Metagenome assembly… in the cloud!!!

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Why assembly?

1. Significantly increases signature for homology searches (BLAST, HMMER).

2. Can dramatically reduce data set size.

3. Not dependent on nearby reference.

4. Long-range correlations (operons, etc.)

   (Eventually, whole genomes from metagenome WGS?)

Why *not* assembly?

1. Fairly strict coverage cutoff (below ~2-5x little assembles)

2. Unknown effect of strain variation on sensitivity of assemblies.

Apart from that, **with our tools**, we get sensitive recovery of spiked-in genomes and highly specific contigs.

The practical barrier - memory

For even relatively small data sets, metagenomic assemblers scale poorly.

Memory usage ~ number of errors

Number of errors ~ size of data set

Size of data set == big!!

This is the problem that we have (mostly) solved.

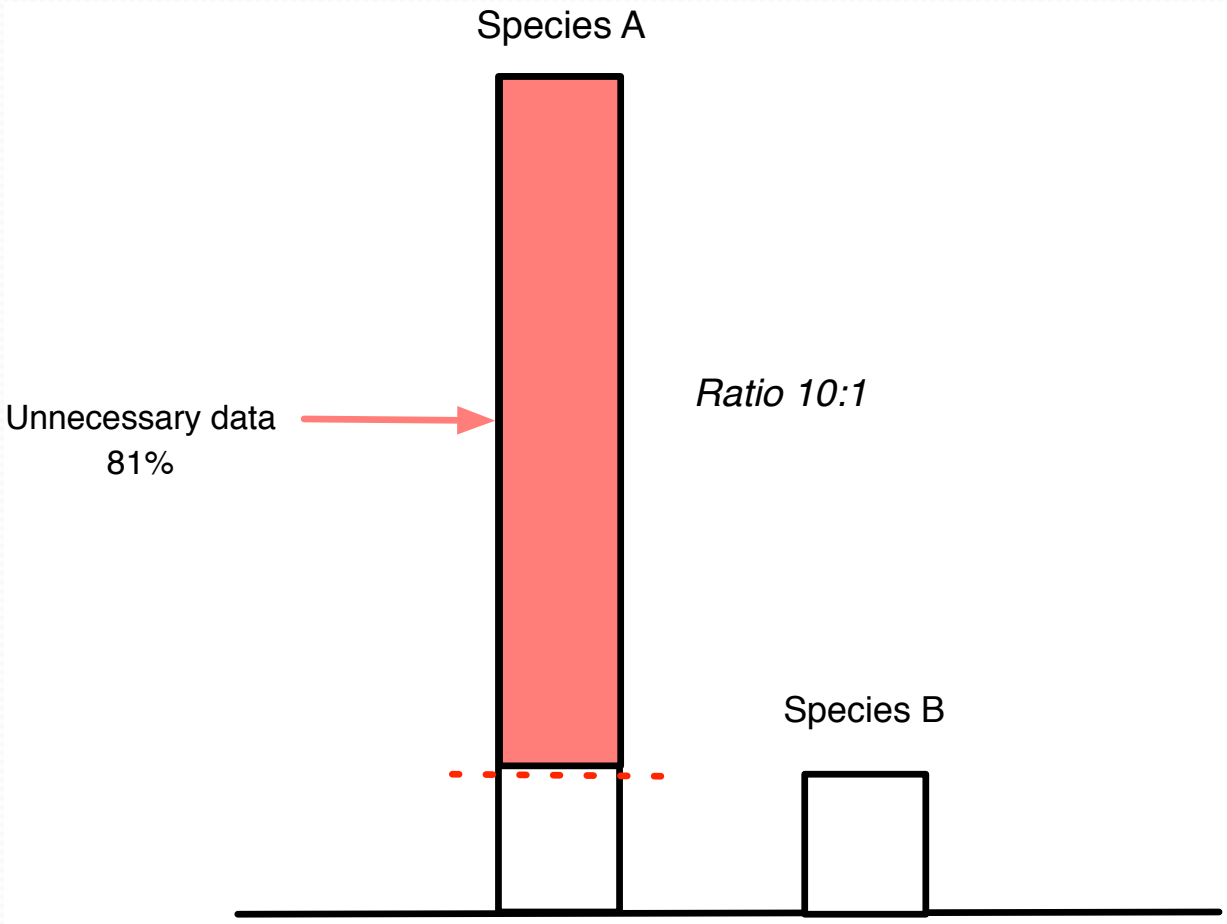
Two basic techniques

Digital normalization

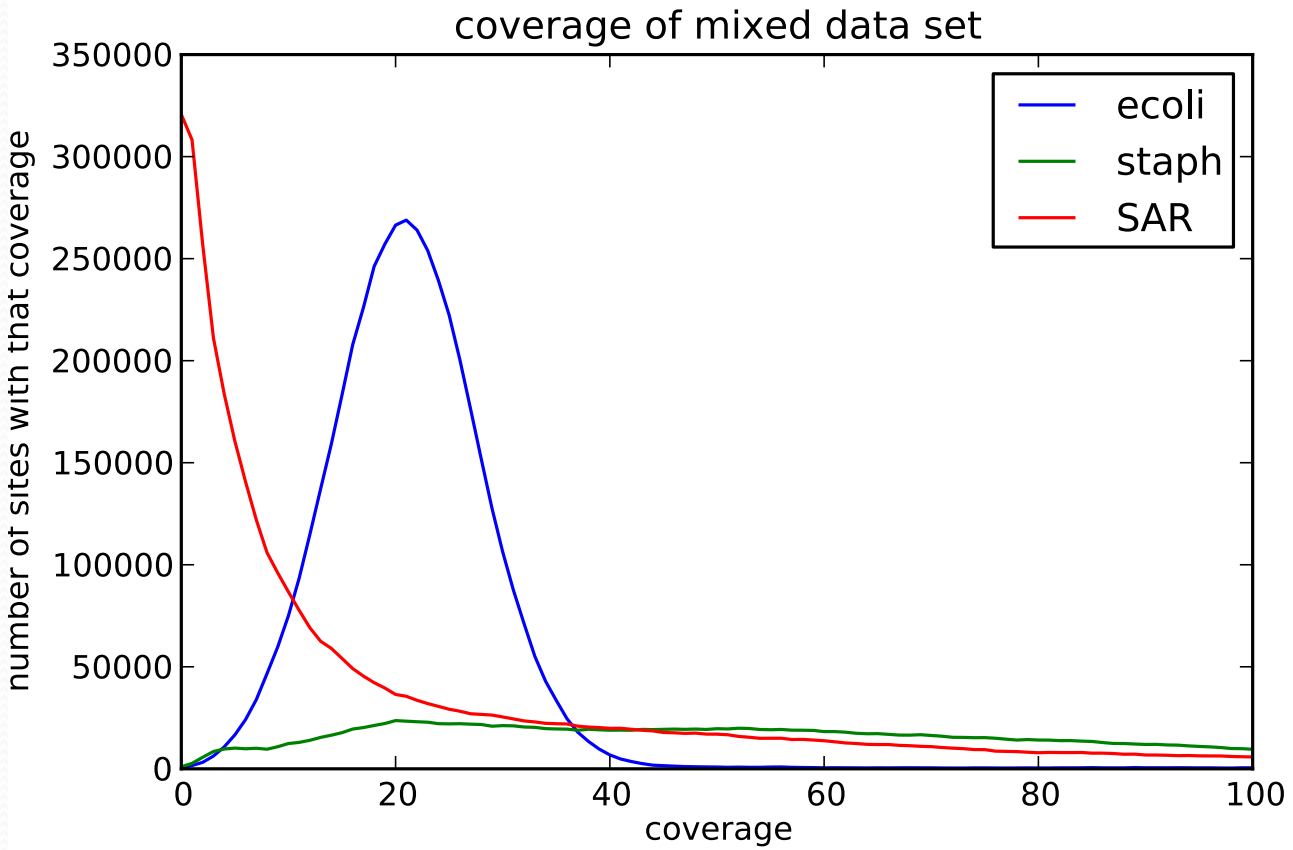
Partitioning

Important note: assembly *after* these techniques are applied is ***inclusive*** of assembly before these techniques – i.e. equivalent or better.

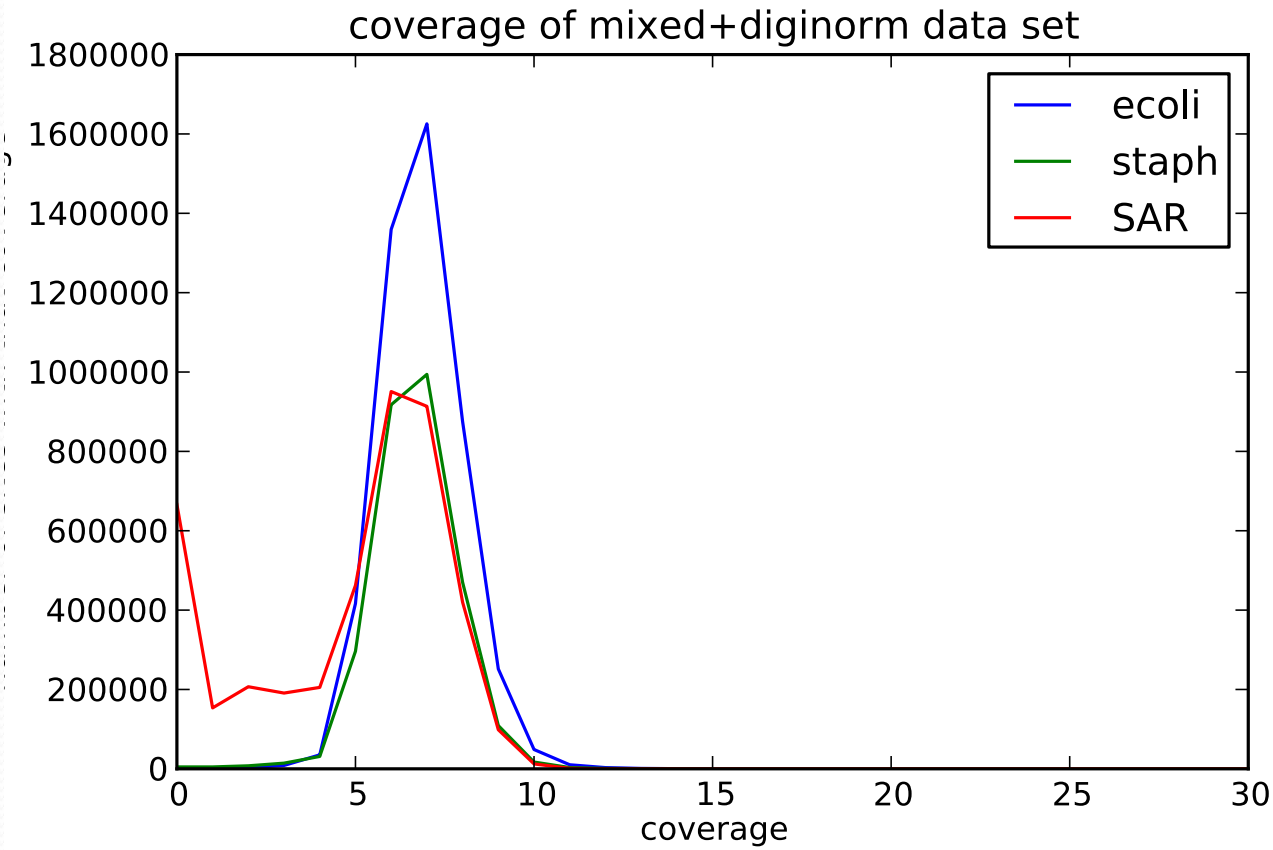
Digital normalization



Coverage before normalization

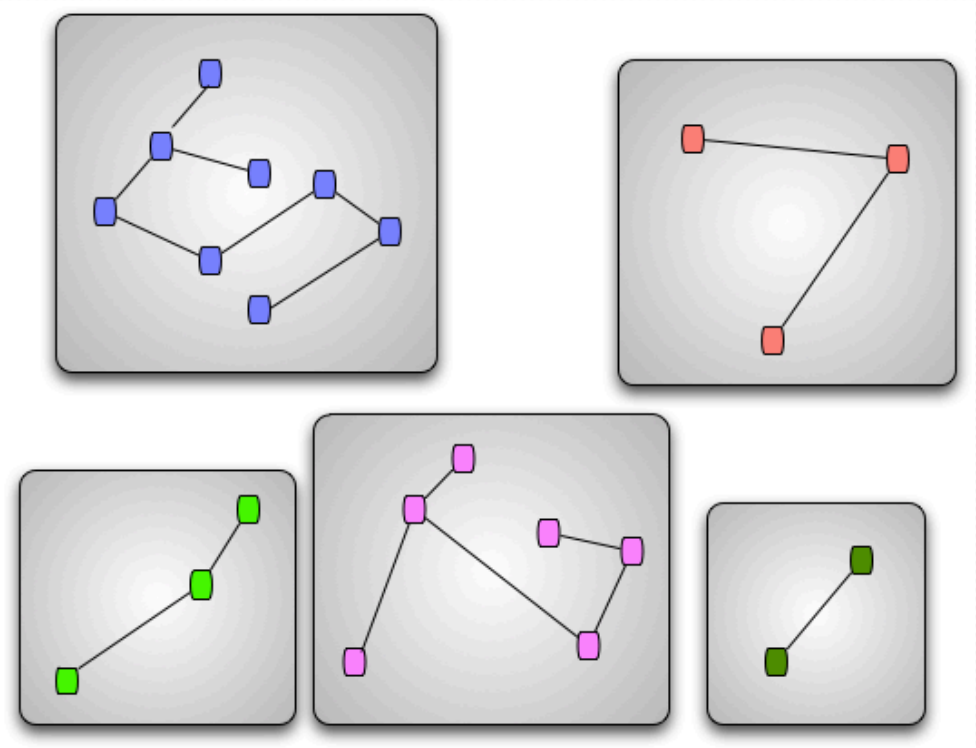


Coverage after normalization



Partitioning

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



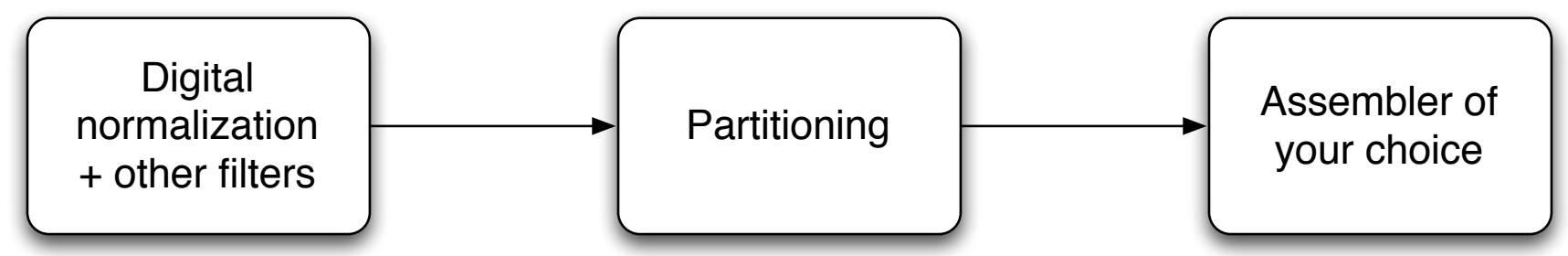
Diginorm + partitioning make small-memory assembly possible.

- Digital normalization is fixed memory, single pass (streaming, online), scales with diversity of underlying sample.

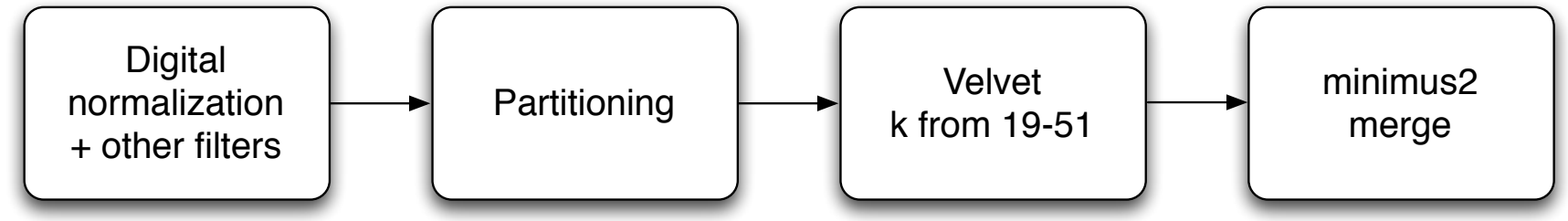
  Note: Diginorm applies equally well to (meta)transcriptome, genome, MDA single-cell.

- Partitioning is ~20x lower memory usage than assembly, and following assembly steps are guaranteed to be <= unpartitioned assembly. After partitioning, remaining steps are pleasantly parallel & small memory.

Pipeline options



Below is what *we* do:



Example

Dethlefsen data set / Relman lab

251 m reads / 16gb FASTQ gzipped

~ 24 hrs, < 32 gb of RAM for full pipeline

(reads => final assembly + mapping)

Assembly stats:

58,224 contigs > 1000 bp (average 3kb)

summing to 190 mb genomic

~38 microbial genomes worth of DNA

~65% of reads mapped back to assembly

Why would you want to assemble?

Some use cases:

- Look for large-scale variation from reference – pathogenicity islands, etc.
- Assemble new “reference”.
- Discriminate between different members of gene families.
- Discover operon assemblages & annotate on co-incidence of genes.