Data-Driven Hypothesis Weighting Increases Detection Power In Genome-Scale Multiple Testing

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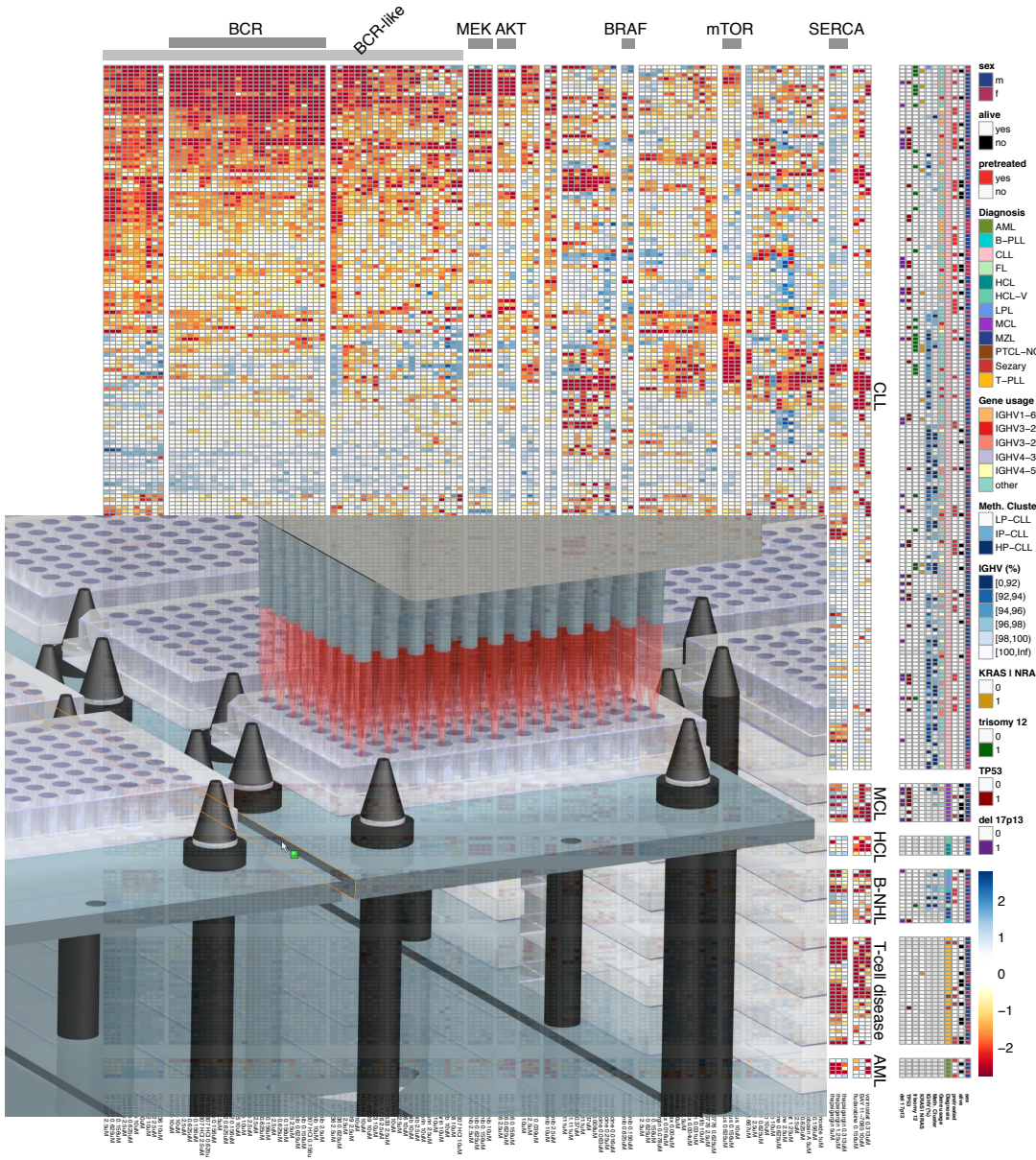
Multiple Testing

Many data analysis approaches in genomics employ item-by-item testing:

- Expression profiling
- ChIP-Seq
- Genetic or chemical compound screens
- Genome-wide association studies
- Proteomics
- Variant calling

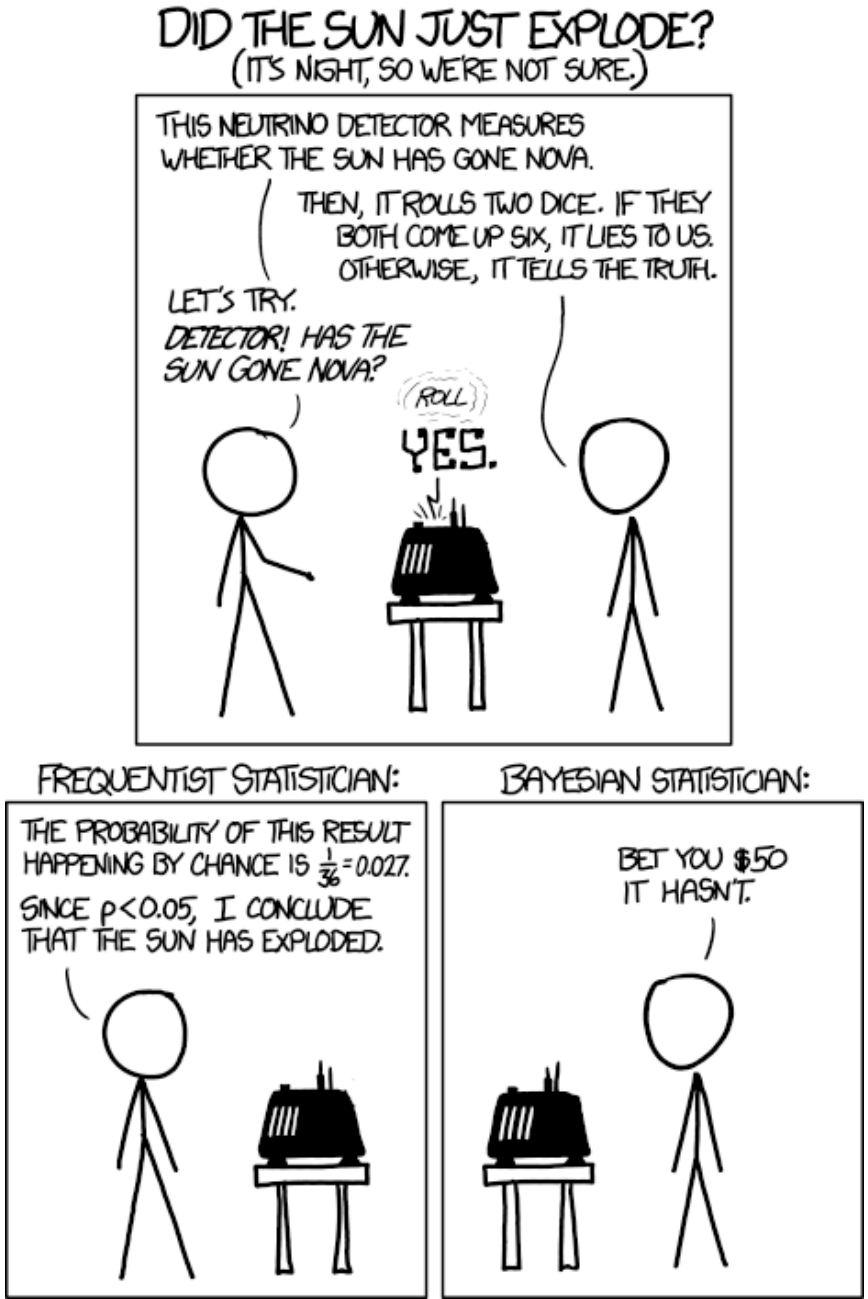


The Multiple Testing Burden

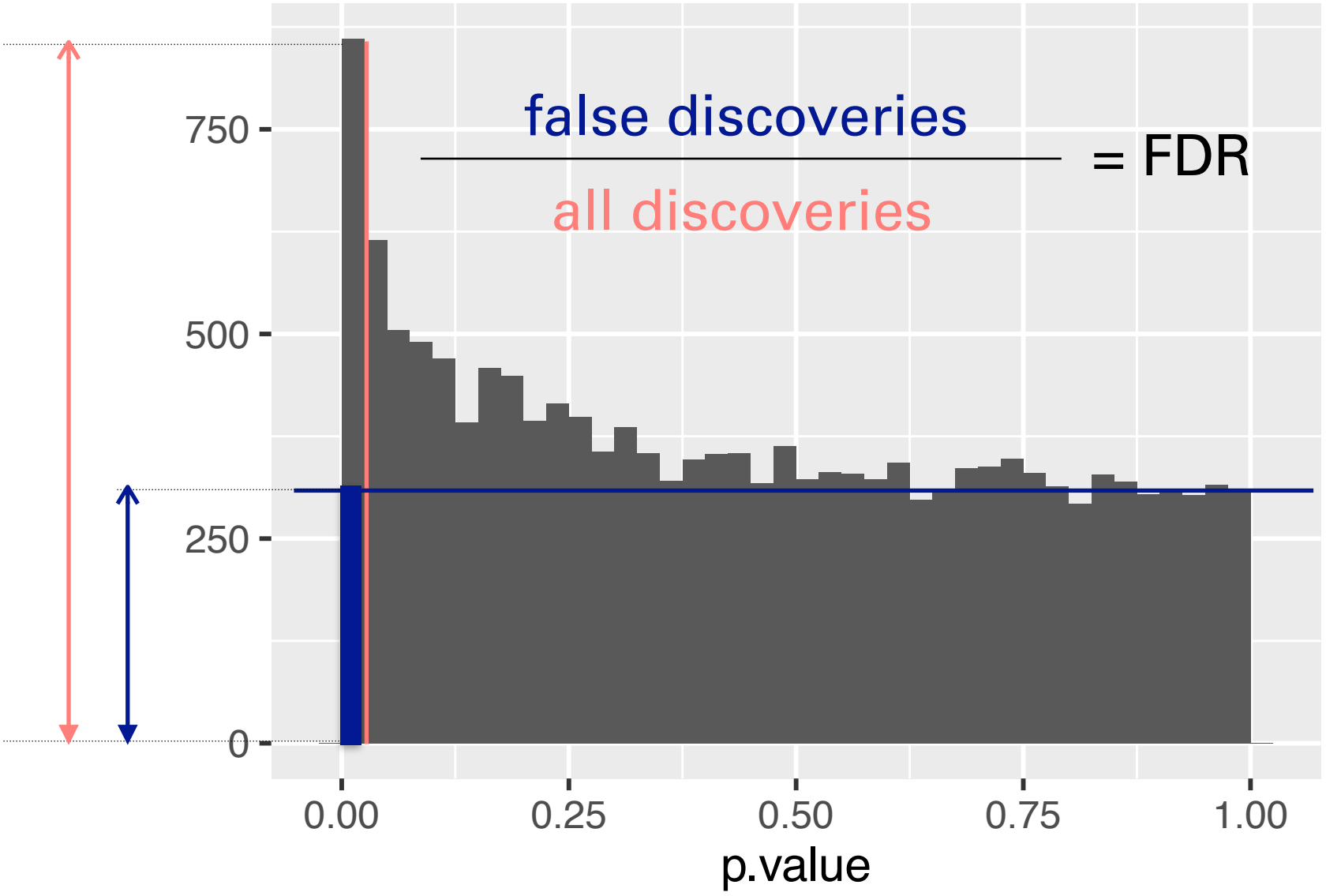
When performing several tests, type I error goes up: for $\alpha = 0.05$ and n independent tests, probability of no false positive result is

$$\underbrace{0.95 \cdot 0.95 \cdot \ldots \cdot 0.95}_{\text{n–times}} \lll 0.95$$

The Multiple Testing Opportunity



False discovery rate



Method of Benjamini & Hochberg (1995)

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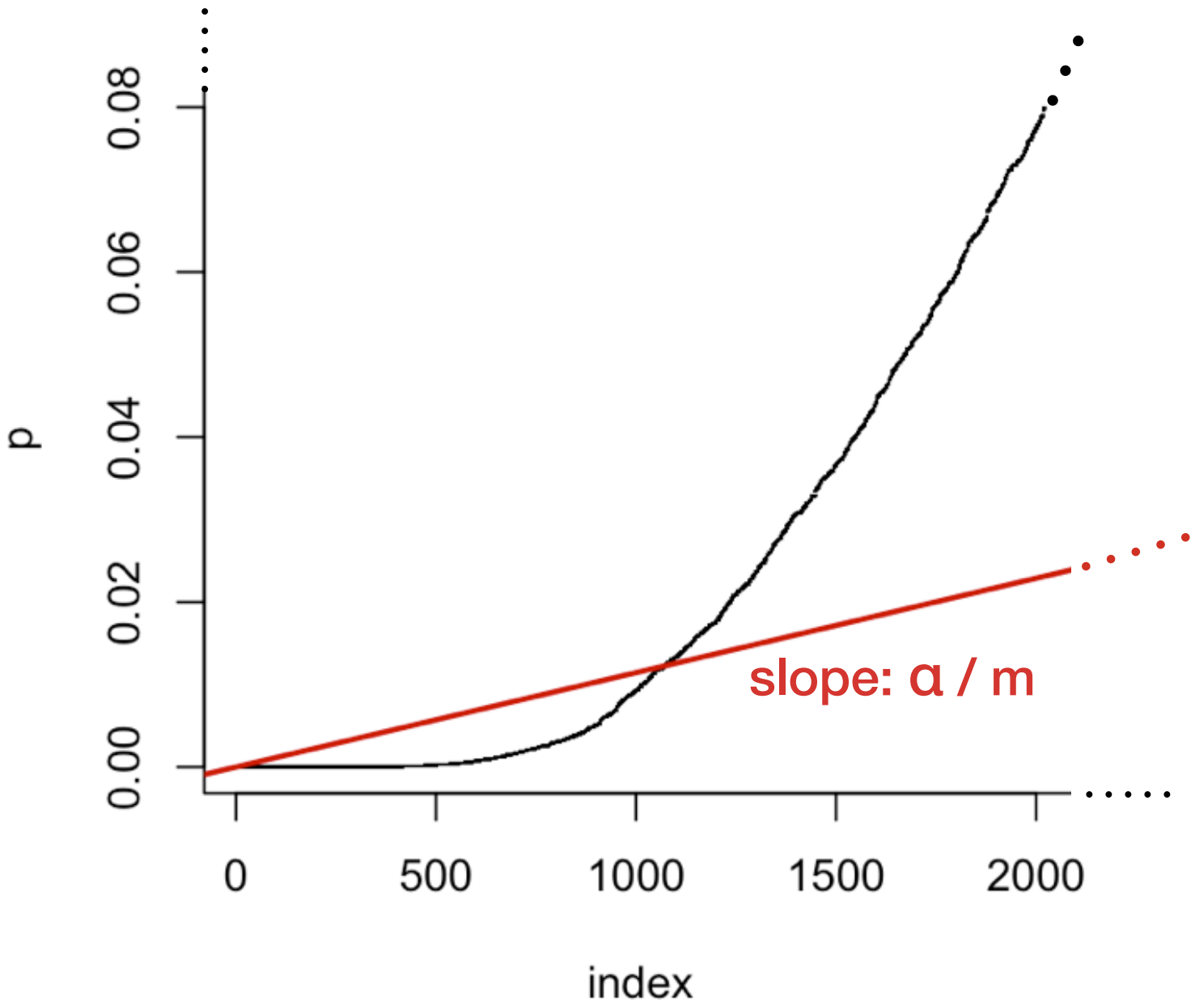
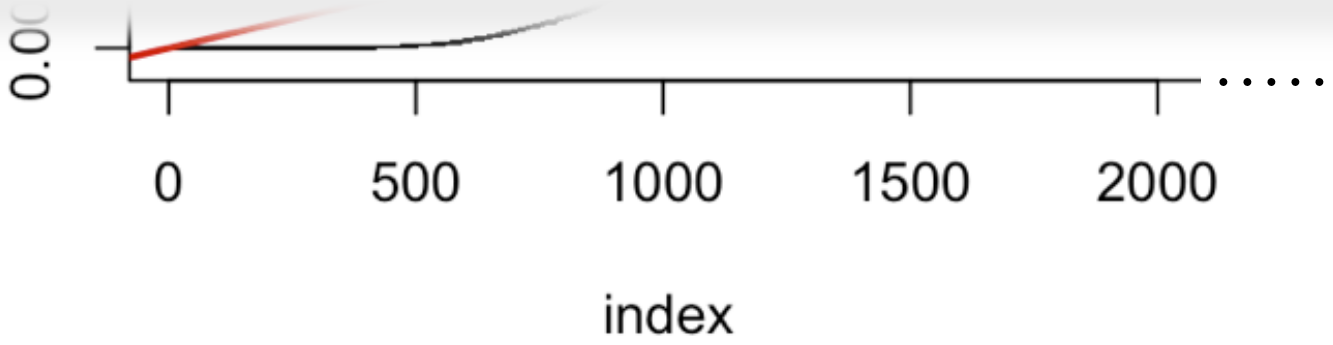


Method of Benjamini & Hochberg

```
BH = {
        i <- length(p):1
        o <- order(p, decreasing = TRUE)
        ro <- order(o)
        pmin(1, cummin(n/i * p[o]))[ro]
    }
```

takes a list of p-values as input and returns a matched list of ‘adjusted’ p-values.




Exchangeability?

Covariates - examples

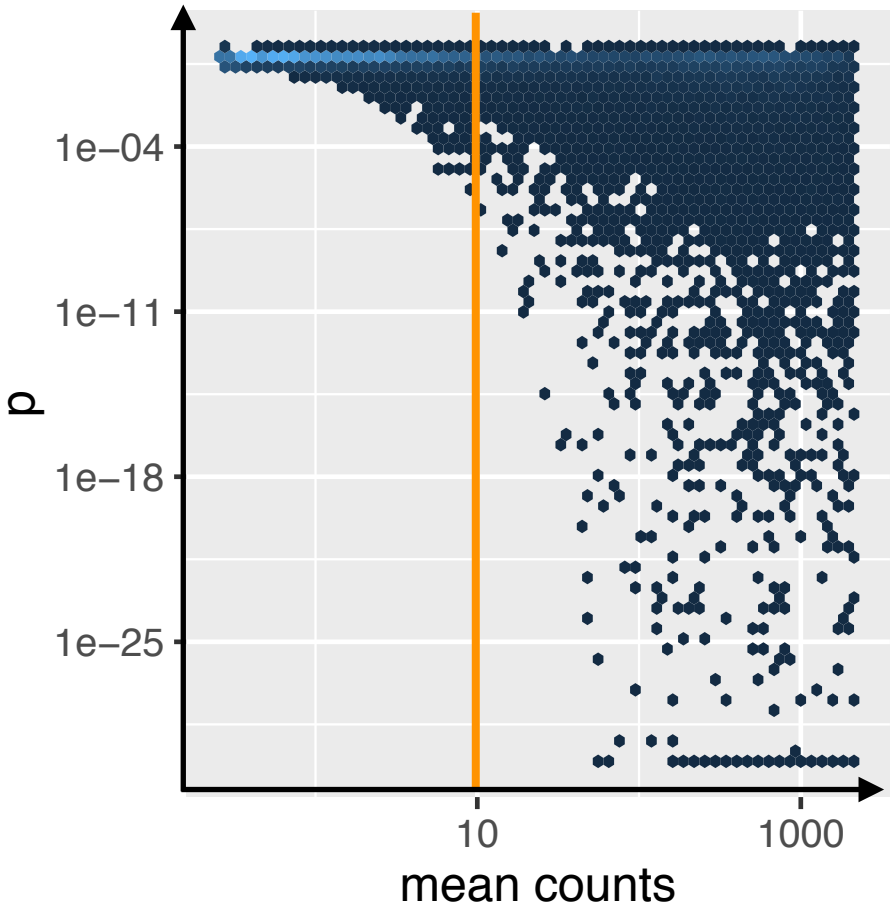
Application	Covariate
Differential RNA-Seq, ChIP-Seq, CLIP-seq, ...	(Normalized) mean of counts for each gene
GWAS	Minor allele frequency
eQTL analysis	SNP – gene distance
t-tests	Overall variance
Two-sided tests	Sign
All applications	Sample size; measures of signal-to-noise ratio

Independent Filtering

Two steps:

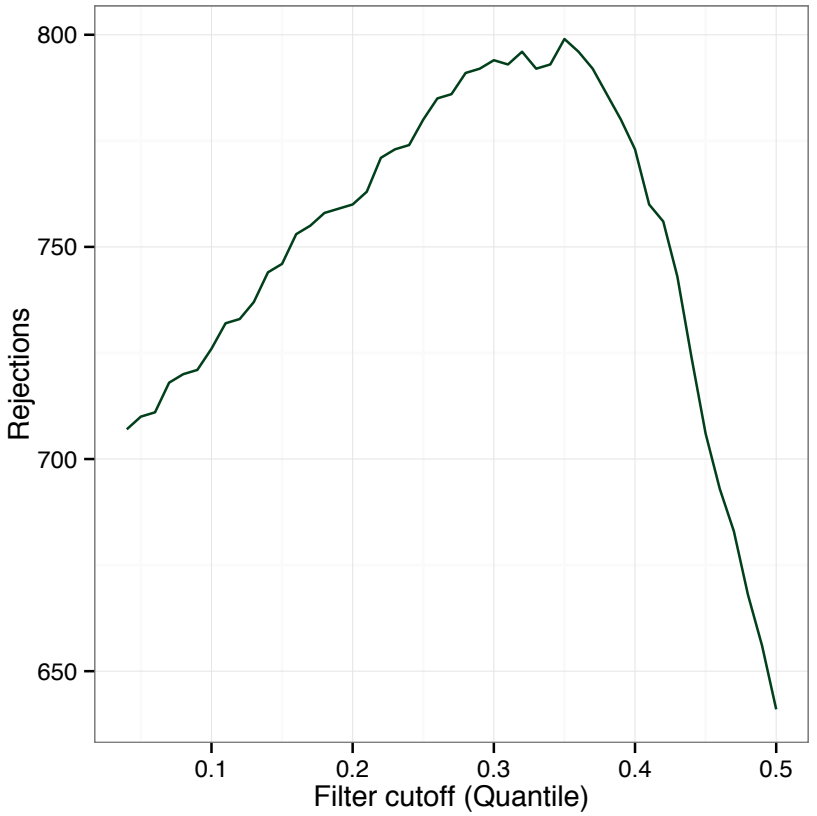
- All hypotheses H_i with $X_i < x$ get filtered.
- Apply BH to remaining hypotheses.

(Bourgon, Gentleman, Huber *PNAS* 2010)



Data-driven choice of filtering threshold

- Do Independent Filtering followed by Benjamini-Hochberg procedure with all possible thresholds.
- Report the result with the optimal threshold.
- We have been doing this in *DESeq2* for the last two years.



Weighted Benjamini-Hochberg method

- Let $w_i \geq 0$ and $\frac{1}{m}\sum_{i=1}^{m} w_i = 1$ ("weight budget").
- Define $Q_i = P_i / w_i$.
- Apply BH to Q_i instead of P_i.
- Proven Type-I error (FDR) control (Genovese, Roeder, Wasserman *Biometrika* 2006).
- If $w_i > 1$, then H_i is easier to reject.
- $Q_i \leq t \Leftrightarrow P_i \leq w_i t =: t_i$

Weighted Benjamini-Hochberg method

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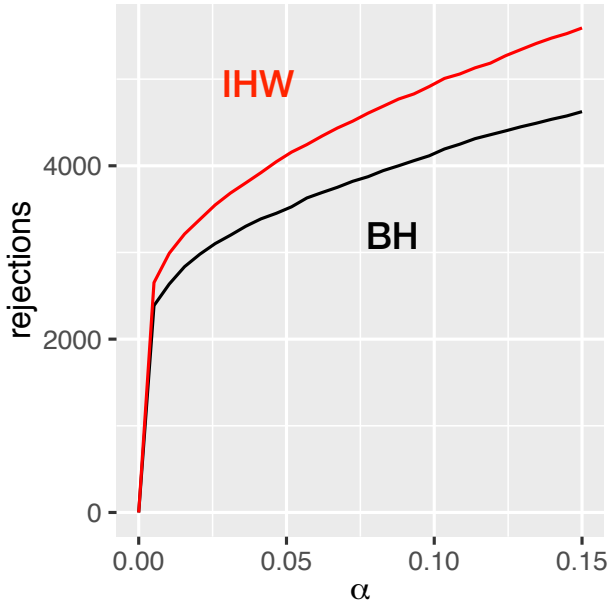
- P r, Wa
- If w_i
- $Q_i \leq t \Leftarrow$

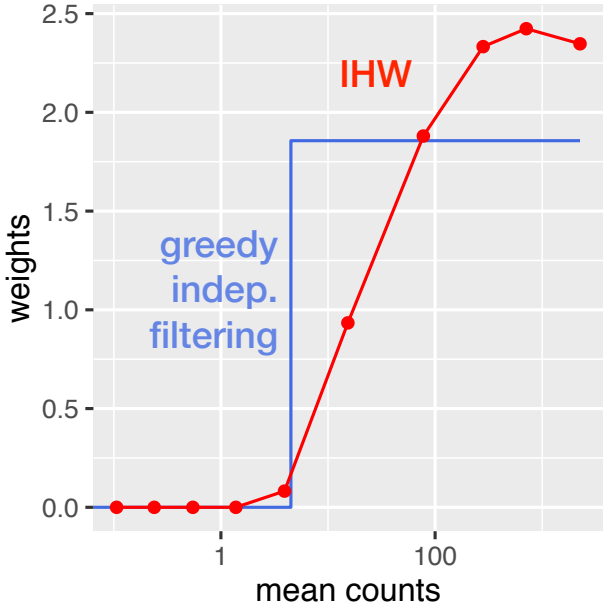
Problem: how to know the weights?

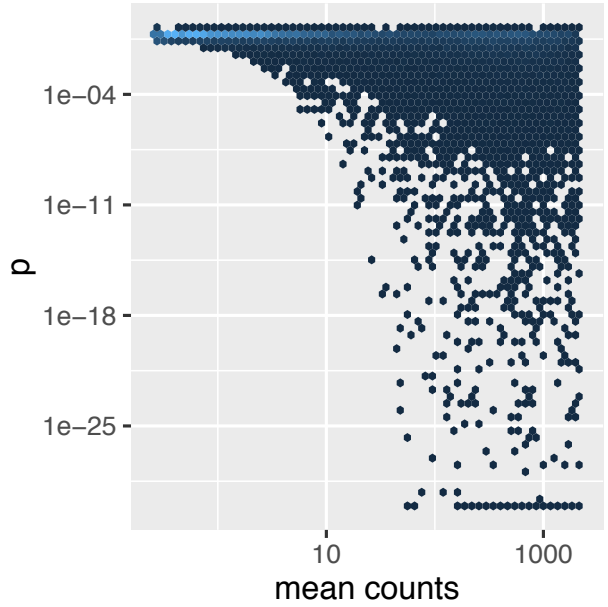
IHW (naive): Independent (data-driven) hypothesis weighting

- Stratify the tests into G bins, by covariate X
- Choose α
- For each possible weight vector $\mathbf{w} = (w_1, \ldots, w_G)$ apply weighted BH procedure. Choose $\mathbf{w}$ that maximizes the number of rejections at level α.
- Report the result with the optimal weight vector $\mathbf{w}^*$.

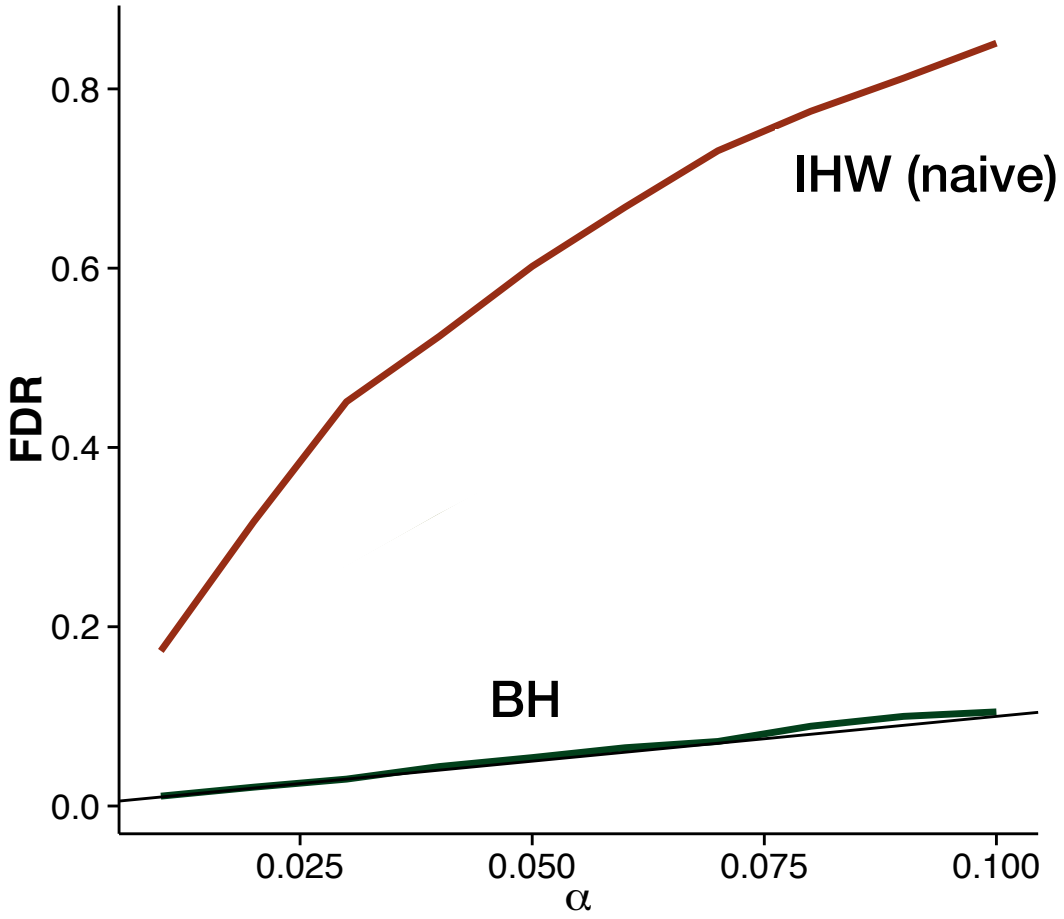
RNA-Seq example (DESeq2)







But naive IHW does not always control the FDR (e.g. $\pi_0 = 1$)



Modified IHW

Nikos Ignatiadis

Data splitting: randomly split hypotheses into k folds. Learn weights for the hypotheses in a fold from the other k–1 folds

Regularisation:

- for ordered covariate: $\Sigma_g |w_g - w_{g-1}| \leq \lambda$
- for categorical covariate: $\Sigma_g |w_g - 1| \leq \lambda$

Convex relaxation: for weight optimisation (only), replace ECDFs of the p-values with Grenander estimators (least concave majorant of the ECDF)

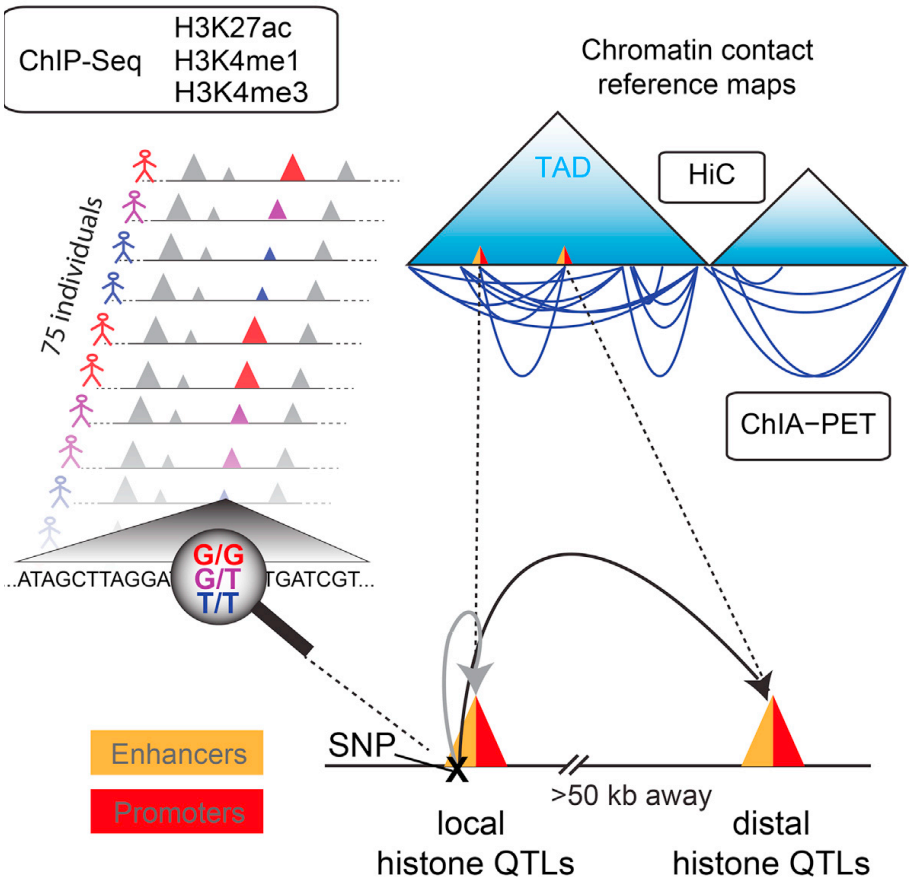
Data set 2: hQTL

ChIP-seq for histone marks in lymphoblastoid cell lines from 75 sequenced individuals.

Local QTLs: find best-correlated SNP within 2kb of peak boundaries/promoters.

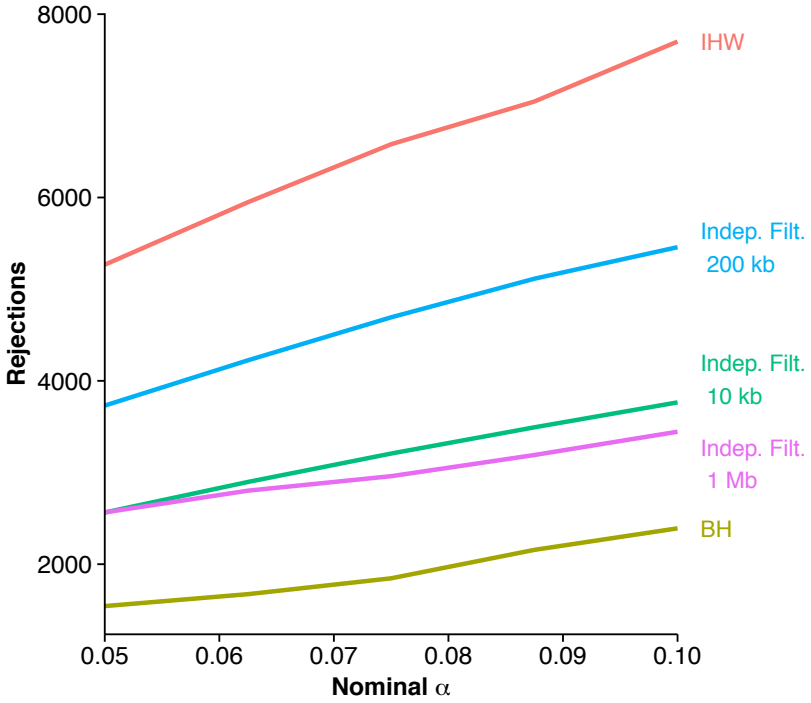
14,142 local hQTLs linked to ~10% of H3K27ac peaks (FDR 10%, permutations)

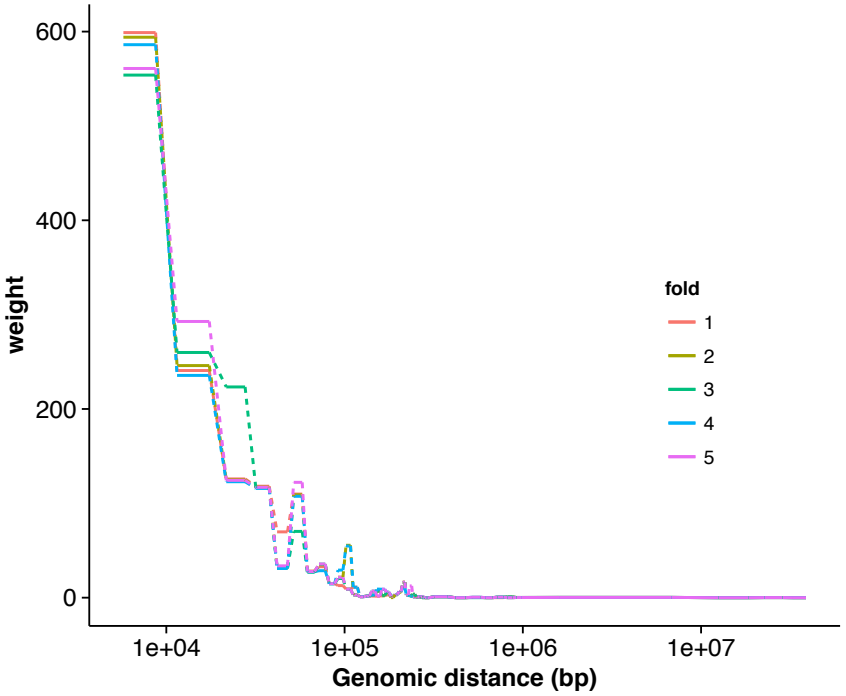
Distal: distance cutoffs from 50 to 300 kb; also HiC



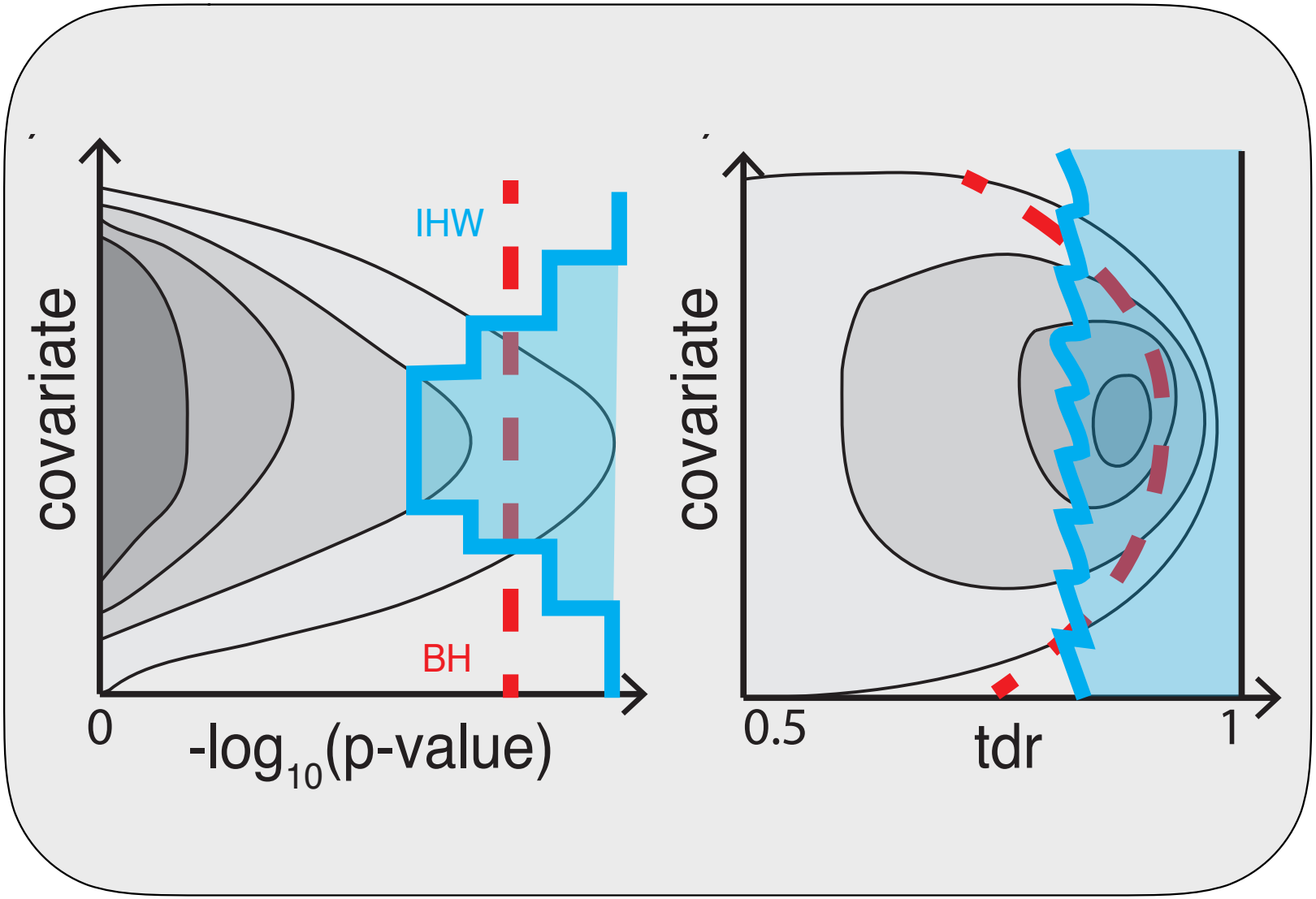
Grubert, Zaugg, Kasowski, et al. Genetic control of chromatin states in humans involves local and distal chromosomal interactions. Cell (2015).

histone-QTL example: H3K27ac

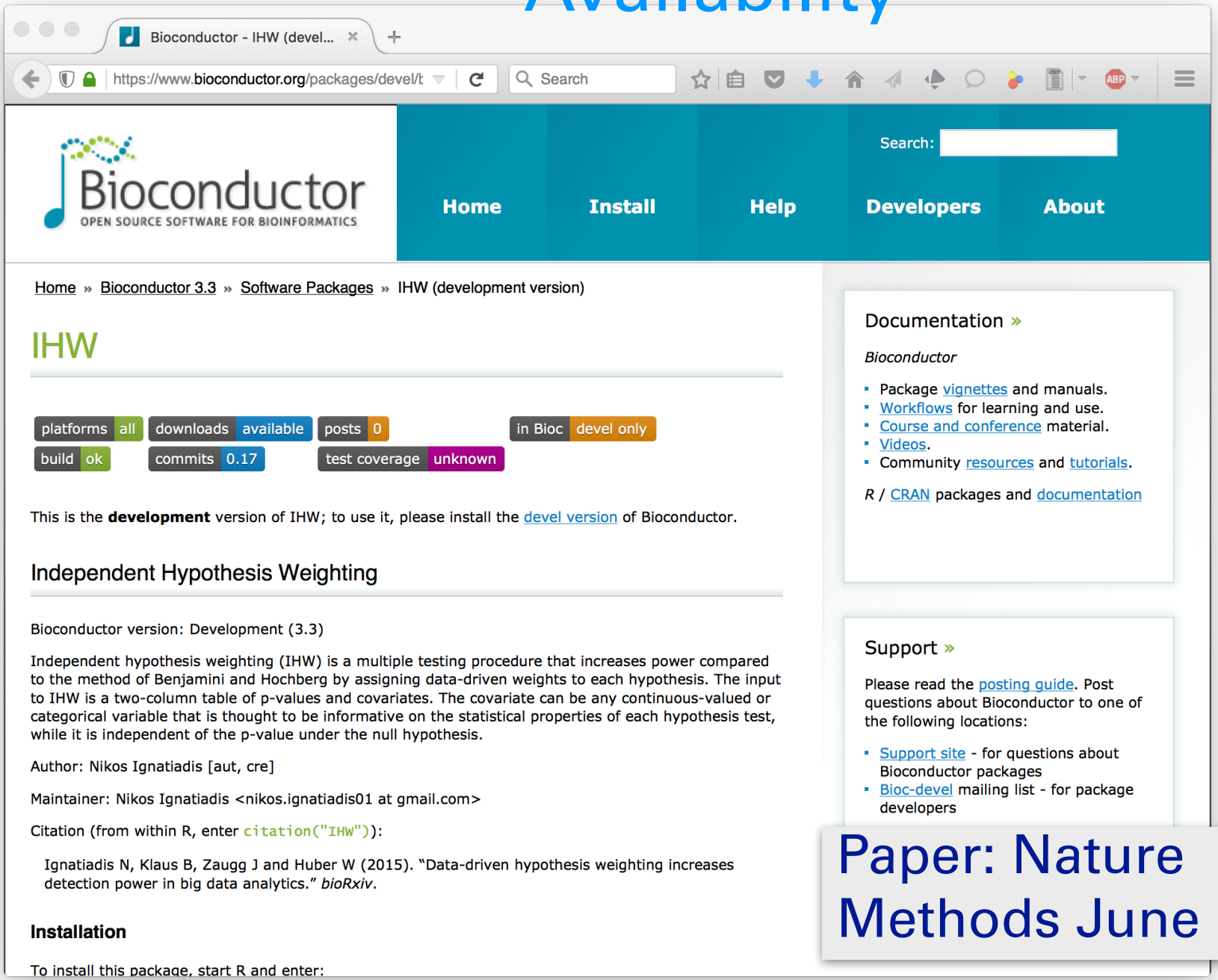




2D decision boundaries



Availability



Paper: Nature Methods June 2016

Summary

Joint work with
Nikos Ignatiadis

- Multiple testing is not a problem but an opportunity
- Heterogeneity across tests
- Informative covariates are often apparent to domain scientists
  - independent of test statistic under the null
  - informative on π_1, F_{alt}

Data-driven weighting

- Scales well to millions of hypotheses
- Controls ‘overoptimism’

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Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

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