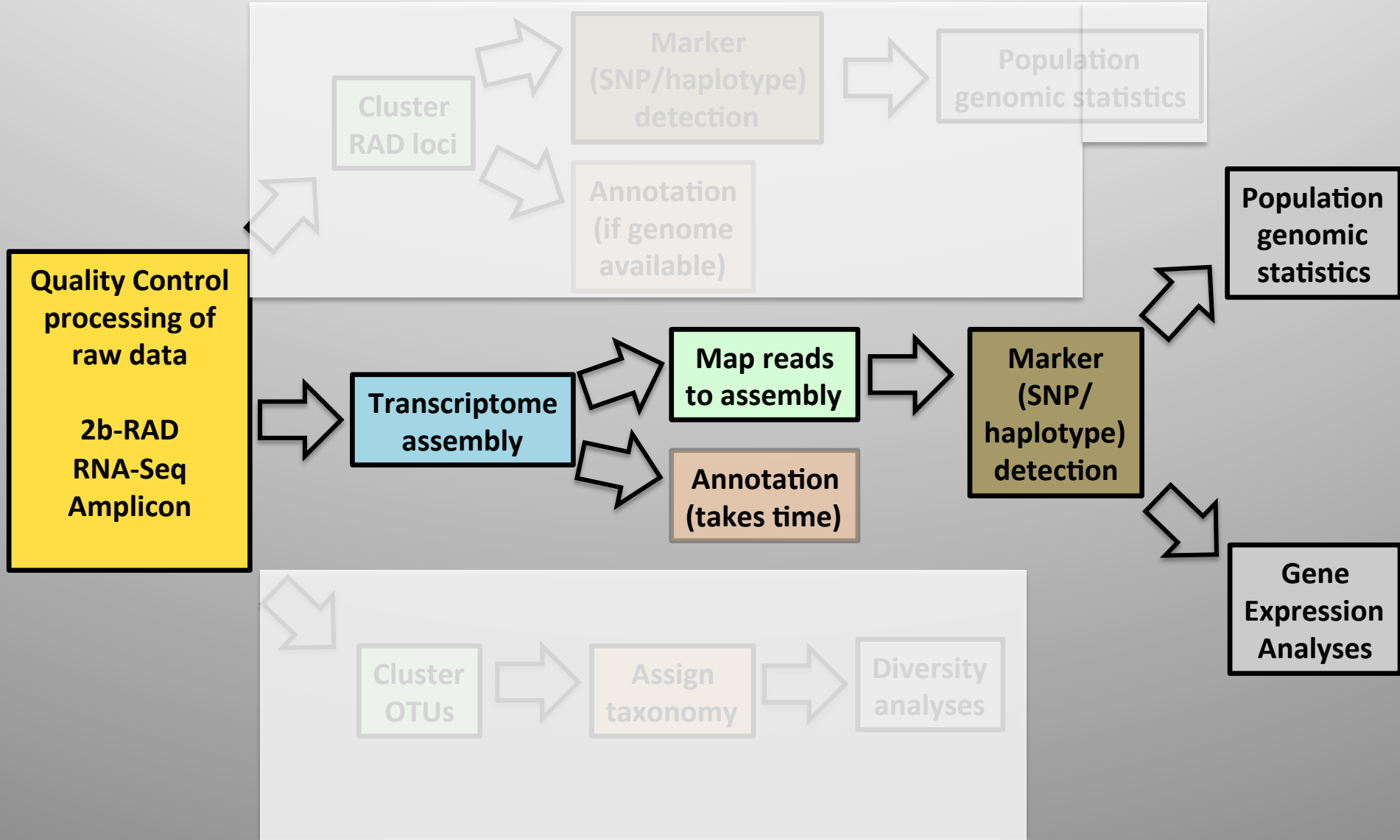

De novo assembly– software and algorithms

Analysis pipeline for RNA-Seq data



De novo genome/transcriptome assembly

Mission: combine hundreds of millions of short reads to a genome/transcriptome (with no pre-existing genomic information available).

Method: Look for regions of overlap – create *Contigs* (=contiguous sequences)

De Bruijn graphs are excellent mathematical tools for this purpose

There are many different algorithms and programs for doing this.
3 main things to weight against each other when choosing:

- Speed
- Memory requirements
- Accuracy

See Martin & Wang 2011. *Nature Reviews Genetics,* for a great review of all caveats / issues with transcriptome assembly

De Bruijn graphs

Consist of nodes (k-mers) of *k* length, connected by edges

k-1 overlap between each node.

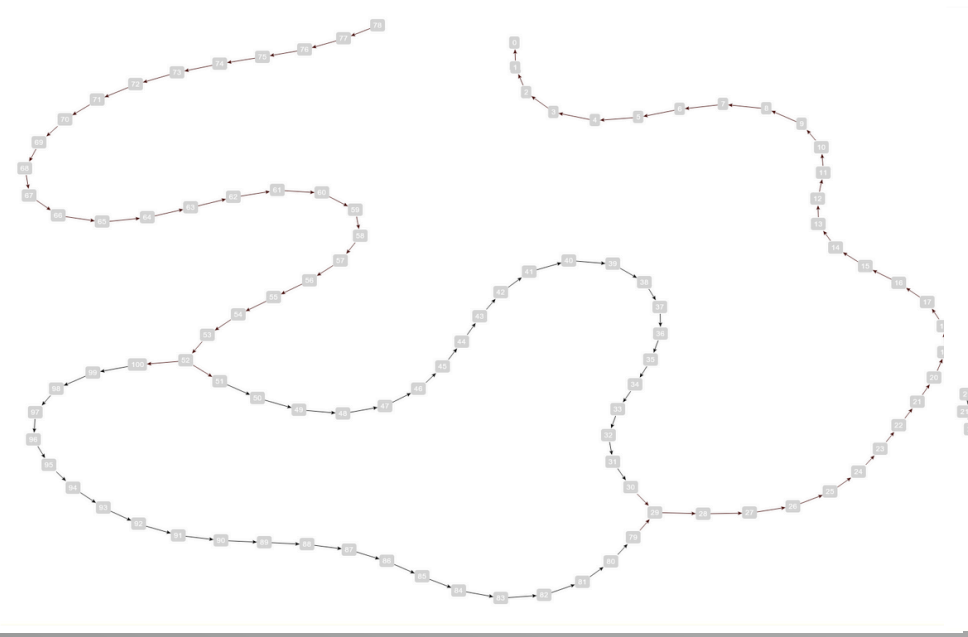
Polymorphisms cause bifurcations in the graph

Nodes connected by one edge can then be merged to save space

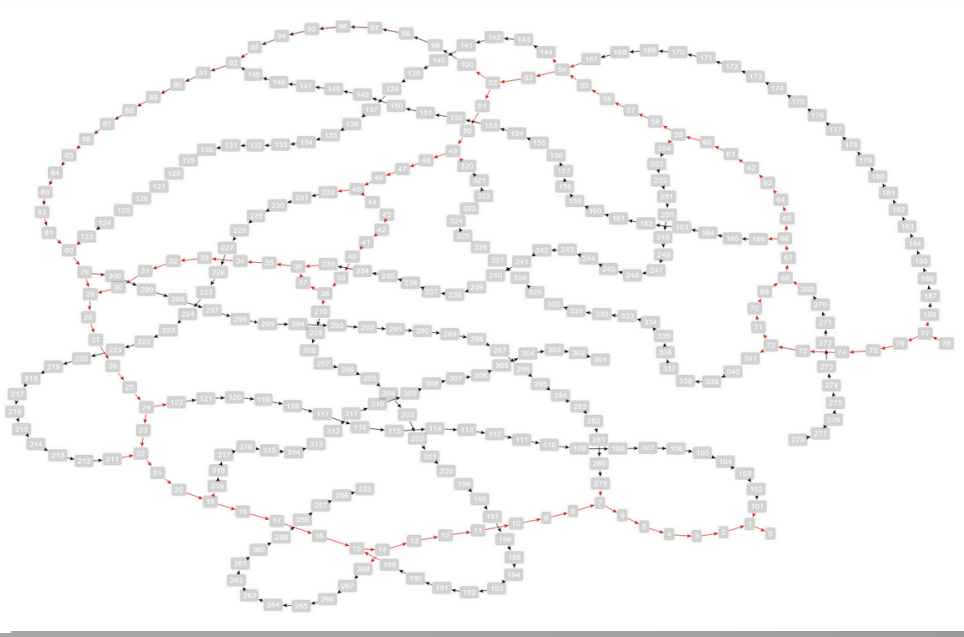
Polymorphisms result in “bubbles” with size dependent on *k*

De Bruijn graphs

1 SNP



5 SNPs



Polymorphisms in transcriptomes

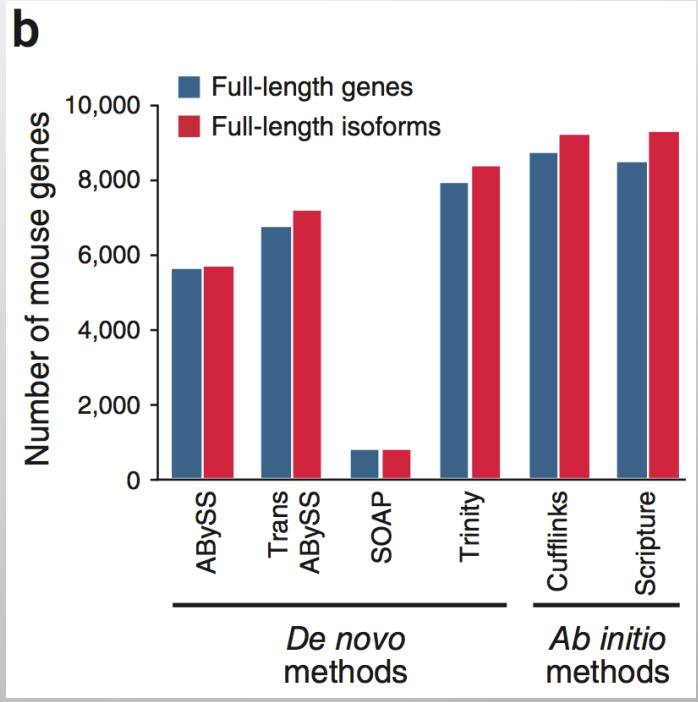
Can be due to

- Sequencing errors
- SNPs /Indels
- Alternative splicing
- Paralogous genes

Can use base frequencies to remove sequencing errors, but there is no perfect way to distinguish true polymorhisms from false positives in some cases.

Currently, Trinity seems to be the best at finding alternative isoforms/paralogs

CLC genomics workbench is a fast low-memory (but high-cost) alternative (Bräutigam et al. 2011)



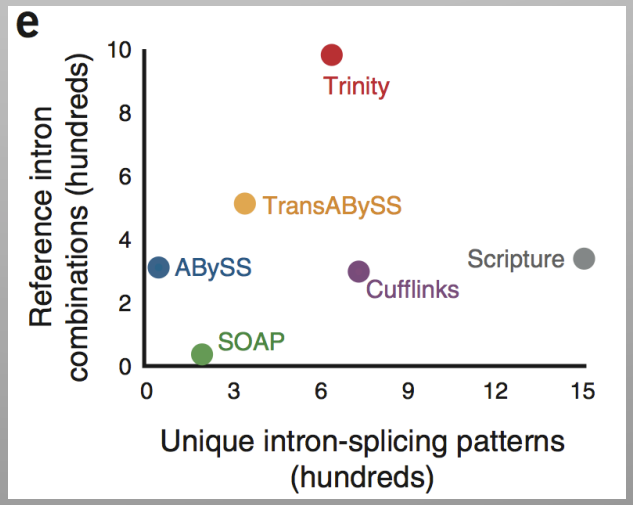


Fig 5; Grabherr et al . 2011

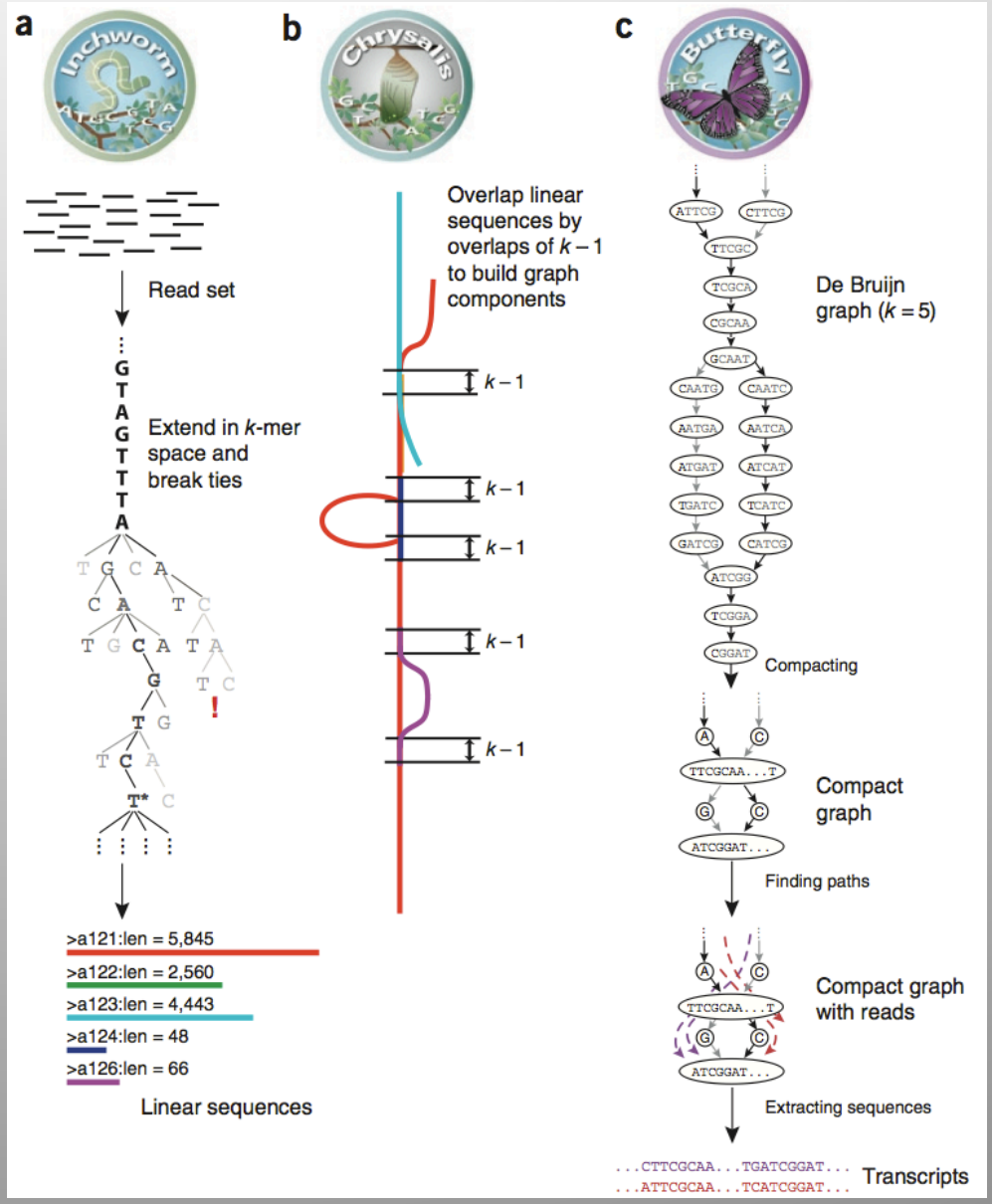
The Trinity algorithm

a. Inchworm creates a table of all k-mers in the read data
b. Chrysalis overlaps k-mers to create De Bruijn graphs
c. Butterfly compacts graphs, saves transcripts/isoforms.

Each part can be run independently, or they can be run combined.

Currently only supports k=25 for combined runs

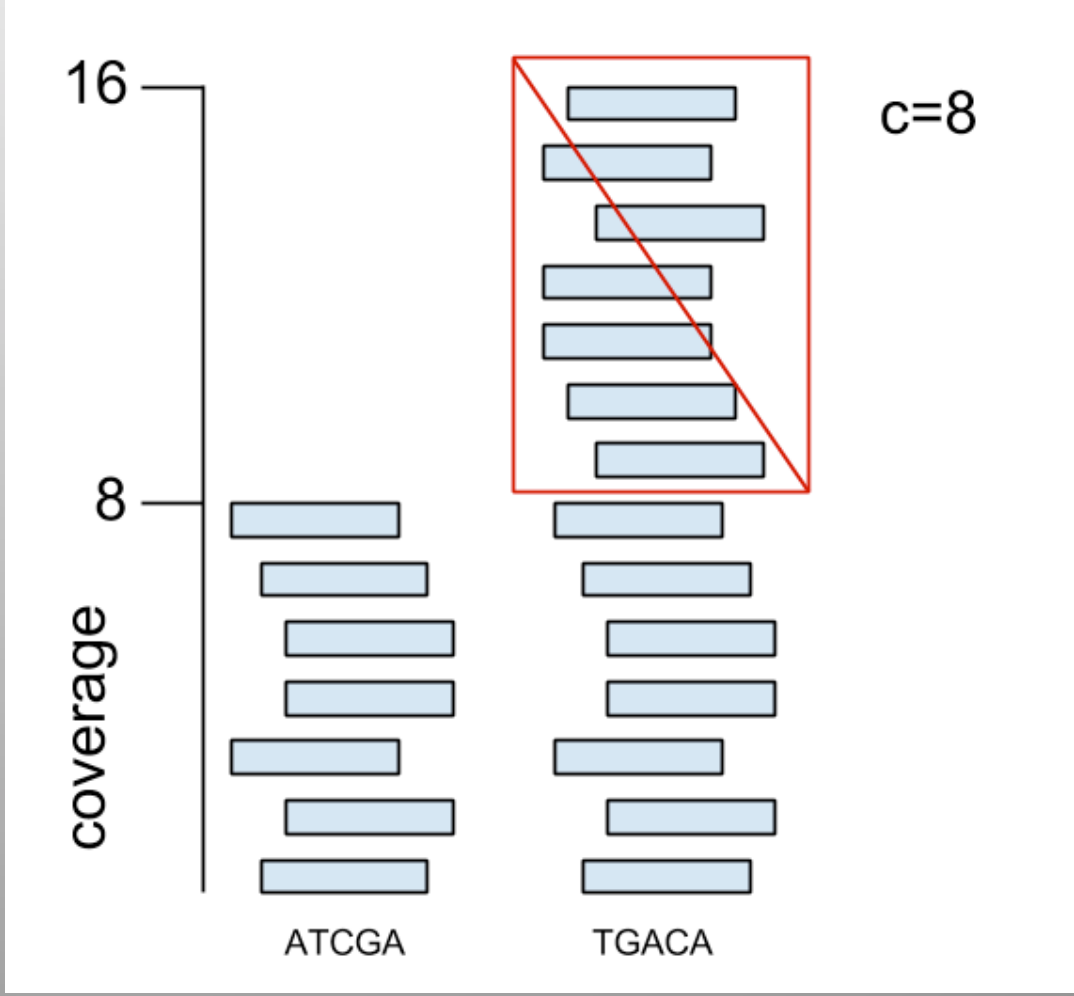
Requires ca. 1 GB RAM per million reads.



High coverage is not useful after a point

Trinity uses a digital normalization step that removes highly repeated sequences called “khmer”

Reduces computational load.

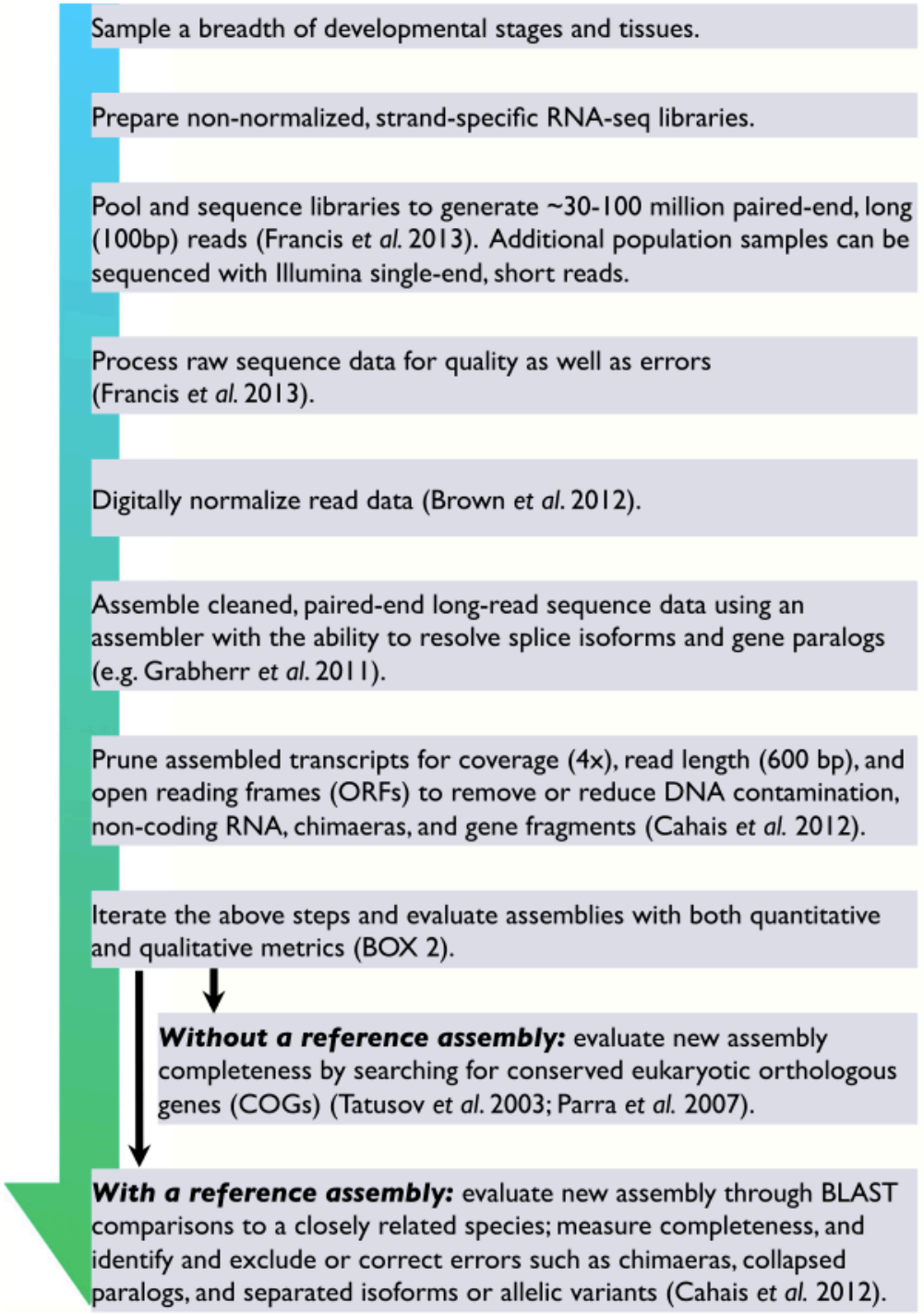


CLC genomics workbench

- Fast – low memory alternative for both genomes and transcriptomes.
- Also does many other things (QC, alignment, SNP detection, etc..)
- Uses De Bruijn graphs, but exact algorithm is proprietary
- License ca. 5000 USD /yr, but 2-week free trial versions are available for download at:

http://www.clcbio.com/products/clc-genomics-workbench/

Optimal workflow for transcriptome assembly (which we will NOT do today)



De Wit et al. (2015)