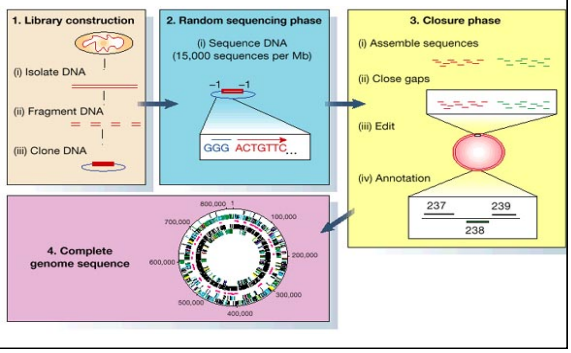
Fraser, Eisen, and Salzman **Shotgun genome sequencing**
Nature **406**, 799-803 (17 August 2000)



SEQUENCE DATA & BIOINFORMATICS

SEQUENCE DATA TYPE (What exactly are you looking at ?)
PCR amplicon, "Metagenome assembly", BAC sequence 454 pyrosequence reads, etc …

SAMPLE METADATA (Context is everything !)
Sample type, collection method, physics, chemistry, biology

DATA STANDARDS
Quality, voucher availability, 'ocean gene ontologies, MIGS/MIMS ?'

DATABASE STRUCTURE/ACCESSIBILITY
Genomic, Proteomic, Environmental, central vs, distributed, linkout, Federated databases, etc…

AVAILABILITY/ACCESSIBILITY of ANALYTICAL TOOLS
Genomic/Proteomic, Environmental, Polymorphs., Metagenome Analyses., Data cross comparisons

GENE EXPRESSION & PHYSIOLOGY

- MIAME-LIKE STANDARDS FOR ENVIRONMENTAL GENE EXPRESSION ?
  (*The MIAME Checklist:* Experiment Design, Samples used, extract preparation & labeling, Hybridization procedures and parameters, Measurement data and specs, Array design)
  (see http://www.mged.org/minseqe_
- CULTURE COLLECTIONS OF RELEVANT MICROBES
- STANDARD 'CHIPSETS', INTERNAL CONTROLS FOR ENVIRON. MONITORING, CROSS Q-PCR STANDARDIZATION, ETC..
- SGDB-LIKE MODEL FOR INTEGRATING HETERGENEOUS DATASETS ?
- CROSS COMPARISON/INTERCALIBRATION GENE X EXPER. (QPCR, ETC)

PROTEOMICS & BIOCHEMISTRY

- GENOME & PEPTIDE SEQUENCE DATABASES (& POLYMORPHISMS)
- ACCURATE MASS TAG DATABASES & EXPERIMENTAL DATA
- SAMPLES, VOUCHERS, AND ANITBODIES
- COORDINATED GENE EXPRESSION & PROTEOMIC STUDIES

DEALING WITH 'IMPEDANCE MISMATCH'

- **Data assimilation, analysis, archiving & integration**
  (Contemporary Biological (and Oceanographic) Science is largely Information Science !)
- **Field verification, process measurement & quantification**
  (Beyond *in silico* Bioinformatics and Towards Environmetnal Quantitative Biology)
- **Instrumentation/methods development - benchtop/in situ**
  (Make New Instruments, Measure New Things - the challenge of *in situ* measurement)
- **Scalar and disciplinary integration (the cultural gap)**
  (Earth Systems Science is Life Systems Science - better cross-talk required !)

How do you make sense of this ???????

TTCAATTGCCAAATCCATCACTAGATGAATGATTCTGAATCAGATATAAAATTACGTAATTGTAATAAGA
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TGCGTAAACAGATAACGAAAAGATTCGAATCAAATTTACAAATATAGTTCCAAGAATTCCTAAAATAAAA

Content Sensor

- **Extrinsic Content Sensors**: Local alignment, BLAST
  - Sequence from SWISSPROT, cDNA, EST
  - Intra- and inter- genomic similarity
- Depends on quality of database

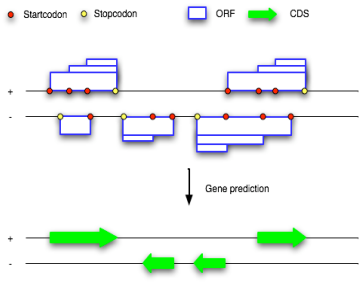
- **Intrinsic Content Sensors**: hexamer count
- HMM: Pr (Xlk previous nt), X = A, T, G, C
- GeneMark, GeneScan: 5 order
- 2 types of content sensors: 1 for coding , other for non-coding sequences.
- (some programs use both - e.g., CRITICA)

Gene Prediction Tools

- GENSCAN/Genome Scan
- TwinScan
- Glimmer
- GenMark
- Critica

ORF = open reading frame = start codon…[GATC]n…..stop codon

CDS = coding sequence (produces the actual protein/RNA species)



Annotation of bacterial and archaeal genomes: improving accuracy and consistency. Overbeek et al., Chem Rev. 2007. 107:3431-3447.

Softberry FGENESB annotation "pipeline". http://softberry.com/berry.phtml

STEP 1. Finds all potential ribosomal RNA genes using BLAST against bacterial and/or archaeal rRNA databases, and masks detected rRNA genes.

STEP 2. Predicts tRNA genes using tRNAscan-SE program (Washington University) and masks detected tRNA genes.

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STEP 6. Uses information about conservation of neighboring gene pairs in known genomes to improve operon prediction.

STEP 7. Runs BLASTP against NR for proteins having no COGs hits.

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STEP 9. Refines operon predictions using predicted promoters and terminators as additional evidences.

Typical softberry output

```
1     1 Op  1  21/0.000  +    CDS       407 -     1747   1311  ## COG0593 ATPase involved in DNA
                         +    Term     1786 -     1823    3.2
                         +    Prom     1847 -     1906   10.5
2     1 Op  2   3/0.019  +    CDS       1926 -    3065   1237  ## COG0592 DNA polymerase
                         +    Term     3074 -     3122    9.1
                         +    Prom     3105 -     3164    4.0
3     2 Op  1   4/0.002  +    CDS       3193 -     3405    278  ## COG2501 Uncharacterized ACR
4     2 Op  2   4/0.002  +    CDS       3418 -     4545    899  ## COG1195 Recombinational DNA
2       Op  3  16/0.000  +    CDS       4578 -     6506   2148  ## COG0187 DNA gyrase (topoisomerase II) B SU
                         +    Term     6516 -     6551    4.7
                         +    Prom     6512 -     6571    2.3
6     2 Op  4     .      +    CDS       6595 -     9066   2957  ## COG0188 DNA gyrase (topoisomerase II) A SU
                         +    Term     9067 -     9098    3.4
                         + SSU_RRNA     9308 -    10861  100.0  # AY138279 [D:1..1554] # 16S rRNA # B cereus
                         +    TRNA     10992 -    11068  101.2  # Ile GAT 0 0
                         +    TRNA     11077 -    11152   93.9  # Ala TGC 0 0
                         + LSU_RRNA    11233 -    14154   99.0  # AF267882 [D:1..2922] # 23S rRNA # Bacillus
7     3 Op  1                     .      -    CDS      14175 -     14363    158
                         + SS_RRNA     14205 -    14315   97.0  # AE017026 [D:165635..165750] # 5S rRNA # Bacil
8     3 Op  2     .      -    CDS       14353 -    15249    351  ## Similar_to_GB
9     3 Op  3     .      -    CDS       15170 -    15352     99
                         -    Prom     15373 -    15432    6.9
```

Expect Value (E) (Karlin-Altschul Statistics)

E = number of database hits you expect to find by chance, in a db of a given size

$E = Kmne^{-\lambda S}$ or $E = mn2^{-S'}$

m = query length

n = subject length (sequence or db)

K = scale for search space

λ = scale for scoring system

S'= bitscore = (λS – lnK)/ln2

More info: www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html

BLAST Executables & Programs

Executables:

blastall, megablast, blastpgp, bl2seq, blastclus

Blastall programs:

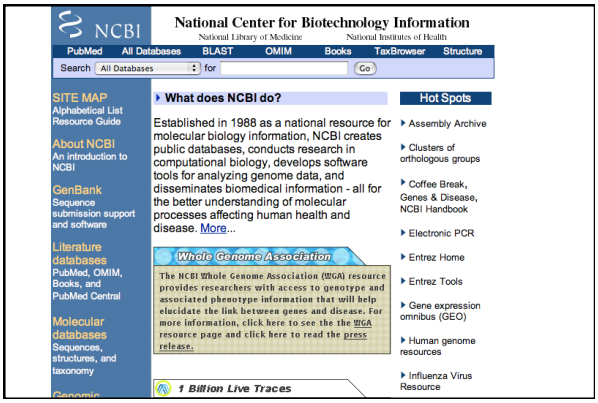
blastp, blastn, blastx, tblastn, tblastx

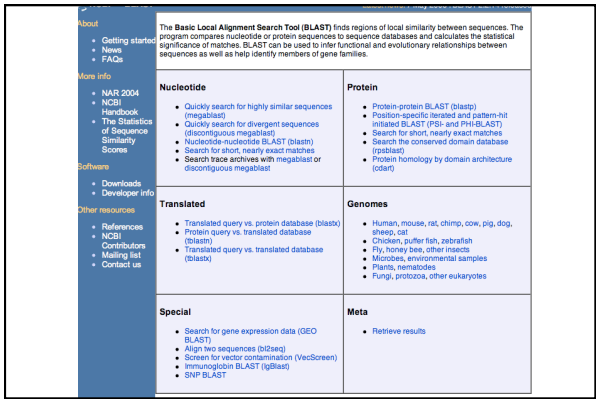
Bare minimum for blastall:

./blastall -p [program] -i [fasta file] -d [database] -o [output]

Several different BLAST programs:

Program	Description
blastp	Compares an amino acid query sequence against a protein sequence database.
blastn	Compares a nucleotide query sequence against a nucleotide sequence database.
blastx	Compares a nucleotide query sequence translated in all reading frames against a protein sequence database. You could use this option to find potential translation products of an unknown nucleotide sequence.
tblastn	Compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames.
tblastx	Compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. Please note that the tblastx program cannot be used with the nr database on the BLAST Web page because it is too computationally intensive.



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finished/draft
JGI
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Archaea
Eukarya
Viruses
Microbiomes
All Genomes
Grand Total
IMG/M is an experimental system that is based on the Integrated Microbial Genomes (IMG) system. IMG/M integrates microbial community (microbiome) genome data with IMG's isolate microbial genomes and provides support for the comparative analysis of the aggregate microbiome genomes (metagenomes) in the context of isolate microbial genomes and other metagenomes.
IMG/M will be presented on Monday, August 7th, at 10:30am and demonstrated on Wednesday, August 9th, at 9:50am, at ISMB 2006.
IMG/M will be also presented on Friday, August 25, 2006 at 12:30pm at ISME 11.
Experimental Version, March 1, 2006
Questions/Comments
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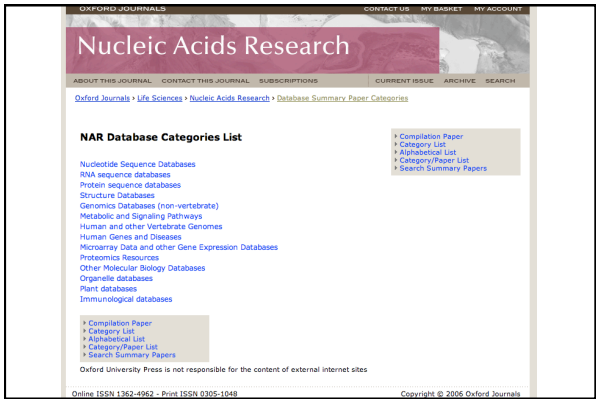
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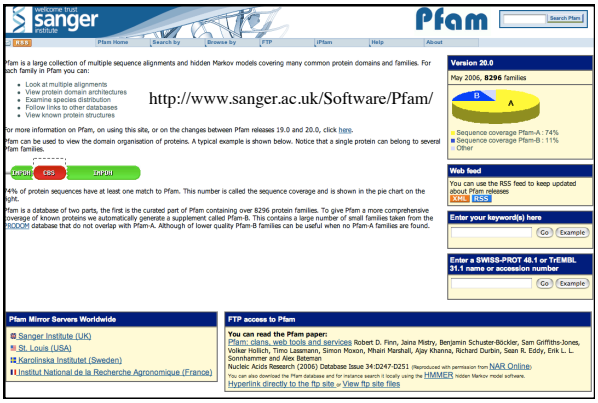
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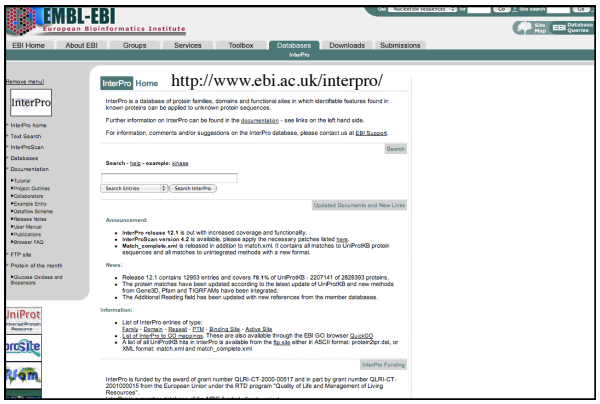
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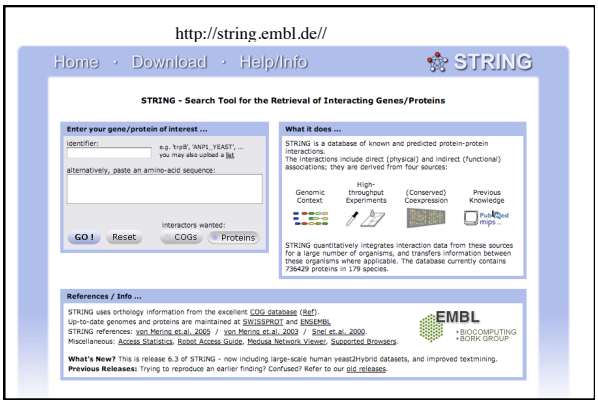
InterPro is a database of protein families, domains and functional sites in which identifiable features found in known proteins can be applied to unknown protein sequences.
Announcement:
News:
Information:
InterPro is funded by the award of grant number QLRI-CT-2000-00517 and in part by grant number QLRI-CT-2001000015 from the European Union under the RTD program "Quality of Life and Management of Living Resources".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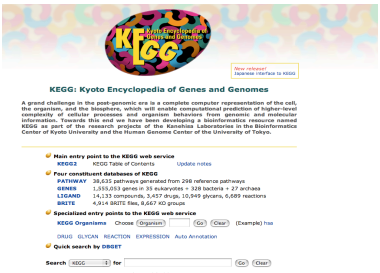
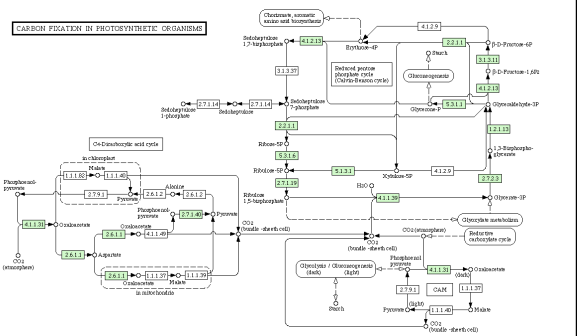
Previous Releases: Trying to reproduce an earlier finding? Confused? Refer to our old releases.

KEGG
Kyoto Encyclopedia of genes and genomes

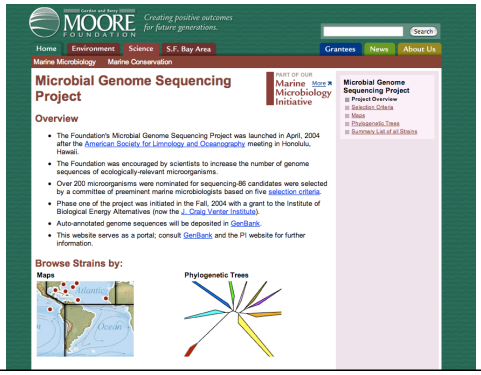
http://www.genome.jp/kegg/



Prochlorococcus MED4 Carbon fixation



http://www.moore.org/microgenome/



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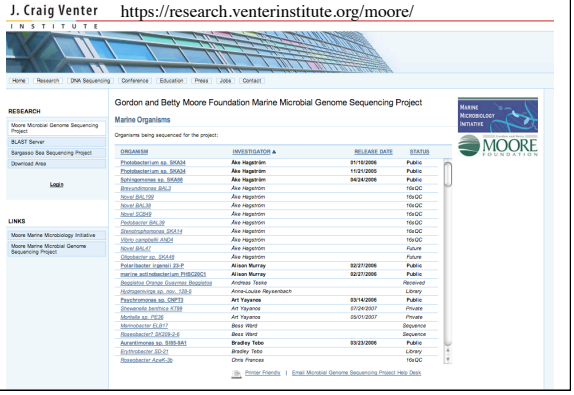

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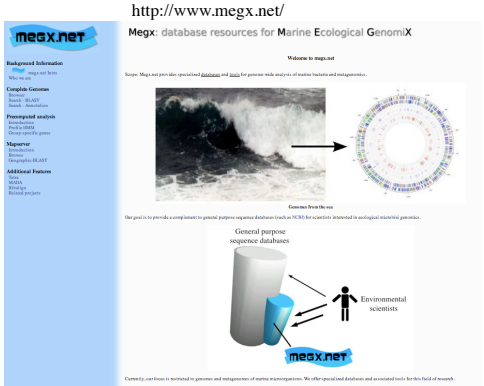

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http://www.ncbi.nih.gov/

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http://img.jgi.doe.gov/cgi-bin/m/main.cgi

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http://www.microbial-ecology.net/probebase/

http://www.arb-home.de/

http://rdp.cme.msu.edu/

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http://www.genome.jp/kegg/

http://www.ncbi.nlm.nih.gov/COG/

http://www.sanger.ac.uk/Software/Pfam/

http://www.ebi.ac.uk/interpro/

http://string.embl.de

Gene Ontology (GO; not a database - rather, sequence semantics !)
http://www.geneontology.org/GO.doc.shtml

FGENESB Suite of Bacterial Operon and Gene Finding Programs

FGENESB automatic annotation of bacterial and archaeal genomes. The FGENESB gene algorithm is based on Markov chain models of coding regions and translation and termination sites.

Features

- Automatic training of gene finding parameters for new bac terial genomes using only genomic DNA as an i nput (optionally, pre-learned parameters from related organism can be used)
- Mapping of tRNA and rRNA genes
- Highly accurate Markov chains-based gene prediction_Prediction of promoters and terminators
- Operon prediction based on distances between ORFs and f requencies of different genes neighboring each other in known bacterial genomes, as we ll as on promoter and terminator predictions
- Automatic annotation of predicted genes by homology with COG and NR databases.
- FGENESB gene prediction algorithm is based on Markov chain models of coding regions and translation and termination sites.

Softberry FGENESB annotation "pipeline". http://softberry.com/berry.phtml

STEP 1. Finds all potential ribosomal RNA genes using BLAST against bacterial and/or archaeal rRNA databases, and masks detected rRNA genes.

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                          +    TRNA    10992 -    11068  101.2  # Ile GAT 0 0
                          +    TRNA    11077 -    11152   93.9  # Ala TGC 0 0
                          + LSU_RRNA   11233 -    14154   99.0  # AF267882 [D:1..2922] # 23S rRNA # Bacillus
7     3 Op  1                       .       -    CDS      14175 -    14363    158
                          + 5S_RRNA    14205 -    14315   97.0  # AE017026 [D:165635..165750] # 5S rRNA # Bacil
8     3 Op  2     .       -    CDS      14353 -    15249    351  ## Similar_to_GB
9     3 Op  3     .       -    CDS      15170 -    15352     99
                          -    Prom     15373 -    15432    6.9
```