‘Not-So-Greedy’ Algorithm for DNA Shotgun Sequencing

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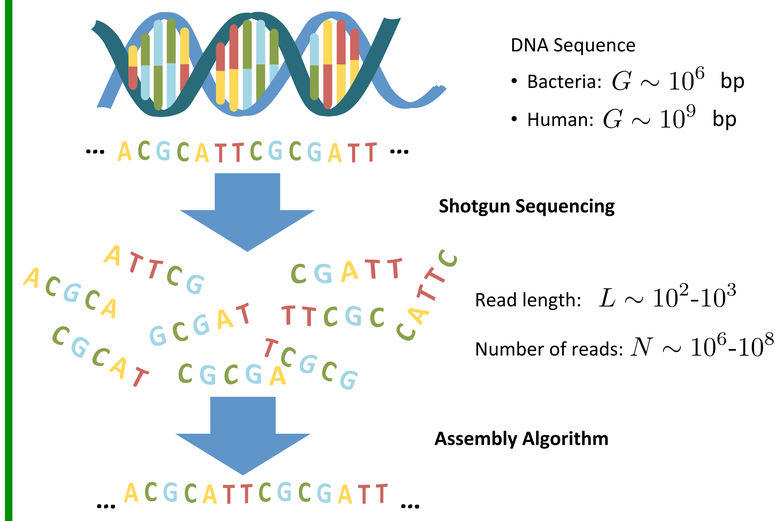
Objective

DNA shotgun sequencing technologies generate short reads from the underlying sequence. Given these reads, an assembly algorithm attempts to reconstruct the original DNA sequence. We present a linear-time assembly algorithm that is near-optimal from an information-theory standpoint.

DNA Shotgun Sequencing



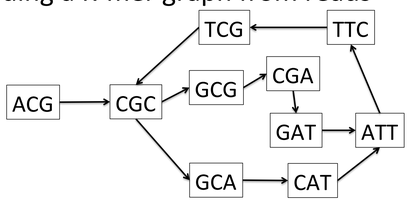
DNA Sequence
- Bacteria: $G \sim 10^6$ bp
- Human: $G \sim 10^9$ bp

Read length: $L \sim 10^2$-10^3

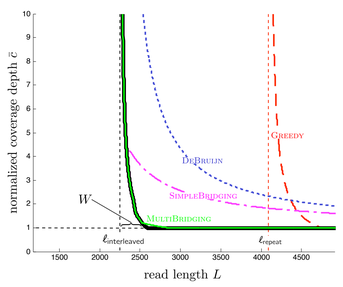
Number of reads: $N \sim 10^6$-10^8

Existing Algorithms

- Most algorithms (EULER, Allpaths, Velvet, ABySS) based on:
  - Building a K-mer graph from reads

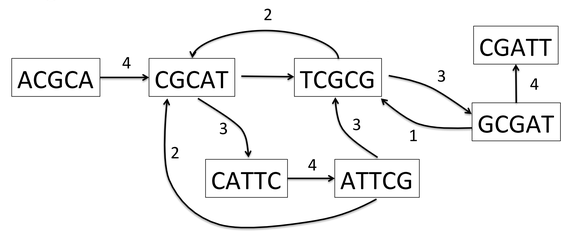


  - Finding an Eulerian path
- MULTIBRIDGING [1]:
  - Picks K dynamically
  - Uses reads to resolve repeats
  - Nearly optimal from information-theoretic perspective
  - Time and memory complexity are $O\left((L-K)NK\right)$



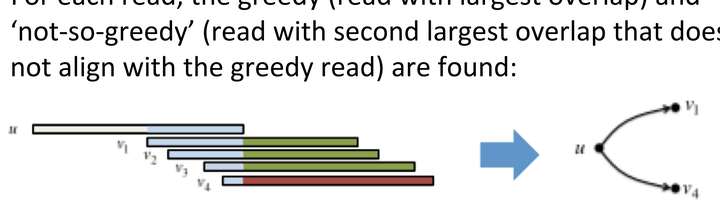
A “Not-so-greedy” Algorithm based on Read-Overlap Graphs

- Read-overlap graph contains one node per read
- Edge weights indicate size of overlap



Graph Construction

- In general, read-overlap graphs have $O(N^2)$ edges
- We generate a sparse subgraph, where each node has in-degree and out-degree at most 2:
- For each read, the greedy (read with largest overlap) and ‘not-so-greedy’ (read with second largest overlap that does not align with the greedy read) are found:



- Read-overlaps are efficiently found using incremental (decremental) Rabin-Karp-type hash to compute all reads’ prefixes and suffixes in order for $O(1)$ look up time on average:

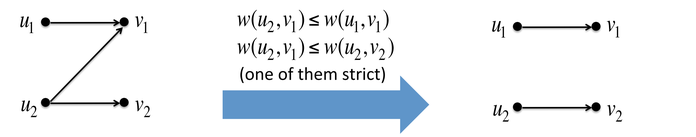
$$H(r) = \sum_{i=1}^{l} \phi(r[i])\alpha^{l-i} \mod p$$

where ϕ is a one-to-one function that assigns different numeric value to each base, *p* = 1000000007, α= 13.

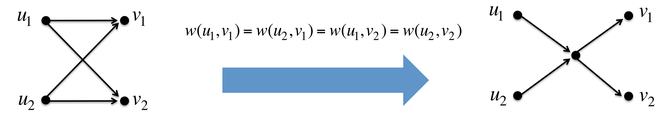
- Time and space complexity to construct graph: $O(LN)$
- Potentially, worst case runtime can be $O(N^2)$ if we end up hashing all reads’ prefixes and suffixes to the same bucket in our hash table, which can occur when finding overlaps at the noisy level.
- To account for this, we limit the number of read prefixes or suffixes in each bucket of the hash table to 2 assuming necessary conditions are satisfied.
- *Why “not-so-greedy”?* It is known that greedy assembly can fail, but this algorithm provably succeeds.

Identifying X and Z Structures

- Algorithm iteratively performs two operations on the graph in order to remove unnecessary transitive edges that have in-degree and out-degree equal to 2:
  - Delete middle edge from a Z-structure:



  - Transform X-structures:



- When these operations are no longer possible, we condense unambiguous edges and look for an Eulerian path
- Same sufficient conditions for success as MULTIBRIDGING:

> **Theorem:** Proposed algorithm correctly reconstructs the sequence, provided:
>
> - Interleaved repeats are bridged
> - Triple repeats are all-bridged

- Assembly is only possible only if [1]:

$$L > \ell_{\text{critical}} = \max(\ell_{\text{triple}}, \ell_{\text{interleaved}})$$

- The sufficient conditions in the theorem are a modest strengthening of the necessary conditions.
- Current C++ implementation needs only ~100ns/character.

Future Goals

- Handle the case when reads are noisy
- Build the “smallest” overlap graph that is guaranteed to contain the true sequence provided *t*-ary repeats are all-bridged

[1] G. Bresler, M. Bresler and D. Tse “Optimal Assembly for High-Throughput Shotgun Sequencing”
[2] J. T. Simpson and R. Durbin “Efficient construction of an assembly string graph using the FM-index”
[3] I. Ben-Bassat and B. Chor “String graph construction using incremental hashing