Virtual Institute of Microbial Stress and Survival

Deduction of Stress Response Pathways in Metal and Radionuclide Reducing Microorganisms

http://vimss.lbl.gov

2004

Project Application Goals

- To understand bacterial stress-response to the unique stressors in metal/radionuclide contamination sites
- To turn this understanding into a quantitative, data-driven model for exploring policies for natural and biostimulatory bioremediation
- To implement proposed policies in the field and compare results to model predictions
- Close the experimental/computation cycle by using discrepancies between models and predictions to drive new measurements and construction of new models

Project Sciences Goals

- Compare physiological and molecular response of three target microorganisms to environmental perturbation.

- Deduce the underlying regulatory pathways that control these responses through analysis of phenotype, functional genomic, and molecular interaction data.

- Use differences in the cellular responses among the target organisms to understand niche specific adaptations of the stress and metal reduction pathways.

- From this analysis derive an understanding of the mechanisms of pathway evolution in the environment.

- Ultimately, derive dynamical models for the control of these pathways to predict how natural stimulation can optimize growth and metal reduction efficiency at field sites.

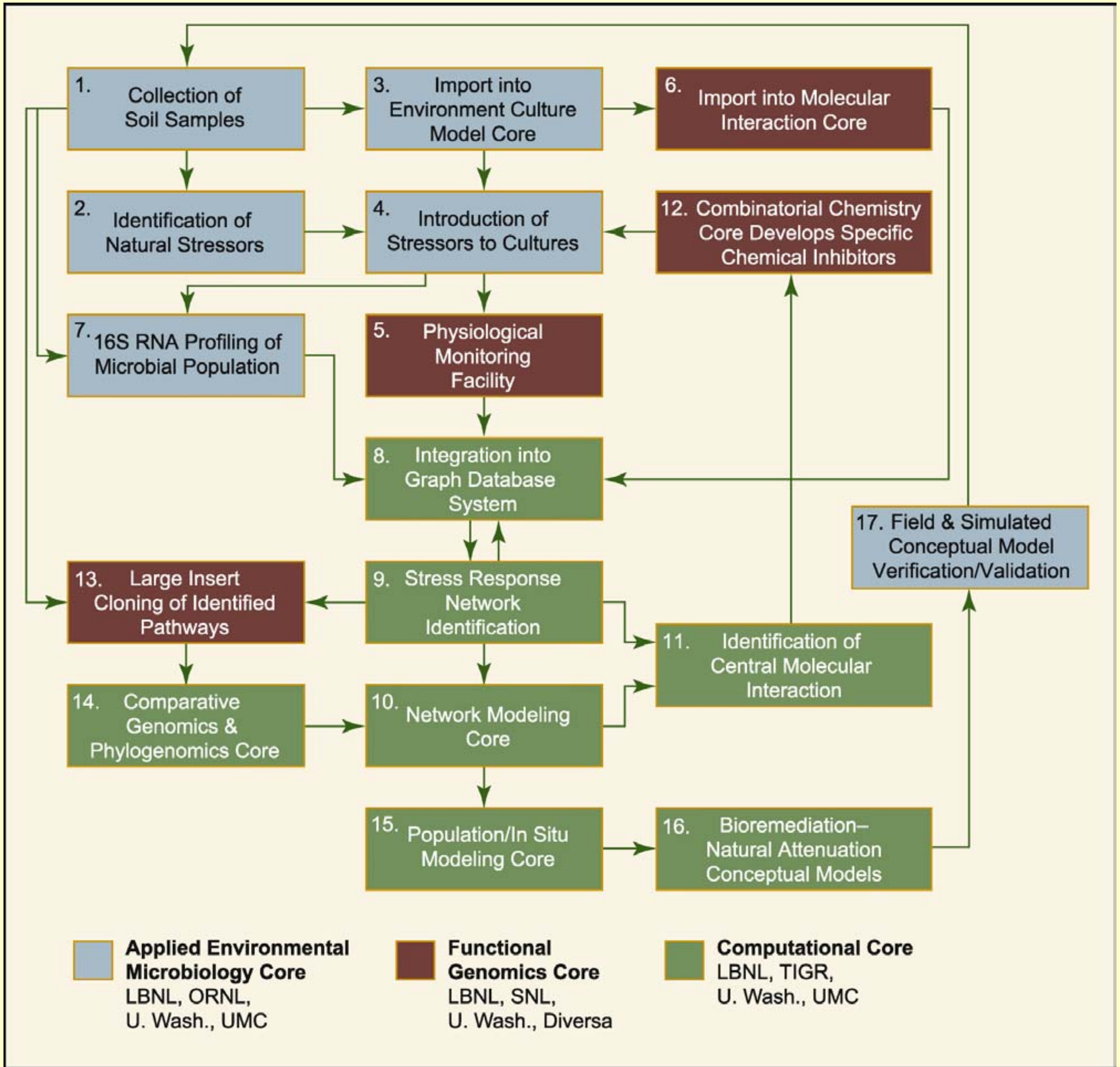
Organisms

- Primary organism:
  - *Desulfovibrio vulgaris*
    - δ-proteobacteria,
    - "Anaerobic"
    - SRB, uses sulfate and sulfite as terminal electron acceptors for growth.
    - Oxygen, iron, nitrite, chromate, and U(VI) can be reduce but growth is not observed.
    - Does not reduce nitrate
    - Has a megaplasmid containing nitrogen fixation genes
    - Has a number of interesting pathogenicity factors: type III-secretion, adhesins, hemagluttin
    - common in eutrophic environments, much less known about this organism

- Comparison organisms:
  - *Shewanella oneidensis* MR-1
    - γ-proteobacteria
    - "facultative anaerobe"
    - Reduces nitrate
    - Does not have nitrogenase
    - more common in oligotrophic environments
  - *Geobacter metallireducens*
    - δ-proteobacteria,
    - "Anaerobic"
    - More common in oligotrophic environments

- Stressors: O_2, metals, TEAs, PO_4, nitrate, nitrite, pH, salt, heat

Design of Project



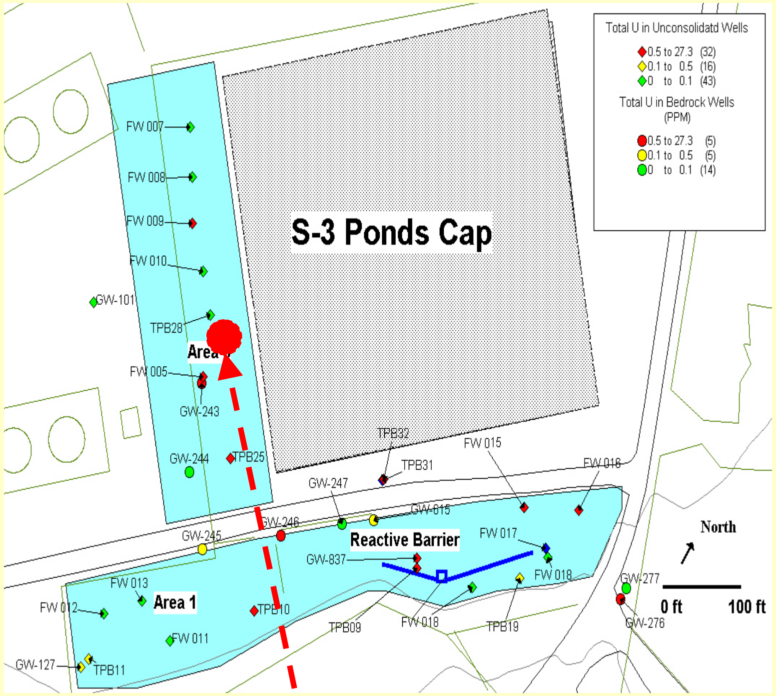
AEMC
Applied Environmental Microbiology Core

- Characterize biogeochemical environment of organisms
- Develop principled environmental simulators for production of biomass and growth characterization
- Cell-biological characterization
- Phenotyping/Environmental Physiology characterization
- Terry Hazen: Core Leader
- LBNL, UMC, UWASH, ORNL,UMO

NABIR Field Research Center Uranium-contaminated site

- –Sulfate reducers common
- –Lactate stimulated soil columns demonstrated increases in sulfate reducers after U reduction, including 4 *Desulfovibrio* spp. using Affymetrix GeneChip microarray

Subsurface sediments from the wells FWB-107 (13.2 m) and FWB-109 (15.4 m) in Area 3 were serially diluted in a basal salts medium (NaCl, $NaHCO_3$, NH_4Cl, minerals, 99%:1% N_2/CO_2) with 5 mM lactate and 5 mM ethanol. The dilutions were provided with nitrate, Fe(III)-citrate, or sulfate and incubated anaerobically at approximately 18 to 20¼C. The results are summarized in the table below:



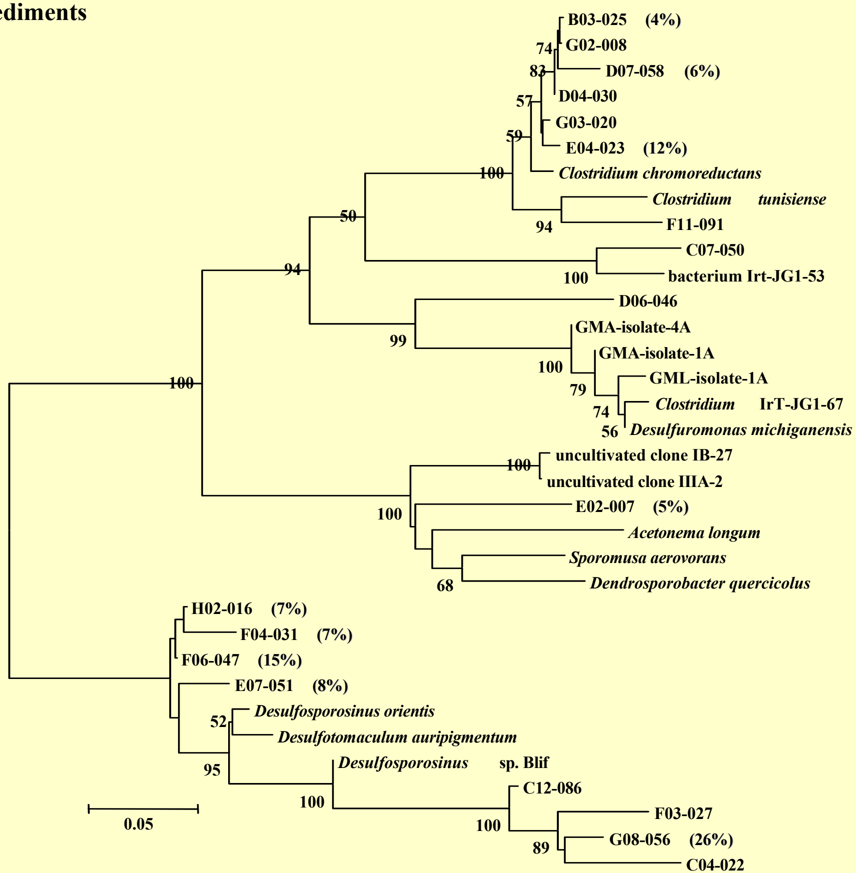
	NO_3	$Fe^{(III)}$	SO_4
FWB-107 (13.2 m)	3500 cells/g	46 cells/g	240 cells/g
FWB-109 (15.4 m)	5400 cells/g	1700 cells/g	1100 cells/g

Matt Field, Terry Hazen, Gary Anderson

OTU number	OTU designation	Representative organism	Control	Lactate Stimulated*
02280401010000.4114	Dhb.retbaense_subgroup soil PBS-21	*Desulfohalobium retbaense*	nd	3234
02280401040100.683	Dsv.halophilus_subgroup Desulfovibrio sp. Ac5.2	*Desulfovibrio halophilus*	nd	2560
02280401040500.4661	Dsv.aminophilus_subgroup clone R2b32	*Desulfovibrio aminophilus*	nd	2772
02280401040600.2471	Dsv.africanus_subgroup delta clone:KB47	*Desulfovibrio africanus*	nd	2640
02280401041100.2700	Dsv.desulfuricans_subgroup delta clone:Rs-N31	*Desulfovibrio desulfuricans*	nd	2921

* Average difference, Sulfate reducing bacteria detected using the Affymetrix GeneChip high-density oligonucleotide array. NABIR FRC Area 2 Sediments

Community analysis of sulfate-reducing enrichment from FW-109. The predominant population comprised approximately 25% of the sampled diversity and had 88% sequence identity with *Desulfosporosinus* Blif. Sub-populations that had 95% to 97% sequence identity with *Desulfosporosinus orientis* constituted for an additional 37% of the library. The clone E04-023 constituted just over 10% of the library, and had 98% sequence identity with *Clostridium chromoreductans*. A small fraction of the enrichment community (5%) appeared to have only 87% sequence identity with previously uncultivated clones that originated from a chlorobenzene-degrading community.



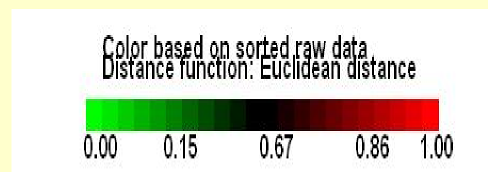
Hanford 100H Chromium-contaminated site

- Sulfate Reducers and *Geobacter* in most sediments initially though densities of all bacteria quite low as indicated by PLFA and dominated by G+ bacteria, shifted to G- after lactate stimulation.
- 16S rDNA genes were only successfully amplified from sediments that had been stimulated with lactate, HRC, or MRC. Further PCR analyses using group specific primers indicated the presence of *Geobacter* sp. and *Desulfovibrio* sp. These amplicons were also assayed with a 16S microarray (Affymetrix GeneChip). The microarray indicated that all five subgroups within the proteobacteria were present, including 2 species of *Desulfovibrio*.
- The biostimulated sediments reduced Cr(VI) from 1000 ppm to non-detect in 1 week.

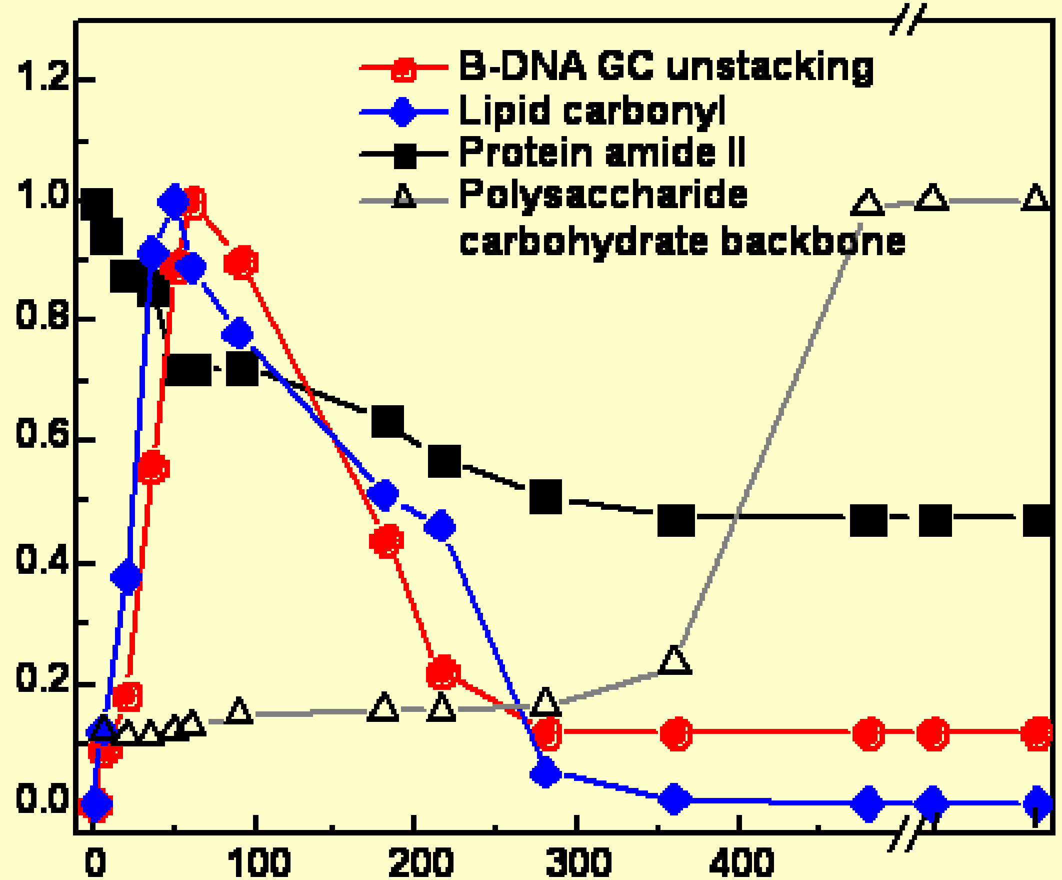
Enterococcus Grp	Gram (+)
B. megaterium Grp	Gram (+)
Brevibac. Grp	Gram (+)
Eubacteria	Gram (+)
Carnobacteria	Gram (+)
B. megaterium	Gram (+)
Eubacteria	Gram (+)
B. sphaericus Grp	Gram (+)
B. sphaericus Grp	Gram (+)
B. megaterium Grp	Gram (+)
B. megaterium Grp	Gram (+)
Eubacteria	Gram (+)
Eubacteria	Gram (+)
Environmental clone	
ε-proteobacteria	
γ-proteobacteria	
γ-proteobacteria	
Sphingobacteria	
β-proteobacteria	
δ–proteobacteria	
γ-proteobacteria	
α-proteobacteria	
γ-proteobacteria	
β-proteobacteria	
Sphingobacteria	
γ-proteobacteria	
β-proteobacteria	
Lewinella	
Lewinella	
Environmental clone	
β-proteobacteria	
δ–proteobacteria	
Lewinella	
δ–proteobacteria	

Exp1 = Control

Exp5 = lactate stimulated



AEMC: FTIR Profiling

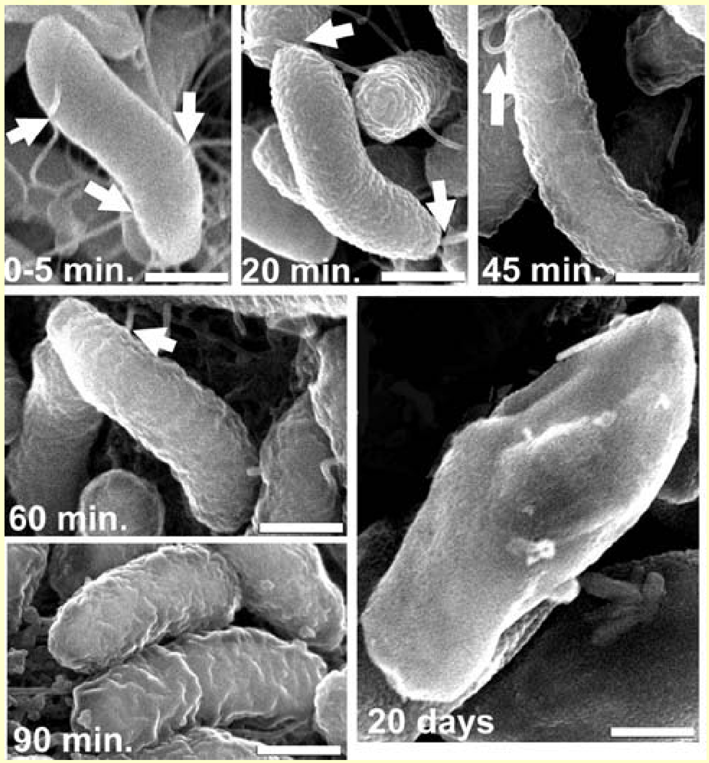


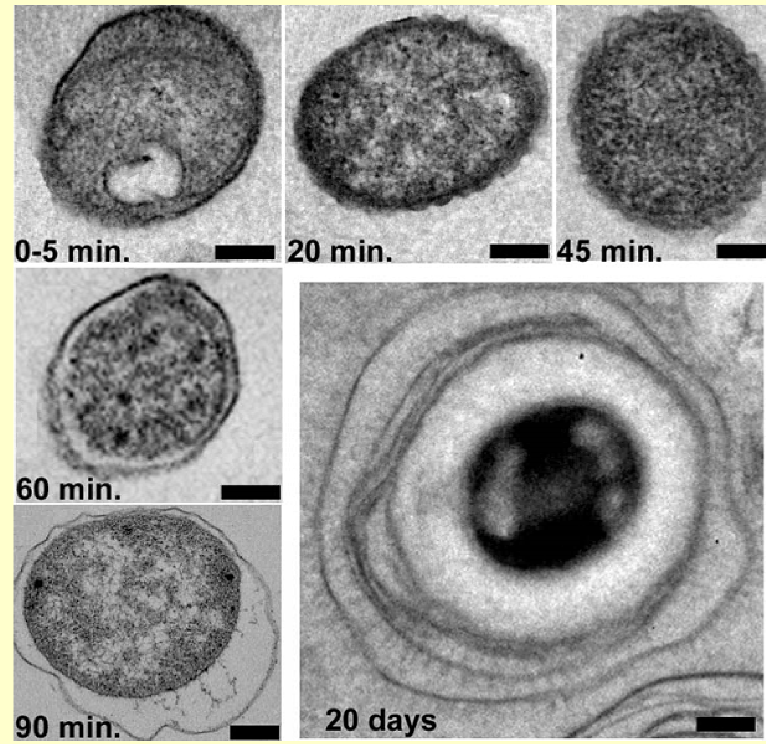
- Synchrotron FTIR t**ime course of infrared absorption intensity, indicative of oxidative stress levels in different biologically important molecules in *Desulfovibrio vulgaris* after exposure to atmospheric oxygen.**

- Also found signatures for Cytochrome B hemes

Hoi-Ying Holman, Terry Hazen

AEMC: Electron Microscopy



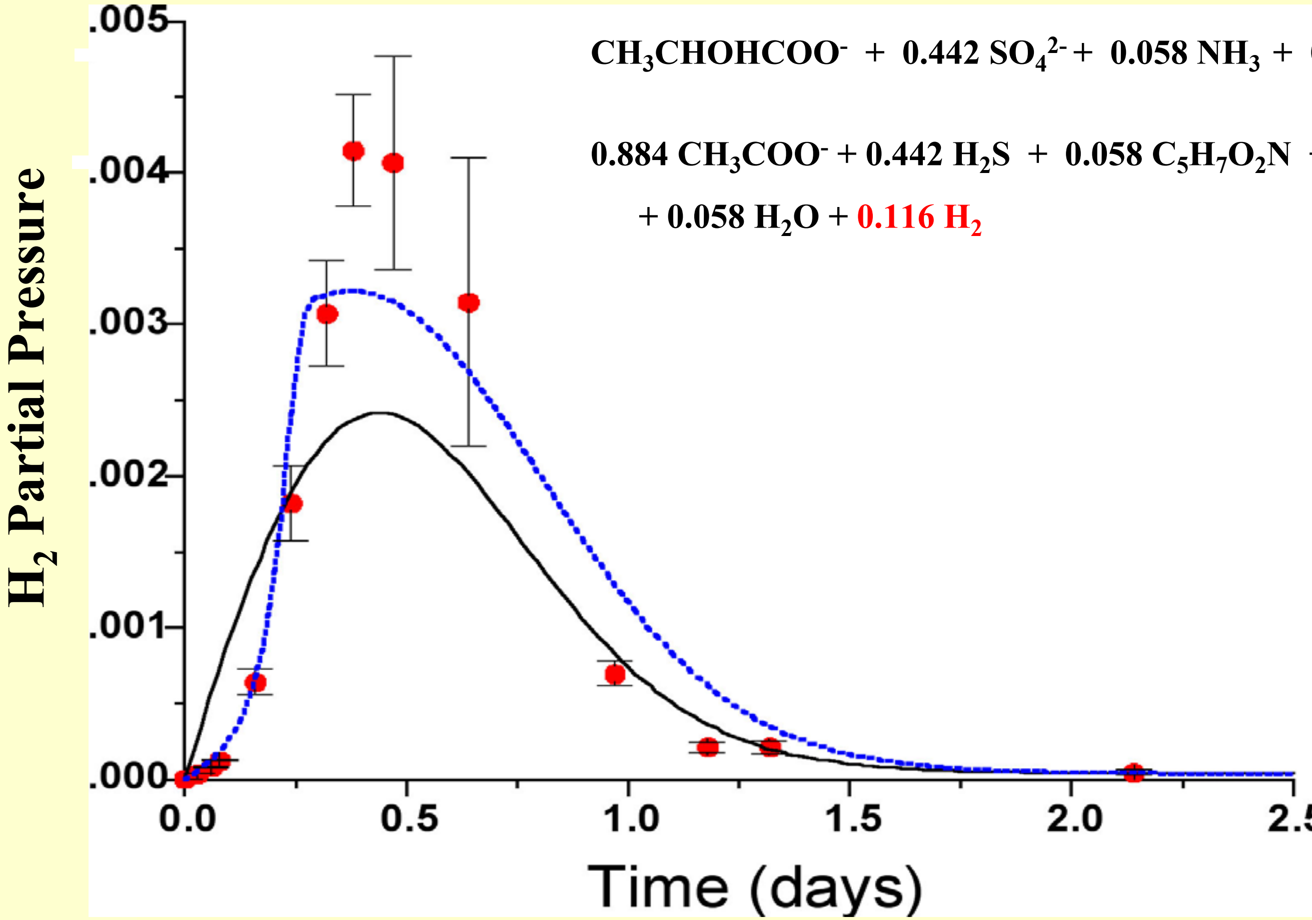


- Electron microscopic images of D.v. under oxygen exposure

Hoi-Ying Holman, Terry Hazen

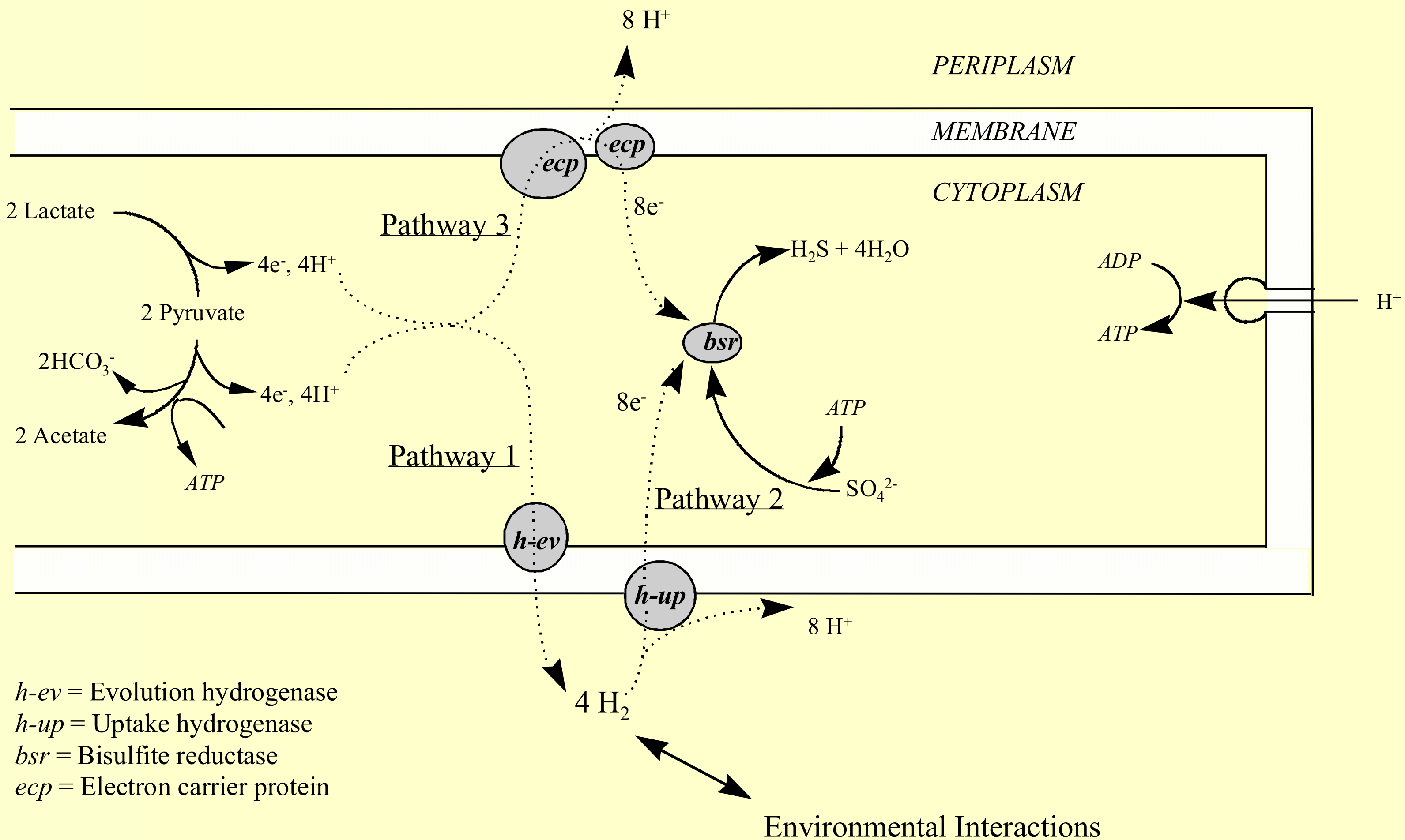
The Hydrogen Burst

Transient H_2 accumulation during growth of *Desulfovibrio* species in <u>batch culture</u>



David Stahl

Modeling of the Hydrogen Burst - *Desulfovibrio* species batch culture



h-ev = Evolution hydrogenase
h-up = Uptake hydrogenase
bsr = Bisulfite reductase
ecp = Electron carrier protein

David Stahl

Mass Balance Equations Used to Model the Growth of *D. vulgaris*. in Sealed Serum Bottles

Lactate
$$\frac{dLac}{dt} = r^{L} + r^{LS} + 0.05 r^{HS}(1-\gamma)$$

Acetate
$$\frac{dAc}{dt} = -0.906 r^{L} - 0.884 r^{LS} + 0.05 r^{HS}(\gamma)$$

Sulfate
$$\frac{d[SO_4^{\ 2-}]}{dt} = 0.442 r^{LS} + 0.262 r^{HS}(1-\gamma) + 0.238 r^{HS}(\gamma)$$

Hydrogen in liquid
$$\frac{d[H_2]}{dt} = -1.906 r^{L} - 0.116 r^{LS} + r^{HS} - \frac{k_{H2}}{R_g T}\left(H_{H2}[H_2] - P_{H2}\right)\frac{V_g}{V_l}$$

Total hydrogen sulfide in liquid
$$\frac{dC_{TH2S}}{dt} = -0.442 r^{LS} - 0.262 r^{HS}(1-\gamma) - 0.238 r^{HS}(\gamma) - \frac{k_{H2S}}{R_g T}\left(H_{H2S}[H_2S] - P_{H2S}\right)\frac{V_g}{V_l}$$

Total Carbonates in liquid
$$\frac{dC_{TCO3}}{dt} = -0.953 r^{L} - 0.942 r^{LS} - 0.025 r^{HS}(1-\gamma) + 0.025 r^{HS}(\gamma) - \frac{k_{CO2}}{R_g T}\left(H_{CO2}[H_2CO_3^{*}] - P_{CO2}\right)\frac{V_g}{V_l}$$

Hydrogen in headspace
$$\frac{dP_{H2}}{dt} = k_{H2}\left(H_{H2}[H_2] - P_{H2}\right)$$

Hydrogen sulfide in headspace
$$\frac{dP_{H2S}}{dt} = k_{H2S}\left(H_{H2S}[H_2S] - P_{H2S}\right)$$

Carbon dioxide in headspace
$$\frac{dP_{CO2}}{dt} = k_{CO2}\left(H_{CO2}[H_2CO_3^{*}] - P_{CO2}\right)$$

Biomass growth
$$\frac{dX}{dt} = -Y^{L} r^{L} - Y^{HS} r^{HS} - Y^{LS} r^{LS} - bX$$

Noguera, et al. 1998. Bioeng.. Biotechnol. ***59:*** *733-746.*

David Stahl

Syntrophic Growth of *Desulfovibrio* and Methanogen

DOE Genomes to Life Initiative

Lactate as electron donor; no electron acceptor

$$2CH_3CHOHCOO^- + 4H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2HCO_3^- + 4H_2$$

$\Delta G^{0\prime}$ = -8.4 kJ (SRB alone)

Hydrogen as electron donor; CO_2 as electron acceptor

$$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3H_2O$$

$\Delta G^{0\prime}$ = -135.6 kJ (Methanogen alone)

Combined equations (syntrophic growth)

$$2CH_3CHOHCOO^- + H_2O \rightarrow 2CH_3COO^- + CH_4 + H^+ + HCO_3^-$$

$\Delta G^{0\prime}$ = -144.0 kJ (SRB plus Methanogen)

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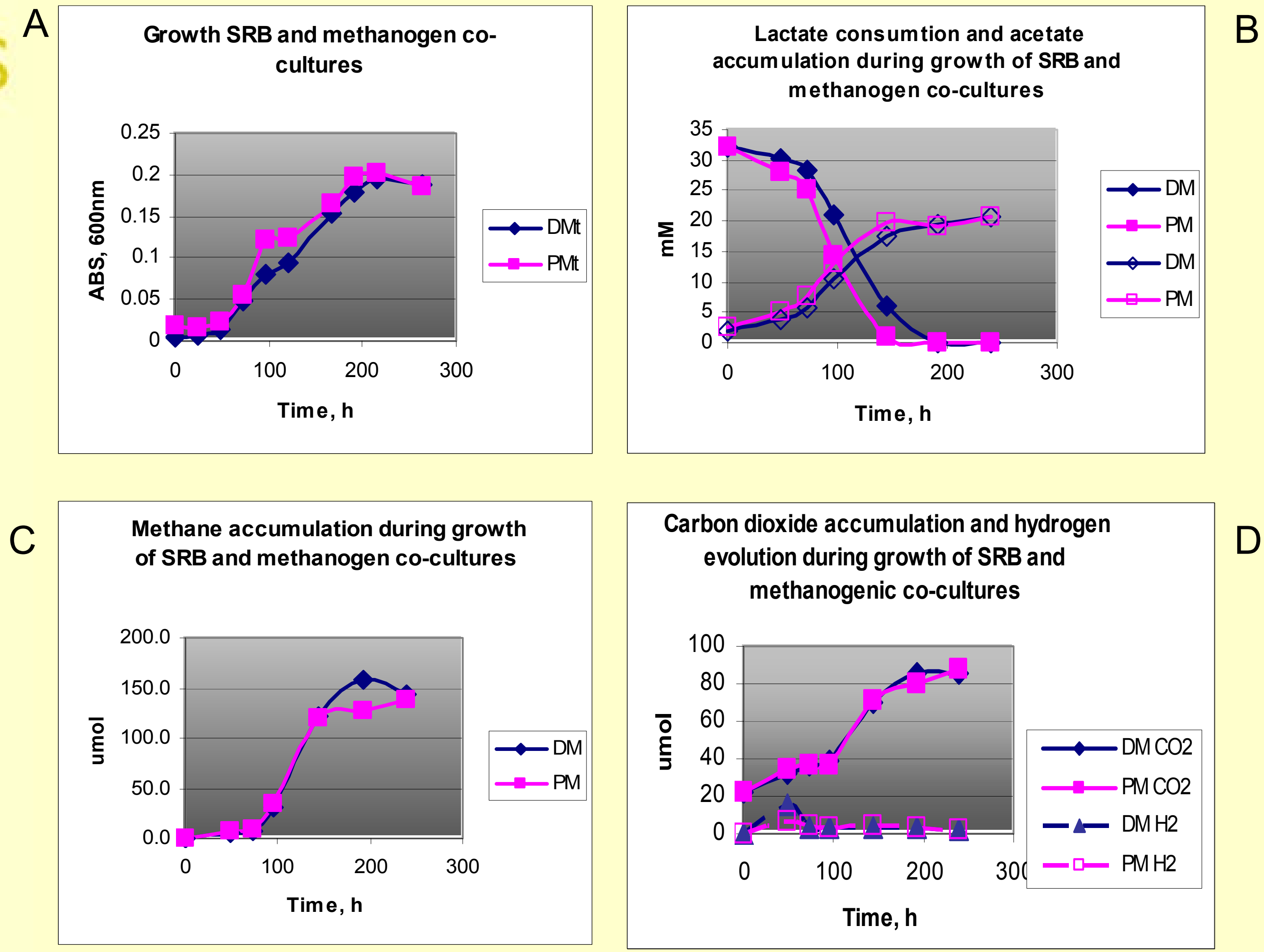
AEMC: Co-Culturing



DM indicate co-culture of *Desulfovibrio vulgaris* with *M. maripulidis*

PM indicate co-culture of *Desulfovibrio sp.* PT2 with *M. maripulidis*

Figures C and D depict total amount of umol of gases in head space of tubes.

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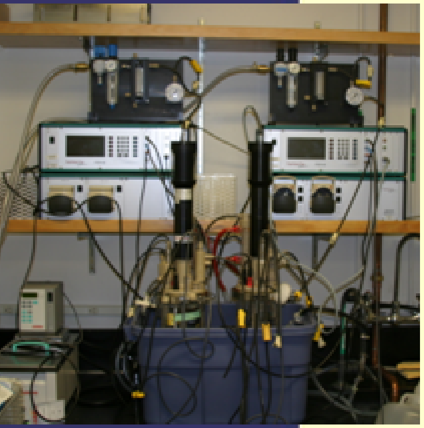
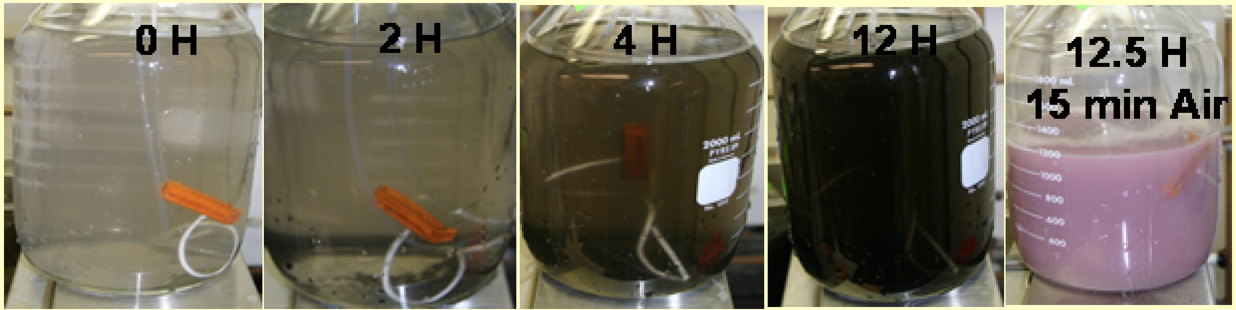
AEMC: BioMass

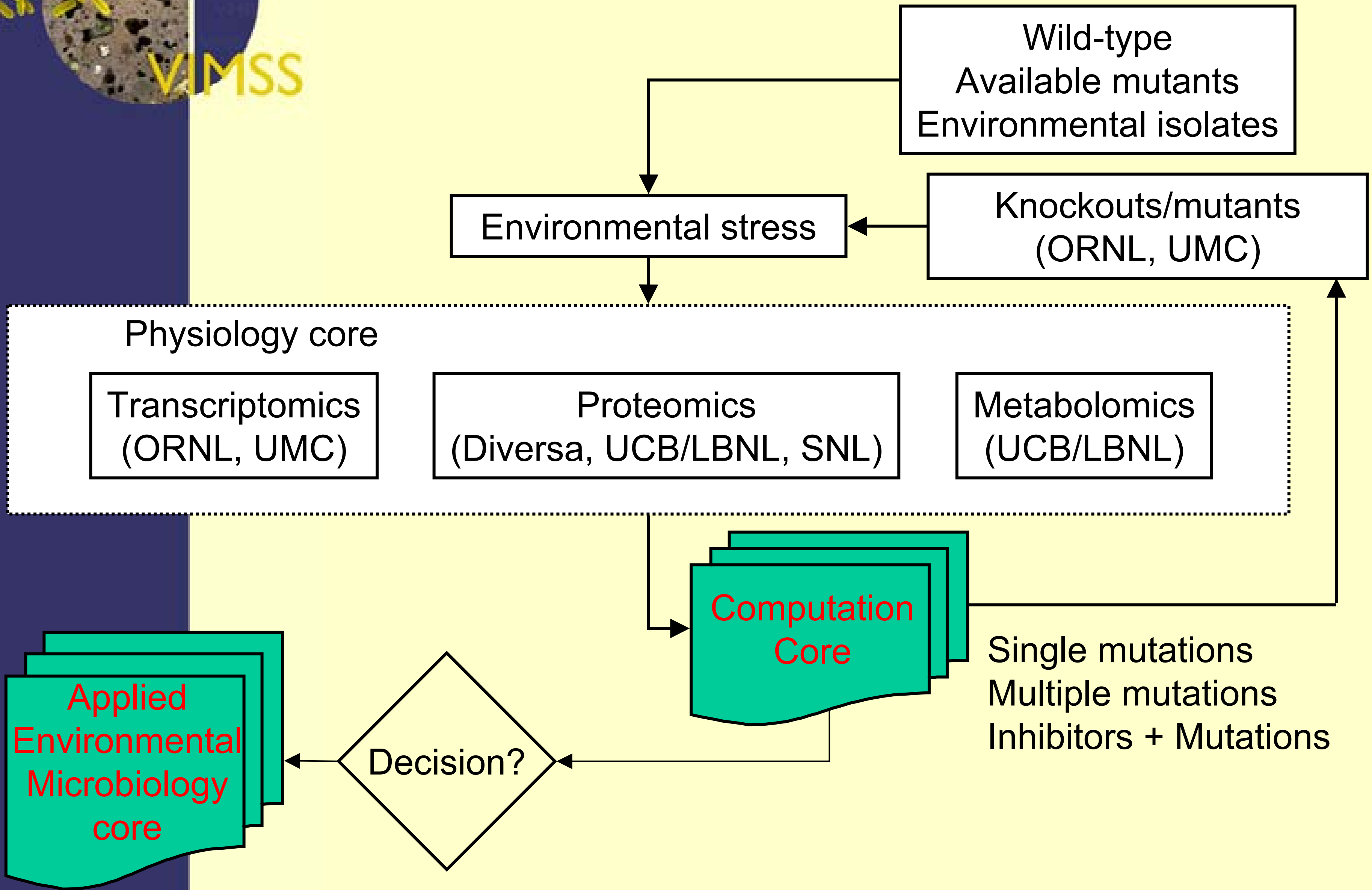
- Maintenance and archiving of *D. vulgaris* experimental strain (ATCC 29579) to minimize ‘culture’ drift from multiple transfers
- Develop and define growth conditions for stress studies using defined media (1000’s of growth curves using automated systems)
- QA/QC on all media components and culture conditions, e.g. pH, DO, temperature, containers, anaerobic chamber, etc.
- Physiological and Morphological typing: PLFA, sFTIR, AODC, Fluorescent Antibody (O&H),Omnilog (Phenotype Microarray), SEM, TEM, x-ray, chemotaxis, protien, optical density, lactate/acetate, sulfate, etc.
- Develop and validate large scale production of biomass under sterile, anaerobic, reproducible conditions for simultaneous comparison of control and stress. Currently up to 2 L in triplicate under 2 conditions. Expanding to multiple 3 L no-metal, anaerobic chemostats.
- Develop techniques for stressing cells that minimize other stress responses: log phase cells, sparge with N and air for comparison of air stress, special porous tubing to maximize DO saturation from air.
- Develop sampling and processing techniques that minimize contamination, processing, shipping, and maximize sample quality and quantity for simultaneous sampling and processing for 5 different labs.





Terry Hazen

FGC Design


Wild-type
Available mutants
Environmental isolates
Environmental stress
Knockouts/mutants
(ORNL, UMC)
Physiology core
Transcriptomics
(ORNL, UMC)
Proteomics
(Diversa, UCB/LBNL, SNL)
Metabolomics
(UCB/LBNL)
Computation Core
Single mutations
Multiple mutations
Inhibitors + Mutations
Applied Environmental Microbiology core
Decision?

FGC: Summary of Experiments

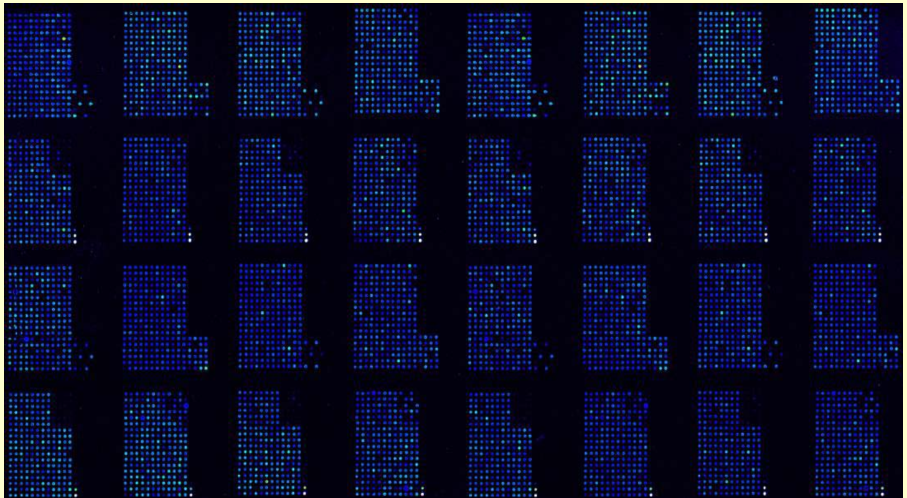
	Shewanella	Desulfovibrio	Geobacter m.
Salt	**T** P M	**T** P M	T P M
pH	**T** P M	T P M	T P M
Heat	**T** P M	T **P** M	T P M
O_2	T P M	**T** **P** **M**	T P M
Strontium	**T** P M	**T** P M	T P M
$H_2 0_2$	**T** P M	T P M	T P M
Nitrite	T P M	**T** P M	T P M

Conditions and experiments chosen to develop the different measurement technologies and for sketching out most of our target stress responses

Transcriptome

Joe Zhou, Dorothea Thompson

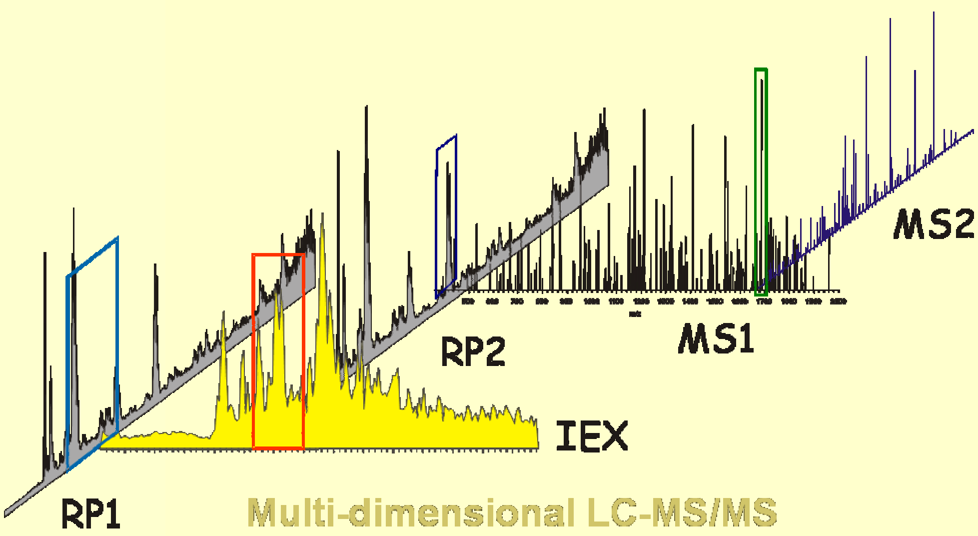
- Full Oligo Arrays for
  - *Shewanella,*
  - *Desulfovibrio vulgaris,*
  - *Geobacter metallireducens*
  - *a combined Desulfovibrio/M. maripulidis array*

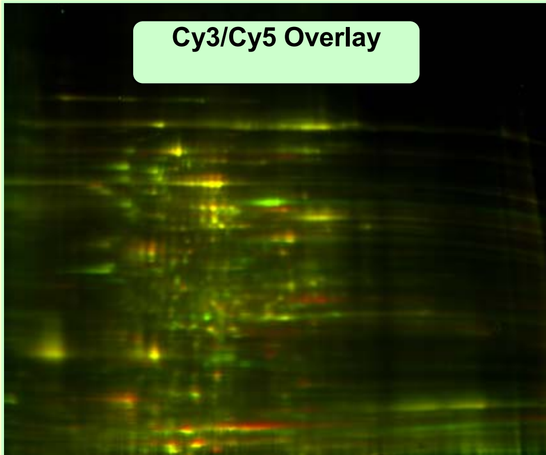


Cover all ORFs in the genome with 3574 oligos, including 3471 (97.1%) unique probes and 103 (2.9%) probes which may cross-hybridize with other ORFs

Proteome

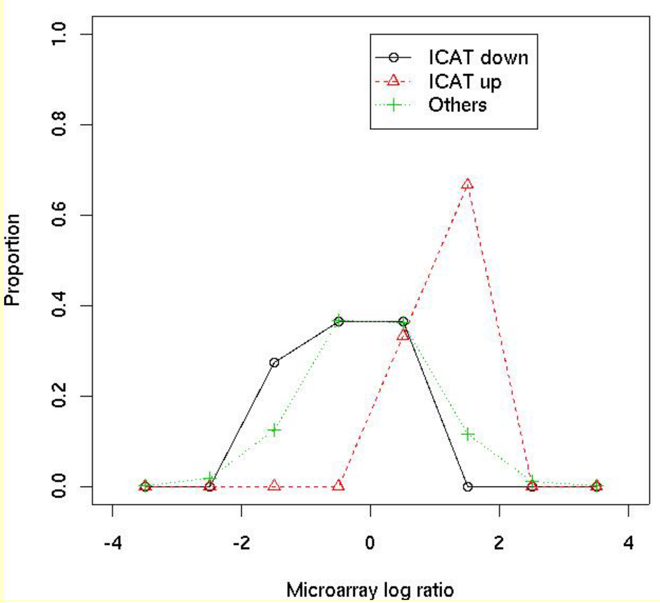
- Three different proteomics methods for detection and quantitation of protein levels
  - 3D LC MS-MS (Martin Keller, Diversa)
  - DIGE-MALDI/TOF MS (Anup Singh, SANDIA)
  - ICAT MS-MS (Jay Keasling, LBNL)

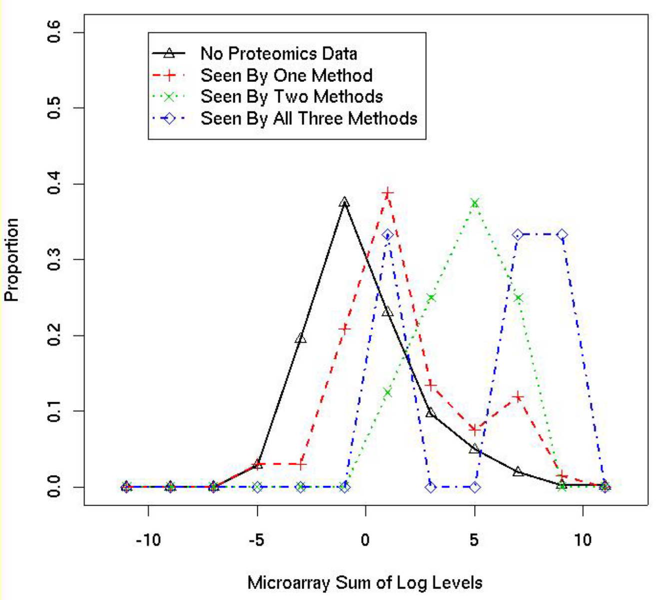




Microarray Proteome Comparison

- Data from D.v. O_2 stress experiments.
- Top: Comparison of ICAT results to MA data after 5 hrs exposure.
- Bottom: Agreement among proteomic and MA methods





Analysis: Alm, Huang, Price, Arkin

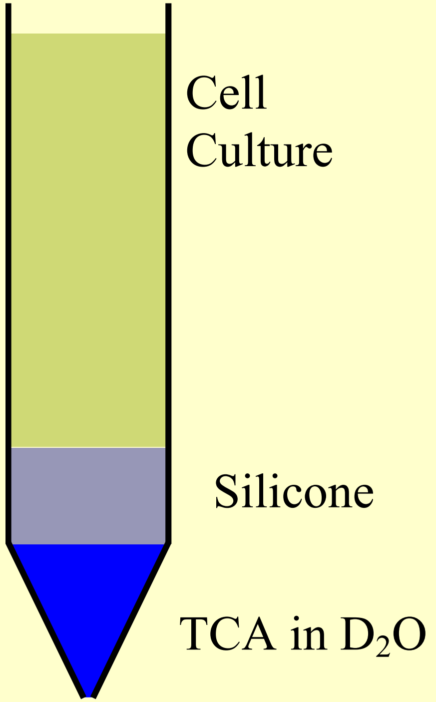
Metabolomic Progress

- GC-MS, LC-MS/MS, CE-MS
  - Linear ion-trap, triple quadrupole, single quadrupole MS

- Rapid quenching of metabolism
  - D_2O with trichloroacetic acid
  - concentrates metabolites
- ATP, ADP, and AMP measured to calculate the energy charge

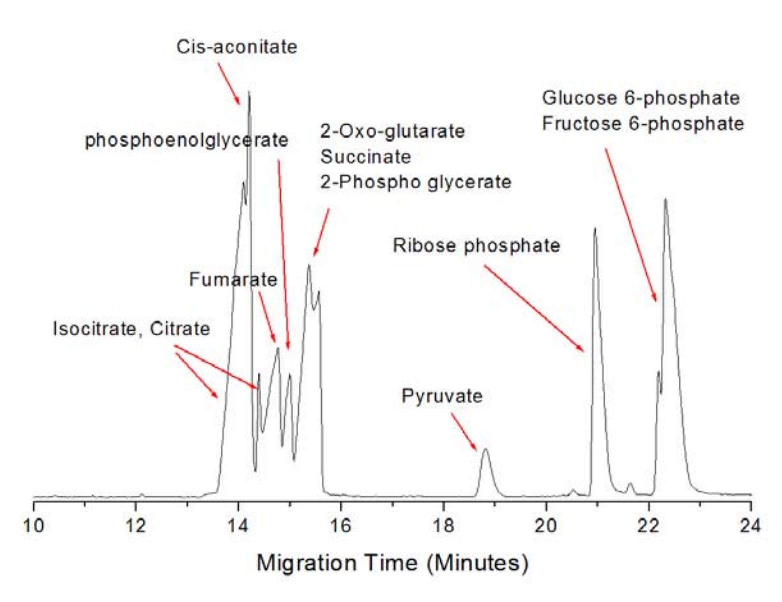
$$EC = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

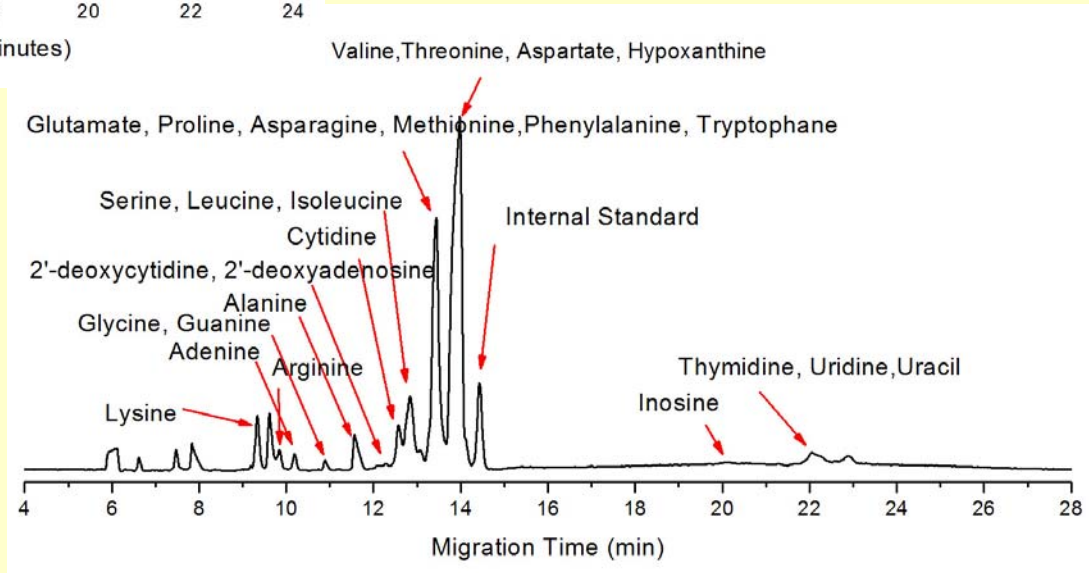
  - control for proper extraction
  - successful if EC = 0.7-0.9



Jay Keasling

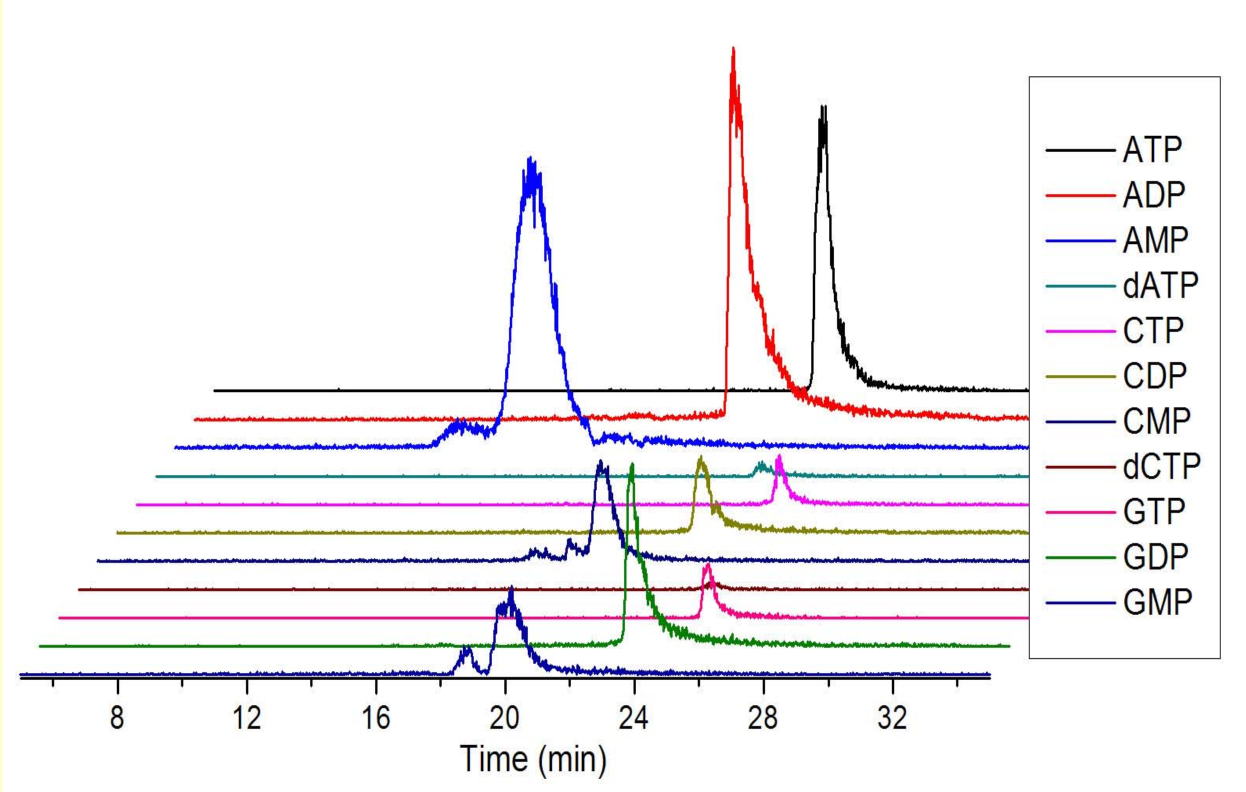
CE-MS of amino acids, nucleosides, and organic acids

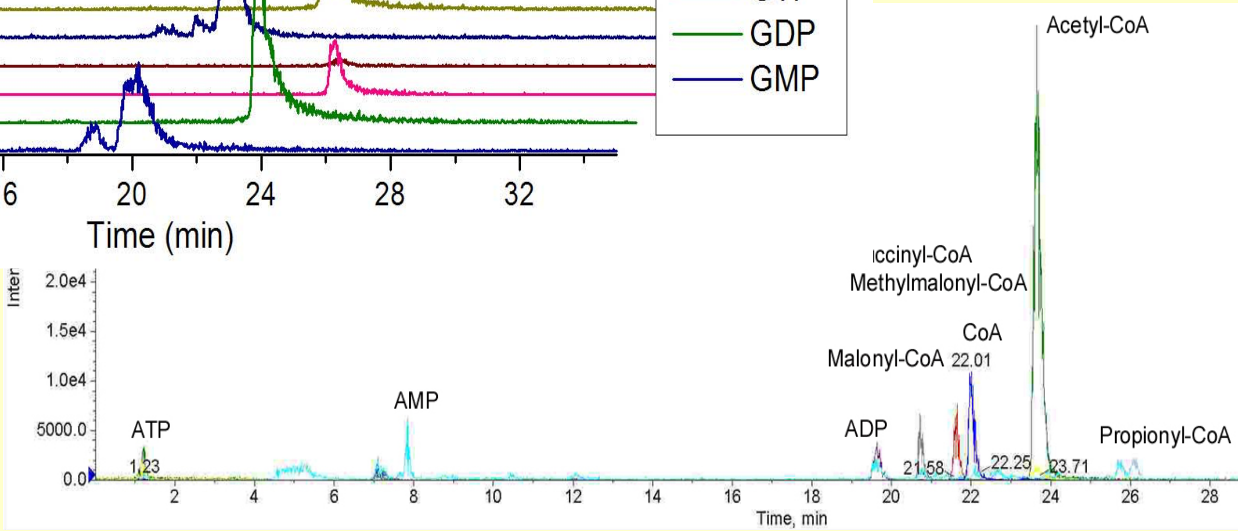




Jay Keasling

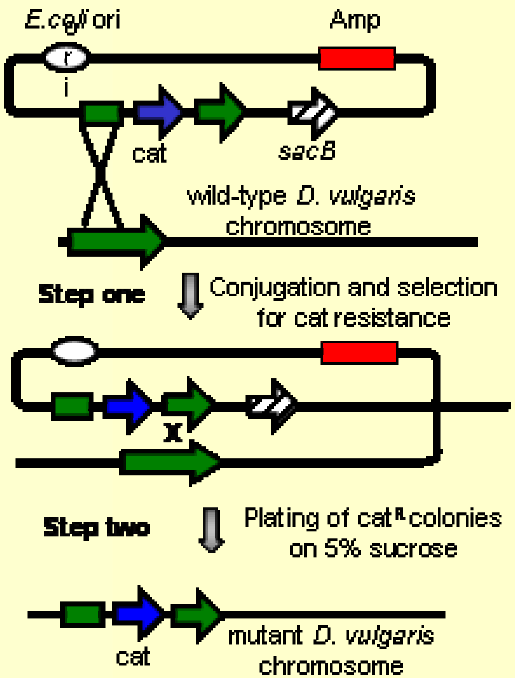
LC-MS^n of nucleotides and CoA's

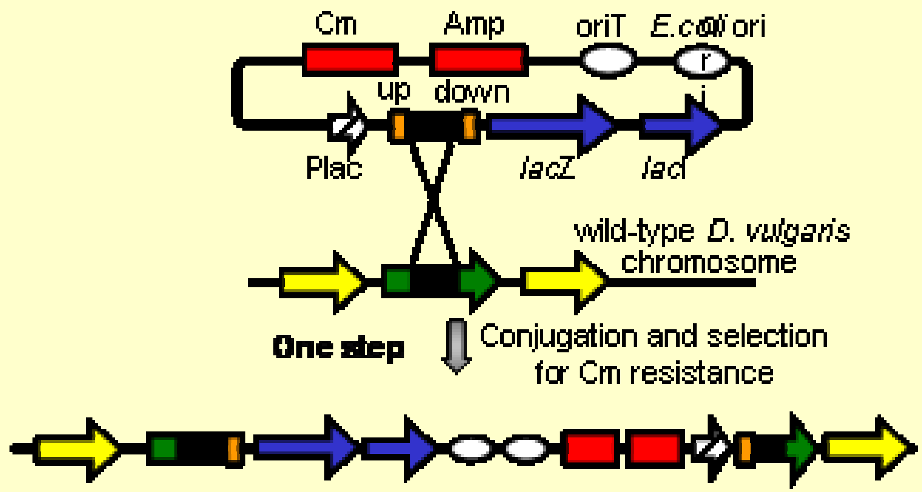




Jay Keasling

FGC: Genetic System Development



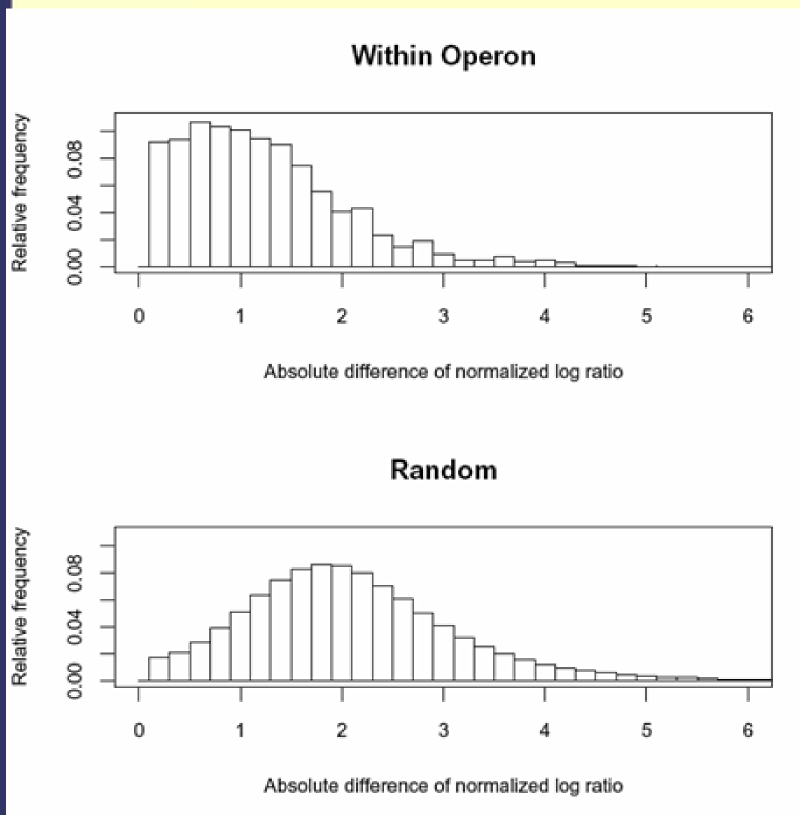


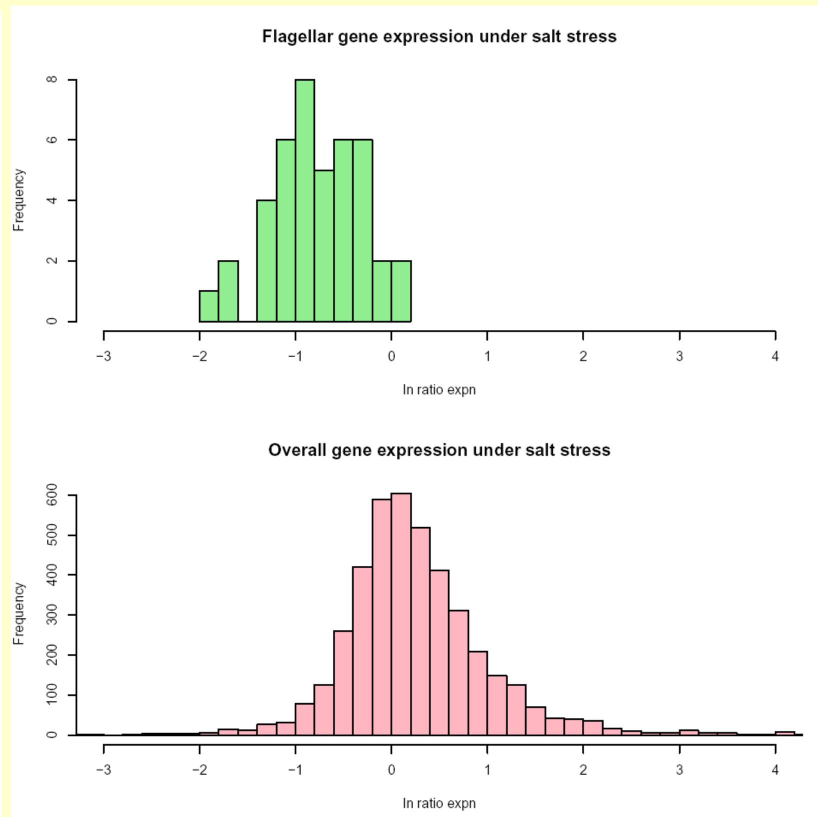
Two step vs. one step knockout by homologous recombination.
Marking deletion and tagged strains using 25-mer molecular bar-codes

Judy Wall, Vince Martin, Jay Keasling

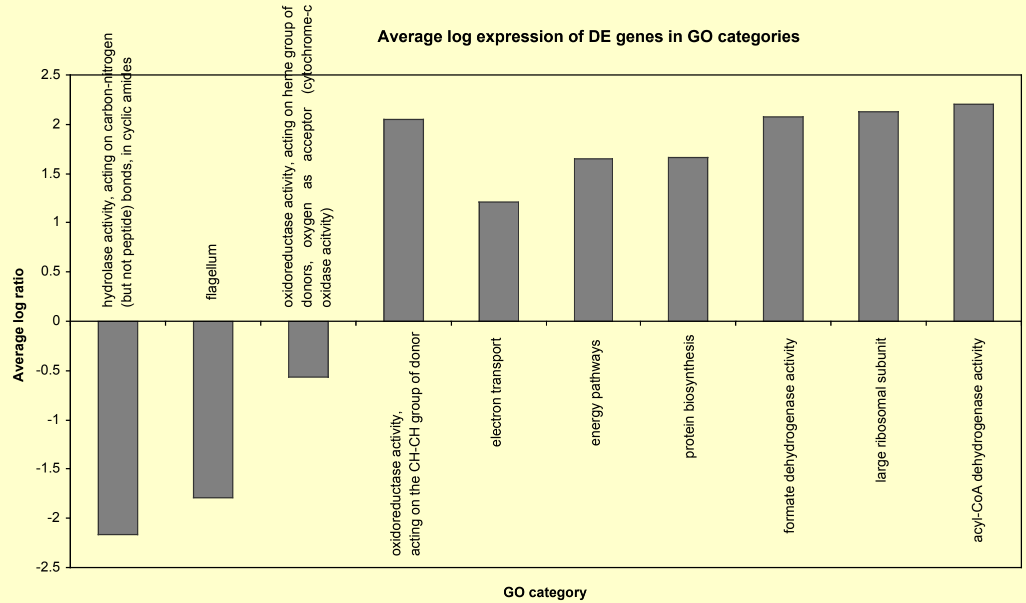
FGC: Shewanella Salt Stress

- Differentially Expressed Genes Detected by B-test.



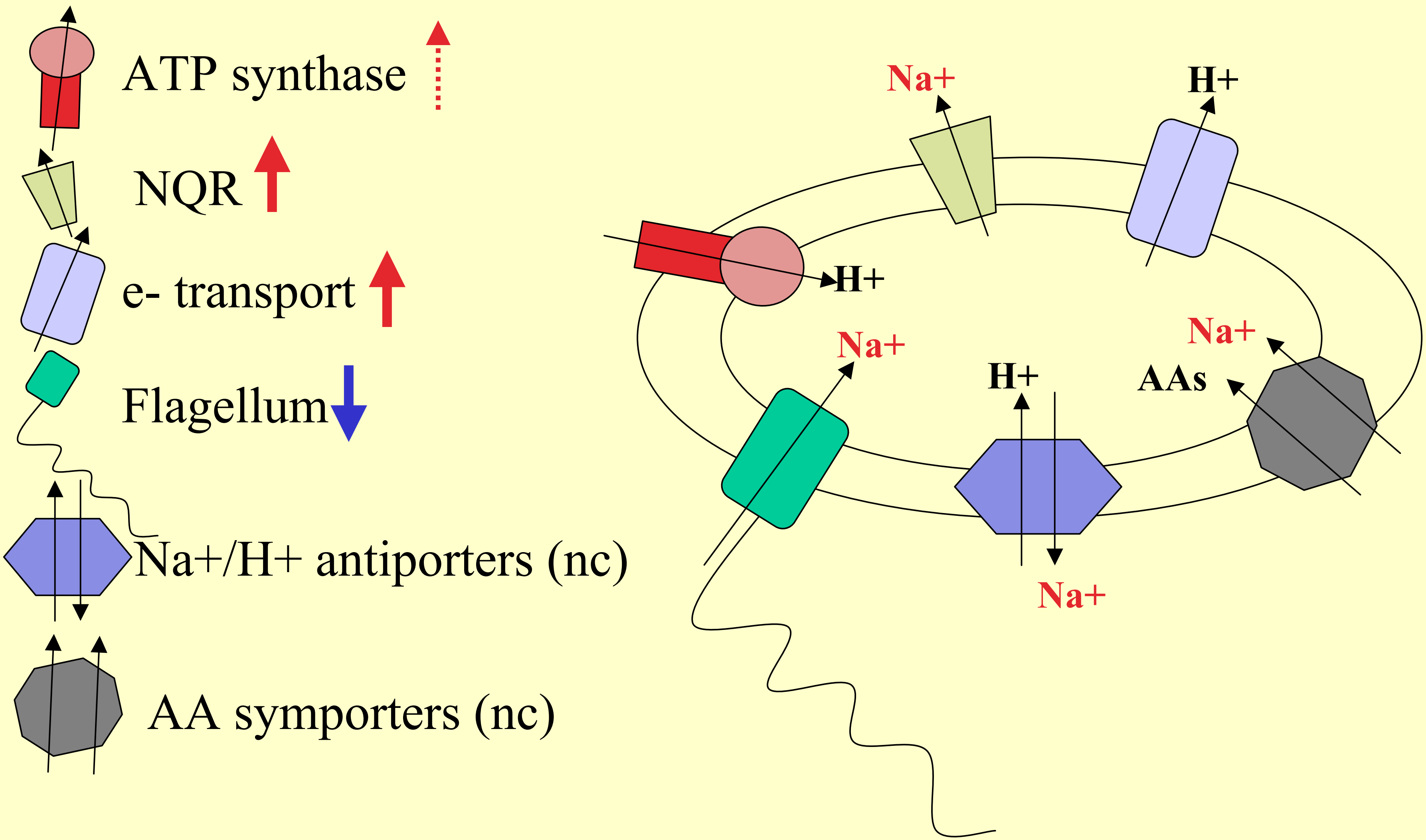


FGC:Shewanella Salt Stress



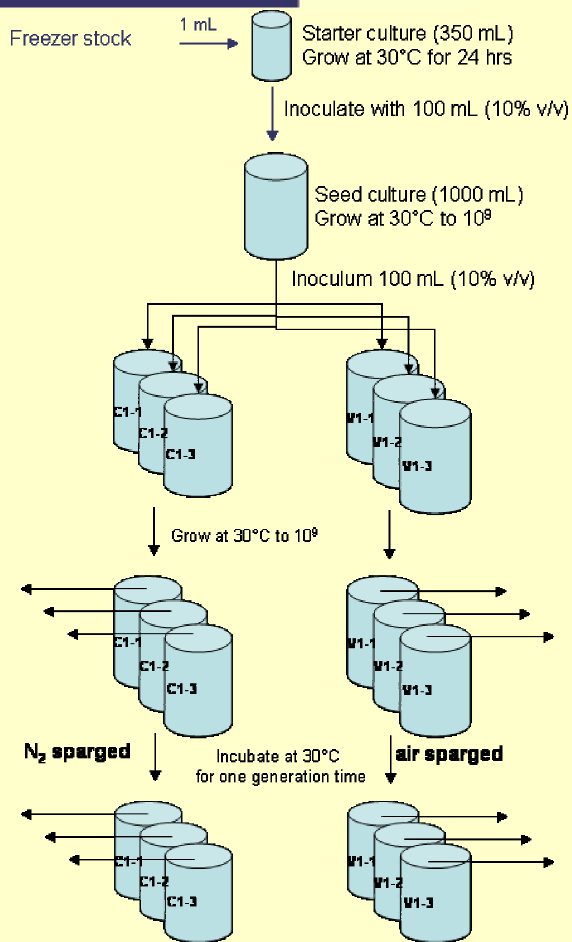
GOaccn#	Fisher's p	GO term
0016812	0.0337	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds , in cyclic amides
0019861	1.42E-05	flagellum
0016676	0.0082	oxidoreductase activity, acting on heme group of donors, oxygen as acceptor (cytochrome-c oxidase acitvity)
0016627	0.0023	oxidoreductase activity, acting on the CH-CH group of donors
0006118	0.0017	electron transport
0006091	0.0365	energy pathways
0006412	0.0192	protein biosynthesis
0008863	0.0021	formate dehydrogenase activity
0015934	0.0135	large ribosomal subunit
0003995	0.0019	acyl-CoA dehydrogenase activity
0005515	0.0337	protein binding

Salt Stress in *Shewanella oneidensis*



FGC: O_2 Stress D.v.

Data just in from the entire team!

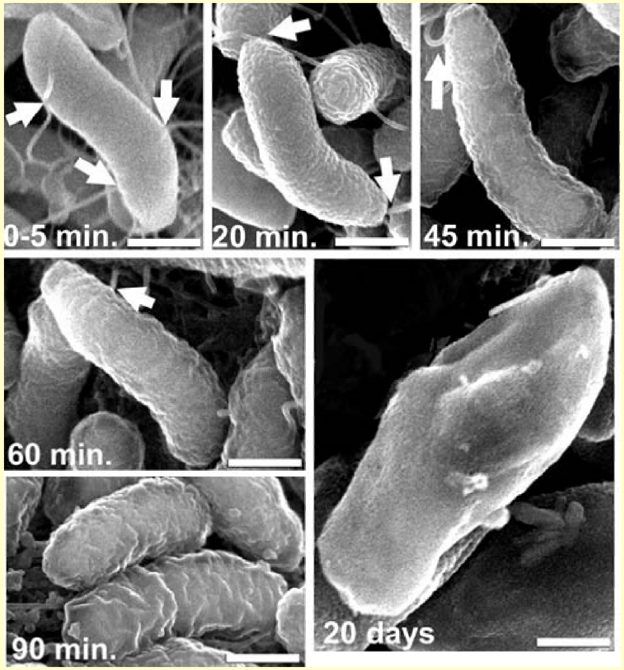


Cells grown batch from log phase starter to mid log phase and then sparged with air and nitrogen for 5 h (one generation time) and harvested at T0 and T5 for 5 VIMSS labs.

0 H | **2 H** | **4 H** | **12 H** | **12.5 H 15 min Air**

O_2 Stress in *Desulfovibrio vulgaris*

nSig	nUarray	p	GOName
26	142	0.0002	transcription termination
4	6	0.0008	4-diphosphocytidyl-2C-methyl-D-erythritol synth
4	6	0.0008	O-acetyltransferase activity
5	11	0.0017	primary active transporter activity
5	11	0.0017	cell wall
11	51	0.0043	proline-tRNA ligase activity
2	2	0.0082	purine base catabolism
2	2	0.0082	adenine catabolism
2	2	0.0082	phenylalanyl-tRNA aminoacylation
2	2	0.0082	prolyl-tRNA aminoacylation
2	2	0.0082	nucleoside triphosphate metabolism
14	77	0.0109	N-acetyltransferase activity
14	77	0.0109	phosphoenolpyruvate-dependent sugar phosph
2	3	0.0233	acyl-CoA or acyl binding
2	3	0.0233	cobalamin [5'-phosphate] synthase activity
2	3	0.0233	chloramphenicol O-acetyltransferase activity
2	3	0.0233	transferase activity, transferring glycosyl groups
2	3	0.0233	transferase activity, transferring hexosyl groups



Fischer exact test of GO terms for DE genes as measured by microarrays at 2hrs revealed numerous up-regulated genes in cell wall and polysaccharide metabolism. Candidates for EPS activity.

Also– why all the sugar activity given D.v. doesn't use hexoses for cell growth?

Differential In-Gel Electrophoresis (DIGE) for Identifying Proteins Involved in Oxygen Stress

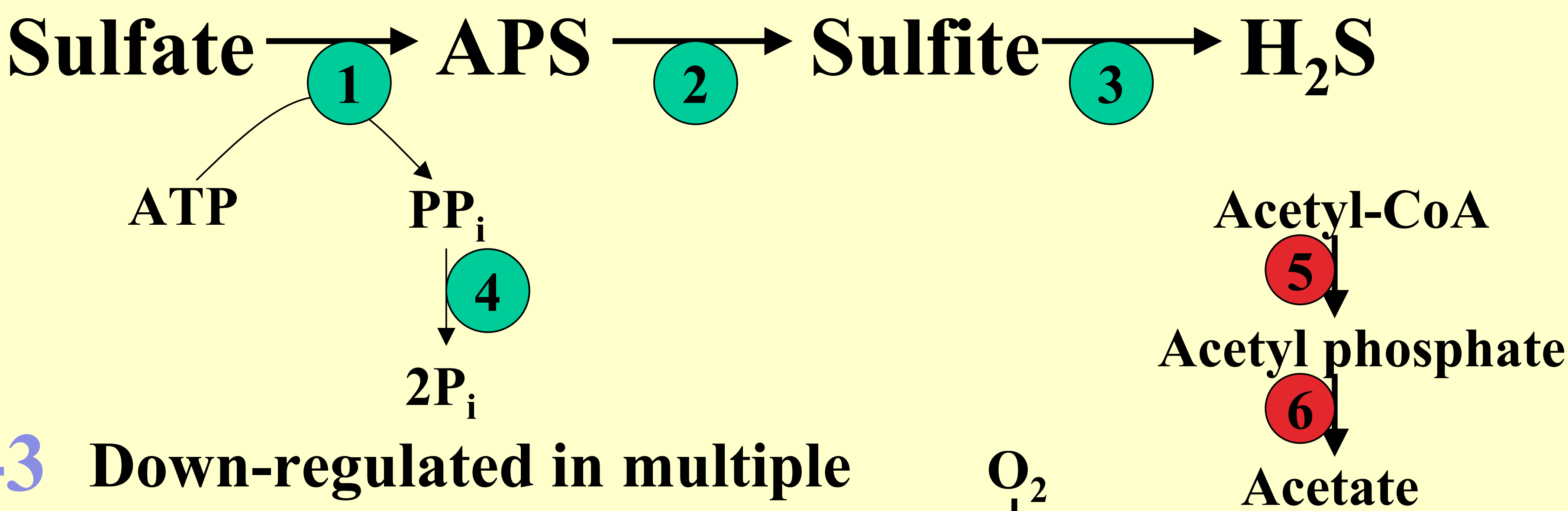
Summary of Results: Unique IDs

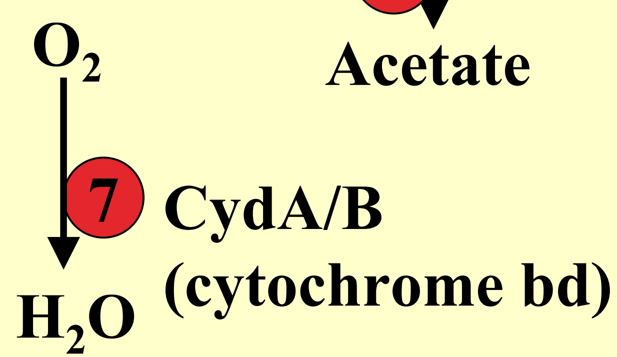
			Experimental		Theoretical		
ORF	Score*	Annotation	MW	pI	MW	pI	Differential Expression
ORF01081	177	sulfate adenylyltransferase	48779	6.22	47469	6.07	2.75
ORF04271	104	desulfoferrodoxin	27632	6.06	13983	5.96	-2.38
ORF00918	89	riboflavin synthase, beta subunit	28427	6.5	23648	5.58	-2.54
ORF00338	119	adenylyl-sulphate reductase, alpha subunit	38698	6.63	74627	6.52	-2.6
ORF01214	105	ketol-acid reductoisomerase	44731	6.39	36066	6.04	-2.7
ORF00530	126	ribosomal protein L9	31561	5.58	17953	5.37	-2.79
ORF03581	82	dissimilatory sulfite reductase, gamma subunit	26461	5.35	11872	5.14	-2.82
ORF04274	213	rubredoxin-oxygen oxidoreductase	47912	6.21	45079	5.97	-3.14
ORF05313	136	dissimilatory sulfite reductase, alpha subunit	52015	5.52	49091	5.42	-3.26
ORF04950	146	conserved hypothetical protein	38181	6.71	29119	6.4	-8.5

*Protein scores greater than 48 are significant ($p<0.05$)

Team: Swapnil Chhabra, Sara Gaucher, Masood Hadi, Anup Singh

Down-regulation of Sulfate Reduction Pathway





1-3 Down-regulated in multiple proteomics methods + MA

4 Strongly down in MA

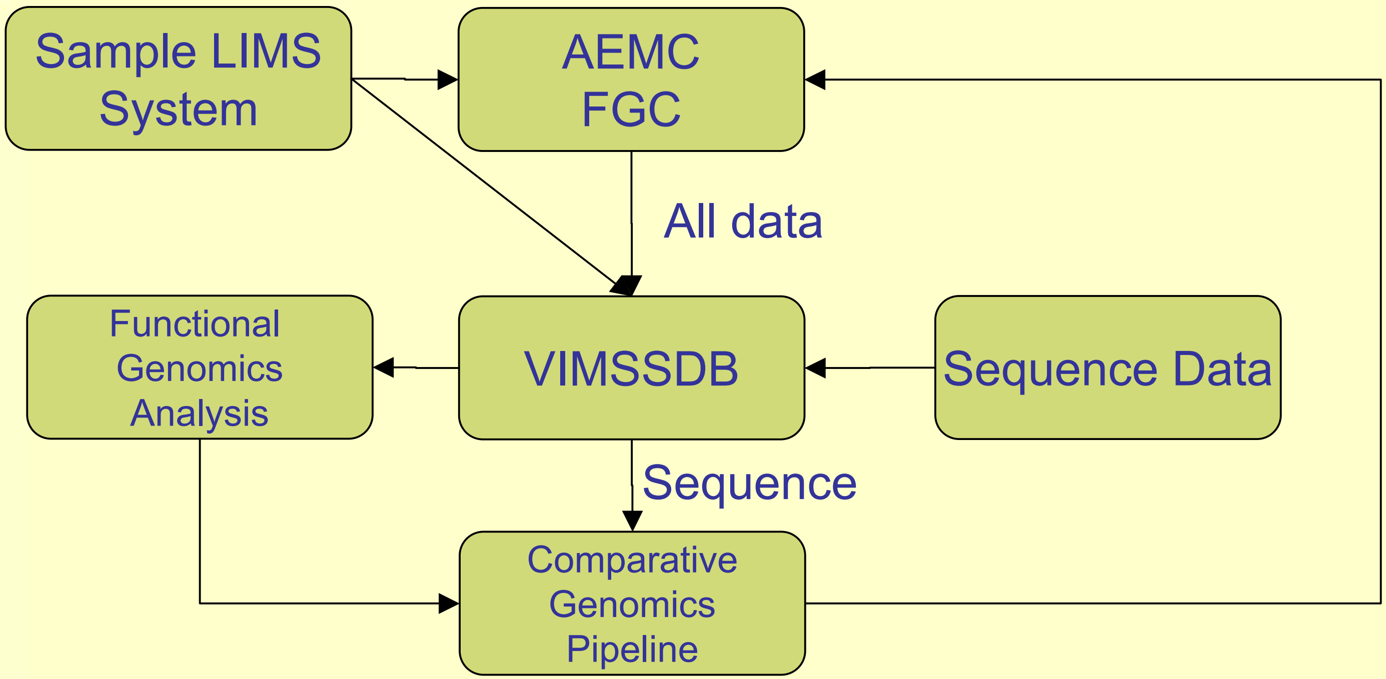
5-7 Up-regulated in MA

O_2 Stress: Summary of Results

- Cell wall and various sugar metabolism categories are upregulated in response to O_2 stress.
- This is consistent with the EPS activity observed in the electron micrographs, giving us an initial seed group for elucidating and further characterizing those pathways.
- Apparent down-regulation of the sulfate-reduction pathway observed in MA, and confirmed by several proteomics methods.
- Additional evidence suggests this may be an actual O_2 related change (rather than growth effect) is that pyrophosphatase is significantly down-regulated (pyrophosphate is a byproduct of the second step in sulfate reduction), and several genes involved in substrate-level phosphorylation of ADP are up-regulated (phosphate acetyltransferase and acetate kinase).
- The attractive speculation resulting from all of this is that Dv may be down-regulating sulfate reduction to increase the amount of reducing power available for O_2 reduction.
- One mechanism for such reduction would be the cydAB operon (cytochrome bd) recently shown to be essential for oxygen consumption in the strict anaerobe Bacteroides fragilis. We note that both cydA and cydB are significantly up-regulated at 2 hours after air sparging compared to t=0.

Baughn AD, Malamy MH. Nature. (2004) **The strict anaerobe Bacteroides fragilis grows in and benefits from nanomolar concentrations of oxygen. 427**(6973):441-4.

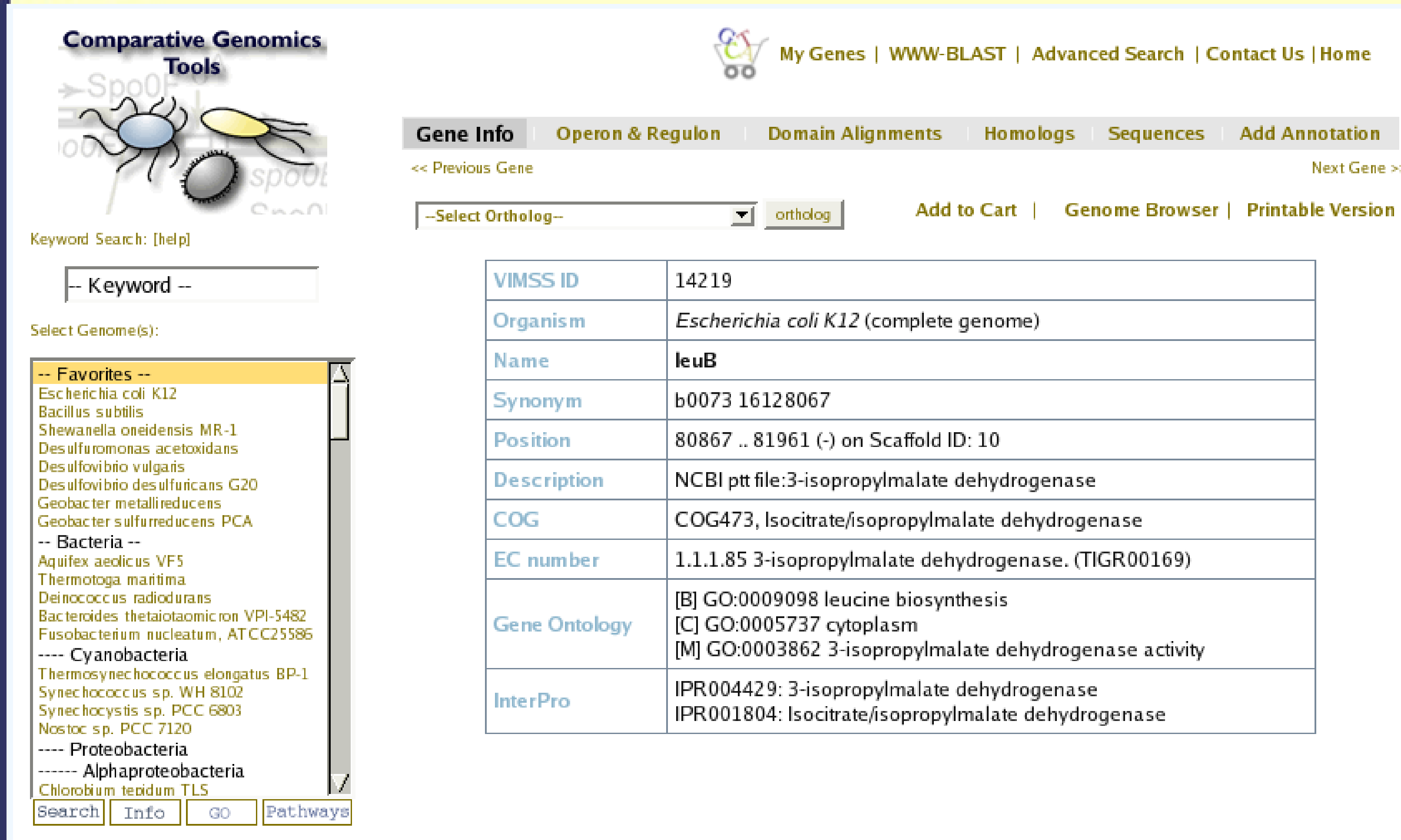
CC: Design of Current Components



CC: VIMSS CGDB

http://escalante.lbl.gov

http://vimss.lbl.gov



Comparative Genomics Tools

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Gene Info | Operon & Regulon | Domain Alignments | Homologs | Sequences | Add Annotation

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Next Gene >>

--Select Ortholog-- ortholog

Add to Cart | Genome Browser | Printable Version

Keyword Search: [help]

-- Keyword --

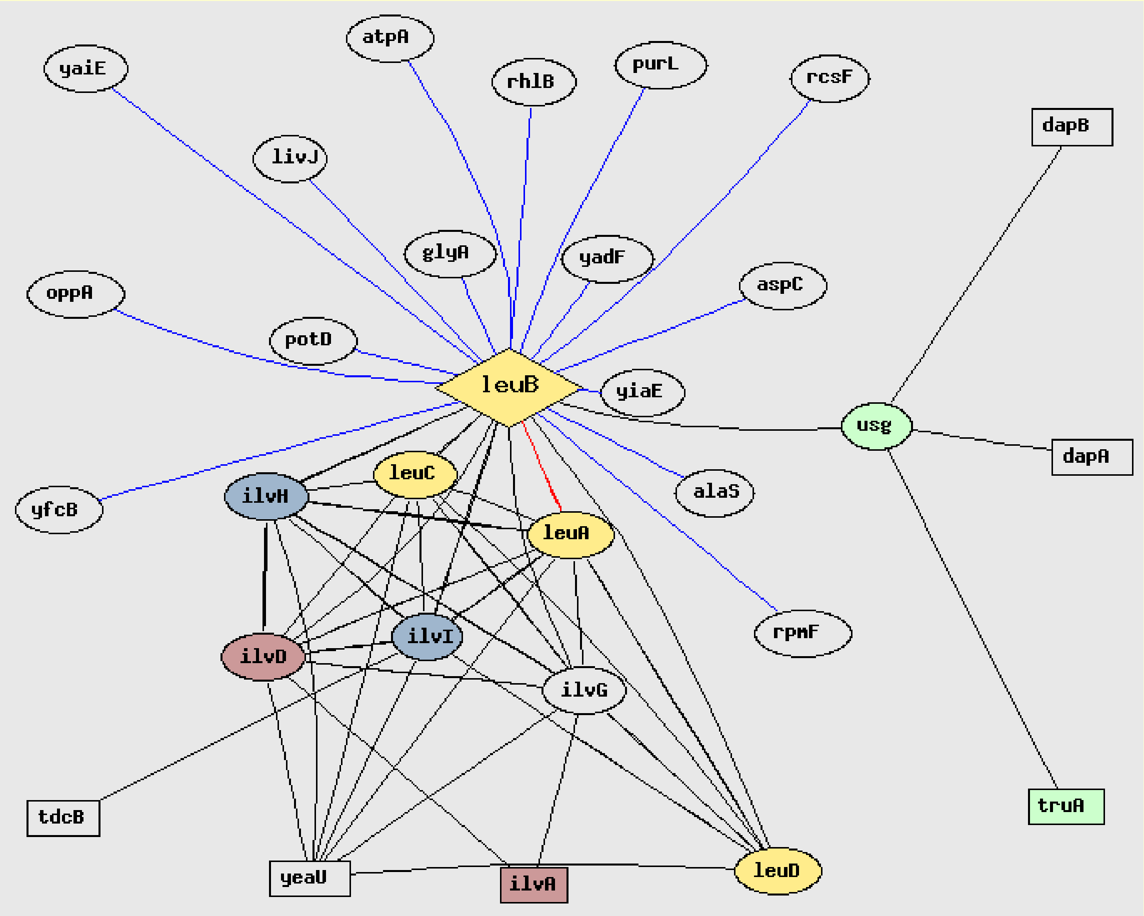
Select Genome(s):

-- Favorites --
Escherichia coli K12
Bacillus subtilis
Shewanella oneidensis MR-1
Desulfuromonas acetoxidans
Desulfovibrio vulgaris
Desulfovibrio desulfuricans G20
Geobacter metallireducens
Geobacter sulfurreducens PCA
-- Bacteria --
Aquifex aeolicus VF5
Thermotoga maritima
Deinococcus radiodurans
Bacteroides thetaiotaomicron VPI-5482
Fusobacterium nucleatum, ATCC25586
---- Cyanobacteria
Thermosynechococcus elongatus BP-1
Synechococcus sp. WH 8102
Synechocystis sp. PCC 6803
Nostoc sp. PCC 7120
---- Proteobacteria
------ Alphaproteobacteria
Chlorobium tepidum TLS

Search | Info | GO | Pathways

VIMSS ID	14219
Organism	*Escherichia coli K12* (complete genome)
Name	**leuB**
Synonym	b0073 16128067
Position	80867 .. 81961 (-) on Scaffold ID: 10
Description	NCBI ptt file:3-isopropylmalate dehydrogenase
COG	COG473, Isocitrate/isopropylmalate dehydrogenase
EC number	1.1.1.85 3-isopropylmalate dehydrogenase. (TIGR00169)
Gene Ontology	[B] GO:0009098 leucine biosynthesis [C] GO:0005737 cytoplasm [M] GO:0003862 3-isopropylmalate dehydrogenase activity
InterPro	IPR004429: 3-isopropylmalate dehydrogenase IPR001804: Isocitrate/isopropylmalate dehydrogenase

VIMSSDB: Regulon Prediction



VIMSS regulon browser allows users to browse the neighborhood of genes predicted to be coregulated based on:

- conserved gene order in distant genomes, black lines
- observed to be coregulated in microarray experiments, blue lines
- red lines indicate connections both predicted and observed).

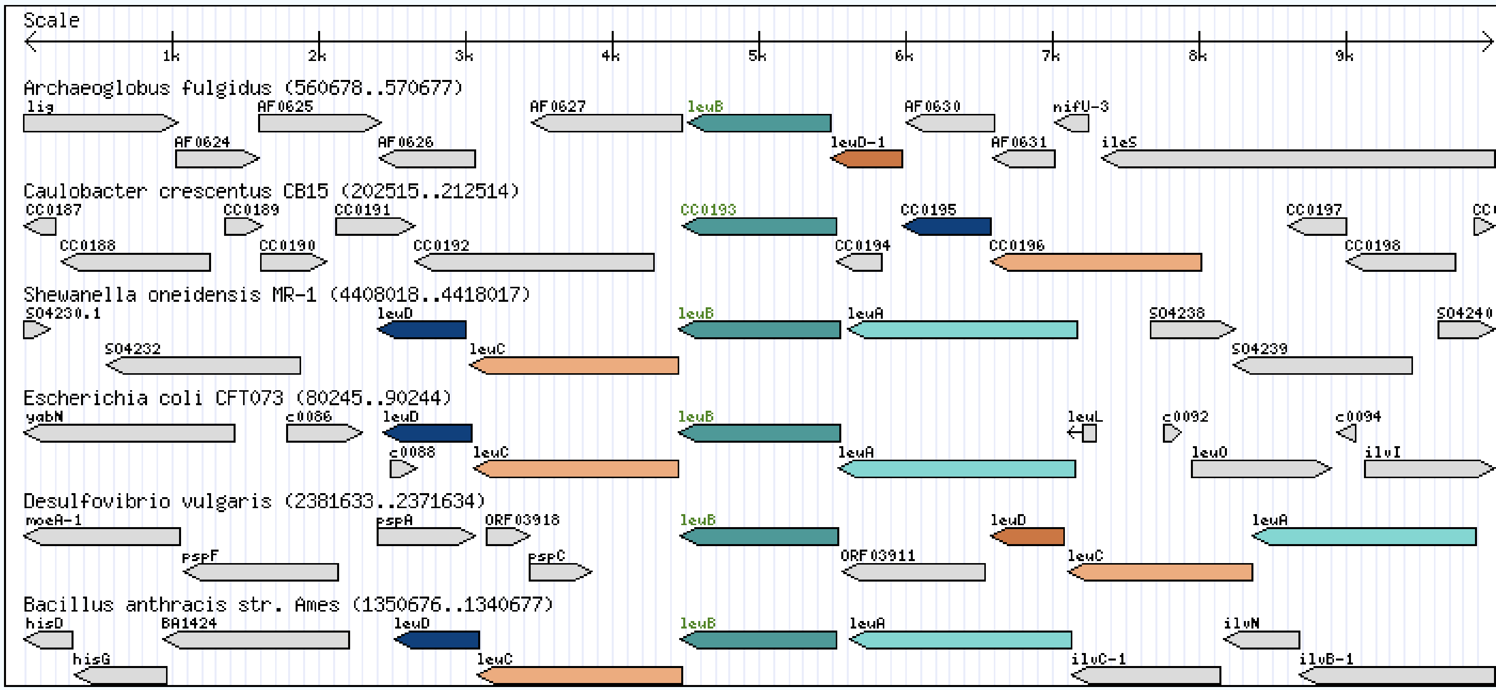
VIMSS: CG Browser

Move Left
<<< (95%)   << (66%)   < (25%)

Zoom In
10x   3x   1.5x

Zoom Out
1.5x   3x   10x

Move Right
(25%) >   (66%) >>   (95%) >>>



Clicking on a feature will:

- Load Protein Page
- Recenter on feature
- Save as feature of interest

View your current cart
View all saved carts

Select Organism(s) to Display (Ctrl-Click to select multiple)

-- Organisms Currently Displayed --
Archaeoglobus fulgidus
Caulobacter crescentus CB15
Shewanella oneidensis MR-1
Escherichia coli CFT073
Desulfovibrio vulgaris
Bacillus anthracis str. Ames
-- Bacteria --
Aquifex aeolicus VF5
Thermotoga maritima

Update Display

CC: Comparative GO Browser

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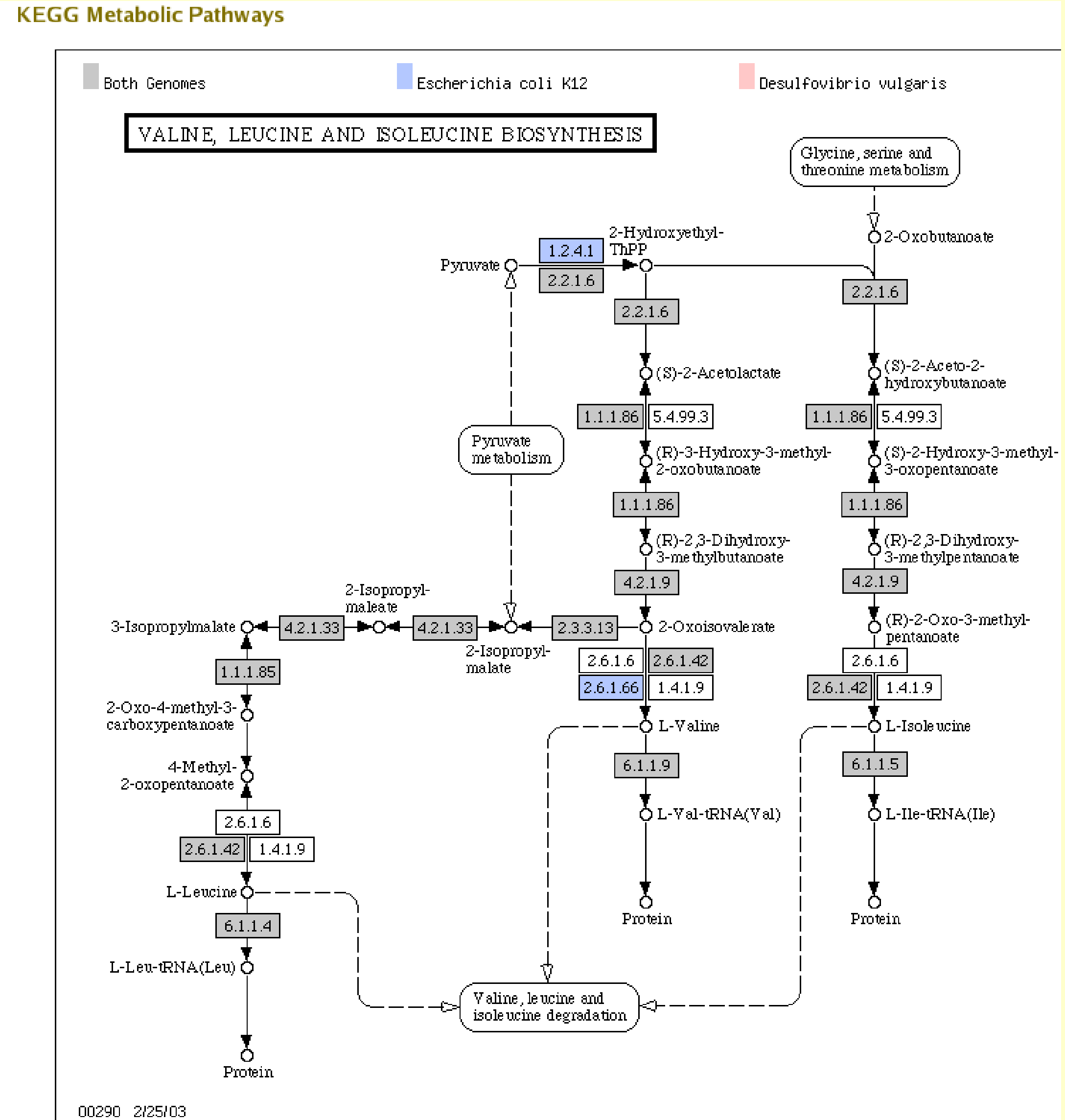
VertiGo: VIMSS to Gene Ontology Browser

(E.col K12)	*(S.one MR-1)*	*(D.ace)*	*(D.vul)*	*(D.des G20)*	*(G.met)*	*(G.sul PCA)*	
2713	2316	3606	1801	1875	1973	1996	+ GO:0003673 : Gene_Ontology
2340	1978	3106	1544	1588	1695	1719	+ GO:0008150 : biological_process [P]
2263	1915	2964	1491	1541	1627	1652	+ GO:0007582 : physiological processes [I]
1666	1526	2330	1152	1194	1377	1336	+ GO:0008152 : metabolism [I]
143	118	168	83	84	102	96	+ GO:0009308 : amine metabolism [I]
129	112	162	78	78	96	91	+ GO:0006520 : amino acid metabolism [I]
16	13	17	10	11	7	8	+ GO:0009081 : branched chain family amino acid metabolism [I]
4	4	3	2	2	1	2	+ GO:0006551 : leucine metabolism [I]
4	4	3	2	2	1	2	− GO:0009098 : leucine biosynthesis [I]
0	0	0	0	0	0	0	− GO:0006552 : leucine catabolism [I]

VIMSS: Comparative Metabolic Maps

KEGG Metabolic Pathways



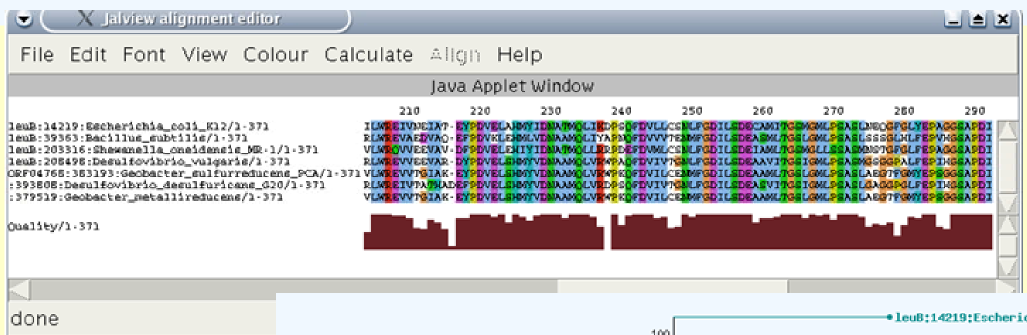
VIMSS: Workbench

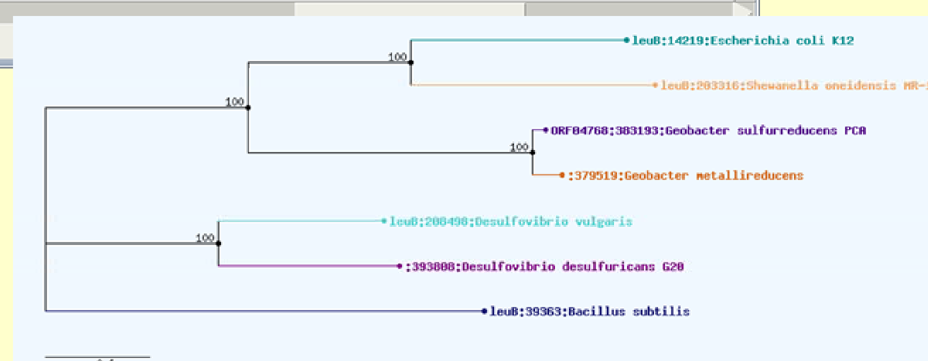
Your Genes of Interest

VIMSS Id	Name	Organism	Remove?
14219	leuB	Escherichia coli K12	Remove
39363	leuB	Bacillus subtilis	Remove
203316	leuB	Shewanella oneidensis MR-1	Remove
208498	leuB	Desulfovibrio vulgaris	Remove
383193	ORF04768	Geobacter sulfurreducens PCA	Remove
393808		Desulfovibrio desulfuricans G20	Remove
379519		Geobacter metallireducens	Remove

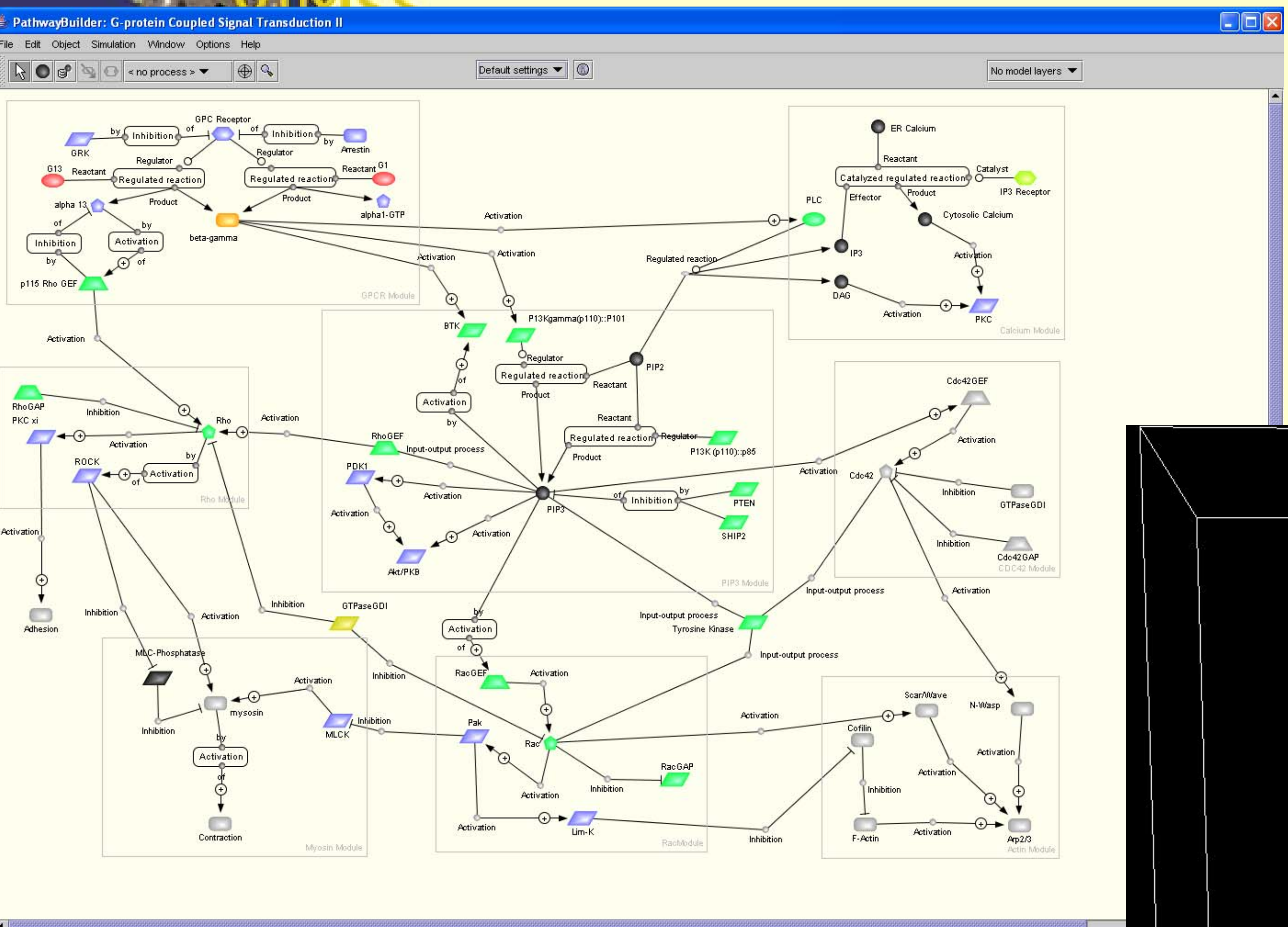
Retrieve Data

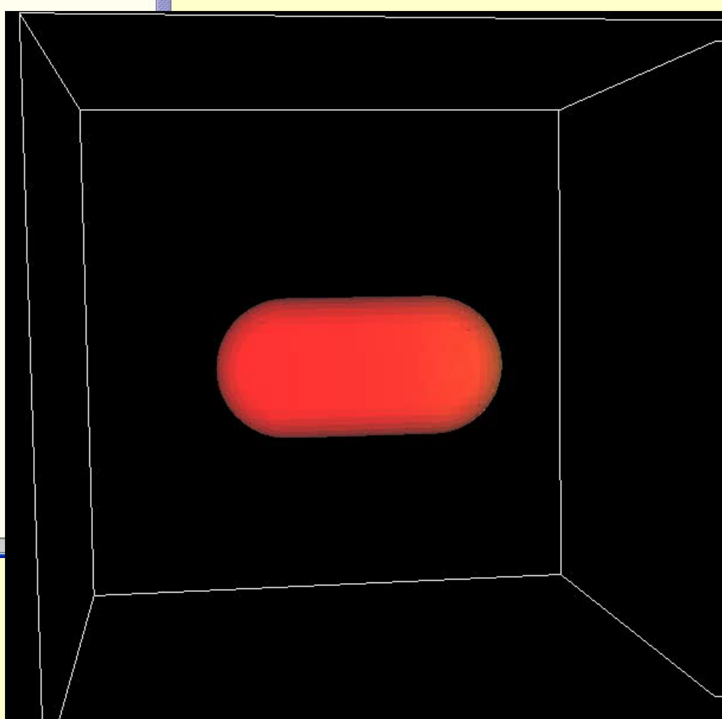
Type: Positional   Format: ASCII   View   Download





VIMSS: Towards Integration with Pathway/Simulation Tools





BioSpice open-source biology

DARPA