Annotation of genomes and transcriptomes

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Annotation is not like annotation

Structural annotation:

The process of identifying genes, their intron-exon structures and for transcriptomes their isoforms. Recently also ncRNA like tRNAs, µRNAs, retroposons, line elements and so forth.

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Functional annotation:
The process of attaching meta-data such as gene names, gene family, gene ontology terms to structural annotations.

Only minimaly necessary for phylogenomics (as off here)
Orthology determination

Most important

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Quality of assembly

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Depends on purpose

Quality parameters

contigs & scaffolds
total bp
shortest & longest contigs | scaffolds
average length of contigs | scaffolds
GC%

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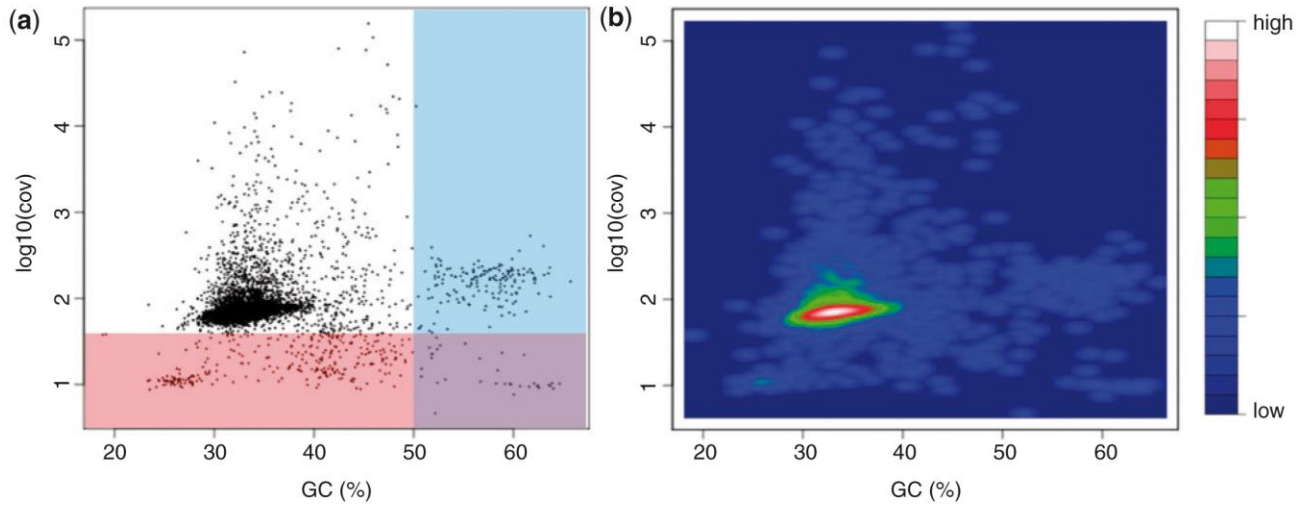

FIG. 1.—Aggregate properties (coverage and GC content) of contigs obtained by Celera 7.0 as (*a*) scatter plot and (*b*) heat map. Colored areas in (*a*) illustrate the aggressive cleaning strategy adopted in this study, that is, the removal of putative nontarget contigs of coverage <40× (red; putative host contamination) and GC content >50% (blue; putative bacterial contamination) (see supplementary file S1, Section A for details, Supplementary Material online).

Hahn et al. (2014) Genome Biology & Evolution

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gene coverage → # genes found/# genes tested

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coverage → total sequenced bp/known genome size
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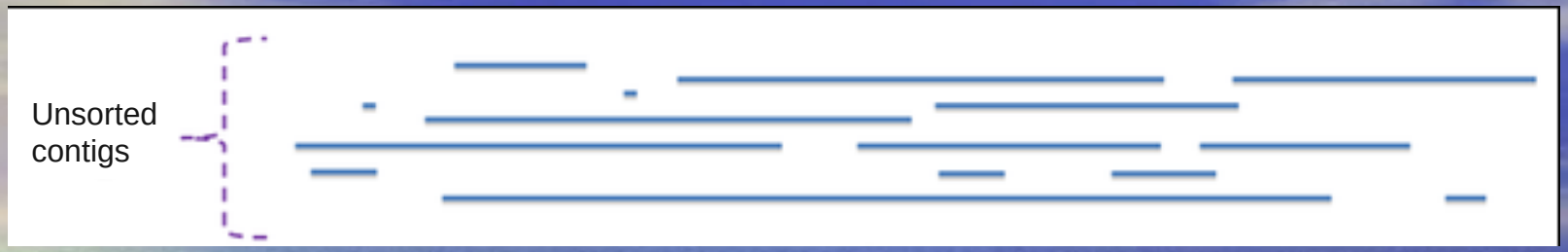
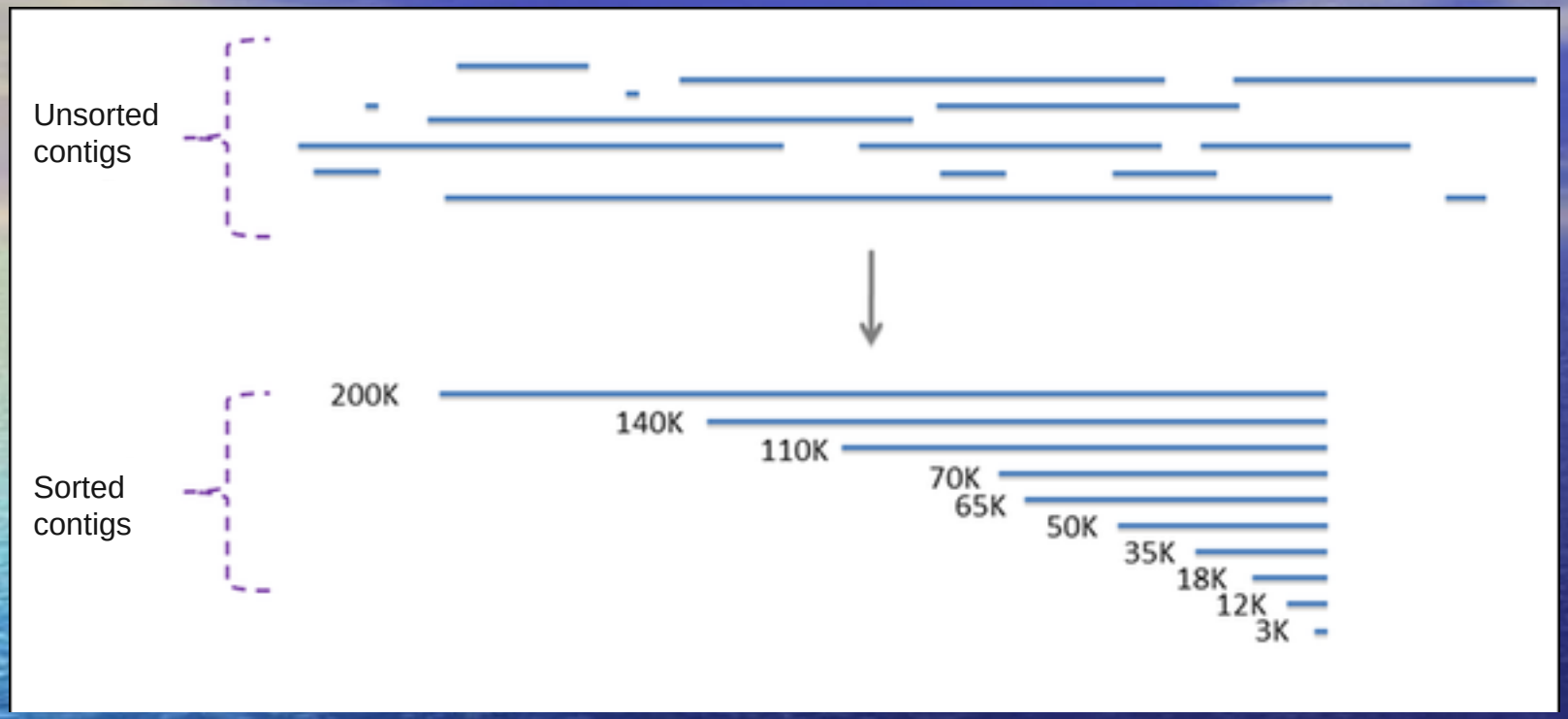
CEGMA → screening set of universal, single-copy genes

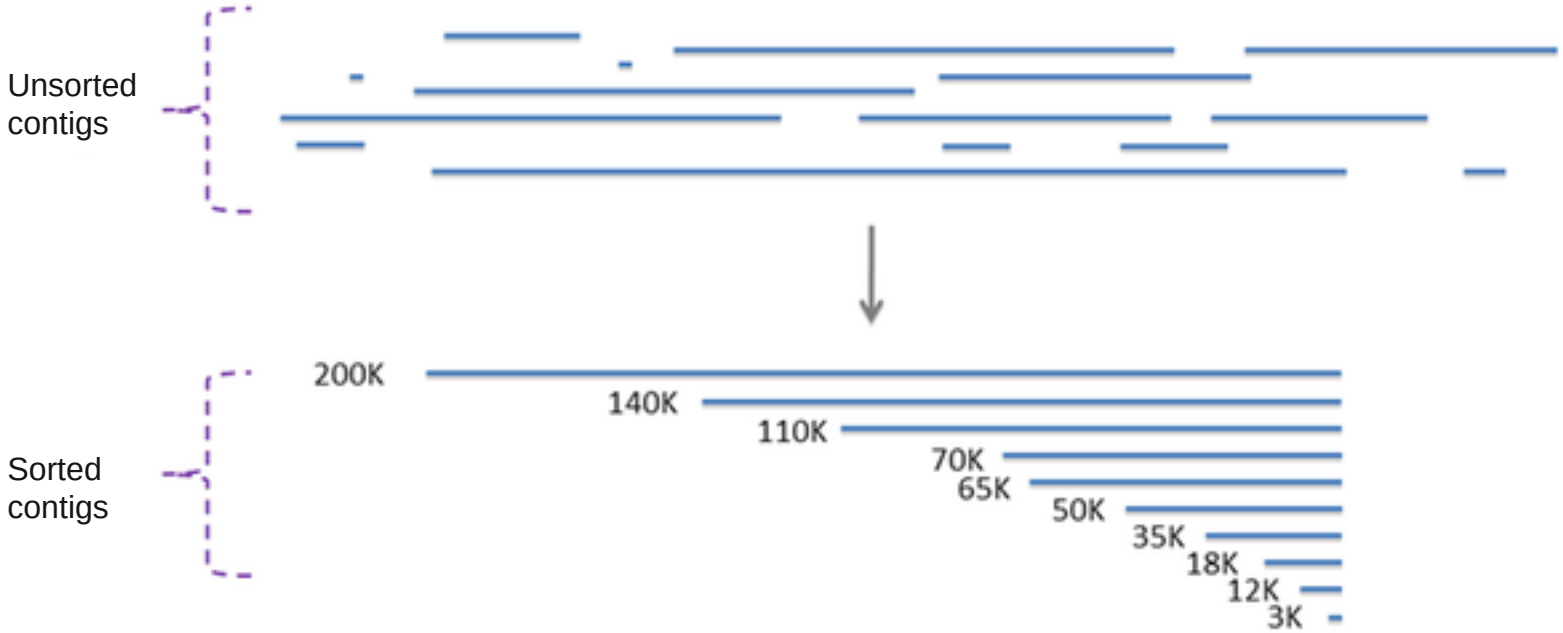
N50 & L50



N50 & L50


Unsorted contigs
Sorted contigs
200K
140K
110K
70K
65K
50K
35K
18K
12K
3K

N50 & L50

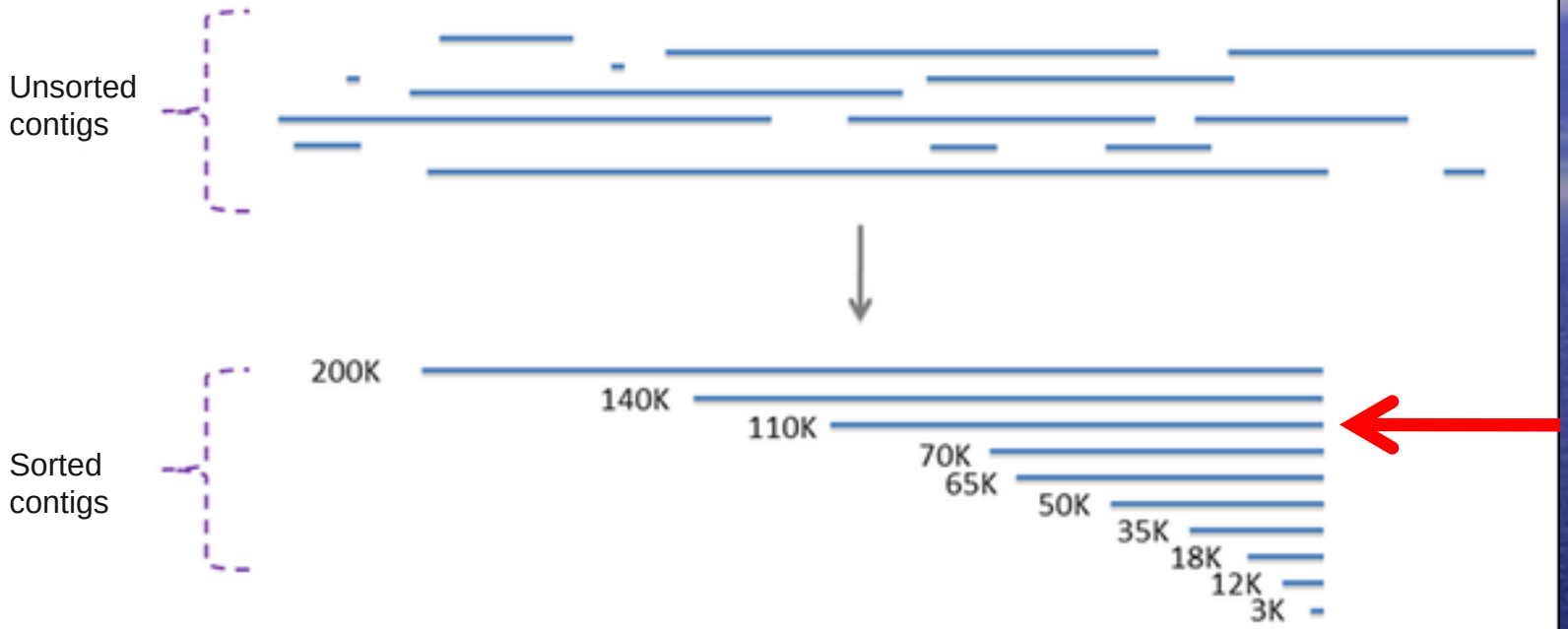


Total contig length= 200K + 140K + 110K + 70K + 65K + 50K + 35K + 18K + 12K + 3K= 703K

50% total contig length= 703K x 50%= 351.5K

200K + 140K + **110K** > 351.5K → N50= 110K

N50 & L50



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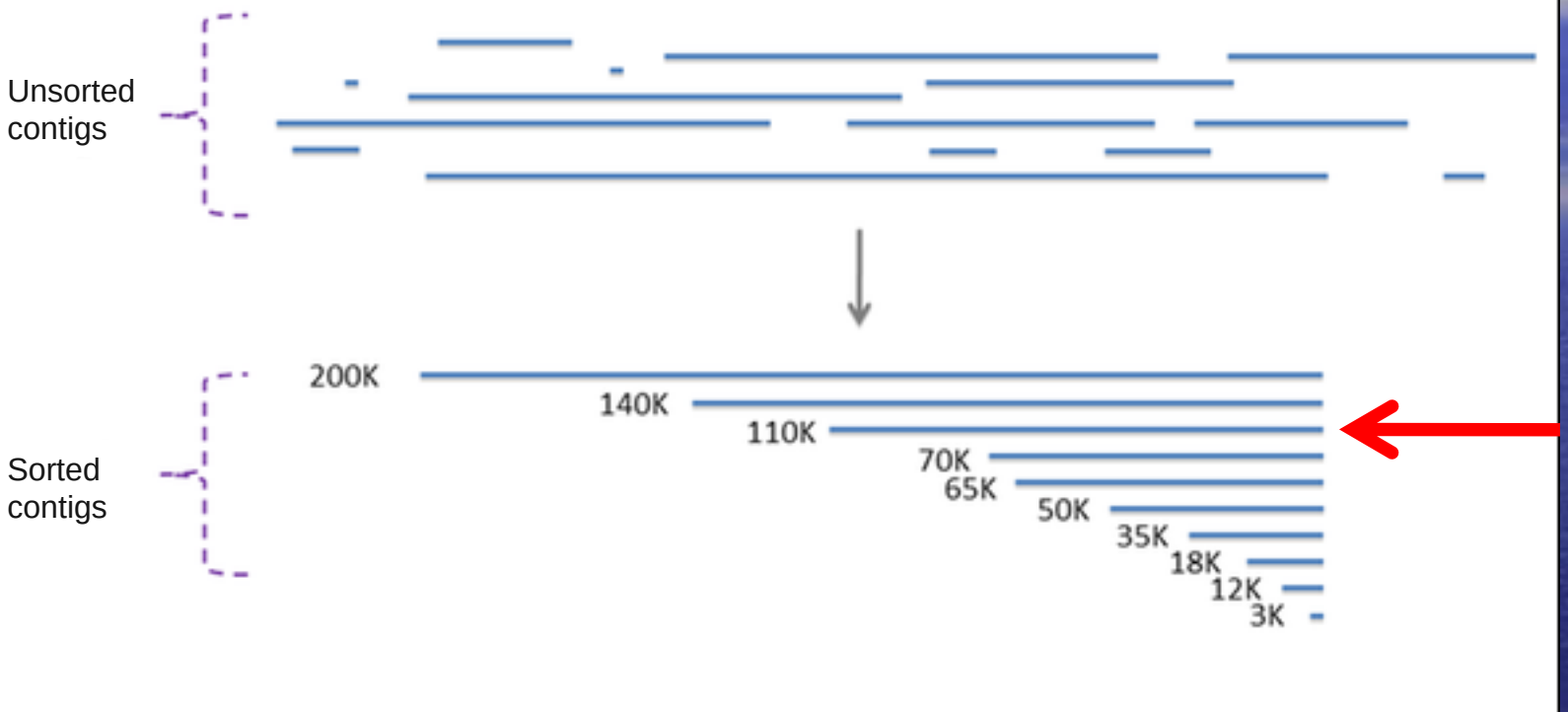

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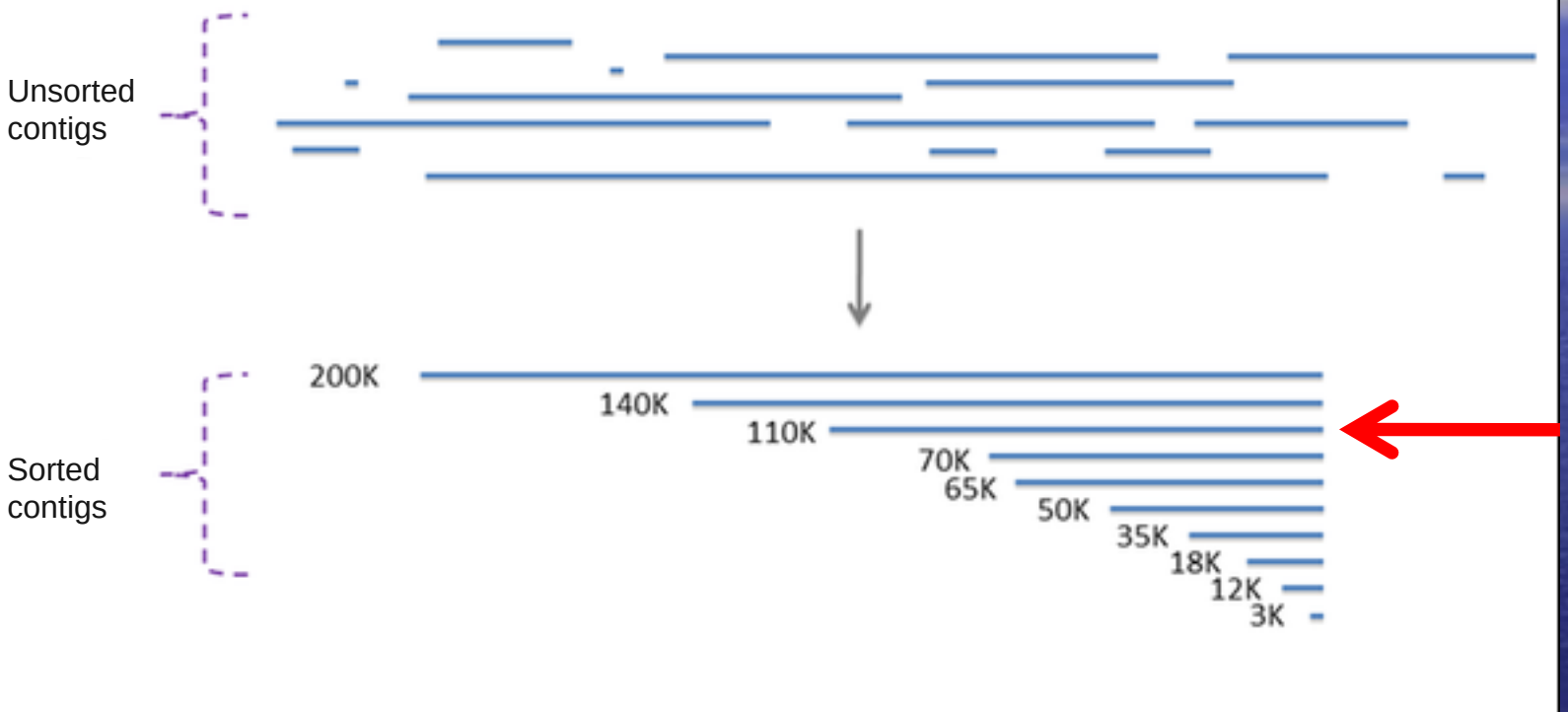

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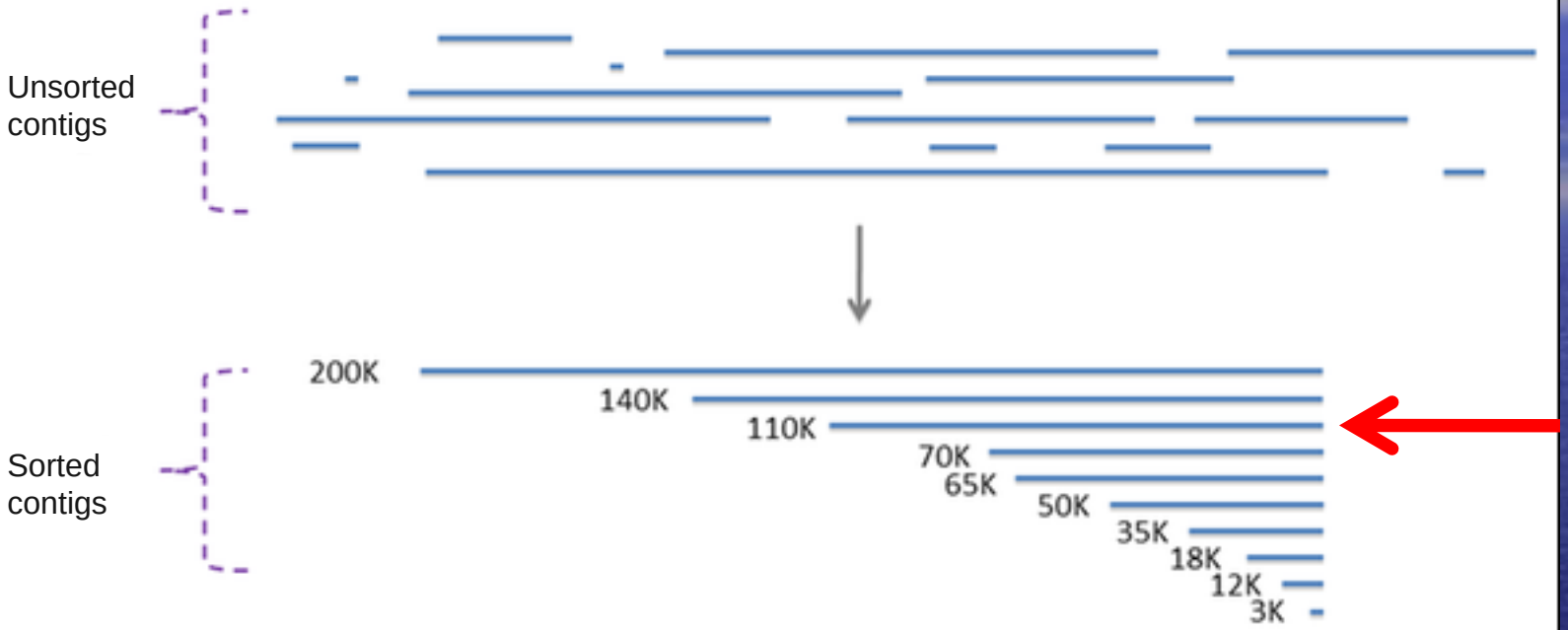
N50 & L50



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Same principals apply to scaffolds

While there are correlations in assembly statistics...

Table 1 Metric trends and consistency

Metric	Trend by sequencing depth	Consistent over sequencing depths	Trend by read length	Consistent over read lengths	Fully consistent metric
Contig count	↗	✓	↘	✓	✗
% of reads used in contigs	↗	✓	↗	✓	✓
BP in contigs	↗	✓	↗	✓	✓
% BP in contigs	↗	✓	↗	✓	✓
Average contig coverage	↗	✗	↗	✗	✗
Average unigene coverage	↗	✓	↗	✓	✓
Contig read count COV	⤵	✓	↘	✓	✗
Unigene read count COV	⤵	✓	↘	✓	✗
Average contig length	↗	✗	↗	✗	✗
Average unigene length	↗	✓	↗	✓	✓
Contig N50 length	↗	✗	↗	✗	✗

O'Neil & Emrich (2013) *BMC Genomics*

Metrics are considered consistent if they consistently ranked perfectly assemblies as better; fully consistent metrics are consistent metrics with similar monotonic trends by sequencing depth and read length for perfect assemblies.

…what represents a "good" assembly is debatable

(what is "truth" when it's unknown to start with?)

Hunt *et al. Genome Biology* 2013, **14**:R47
http://genomebiology.com/2013/14/5/R47

Genome Biology

SOFTWARE — Open Access

REAPR: a universal tool for genome assembly evaluation

Martin Hunt[1], Taisei Kikuchi[1,2], Mandy Sanders[1], Chris Newbol

BIOINFORMATICS APPLICATIONS NOTE
Vol. 29 no. 8 2013, pages 1072–1075
doi:10.1093/bioinformatics/btt086

Genome analysis

Advance Access publication February 19, 2013

QUAST: quality assessment tool for genome assemblies

Alexey Gurevich[1,*], Vladislav Saveliev[1], Nikolay Vyahhi[1] and Glenn Tesler[2]
Academic University, Russian Academy of Sciences, St. Petersburg
s, University of California, San Diego, La Jolla, CA 92093-0112, USA

O'Neil and Emrich *BMC Genomics* 2013, **14**:465
http://www.biomedcentral.com/1471-2164/14/465

BMC Genomics

RESEARCH ARTICLE — Open Access

Assessing *De Novo* transcriptome assembly metrics for consistency and utility

Shawn T O'Neil[1,2] and Scott J Emrich[2*]

Bradnam *et al. GigaScience* 2013, **2**:10
http://www.gigasciencejournal.com/content/2/1/10

(GIGA)^n SCIENCE

RESEARCH — Open Access

Assemblathon 2: evaluating *de novo* methods of genome assembly in three vertebrate species

EBP standard based on vertebrate genomes

1) Species with sufficient DNA and tissue:
   Minimum reference standard of **6.C.Q40**
   - N50 contig > 1 Mb
   - N50 scaffolding = chromosomal scale
   - error rate < 1/10,000 (i.e., Q40)
   - Additional criteria:
     - < 5% false duplications
     - > 90% kmer completeness
     - > 90% sequence assigned to candidate chromosomal sequences
     - > 90% single copy conserved genes (e.g. BUSCO)
     - > 90% transcripts from the same organism mappable

2) Challenging species with limited DNA or material (<~100ng DNA)
   Minimum reference standard of **4.5.Q40**
   - N50 contig > 10kb
   - N50 scaffold > 100kb
   - error rate < 1/10,000 (i.e., Q40)

BLAST

Target specific contigs & “fish” them out

– BLAST and its variants (Altschul et al. (1990) J. Mol. Biol.)

– Cited 50K+ times

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Query/Queries

– What is being compared to the DB sequences

- Nucleotides or amino acids coming from assemblies

Subject/Subjects

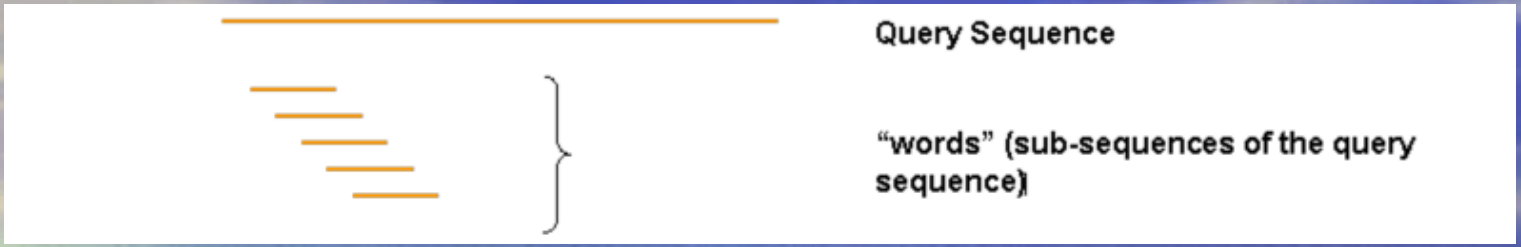
– DB sequences being compared to

- Nucleotides or amino acids potentially coming from other sources

BLAST – how it works

Query Sequence

BLAST – how it works



BLAST – how it works

The BLAST Search Algorithm

query word (*W* = 3)

```
Query:  GSVEDTTGSQSLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVEDAELRQTLQEDL
```

neighborhood words

PQG	18
PEG	15
PRG	14
PKG	14
PNG	13
PDG	13
PHG	13
PMG	13
PSG	13
PQA	12
PQN	12
etc...	

neighborhood score threshold (*T* = 13)

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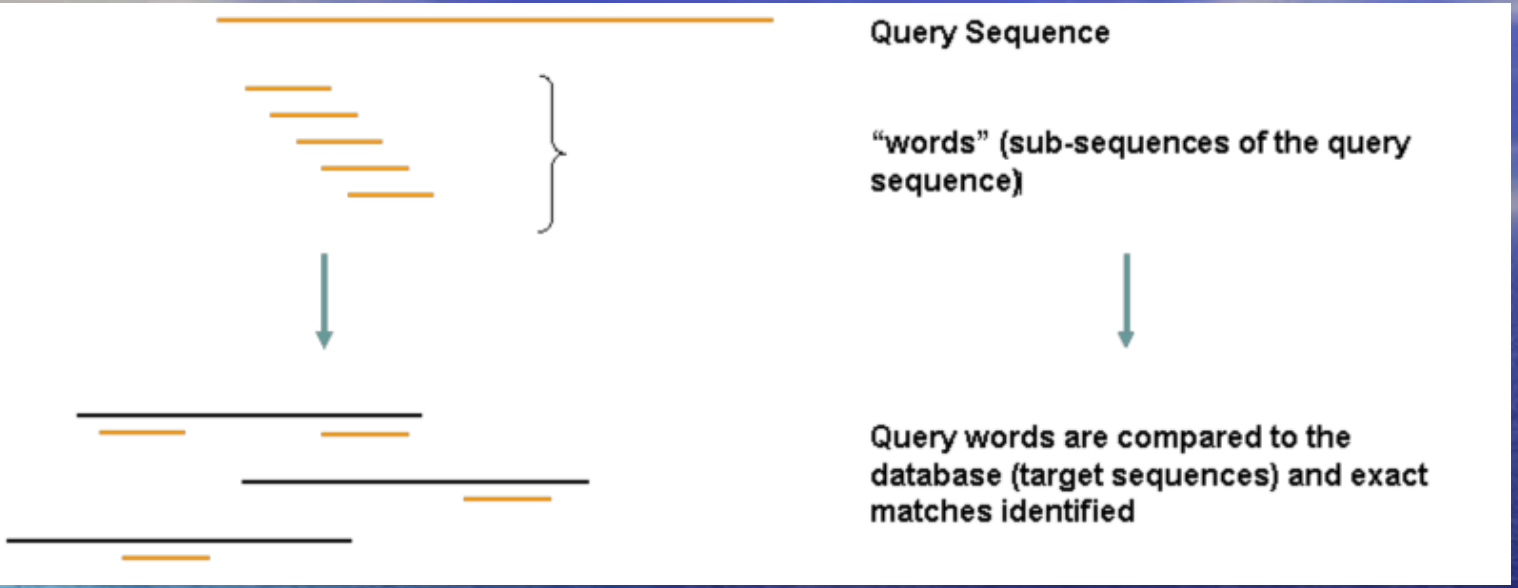

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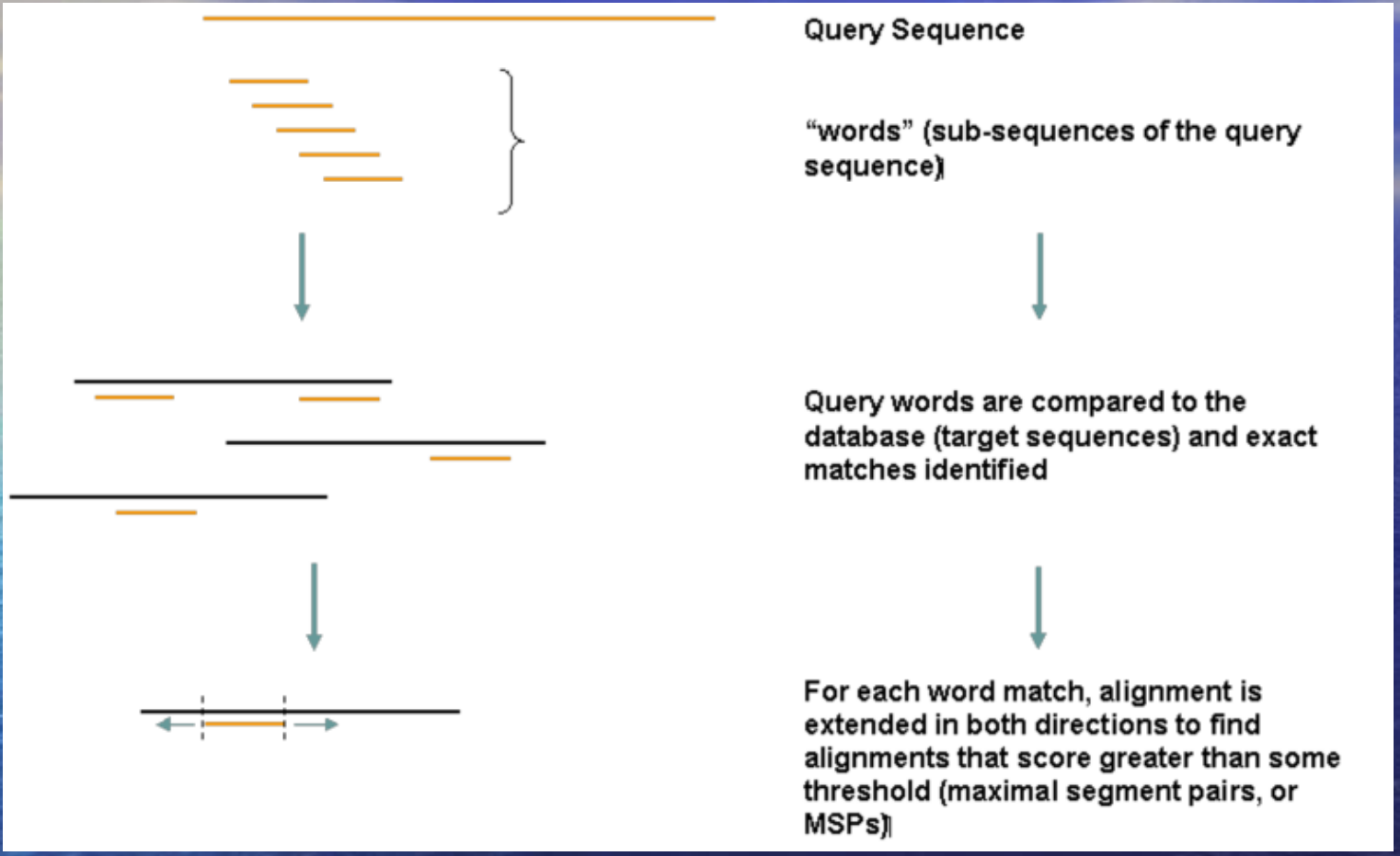

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etc...	

neighborhood score threshold (*T* = 13)

```
Query:  325 SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA 365
            +LA++L+   TP G R++ +W+  P+ D   + ER   + A
Sbjct:  290 TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA 330
```

High-scoring Segment Pair (HSP)

BLAST – on the command line

Tools in the BLAST+ CLI suite

Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast, delta-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

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blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

makeblastdb = to make BLAST DBs from subject sequences