Statistical analysis of genome-scale data

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The way we do this

- "Try out yourself" means try out yourself
  - No use in me demonstrating one step at a time
  - I provide the key terms - you sort out the clicking
  - If you're stuck - ask us or neighbor
  - I will anyway demo each task afterwards
- And science is more than clicking, anyway
  - We will focus just as much on the underlying concepts

Outline of session

- Analyzing genomic tracks
- The gospel:
  powerful analyses through simple means
- The cautionary tale:
  challenges with data and assumptions
- Conclusion

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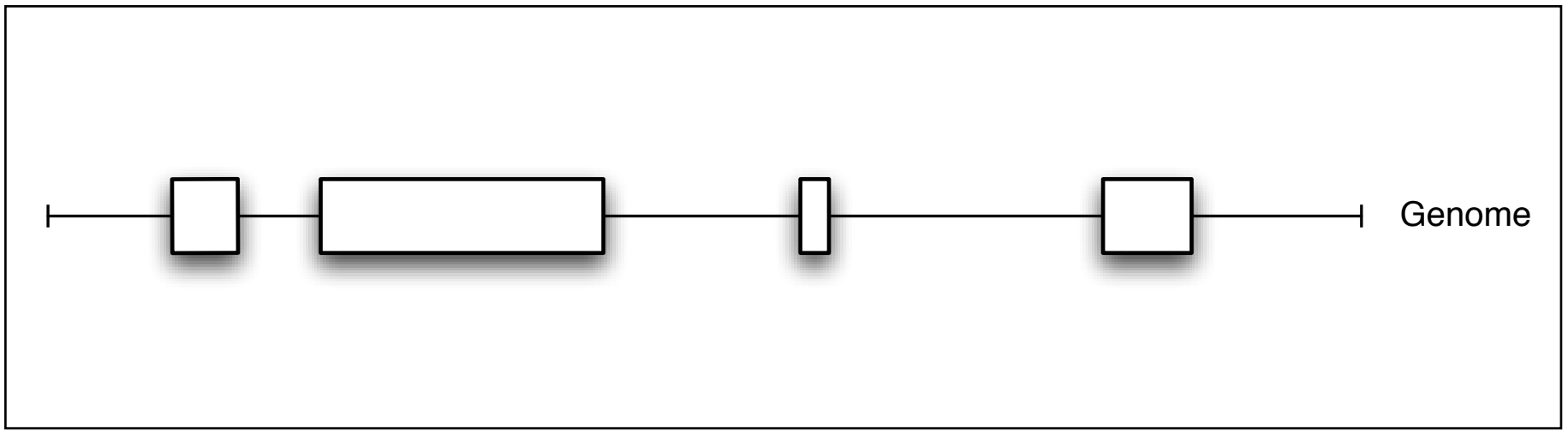
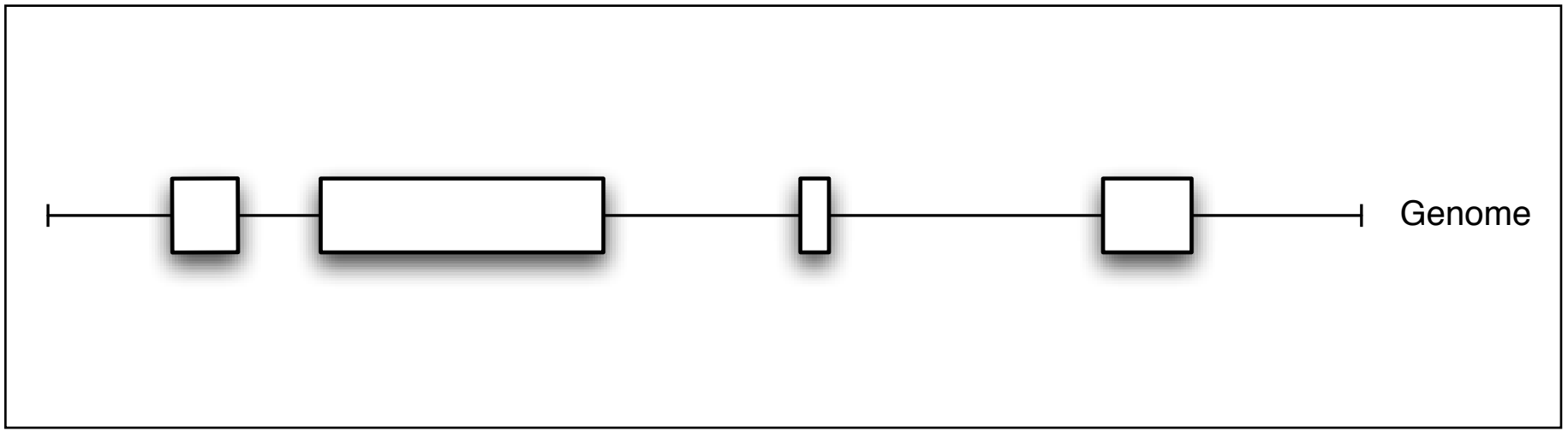
What are genes?

This! :



What are genes?



Reference genome acts like coordinate system for genomic data

```
chr21 10079666 10120808 NM_001187
chr21 13332357 13412442 NR_026916
chr21 13700575 13700652 NR_036164
chr21 13904368 13935777 NM_174981
chr21 14137324 14142556 NR_026755
```

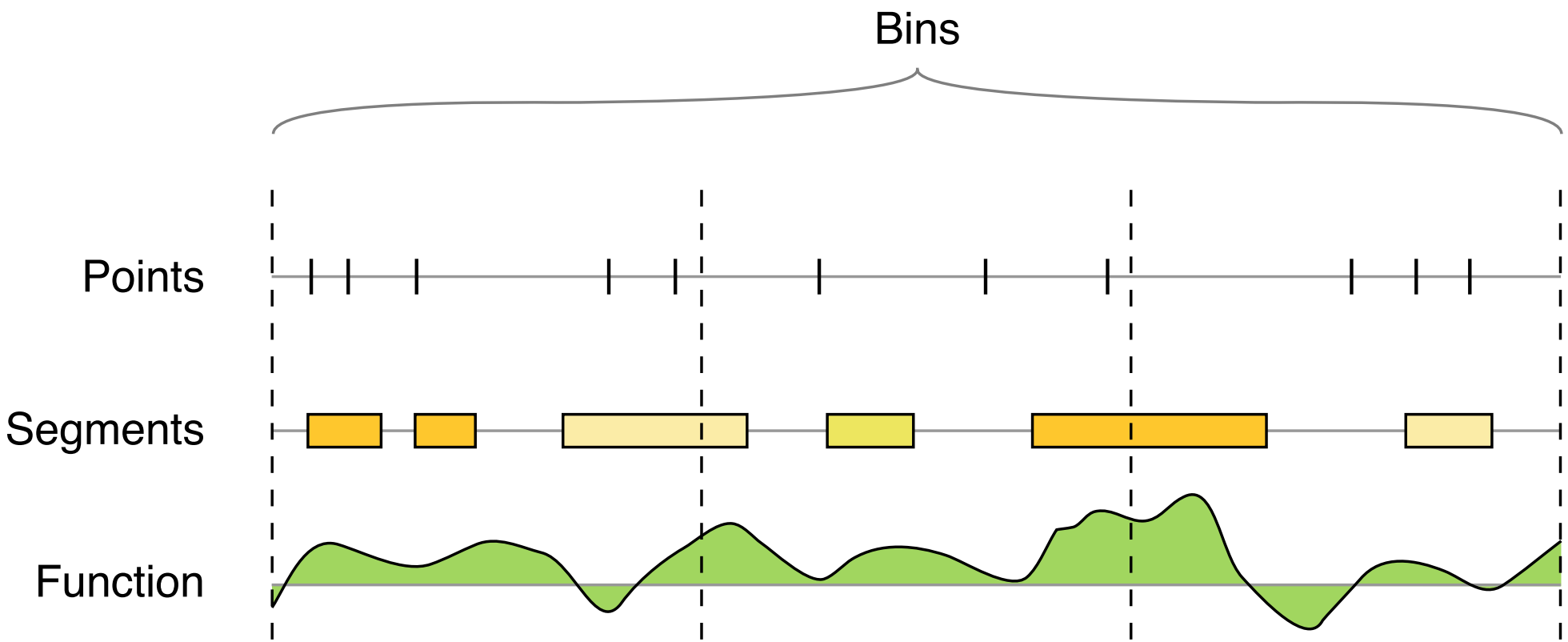
Classic examples of genomic track data

- Gene locations, gene expression
- Evolutionary conserved regions
- Repeating elements

ENCODE, FANTOM, GEO, Roadmap Epigenomics ..

- By now, Big Science provides:
  - Chromatin accessibility (DHSs) for ~350 cell samples
  - Binding of ~100 TFs in several cell types
  - Most histone modifications in several cell types
  - Gene expression for thousands of setups
  - TSS and active promoters in ~950 cell samples
  - DNA methylation, 3D genome structure, ...

Delineating basic types of genomic tracks



And what about analysis?

Example analyses

- A relation between methylation patterns and repeating elements? (Genome Res. 2009 19: 221-233)
- Distinct methylation for tissue-specific genes?(Genome Res. 2010 20: 1493-1502)
- Cooperative histone modifications? (Nat Genet 2008 40:897-903)

Example analyses (cont.)

- Fragile sites, breakpoints and repeats? (Genome Biology 2006 7:R115)
- Copy number variation, repeats, duplications and genes? (Genome Res. 2009 19: 1682-1690)
- Methylation and active genes at T-Cell G0->G1 (Genome Res. 2009 19: 1325-1337)

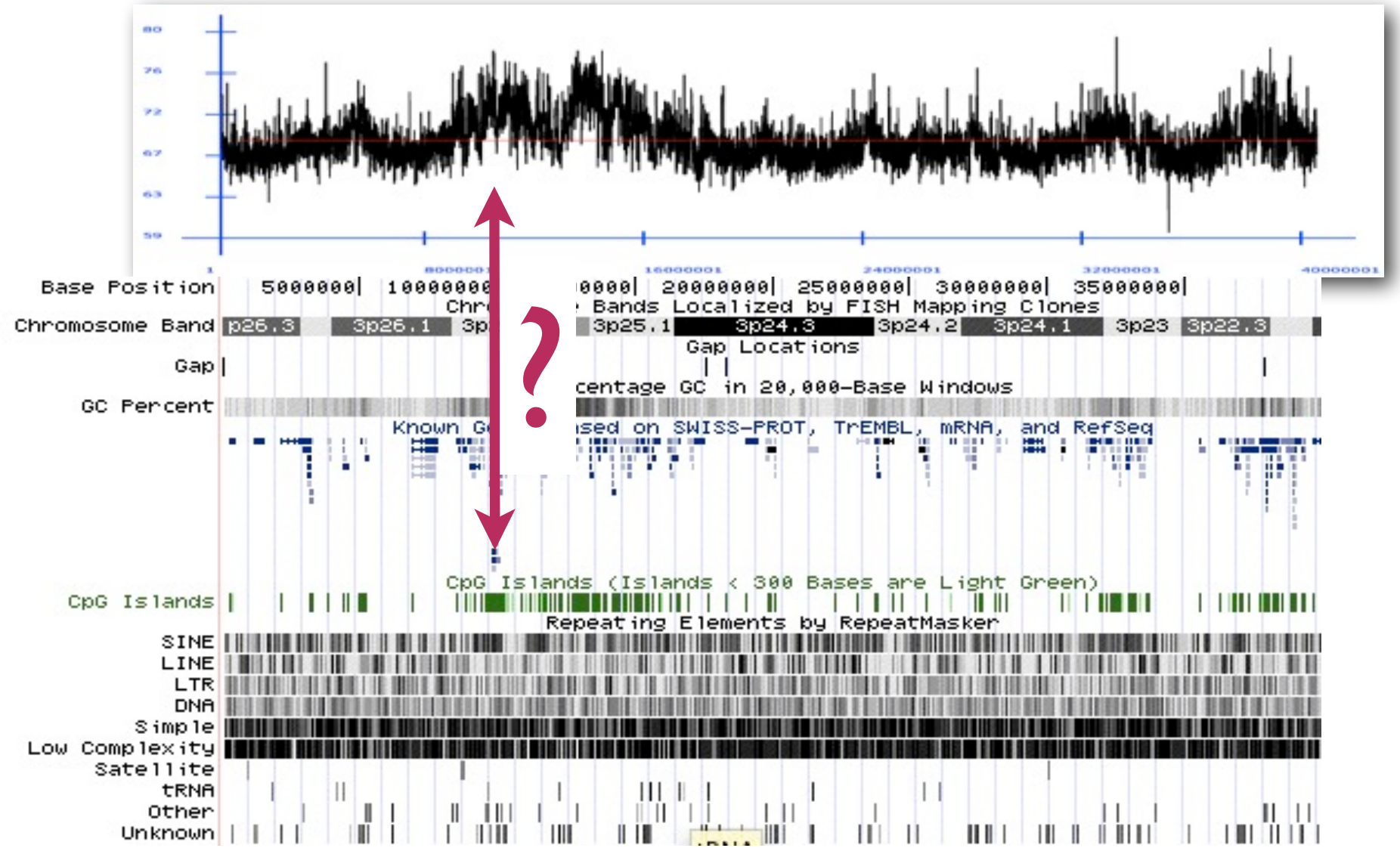
Example analyses (cont.)

- Virus integration vs genes, CpG, GC-content
  (Journal of Virology 2007 6731–6741)
- Methylation patterns in embryonic cells
  (PNAS 2010 107:10783–10790)

Example analyses (cont.)

- 80.4% of the genome participates in at least one biochemical/chromatin-associated event (Nature 2012, 489:57)
- Motifless TF ChIP-seq peaks vs high TRF occupancy (HOT) (Genome Biol 2012, 13:R48)
- [Almost every ENCODE article has many examples, really]

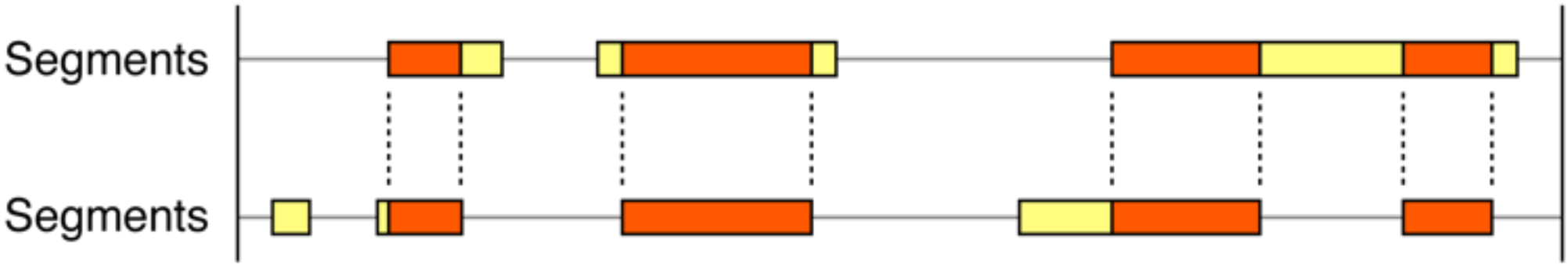
This can't be it?!



Co-location of genomic features

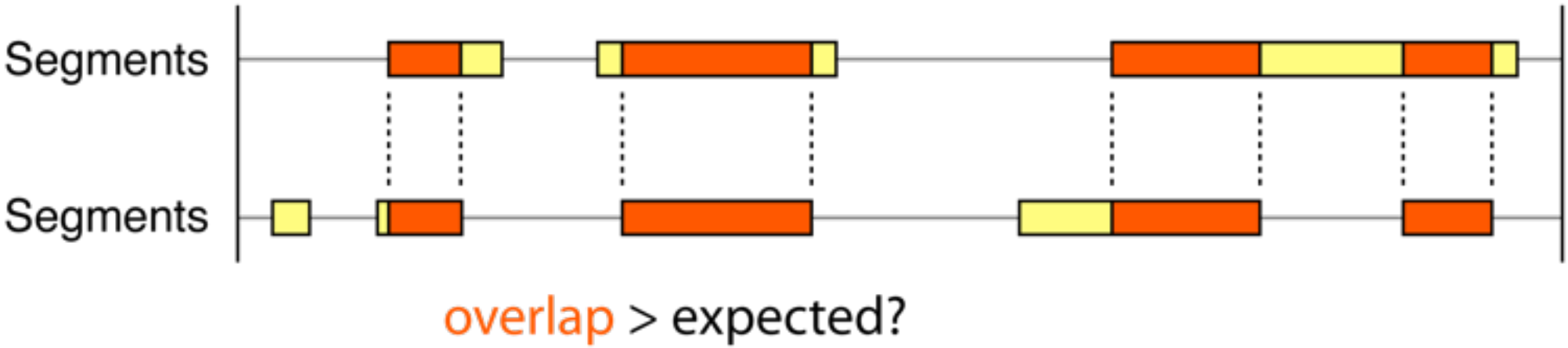
- Common question:
  do genomic feature X and Y occur
  (more than expected)
  at the same locations in the genome?
- Used to discover novel relations
- May indicate a direct causal relation, or hint to indirect association.

How does this look at the drawing board?



overlap > expected?

How does this look at the drawing board?



- Issues in practice:
  - How to represent data (easy)
  - How to count overlap (easy)
  - How to conclude on relation or not (challenging)

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Technical note (1): Wifi access

- Network name (ssid): **`conferences`**
- Password (WPA2): uio202aar

Technical note (2): Server load

- We will today be 40 people running on a 32-CPU server
  - Might be some queueing of runs or slow GUI
  - Might be some DB operational errors (just refresh..)
  - [we are in transition to a larger server]

B-cells important for multiple sclerosis?

- "Results suggest an important role of B cells in the pathology of MS"
- "MS associated genomic regions co-localized with regions which are functionally active in B cells"
- "MS SNPs including 0.25cM flanks overlap more than expected with regions of chromatin state AP in gm12878"

Getting to know MS

- Go to HyperBrowser:
  - `"hyperbrowser.uio.no"`
- Import MS from published Page:
  - `"Training material"`
- Expand history element, and:
  - `"Perform HyperBrowser analysis"`
- Keep defaults, and `"Start analysis"`

Do MS overlap unexpectedly with AP regions in gm12878?

- Select tool: "**Analyze genomic tracks**"
- Genome: "**hg18**" (!)
- Track1: "**--From history..--**" -> **MS**
- Track2: "**Chromatin / Chromatin state../ ..Gm12878.. / 1 Active Promoter**"
- Analysis: "**Overlap?**"
- Keep defaults and "**Start analysis**"

But, something isn't right!

- Unexpected overlap between MS and B-cell AP does not confirm a role of B-cells in MS!
  - (why not?)
- Restrict to AP regions specific to B-cell
  - "Subtract" intervals of other cell from B-cell
  - But still not right! (and why not?)
- Must instead use a case-control analysis..

Track customized for analysis: Create B-cell AP vs hepatocyte AP

- Menu "**HyperBrowser track repository**":
  - "**Extract track from HyperBrowser repository**"
  - "**hg18**"

1. "**Chromatin / Chromatin state../..Gm12878../.. AP**
2. "**Chromatin / Chromatin state../..Hepg2../.. AP**

- Menu "**Customize tracks**":
  - "**Combine two BED files into single case-control track**"

Do MS overlap preferentially with B-cell AP vs hepatocyte AP?

- Tool: **"Analyze genomic tracks"**
- Genome: **hg18**, Track1: **MS**
  - ***shortcut***:
    "perform HyperBrowser analysis"
- Track2: **Customized case-control track**
- Question: **"Preferential overlap?"**
- Keep defaults and **"Start analysis"**

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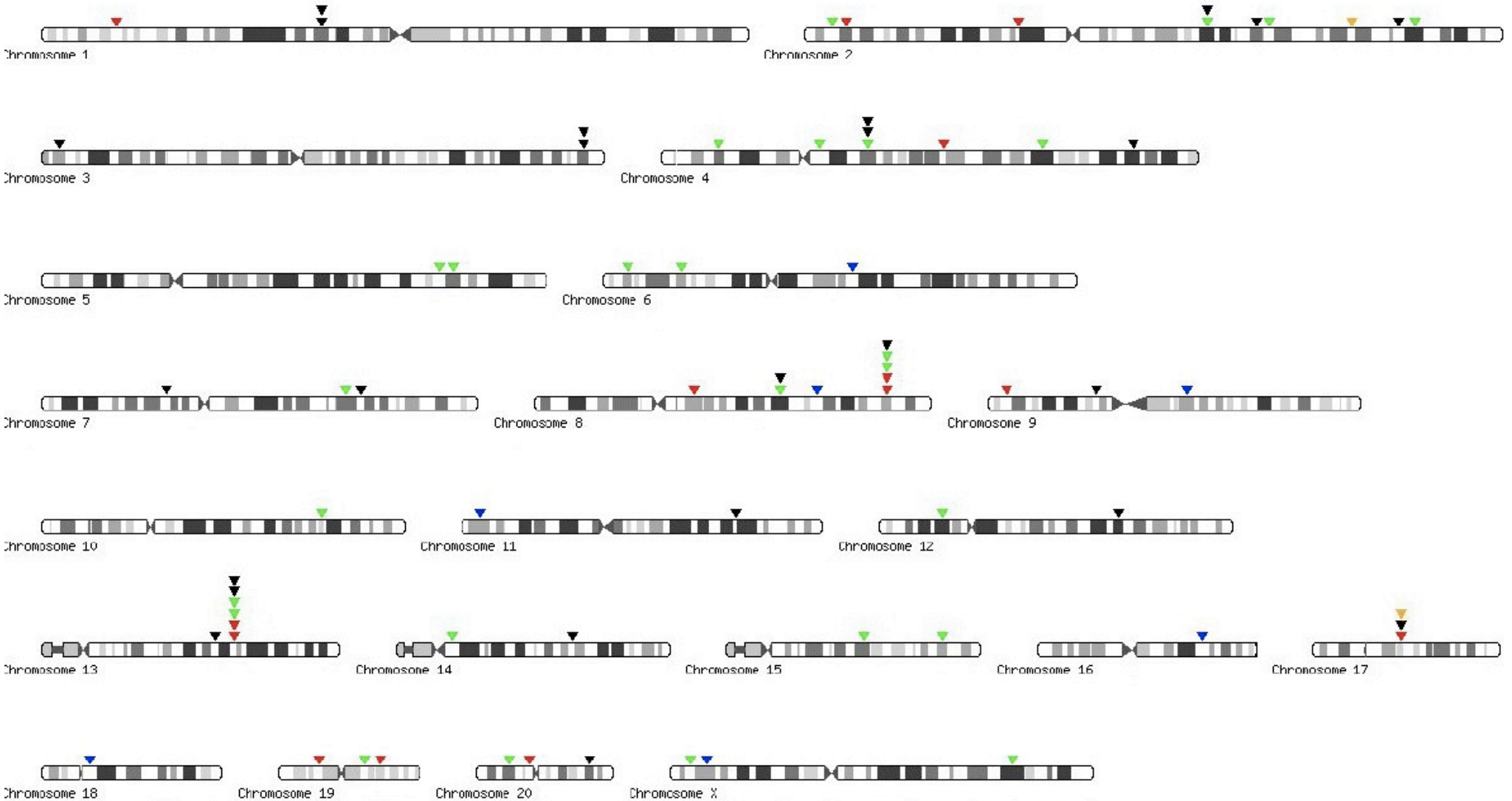
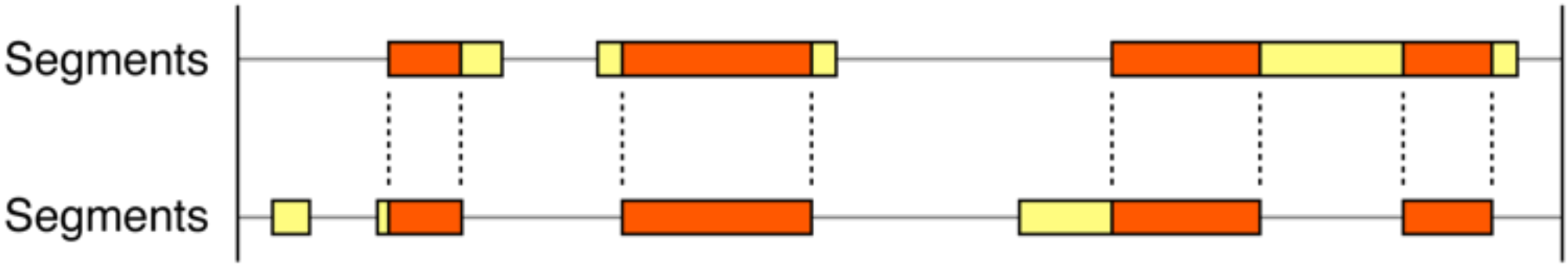
A second case:
Do HPVs integrate preferentially inside genes?



Do HPV and genes overlap?

- Import HPV from (the same) published Page:
  - **"Training material"**
- **"Analyze genomic tracks", "hg19" (!)**
  - **(*shortcut*: "perform HyperBrowser analysis")**
- Track1: **"--From history..--" -> HPV**
- Track2: Find genes in track collection ..
- **"Located inside?", "Start analysis"**

How does this look at the drawing board?



overlap > expected?

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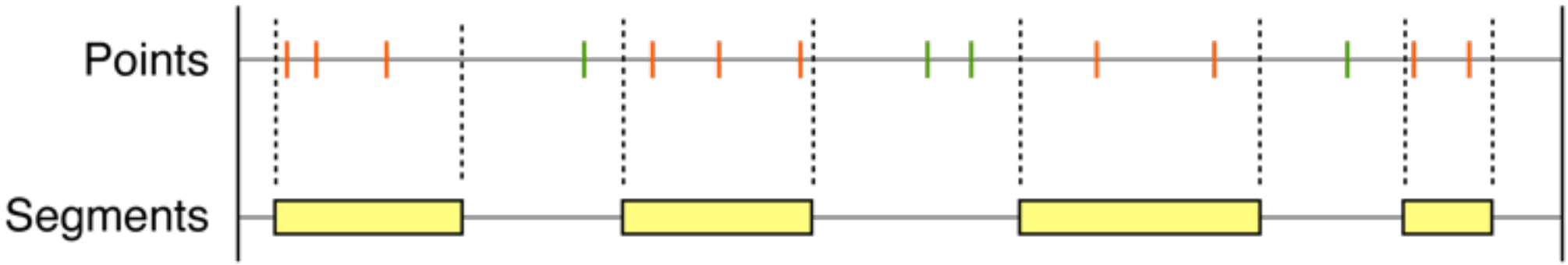


inside > outside ?

P	P	Different frequencies?
P	P	Located nearby?
P	S	Located inside?
P	S	Located nonuniformly inside?
P	S	Located nearby?
S	S	Similar segments?
S	S	Overlap?
S	S	Located nearby?
F	F	Correlated?
P	F	Higher values at locations?
S	F	Higher values inside?
P	VS	Located in segments with high values?
S	VP	Higher values inside segments?
VP	VP	Nearby values similar?
P	VS (c/c)	Located in case segments
VS (c/c)	S	Preferential overlap?
VP (cat)	VS (cat)	Category pairs differentially co-located?
LGP	P	Colocalized in 3D?

Making justified choices is indeed hard!

- The choice of data may influence results
  - Both source and exact version of genes might matter
  - Can sometimes justify, e.g. based on sensitivity/ specificity trade-off
  - Should ideally show how results vary with choice of data
  - Should at least be very precise in what was done (accessibility, transparency, reproducibility)

Making justified choices is indeed hard (2)

- There is usually more than one possible test for a given biological question
  - The choice has to be made,
    and can't be resolved automatically
  - Statistical and biological implications play together to determine what may be reasonable
  - Should at least expose the different possibilities

Hypothesis testing

- Alternative hypothesis (H1)
  - What you really want to show (more HPV in genes)
- Null hypothesis (H0)
  - A neutral baseline (HPV equally inside/outside)
- P-value
  - How likely is observation (or more extreme), given H0
  - Observation unlikely -> reject H0, left with H1

Hypothesis testing: the challenges

- Alternative hypothesis (H1)
  - What you really want to show (more HPV in genes)
- Null hypothesis (H0)
  - A neutral baseline (HPV equally inside/outside)
- P-value
  - How likely is observation (or more extreme), given H0
  - Observation unlikely -> reject H0, left with H1

Mathematically imprecise?

Is it easy to define?

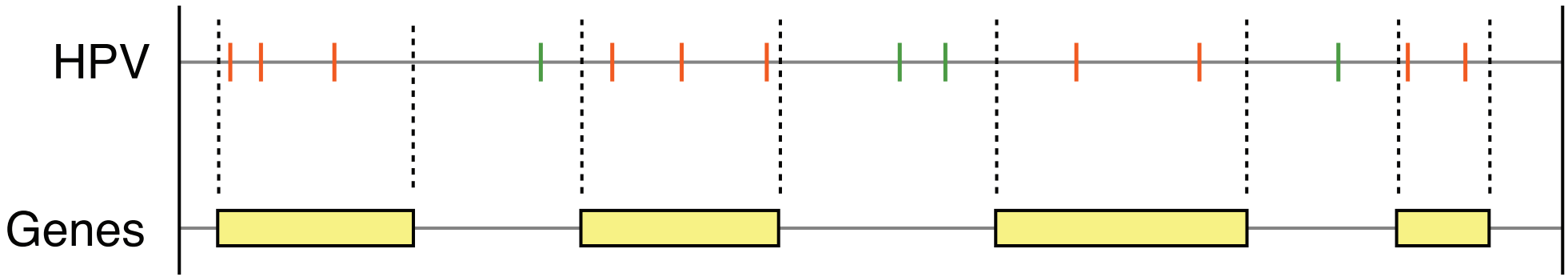
How to compute?

Or maybe unlikely for other reason?

How to compute p-value?

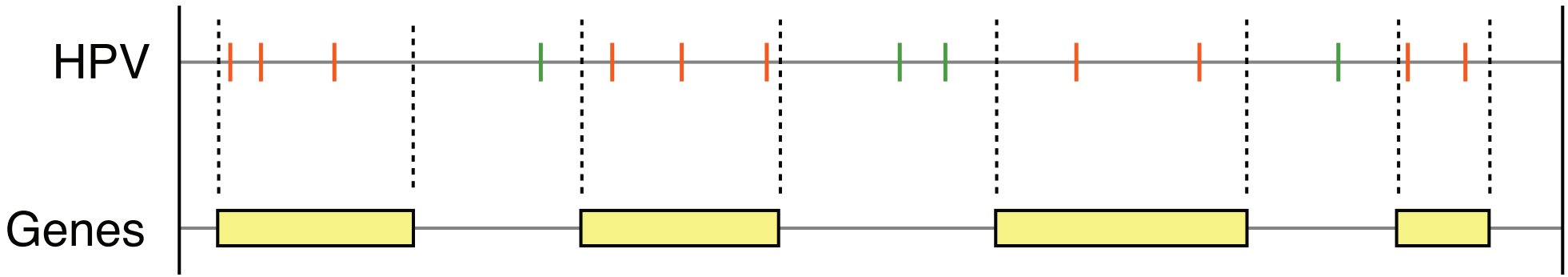
- Look at normal distribution table? Run a t-test?
  - But where to put in HPV and genes?

The quest for a distribution

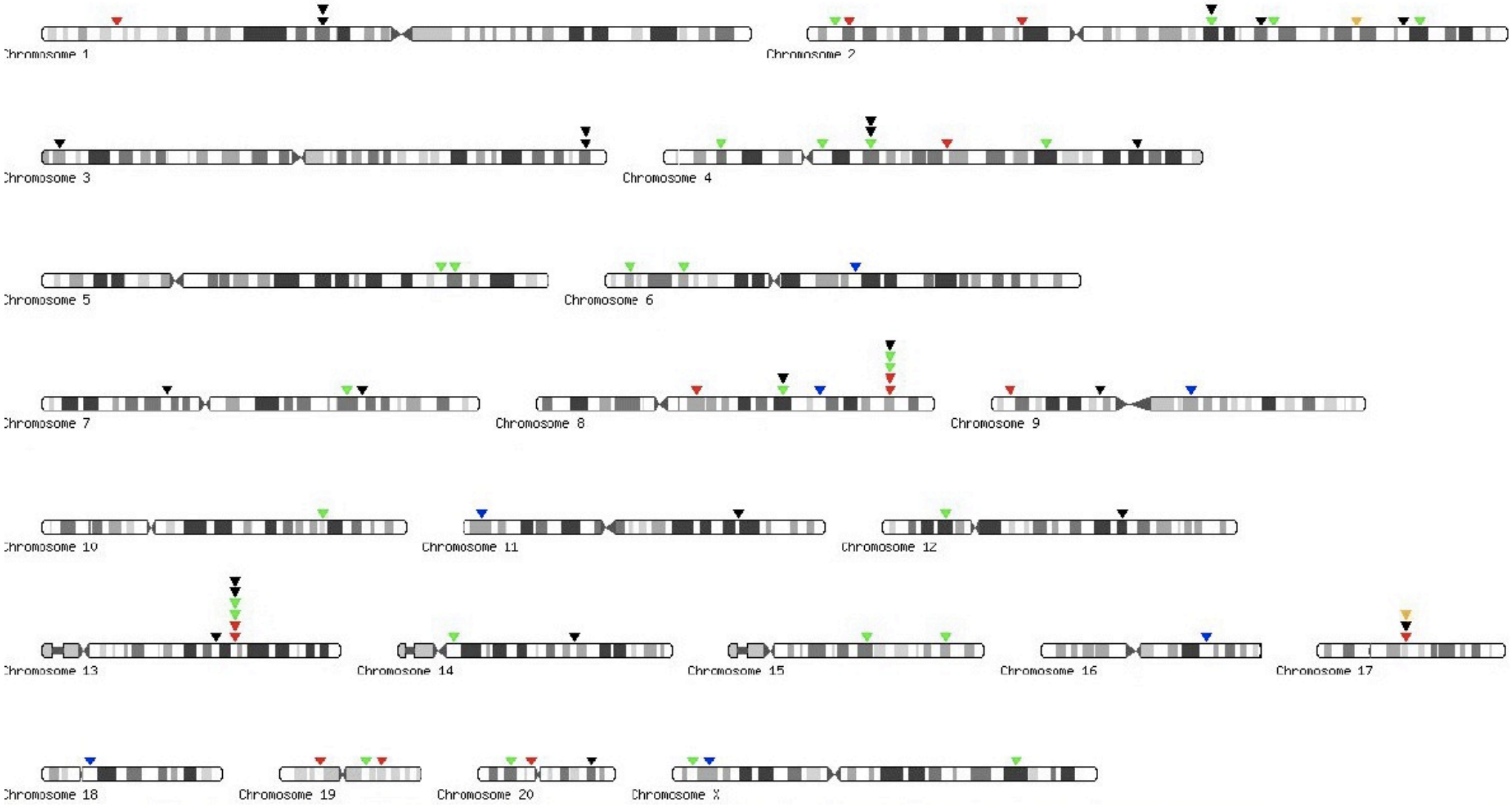


- Can we find a suited distribution?
  (for number of HPV sites inside genes under H0)
  - Statistician may find that "yes: a binomial distribution"
  - Would you be comfortable assuming a binomial distribution?
    Or better: Would you have any clue on the implications?

The quest for a distribution



- The implication of using a binomial distribution
  - What is binomially distributed - HPV or genes?
  - Neither.. This only applies to the measure.
  - Instead, HPV assumed independently and uniformly distributed
  - Not trivial to see, and if found: is this acceptable?



HPV integration sites

How to compute p-value?

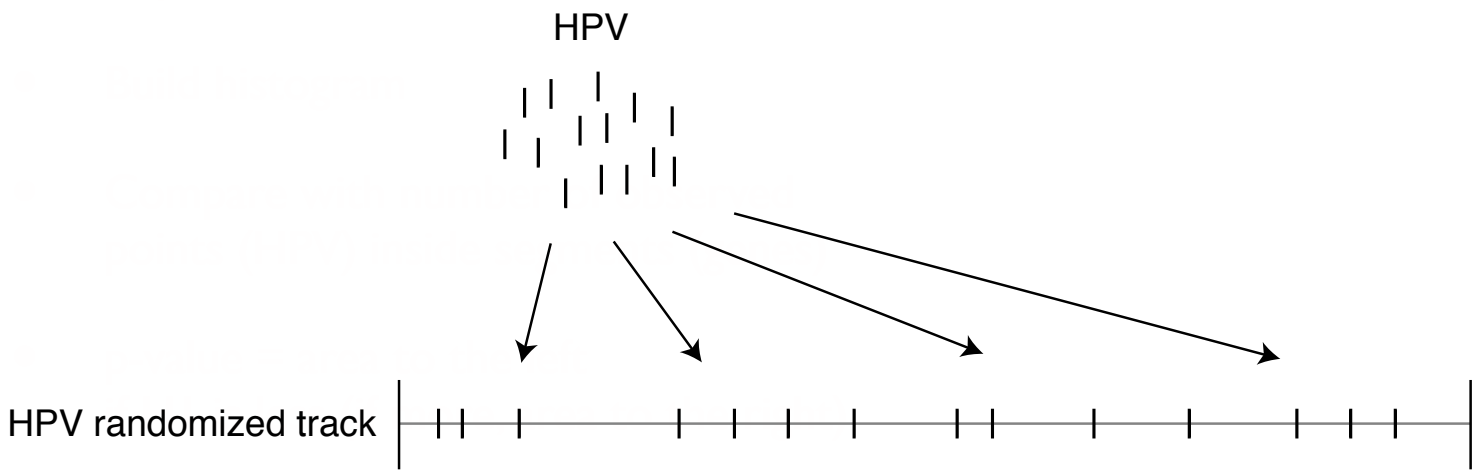
- Look at normal distribution table? Run a t-test?
  - But where to put in HPV and genes?
- Turns out that thinking about standard tests and distributions becomes awkward
  - Instead, do it the modern way..

Meet Monte Carlo

- Null model:
  - How to randomize data (precise rendition of H0)
  - Where could HPV be located under H0..
- Test-statistic:
  - How to measure aspect of interest
  - Number of HPV sites located inside genes
- P-value:
  - How often is ***test-statistic*** from ***null model*** more extreme than for observation?
  - How often are 78 or more random HPV inside genes?

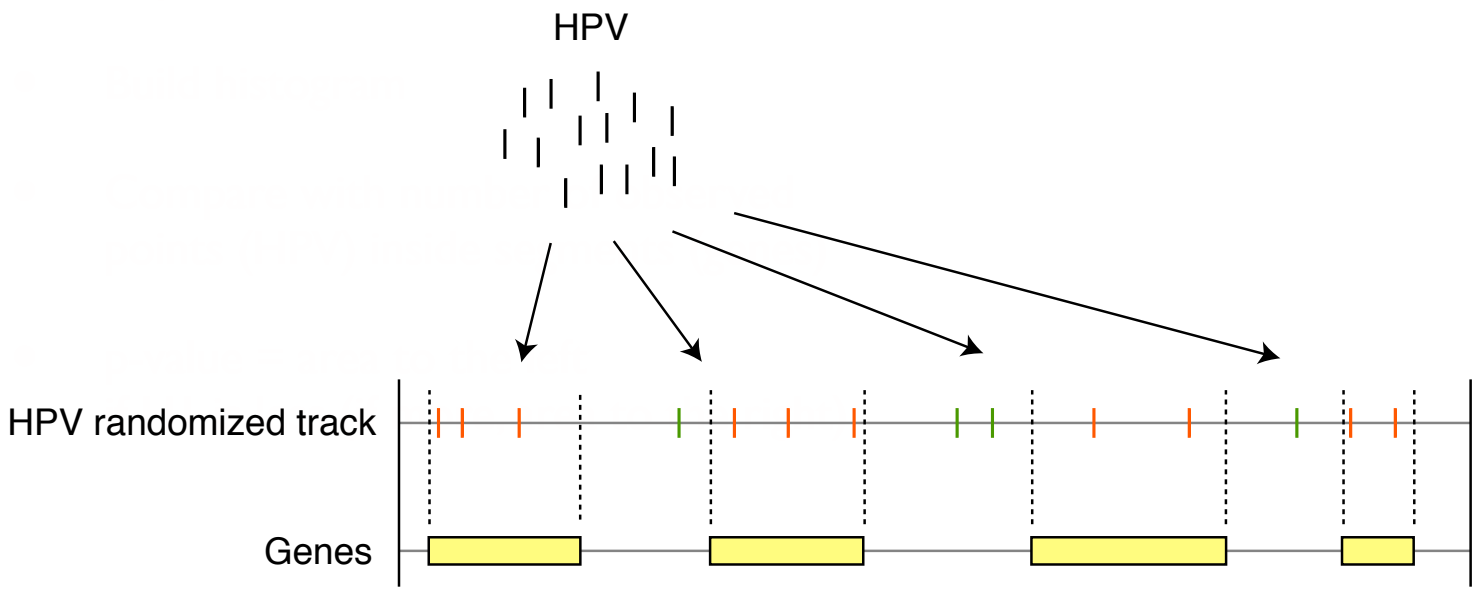
Monte Carlo test on "points inside segments"

- Randomize point (HPV) locations (null model)



Monte Carlo test on "points inside segments"

- Randomize point (HPV) locations (null model)
- Count random points (HPV) inside segments (genes) - test statistic



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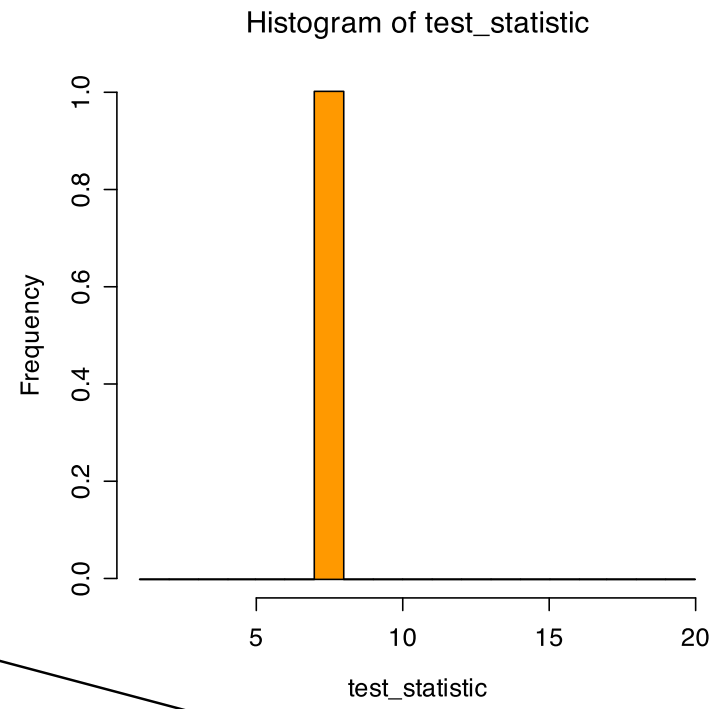
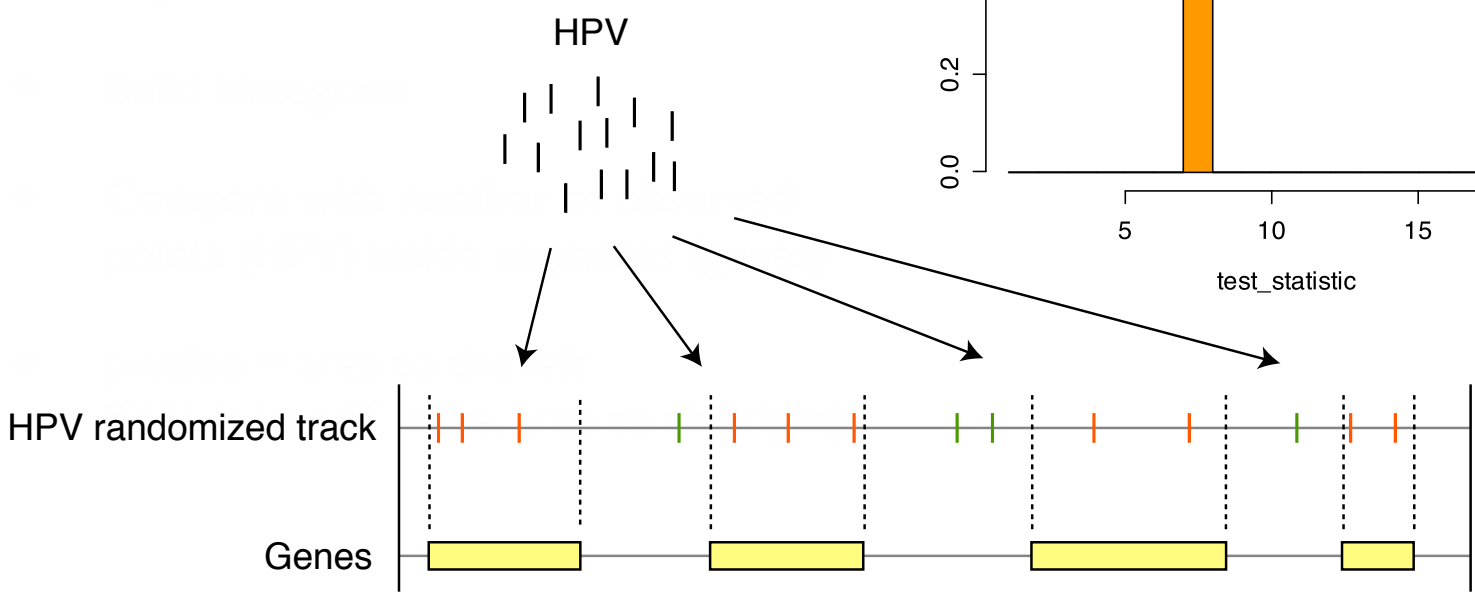
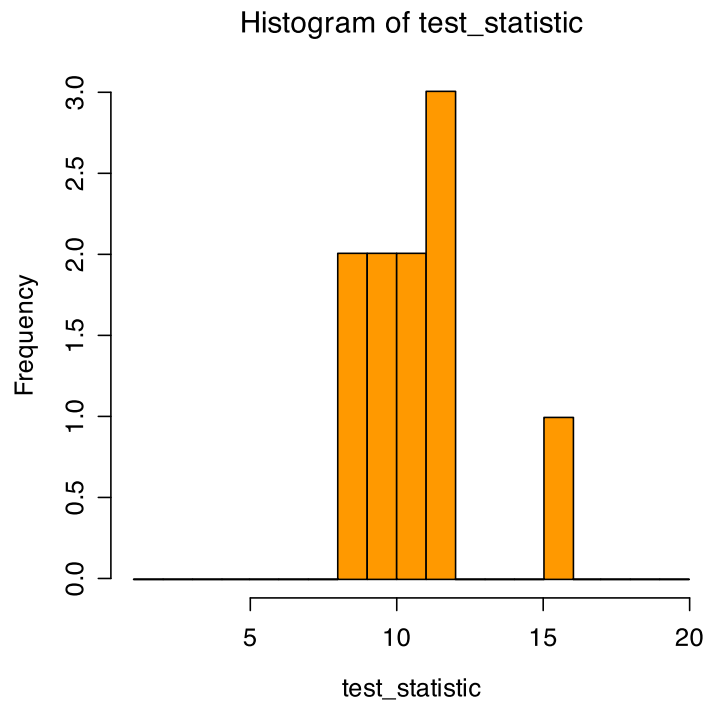




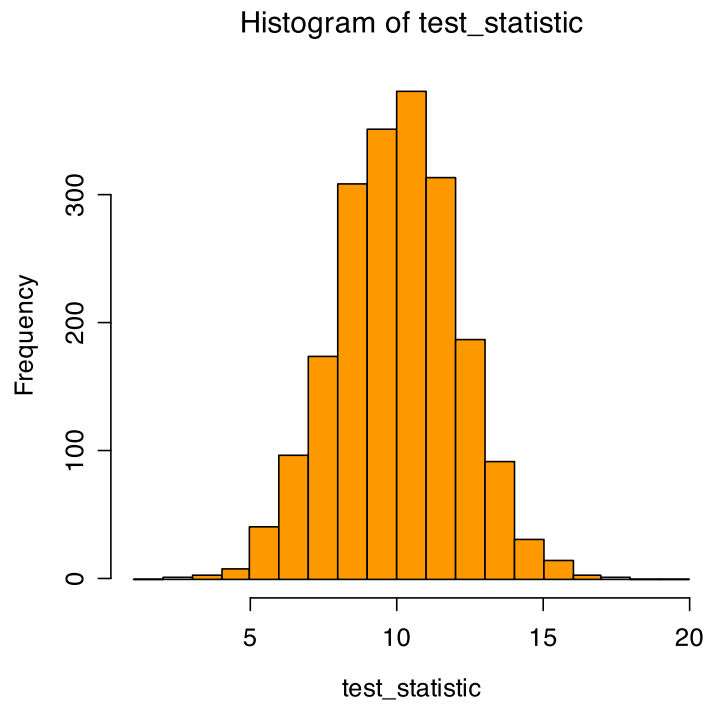
Monte Carlo test on "points inside segments"

- Randomize point (HPV) locations (null model)
- Count random points (HPV) inside segments (genes) - test statistic
- Repeat a number of times



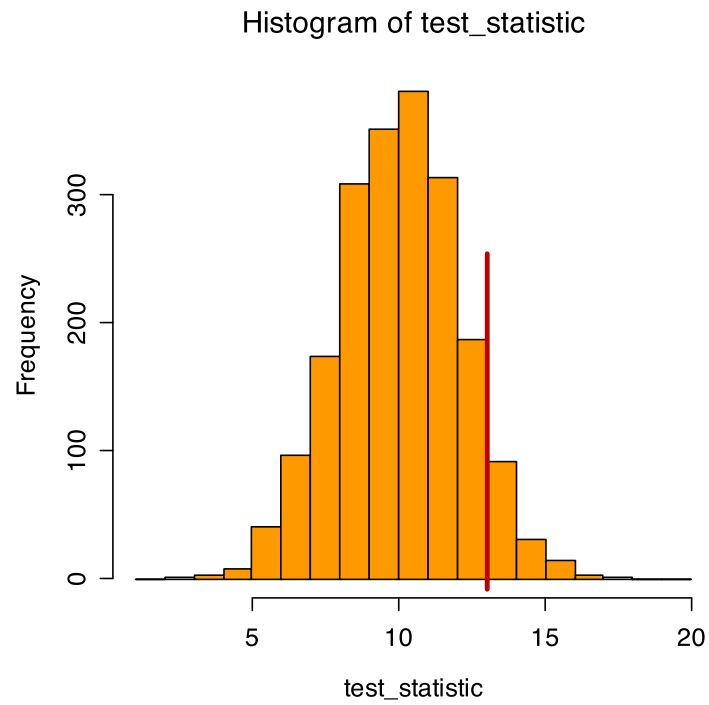
Monte Carlo test on "points inside segments"

- Randomize point (HPV) locations (null model)
- Count random points (HPV) inside segments (genes) - test statistic
- Repeat a number of times
- Build histogram



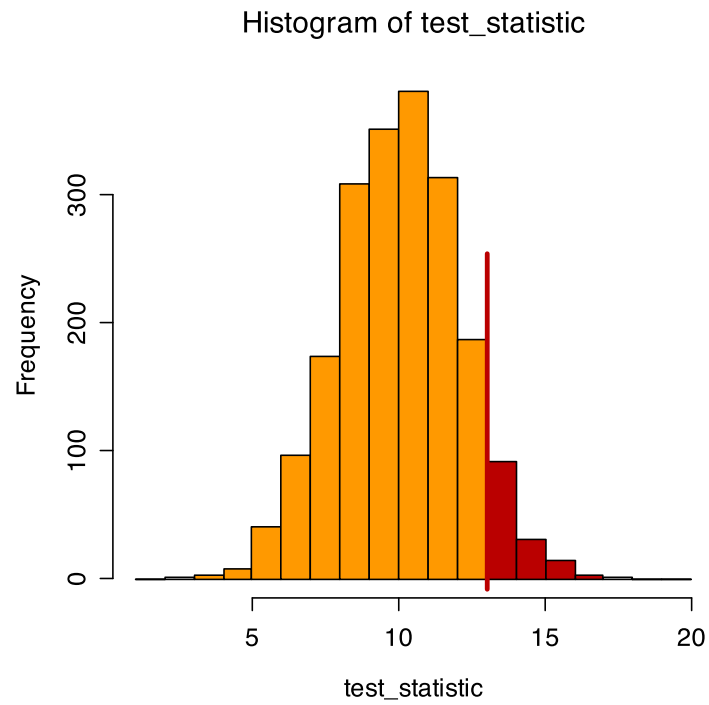
Monte Carlo test on "points inside segments"

- Randomize point (HPV) locations (null model)
- Count random points (HPV) inside segments (genes) - test statistic
- Repeat a number of times
- Build histogram
- Compare with number of observed points (HPV) inside segments (genes)



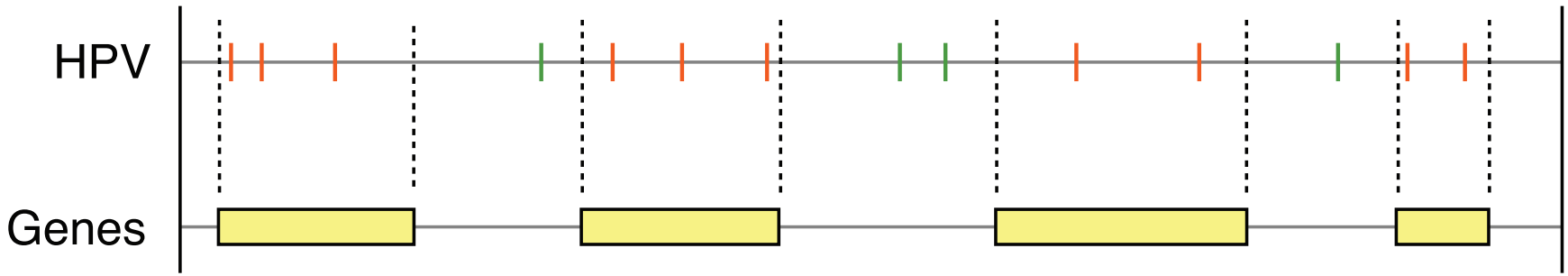
Monte Carlo test on "points inside segments"

- Randomize point (HPV) locations (null model)
- Count random points (HPV) inside segments (genes) - test statistic
- Repeat a number of times
- Build histogram
- Compare with number of observed points (HPV) inside segments (genes)
- p-value = area to the right if H1 is more (if less, area to the left)



p-value = 0.08

Back to HPV and genes



- Didn't like implications of binomial distribution?
- With Monte Carlo, you can shuffle how you like
  - Throw HPV around uniformly and independently (like binomial)
  - Keep clustering tendency of HPV (shuffle HPV spacings)
  - Keep HPV as is, only shuffle genes (in various ways)

Exploring alternative data and assumptions

- Try different gene data sources and assumptions (null models) on HPV-gene relation
- Use back button or redo functionality ()
- Who get's the best p-value;)

Data and assumptions matter!

- HPV inside Ensembl genes? *(default assumptions)*
  - Yes! *(p-value=0.006)*
- HPV inside Refseq genes? *(default assumptions)*
  - No! *(p-value=0.5)*
- Inside Ensembl (v2)? *(Preserve inter-HPV distances)*
  - Still yes (p-value=0.005)
- Inside Ensembl (v3)? *(Randomize genes)*
  - Maybe.. (p-value=0.02)

An example of alternative assumptions

- Duan, [...] and Noble (Nature, 2011):
  - Extensive significant 3D co-localization of functional elements, assessed by hypergeometric distribution
- Witten and Noble (NAR, 2012):
  - Hypergeometric test had unrealistic implications. Telomeres and breakpoints may not be co-located after all.. (cancelled 4 of 11 findings)

Other important issues

- Selecting appropriate test statistic
- Handling confounders

Conclusion

- Genomic tracks provide a powerful, generic basis for statistical analysis
- Sophisticated statistical testing can be performed through simple means (web GUI)
- The devil may be in the details, and selection of data and assumptions can't be outsourced

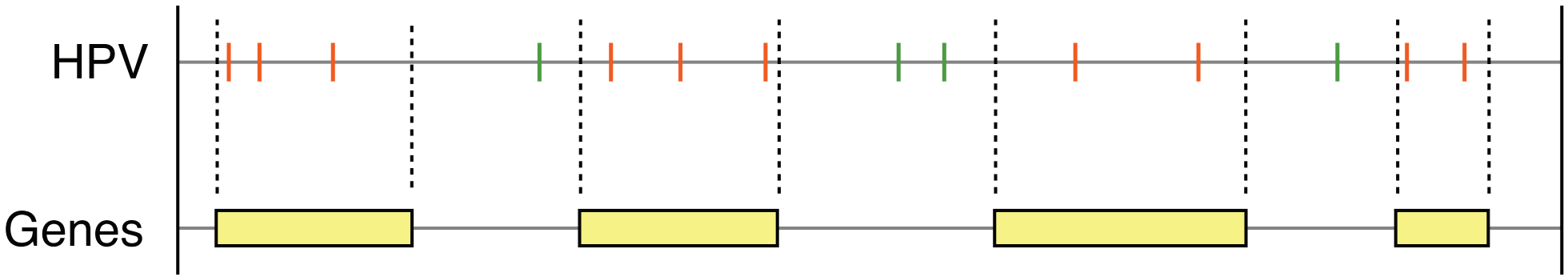
Further into statistical details: the test-statistic

- Maybe viruses integrate close, rather than inside?
  - Let's instead analyze distance to TSS!

HPV close to genes?

- Same data as for last analysis!
  - Use redo - only slight changes are needed..
- Question: **"Located nearby?"**
- Options looks okay !?
- **"Start analysis"**

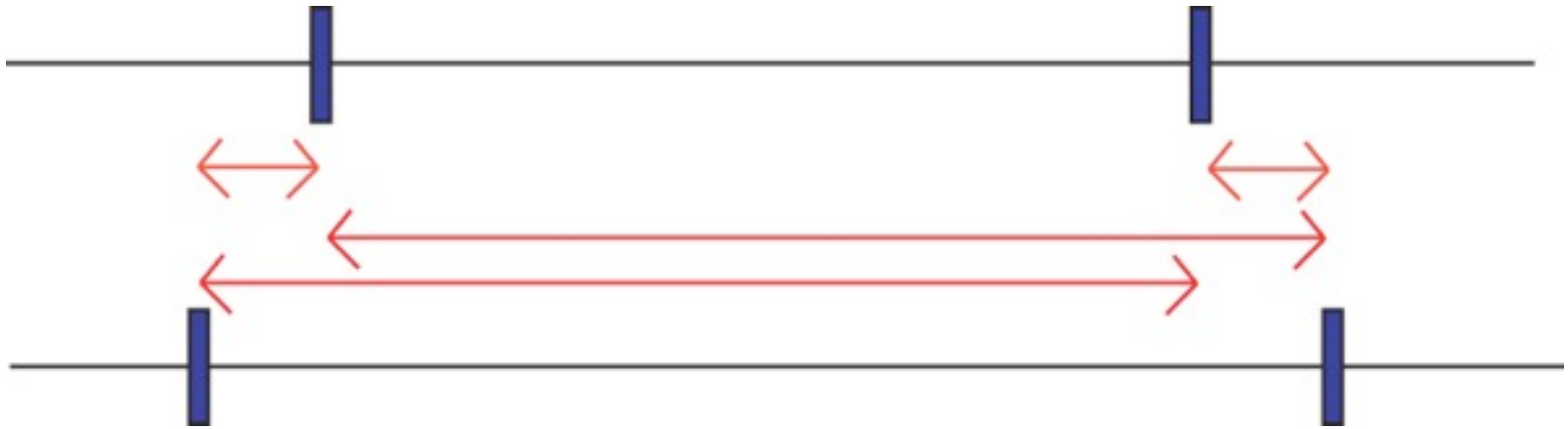
Back to drawing board: the test-statistic



- For "located inside":
  - Could simply count the number of HPV sites falling inside genes

Back to drawing board: Must quantify “close”

But that's trivial, sure: Just count bp distance!?



- But which distances - not all vs all?!

But that's trivial, sure: Just count bp distance!?

- But which distances - not all vs all?!
  - Only shortest!

But that's trivial, sure: Just count bp distance!?

- But which distances - not all vs all?!
  - Only shortest! From 1 to 2!

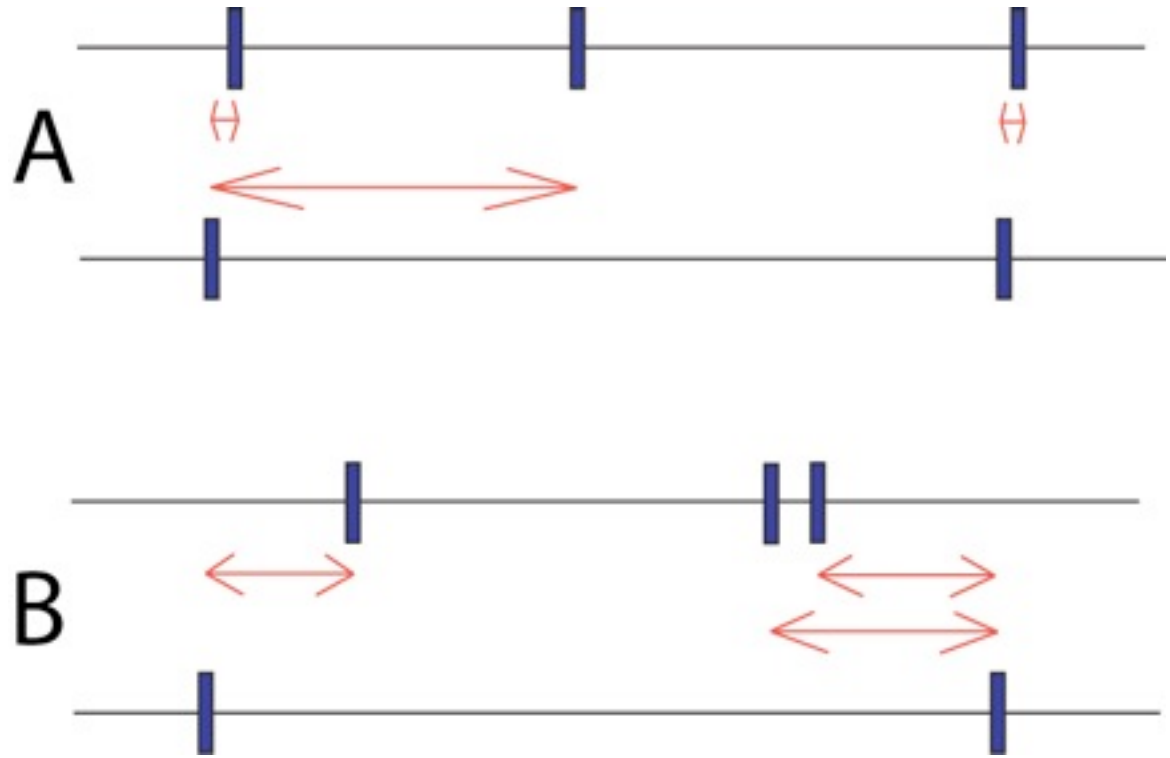
But that's trivial, sure: Just count bp distance!?

- But which distances - not all vs all?!
  - Only shortest! From 1 to 2! But MC needs a single number..

But that's trivial, sure: Just count bp distance!?

- But which distances - not all vs all?!
  - Only shortest! From 1 to 2! But MC needs a single number..
  - Just use sum/average of distances!?

Same degree of close?!



- Two scenarios with same (arithmetic) average..
  - Scenario A indicates relation, but not B !?
  - If so, can be captured by instead using geometric average

You try!

- Can you find a significant HPV-gene relation?
- Would you be comfortable reporting (publishing) this relation?
- If so, what would be an acceptable way to report it?

Any rules of thumb?

(for the statistical testing)

- Maybe:
  - Use test-statistic that gives best (lowest) p-value
  - Use null model that gives worst (highest) p-value
- Reasoning:
  - Use measure that best catches relation of interest
  - Use the most realistic model of nature (null model)

Conclusion

- Genomic tracks provide a powerful, generic basis for statistical analysis
- Sophisticated statistical testing can be performed through simple means (web GUI)
- The devil may be in the details, and selection of data and assumptions can't be outsourced

Handling confounders

- Exons are associated with heightened DNA melting temperature
  - But both exons and DNA melting are also directly associated with GC content
  - Are exon regions really associated mechanistically with DNA melting, beyond the relation through a common association with GC?
- Analyzing exon-melting while controlling for confounders

Controlling for confounders

- Tutorial 5 of "**`Analyze genomic tracks`**"

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