ChIP-seq

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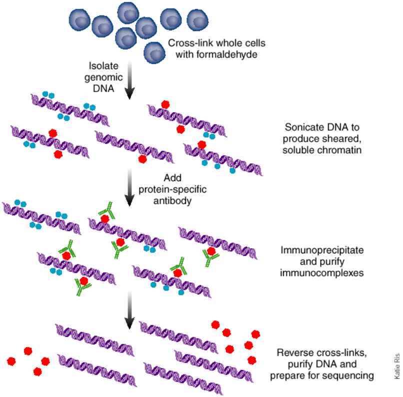
ChIP-seq


Cross-link whole cells with formaldehyde
Isolate genomic DNA
Sonicate DNA to produce sheared, soluble chromatin
Add protein-specific antibody
Immunoprecipitate and purify immunocomplexes
Reverse cross-links, purify DNA and prepare for sequencing
Katie Ris

Chromatin immunoprecipitation, followed by sequencing

- Determine location of proteins bound to DNA

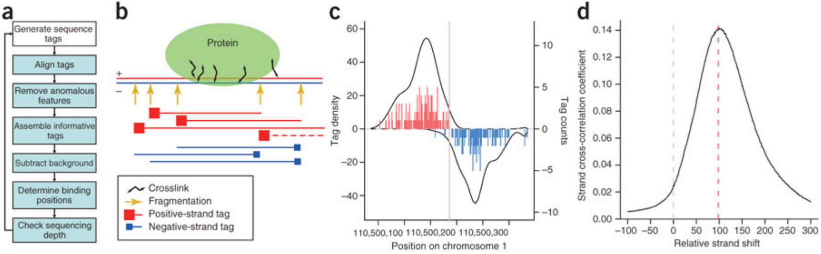
Useful for detecting

- Transcription factor binding sites
- Histone modification patterns

Common questions

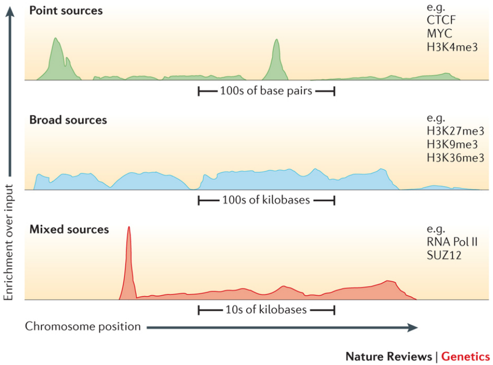
- Which genes is this TF regulating?
- How do histone modifications affect expression?

ChIP-seq: peak calling



- Peaks and strand cross-correlation, Kharchenko et al. (2008)
- Broad vs. narrow peaks, Sims et al. (2014)

ChIP-seq: peak calling



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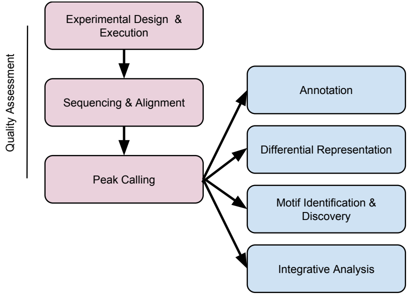
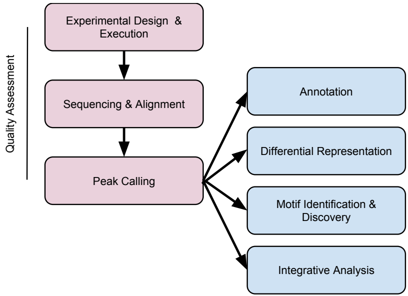
Work flow


Experimental Design & Execution
Sequencing & Alignment
Peak Calling
Quality Assessment
Annotation
Differential Representation
Motif Identification & Discovery
Integrative Analysis

Analysis overview

- Bailey et al. (2013)

Work flow: experimental design & execution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Analysis overview

- Bailey et al. (2013)

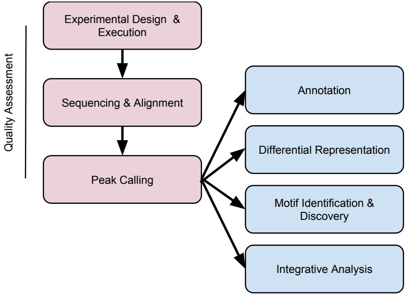
Single sample

- ChIPed transcription factor and…
- Input (fragmented genomic DNA) or control (e.g., IP with non-specific antibody such as immunoglobulin G, IgG)

Designed experiments

- Replication of TF / control pairs

Work flow: sequencing & alignment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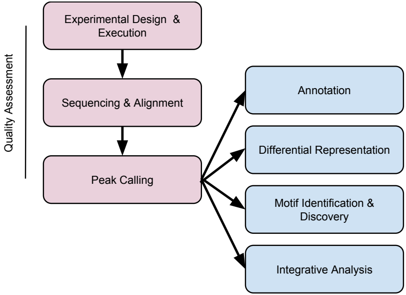
- Sequencing depth rules of thumb: $> 10M$ reads for narrow peaks, $> 20M$ for broad peaks
- Long & paired end useful but not essential – alignment in ambiguous regions
- Basic aligners generally adequate, e.g., no need to align splice junctions
- Sims et al. (2014)

Work flow: peak calling



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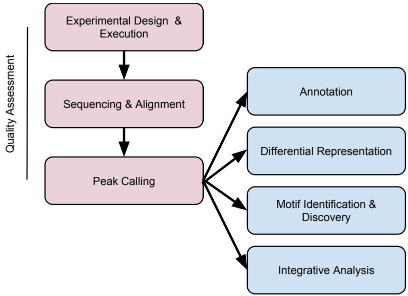
- Very large number of peak calling programs; some specialized for e.g., narrow vs. broad peaks.
- Commmonly used: MACS, PeakSeq, CisGenome, . . .

Work flow: down-stream analysis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- Annotation: what genes are my peaks near?
- Differential representation: which peaks are over- or under-represented in treatment 1, compared to treatment 2?
- Motif identification (peaks over known motifs?) and discovery
- Integrative analysis, e.g., assoication of regulatory elements and expression

Peak calling: MACS

MACS: Model-based Analysis for ChIP-Seq, Zhang et al. (2008)
`http://liulab.dfci.harvard.edu/MACS/`

- Scale control tag counts to match ChIP counts
- Center peaks by shifting $d/2$
- Model occurrence of a tag as a Poisson process
- Look for fixed width sliding windows with excess number of tag enrichment

Empirical FDR

- Swap ChIP and control samples; FDR is # control peaks / # ChIP peaks

Output: BED file of called peaks

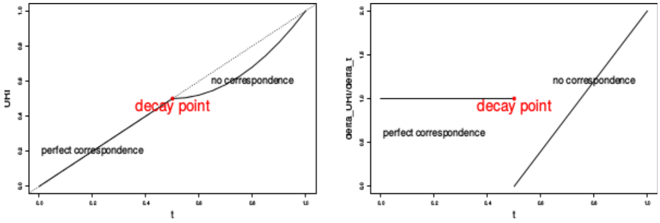
Peak calling: Irreproducible Discovery Rate

When replicates present:

- Peak callers often consistent on most confidently called peaks, but disagree on more ambiguous peaks
- When should one stop calling peaks?

Answer: Li et al. (2011) (also IDR101)

- Ranking of significance coupled with consistency between replicates



a. Ψ

b. Ψ′

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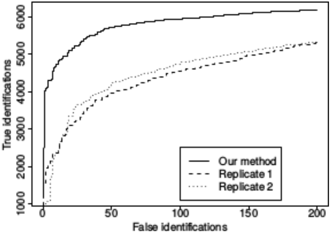
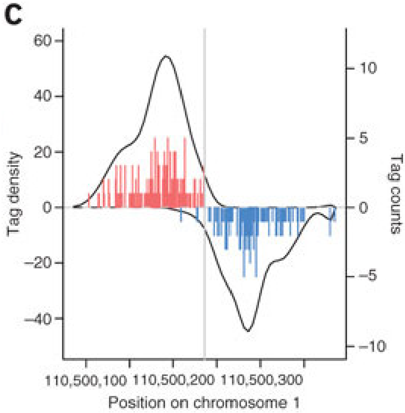


Quality Assessment

ENCODE guidelines: Landt et al. (2012)

- *Sequencing depth* relevant to TF site occupancy; $> 12M$ reads
- *Library complexity* diverse libraries indicate better sample prep, e.g., low complexity if original library contained only a few distinct reads
- *Cross-correlation* height: quality of ChIP; offset: length of fragments; ‘phantom’ peak: overlapping singletons



Kharchenko et al. (2008)

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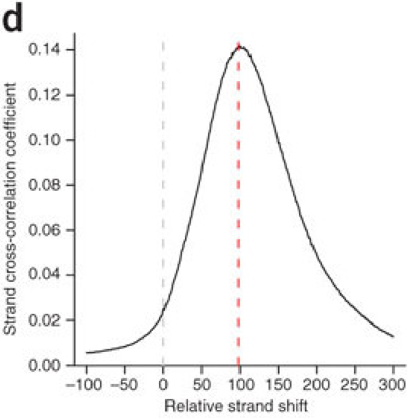

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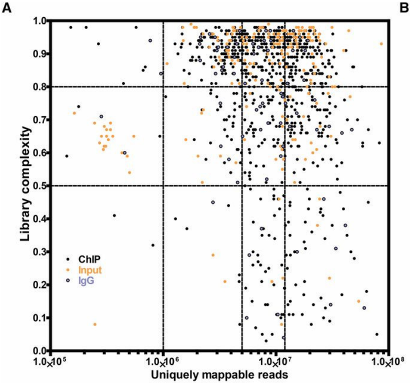
Quality Assessment



Marinov et al. (2014)

- Large-scale assessment of published ChIP-seq experiments
- 191 GEO experiments
- 55% highly successful; 20% poor

Quality Assessment: *ChIPQC*

Inputs: BAM files (raw data) and BED files (called peaks)

```r
experiment <- ChIPQC(samples)
ChIPQCreport(experiment)
```

Output: HTML report — `http://starkhome.com/ChIPQC/Reports/tamoxifen/ChIPQC.html`

Annotation: *ChIPpeakAnno*

Inputs

- Peaks: *RangedData* (*GRanges*-like) peaks, e.g., from `rtracklayer::import()` BED files
- Annotation: *RangedData* representing gene boundaries, or query to *biomaRt*

```
library(ChIPpeakAnno)
## ...
annotated <- annotatePeakInBatch(peaks,
    AnnotationData=annotation)
```

Output: *RangedData* with annotations about near-by peaks.

Differential Representation: *DiffBind*

Inputs: called peaks and raw BED or BAM files

```
library(DiffBind)
tamoxifen = dba(sampleSheet="tamoxifen.csv")
tamoxifen = dba.count(tamoxifen)
tamoxifen = dba.contrast(tamoxifen,
    categories=DBA_CONDITION)
tamoxifen = dba.analyze(tamoxifen)
tamoxifen.DB = dba.report(tamoxifen)
```

Outputs: diagnositics, visiualizations, and ‘top table’ of differentially expressed regions.

Motifs

Identification

- JASPAR and other motif catalogs
- Position Weight Matrix describing probability of nucleotide(s) at each position
- Scan genome / under peaks for known motifs
- *MotifDb*, `matchPWM` (*Biostrings*);
- FIMO, etc

Discovery

- Collate sequences under peaks, search for recurrent sequences
- e.g., DREME / MEME-ChIP

Also: enrichment, regulatory modules (2+ motifs co-occurring), function, . . .

ChIP-seq in *Bioconductor*: resources

- EdX MOOC ‘Data Analysis for Genomics’, chapter on ChIP-seq analysis
- biocViews terms: ChIPSeq, MotifAnnotation, MotifDiscovery
- Work flows: Candidate Binding Sites for Known Transcription Factors

ChIP-seq in *Bioconductor*: packages

Sample packages

- Quality assessment – *ChIPQC*;
- (Peak calling) – *chipseq*, *PICS*, *triform*, *ChIPseqR*, *iSeq*, ...
- Single sample summary / exploration – *ChIPpeekAnno*, *chIPseeker*
- Differential representation – *DiffBind*, *MMDiff*, ...
- Motifs – *MotifDb*, *TFBSTools* (matching known motifs), *motifRG*, *MotIV*, *rGADEM* *BCRANK* (motif discovery)
- Integration with expression data – *Rcade*, *epigenomix*

References I

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