Downstream analysis of ChIP-seq data

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ChIP-seq workflow overview II

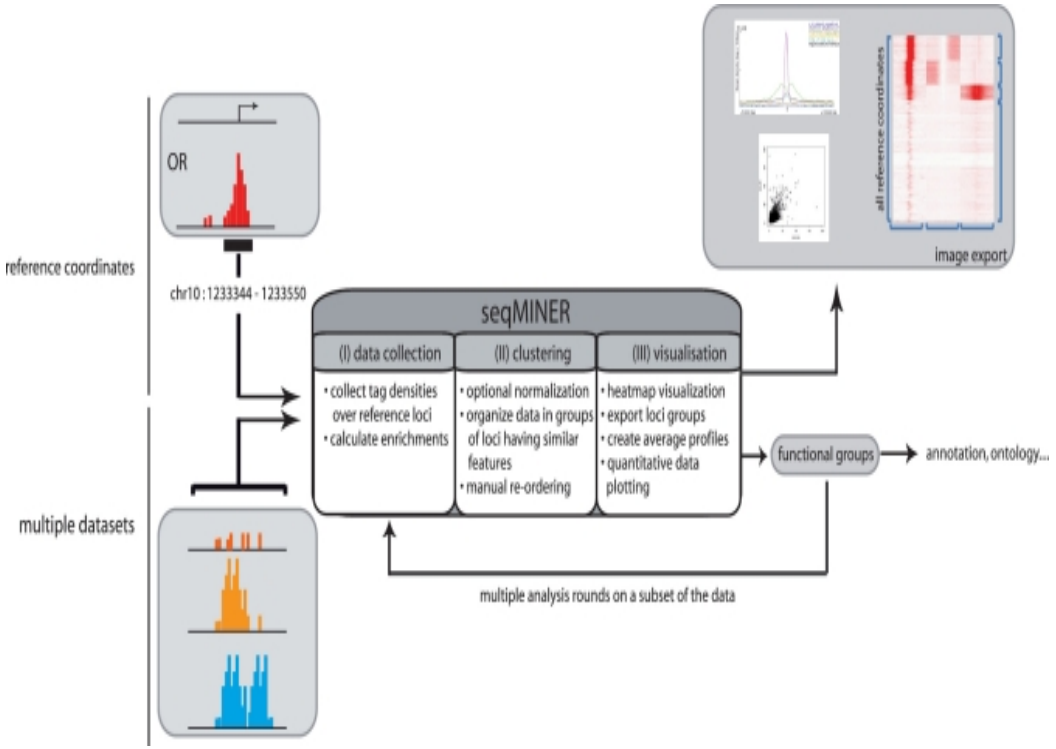
Downstream analysis for extracting meaningful biology :

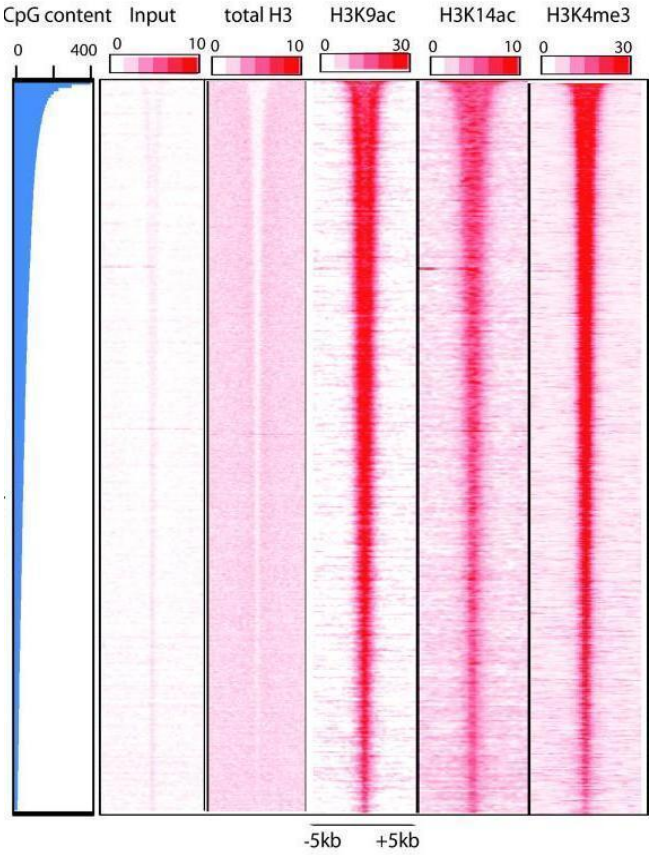
- Annotation of genomic features to peaks
- Binding site distributions
- Motif identification and Motif Enrichment Analysis
- Feature overlap analysis
- Functional enrichment analysis: Ontologies, Gene Sets, Pathways
- Differential binding analysis
- Integration with transcriptomic data to Identify direct targets
- Network Biology applications

seqMINER

- Enables qualitative comparisons between a reference set of genomic positions and multiple ChIP-seq data-sets.
- Useful for comparing and visualizing replicates or conditions.

Ye *et al*., 2011 Nucleic Acids Res. PMID: 21177645





Peak annotation 1

- **ChIPpeakAnno (BioC)** (Zhu *et al.*, 2010, BMC Bioinformatics)
- map peaks to nearest feature (TSS, gene, exon, miRNA or custom features)
  - extract peak sequences
  - find peaks with bidirectional promoters
  - obtain enriched gene ontology
  - map different annotation and gene identifiers to peaks
- can use **biomaRt** package to get annotation.
- **IRanges, GenomicFeatures, GO.db, BSgenomes, multtest**
- converts BED and GFF data formats to RangedData before calling peak annotate function.

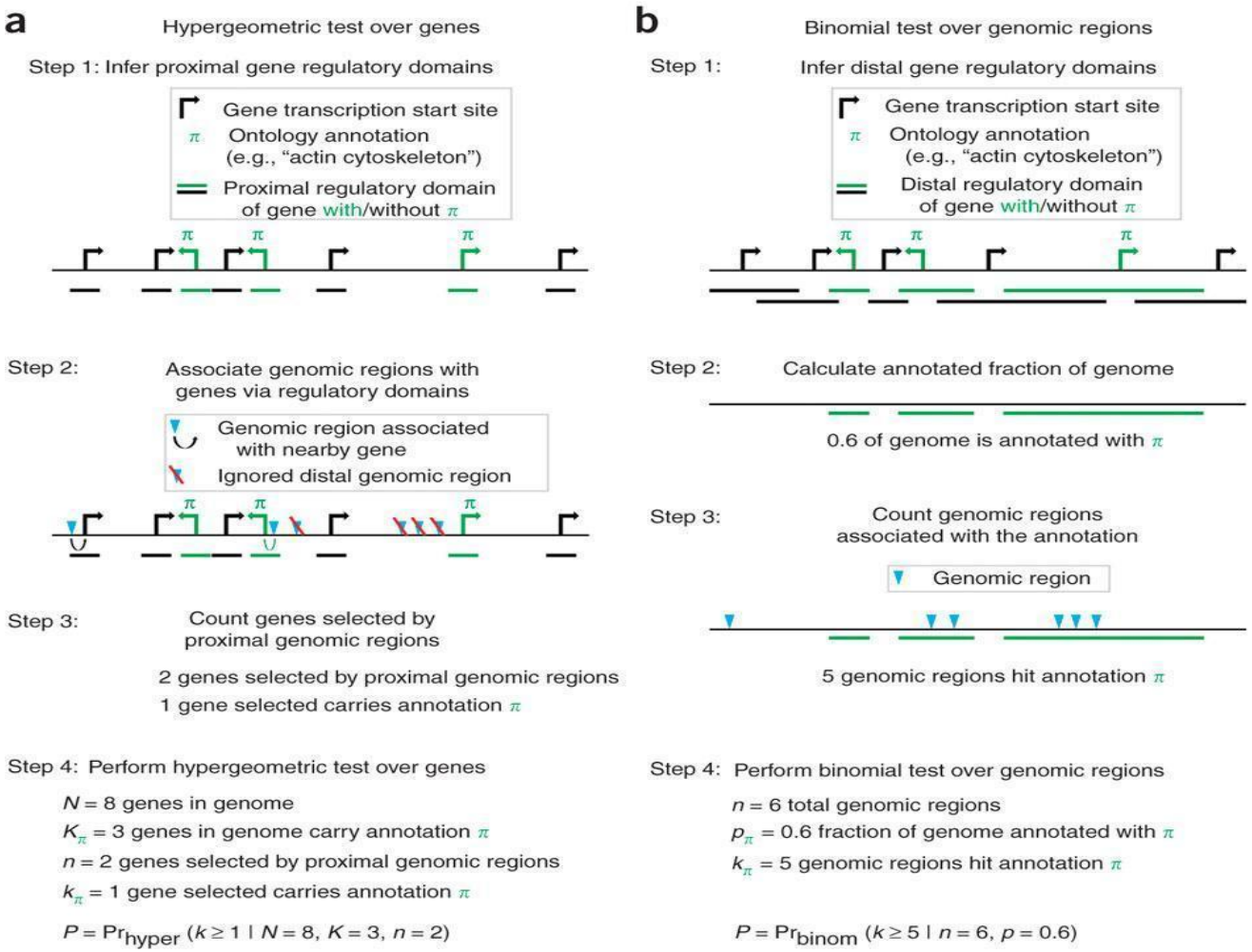
Peak annotation 2

PeakAnalyzer (Salmon-divon *et al.*, 2010, BMC Bioinformatics)

- A set of high-performance utilities for the automated processing of experimentally-derived peak regions and annotation of genomic loci.
- Consists of PeakSplitter and PeakAnnotator.
- Biologist' friendly tool.
- Get latest genome annotation files from Ensembl (gtf format) or UCSC (BED format).
- Map to either nearest downstream gene, TSS or user defined annotation.
- Determine overlap between peak sets.
- Split peaks to sub-peaks. May be useful for *de novo* motif analysis.

Functional Enrichment Analysis

GREAT & **rGREAT:** Genomic Regions Enrichment of Annotations Tool



McLean C.Y. et al., "GREAT improves functional interpretation of *cis*-regulatory regions". *Nat.Biotechnol.* **28**(5):495-501, 2010.

Motif detection

Don't scan a sequence with a motif and expect all sites identified to be biologically active. Random matches will swamp the biologically relevant matches! This is a well known problem in motif searching, amusingly called the **"Futility Theorem"** of motif finding. -Wasserman WW, Sandelin A. Nat Rev Genet 2004;5:276-87.

1. PWM based sequence scanning or word search methods. These methods uses prior information about TF binding sites and therefore can only be used to detect known Transcription Factor Binding Sites (TFBS).

2. *De novo* motif identification –Pattern discovery methods:

- **Word based** – occurrence of each 'word' of nucleotides of a certain length is counted and compared to a background distribution.
- **Probabilistic**- seek the most over-represented pattern using algorithmic approaches like Gibbs sampling and Expectation maximization. These iteratively evolve an initial random pattern until a more specific one is found.

Use *de novo* motif calling and alignment to build your own PWMs! Biostrings & Motiv packages have PFM to PWM conversion methods.

BioConductor motif analysis packages

- rGADEM -motif discovery
- MotifRG -motif discovery
- MotIV -map motif to known TFBS, visualize logos
- motifStack -plot sequence logos
- MotifDb -motif database
- PWMenrich -motif enrichment analysis
- TFBSTools – R interface to the JASPAR database

Position Weight Matrices

a

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Site 1	G	A	C	C	A	A	A	T	A	A	G	G	C	A
Site 2	G	A	C	C	A	A	A	T	A	A	G	G	C	A
Site 3	T	G	A	C	T	A	T	A	A	A	A	G	G	A
Site 4	T	G	A	C	T	A	T	A	A	A	A	G	G	A
Site 5	T	G	C	C	A	A	A	A	G	T	G	G	T	C
Site 6	C	A	A	C	T	A	T	C	T	T	G	G	G	C
Site 7	C	A	A	C	T	A	T	C	T	T	G	G	G	C
Site 8	C	T	C	C	T	T	A	C	A	T	G	G	G	C

Source binding sites

b

B	R	M	C	W	A	W	H	R	W	G	G	B	M

Consensus sequence

c Position frequency matrix (PFM)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	4	8	0	0	0	3	0	0	0	0	2	4
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	4	2	2	4	0	0	1	0

d Position weight matrix (PWM)

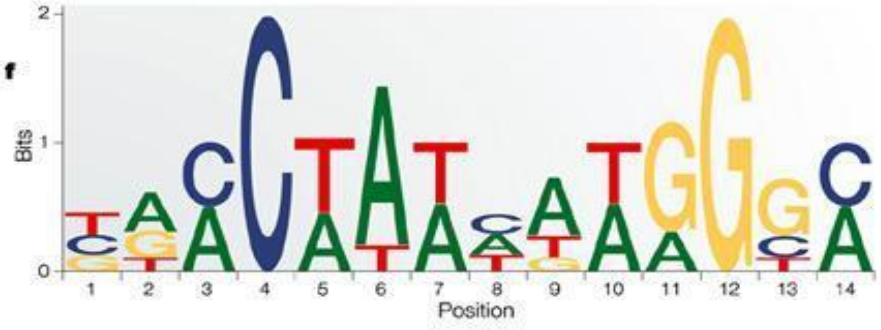
A	−1.93	0.79	0.79	−1.93	0.45	1.50	0.79	0.45	1.07	0.79	0.00	−1.93	−1.93	0.79
C	0.45	−1.93	0.79	1.68	−1.93	−1.93	−1.93	0.45	−1.93	−1.93	−1.93	−1.93	0.00	0.79
G	0.00	0.45	−1.93	−1.93	−1.93	−1.93	−1.93	−1.93	0.66	−1.93	1.30	1.68	1.07	−1.93
T	0.15	0.66	−1.93	−1.93	1.07	0.66	0.79	0.00	0.00	0.79	−1.93	−1.93	−0.66	−1.93

e Site scoring

0.45	−0.66	0.79	1.68	0.45	−0.66	0.79	0.45	−0.66	0.79	0.00	1.68	−0.66	0.79
T	T	A	C	A	T	A	A	G	T	A	G	T	C

Σ = 5.23, 78% of maximum

f



Nature Reviews | Genetics

PWM conversion:

$$W_{b,i} = \log_2 \frac{p(b,i)}{p(b)}$$

TFBS PWM/PFM sources

TRANSFAC public	Matys et al., 2006	Multiple species	v7.0 2005, Not been updated for a while!
TRANSFAC professional	Matys et al., 2006	Multiple species	v2015
JASPAR 2014	Mathelier et al., 2014	Multiple species	(656)
ORegAnno		Multiple species	Curated collection from different sources.
hPDI	Xie et al., 2010	Human	(437)
SwissRegulon	Pachkov et al., 2010	mammalian	(190)
HOMER	Heinz et al., 2010	Human	(1865)
UniPROBE	Newburger & Bulyk, 2009	Multiple species	
Dimers	Jonawski et al., 2013	Human	(603) predicted dimers
FactorBook	Wang et al., 2012	Human	(79) ENCODE ChIP-seq motifs
SCPD, YetFasco		Yeast	
Elemento, Redfly FlyFactorSurvey,Tiffin		Drosophila	

Motif Enrichment Analysis

MEA identifies over- and under-represented known motifs in a set of genes.

The regulatory proteins whose DNA binding motifs are enriched in a set of regulatory sequences are candidate transcription regulators of that gene set.

Identifying co-regulated gene sets is difficult. Use Ontologies, pathways, GSEA etc.

Picking the right background model will determine the success of the motif enrichment analysis:

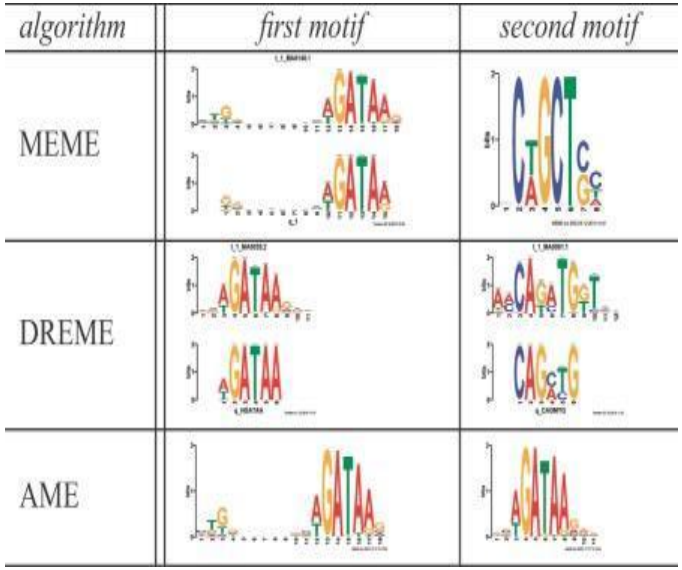
- All core-promoters from protein coding or non-coding genes etc,
- Higher order Markov model based backgrounds,
- A sequence set similar in nucleotide composition, length and number to the test set,
- Open chromatin regions,
- A shuffled test sequence set.

MEME-Chip

- http://meme.nbcr.net

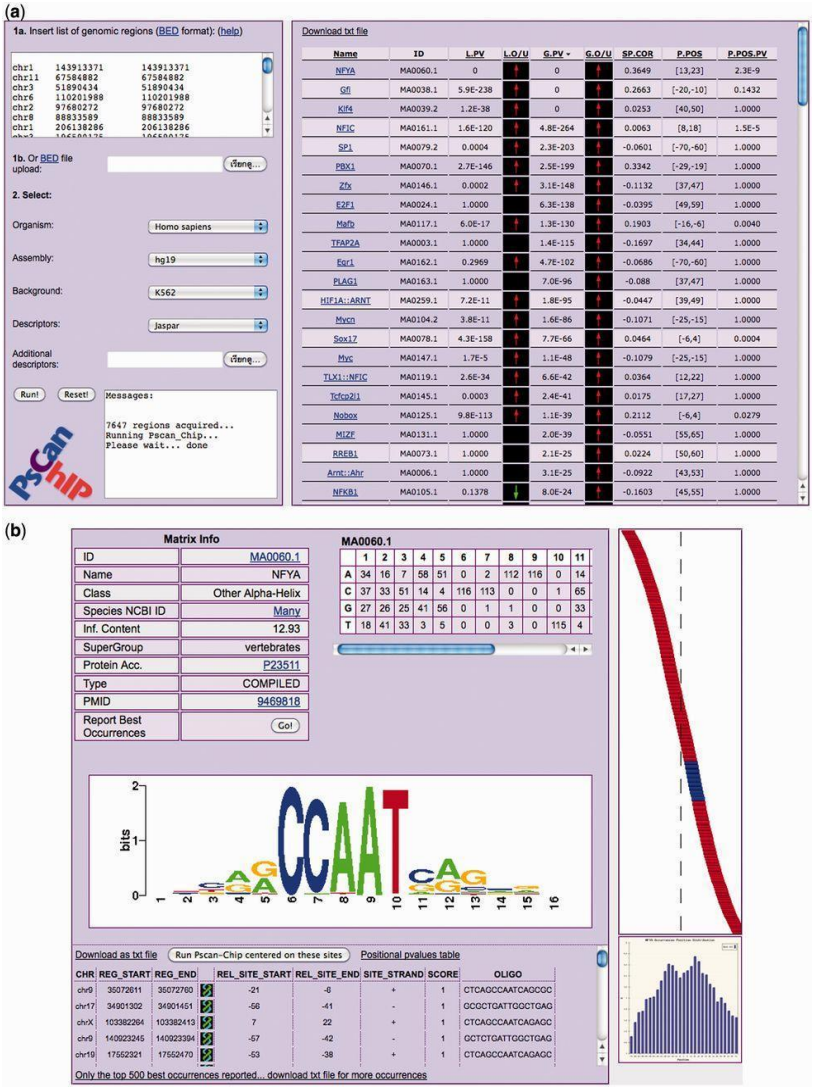
"MEME-ChIP: motif analysis of large DNA datasets." Machanick and Bailey, 2011 Bioinformatics

- Given a set of genomic regions, it performs
  - *ab initio* motif discovery -novel TF binding sites (MEME, DREME)
  - motif enrichment analysis -known TF enrichment (Centrimo/AME)
  - motif visualization (MAST and AMA)
  - binding affinity analysis
  - motif identification -compare to known motifs (TOMTOM)
- Uses two algorithms for motif discovery:
- MEME -expectation maximization (EM) to discover probabilistic models of DNA-binding by single TFs or TF complexes.
- DREME -simpler, non-probabilistic model (regular expressions) to describe the short binding motifs.



Pscan-Chip

- http://159.149.160.51/pscan_chip_dev/
- Motif enrichment analysis using PWM databases and user defined background models.
- Optimized for ChIP-seq.
- Ranked lists of enriched motifs.
- Sequence logo's and motif enrichment distribution plots.



"PscanChIP: Finding over-represented transcription factor-binding site motifs and their correlations in sequences from ChIP-Seq experiments." Zambelli *et al.*, 2013 *Nucleic Acids Res.*

HOMER (Hypergeometric Optimization of Motif EnRichment) v4

- http://homer.salk.edu/homer/index.html
- Large number of (Perl & C++) tools for ChIP-seq analysis.
- Provides both *de novo* and PWM scanning based motif identification and enrichment analysis.
- User can specify custom background. (Randomly selected, GC or CGI matched backgrounds.)
- Uses a collection of ChIP-seq derived PWMs or user can specify PWM.
- Peak annotation, GO enrichment analysis, Extract peak sequences, Visualization.

Meta-Motif Analyzers

http://131.174.198.125/bioinfo/gimmemotifs/

GimmeMotifs: a *de novo* motif prediction pipeline, especially suited for ChIP-seq datasets. It incorporates several existing motif prediction algorithms in an ensemble method to predict motifs and clusters these motifs using the weighted information content (WIC) similarity scoring metric.

BioProspector http://motif.stanford.edu/distributions/bioprospector/

GADEM http://www.niehs.nih.gov/research/resources/software/gadem/index.cfm

Improbizer http://users.soe.ucsc.edu/~kent/

MDmodule (included in the MotifRegressor Package) http://www.math.umass.edu/~conlon/mr.html

MEME http://meme.sdsc.edu/

MoAn http://moan.binf.ku.dk/

MotifSampler http://homes.esat.kuleuven.be/~sistawww/bioi/thijs/download.html

Trawler http://ani.embl.de/trawler/

Weeder http://159.149.160.51/modtools/

Network Biology: Integrating TF binding with transcriptomic data

- Not all TF binding sites are transcriptionally active. The collection of transcriptionally active targets of a TF is its regulome.
- Regulomes can be used to "explain" the phenotype under consideration and understand aspects of biological systems.
- Regulomes in combination with pathway and network modelling approaches can then be used decipher the networks underlying phenotypes.
- These networks provide information on connectivity, information flow, and regulatory, signaling and other interactions between cellular components.
- **BioNet, GeneNetworkBuilder**

Rcade

R-based analysis of ChIP-seq And Differential Expression

- Rcade is a Bioconductor package developed by **Cairns *et al.***, that utilizes **Bayesian** methods to integrates ChIP-seq TF bindng, with a transcriptomic Differential Expression (DE) analysis.
- The method is read-based and independent of peak-calling, thus avoids problems associated with peak-calling methods.
- A key application of Rcade is in inferring the direct targets of a transcription factor (TF).
- These targets should exhibit TF binding activity, and their expression levels should change in response to a perturbation of the TF.

$$P(A \mid B) = \frac{P(B \mid A)P(A)}{P(B)}$$

Rcade

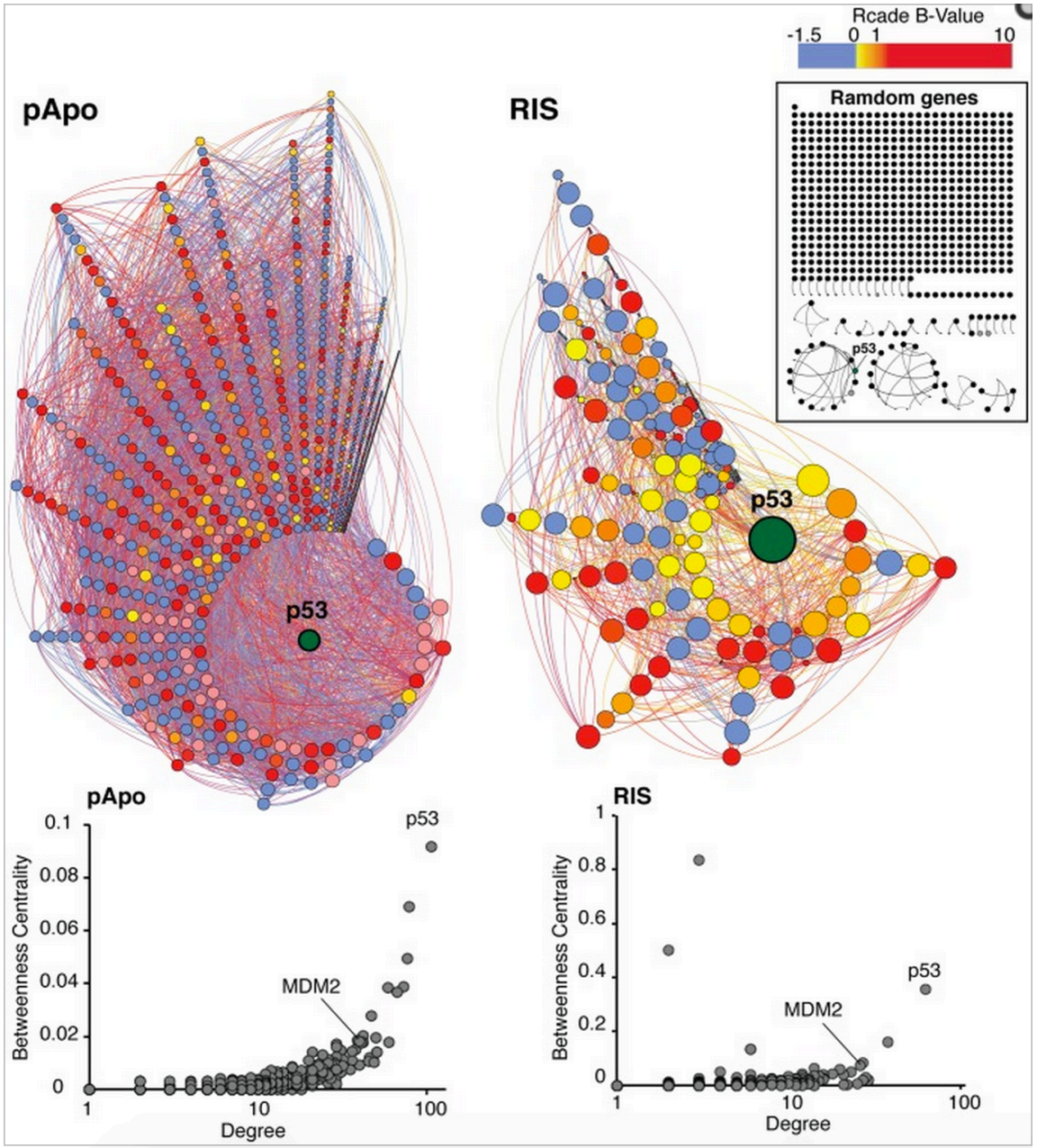
- Rcade integrates posterior probabilities of binding (determined via the baySeq package) with those of differential expression (determined via the limma package).

$$B = \log\left(\frac{PP}{1 - PP}\right)$$

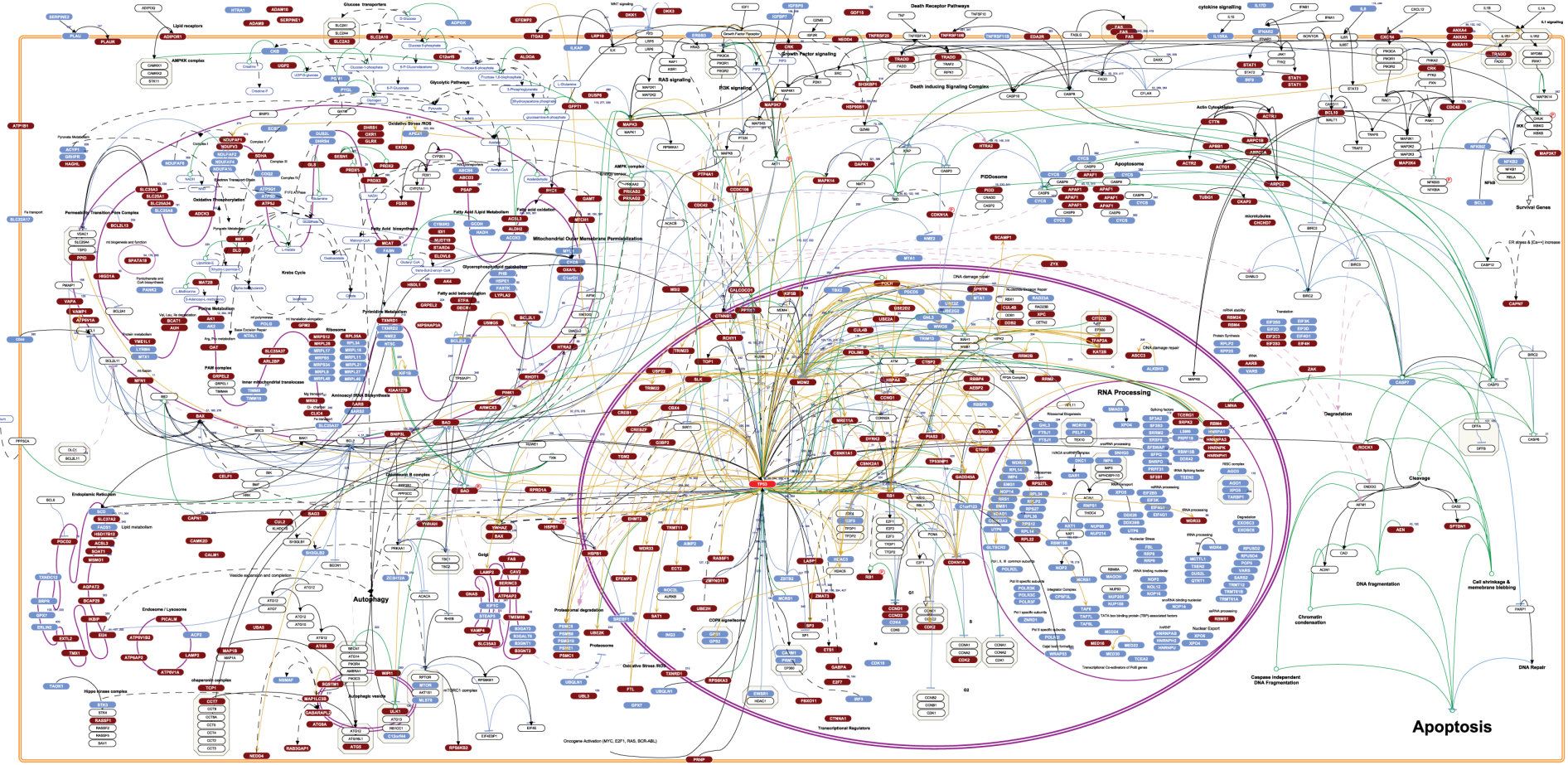
- Rcade uses a fully Bayesian modelling approach. In particular, it uses log-odds values (a measure of probability), or B-values, in both its input and output. The log-odds value is related to the posterior probability (PP) of an event, as per the formula above.
- Priors need to be defined.
- A number of output files are generated by Rcade. Usually, the file of interest is "DEandChIP.csv", which contains a list of genes most likely to have both DE and ChIP signals ranked by their B-value.
- More on Rcade @ the practical!

Functional Association Networks

Network Topology



Using TF direct targets to build Pathway and Network Models



http://australian-systemsbiology.org/tp53

DiffBind

BioConductor package by **Stark *et al.,*** for identifying sites that are differentially bound between two sample groups.

It includes functions to support the processing of peak sets, including overlapping and merging peak sets, counting sequencing reads overlapping intervals in peak sets, and identifying statistically significantly differentially bound sites based on evidence of binding affinity (measured by differences in read densities).

More on DiffBind @ the practical!