Welcome!

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Goals!

- Drive-by introduction to:
  - Cloud computing
  - Basic Illumina sequence quality evaluation & control
  - De novo mRNAseq assembly
  - A (our) "protocol" for mRNAseq analysis => diff expr
  - Variant calling protocol, too

- This will let you explore other online resources to your heart's content, we hope!
  - Other protocols & tutorials
    - khmer-protocols
    - ged.msu.edu/angus/tutorials-2013

Our goals:

Answer your questions!

Help you figure out what questions to ask!

Point to further materials!

Structure of day

- Start by logging into cloud machines, grabbing data, running analyses.
- Coffee break at 10:30
- After lunch, check out variant calling.
- Coffee break at 2:30
- Some open time, if possible
  - Starting your own cloud machine (costs $$, but: freedom!).

Strategy

- Run stuff!
- Talk while it's running.
- Ask questions whenever!

Technology!

- Stickies
- Minute cards
- …Dropbox?

Etherpad?

Why... the cloud?

- Rental computers for small and BIG problems.
- Completely reproducible; independent of institution; so I can write tutorials!
- Once you get something working in the cloud, your local sysadmins can often help you get it running at your institution. If not, well, you can always pay $$.
- (How much? est $150 compute/$1000 mRNAseq sample)

The challenges of non-model transcriptomics

- Missing or low quality genome reference.
- Evolutionarily distant.
- Most extant computational tools focus on model organisms –
  - Assume low polymorphism (internal variation)
  - Assume reference genome
  - Assume somewhat reliable functional annotation
  - More significant compute infrastructure

…and cannot easily or directly be used on critters of interest.

The problem of lamprey...

- Diverged at base of vertebrates; evolutionarily distant from model organisms.
- Large, complicated genome (~2 GB)
- Relatively little existing sequence.
- We sequenced the liver genome…

Assembly

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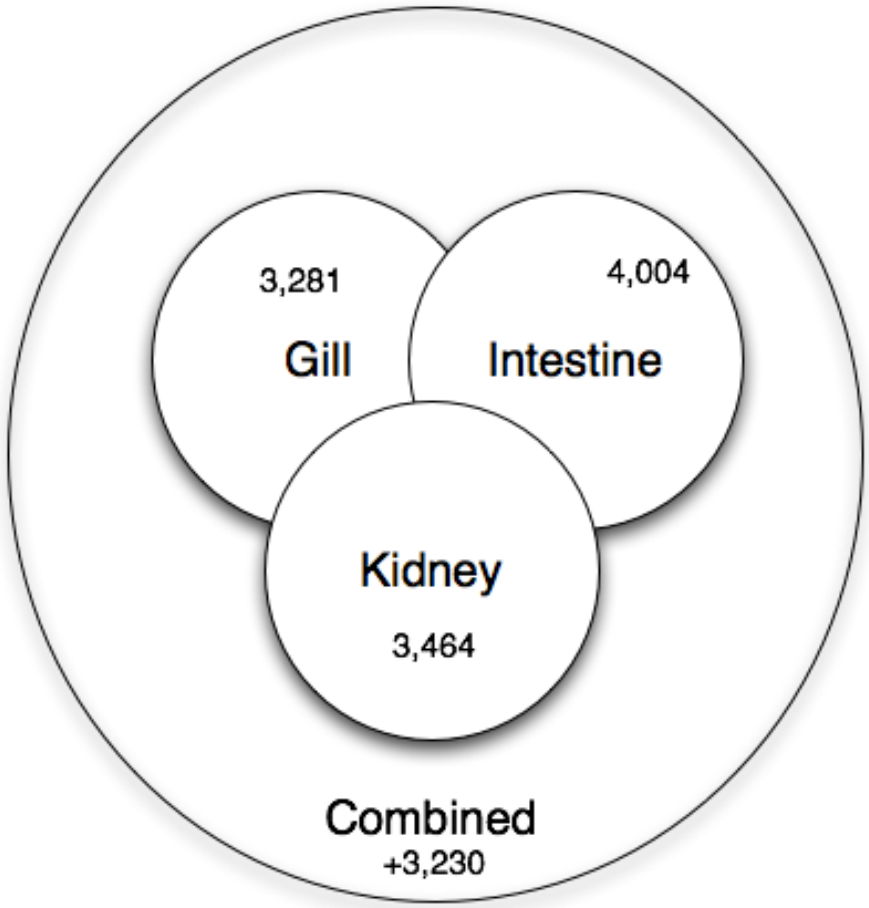
…but for lots and lots of fragments!

Co-assembly is important for sensitivity

Shared low-level transcripts may not reach the threshold for assembly.



Two problems:

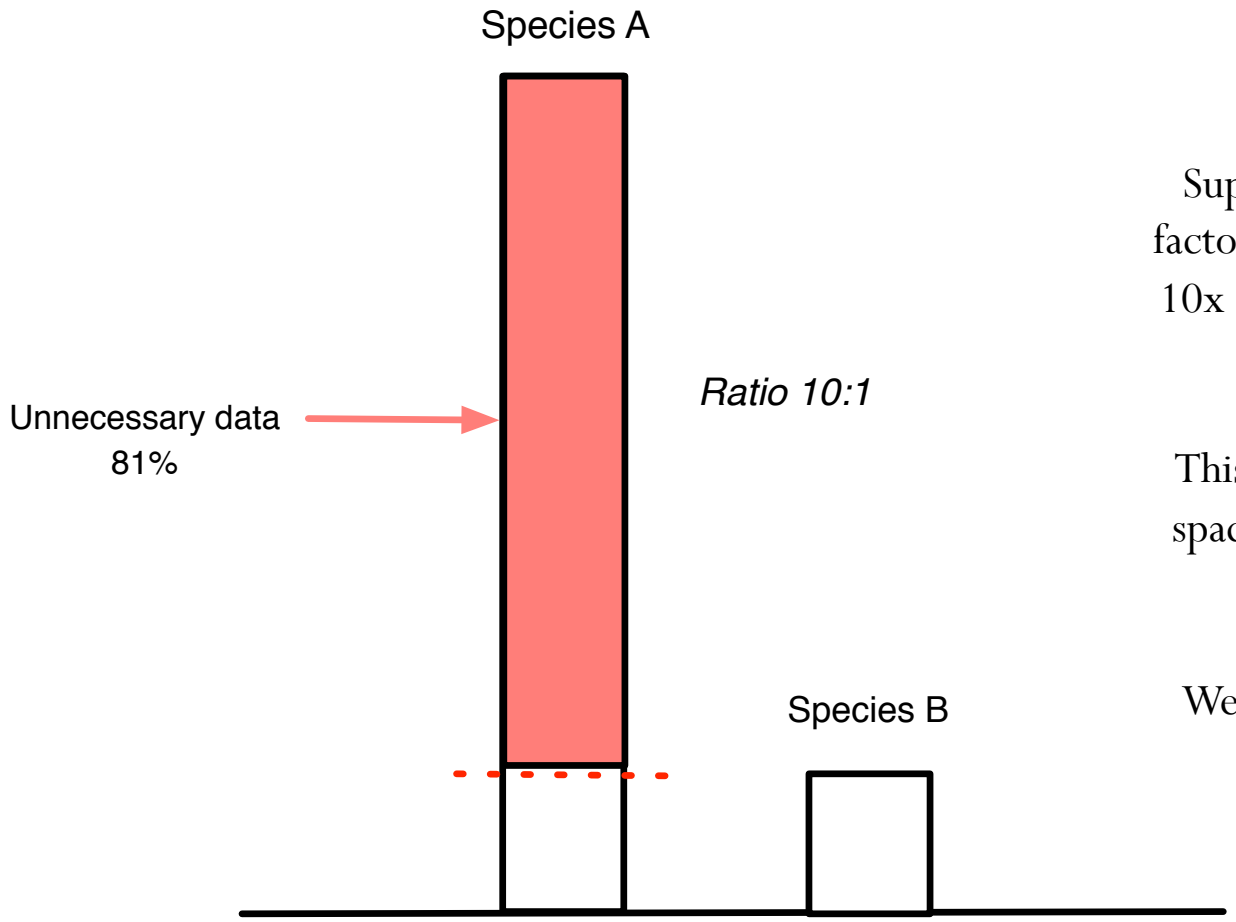
- We want to assemble a lot of stuff together.

- We need to construct transcript families (to collapse isoforms) without having a reference genome.

Diginorm

Solution: Digital normalization

(a computational version of library normalization)



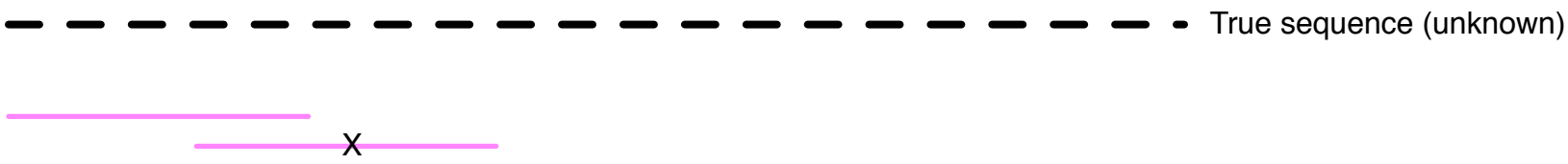
Suppose you have a dilution factor of A (10) to B(1). To get 10x of B you need to get 100x of A! Overkill!!

This 100x will consume disk space and, because of errors, **memory**.

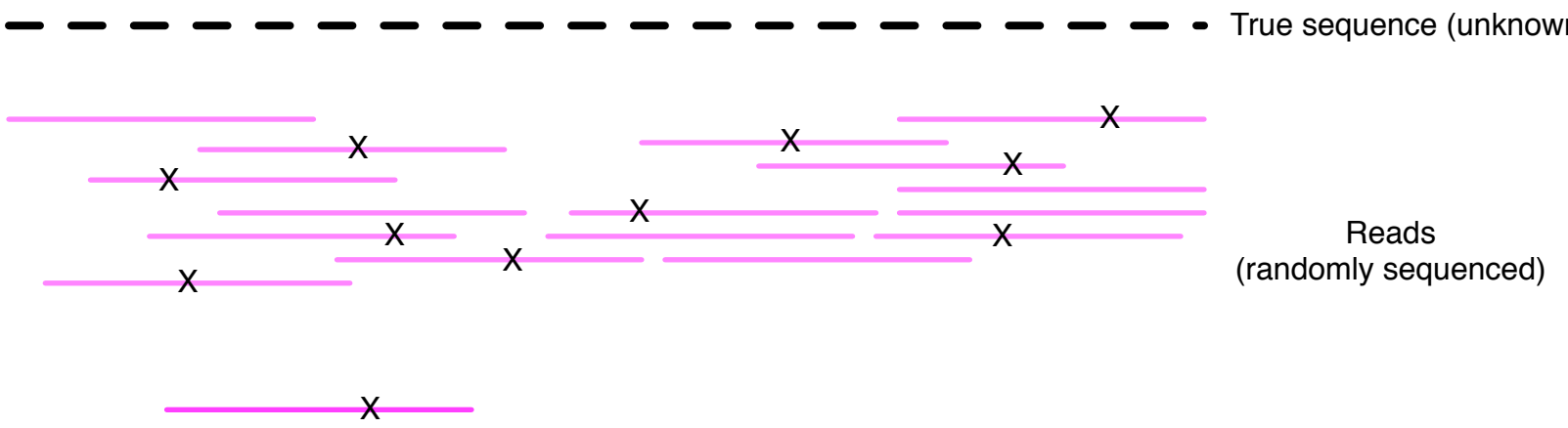
We can discard it for you…

Digital normalization

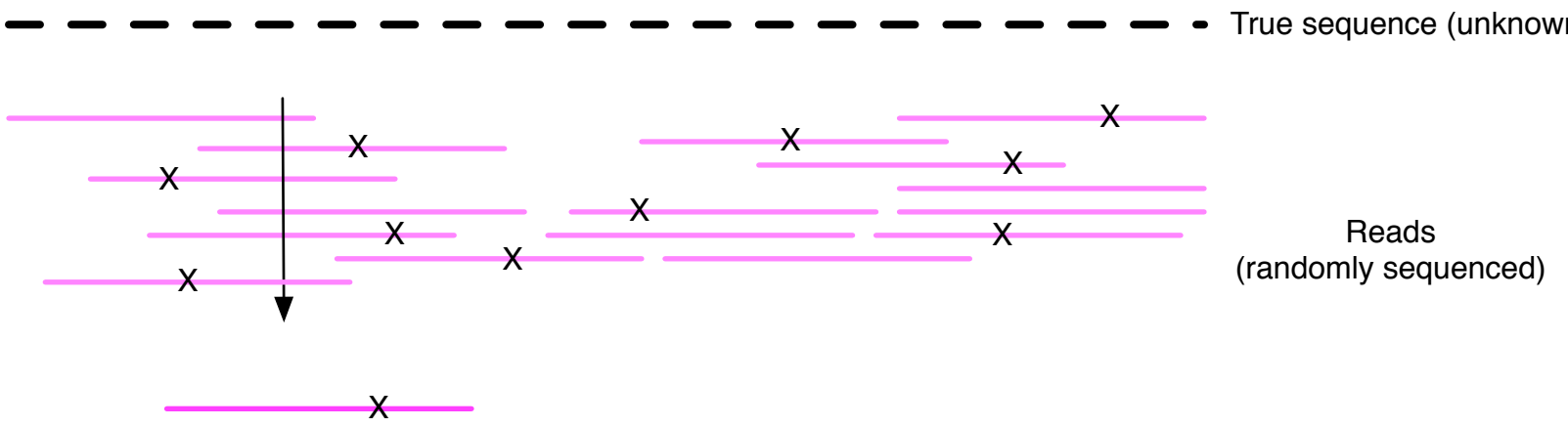
Digital normalization



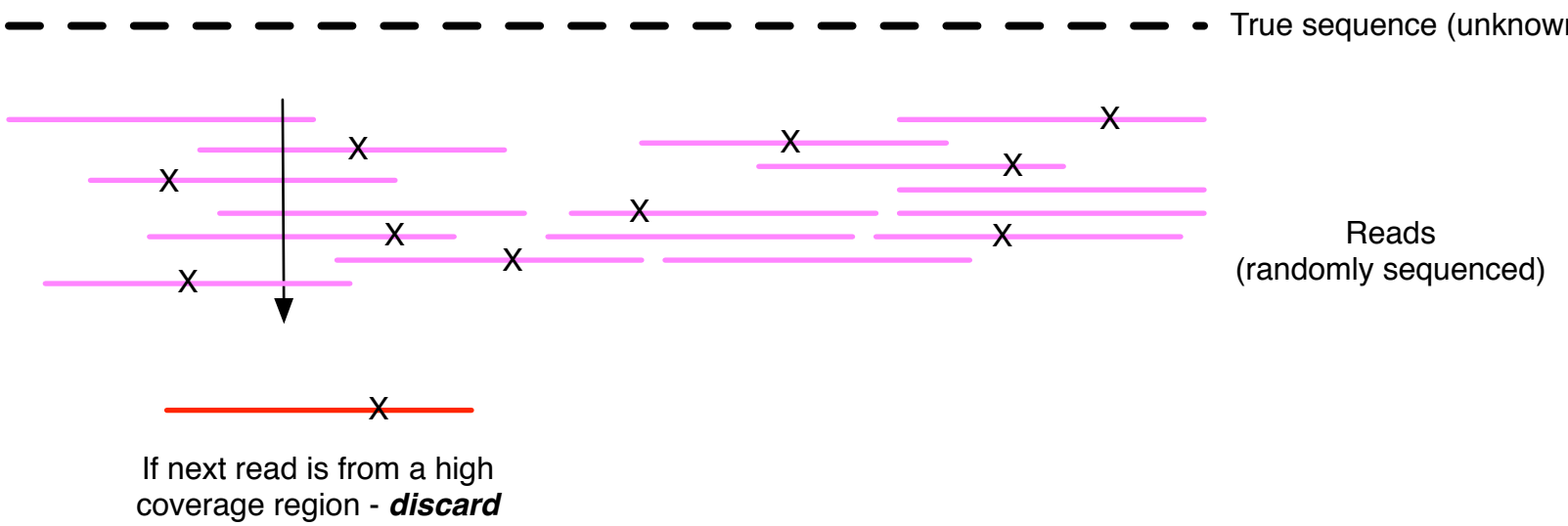
Digital normalization



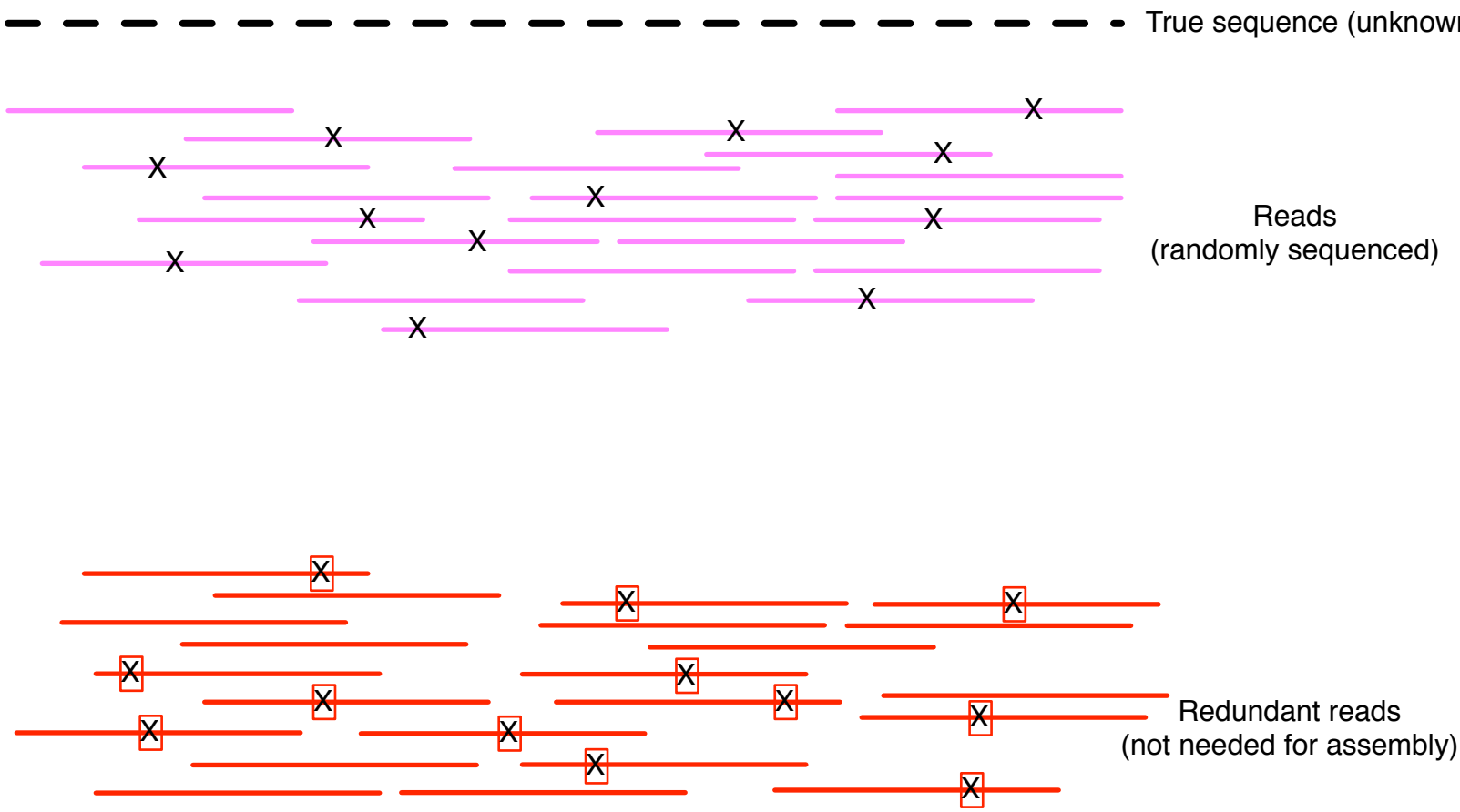
Digital normalization



Digital normalization



Digital normalization



Digital normalization approach

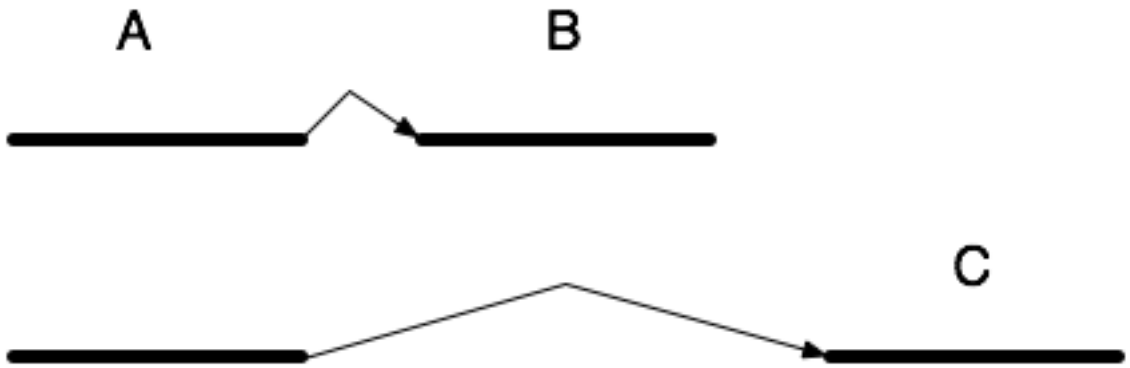
A *digital* analog to cDNA library normalization, diginorm:

- Is single pass: looks at each read only once;
- Does not "collect" the majority of errors;
- Keeps all low-coverage reads;
- **Smooths out coverage of regions.**

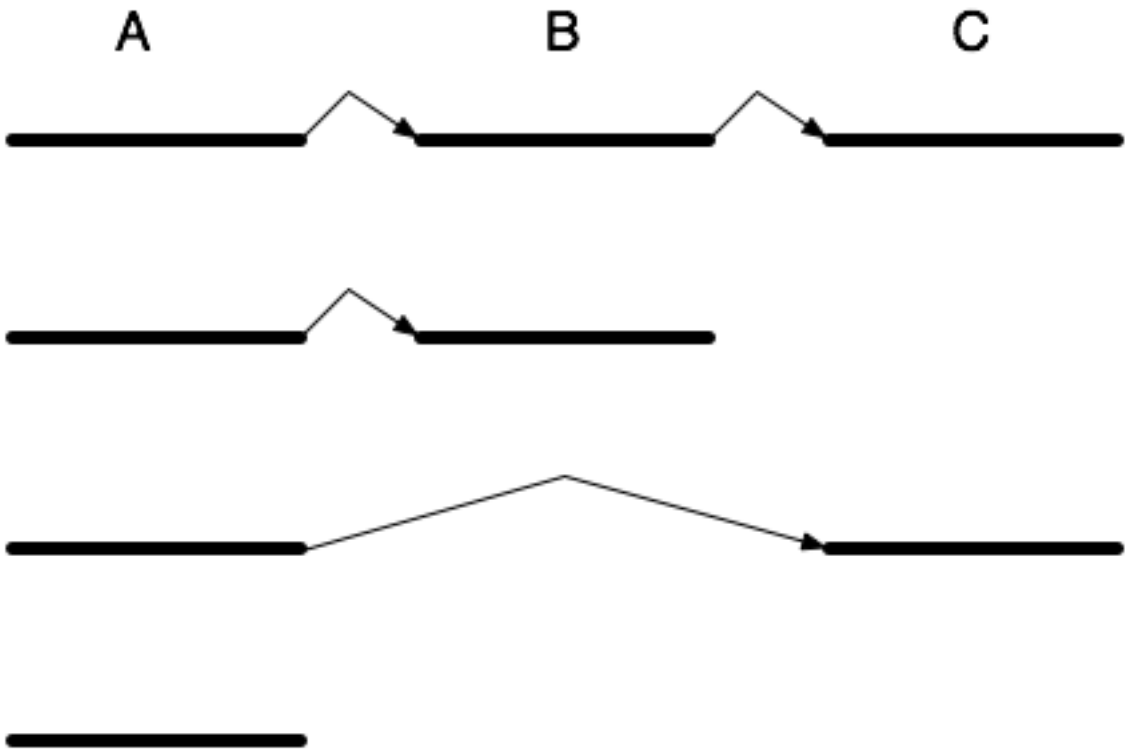
=> Enables analyses that are otherwise completely impossible.

Partitioning transcripts into families based on overlap

Isoform analysis – some easy...

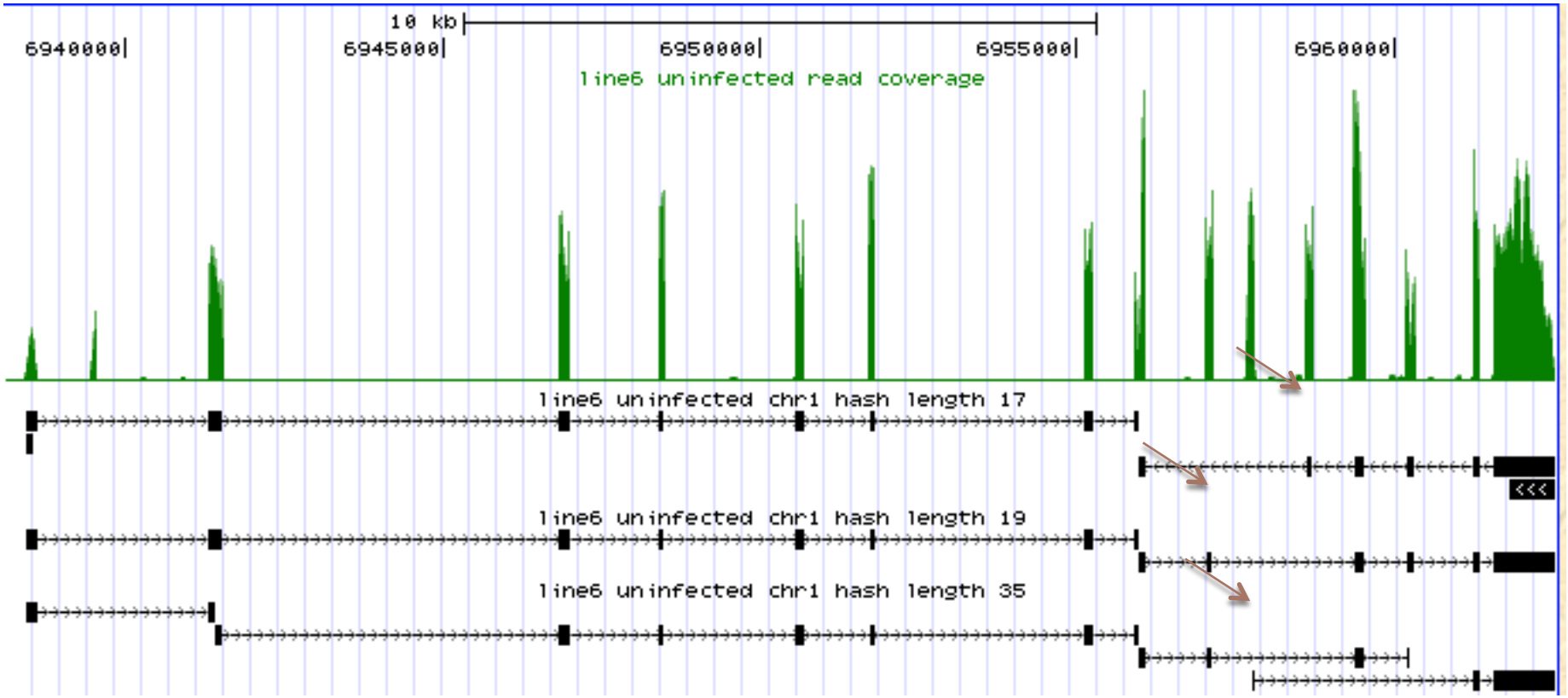


Isoform analysis – some hard

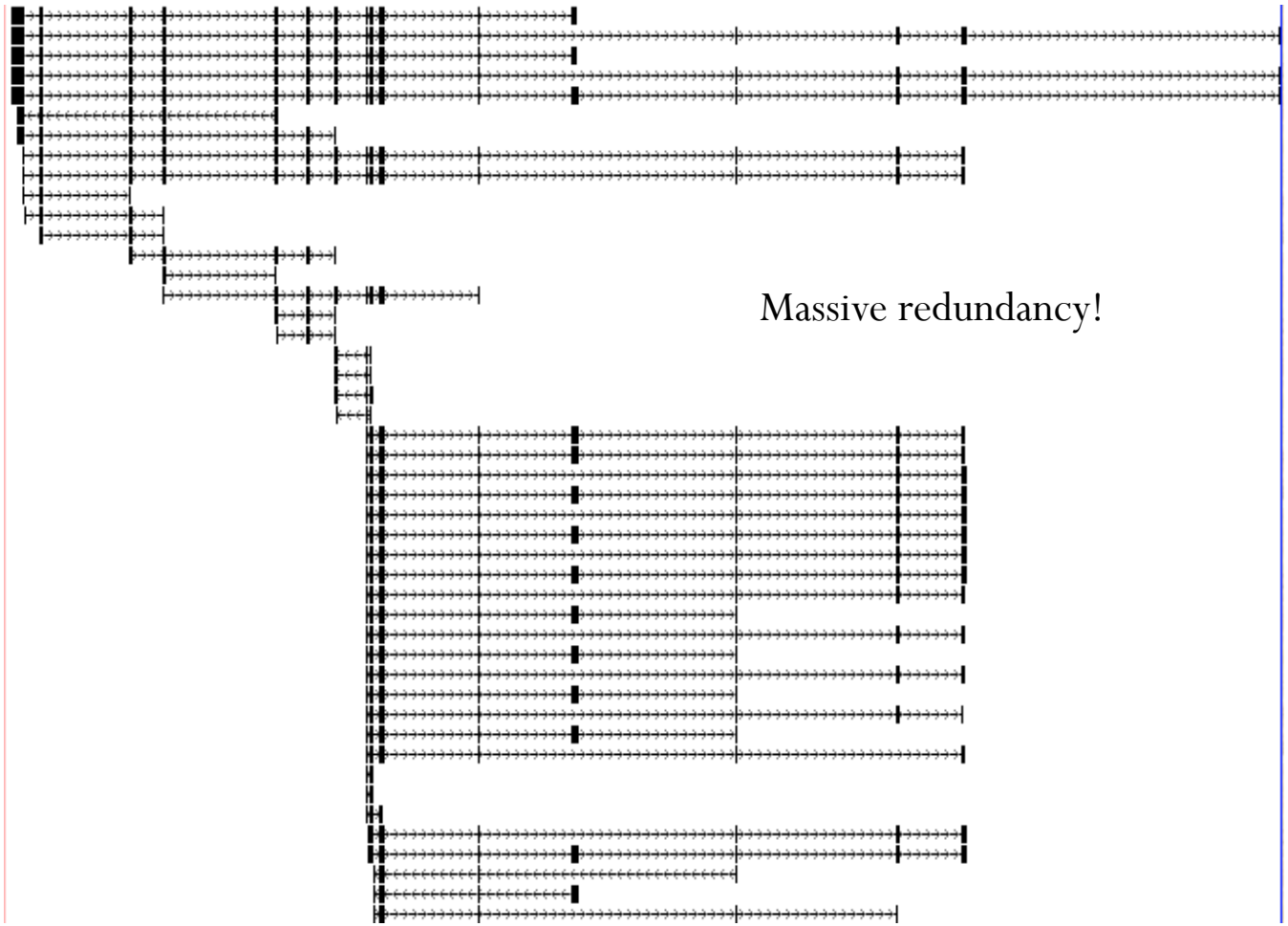


Counting methods mostly rely on presence of *unique sequence* to which to map.

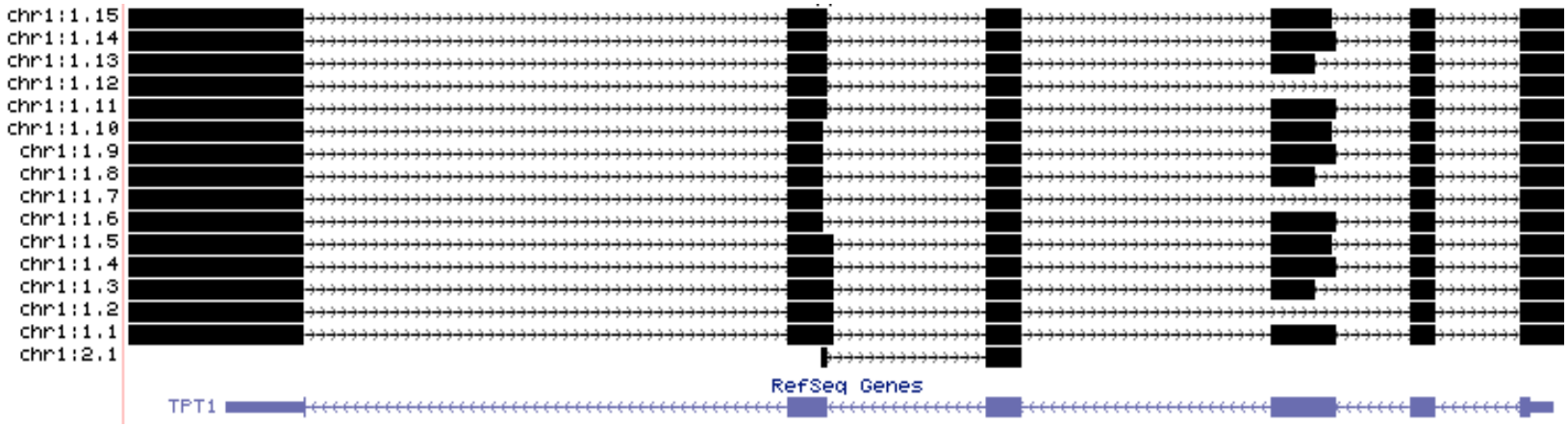
Exons are easy to locate, given genomic sequence



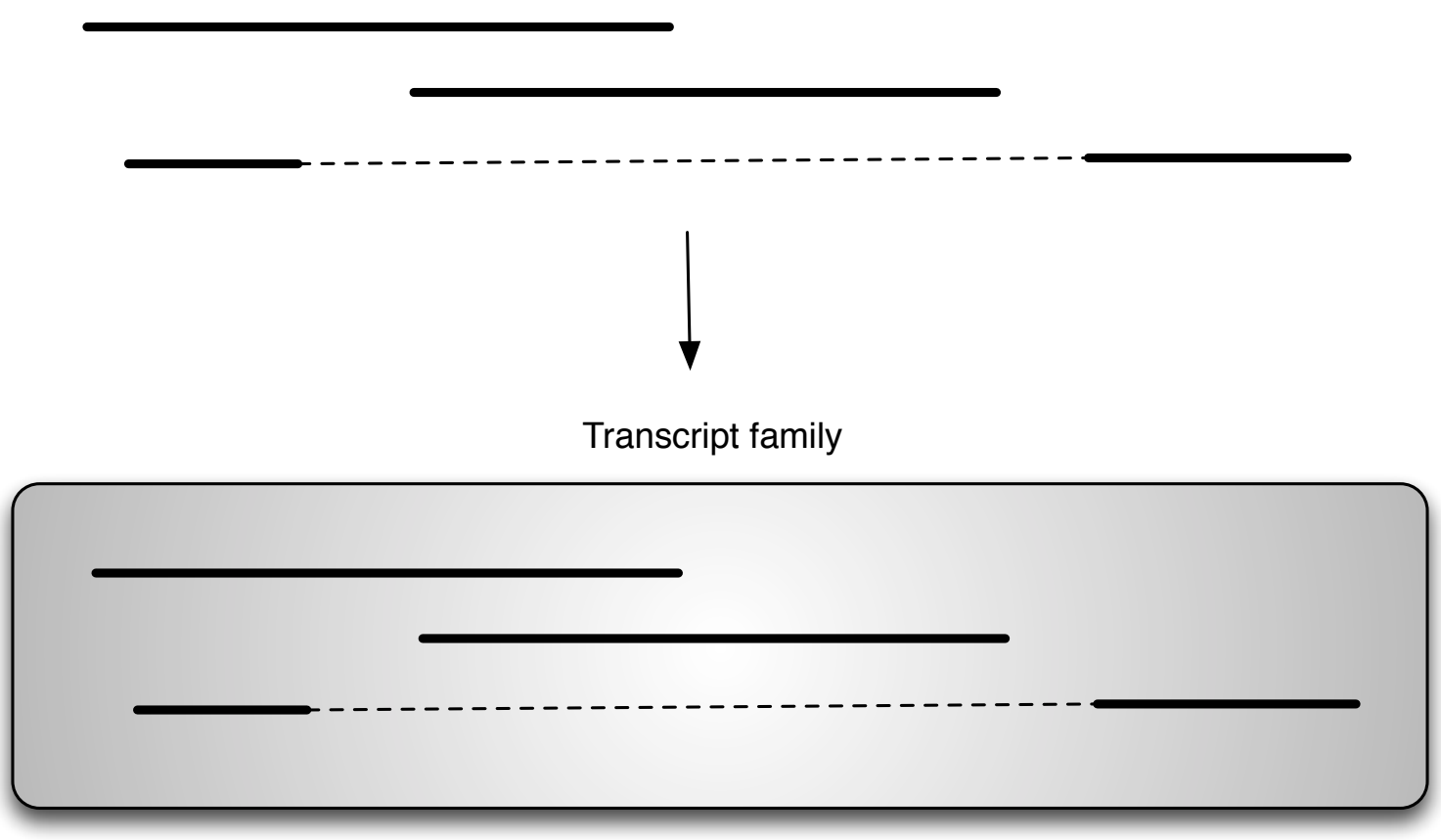
Genome-reference-free assembly leads to many isoforms.



Gene models can be “collapsed” given genomic sequence… But don’t always have.



Solution: Partitioning transcripts into "transcript families"



Pell et al., 2012, PNAS