Chip-seq - Analysis

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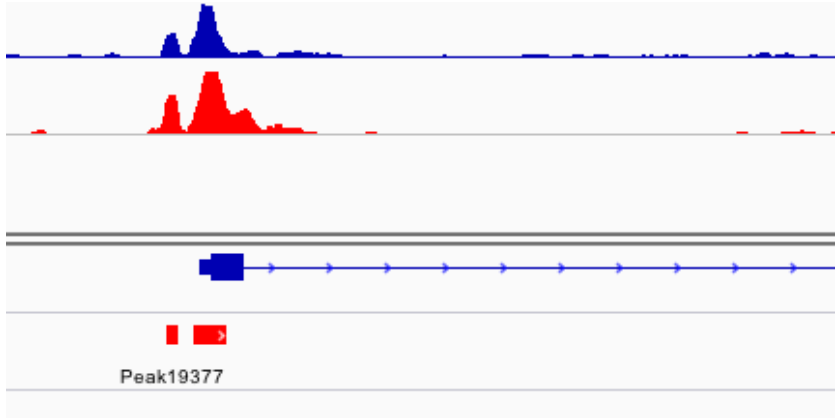
Bioinformatics analysis

Sequence file

`fastq`

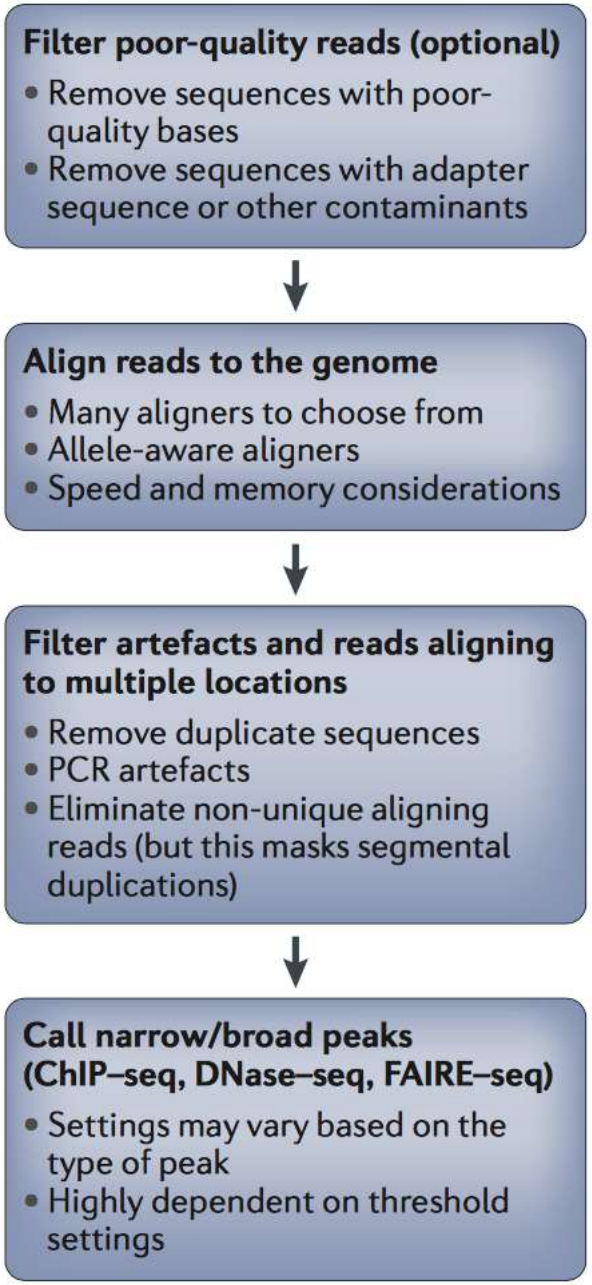
```
@SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA
+SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=
@SRR038845.53 HWI-EAS038:6:1:1:360 length=36
GTTCAAAAAGAACTAAATTGTGTCAATAGAAAACTC
+SRR038845.53 HWI-EAS038:6:1:1:360 length=36
BBCBBBBBB@@BAB?BBBBCBC>BBBAA8>BBBAA@
```

Peak file



what this course is about

Steps



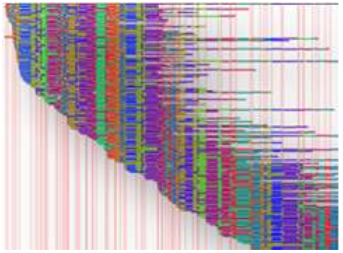
Furey 2012.
Nat. Genet. Rev.

Steps, graphics

Quality control

Cleaning

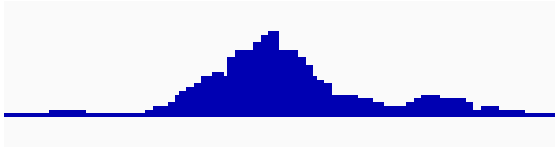
Mapping



Differential peak calling

Motif discovery

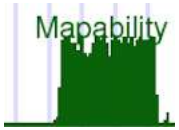
Peak calling

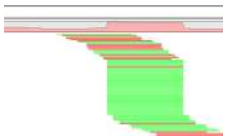


Signal normalization

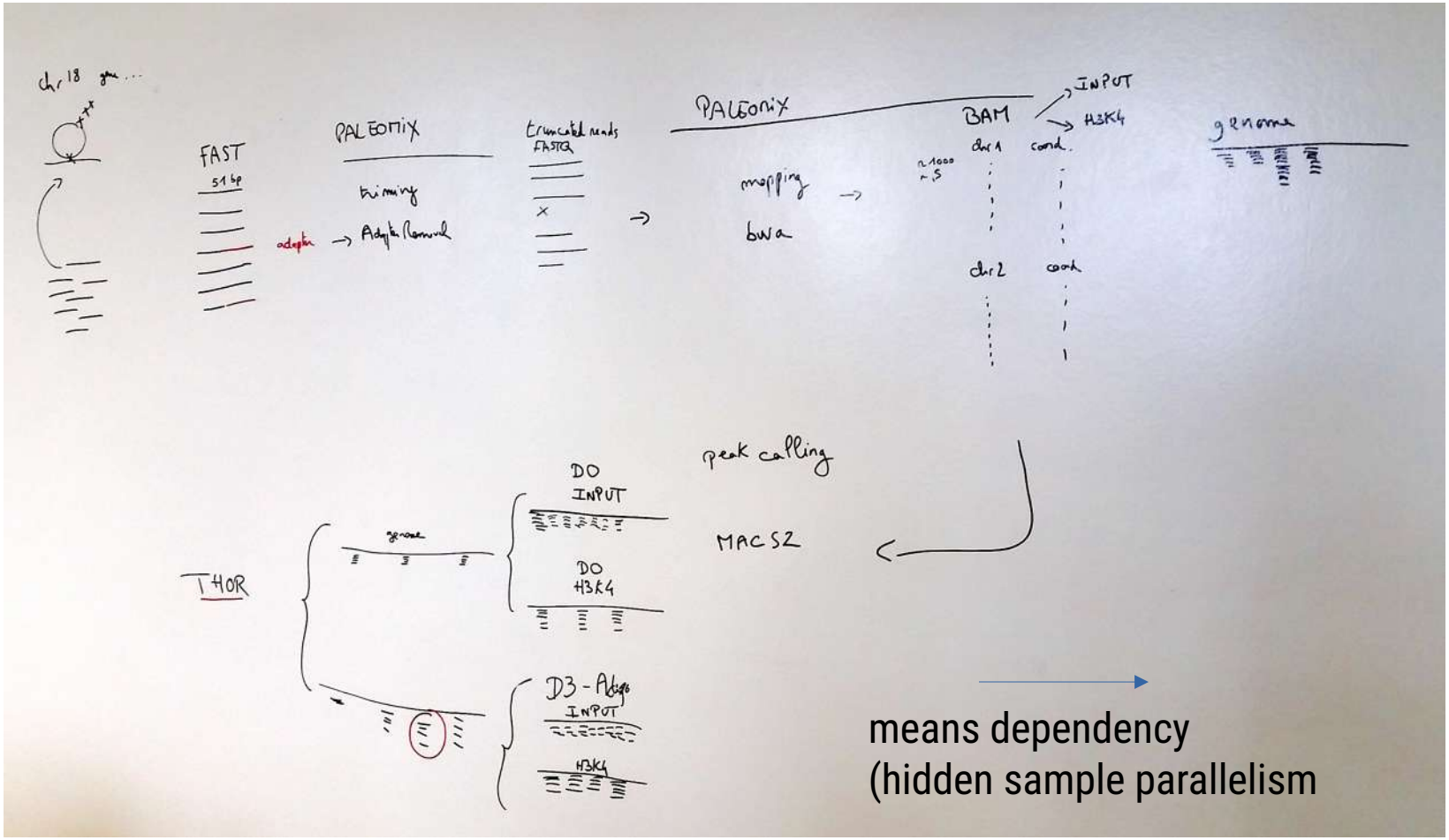
1 input / 1 IP

Controls





White board implementation



Computer implementation

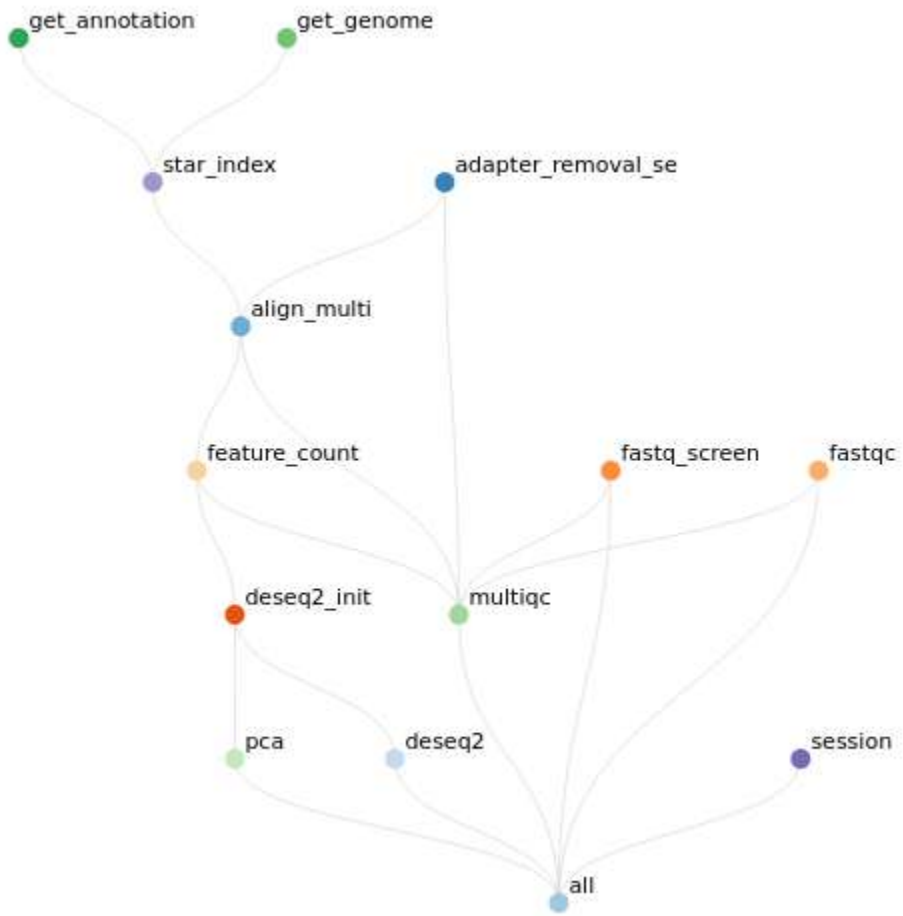
```
#!/bin/csh
# Created by: Chandra Sekhar Pedamallu @ DFCI, The Broad Ins
# Date: June 2016
# Full PathSeq pipeline
# time $pdir/FullPathSeq_June2016.sh $bamloc/SN218_Run0771_L
#
set start_time=`date +%s`

@ noargs=$#
##############################PLEASE SET THESE ENVIRONMENTAL
# Program Settings
set Institute="BROAD"
[...]
if($noargs < 4) then
    echo "Please check your argunments"
    echo "Usage : ./FullPathSeq_xxxx.sh <Input file in BAM c
    echo "Example : ./FullPathSeq_xxxx.sh unmappedreads.10K.
    exit
endif
####################INPUT FILES##############################
# Present Directory
set pdir = `pwd`
rm $pdir"/clean.files"
rm -r $pdir"/Commands/"
[...]
```

Issues

- mix of code and parameters
- common actions are mingled
- software/input defined as **absolute** paths
- comments are instructions
- software dependencies not included (`csh`!)
- on HPC, no admin rights
- any issue implies to start over
- version in filename (see the usage with **xxxx**)
- file management (here: `rm` recursive!)
- requires many effort to port over

Source: https://github.com/ChandraPedamallu/PathSeq/blob/4de7f350575404c9206b1e1c2d3f1fc81884908b/FullPathSeq_June2016.sh

What we need, dependencies



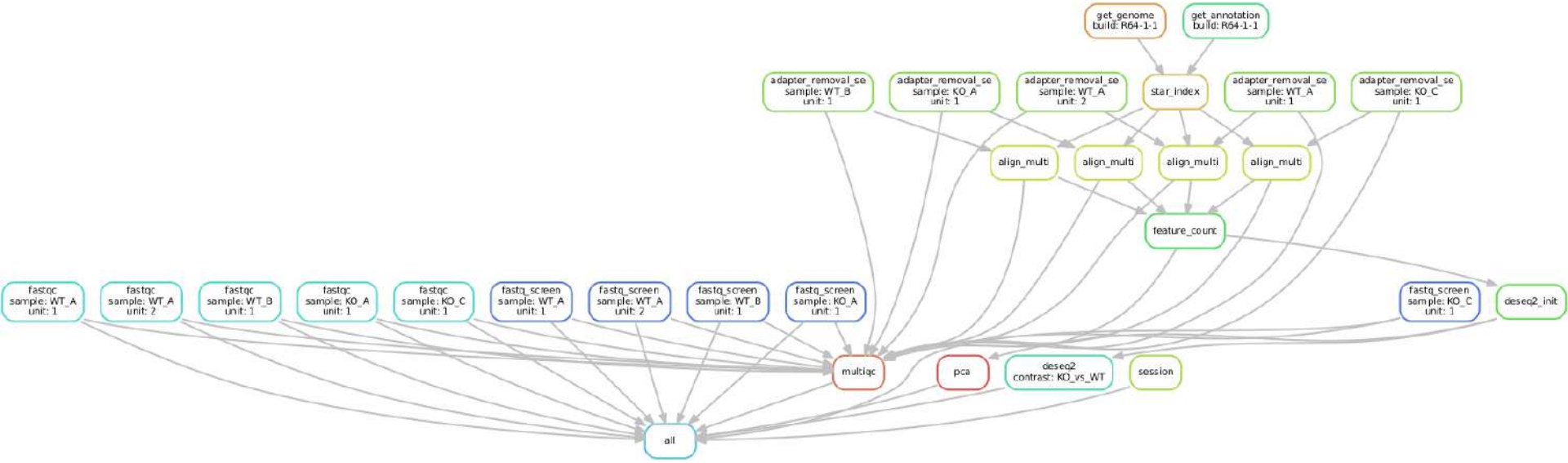
Solved issues

- run only what needs to be, stop micromanaging your analyses
- use **relative** paths
- clear instructions in a `README`
- code in one folder, users edit 3 text files
- software installed in `singularity` image
- makes seamless deployment on HPC
- singularity images are versioned
- code and reports also versioned
- ongoing work in temporary folders
- multi-platform (Windows, MacOS, GNU/Linux)

Source: Snakemake RNA-seq workflow,
https://gitlab.lcsb.uni.lu/aurelien.ginolhac/snakemake-rna-seq

Python

- This is the workflow being **used**: not just a diagram
- **Explicit** dependencies
- **Parallelization**: independent branches, like `fastqc` and `adapter_removal` and samples



Why you don't need/want to install any software?

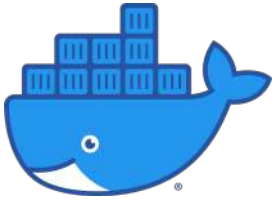
- it's boring
- On HPC one doesn't have admin rights
- modules prepared by the HPC teams are great, but more specific software are missing

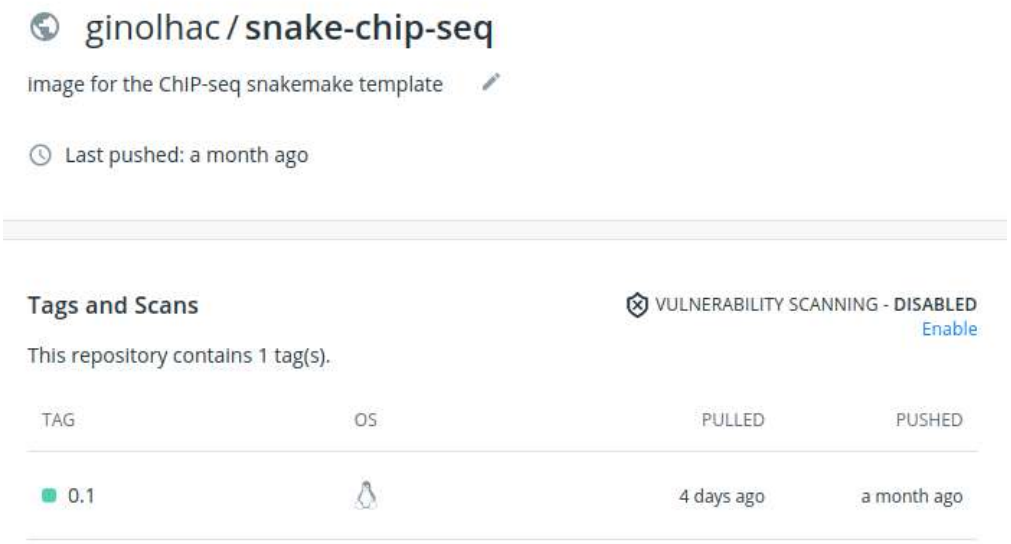
Docker

- is great but requires admin rights
- `singularity` is kind of docker for High Performance Computers

Of course, someone has to install software, it doesn't have to be you

— Aurélien adapted from **Jenny Bryan**

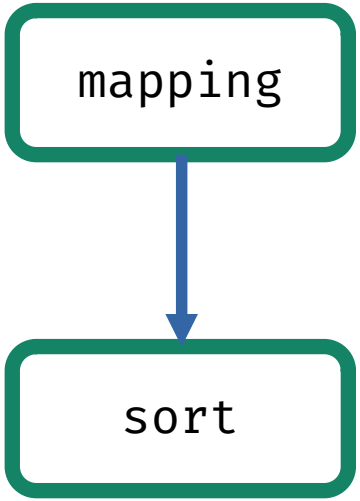




Snakemake basic

```
rule mapping:
  input: "A.fastq.gz"
  output: "A.bam"
  shell:
    """
    bowtie2 -x GRCm38 -U {input} | \
    samtools sort - > {output}
    """

rule sort:
  input: "A.bam"
  output: "A.bam.bai"
  shell:
    """
    samtools index {input}
    """
```

Deduced DAG



Snakemake workshop

Johannes Köster
@johanneskoester

Registration for the 2021 edition of the Boston #Snakemake tutorial days (21st and 22nd Sep) is open now. It will be virtual, via Zoom, participation is free, and of course also open to non Bostonians :-): koesterlab.github.io/bsd2021.html

- free
- by Snakemake creator/maintainer
- online

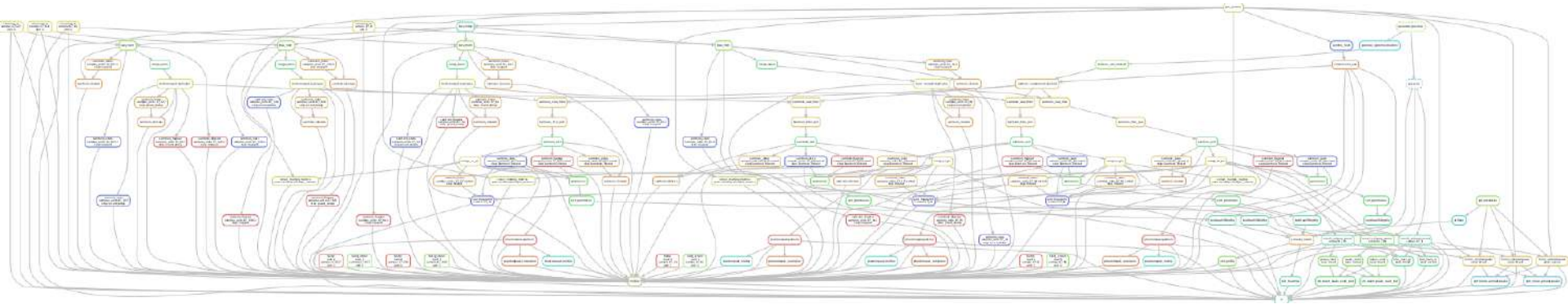
https://koesterlab.github.io/bsd2021.html

LCSB biocore: Sarah Peter

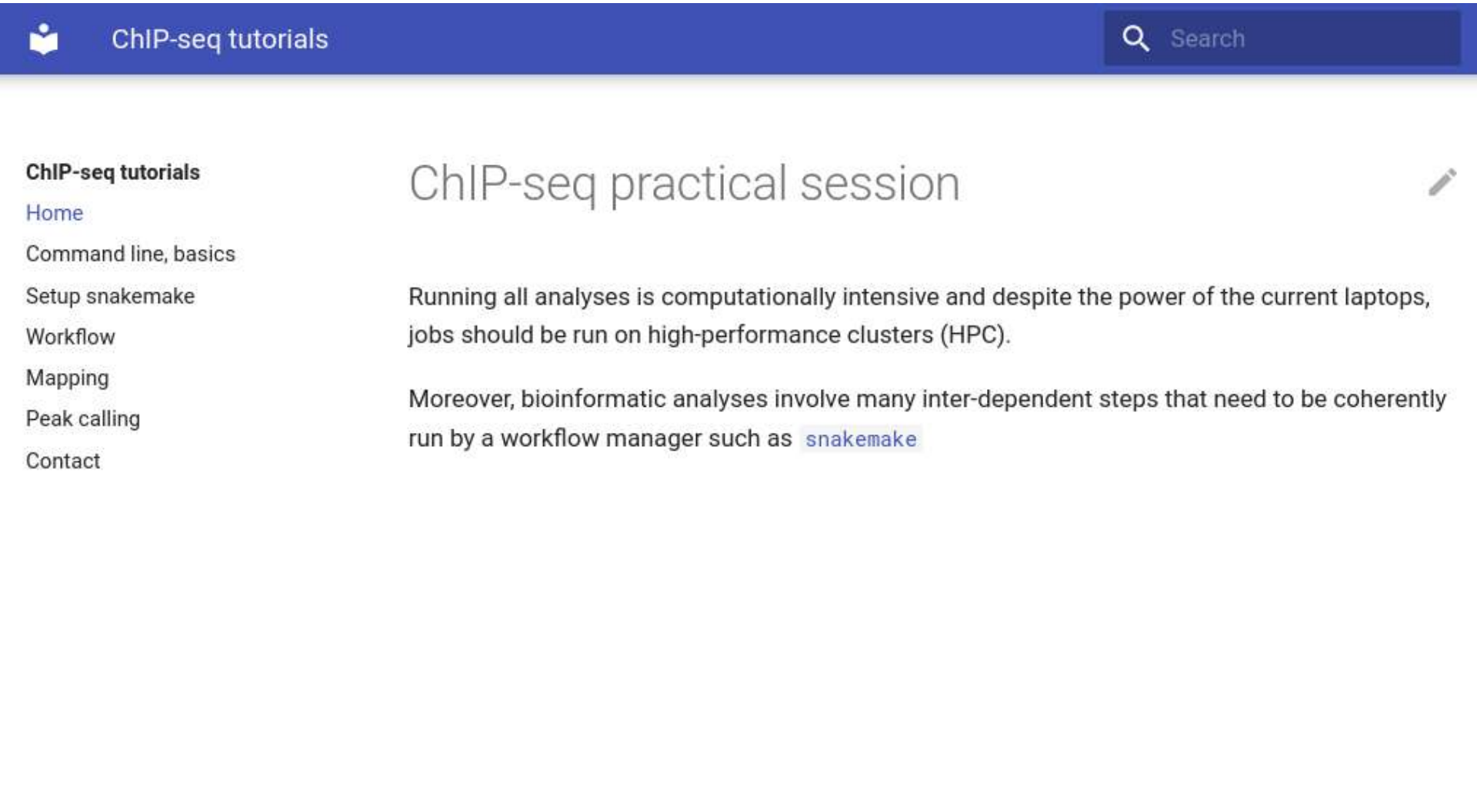
https://r3.pages.uni.lu/school/snakemake-tutorial/

ChIP-seq template

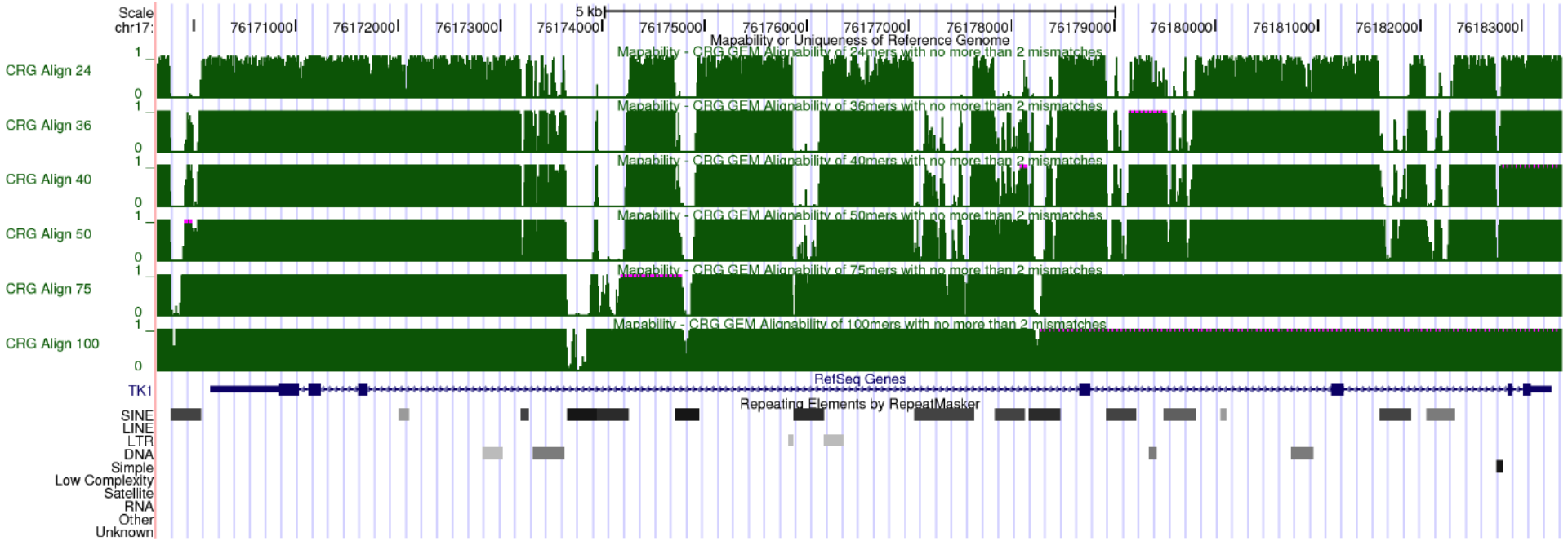
Direct Acyclic Graph



https://ginolhac.github.io/chip-seq/

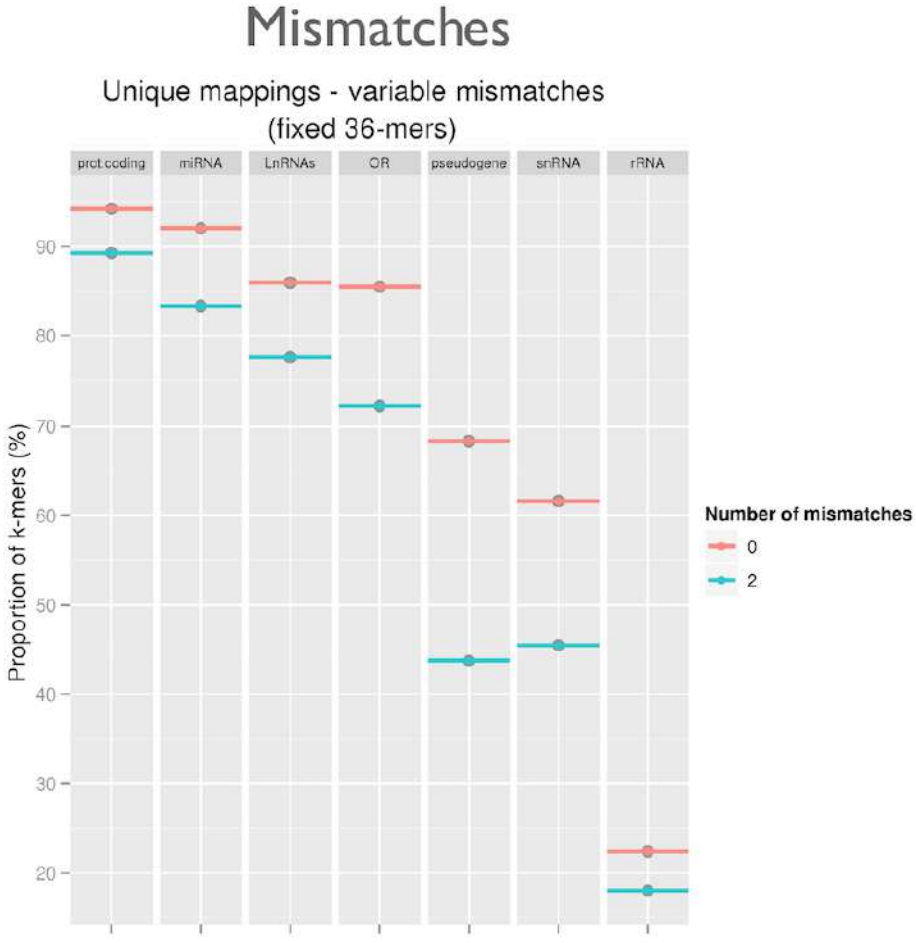


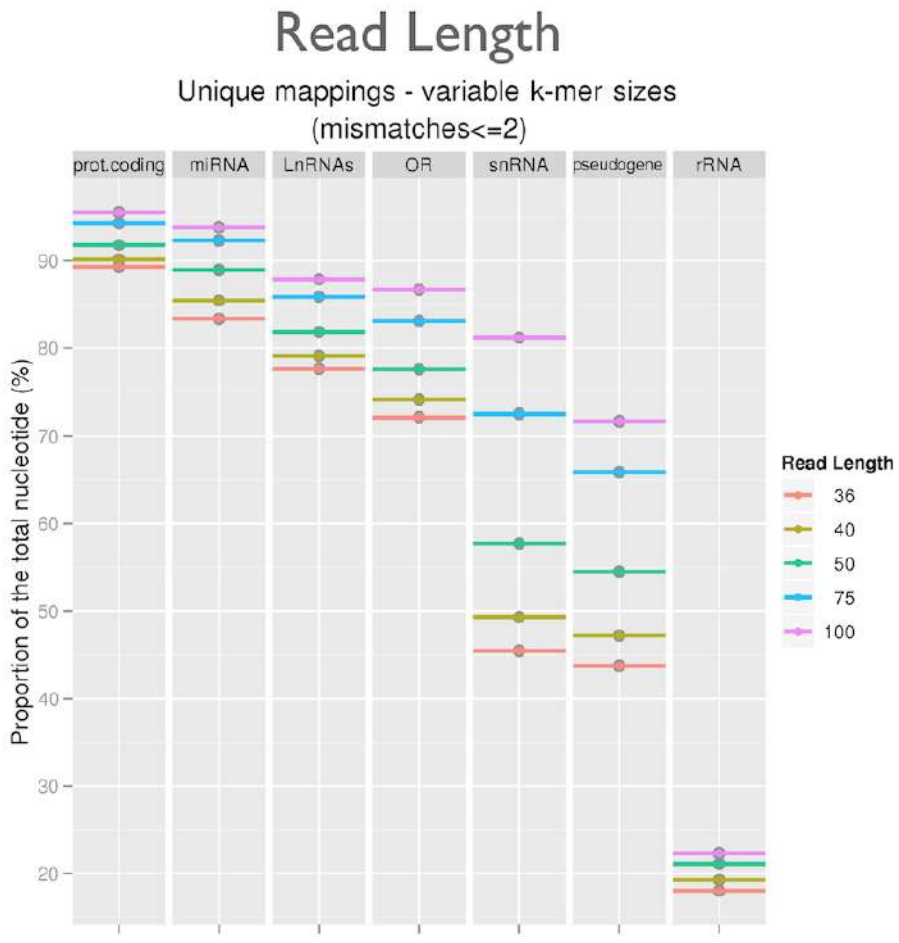
Mappability, causes



Mappability, consequences

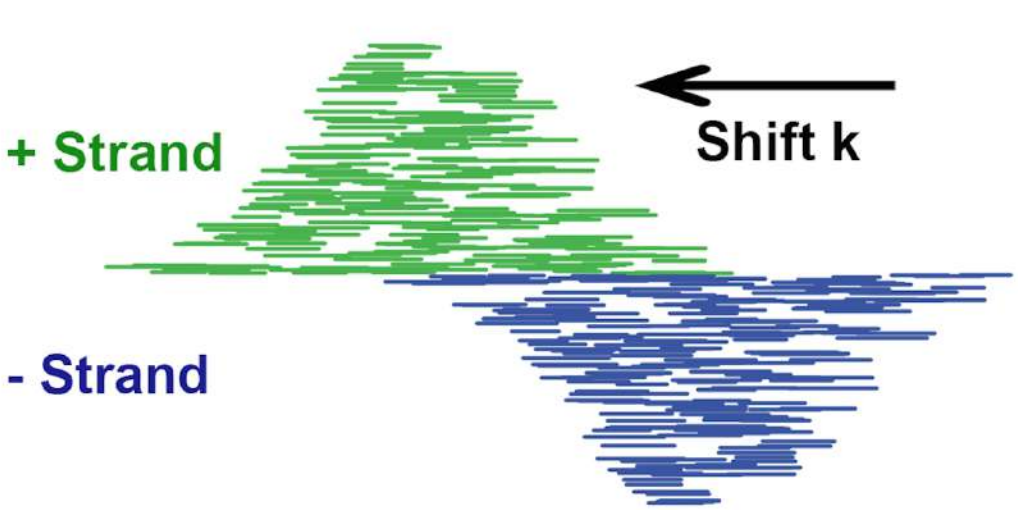
Uniquely mapped

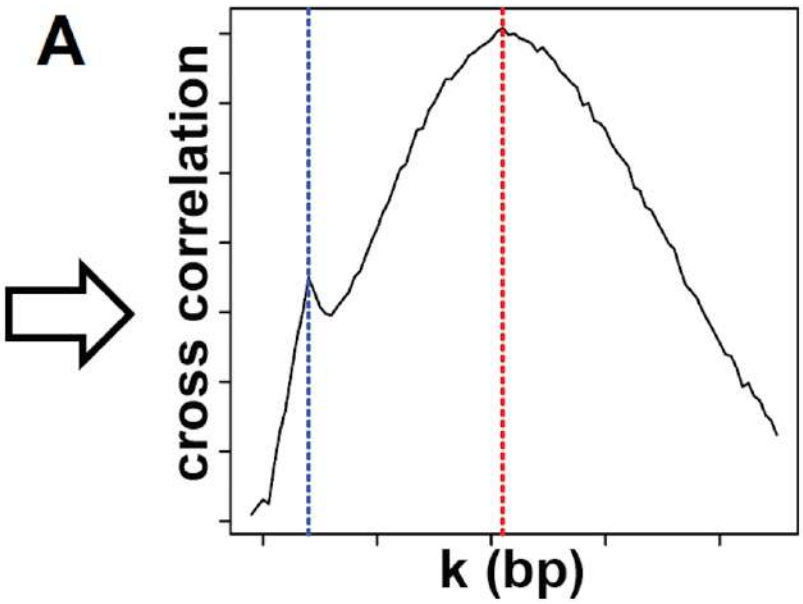




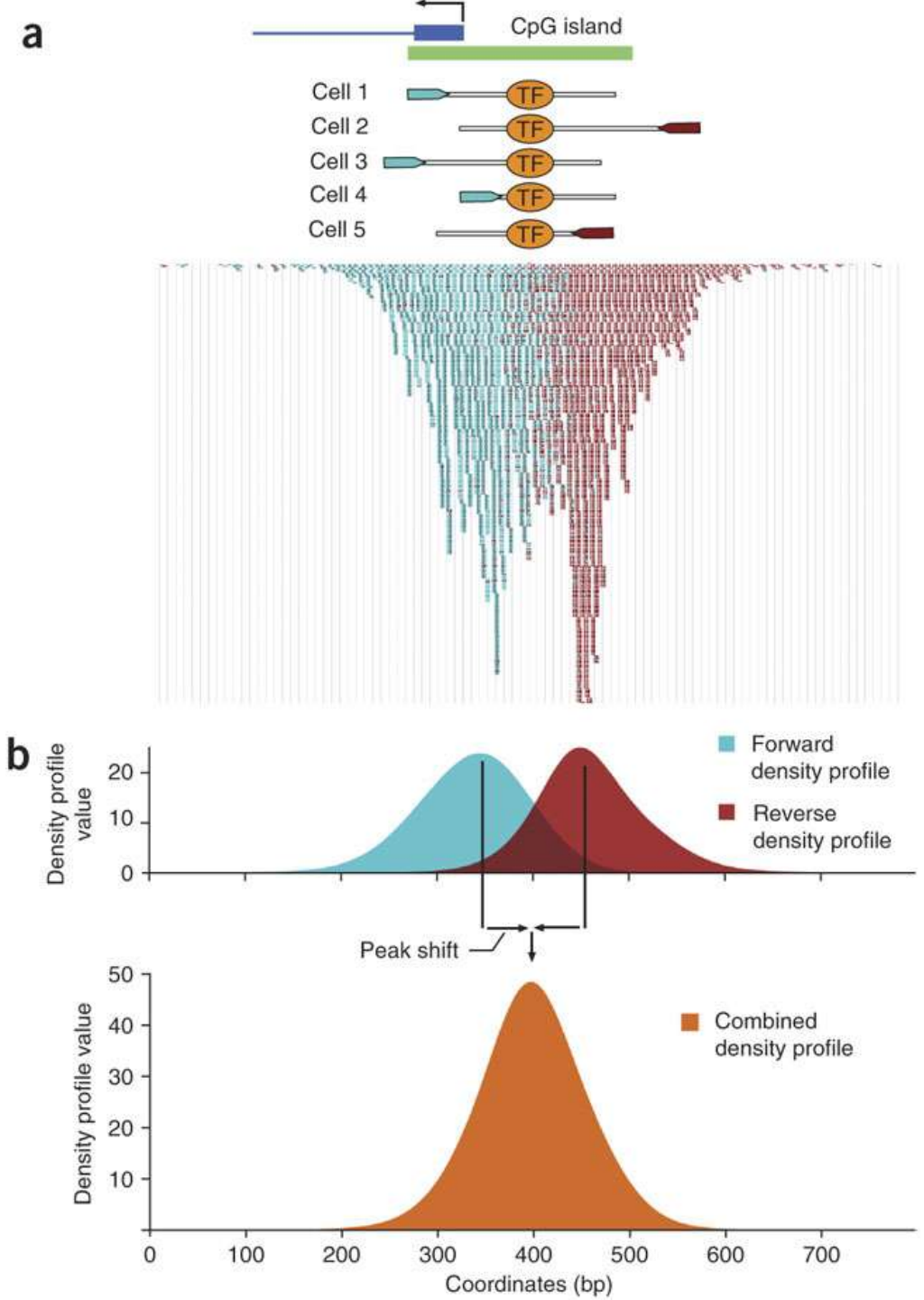
Derrien et al. 2013. *PLoS ONE*

Peal calling, infer the shift size





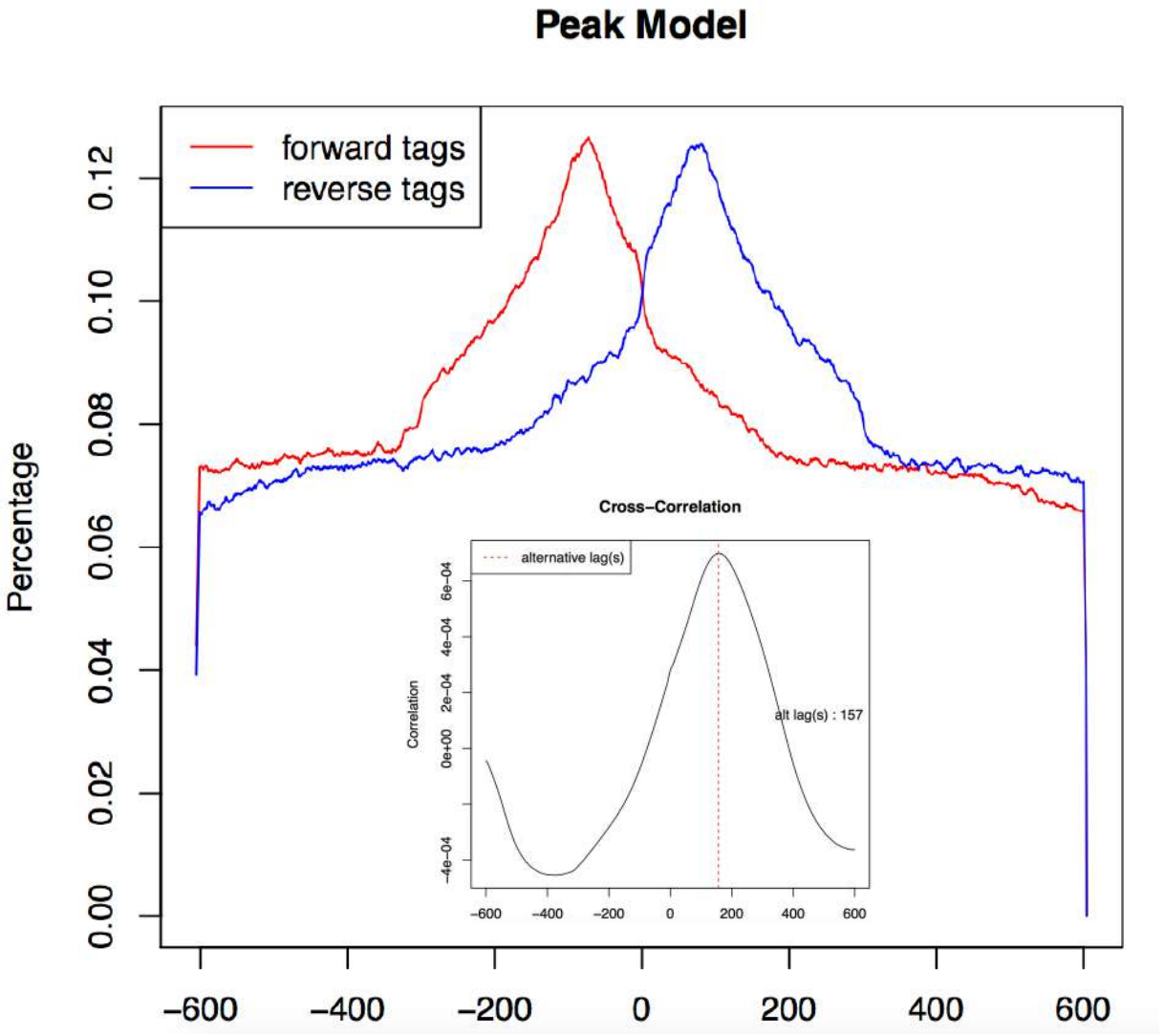
Bailey et al. 2013.
PLoS Comp. Biol.



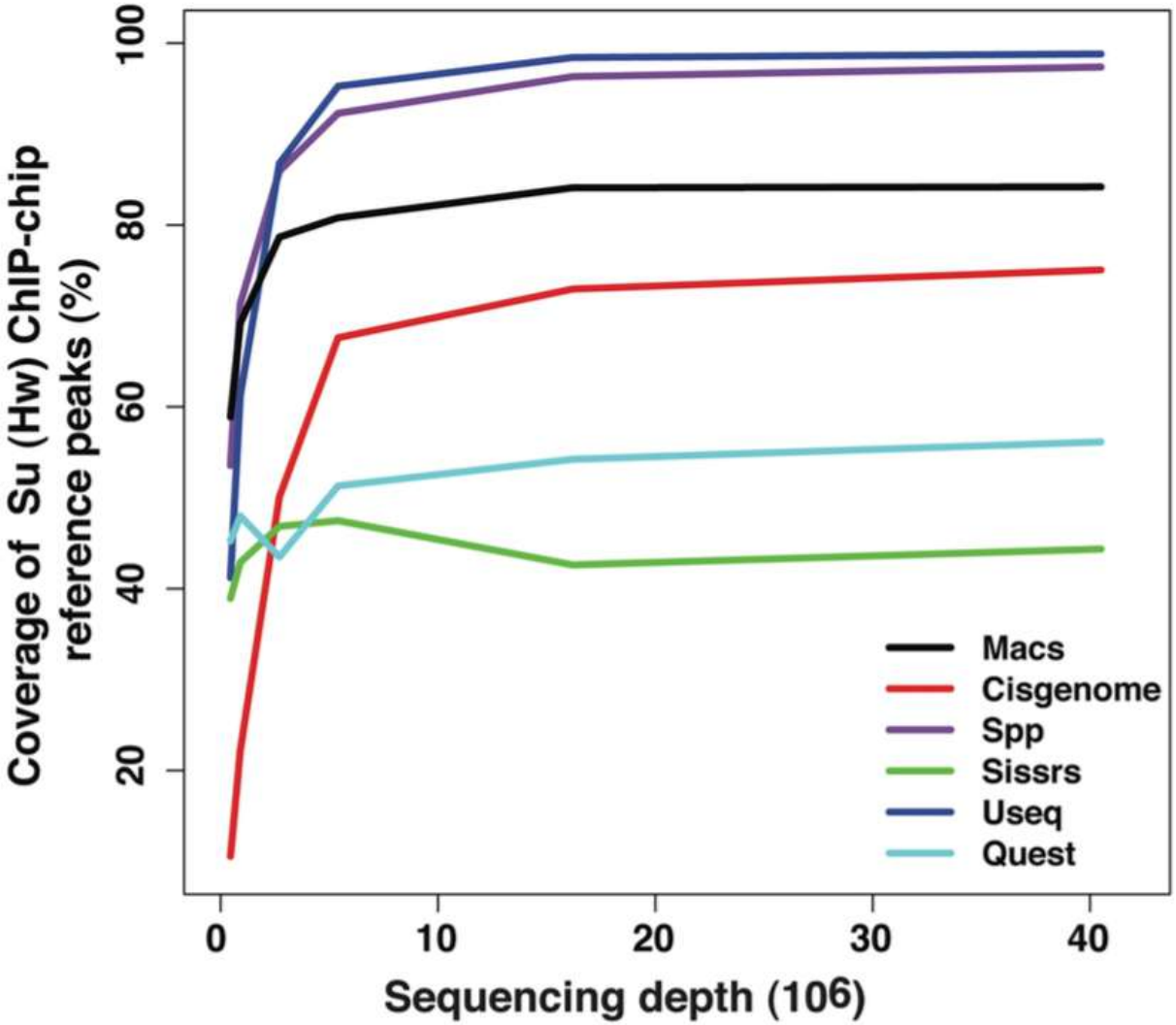
Valouev et al. 2008.
Nat. Methods

Shift modeling: MACS2

Given a sonication size (*bandwidth*) and a high-confidence fold-enrichment (*mfold*), **MACS** slides *2 bandwidth* windows across the genome to find regions with tags more than *mfold* enriched relative to a random tag genome distribution

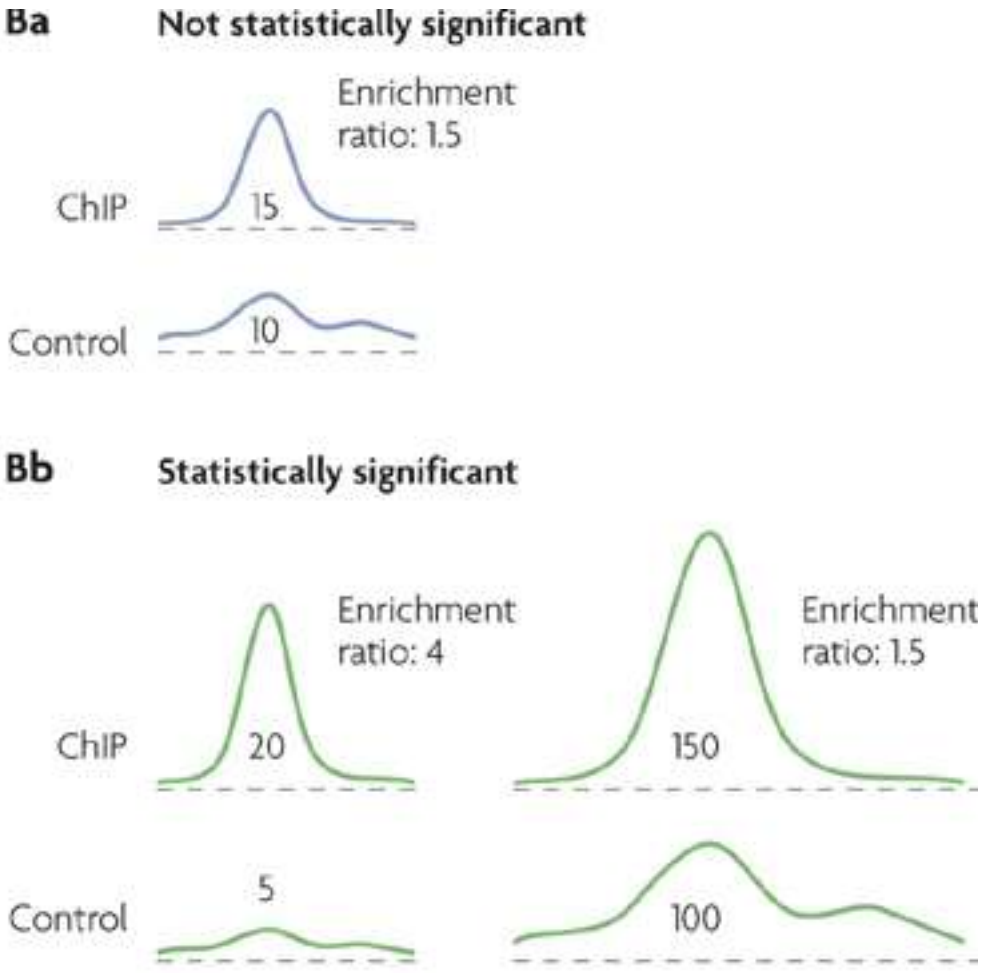


Sequencing depth

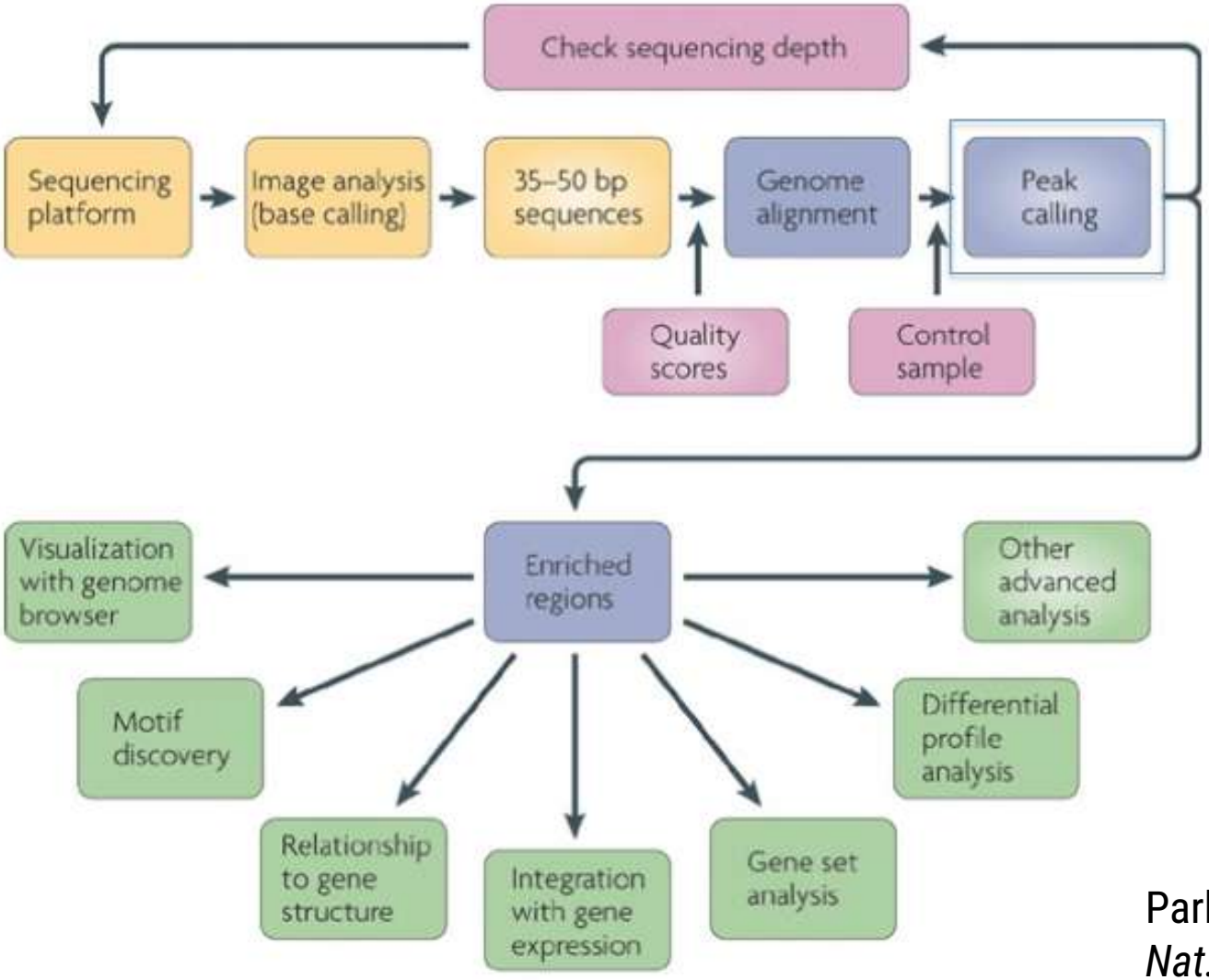


Chen et al. 2013.
Nat. Methods

Sequencing depth



Park et al. 2009.
Nat. Rev. Genet.



Park et al. 2009.
Nat. Rev. Genet.