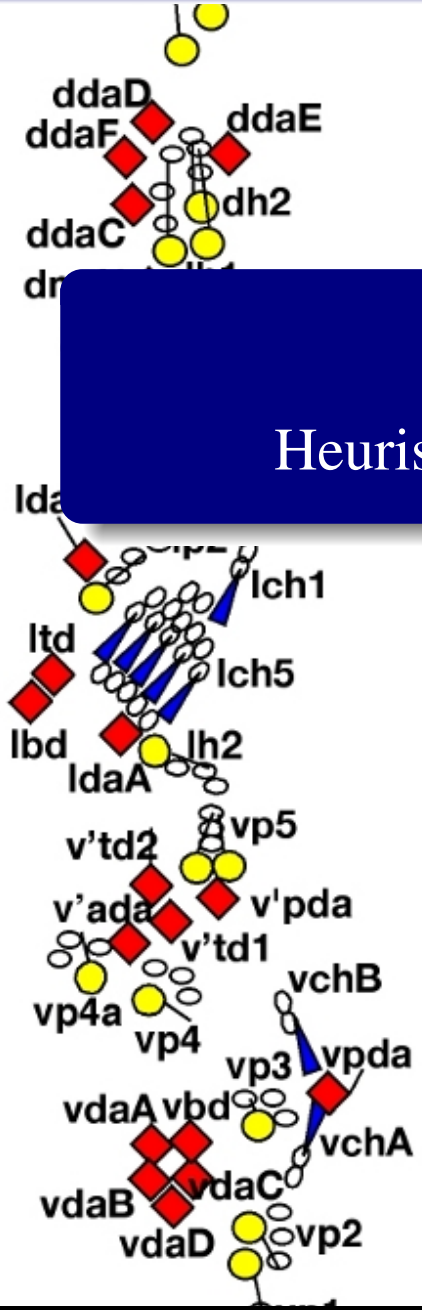

Bioinformatics 2 - Lecture 7

Heuristic methods, clustering and gene feature finding

Dr. Ian Simpson

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University of Edinburgh

Structure of the Lecture

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1. Heuristics
   - Introduction to heuristics
   - Heuristics and Computational Biology
   - FASTA (fast all) - the original heuristic
   - Summary
2. Clustering
   - Introduction to clustering in Biology
   - Clustering example - Drosophila PNS development
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   - Heuristics and Computational Biology
   - FASTA (fast all) - the original heuristic
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2. Clustering
   - Introduction to clustering in Biology
   - Clustering example - Drosophila PNS development
   - Summary
3. Gene feature finding
   - Primer on gene regulation
   - DNA sequence searching
   - Transcription factor binding site prediction
   - Summary

What is a Heuristic?

a heuristic is

"..a method for problem solving...often involving experimentation and trial and error.."

and a heuristic algorithm is

"a heuristic, is an algorithm that is able to produce an acceptable solution to a problem in many practical scenarios, but for which there is no formal proof of its correctness"

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- Allow us to incorporate knowledge about a problem or system to reduce the overall complexity of the task
- Can help to constrain search space and/or possible solution space to avoid erroneous solutions

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- do not perform well when the underlying sample is small or the problem is ill defined
- need to develop customised statistical models to go alongside the analysis to have confidence, normally randomisation based with it's associated sampling problems

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- First heuristic algorithms developed in sequence analysis used both heuristics and dynamic programming
  - FASTA - Lipman and Pearson 1985,1988
  - Clustal - Higgins et al. 1988
  - BLAST - Altschul et al. 1990
- Heuristics are now epidemic in Bioinformatics applied to
  - classic alignment and sequence search problems
  - cluster editing, partitioning problem solving
  - phylogenetic parsimony
  - motif detection
  - protein docking
  - protein structure resolution

FASTA - a heuristic sequence searching algorithm

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- can perform gapped local alignments
- performs optimized searches for local alignment using substitution matrices (identity for DNA, BLOSUM/PAM for protein)
- slower than BLAST, but more sensitive for nucleotides and particularly good for repetitive sequence

FASTA - a heuristic sequence searching algorithm

- Variables
  - ktup: word-length (similar to BLAST)
    1-2 for proteins, 4-6 for nucleotides
  - gap opening penalties : -12 (protein) and -16 (DNA)
  - gap extension penalties : -2 (protein) and -4 (DNA)

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  - gap opening penalties : -12 (protein) and -16 (DNA)
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- Statistics
  - Z-scores : calculated normalised by sequence length
  - E (expectation) scores : number of sequences expect with same score by chance

The main steps of the FASTA algorithm

- Step One
  - Find exact matches of word size between query and target, record in a hash/lookup table
  - hash/lookup can be pre-computed for different searches k=1 (oligo 20nt), k=6 (normal 100-500nt)

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- Step Two
  - cluster the 'hot-spots' into diagonals by making a matrix of 1s and 0s by position
  - score all of the diagonals with each region + and each gap -
  - find the 10 best diagonals and then perform a local alignment with no indels
  - the best partial alignment is called init1 and is used in Step Four

The main steps of the FASTA algorithm

- Step Three
  - going back to the 10 partial alignments, the algorithm now takes any that exceed a certain score cut-off and tries to make them into longer alignment runs
  - if a longer partial can be made (and this is a graph theoretic problem) it is optimally aligned and returned as one result from the algorithm

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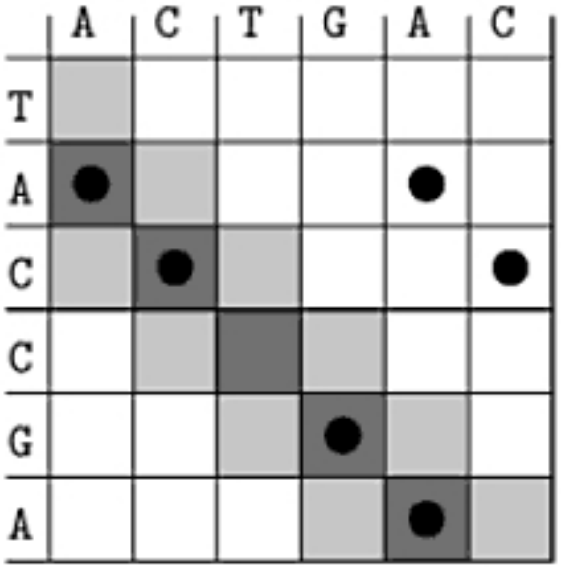
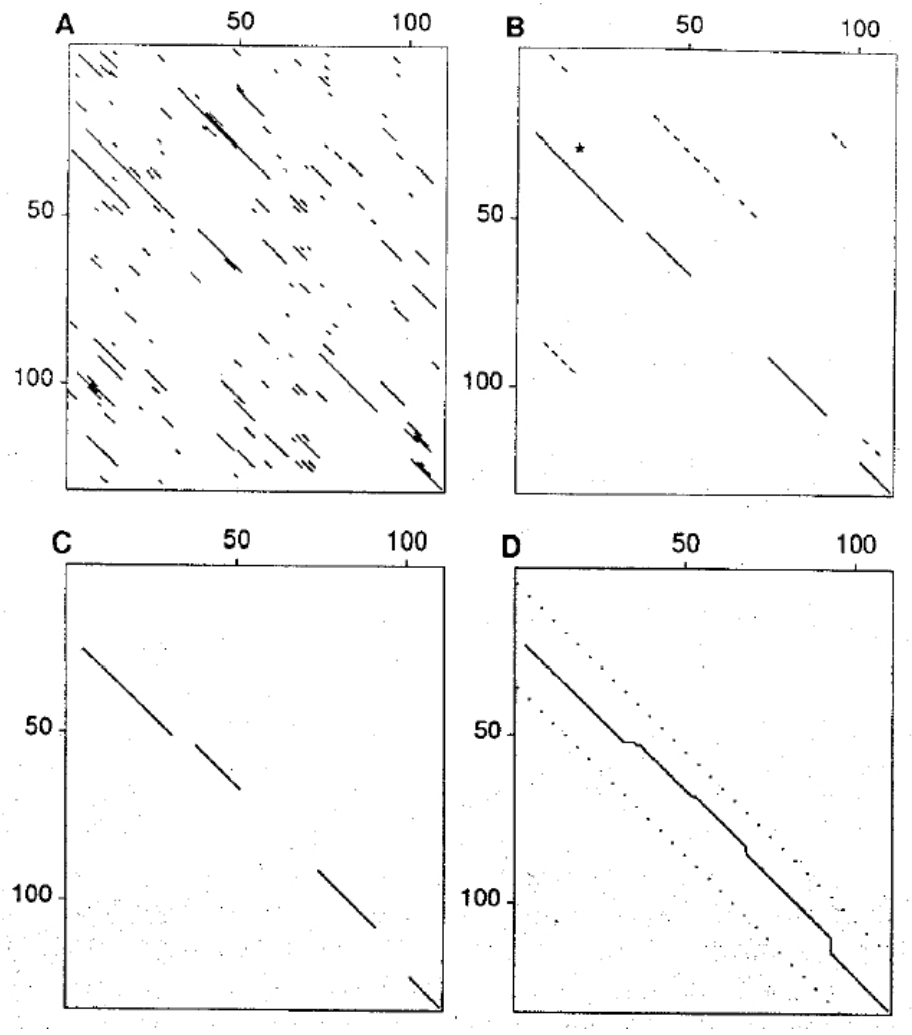
- Step Four
  - picking up the init1 partial alignment from Step Two the algorithm performs a banded Smith Waterman
  - a window of alignment space either side of the init1 diagonal is identified and optimal local alignments are performed throughout the space
  - the alignments are scored by matrix and statistics are calculated, normalised Z-score and E value

Schematic of the Fasta matrix process





Example FASTA result

```
>>UNIPROT:FOSB_MOUSE P13346 Protein fosB.                    (338 aa)
 initn: 2268 init1: 2268 opt: 2268  Z-score: 1866.3  bits: 353.7 E(): 3.3e-96
Smith-Waterman score: 2268;  100.000% identity (100.000% ungapped) in 338 aa overlap (1-338:1-338)

               10        20        30        40        50        60
FOSB_M MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVPTVTA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
UNIPRO MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVPTVTA
               10        20        30        40        50        60

               70        80        90       100       110       120
FOSB_M ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYSTGGASGS
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
UNIPRO ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYSTGGASGS
               70        80        90       100       110       120

              130       140       150       160       170       180
FOSB_M GGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRRRELT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
UNIPRO GGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRRRELT
              130       140       150       160       170       180

              190       200       210       220       230       240
FOSB_M DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPLAEVRD
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
UNIPRO DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPLAEVRD
              190       200       210       220       230       240
```

Example FASTA histogram output

```
       opt      E()
< 20  1040     0:=
  22     0     0:           one = represents 1534 library sequences
  24     4     1:*
  26    14    19:*
  28    53   201:*
  30   227  1223:*
  32  1161  4728:=  *
  34  5065 12823:====    *
  36 16019 26336:==========      *
  38 33416 43523:======================      *
  40 58656 60711:=======================================*
  42 81562 74211:================================================*=====
  44 87125 81862:=======================================================*===
  46 92000 83378:=======================================================*=====
  48 83023 79825:=====================================================*==
  50 83389 72841:=================================================*=======
  52 68683 64039:=========================================*===
  54 58489 54701:===================================*===
  56 48841 45692:=============================*==
  58 36330 37512:======================*
  60 26755 30387:================= *
  62 21306 24361:============== *
  64 17453 19374:============*
  66 13701 15313:=========*
  68 10436 12045:=======*
  70  8222  9439:======*
  72  6047  7376:====*
```

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- Heuristics are used widely in computational biology especially in studies using genome scale data, proteomics, transcriptomics, phylgenetics etc..

Finding groups in data

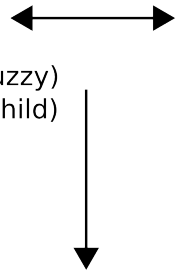
- finding trends and groupings within high order complex datasets is fundamental to many computational biology projects
  - Proteomics - protein-protein interaction data
  - Functional annotation clustering - grouping genes by function
  - Transcriptomics - grouping genes by expression profile by condition
- in large datsets the ditinction between groupings can be obtuse and many parallel methods are often used to try to validate the clustering results
  - protein-protein interactions tend to be multplicitous and single group membership may not be appropriate
  - functional annotation clustering is constrained by ontologies and provides a unique, and unsolved? problem
  - gene expression data - expression on a continuous scale with high noise, often need to pre-transform data to reduce dimensionality and/or exacerbate distinctions between groups

Classic clustering methodologies

- Slides adapted from to Dr. Dirk Husmeier, BioSS Scotland
- Would take a long time to do this in Beamer.....

MAGIC - multi algorithmic grouping with integrity checking

multi-algorithm clustering
agnes - heirarchical (agg)
PAM - partional(medoid)
fuzzy-c - partial membership (fuzzy)
hpach - combnination (parent-child)

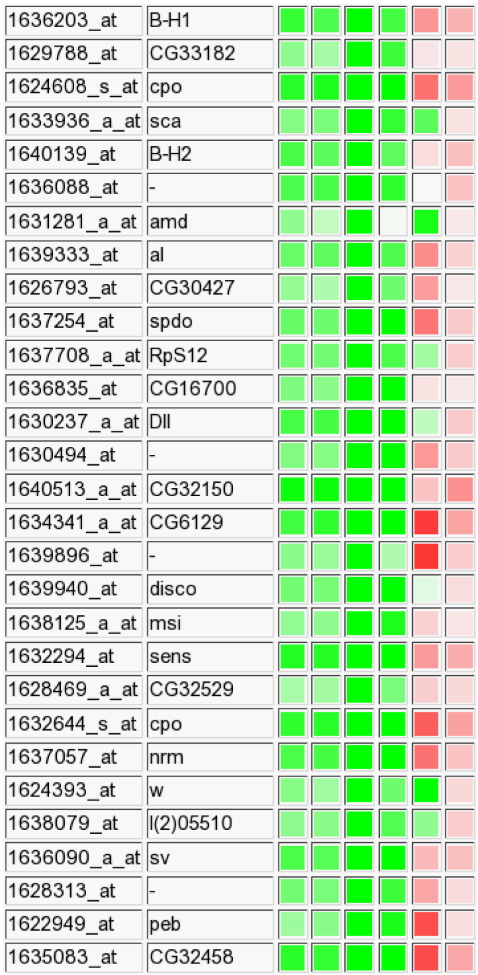


parameter optimisation
average silhouette width
median split silhouette
elbow-plot

experimental cluster sets

cluster comparison
set-operation cluster list comparison
matrix of unit sets
expands into dimensions (robustness)
conservation scoring by membership
cluster ranking
bootstrap resampling - confidence intervals

ranked conserved cluster sets



hopach5 - bristle morphogenesis

MAGIC - finishing off the pipeline with a statistical analysis

- Determine the statistical significance of the scores for each cluster - bootstrap confidence estimation
  - generate many random cluster sets with the same pool of members and the same structure for each cluster and each clustering experiment
  - score each of the random sets to build up a distribution that estimates the true distribution of scores
  - fit a probability density function to the bootstrap distribution
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- rank clusters by score with an associated p-value that is a measure of how far away the cluster membership is than randomly populated clusters of the same structure

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  - heirarchical, partitioning, fuzzy, combinatorial

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- Many parameter optimisation methods have been developed
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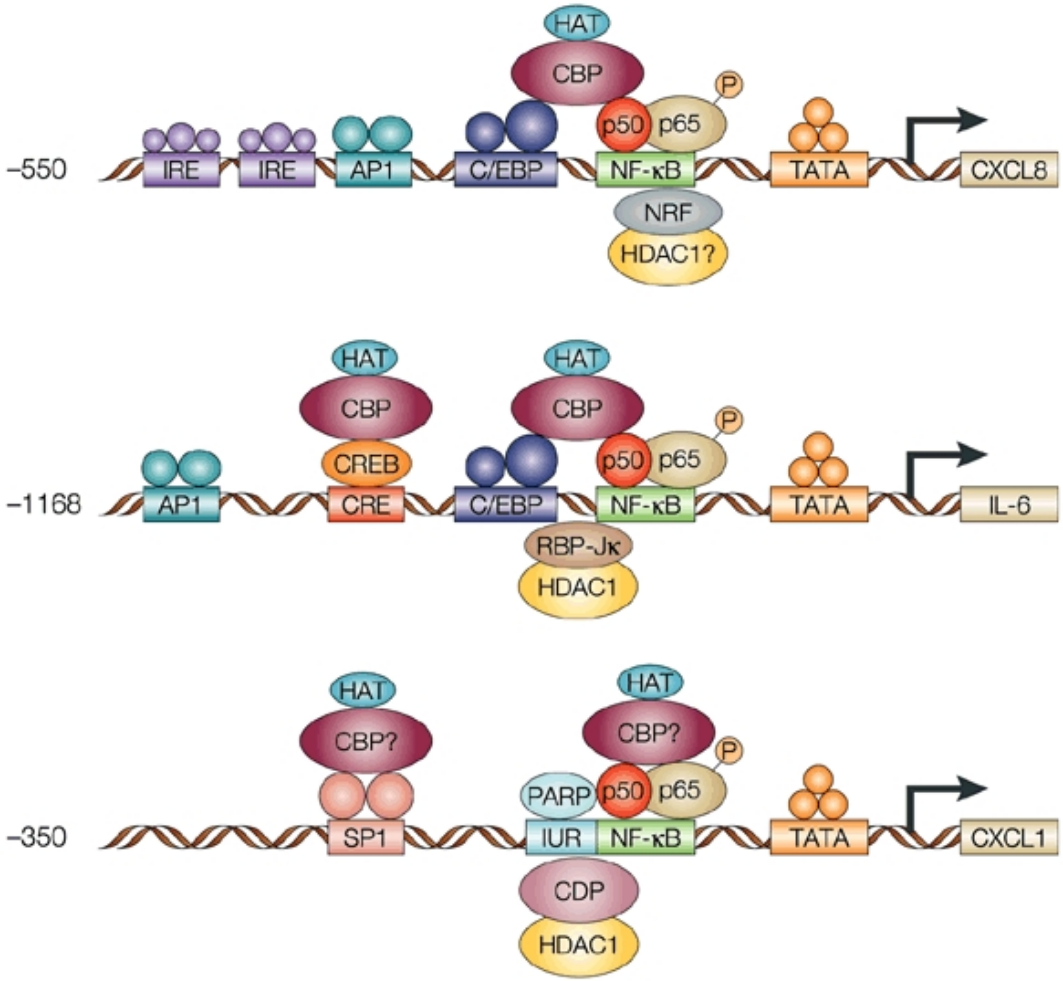
- Now integrative pipelines are being developed to cross-compare results from clustering using a whole range of algorithms, variables and measures - so called consensus clustering

Anatomy of a promoter/enhancer



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Promoter/enhancer features

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- promoters are close to the transcriptional start of the gene
- enhancers can be very far away from the gene
- TFBS sites are used in complex combinations to modulate the time, location and level of expression of genes
- TFBS sites are generally small 6-8nt and are also degenerate (more than one sequence can perform the same or similar task)

Examples of TFBS binding sites

Atonal E-box

Scute E-box

Problems finding TFBS sites in promoters and enhancers

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- reg exps produce binary results (0,1), but searches with PWMs produce continuous scores and probabilities, i.e. uncertainty
- need ways to reduce complexity and or search space

Regular expressions

- a simple consensus is essentially a regular expression such as for example CANNTG, the E-box consensus
  - possibilities are CAAATG, CAATTG...etc
  - you could express this as a regular expression CA[ACTG]{2}TG and search for matches
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  - the result is a hit sequence and a location, it's binary
- Problems with regular expressions
  - assume that all possible permutations are equal
  - in order to be informative you have to exclude what could be informative, but low frequency, sequences from the consensus (so that you don't have an E-box of [ACTG]{6} for example !
  - there are currently two main solutions to this problem, position weight matrices and hidden markov model profiles

Weight matrices, PWMs

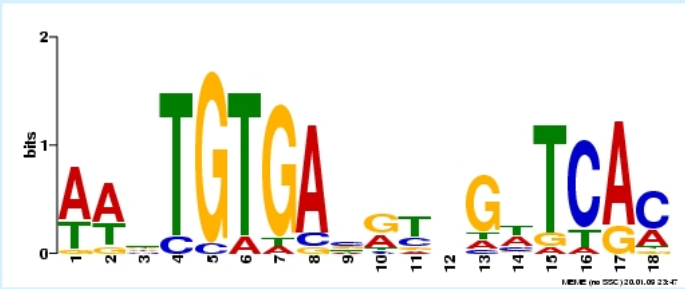
	A	C	T	G
1	101	-1081	-182	13
2	87	-1081	-82	13
3	-45	-23	18	35
4	-1081	-82	-1081	155
5	-1081	-182	227	-1081
6	-145	-1081	-1081	155
7	-245	-1081	218	-245
8	145	-82	-182	-1081

SEQUENCE LOGO

Information Content
14.7 (bits)

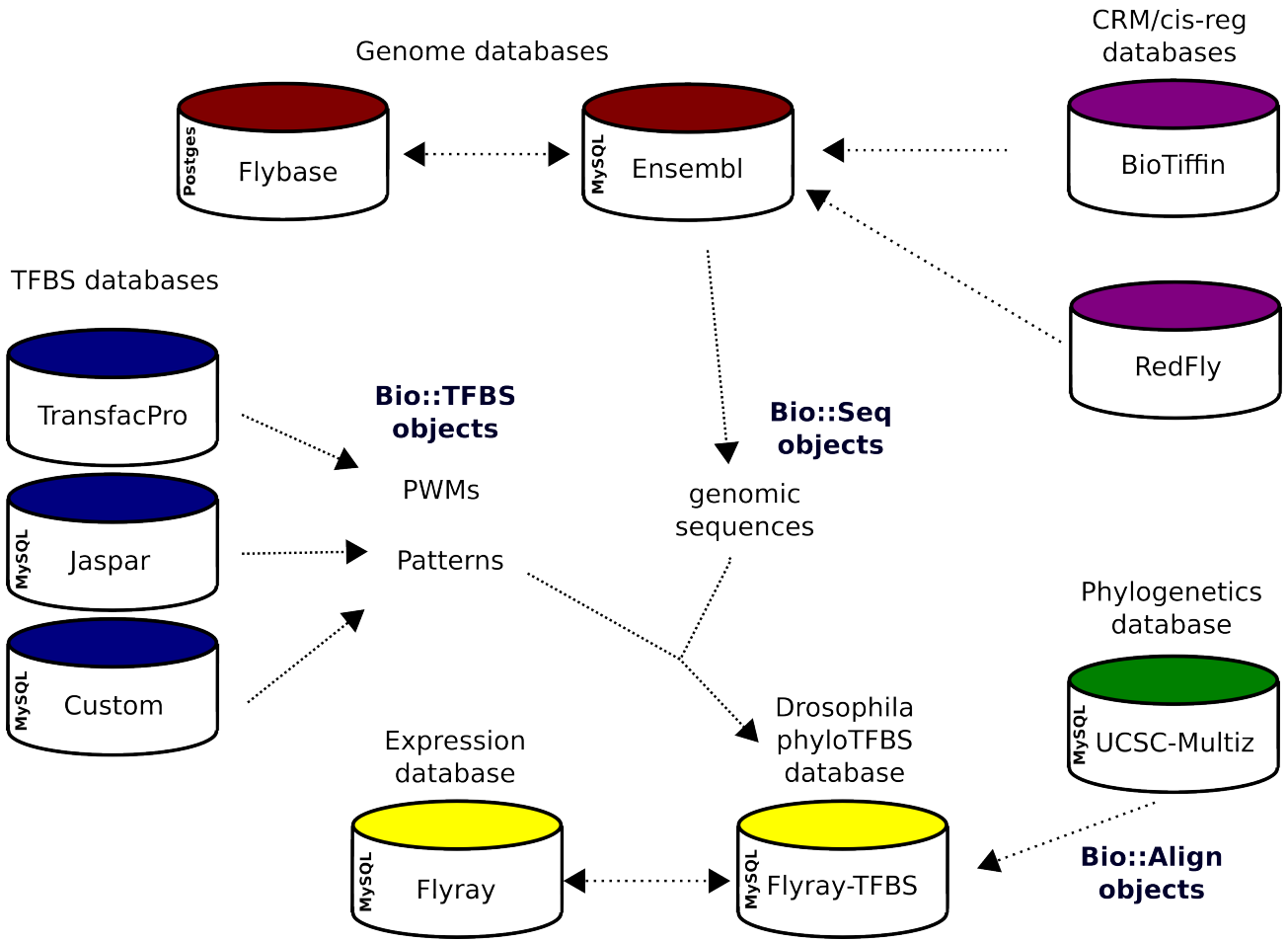
Relative Entropy
14.4 (bits)

Download LOGO
Without SSC:[EPS][PNG]
With SSC:[EPS][PNG]



NAME	STRAND	START	P-VALUE	SITES
ara	-	58	2.51e-07	TGGCATAGCA AAGTGTGACGCCGTGCAA ATAATCAATG
lac	+	8	5.35e-07	AACGCAAT TAATGTGAGTTAGCTCAC TCATTAGGCA
malt	+	40	8.61e-07	AAAGATTTGG AATTGTGACACAGTGCAA ATTCAGACAC
ilv	-	42	1.69e-06	GCAAAGGGAA AATTGAGGGGTTGATCAC GTTTTGTACT
pbr322	-	56	2.85e-06	CTCCTTACGC ATCTGTGCGGTATTTCAC ACCGCATATG
deop2	+	59	2.85e-06	AGATTTCCTT AATTGTGATGTGTATCGA AGTGTGTTGC
uxu1	+	16	5.17e-06	AGAGTGAAAT TGTTGTGATGTGGTTAAC CCAATTAGAA
trn9cat	+	83	5.69e-06	CTTTTGGCGA AAATGAGACGTTGATCGG CACG
ce1cg	-	64	7.54e-06	GGACTTCCAT TTTTGTGAAAACGATCAA AAAAACAGTC
ompa	+	47	9.04e-06	TTTTTTTCAT ATGCCTGACGGAGTTCAC ACTTGTAAGT

Apply a PWMSearch but with databases



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  - Total of 20 percent of the Drosophila melanogaster genome screened producing $3.5x10^6$ hits

Calculating TFBS site conservation, phylogenetics

- UCSC 15-way Multiz alignments - phylogenetic analysis
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  - retrieved all TFBS site hit data from screen and scored every site to every pairwise alignment

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  - Calculate on a per site basis (i.e. randomise per site not for all sites) some sites will be more informative than others, drop the uninformative ones

Rfx, the X-box and ciliated sensory neuron development

- Ciliated sensory neurons
  - Most sensory neurons have cilia at their dendritic tips
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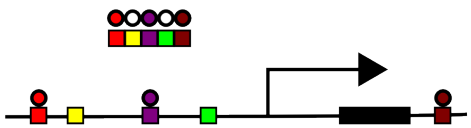
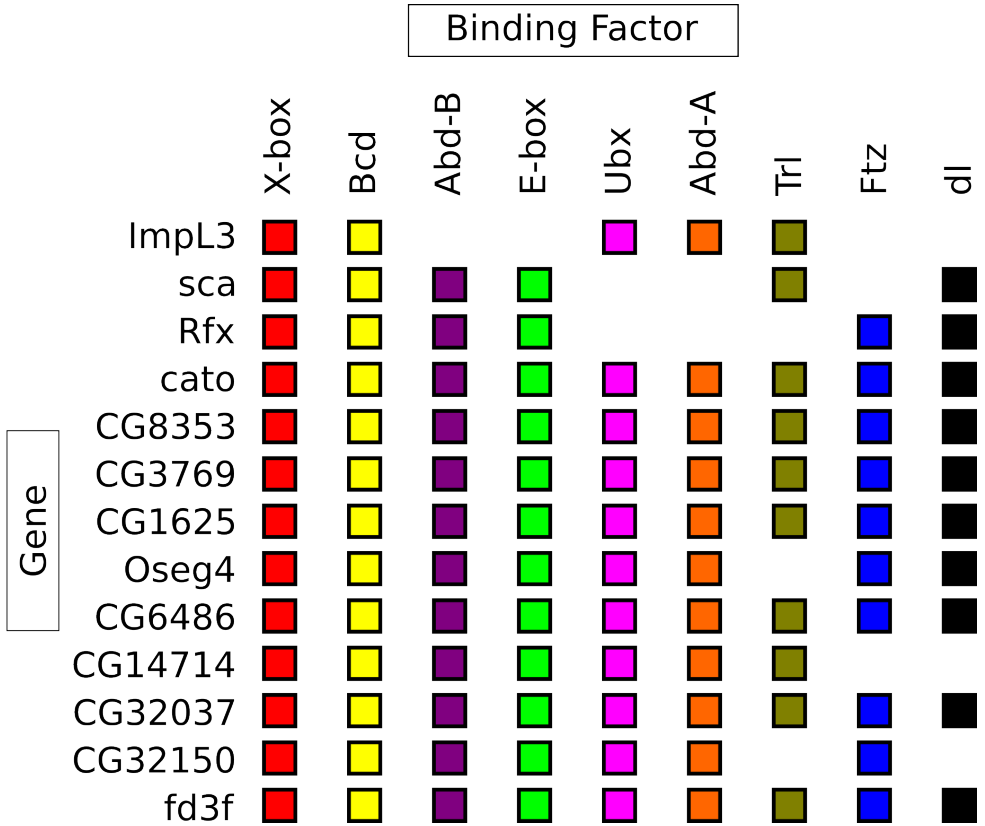
- The X-box, comparative genetics and the ciliome
  - Rfx proteins bind to the X-box RYYNYYN[1-3]RRNRAC is bound by Rfx proteins
  - Genome screens for conserved X-boxes have recently been used to identify novel targets of Rfx proteins in Drosophila (Laurencon et al. Genome Biology(2007)**8**,R195)
  - Compared D.mel and D.pse common ancestor 40-60 mya
  - intron sequences 40% identical, known binding sites from the literature mapped on are 63% identical

cis-regulatory modules (CRMs) an entry point for network assembly



Sites >=75% identical between D.mel and D.Pse that for genes that also contain an X-box (13/27) from the sensory cilium biogenesis cluster.



- based on 75% conservation there are 7823 X-boxes in the fly genome (0.5/gene) so we expect 13 in list of 27
- sensory cluster has 50 conserved X-boxes an enrichment of x3.8

Gene feature finding summary

- heuristics, Gibbs samplers, dynamic programming, Markov chains and randomisation/bootstrap methods are commonly integrated into pipelines to study a series of connected processes from beginnning of analysis to the end

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- heuristics, Gibbs samplers, dynamic programming, Markov chains and randomisation/bootstrap methods are commonly integrated into pipelines to study a series of connected processes from beginnning of analysis to the end
- here we have looked at a specific (and unsolved) instance of TFBS searching (and prediction)
- these methods are rapidly evolving in all areas, most are useable on a standard workstation and most have programmatic access through BioJava, BioPerl and of course C