CSE 428

Spring 2019

Overview

Course Web Pages:

https://courses.cs.washington.edu/courses/cse428/19sp/

TAs:

Daniel Jones

Group-Project-oriented:

Typically teams of ~3-4 students

I will offer some projects ideas

I am open to student-generated ideas

"computers" + "biology"

(+ reasonable scope + something I can facilitate)

Project Challenges

Organization & Scheduling

Bio Jargon

Tools from elsewhere

Did I mention Organization & Scheduling?

What I hope you will learn

See previous slide!

You'll see real DNA/RNA seq data in all of them, plus

Some mixture of:

- data structures,
- algorithms,
- data analytics,
- statistics,
- biology,
- HCI,
- ML, …

Project Evaluation

Weekly Goals + Progress reports

Some midcourse checkpoint

Final written reports + oral presentations

  Including evaluation of code, test results, etc.

Peer comments

Project Ideas

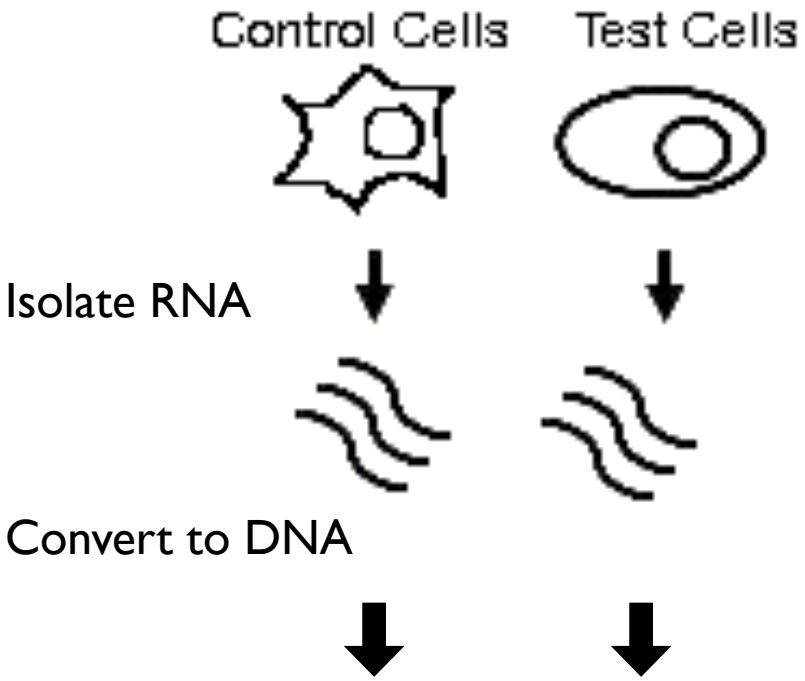
Our suggestions grow out of technical issues ("bias" and "dropout") in RNA sequencing, outlined in the following few slides. For today, at least, the details are not critical; key points I hope you get are that
a) we can sequence RNA from cells
b) it's informative
c) it's quantitative, but
d) technical artifacts bias that quantitative information, and

e) *there are unexplored issues surrounding this,* hence, project ideas: understanding the sources and extent of the biases and their impact on various downstream analyses.

Some Background

RNA sequencing

RNAseq Example



DNA Sequencer

Millions of reads,
say, 100 bp each

map to genome,
compare & analyze

Goals of RNAseq

1. Which genes are being expressed?
   How? *Map* them to a reference genome and/or *assemble* reads (fragments of mRNAs) into (nearly) full-length mRNAs

2. How highly expressed are they?
   How? C*ount* how many fragments come from each gene–expect more-highly-expressed genes to yield more reads per unit length

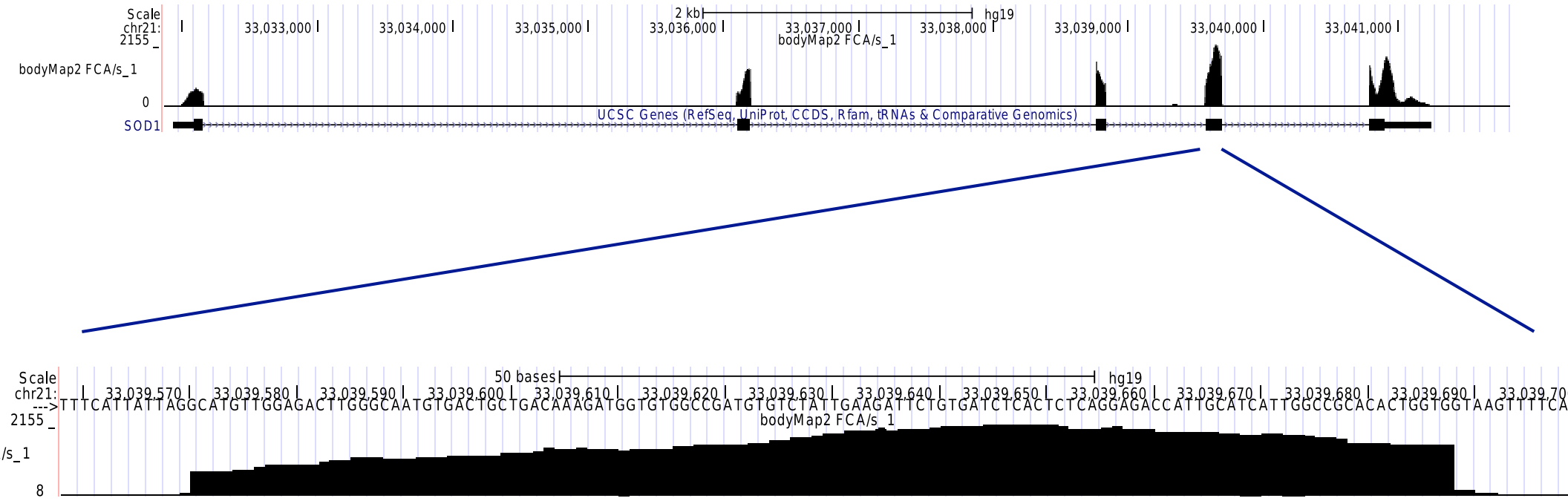
3. What's same/diff between, e.g., tumor/normal?

4. Which *alleles* are being expressed? Differentially expressed? Which cell types? How variable are they?
   … … …

RNAseq

What does it look like?

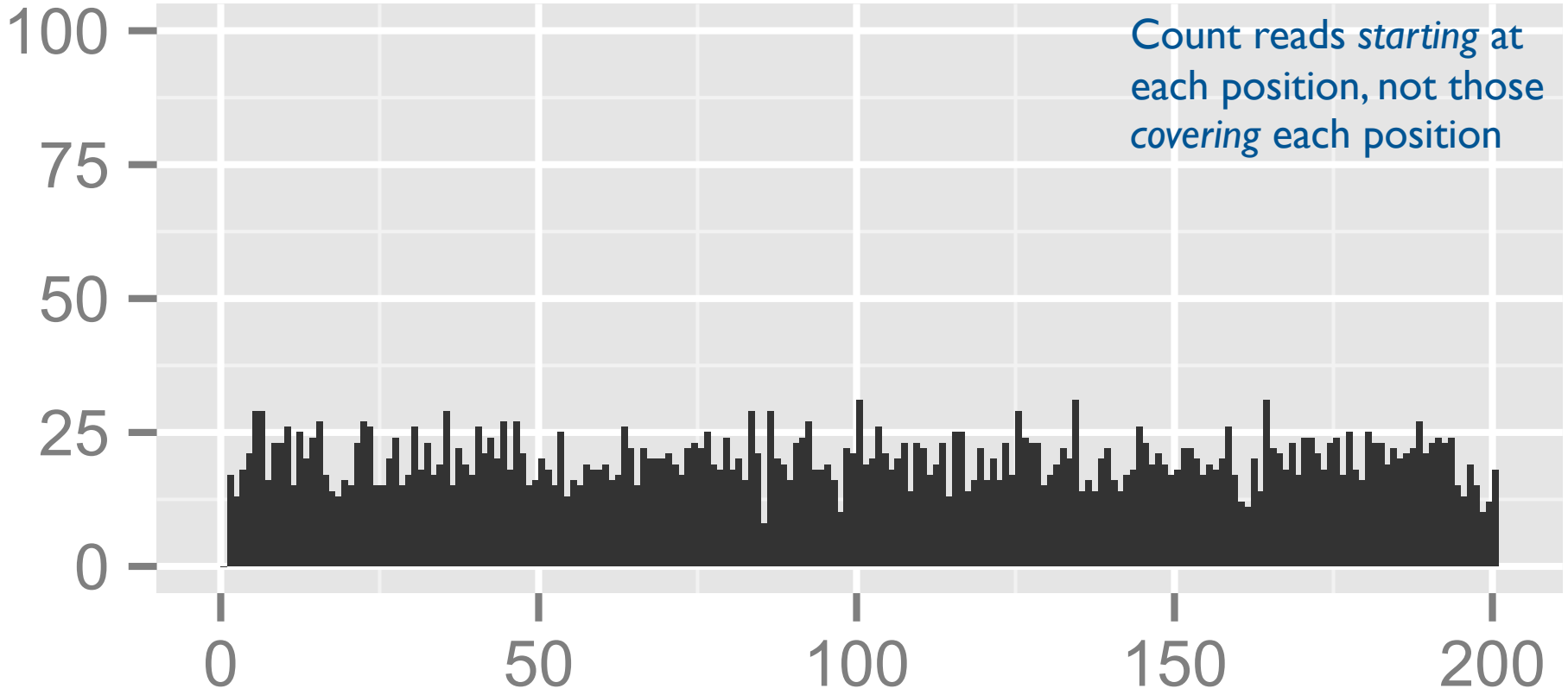
RNA seq



RNA → cDNA, fragment, end repair, A-tail, ligate, PCR, … → Sequence → QC filter, trim, map, … → Count

It's so easy, what could possibly go wrong?

What we expect: Uniform Sampling



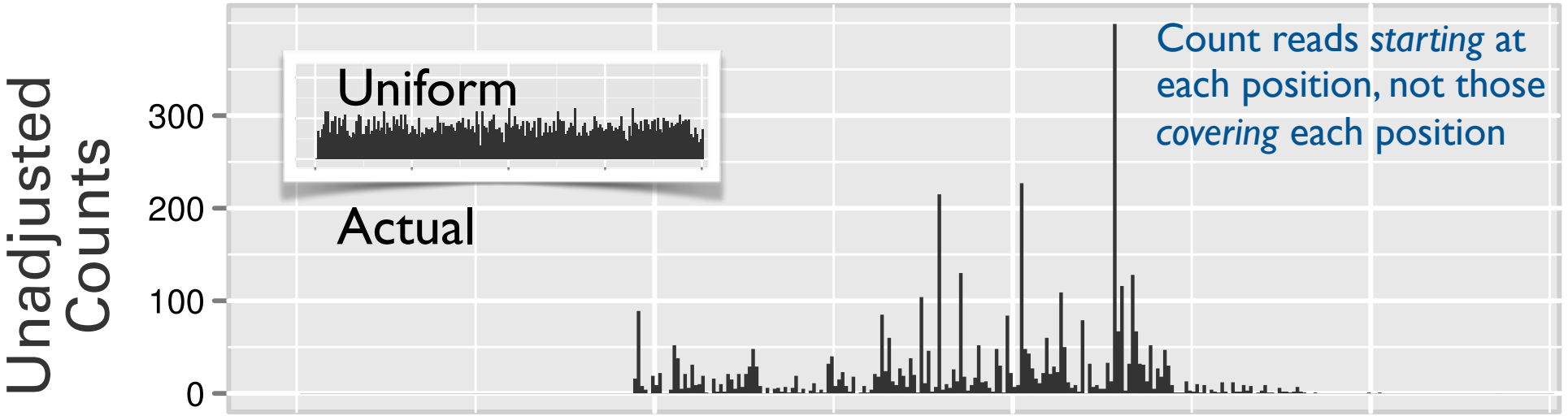
Uniform sampling of 4000 "reads" across a 200 bp "exon."

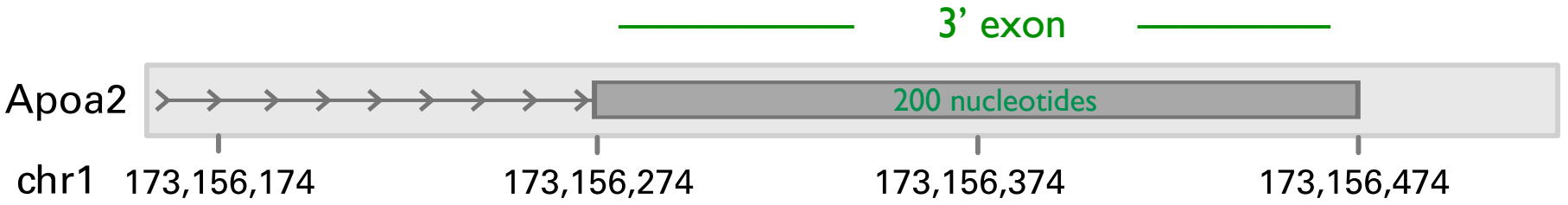
Average 20 ± 4.7 per position, min ≈ 9, max ≈33

I.e., as expected, we see ≈ μ ± 3σ in 200 samples

What we get: *highly* non-uniform coverage

E.g., assuming uniform, the 8 peaks above 100 are ≳ +10σ above mean

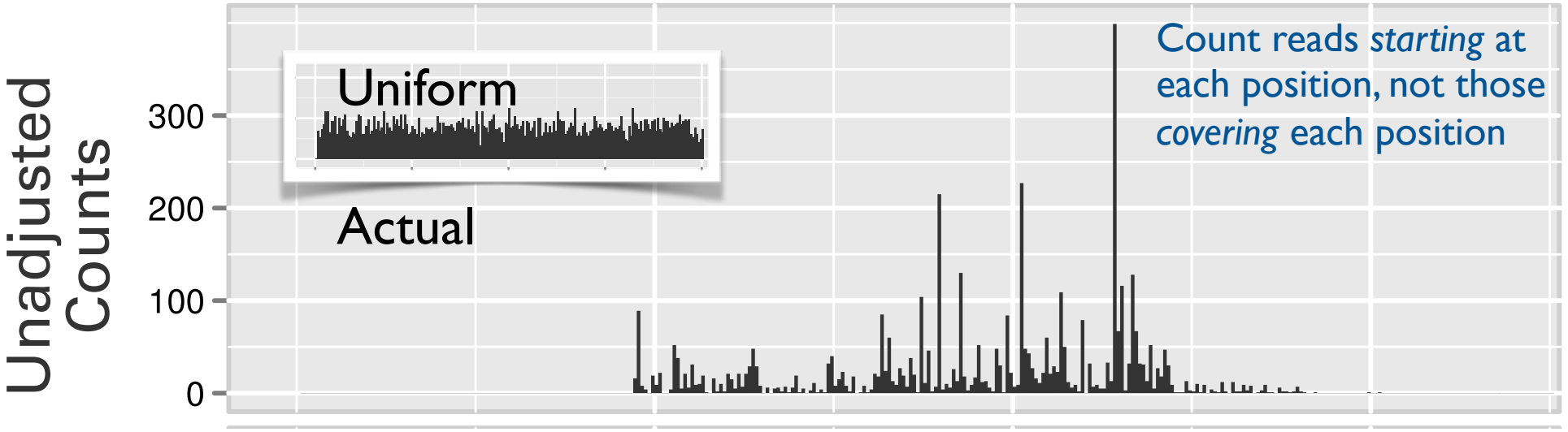




Mortazavi data

What we get: *highly* non-uniform coverage

E.g., assuming uniform, the 8 peaks above 100 are ≳ +10σ above mean



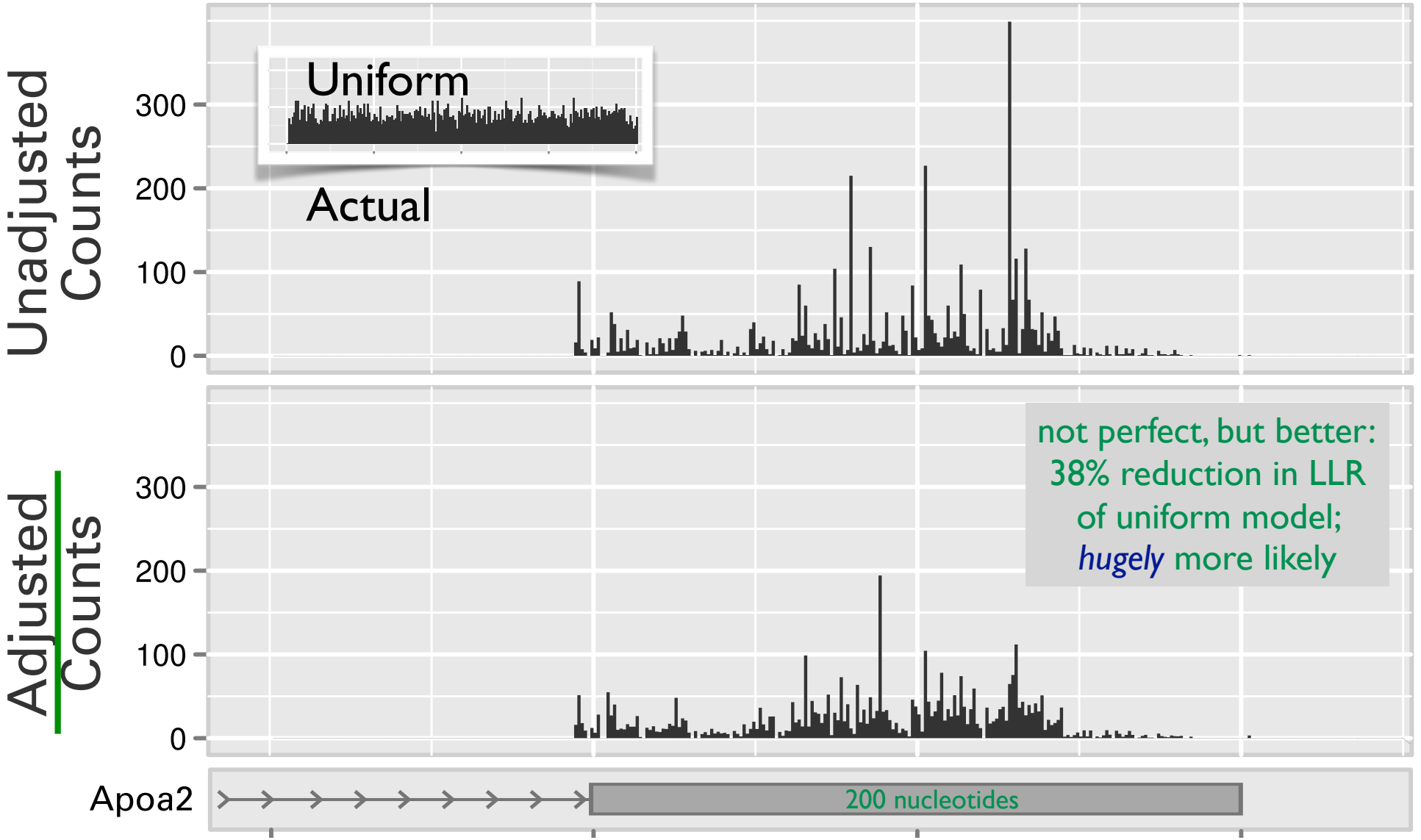
How to make it more uniform?

A: Math tricks like averaging/smoothing (e.g. "coverage") or transformations ("log"), …, or

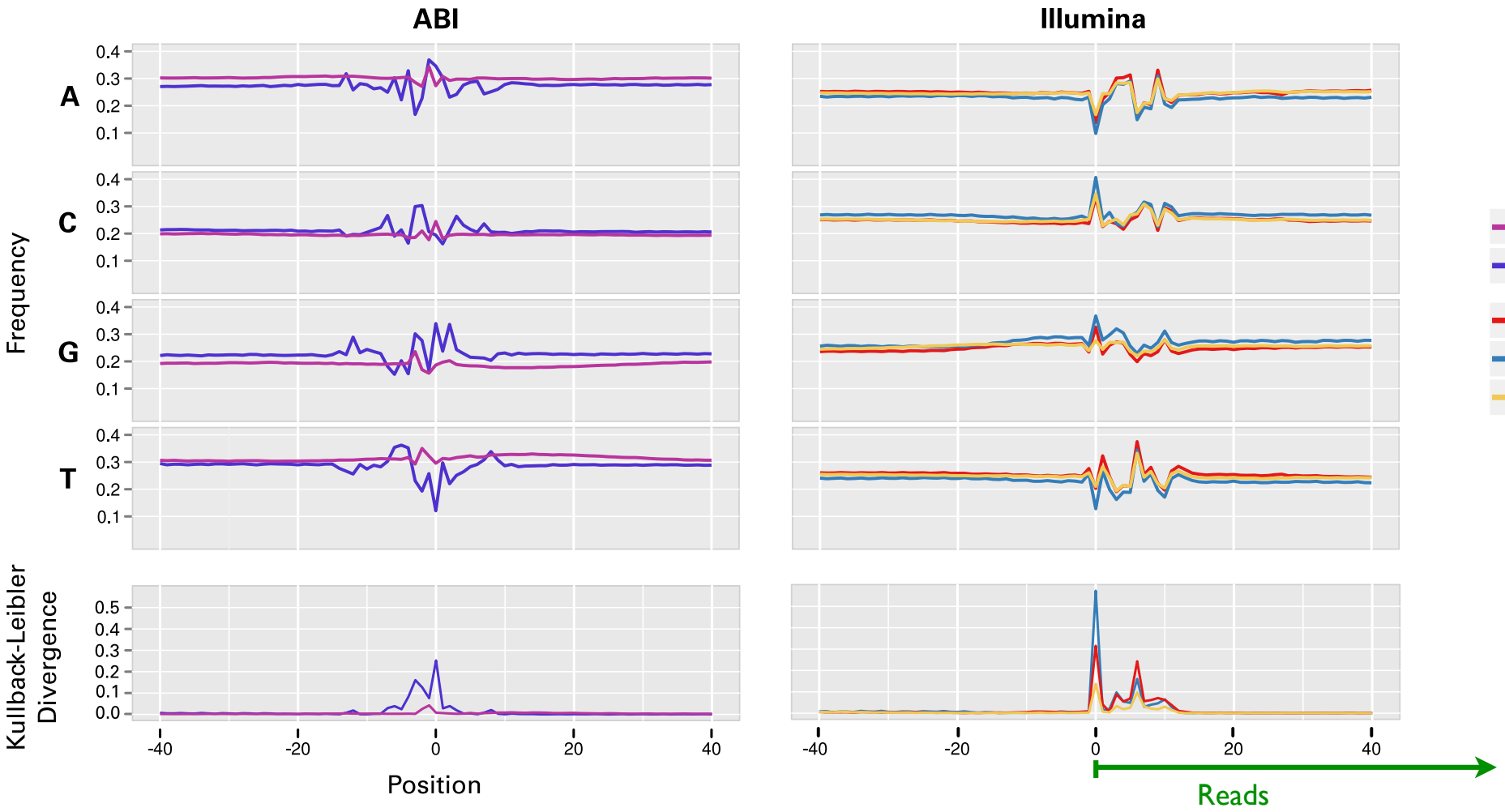
B: Try to model (aspects of) causation ← *WE DO THIS*

(& use increased uniformity of result as a measure of success)

The Good News: we can (partially) correct the bias



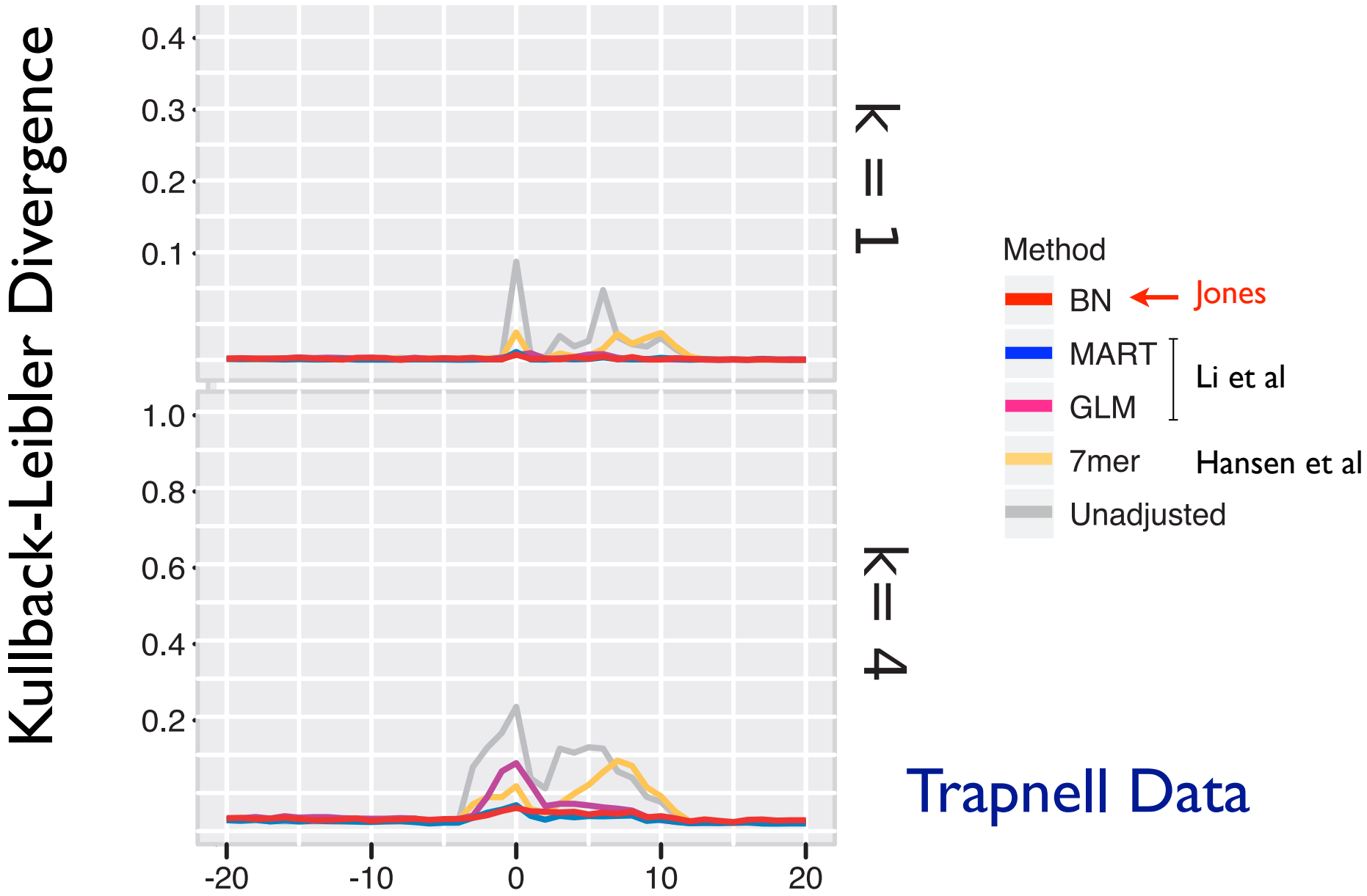
Bias is (in part) sequence-dependent



and platform/sample-dependent

Fitting a model of the sequence surrounding read starts lets us predict which positions have more reads.

Result – Increased Uniformity



Trapnell Data

Project Idea: Next Few Slides

Open-ended, underspecified; as you think about them, both let your imagination run free, *and* think carefully about how to scale and stage your project so you can collect low-hanging fruit before potentially getting lost in the open-ended weeds. (Fortunately, I don't think mixing metaphors is a crime in this state–at least not yet.)

Idea #1

Idea #1: Bias Distorts Allele Specific Expression Analysis?

Background: An *allele* is one variant of a gene, e.g., the A/B/O alleles that determine "Blood Type." You have 2 alleles of every gene (partially excluding those on X,Y chromosomes). E.g., if you got A from mom & B from dad, you have AB blood-type; if you have O from both, you have O blood-type.

Usually, both alleles are "expressed", i.e., made into proteins, as in the case above, but there are exceptions where only one of the two alleles is expressed ("allele specific expression" or ASE, with dozens of examples known in humans), and potentially severe consequences for disrupting this (e.g., see "Prader-Willi/Angelman syndromes").

How do you detect ASE? One way: compare DNAseq to RNAseq in an individual; if DNA shows 2 alleles, but RNA only sees one of them (or much more of one than the other), then you call it ASE.

Idea #1: Bias Distorts Allele Specific Expression Analysis?

Alleles differ in a small number of positions; bias is sensitive to sequence; so a change in bias at a few changed positions might falsely appear to be ASE, or falsely mask true ASE.

Goal: Explore the effect of SeqBias on ASE prediction. If deemed significant, develop a tool to automatically "correct" for it and apply this too a variety of data sets.

Motivating Questions: Does bias compromise our ability to detect ASE from RNAseq data? What can we do about it?

Some Suggested Steps:

Make a *basic* ASE pipeline; what do you see?

Learn state-of-the-art in ASE discovery; refine your pipeline

Add SeqBias correction to that pipeline

Assess whether it makes a difference

Apply to a variety of data?

Idea #2

Idea #2: in single-cell RNAseq, bias from fragment-dropout?

Say 10^7 reads from 10^4 genes; in *bulk* RNAseq, = 10^3 reads per gene–good statistics.

But in *single-cell* RNAseq, say, for 10^3 cells, only ≈1/gene/cell

I.e., *dropout*: many zeros for *expressed* genes.

Common approaches to ameliorate this bias:

a) "Impute" missing data from "similar" cells

b) "Model" dropout via "zero-inflated distribution"

Motivating Q: for "fragment-based" sequencing protocols, i.e., we randomly fragment full-length transcripts and sequence the fragments, is "dropout" a problem? What should we do about it?

Misc. Projects From 428's Past

428 Past Projects

Just to give you some idea of scope, here are some projects from previous iterations of 428:

- Convenient web interface for "phylogenetic footprinting" in prokaryotes
- Build a genome assembler
- Machine learning applied to cancer genomics
- Convenient web interface for exploring "Foldit" results
- # 0, 3, 4 below

Idea #0: Visualizing and Exploring SeqBias

It's hard to think about it if you can't visualize it.

Goal: Develop a tool to automatically measure, quantify, and display summaries of bias in specific RNAseq data sets, and apply this too a variety of them.

Motivating Questions: How does bias vary from one data set to another? Is more modern data less biased? How does it impact down-stream analyses?

Some Suggested Steps:

- Learn state-of-the-art in RNAseq Quality Control
- Add SeqBias, starting with figures like those in Daniel's paper
- Other metrics?
- Apply to a variety of data?
- HCI issues in presenting such data to potential users?
- Very Speculative: can we implicate *cause*s of bias?

Idea #3: Impact of bias in other RNAseq use cases

Other RNAseq applications may be even more susceptible to distortion due to seqbias, e.g. ribosome foot-printing and RNA structure prediction (SHAPE).

Goal: Explore the effect of SeqBias on these tasks. If deemed significant, develop a tool to automatically "correct" for it and apply this too a variety of data sets.

Motivating Questions: Does bias compromise accuracy of our predictions from RNAseq data? What can we do about it?

Some Suggested Steps:

- Learn state-of-the-art in these applications
- Add SeqBias correction to that pipeline; a key is defining an appropriate "background"
- Assess whether it makes a difference
- Apply to a variety of data?

Idea #4: Improved crossover detection–Background

Jargon: A position in your genome where your mom's nucleotide agrees with your dad's is called *homozygous* (~99.9%); places where they disagree are *heterozygous* (the other .1%).

How might you find heterozygous sites? Perhaps DNAseq will give you "coverage" ~100 at a site, with, say 60 A's and 40 G's:

...GATAGCGATATGGAGTAGAATACCAGGAGCATTTGACCATACTAC...

Idea #4: Improved crossover detection–Background

The *phasing* problem: Given a pair of nearby heterozygous sites, say A/G at position i and G/T at position j > i, does the G at pos j appear on the same chromosome as the A at i or the G at i? I.e., do we have this:

```
          i           j
- - - A - - - - G - - -
- - - G - - - - T - - -
```

or this:

```
- - - A - - - - T - - -
- - - G - - - - G - - -
```

?

How could we tell? Again, maybe DNAseq: If there are single reads covering both pos i and pos j, do they show a mixture of `A--G` with `G--T` or a mixture of `A--T` with `G--G`?

Potential confusion to avoid: each cartoon shows one strand on each of the 2 chromosomes, not "base pairs" on one chromosome (A:T and G:C base pairs.)

Idea #4: Improved crossover detection–Background

The *crossover* problem: Given the same setup, but looking at *two individuals*, perhaps siblings, if we see this in one:

```
          i           j
- - - A - - - - G - - -
- - - G - - - - T - - -
```

and this in the other:

```
- - - A - - - - T - - -
- - - G - - - - G - - -
```

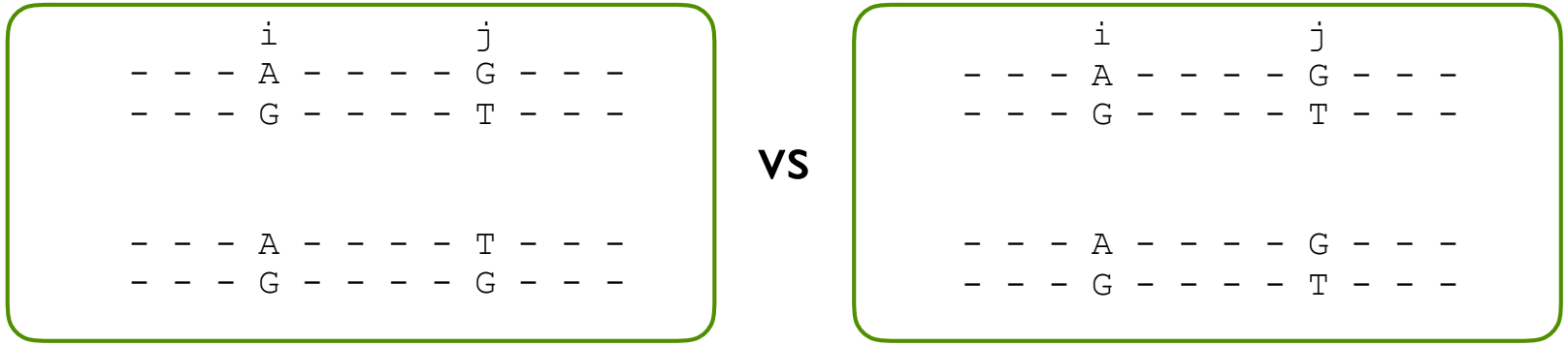
how could that be?

One likely answer: crossover/recombination (in meiosis)

Another possibility: a phasing error!

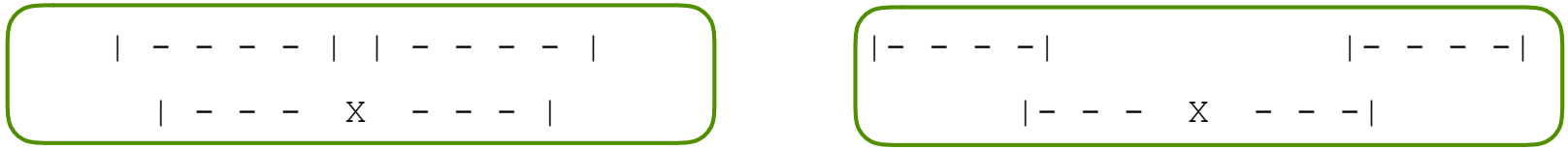
Idea #4: Improved crossover detection–Background

Is crossover distinguishable from a phasing error? Probably not in isolation, but what if we have several *overlapping* i-j pairs that are phased in both individuals? Then we can try for a probabilistic assessment. E.g., abstracting:



as :

What does this suggest?:



(If top gap is short vs long, error in "X" is more/less likely)

Idea #4: Improved crossover detection

Data from a pair of closely related individuals, after being (separately) phased, may/will show crossovers. Are they real/ how many of them are real?

Goal: build a tool to find maximum likelihood estimate of # crossovers, based simple models of xover/error.

Motivating Questions: Can we do better than blindly trusting the phasing results.

Some Suggested Steps:

- Learn state-of-the-art in these applications
- Model as max likelihood solution to system of linear eqns.

$$x_1+x_2+e_1 \equiv 0 \pmod 2$$
$$x_4+x_5+e_2 \equiv 0 \pmod 2$$
$$x_2+x_3+x_4+e_3 \equiv 1 \pmod 2$$

- Good Alg? NP-hard? Good heuristics? Decomposes?
- Apply to a variety of data (especially mine; phasing on up)?

Next steps

review slides

which (if any) appeals?

form groups

skim references on web

talk to/email me/Daniel

we may have fragments of code for parts of this

(may or may not be useful…)

Form a Group/Form a Plan!