SRI International

Pathway Tools | BioCyc

Pathway Tools Software and BioCyc.org Genome and Metabolic Pathway Web Portal

SRI International offers innovative tools for modeling and analyzing genomes, metabolic pathways, and regulatory networks to support activities in drug discovery, agriculture, and biotechnology. These tools accelerate research and lead to a greater understanding of biological systems.

SRI's BioCyc.org web portal contains 19,000 Pathway/Genome Databases (PGDBs) for sequenced genomes. A PGDB contains the entire genome of an organism, as well as its biochemical pathways and (when curated) its regulatory network. Two BioCyc databases, EcoCyc[1] and MetaCyc[2] are derived from more than three decades of literature-based curation of genome and pathway data. The HumanCyc database provides a unique collection of human metabolic pathway data. The BioCyc.org website is used each year by more than 600,000 people worldwide.

The downloadable Pathway Tools software,[3] licensed to date to more than 11,000 groups, is apathway/genome data management system that enables creation of a private BioCyc portal, and/or creation of BioCyc-like portals containing custom genome data. The software can ingest proprietary genome data, compute a metabolic reconstruction, then make the genome accessible through a large number of search, visualization, and comparative-analysis tools. Data can be accessed using a web browser and through a desktop application. Multiple tools for transcriptomics and metabolomics data analysis are provided.

Pathway Tools is free for academic research and teaching purposes.

> *"For Ginkgo scientists, BioCyc and Pathway Tools are the go-to resources of knowledge and software for exploring ideas, answering questions, and analyzing data."*
>
> —**Ginkgo Bioworks**

> *"I'm impressed with the level of detail and care in BioCyc annotations. It is a tremendous resource for pathway analysis in metabolomics."*
>
> — **Prof. Art Edison, University of Georgia**

APPLICATIONS OF BIOCYC AND PATHWAY TOOLS

BioCyc and Pathway Tools span genome informatics, pathway informatics, and regulatory informatics. Applications include:

- **Enterprise Genome Data Management:** Extensive search tools to speed information finding; multiple visualization tools expedite user uptake of information.
- **Computational Inferences:** Predict metabolic pathways, genes coding for missing enzymes in metabolic pathways, protein complexes, and operons, from an annotated genome or metagenome.

PATHWAY TOOLS

- **Enterprise pathway/genome data management**
- **Desktop and web operation**
- **Computational prediction of metabolic pathways, operons, protein complexes**
- **Incorporate selected BioCyc databases**
- **Quantitative metabolic modeling**
- **Includes BioCyc tools**

BIOCYC

- 19,000 Pathway/Genome Databases
- 60 databases curated from 130,000 publications
- Extensive mini-reviews for genes and pathways
- Most comprehensive pathway database: MetaCyc
- Three releases per year
- BioCyc.org Tools:
  - Search, visualization, and comparative analysis of genome and pathway information
  - Transcriptomics data analysis
  - Metabolomics data analysis
  - Metabolic route search

- **Microbiome Analysis:** Create PGDBs from metagenome-assembled genomes (MAGs); search and compare community members.
- **Metabolic Flux Analysis: Generate quantitative metabolic** flux models using flux balance analysis.
- **Omics Data Analysis:** Display gene expression, metabolomics, and proteomics data in the Omics Dashboard (Figure 1), on a metabolic map diagram configured for each organism (Figure 2), and on a genome map diagram (Figure 3).
- **Drug Discovery:** BioCyc databases facilitate discovery of new drugs through improved pathway-based target selection and validation[4]:
  - **Target selection:** Pathway Tools finds drug targets by identifying essential genes using chokepoints and metabolic modeling, identifying enzymes present in multiple pathways, and identifying previously uncharacterized genes filling holes in the metabolic network.
  - **Lead generation:** Extensive data on enzyme inhibitors in EcoCyc and MetaCyc
  - **Target and lead evaluation:** Improved analysis of omics data
- **Metabolic Engineering:**
  - Fast characterization of cellular metabolism for sequenced industrial microorganisms
  - RouteSearch Tool: Searches for routes within existing metabolic network; designs novel pathways by combining native reactions with MetaCyc reactions.
  - Use quantitative metabolic modeling to guide alternative strain designs
  - Comprehensive catalog, through MetaCyc, of known metabolic reactions and metabolic enzymes
- Comparative Genome and Pathway Analyses

Pathway Tools is free to academic groups for research and teaching.

THE BIOCYC DATABASE COLLECTION

Each PGDB in the BioCyc.org collection contains the genome and metabolic network of a single organism, and its regulatory network, when curated. The following are the most highly curated databases in BioCyc:

- **EcoCyc:** Pathway/Genome Database for *Escherichia coli* K-12 MG1655. EcoCyc data have been gathered during two decades of literature-based curation from more than 41,000 articles.[1] EcoCyc provides the equivalent of 3,600 textbook pages of mini-review summaries for 4,090 *E. coli* genes.
- **HumanCyc:** HumanCyc includes literature- based curation of human enzymes and metabolic pathways and is frequently used for metabolomics data analysis.
- **MetaCyc:** Contains 2,900 metabolic pathways and 17,000 reactions from all domains of life. MetaCyc data and commentary were gathered from 70,000 publications to provide a comprehensive metabolic encyclopedia whose mini-review summaries encompass the equivalent of 9,900 textbook pages.[2]

The PGDBs are organized into three tiers:

- **Tier 1 databases** have received extensive manual curation, and include EcoCyc, MetaCyc, and HumanCyc.
- **Tier 2 databases** are computationally generated from RefSeq entries, followed by up to one person-year of subsequent curation. Tier 2 databases include *Bacillus subtilis, Saccharomyces cerevisiae, Mycobacterium tuberculosis, Staphylococcus aureus, Clostridioides difficile, Bacillus anthracis, Helicobacter pylori, and Vibrio cholerae.*
- **Tier 3 databases** were computationally generated from RefSeq entries with no subsequent curation.

All PGDBs include the genome, predicted metabolic pathways, predicted pathway hole fillers (genes coding for missing (unannotated) enzymes in metabolic pathways) and, for bacteria, predicted operons.

Additional types of data present in BioCyc include:

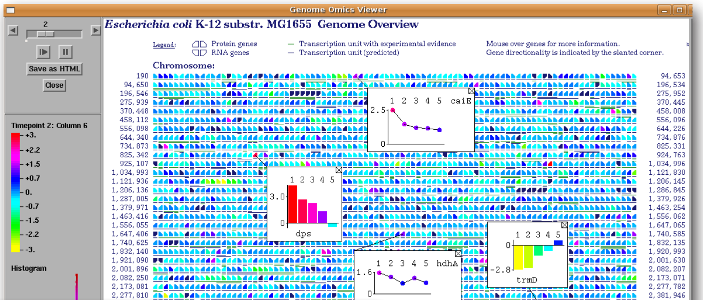
- Protein subcellular locations, enzyme kinetics data, protein features, Gene Ontology terms, predicted Pfam domains
- Curated regulatory information including promoters, operons, transcription-factor binding sites
- Gene essentiality data
- Reaction atom mappings
- Gibbs free energies of formation for metabolites
- Growth media
- Ortholog relationships to other BioCyc genomes
- Database links (e.g., to UniProt and RefSeq)

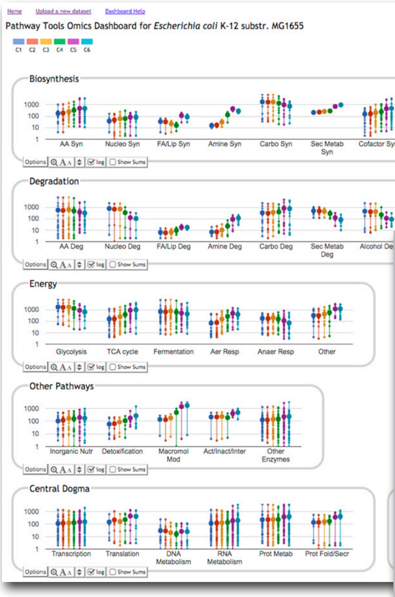
PATHWAY TOOLS SOFTWARE

Pathway Tools can operate on the BioCyc PGDBs available through SRI, and on locally created PGDBs.

GENOME INFORMATICS TOOLS

- Search for genomes by name, taxonomy, phenotypic properties
- Gene information page
  - Retrieve amino-acid sequence and nucleotide sequence of arbitrary genome region
  - Search genes by name, accession number, sequence length, replicon position, GO terms, and protein properties (pI, MW, protein features, subcellular location, ligand)
- Genome Browser (Figure 4) depicts genomic regions at user-selected resolution with semantic zooming (new features appear as zoom increases). Visible features include pseudogenes, promoters, transcription-factor binding sites, repeats, terminators, and nucleotide sequence. Genome posters can be generated.
- Transcription-unit information page
- BLAST search, sequence-pattern searching, map SNPs to genes and show effects on translation


Genome Omics Viewer
Escherichia coli K-12 substr. MG1655 Genome Overview
Save as HTML
Close
Legend:
Protein genes
RNA genes
Transcription unit with experimental evidence
Transcription unit (predicted)
Mouse over genes for more information.
Gene directionality is indicated by the slanted corner.
Chromosome:
Timepoint 2: Column 6
Histogram
caiE
dps
hdhA
trmD
katG



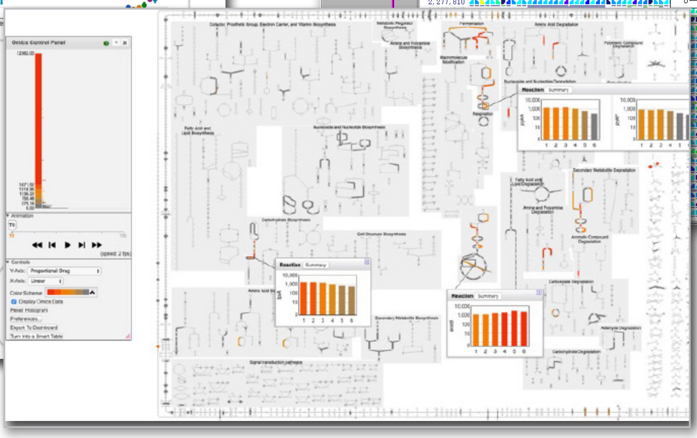


Figure 3: The Genome Omics Viewer depicts an entire chromosome in one window. Genes are colored according to a gene expression dataset.

Figure 1: The Omics Dashboard provides a visual read-out of the cellular state within an omics dataset.

Figure 2: The Cellular Omics viewer paints omics datasets onto a diagram of the cellular biochemical network. Reaction lines can be colored with gene expression, proteomics, or reaction flux data; compound nodes can be colored with metabolomics data. Multi-omics data can be analyzed by coloring data onto reactions and metabolites simultaneously. Omics pop-ups graph omics data values using bar graphs, heat maps, or X-Y plots.

PATHWAY AND REGULATORY INFORMATICS TOOLS

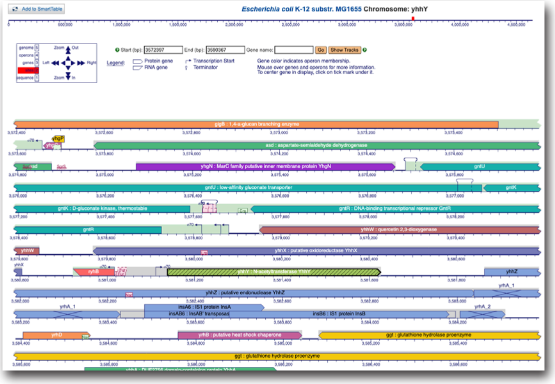
- Pathway information page
  - Each pathway shows a detailed mini-review from MetaCyc
  - Search pathways by name, substrates, and length
- Reaction information page includes atom mappings
- Metabolite information page
  - Search metabolites by name, accession numbers, substructure, mass, monoisotopic mass, elemental composition
- Customize pathway diagrams for publication or assemble groups of pathways into a pathway collage.
- Cellular Overview diagrams (Figure 2) are organism-specific depictions of metabolic and transporter networks that are zoomable and searchable.
- Route Search tool finds minimum-cost paths between metabolites in the metabolic network. Route Search paths maximize the number of atoms conserved from source metabolite to target metabolite by using an extensive library of reaction atom mappings. Route Search can find paths across multiple organisms from a microbiome.
- The regulatory Overview presents the genetic regulatory network stored in a PGDB.

ANALYSIS TOOLS FOR GENE EXPRESSION AND METABOLOMICS DATA

- SmartTables store lists of genes, metabolites, pathways, and more. Browse database attributes, share with colleagues, transform to pathway lists, perform enrichment analysis.
- The Omics Dashboard presents a visual read-out of the expression status of all cellular systems to facilitate a rapid top-down user survey of cellular responses (Figure 1)
- Paint omics data onto individual pathways, pathway collage, and full metabolic networks (Figure 2) (example animation at http://biocyc.org/ov-expr.shtml).
- Regulatory Omics Viewer paints omics datasets onto the regulatory network to enable comparisons of expression measurements with regulatory mechanisms.

COMPARATIVE GENOMICS TOOLS

- Comparative genome browser (Figure 5) aligns chromo-somal regions from multiple genomes at orthologous genes
- Sequence alignments
- Compare pathway, reaction, metabolite, and protein complements of specified organisms
- Quick navigation between corresponding entities (e.g., genes, pathways, metabolites) in different organisms
- Cross-organism search finds genes, metabolites, and pathways across organisms


Add to SmartTable
Escherichia coli K-12 substr. MG1655 Chromosome: yhhY
Zoom Out
Left
Right
Zoom In
Legend:
Protein gene
RNA gene
Transcription Start
Terminator
Gene color indicates operon membership.
Mouse over genes and operons for more information.
To center gene in display, click on tick mark under it.
Go
Show Tracks
glgB : 1,4-α-glucan branching enzyme
asd : aspartate-semialdehyde dehydrogenase
yhgN : MarC family putative inner membrane protein YhgN
gntU
gntK : D-gluconate kinase, thermostable
gntR : DNA-binding transcriptional repressor GntR
yhhW : quercetin 2,3-dioxygenase
yhhX : putative oxidoreductase YhhX
yhhZ : putative endonuclease YhhZ
insA6 : IS1 protein InsA
insB6 : IS1 protein InsB
yhhA
yrhB : putative heat shock chaperone
ggt : glutathione hydrolase proenzyme

▲ *Figure 4. Above: Genome browser depiction of a region of the* E. coli *chromosome. Gene colors indicate operon organization. Promoters and terminators are depicted when known. Pseudogenes are marked with X's.*

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ADVANCED DATABASE ACCESS

- Web services API provided.
- Python, Perl, Java, and Lisp APIs.
- Author advanced queries of SQL power using the intuitive Structured Advanced Query Form.
- Interoperability: Export PGDBs to the BioPAX, SBML, GFF, and Genbank formats.

METABOLIC MODELING IN PATHWAY TOOLS

The MetaFlux module generates a flux balance analysis (FBA) model automatically from a PGDB, enabling quantitative modeling of steady-state metabolic fluxes. Combine models for multiple organisms to model organism communities.

Simulate spatial interactions by situating organisms within a spatial grid. Dynamic FBA enables temporal simulations.

By combining pathway databases with FBA, MetaFlux achieves close coupling of the FBA model to genome and metabolite data; and high accessibility of the FBA model via the query and visualization features of Pathway Tools. MetaFlux speeds comprehension of simulation results by painting computed fluxes on metabolic-map diagrams and on individual pathways, and MetaFlux will plot metabolite concentrations and organism biomass changes.

Development of FBA models is accelerated by a multiple gap-filling tool that postulates additional reactions to add to an FBA model to complete it, and that identifies what subset of biomass components can be produced by the current model. Modeling of gene knock-outs is supported.

TECHNICAL SPECIFICATIONS/CONFIGURATIONS

1. Pathway/Genome Navigator Bundled with BioCyc Databases

All configurations provide query, visualization, and analysis of existing BioCyc databases. The same binary application can run as both a desktop application and as a Web server within an organization's intranet.

Configurations available:

1) Pathway/Genome Navigator plus as many as 500 user-selected BioCyc databases

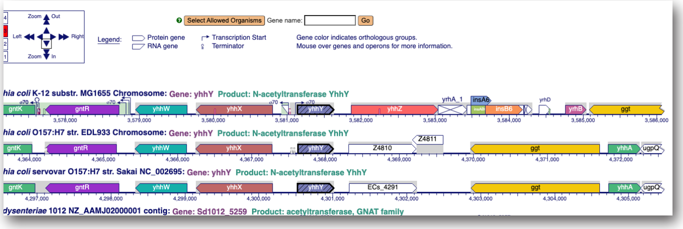
- Platforms supported: Linux, Windows (Web mode not available), Macintosh
- Hardware: 2.4 GHz processor, 16 GB RAM, 5 GB disk

2) Pathway/Genome Navigator plus all BioCyc databases

- Platforms supported: Linux
- Hardware: 2.4 GHz processor, 128 GB RAM, 3 TB disk

2. Add Editors, PathoLogic, MetaFlux

- To edit existing BioCyc PGDBs, add the Pathway/Genome Editors alone to configuration (1).
- To create and edit new Pathway/Genome Databases, add PathoLogic and Pathway/Genome Editors to configuration (1).


Zoom Out
Left
Right
Zoom In
Select Allowed Organisms
Gene name:
Go
Legend:
Protein gene
RNA gene
Transcription Start
Terminator
Gene color indicates orthologous groups.
Mouse over genes and operons for more information.
hia coli K-12 substr. MG1655 Chromosome: Gene: yhhY Product: N-acetyltransferase YhhY
gntK
gntR
yhhW
yhhX
yhhY
yhhZ
yhhA_1
insA6
insB6
yhhD
ggt
hia coli O157:H7 str. EDL933 Chromosome: Gene: yhhY Product: N-acetyltransferase YhhY
Z4810
Z4811
yhhA
ugpQ
hia coli servovar O157:H7 str. Sakai NC_002695: Gene: yhhY Product: N-acetyltransferase YhhY
ECs_4291
dysenteriae 1012 NZ_AAMJ02000001 contig: Gene: Sd1012_5259 Product: acetyltransferase, GNAT family

◀ *Comparative genome browser showing alignments with respect to the yhhY gene in one* E. coli *genome, a* Shigella flexneri *genome, and a* Salmonella enterica *genome. Colors indicate orthologs; there is strong conservation to the left of the yhhY gene, but not to the right of that gene (genes in white are not conserved).*

- PathoLogic requires the EcoCyc and MetaCyc PGDBs.
- To run quantitative metabolic flux models, add MetaFlux to configuration (1).

COMPREHENSIVE LIST OF FEATURES IN THE PATHWAY TOOLS SOFTWARE SUITE

Genome Informatics

- Gene/protein/RNA searches, gene information page
- Genome browser
- BLAST search, sequence pattern search
- Multiple sequence alignment
- Sequence retrieval

Pathway Informatics

- Reaction/metabolite/pathway searches
- Reaction, metabolite, pathway pages
- Single and multi-pathway diagrams
- Zoomable metabolic network diagrams
- Pathway inference from annotated genome
- Quantitative metabolic modeling via flux- balance analysis: FBA, dFBA, FVA, gap filling, blocked metabolites
- Pathway-based inference of nutrient requirements
- Metabolic route search for metabolic engineering
- Metabolic route search for microbial communities
- Metabolic network explorer

Regulatory Informatics

- Operon inference from genome
- Capture/visualize promoters, transcription factor binding sites
- Visualize regulon of transcription factor
- Regulatory network viewer

Transcriptomics Data Analysis

- Enrichment analysis
- Paint transcriptomics data onto single pathways, zoomable metabolic network
- Paint transcriptomics data onto regulatory network
- Paint transcriptomics data onto genome diagram
- Omics Dashboard
- Sort pathways by pathway activation score
- Multi-omics Explainer

Metabolomics Data Analysis

- Enrichment analysis
- Paint metabolomics data onto single pathways, zoomable metabolic network
- Omics Dashboard
- Sort pathways by pathway activation score
- Pathway covering analysis

Microbiome Informatics

- Calculate pathway abundances across metagenome samples
- Create PGDBs for an organism community
- Search across multiple PGDBs, compare PGDBs
- Analysis of meta-omics data

Advanced Data Manipulation

- SmartTables
- Interactive editing tools
- Advanced Query Interface

APIs and Data Import/Export

- Web services, Python, Java, Perl, R, Lisp
- Import/Export: SBML, GenBank, GFF, BioPAX, tab-delimited files

ABOUT SRI'S BIOINFORMATICS RESEARCH GROUP

SRI International, an independent research institute, is a key player in the field of computational biology. SRI's Bioinformatics Research Group, which produces BioCyc and Pathway Tools, is a leader in the development of database content and software tools for bioinformatics.

REFERENCES

[1] The EcoCyc Database. EcoSal Plus. 2018. http://www.asmscience.org/content/journal/ecosalplus/10.1128/ecosalplus.ESP-0006-2018

[2] MetaCyc. Nucleic Acids Research 2020. doi: 10.1093/nar/gkz862

[3] Pathway Tools version 24.0:Integrated Software for Pathway/Genome Informatics and Systems Biology, 2020. http://arxiv.org/abs/1510.03964v4

[4] The Pathway Tools Software and Its Role in Anti-Microbial Drug Discovery, Microbial Genomics and Drug Discovery, T.J. Dougherty and S.J. Projan eds., Marcel Dekker Inc., New York, 2003

Additional publications: http://biocyc.org/publications.shtml

FOR MORE INFORMATION

Downloadable Pathway Tools Software

- Freely available to academics and government laboratories for research purposes; see http://biocyc.org/download.shtml
- For commercial use contact ptools-commercials@ai.sri.com.

BioCyc.org Website Subscriptions

- Subscriptions provide access to more than 19,000 organism databases and extensive online software tools
- The EcoCyc and MetaCyc databases are free to all users.
- Subscriptions in USA and other countries: contact Phoenix Bioinformatics at biocyc@phoenixbioinformatics.org.

SRI International

333 Ravenswood Avenue
Menlo Park, CA 94025-3493
650.859.2000
www.sri.com