Dissection of the Yeast Redox Cycle

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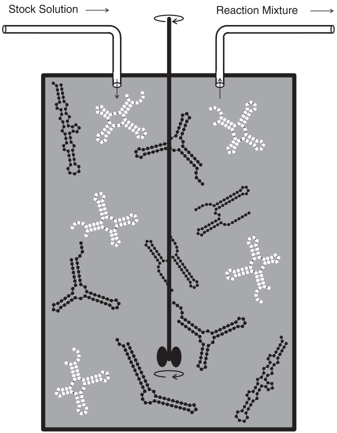
Keio University, Japan

&

RICAM Biology Group
Balkan, Vienna

Bled 2010

Spontaneous Synchronization in Continuous Culture (of baker’s yeast)



Auto-catalytic RNA

pivoRNA (+ other stuff)

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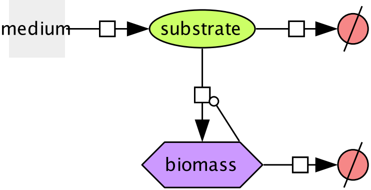


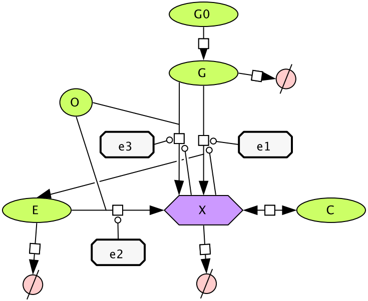
Monod 1942 - Bacterial Growth in Continuous Culture

Dilution rate	ϕ, **here:** $\phi \sim 0.1h^{-1}$
Substrate S	$\dot{S} = -v\mu B + \phi(S_{in} - S)$
Biomass B (or cell number C)	$\dot{B} = \mu B - \phi B$
Average growth rate	$\mu = \mu_{max} \frac{S}{K+S}$
Steady State @ $\dot{B} = 0$	$\mu = \phi$, **here:** $C \sim 10^8$ **cells** mL^{-1}
average cell doubling time	$\overline{T_2} = \frac{ln(2)}{\mu}$, **here: 7-8** h
cell size e.g. 33 fL	$\Rightarrow$ ca 0.3% of volume

Monod, J: La technique de culture continue, théorie et applications.
Ann Inst Pasteur 1950

pivoRNA (+ other stuff)

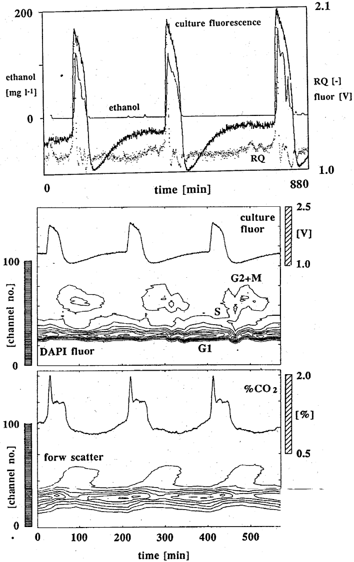
Spontaneous Synchronization in Continuous Culture (of baker's yeast): slow - $T_R > \frac{\overline{T_2}}{2}$



O: Oxygen, G: Glucose, C: storage carbohydrates,
E: Ethanol, X: biomass
Regulated Enzymes:
*e*1: fermentation (glycolysis, reductive metabolism)
*e*2: ethanol oxidation
*e*3: glucose oxidation

Simpson, Kompala and Meiss: *Discontinuity induced bifurcations in a model of Saccharomyces cerevisiae.* Math Biosci 2009

Münch, Sonnleitner and Fiechter: *The decisive role of the Saccharomyces cerevisiae cell cycle behaviour for dynamic growth characterization.* J Biotech 1992



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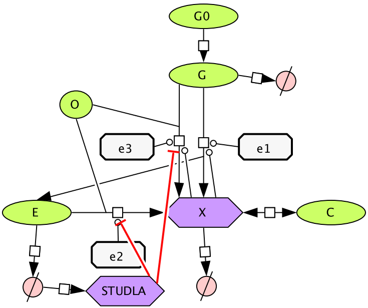

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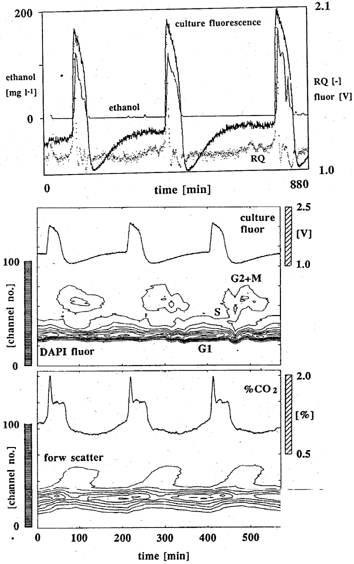

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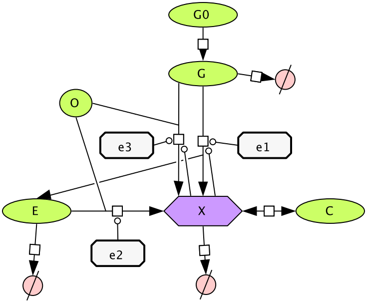

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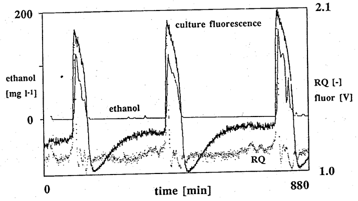
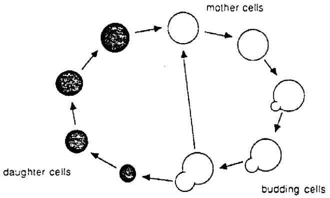
Strässle, Sonnleitner and Fiechter: *A predictive model for the spontaneous synchronization of Saccharomyces cerevisiae grown in continuous culture. I. Concept.* J Biotech 1988

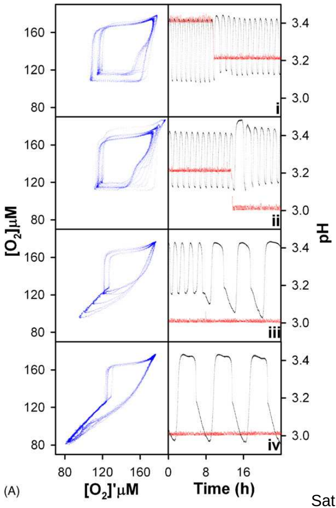


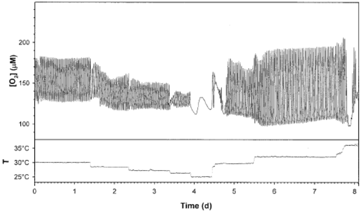


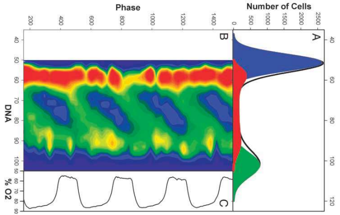
POPULATION STRUCTURE MODEL:

- **HOW? - Respiratory bottle-neck & Cell Cycle Cross-Synchronization**
- **WHY? - Brood Care? Optim.?**

Spontaneous Synchronization in Continuous Culture (of baker's yeast): **fast** - $T_R \sim \frac{\overline{T_2}}{10}$







Satroutdinov, Kuriyama & Kobayashi 1992 FEMS Microbiol. Lett.
Murray et al. 2001 J Bacteriol, Murray & Lloyd 2006 Biosystems
Klevecz et al. 2004 PNAS, **Li & Klevecz 2006 PNAS**, Tu et al. 2005 Science

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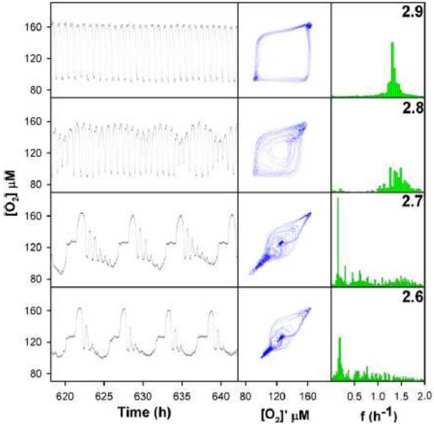
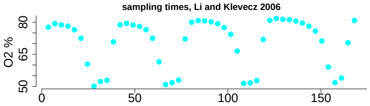


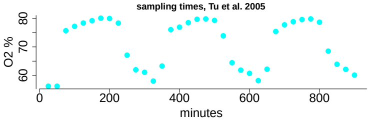
$\sim$ 5h envelopes of .7h redox oscillation
(also e.g. hysteresis after entrainment to 24h light cycle)

cells dividing per redox cycle:

	$\phi \sim 0.1h^{-1}$	
	$\overline{T_2} \sim 7 - 8h$	
Tu	$T_R \sim 5h$	**ca. 60%**
Li	$T_R \sim .7h$	**ca. 10%**
$GTS1^{-/-}$	$T_R \sim .35h$	**ca. 5%**

$\Rightarrow$ **quantized cell division times:** $i * T_R \Leftarrow$


sampling times, Li and Klevecz 2006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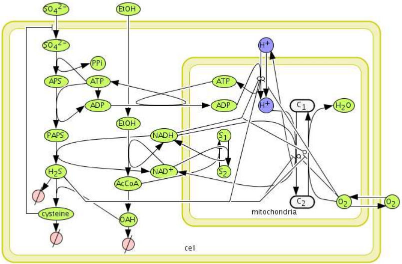

Jana Wolf et al. (Heinrich group) 2001 FEBS Letters

H_2S inhibition of COX1

- H_2S blocks H_2O binding site in COX1 - ancient! (might be predecessor of H_2O)
- Sulfate uptake: all eukaryotes
- e.g. H_2S induces hibernation in mice (NASA)
- Thiols (C-SH) central in Redox Metabolism

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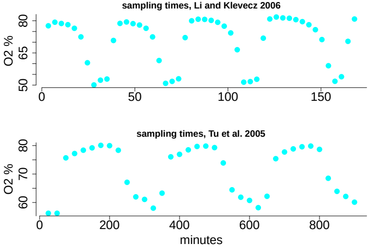

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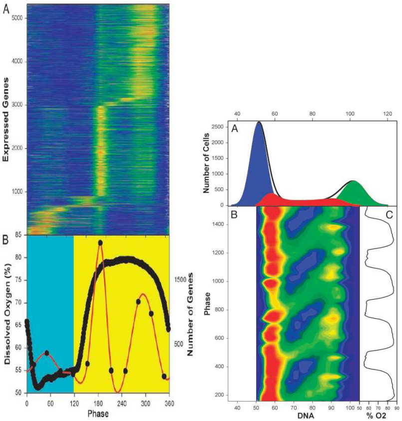
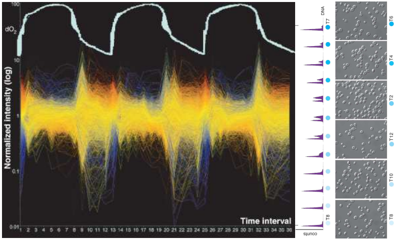
Temporal Separation of Anabolism and Catabolism



Short-period oscillation - 40 minutes
Klevecz et al. 2004 PNAS, **Li and Klevecz 2006 PNAS**



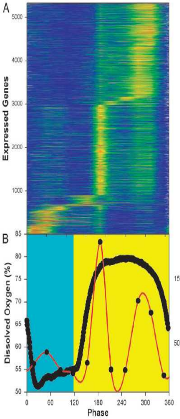
Long-period oscillation - 300 minutes
Tu et al. 2005 Science

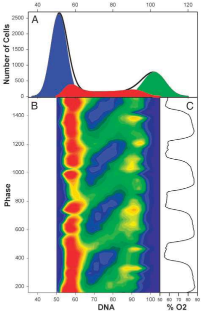
	Li & Klevecz 2006	**Tu et al. 2005**
STRAIN	IFO 0233	CEN.PK122
GLUCOSE	100 mmol/L	50 mmol/L
H2SO4	13 mmol/L	6.5 mmol/L
pH	4	3.4
REDOX PERIOD	**40-45’**	**300’**
DIVISIONS PER REDOX CYCLES	10%	60%

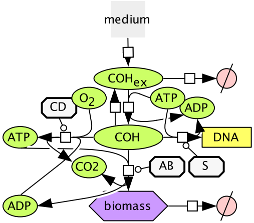
⇒ **TRANSCRIPTOME OSCILLATION**
⇒ **S-PHASE** ***GATING***

A Comparative Re-Evaluation
what oscillates, how and why?

Temporal Separation of Anabolism and Catabolism







COH: Carbohydrate units
AB: Anabolism/Biosynthesis
CD: Catabolism/Degradation
S: S-phase

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what oscillates, how and ~~why?~~

WHAT? - **Time-course Analysis:**

- DFT-based clustering
  - Normalization: **? !**
  - Discrete Fourier Transform

1. Comparative time-course profile analysis
2. GO/SBML analysis & Correlation to Metabolome

HOW? - **Cluster Analysis:**

- Statistical Scans:
  - t-test, Wilcox-test, hypergeometric distribution
  - Scanning diverse ‘gene/protein’ properties
  - Scans along aligned DNA sequences

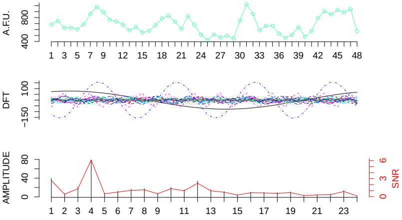
1. Transcriptome Meta-analysis
2. General Properties & Promotor Structure
3. Motifs: Specific RNA/DNA-binding Proteins
4. Chromosomal Domains

WHY? - **Modeling:**

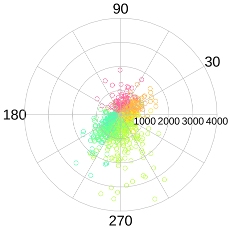
- *ODE* Models of
  metabolism ↔ gene expression feedback

1. Coupled *Oscillatory* Loops?
2. Avoid *Futile Cycles*?
3. Control *Mutation Rates*?

DFT-BASED CLUSTERING



gene YAL019W , cluster 1 , SNR 6.8 , phase 218



Amplitude A_4, Li and Klevecz 2006

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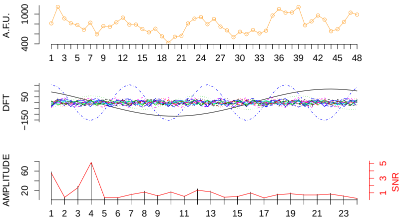
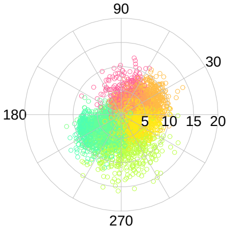


gene YDR329C , cluster 7 , SNR 5.9 , phase 360



SNR_4, Li and Klevecz 2006

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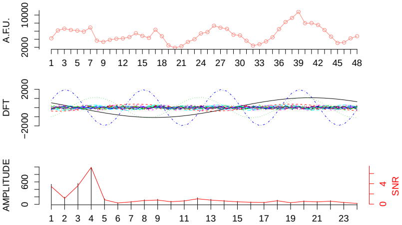
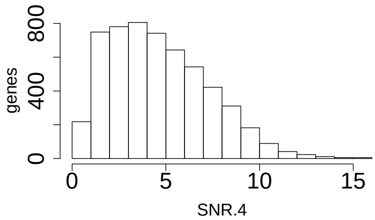


gene YPR184W , cluster 8 , SNR 8.3 , phase 66


Li and Klevecz 2006

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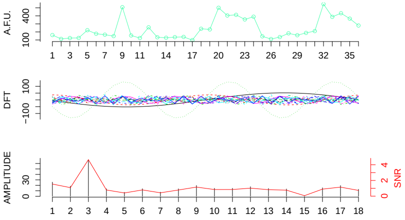
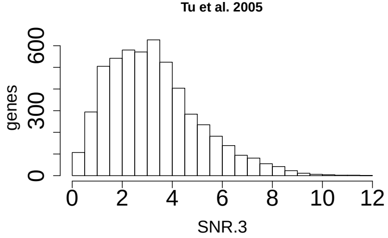


gene YAL019W , cluster 1 , SNR 4.9 , phase 248



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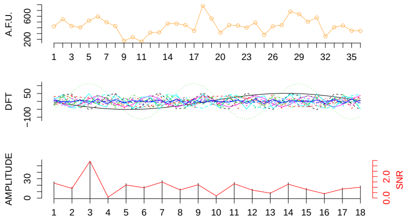
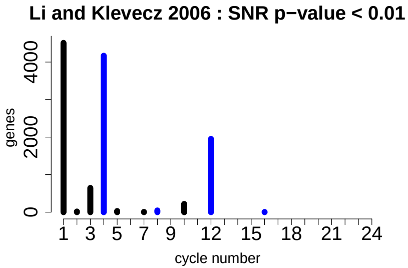


gene YDR329C , cluster 7 , SNR 3.6 , phase 119



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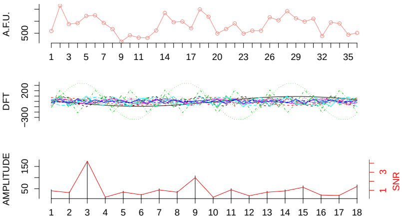
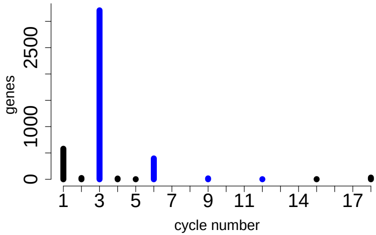


gene YPR184W , cluster 8 , SNR 4.2 , phase 104

Tu et al. 2005 : SNR p−value < 0.01



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Amplitude: $A_K = \frac{|X_K|}{N}$ (DC *component* at $k = 0$)

AMPLITUDE SCALES

cycle number: $cyc = 1, \ldots, N/2$

Relative Amplitude: $rA_{cyc} = A_{cyc}/A_0$ (similar to log2-ratio)

Signal-to-Noise Ratio: $SNR_{cyc} = A_{cyc} / \frac{1}{N/2-1} \sum_{i \neq cyc} A_i$

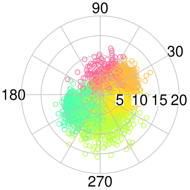
Oscillation Strength/Width: $OS_{cyc} = 1/OW_{cyc} = A_{cyc} / \frac{1}{N} \sum_{n=1}^{N} (x_n - min(X_N))$

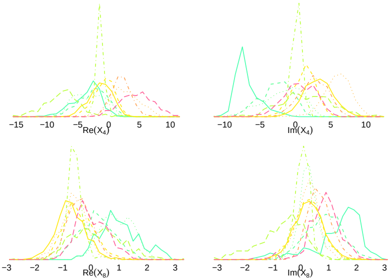
PERMUTATION TEST: calculate amplitude (scales) for 5.10^4 permutations of X_N

CLUSTERING OF SELECTED DFT COMPONENTS: $h \in cyc$ **(e.g. harmonic frequencies)**

amplitude-scaled $Im(X_h), Re(X_h)$

DFT-BASED CLUSTERING





Lo, K., Brinkman, R. R. and Gottardo, R. Cytometry A (2008),321-332. *Automated Gating of Flow Cytometry Data via Robust Model-based Clustering.*
“based on t-mixture models [...] generalizes the popular Gaussian mixture models [...] allow for nonelliptical clusters.”

N time-series measurements: $X_N = x_1, \ldots, x_N$

Discrete Fourier Transform: $X_K = \sum_{n=1}^{N} x_n e^{-2\pi i \frac{k(n-1)}{N}} \quad k = 0, \ldots, N-1$

Phase: $\phi_K = -atan2(Im(X_K), Re(X_K))$

Amplitude: $A_K = \frac{|X_K|}{N}$ (*DC component* at $k = 0$)

AMPLITUDE SCALES

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CLUSTERING OF SELECTED DFT COMPONENTS: $h \in cyc$ **(e.g. harmonic frequencies)**
amplitude-scaled $Im(X_h), Re(X_h)$

Data Disclaimer - *Microarrays*

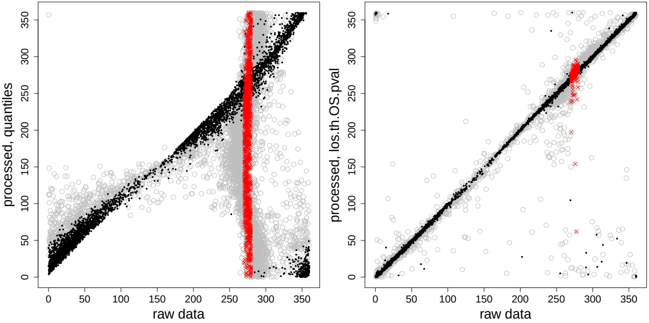
Microarray Experiment:

cell culture → RNA → microarray → time-series data

Yeast Redox Cycle:

violation of basic assumptions at each processing level

⇒ see chapter *Normalization* ⇐



Data Disclaimer - *Microarrays*

Microarray Experiment:

cell culture → RNA → microarray → time-series data

Yeast Redox Cycle:

violation of basic assumptions at each processing level

⇒ see chapter *Normalization* ⇐

Time-course Analysis:

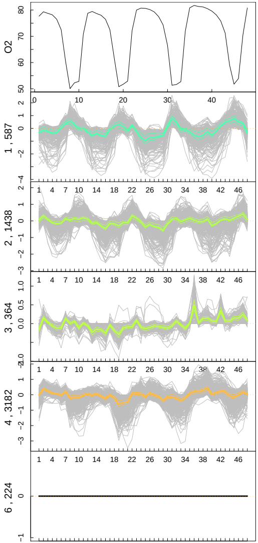
raw fluorescence data + *summarization*

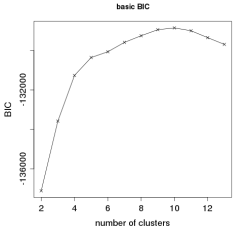
(probe→transcript mapping)

Visualization & Interpretation:

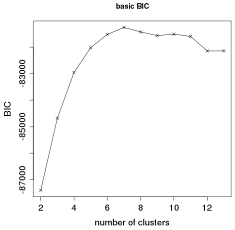
normalization by *‘least-oscillating’* probes

Clustering by Phase and Waveform

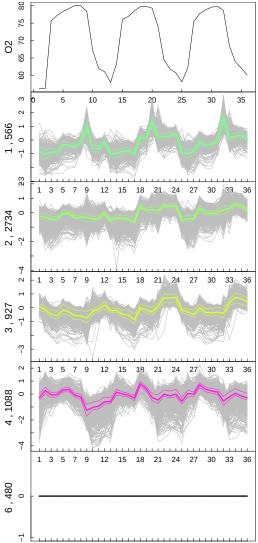




⇐ Li and Klevecz 2004

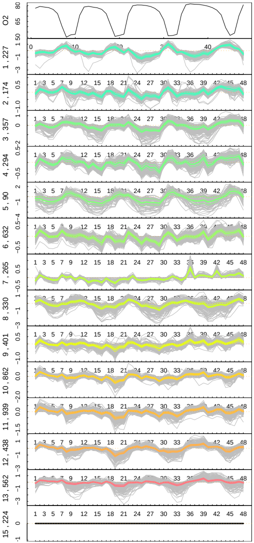


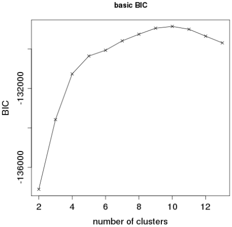
Tu et al. 2005 ⇒



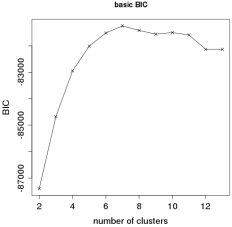
Model-based Clustering:
BIC: Bayesian Information Criterion

Clustering by Phase and Waveform

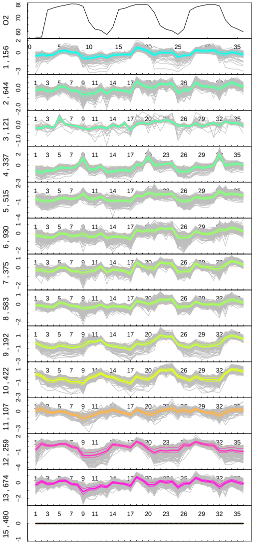




⇐ Li and Klevecz 2004

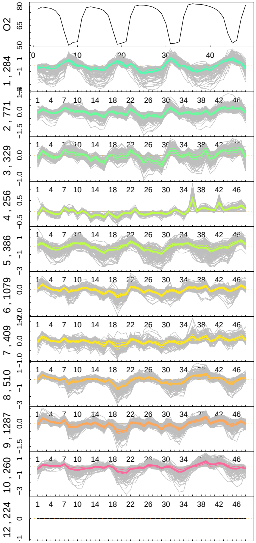


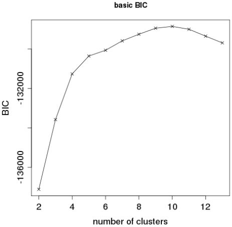
Tu et al. 2005 ⇒



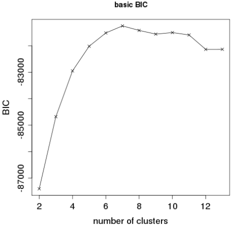
Model-based Clustering:
BIC: Bayesian Information Criterion

Clustering by Phase and Waveform

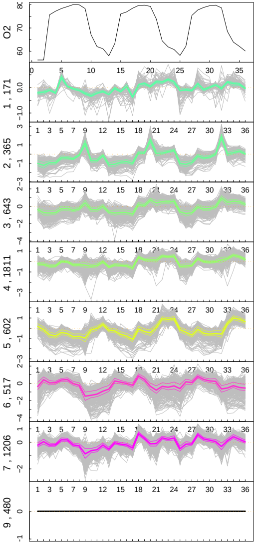




⇐ Li and Klevecz 2004

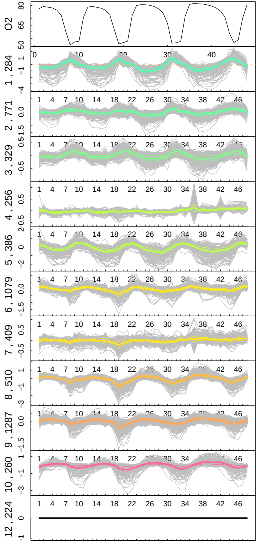


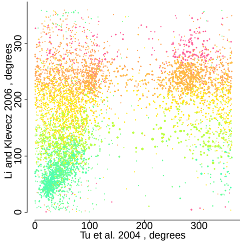
Tu et al. 2005 ⇒



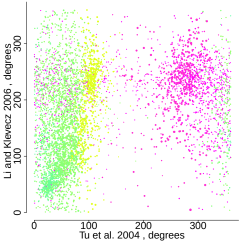
Model-based Clustering:
BIC: Bayesian Information Criterion

Clustering by Phase and Waveform

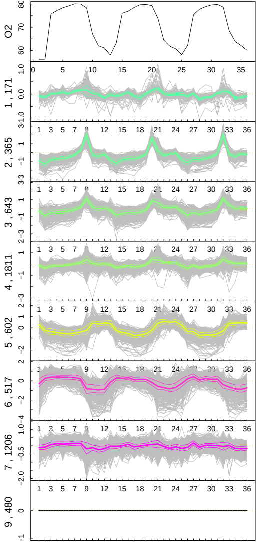




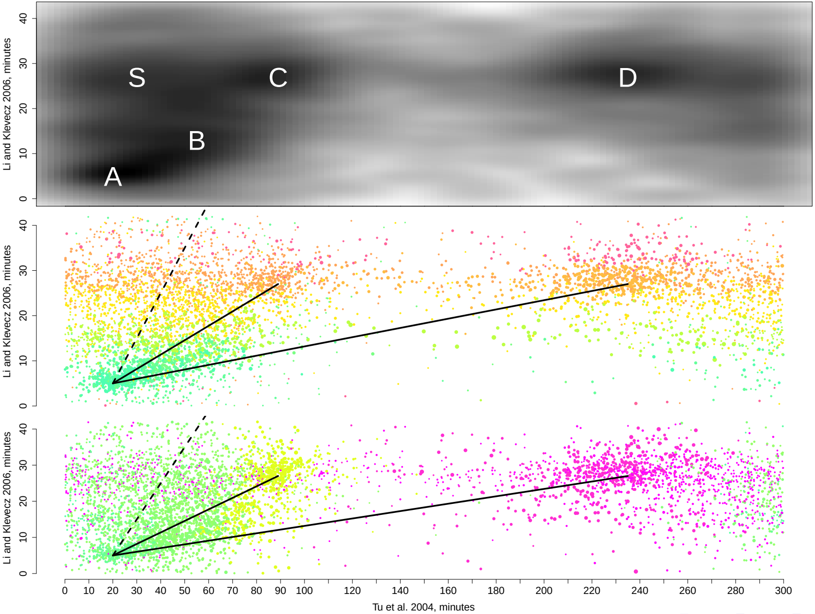
⇐ Li and Klevecz 2004, y-axis (40')



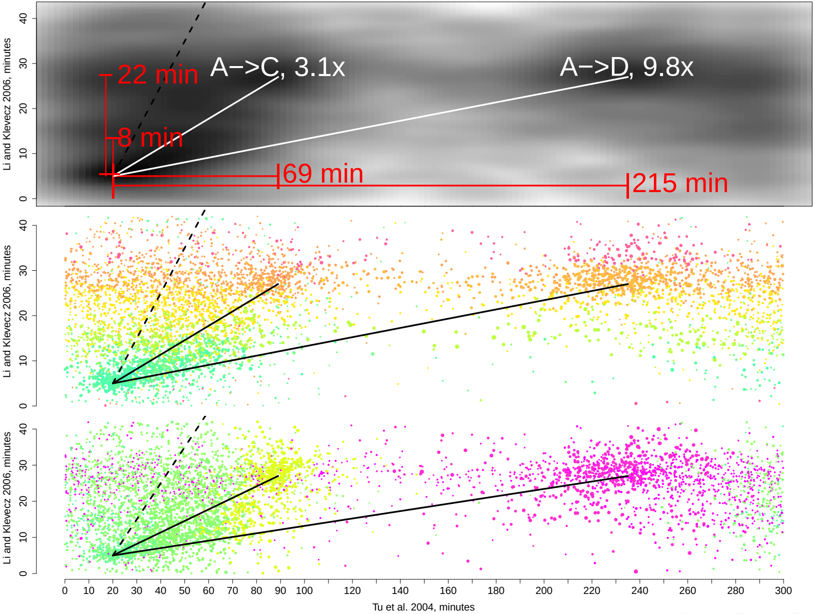
x-axis (300'), Tu et al. 2005 ⇒



Short- vs. Long-Period Times *(still not to scale)*



Short- vs. Long-Period Times *(still not to scale)*

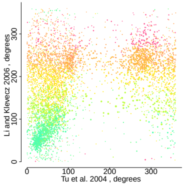


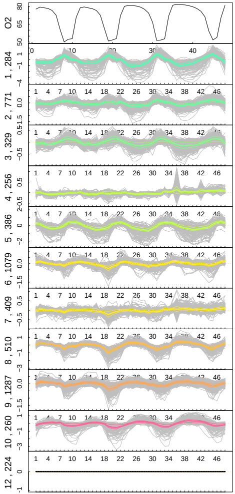
li06 clustering - GO RESULTS
CLUSTER 1 - 284 GENES:

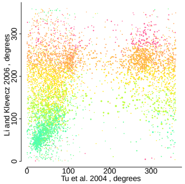
GO	total	% in cluster	p-value
ribosome biogenesis	199	73.4	8e-163
rRNA processing	120	60.8	2e-67
snoRNA binding	21	100	2e-28
DNA-directed RNA polymerase I complex	14	64.3	2e-09
rDNA heterochromatin	6	100	1e-08
DNA-directed RNA polymerase activity	32	37.5	1e-08
sulfur metabolic process	7	57.1	2e-04
tRNA modification	27	18.5	0.009

li06 clustering - GO RESULTS
CLUSTER 2 - 771 GENES:

GO	total	% in cluster	p-value
exosome (RNase complex)	12	83.3	8e-08
rRNA export from nucleus	26	57.7	1e-07
nuclear pore	51	43.1	1e-07
ribosomal protein import into nucleus	23	56.5	1e-06
RNA splicing factor activity, transester	53	39.6	1e-06
nuclear pore organization	27	51.9	2e-06
DNA replication origin binding	26	46.2	5e-05
tRNA modification	27	44.4	7e-05
tRNA export from nucleus	28	42.9	1e-04
mRNA export from nucleus	58	32.8	1e-04
chromatin silencing at rDNA	18	50	2e-04
RNA elongation from RNA polymerase II pr	54	31.5	4e-04



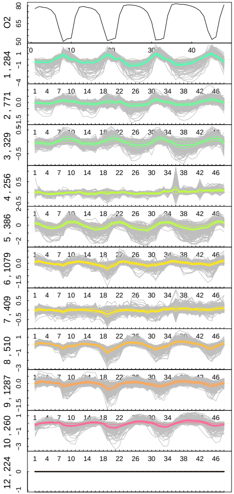




li06 clustering - GO RESULTS

CLUSTER 3 - 329 GENES:

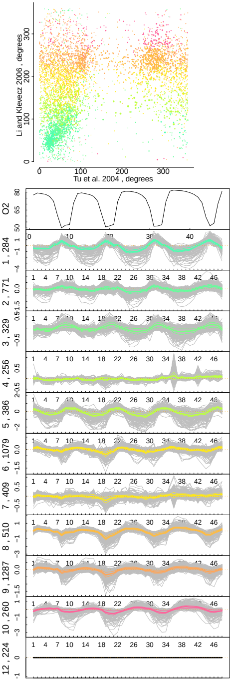
GO	total	% in cluster	p-value
structural constituent of ribosome	216	53.2	2e-91
translation	270	44.8	5e-85
cytosolic large ribosomal subunit	87	75.9	2e-66
cytosolic small ribosomal subunit	62	80.6	1e-52
glycolysis	16	62.5	2e-09
gluconeogenesis	15	46.7	8e-06
fatty acid biosynthetic process	8	37.5	0.008
mitochondrial respiratory chain complex	10	80	4e-09
mitochondrial nucleoid	23	43.5	2e-07
mitochondrion	988	9.3	2e-07



li06 clustering - GO RESULTS

CLUSTER 5 - 386 GENES:

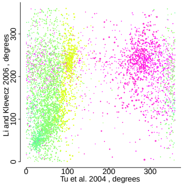
GO	total	% in cluster	p-value
'de novo' IMP biosynthetic process	14	42.9	2e-04
purine nucleotide biosynthetic process	11	63.6	1e-06
arginine biosynthetic process	10	100	2e-12
lysine biosynthetic process via aminoadi	8	75	2e-06
regulation of nitrogen utilization	8	62.5	6e-05
nitrogen utilization	9	66.7	6e-06
ammonium transport	6	83.3	7e-06
histidine biosynthetic process	14	50	1e-05
ornithine biosynthetic process	4	100	2e-05
allantoin catabolic process	7	71.4	2e-05
branched chain family amino acid biosynt	7	71.4	2e-05
aromatic amino acid family biosynthetic	5	80	9e-05
leucine biosynthetic process	5	80	9e-05
threonine metabolic process	5	80	9e-05
isocitrate metabolic process	5	80	9e-05
amino acid transmembrane transporter act	9	55.6	1e-04
amino acid transport	14	42.9	2e-04
acetate-CoA ligase activity	2	100	0.004
acetyl-CoA biosynthetic process	2	100	0.004

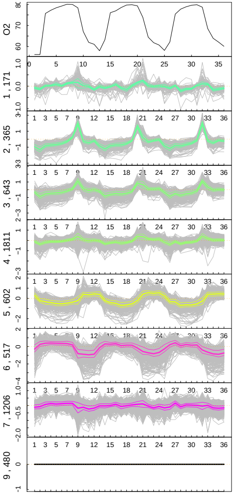


tu05 clustering - GO RESULTS

CLUSTER 5 - 602 GENES:

GO	total	% in cluster	p-value
mitochondrion	988	30.4	4e-88
mitochondrial large ribosomal subunit	44	95.5	1e-39
mitochondrial small ribosomal subunit	33	90.9	7e-27
structural constituent of ribosome	216	33.8	2e-21
mitochondrial matrix	61	50.8	2e-15
mitochondrial inner membrane	93	40.9	1e-14
mitochondrial outer membrane translocase	8	100	1e-08
mitochondrial intermembrane space	31	48.4	9e-08
aerobic respiration	69	46.4	2e-14
tricarboxylic acid cycle	15	73.3	1e-08
axial cellular bud site selection	22	45.5	3e-05
cellular bud neck	99	19.2	0.006
proteolysis	24	29.2	0.009
mitotic sister chromatid cohesion	22	54.5	3e-07
replication fork	14	64.3	2e-06

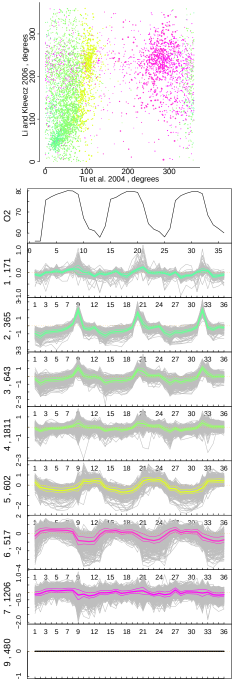




tu05 clustering - GO RESULTS

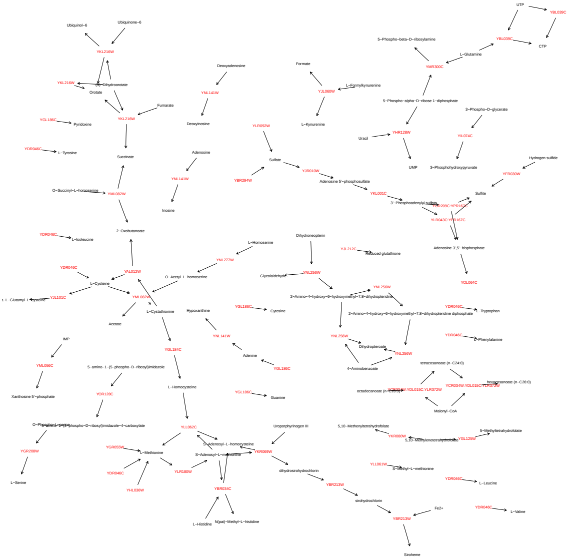
CLUSTER 6 - 517 GENES:

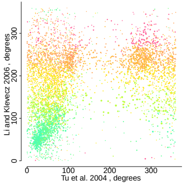
GO	**total**	**% in cluster**	**p-value**
response to stress	68	38.2	3e-11
peroxisome	27	40.7	9e-06
peroxisomal matrix	12	91.7	3e-11
fatty acid beta-oxidation	9	100	3e-10
cytokinesis, completion of separation	11	81.8	2e-08
structural constituent of cell wall	13	61.5	3e-06
regulation of pH	4	75	0.003
ethanol metabolic process	4	75	0.003

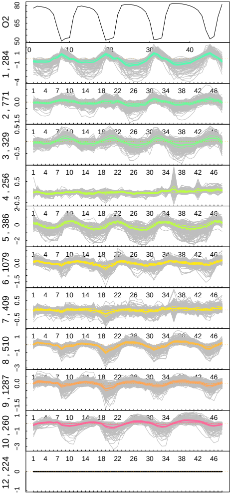


Extracting catalyzed reactions from metabolic reconstruction in Herrgård et al. 2008 Nat Biotechnol; **hub metabolites (>26 reactions) removed**

red: enzymes (complexes), **black: metabolites**







Li06 CLUSTER 1 - 284 GENES

Extracting catalyzed reactions from metabolic reconstruction in Herrgård et al. 2008 Nat Biotechnol; **hub metabolites (>26 reactions) removed**

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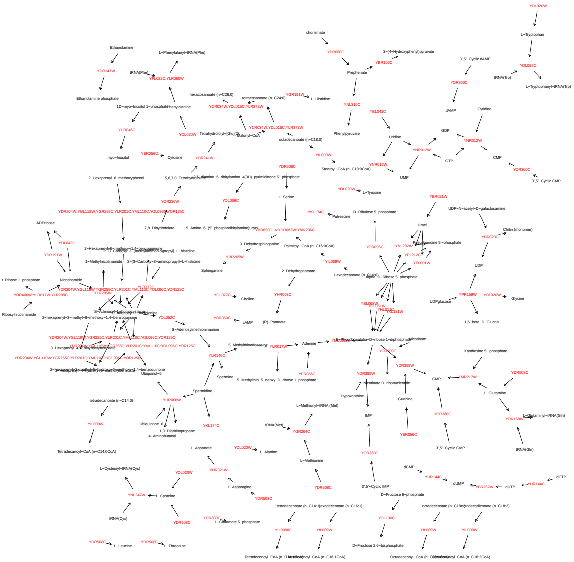

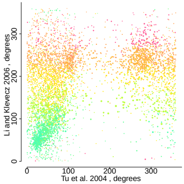

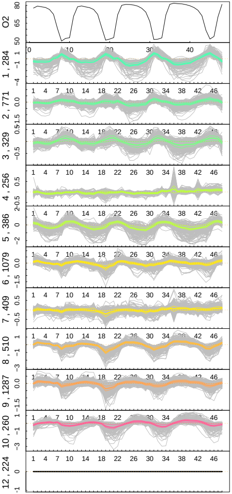

Li06 CLUSTER 2 - 771 GENES

Extracting catalyzed reactions from metabolic reconstruction in Herrgård et al. 2008 Nat Biotechnol; **hub metabolites (>26 reactions) removed**

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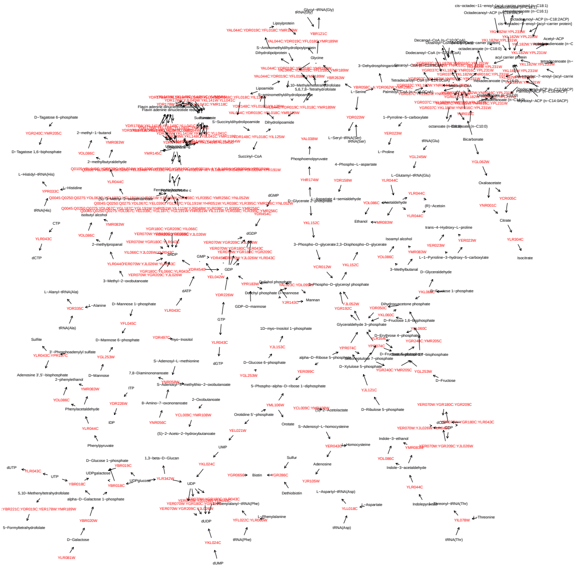
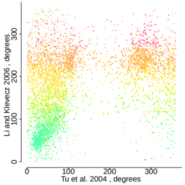
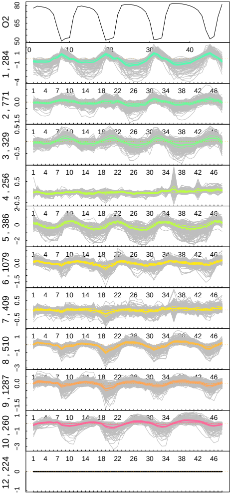


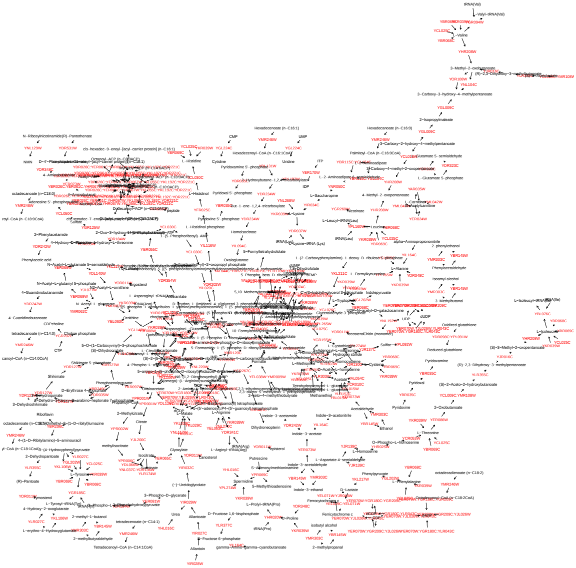


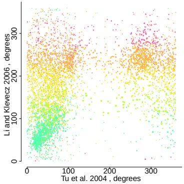


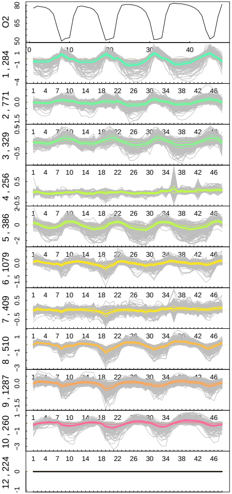
Li06 CLUSTER 3 - 329 GENES

Extracting catalyzed reactions from metabolic reconstruction in Herrgård et al. 2008 Nat Biotechnol; **hub metabolites (>26 reactions) removed**

red: enzymes (complexes), **black: metabolites**



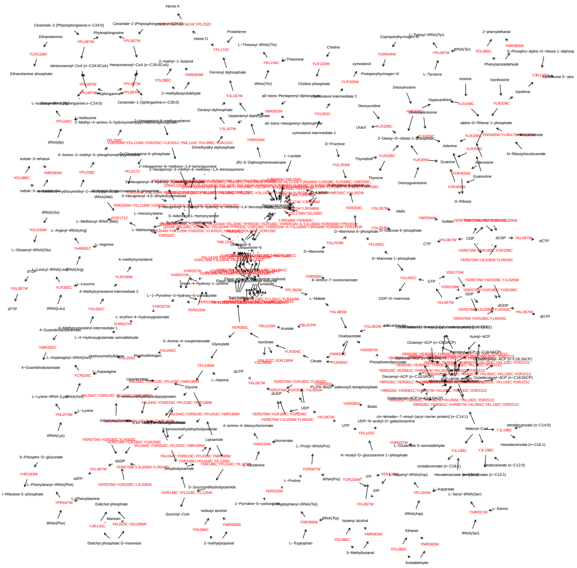


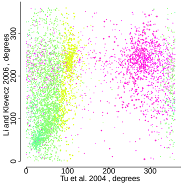


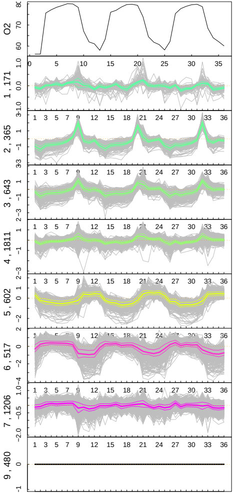
Li06 CLUSTER 5 - 386 GENES

Extracting catalyzed reactions from metabolic reconstruction in Herrgård et al. 2008 Nat Biotechnol; **hub metabolites (>26 reactions) removed**

red: enzymes (complexes), **black: metabolites**



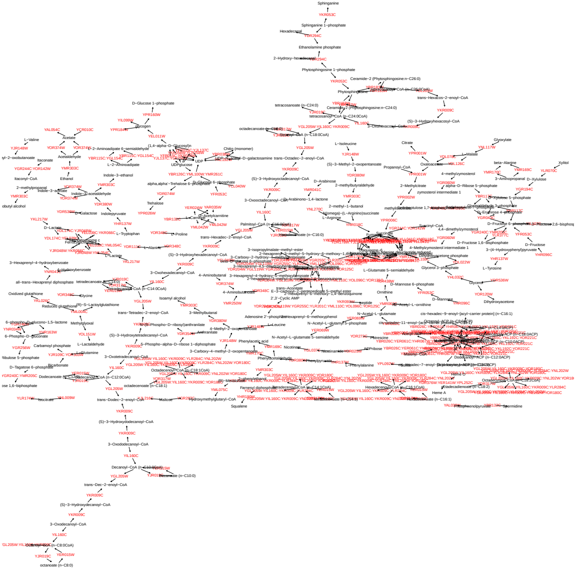




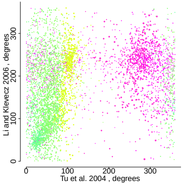
Tu05 CLUSTER 5 - 602 GENES

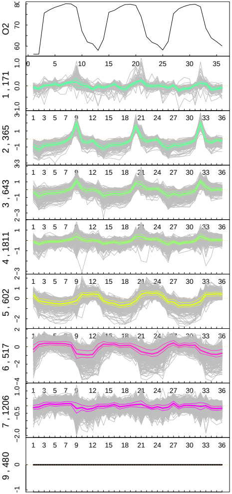
Extracting catalyzed reactions from metabolic reconstruction in Herrgård et al. 2008 Nat Biotechnol; **hub metabolites (>26 reactions) removed**

red: enzymes (complexes), **black: metabolites**

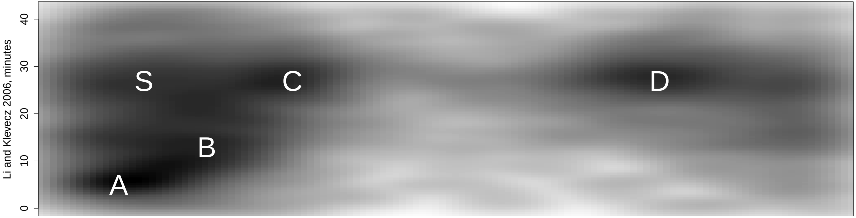


Tu05 CLUSTER 6 - 517 GENES





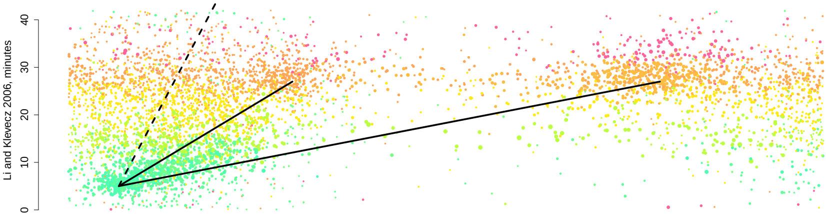
Cluster Functions



- A: cytosolic ribosomes
- B: anabolic metabolism - cell growth
- C: mitochondrial ribosomes
- D: catabolic metabolism - mitochondrial activity

Dual Dichotomy:
Informational vs. Structural
Host cell vs. Endosymbiont

Cluster Functions



- A: cytosolic ribosomes
- B: anabolic metabolism - cell growth
- C: mitochondrial ribosomes
- D: catabolic metabolism - mitochondrial activity

Cluster S: weak oscillation ...
Short period: with clusters CD
Long period: with clusters AB

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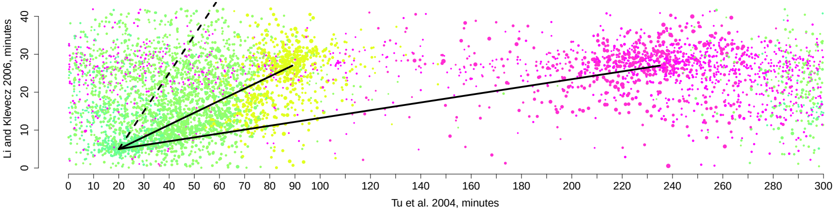

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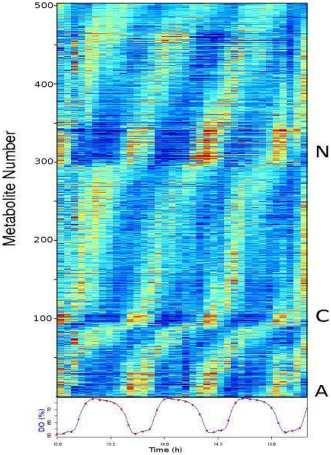
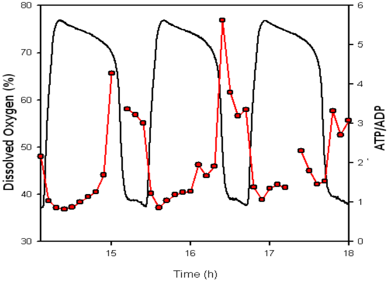
(NOT YET) Metabolome Analysis

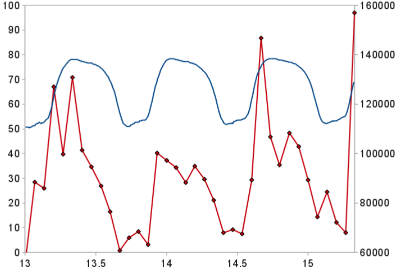


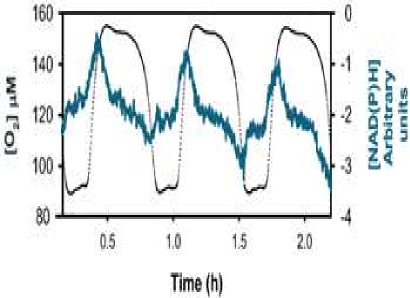
- Capillary Electrophoresis-MS (Tsuruoka Metabolome Campus)
- Anionic, Cationic and Neutral Metabolites
- Currently: mapping m/z peaks to yeast metabolism (...)

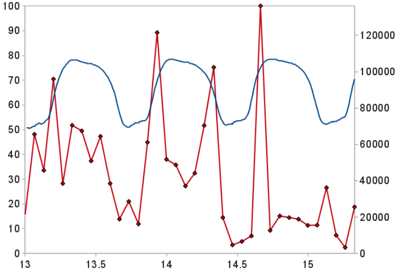
Douglas Murray, Kalesh Sasidharan
2009-: work in progress

Preliminary Metabolome Results



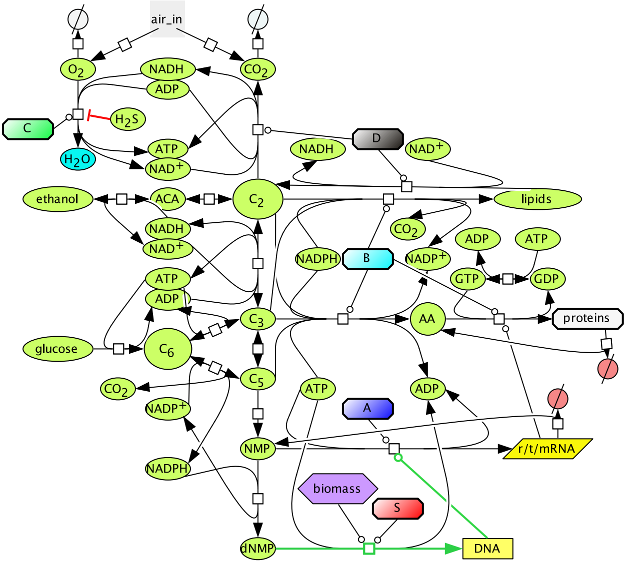






NOTE: (not only) histone modification co-substrates!

Anabolism vs. Catabolism



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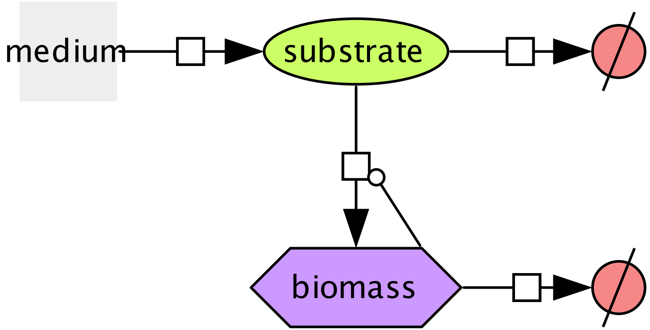


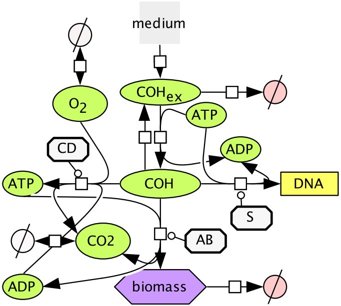
Coarse-graining metabolism in terms of

- Redox Clusters and
- Adenosine-based co-substrates (ATP, NADH, NADPH, SAM, CoA, ...)

Missing:

- Metabolome & Stoichiometries (map from full model)
- Metabolism-internal Feedbacks
- Feedback on Transcription

Anabolism vs. Catabolism



+ ATP: ⇒ AB + CD

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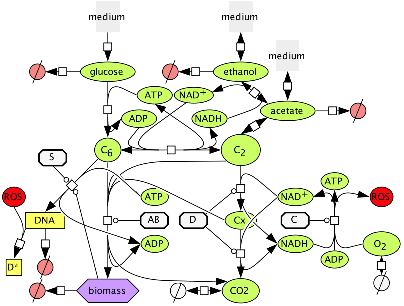

+ NADH: ⇒ AB + C + D

Coarse-graining metabolism in terms of

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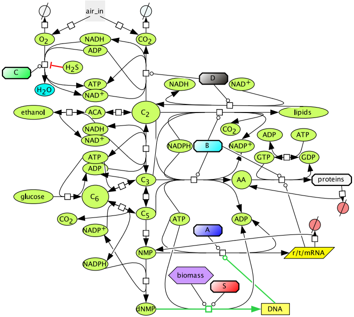

+ NADPH: $\Rightarrow$ A+B+C+D

Coarse-graining metabolism in terms of

- Redox Clusters and
- Adenosine-based co-substrates (ATP, NADH, NADPH, SAM, CoA, ...)

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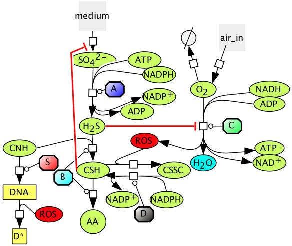
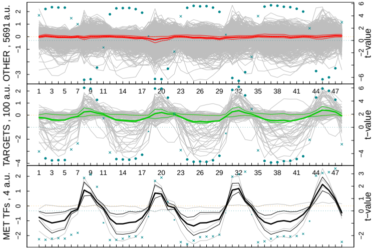
Coarse-graining Metabolism - constructing *Fluchtlinien*



Competition for nitrogen?
Sequential expression?



target genes: MacIsaac et al. 2006: ChIP-chip, **$p<0.05$**, TODO: H_2S

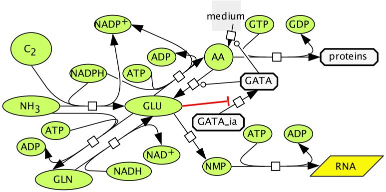
WHAT? - *transcriptome & metabolome*

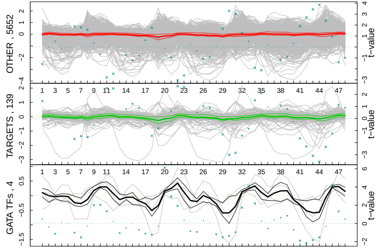
1. Temporal separation of fundamental cellular functions
2. Specific metabolic subnetworks active sequentially, global pattern: anabolic vs. catabolic pathways

HOW? - *metabolic feedback, e.g.:*

1. **All Eukaryotes:** H_2S is an intermediate product in *thiol* synthesis, but inhibits H_2O binding site in COX1
2. **Yeast Redox Cycle:** H_2S acts as *synchronizing agent*
3. **S-phase** might drain nitrogen, H_2S accumulates and diffuses
4. **MET** *transcription factor system* and target genes are strong cluster A-like oscillators

Coarse-graining Metabolism - constructing *Fluchtlinien*





ChIP-chip, **p<0.01**, TODO: GLN/GLU/AA,
NOTE: biphasic GAT1 and target expression!

e.g. **Nitrogen Metabolism**

⇒ **If any major REDOX reaction is disturbed (e.g.** H_2S **vs. COX1) 'everything' is affected**
cell-cell synchronization *via* **diverse intermediates (**H_2S, *acetaldehyde***)**

WHAT? - *transcriptome & metabolome*

1. Temporal separation of fundamental cellular functions
2. Specific metabolic subnetworks active sequentially, global pattern: anabolic vs. catabolic pathways

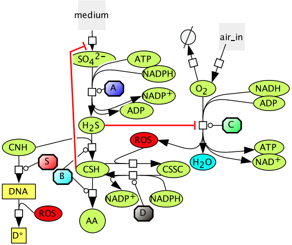
HOW? - *transcriptional feedback*

1. MET (sulfate uptake), **GATA (nitrogen)**, GCN4 (charged tRNA) regulatory networks, *etc. etc.*
2. **auto-synchronization of multiple enzymatic & transcriptional feedback loops**

~~WHY?~~ - *NULL hypothesis*

1. *'The selfish cycle':* **stable limit-cycle(s) with no further 'function'**

Coarse-graining Metabolism - constructing *Fluchtlinien*



H_2S **production during S-phase**

WHAT? - *S-phase gating*

~~WHY~~? - *cell integrity*

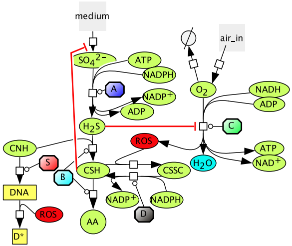
1. *Mutations:* **ROS-induced mutations in OX Phase?**
2. *Recombinations:* **DNA replication might stall at low ATP?**

$\Rightarrow$ **maximal** $dATP/dt$ $\leftrightarrow$ **maximal RESPIRATION** $\leftrightarrow$ **maximal** $dROS/dt$ **?**

HOW? - *redox metabolism*

- **Thiols are essential for redox metabolism (glutathione, thioredoxin)**

Coarse-graining Metabolism - constructing *Fluchtlinien*



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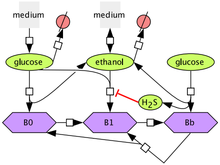


Population Structure

WHAT? - *metabolome & cell cycle*

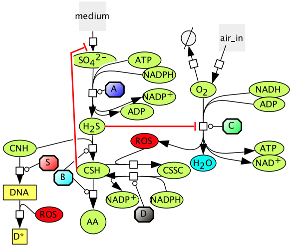
1. Budding Cells produce ethanol, e.g. by nitrogen-drain in S-phase
2. Small daughter cells lacks mitochondria
3. Growing cells consume both glucose & ethanol

HOW? - *auto-synchronization via feedforward loops*

~~WHY?~~ - *growth optimization*

1. *Brutpflege:* **temporal multi-cellularity**
2. *‘Side-effect’:* **ethanol inhibits competitors**

Fluchtlinien - constructing *PubMed Narratives*



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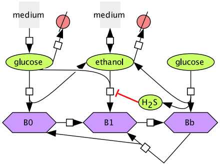

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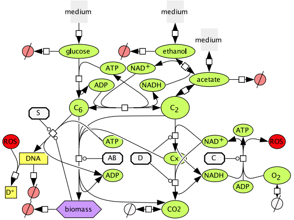

Respiratory Bottleneck

WHAT? - *temporal separation of fundamental cell ‘functions’*

~~**WHY?**~~

1. **Evolutionary Accident & Global Synchronization** *(H_2S)*
2. **Control DNA & Cell Integrity** *(ROS, thiols)*
3. **Flux Optimization** *(ATP, NAD(P)H, etc.)*
4. **Brutpflege** *(ethanol)*

WHAT? - **Time-course Analysis:**

- DFT-based clustering
  - Normalization: **? !**
  - Discrete Fourier Transform

1. Comparative time-course profile analysis
2. GO/SBML analysis & Correlation to Metabolome

HOW? - **Cluster Analysis:**

- Statistical Scans:
  - t-test, Wilcox-test, hypergeometric distribution
  - Scanning diverse ‘gene/protein’ properties
  - Scans along aligned DNA sequences

1. Transcriptome Meta-analysis
2. General Properties & Promotor Structure
3. Motifs: Specific RNA/DNA-binding Proteins
4. Chromosomal Domains

WHY? - **Modeling:**

- *ODE* Models of
  metabolism ↔ gene expression feedback

1. Coupled *Oscillatory* Loops?
2. Avoid *Futile Cycles*?
3. Control *Mutation Rates*?

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Cluster Analysis - Data Types and Statistics

Basic Data Types

- Continuous
  - arbitrary: some property
  - dimensional: time, space
  - circular: phase angles
- Categorical
  - binary
  - classifications (clusters)
  - binned continuous dist.

Basic Tests

- Categorical vs. Continuous
  - normal distribution
    Gaussian and *von Mises*
  - non-normal distribution
- Categorical vs. Categorical
  - hypergeometric distribution

LARGE DATA-SETS for both GENES and GENOME
⇒ STATISTIC SCANS ALONG PHASE ANGLE OR DNA STRUCTURES ⇐
FILTER: p-value, INTERPRETATION: statistic (t, U, E)

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Welch's t-test:

1. calculate sample mean and variance of X and Y
2. calculate difference of means: *t-statistic*
3. p-value by theoretical t distribution

mean	$\overline{X} = \frac{1}{N}\sum_{k=1}^{N} x_k$
variance	$s^2 = \frac{\sum_{k=1}^{N}(x_k - \overline{x})^2}{N-1}$
t-statistic	$t = \frac{\overline{X}-\overline{Y}}{\sqrt{\frac{s_x^2}{N_x}+\frac{s_y^2}{N_y}}}$
expected t	$E(t) = 0$
p-value	integral of Student's probability density function, between -t and t

LARGE DATA-SETS for both GENES and GENOME

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Wilcoxon rank-sum test/Mann-Whitney U test:

1. sort and rank all values $X \cup Y$
2. calculate sum of ranks of subset (W_m) or *U-statistic*
3. p-value by permutation tests

rank-sum $W_m = \sum_{i=1}^{m} R(X_i)$

U-statistic $U_m = \sum_{i=1}^{m} \sum_{j=1}^{n} S(X_i, Y_j)$

$$S(X, Y) = \begin{cases} 1, & \text{if Y < X} \\ 0, & \text{if Y >= X} \end{cases}$$

$$W_m = U_m + \frac{n(n+1)}{2}$$

$$U_m + U_n = mn$$

$$0 \le \frac{U}{mn} \le 1$$

expected U $E(\frac{U}{mn}) = 0.5$

p-value Shift-Algorithm by Streitberg & Röhmel
for both tied and untied samples

LARGE DATA-SETS for both GENES and GENOME
$\Rightarrow$ STATISTIC SCANS ALONG PHASE ANGLE OR DNA STRUCTURES $\Leftarrow$
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Cumulative Hypergeometric Distribution:

1. count overlaps
2. calculate relative *enrichment*
3. calculate p-value directly

all genes	N
genes with motif	$m \leq N$
genes in cluster	$n \leq N$
genes in cluster with motif	$k \leq n \quad , k \leq m$
enrichment	$R = \frac{k}{n} \frac{N}{m}$
expected	$E(R) = 1 \quad , \frac{k}{n} = \frac{m}{N}$
p-value, **exactly** k genes	$P(k) = \frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}}$
p-value, **at least** k genes	$P_c(k) = 1 - \sum_{i=0}^{k-1} \frac{\binom{m}{i}\binom{N-m}{n-i}}{\binom{N}{n}}$

LARGE DATA-SETS for both GENES and GENOME

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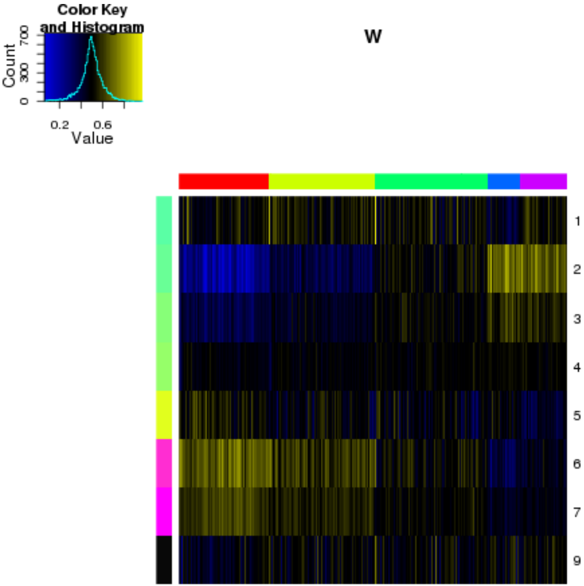
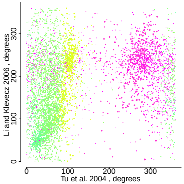
TRANSCRIPTOME META-ANALYSIS:

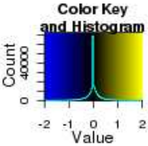
- Columns: >1300 transcriptome experiments
- Rows: redox clusters (here: tu05), U-statistic from Wilcox ranking test
- Column-sorting: SOTA clusters of test statistic U

DATA-SET: 1327 from McCord et al. 2007 Mol Syst Biol
+ some manually collected

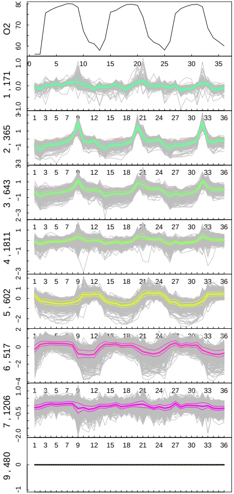
SOTA: self-organizing tree algorithm
Yin, Huang & Ni 2006, BMC Bioinformatics

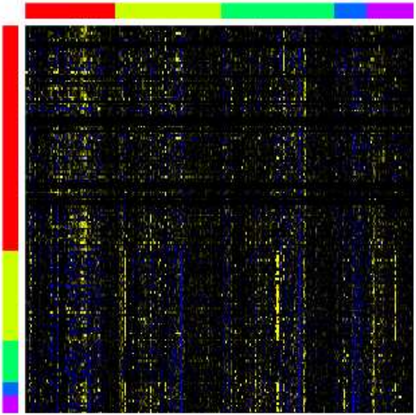




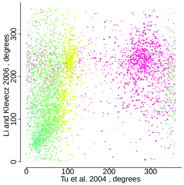


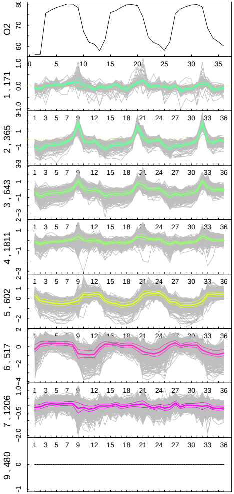
cluster 1 - 171 genes

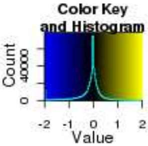


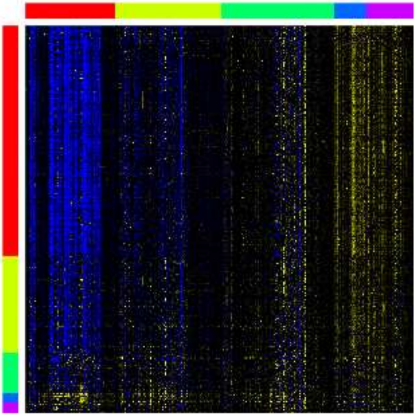


cluster 2 - 365 genes

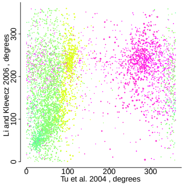


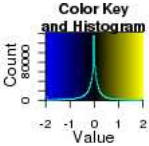


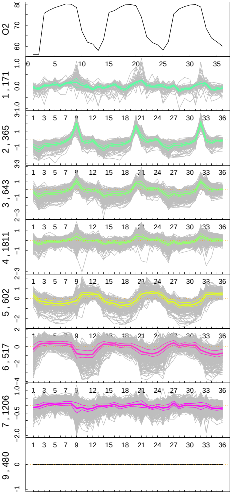


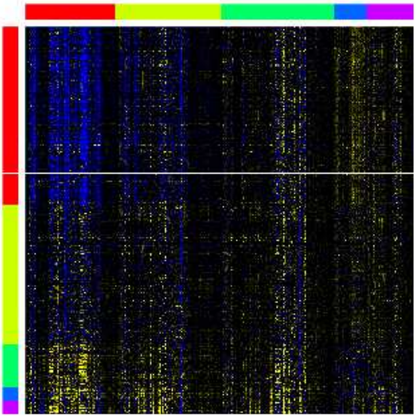


cluster 3 - 643 genes

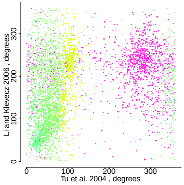


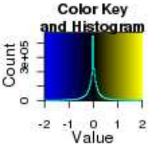


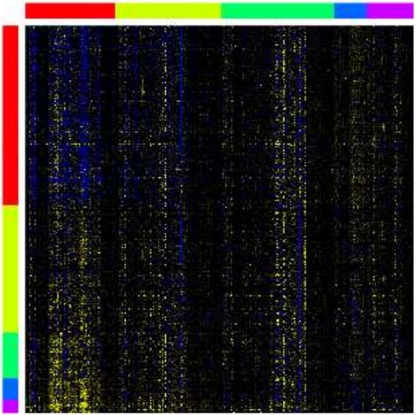


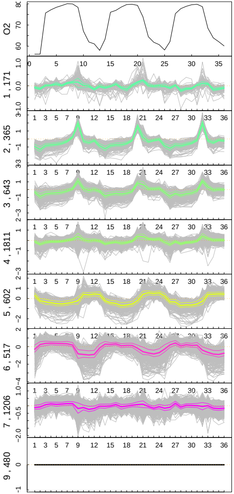


cluster 4 - 1811 genes

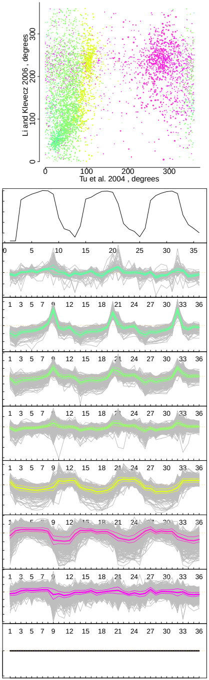


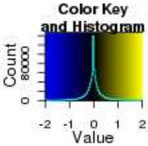


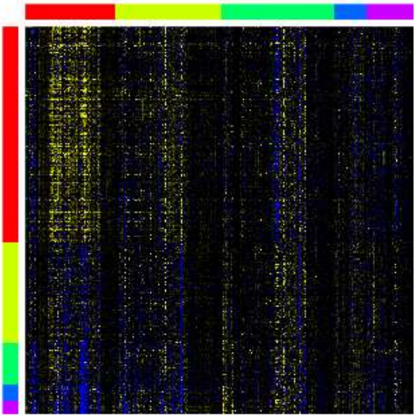




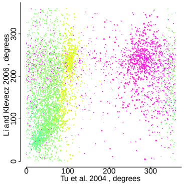
cluster 5 - 602 genes

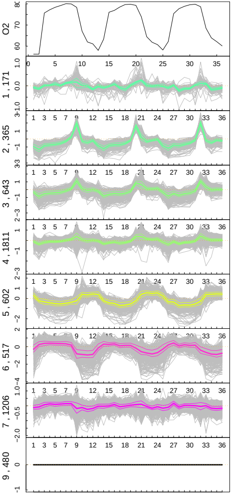


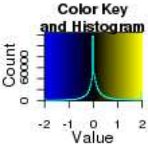


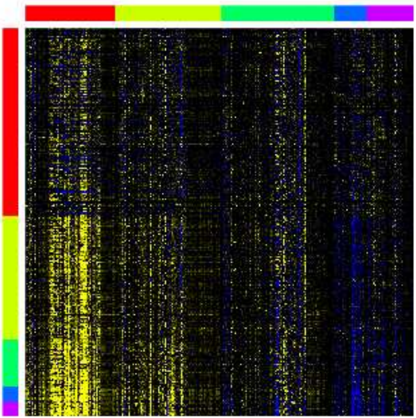


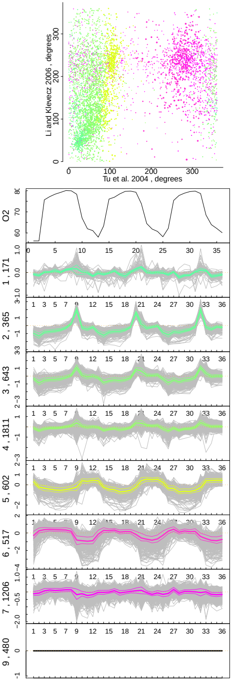
cluster 6 - 517 genes

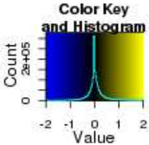




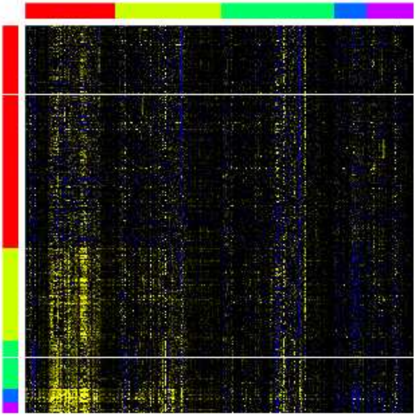




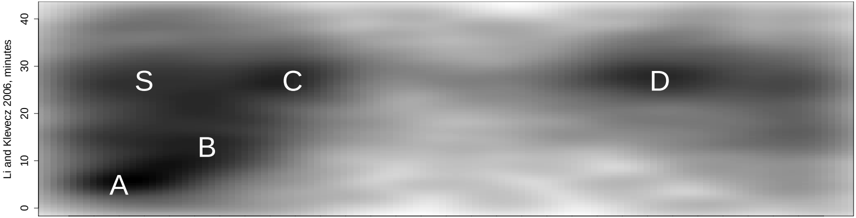




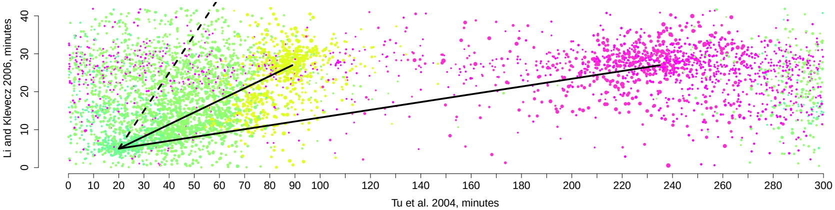
cluster 7 - 1206 genes



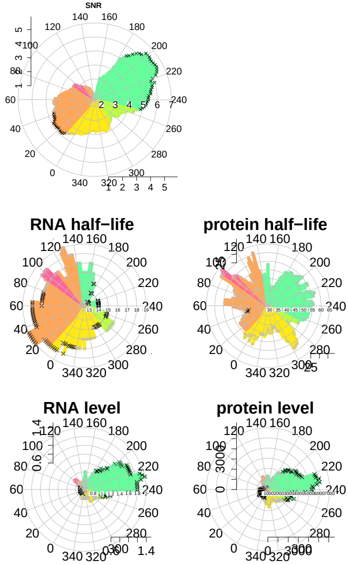
Transcriptome Meta-Analysis



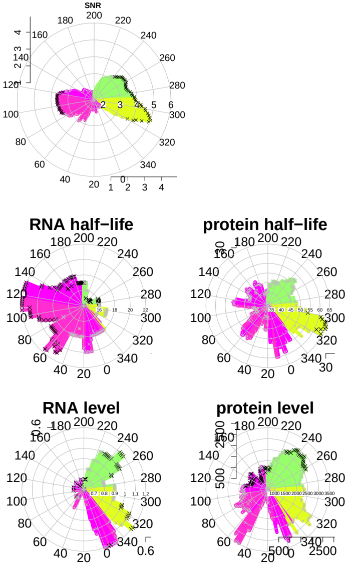
Antagonistic Transcription: A ⇔ D :Natural Cycle in Yeast CC!



General Props - half-lives and levels

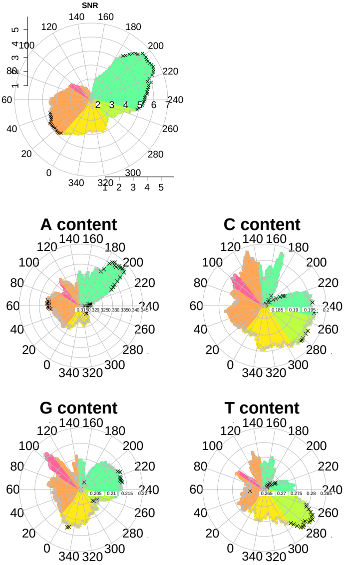


Li and Klevecz 2006

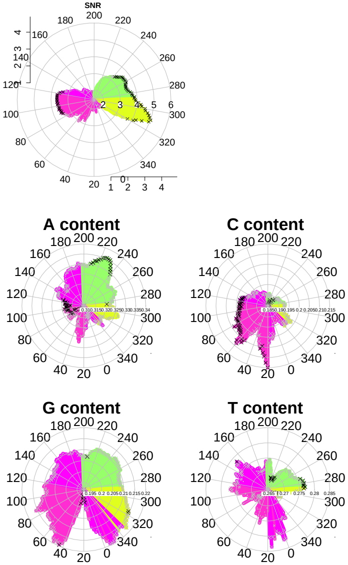


Tu et al. 2005

General Props - nucleotide content

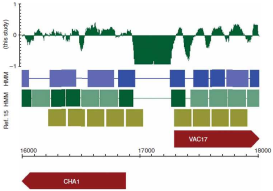


Li and Klevecz 2006

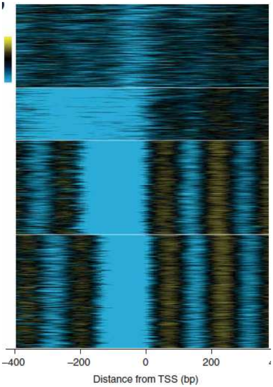


Tu et al. 2005

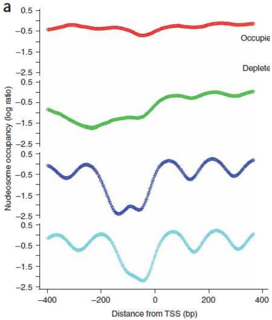
Nucleosomes and RNA Transcription



Nucleosome occupancy (ChIP, tiling array, 5bp) around single genes



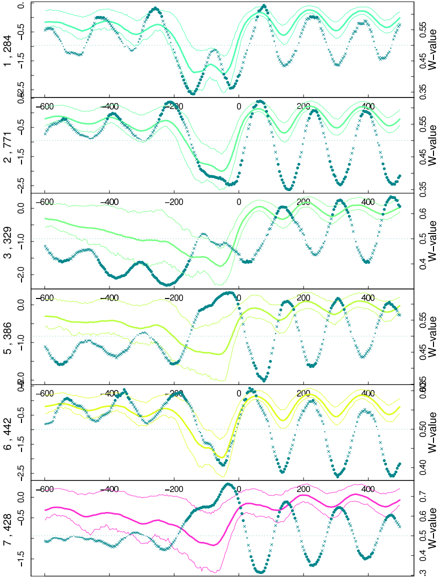
All genes as heatmap, aligned at transcription start site (TSS), k-means clustered



Average nucleosome occupancy of k-means clusters

Lee et al.: *A high-resolution atlas of nucleosome occupancy in yeast.* Nature Genetics 2007

Promotor Structures

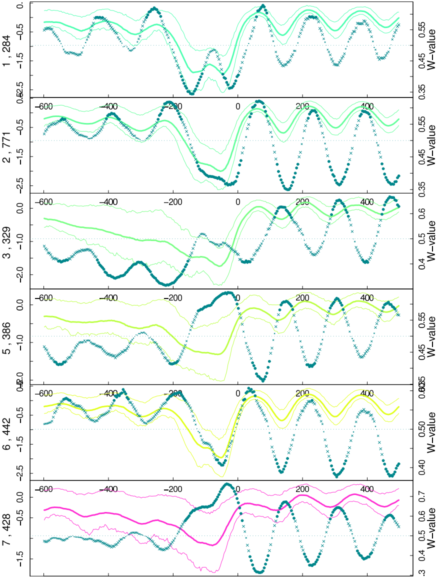


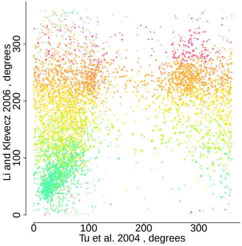
Promotor Profiles:

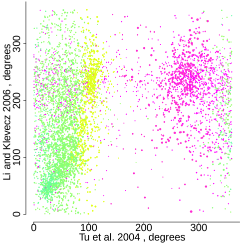
- thick middle line: mean
- thin lines: quartiles
- blue dots: U-statistic of Wilcox Rank Test or t-statistic of t-Test
  - circle: p-value < 0.01
  - cross: p-value >= 0.01
  - size ∼ 1 - p-value

The statistic profile (wrt Mean) provides information how a cluster differs from genomic average.
(U > 0.5: larger then average)

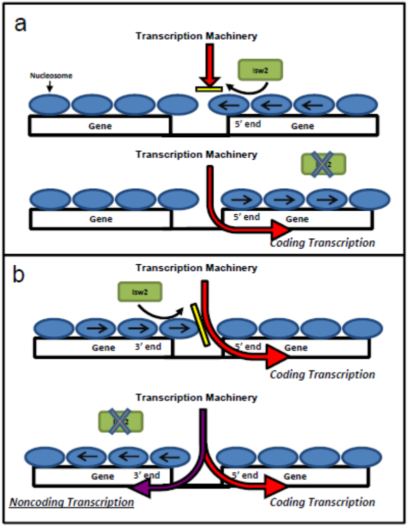
Promotor Structures

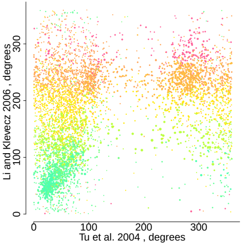






Promotor Structures





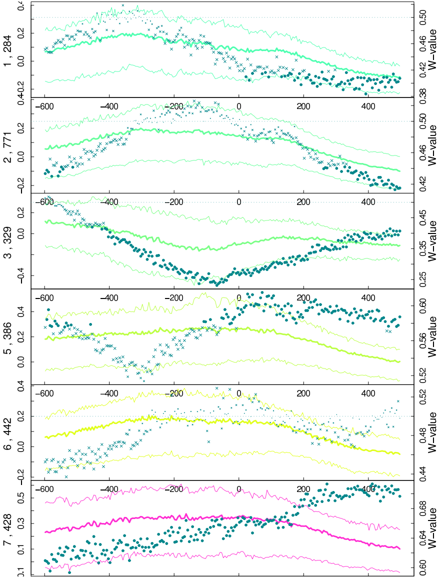
Isw2: ATP-dependent nucleosome remodelling

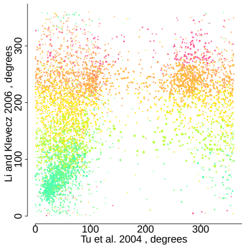
1. Isw2 ChIP-tiling (25bp)
2. Nucl. ChIP-tiling (5bp) in WT and Isw2$^{-/-}$
3. *Remodelling Score* (5bp)

Whitehouse et al. 2007 Nature

Chromatin remodelling at promoters suppresses antisense transcription.

Promotor Structures





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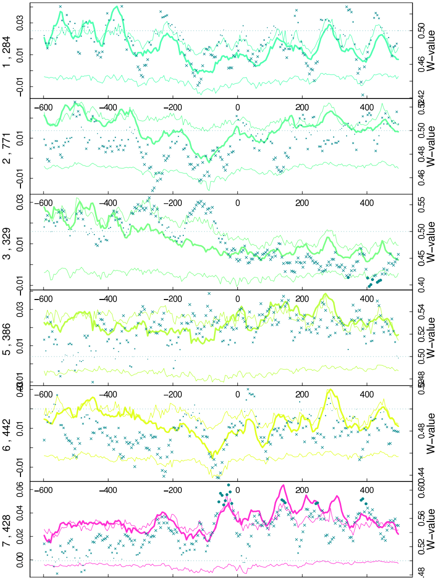

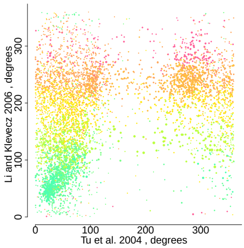

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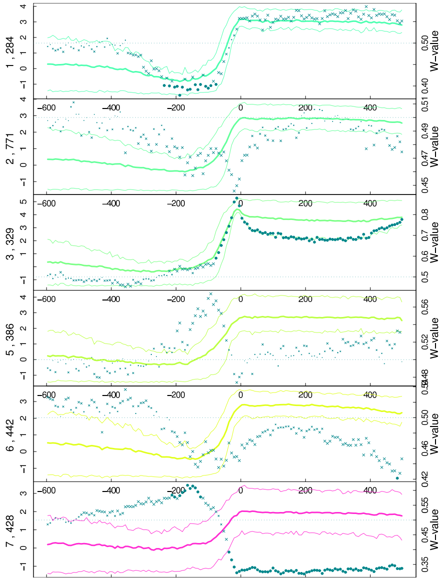
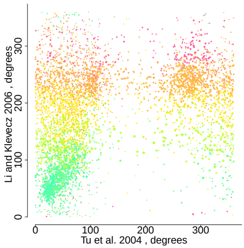


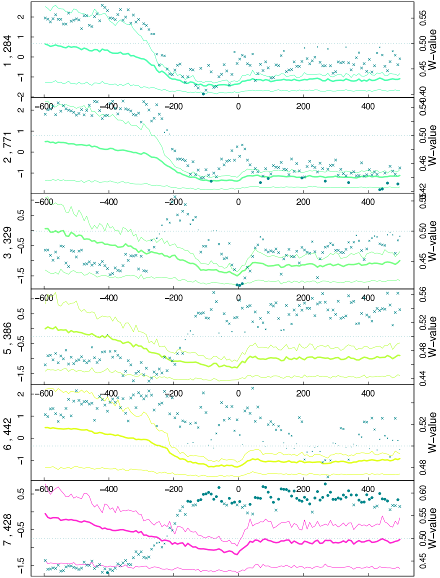


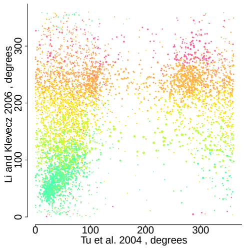
Transcriptome Tiling (8bp)

1. Plus-strand
2. Minus-strand

David et al. 2006 Nature
Perocchi et al. 2007 NAR (anti-sense correction)

Promotor Structures





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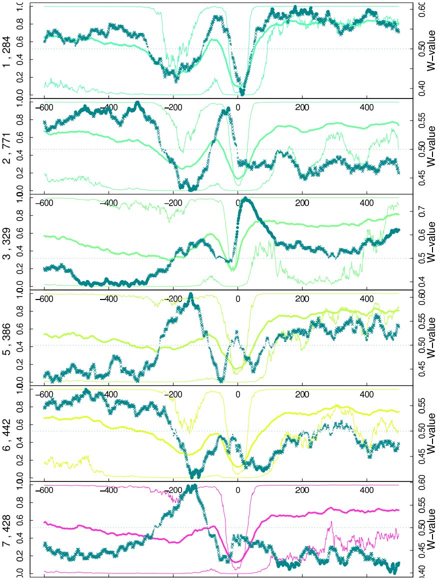
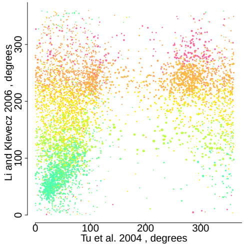


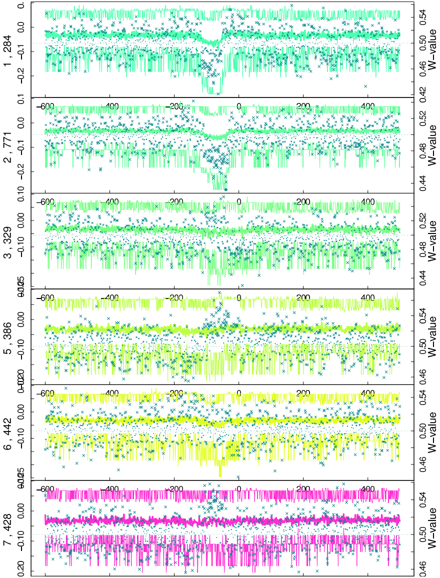


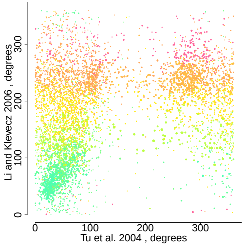
Conservation

1. Phastcons track @UCSC: 7 yeast species

e.g. cluster 2: low mutation rates in linker region of nucleosomes (Washietl, Machne, Goldman 2008 Trends Genet) at 5'/3' gene ends and introns

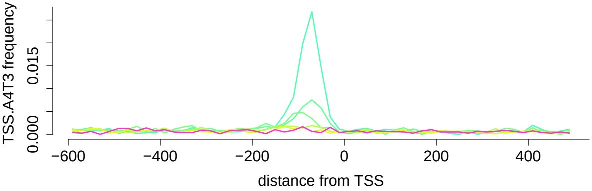
Promotor Structures



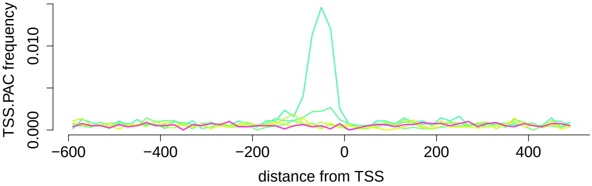


DNA trinucleotide *bendability* ($\sim$ DNAseI cutting frequency) Brukner et al. 1995 J Biomol Struct Dyn

5’ and 3’ Motifs



RRPE Element: **TGAAA[AT]TTT**
rRNA processing

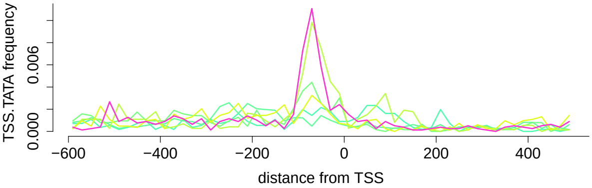


PAC Element: **GATGAG**
Polymerase A & C - associated with RRPE

	A4T3	**PAC**
1	**0.77**	**0.73**
2	**0.5**	0.37
3	0.44	0.25
5	0.31	0.29
6	0.27	0.24
7	0.29	0.21

fractions of genes per cluster carrying the resp. motif within -500 (+500) of ATG START (STOP) Codon

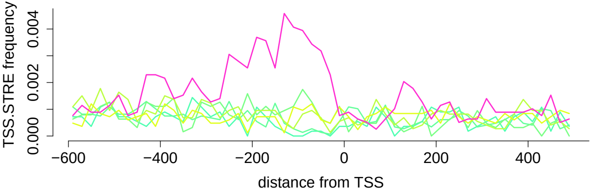
5’ and 3’ Motifs



TATA Box: **TATA[TA]A[TA][AG]**
regulated (as opposed to *house-keeping*) genes

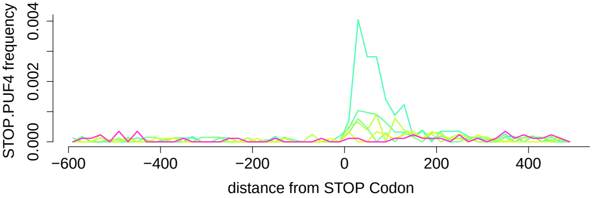
	TATA	STRE
1	0.48	0.24
2	0.44	0.26
3	0.49	0.36
5	**0.65**	0.33
6	0.51	0.3
7	**0.61**	**0.62**

fractions of genes per cluster carrying the resp. motif within -500 (+500) of ATG START (STOP) Codon



STRE Motif: **AGGG**
several binding proteins known, e.g. Msn2p/Msn4p

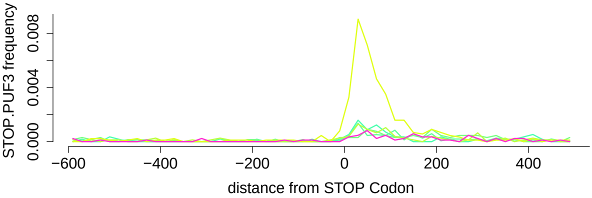
5’ and 3’ Motifs



3’ UTR, PUF4: **TGTA[ACT]A[ACT]TA**
Puf4 protein binds mRNA, controls degradation, function unknown

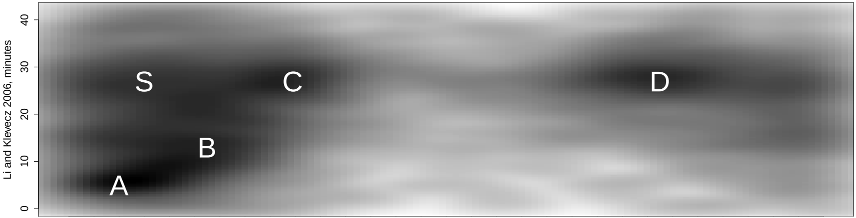
	PUF4.3p	**PUF3.3p**
1	**0.39**	0.31
2	0.19	0.28
3	0.15	0.28
5	0.17	0.29
6	0.1	**0.67**
7	0.11	0.25

fractions of genes per cluster carrying the resp. motif within -500 (+500) of ATG START (STOP) Codon



3’UTR, PUF3: **TGTA[ACT]ATA**
Puf3p sequesters mRNAs @ mitochondria & controls degradation (signal unknown)
(translation into mito. through ancient pores)

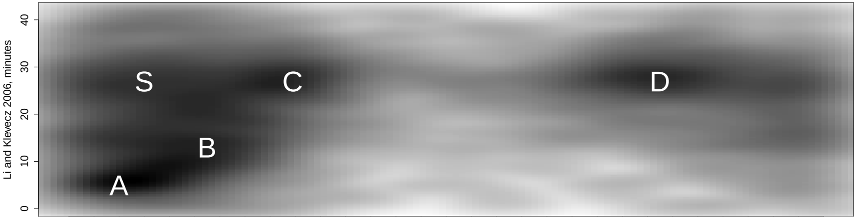
Cluster Properties



- A: cytosolic ribosomes
- B: anabolic metabolism - cell growth
- C: mitochondrial ribosomes
- D: catabolic metabolism - mitochondrial activity

Dual Dichotomy:
Informational vs. Structural
Host cell vs. Endosymbiont

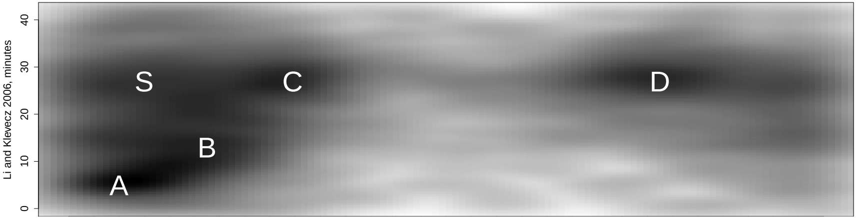
Cluster Properties



- A: Large NFR - well-positioned Nucl.s 5’ & 3’ - no remod.
- B: Promotor Nucl.s - fuzzy-positioned - remodelling
- C: Small NFR - well-positioned Nucl.s 5’ & 3’ - no remod.
- D: Promotor Nucl.s - fuzzy-positioned - remodelling

Dual Dichotomy:
Informational vs. Structural
Host cell vs. Endosymbiont

Cluster Properties



- A: RRPE/PAC - PUF4 mRNA regulation - short RNA $T_{1/2}$
- B: TATA - many TFs
- C: no 5’ motifs - PUF3 mRNA regulation - short RNA $T_{1/2}$
- D: TATA/STRE - long RNA $T_{1/2}$

Dual Dichotomy:
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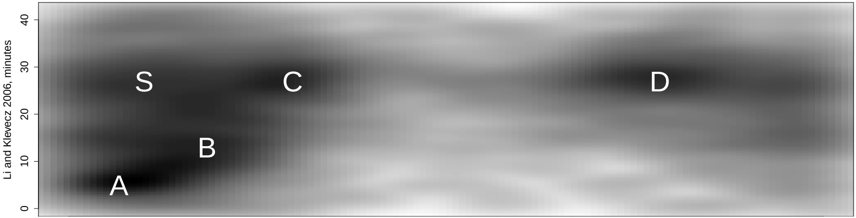
Host cell vs. Endosymbiont

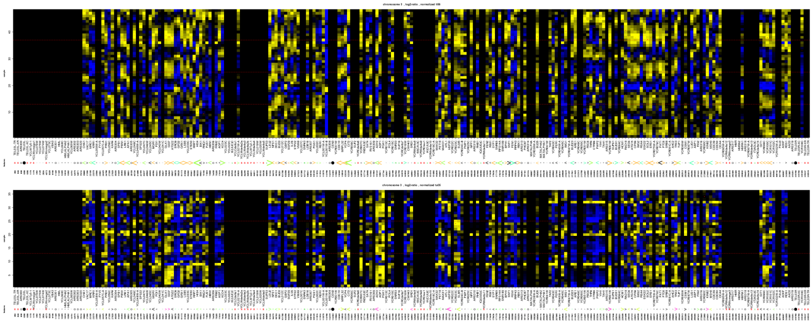
Cluster Properties



- A: A-rich coding region
- B: T-rich coding region
- C: G-rich coding region (slightly)
- D: C-rich coding region

Dual Dichotomy:
Informational vs. Structural
Host cell vs. Endosymbiont

Chromosomal Domains?



Chromosome 3:

- x-axis: transcribed entities, centromer, telomers
- y-axis: redox time-course
- colors: expression in log2-ratio, blue: low, yellow: high

CHROMOSOMAL NEIGHBORHOODS:

1. for each cluster (here: li06)
2. count other clusters in neighboring genes
3. do cumul. hypergeometric distribution test

Color: -log2(p-value)
(p-value 0.01 → 6.6)
T: tRNA
S: snRNA, snoRNA, ncRNA
G: pseudogene, dubious gene, transposon gene
A: autonomous replication sequence!

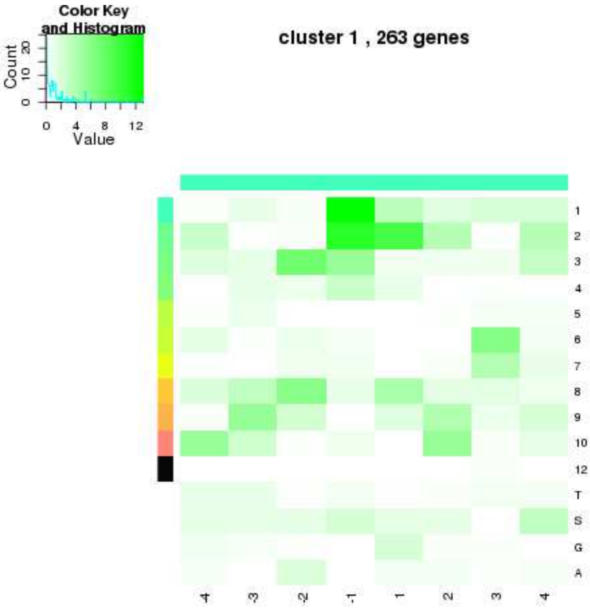
INTERPRETATION:

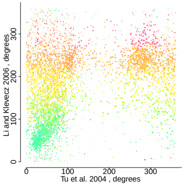
- @ -1: could be shared promotor
  (currently not controlled for)
- **+1, -2: can only be chromosomal domain!**

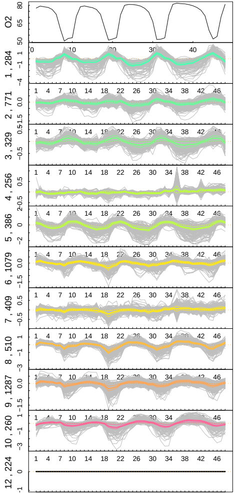
also:

- count all k-mers
  (w or w/o collapsing same clusters)
- permutate sequence
  (w or w/o conservation of di-‘nucleotide’ content)

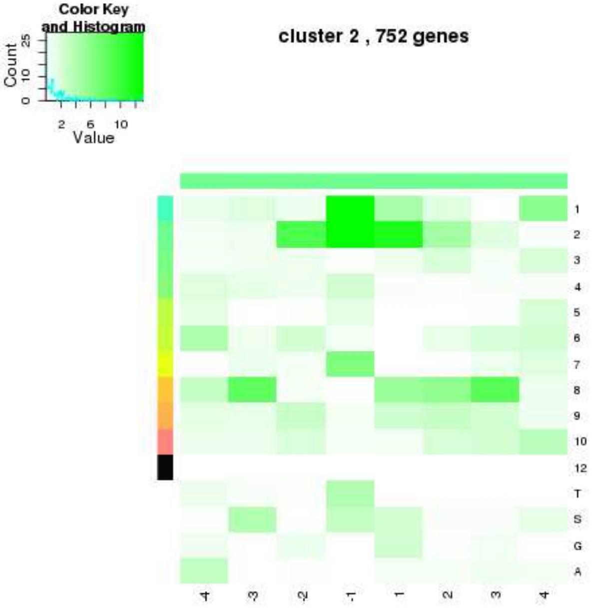
IDEAS??

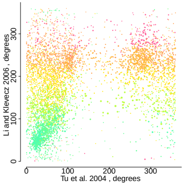


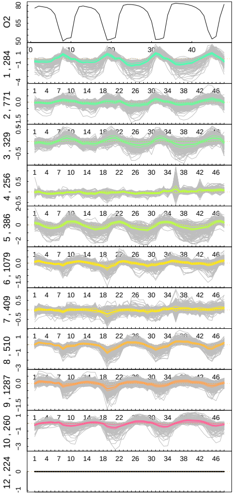


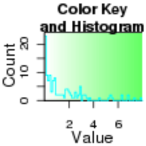


cluster 2 , 752 genes

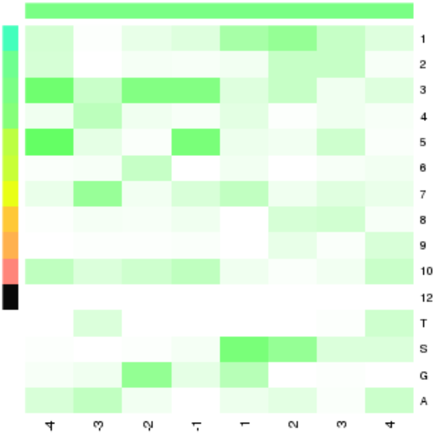


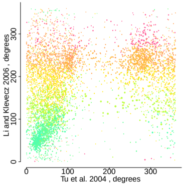


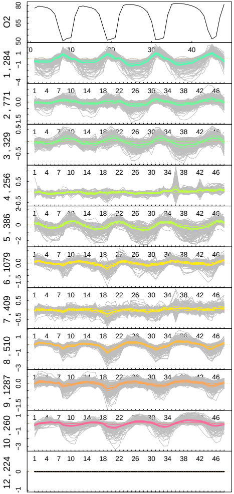


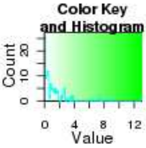


cluster 3 , 286 genes

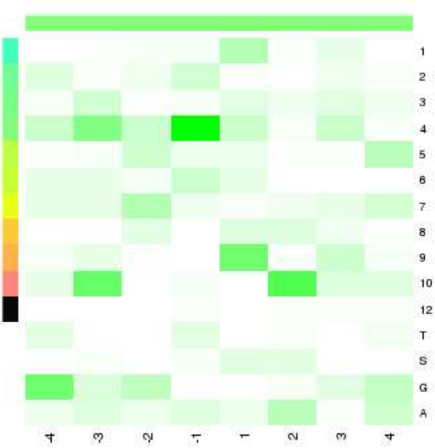




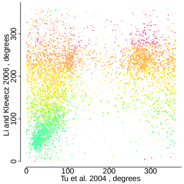


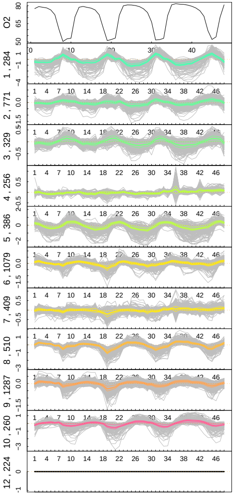


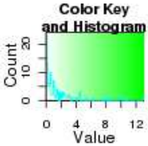
cluster 4 , 101 genes

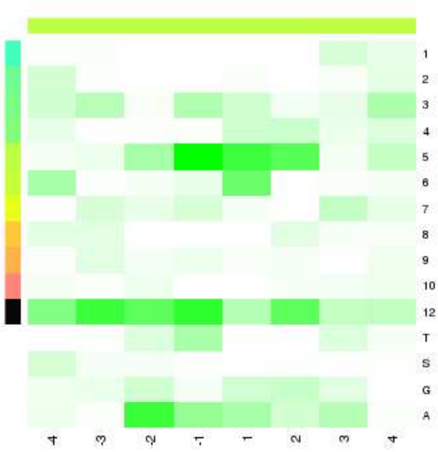


cluster 5 , 275 genes

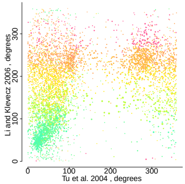


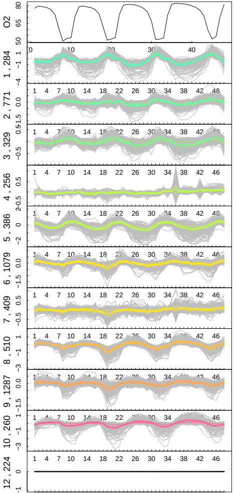


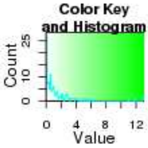


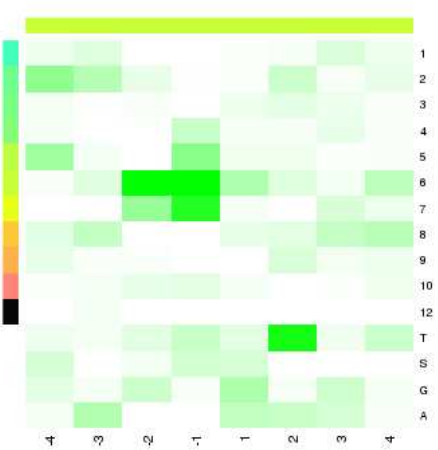


cluster 6 , 331 genes

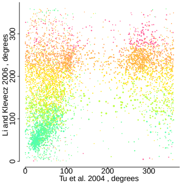


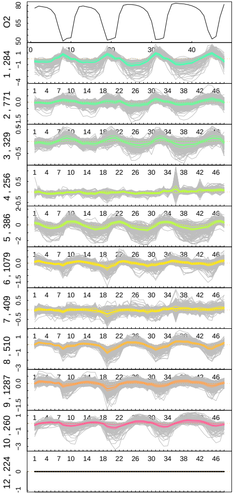


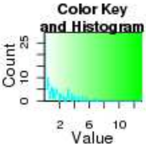


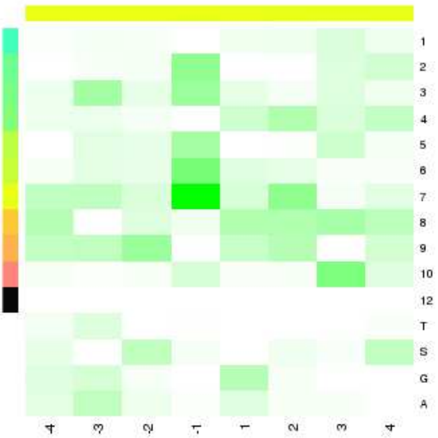


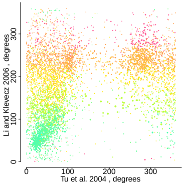
cluster 7 , 740 genes

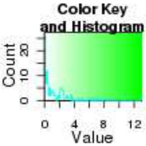




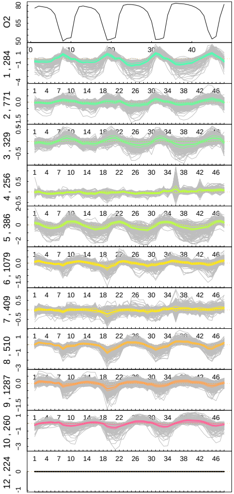


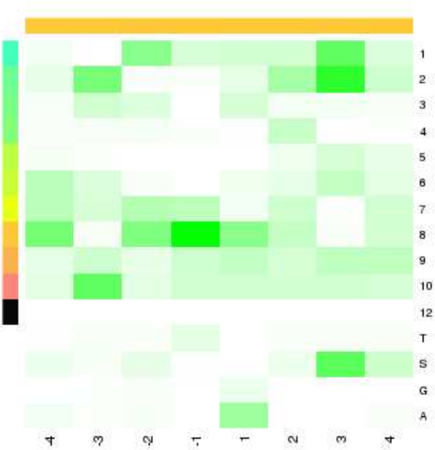




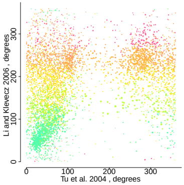


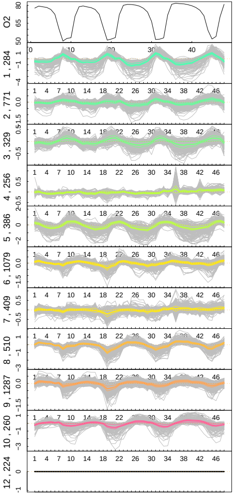
cluster 8 , 1498 genes

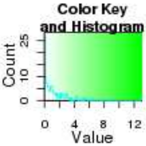


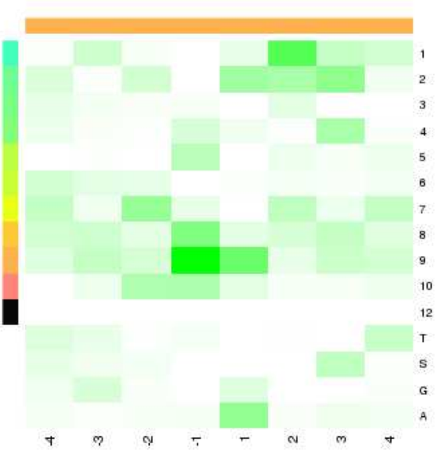


cluster 9 , 589 genes

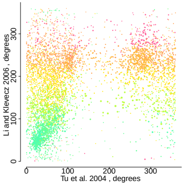


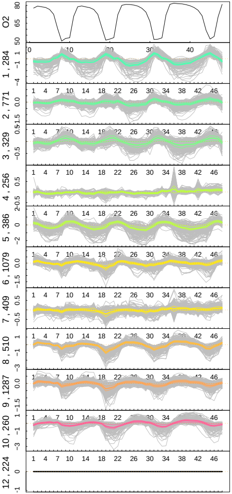


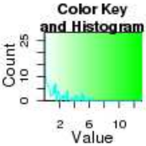


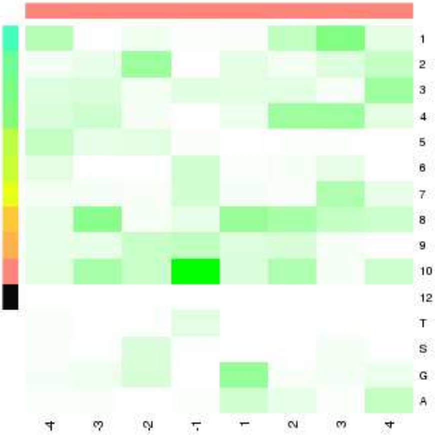


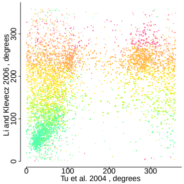
cluster 10 , 736 genes

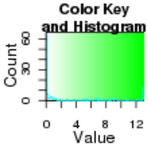




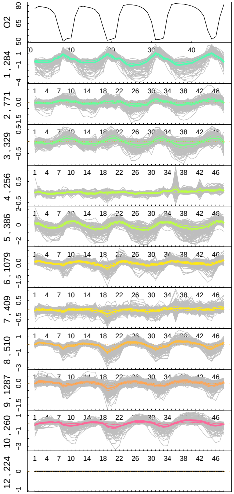


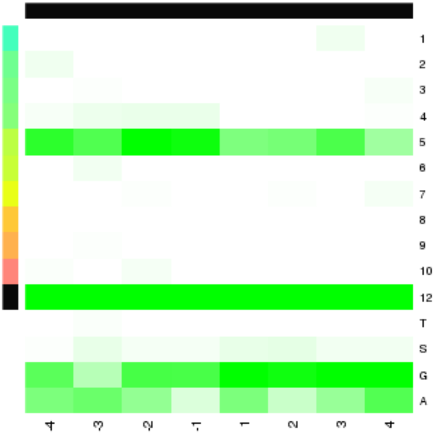




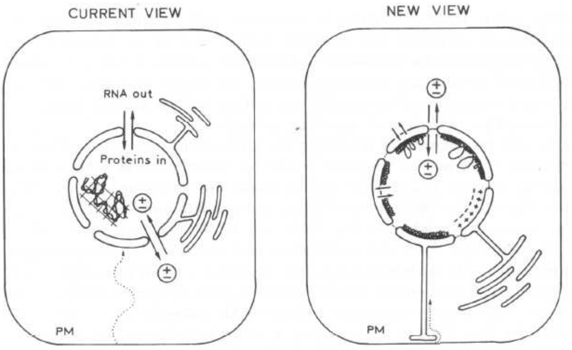


cluster 12 , 224 genes





Cluster Analysis Conclusion



Matzke & Matzke 1991 Bioelectrochemistry & Bioenergetics; and see recent work by Susan Gasser (Bern, Suisse)

1. Dualistic Meta-Transcriptome
2. Gene Properties: half-lives, Nt. content
3. Promotor Structure & Classes
4. 5’ and 3’ Motifs
5. Chromosomal Domains

WHAT? - **Time-course Analysis:**

- DFT-based clustering
  - Normalization: **? !**
  - Discrete Fourier Transform

1. Comparative time-course profile analysis
2. GO/SBML analysis & Correlation to Metabolome

HOW? - **Cluster Analysis:**

- Statistical Scans:
  - t-test, Wilcox-test, hypergeometric distribution
  - Scanning diverse ‘gene/protein’ properties
  - Scans along aligned DNA sequences

1. Transcriptome Meta-analysis
2. General Properties & Promotor Structure
3. Motifs: Specific RNA/DNA-binding Proteins
4. Chromosomal Domains

WHY? - **Modeling:**

- *ODE* Models of metabolism ↔ gene expression feedback

1. Coupled *Oscillatory* Loops?
2. Avoid *Futile Cycles*?
3. Control *Mutation Rates*?

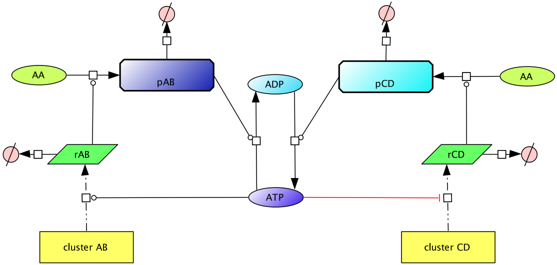
Gene Regulation Hypotheses

Minimal Hypothesis: ATP (directly or indirectly) required for

- **expression of anabolic** and / or
- **repression of catabolic**

gene programs, respectively

entia non sunt multiplicanda praeter necessitatem

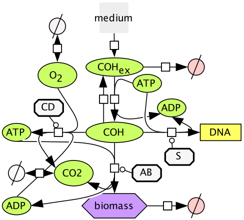


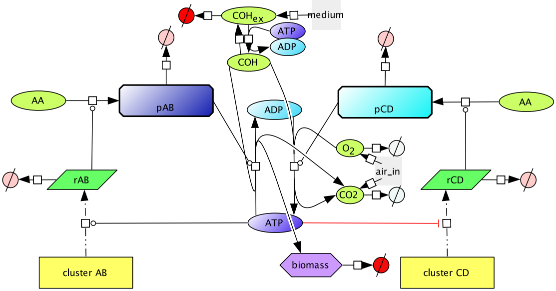
HOW?

- cf. Superhelical DNA in circular genomes: helicases vs. topoisomerases (ATP), GC-content
  - Circadian *oscilloid* in cyanobacteria (Vijayan et al. 2009 PNAS, Woelfle et al. 2007 PNAS)
  - Metabolic vs. Anabolic genes in *E.coli*
- *Saccharomyces cerevisiae*: **metabolism ↔ chromatin dynamics**
  - ATP-dependent remodeling
  - Histone modification (AcCoA, SAM, ATP, UbiQ, ...)

1. **ATP inhibits catabolic gene (CD) expression but is required for anabolic genes (AB)**
2. **Parameterize by data from 40' cycle - Cont. Culture allows to Quantify our Hypotheses!**
3. **Test simple nucleosome remodelling dynamics**
4. **Split ATP/NAD(P)H, DNA/biomass, include histone modification**

Gene Regulation Hypotheses





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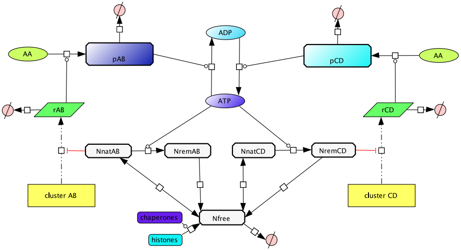

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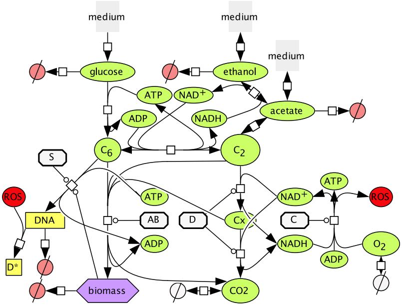

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Conclusion

the times are a’swinging

Arigato-gozaimasu:
Douglas B. Murray
Cornelia Amariai (nucleosomes: remodelling and modifications)
Kalesh Sasidharan (tRNA and amino acid regulation)

Danke Schön:
Xtof Flamm
Lukas Endler
Stefan Müller
Clemens Zarzer
James Lu