Chip-seq - Analysis

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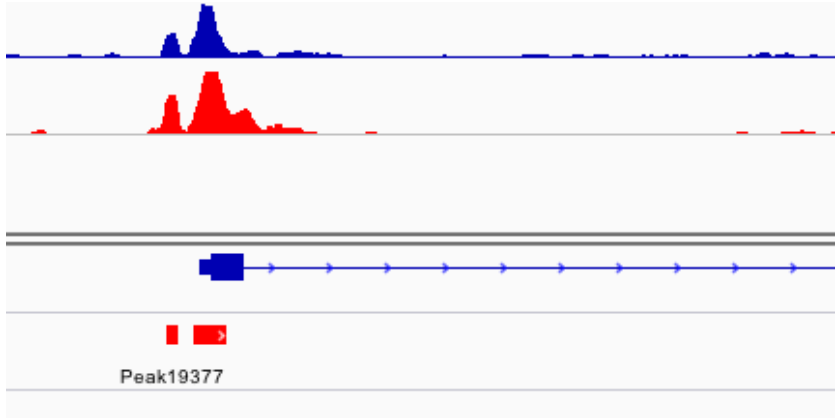
Bioinformatics analysis

Sequence file

`fastq`

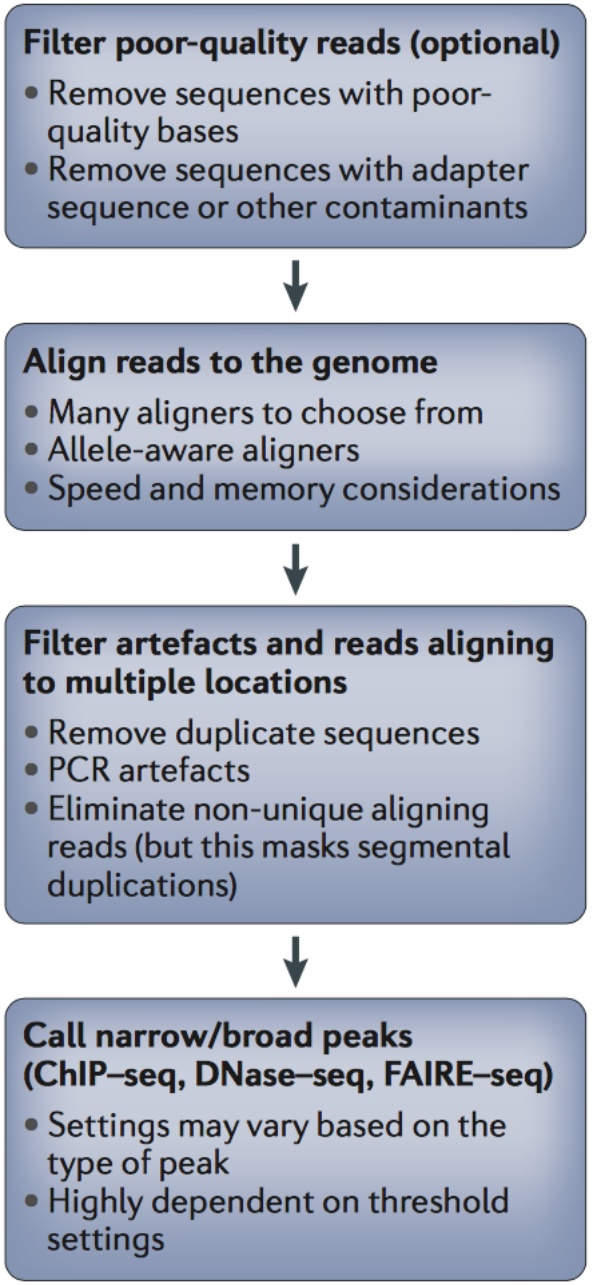
```
@SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
CAACGAGTTCACACCTTGGCCGACAGGCCCGGGTAA
+SRR038845.3 HWI-EAS038:6:1:0:1938 length=36
BA@7>B=>:>>7@7@>>9=BAA?;>52;>:9=8.=A
@SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
CCAATGATTTTTTTCCGTGTTTCAGAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474 length=36
BCCBA@BB@BBBBAB@B9B@=BABA@A:@693:@B=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

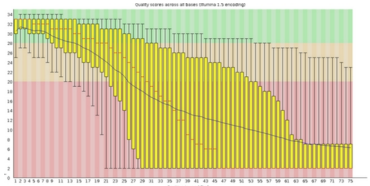
Steps



Furey 2012.
Nat. Genet. Rev.

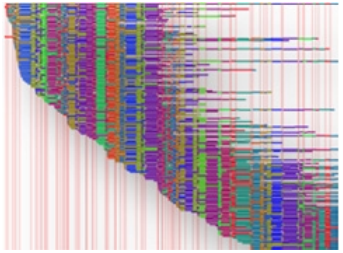
Steps, graphics

Quality control



Cleaning

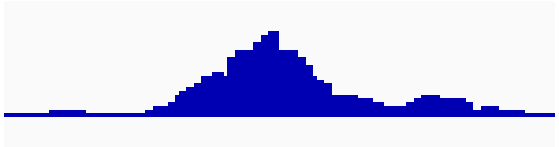
Mapping



Differential peak calling

Motif discovery

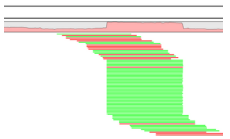
Peak calling



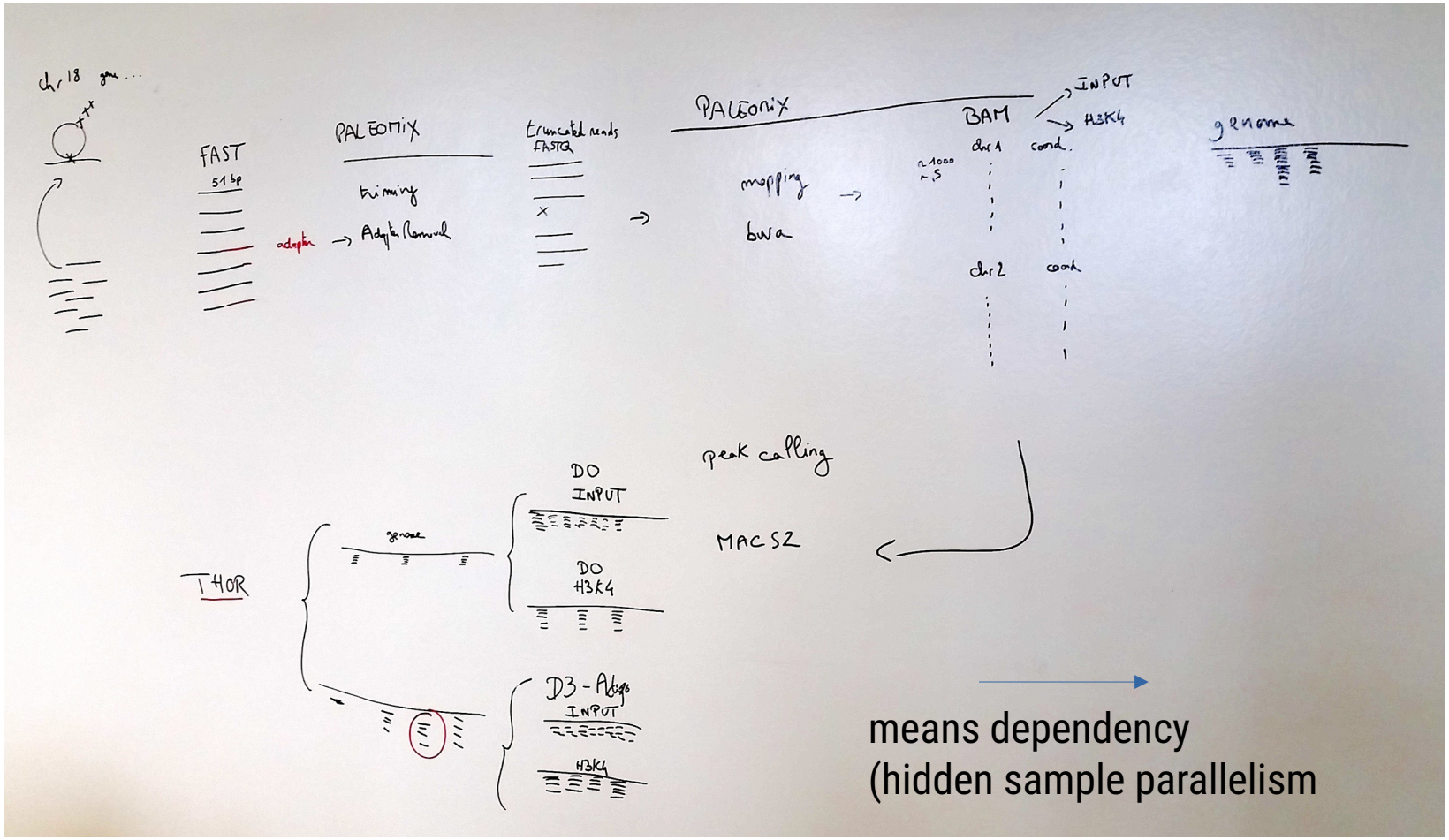
Signal normalization

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

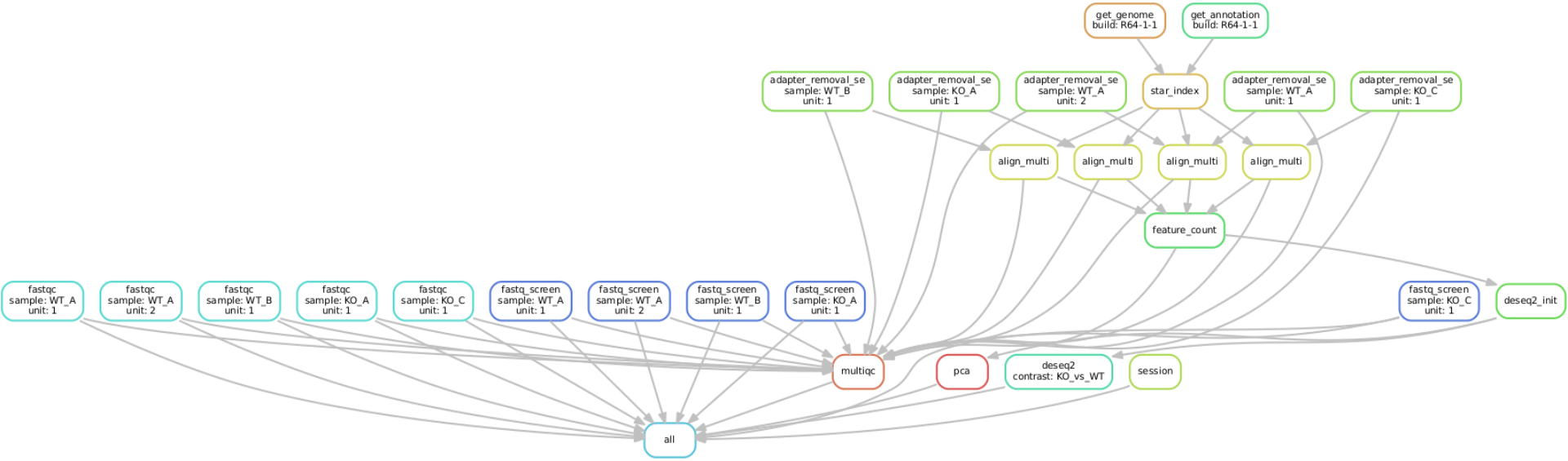
Solved issues

- 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 too
- 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?

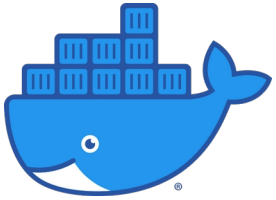
- it's boring
- On HPC one doesn't have admin rights
- modules prepared by the HPC teams are great, but more specific software are missing

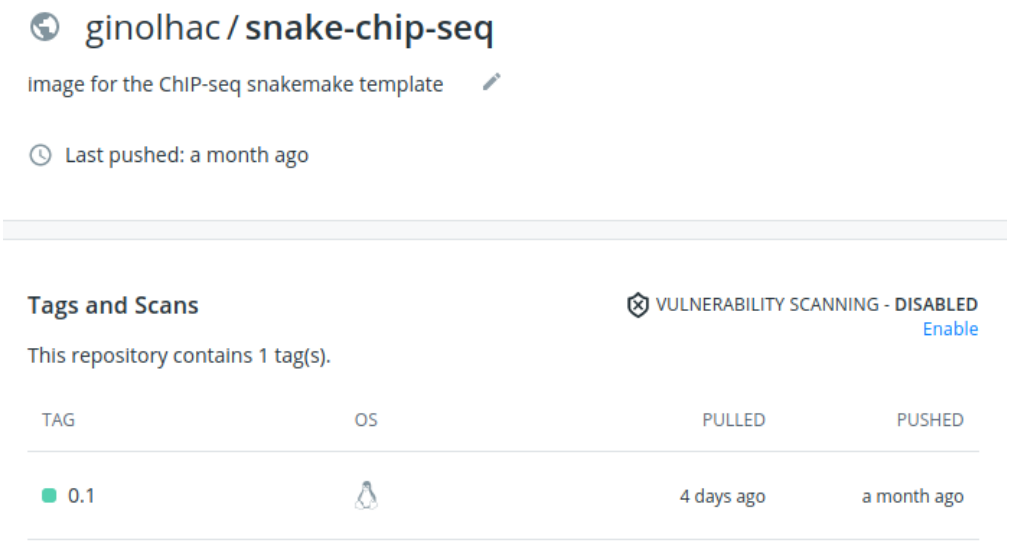
Docker

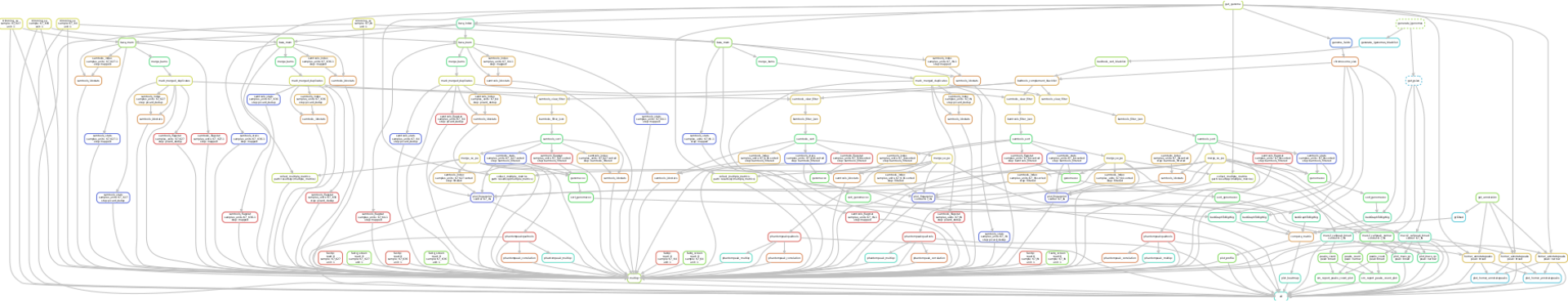
- is great but requires admin rights
- `singularity` is kind of docker for High Performance Computers

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

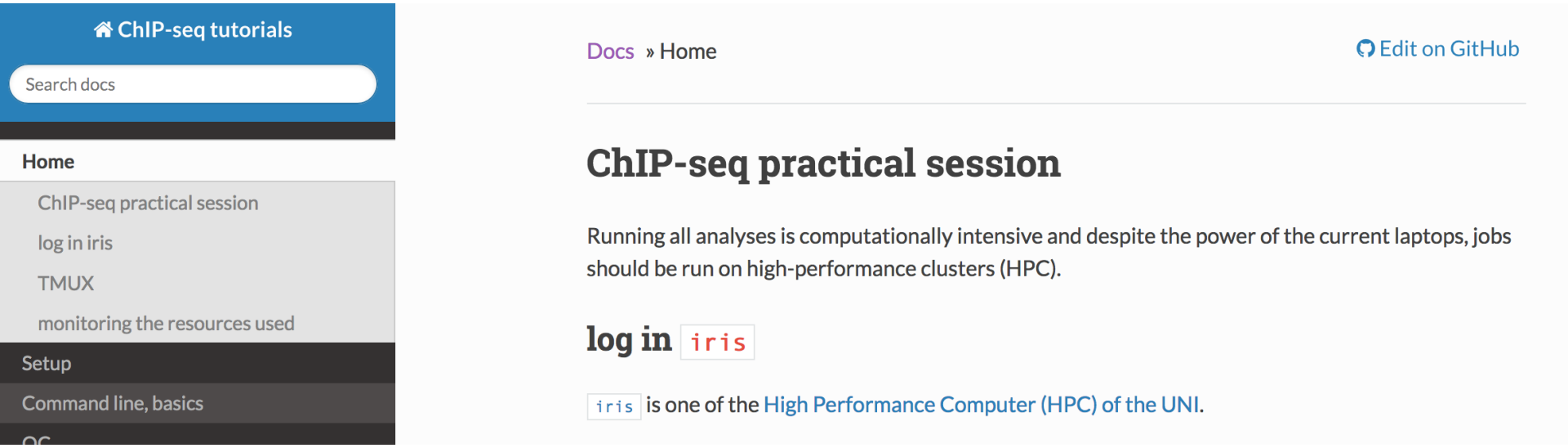
— Aurélien adapted from **Jenny Bryan**



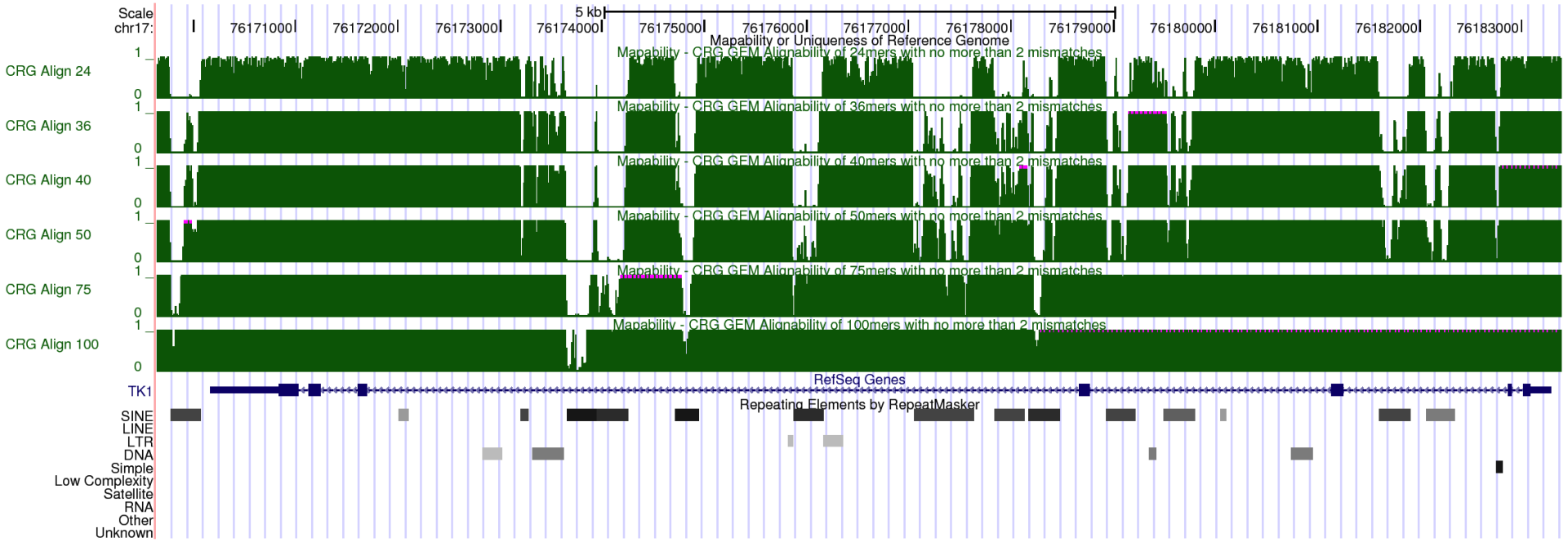




https://ginolhac.github.io/chip-seq/



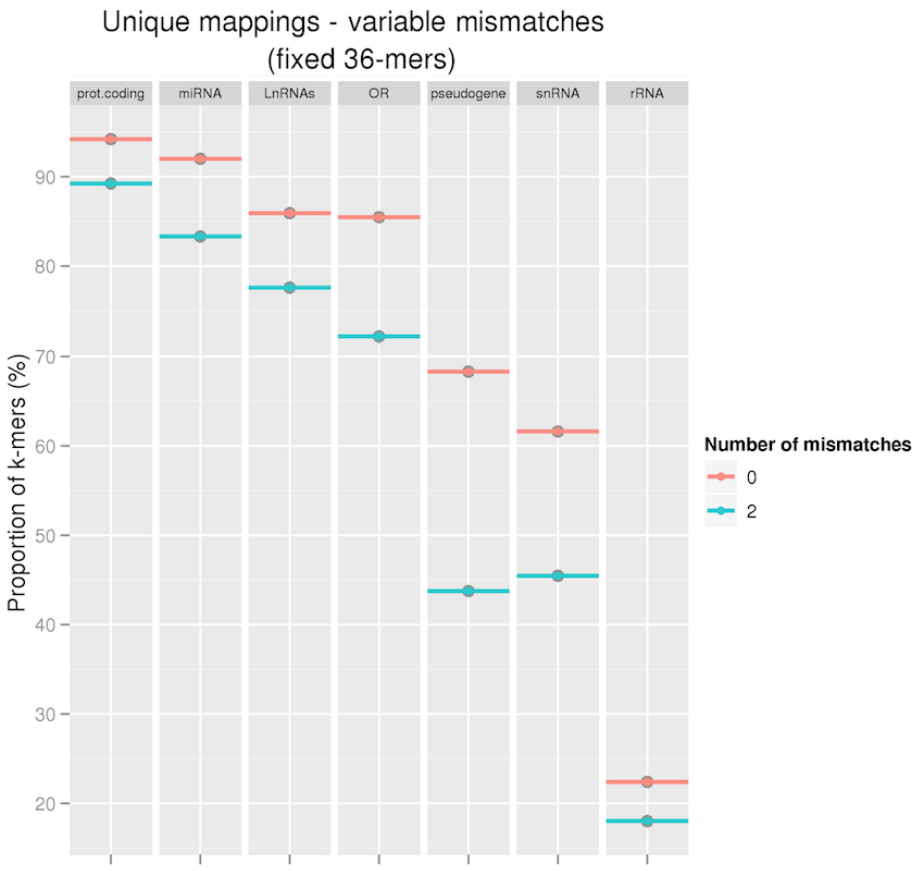
Mappability, causes



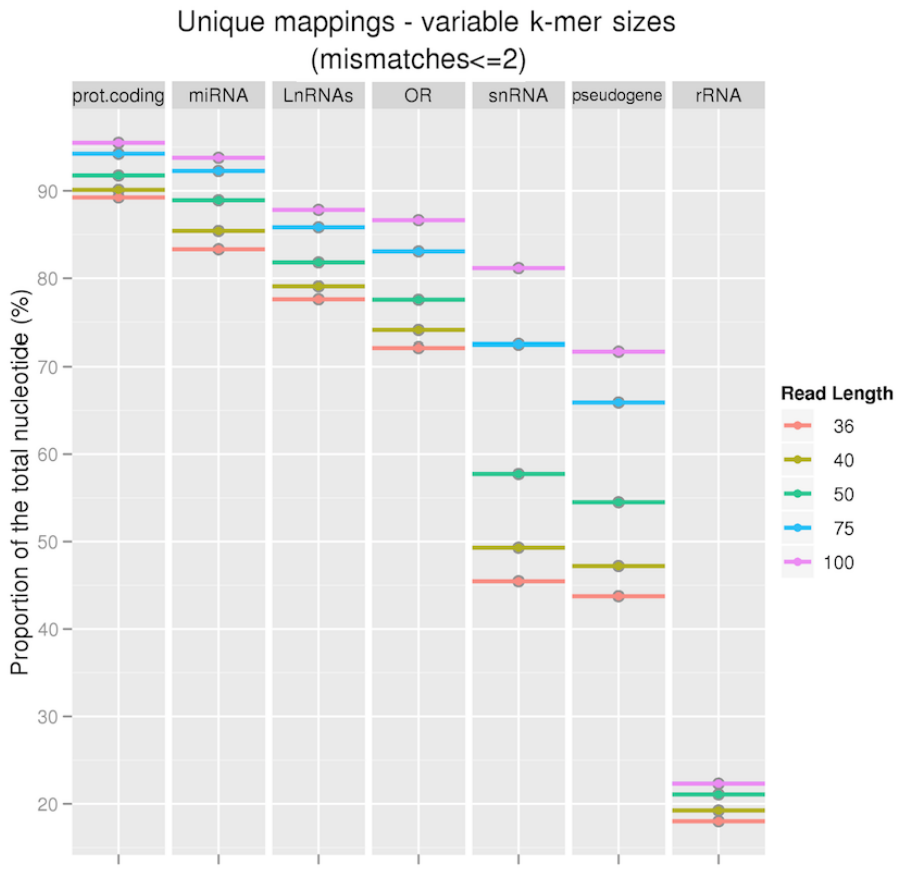
Mappability, consequences

Uniquely mapped

Mismatches

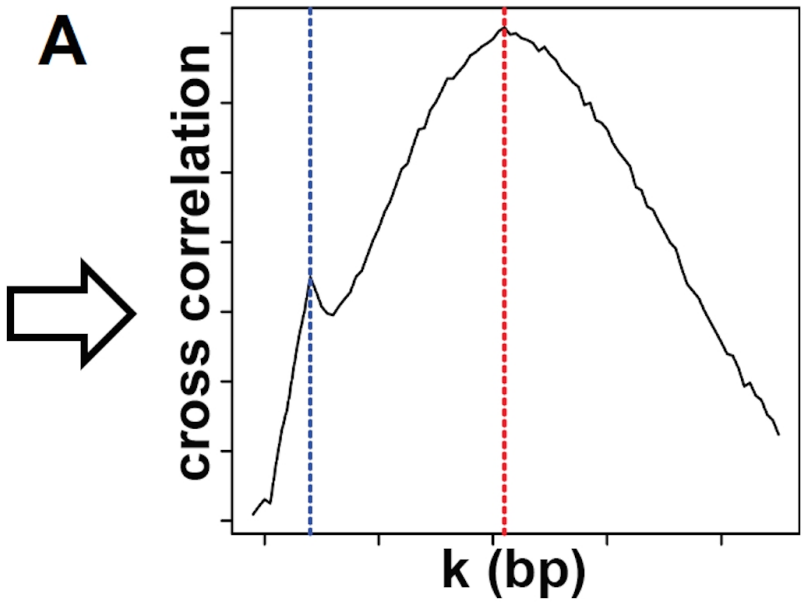


Read Length

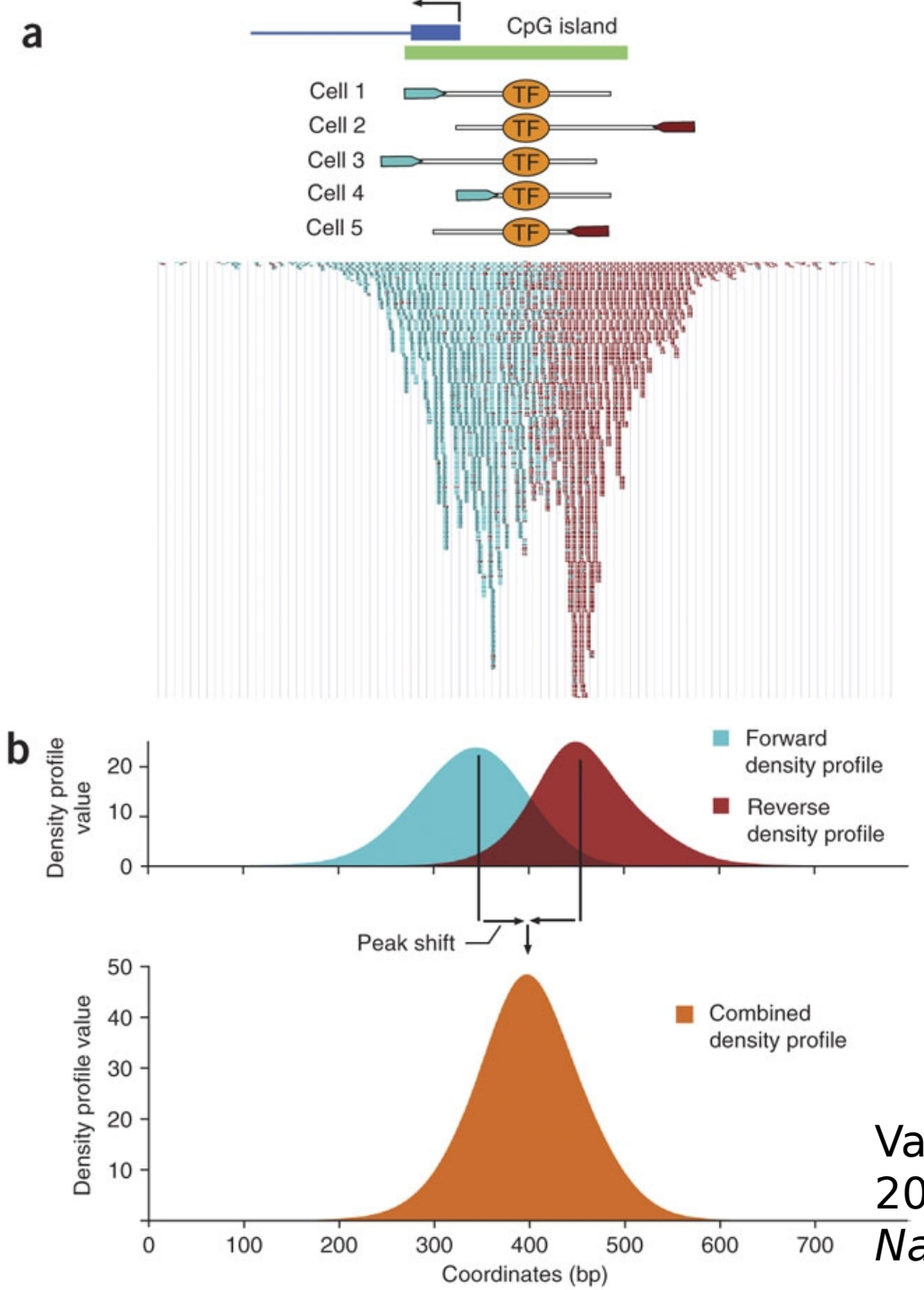


Derrien et al. 2013.
PLoS ONE

Peal calling, infer the shift size



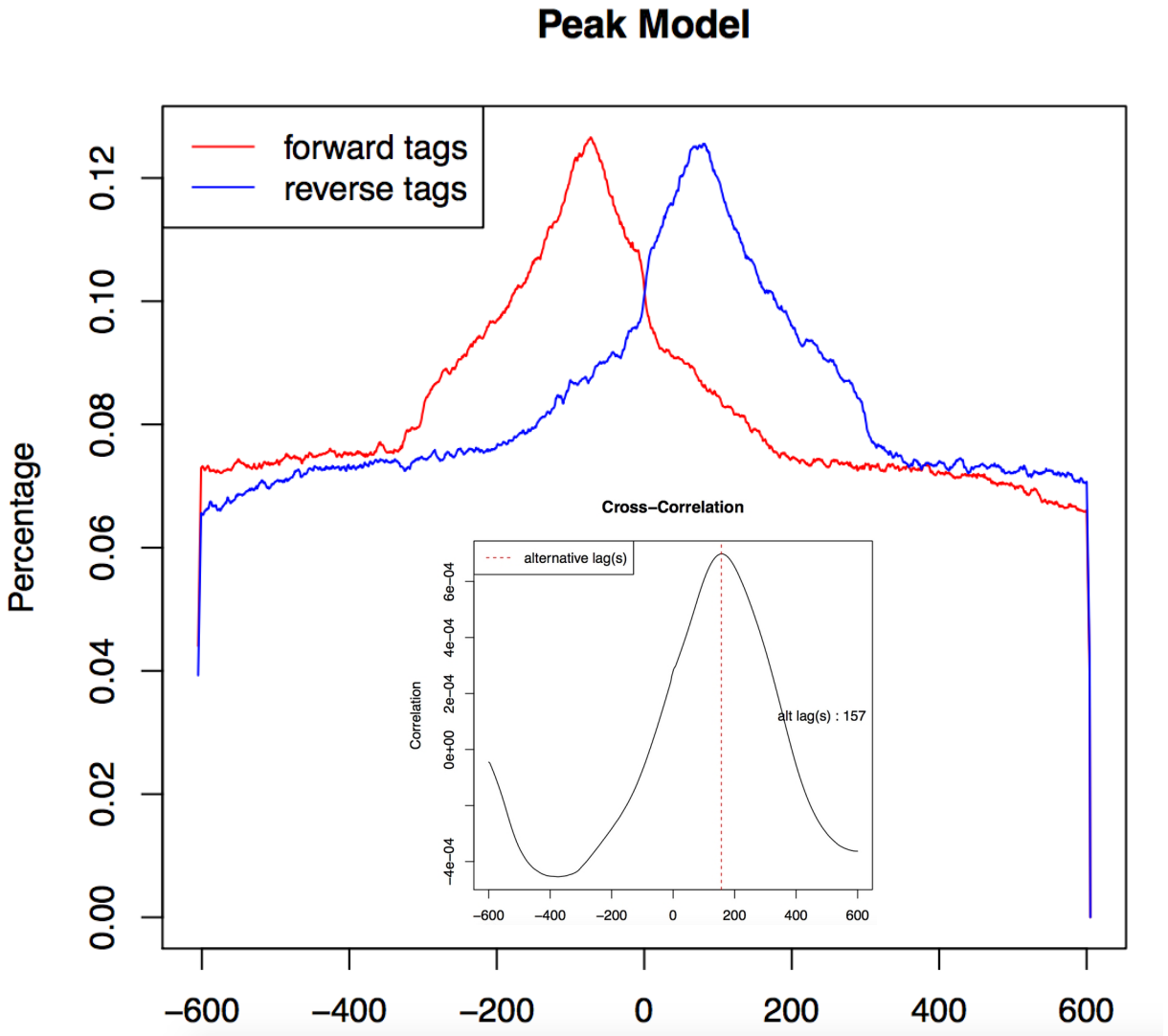
Bailey et al.
2013.
PLoS Comp. Biol.



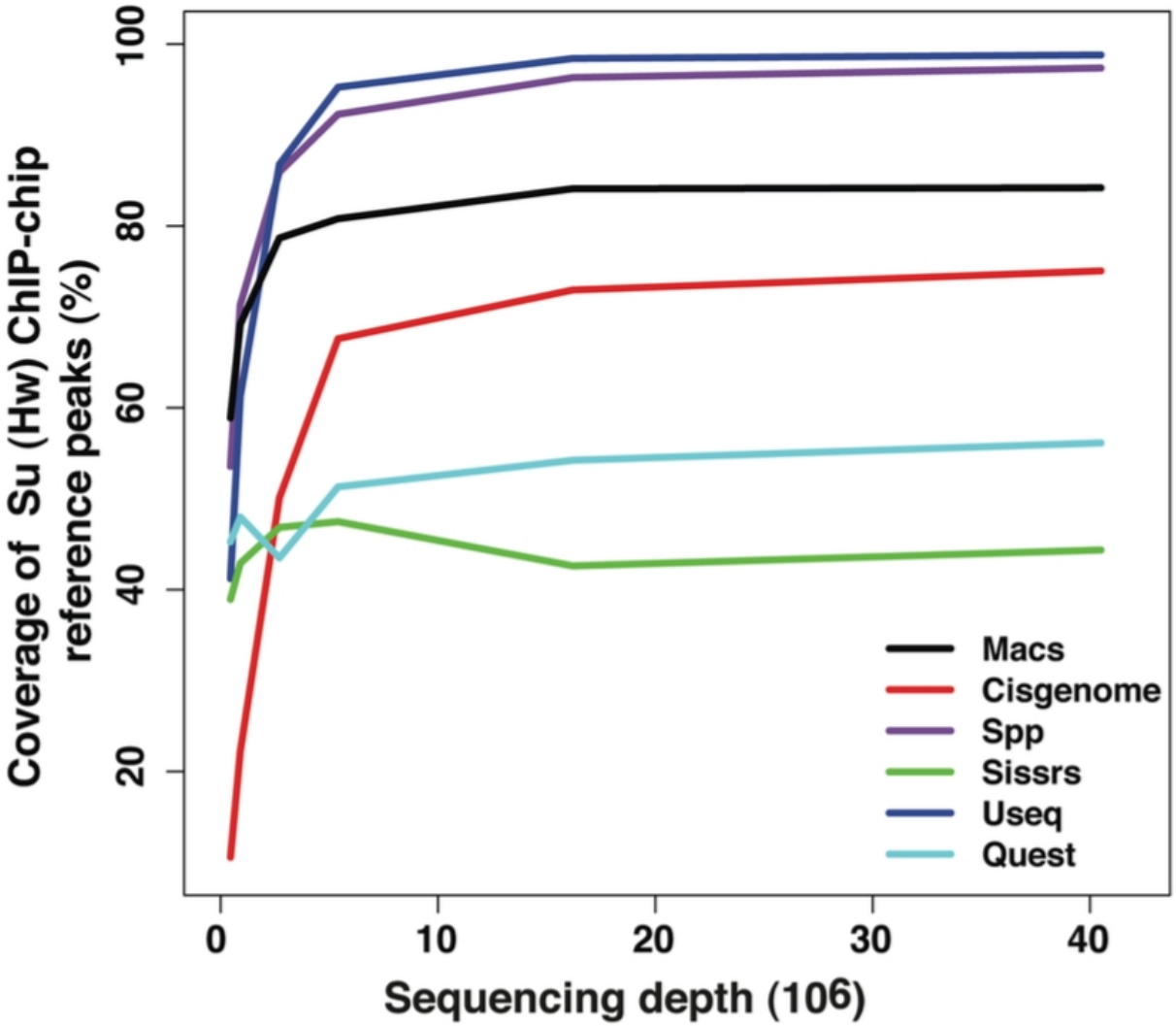
Valouev et al. 2008. *Nat. Methods*

Shift modeling: MACS2

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

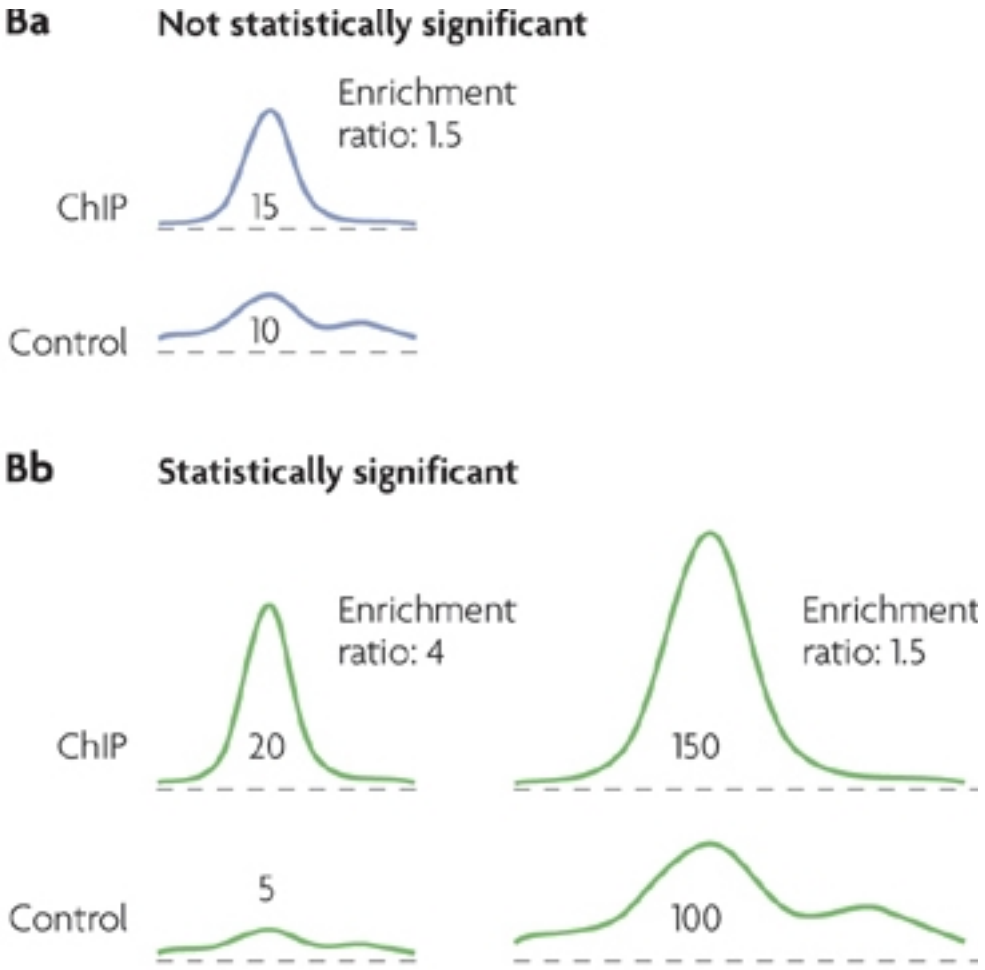


Sequencing depth

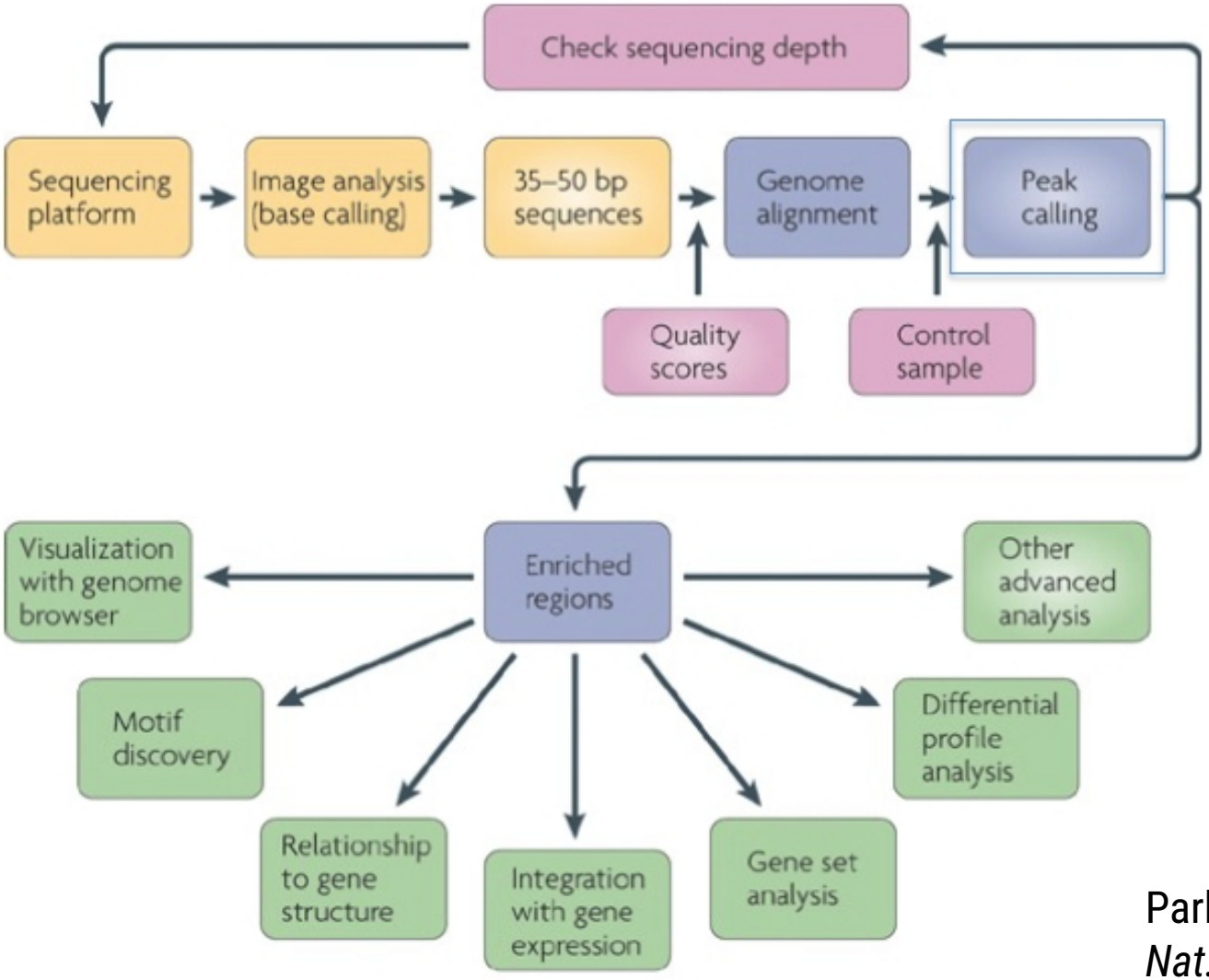


Chen et al. 2013.
Nat. Methods

Sequencing depth



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



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