Statistical Methods for Epigenetics

Yongjin Park (slide credit: Keegan Korthauer, Manolis Kellis)

What is epigenetics?

High-dimension?

Curse or blessing?

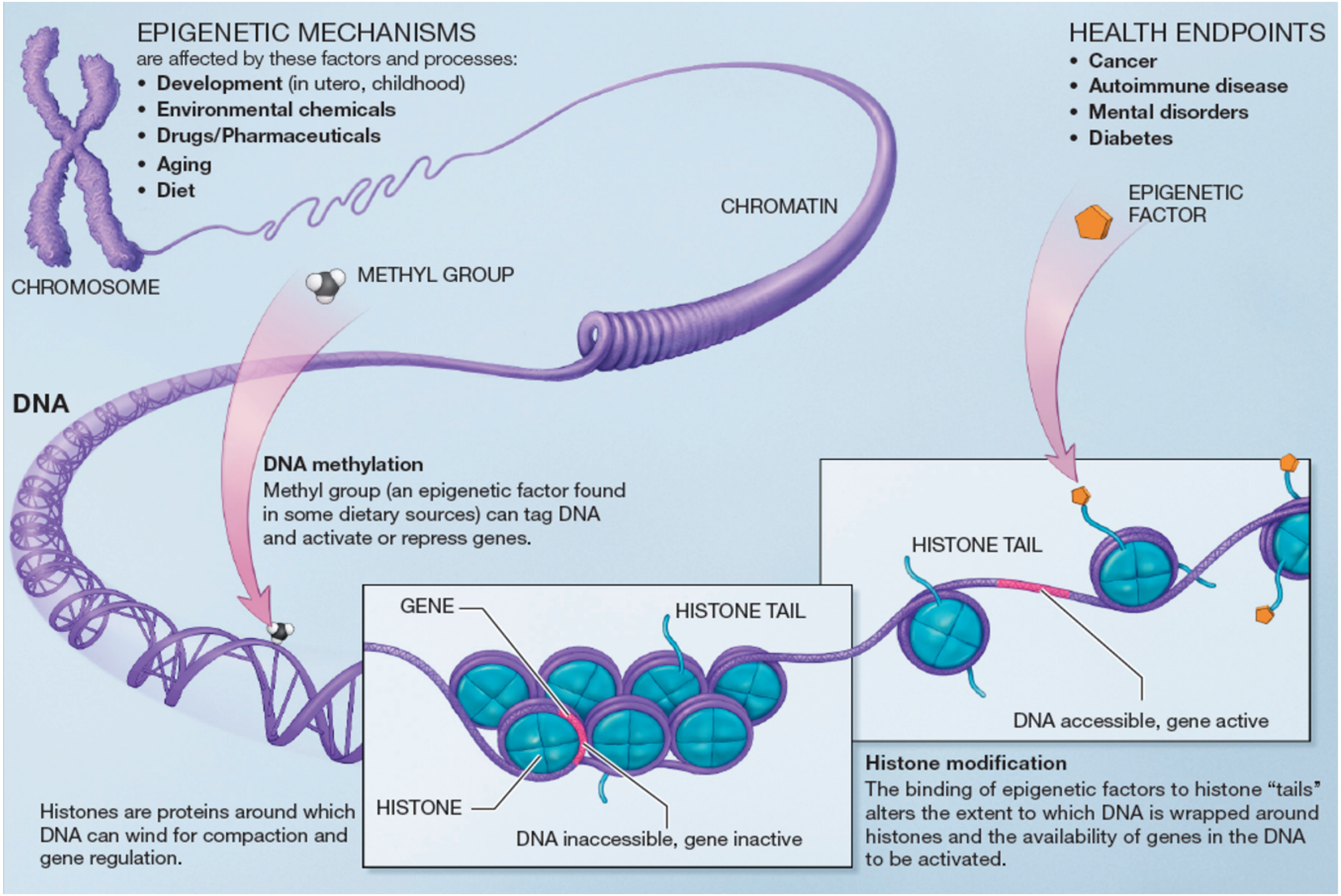
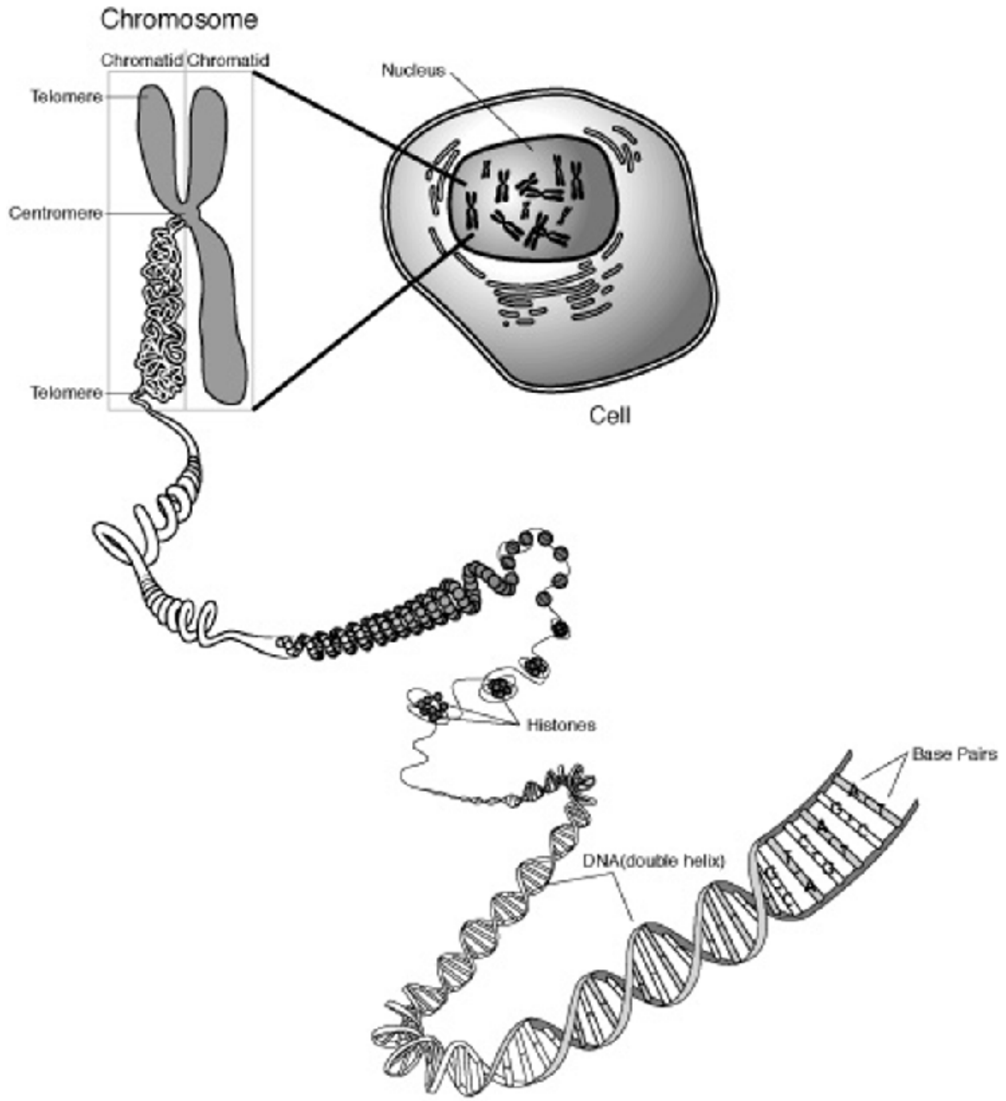


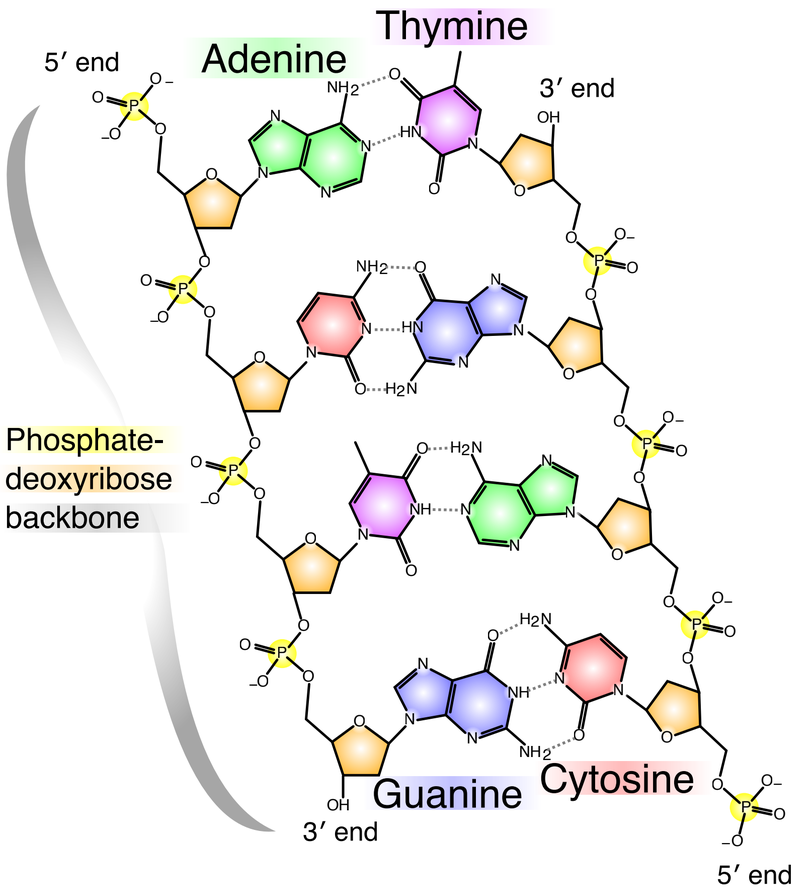
Image source: http://nihroadmap.nih.gov/epigenomics/

Statistical Methods for Epigenomics

- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)

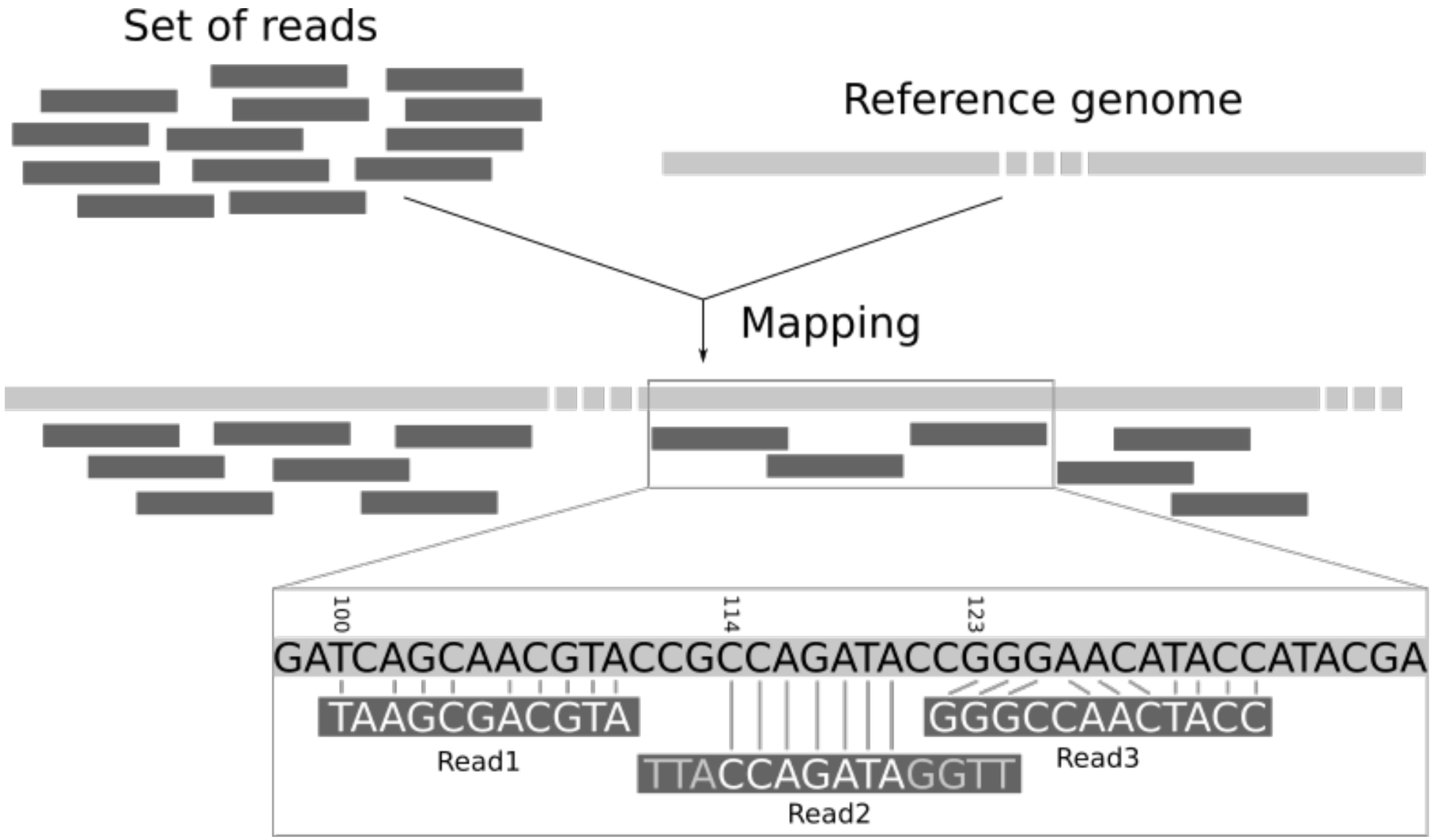
A reference genome can serve as an efficient computing tool (a template for matching)



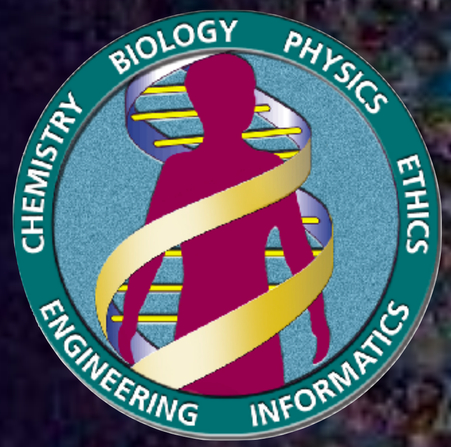


Given reference, we can look up locations

Short fragments/reads to reference genome



Human Genome Project



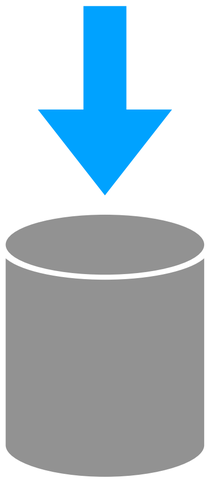
A reference DNA sequence for
3.2B basepairs x diploid for each individual

https://www.genome.gov/human-genome-project

How do we align/map/match a short read to known genomic DNAs?

Computational Method

```
TTATATTGAATTTTCAAAAATTCTTACTTTTT
TTTTGGATGGACGCAAAGAAGTTTAATAAT
CATATTACATGGCATTACCACCATATACATA
TCCATATCTAATCTTACTTATATGTTGTGGA
AATGTAAAGAGCCCCATTATCTTAGCCTAA
AAAAACCTTCTCTTTGGAACTTTCAGTAAT
ACGCTTAACTGCTCATTGCTATATTGAAGT
```



Index reference genome for a quick lookup (e.g., BWT, Bloom filter)

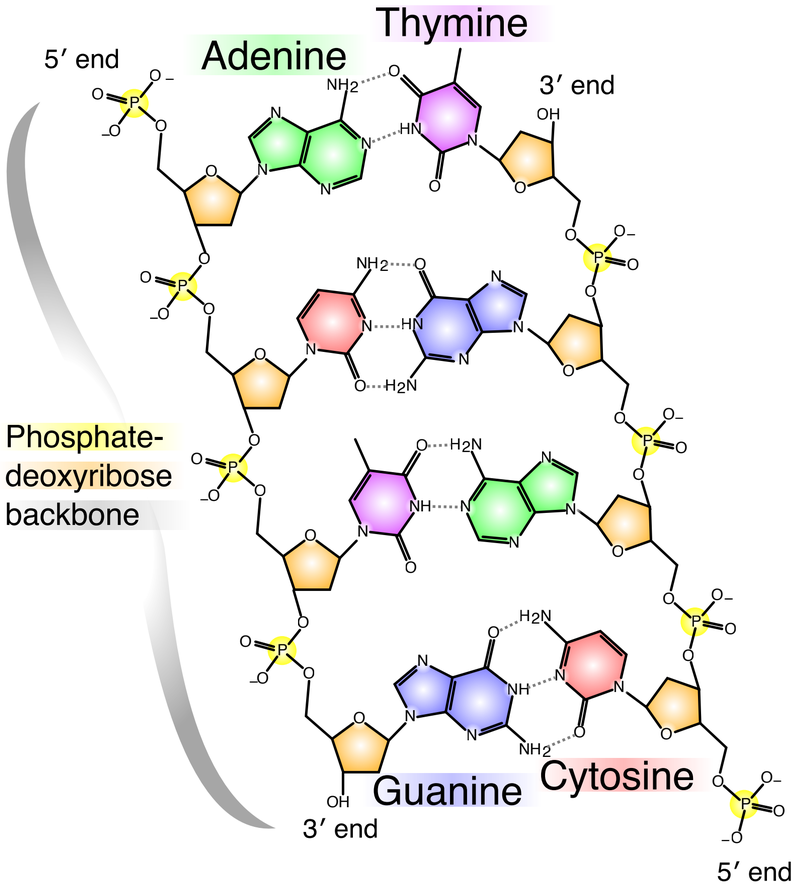
GCCCCATTATCTT?

vs.

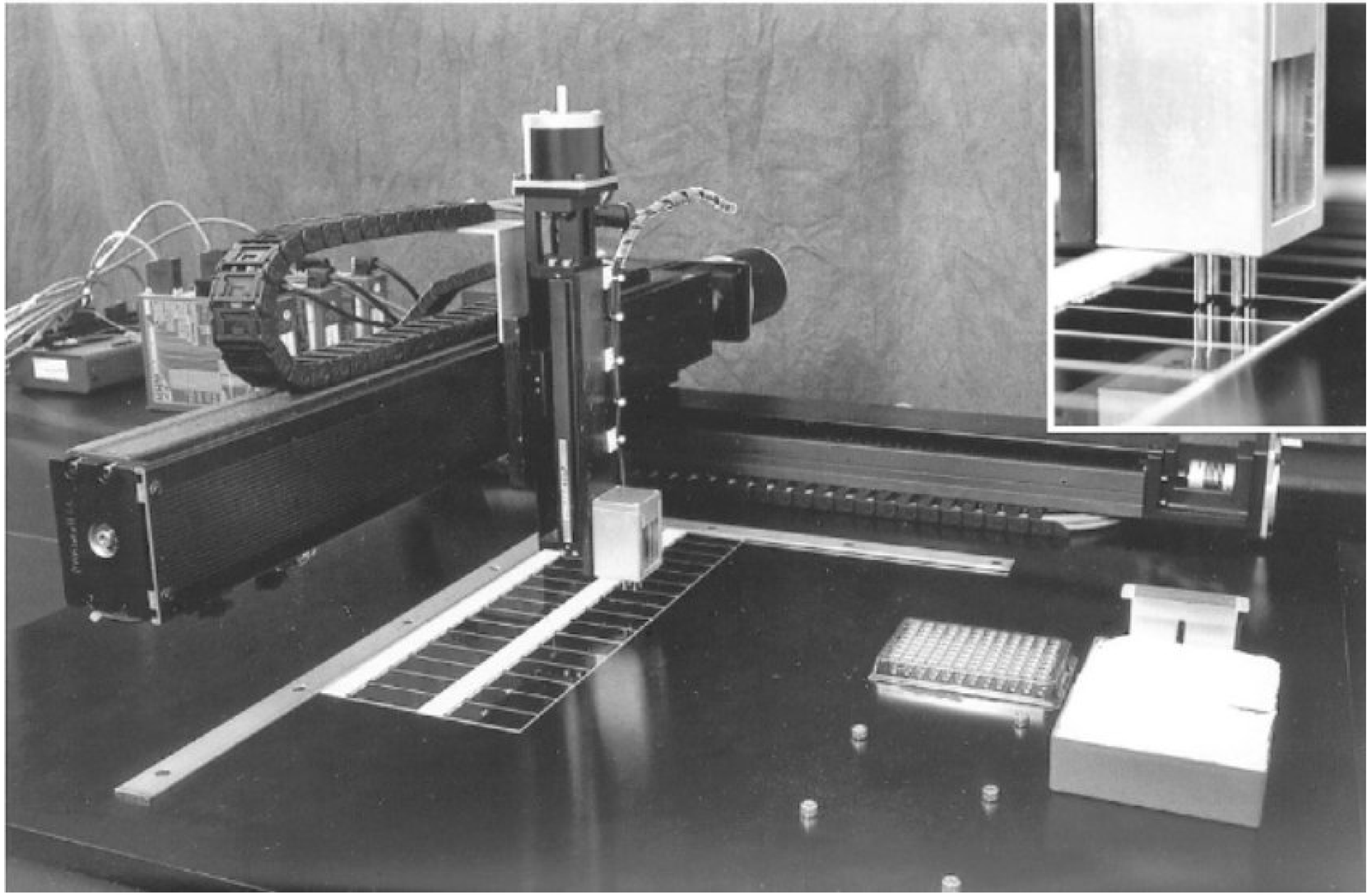
GCCTTTTTATCTT?

Can we fine this short read? If so, where?

Biochemical Method

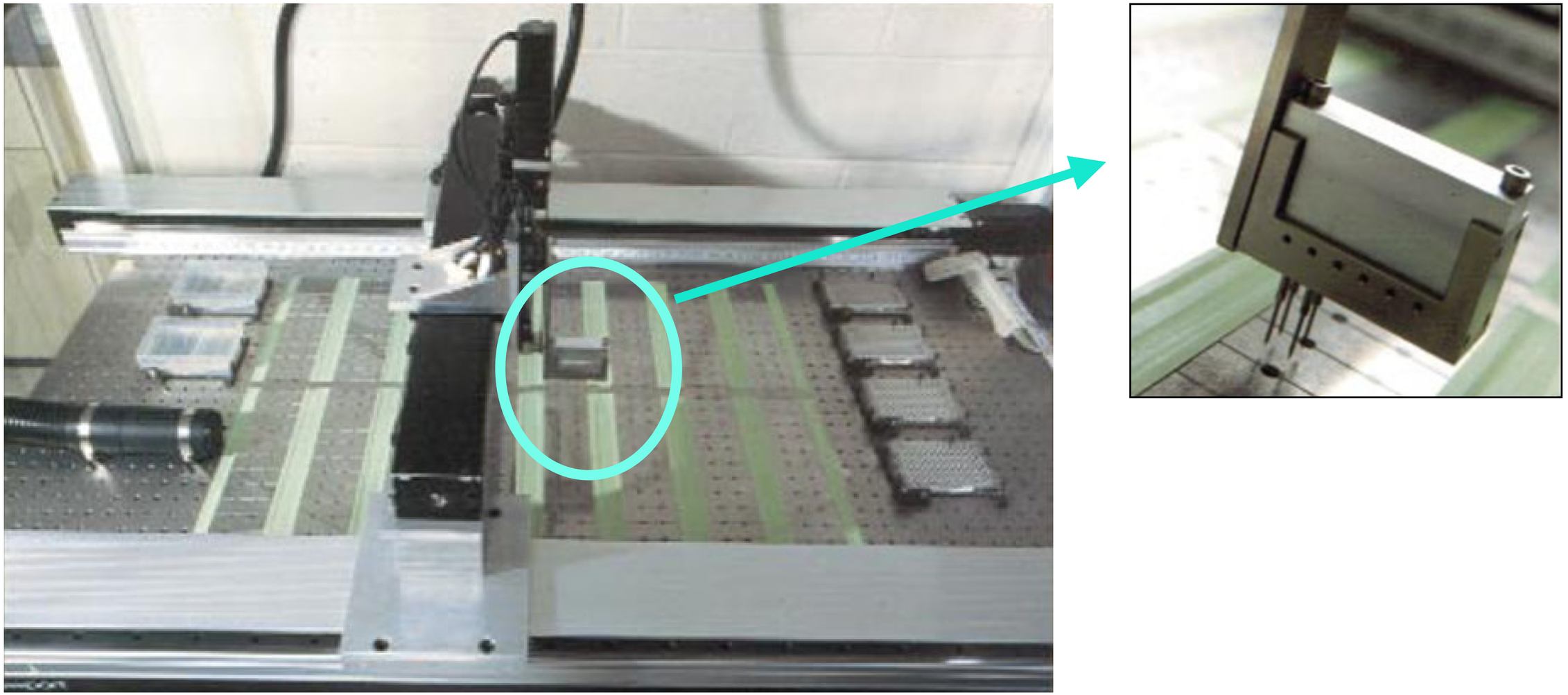


A long time ago…
Array technology (Pat Brown's pipetting machine)



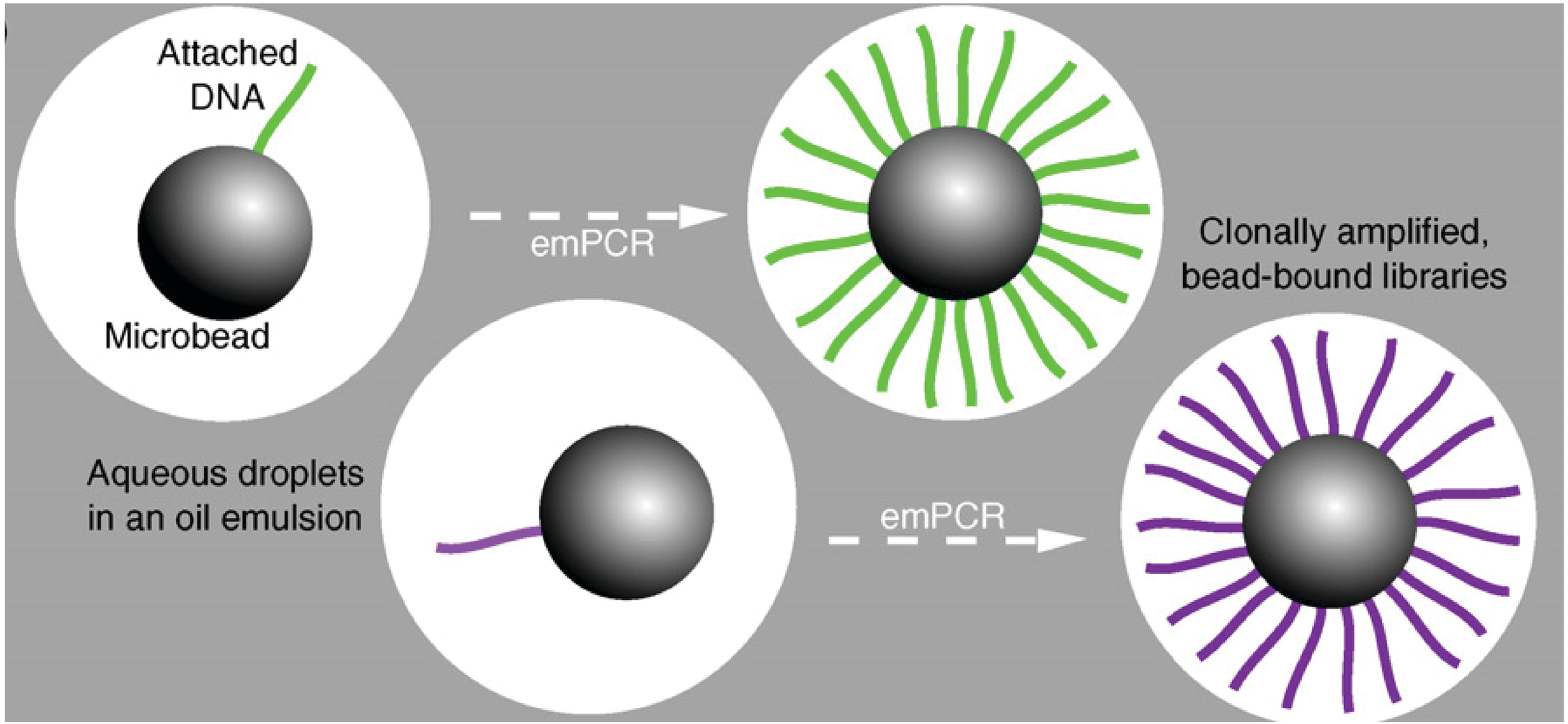
Cheung *et al*. (1999)

Pat Brown's pipetting machine



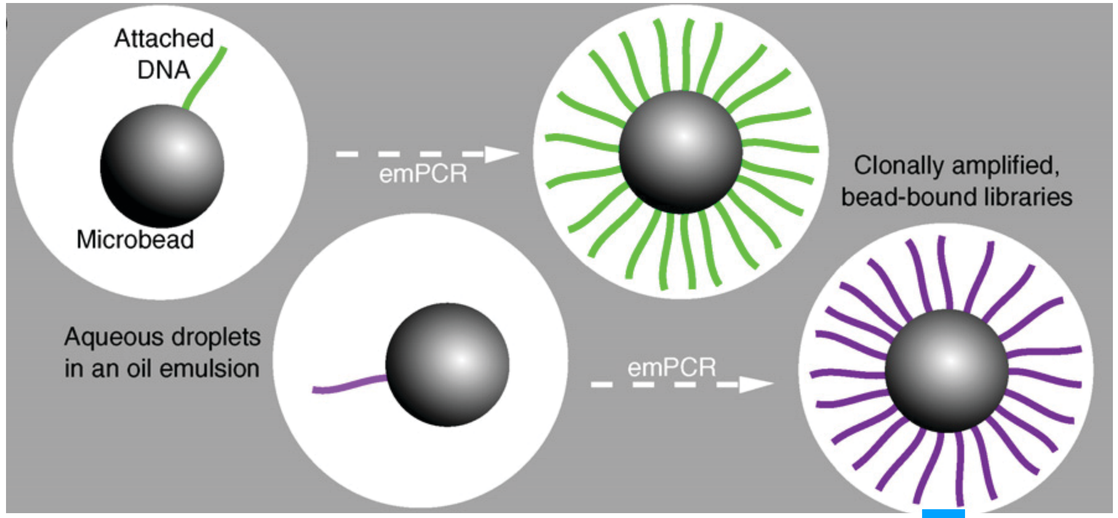
Cheung *et al*. (1999)

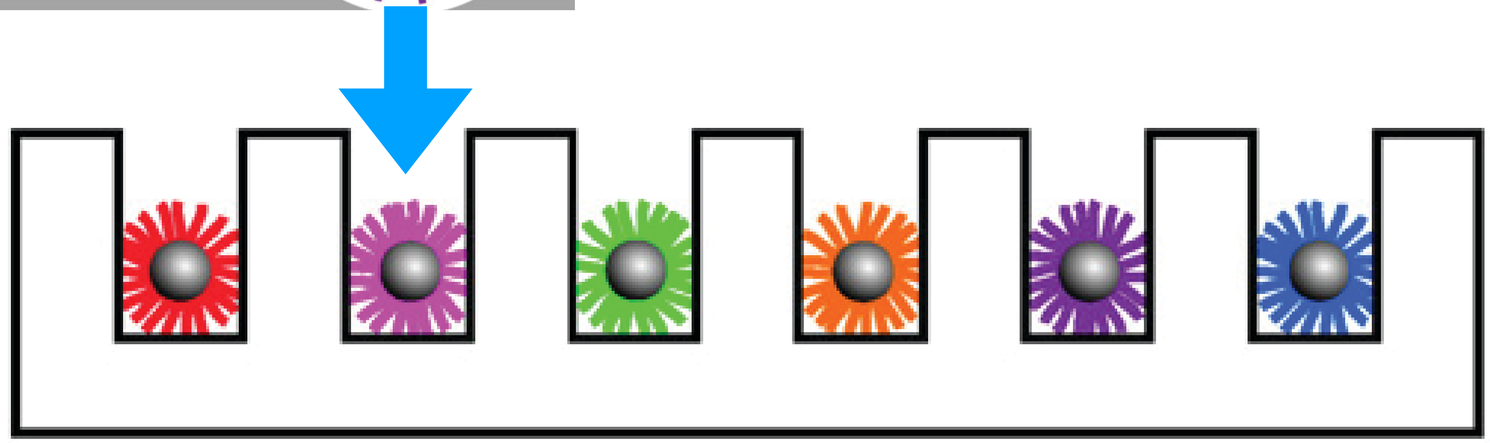
Micro bead-based applications followed by massively parallel short read sequencing



J.M. Heather, B. Chain /Genomics 107 (2016) 1–8

Micro bead-based applications followed by massively parallel short read sequencing

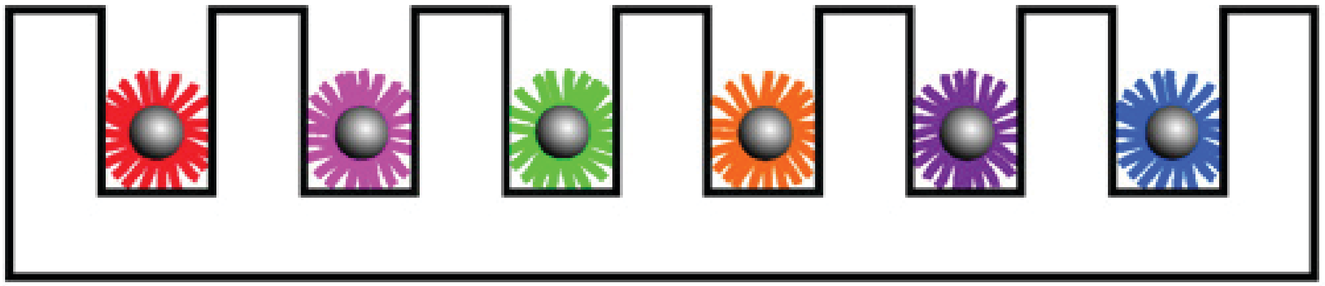




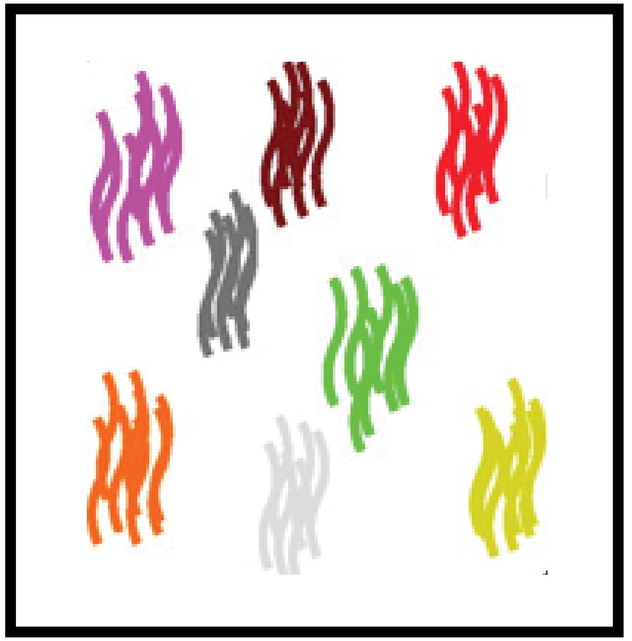
Picotitre wells containing DNA-bearing microbreads

J.M. Heather, B. Chain /Genomics 107 (2016) 1–8

Micro bead-based applications followed by massively parallel short read sequencing



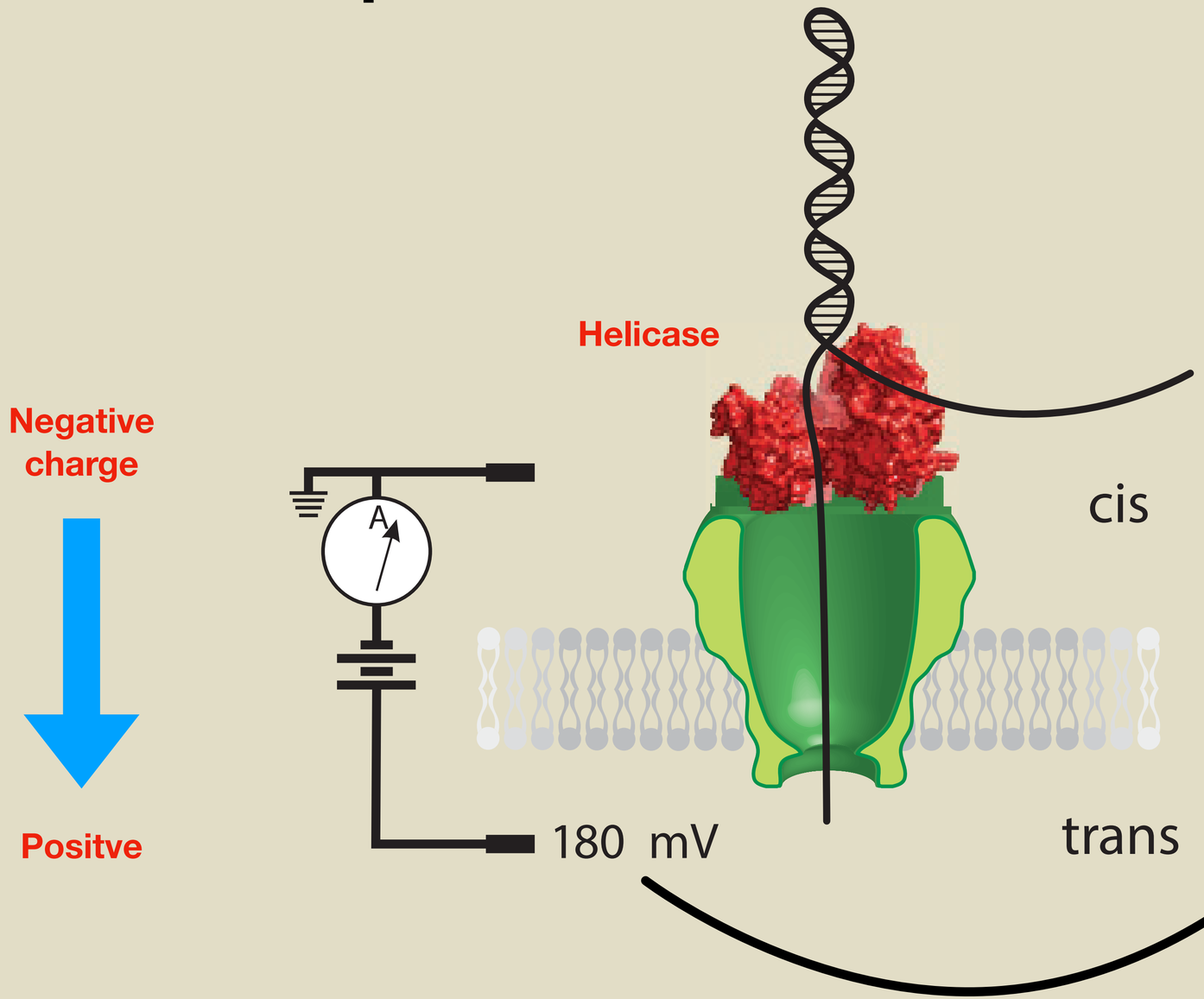
Picotitre wells containing DNA-bearing microbreads

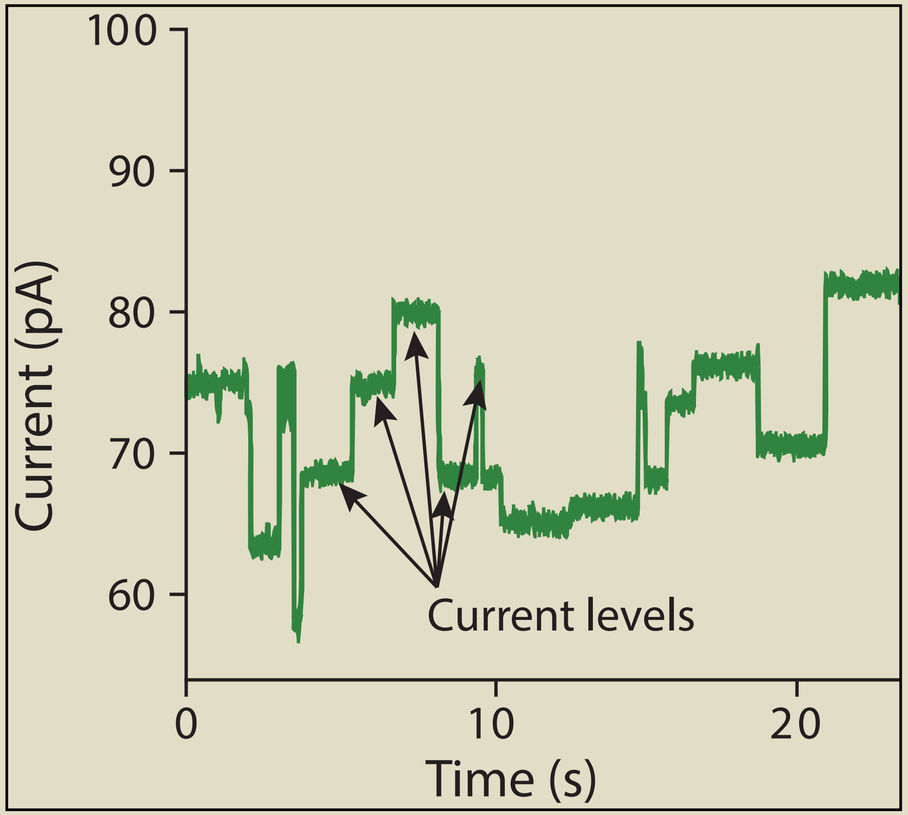


Flow cell-bound clusters

J.M. Heather, B. Chain /Genomics 107 (2016) 1–8

Nanopore: Yet another method for sequencing

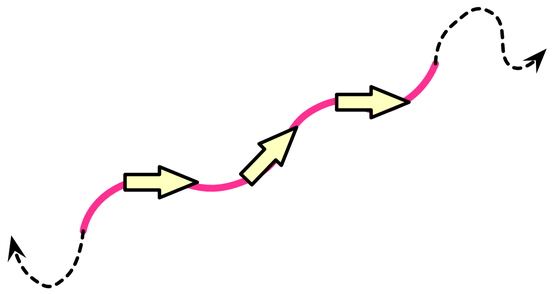




- The ionic conductivity is sensitive to the presence of the nucleotide.
- Charatersitic "squiggle"

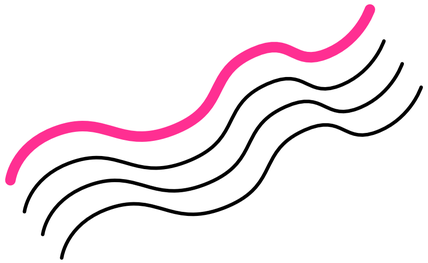
Deamer, Akeson, Branton, Nature Biotech. (2016)

The basic idea of High-throughput methods: selection followed by sequencing



some region
of interest
(gene, regulatory
elements, etc.)

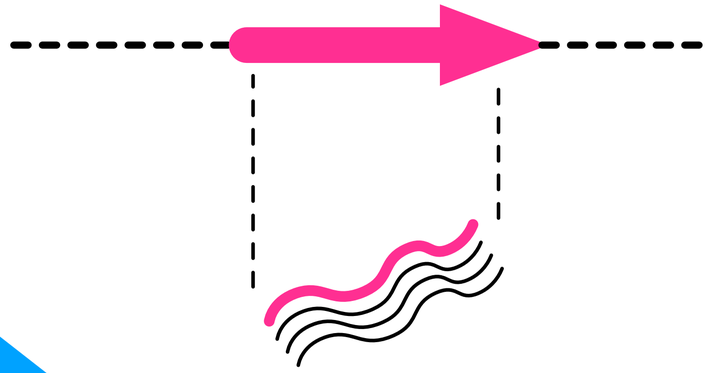
Genome ≈ an anchor point



some technology
to selectively
pool & copy the
region of interest

How many unique short reads were found?

Which regions and how do we select the region?



Align/match
to locate this region
in the genome

Statistical Methods for Epigenomics

- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)

Epigenetic modifications

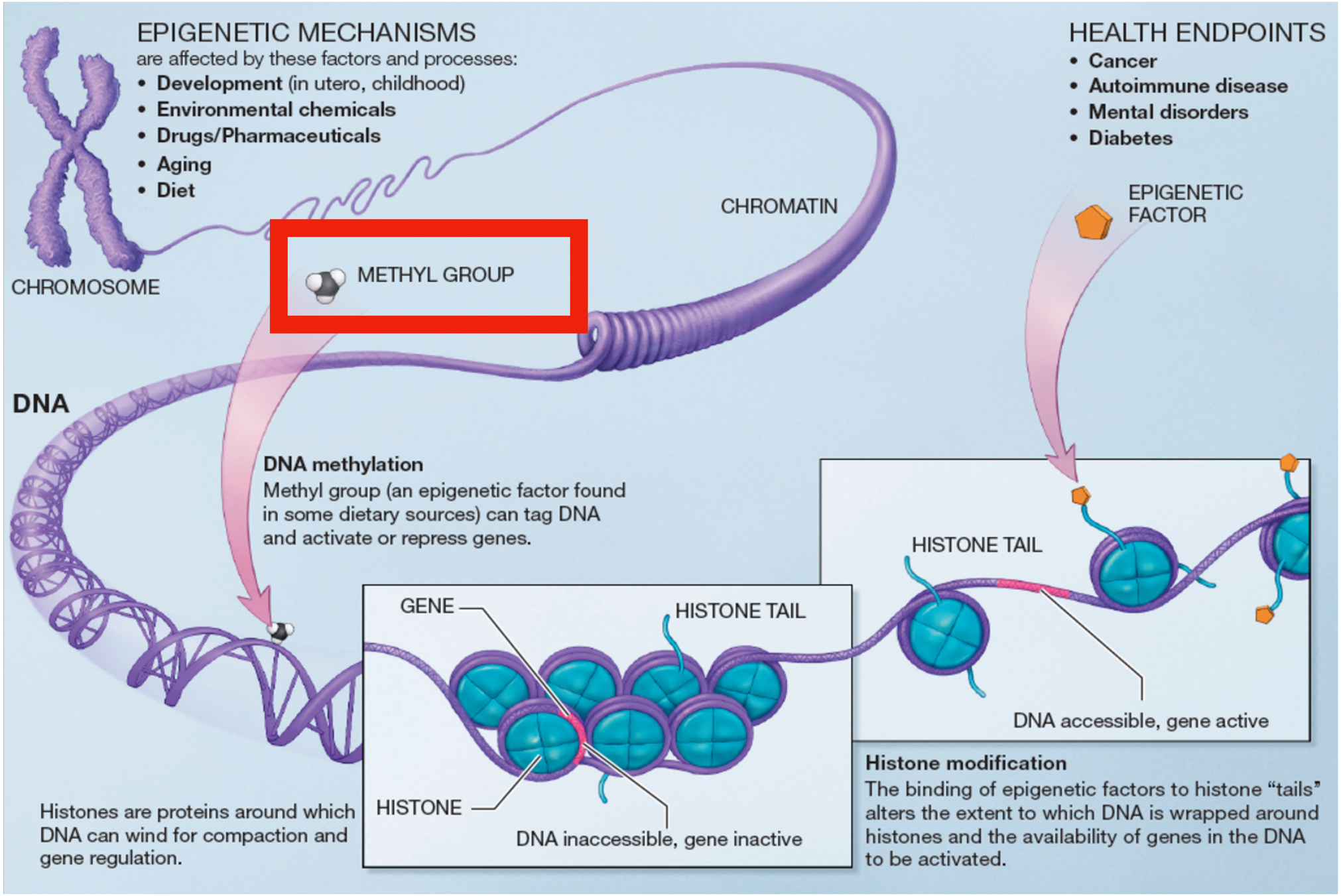
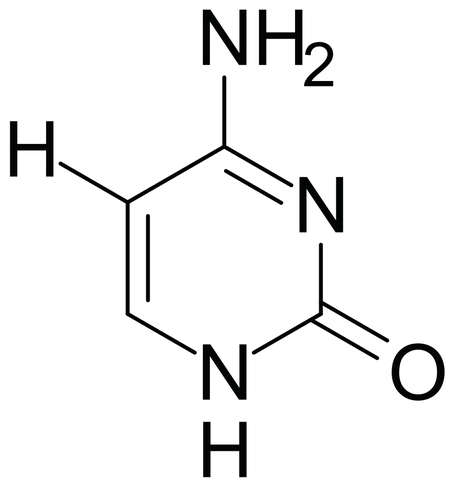
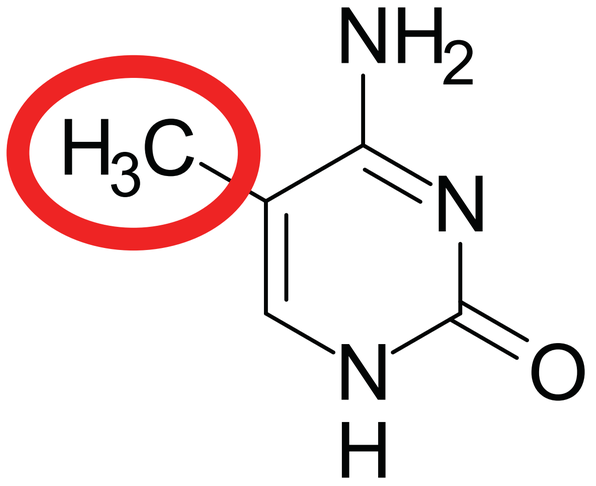


Image source: http://nihroadmap.nih.gov/epigenomics/

DNA methylation modifies the biochemical properties of a particular base



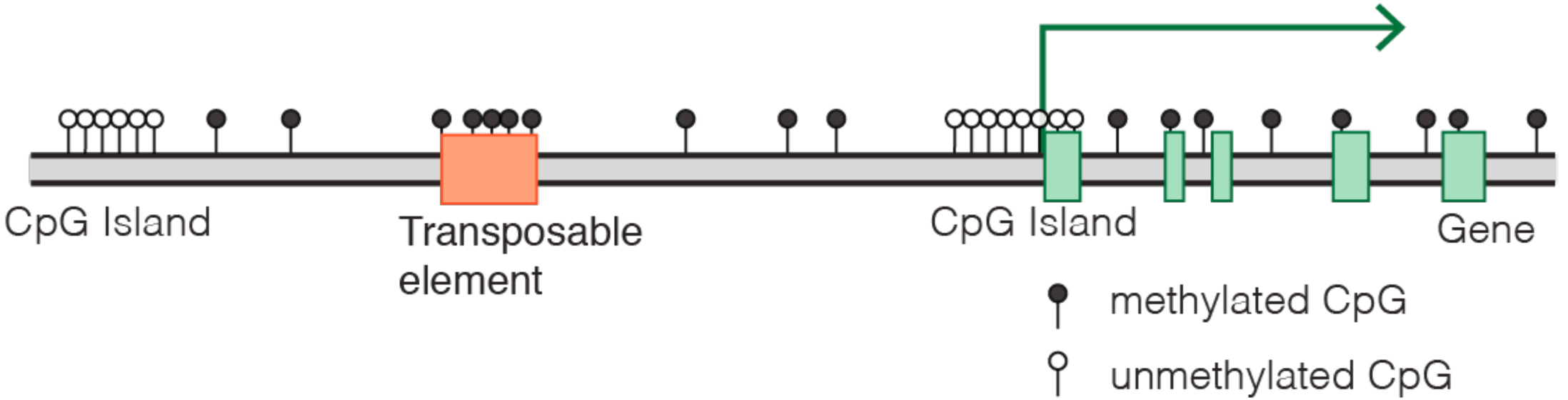
cytosine



methylated cytosine

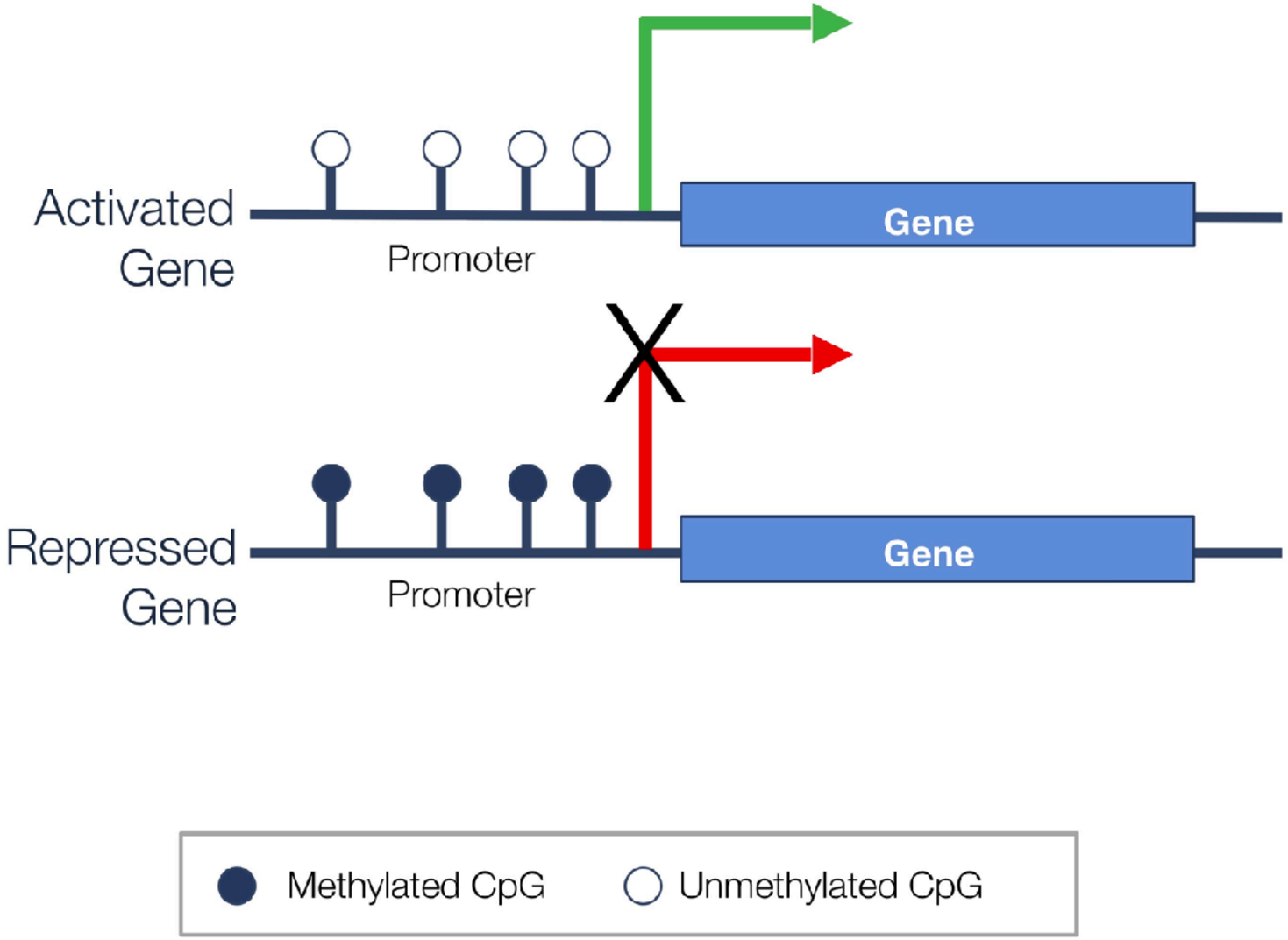
https://en.wikipedia.org/wiki/DNA_methylation

Typical mammalian DNA methylation landscape



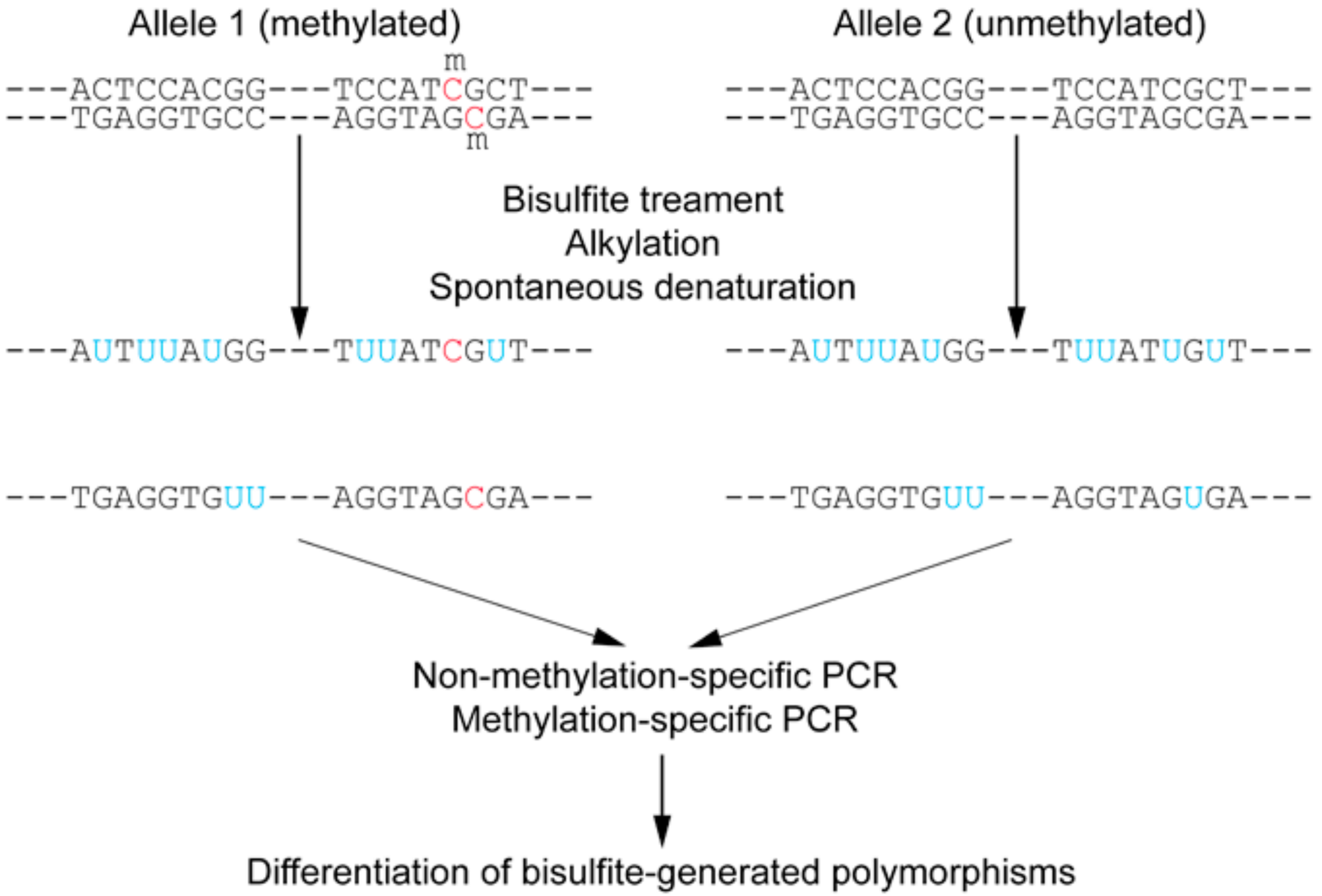
https://en.wikipedia.org/wiki/DNA_methylation

Regulatory functions of DNA methylation



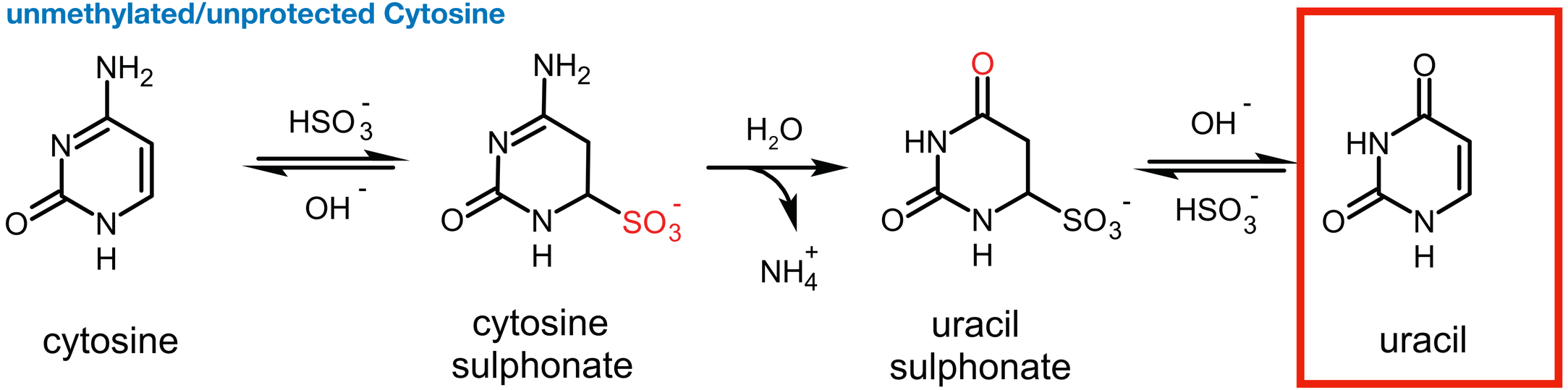
Slide: Keegan Korthauer

How do we know if a certain "C" is methylated?

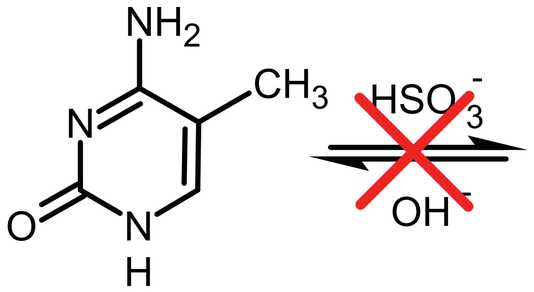


Bisulfite conversion: "unmethylated" C ☞ U

For unmethylated/unprotected Cytosine

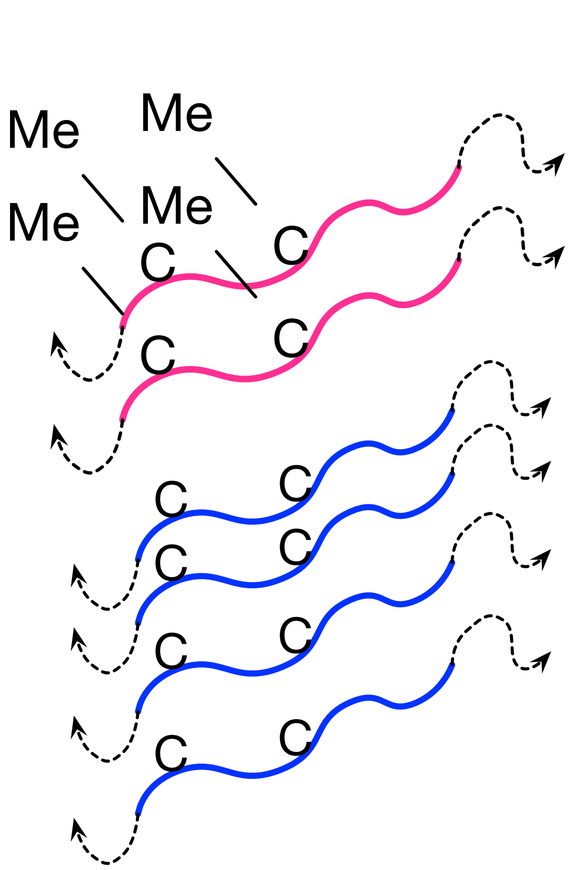


For methylated Cytosine

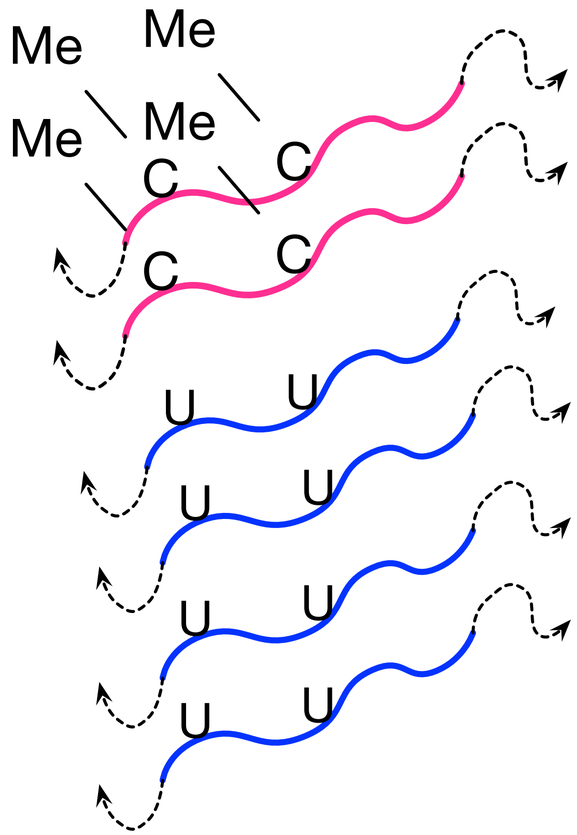


https://en.wikipedia.org/wiki/Bisulfite_sequencing

Bisulfite sequencing/array: Distinguish DNAs methylated vs. un-methylated



bisulfite treatment



sequencing & alignment

Where in the genome?

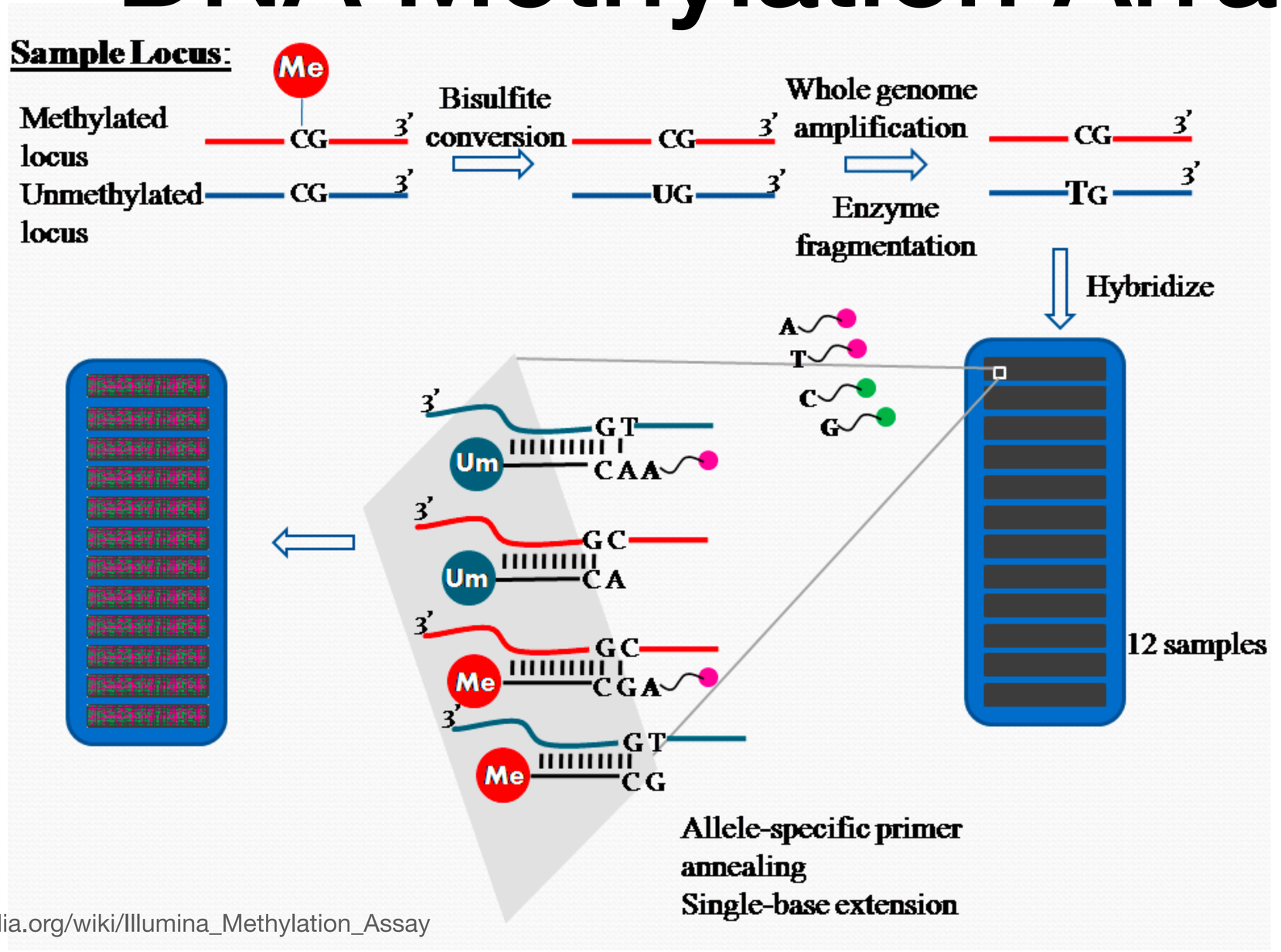
How many with C?

How many with T/U?

Quantify β-value:

$$\beta = \frac{\#\textbf{meth}}{\#\textbf{meth} + \#\textbf{unmeth}}$$

DNA Methylation Arrays



https://en.wikipedia.org/wiki/Illumina_Methylation_Assay

Source: Illumina

Statistical Methods for Epigenomics

- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)

Beta values and M values

Need some way to summarize the two relative measures for each probe:

1. methylated intensity
2. unmethylated intensity

Beta (β) values

- Combine two measurements for each probe, and measure level of methylation:

$$\beta = \frac{\text{methylated intensity}}{(\text{methylated intensity} + \text{unmeth intensity})}$$

- Range from 0 to 1
- Easily interpretable as a methylation proportion
- Best used for visualization

M-values

- Transformed β values:

$$M = \log_2 \left(\frac{\text{methylated intensity}}{\text{unmethylated intensity}} \right) = \log_2 \left(\frac{\beta}{1-\beta} \right)$$

- This type of transformation is referred to as the *logit*
  - maps a proportion onto real line
- alleviate heteroscedasticity of β values (recall that Binomial(n,p) variance = np(1-p))

Du et al. (2010)

Methylation array analysis

- Analysis strategy: linear regression on M-values
- R Packages:
  - minfi for preprocessing and normalization
  - limma for individual cytosine analysis
  - bumphunter or dmrcate for region-level analysis
  - Workflow that combines these: methylationArrayAnalysis - explore in Seminar 7[1]

[1] Note that this seminar requires installation of a couple of packages that download data and can take some time - please try run the installation step before class time!

A working Example - DNA Methylation Arrays in sorted T cells

"Genome-wide DNA methylation analysis identifies hypomethylated genes regulated by FOXP3 in human regulatory T cells" from Zhang et al. (2013)

- GEO accession GSE49667
- Here we use filtered and normalized Bioc objects that were created by this workflow, with details described in Seminar 7

```
mSet <- readRDS("data/mSet.rds")        # see Seminar 7
targets <- readRDS("data/targets.rds") #
```

```
## class: GenomicRatioSet
## dim: 439918 10
## metadata(0):
## assays(2): M CN
## rownames(439918): cg13869341 cg24669183 ... cg08265308 cg14273923
## rowData names(0):
## colnames(10): naive.1 rTreg.2 ... act_naive.9 act_rTreg.10
## colData names(13): Sample_Name Sample_Well ... yMed predictedSex
## Annotation
##   array: IlluminaHumanMethylation450k
##   annotation: ilmn12.hg19
## Preprocessing
```

Metadata values

```
##   Sample_Name Sample_Well Sample_Source Sample_Group Sample_Label
## 1           1          A1           M28        naive        naive
## 2           2          B1           M28        rTreg        rTreg
## 3           3          C1           M28    act_naive    act_naive
## 4           4          D1           M29        naive        naive
## 5           5          E1           M29    act_naive    act_naive
## 6           6          F1           M29    act_rTreg    act_rTreg
```

```
##
## act_naive act_rTreg     naive     rTreg
##         3         2         3         2
```

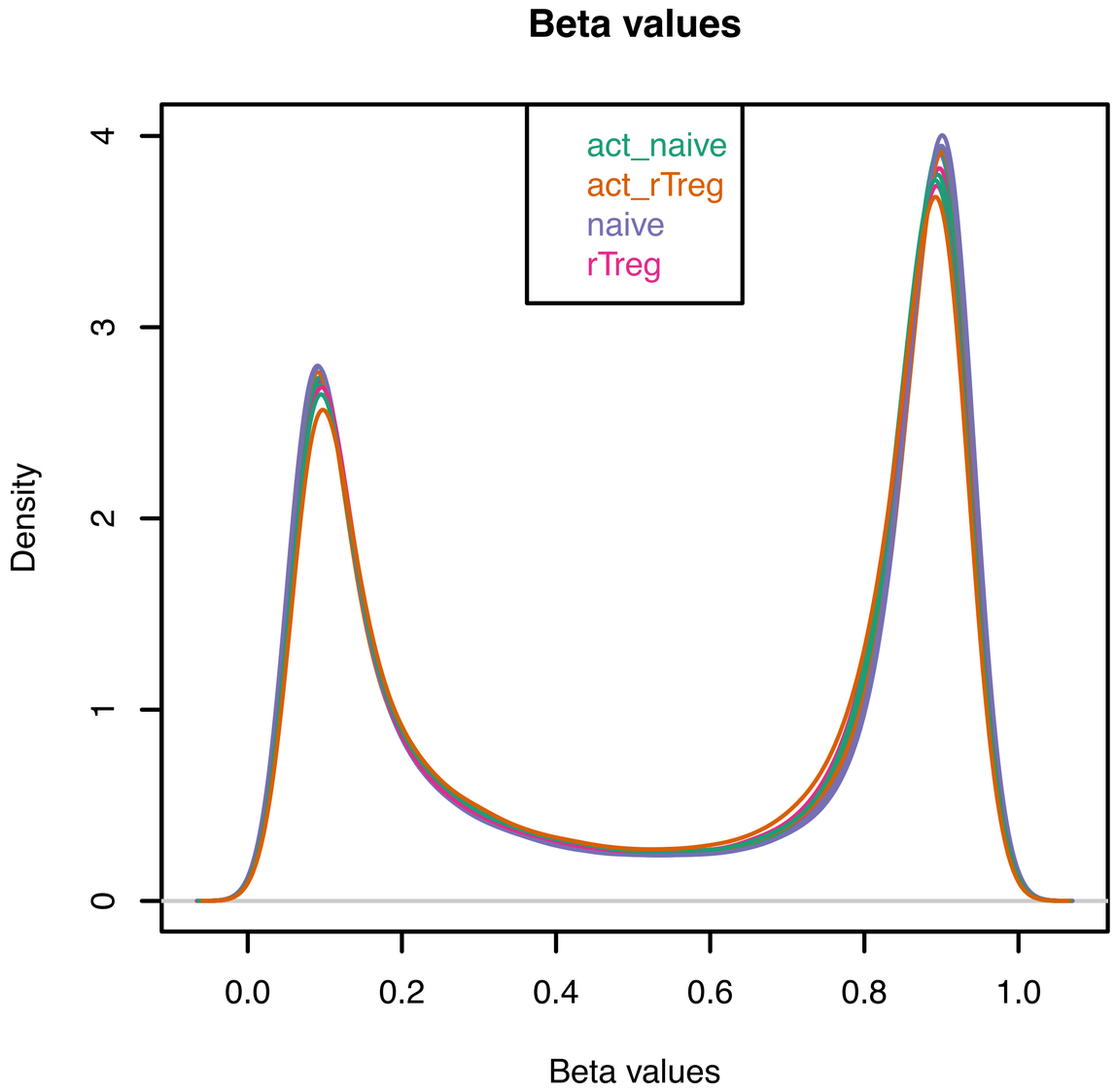
Beta values

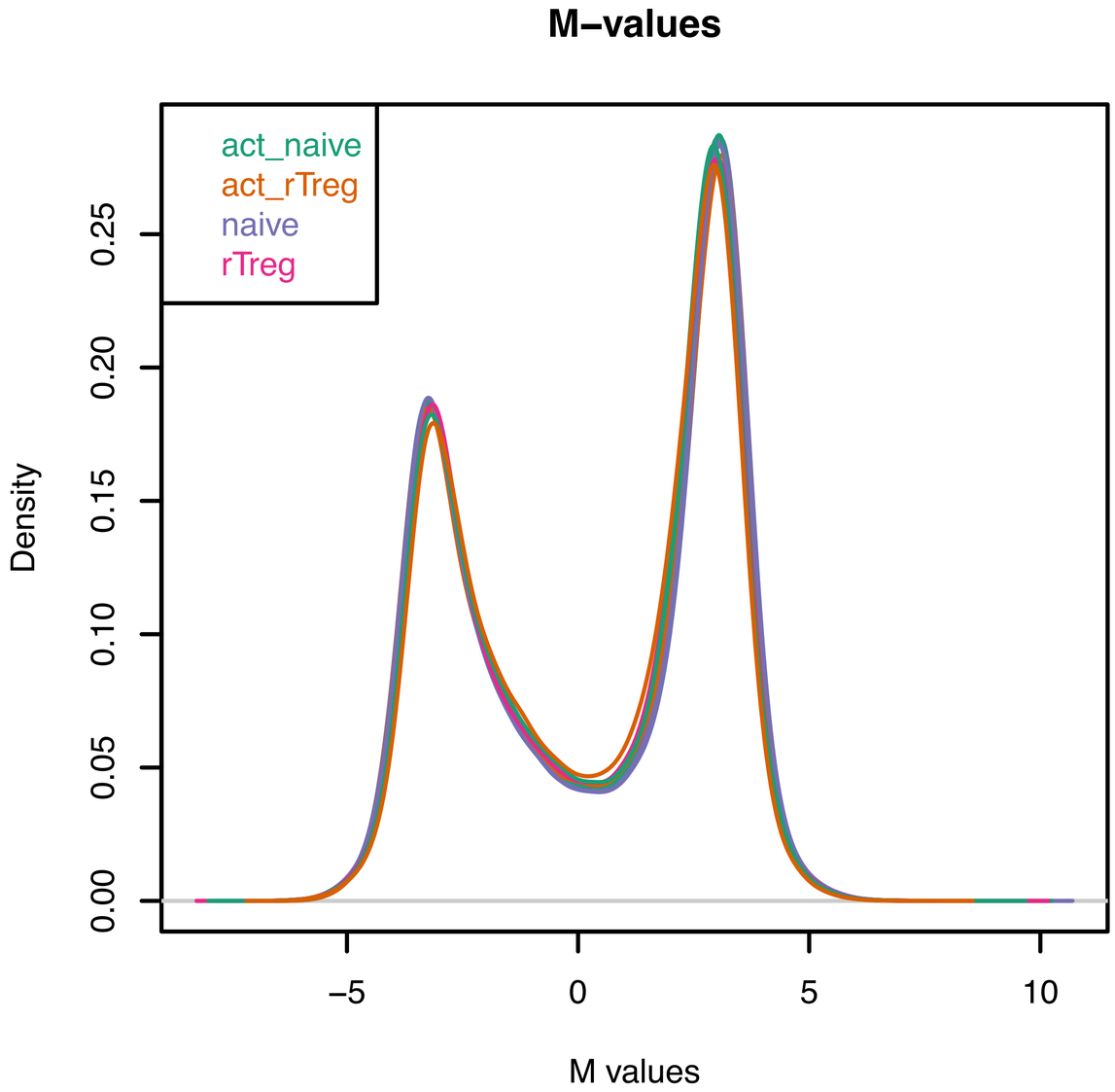
```
## [1] 439918     10

##                naive.1    rTreg.2 act_naive.3    naive.4 act_naive.5 act_rTreg.6
## cg13869341 0.84267937 0.85118462   0.8177504 0.82987650  0.81186174   0.8090798
## cg24669183 0.81812908 0.82489238   0.8293297 0.75610281  0.81967323   0.8187838
## cg15560884 0.77219626 0.74903910   0.7516263 0.77417882  0.77266205   0.7721528
## cg01014490 0.08098986 0.06590459   0.0233755 0.04127262  0.04842397   0.0644404
## cg17505339 0.89439216 0.93822870   0.9471357 0.90520570  0.92641305   0.9286016
## cg11954957 0.74495496 0.79008516   0.7681146 0.84450764  0.75431167   0.8116911
##               naive.7    rTreg.8 act_naive.9 act_rTreg.10
## cg13869341 0.8891851 0.88537940  0.90916748   0.88334231
## cg24669183 0.7903763 0.85304116  0.80930568   0.80979554
## cg15560884 0.7658623 0.75909061  0.78099397   0.78569274
## cg01014490 0.0245281 0.02832358  0.07740468   0.04640659
## cg17505339 0.8889361 0.87205348  0.90099782   0.93508348
## cg11954957 0.7832207 0.84929777  0.84719430   0.83350220
```

M values

```
## [1] 439918     10

##                naive.1   rTreg.2 act_naive.3   naive.4 act_naive.5 act_rTreg.6
## cg13869341  2.421276  2.515948    2.165745  2.286314    2.109441    2.083313
## cg24669183  2.169414  2.235964    2.280734  1.632309    2.184435    2.175771
## cg15560884  1.761176  1.577578    1.597503  1.777486    1.764999    1.760819
## cg01014490 -3.504268 -3.825119   -5.384735 -4.537864   -4.296526   -3.859792
## cg17505339  3.082191  3.924931    4.163206  3.255373    3.654134    3.701096
## cg11954957  1.546401  1.912204    1.727910  2.441267    1.618331    2.107829
##                naive.7   rTreg.8 act_naive.9 act_rTreg.10
## cg13869341  3.004332  2.949430    3.323265     2.920691
## cg24669183  1.914738  2.537203    2.085423     2.090007
## cg15560884  1.709728  1.655782    1.834341     1.874285
## cg01014490 -5.313593 -5.100400   -3.575205    -4.360973
## cg17505339  3.000690  2.768876    3.185991     3.848438
## cg11954957  1.853192  2.494570    2.470994     2.323683
```

Comparing beta and M values





Differentially methylated cytosines (DMC)

Which cytosines/probes are differentially methylated between naive & activated naive T cells?

```
## this is the factor of interest
cellType <- factor(targets$Sample_Group)

## this is the individual effect that we need to account for
individual <- factor(targets$Sample_Source)

## use the above to create a design matrix
design <- model.matrix( ~ cellType + individual,
                        data = targets)
design
```

```
##    (Intercept) cellTypeact_rTreg cellTypenaive cellTyperTreg individualM29
## 1            1                 0             1             0             0
## 2            1                 0             0             1             0
## 3            1                 0             0             0             0
## 4            1                 0             1             0             1
## 5            1                 0             0             0             1
## 6            1                 1             0             0             1
## 7            1                 0             1             0             0
## 8            1                 0             0             1             0
## 9            1                 0             0             0             0
```

Differential methylation: limma on M-values

Here, the reference cell type is `act_naive` cells

```
## fit the linear model (one for each probe)
mfit <- lmFit(mVals, design)
cfit <- eBayes(mfit)

## look at top DM CpGs between naive and ref (act_naive)
topTable(cfit, coef="cellTypenaive")
```

```
##                logFC     AveExpr         t      P.Value   adj.P.Val        B
## cg15459165 -3.892074 -1.47895212 -22.09541 1.993958e-08 0.002793543 6.810420
## cg19704755 -4.139885  0.09100138 -20.35779 3.787335e-08 0.002793543 6.594795
## cg13531460  3.484448 -0.63132888  20.23020 3.978221e-08 0.002793543 6.577256
## cg17137500 -3.007052 -2.47635924 -20.21569 4.000601e-08 0.002793543 6.575245
## cg17048073 -3.650550 -0.87247048 -20.04737 4.270889e-08 0.002793543 6.551674
## cg03899643  4.603864  0.02760746  19.86354 4.589787e-08 0.002793543 6.525407
## cg19827923  3.316270  0.15932891  19.82438 4.661126e-08 0.002793543 6.519739
## cg26800893 -3.080184 -2.39958457 -19.60718 5.080116e-08 0.002793543 6.487839
## cg14688905 -3.119261 -1.31909419 -18.94774 6.635412e-08 0.003243375 6.385901
## cg21837189 -3.717982 -1.08764112 -18.00052 9.895908e-08 0.003769603 6.224845
```

Summary for all coefficients

Significant tests (BH-adjusted p-value less than 0.05) of the null hypotheses: each coefficient equal to zero

```
##         (Intercept) cellTypeact_rTreg cellTypenaive cellTyperTreg individualM29
## Down         184747               919           400           827          1367
## NotSig        14791            438440        439291        438570        437499
## Up           240380               559           227           521          1052
##         individualM30
## Down             1245
## NotSig         437716
## Up                957
```

Note that the first column tests whether the reference group (`act_naive`) has an M value of zero (not usually of interest)

Interpretation of parameters and effect sizes

- To interpret parameter estimates from M-values on β scale, need to back-transform:
  - Recall: $M = log_2(\frac{\text{methylated intensity}}{\text{unmethylated intensity}}) = log_2(\beta/(1-\beta))$
  - Solving for beta gives $\beta = 2^M/(1+2^M)$

```
# fitted mean M-value for cg15459165 in ref group:
(M_act_naive <- cfit$coefficients["cg15459165", "(Intercept)"])
```

```
## [1] 0.4121952
```

```
(M_naive <- M_act_naive + cfit$coefficients["cg15459165", "cellTypenaive"])
```

```
## [1] -3.479879
```

```
# back transform to beta values
inv_logit <- function(x){ 2^x / (1+2^x) }
inv_logit(M_act_naive)
```

```
## [1] 0.570946
```

```
inv_logit(M_naive)
```

```
## [1] 0.08225703
```

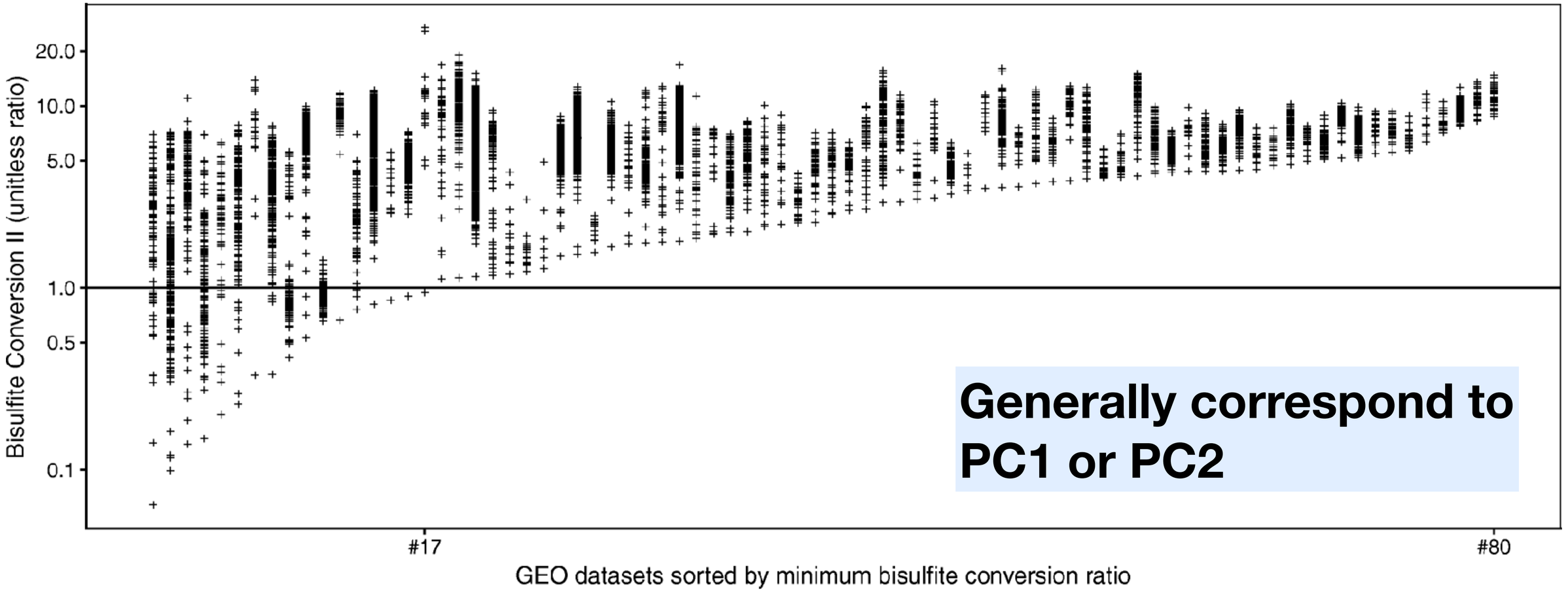
Summary of array DMC analysis

- **Just like for limma analysis of gene expression microarray**: Can use additive models, models with interactions, continuous and categorical variables
  - **interpretation of hypothesis tests, contrasts** with `makeContrasts` (for other comparisons not represented by coefficients in our design matrix): follows as before
  - **interpretation of parameters/effect sizes**: keep in mind that M-values are *transformed* proportions

Summary of array DMC analysis

- **Just like for limma analysis of gene expression microarray**: Can use additive models, models with interactions, continuous and categorical variables
  - **interpretation of hypothesis tests, contrasts** with `makeContrasts` (for other comparisons not represented by coefficients in our design matrix): follows as before
  - **interpretation of parameters/effect sizes**: keep in mind that M-values are *transformed* proportions
- Annotation packages (e.g. `IlluminaHumanMethylation450kanno.ilmn12.hg19`) allow for adding information about each CpG
  - chromosome and position
  - strand
  - whether in CpG Island

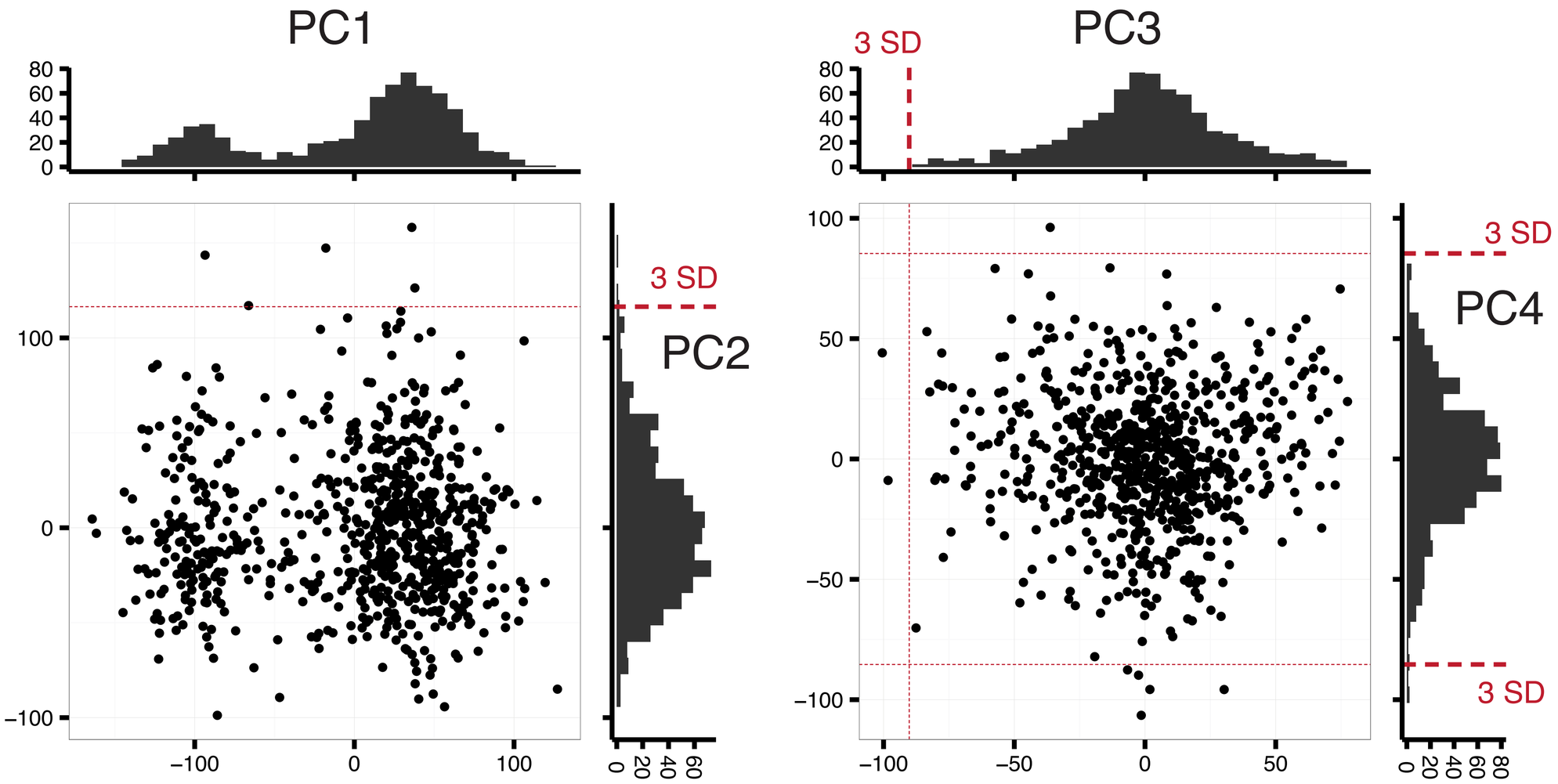
Bisulfite conversion rates can vary



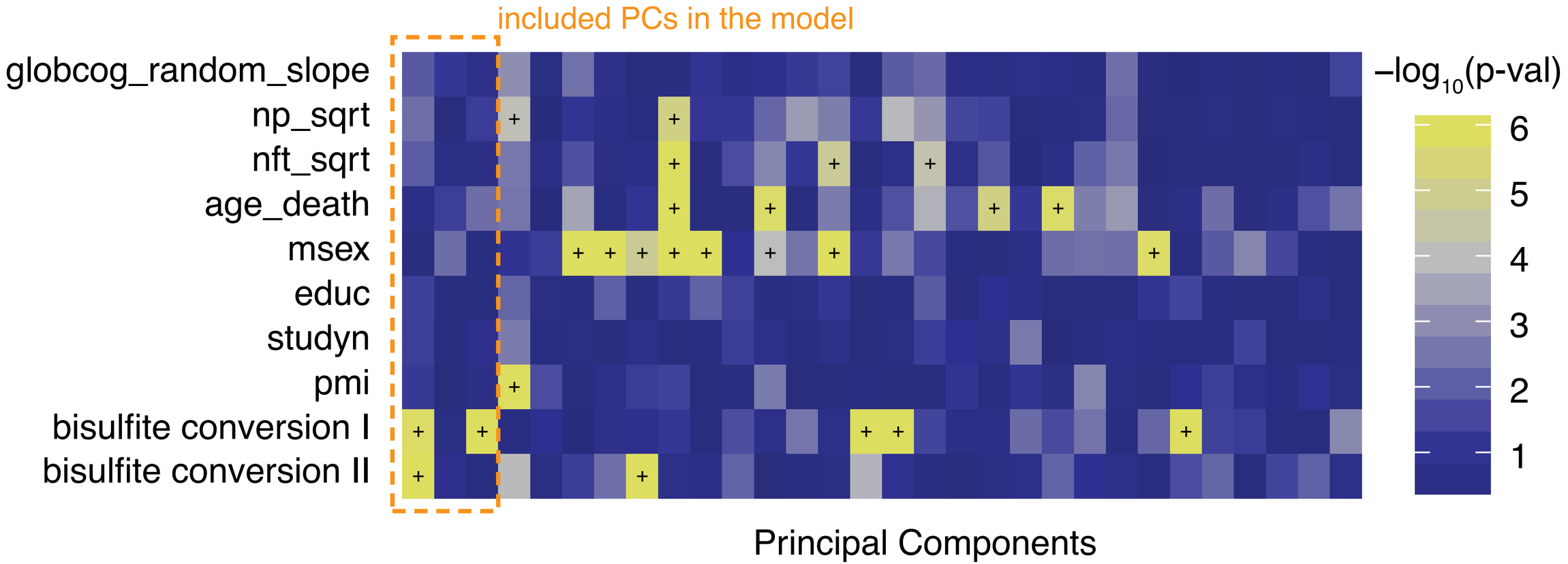
Generally correspond to PC1 or PC2

Heiss & Just (2018)

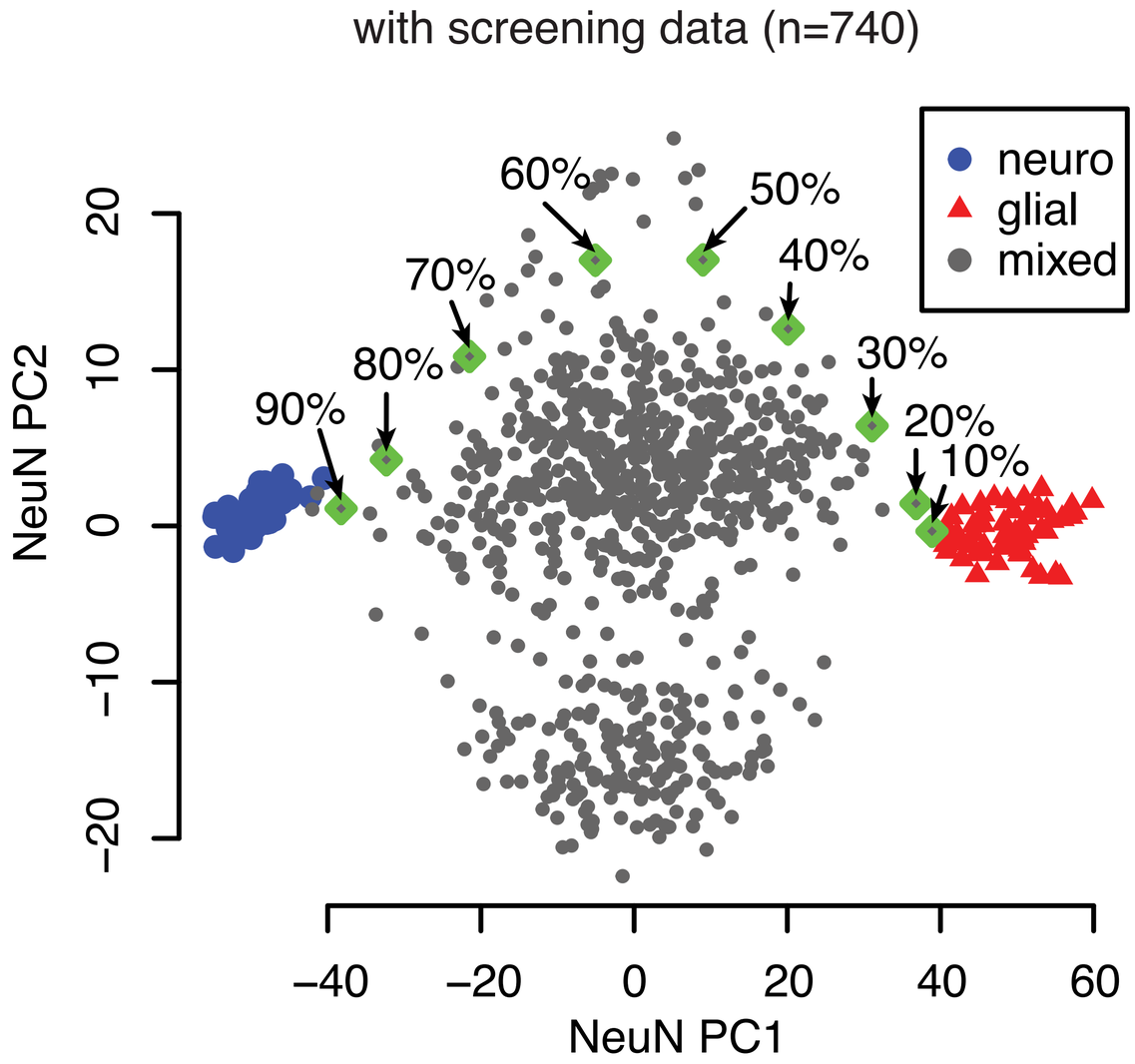
EDA in DNAmeth analysis could be useful



PCs ☞ known biological/technical biases



DNA methylation is well-known to capture cell type variability in bulk samples

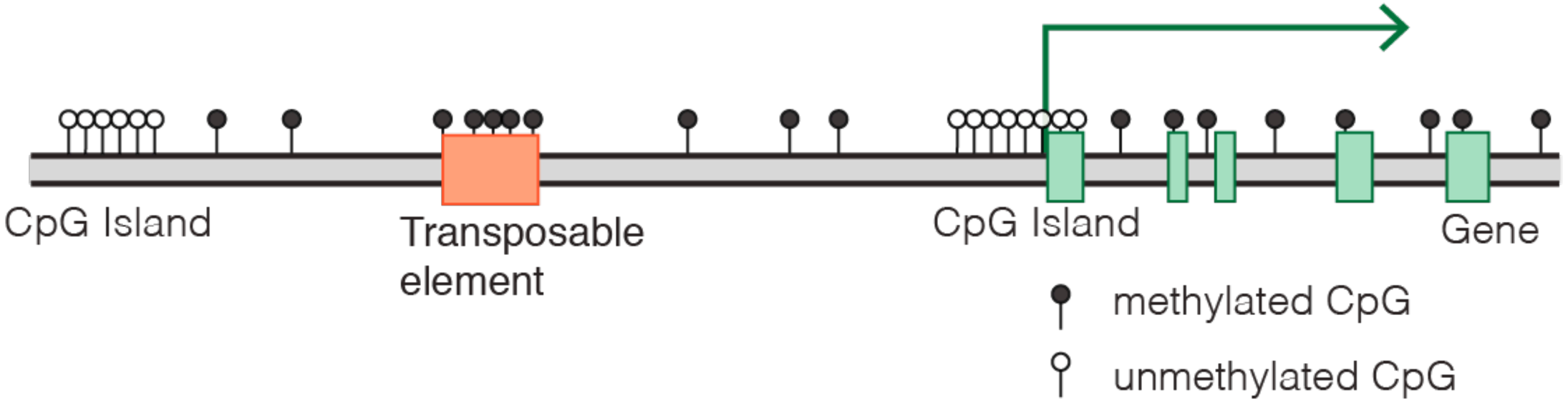


Statistical Methods for Epigenomics

- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)

Isn't DNA methylation anlaysis the same as RNA?

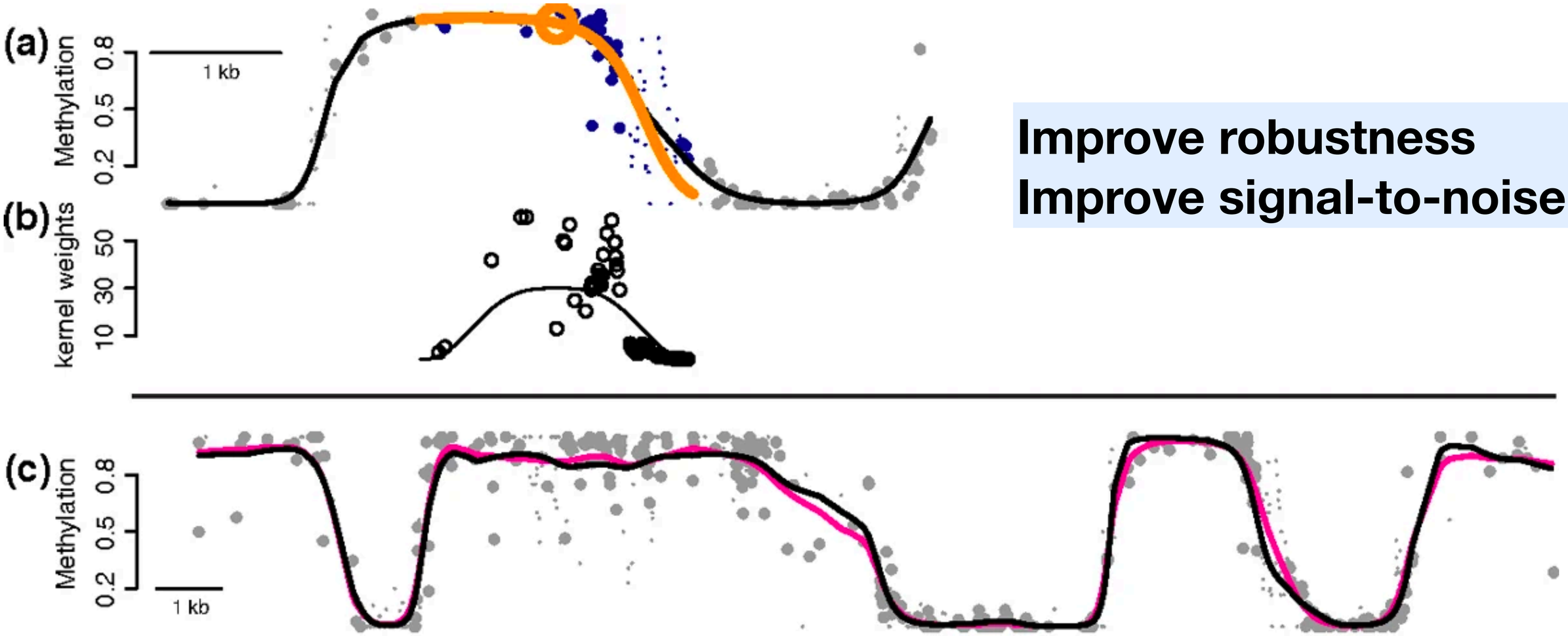
No, so many CpGs exist in the vicinity of a gene



Who are your neighbours?

https://en.wikipedia.org/wiki/DNA_methylation

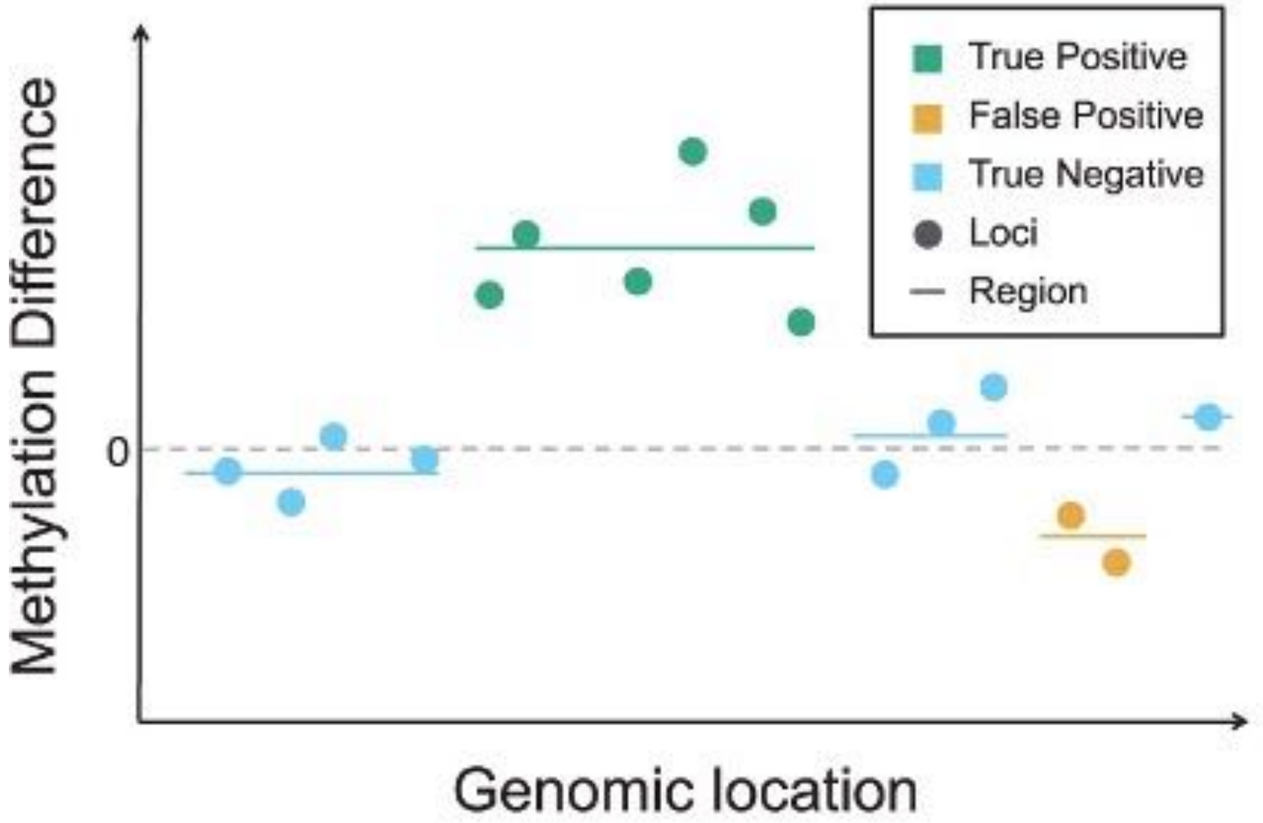
Why DMR: borrow information across genome



Improve robustness
Improve signal-to-noise

Hansen .. Irizarry (2012)

CpG-level analysis may be too optimistic

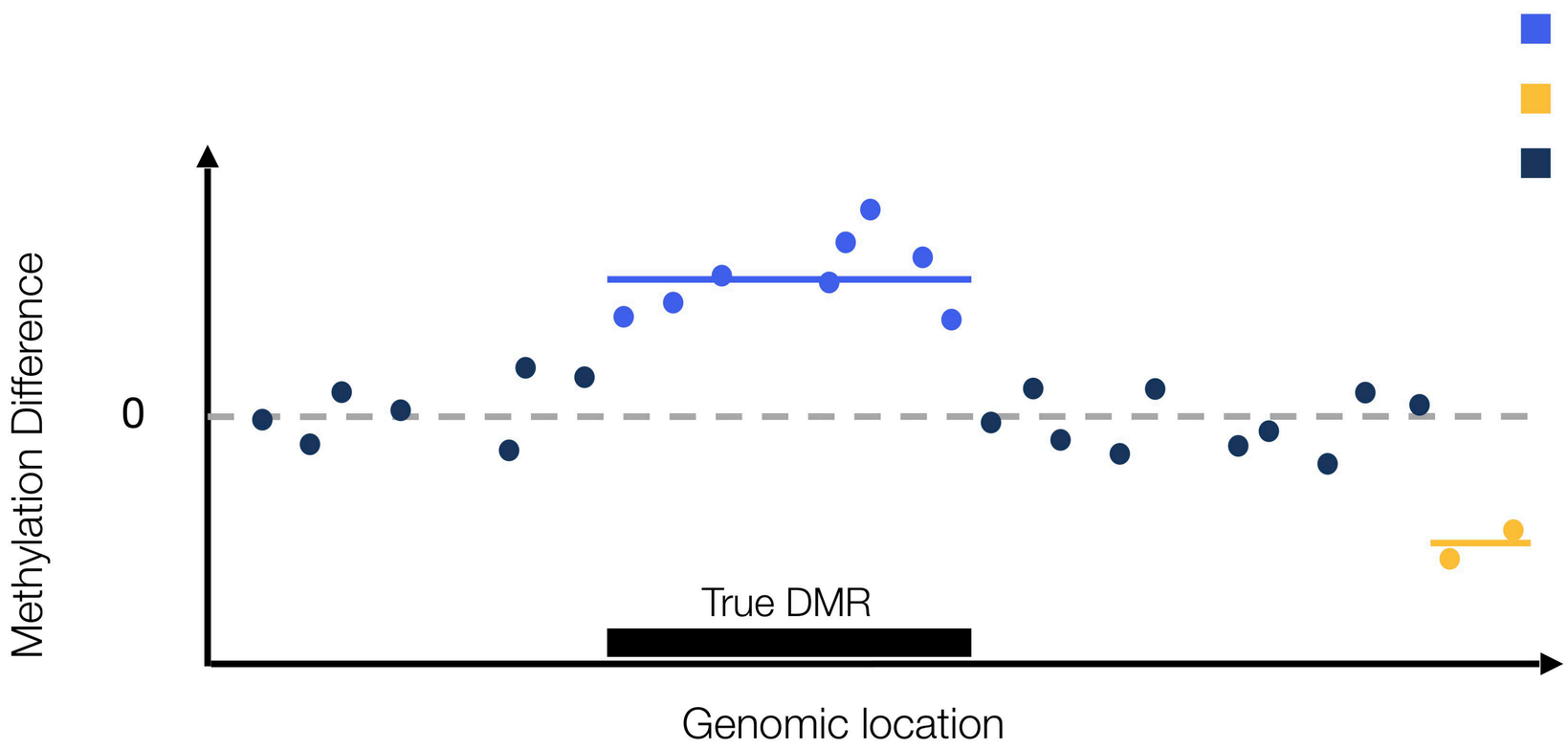


What is the fundamental assumption of multiple hypothesis correction?

Can you treat each CpG and the corresponding hypothesis test independently?

Korthauer *et al.* (2019)

Why DMR: correct false discovery calibration



FDR @ CpG = 2/10

FDR @ DMR = 1/2

$$\text{False Discovery Rate (FDR)} = E\left[\frac{FP}{FP+TP}\right]$$

Korthauer *et al.* (2019)

First of all, how do we find the regions?

Published by Oxford University Press on behalf of the International Epidemiological Association
International Journal of Epidemiology 2012;**41**:200–209
doi:10.1093/ije/dyr238

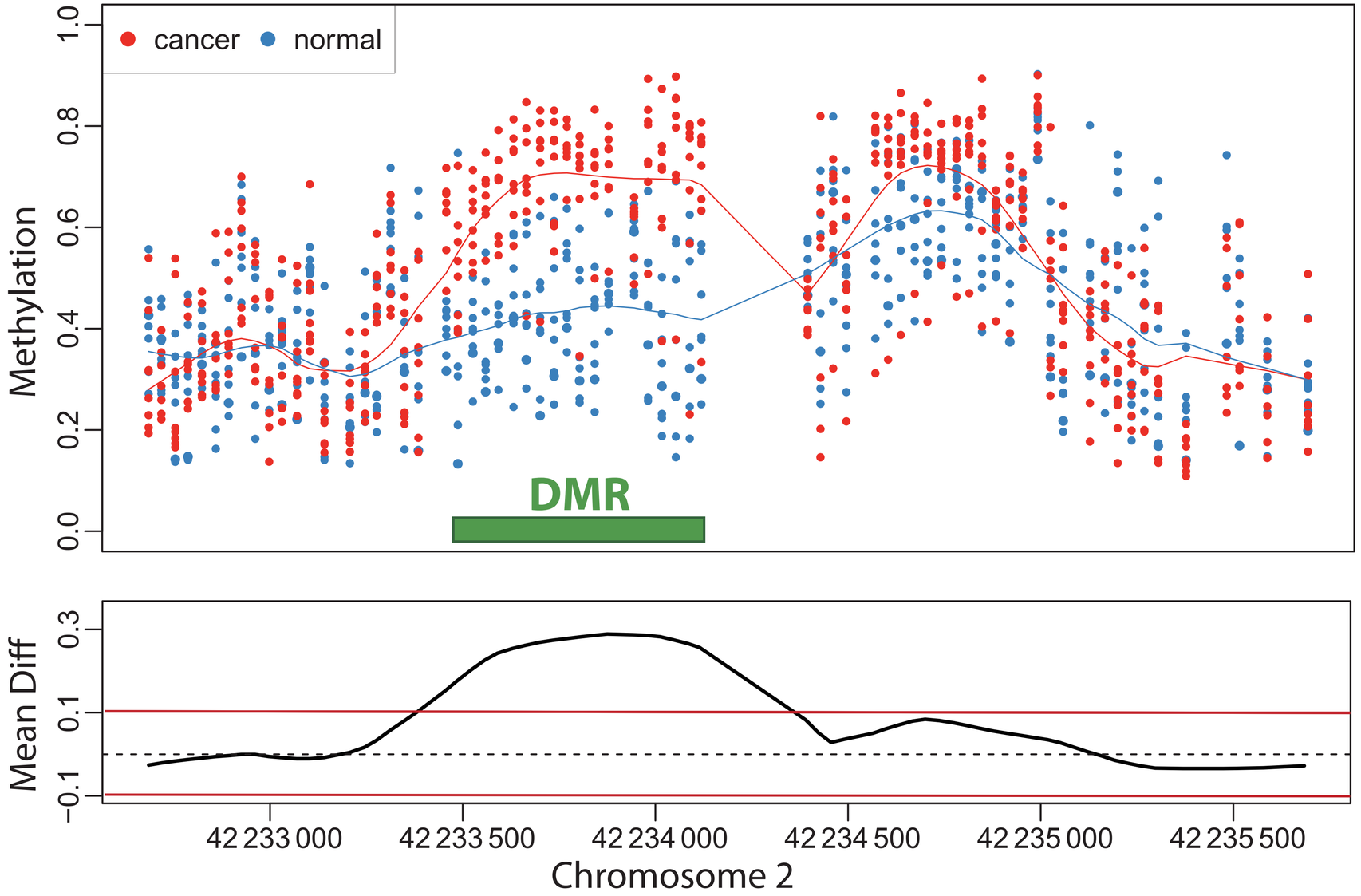
Bump hunting to identify differentially methylated regions in epigenetic epidemiology studies

Andrew E Jaffe,[1,2,3] Peter Murakami,[3] Hwajin Lee,[3] Jeffrey T Leek,[1] M Daniele Fallin,[1,2,3,4] Andrew P Feinberg[1,3,4] and Rafael A Irizarry[1,3]*

[1]Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, [2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health. Baltimore, MD, USA, [3]Center for Epigenetics, Johns Hopkins School of Medicine, Baltimore, MD, USA and [4]Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD, USA

*Corresponding author. Department of Biostatistics, 615 N. Wolfe St E3620, Baltimore, MD 21205. E-mail: rafa@jhu.edu

Goal: Identify differentially methylated regions

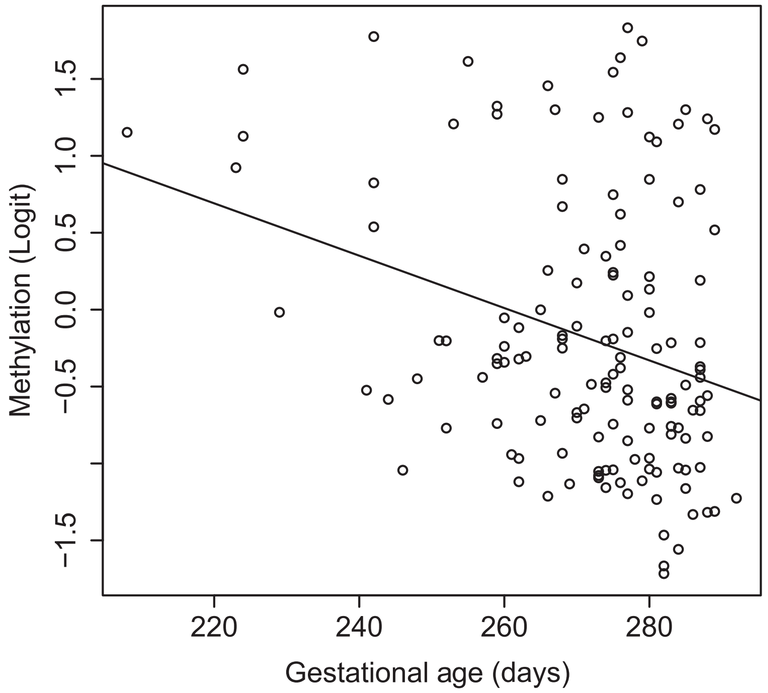


Jaffe *et al.* (2012)

DMR bump hunter

Treat each CpG independently

Perform association, thus get **β**



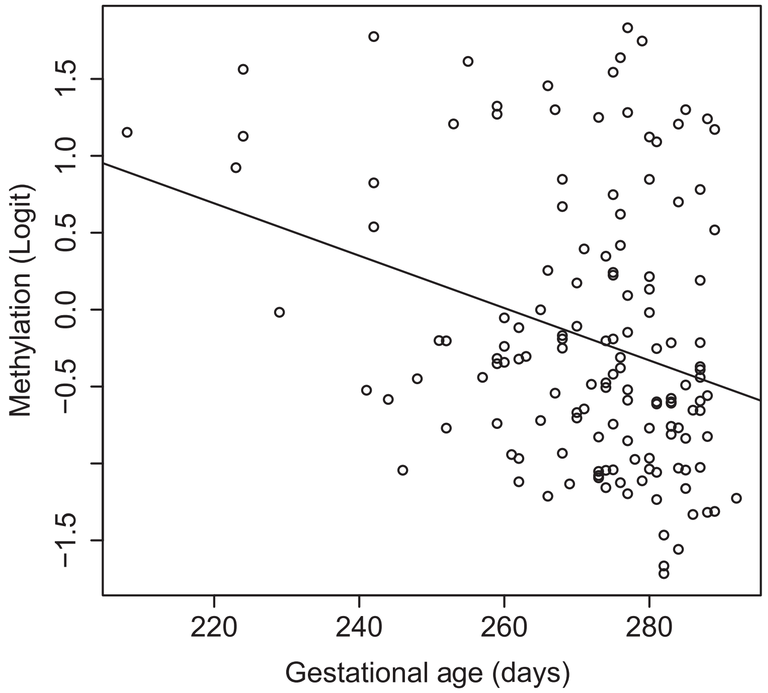
$$M_{ig} \sim \beta_g \times X_i + \cdots$$

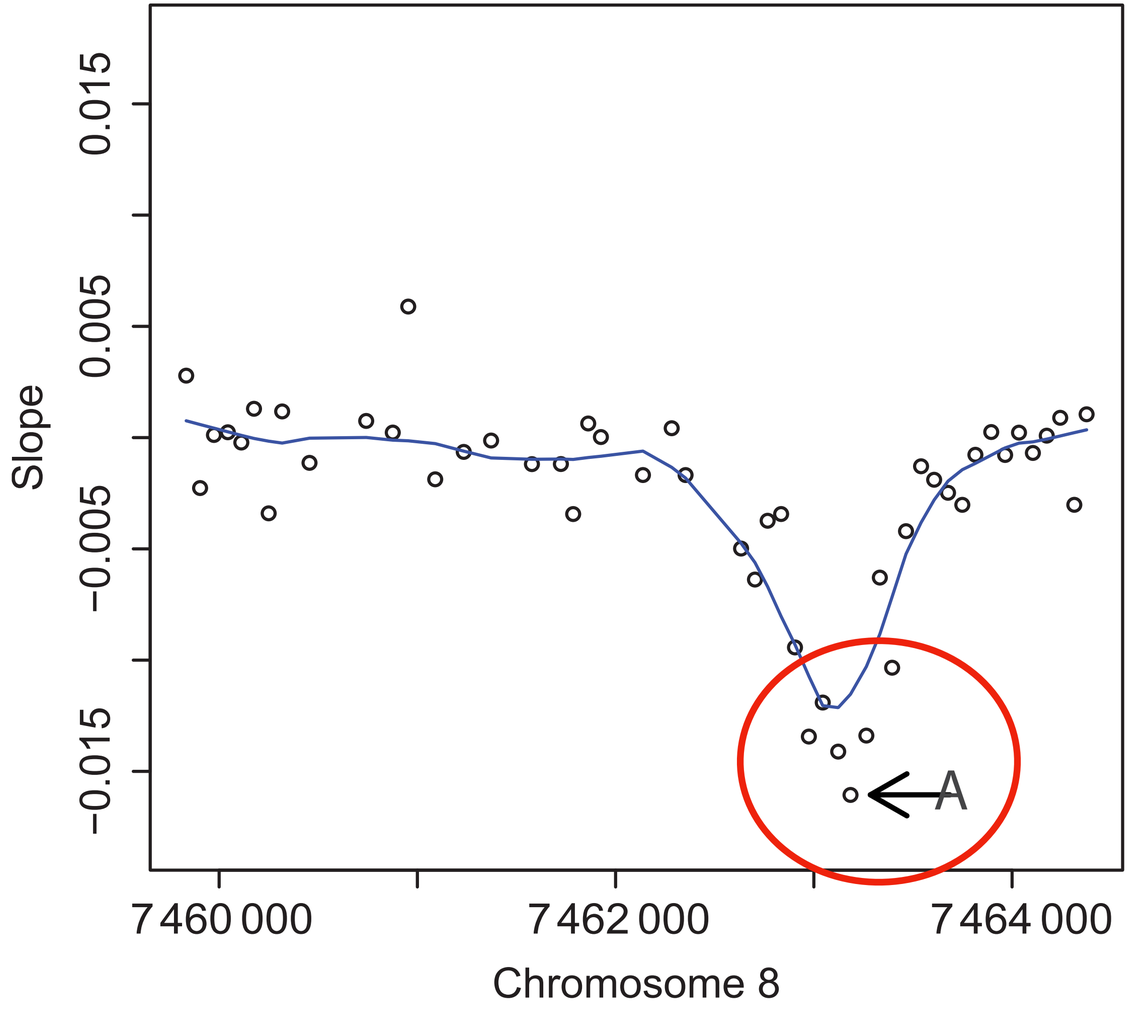
Jaffe *et al.* (2012)

DMR bump hunter

Treat each CpG independently

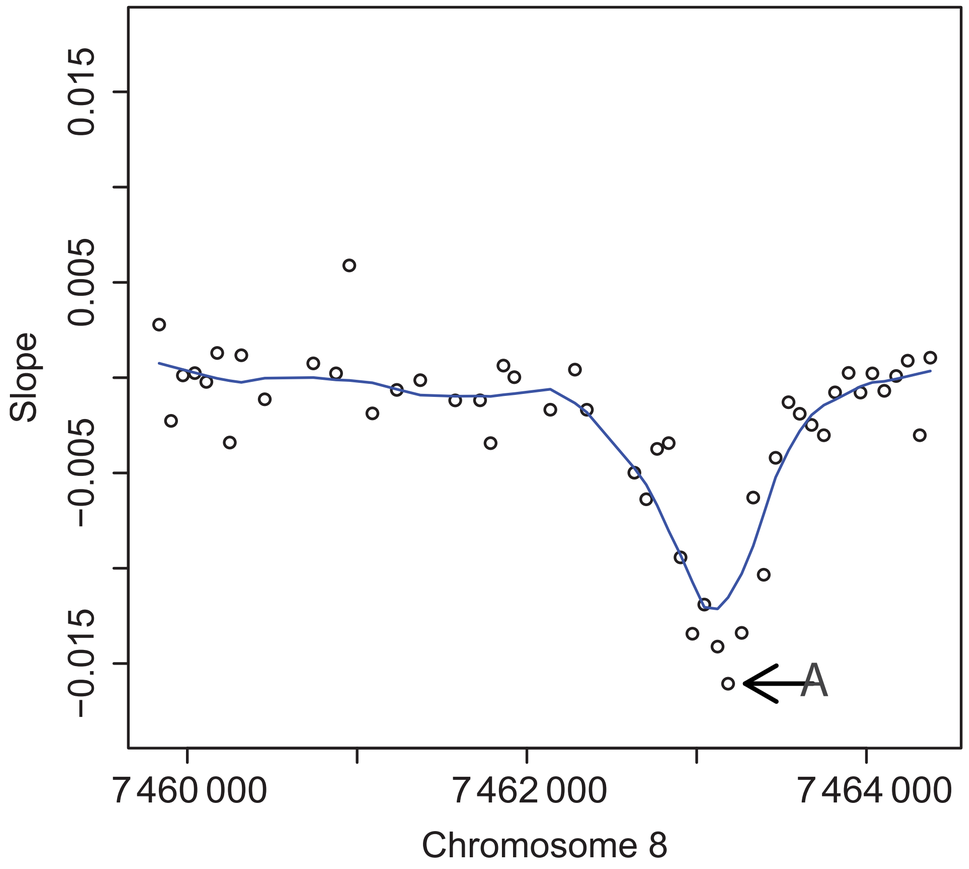
Perform association, thus get **β**



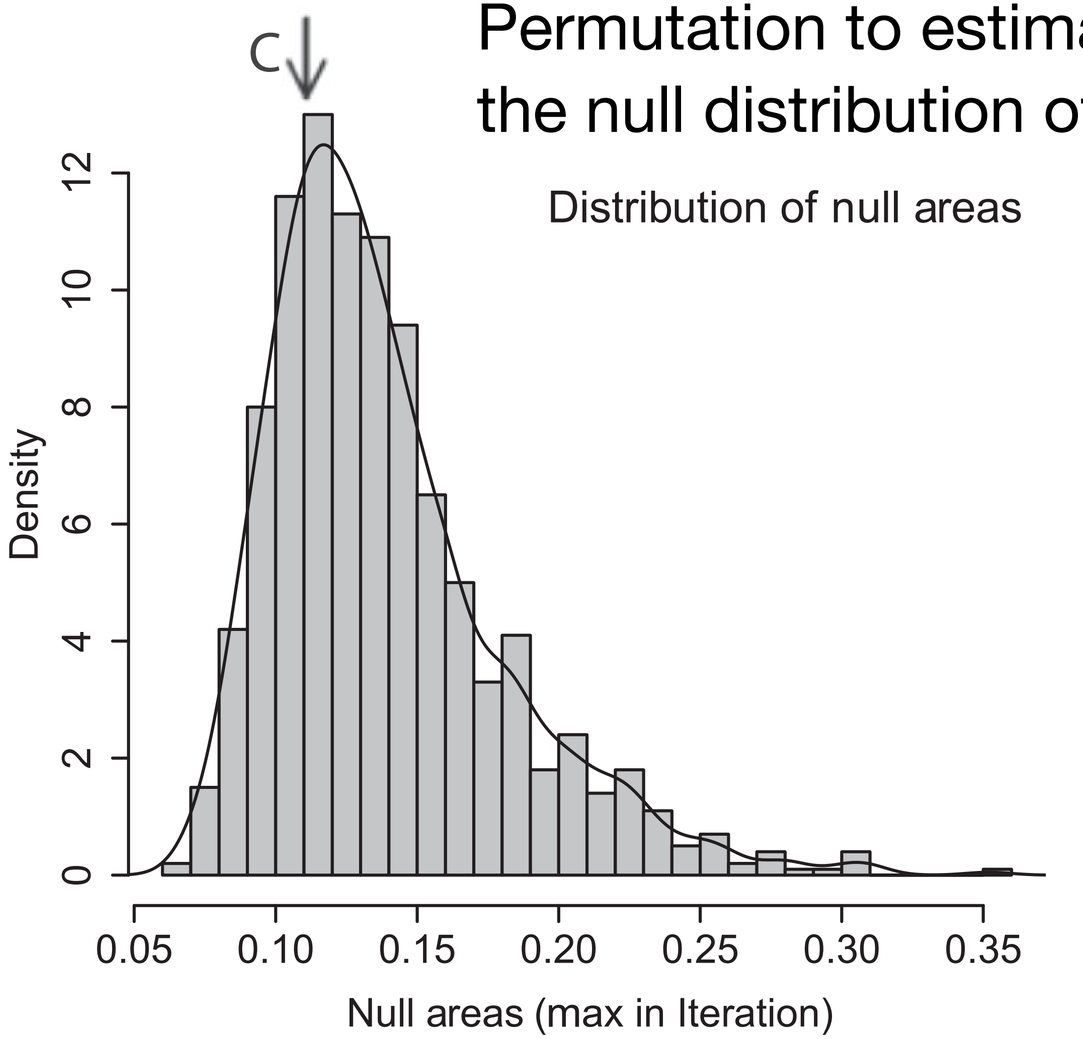


Jaffe *et al.* (2012)

DMR bump hunter

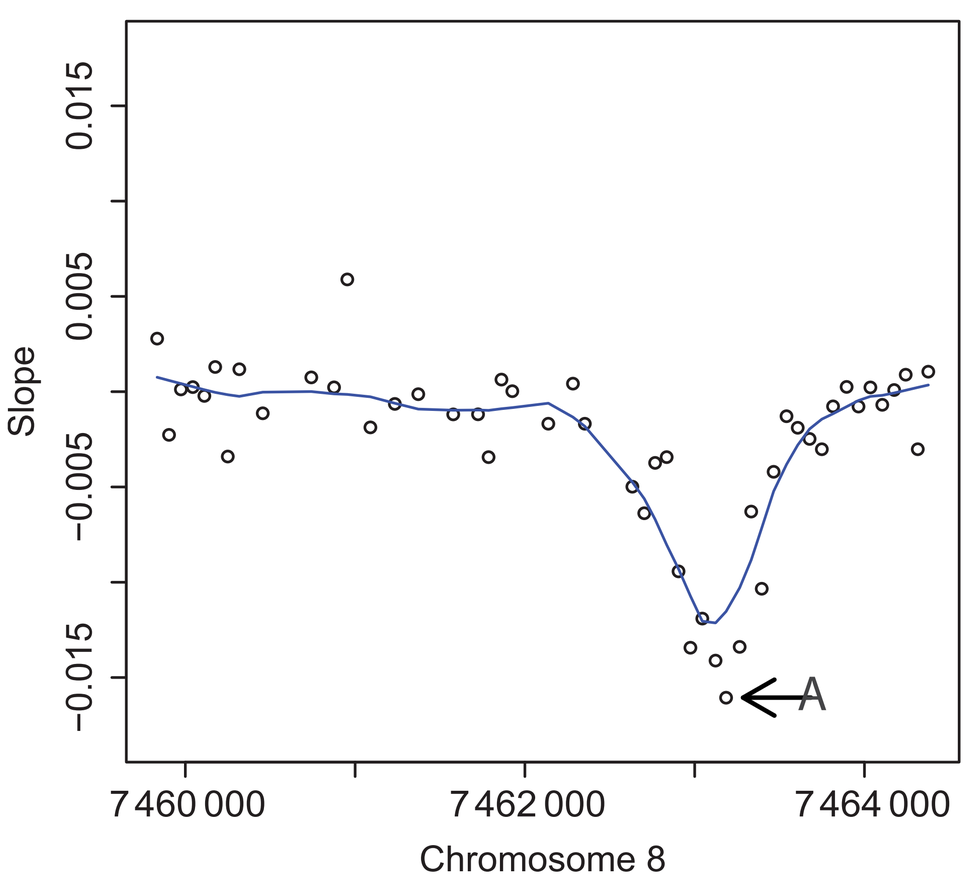


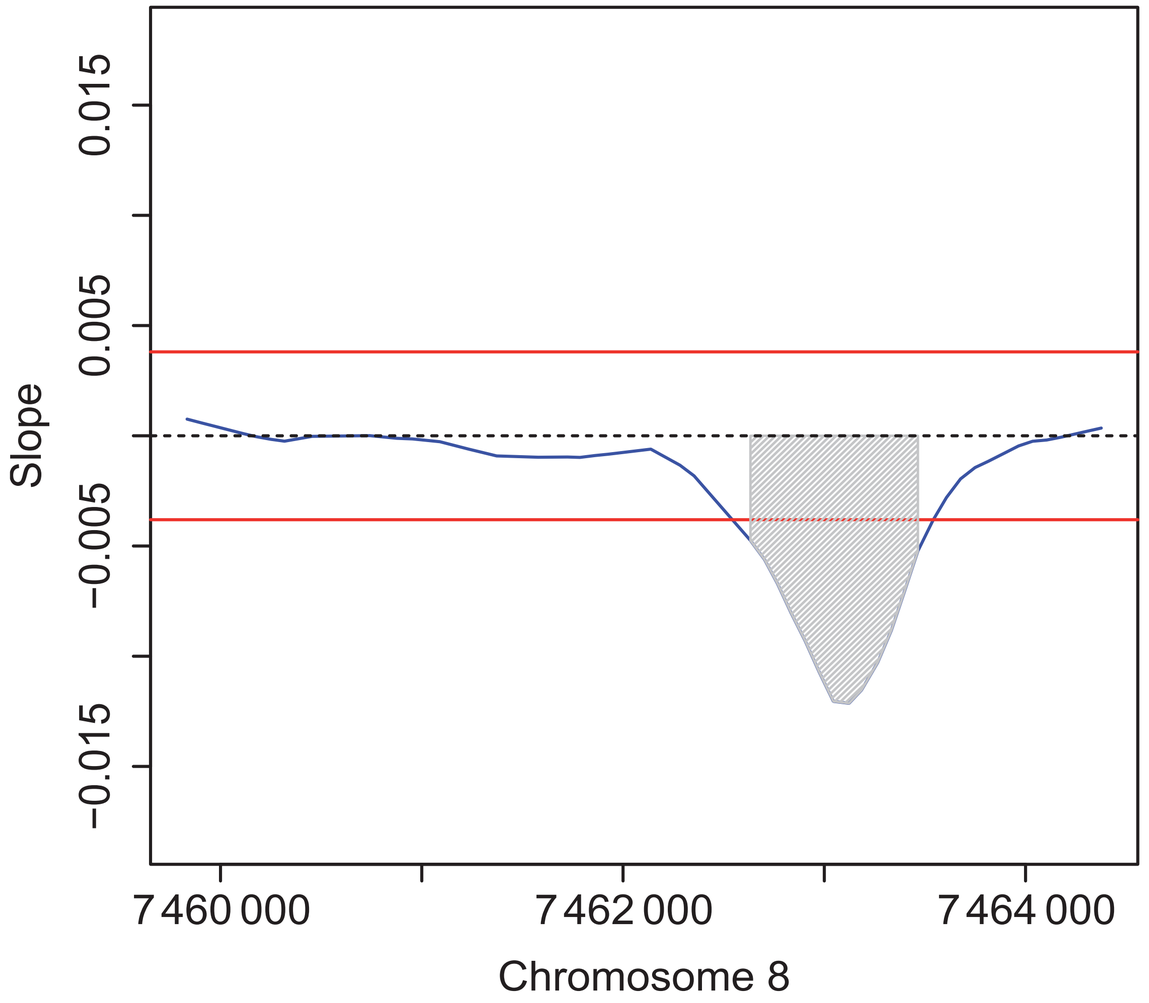
Permutation to estimate the null distribution of β



Jaffe *et al.* (2012)

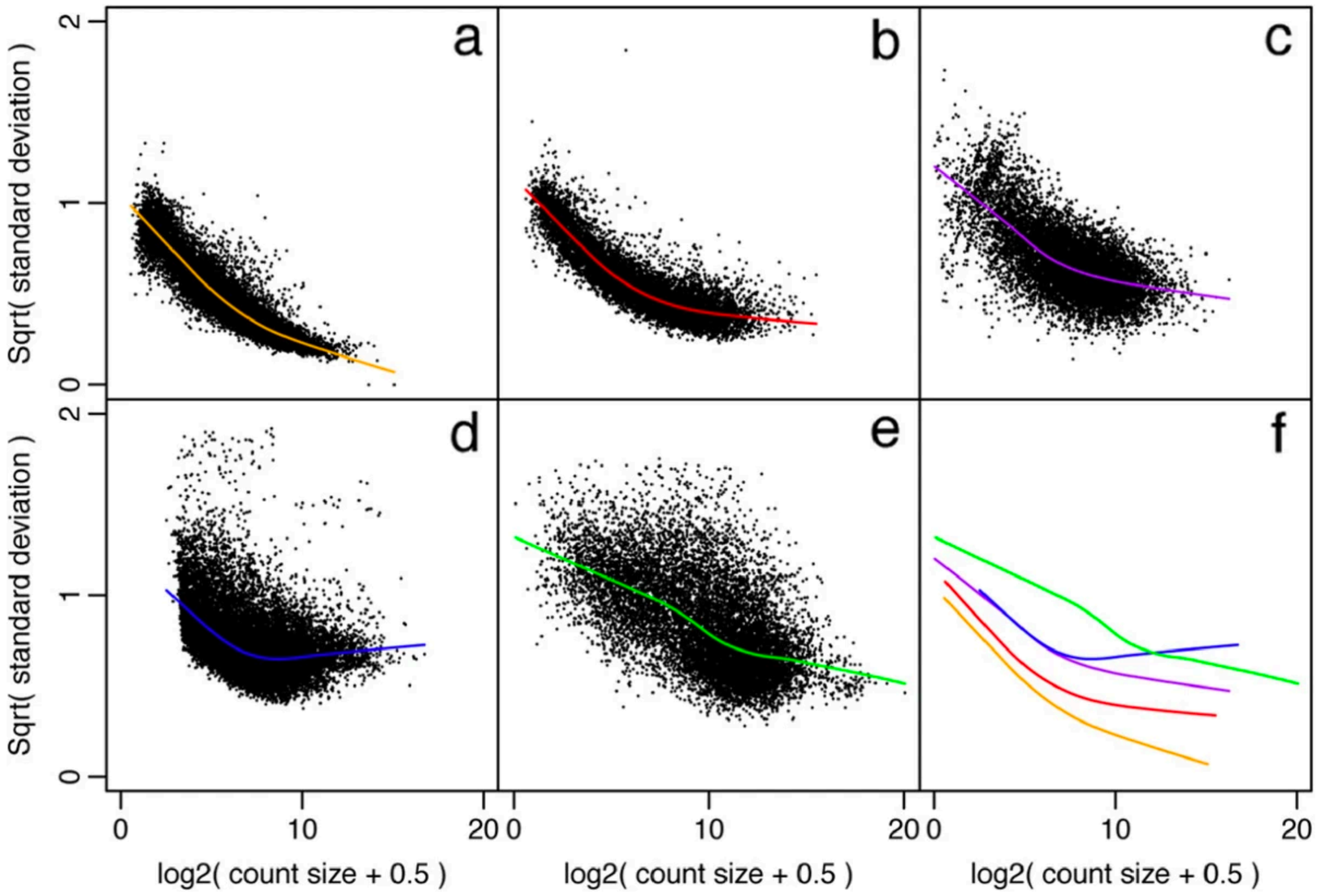
DMR bump hunter





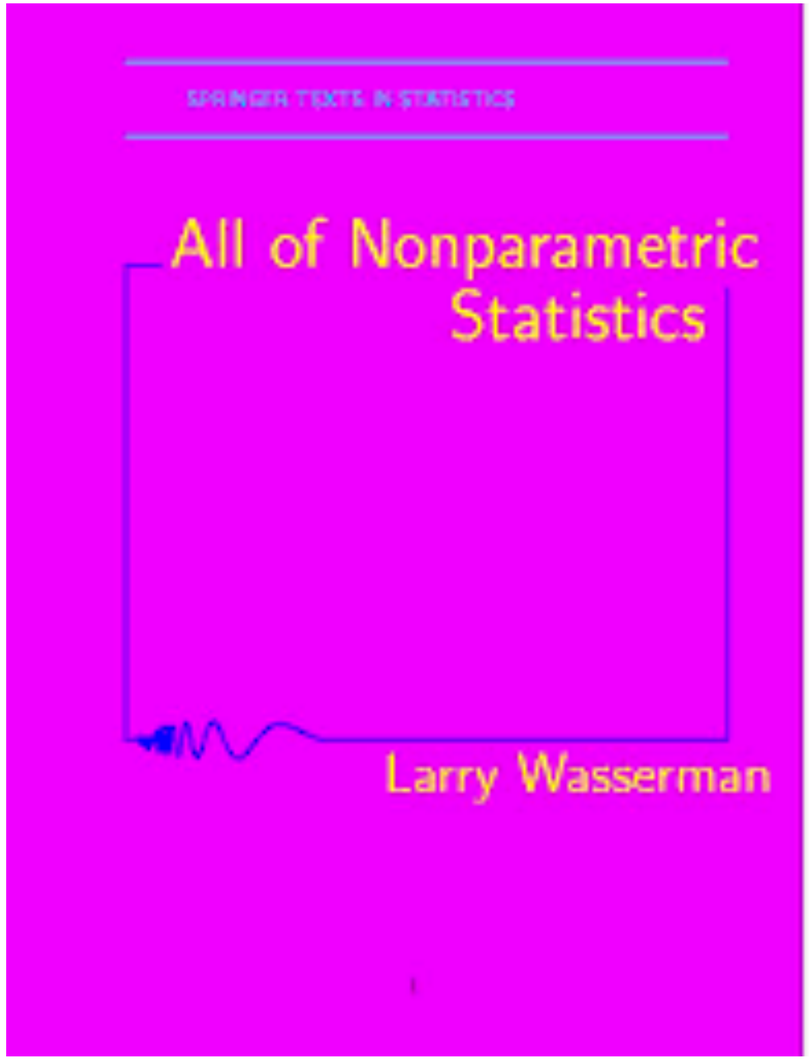
Jaffe *et al.* (2012)

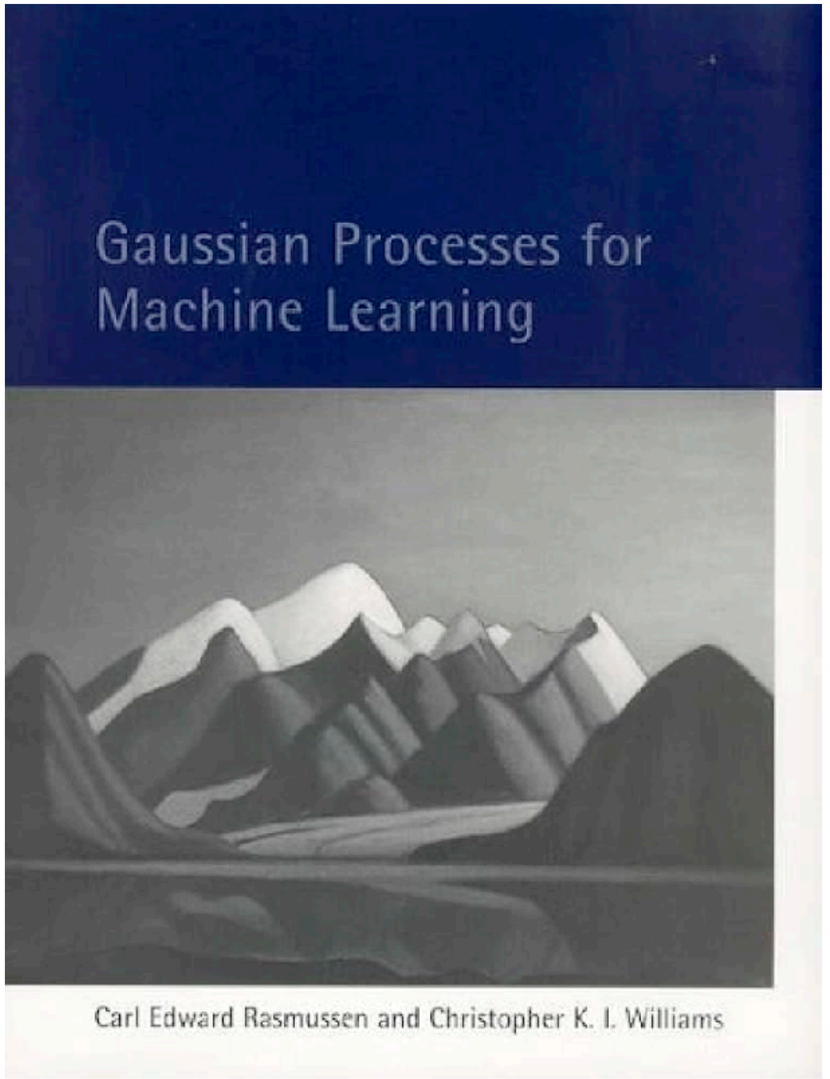
How can we model the wiggly patterns in 1D?



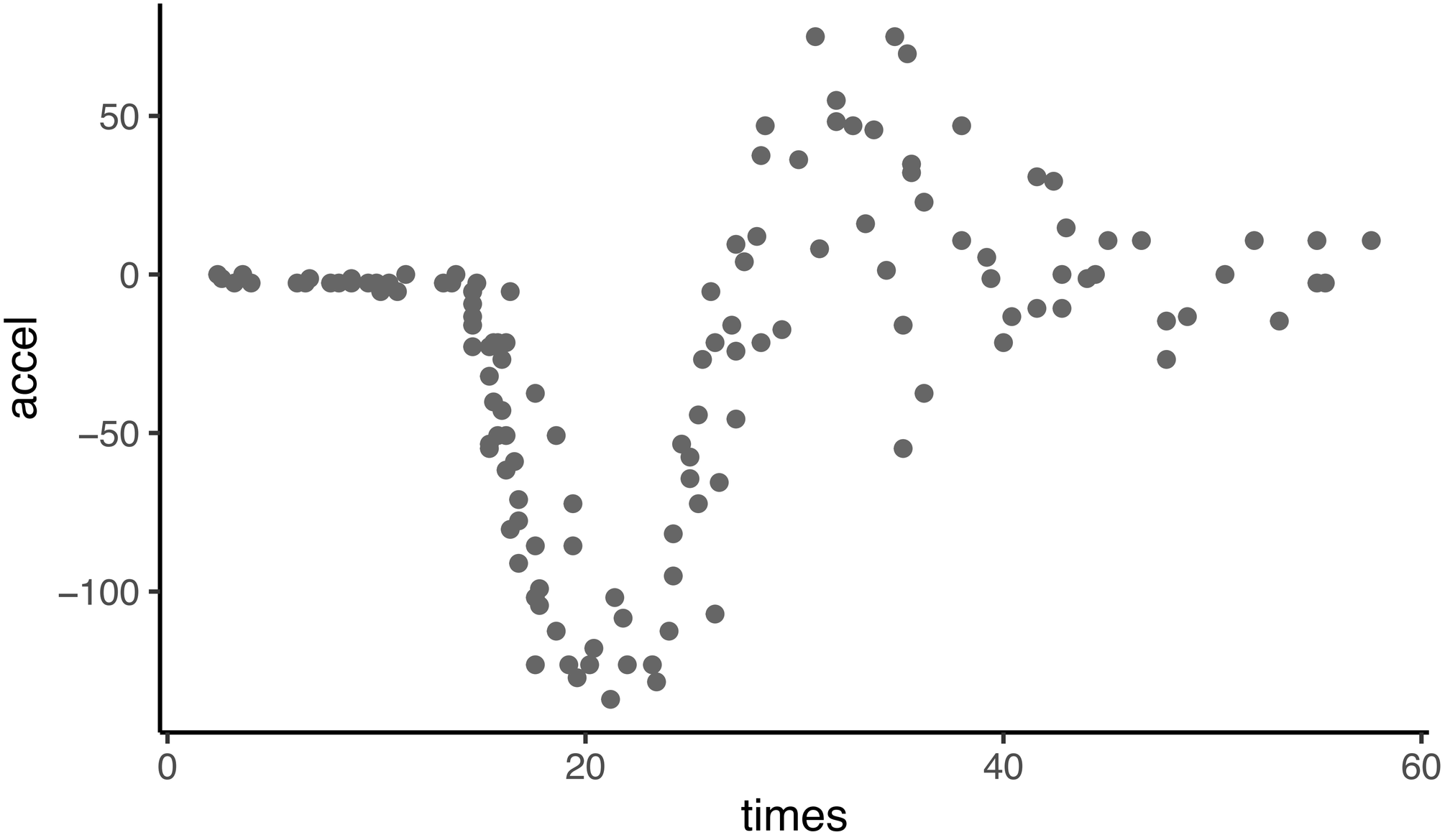
Law .. Smyth,
Genome Biology
(2014)

In case you want to know more:

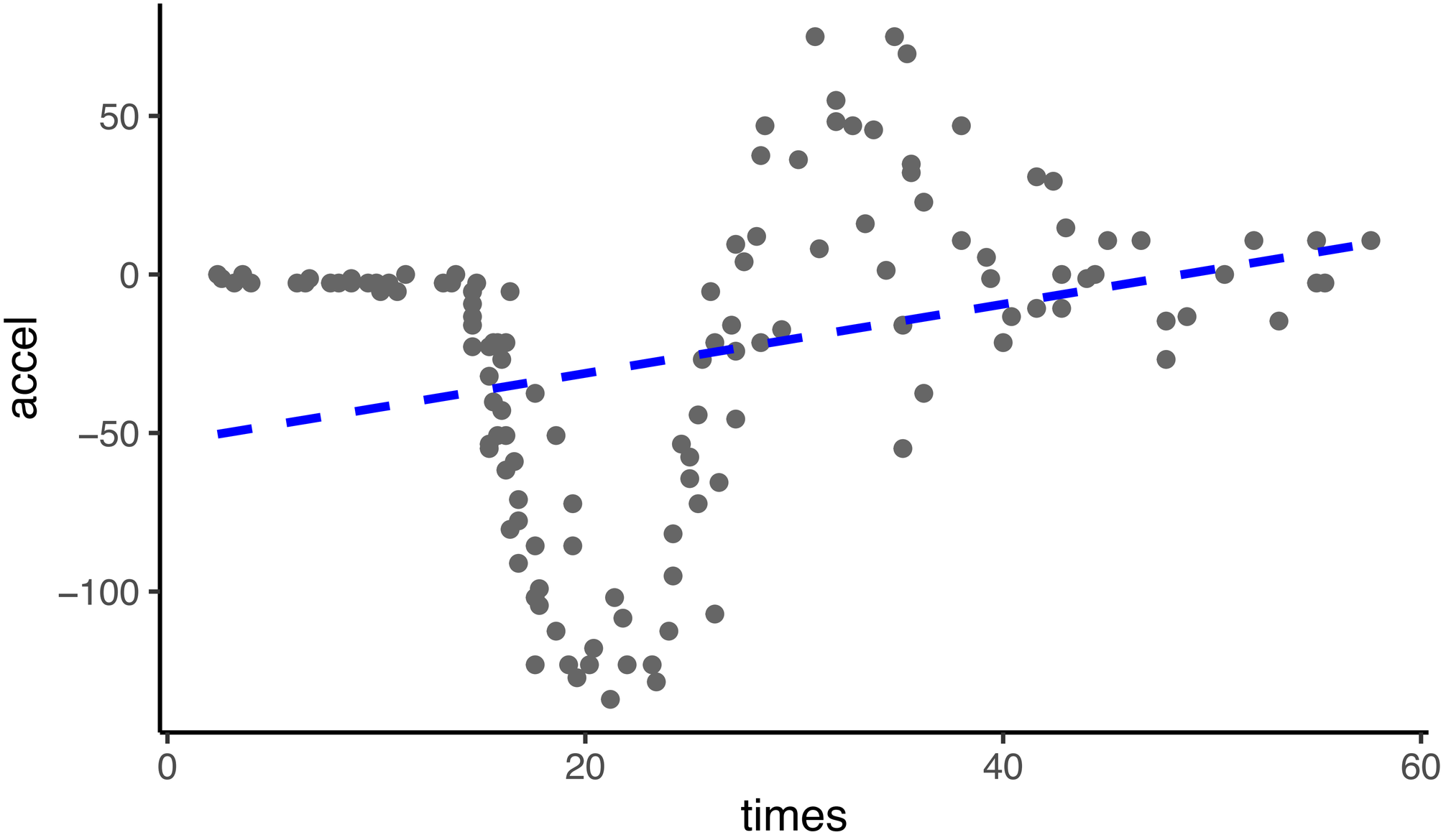




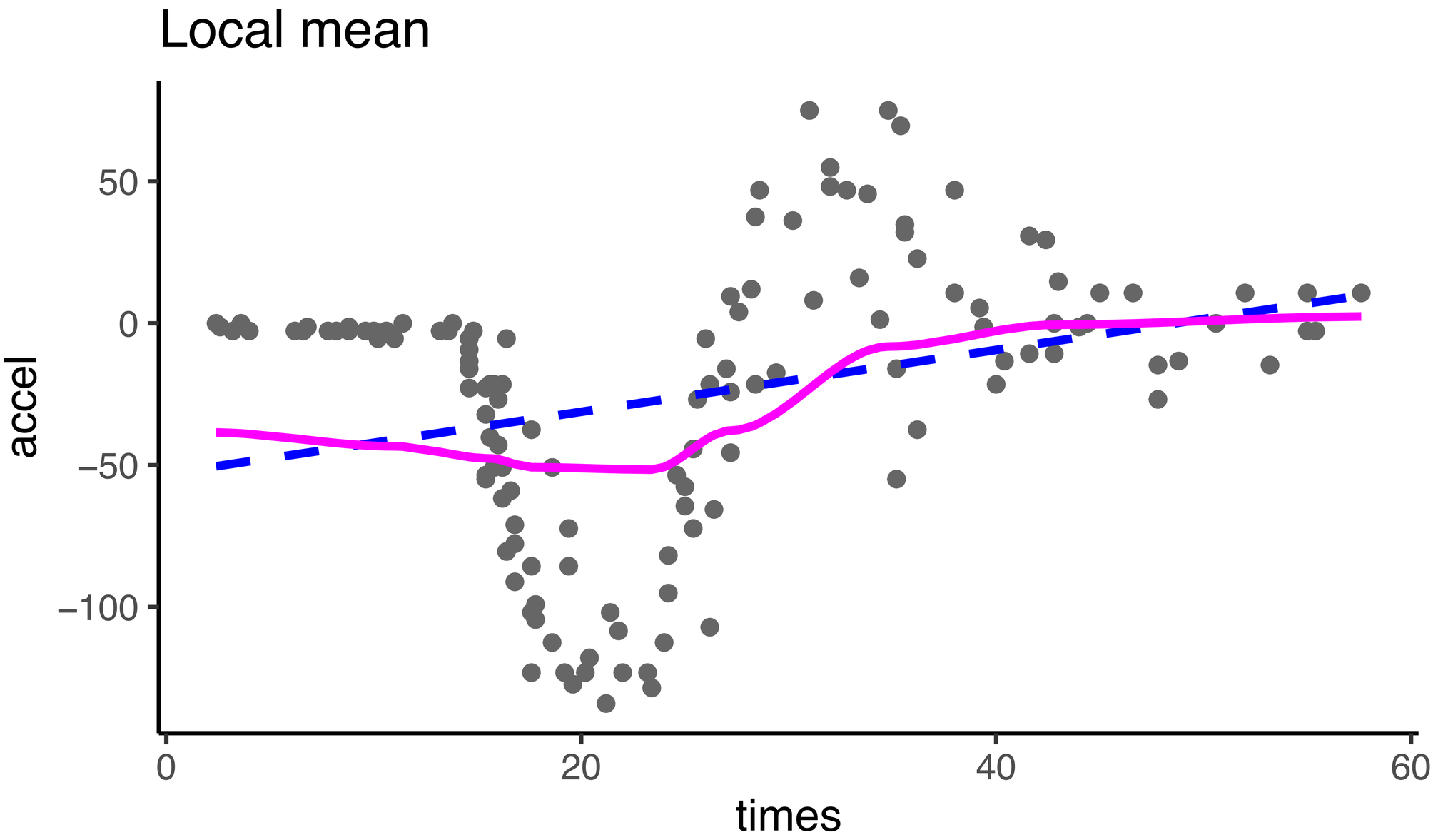
There are many things that a linear model cannot capture



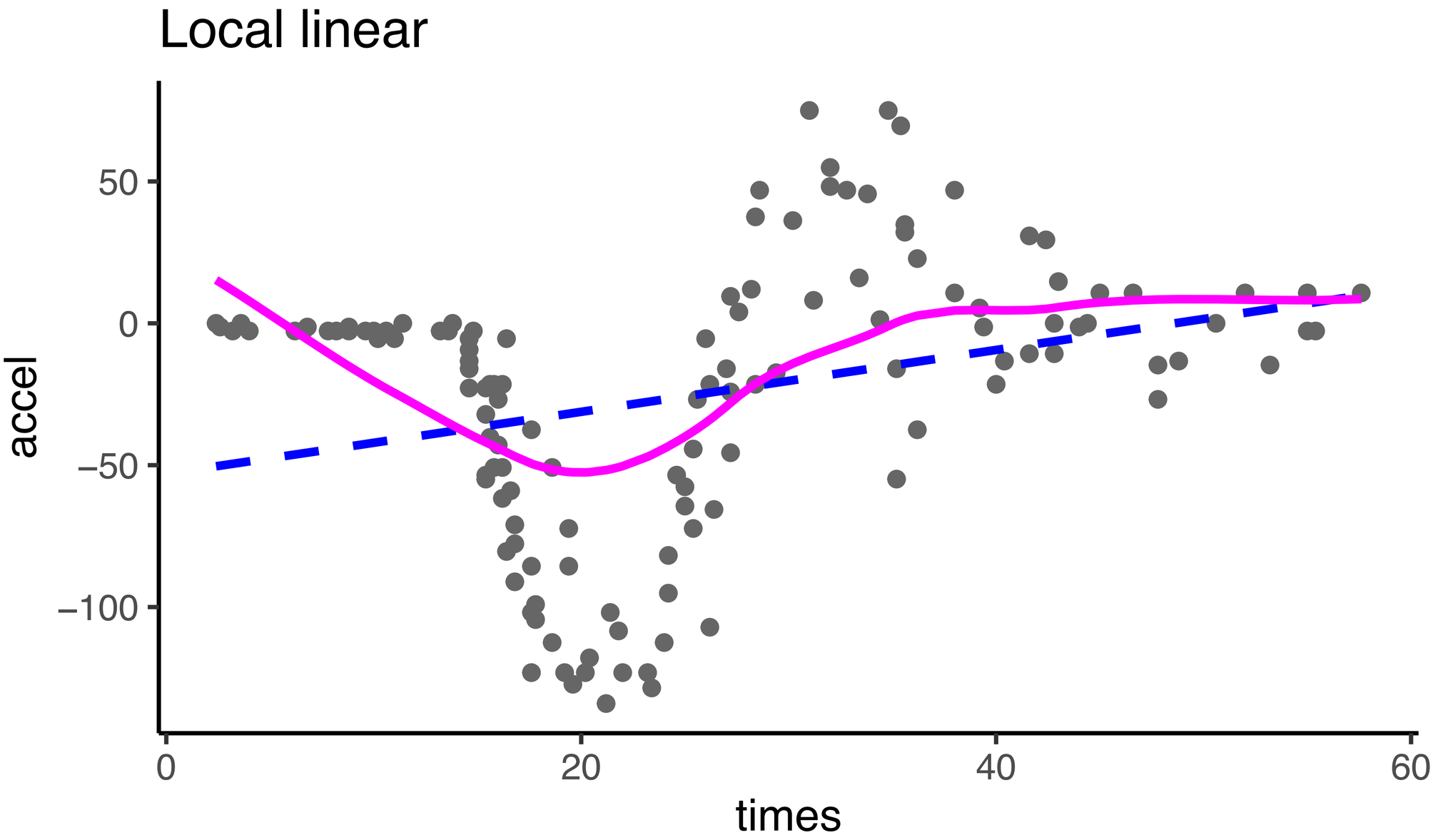
There are many things that a linear model cannot capture



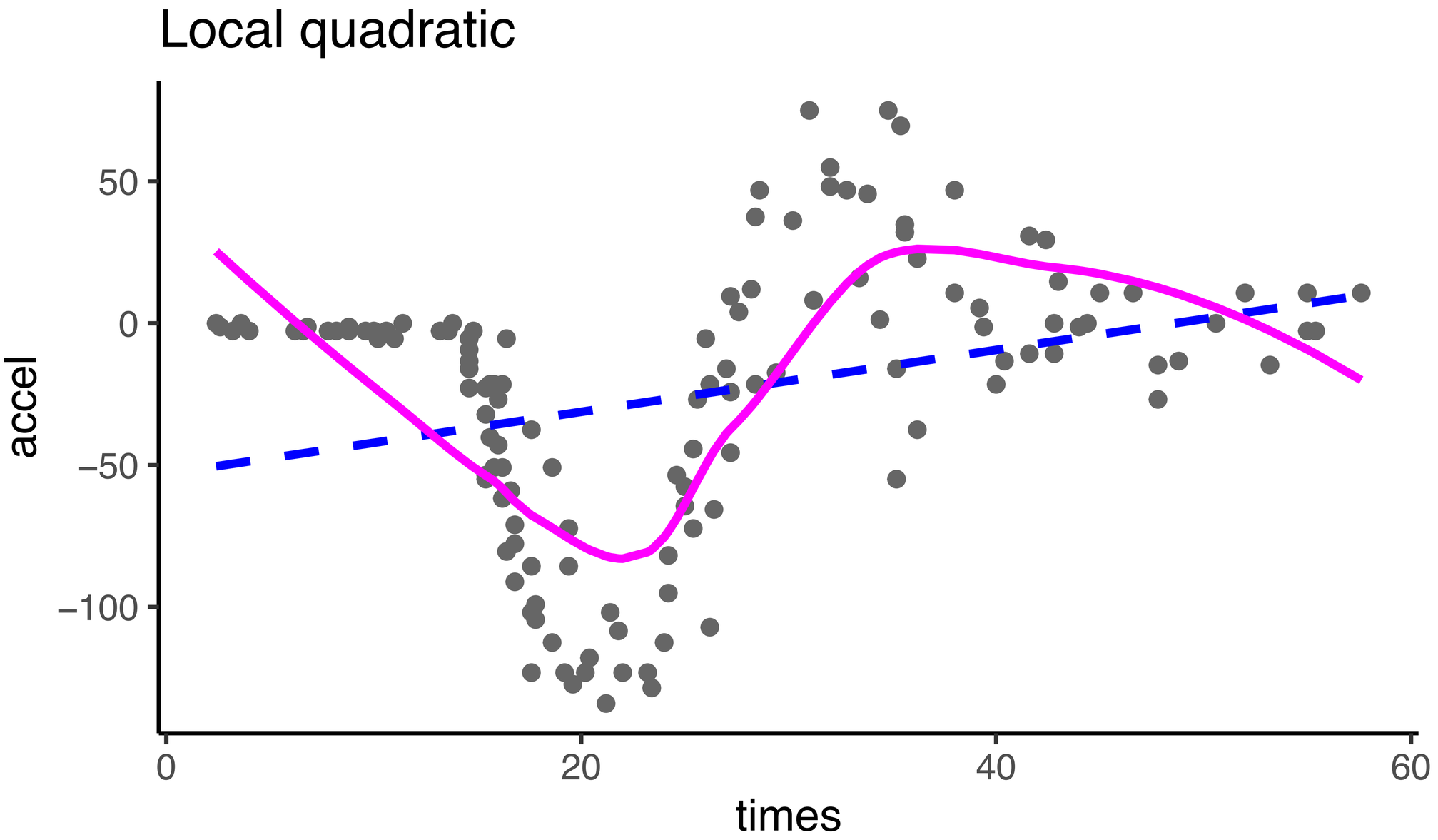
Let's think locally ... local polynomial regression `loess`



Let's think locally ... local polynomial regression `loess`



Let's think locally ... local polynomial regression `loess`

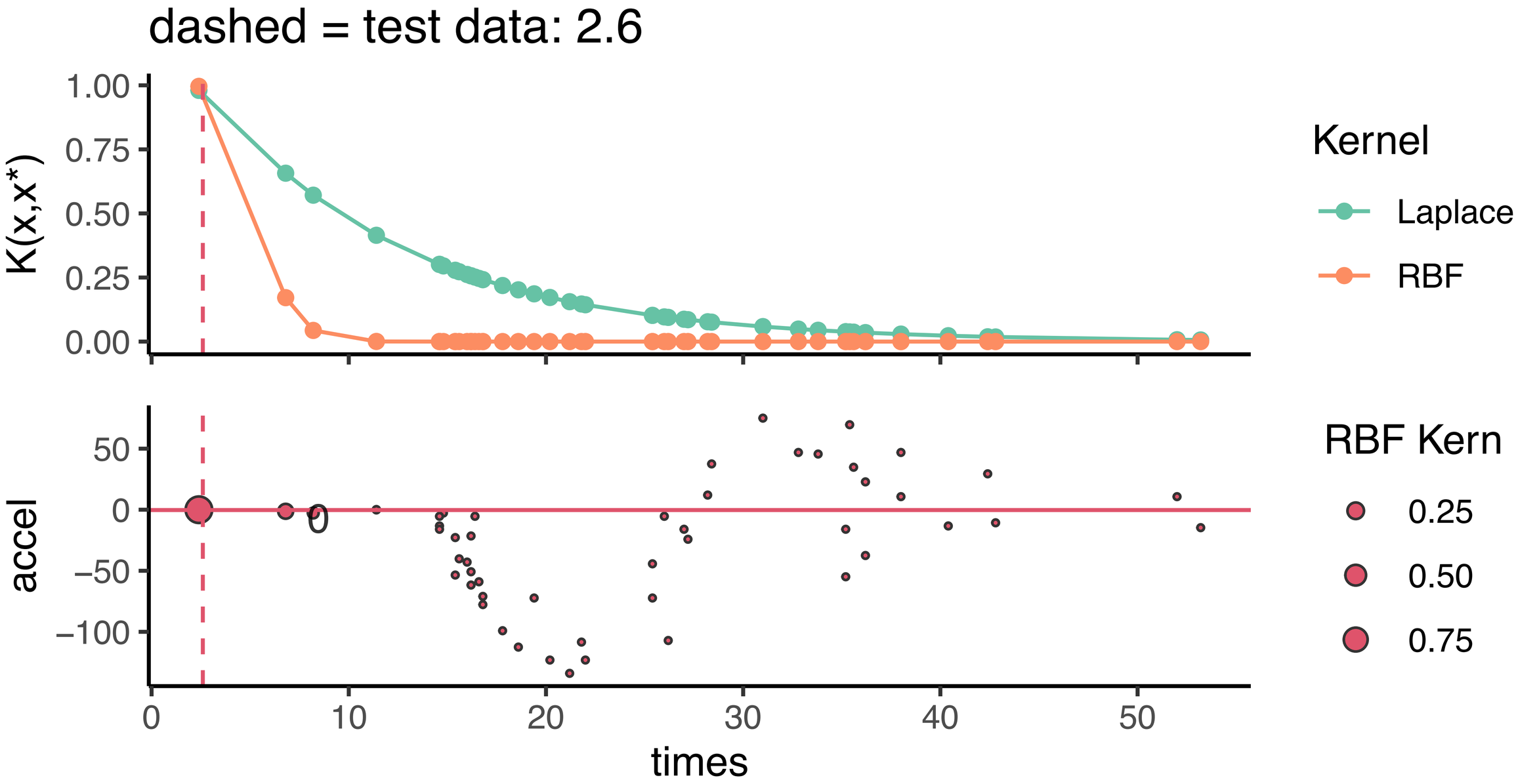


Nadaraya-Watson (kernelized local) estimator

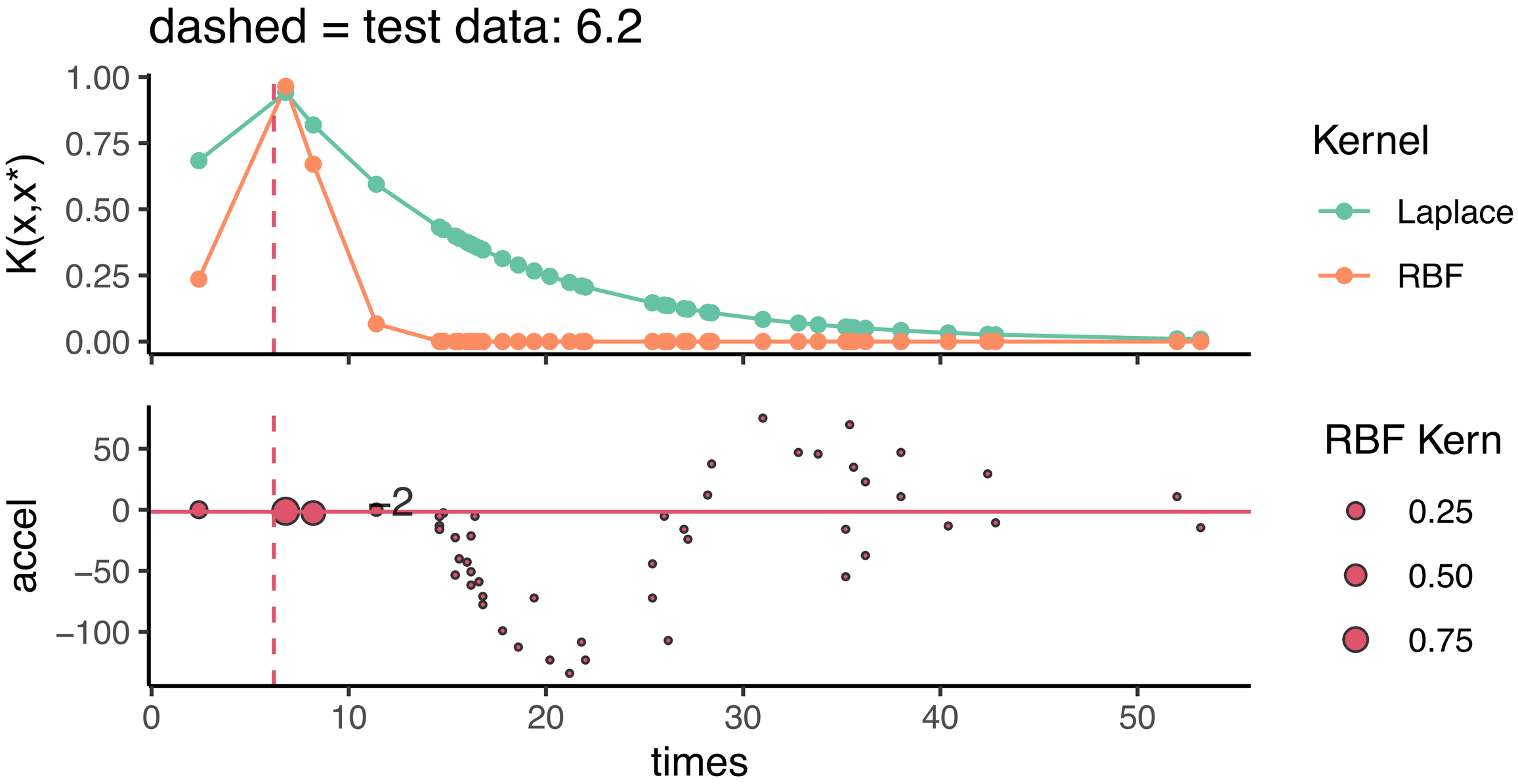
```
nn <- nrow(mcycle)
train.idx <- sample(nn, 50)
x.test <-
    mcycle[-train.idx, 1, drop = FALSE] %>%
    as.matrix
y.test <-
    mcycle[-train.idx, 2, drop = FALSE] %>%
    as.matrix
x.train <-
    mcycle[train.idx, 1, drop = FALSE] %>%
    as.matrix
y.train <-
    mcycle[train.idx, 2, drop = FALSE] %>%
    as.matrix
```

```
#' @param x.test testing data points
#' @param X training X
#' @param Y training Y
#' @param h bandwidth h
nw.rbf <- function(x.test, X, Y, h) {
    rbf <- kernlab::rbfdot(sigma=h)
    K <- kernlab::kernelMatrix(rbf, x.test, X)
    W <- K / rowSums(K)
    W %*% as.matrix(Y)
}
```

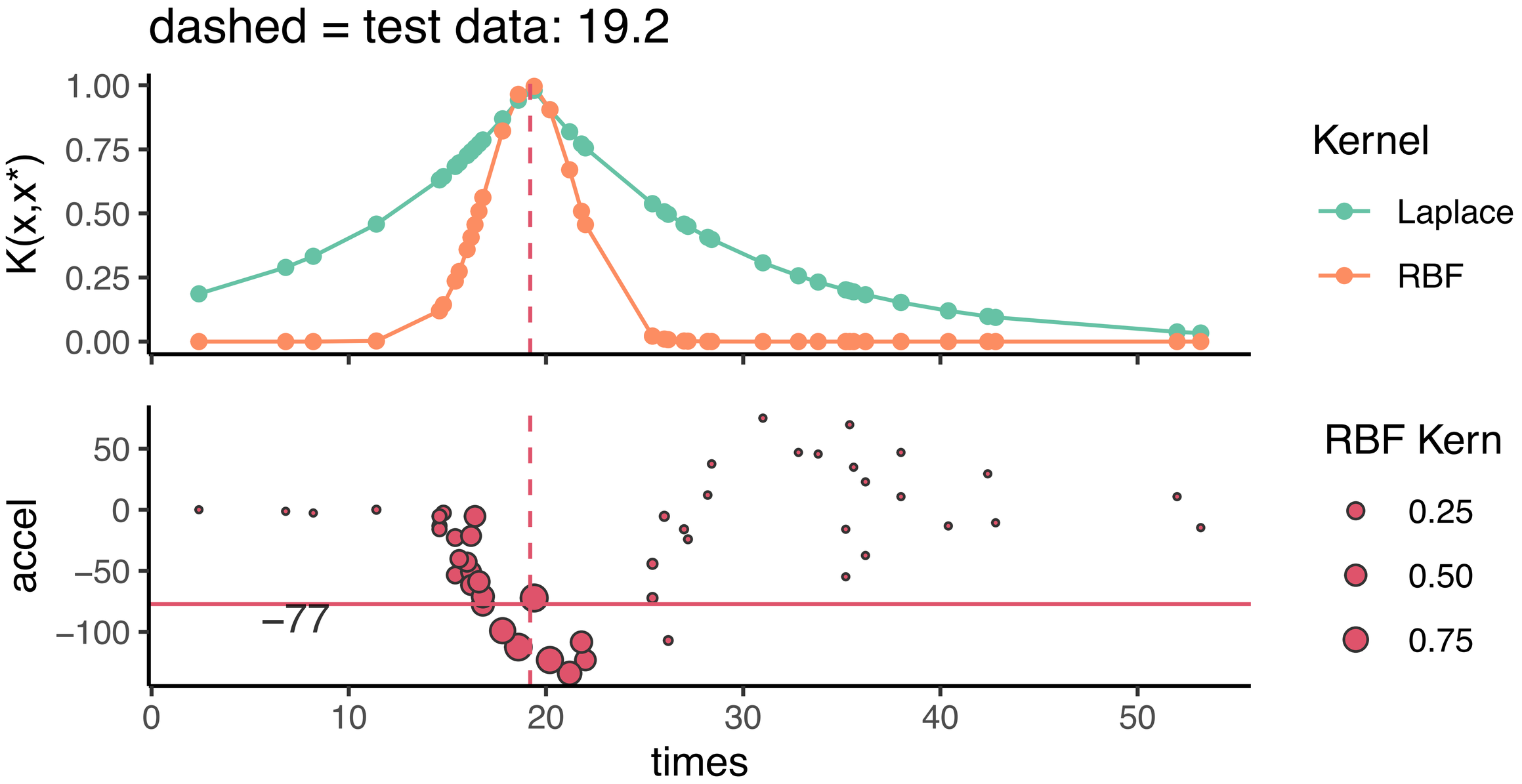
Basic idea: borrow neighbours' Y values in training data



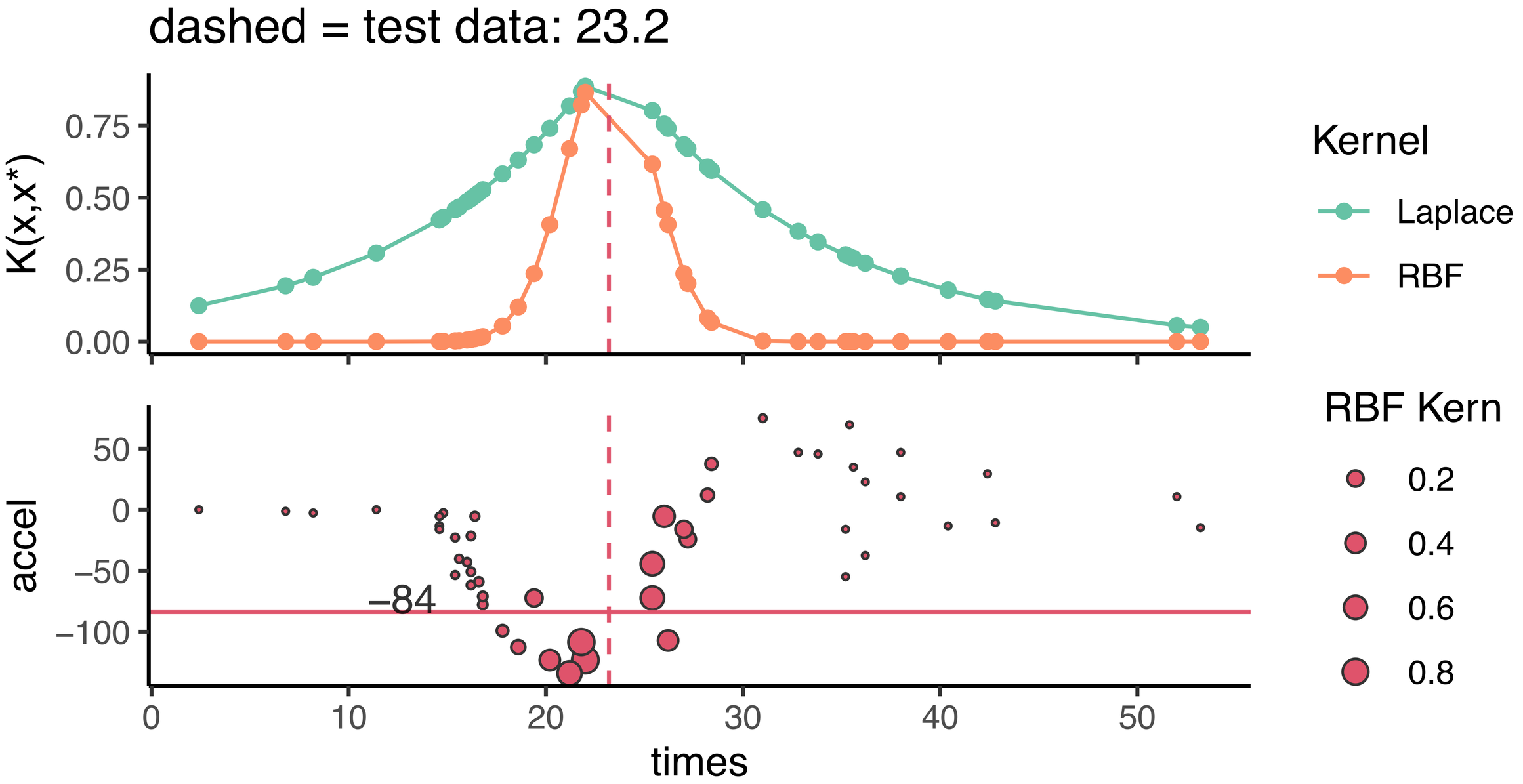
Basic idea: borrow neighbours' Y values in training data



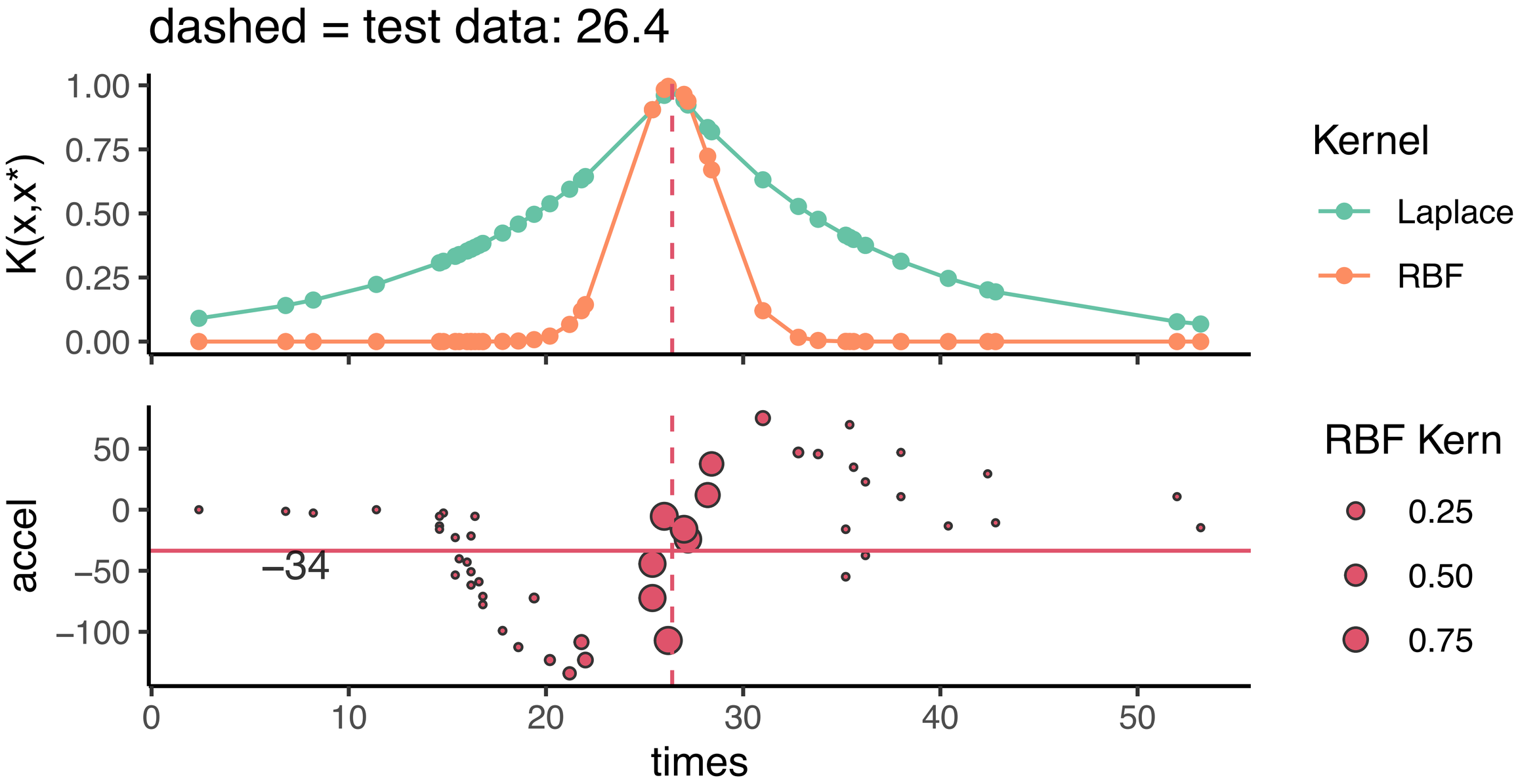
Basic idea: borrow neighbours' Y values in training data



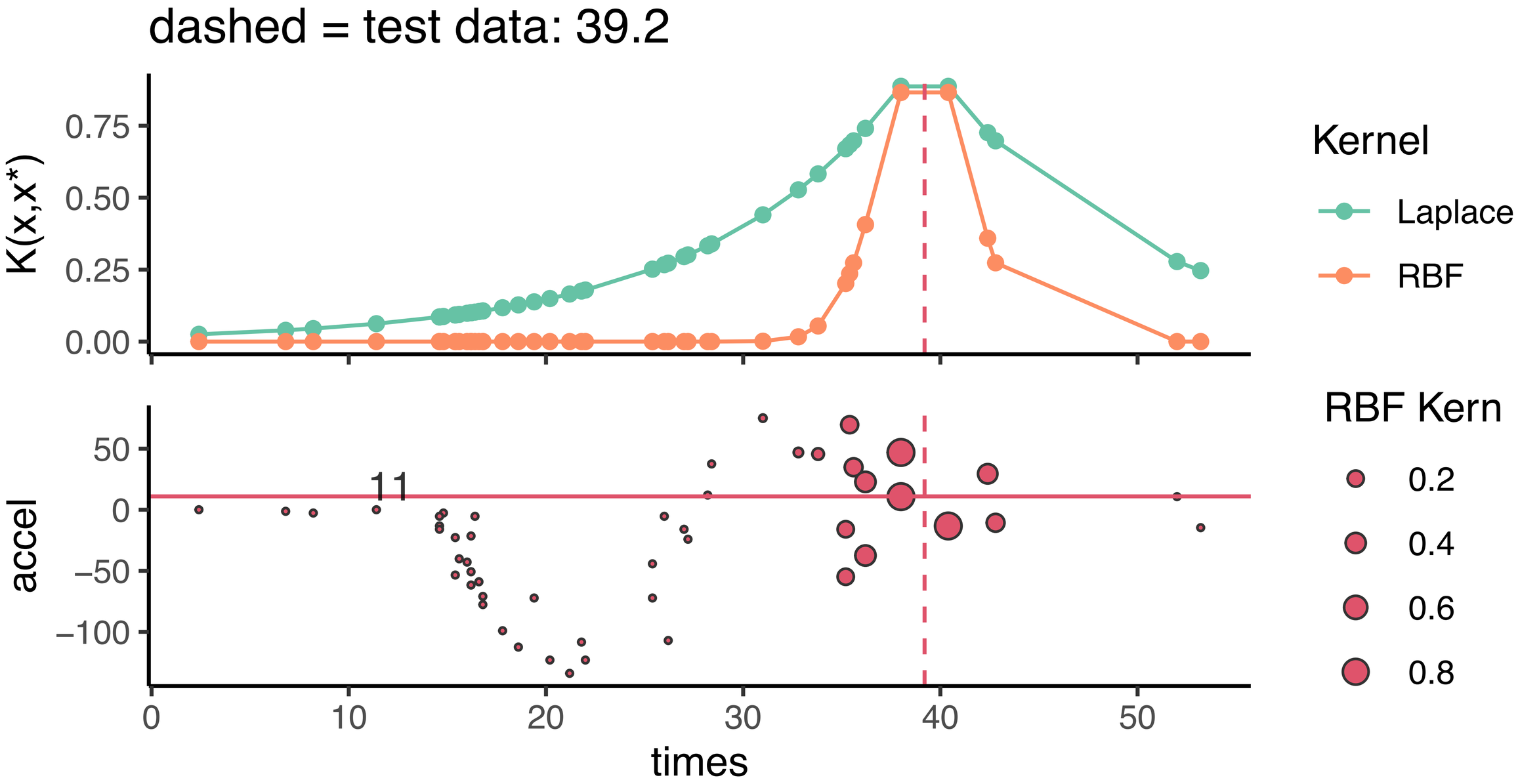
Basic idea: borrow neighbours' Y values in training data



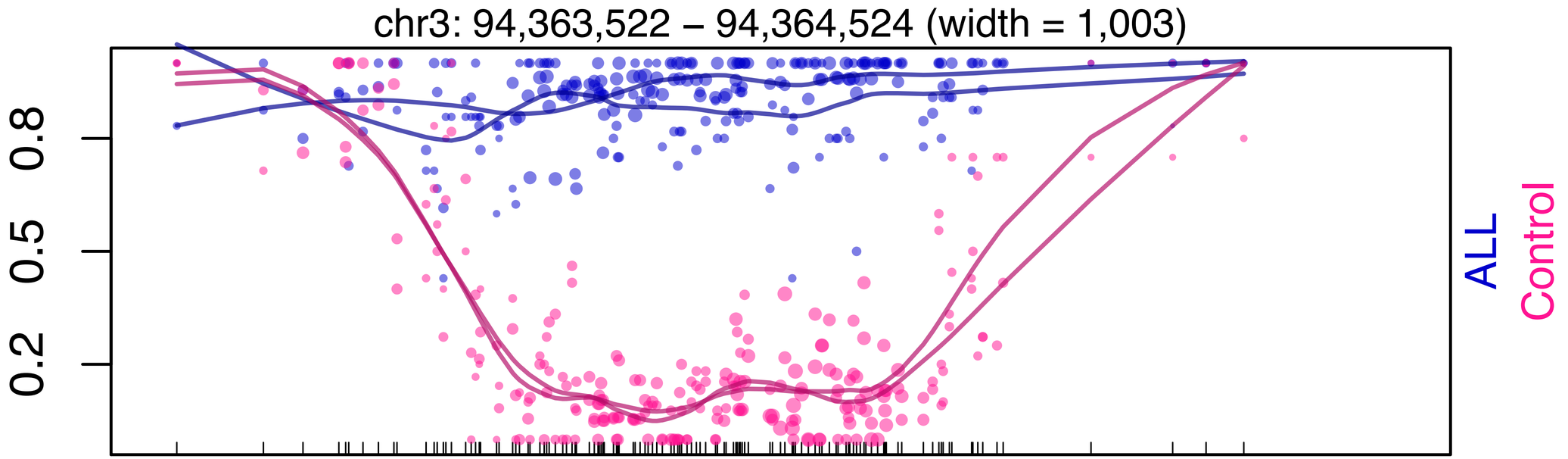
Basic idea: borrow neighbours' Y values in training data



Basic idea: borrow neighbours' Y values in training data

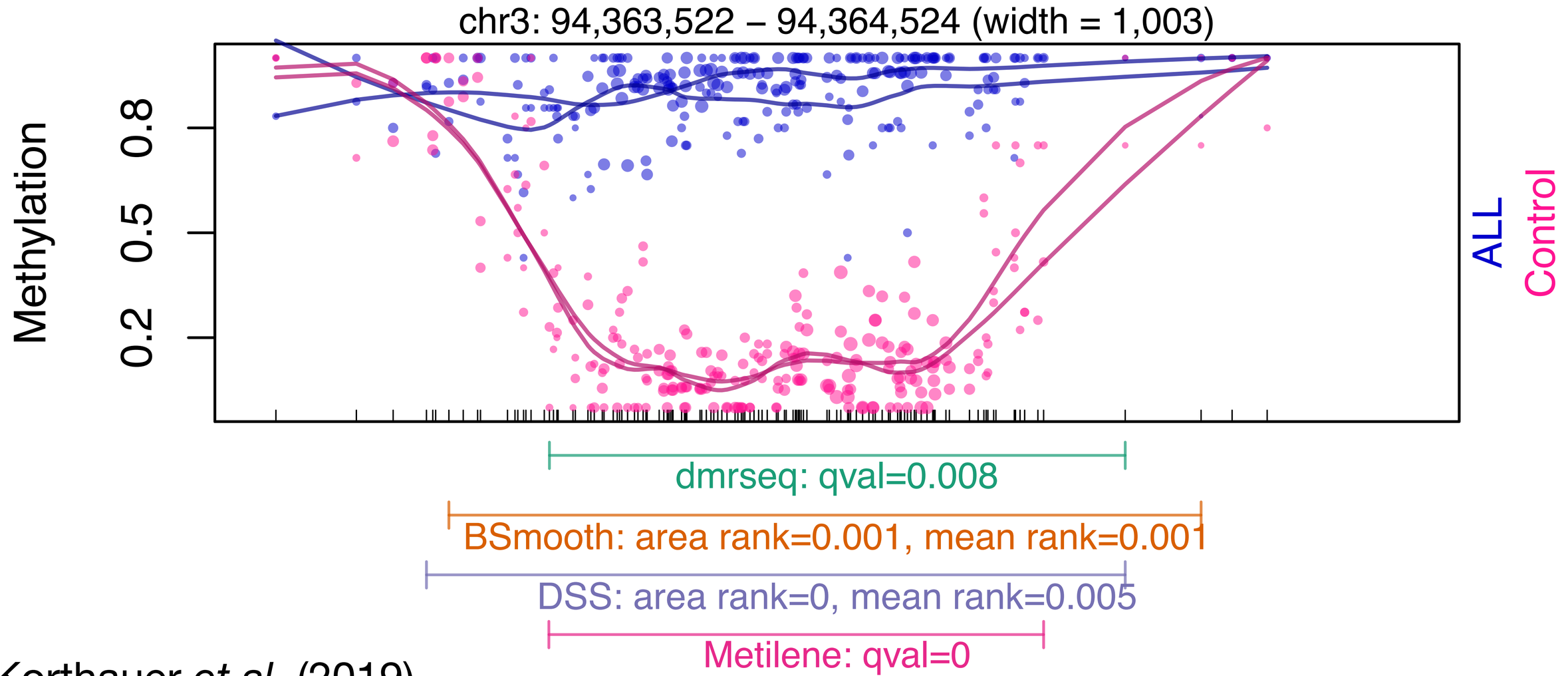


Calibrating DMR considering FDR



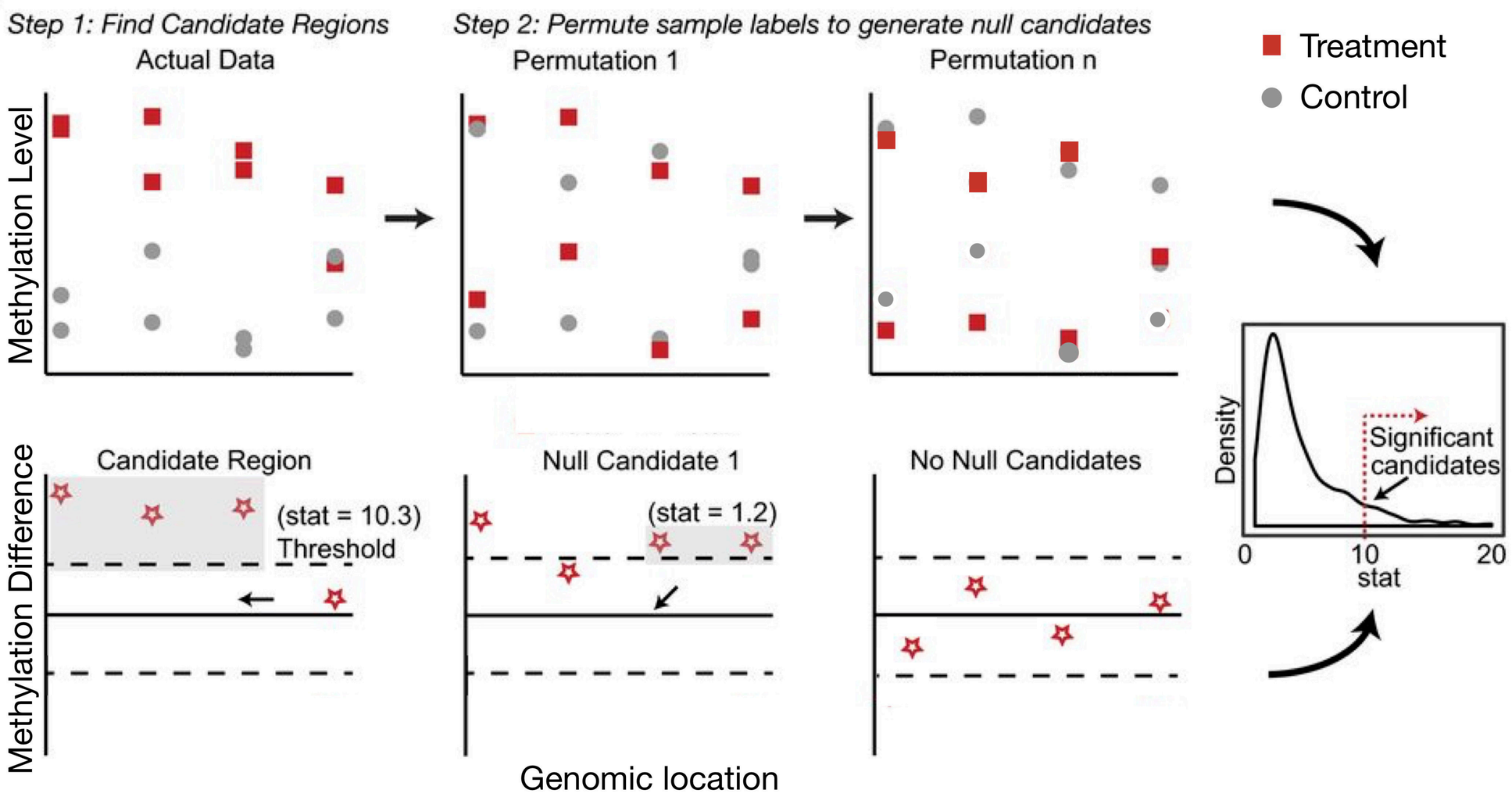
Korthauer *et al.* (2019)

dmrseq improves statistical power with tight FDR clibration



Korthauer *et al.* (2019)

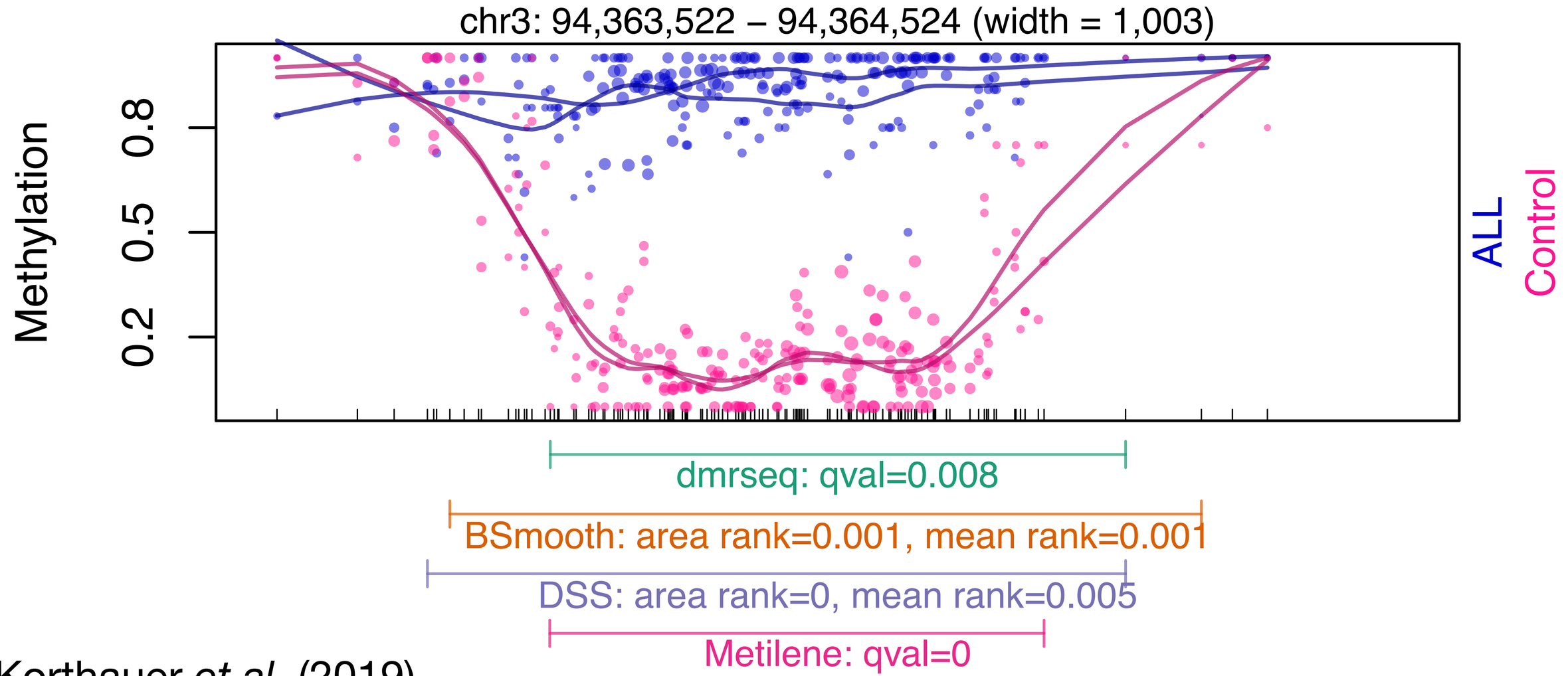
How can we tighten up FDR correction?



Korthauer *et al.* (2019)

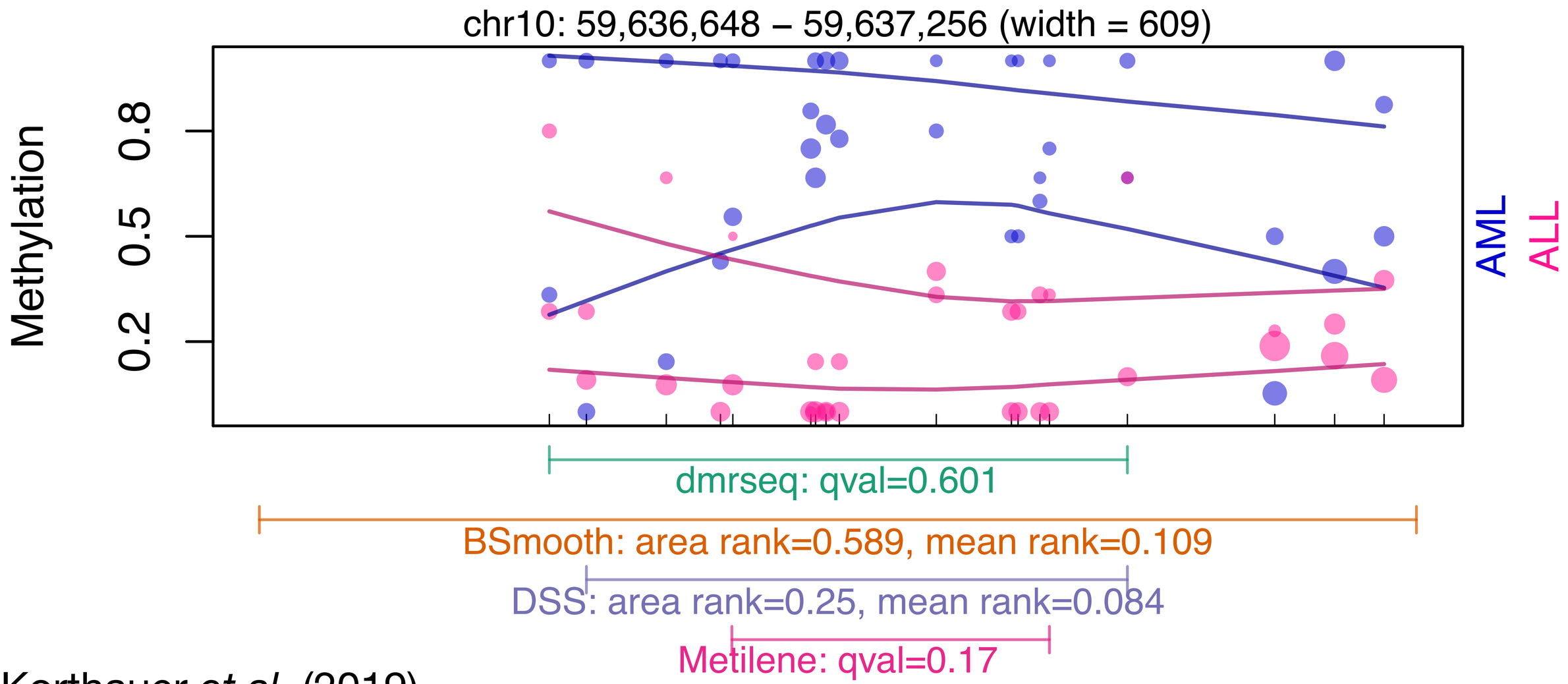
dmrseq

Do we see a DMR?



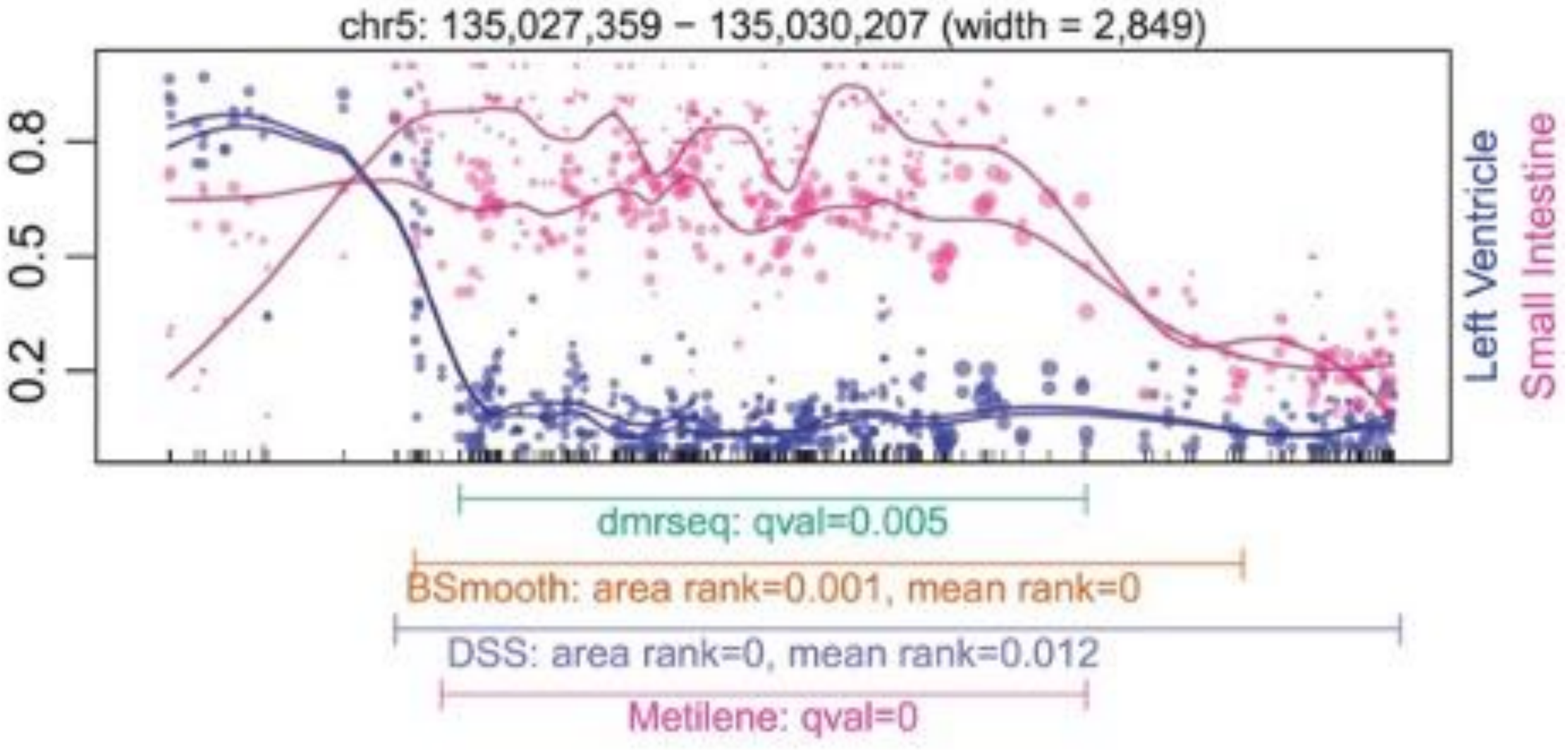
Korthauer *et al.* (2019)

Do we see a DMR?

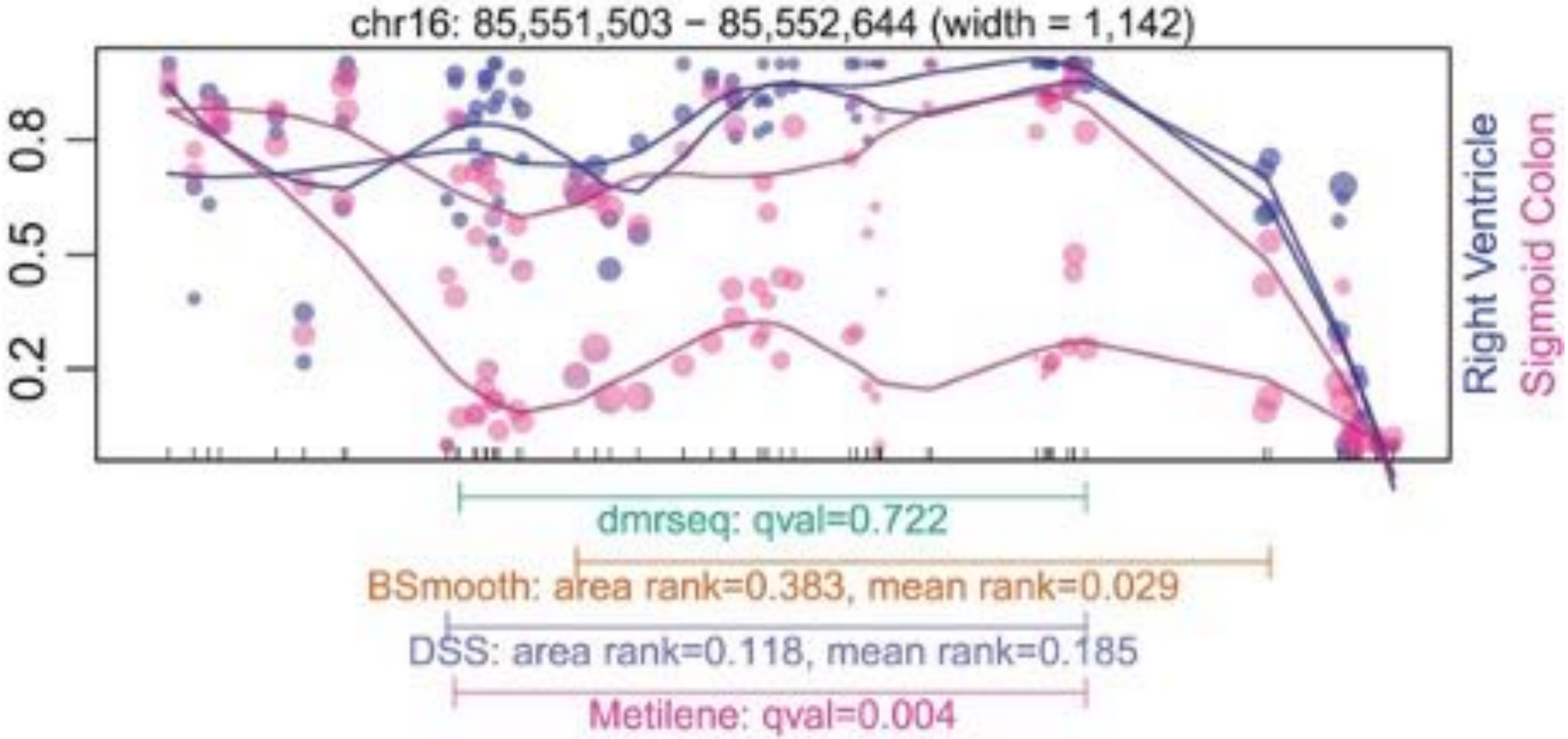


Korthauer *et al.* (2019)

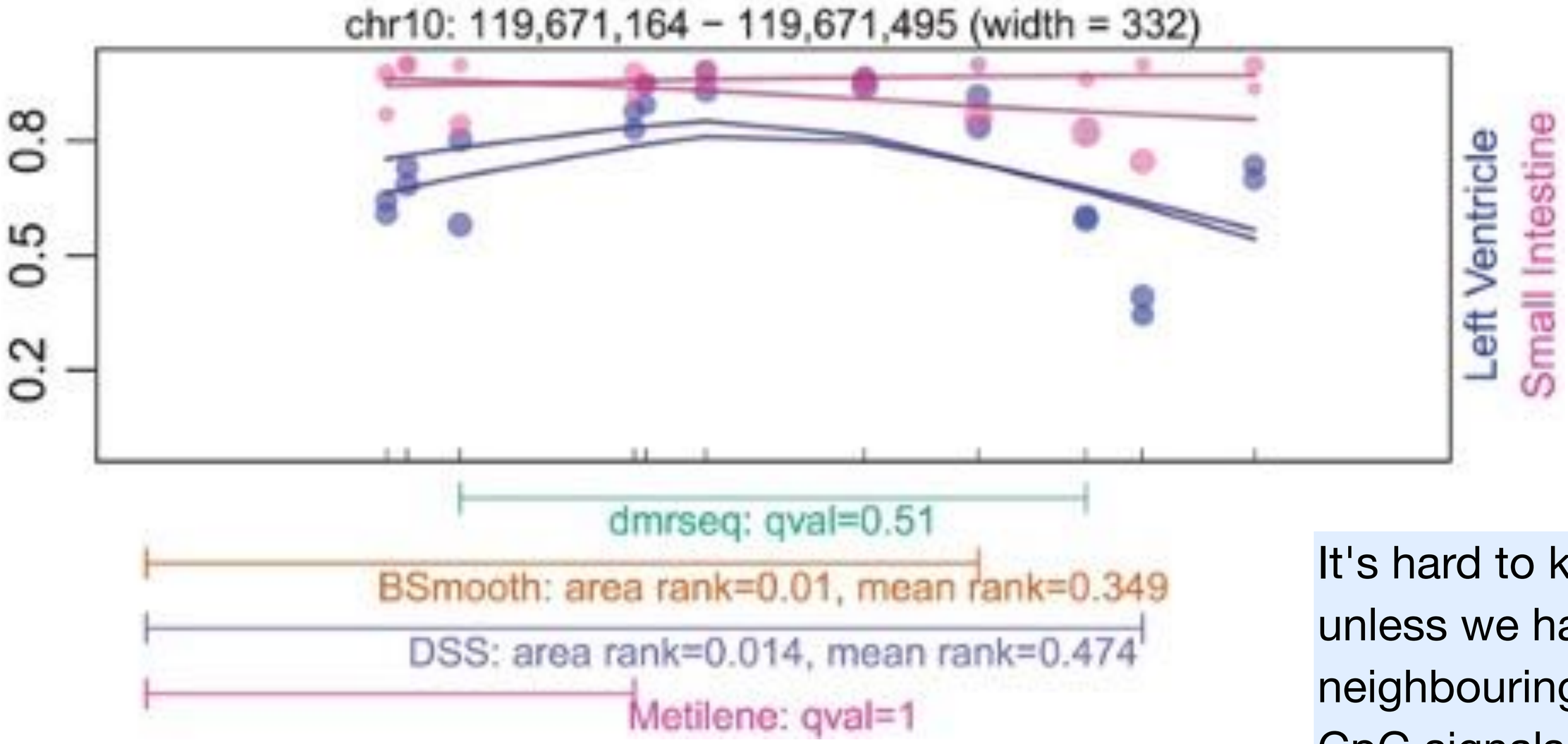
An obvious case where most methods work fine



Some start breaking down in a harder case



Heuristic approaches report wrong



It's hard to know unless we have neighbouring CpG signals

Statistical Methods for Epigenomics

- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)

Epigenetic modifications

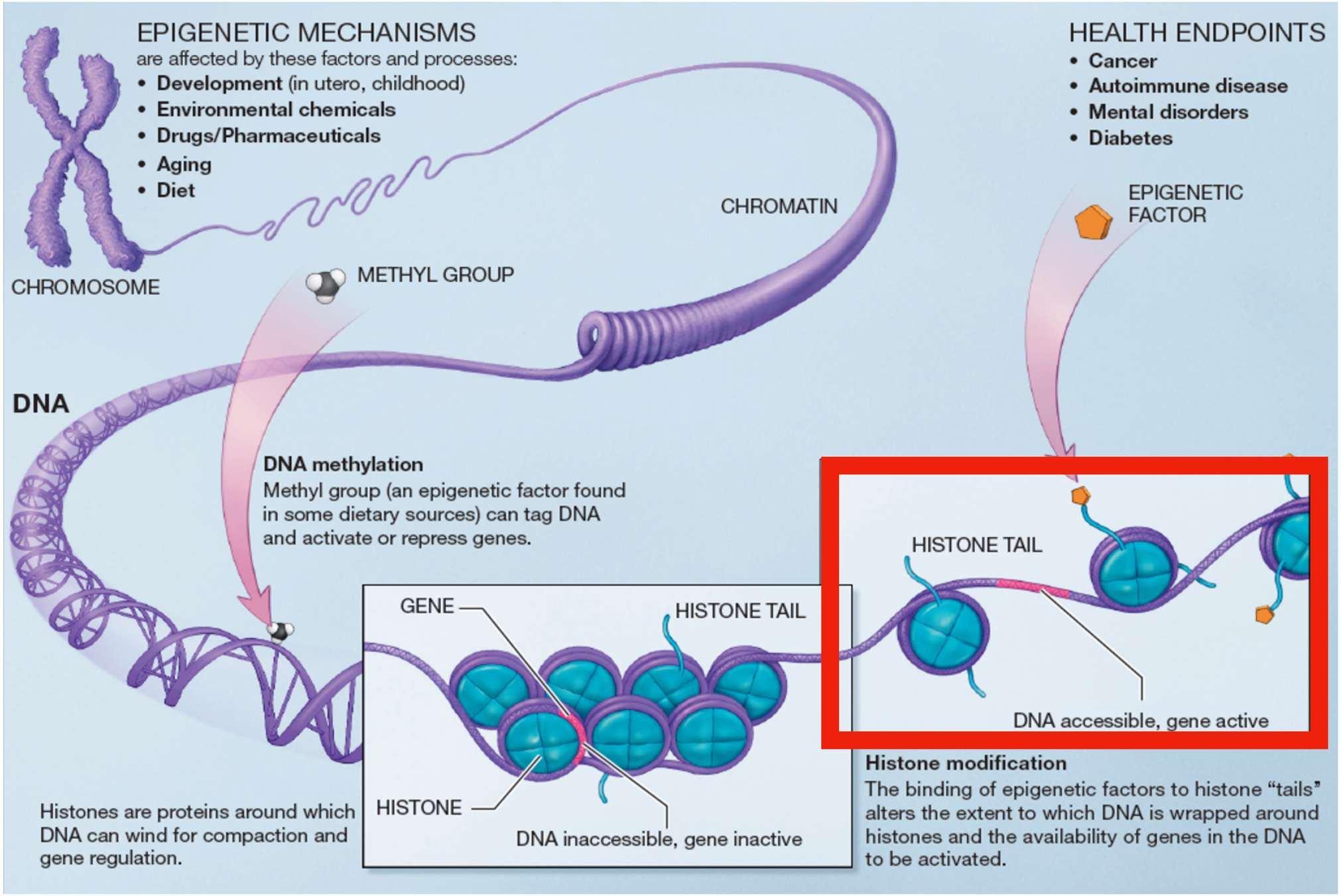
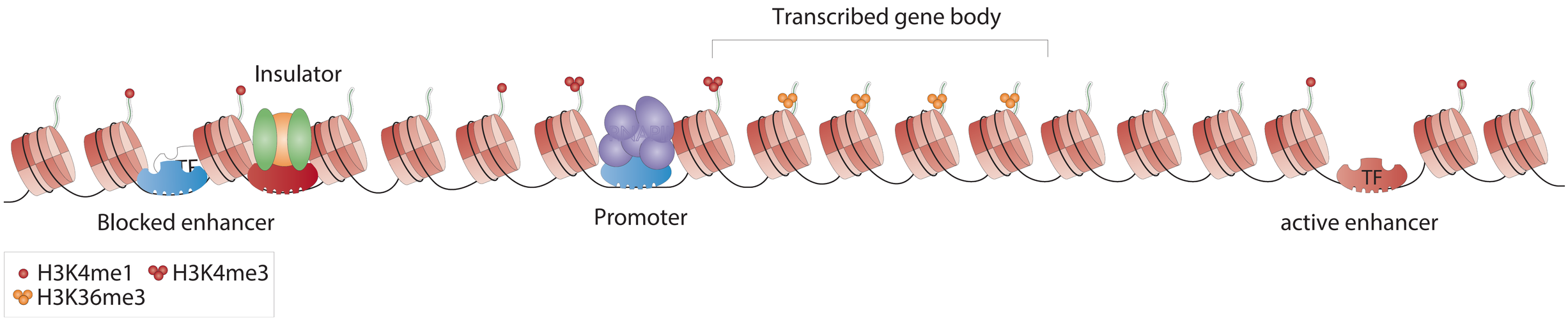


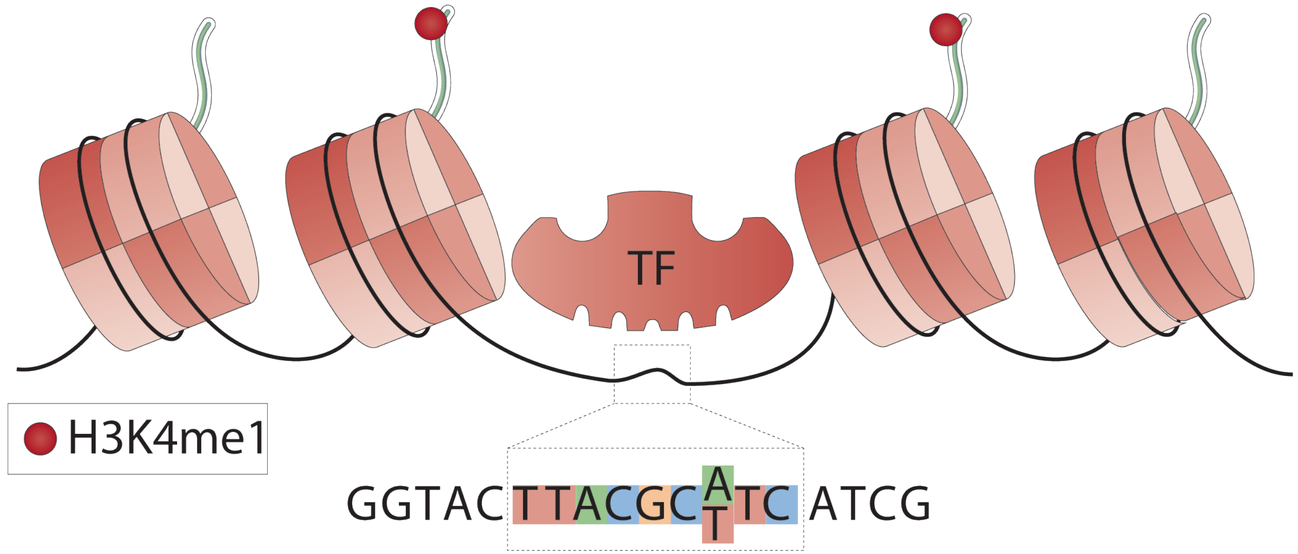
Image source: http://nihroadmap.nih.gov/epigenomics/

Different histone code marks different types of regulatory elements



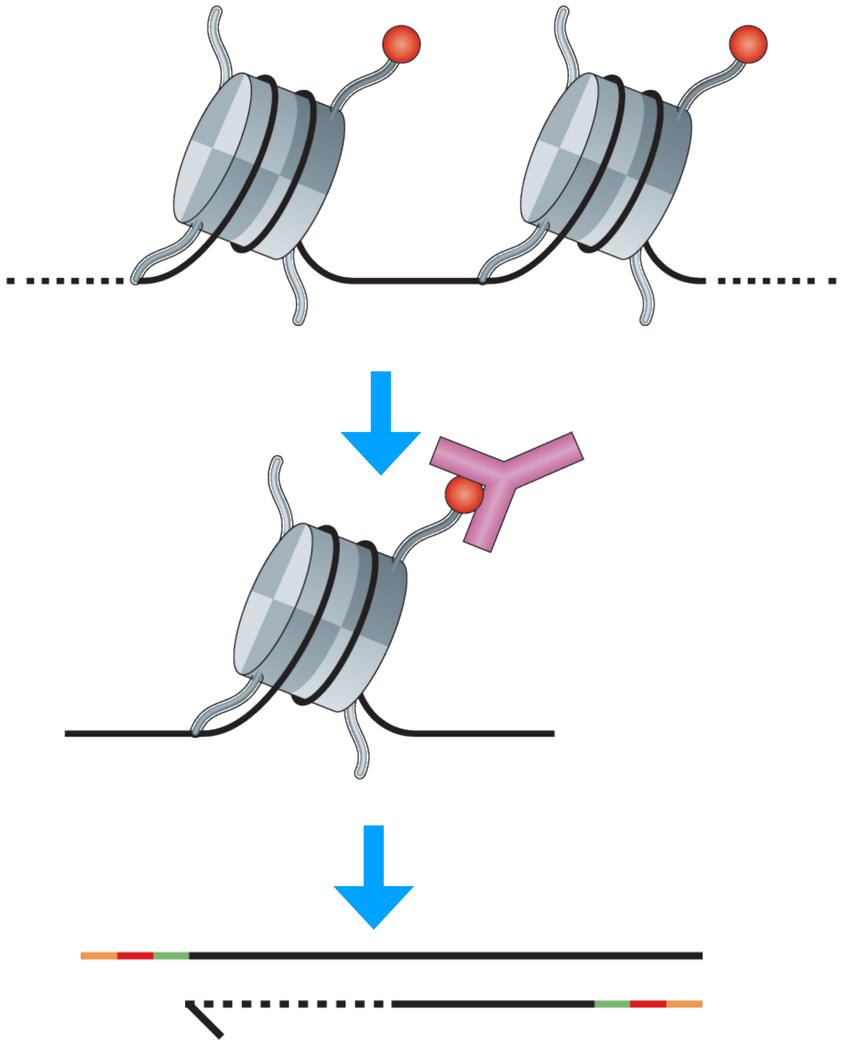
Hawkins, Hon, Ren, *Nature Review Genetics* (2010)

One of the goals is to understand the logic of non-coding DNA sequences



Hawkins, Hon, Ren, *Nature Review Genetics* (2010)

ChIP-seq: Quantifying histone modifications



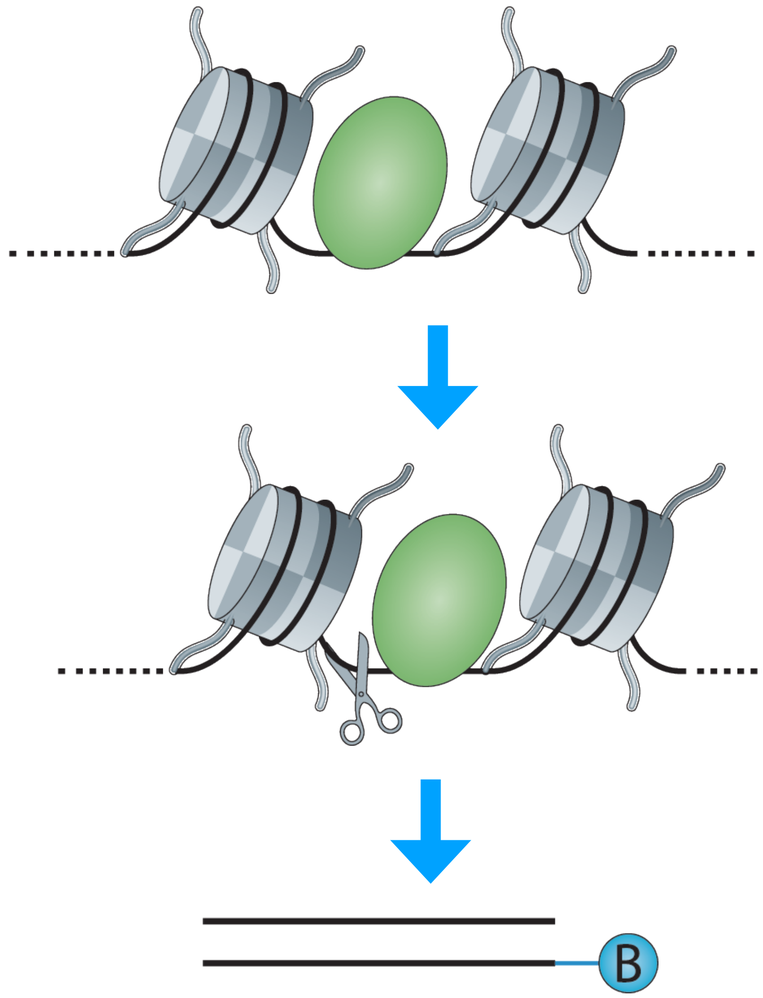
Crosslink proteins & DNA

Immunoprecipitate &
purify DNA bound

Amplify/duplicate
the extracted
DNA segments

Furey, *Nature Reviews Genetics* (2012)

DNase-seq: Quantifying DNA accessibility



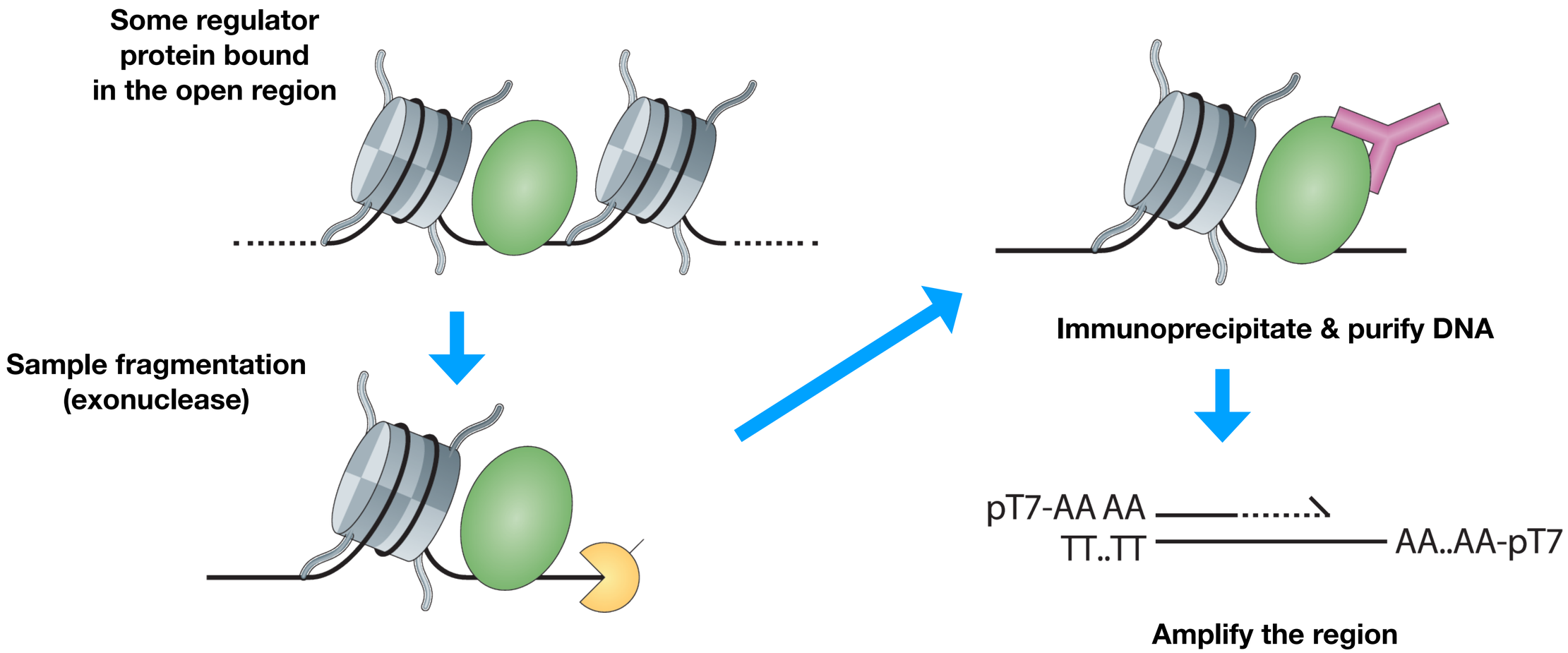
Some regulator protein bound
in the open region

Sample fragmentation
(DNaseI digestion)

Add biotynylated linkers
and extract

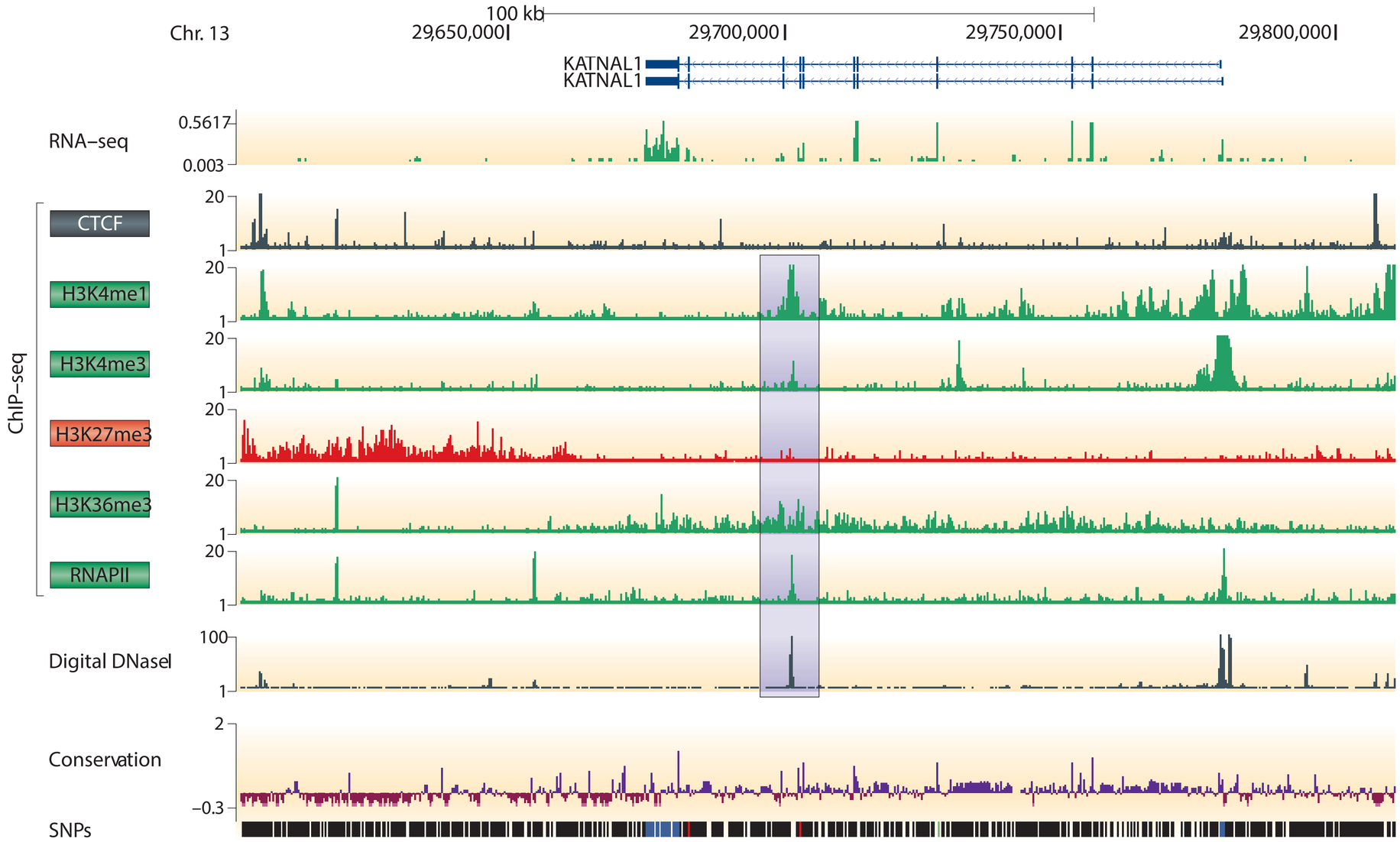
Furey, *Nature Reviews Genetics* (2012)

ChIP-seq: Quantifying regions bound by a particular protein



Furey, *Nature Reviews Genetics* (2012)

An example of multiple ChIP-seq tracks

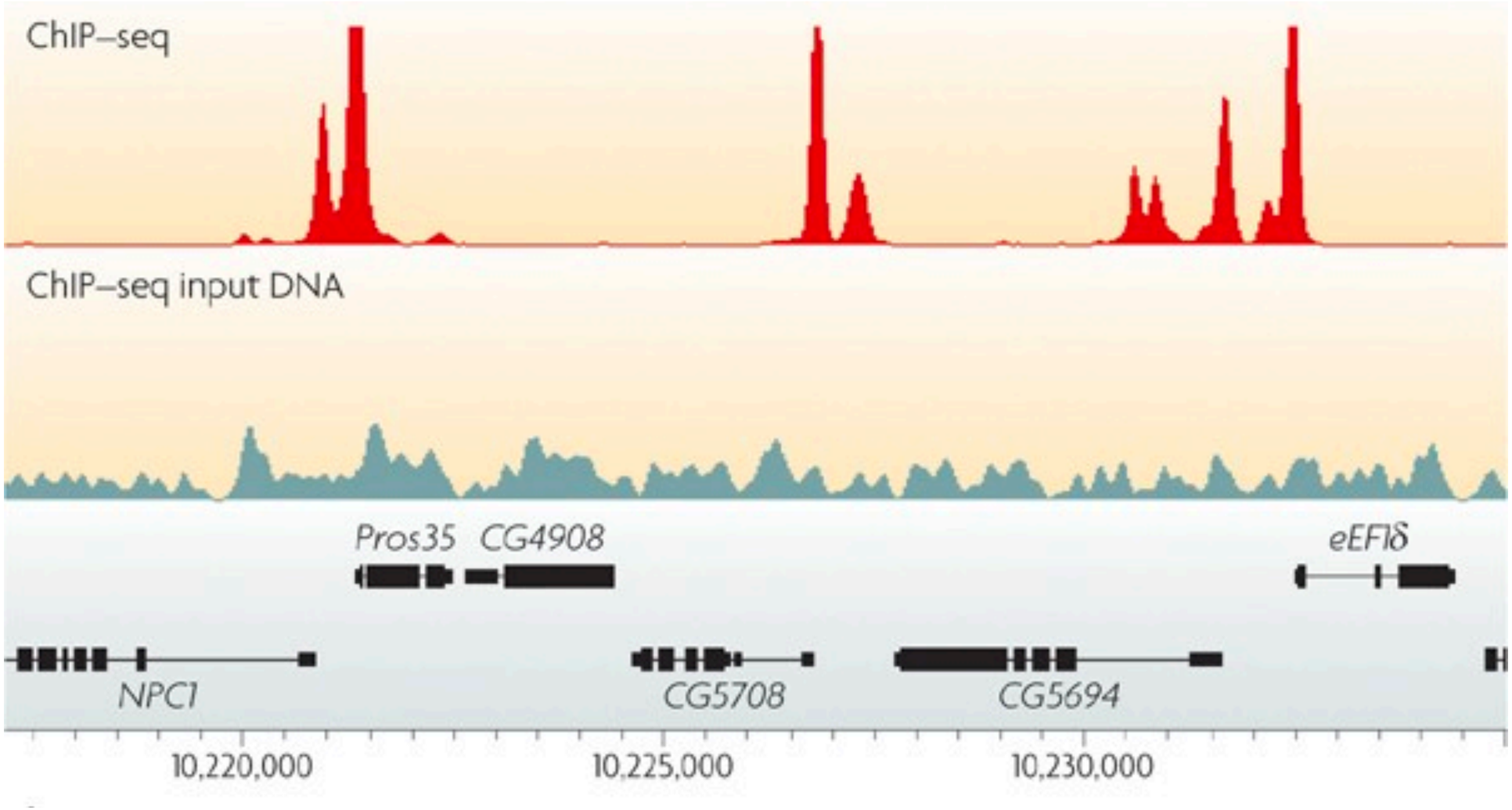


Hawkins, Hon, Ren, *Nature Review Genetics* (2010)

Statistical Methods for Epigenomics

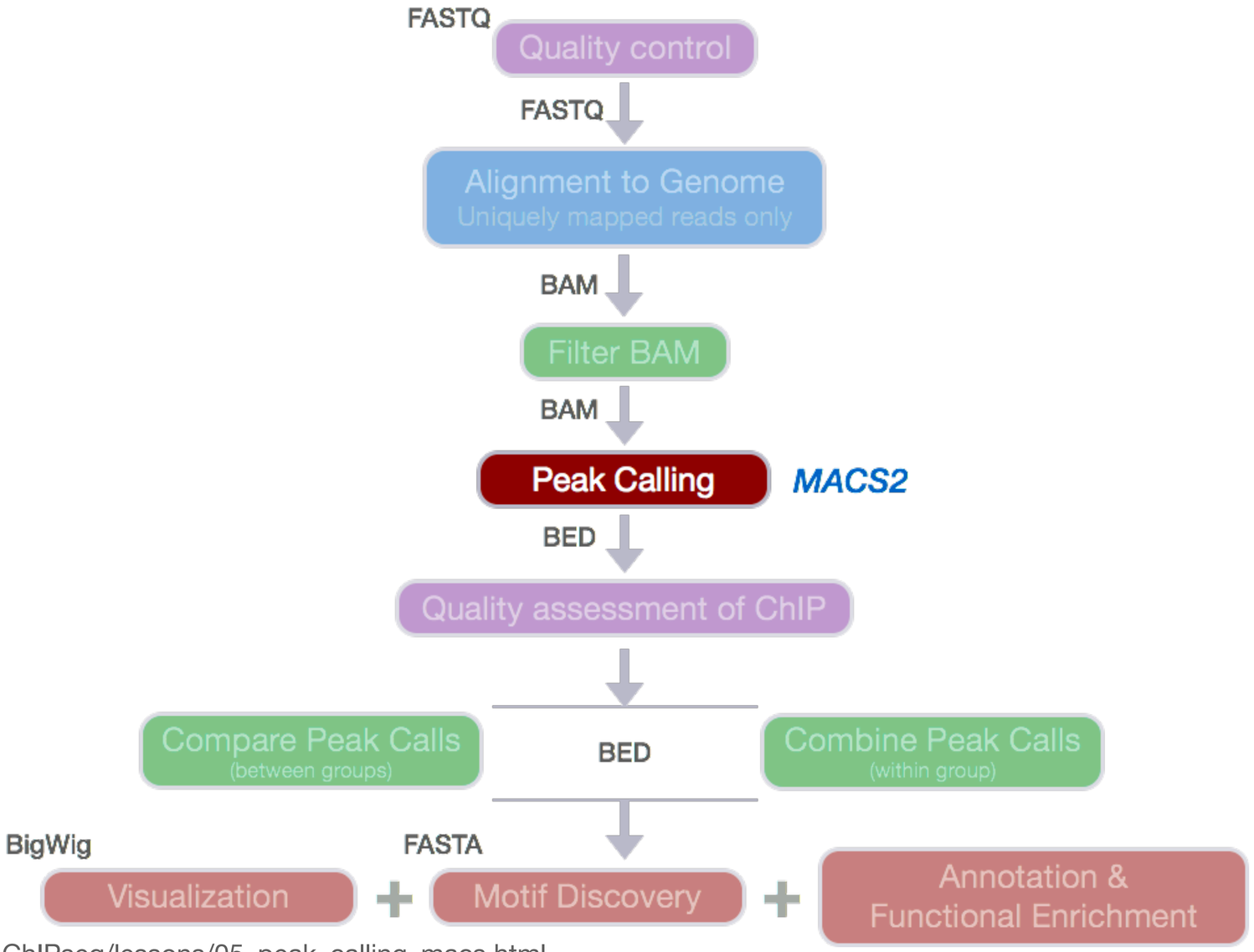
- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)

How do we know which regions are "peaks?"



Park, Nature Review Genetics (2009)

ChIP-seq analysis pipeline



https://hbctraining.github.io/Intro-to-ChIPseq/lessons/05_peak_calling_macs.html

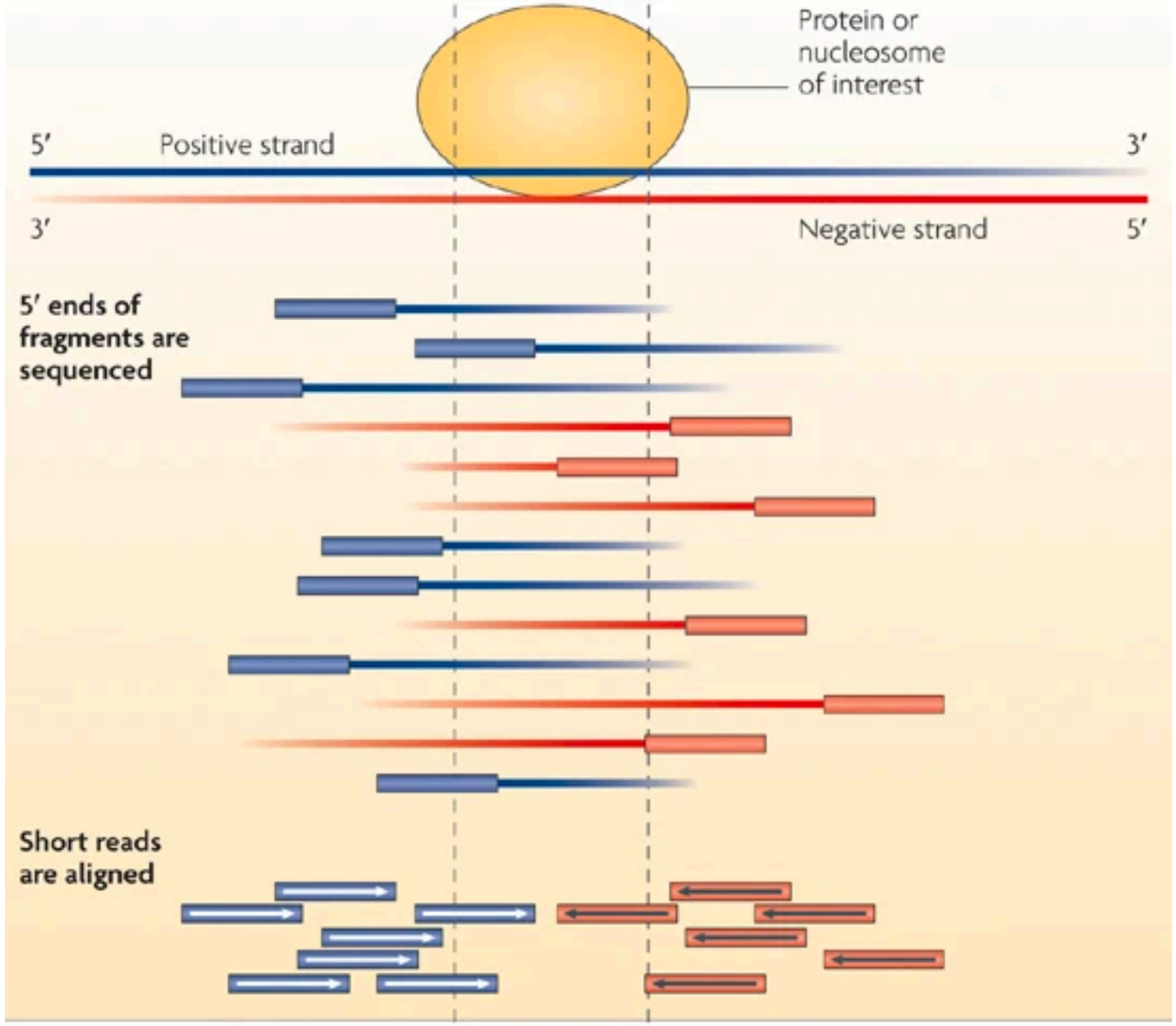
Method | Open access | Published: 17 September 2008

Model-based Analysis of ChIP-Seq (MACS)

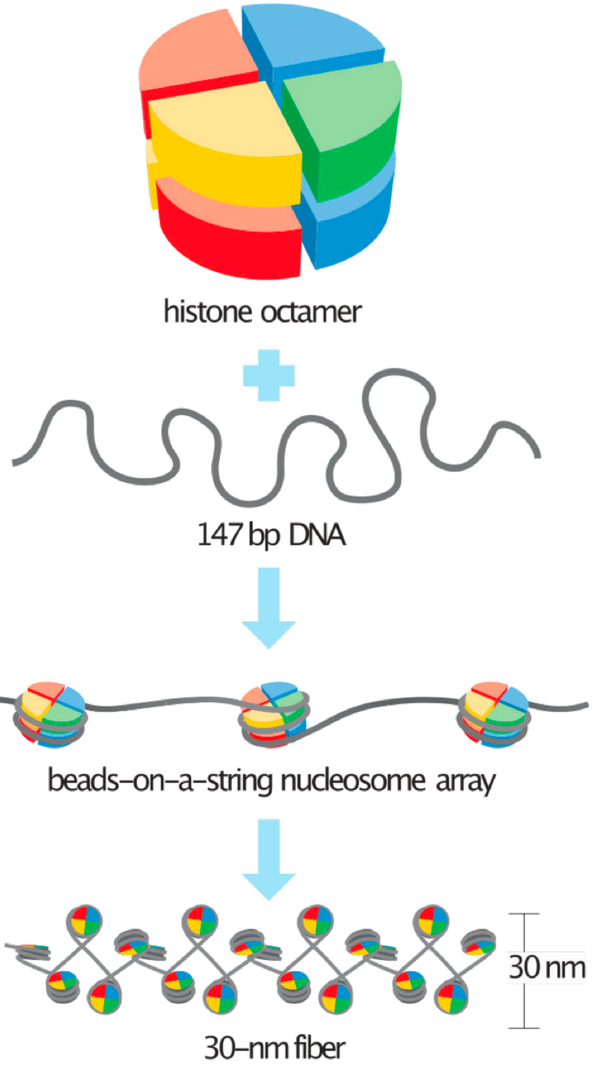
Yong Zhang, Tao Liu, Clifford A Meyer, Jérôme Eeckhoute, David S Johnson, Bradley E Bernstein, Chad Nusbaum, Richard M Myers, Myles Brown, Wei Li ✉ & X Shirley Liu ✉

Genome Biology **9**, Article number: R137 (2008) | Cite this article

Why not using the same type of bump hunter?

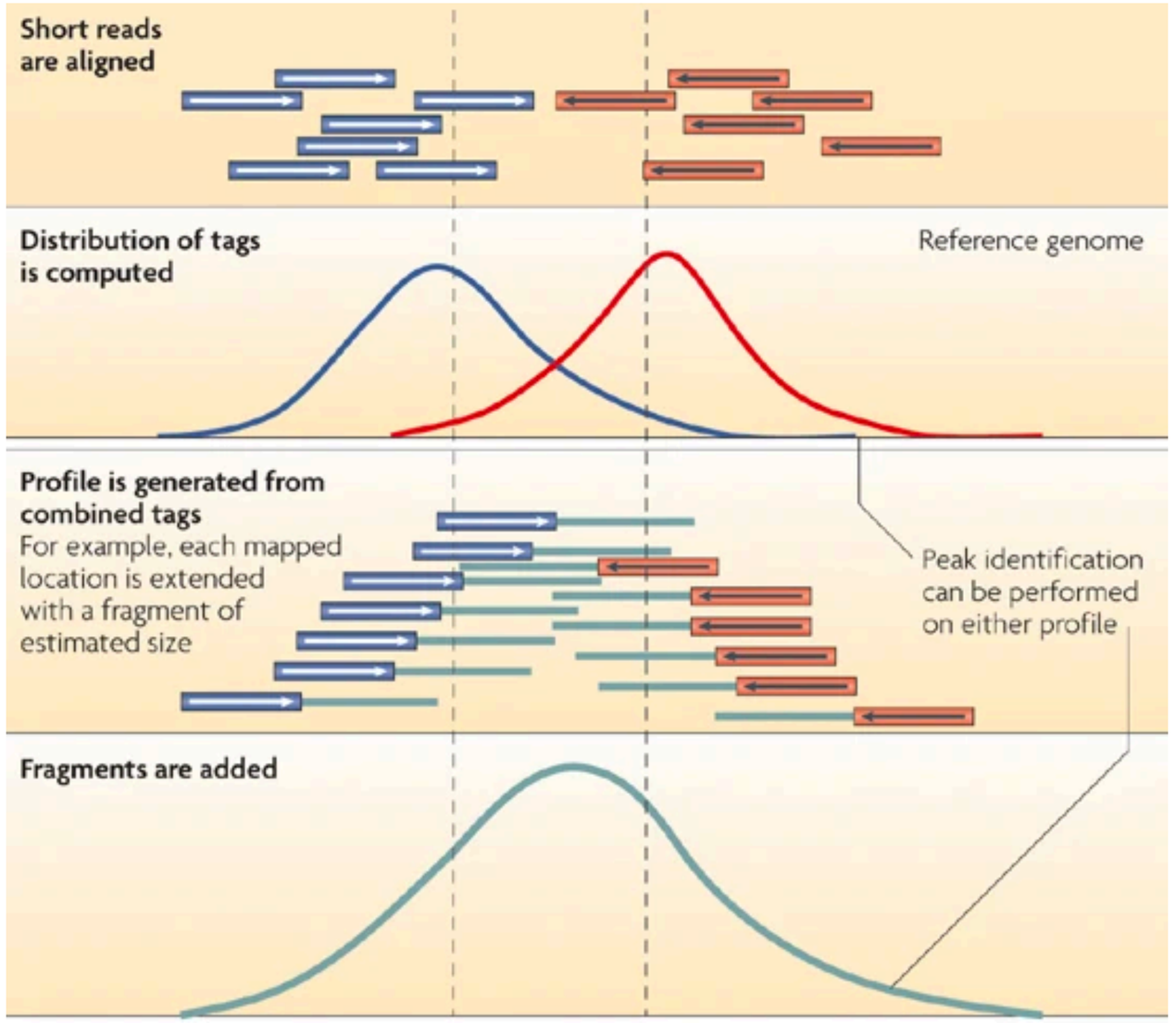


~150bp



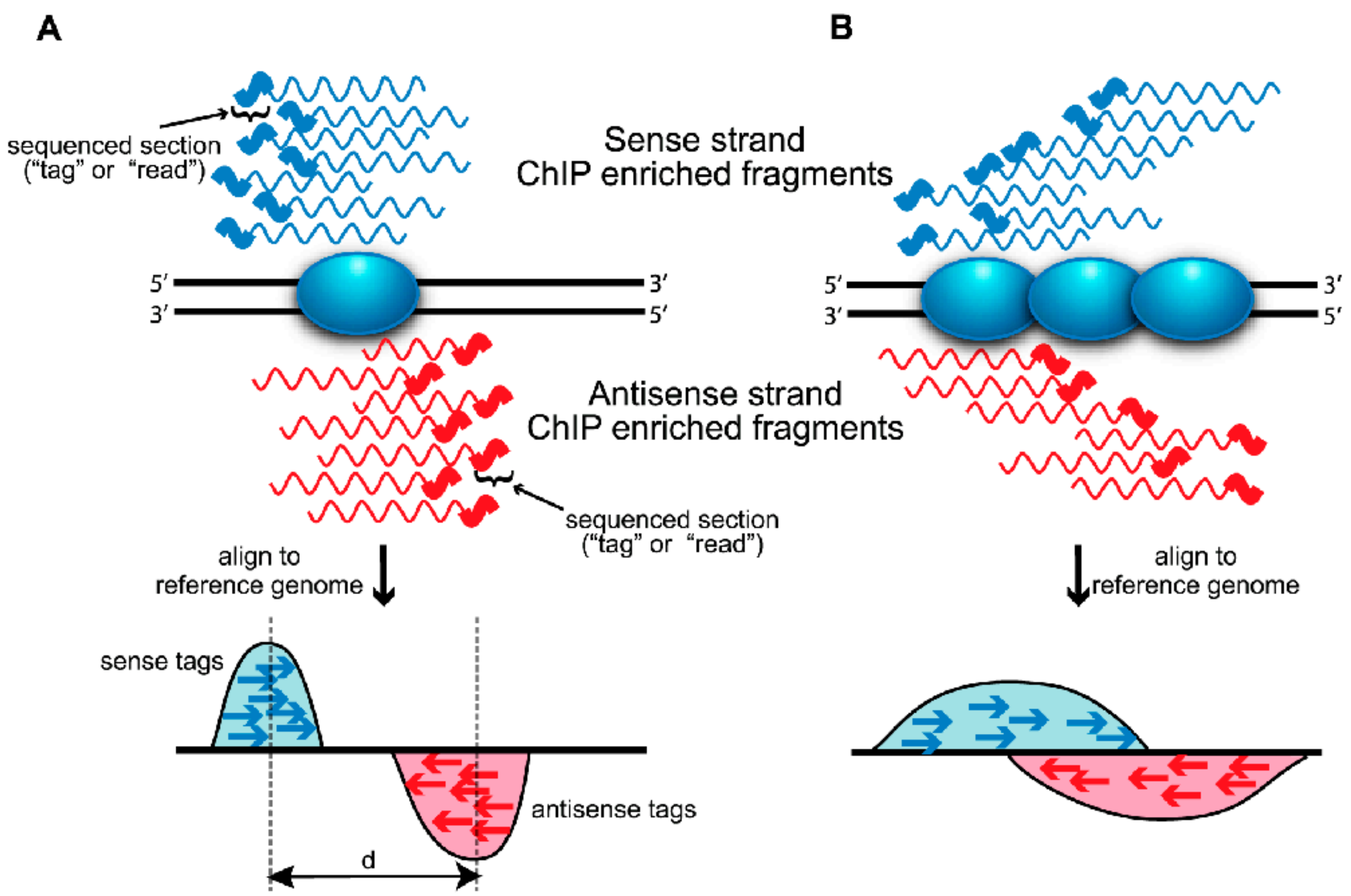
Park, Nature Review Genetics (2009)

Paired-end, sense, anti-sense seq = more accurate nucleosome positioning

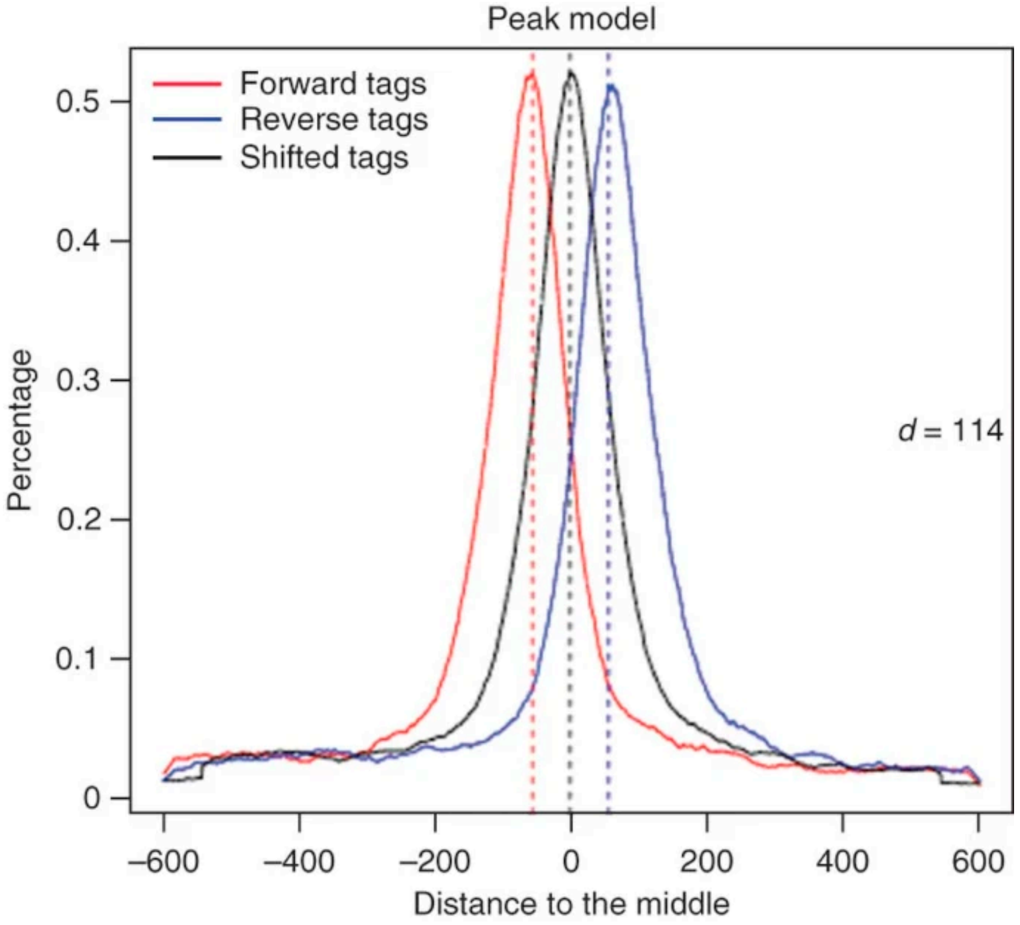


Park, Nature Review Genetics (2009)

MACS: shift reads toward 3'end



Wilbanks & Facciotti (2010)



Feng *et al.* (2012)

MACS: multiple Poisson p-values

Poisson p-value thresholds

- Read count model: Locally-adjusted' Poisson distribution

$$P(\text{count} = x \mid \lambda) = \frac{\lambda^x \exp(-\lambda)}{x!}$$

- $\lambda_{local} = \max(\lambda_{BG}, [\lambda_{1k},] \lambda_{5k}, \lambda_{10k})$ estimated from control data

- Poisson *p*-value = $P(\text{count} \geq x)$

- *q*-value : Multiple hypothesis correction

Peaks: Genomic locations that pass a user-defined *p*-value (e.g. 1e-5) or *q*-value (e.g. 0.01) threshold

Slide: Manolis Kellis

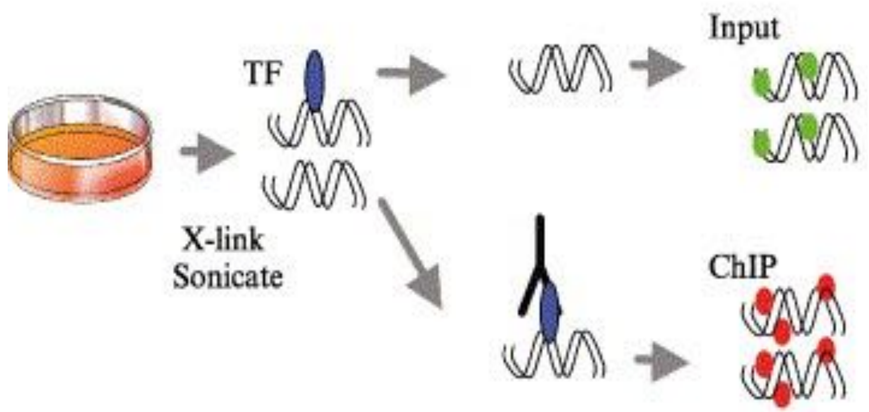
MACS: How do get the null distribution?

Empirical False discovery rates

- Swap ChIP and input-DNA tracks
- Recompute *p*-values
- At each *p*-value, eFDR = Number of control peaks / Number of ChIP peaks
- Use an FDR threshold to call peaks

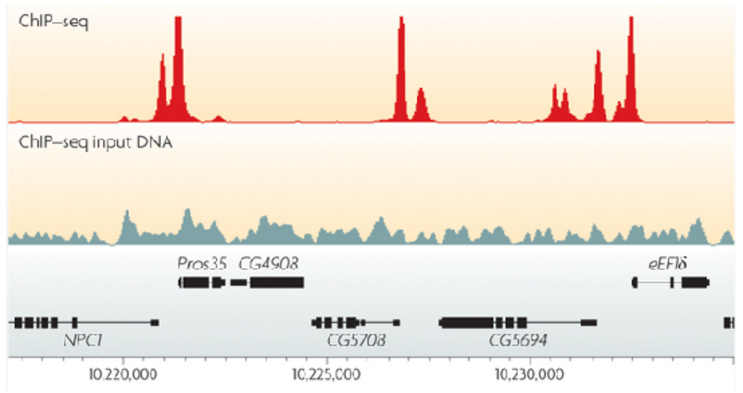
Slide: Manolis Kellis

Q/C: Use of input DNA as control dataset



signal

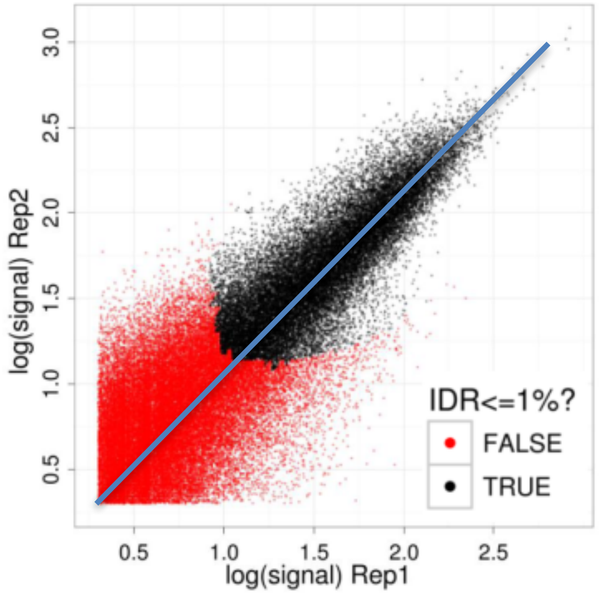
background

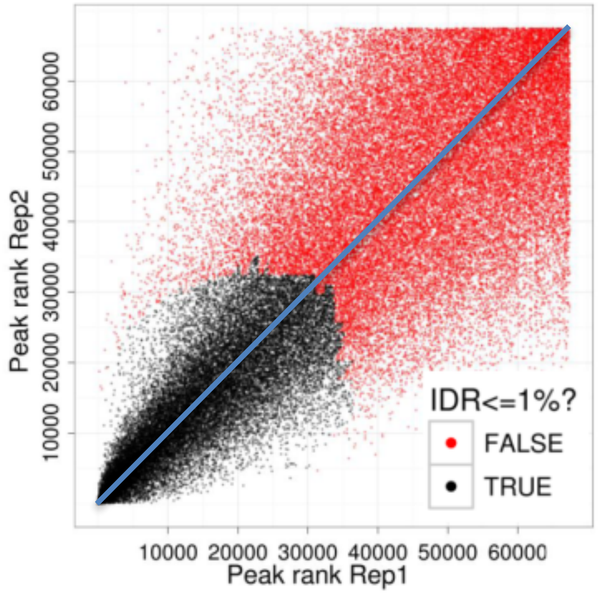


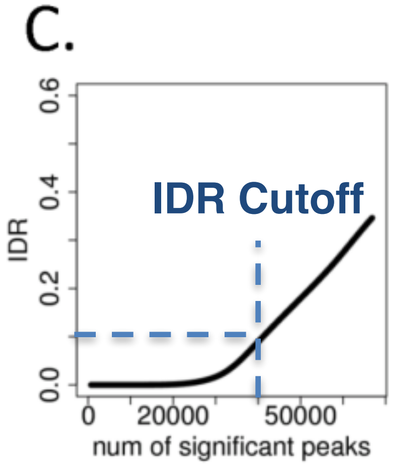
- **Challenge:**
  - Even without antibody: Reads are **not** uniformly scattered
- **Sources of bias in input dataset scatter:**
  - Non-uniform fragmentation of the genome
  - Open chromatin fragmented more easily than closed regions
  - Repetitive sequences over-collapsed in the assembled genome.
- **How to control for these biases:**
  - Remove portion of DNA sample before ChIP step
  - Carry out control experiment without an antibody (input DNA)
  - Fragment input DNA, sequence reads, map, use as background

Slide: Manolis Kellis

How do we know peaks are good enough? Measure Irreproducibility!







- Key idea: True peaks will be highly ranked in both replicates
  - Keep going down rank list, until ranks are no longer correlated
  - This cutoff could be different for the two replicates
  - The actual peaks included may differ between replicates
- Adaptively learn optimal peak calling threshold
  - **F**DR threshold of 10% ➔ 10% of peaks are false (widely used)
  - **I**DR threshold of 10% ➔ 10% of peaks are not reproducible

Slide: Manolis Kellis

The IDR model: A two component mixture model

- Looking only at ranks means that the marginals are uniform, so all the information is encoded in the joint distribution.
- Model the joint distribution of ranks as though it came from a two component Gaussian mixture model:

$$(x, y) \sim pN(\mu, \mu, \sigma, \sigma, \rho) + (1 - p)N(0,0,1,1,0)$$

- 2D Copula mixture

Slide: Manolis Kellis

Read & use IDR if interested in

The Annals of Applied Statistics
2011, Vol. 5, No. 3, 1752–1779
DOI: 10.1214/11-AOAS466

MEASURING REPRODUCIBILITY OF HIGH-THROUGHPUT EXPERIMENTS[1]

BY QUNHUA LI, JAMES B. BROWN, HAIYAN HUANG AND PETER J. BICKEL

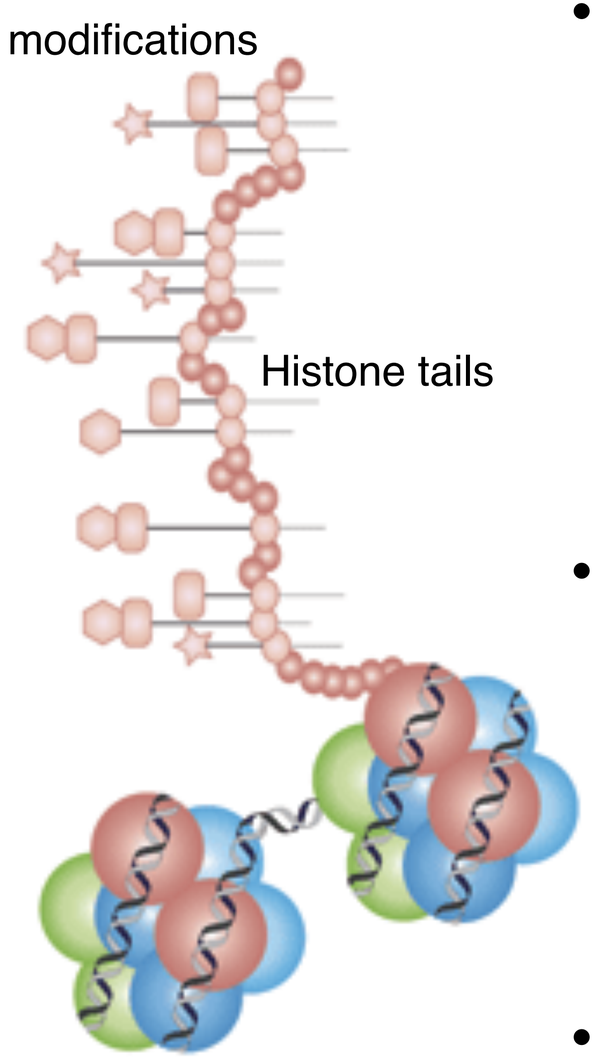
University of California at Berkeley

idr: Irreproducible Discovery Rate

This is a package for estimating the copula mixture model and plotting correspondence curves. Details are in "Measuring reproducibility of high-throughput experiments" (2011), Annals of Applied Statistics, Vol. 5, No. 3, 1752-1779, by Li, Brown, Huang, and Bickel.

Version:	1.3
Imports:	stats
Published:	2022-06-21
Author:	Qunhua Li
Maintainer:	Qunhua Li <qunhua.li at psu.edu>
License:	GPL-2 | GPL-3 [expanded from: GPL (≥ 2.0)]
NeedsCompilation:	no
CRAN checks:	idr results

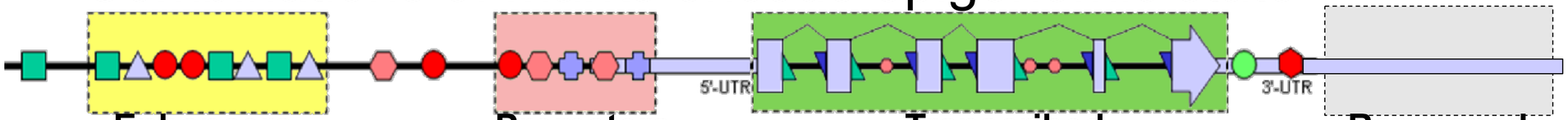
100s of histone tail modifications



- 100+ different histone modifications
  - Histone protein → H3/H4/H2A/H2B
  - AA residue → Lysine4(K4)/K36…
  - Chemical modification → Met/Pho/Ubi
  - Number → Me-Me-Me(me3)
  - Shorthand: H3K4me3, H2BK5ac
- In addition:
  - DNA modifications
  - Methyl-C in CpG / Methyl-Adenosine
  - Nucleosome positioning
  - DNA accessibility
- The constant struggle of gene regulation
  - TF/histone/nucleo/GFs/Chrom compete

Slide: Manolis Kellis

Combinations of marks encode epigenomic state

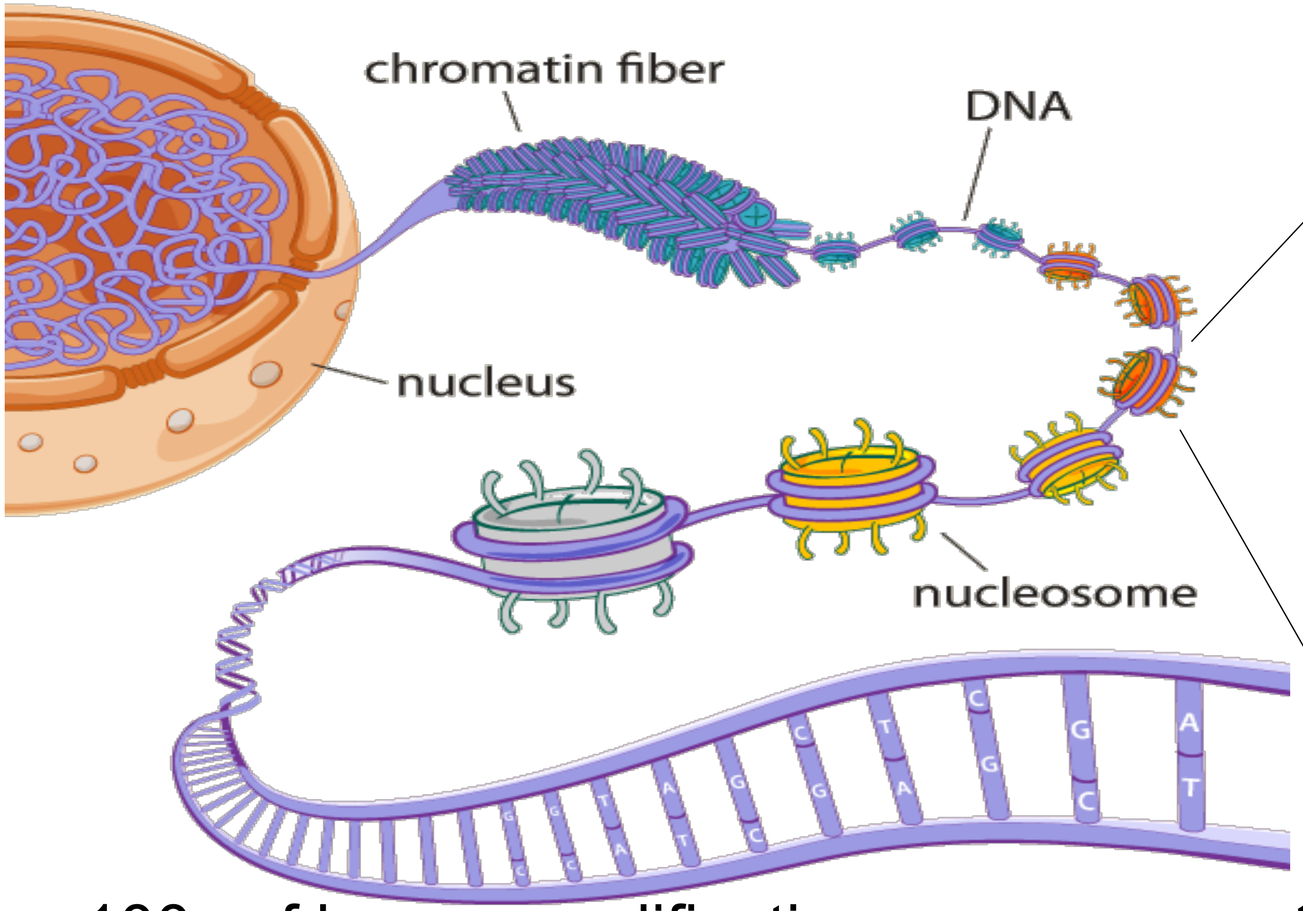


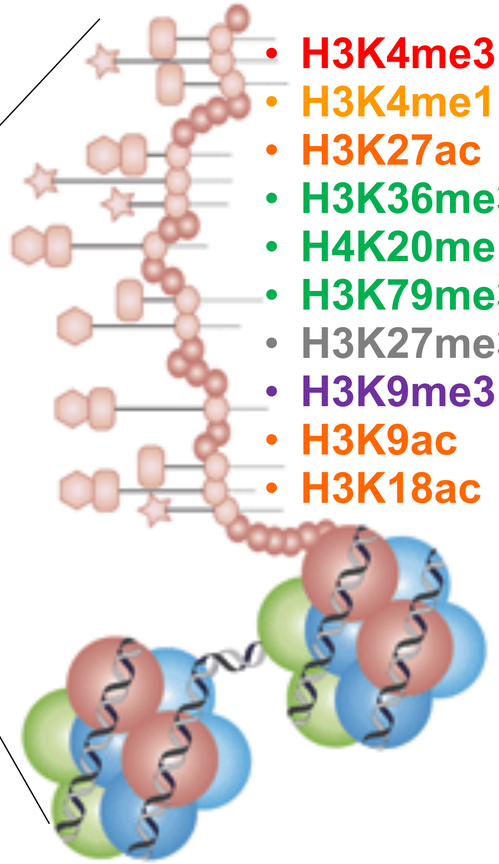
Enhancers
- H3K4me1
- H3K27ac
- DNase

Promoters
- H3K4me3
- H3K9ac
- DNase

Transcribed
- H3K36me3
- H3K79me2
- H4K20me1

Repressed
- H3K9me3
- H3K27me3
- DNAmethyl



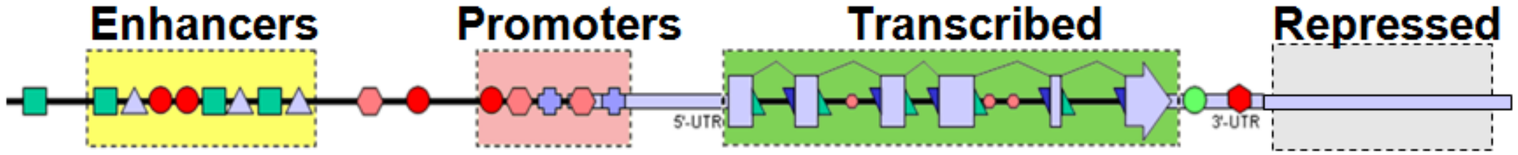


- H3K4me3
- H3K4me1
- H3K27ac
- H3K36me3
- H4K20me1
- H3K79me3
- H3K27me3
- H3K9me3
- H3K9ac
- H3K18ac

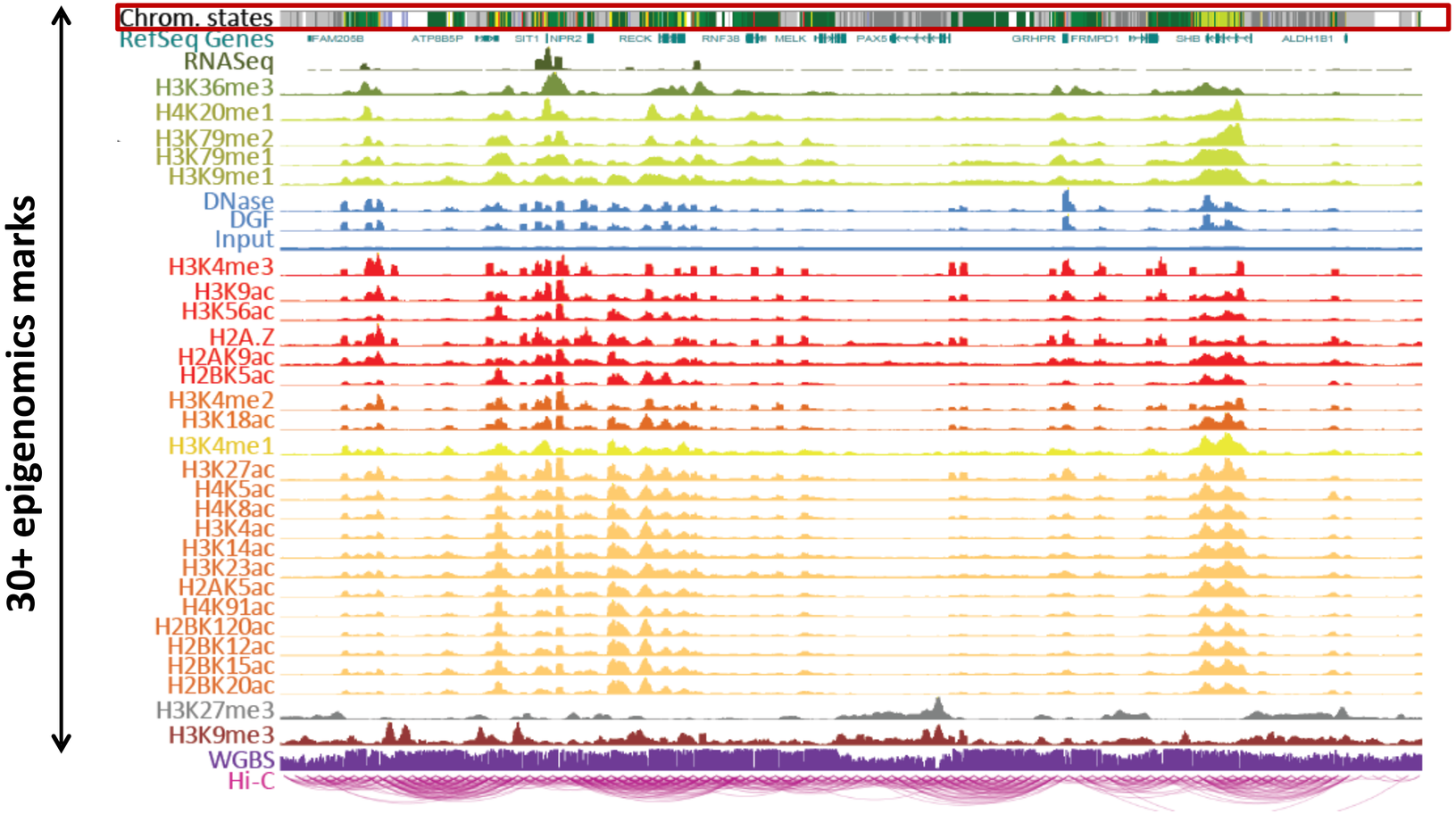
Slide: Manolis Kellis

- 100s of known modifications, many new still emerging
- Systematic mapping using ChIP-, Bisulfite-, DNase-Seq

Summarize multiple marks into chromatin states



Chromatin state track summary

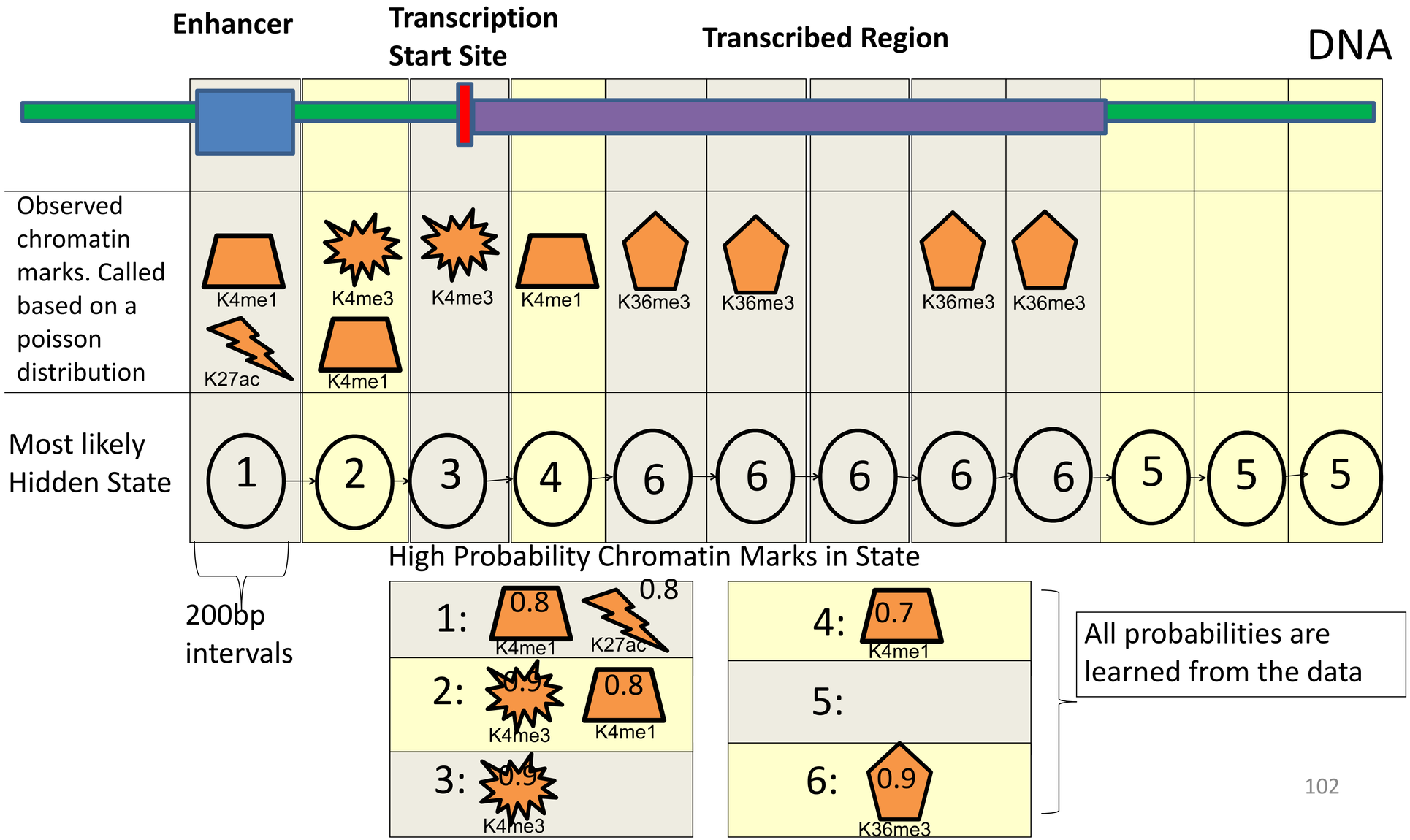


WashU Epigenome Browser

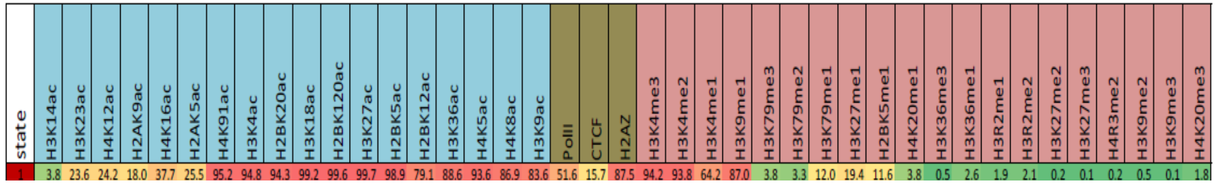
Slide: Manolis Kellis

ChromHMM: multi-variate hidden Markov model

Multivariate HMM for Chromatin States



Slide: Manolis Kellis



Ernst and Kellis Nature Biotech 2010

Statistical Methods for Epigenomics

- **Review: a set-up for epigenomics profiling**
  - Importance of Reference Genome
- **DNA methylation--basics**
  - Why do we investigate DNA methylation?
  - Bisulfite conversion: methyl-CpG tagging
- **Statistical methods for DNA methylation analysis**
  - A method treating each CpG as a variant
  - A method treating aggregating signals across genome
- **A brief overview of other ChIP-seq analysis**
  - Technology and biology
  - Peak calling
  - A step forward (too big for one person's project)