Differential binding

DiffBind, THOR

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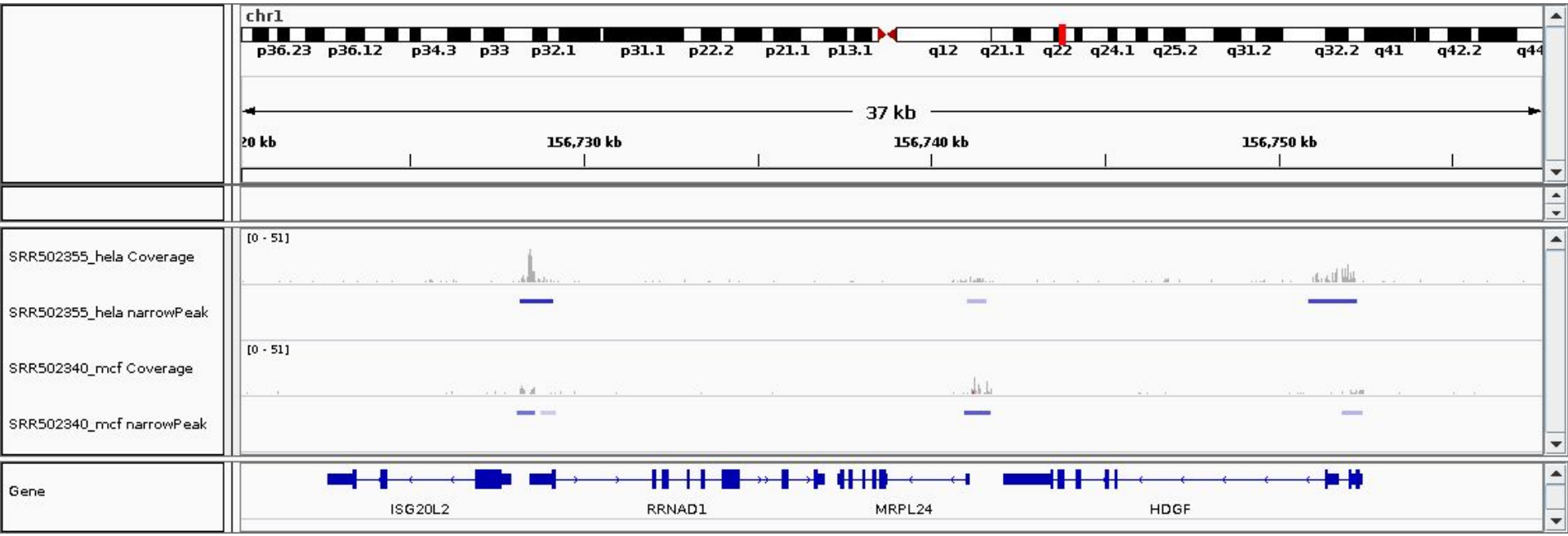
Overview

- Introduction to differential binding
- DiffBind - Bioconductor package
- THOR and ODIN - standalone tools

Differential binding



Differential binding

- The aim of differential binding analysis is to compare changes in protein-DNA interactions measured by ChIP-seq
- Two main types:
  - Two-stage methods (DiffBind):
    - Identify candidate peaks using peak callers like MACS2
    - Apply methods tailored for differential expression analysis like DESeq2 and edgeR
  - One-stage methods (THOR):
    - Based on segmentation methods like hidden Markov models (HMM) or sliding window approaches

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DiffBind

- https://bioconductor.org/packages/release/bioc/html/DiffBind.html

Main steps:

- Reads in peak sets generated by peak callers like MACS2
- Quality control
- Identifies consensus peak set for further analysis
- Counts reads based on .bam files provided
- Generates affinity matrix: a normalised read count matrix
- Differential binding affinity analysis
- Generates plots and reports results

DiffBind - Differential binding with DESeq2

- DESeq2 is a Bioconductor package for differential expression analysis
- https://bioconductor.org/packages/release/bioc/html/DESeq2.html
- It normalises raw read counts for the number of reads in peaks (default) or the total library size
- Calculates dispersion
- Uses negative binomial generalised linear models (GLMs) to model counts
- P-values and differentially expressed genes are calculated by Wald test

DiffBind - Differential binding with edgeR

- edgeR is also a Bioconductor package for differential expression analysis
- http://bioconductor.org/packages/release/bioc/html/edgeR.html
- edgeR stands for Empirical analysis of Digital Gene Expression data in R
- It normalises raw read counts for the total library size (default) or the number of reads in peaks using TMM (trimmed mean of M-values)
  - Define gene-wise log-fold changes compared to samples which have expression closest to average
  - Trim highest and lowest expressed genes (genes with higher read counts have lower variance on log scale)
- Builds a model based on your experimental setup
- Calculates dispersion based on an empirical Bayes method
- Uses negative binomial GLMs to identify differentially expressed/bound sites

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ODIN - One-stage DIffereNtial peak caller

- Capable of detecting differential peaks (DPs) in pairs of ChIP-seq data
- Performs
  - Genomic signal processing
  - Peak calling
  - Post processing
  - P-value calculation

1. Main steps of genomic signal processing
   a. Fragment the DNA into bins, count reads in each bin
   b. Ignore reads with poor mappability
   c. Determine fragment size (based on cross correlation)
   d. Input subtraction
   e. Normalize based on sequencing depth
   f. Filter bins with low number of reads

[1] M Allhoff et al, *Bioinformatics*, 2014.

ODIN - One-stage DIffereNtial peak caller

2. HMM-based peak calling
   a. Three state HMM model:
      i. DP gained in first sample
      ii. DP gained in second sample
      iii. Background
3. Post processing
   a. Ignore DPs with size smaller than the estimated fragment size
   b. Merge DPs that have distance less than the estimated fragment size
4. P-value calculation

[1] M Allhoff et al, *Bioinformatics*, 2014.

THOR

- Extension of ODIN:
  - Allows the analysis of multiple replicates of two conditions based on a negative binomial distribution
  - Two additional normalisation of ChIP libraries
    - TMM approach
    - Housekeeping genes based normalisation
- Python package
- Inputs:
  - .bam files for the two conditions
  - Chromosome sizes
- Outputs:
  - Post-processed bigWig files
  - DPs in .bed and .narrowPeak format

[1] M Allhoff et al, *Oxford Journals*, 2016.

Comparison of DiffBind and THOR

- Both tools are capable of handling replicated ChIP-seq peak sets
- The methods used by DiffBind
  - Were originally designed for differential expression analysis on RNA-seq data that assumes that most of the genes between conditions are not differentially expressed - this might not be true for differential binding
- DiffBind is usually more stringent resulting in less DPs
  - This might as well be a limitation in finding true differences between conditions
- It is usually a good practice to use different tools and come up with consensus solutions
- Other tools you can use for differential binding analysis:
  - SICER
  - MACS2
  - RSEG
  - MAnorm
  - HOMER
  - MMDiff
  - etc.