

# Differential binding

DiffBind, THOR

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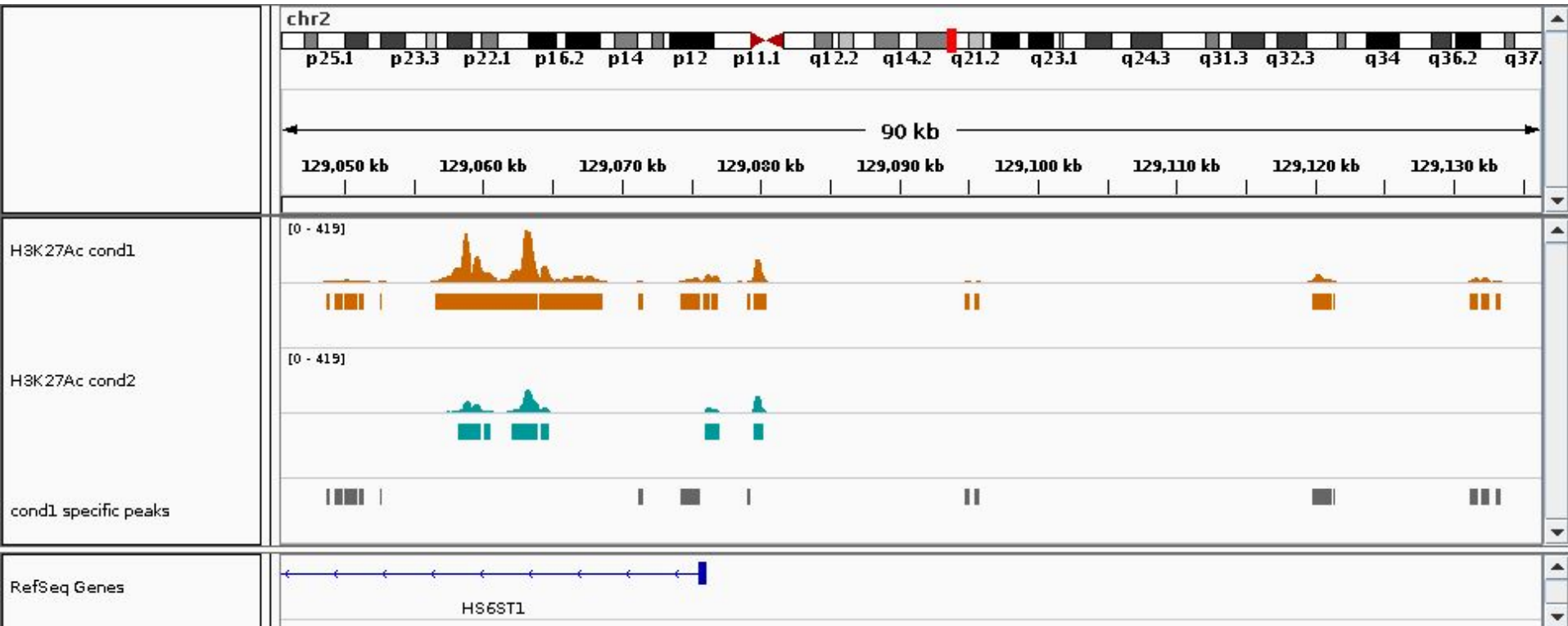
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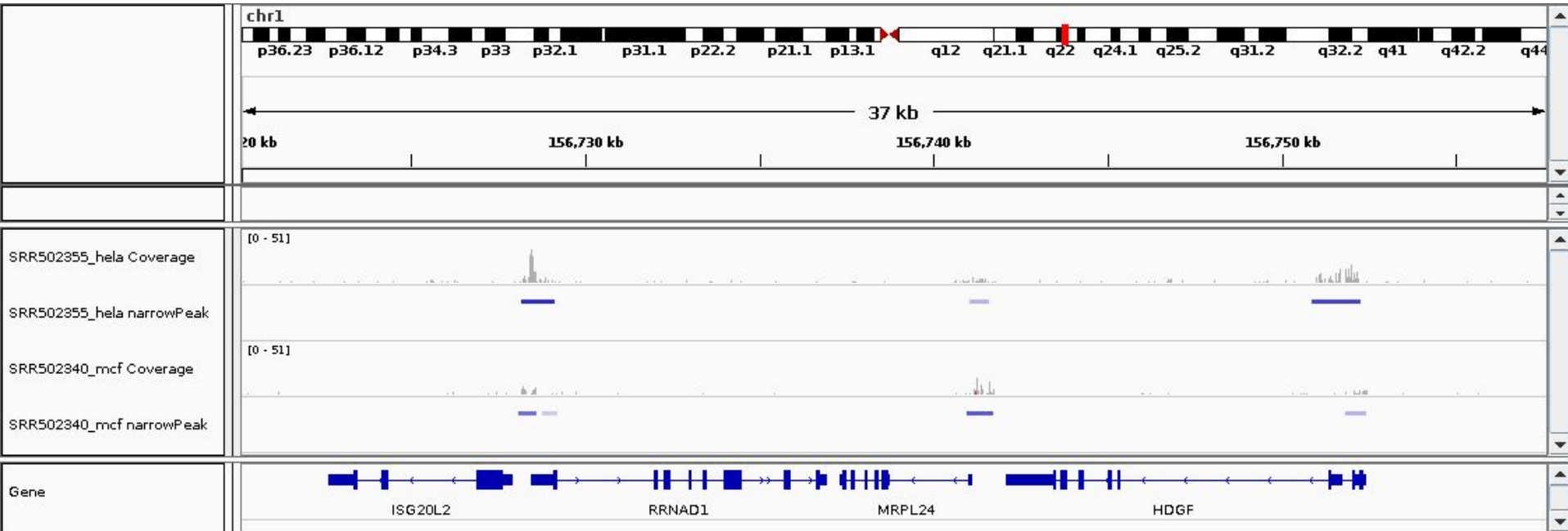
# Overview

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

# Peak intersection vs. differential binding



# 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

# 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

# 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, normalised bigWig files
  - DPs in .bed and .narrowPeak format

# 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 multiple tools and come up with a consensus solution by combining those results
- Other tools you can use for differential binding analysis:
  - SICER
  - MACS2
  - RSEG
  - MAnorm
  - HOMER
  - MMDiff
  - etc.