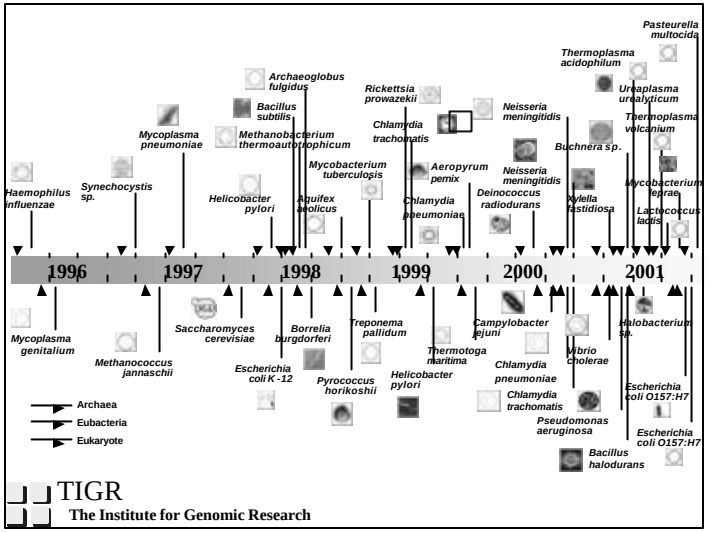
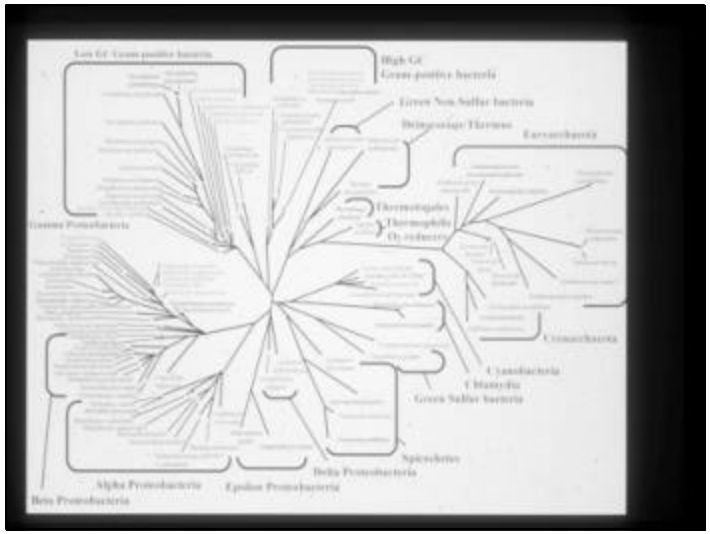
Microbial Genomics

Karen E. Nelson, Ph.D.
The Institute for Genomic Research
Rockville, MD.

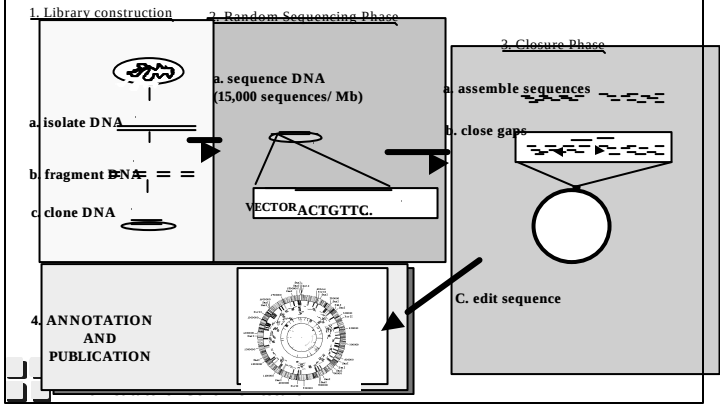
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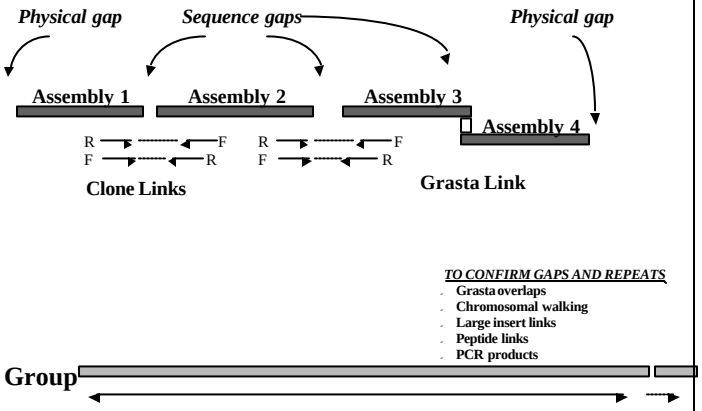


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Random Shotgun Sequencing





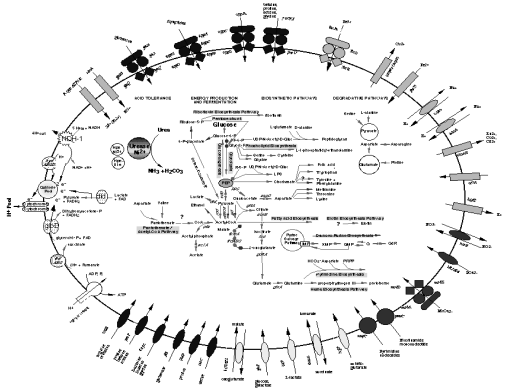
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Main Accomplishments from Sequencing :

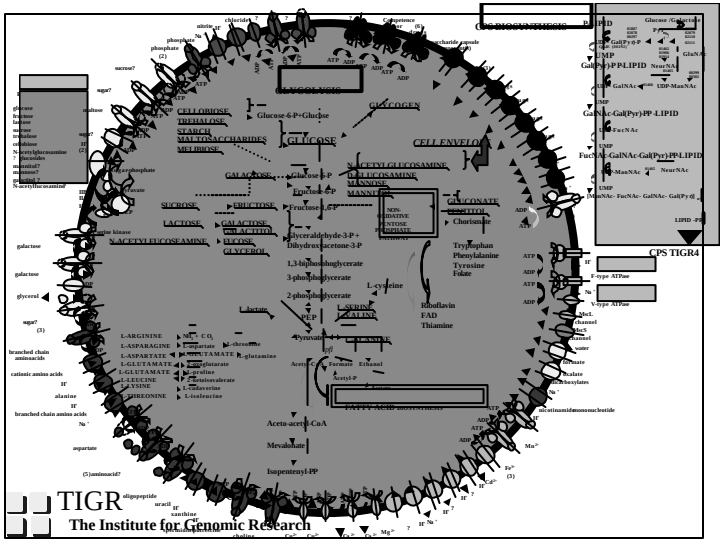
- Ability to reconstruct physiology profiles (growth requirements?)
- Identification of differences in metabolism and transporter profiles
- Transport capabilities dependent on niche
- Lateral/Horizontal Gene Transfer
- Genome rearrangements and Genome plasticity
- Genomic differences between closely related strains and species
- Initiation of proteomic and microarray studies

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Helicobacter pylori: Metabolism and Transport

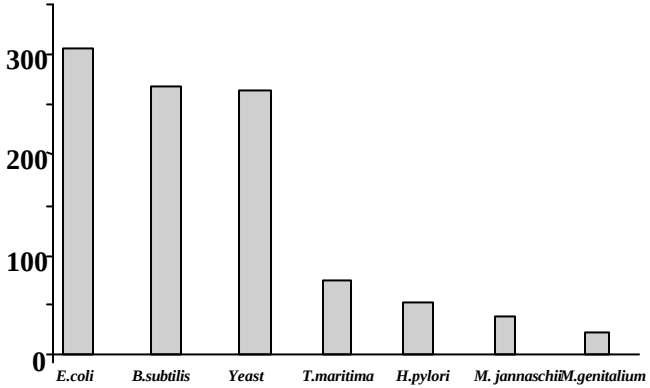


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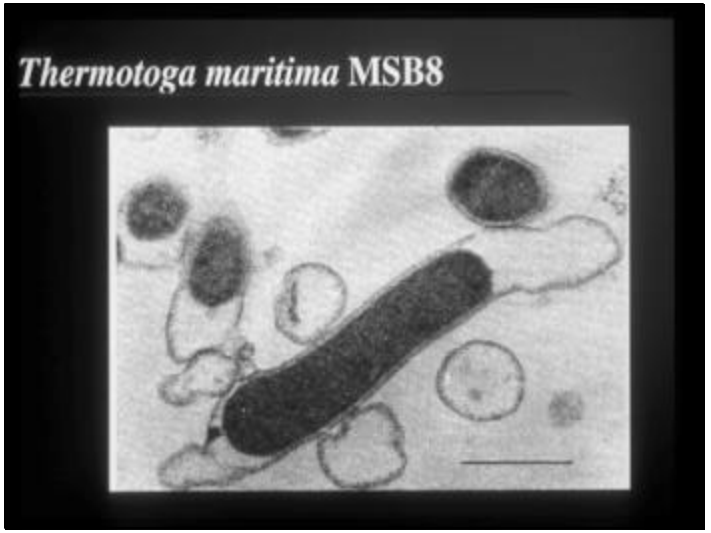


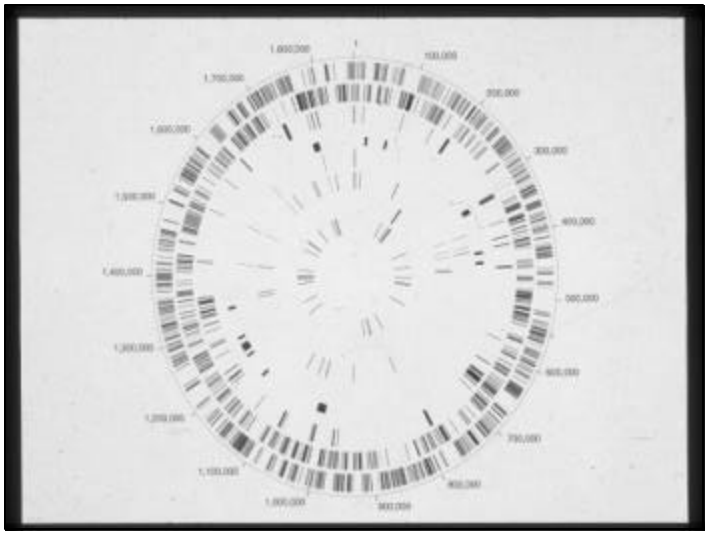
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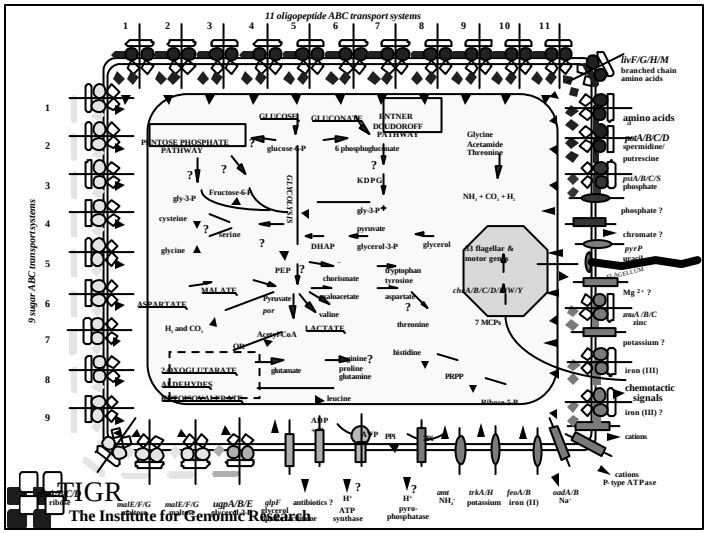
Number of Transport Systems



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Transporter profiles:

Archaea

- Phosphotransferase system completely lacking in Archaea
- Exhibit fewer net transporters
- Highest proportion of inorganic ion transporters
- Corresponding lack of transporters for organic carbon compounds
- Primarily a reflection of environmental niche

Thermotoga maritima displays highest percentage of ABC transporters for all completed genomes

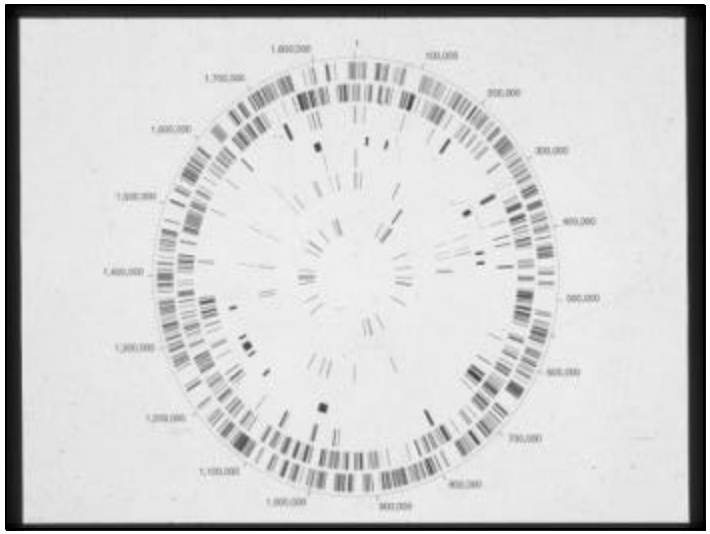
- Large number for sugars and oligopeptides
- Few for amino acids

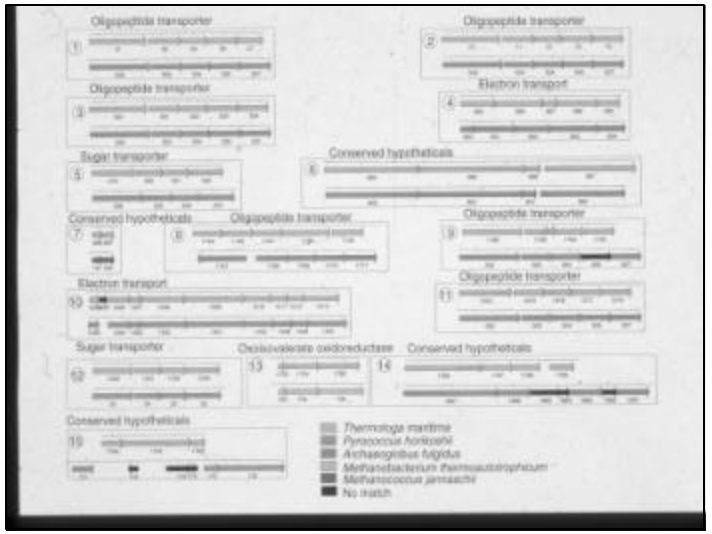
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? ^{2} analysis

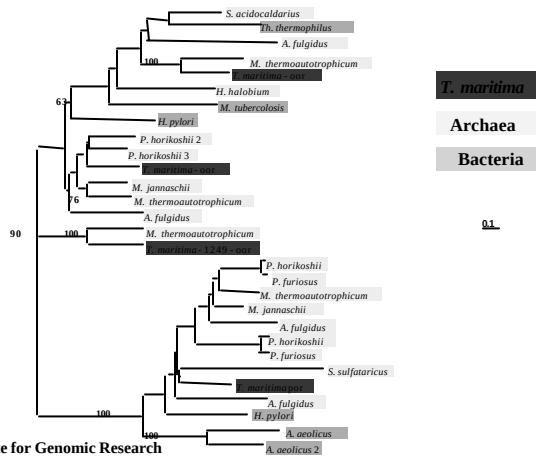
- 110 peaks/areas ($p < 1.9 \times 10^{-9}$)
- 51 regions of chromosome
- 42 regions have highest similarity to thermophiles
- 8 correspond to the 8 stretches of 30 bp short repeats
- 5 correspond to the 4 - 20 kb archaeal islands
- 1 to the ribosomal operon

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Thermotoga maritima (2-oxoisovalerate oxidoreductase, (? subunit) and Homologs



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Gene Transfer: *T.maritima*

- According to rRNA, *T. maritima* is a deep branching bacteria
- Other genes do not agree with rRNA tree
- From genome sequence, 24% of *T. maritima* genes are most similar to archaeal genes (many to *Pyrococcus* spp.)
- 81 of these archaeal-like genes are clustered, and share conserved gene order with archaeal counterparts
- Many regions of archaeal-like genes have atypical trinucleotide composition
- Some of these regions share a 30 bp repeat only found in thermop hiles
- Phylogenetic reconstructions support gene transfer

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Phylomes* of 7 Microbial Genomes

Organism	Genes/genome	Genes/Tree	Archaea only	Bacteria only	Eukarya only
R. prowazekii	837	47	16	464	121
C. pneumoniae	1052	29	18	421	85
B. burgdorferi	850	38	24	492	85
Synechocystis	3166	39	121	1131	471
A. aeolicus	1522	46	173	812	74
T. maritima	1849	47	318	859	59
P. abysiii	1765	35	893	341	43

* Phylome: the complete set of phylogenetic trees derived from the genome sequence

Sicheritz-Ponten and Anderssen , 2001

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Recent example of Horizontal Gene Transfer:

Maltose / Trehalose Region 16,583 nucleotides, only 173 nucleotide differences.

Pyrococcus furiosus and *Thermococcus litoralis*

J. Diruggiero, D. Dunn , D.L. Maeder, et al. (2000).
Evidence of recent lateral gene transfer among hyperthermophilic archaea.
Mol Microbiol. **38(4):** 684-93.

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Implications of Gene Transfers

- Web of Life; not a Tree of Life
- Genetic engineering is common in nature and can be extremely precise

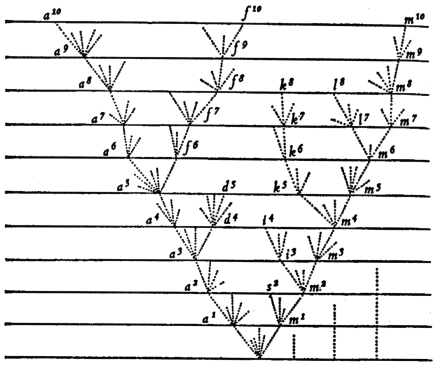
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Evolution and Lateral Gene Transfer

- Most early studies assumed species evolved primarily by *Vertical Inheritance* from parent or parents
- *Horizontal Inheritance* is now considered to be quite common
- Comparison of genome sequences is the primary way to study evolution of microbes and gives new light

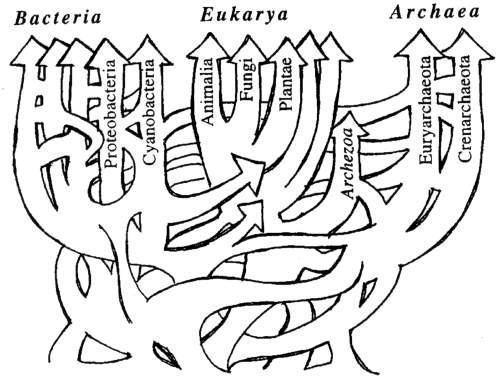
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Vertical Inheritance



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Horizontal Gene Transfer



Doolittle, 1999

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Known Examples of Gene Transfers:

- Antibiotic resistance genes on plasmid
- Insertion sequences
- Pathogenicity islands
- Toxin resistance genes on plasmids
- *Agrobacterium* Ti plasmid
- Viruses and viroids
- Organelle to nucleus transfers

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Examples of Horizontal Transfers from New Genome Sequences:

- *T. maritima* and Archaea
- *A. thaliana* chloroplast, mitochondria
- *D. radiodurans* chromosome II and megaplasmid
- *V. cholerae* chromosome II
- *N. meningitidis* and *H. influenzae* and *S. pneumoniae*

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Mechanisms of Horizontal Transfers

- Plasmid exchang
- Mating/conjugation
- Viruses and viroids
- Transposable elements
- Organelle to nucleus exchange
- Scavenging from environment
- Passive absorption
- Fusion of cells

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Implications of Horizontal Transfer

- Natural genetic engineering is very common, even across large evolutionary distances
- Genetically modified organisms will likely exchange genes with non GMOs
- More information on the frequency,the regulation, and the mechanisms of horizontal transfer would be beneficial

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Gene Evolution ≠ Species Evolution

- **Horizontal gene transfer**
- **Differences in mutation rates**
- **Gene duplication and extinction**

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Genome Plasticity:

***Sulfolobus solfataricus* genome**

200 diverse IS elements (10% of genome)
numerous mobile elements
evidence of integrase-mediated insertion events
long tandem repeats

She et al., 2001

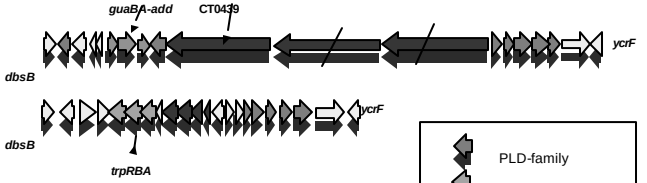
***Pyrococcus* spp. (*abyssi*, *horikoshii*, *furiosus*)**

differential conservation of genes
genetic elements mediating DNA reorganization
replication terminus rearrangements

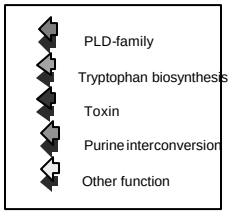
Lecompte et al., 2001

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***C. trachomatis* MoPn Plasticity Zone**



***C. trachomatis* serovar D Plasticity Zone**

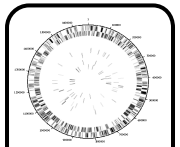


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Genomic potential for vaccine development

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STRATEGIES FOR MICROBIAL VACCINES



ALL POTENTIAL ANTIGENS

Microbe

3-12 months

HOURS

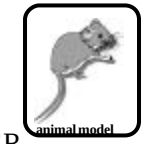
SELECTION OF VACCINE TARGETS



CD4⁺ EPITOPES
CD8⁺ EPITOPES
SECRETED PROTEINS
SURFACE PROTEINS

few months

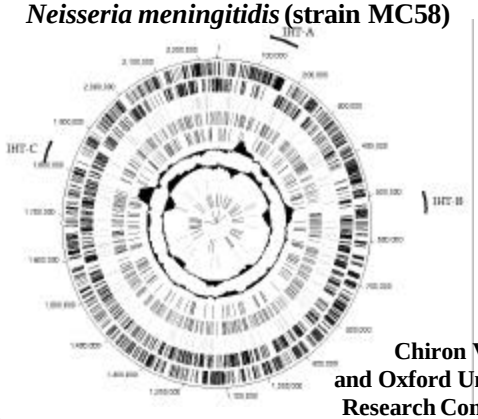
PURIFIED RECOMBINANT PROTEINS
SYNTHETIC PEPTIDES
LIVE RECOMBINANT INFECTIOUS AGENTS
DNA VACCINES



animal model

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Selected features of complete genome of Group B *Neisseria meningitidis* (strain MC58)

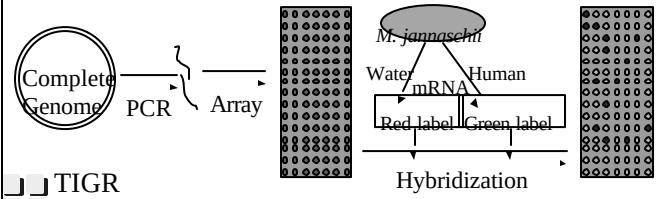


TIGR, Chiron Vaccines and Oxford University Research Consortium

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Microarray Technology to understand physiology

- Distinct spots of DNA that correspond to each gene are placed on a single microscope slide (up to about 10,000/slide)
- This can be used to detect the expression of message RNA (in bacteria this is often a good indication of protein synthesis).



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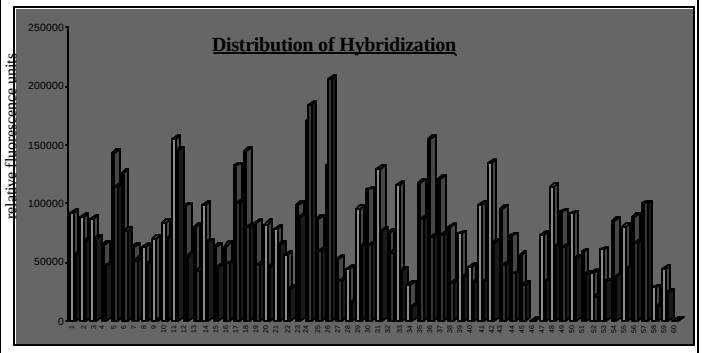
Microarray studies:

Thermotoga maritima
Methanobacterium thermoautotrophicum
Methanococcus jannaschii

- **Genes involved in thermophily**
- **Genes involved in methane production**
- **Life under saturated salt conditions**
- **Genes involved in carbohydrate metabolism**
- **Mechanisms for gene expression**
- **Detailed physiology of each organism**

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Microarray expression analysis of fifty-six selected *T. maritima* genes with potential involvement in carbohydrate metabolism.



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Gill et al., in press

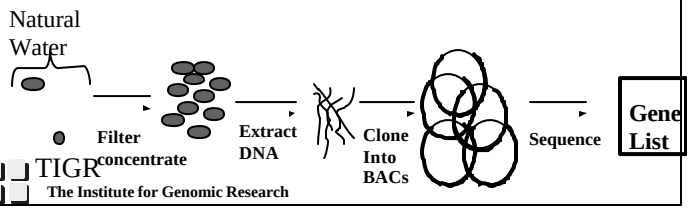
Potential for Exploring Microbial Diversity

- Problem: The vast majority of environmental microorganisms are not able to be cultured.
  - But they are the most abundant organisms on the planet, and are important in all biogeochemical cycles.
  - We need to better understand uncultured species to better determine how planetary scale anthropogenic changes will effect the Earth.

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Genomics that does not an require initial culturing step:

- Isolate, by filtration, all microorganisms in a water sample
- Extract total DNA in very large pieces
- Clone those pieces as BACs into *E.coli* to get enough.
- Sequence the BACs like a microbial genome.



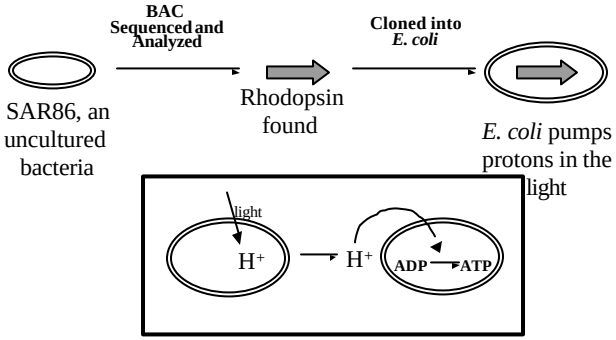
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Gene Discovery in Uncultured Bacteria

- **A completely sequenced BAC can be put through the standard microbial gene finding pipeline.**
  - **Genes can be assigned a function**
  - **Expressed in *E. coli***
  - **Gene function can be then experimentally determined (and we never grew the original species!)**

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Bacterial Rhodopsin:
a new photosynthesis system in the oceans



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Future Potential of Microbial Genomics: Pathogens

- Detection
  - Typing of strain variations and relationships of clinical isolates
  - More sensitive test for diagnosis
  - Detection of drug resistance
- Therapy
  - Identification of virulence factor genes
  - Identification of novel anti-microbial targets
  - Rational drug design
  - Organism-specific drug design
- Vaccine development
  - Attenuated strains
  - Subunit vaccines
  - DNA vaccines

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The Comprehensive Microbial Resource (CMR)

- Designed to facilitate comparative genomic studies on completed organisms
- Includes the genome and annotation of each completed genome
  - Associated information, Gram stain, taxon etc.
  - Structure and composition of the DNA molecule
  - Protein attributes eg. pI and molecular weight
- User can query all genomes at once or any desired subset
- Can make complex queries about any property of the organism
- Can be used to mine the databases in ways not possible with single genome databases

(http://www.tigr.org)

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