Downstream analysis of ChIP-seq data

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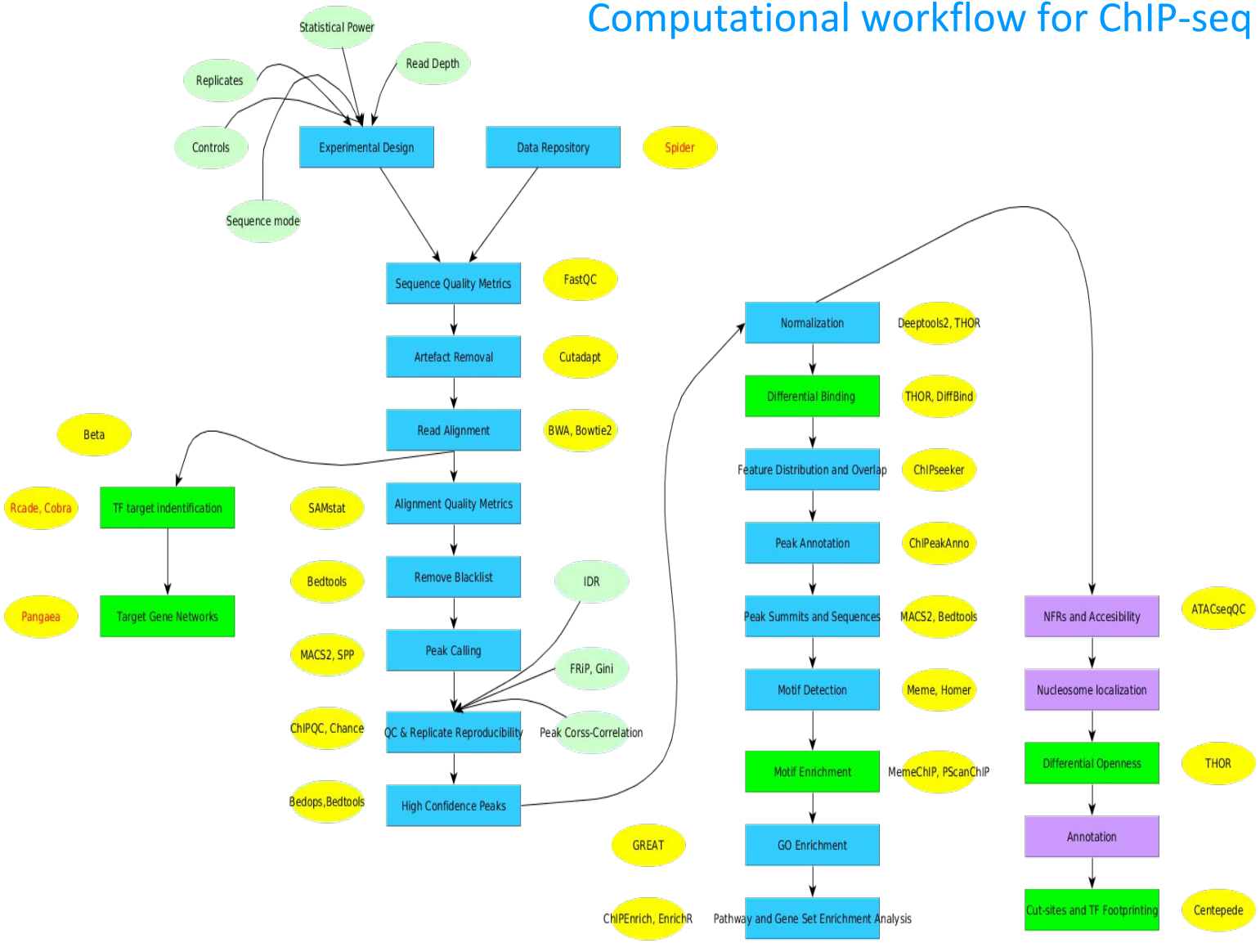
Summary

Downstream analysis for extracting meaningful biology :

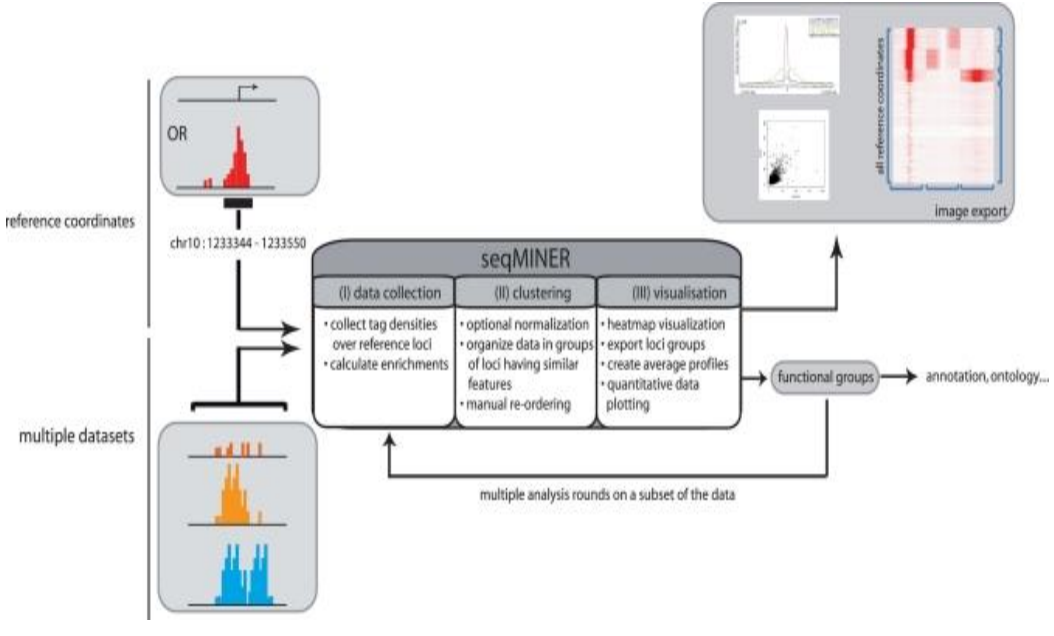
- Normalization and Visualization
- Annotation of genomic features to peaks
- Feature distribution of binding sites
- Feature overlap analysis
- Functional enrichment analysis: Ontologies, Gene Sets, Pathways
- Motif identification and Motif Enrichment Analysis
- Integration with transcriptomic data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

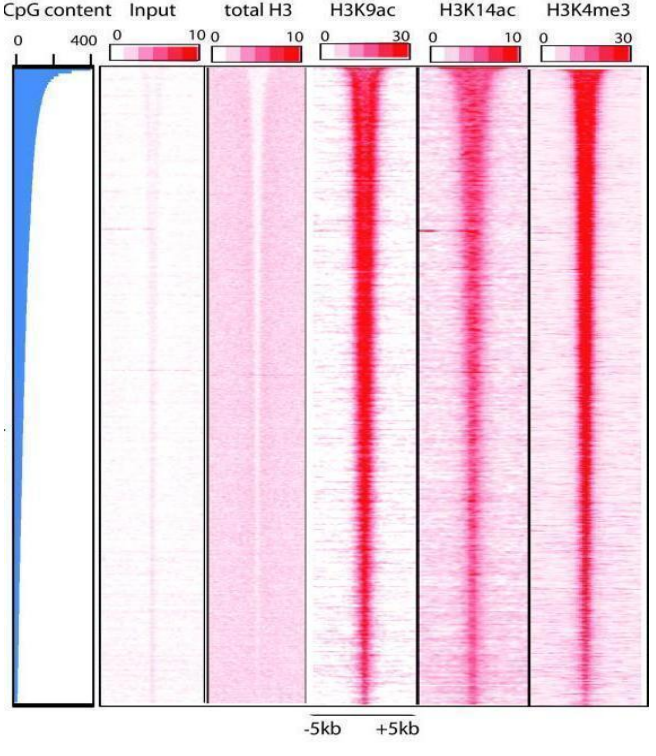
Computational workflow for ChIP-seq and ATAC-seq



Compare, Normalize & Visualize 1

- **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.

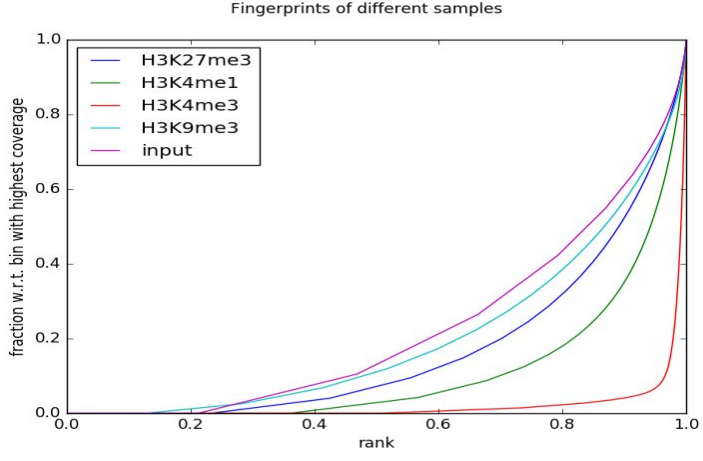


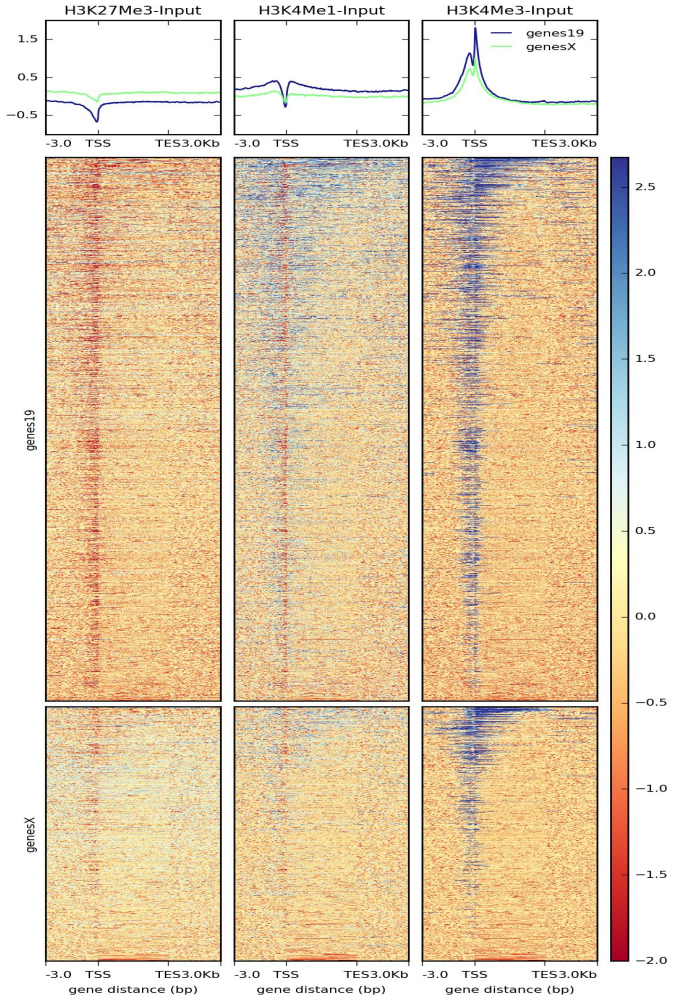


Ye *et al*., 2011, Nucleic Acids Res.

Compare, Normalize & Visualize 2

- **deepTools2** sequence depth or input normalization, GC bias correction
- Plot signal profiles
- Customized heat-maps
- PCA, correlation and fingerprint plots (chip





Ramírez et al., 2016, Nucleic Acids Res.

Peak annotation 1

- **ChIPpeakAnno (BioC)** 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
- Use **biomaRt** package to get annotation from Ensembl.
- **IRanges, GenomicFeatures, GO.db, BSgenomes, multtest (BioC)**
- converts BED and GFF data formats to *RangedData* object before calling *peak annotate* function.

Zhu et al., 2010, BMC Bioinformatics

Peak annotation 2

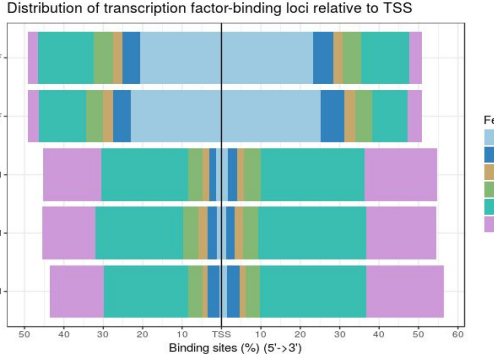
PeakAnalyzer

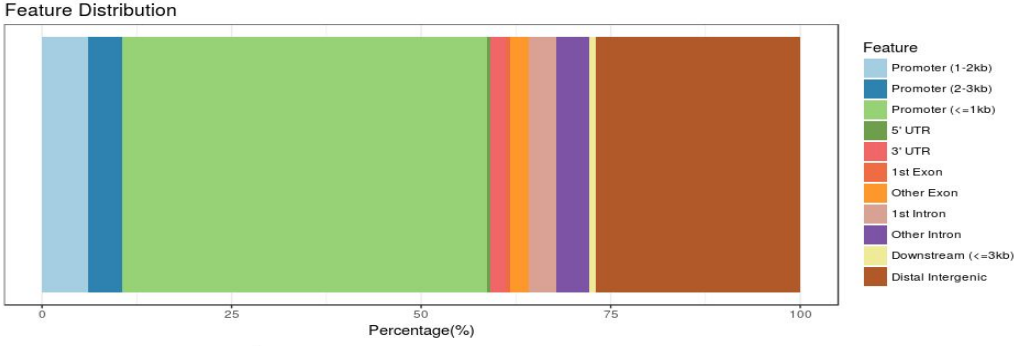
- A set of high-performance utilities for the automated processing of experimentally-derived peak regions and annotation of genomic loci.
- Consists of PeakSplitter and PeakAnnotator.
- Biologist' friendly tool.
- Get latest genome annotation files from Ensembl (gtf format) or UCSC (BED format).
- Map 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.

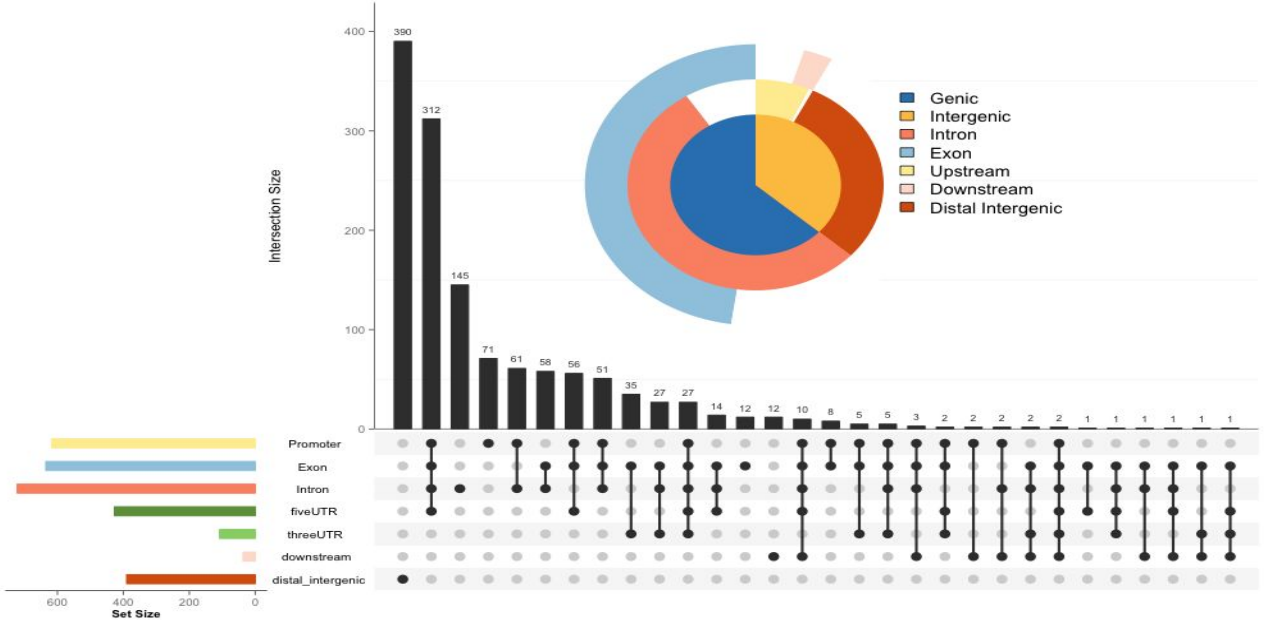
Salmon-Divon et al., 2010, BMC Bioinformatics.

Peaks distribution across features

ChIPseeker (BioC)



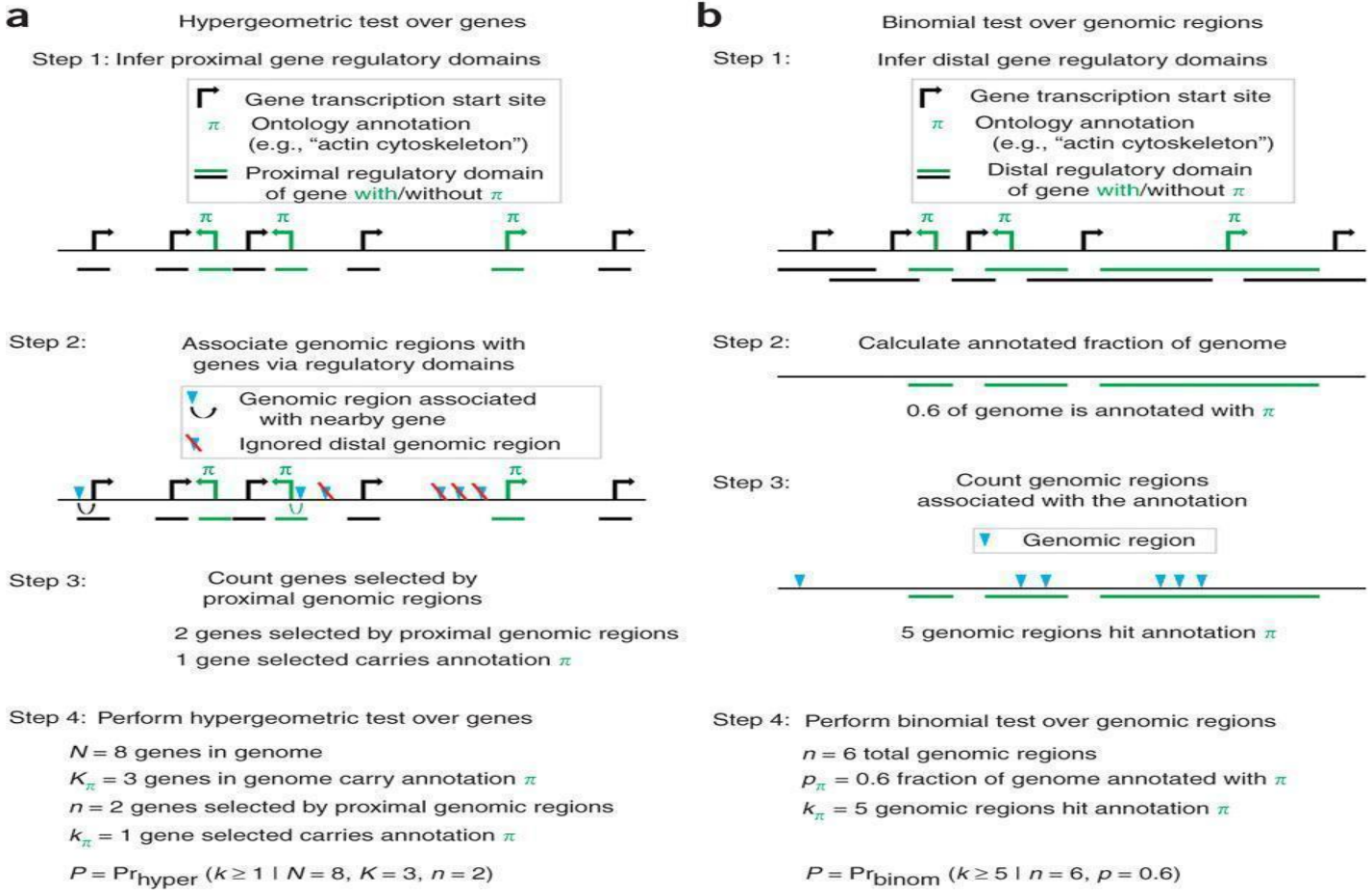




Yu et al., 2015, Bioinformatics

Functional Enrichment Analysis 1

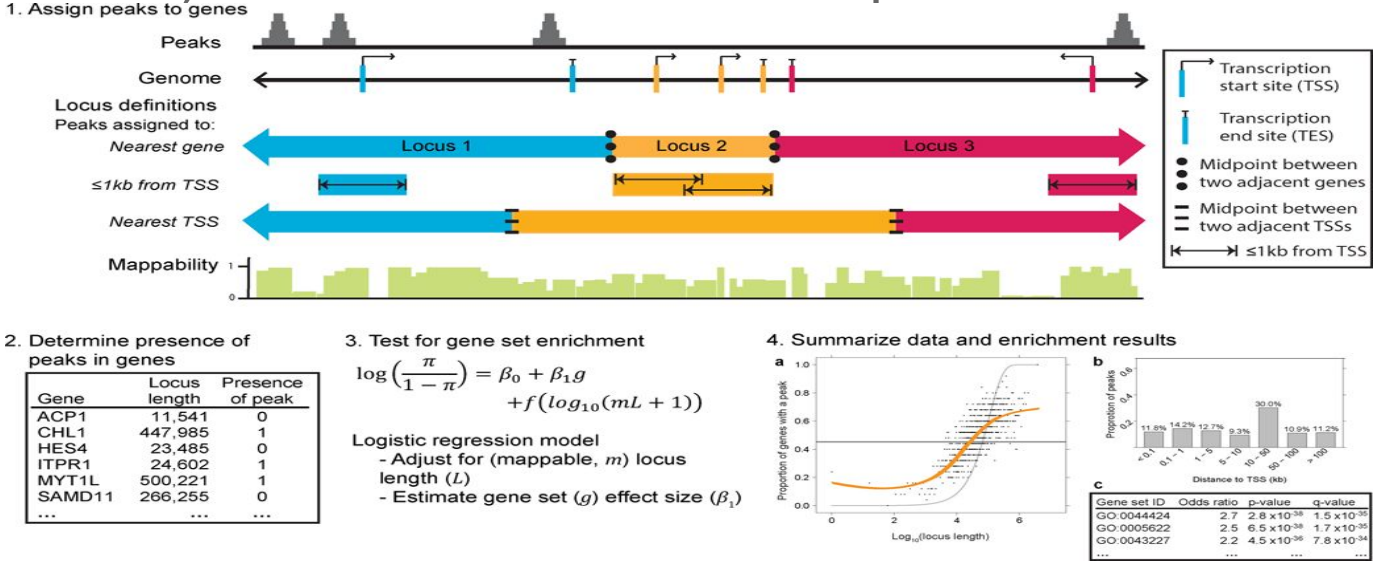
GREAT & rGREAT: Genomic Regions Enrichment of Annotations Tool



Functional Enrichment Analysis 2

chipenrich

- Includes 3 different enrichment methods:
  - Broadenrich - broadpeaks or histone modifications
  - Chipenrich -TF narrow peaks 1000-10000's
  - Polyenrich -TF >100,000
- Includes annotation, and can use custom user provided annotation



Welch et al., 2014, Nuc. Acids Res.

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 & Sandelin, 2004, Nat Rev Genet.*
- 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 overrepresented 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

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
	1	2	3	4	5	6	7	8	9	10	11	12	13	14

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)

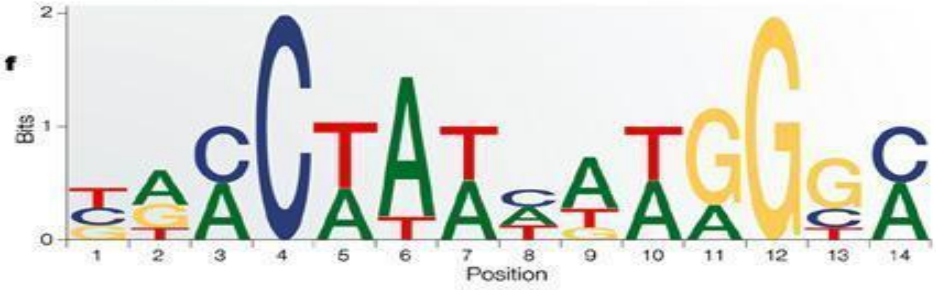
A	−1.93	0.79	0.79	−1.93	0.45	1.50	0.79	0.45	1.07	0.79	0.00	−1.93	−1.93	0.79
C	0.45	−1.93	0.79	1.68	−1.93	−1.93	−1.93	0.45	−1.93	−1.93	−1.93	−1.93	0.00	0.79
G	0.00	0.45	−1.93	−1.93	−1.93	−1.93	−1.93	−1.93	0.66	−1.93	1.30	1.68	1.07	−1.93
T	0.15	0.66	−1.93	−1.93	1.07	0.66	0.79	0.00	0.00	0.79	−1.93	−1.93	−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	v2017
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	
Prodoric		Prokaryotic	

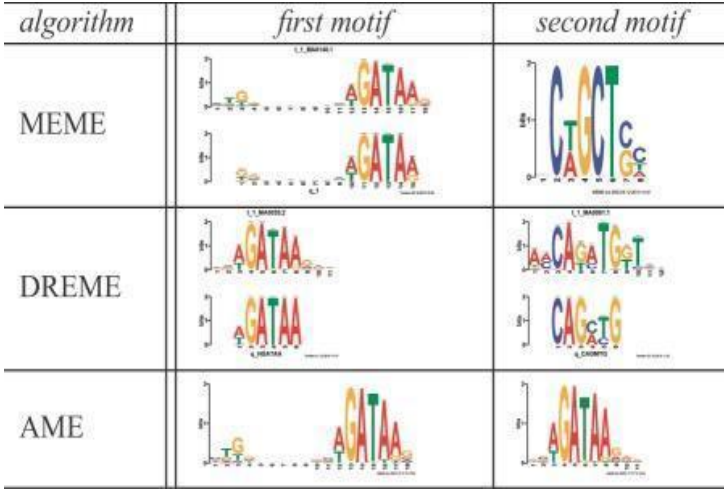
Motif Enrichment Analysis

- Identifies over and under-represented known motifs in a set of regions
- The TFs whose DNA binding motifs are enriched in a set of regulatory regions are candidate transcription regulators of that gene/promoter/enhancer set.
- Without ChIP-seq, identifying a 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 or a shuffled test sequence set.

Motif detection and enrichment analysis

- **MEME Suite and MEME-Chip** http://meme.nbcr.net

- Given a set of genomic regions, it performs
  - Motif detection (FIMO)
  - *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)
- 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.



Machanick and Bailey, 2011, Bioinformatics

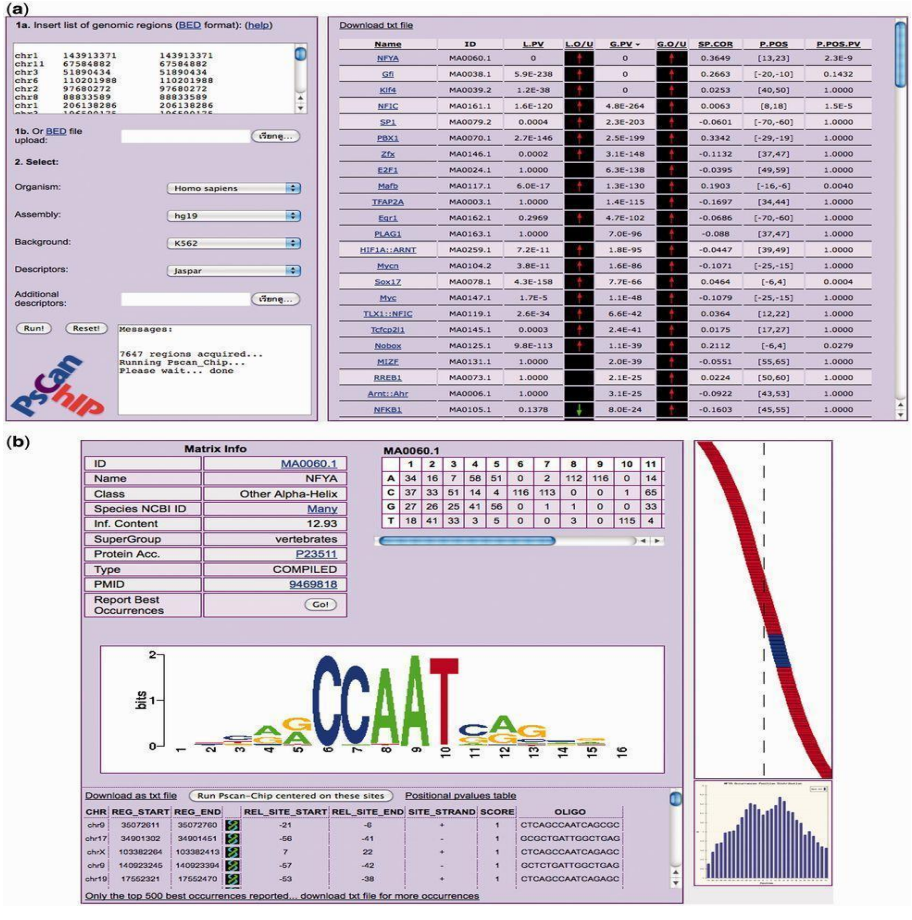
Motif detection

- HOMER v4 http://homer.salk.edu/homer/index.html
- Large number of (Perl and C++) tools for ChIP-seq analy
- 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.
- Can help with Peak annotation, GO enrichment analysis, Extract peak sequences, Visualization.

Motif Enrichment Analysis

Pscan-Chip

- 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.



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/

Differential binding analysis 1

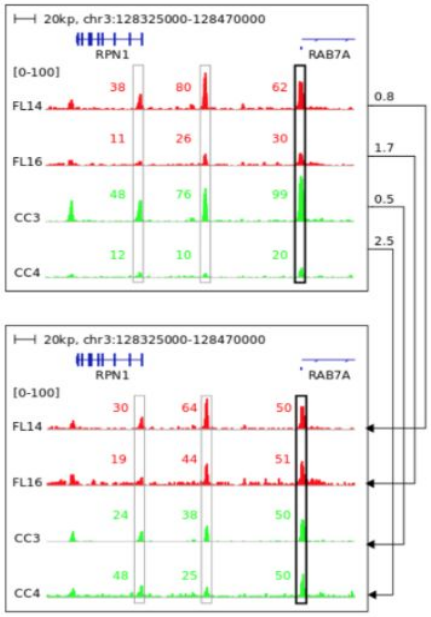
- **THOR** is an HMM-based approach to detect and analyze differential peaks in two sets of ChIP-seq data from distinct biological conditions with replicates.

- Performs genomic signal processing and normalization, peak calling and p-value calculation in an integrated framework.

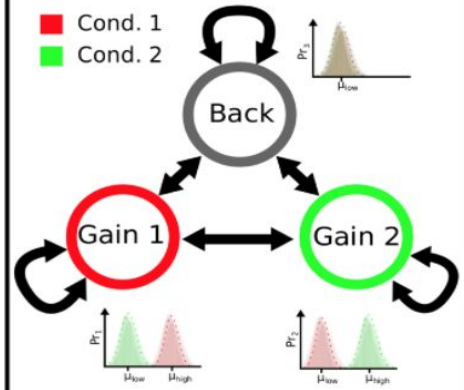
A - THOR

1 - preprocessing
- fragment size estimation
- GC-content normalization
- input-DNA normalization
- input-DNA subtraction

2 - signal normalization

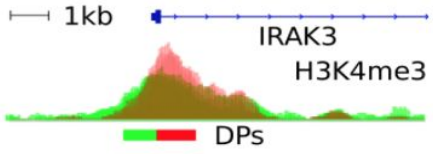


3 - HMM



4 - postprocessing
- *P*-value estimate
- strand lag filter

5 - DP estimate example

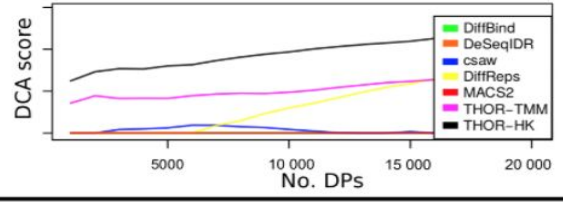


B - Competing Methods

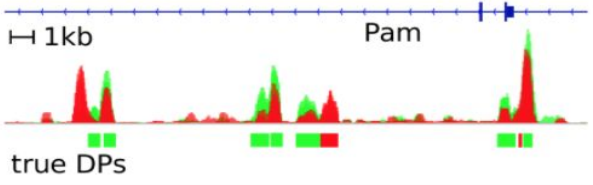
One-Stage DPC	Two-State DPC
PePr	MACS2
DiffReps	DESeq-IDR
csaw	DESeq-JAMM
	DiffBind

C - Evaluation

1 - biological data
- 4 studies and 13 DPC problems
- evaluation with expression/histones (DCA)



2 - simulated data
- 12 scenarios: no. of replicates, within condition variance, ...



Differential binding analysis 2

- **Diffbind** is a 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, 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 difference in read densities).
- More on THOR and

  DiffBind @ the practical!