Streaming and Compression Approaches for Terascale Biological Sequence Data Analysis

C. Titus Brown
Assistant Professor
CSE, MMG, BEACON
Michigan State University
September 2012
ctb@msu.edu

Outline

- Acknowledgements
- Big Data and next-gen sequence analysis
- Sweeping generalizations about physics and biology
- Physics ain't biology, and vice versa

Acknowledgements

Lab members involved

- **Adina Howe (w/Tiedje)**
- **Jason Pell**
- **Arend Hintze**
- **Rosangela Canino-Koning**
- **Qingpeng Zhang**
- Elijah Lowe
- Likit Preeyanon
- Jiarong Guo
- **Tim Brom**
- Kanchan Pavangadkar
- **Eric McDonald**

Collaborators

- Jim Tiedje, MSU
- Janet Jansson, LBNL
- Susannah Tringe, JGI

Funding

USDA NIFA; NSF IOS; BEACON.

We practice open science!

See blog post accompanying talk: 'titus brown blog'

Everything discussed here:

- Code: github.com/ged-lab/ ; BSD license
- Blog: http://ivory.idyll.org/blog
- Twitter: @ctitusbrown
- Grants on Lab Web site: http://ged.msu.edu/interests.html
- Preprints: on arXiv, q-bio:

  'diginorm arxiv'

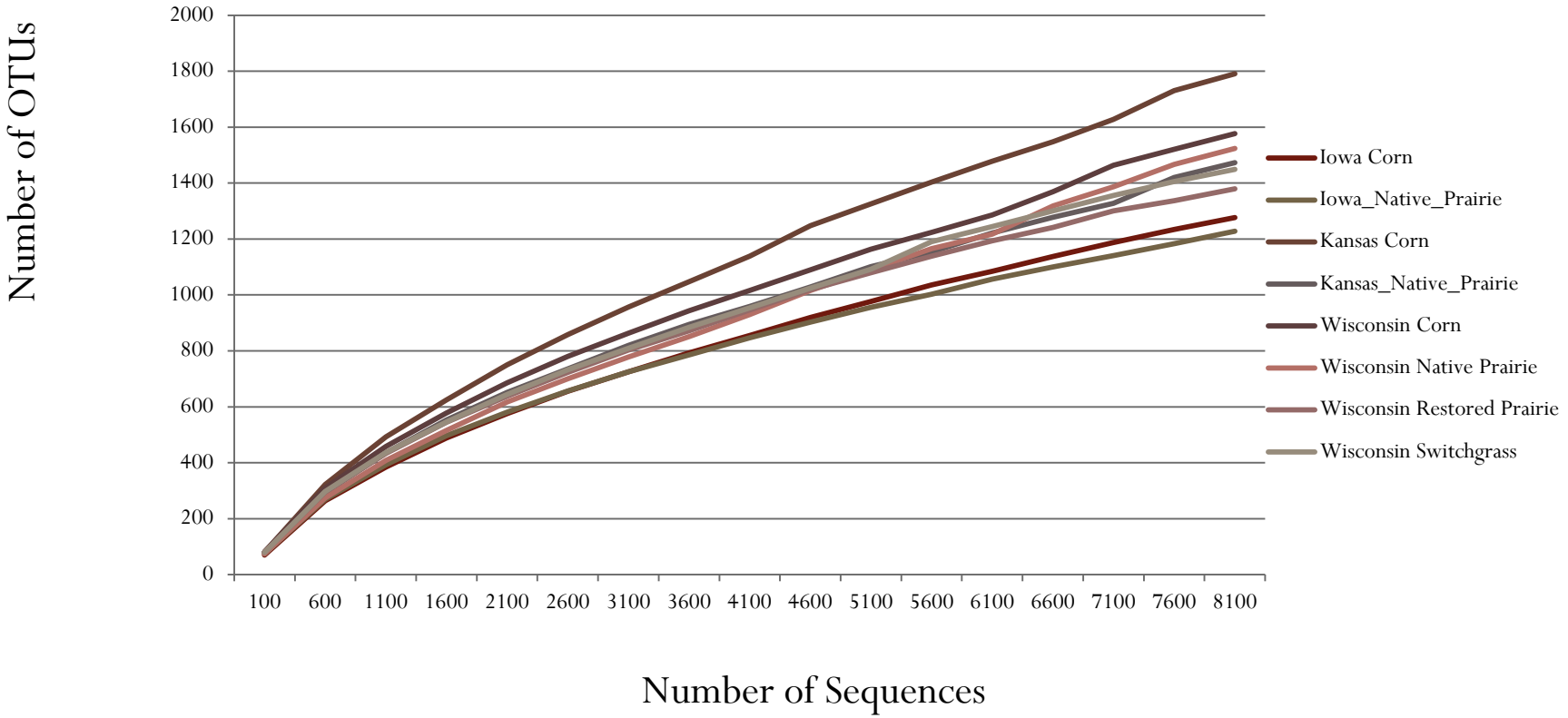
Soil is full of uncultured microbes

Randy Jackson

Soil contains thousands to millions of species

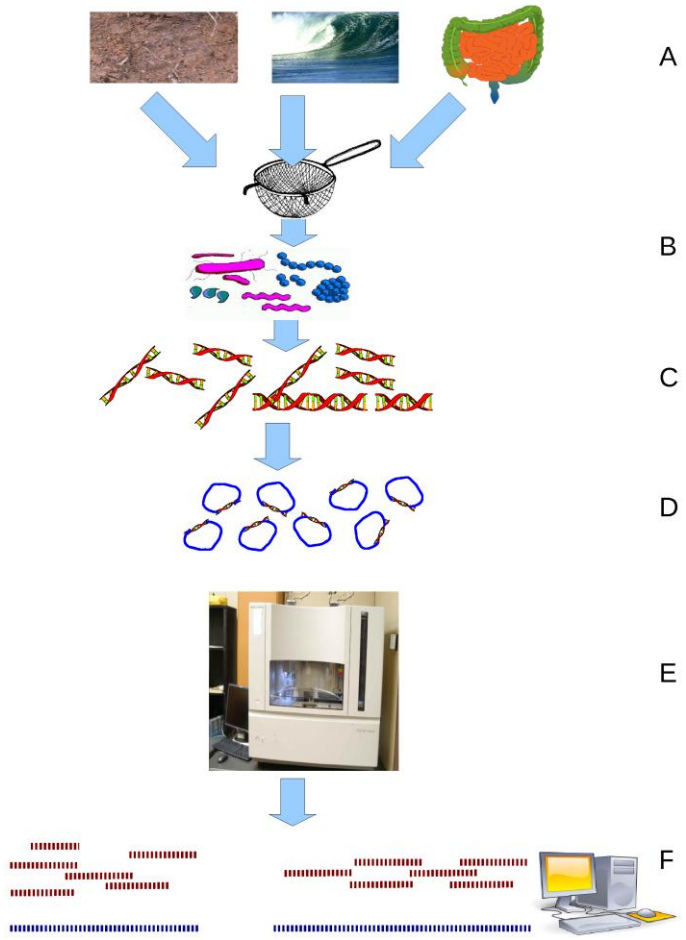
("Collector's curves" of ~species)

99% of microbes cannot easily be cultured in the lab.



Shotgun metagenomics

- Collect samples;
- Extract DNA;
- Feed into sequencer;
- Computationally analyze.



Wikipedia: Environmental shotgun sequencing.png

Task: assemble original text from random, error prone observations

It was the Gest of times, it was the wor

, it was the worst of timZs, it was the

isdom, it was the age of foolisXness

, it was the worVt of times, it was the

mes, it was Ahe age of wisdom, it was th

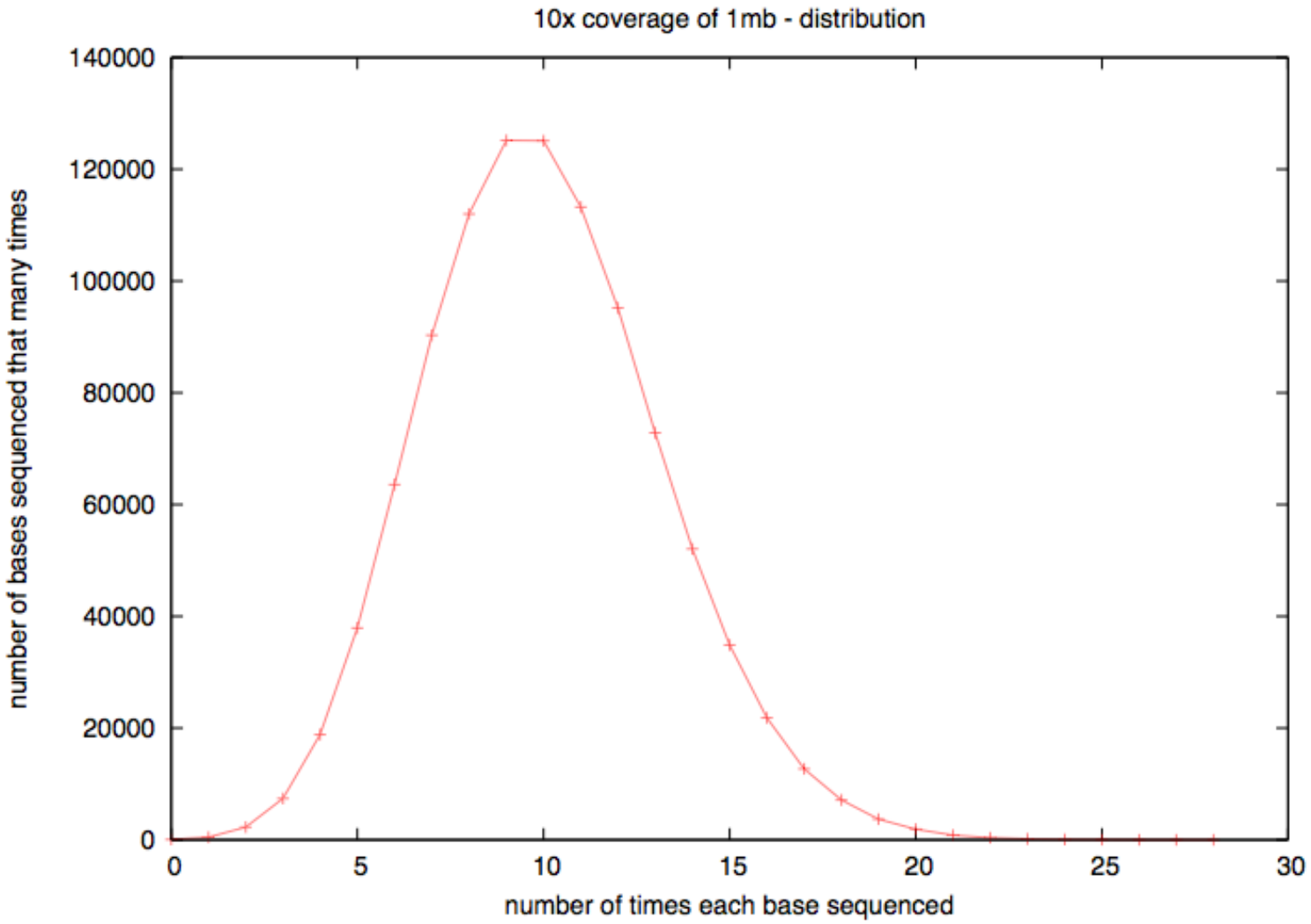
It was the best of times, it Gas the wor

mes, it was the age of witdom, it was th

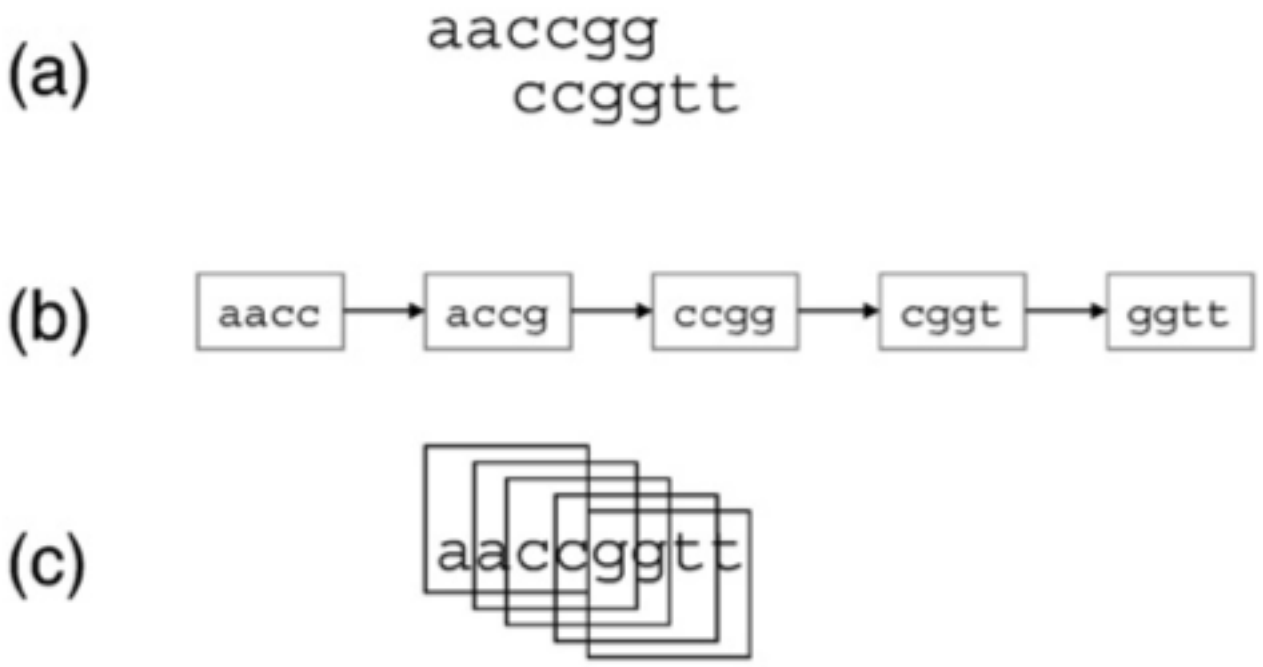
isdom, it was tIe age of foolishness

It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness

Actual coverage varies widely from the average.

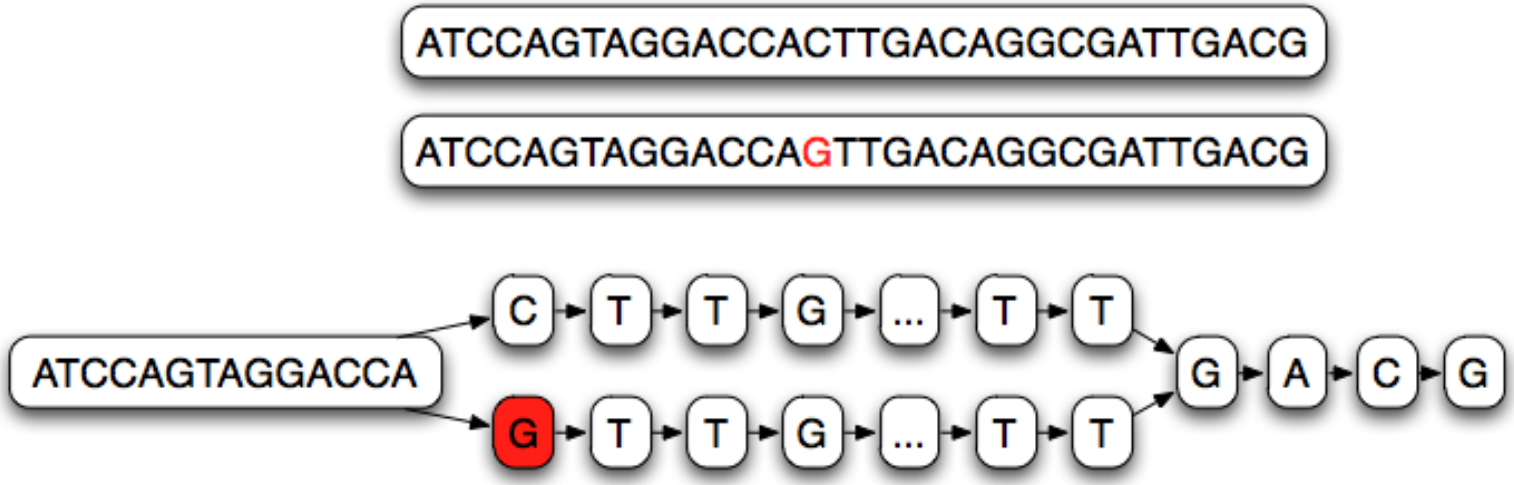


Assembly via de Bruijn graphs – k-mer overlaps



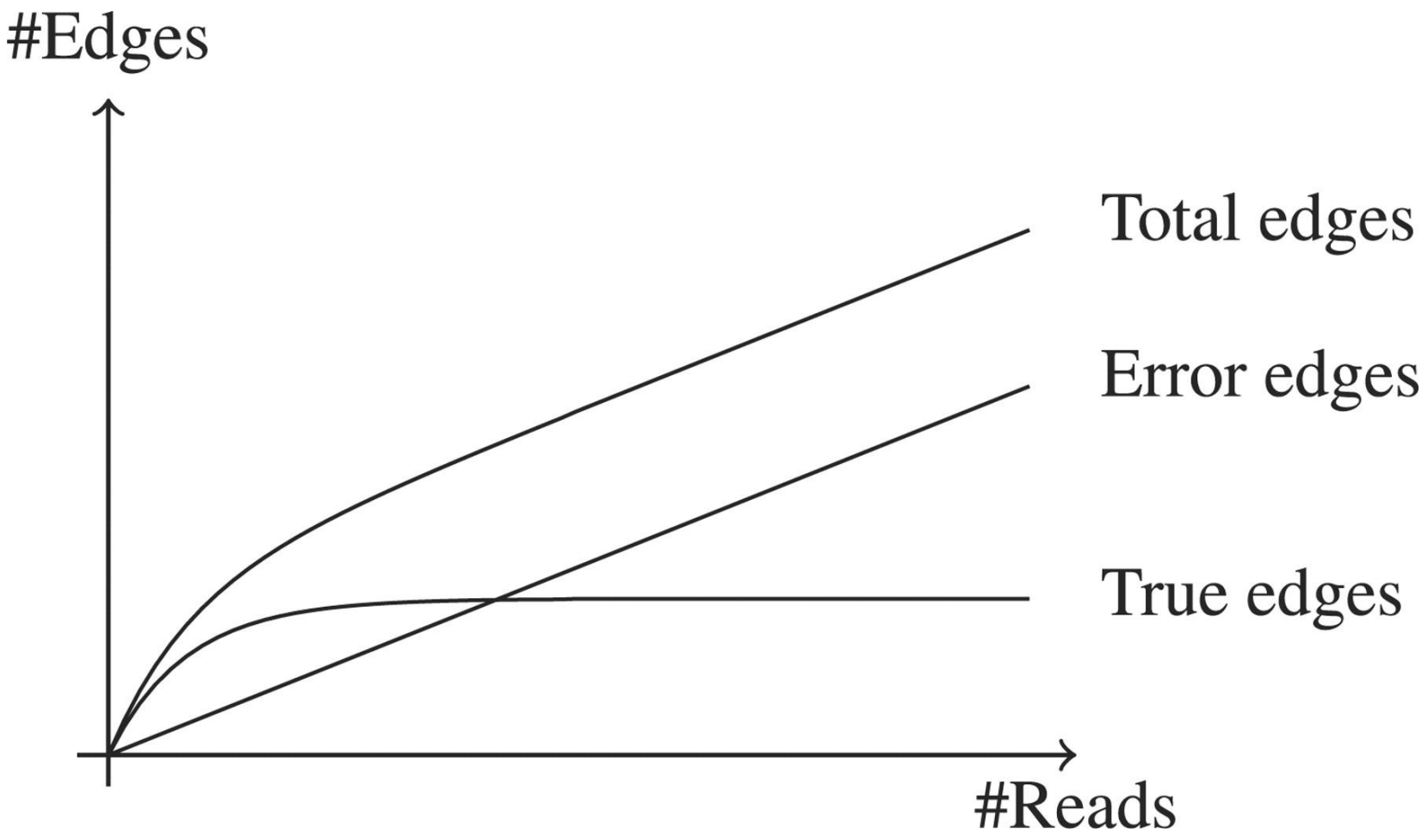
J.R. Miller et al. / Genomics (2010)

K-mer graph (k=14)



Single nucleotide variations cause long branches;
They don't rejoin quickly.

Reads vs edges (memory) in de Bruijn graphs



Conway T C , Bromage A J Bioinformatics 2011;27:479-486

The scale of the problem is *stunning*.

- I estimate a worldwide capacity for DNA sequencing of 15 petabases/yr (it's probably larger).
- Individual labs can generate ~100 Gbp in ~1 week for $10k.
- This sequencing is at a *boutique* level:
  - Sequencing formats are semi-standard.
  - Basic analysis approaches are ~80% cookbook.
  - Every biological prep, problem, and analysis is different.
- **Traditionally, biologists receive no training in computation.** (And computational people receive no training in biology :)
- …*and* our computational infrastructure is optimizing for high *performance* computing, not high *throughput*.

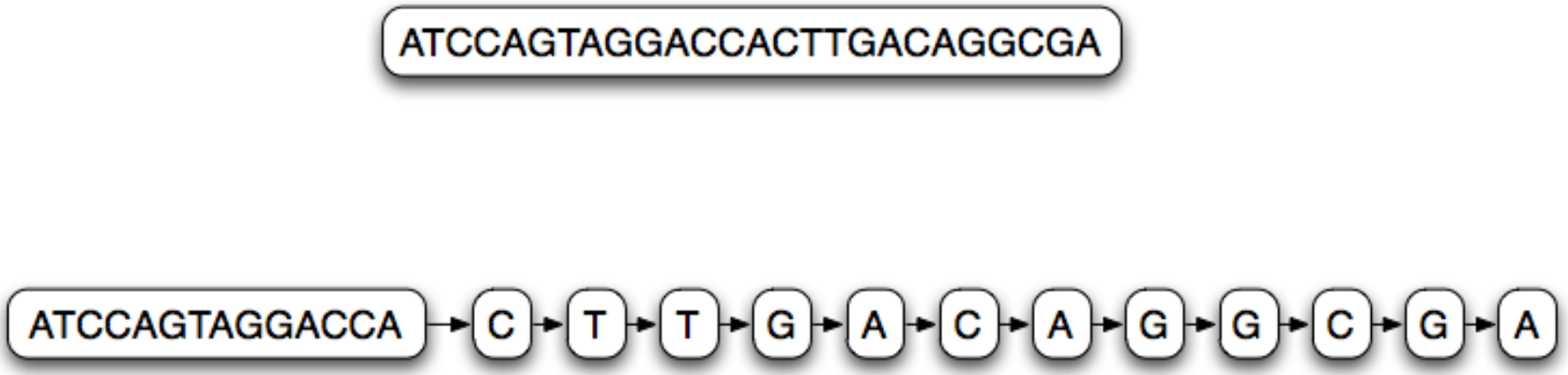
My problems are also very annoying...

- Est ~50 Tbp to comprehensively sample the microbial composition of a gram of soil.
- Currently we have approximately 2 Tbp spread across 9 soil samples, for one project; 1 Tbp across 10 samples for another.

- Need 3 TB RAM on single chassis to do assembly of 300 Gbp.
- …estimate 500 TB RAM for 50 Tbp of sequence.

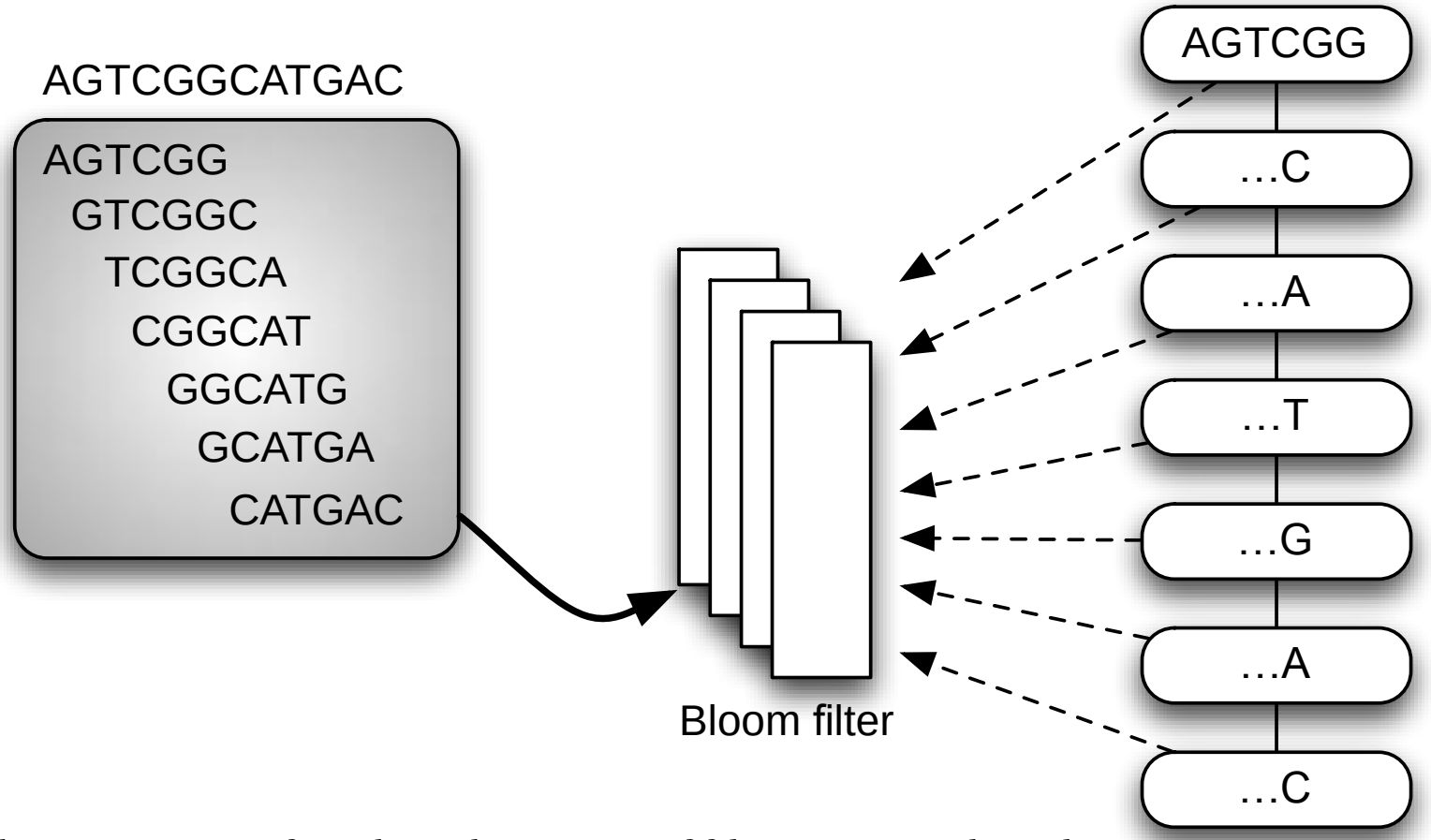
That just won't do.

1. Compressible de Bruijn graphs



Each node represents a 14-mer;
Links between each node are 13-mer overlaps

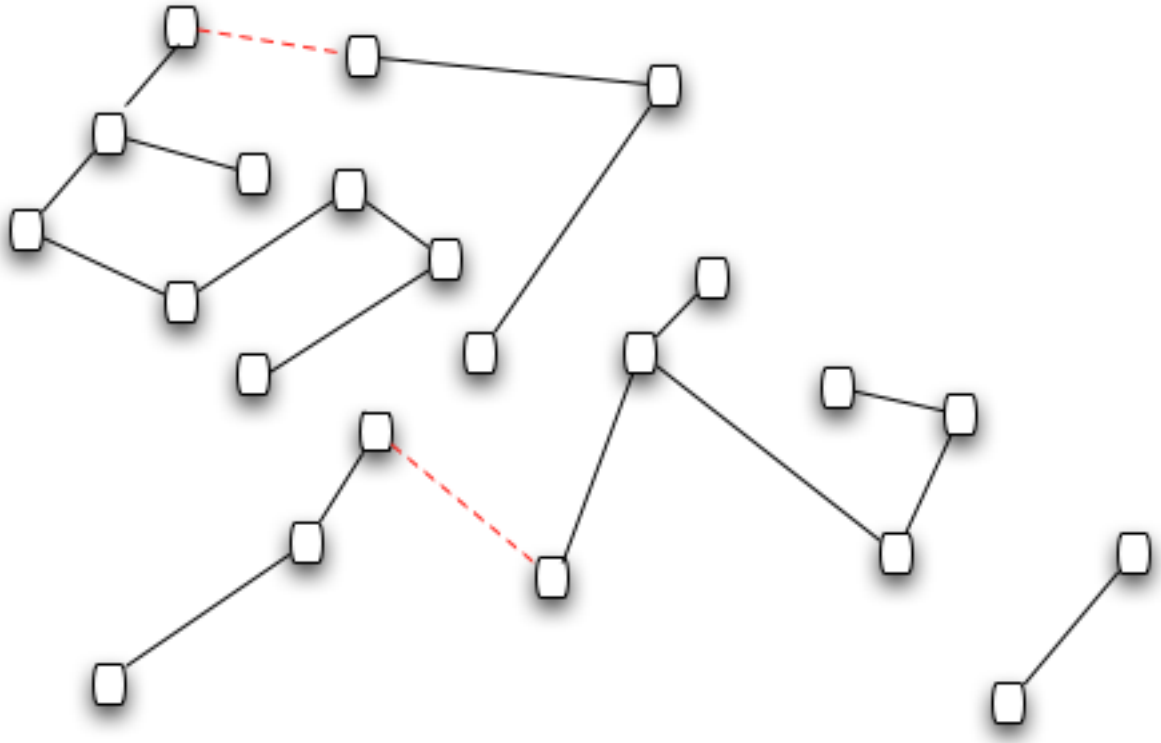
Can store *implicit* de Bruijn graphs in a Bloom filter



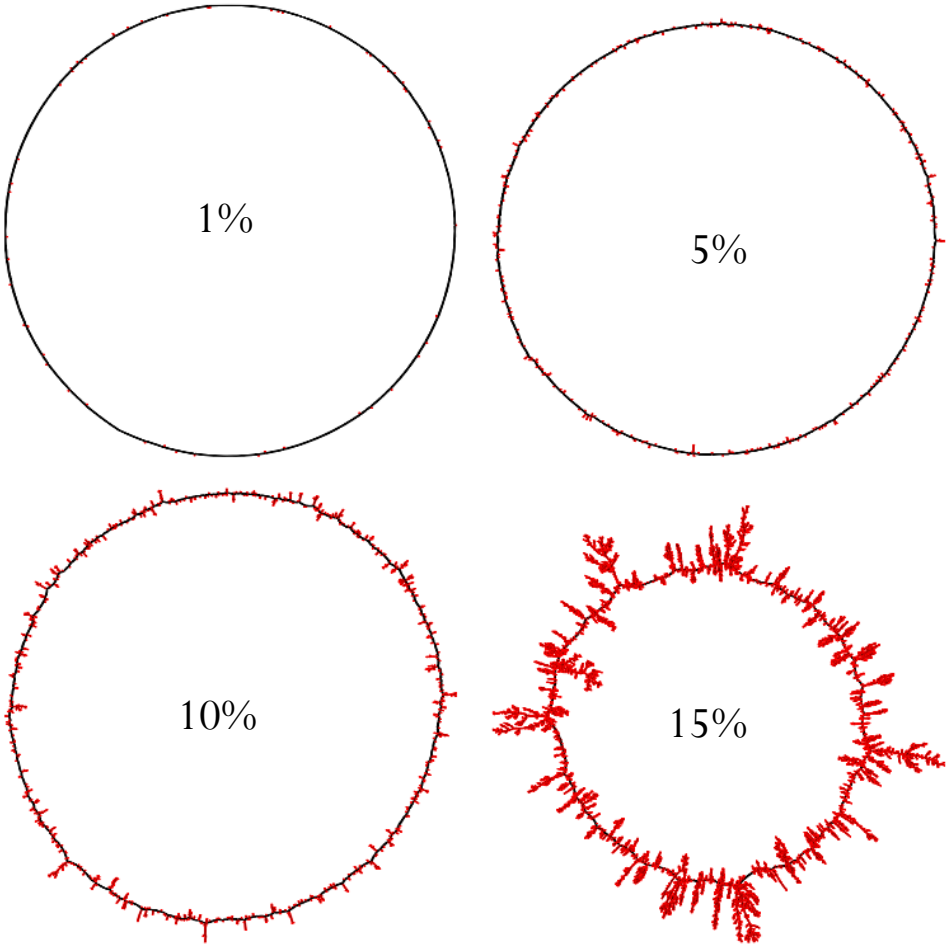
This allows *compression* of graphs at the expense of false positive nodes/edges.

False positives introduce false nodes/edges.

When does this start to distort the graph?

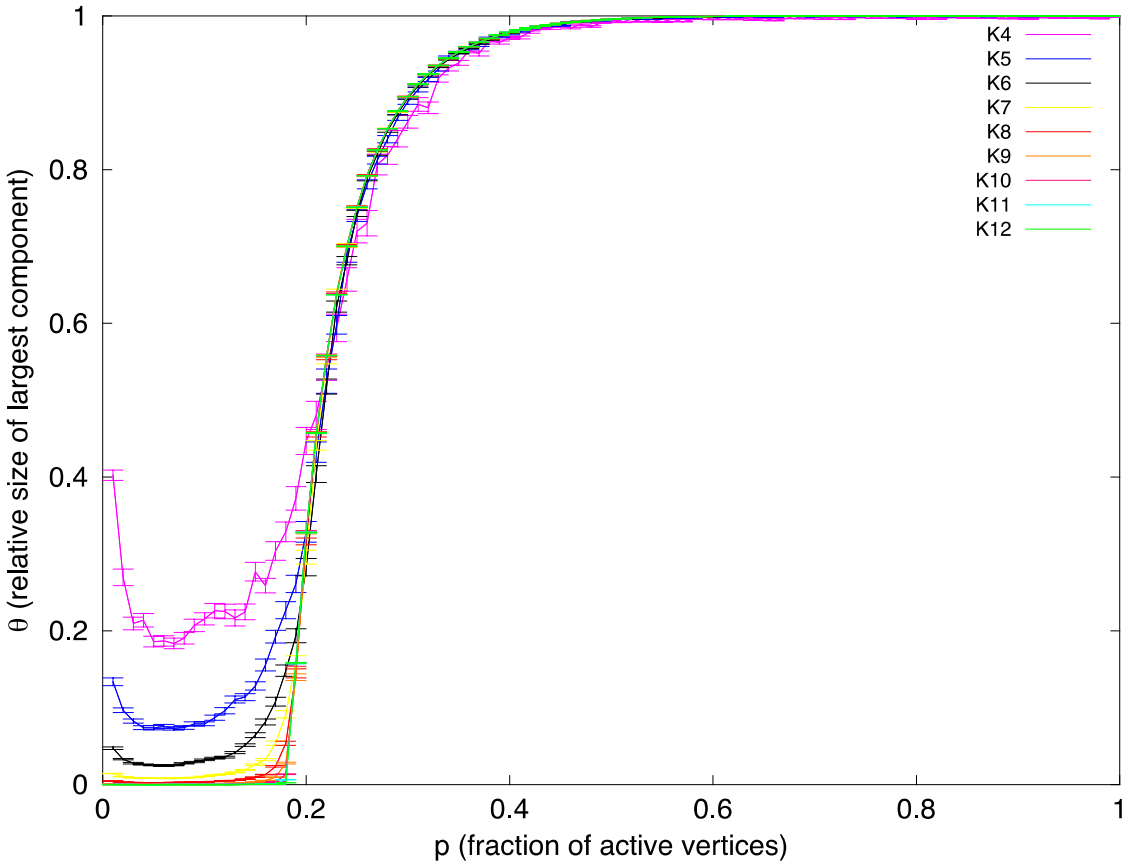


Global graph structure is retained past 18% FPR



Jason Pell & Arend Hintze

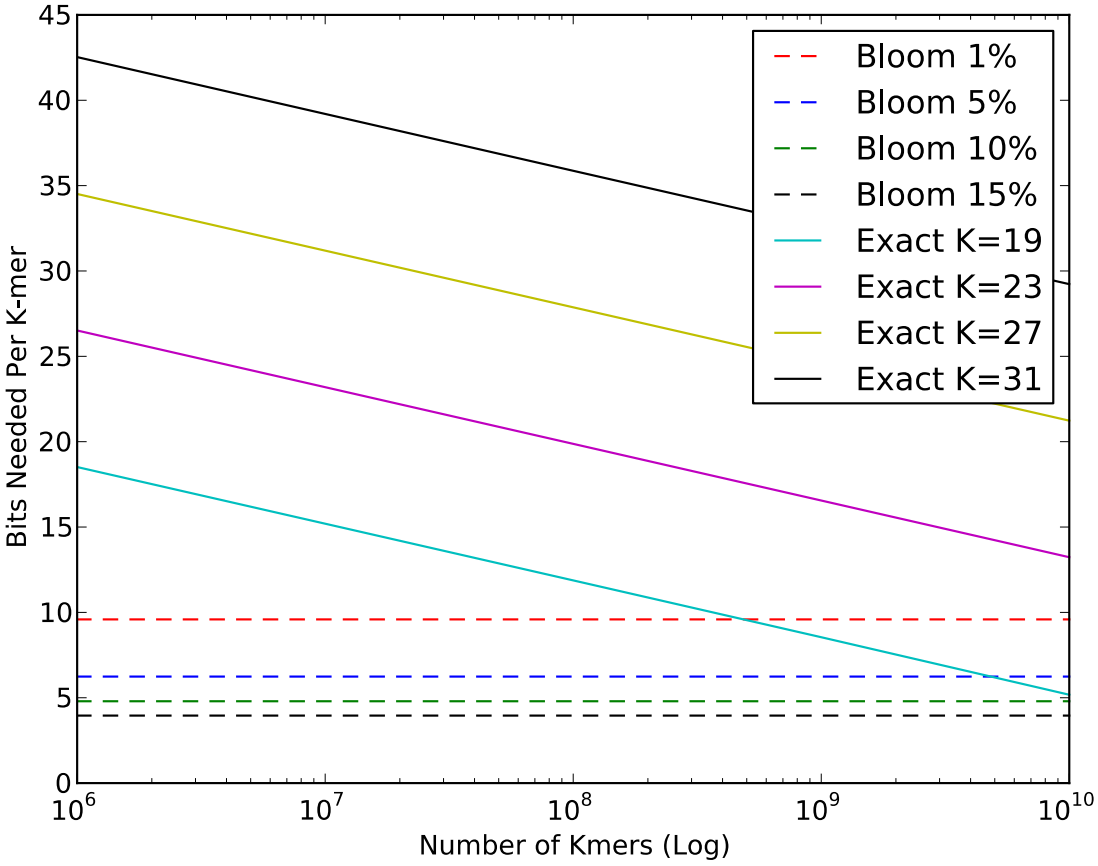
Equivalent to *bond percolation* problem; percolation threshold independent of k (?)



Jason Pell & Arend Hintze

This data structure is strikingly efficient for storing sparse k-mer graphs.

"Exact" is for best possible information-theoretical storage.

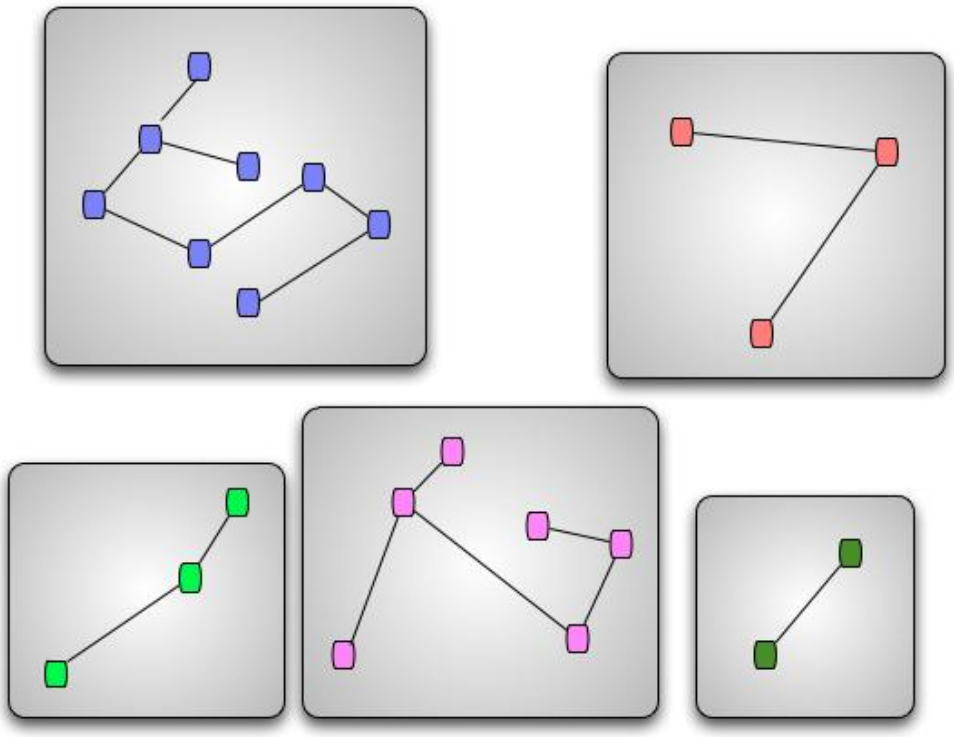


Jason Pell & Arend Hintze

We implemented *graph partitioning* on top of this probabilistic de Bruijn graph.

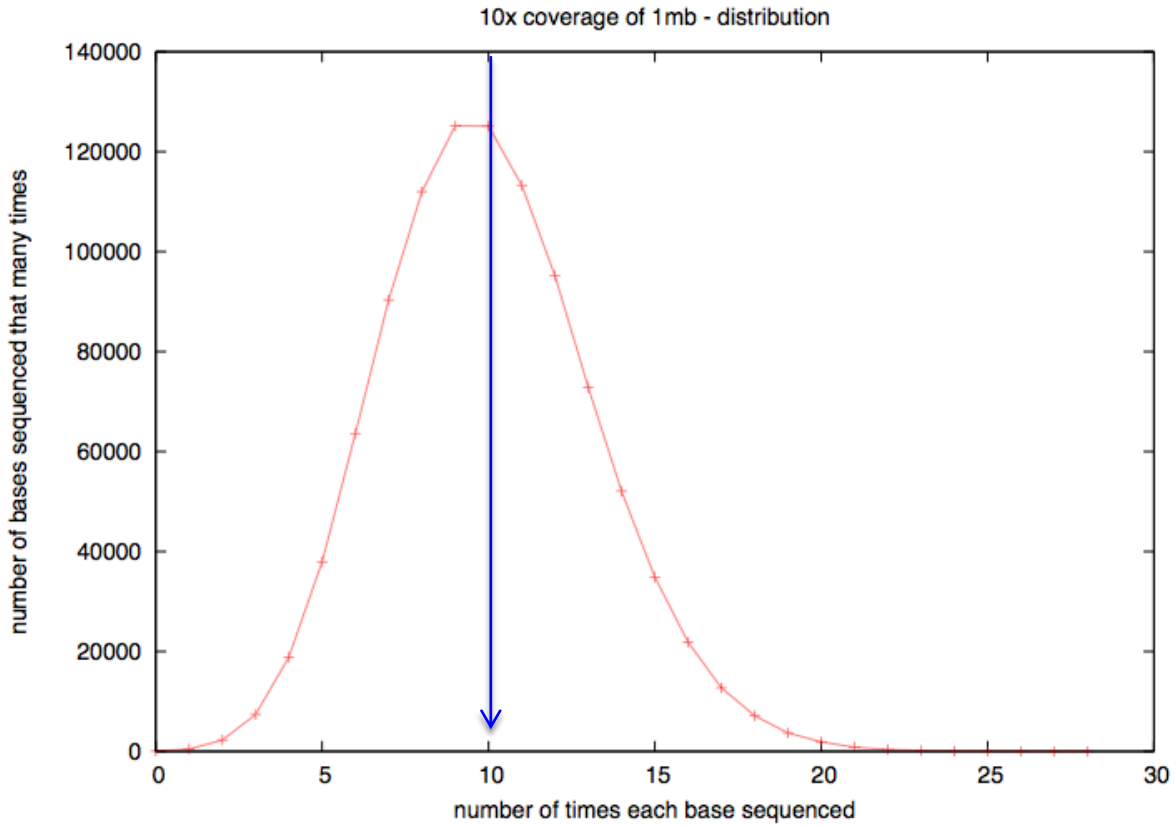
Split reads into "bins" belonging to different source species.

Can do this based almost entirely on connectivity of sequences.

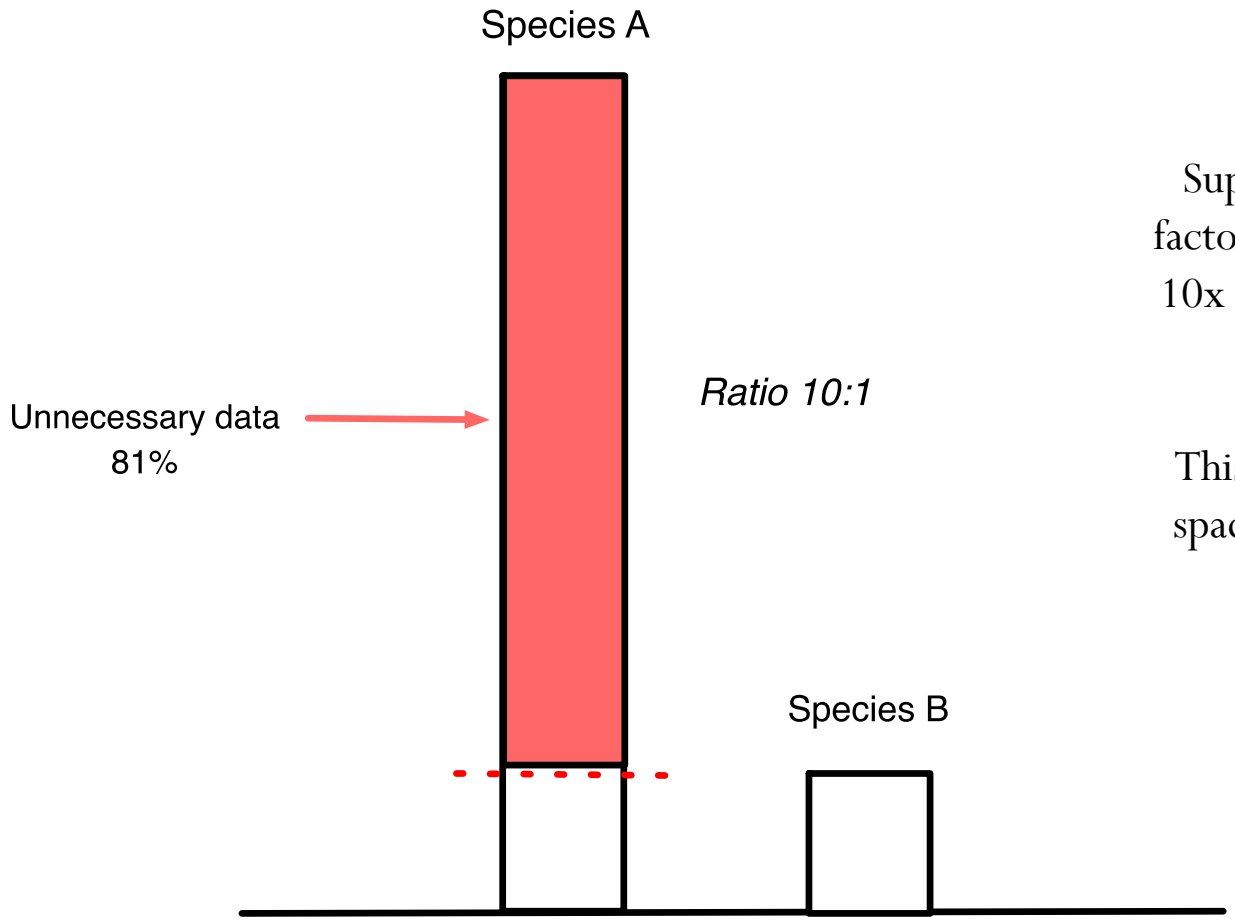


2. Online, streaming, lossy compression.

Much of next-gen sequencing is *redundant*.



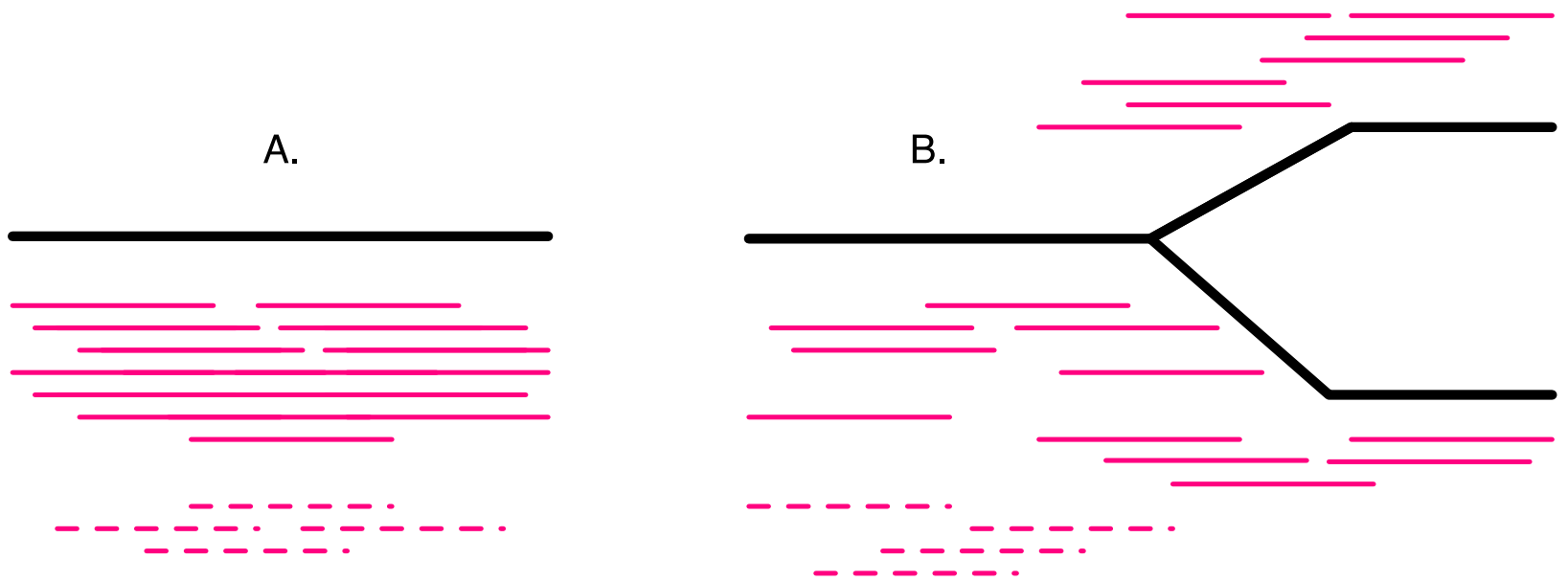
Uneven coverage => even more redundancy



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**.

Downsample based on de Bruijn graph structure; this can be derived via an *online* algorithm.



Digital normalization algorithm

```
for read in dataset:
   if estimated_coverage(read) < CUTOFF:
         update_kmer_counts(read)
         save(read)
   else:
         # discard read
```

Note, single pass; fixed memory.

Digital normalization retains information, while discarding data and errors

Table 1. Digital normalization to C=20 removes many erroneous k-mers from sequencing data sets. Numbers in parentheses indicate number of true k-mers lost at each step, based on reference.

Data set	True 20-mers	20-mers in reads	20-mers at C=20	% reads kept
Simulated genome	399,981	8,162,813	3,052,007 (-2)	19%
Simulated mRNAseq	48,100	2,466,638 (-88)	1,087,916 (-9)	4.1%
E. coli genome	4,542,150	175,627,381 (-152)	90,844,428 (-5)	11%
Yeast mRNAseq	10,631,882	224,847,659 (-683)	10,625,416 (-6,469)	9.3%
Mouse mRNAseq	43,830,642	709,662,624 (-23,196)	43,820,319 (-13,400)	26.4%

Table 2. Three-pass digital normalization removes most erroneous k-mers. Numbers in parentheses indicate number of true k-mers lost at each step, based on known reference.

Data set	True 20-mers	20-mers in reads	20-mers remaining	% reads kept
Simulated genome	399,981	8,162,813	453,588 (-4)	5%
Simulated mRNAseq	48,100	2,466,638 (-88)	182,855 (-351)	1.2%
E. coli genome	4,542,150	175,627,381 (-152)	7,638,175 (-23)	2.1%
Yeast mRNAseq	10,631,882	224,847,659 (-683)	10,532,451 (-99,436)	2.1%
Mouse mRNAseq	43,830,642	709,662,624 (-23,196)	42,350,127 (-1,488,380)	7.1%

For soil… what do we assemble?

Total Assembly	Total Contigs	% Reads Assembled	Predicted protein coding	*rplb* genes
2.5 bill	4.5 mill	19%	5.3 mill	391
3.5 bill	5.9 mill	22%	6.8 mill	466

This estimates number of species ^

Putting it in perspective:
Total equivalent of ~1200 bacterial genomes
Human genome ~3 billion bp

Adina Howe

Concluding thoughts

- Our approaches provide significant and substantial *practical* and *theoretical* leverage to one of the most challenging current problems in computational biology: assembly.
- They provide a path to the future:
  - Many-core compatible; distributable?
  - Decreased memory footprint => cloud computing can be used for many analyses.
  - At an algorithmic level, provide a noise-filtering solution for most of the current sequencing Big Data problems.
- They are *in use*, ~dozens of labs using digital normalization.
- …although we're still in the process of publishing them.

Physics ain't biology

The following observations are **for discussion**… they are not-so-casual observations from a lifetime of interacting with physicists.

(Apologies in advance for the sweeping generalizations.)

Important note: I don't hate physicists!

Significant life events involving physicists:

Birth – Gerry Brown

First UNIX account – Mark Galassi

First publication – w/Chris Adami

Grad school plans – Hans Bethe et al.

Earthshine research (~8 pubs) – w/Steve Koonin and Phil Goode

2nd Favorite publication – w/Curtis Callan, Jr.

I am very physicist-positive!

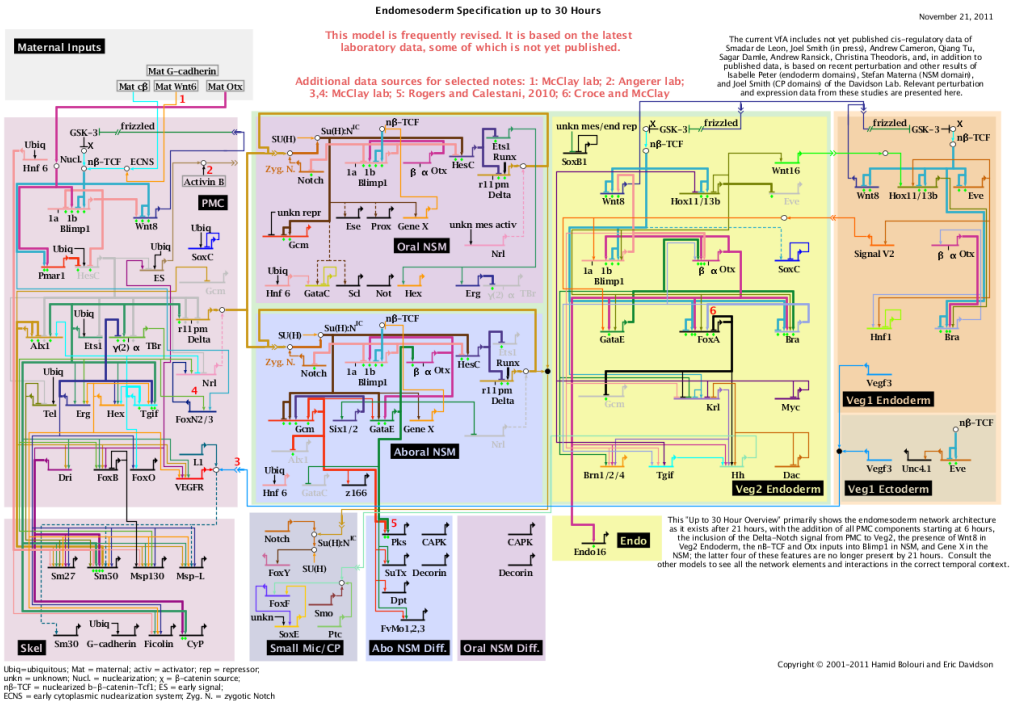
1. Models play a very different role.

- Physics models are often *predictive* and *constraining*.
  - Model specifies dynamics or interaction.
  - Make specific measurements to obtain initial conditions.
  - Model can then be used to predict fine-grained outcomes.

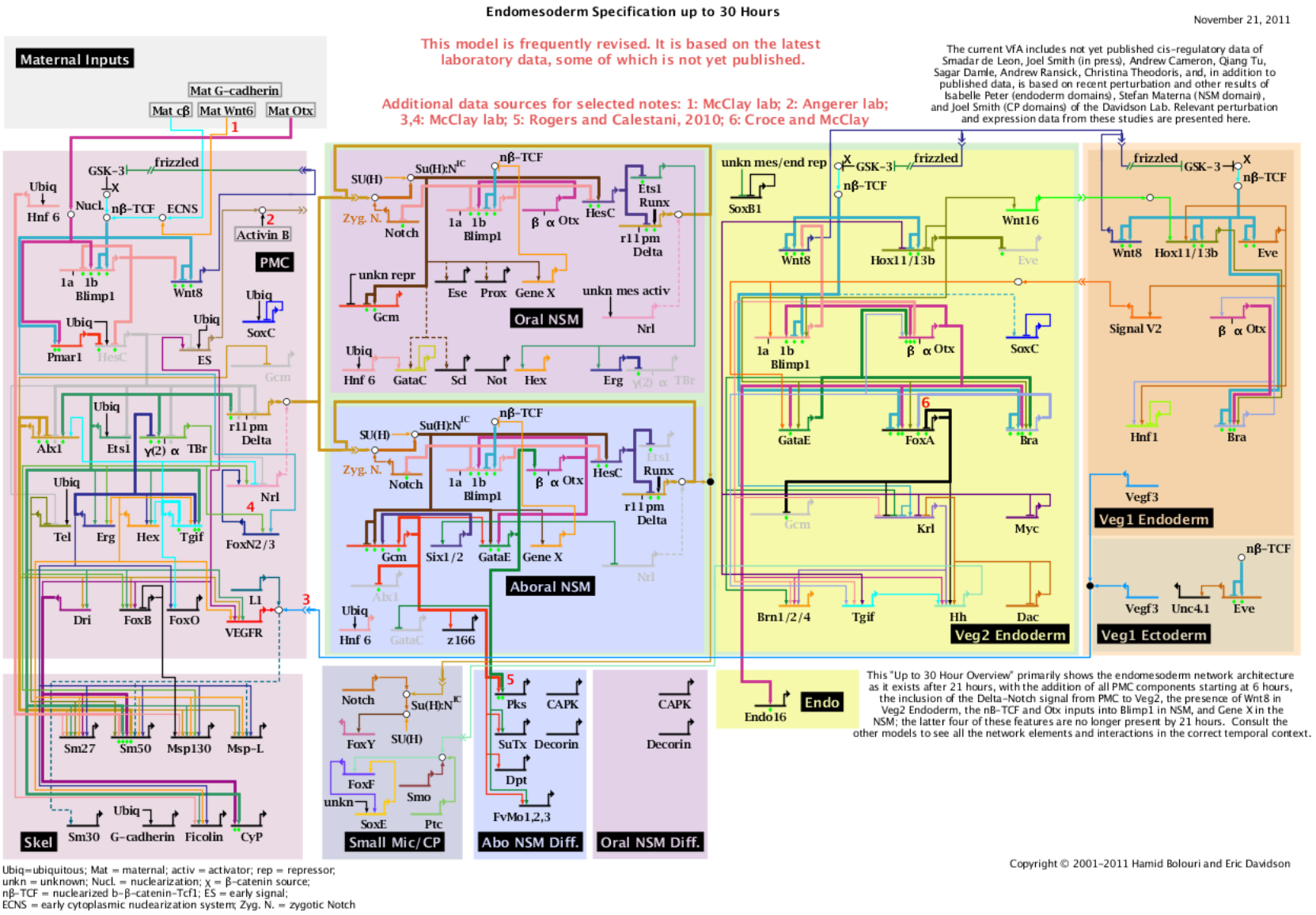
- Biology models can rarely be built in the first place…
  - Models are dominated by unknowns.
  - In a few cases, can be used to determine *sufficiency* of knowledge ("the observations can be explained by our model"); this does not mean the model is correct, merely that it *could* be.
  - Models are rarely *predictive* of specific observations.

Endomesoderm network

Approximately 15 years and probably 200 man-years of research to assemble a map of gene interactions for the first 30 hours of sea urchin development.



http://sugp.caltech.edu/endomes/



http://sugp.caltech.edu/endomes/

2. Little or no tradition of computation in biology

- Until ~last decade, not too much in the way of big data.
- Models are rarely built for the purpose of understanding computational data, although that is changing.
- Ecological and evolutionary models are regarded with suspicion: guilty until proven innocent.
- Essential zero computational training at UG/G (although some math).

- "Sick" culture of computation in biology:
  - Development of computational methods not respected as independent scientific endeavor in biology.
  - Biologists want push-button software that "just works".
  - Sophisticated evaluation/validation of software by users is rare.

(It is hard for me to explain to biologists how big a problem this is.)

3. Biology is built on *facts*, not *theory*.

- Experience with Callan:
  - Constrained optimization of DNA binding model to 48 known CRP binding sites => inability to eliminate 300-3000 extra sites in *E. coli* genome.
  - Ohmigod their binding signature is preserved by evolution => they're probably real! How can this be!?
  - …well, it turns out we don't know that much about *E. coli*.

- A nice damning quote from Mark Galassi:

"Biology and bioinformatics seem interesting. Is there any way I can take part in the research without learning all the details?"

NO. **Biology is all about the details! The more the better!**

My career path

- Undergrad in Math (Reed)
  - Research on evolution model (Avida) ~1992
  - Earthshine observations of global albedo ~1994
- PhD in Molecular Developmental Biology (Caltech)
  - Molecular biology, genomics, gene regulation ~1997-2008
  - Bioinformatics ~2000-
- Faculty position in CSE and Microbiology (MSU), 2008
  - Molecular developmental biology
  - Bioinformatics
  - Metagenomics & next-gen sequence analysis more generally
  - Moving towards integration of data + modeling…

My career path

- Undergrad in Math (Reed)
  - Research on evolution model (Avida) ~1992
  - Earthshine observations of global albedo ~1994
- PhD in Molecular Developmental Biology (Caltech)
  - **Molecular biology, genomics, gene regulation ~1997-2008**
  - Bioinformatics ~2000-
- Faculty position in CSE and Microbiology (MSU), 2008
  - Molecular developmental biology
  - Bioinformatics
  - Metagenomics & next-gen sequence analysis more generally
  - Moving towards integration of data + modeling…

Concluding thoughts on this stuff

- Biologists simply don't trust models and data analysis approaches that come from "outside" biology.
- They're not necessarily wrong!
- Physicists can bring an important *skill set* and *attitude* to biological research, but their *knowledge* is useless. They have to meet the biology *more* than halfway.
- Biologists need more cross-training so we don't retrace the same software development, data analysis, and modeling mistakes that physics et al. has spent 30 years figuring out.

But if you disagree with any of this, I'm happy to chat.