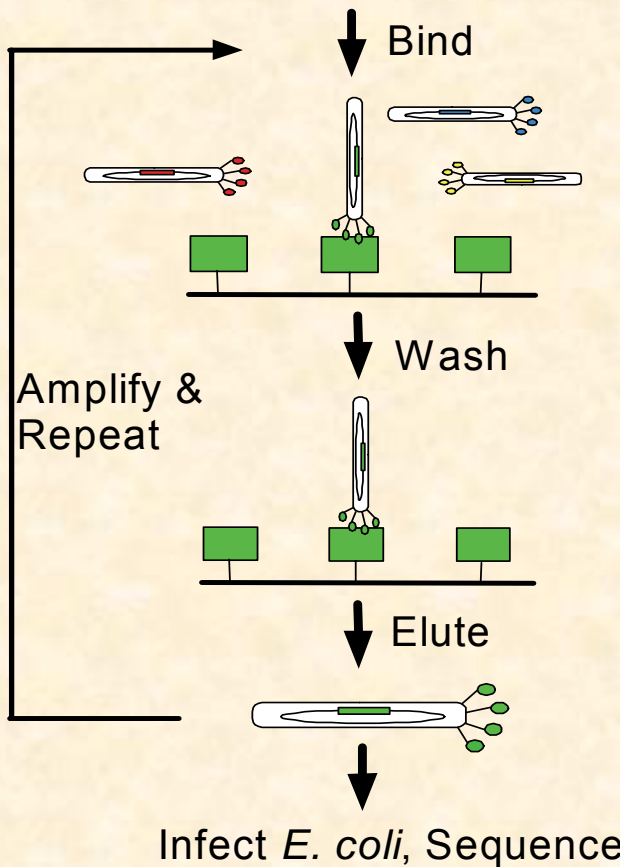
DOUBLE MUTANTS: *etrA-fur*, *etrA-crp*, *cpxR-cpxA*



Phage display for protein-ligand interaction



Random phage library



Phage Display Libraries Constructed with Randomly Sheared Genomic DNA

Organism

Library size

Escherichia coli MG1655

5.4×10^6

Shewanella oneidensis MR-1

1.0×10^7

*Insert size: 350-1000 base pairs

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



The 11th International Conference on Microbial Genomes

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