Assembly of large genomes from short WGS reads

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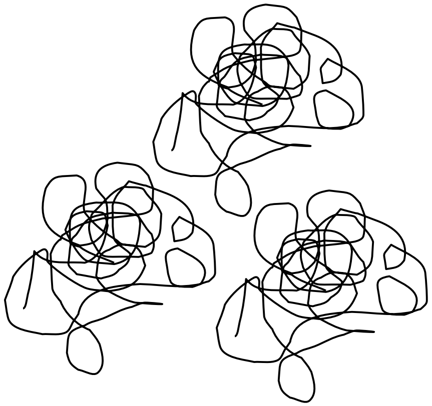
USDA

PineRefSeq
http://pinegenome.org/pinerefseq/

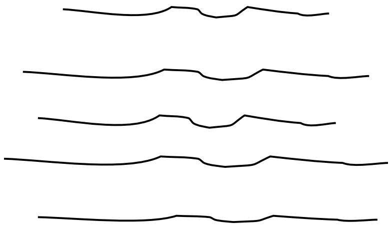
Whole Genome Shotgun reads

Multiple copies of DNA



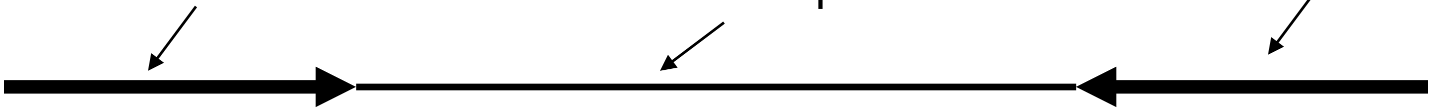
Shred
&
Size
select
→

Fragments of
150 - 200,000 bases



Sequence
the ends
→

Pairs of Reads of 150 – 500 bases each



CAAGCTGAT...

Unknown sequence

...GTTTGGAAC

Recently developed assemblers for NGS data

- MSR-CA
  - handles 454, Illumina, and Sanger reads
- Allpaths-LG
- SOAPdenovo
- Velvet
- ABySS
- Contrail
- SGA (also overlap-based)

Two assembly approaches

- Overlap-Layout-Consensus (OLC)
  - Used by most assemblers for previous generation (Sanger) sequencing
  - Celera Assembler, PCAP, Phusion, Arachne, etc
- Graph
  - Used by most assemblers for Illumina data
  - SOAPdenovo, Allpaths-LG, Velvet, Abyss, etc
- We use a combined approach that combines the benefits of both OLC and Graph in our MSR-CA assembler

Assembly approaches: OLC

- OLC: Overlap-Layout-Consensus
  - Compute overlaps of reads

```
AGTGATTAGATGATACTAGA
        ||||||| ||||
        GATGATAGTAGAGGATAGATTTA
```

  - Create layout of *contigs* from overlapping reads

```
AGTGATTAGATGATAGTAGA
       AGATGATACTAGAGATAGATAGACC
            ATAGTAGAGATAGATAGACCACTCATCATAC
```

  - Create consensus sequence of contigs

```
AGTGATTAGATGATAGTAGAGATAGATAGACCACTCATCATAC
```

5 billion reads?

... that’s 12.5 quadrillion overlaps

at 1 million overlaps/second, that would take 400 years

Two assembly approaches

- Overlap-Layout-Consensus (OLC)
  - Used by most assemblers for previous generation (Sanger) sequencing
  - Celera Assembler, PCAP, Phusion, Arachne, etc
- DeBruijn Graph
  - Used by most assemblers for Illumina data
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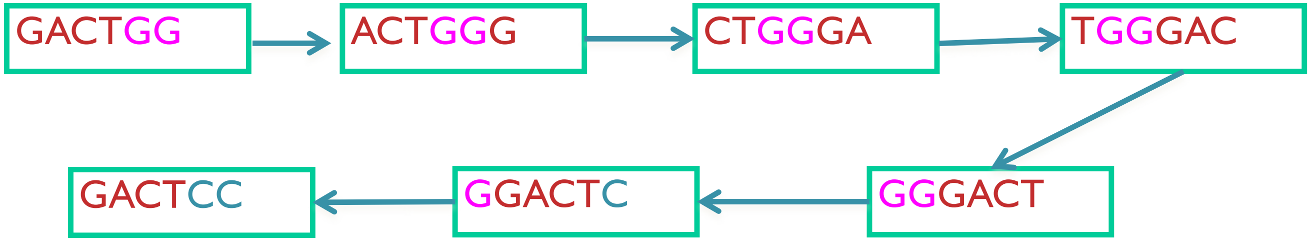
- We use a combined approach that combines the benefits of both OLC and Graph in our MSR-CA assembler

De Bruijn Graph Strategy

- Find all k-mers, build a graph
  - Every k-mer is a node
  - Two nodes are linked with an edge if they share k-1 mer

GACTGGGACTCC



- An assembly is a path through the graph that visits each edge at least once
- We can only roughly estimate the graph of the genome from reads due to sequencing errors and lack of coverage

Two assembly approaches

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- Graph
  - Used by most assemblers for Illumina data
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- We propose to use a combined approach that combines the benefits of both OLC and Graph in our MSR-CA assembler

Benefits and drawbacks of OLC and Graph

- Benefits of OLC
  - Can deal with variable length reads and reads from different sequencing platforms
  - Overlaps can be long and thus more reliable
  - Overlaps do not have to be exact
  - Can resolve repeats of up to read size
- Drawbacks of OLC
  - Computationally intensive, number of overlaps grows quickly with the number of reads and coverage

- **Benefits of Graph**
  - **Computationally efficient**
- **Drawbacks of Graph**
  - **Errors in the reads create spurious branches in the graph – requires error correction**
  - **Max. size of k-mer is limited by the shortest read size**
  - **All overlaps in the graph are exact overlaps of k-1 bases**
  - **Repeats of longer than k-bases cannot be resolved**

MSR-CA combines benefits of OLC and Graph

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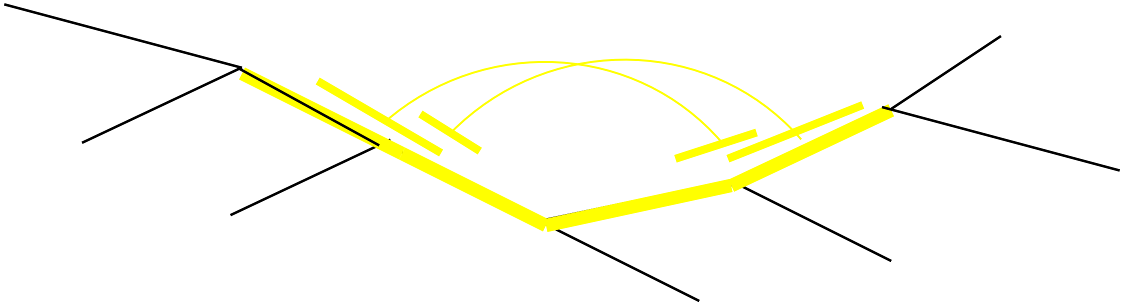
MSR-CA strategy

- Error correct Illumina reads
- Create a deBruijn graph: each k-mer is unique in the graph



  - Many pairs will extend to the same Super-read
  - Replace the pairs by the corresponding Super-reads
- Assemble the Super-reads with an OLC assembler (CABOG) using additional long mate pairs for linking the contigs

MSR-CA design

- Efficient multi-threaded code
- Designed to handle data sets with up to 12B reads
- Development is aimed at WGS assembly of the 24Gb Loblolly Pine genome on a computer with 48 cores and 512Gb of RAM in 1-2 months
- Current version 1.4 and being continually improved

Results on a pool of 500 Pine fosmids

	Sequence in assembly, bp	N50 contig size, bp	N50 scaffold size	Number of scaffolds>30 kb
Allpaths-LG	14,050,574	10,324	26,298	248
SOAPdenovo	13,470,572	1,632	33,389	322
MSR-CA	14,604,209	7,640	22,740	218

Notes: N50 computed from estimated 19.25Mb total sequence, each fosmid was estimated at 38Kb, assemblies used short pairs and jumping library pairs

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  - Brian Walenz
- UMD
  - Carl Kingsford