Gene Regulation: Bioinformatic aspects

Jaak Vilo

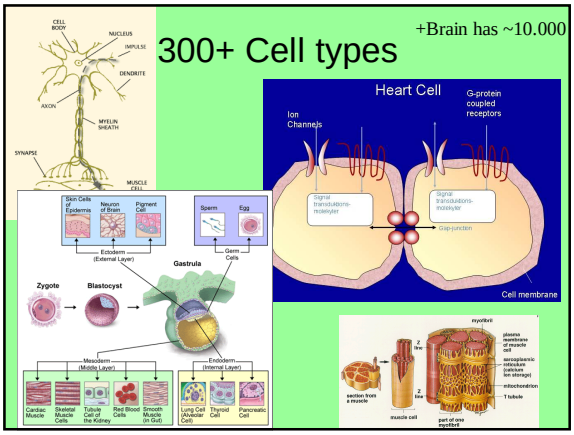
CS theory days, Koke, 4.2.04

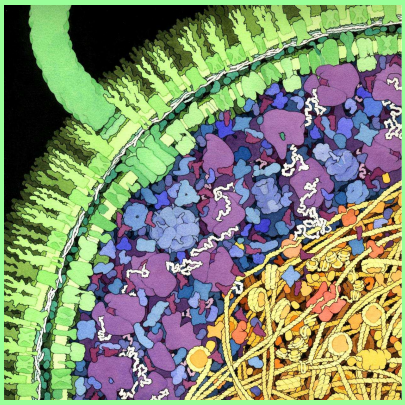
Topics

- Biological background
- Computational methods/challenges
- Current projects

300+ Cell types

+Brain has ~10.000





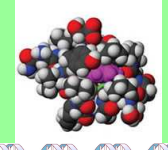
David S. Goodsell
http://www.scripps.edu/pub/goodsell/

Central dogma

TTAAGCTCCGTAGCA DNA

UUAAGCUCCGUAGCA mRNA

Leu Ser Ser Val Ala valk



Level 0	ATCGCTGAATTCCAATGTG
Level 1	
Level 2	
Level 3	
Level 4	
Level 5	
Level 6	

A eukaryotic genome can be thought of as six Levels of DNA structure.

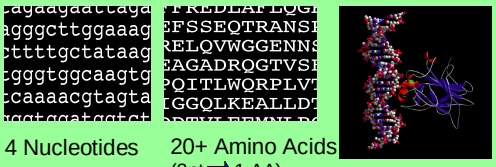
The loops at Level 4 range from 0.5kb to 100kb in length.

If these loops were stabilized then the genes inside the loop would not be expressed.

DNA determines function (?)

DNA → **Protein** → **Structure**

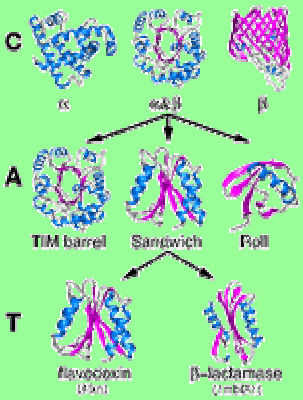
GenBank / EMBL Bank — SwissProt/TrEMBL — PDB/Molecular Structure Database



4 Nucleotides — 20+ Amino Acids (3nt → 1 AA)

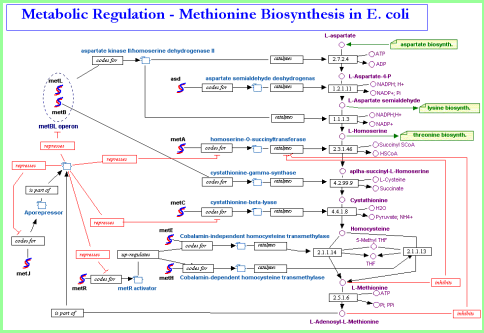
Function?

CATH hierarchy



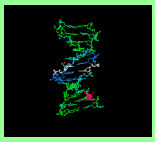
Fold prediction
Threading

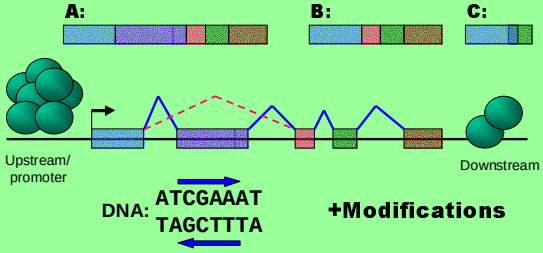
A Simple Metabolic Pathway



Shoshanna Wodak, Jacques van Helden

A Simple Gene



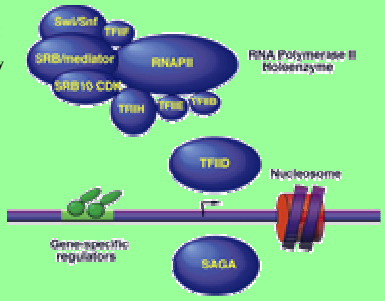


DNA: ATCGAAAT
TAGCTTTA

+Modifications

Regulation of gene expression (transcription)

Model of RNA Polymerase II Transcription Initiation Machinery. **The machinery depicted here encompasses over 85 polypeptides in ten (sub) complexes**: core RNA polymerase II (RNAPII) consists of 12 subunits; TFIIH, 9 subunits; TFIIE, 2 subunits; TFIIF, 3 subunits; TFIIB, 1 subunit, TFIID, 14 subunits; core SRB/mediator, more than 16 subunits; Swi/Snf complex, 11 subunits; Srb10 kinase complex, 4 subunits; and SAGA, 13 subunits.

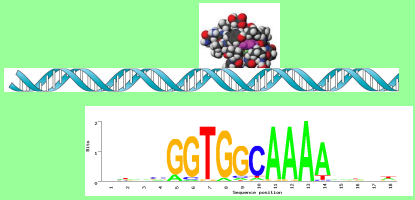


F.C.P. Holstege, E.G. Jenning, J.J. Wyrick, Tong Ihn Lee, C.J. Hengartner, M.R. Green, T.R. Golub, E.S. Lander, and R.A. Young
Dissecting the Regulatory Circuitry of a Eukaryotic Genome
Cell 95: 717-728 (1998)

Gene regulation

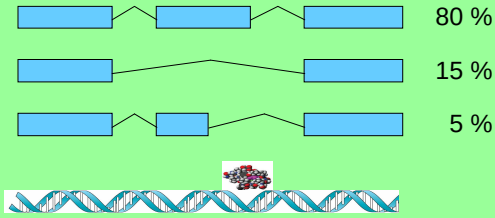
- Determines
  - the development (from embryo)
  - cell types
  - processes of the cell
  - response to the environment
  - …
  - Regulation happens at different levels

Regulation by binding to DNA/RNA



4^6= 4096, 4^8=65.000

Regulation of splicing



Valgu seondumine võib mõjutada splaissingut

Regulation of Alternative Splicing

- Which splice variants in which cells?
- Are there cell type specific splicing regulators and signals in DNA/RNA?

- Find genes that have an exon switched on specifically in tissue X
- Is there a common signal for all such exons or splicing events?

Tissue specific alternative splicing

EST-tehnoloogial baseeruvad andmed (Meelis Kull)

		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	sum
Geen 1	**V1**	1	1	0	0	3	0	1	2	1	6	**15**
	V2	5	0	1	3	3	2	2	9	4	9	**38**
	V3	3	0	1	2	1	0	0	1	0	1	**9**
	V4	1	0	0	1	0	0	0	0	1	0	**3**
	V5	0	0	0	0	1	0	0	0	0	3	**4**
Geen 2	**V1**	8	1	3	4	1	1	2	11	3	12	**46**
	V2	3	0	3	0	0	0	2	7	0	4	**19**
	V3	0	0	0	0	1	1	1	0	0	1	**4**
	V4	2	0	0	0	0	0	1	2	1	2	**8**
	V5	0	0	0	0	0	0	0	1	0	1	**2**
	V6	0	0	1	0	0	0	1	0	0	2	**4**
Geen 3	**V1**	16	1	3	5	2	4	3	17	7	18	**76**
	V2	7	0	1	2	0	2	2	6	4	8	**32**
	V3	1	0	0	0	0	1	2	1	1	1	**7**

How to study the gene regulation with computational methods?

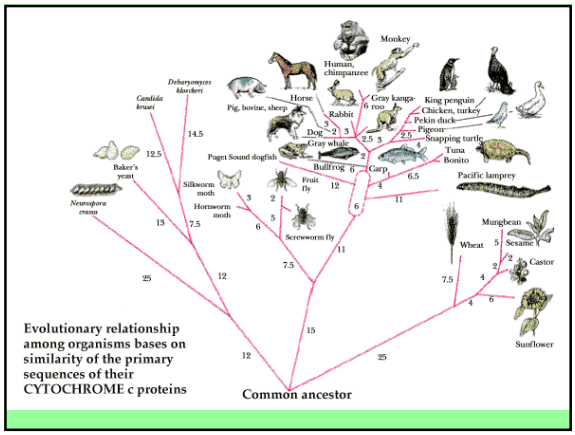
- What data is available?
- How to combine them meaningfully?

- Algorithms (is the analysis feasible)?
- Actual analysis
- Interpret the results

Core data (Static)

- **DNA sequence(s)**
  - Genes
  - Protein sequences
  - Relation to other species
  - Protein structure (???)

- Partial knowledge about function
  - how to capture this formally?



Phylogenetic tree

www.tolweb.org

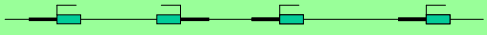
Expression data (dynamics)

- Low-throughput methods
- Expressed Sequence Tags (EST)
  - RNA sequences
- DNA microarrays for gene expression
  - Relative abundance of RNA in cell
- Genome-wide localization studies
  - binding of proteins to DNA
- Proteomics
  - Amount of proteins in cells

Veel pole piisavalt informatsiooni:

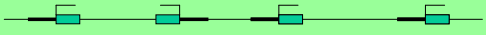
- Alternatiivne splaissing
- Valkude modifikatsioonid
- RNA geenid, lühikesed geenid, …
- geenide regulatsioon ja võrgustikud
- DNA ja RNA struktuur ja nende mõjud
- **Valkude struktuur ja täpne funktsioon ning roll bioloogilistes protsessides**
- metaboolsed ja signaali ülekande rajad
- Variatsioonid populatsioonis
- Rääkimata selle kõige arvesse võtmisest organismi tasemel…

Study of sequence features

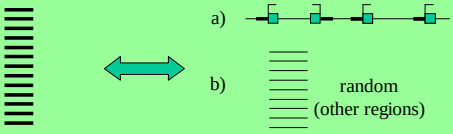


Is there something unique in the promoter regions?

Study of sequence features



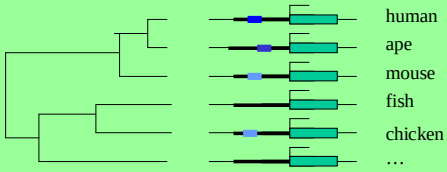
Promoters **vs.** **background**



Upstream vs genomic random

Phylogenetic footprinting

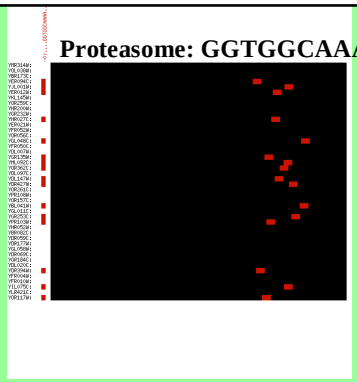
Study the same gene in many species

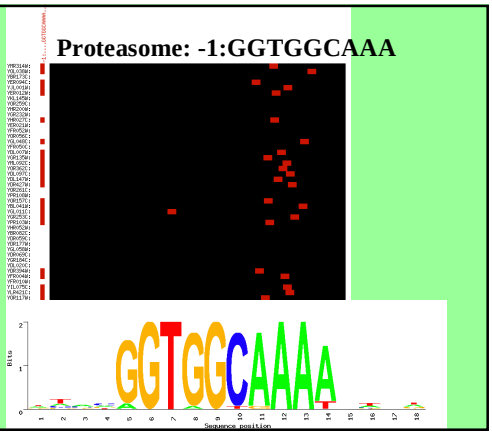


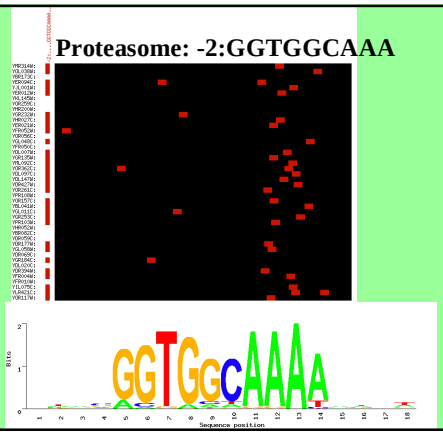
If preserved during evolution then must be important for something!!!

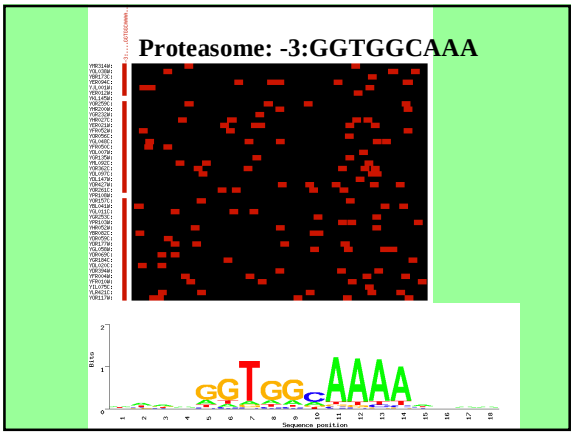
Similar function or role ⇒ same regulation?

- This may or may not be true
- How do we actually know that they are behaving similarly?
- Different regulation mechanisms may achieve the same effect



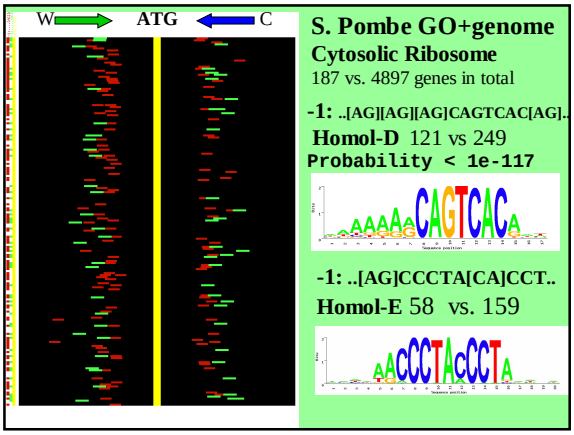


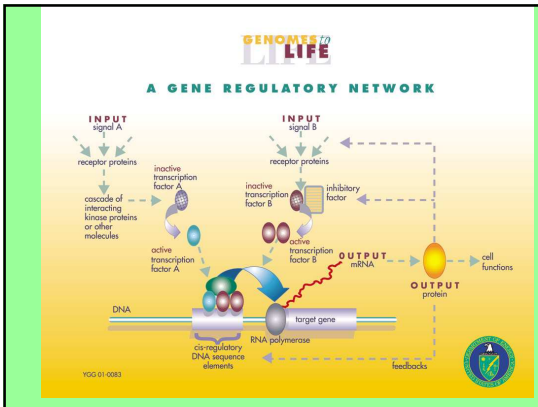




Proteasome movie

- Movies\proteasome.wmv





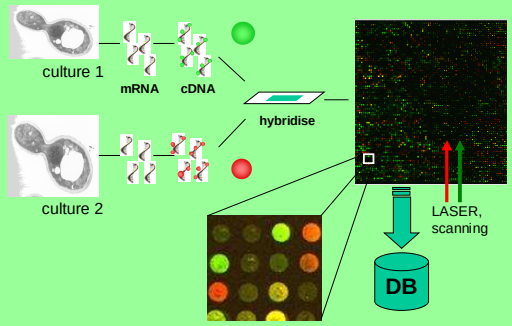
Dynamics?

- Which genes regulate others
- When and how genes are ‘switched on or off?’
- What is the global relationship between genes
- How to model the gene regulation?
  - Continuous stochastic processes responding to the external stimuli

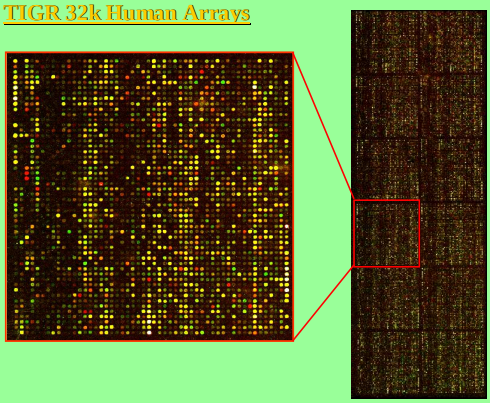
Experimental data?

- What data can we start with?
- What is known or hypothesised so far?
- Can one test the new hypotheses in practice?

Analysis of biological samples with microarrays



TIGR 32k Human Arrays

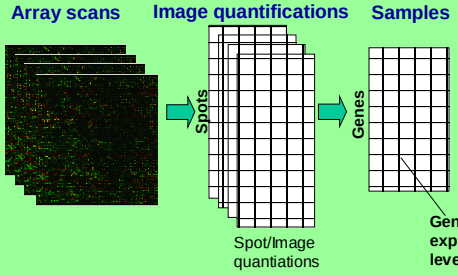


From microarray images to gene expression data

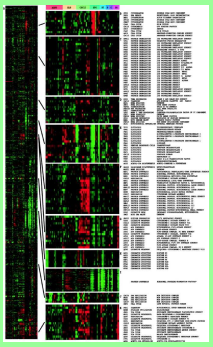
Raw data — **Array scans**

Intermediate data — **Image quantifications**

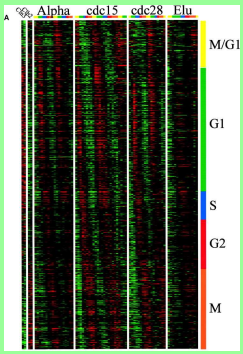
Final data — **Samples**



Eisen *et.al*, PNAS 98

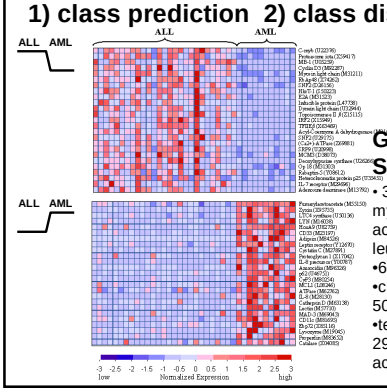


Spellman *et.al*. Mol Biol Cell 98



Tumor classification:

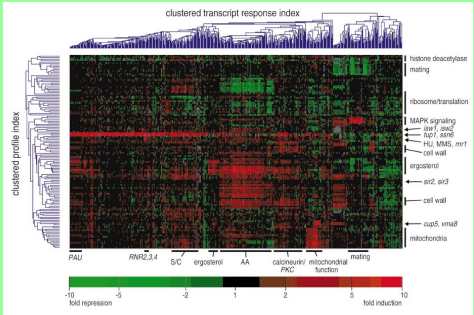
1) class prediction 2) class discovery



Golub et al, Science Oct 15th 1999

- 38 samples of acute myeloic leukemia (AML) and acute lymphoblastic leukemia (ALL)
- 6817 genes
- classificator built based on 50 best correlated genes
- tested on 34 new samples, 29 of them predicted accurately

Hughes, T. R. et al: "Functional Discovery via a Compendium of Expression Profiles", Cell 102 (2000), 109-126.

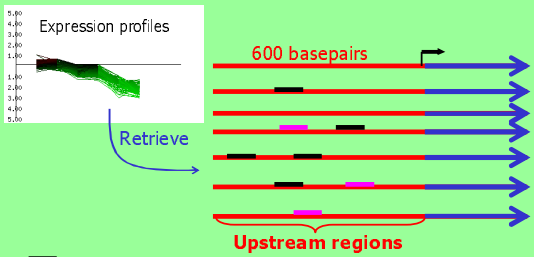


Gene expression data

- Snapshots in time to various stimuli, conditions, tissues, time,
- Approximate information about the level of gene expression (RNA transcripts)

- Limited granularity of time
- Limited accuracy
- Data size is large => need fast methods
  - Algorithm: Meelis Kull and J.V.

Cluster of co-expressed genes, pattern discovery in regulatory regions



Find patterns over-represented within cluster

Genome Research 1998;
ISMB (Intelligent Systems in Mol. Biol.) 2000

Pattern selection criteria Binomial distribution

Background - ALL upstream sequences

Cluster:

π occurs 3 times

P(3,6,0.2) is probability of having ≥3 matches in 6 sequences

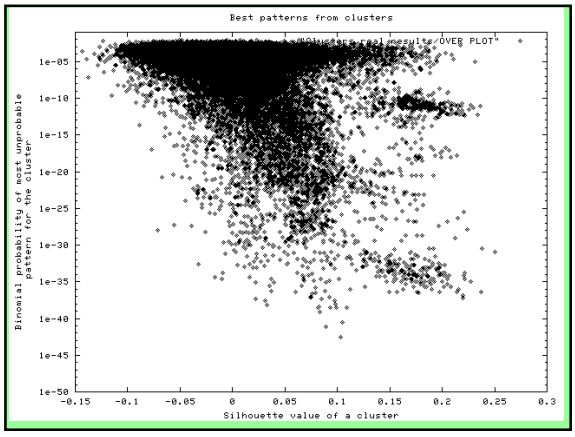
P(π,3,6,0.2) =0.0989

5 out of 25, ***p* = 0.2**

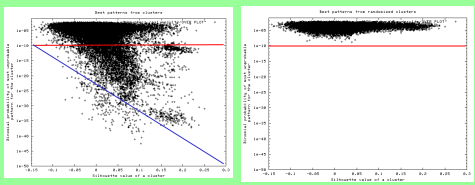
The most unprobable pattern from best clusters

Pattern	Probability	Cluster size	Occurrences in cluster	Total nr of occurrences	K in K-means
AAAATTTT	2.59E-43	96	72	830	60
ACGCG	6.41E-39	96	75	1088	50
ACGCGT	5.23E-38	94	52	387	40
CCTCGACTAA	5.43E-38	27	18	23	220
GACGCG	7.89E-31	86	40	284	38
TTTCGAAACTTACAAAAAT	2.08E-29	26	14	18	450
TTCTTGTCAAAAAGC	2.08E-29	26	14	18	325
ACATACTATTGTTAAT	3.81E-28	22	13	18	280
GATGAGATG	5.60E-28	68	24	83	84
TGTTTATATTGATGGA	1.90E-27	24	13	18	220
GATGGATTTCTTGTCAAAA	5.04E-27	18	12	18	500
TATAAATAGAGC	1.51E-26	27	13	18	300
GATTTCTTGTCAAA	3.40E-26	20	12	18	700
GATGGATTTCTTG	3.40E-26	20	12	18	875
GGTGGCAA	4.18E-26	40	20	96	180
TTGTTGTCAAAAAGCA	5.10E-26	29	13	18	250
CGAAACTTACAAA	5.10E-26	29	13	18	290
GAAACTTACAAAAATAAA	7.92E-26	21	12	18	650
TTTGTTTATATTG	1.74E-25	22	12	18	600
ATCAACATACTATTGT	3.62E-25	23	12	18	375
ATCAACATACTATTGTTA	3.62E-25	23	12	18	625
GAACGCGCG	4.47E-25	20	11	13	260
GTTAATTTCGAAAC	7.23E-25	24	12	18	400
GGTGGCAAAA	3.37E-24	33	14	31	475
ATCTTTTGTTTATATTGA	7.19E-24	19	11	18	675
TTTGTTTATATTGATGGA	7.19E-24	19	11	18	475
GTGGCAAA	1.14E-23	28	18	137	725

Vilo *et.al.* ISMB 2000



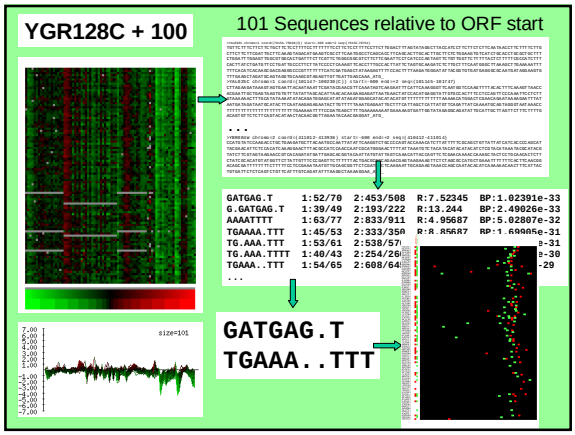
Significance of the patterns

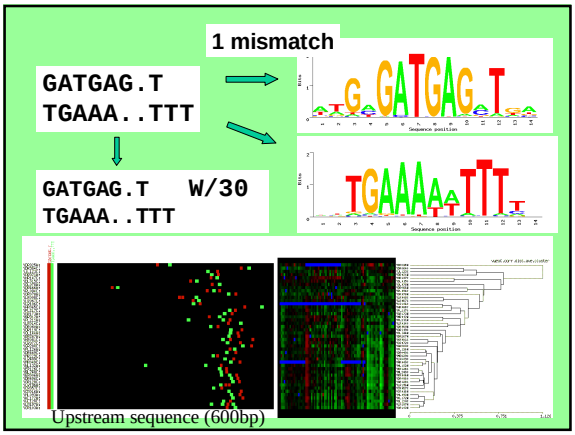


The pattern probability vs. the average silhouette for the cluster

The same for randomised clusters

Vilo *et.al.* ISMB 2000





Problems

- Many motifs are statistically significant
- Many of them similar to each other

⇨ Summarize meaningfully!
⇨ Create probabilistic models

- Algorithms: J.V. and Triinu Tasa

Annotation of clusters

- **Map gene sets to GeneOntology categories.**

GO:0042254 <U-L> **Process: ribosome biogenesis and assembly (+2:15) (depth=7) [sgd:2:187]**
GO:0042254: 47 from cluster (size 98) vs 187 in this class (including subclasses)

GO:0006364 <U-L> **Process: rRNA processing (+3:3) (depth=8) [sgd:50:126]**
GO:0006364: 35 from cluster (size 98) vs 126 in this class (including subclasses)

GO:0006360 <U-L> **Process: transcription from Pol I promoter (+6:14) (depth=8) [sgd:23:155]**
GO:0006360: 38 from cluster (size 98) vs 155 in this class (including subclasses)

GO:0005730 <U-L> **Component: nucleolus (+10:17) (depth=6) [sgd:154:210]**
GO:0005730: 45 from cluster (size 98) vs 210 in this class (including subclasses)

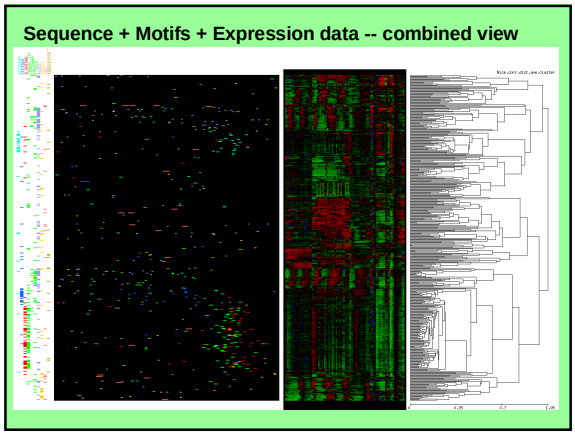
GO:0030515 <U-L> **Function: snoRNA binding (depth=6) [sgd:23:23]**
GO:0030515: 17 from cluster (size 98) vs 23 in this class (including subclasses)

GO:0030490 <U-L> **Process: processing of 20S pre-rRNA (depth=9) [sgd:33:33]**
GO:0030490: 18 from cluster (size 98) vs 33 in this class (including subclasses)

GO:0005732 <U-L> **Component: small nucleolar ribonucleoprotein complex (depth=6) [sgd:30:30]**
GO:0005732: 16 from cluster (size 98) vs 30 in this class (including subclasses)

GO:0006396 <U-L> **Process: RNA processing (+7:52) (depth=7) [sgd:7:370]**
GO:0006396: 40 from cluster (size 98) vs 370 in this class (including subclasses)

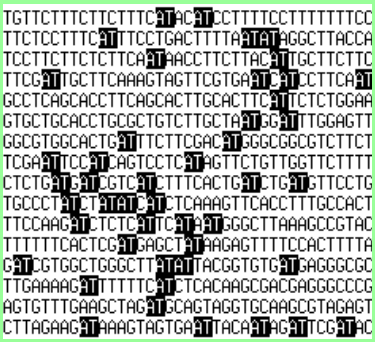
- ...
- **Algorithms: J.V. and Jüri Reimand**



Pattern Discovery

1. Choose the language (formalism) to represent the patterns
2. Choose the rating for patterns, to tell that one pattern is “better” than other
3. Design an algorithm that **finds the best patterns** from the pattern class, **fast.**

Patterns: AT



Patterns: WHAT ([AT][ACT]AT)

SPEXS - **S**equence **P**attern **EX**haustive **S**earch

Jaak Vilo, 1998

- **User-definable pattern language**: substrings, character groups, wildcards, flexible wildcards (c.f. **PROSITE**)
- Fast exhaustive search over pattern language
- "Lazy suffix tree construction"-like algorithm
- **Analyze multiple sets of sequences simultaneously**
- Restrict search to most frequent patterns only (in each set)
- **Report** most frequent patterns, patterns over- or underrepresented in selected subsets, or patterns significant by various statistical criteria, e.g. by binomial distribution

Regular patterns

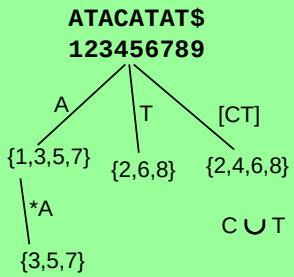
- Substrings ATCGA
- Add groups ATC[GC][AT]
- Add (unrestricted) wildcards AT*CG
- Add restricted wildcards AT*(2,5)CG
- Combine all above

AT[GC]*(1,3)[GT]AC

TGC……GCA

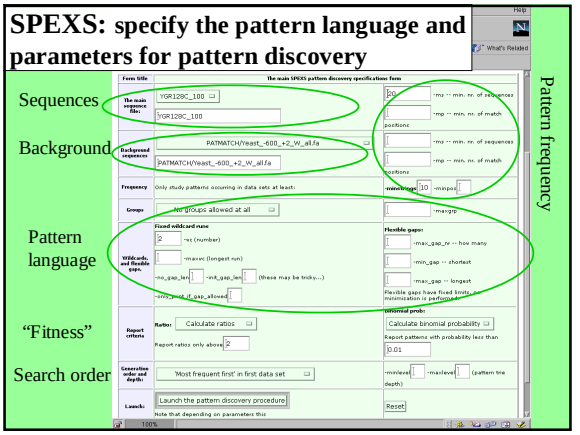
SPEXS: pattern discovery based on pattern trie.

- Substrings
- Group characters
- Wildcard positions
- Variable length wildcards
- Restrictions on the number on each separately
- **At least k occurrences**
- Exact occurrences locations for each pattern



Vilo 1998

SPEXS: specify the pattern language and parameters for pattern discovery



How to improve?

- **Simple vs complex patterns/profiles**
  - What is the best representation?
  - What is the best algorithmic approach
- Can we prove/disprove expression data clustering methods or distance measures by systematic promoter analysis?
- Lots of computations to perform…
- Tools for non-algorithm persons - how to maintain the simplicity vs desired results vs computational complexity

Implant k,d-patterns

(The Challenge Problem, P.Pevzner, 2000)

Length k=15

TGATTTCTTCGACAT

d=4, nr of changed characters

TGTTATCTTGGAGAT

TGAATTGTTCCACAC

Such motifs can differ in up to 8 positions out of 15!

```
TGTTCTTTCTTCTTTCATACATCCTTTTCCTTTTTTTCC
TTCTCCTTTCATTTCCTGACTTTTAATATAGGCTTACCA
TCCTTCTTCTCTTCAATAACCTTCTTACATTGCTTCTTC
TTCGATTGCTTCAAAGTAGTTCGTGAATCATCCTTCAAT
GCCTCAGCACCTTCAGCACTTGCACTTCATTCTCTGGAA
GTGCTGCACCTGCGCTGTCTTGCTAATGGATTTGGAGTT
GGCGTGGCACTGATTTCTTCGACATGGGCGGCGTCTTCT
TCGAATTCCATCAGTCCTCATAGTTCTGTTGGTTCTTTT
CTCTGATGATCGTCATCTTTCACTGATCTGATGTTCCTG
TGCCCTATCTATATCATCTCAAAGTTCACCTTTGCCACT
TTCCAAGATCTCTCATTCATAATGGGCTTAAAGCCGTAC
TTTTTTCACTCGATGAGCTATAAGAGTTTTCCACTTTTA
GATCGTGGCTGGGCTTATATTACGGTGTGATGAGGGCGC
```

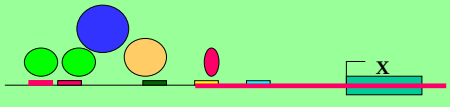
Approximate all against all

- Assume at least one perfect occurrence exists
- Only O(kn) different substrings of length k
- Match all of them approximately
- Find the one that has most significant nr of approximate occurrences
- Trie-index the sequences first, then search
- Algorithm: J.V. and **Hendrik Nigul**

Gene regulation is affected by

- DNA/RNA sequence
  - signals along that sequence
  - DNA structure and state
- State of the cell
  - i.e. all the other molecules and
- Environment

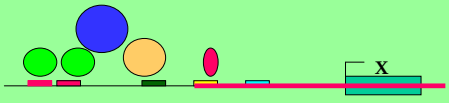
Binding sites: individually and in combination



Episode rules: A followed by (C D or D C)
Asko Tiidumaa

Conservation of distances between sites
Jelena Zaitseva

Goals:

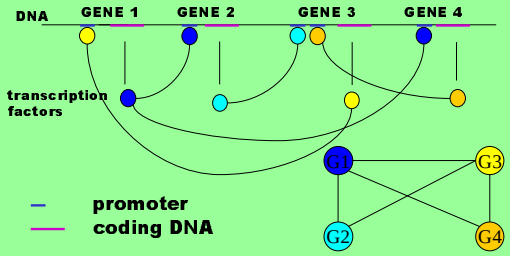


Given the sequence (signals) and gene expression levels of other genes: predict expression level of gene X

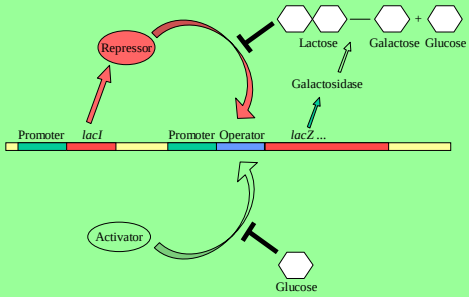
Given the chromosomal sequence predict locations of promoters and genes

Predict dependencies between all genes, and study gene regulation networks

Gene regulation by transcription factors

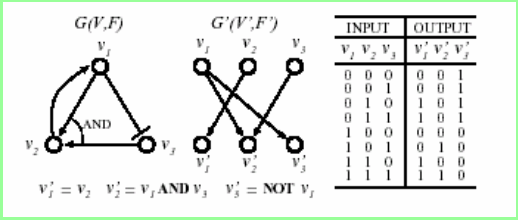


Lac-Operon



Thomas Schlitt

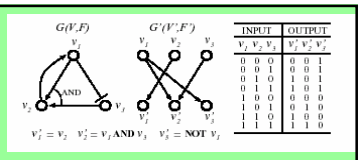
Boolean networks

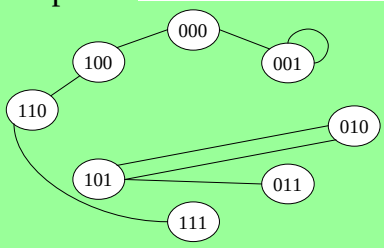


Synchronous Boolean networks - assumptions in gene network modelling

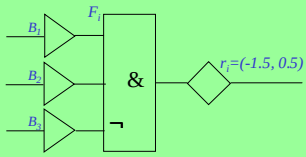
- Each gene the system (cell) can be in one of two states –
  - 'expressed' – 1,
  - 'not expressed' – 0
- The genes can switch from state to state all simultaneously in synchronous manner
- The next state of each gene is determined by previous states of all genes by Boolean functions describing the network

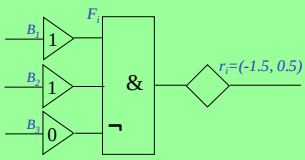
State space

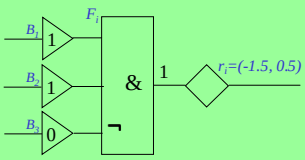


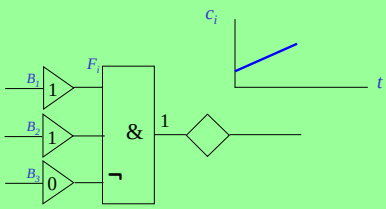


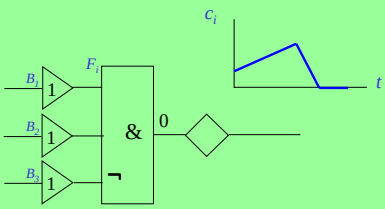
Finite state linear model





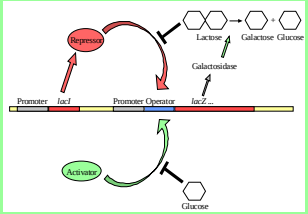


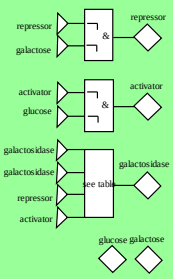




Lac-Operon

FSLM representation





Main related sub-projects

- Clustering – **Meelis Kull**
- Motif discovery – **Hendrik Nigul, Triinu Tasa**, **…**
- Site combinations: **Jelena Zaitseva, Asko Tiidumaa**
- Database of Gene Regulation - **Hedi Peterson, Eero Raudsepp, …**
- Annotate sets of genes based on quilt by association – **Jüri Reimand**
- Alternative Splicing – Meelis Kull

- Software development, visualization, GRID, Web Services, etc.