A source of biological parts for Synthetic Biology. Xenologs?

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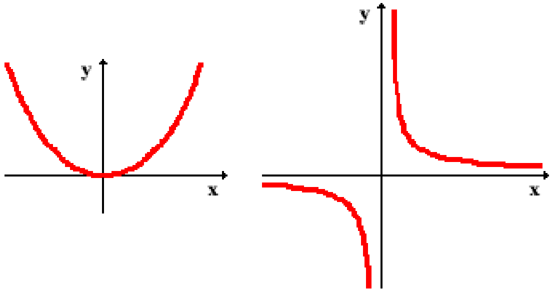
Content

- (1) Design principles in SB
    - Dissatisfaction with bio-bricks
- (2) Dialectical cycle: analysis <> synthesis
- (3) Environmental scanning. Sulfur metabolism in bacteria (an example)
    - reference microorganism
    - min set of genes for sulfur metabolism
    - mapping to environments
    - xenologs vs. orthologs
    - design and simulation

Design principles – MIT & Co

- An idea of SB is to build organisms that perform desired tasks, rather than to modify existing ones
- A major challenge for SB is to tackle complexity
  - top-down
  - bottom-up



- “Cell as an agent” may be a very suitable concept
  - design of agents with adaptive strategies
  - swarm algorithms
- Molecular logic and DNA embedded programming on the different levels
  - recombination
  - transcription
  - RNA interference
  - GTPases
  - Phosphorylation
- Separated genetic programs within different types of cells that work together can be used to avoid a cross talk
  - partition of genomes (incompatible plasmids)
  - compartments (membranes)
  - scaffold (DNA, RNA, proteins)

- Karman-Knight’s strategy:
  information is turned off → abstraction → design

Dissatisfaction with the current bio-brick strategy

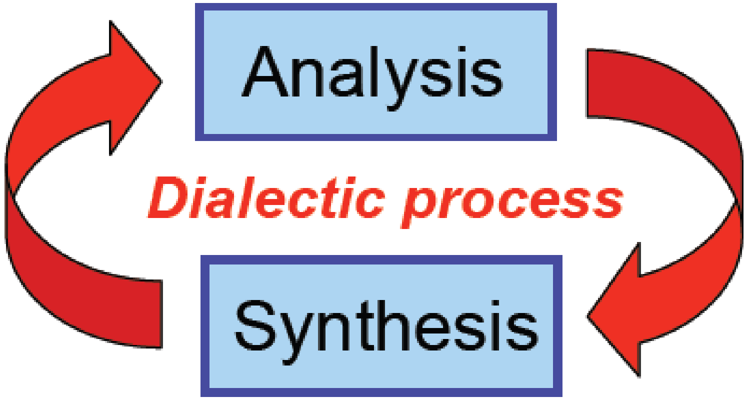
- new biological parts will lead to exponential growth of unspecific interactions in a target system – <u>a cross talk</u>

- standardization will lead to repeats in synthetic DNA and finally to recombinations – <u>a genetic instability</u>

Dialectical cycle in Freiburg

bioss

CENTRE FOR BIOLOGICAL SIGNALLING STUDIES


Analysis

Dialectic process

Synthesis

- “The central research idea of *bioss* is to initiate and promote a dialectic process between scientists using analytical (dissecting) and synthetical (rebuilding) approaches in signalling research.”

Possible impact of bioinformatics on synthetic biology

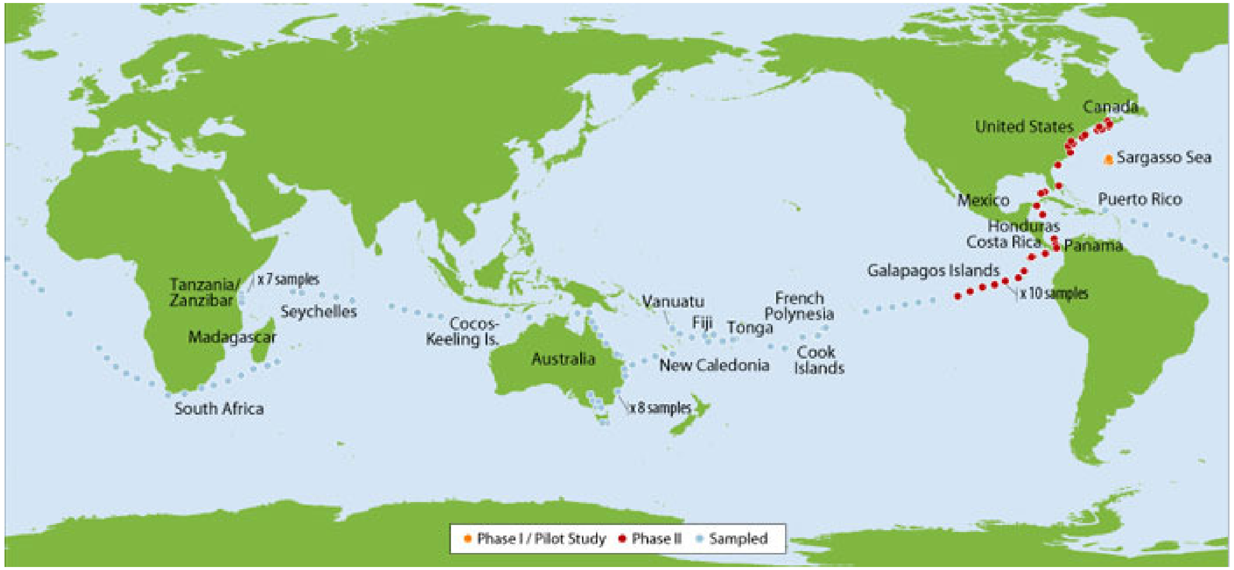
- X-omics
- High-throughput sequencing technologies
  - 2d generation
    - Roche/454 FLX
    - Illumina/Solexa Genome Analyzer
    - Applied Biosystems SOLiD^{TM} System
  - 3d generation
    - Helicos Heliscope^{TM}
    - Pacific Biosciences SMRT
- Understanding of biological complexity

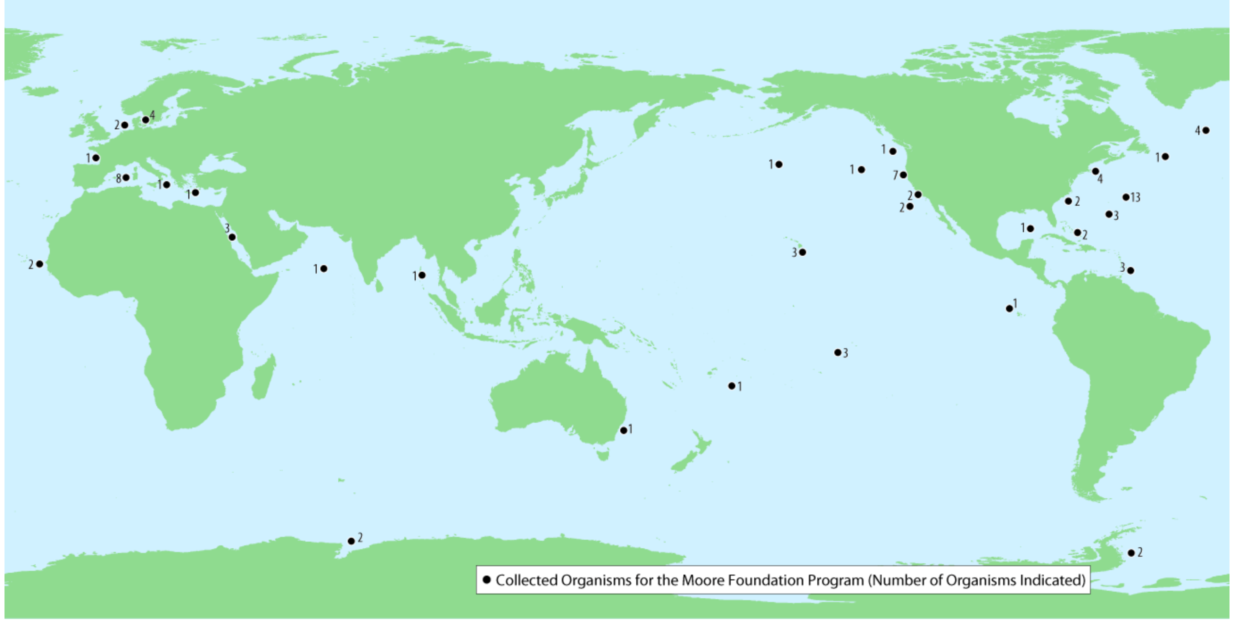
CAMERA v1.3.2

Community Cyberinfrastructure for Advanced Microbial Ecology Research & Analysis

The Project was initiated by the Gordon and Betty Moore Foundation, beginning in Jan 2006

Sampling (‘Sorcerer II’, and other)

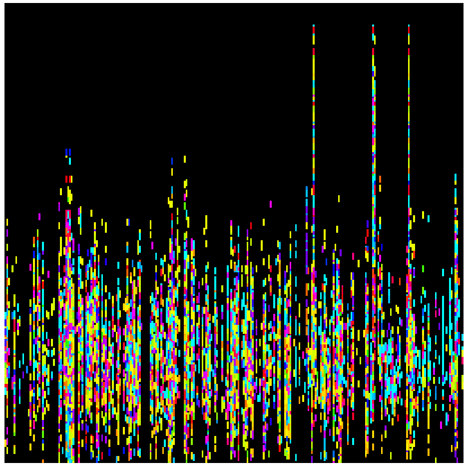




Databases that were used

- Collection from the Sorcerer II expedition
- Hawaii Ocean Time Series station which presents microbial genomes down to 4000 m
- Ionian abyssal plain, a deep flat basin between Sicily and Greece in the Eastern Mediterranean that is reached by H_2S
- Microbial community from deep-sea hydrothermal vent polychaete worm *Alvinella pompejana*
- Symbionts from another worm, Mediterranean gutless oligochaete *Olavius algarvensis*
- Microbes from Acid Mine Drainage biofilm at Iron Mountain, California
- Soil bacteria from Waseca County, Minnesota
- And other

Fragment requirement plots of reference microorganisms

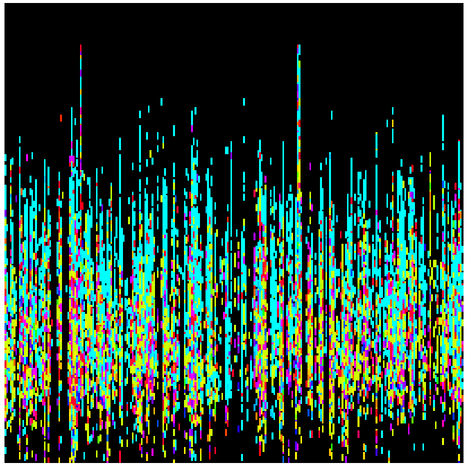


Thiomicrospira crunogena XCL-2

free-living bacterium

genome 2 427 674 nt

63 134 hits

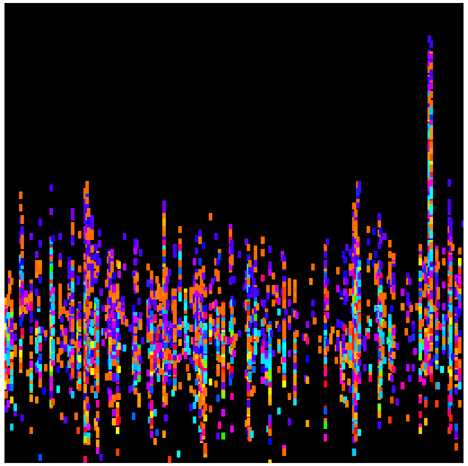


Thiobacillus denitrificans ATCC 25259

free-living bacterium

genome 2 909 749 nt

42 275 hits

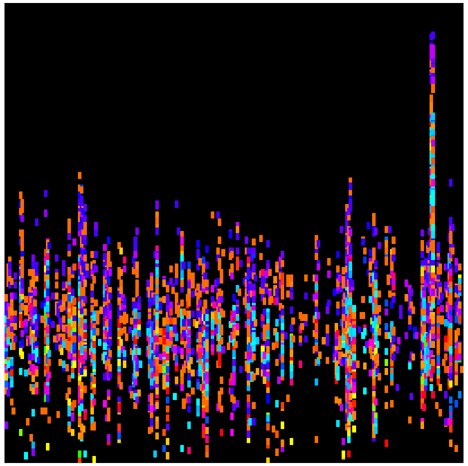


Vesicomyosocius okutanii HA

host *Calyptogena okutanii*

genome 1 022 154 nt

11 443 hits



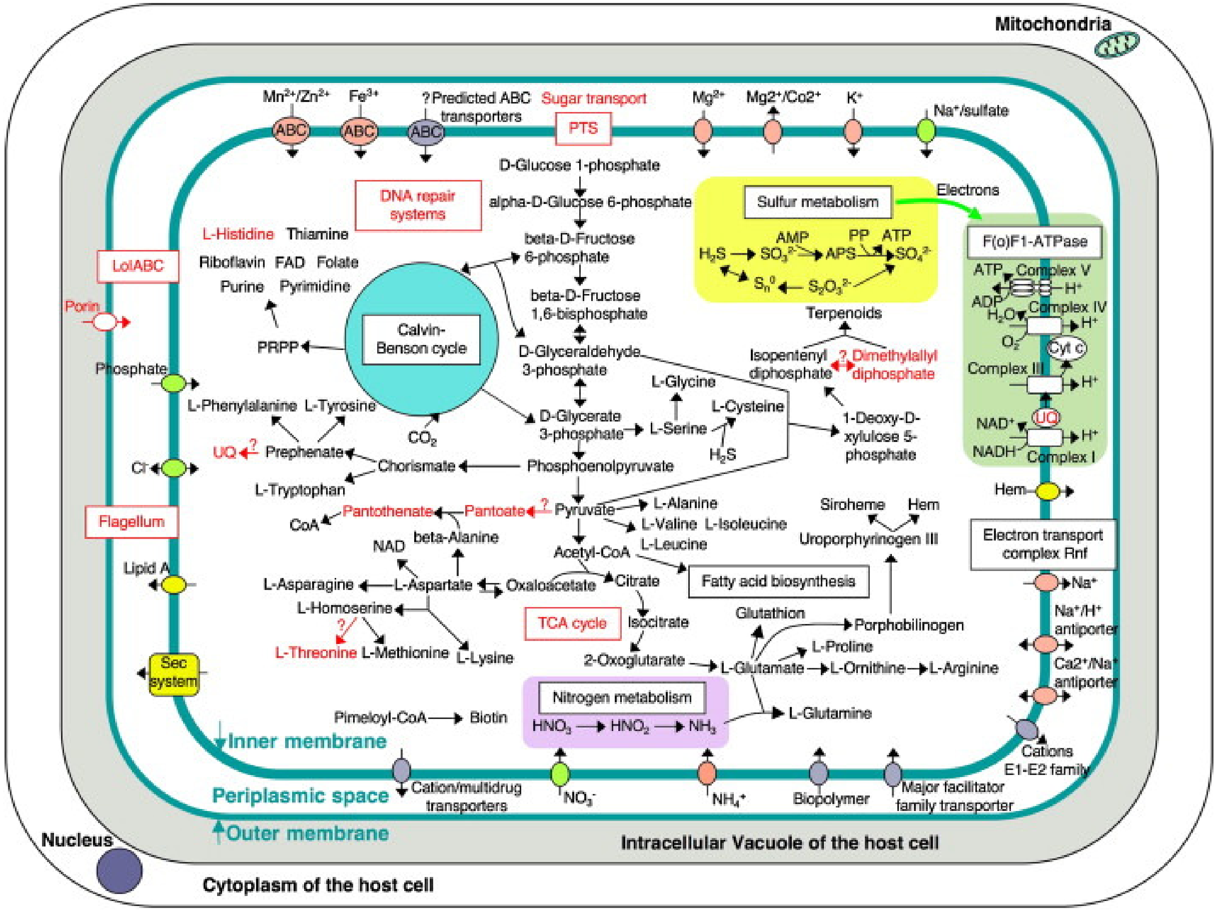
Ruthia magnifica Cm

host *Calyptogena magnifica*

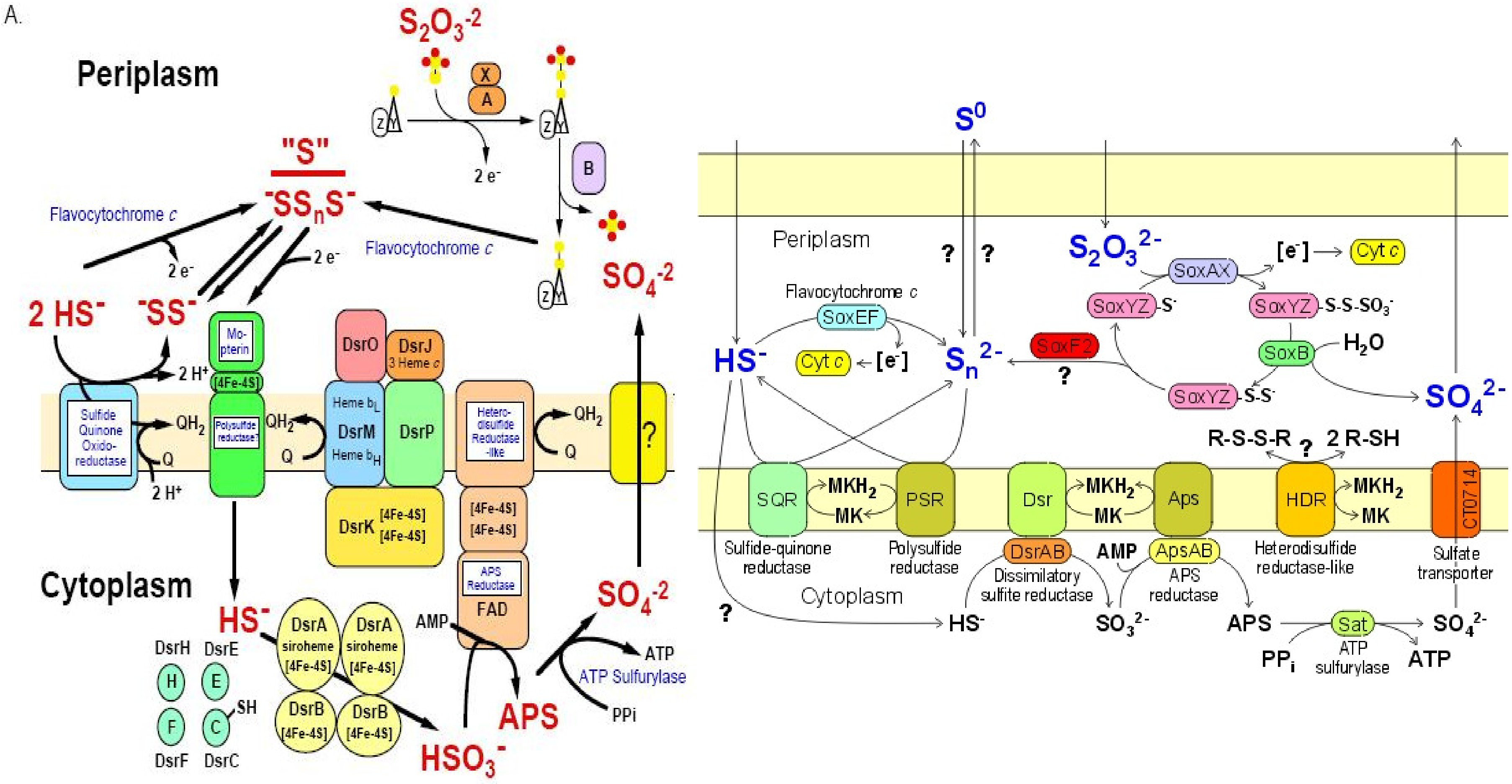
genome 1 160 782 nt

11 160 hits

V.okutanii is a symbiont in a deep-sea clam, *Calyptogena okutanii* [Kuwahara et al, 2007]



Sulfur metabolism in bacteria [lit. overview, KEGG, ERGO]



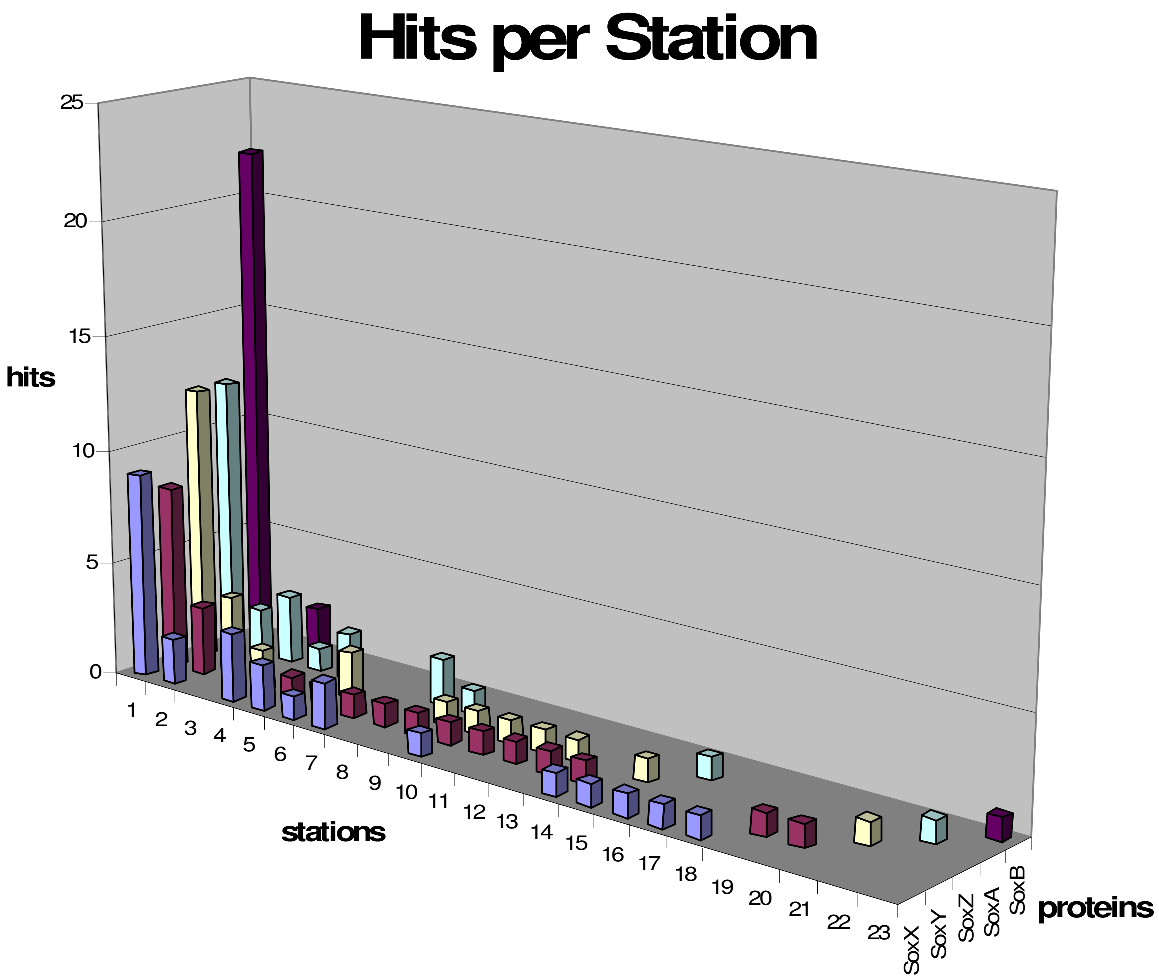
dissimilatory sulfite reductase (DsrAB), adenosine-5'-phosphosulfate reductase (APS), ATP sulfurylase (Sat) in cytoplasm, and Sox proteins in periplasm

The gene set from *V.okutanii* for a minimal sulfur metabolism

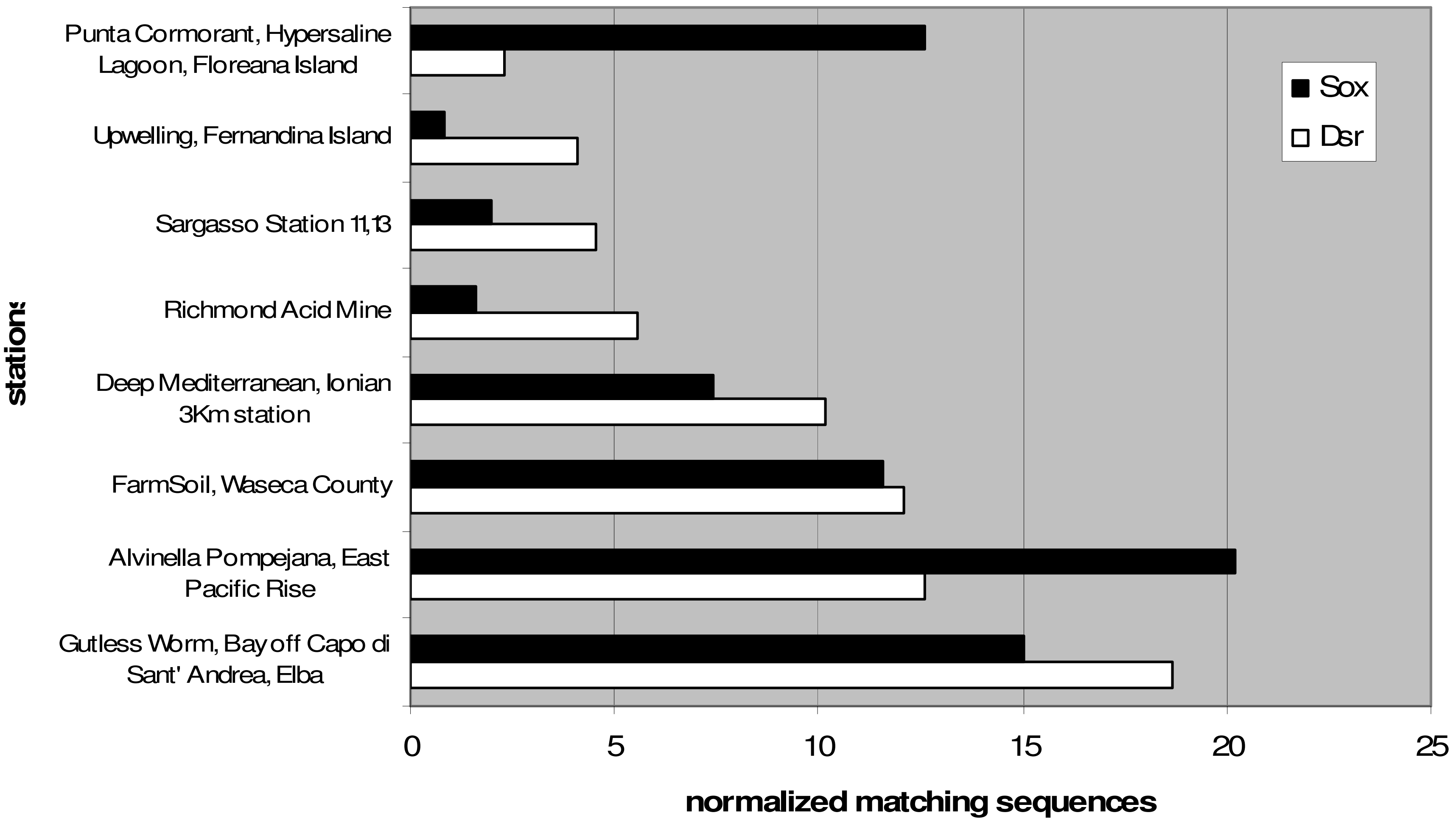
Product Name	**Start**	**End**	**Strand**	**Length**	**Locus**
ATP sulfurylase	98093	99301	+	402	*sat*
adenylylsulfate reductase membrane anchor	99516	100385	+	289	*aprM*
adenylylsulfate reductase β-subunit	100417	100896	+	159	*aprB*
adenylylsulfate reductase	100896	102779	+	627	*aprA*
sulfur oxidation protein SoxB	172596	174485	+	629	*soxB*
sulfur oxidation protein SoxA	770792	771607	-	271	*soxA*
sulfur oxidation protein SoxZ	771635	771937	-	100	*soxZ*
sulfur oxidation protein SoxY	771971	772414	-	147	*soxY*
sulfur oxidation protein SoxX	772425	772772	-	115	*soxX*
intracellular sulfur oxidation protein DsrR	817196	817537	-	113	*dsrR*
intracellular sulfur oxidation protein DsrP	818938	820140	-	400	*dsrP*
intracellular sulfur oxidation protein DsrO	820166	820897	-	243	*dsrO*
intracellular sulfur oxidation protein DsrJ	820894	821277	-	127	*dsrJ*
putative glutamate synthase (NADPH) small subunit	821307	823271	-	654	*dsrL*
intracellular sulfur oxidation protein DsrK	823327	824892	-	521	*dsrK*
intracellular sulfur oxidation protein DsrM	824894	825667	-	257	*dsrM*
intracellular sulfur oxidation protein DsrC	825744	826067	-	107	*dsrC*
intracellular sulfur oxidation protein DsrB	827224	828297	-	357	*dsrB*
intracellular sulfur oxidation protein DsrA	828373	829674	-	433	*dsrA*
rhodanese family protein	950273	950752	-	159	-
sulfide-quinone reductase	995954	997240	+	428	*sqr*

sox operon: SoxX, SoxY, SoxZ, SoxA, SoxB (CAMERA search)

1	Punta Cormorant, Hypersalin...
2	Sargasso Station 11; Sargas...
3	Mangrove on Isabella Island
4	Yucatan Channel
5	Warm seep, Roca Redonda
6	Upwelling, Fernandina Island
7	Newport Harbor, RI
8	Gulf of Maine
9	Sargasso Stations 3
10	South of Charleston, SC
11	30 miles from Cocos Island
12	Hydrostation S
13	Cabo Marshall, Isabella Island
14	Off Key West, FL
15	Cape May, NJ
16	Wolf Island
17	Northern Gulf of Maine
18	134 miles NE of Galapagos
19	Outside Halifax, Nova Scotia
20	Rosario Bank
21	Sargasso Station 13
22	Gulf of Panama
23	Northeast of Colon



Stations with multiple Sox and Dsr hits, the normal values



location of stations with a plenty of Dsr matching sequences

GUTLS_WRM_ELBA_SITE_20040501
Bay off Capo di Sant' Andrea, Elba

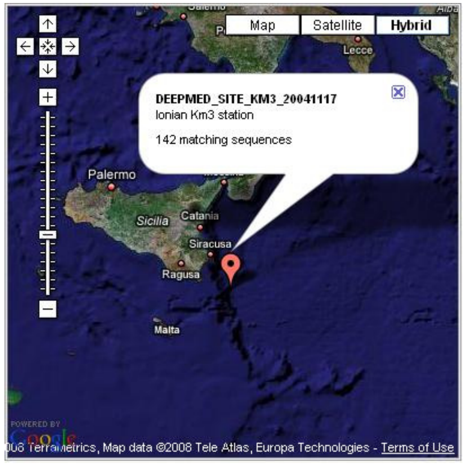
261 matching sequences

ALVINELLA_SITE_20041130
East Pacific Rise

176 matching sequences

WASECA_FRM_SOIL_SITE_20010901
Waseca County

169 matching sequences



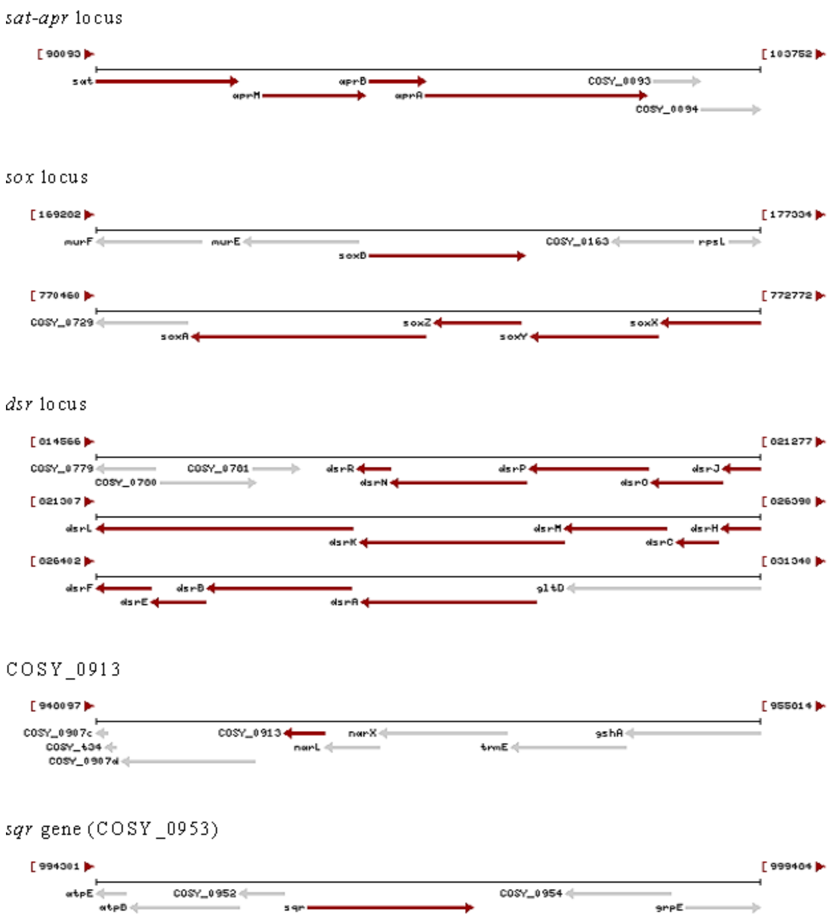
DEEPMED_SITE_KM3_20041117
Ionian Km3 station

142 matching sequences

Xenologs vs. Orthologs

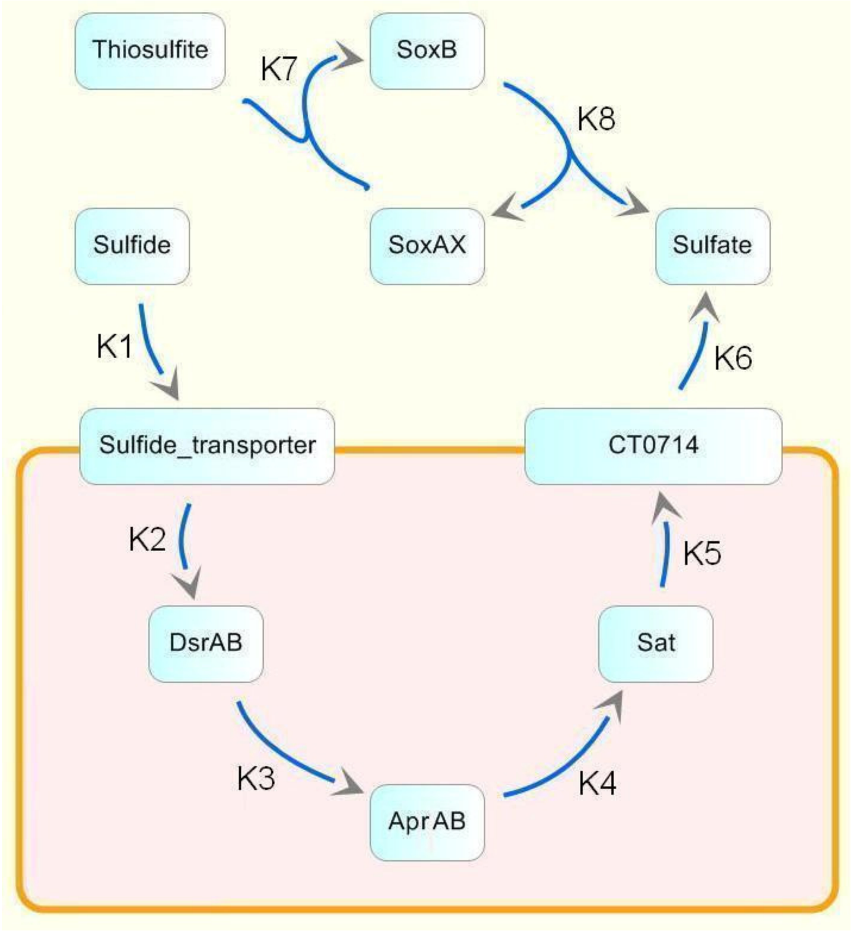
- *dsr* reads generated trees incompatible with the corresponding 16S rRNA phylogeny
- due to the low quality of databases (random and partial DNA reads)?
- due to BLAST local alignment removes the most divergent regions from the sequences?
- or due to a lateral gene transfer? That is in agreement with [Klein et al, 2001; Boucher et al, 2003]

Bio-bricks for sulfur metabolism

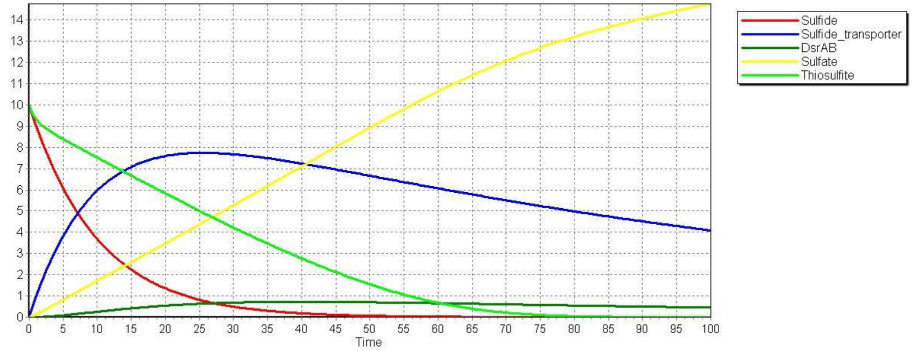


- Fragments of *V.okutanii* HA genetic map with genes coding sulfur metabolism

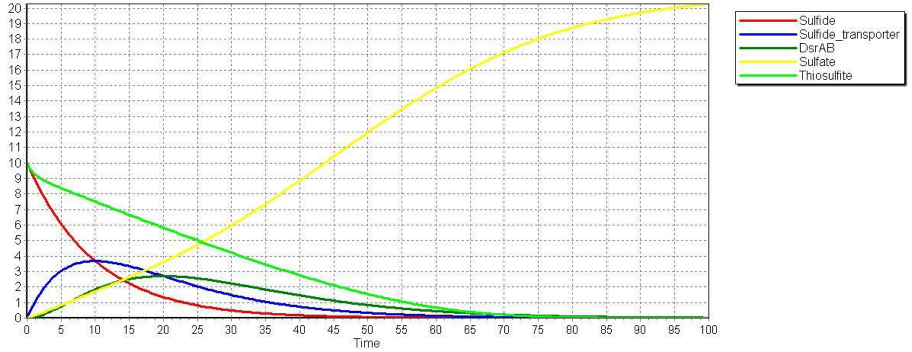
Simulation (1) within JDesigner



Thiosulfite
K7
SoxB
K8
Sulfide
SoxAX
Sulfate
K1
K6
Sulfide_transporter
CT0714
K2
K5
DsrAB
Sat
K3
K4
AprAB

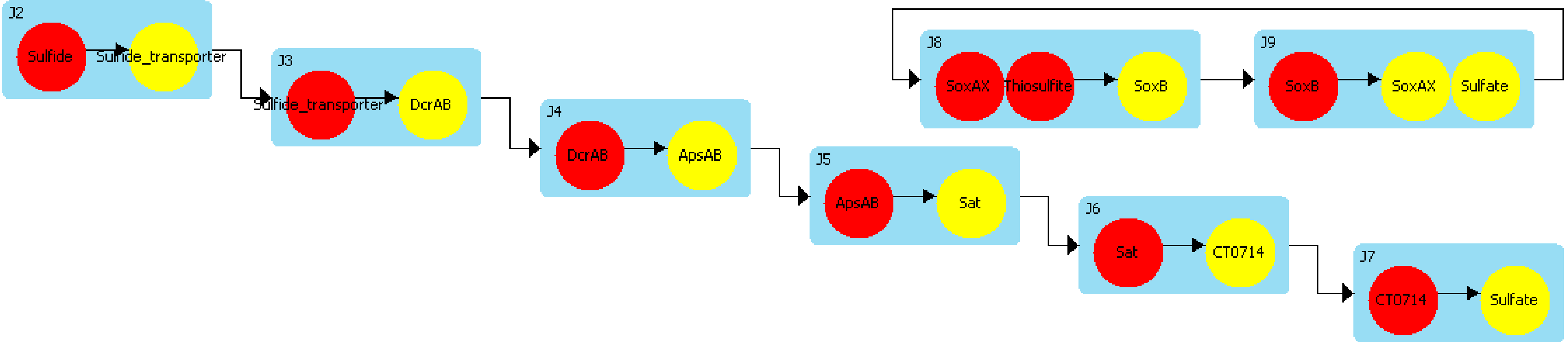


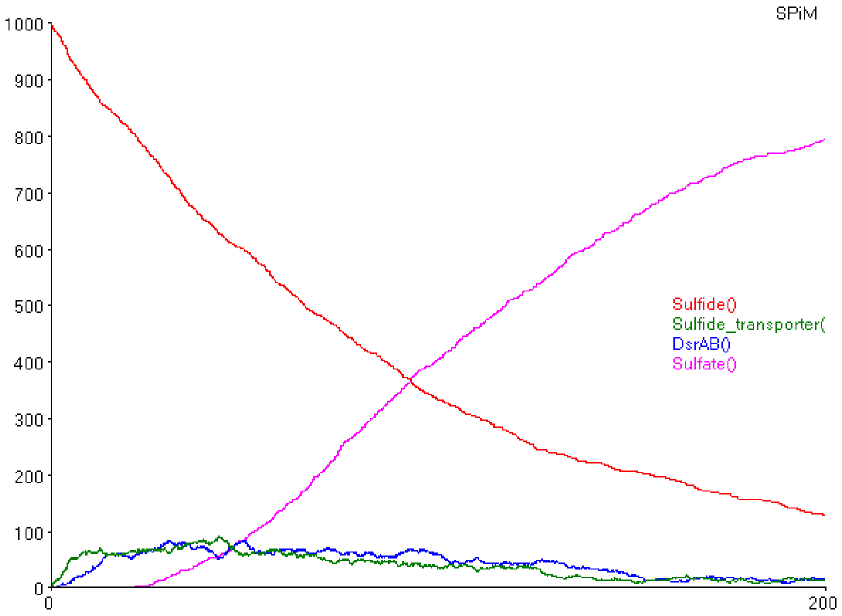
k2 = 0.01



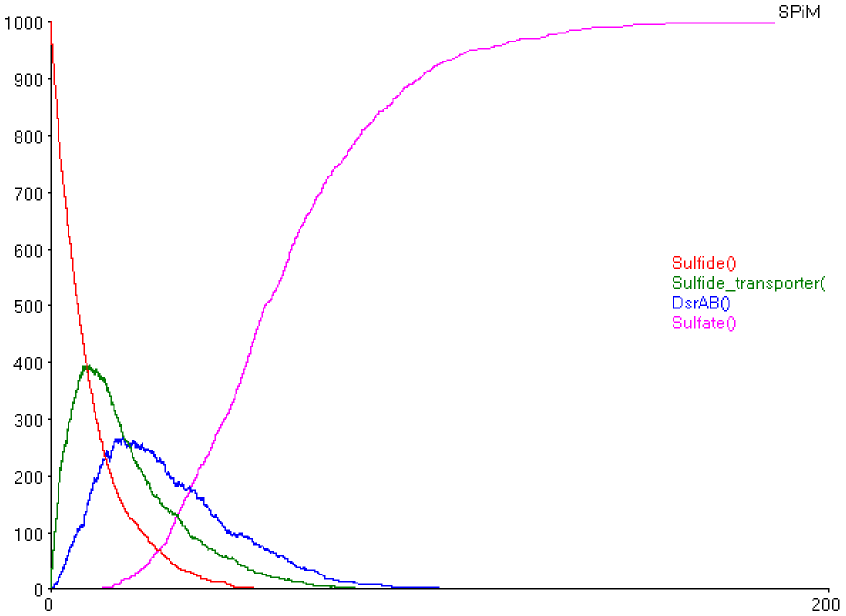
k2 = 0.1

(2) within the stochastic pi-Machine





Sulfide delay 0.01



Sulfide delay 0.1

Conclusion

- *sat-apr*, *sox* and *dsr* locuses from *V.okutanii* are very suitable for design of sulfur metabolism
- xenologs as bio-bricks is an attractive idea which needs more detailed analysis

Thanks

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