Automated annotation

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Automated annotation

- Many programs available that vary considerably in focus:
  - Some are taxon or species specific (e.g. specific to microbes, Arabidopsis)
  - Some are very specific in what they predict
    - Genes
    - Promotors / Regulatory regions
    - Repeat elements
    - CpG islands
    - Transposable elements

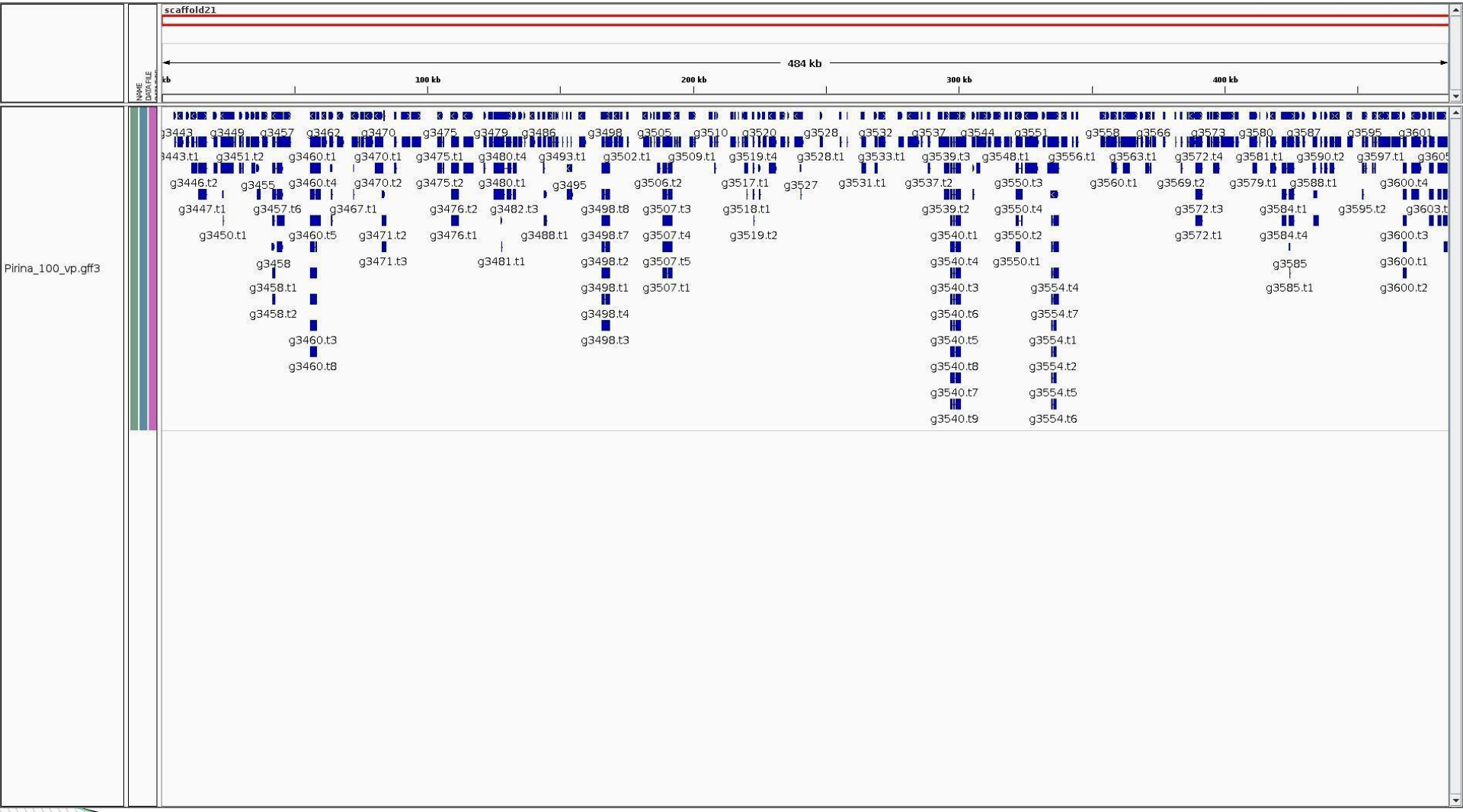
Automated annotation

- Generally, **gene prediction** programs require a set of parameters, which tell you:

  "This is what a gene tends to look like in this species"

  (Intron / exon size, splice sites, UTRs)

- These are derived from known-to-be-good gene models
  - If you don't have any known-to-be-good gene models, you can use parameters from a related species, but take it with a grain of salt
  - An RNAseq run is a good option to get data to make your own known-to-be-good gene models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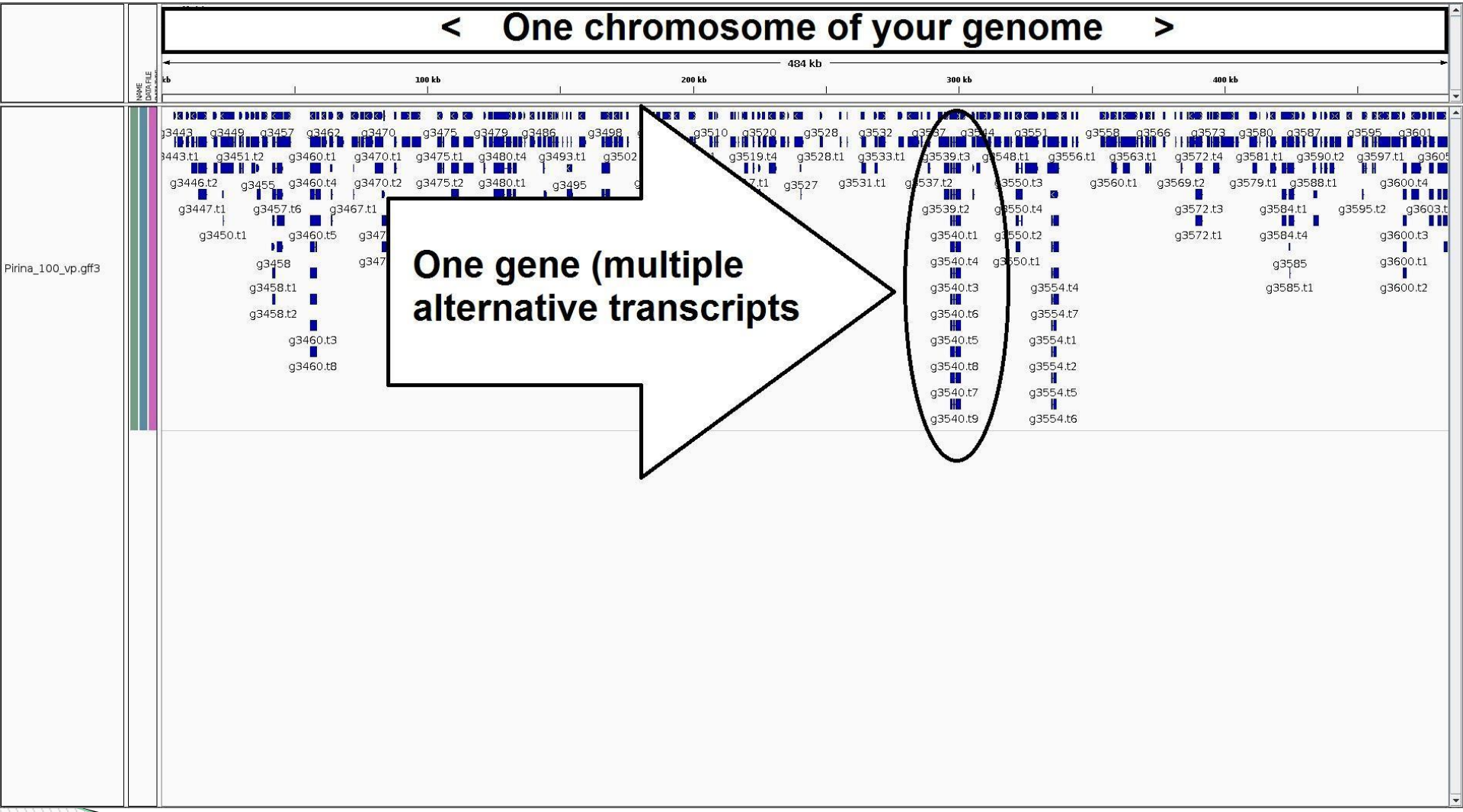
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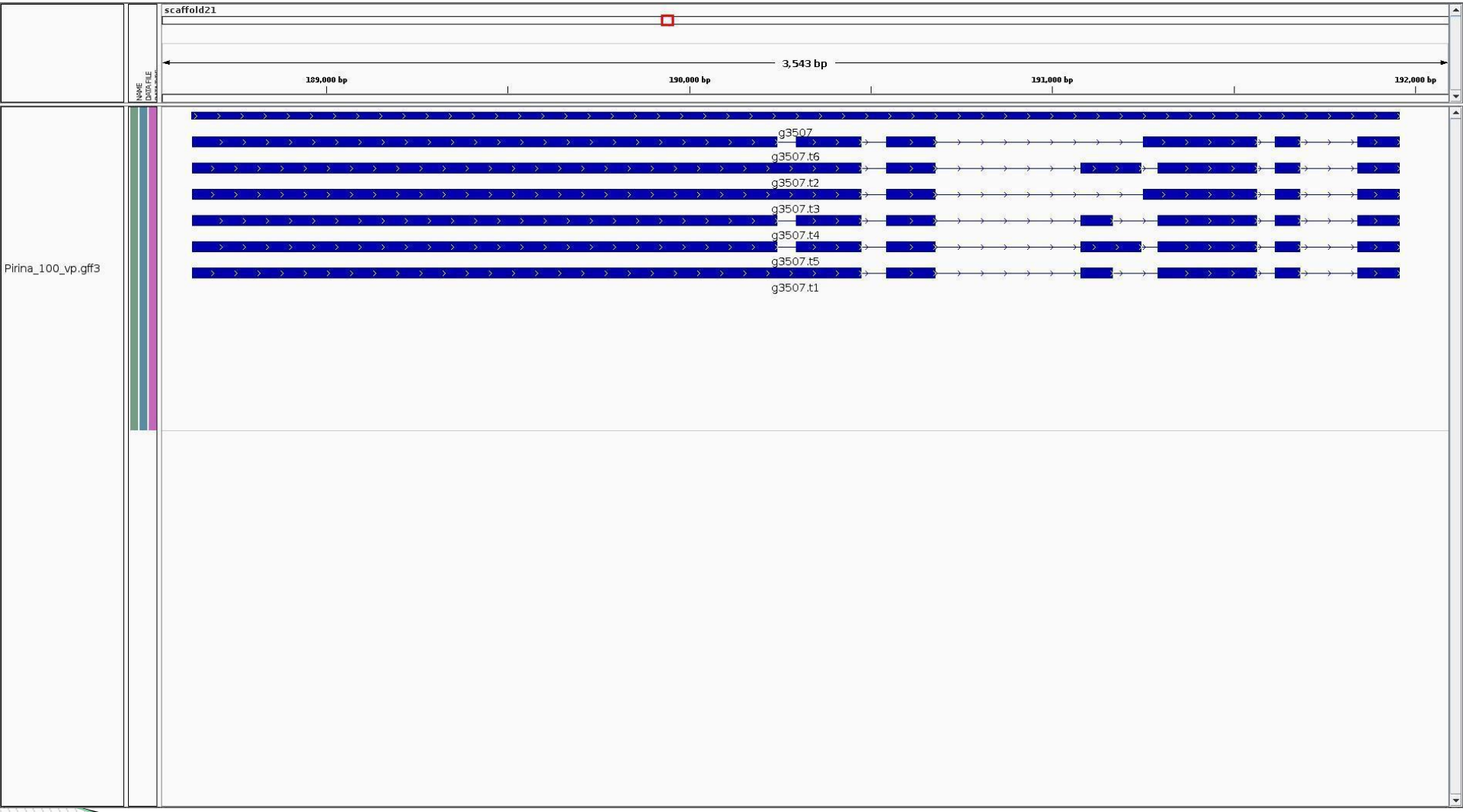
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g3507.t2
g3507.t3
g3507.t4
g3507.t5
g3507.t1

Automated annotation - other genomic features

Repeats

RepeatScout

REPET

Telomeres

TelSeq (predicts using defined telomeric repeats, requires you to map your reads to your genome)

Automated annotation - using RNAseq to improve your gene calls

Augustus can use two sources of information to improve gene calls

- cDNA sequences

ESTs, assembled cDNAs from RNAseq projects, known genes

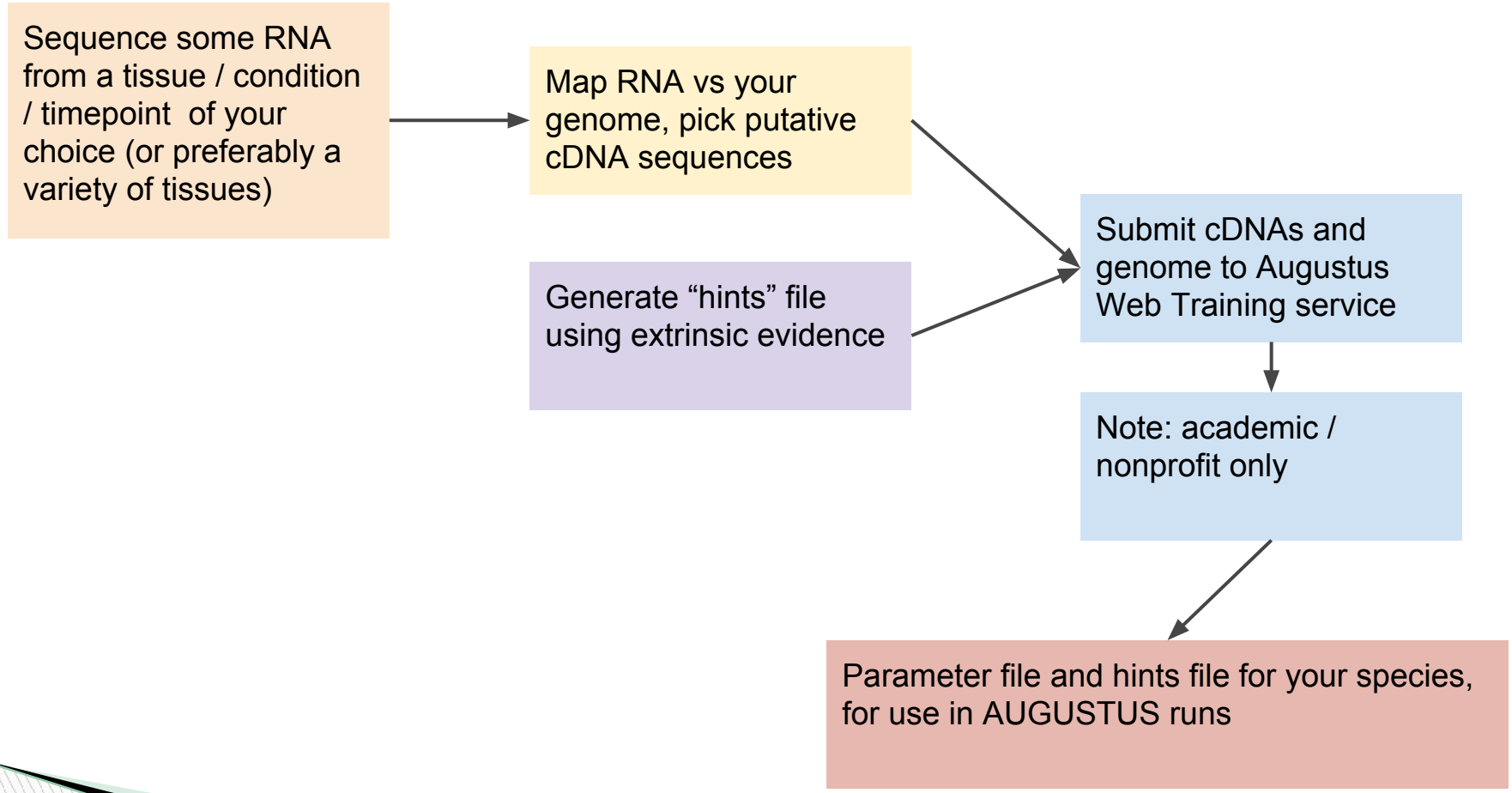
- Raw RNA reads (but not if you're using the web version)

- "Hints"

Hints are extrinsic information on other gene features, e.g. Splice sites, UTRs, non-coding regions, transcription start/stop sites.

Different types of hints can be generated from proteins (using EXONERATE), cDNA (using BLAT), or information on repeat regions (RepeatScout/REPET)

Automated annotation - using RNAseq to improve your gene calls



Viewing and using

Dan Jones

Outputs of a well-designed *de novo* genomics program

- An assembled genome, with an appropriate level of coverage (at least 30x)
- Metrics to assess the assembly quality
- Predicted genes, repeats (low-complexity regions, transposable elements)
- Annotation of the annotations: what are my predicted genes most similar to?
- Mitochondrial and (Chloroplast) genomes
  - Depending on your tissue of origin, organelle genomes will often have a much higher level of coverage than the nuclear genome
- All of the above, in a searchable form, and visualised in a genome browser

Browsers

Free: **IGV**

Tablet (but only for mapped reads)

Gbrowse (not simple to setup, but useful, fast and can be web-facing)

ENSEMBL browser (they do workshops on it!)

ARGO (Broad institute)

Commercial: **Geneious**

CLC Bio

Making your genome searchable

You can (and should) make your genome into a BLAST database to enable searching against this

- Easy to do on command-line (see exercise)
- Many programs like Geneious can do this for you

Collaboration

- Many organisations offering to host your genome in their browser
  - IGV hosts several genomes
  - 1000 fungal genomes

..although microbial genomes + annotations are probably small enough to email around!

- Web-facing browsers like Gbrowse enable you to setup your own website/browser
  - PFR Strawberry genome is a good example

Where to go for help

- SeqAnswers!

www.seqanswers.com

How can we tell if a genome assembly is any good?

N50 (or L50) and L50 (or N50)

N50: Given a set of sequences of varying lengths, the N50 length is defined as the length N for which 50% of all bases in the sequences are in a sequence of length L < N.

L50: The number of contigs that are smaller than the N50 contig

(Why is the N50 a length, and the L50 a number?)
(Contradictory definitions and arguments in SeqAnswers)
(http://seqanswers.com/forums/showthread.php?p=41420)

"Old" methods

Other metrics

Number of contigs
Mean contig length
Average contig length
Average / Mean / Number of contigs of all contigs above a certain size (not always explicitly reported!!)

“Old” methods

Other metrics

Mate-pair analysis: are mate-pairs acting as expected?

Coverage analysis: what's the coverage and how variable is it?

Repeat analysis: do de novo repeat tools (like TRF) match the expected characteristics of the genome?

Breakpoint analysis: where do scaffolds end and why?

"Old" methods

ALE: Assembly Likelihood Evaluator (http://www.ncbi.nlm.nih.gov/pubmed/23303509)

CGAL: Computing Genome Assembly Likelihoods (http://genomebiology.com/2013/14/1/R8)

QUAST: Quality Assessment Tool for Genome Assemblies (http://www.ncbi.nlm.nih.gov/pubmed/23422339)

REAPR (http://www.ncbi.nlm.nih.gov/pubmed/23710727)

LAP (http://www.biomedcentral.com/content/pdf/1756-0500-6-334.pdf)

Mauve (http://www.ncbi.nlm.nih.gov/pubmed/21810901)

AMOSvalidate (http://www.ncbi.nlm.nih.gov/pubmed/18341692)

Plantagora (http://www.plantagora.org/tools_downloads/)

New methods

ALE: Assembly Likelihood Evaluator
(http://www.ncbi.nlm.nih.gov/pubmed/23303509)

Can be used for metagenome or genome assemblies

Does not require reference

Produces a single “ALE” score of likelihood

New methods

CGAL: Computing Genome Assembly Likelihoods
(http://genomebiology.com/2013/14/1/R8)

Can be used for genome assemblies

Does not require reference

Produces a single “score” of likelihood

New methods

QUAST: Quality Assessment Tool for Genome Assemblies (http://www.ncbi.nlm.nih.gov/pubmed/23422339)

Can be used with or without a reference genome

Produces a number of stats in graphical form

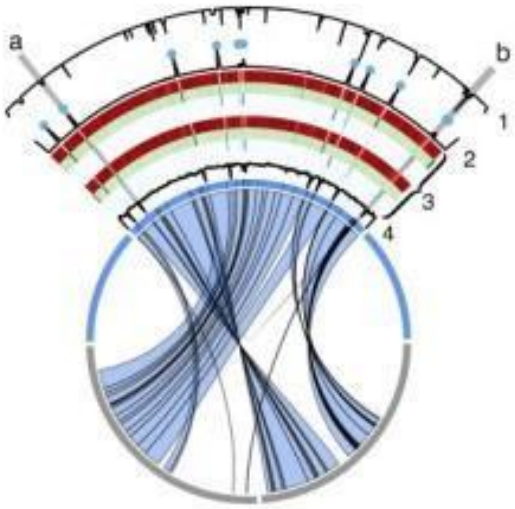
- Trends of stats across the genome (mismatches, indels)
- No. of predicted genes (using GeneMark or Glimmer)

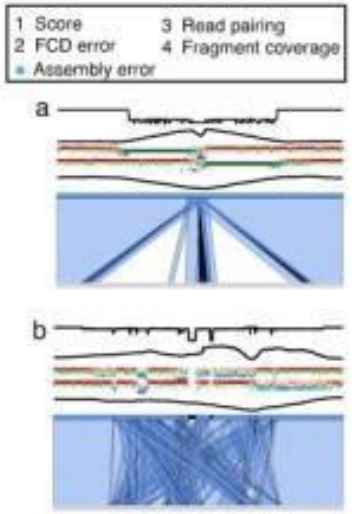
New methods

REAPR (http://www.ncbi.nlm.nih.gov/pubmed/23710727)

Does not require a reference genome

Produces a number of stats in graphical form, some of which are very groovy





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