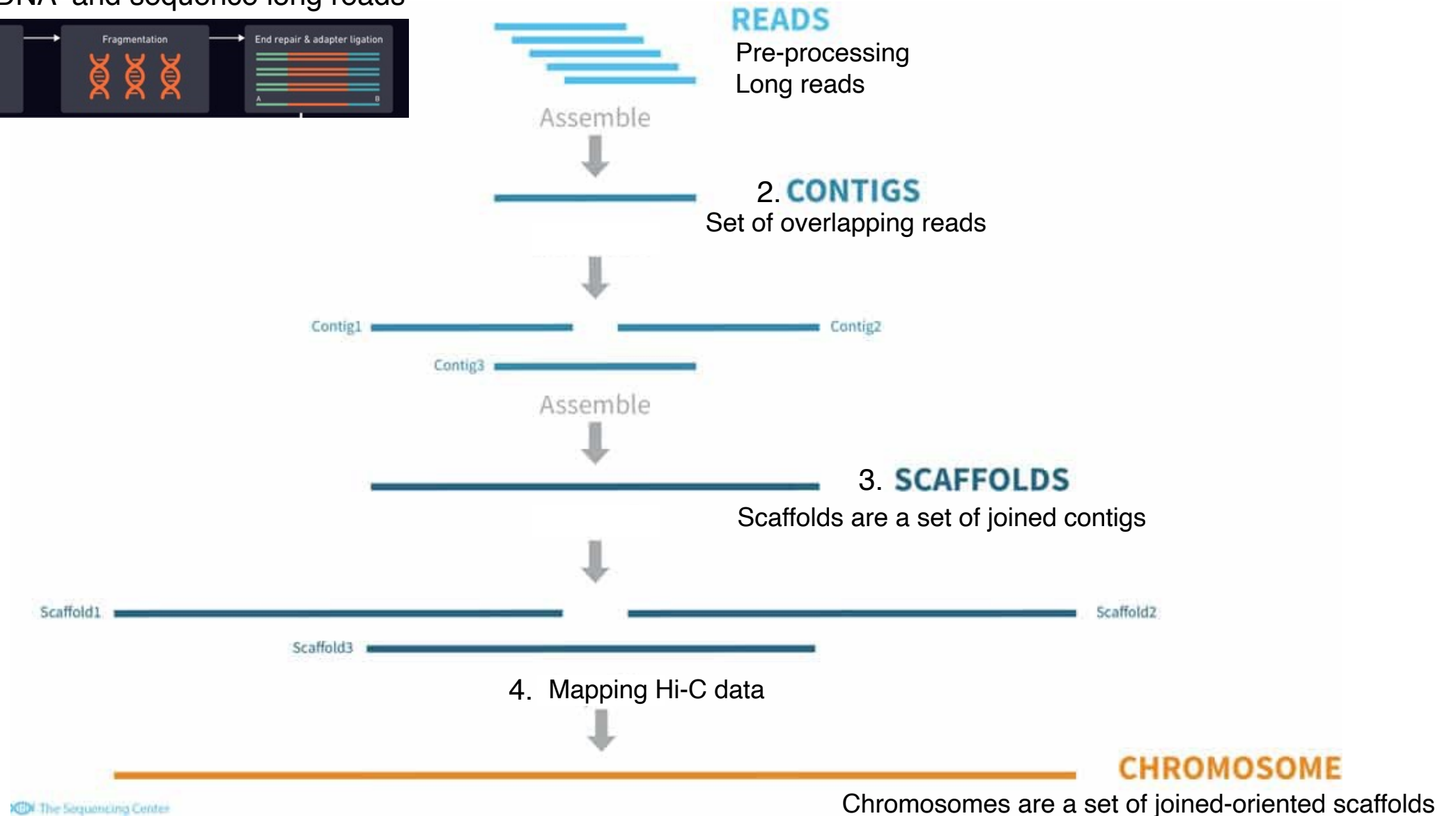
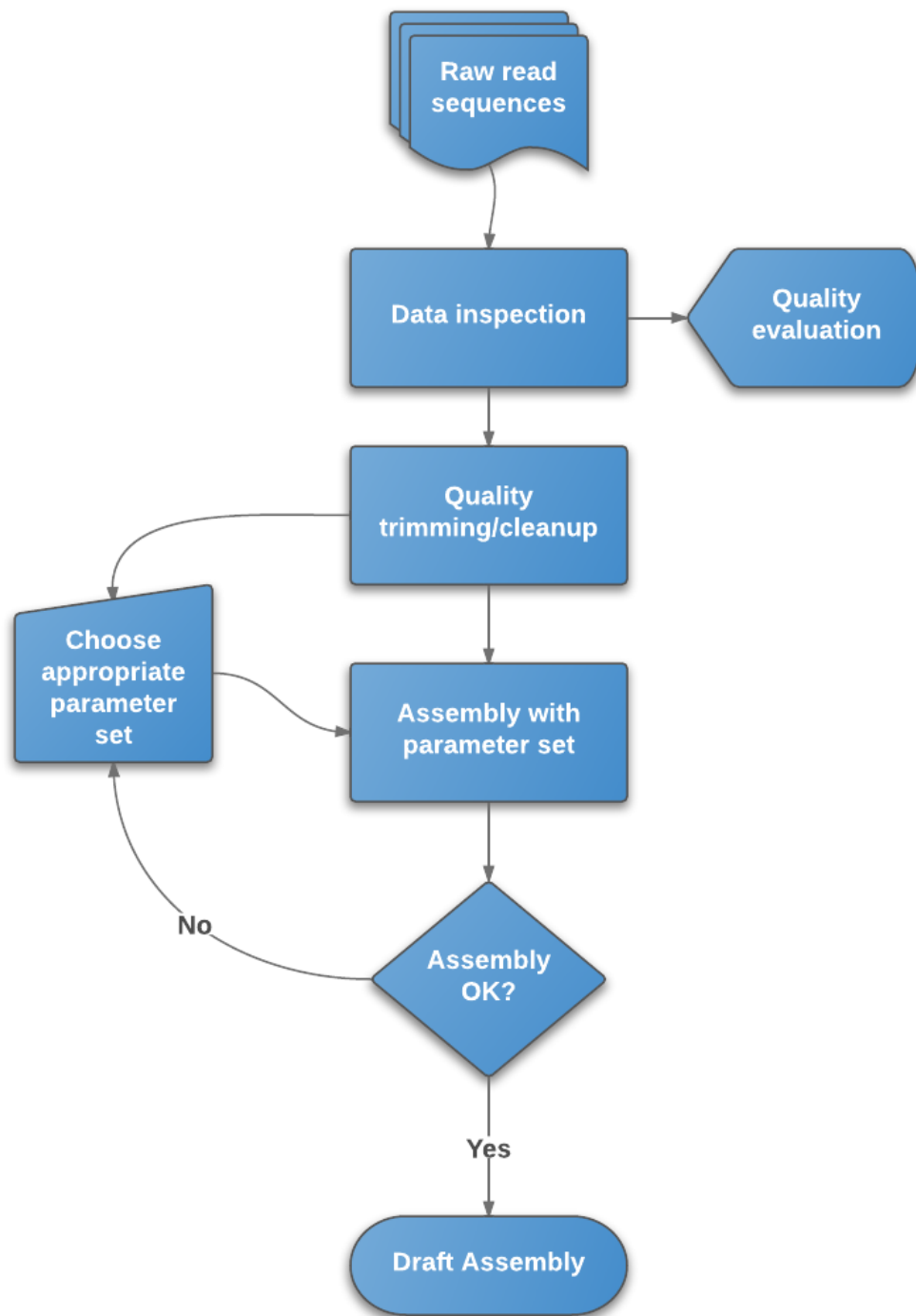


Genome assembly
Biodiversity
genomics
course
Tena-Ecuador 2024



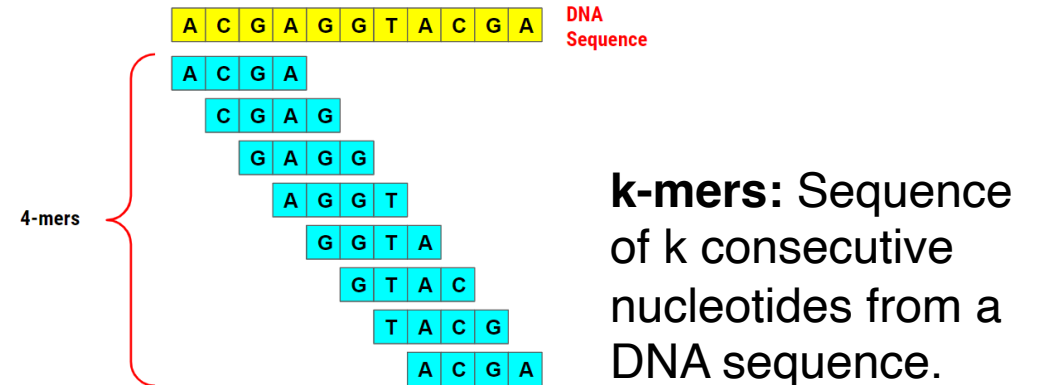
1. Extract DNA and sequence long reads





1. Sequencing of the reads

- Min. length is **500-1000 bp** for long reads (e.g., PacBio, Oxford Nanopore).
- Raw read sequences is usually stored in a **FastQ** file.
- Reads must overlap by a minimum number of base pairs, or **k-mers**, before they can be mapped together.



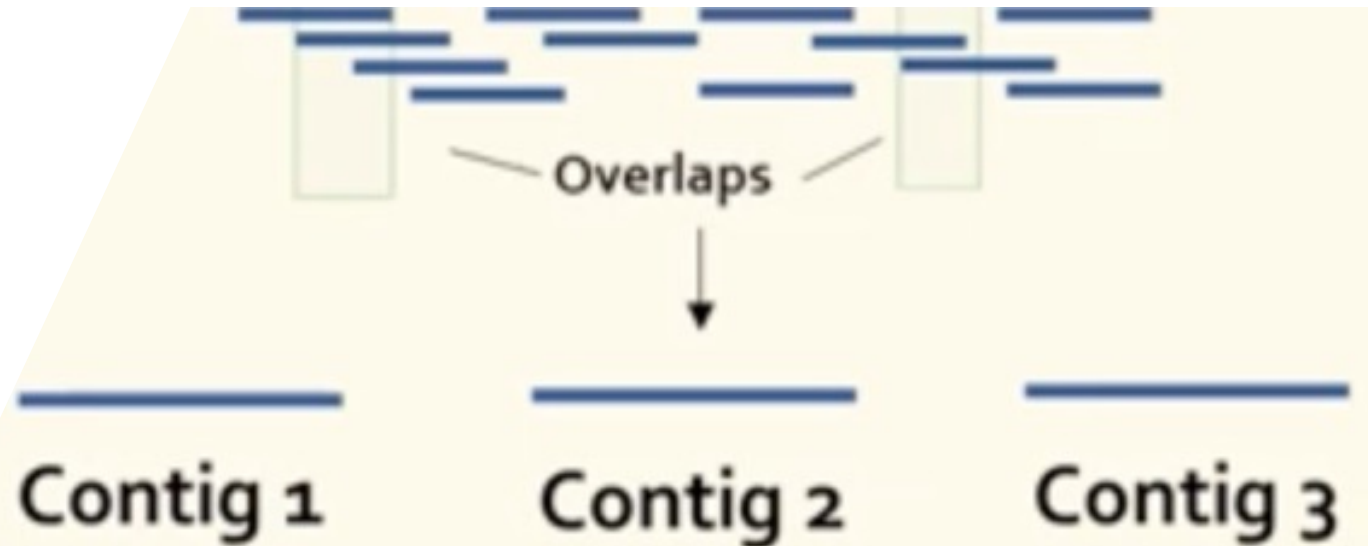
2. Assembling the reads

Aligned reads

```
TGAAGTCCTACAGTCATAGTC
AAGTCCTACAGTCATAGTCGA
GTCCTACAGTCATAGTCGATA
CCTACAGTCATAGTCGATATT
TACAGTCATAGTCGATATT
```

Consensus contig TGAAGTCCTACAGTCATAGTCGATATT

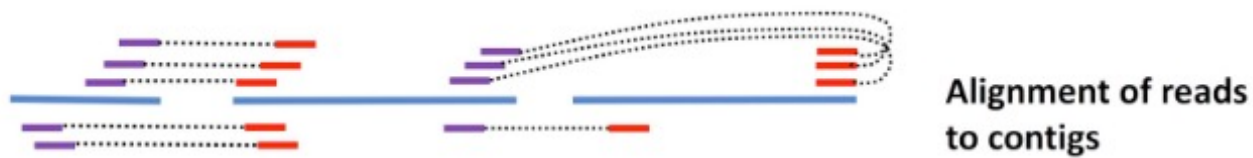
Although the contigs are longer than reads and do not have gaps, they are not large enough to cover or represent entire chromosomes.



Canu
Trinity
SPAdes
Flye
...

3. Scaffolding (Assembling the contigs)

1. Mapping HiC data to Contigs:



This mapping process aims to:

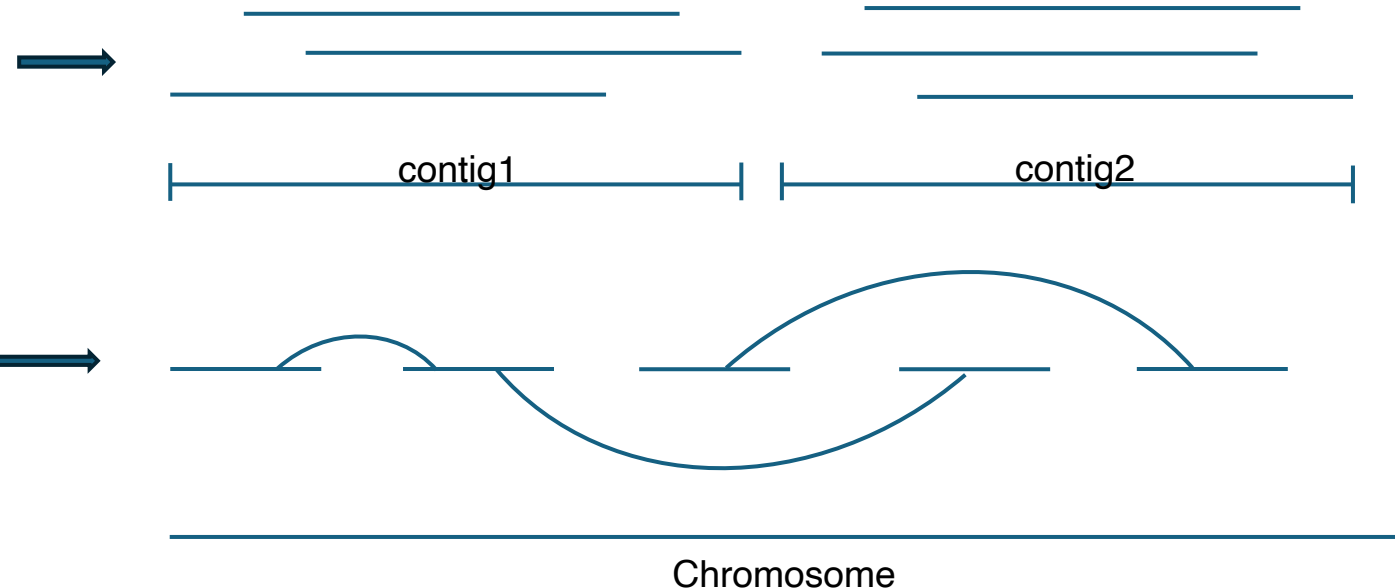
- Validate the contigs by confirming that the reads used to assemble them align correctly.
- Identify any errors in the contig sequences.
- Calculate coverage depth (how many reads cover each position of the contig).



Long reads (PacBio
/Nanopore)

+


Hi-C data



https://pipelines.tol.sanger.ac.uk/

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A Nextflow DSL2 pipeline for pretext generation in curation

Version 1.0.0

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[curation](#) [genome-alignment](#) [genome-assembly](#) [genomics](#) [quality-control](#) [synteny](#)

Pipelines for the production of Treeval data

Version 1.1.1

Published 2 weeks ago

[sanger-tol/ensemblgenedownload](#)

1

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Nextflow pipeline to download gene annotations from Ensembl onto the Tree of Life directory structure

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[sanger-tol/ensemblrepeatdownload](#)

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Nextflow pipeline to download repeat annotations from Ensembl onto the Tree of Life directory structure

Version 2.0.0

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[sanger-tol/insdcdownload](#)

1

[download](#) [genomics](#) [indexing](#)

Nextflow pipeline to download assemblies from INSDC and add them to the Tree of Life directories

Version 2.0.0

Published 2 months ago

[sanger-tol/variantcalling](#)

[alignment](#) [genomics](#) [variant-calling](#)

This Nextflow DSL2 pipeline calls variants on long read alignment. It is run after sanger-tol/readmap in the Sanger ToL production suite but with options to run on unaligned reads.

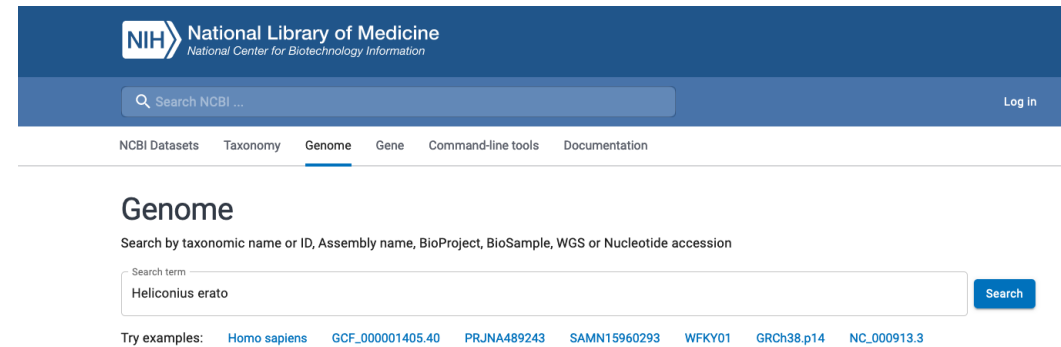
Version 1.1.3

Published 2 months ago

Where can we find genomes?

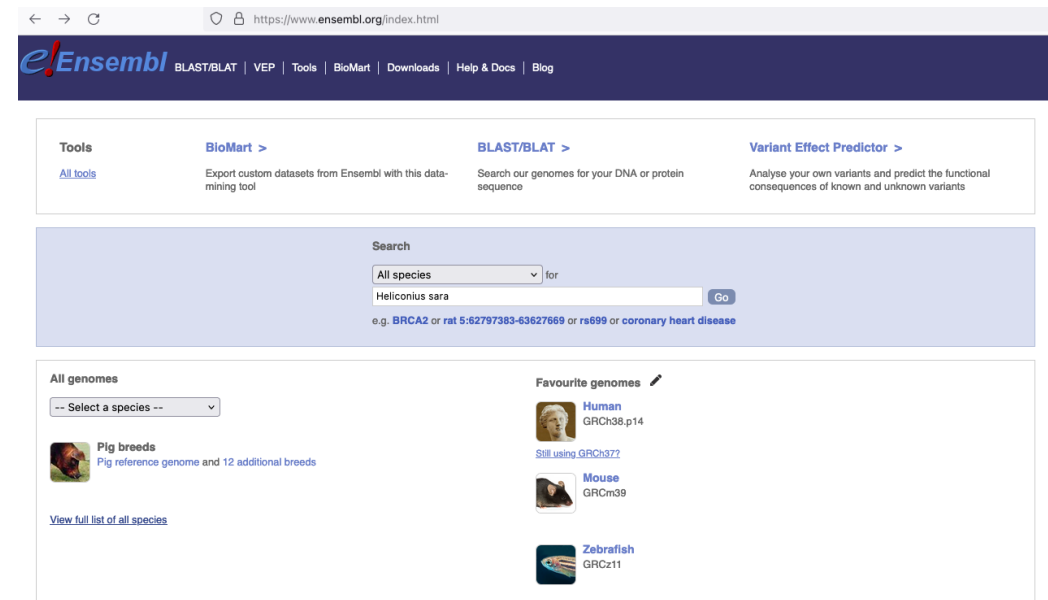
- NCBI (National Center for Biotechnology Information):

<https://www.ncbi.nlm.nih.gov/datasets/genome/>



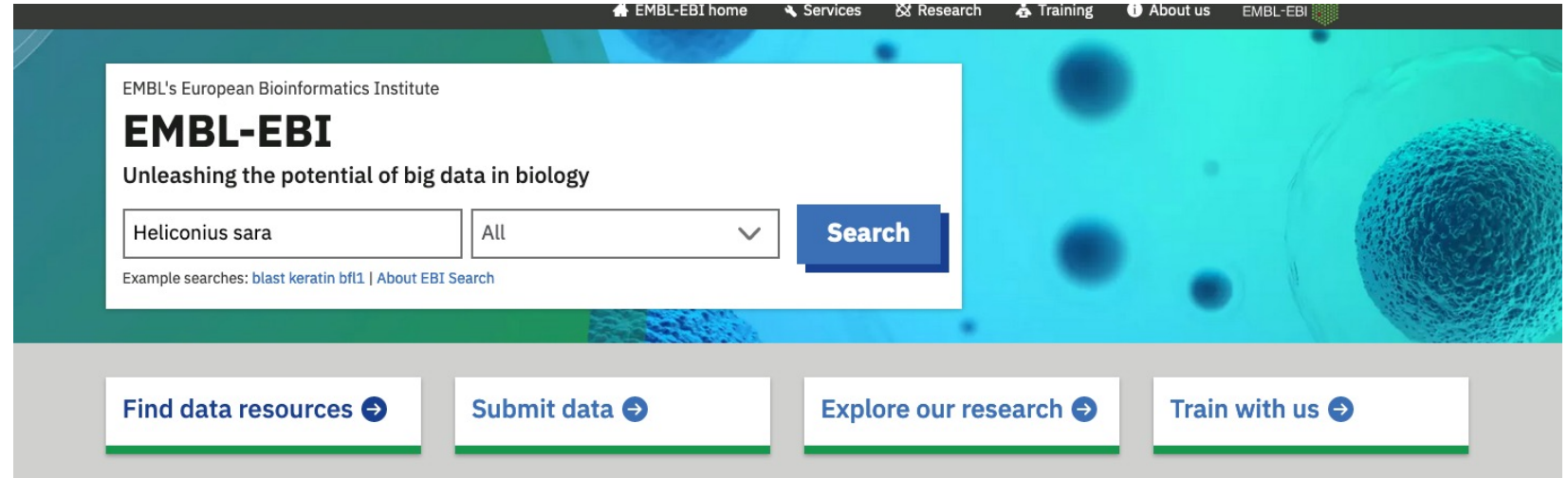
- ensembl

<https://www.ensembl.org/index.html>



➤ EMBL-EBI (European Molecular Biology Laboratory - European Bioinformatics Institute):

<https://www.ebi.ac.uk/>



Example:

https://www.ncbi.nlm.nih.gov/datasets/genome/

An official website of the United States government

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Genome

Gene

Command-line tools

Documentation

Genome

Search by taxonomic name or ID, Assembly name, BioProject, BioSample, WGS or Nucleotide accession

Search term

Heliconius sara

×

Search

Try examples: [Homo sapiens](#) [GCF_000001405.40](#) [PRJNA489243](#) [SAMN15960293](#) [WFKY01](#) [GRCh38.p14](#) [NC_000913.3](#)

https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_917862395.2/

An official website of the United States government

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Genome assembly iHelSar1.2 reference

Download	<div><div>datasets</div><div>URL</div><div>FTP</div></div>	Actions
Submitted GenBank assembly	GCA_917862395.2	⋮
Taxon	Heliconius sara	
WGS project	CAKJTV02	
Assembly type	haploid	
Submitter	WELLCOME SANGER INSTITUTE	
Date	Apr 6, 2023	

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Additional genomes

[Browse all Heliconius sara genomes \(2\)](#)

BioProject

[PRJEB46449](#)

Heliconius sara (Sara longwing) genome assembly iHelSar1

https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=33443

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Selected taxa

Heliconius sara

Enter one or more taxonomic names

⌵

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Select columns

2 Genomes 1 selected

Rows per page

20

1-2 of 2

<

>

<input type="checkbox"/>	Assembly	GenBank	RefSeq	Scientific name	Modifier	Annotation	Action
<input checked="" type="checkbox"/>	iHelSar1.2	GCA_917862395.2		Heliconius sara			⋮
<input type="checkbox"/>	iHelSar1.1 alternate haplotype...	GCA_911392485.1		Heliconius sara			⋮

https://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/917/862/395/GCA_917862395.2_iHelSar1.2/

Index of /genomes/all/GCA/917/862/395/GCA_917862395.2_iHelSar1.2

Name	Last modified	Size
Parent Directory		-
GCA_917862395.2_iHelSar1.2_assembly_report.txt	2023-04-18 17:05	34K
GCA_917862395.2_iHelSar1.2_assembly_stats.txt	2023-04-18 17:05	31K
GCA_917862395.2_iHelSar1.2_feature_count.txt	2024-02-15 01:58	168
GCA_917862395.2_iHelSar1.2_genomic.fna.gz	2023-04-18 17:05	107M
GCA_917862395.2_iHelSar1.2_genomic.gbff.gz	2023-04-18 17:05	138M
GCA_917862395.2_iHelSar1.2_genomic_gaps.txt.gz	2023-04-18 17:05	1.4K
GCA_917862395.2_iHelSar1.2_wgsmaster.gbff.gz	2023-04-18 17:05	1.4K
README.txt	2024-04-11 16:11	54K
annotation_hashes.txt	2024-02-17 10:02	410
assembly_status.txt	2024-07-15 13:53	14
md5checksums.txt	2024-02-17 10:02	625
uncompressed_checksums.txt	2024-06-14 01:21	167

[HHS Vulnerability Disclosure](#)

wget

https://ftp.ncbi.nlm.nih.gov/genomes/all/GC
A/917/862/395/GCA_917862395.2_iHelSar1.2/GC
A_917862395.2_iHelSar1.2_genomic.fna.gz

FASTA Format

It is a text-based format for representing nucleotide sequences (DNA or RNA)

```
>NG_008679.1:5001-38170 Homo sapiens paired box 6 (PAX6) ← Description Line  
ACCTCTTTTCTTATCATTGACATTTAACTCTGGGGCAGGTCCTCGCGTAGAACGCGGCTGTCAGATCT  
GCCACTTCCCCTGCCGAGCGGCGGTGAGAAGTGTGGGAACCGGCGCTGCCAGGCTCACCTGCCTCCCCGC  
CCTCCGCTCCCAGGTAACCGCCCCGGGCTCCGGCCCCGGCCCCGGCTCGGGGCCCGCGGGGCCTCTCCGCTG  
CCAGCGACTGCTGTCCCCAAATCAAAGCCCCGCCCAAGTGGCCCCGGGGCTTGATTTTTTGCTTTTAAAAG  
GAGGCATACAAAGATGGAAGCGAGTTACTGAGGGAGGGATAGGAAGGGGGGTGGAGGAGGGACTTGCTCTT  
TGCCGAGTGTGCTCTTCTGCAAAAGTAGCAAAATGTTCCACTCCTAAGAGTGGACTTCCAGTCCGGCCCT ← Sequence Lines  
GAGCTGGGAGTAGGGGGCGGGAGTCTGCTGCTGTCTGCTAAAGCCACTCGCGACCGCGAAAAATGCA  
GGAGGTGGGGACGCACTTTGCATCCAGACCTCCTCTGCATCGCAGTTCACGACATCCACGCTTGGGAAAG  
TCCGTACCCGCGCCTGGAGCGCTTAAAGACACCCTGCCGCGGGTCGGGCGAGGTGCAGCAGAAGTTTCCC  
GCGGTTGCAAAGTGCAGATGGCTGGACCGCAACAAAGTCTAGAGATGGGGTTCGTTTCTCAGAAAGACGC
```

Description Line: Begins with a > character followed by an identifier and optional description.

Sequence Lines: The actual sequence data, typically represented over multiple lines for readability.

Assembly statistics

	GenBank
Genome size	562.2 Mb
Total ungapped length	562.2 Mb
Number of contigs	203,084
Contig N50	9.9 kb
Contig L50	14,088
GC percent	33.5
Genome coverage	1.9x
Assembly level	Contig

It is the median of the contig lengths.

It is the number of contigs (or scaffolds) that together contain at least 50% of the total assembly length.

Significance

N50: A higher N50 value indicates that the assembly contains longer contigs, suggesting better assembly quality and continuity.

L50: A lower L50 value indicates that fewer contigs are needed to cover 50% of the genome, suggesting higher assembly contiguity.

★ **Quick exercise:** Imagine you have contigs of the following lengths (in kb): 10, 15, 25, 30, 50, 70.

1. Sort the contigs in descending order
2. Calculate the total length
3. Find the N50
4. Find the L50

1. Sort the contigs in descending order: 70, 50, 30, 25, 15, 10.
2. Calculate the total length: $70 + 50 + 30 + 25 + 15 + 10 = 200$ kb.
3. Find the N50:
 - 50% of 200 kb is 100 kb.
 - Cumulative lengths: 70 (70 kb), $70 + 50 = 120$ kb (reaches 100 kb threshold).
 - The contig length where we first exceed 100 kb cumulative is 50 kb.
 - **N50 = 50 kb.**
4. Find the L50:
 - Cumulative lengths: 70 (70 kb), $70 + 50 = 120$ kb (reaches 100 kb threshold).
 - Number of contigs used to reach this point: 2.
 - **L50 = 2 contigs.**

References

- Jay Ghurye and Mihai Popl. 2019. Modern technologies and algorithms for scaffolding assembled genomes. Plos computational biology
- Edward S. Rice and Richard E. Green. 2018. New Approaches for Genome Assembly and Scaffolding. Annual Review of Animal Biosciences.
- https://ucdavis-bioinformatics-training.github.io/2020-Genome_Assembly_Workshop/kmers/kmers
- <https://www.youtube.com/watch?v=8w-CbyGV-pgv>
- <https://star-protocols.cell.com/protocols/1799>
- <https://training.galaxyproject.org/training-material/topics/sequence-analysis/tutorials/quality-control/tutorial.html#per-sequence-quality-scores>