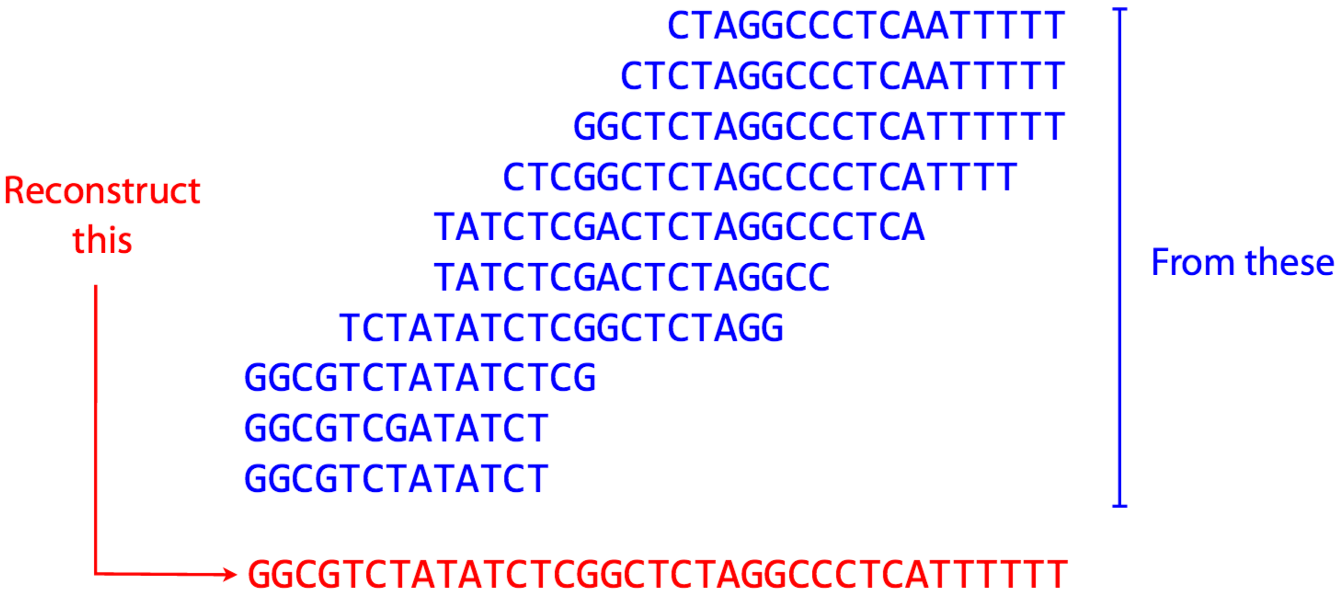
Genome Assembly

21 Oct 15

Announcements

Assembly

Assume sequencing produces such a large # fragments that almost all genome positions are *covered* by many fragments...



CTAGGCCCTCAATTTTT

CTCTAGGCCCTCAATTTTT

GGCTCTAGGCCCTCATTTTTT

CTCGGCTCTAGCCCTCATTTT

TATCTCGACTCTAGGCCCTCA

TATCTCGACTCTAGGCC

TCTATATCTCGGCTCTAGG

GGCGTCTATATCTCG

GGCGTCGATATCT

GGCGTCTATATCT

From these

Reconstruct this

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

ASSEMBLY

...but we don't know what came from where Or what the reference looks like

CTAGGCCCTCAATTTTT
GGCGTCTATATCT
CTCTAGGCCCTCAATTTTT
TCTATATCTCGGCTCTAGG
GGCTCTAGGCCCTCATTTTTT
CTCGGCTCTAGCCCCTCATTTT
TATCTCGACTCTAGGCCCTCA
GGCGTCGATATCT
TATCTCGACTCTAGGCC
GGCGTCTATATCTCG

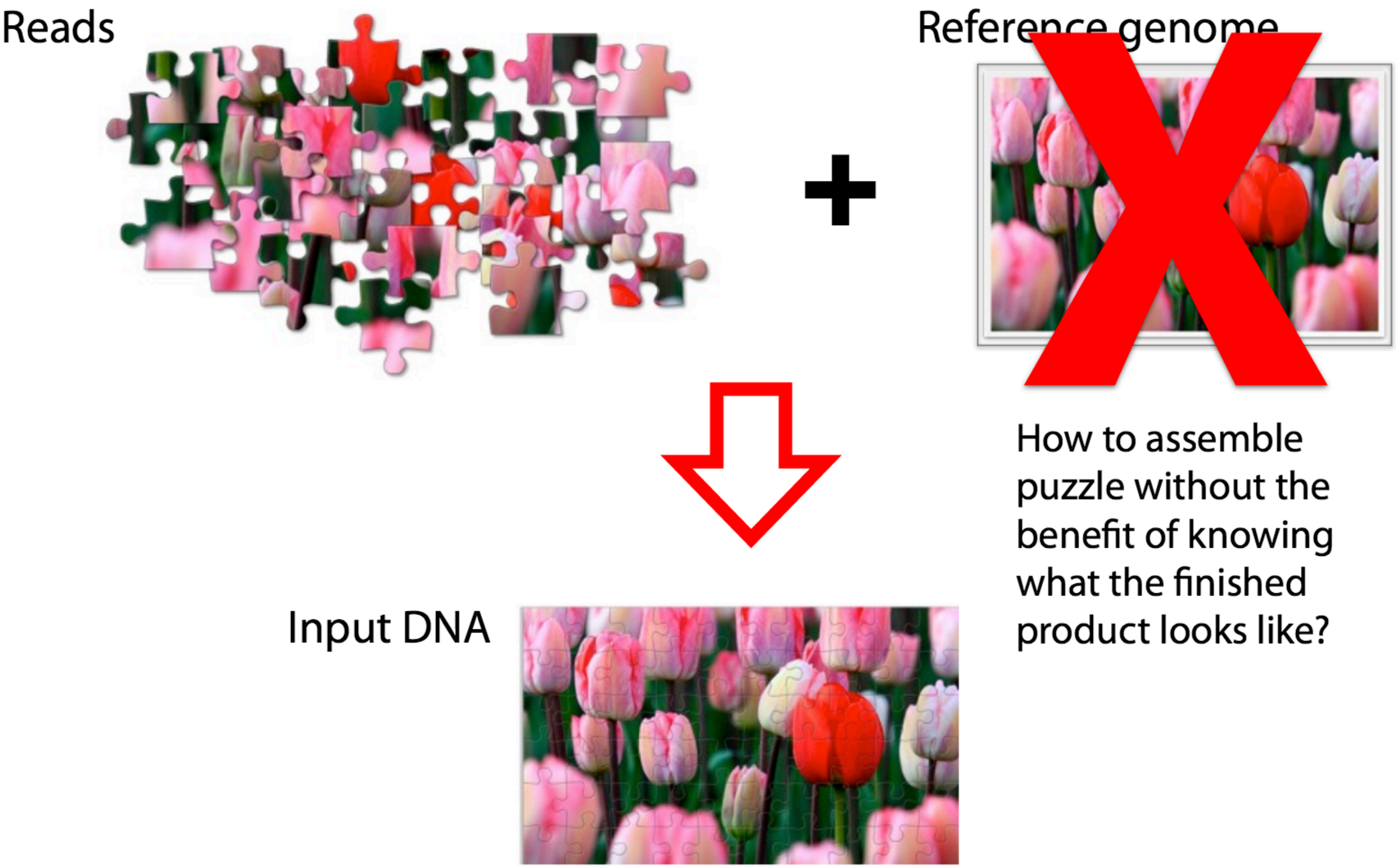
From these

Reconstruct this

GGCGTCTATATCTCGGCTCTAGGCCCTCATTTTTT

Assembly

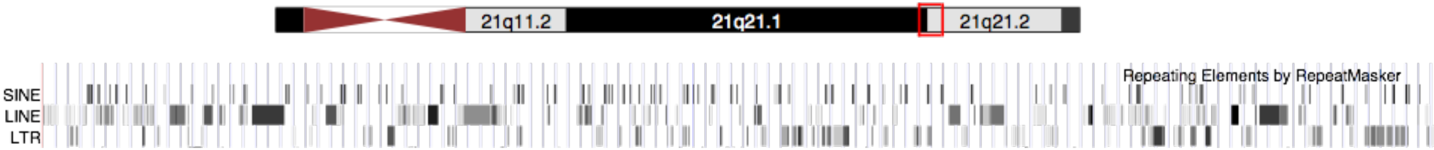
- Complicated by:



How to assemble puzzle without the benefit of knowing what the finished product looks like?

ASSEMBLY

- Complicated by:



ASSEMBLY

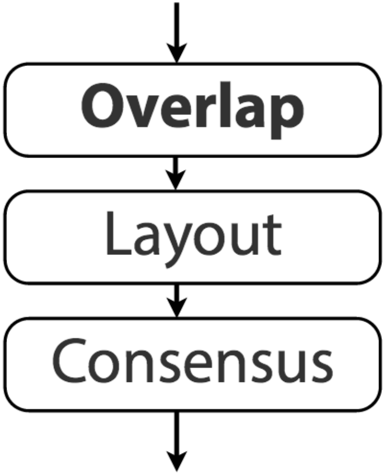
- Workflow:

Assembly

- 3 assembly strategies:

ASSEMBLY

- OLC Assembly



Overlap — **Build overlap graph**

Layout — Bundle stretches of the overlap graph into *contigs*

Consensus — Pick most likely nucleotide sequence for each contig

ASSEMBLY

- OLC Assembly: Characteristics

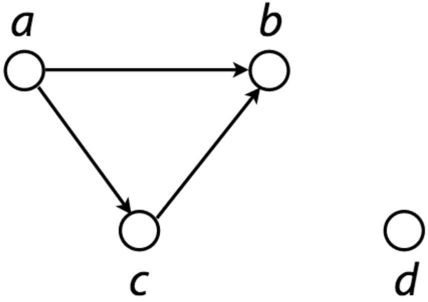
Assembly

Directed graph *G*(*V*, *E*) consists of set of *vertices, V* and set of *directed edges, E*

Directed edge is an *ordered pair* of vertices. First is the *source*, second is the *sink*.

Vertex is drawn as a circle

Edge is drawn as a line with an arrow connecting two circles



Vertex also called *node* or *point*

Edge also called *arc* or *line*

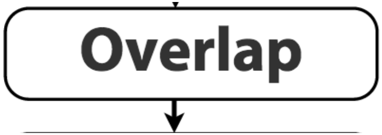
Directed graph also called *digraph*

$V = \{ a, b, c, d \}$

$E = \{ (a, b), (a, c), (c, b) \}$

Source Sink

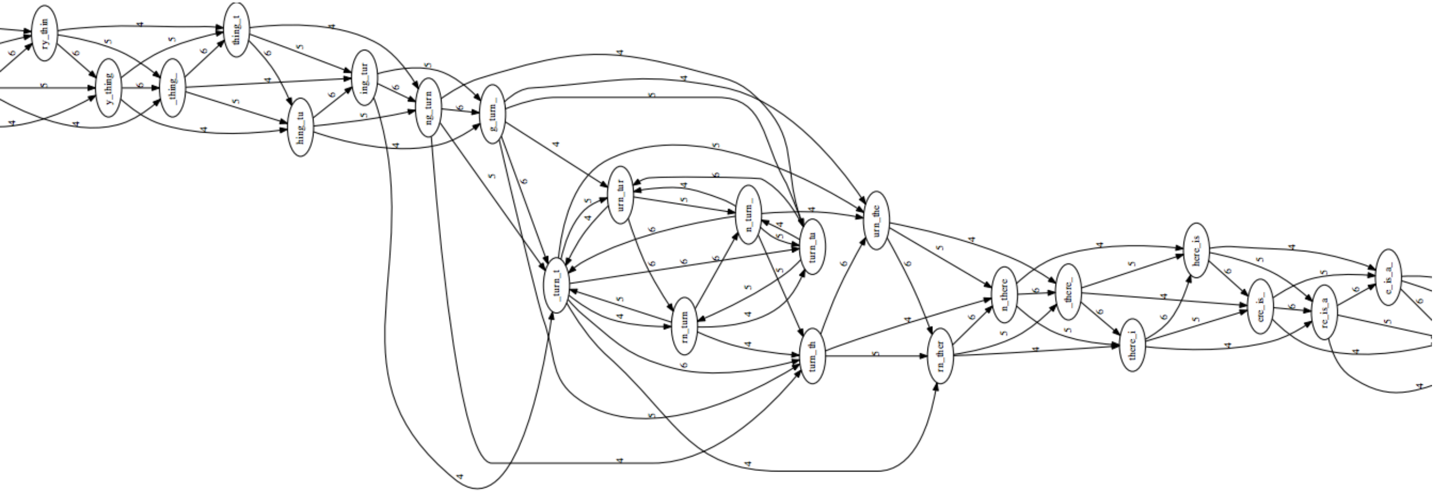
ASSEMBLY - OLC



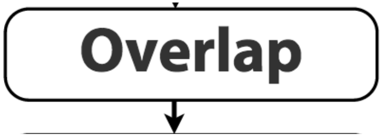
Overlap

Build overlap graph

to_every_thing_turn_turn_turn_there_is_a_season
L=4, k=7



Assembly - OLC

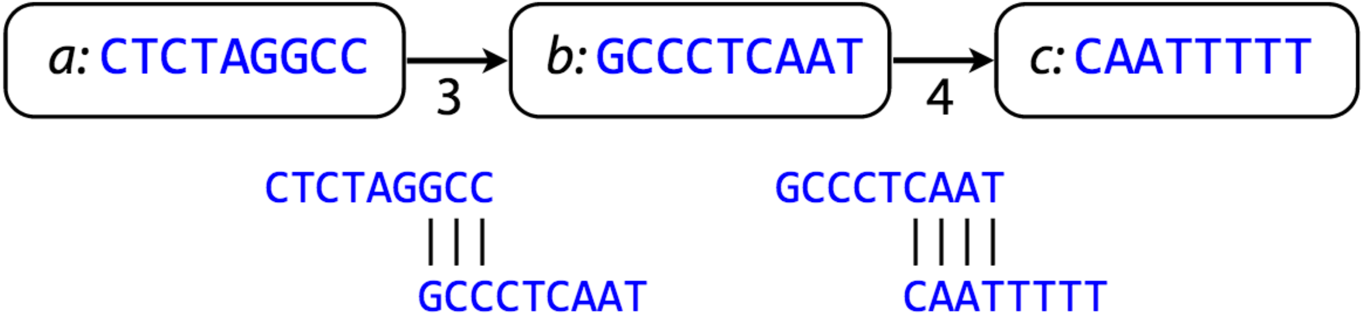


Overlap

Build overlap graph

Vertices (reads): { *a*: CTCTAGGCC, *b*: GCCCTCAAT, *c*: CAATTTTT }

Edges (overlaps): { (*a*, *b*), (*b*, *c*) }



a: CTCTAGGCC

3

b: GCCCTCAAT

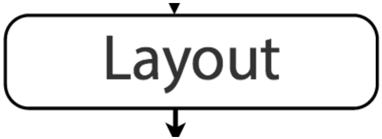
4

c: CAATTTTT

```
CTCTAGGCC
      |||
      GCCCTCAAT
```

```
GCCCTCAAT
     ||||
     CAATTTTT
```

Assembly - OLC



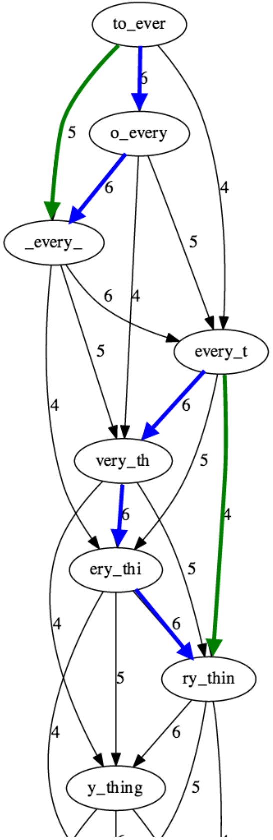
Layout

Bundle stretches of the overlap graph into *contigs*

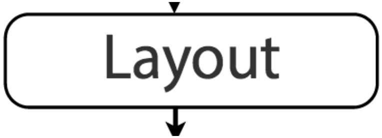
Anything redundant about this part of the overlap graph?

Some edges can be *inferred* (*transitively*) from other edges

E.g. green edge can be inferred from blue



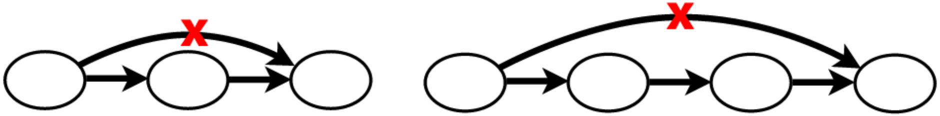
Assembly - OLC



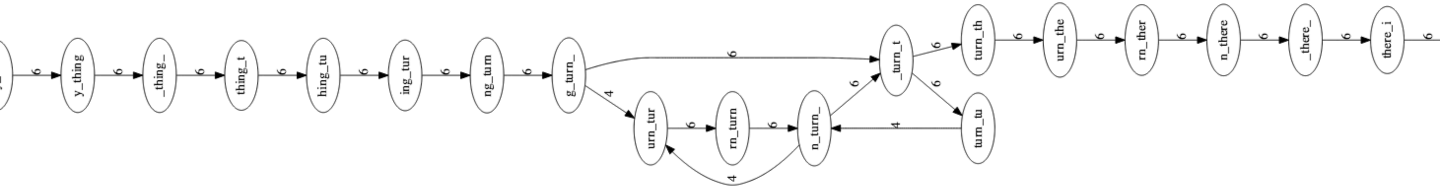
Layout

Bundle stretches of the overlap graph into *contigs*

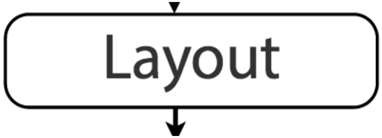
Remove transitively-inferrible edges, starting with edges that skip one *or two* nodes:



After:



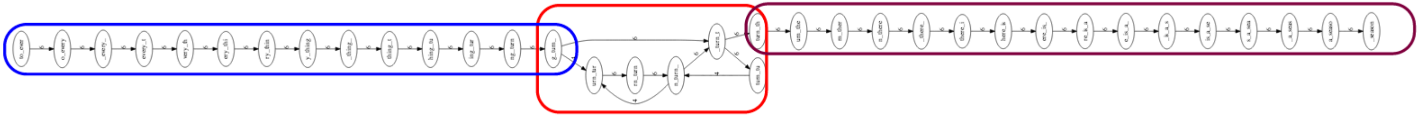
Assembly - OLC



Layout

Bundle stretches of the overlap graph into *contigs*

Emit *contigs* corresponding to the non-branching stretches



Contig 1
to_every_thing_turn_

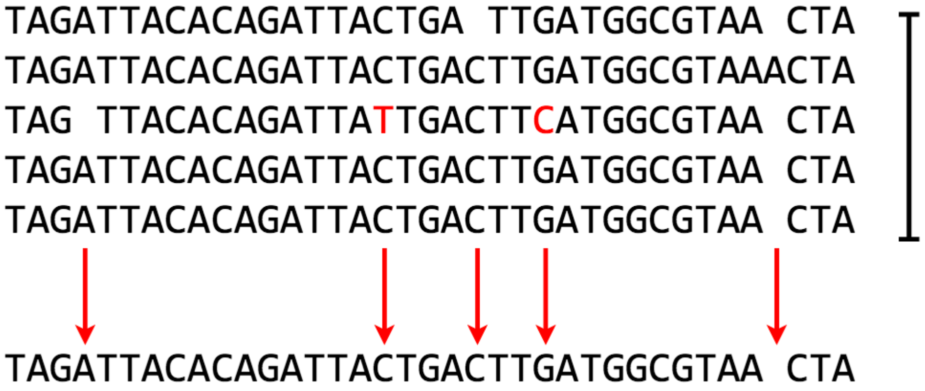
Contig 2
turn_there_is_a_season

Unresolvable repeat

Assembly - OLC

Consensus

Pick most likely nucleotide sequence for each contig



```
TAGATTACACAGATTACTGA TTGATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAAACTA
TAG TTACACAGATTATTGACTTCATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAA CTA
TAGATTACACAGATTACTGACTTGATGGCGTAA CTA

TAGATTACACAGATTACTGACTTGATGGCGTAA CTA
```

Take reads that make up a contig and line them up

Take *consensus*, i.e. majority vote

At each position, ask: what nucleotide (and/or gap) is here?

Complications: (a) sequencing error, (b) ploidy

Say the true genotype is AG, but we have a high sequencing error rate and only about 6 reads covering the position.