The Value of Mate-pairs for Repeat Resolution

An Analysis on Graphs Created From Short Reads

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Next Generation Sequencing

Approx. 25 to 250 bp per read

Advantages:

- high throughput
- relatively low cost

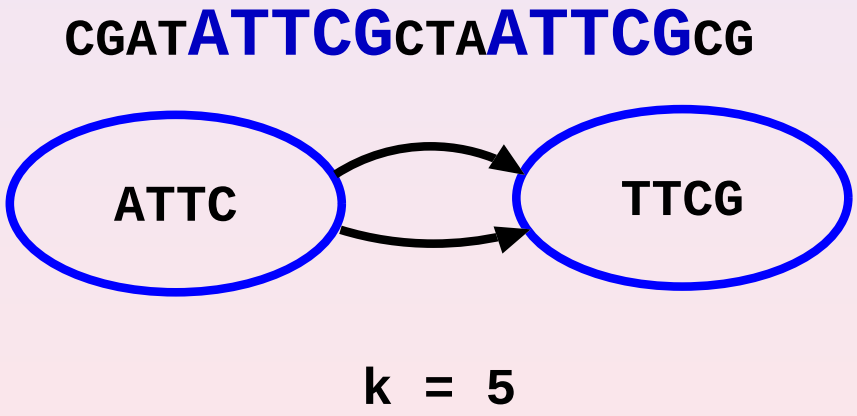
Disadvantages:

- less certainty with error detection
- greater difficulty with resolution of repeats
  - lower likelihood of spanning an entire repeat within one read

The Assembly Problem and the De Bruijn Graph

1. Choose a value k
2. 1 Node for each length $k-1$ substring that exists in any read
3. Length k substrings are represented by directed edges between $k-2$ suffix/prefix overlap
   - Edge multiplicity rep. # of times a substring appears

Example



Pevzner et al.

Eulerian Paths

- Every length k substring of read $\rightarrow$ 1 edge
- With perfect data, graph is Eulerian
- Any Eulerian tour $\rightarrow$ valid assembly of the reads

Good News:

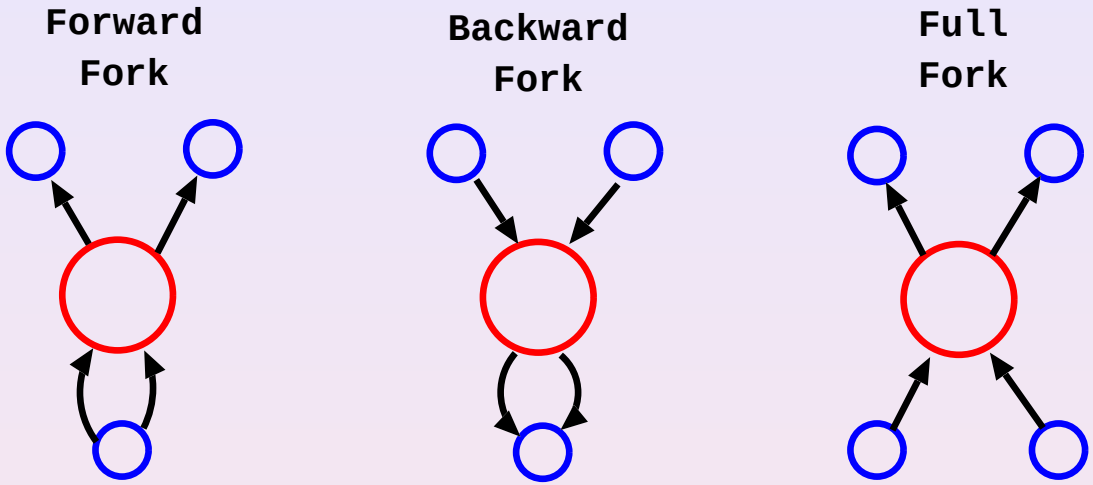
- Eulerian Tour can be found quickly

Bad News:

- Repeats $\rightarrow$ many Eulerian tours

Eulerian Simplifications

Three types of forks:



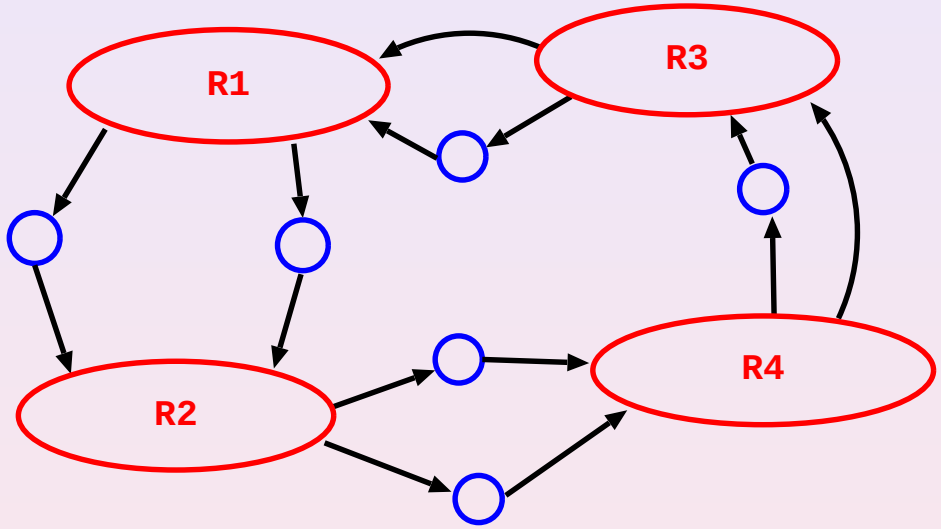
Reduce Unambiguous Traversal Regions into Single Nodes

- Simple Path Compression
- Compressing Tree-like Regions
- Splitting Half-decision Nodes

Kingsford

What Remains Now

A graph of only full-fork nodes (any two of which may be separated by a non-decision node)



We need additional information for finishing

Best known avenue: Use matepairs

Mate-pairs

Random fragment with a approximately known size

Both ends are sequenced

Unique path of same approximate size in the assembly graph $\rightarrow$ partial traversal of the graph

Widely accepted empirically, but no work has been done to assess their theoretical usefulness with short reads

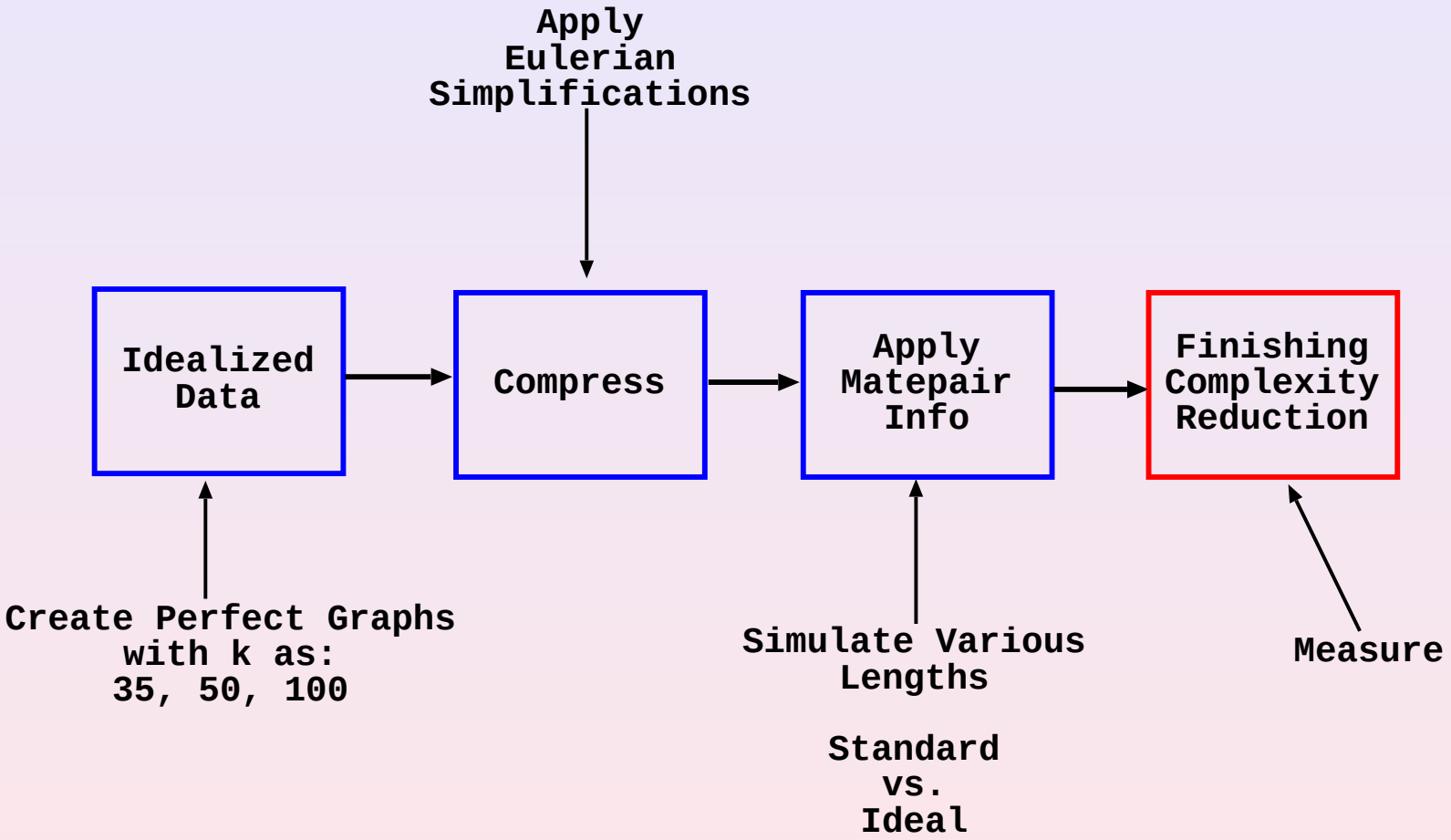
Before We Spend Our Money

Mate-pair libraries are expensive.
How much are they worth?

Can Repeats Be Resolved By Mate-pairs
If Data Is Perfect?

- No sequencing errors
- Perfect coverage of the genome - *exactly one* edge in the graph for every length k substring of the genome
- Assume we know *exact* length of each insert

The Plan


Apply
Eulerian
Simplifications
Idealized
Data
Compress
Apply
Matepair
Info
Finishing
Complexity
Reduction
Create Perfect Graphs
with k as:
35, 50, 100
Simulate Various
Lengths
Standard
vs.
Ideal
Measure

Simulating Mate-pairs

1. Choose an insert size, j
2. Choose a random starting point, s, in the known sequence
3. Choose an ending point based on a Gaussian dist. using j as the mean and a 10% stand. dev.
4. First and last k letters are pairs with known distance

Reducing Finishing Complexity Using Mate-pairs

Use a shortest path heuristic

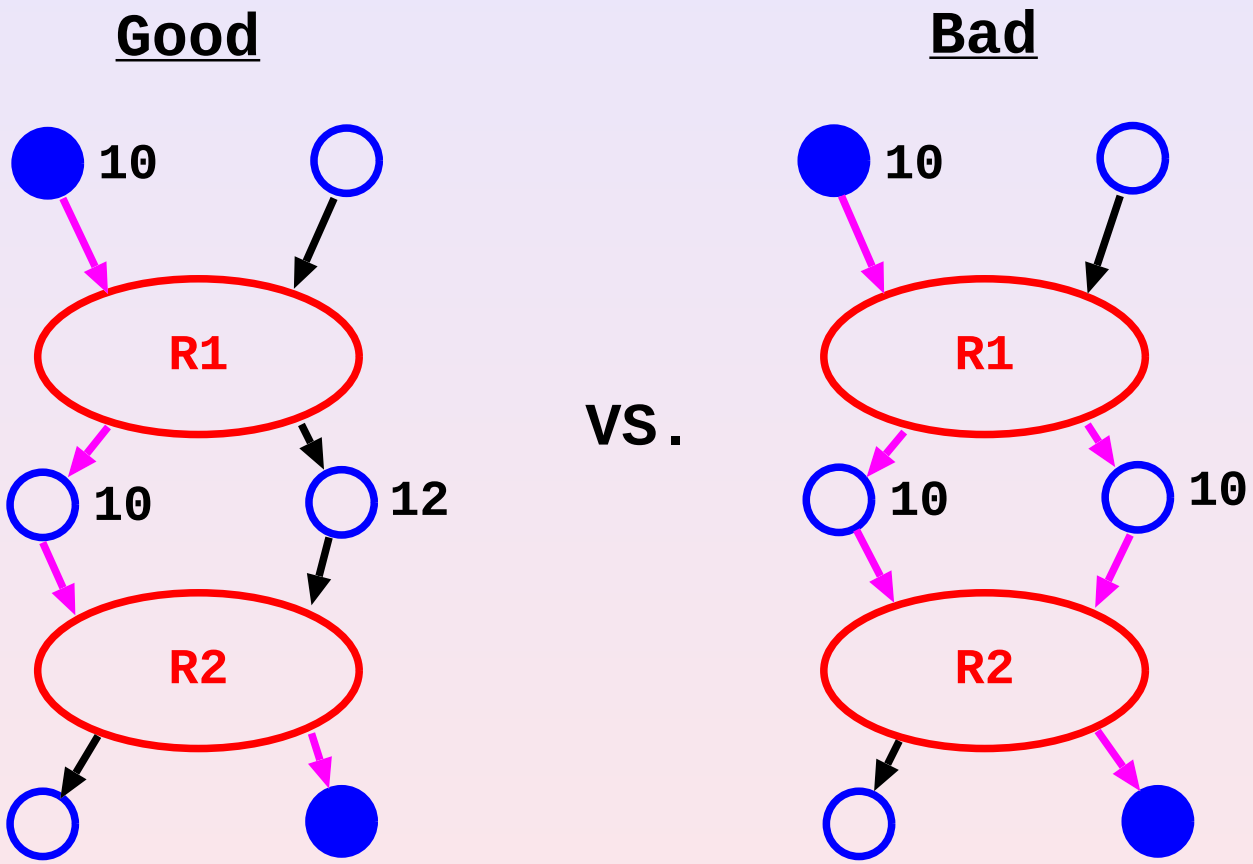
- Find a pair of nodes where the mate sequences are located
- If shortest path = insert size $\rightarrow$ assume path is correct

- We can verify correctness by comparison to known sequence
- An assembler cannot do this

Solution:

Only use mates if shortest path is unique

Which Mate Pairs Can Be Used?



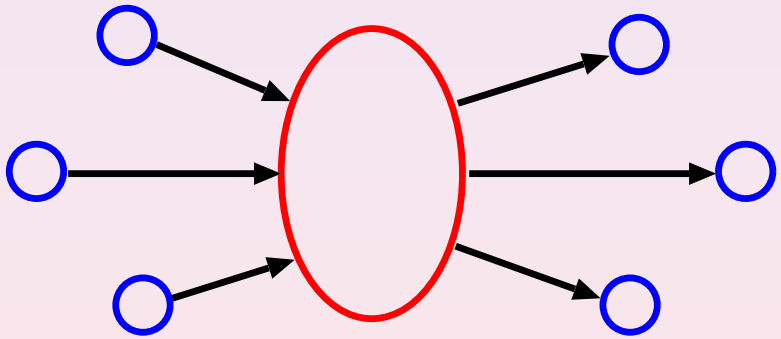
Finishing Complexity

Goal: Reduce manual finishing efforts

Define Finishing Complexity:

- A value proportional to # of experiments needed to finish the genome

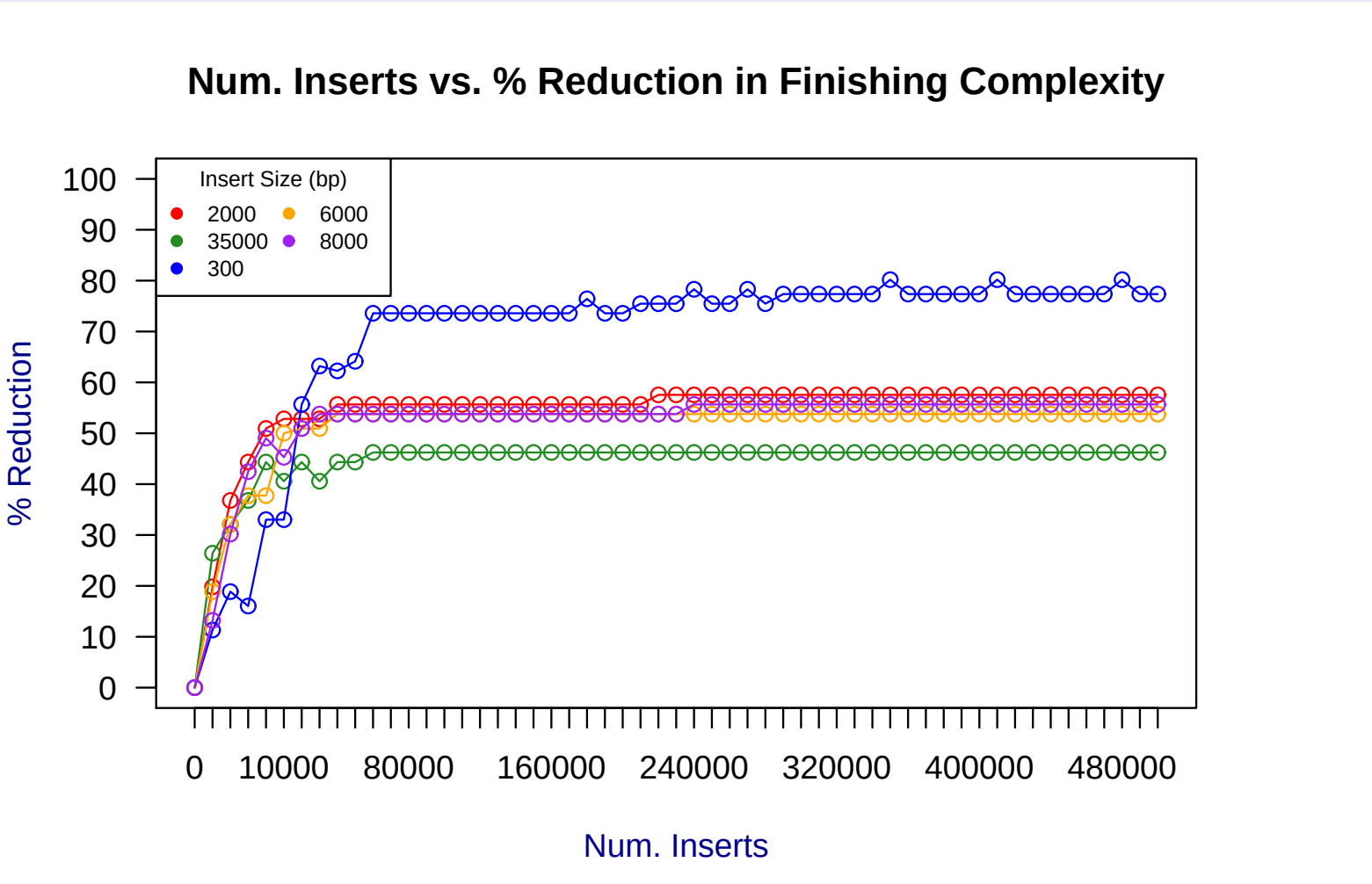
  Fin. Comp. of one node is $\sum_{i=2}^{d} i = \frac{d^2+d}{2} - 1$



FinComplex(G) = Sum over all nodes

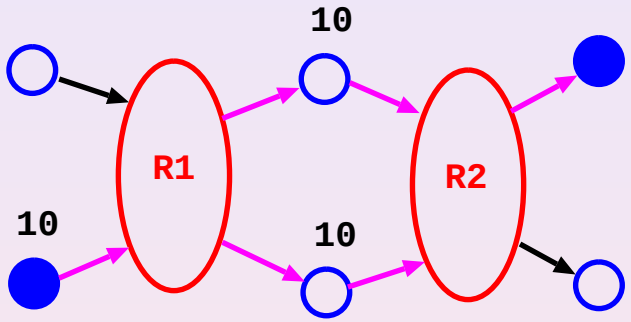
Increasing Clone Coverage to Infinity

Bartonella quintana Toulouse: Genome Size $\approx$ 1.5 Mb



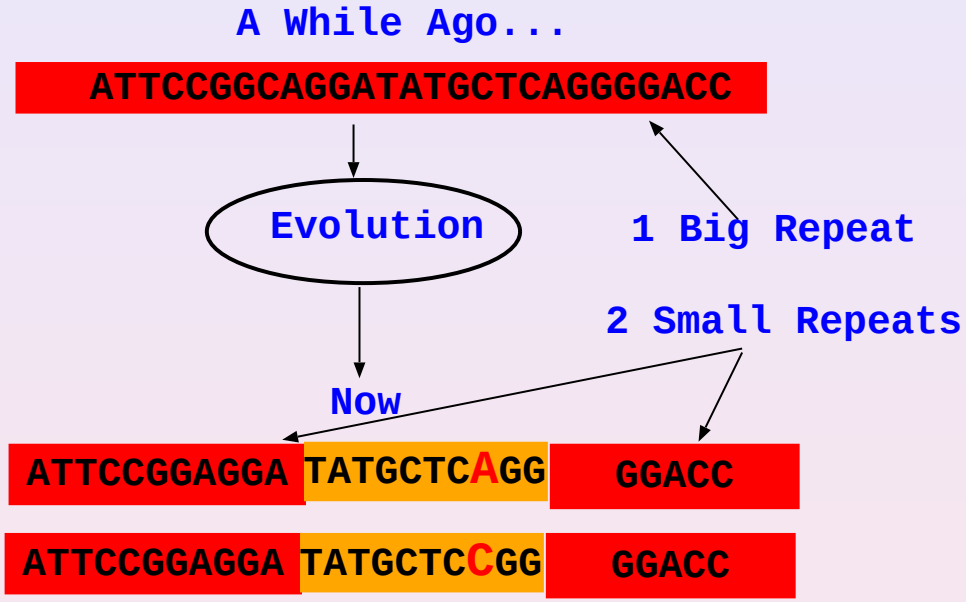
An Important Observation

Many areas of these Graphs Look Like This



- **These repeats can only be resolved by inserts that span exactly 1 repeat**

What Causes Localized Complexity?



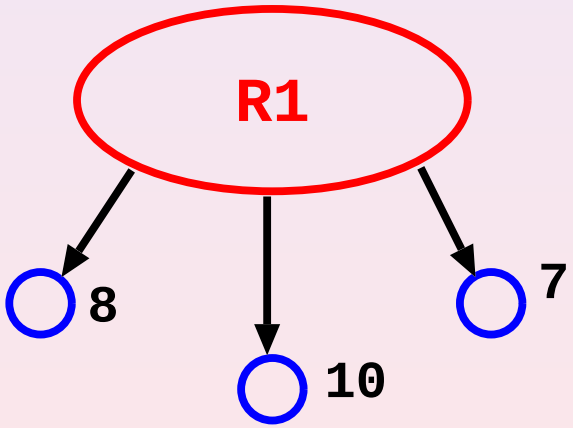
How often does this situation arise in the graph?

Graph Complexity Statistic: Concept

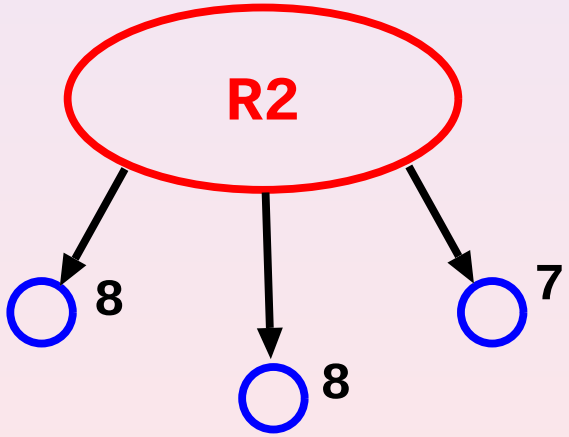
- **Goal:** Measure the amount of *localized* complexity in the graph (C-Statistic)

Label each fork in the graph as either **trivial** or **non-trivial**

Trivial



Non-Trivial



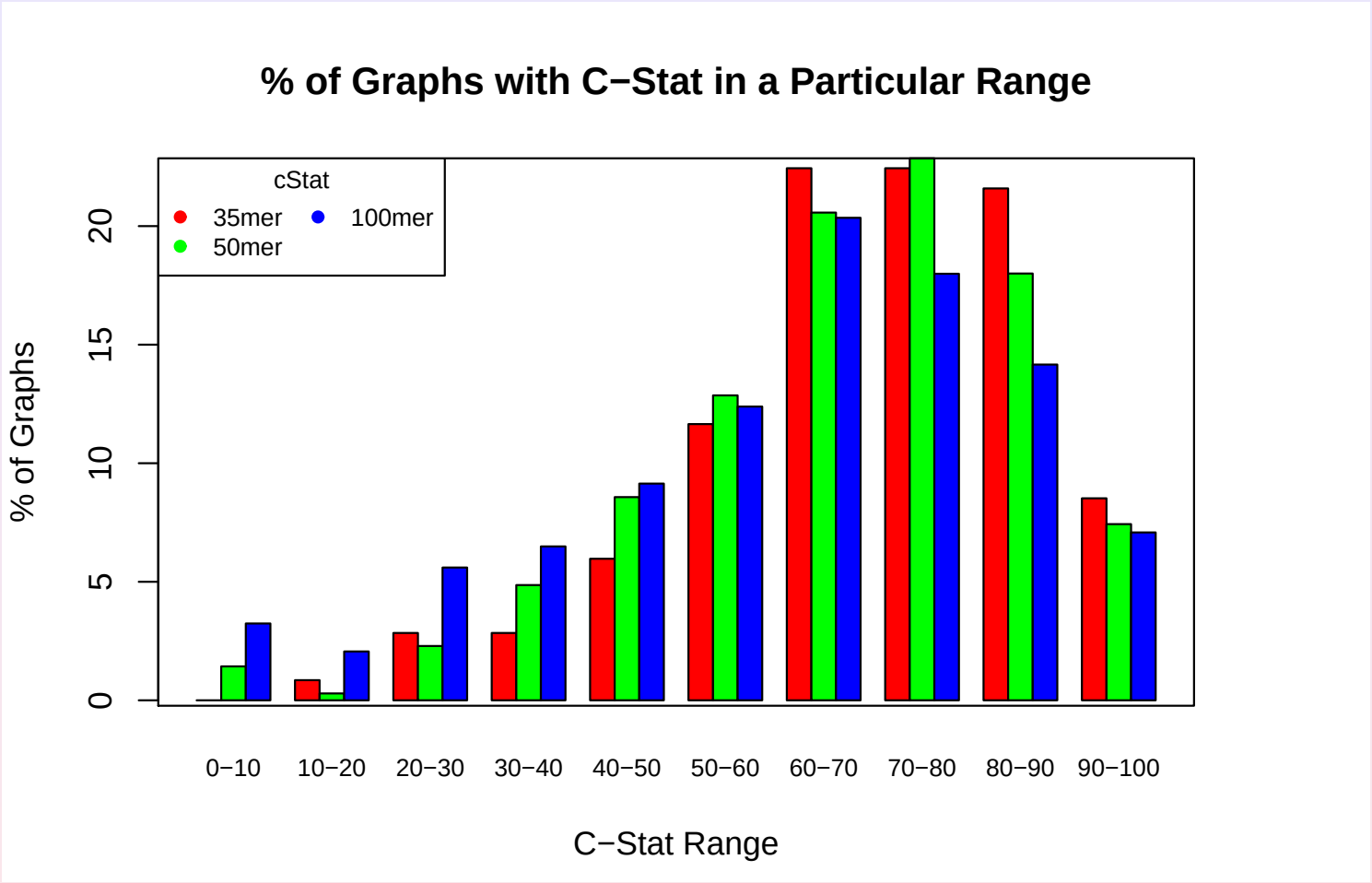
Graph Complexity Statistic

- Let S be the set of all forks in the graph, G
- Let N be the set of all non-trivial forks in G
- Let C_v be the finishing complexity of node v

$$cstat(G) = \frac{\sum_{v \in N} C_v}{\sum_{v \in S} C_v} * 100$$

- **Hypothesis:** Graphs with a high C-Stat will not experience good reductions in finishing complexity using traditional mate-pair libraries

C-Statistic Across All Graphs



Choosing Graph-Specific Insert Sizes is Important

Tailoring Mate-pair Size to the Graph

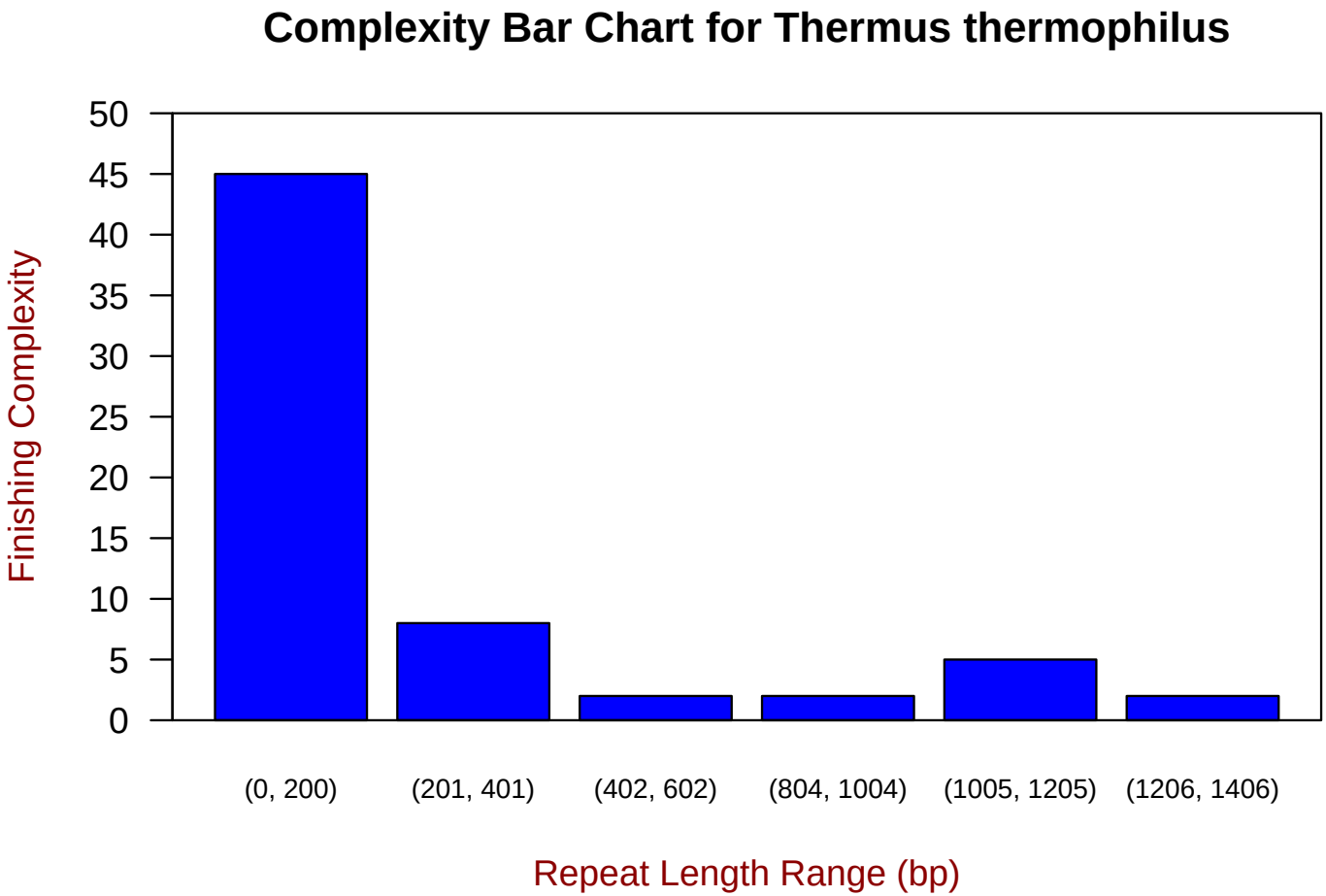
Typical sequencing experiments choose 2 common size mate-pair libraries without considering repeat size

- Typical sizes: 2000, 6000, 8000, 35000 (bp)

Hypothesis:
Given the prevalence of non-trivial repeats, we can likely reduce finishing complexity more by choosing 2 library sizes targeted at just barely crossing the forks of greatest complexity

How do we choose what size inserts to use?

Complexity Bar Chart for Repeats



How the Complexity Bar Chart is Used

Two tallest bins (most combined complexity)

For of these two bins:

1. Take the mean repeat length of all the repeats in the bin
2. Add $3 * k$ to the avg.
3. Create inserts of this approximate size

Average optimal mate-pair length to cover nodes of most complexity was between $4.5k$ **and** $6k$

- Small σ for graphs with C-Stat ≥ 50
- Large σ for graphs with C-Stat < 50

How Many Mate-pairs? - Poisson Process

- Want mate-pair to begin in a non-decision node and cross exactly one repeat
- In worst case non-decision node maybe be as short as k
  - Want at least one insert for every k bps in the genome
  - If average arrival rate, λ, is 10 across k bp

$$
\begin{aligned}
P[X = x] &= \frac{\lambda^x e^{-\lambda}}{x!} \\
P[X = 1] &= \frac{10}{e^{10}} \approx 0.00045 \\
P[X < 1] &\leq 0.00045
\end{aligned}
$$

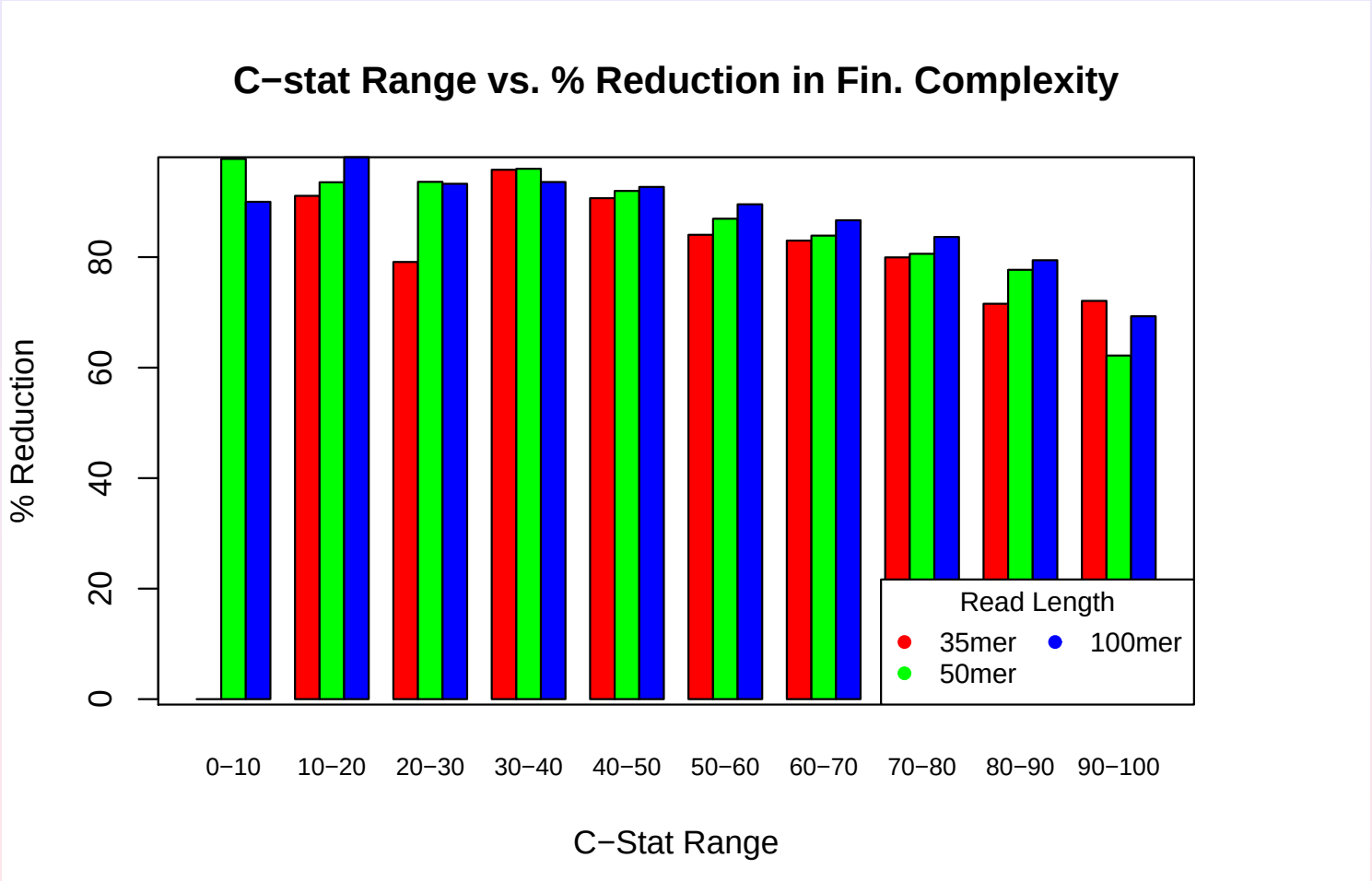
To get $\lambda = 10$ across k bp we need $10\frac{G}{k}$ inserts

Simulations Across 360 Organisms

How Much Can We Reduce Finishing Complexity Using 2 libraries each with $10\frac{G}{k}$ # of inserts if we use:

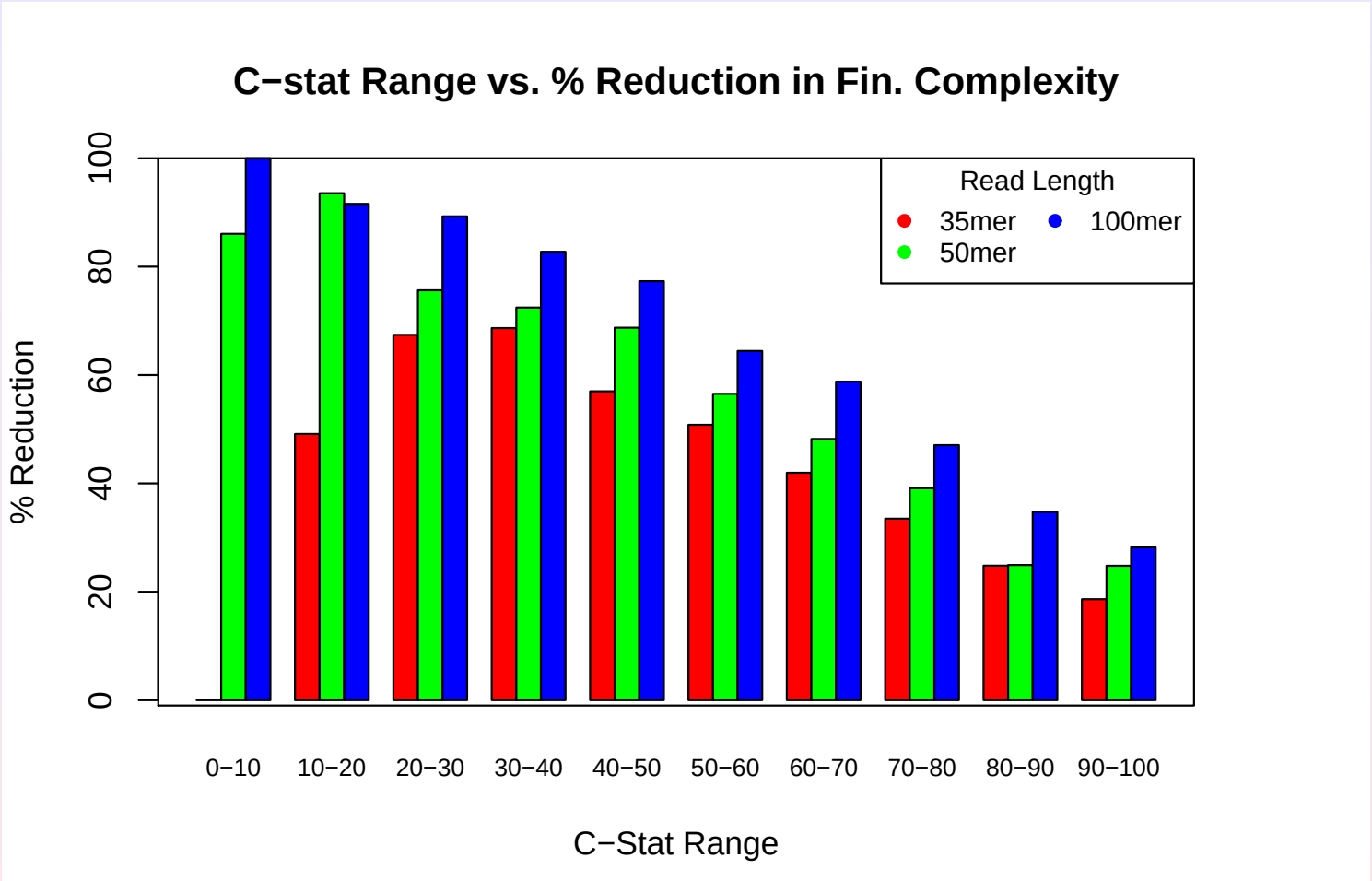
- 2 standard mate-pair libraries
- The 2 'ideal' libraries based on the complexity chart

Using Two 'Ideal' Mate-Pair Libraries (Avgs: 4.5k to 6k for smallest)



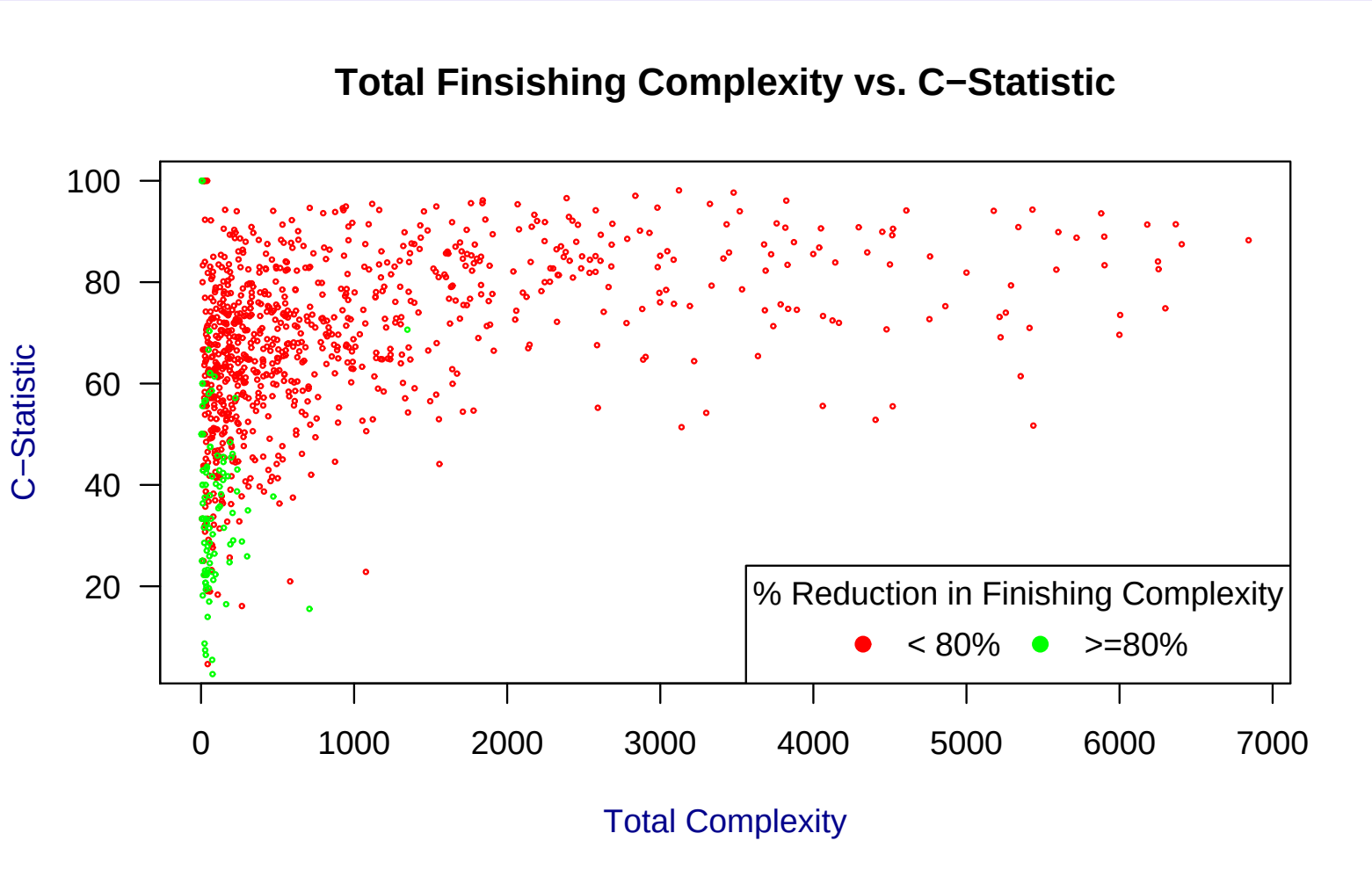
Overall Avg Reduction: 82.70%, $\sigma \approx$ 17%

Using Standard Libraries: 2000 and 8000



Overall Avg Reduction: 47.52%, $\sigma \approx$ 22%

Local Complexity Vs. Global Complexity (Using 2000 and 8000)



Some Conclusions

- **Short inserts are better for repeat resolution**
- A large confounding factor in repeat resolution is localized complexity caused by intra-repeat nucleotide mutations
- The C-Stat is simple and useful measurement of localized complexity
  - Most graphs have a C-Stat ≥ 60
  - If C-Stat ≥ 50, then standard mp libs. are not useful for resolving repeats
- Tailoring mp libs. to the repeat structure of the assembly graph is a useful technique

Are Mate-pairs Worth the Money?

- Even with perfect data and an ideal approach to creating mate-pair libs, we can only resolve an avg. of 83% of finishing complexity ($\sigma \approx 17\%$)

  Does not bode well for noisy data

- *On the other hand:*

  The only known technique for better resolving localized complexity is use of longer reads

Thanks