UNIVERSITÄT BONN

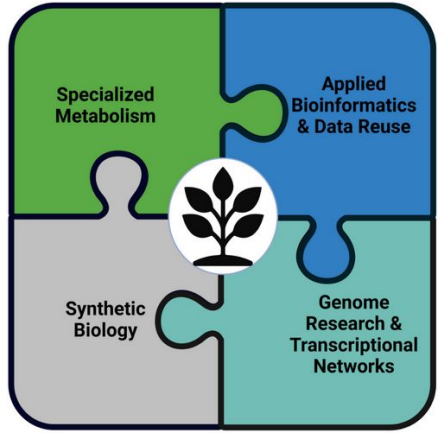
PuckerLab

de.NBI course 1: Plant Genome Sequence Assembly and Annotation

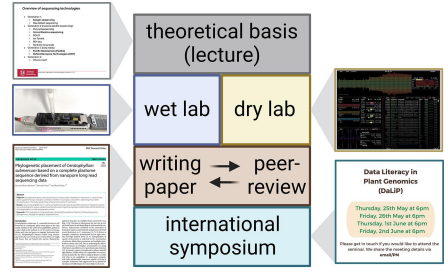
@PuckerLab

@puckerlab

- Flavonoids: Anthocyanins, Flavonols, Flavones, Proanthocyanidins
- Withanolides
- Carotenoids
- …



Tools: KIPES, NAVIP, MGSE, MYB/bHLH_annotator, DupyliCate…



Wolff et al., 2023: 10.1515/jib-2023-0033
Dassow et al., 2025: 10.1101/2025.01.31.635922

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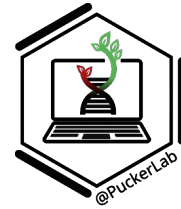
Availability of Slides



All materials will be shared with participants at the end of this course. Background information is available here:

https://www.preprints.org/manuscript/202508.1176

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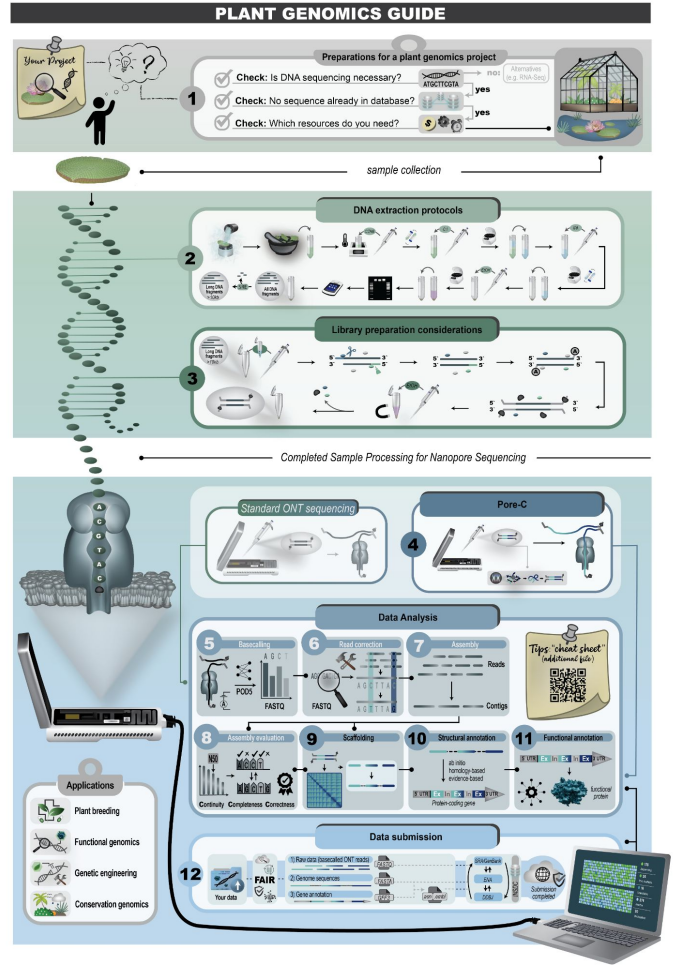
Course Overview

Plant Genome Sequence Assembly and Annotation

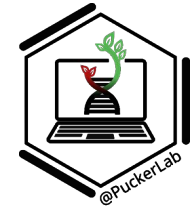
- Plant genomics
- Long read sequencing (ONT)
- Read acquisition
- Genome assembly & evaluation
- Structural annotation

From L to R: Shakunthala, Samuel, Nancy, Julie, and Boas

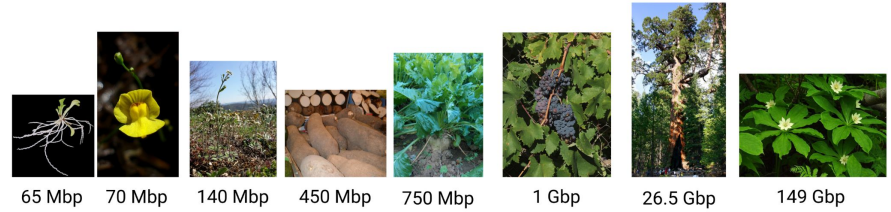
Picture credits: Jakob Horz



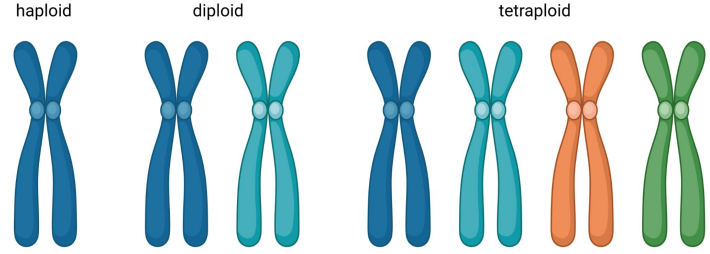
Oliveira et al., 2025: 10.20944/preprints202508.1176.v2



- Genome size: variation from 65 Mbp to 149 Gbp
- Ploidy: haploid/diploid genomes are much easier to analyze than polyploid genomes
- Computational costs increase with genome size



Genlisea violacea: https://commons.wikimedia.org/wiki/File:Genlisea_violacea_giant.jpg
Utricularia gibba: https://commons.wikimedia.org/wiki/File:Utricularia_gibba_flower_01.jpg
Arabidopsis thaliana: https://commons.wikimedia.org/wiki/File:Arabidopsis_thaliana_sl10.jpg
Dioscorea dumetorum: https://commons.wikimedia.org/wiki/File:YamsatBrixtonMarket.jpg
Beta vulgaris: https://de.wikipedia.org/wiki/Zuckerr%C3%BCbe#/media/Datei:Zuckerr%C3%BCbe.jpg
Vitis vinifera: https://commons.wikimedia.org/wiki/File:Vitis_vinifera_20120823.jpg
Sequioa giganteum: https://commons.wikimedia.org/wiki/File:Grizzly_Giant_Mariposa_Grove.jpg
Paris japonica: https://commons.wikimedia.org/wiki/File:Paris_japonica_Kinugasasou_in_Hakusan_2003_7_27.jpg



Kress et al., 2022: 10.1073/pnas.2115640118

Long read sequencing


Aquilegia vulgaris


Urtica dioica

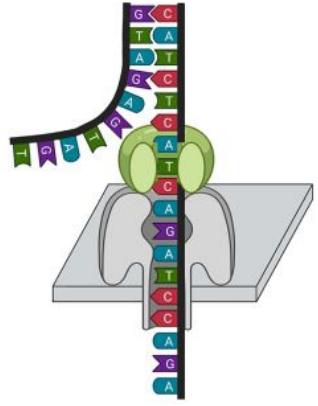

Nymphaea spec.

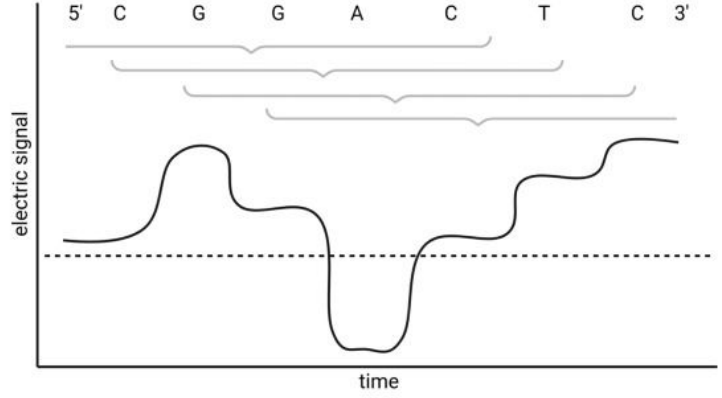

Theobroma cacao

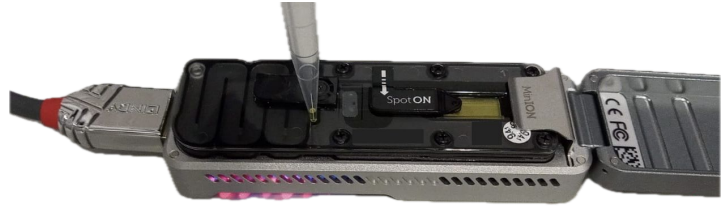

Tacca chantrieri


Corylus avellana


Solanaceae species







Introduction to Virtual Machines (VM)

- high performance computing => server farm

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- de.NBI offers VMs (VM = virtual machine)
  - running Linux
  - access through SSH

- de.NBI connected LifeScience AAI Account needed

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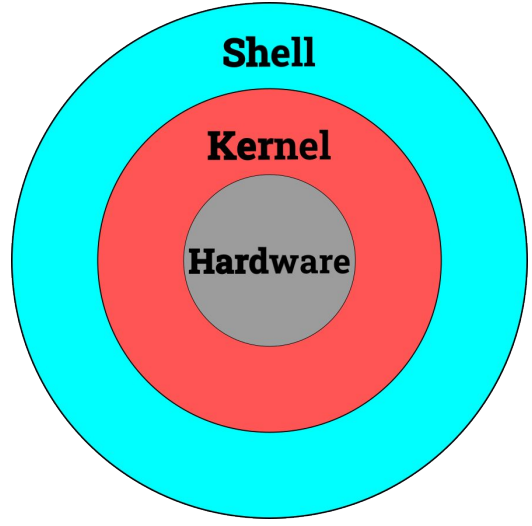
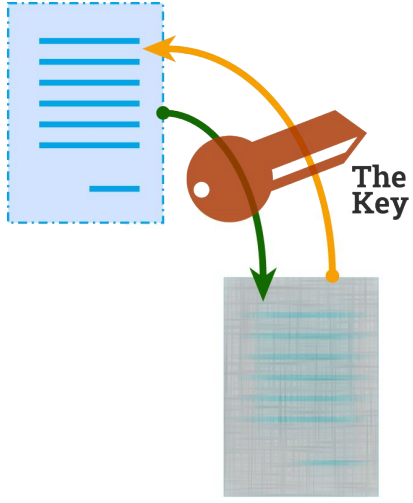
- Tasks:
  1. Register: https://cloud.denbi.de/wiki/registration/
  2. Apply for the course de.NBI project: https://signup.aai.lifescience-ri.eu/fed/registrar/?vo=denbi&group=pbbcourse20251

Accessing VMs: SSH

- SSH = Secure Shell
- Linux is a kernel
- shell ≈ Interface to the Computer
- CLI = command line interface

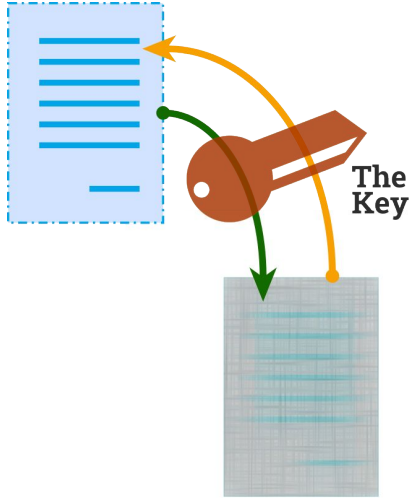


SSH keys - asymmetric encryption

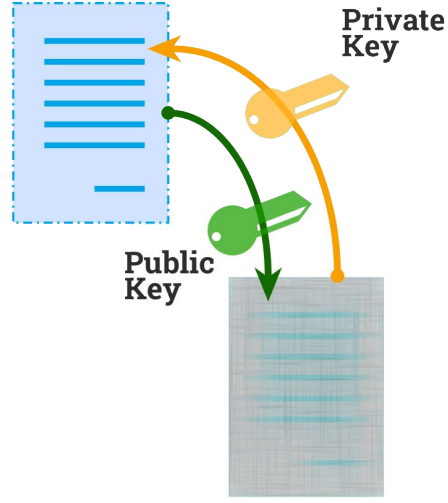


Symmetric encryption

SSH keys - asymmetric encryption

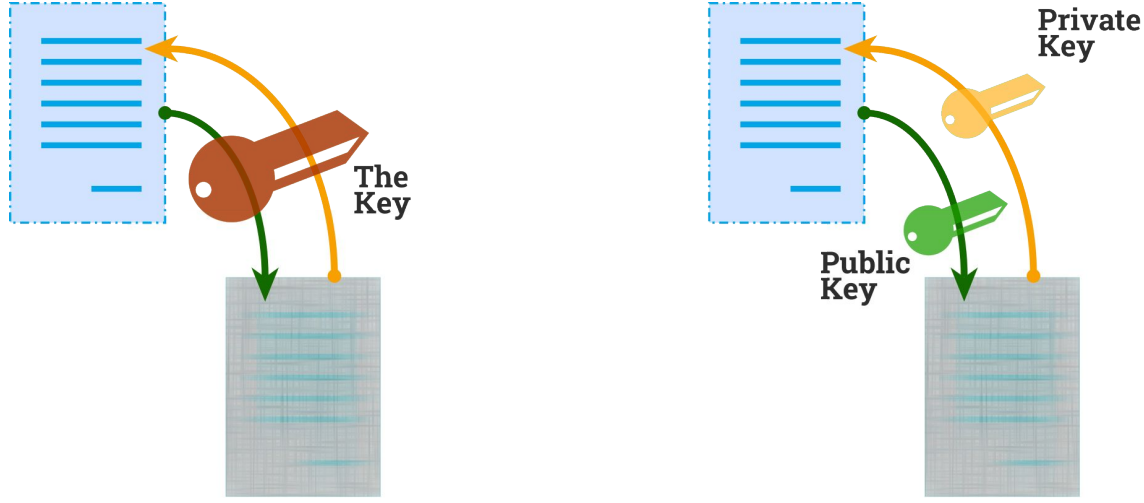


Symmetric encryption



Asymmetric encryption

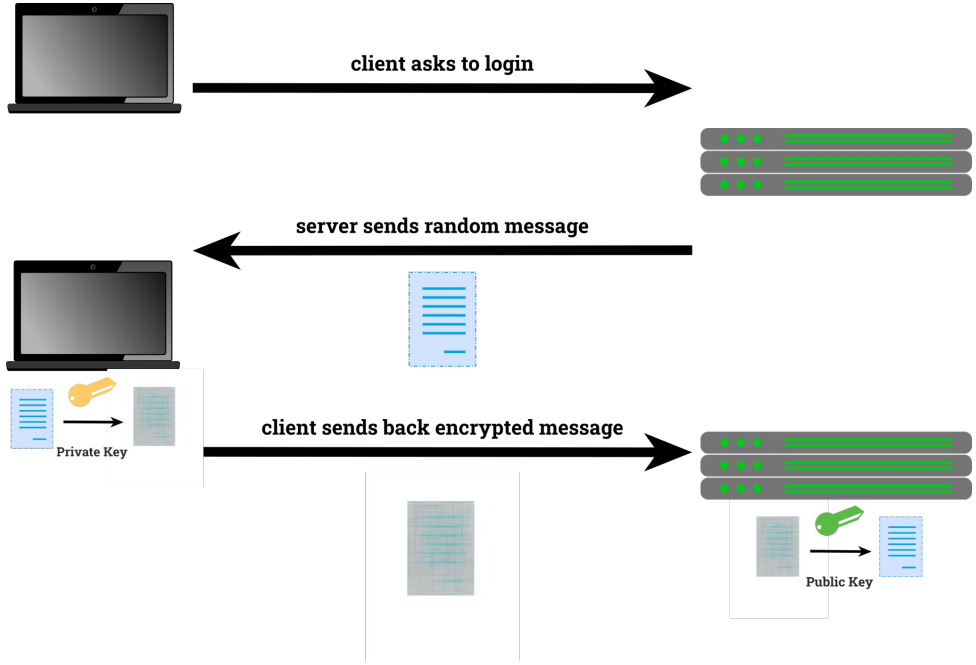
SSH keys - asymmetric encryption



Symmetric encryption **Asymmetric encryption**

ecdsa-sha2-nistp256
AAAAE2VjZHNhLXNoYTItbmlzdHAyNTYAAAAIbmlzdHAyNTYAAABBBJHT0aW+Q2bjkfvVM7kN2ucaf8eltoaIV6V0CDBgpWF+dKV1HKkvGWWfjtYMOdLDUKlAzJc5Q6bKs6bkYiqqtUQ=

SSH keys - asymmetric encryption



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  2. Apply for the course de.NBI project: https://signup.aai.lifescience-ri.eu/fed/registrar/?vo=denbi&group=pbbcourse20251
  3. create SSH key pair in de.NBI cloud portal and confirm unix user name

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Groups of two for VM creation

- We will create the VMs
  - tell us your group members names

- Tasks:
  1. open a Terminal and login via ssh

SSH command and CLI

- SSH key might need the correct permissions
- needed information:
  - username
  - ip address/domain
  - port number (usually optional but mandatory for de.NBI VMs)
  - ssh private key
  - exemplary commands:

```
ssh -i /path/to/key username@ip-address -p port-number
```

```
ssh -i C:\Users\User\de.NBI_course\key.txt ubuntu@134.176.27.78 -p 23457
```

CLI - basic commands

```
sam@dhcp068:~$ ssh -i /home/TUBS_NC/13.DenBiCloud_access_scripts/meckoni_ecdsa.txt ubuntu@134.176.27.78 -p 30235
     _                 _ _ ___ ___
  __| |___   | \| | _ )_ _|     de.NBI cloud
 / _` / -_)_| .` | _ \| |      http://cloud.denbi.de
 \__,_\___(_)_|\_|___/___|     cloud@denbi.de

Welcome to Ubuntu 24.04.3 LTS (GNU/Linux 6.8.0-87-generic x86_64)

 System information as of Sun Dec  7 14:28:58 UTC 2025

  System load:  0.14               Processes:             422
  Usage of /:   13.5% of 18.33GB   Users logged in:       0
  Memory usage: 0%                 IPv4 address for ens3: 192.168.0.235
  Swap usage:   0%

Last login: Sun Dec  7 14:18:21 2025 from 192.168.0.143
ubuntu@snmtestdeletesoon-9b08e:~$
```

```
ubuntu@snmtestdeletesoon-9b08e:~$ cd /vol/data/
ubuntu@snmtestdeletesoon-9b08e:/vol/data$
```

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CLI - basic commands


```
sam@dhcp068:~$ ssh -i /home/TUBS_NC/13.DenBiCloud_access_scripts/meckoni_ecdsa.txt ubuntu@134.176.27.78 -p 30235
      _                 _ ___ ___
   __| |___   | \| | _ )_ _|     de.NBI cloud
  / _` / -_)_| .` | _ \| |       http://cloud.denbi.de
  \__,_\___(_)_|\_|___/___|      cloud@denbi.de

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```

https://github.com/bpucker/teaching/tree/master/deNBI2025

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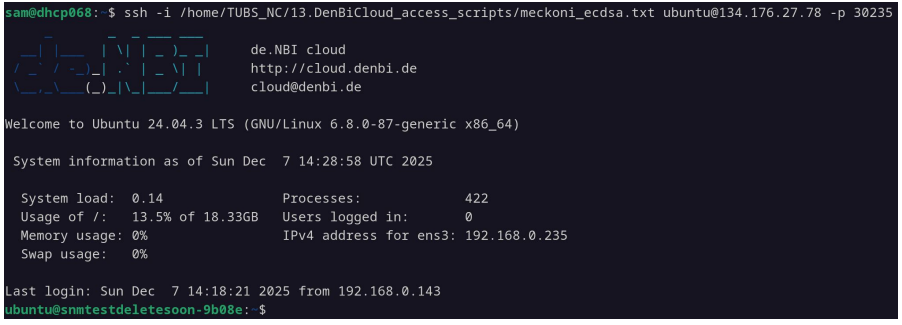
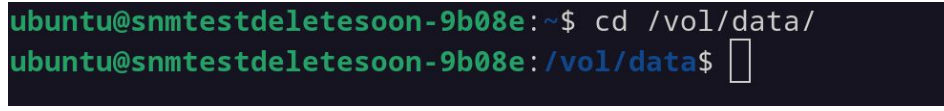
CLI - basic commands





- Tasks:
  1. create your own folder in the 1 TB volume

https://github.com/bpucker/teaching/tree/master/deNBI2025

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1. Downloading DNA reads

- SRA = Sequence Read Archive
- downloading tools:
  1. prefetch (compressed file)
  2. fasterq-dump (un- compresses the files)
- December 2, 2025: **SRA Toolkit** Release 3.3.0 -> **GitHub** repository (apt version is outdated)

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- Tasks:
  1. install the tools
  2. get the reads with the SRA/ENA ID: ERR4760286

1. Downloading DNA reads

- SRA = Sequence Read Archive
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  1. prefetch (compressed file)
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- December 2, 2025: **SRA Toolkit** Release 3.3.0 -> **github** repository (apt version is outdated)

SRA/ENA ID: ERR4760286

wget https://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/3.3.0/sratoolkit.3.3.0-ubuntu64.tar.gz
tar -xvf sratoolkit.3.3.0-ubuntu64.tar.gz

```
prefetch -p --max-size 250g --output-directory /vol/data/DNA_reads ERR4760286
```

no. of threads (CPU)

SRA/ENA read ID

```
fasterq-dump -e 15 /vol/data/DNA_reads/ERR4760286 --outdir /vol/data/DNA_reads/raw_reads
```

Reads Statistics

- Numerical summaries that describe the quality, length, composition, and coverage of your reads

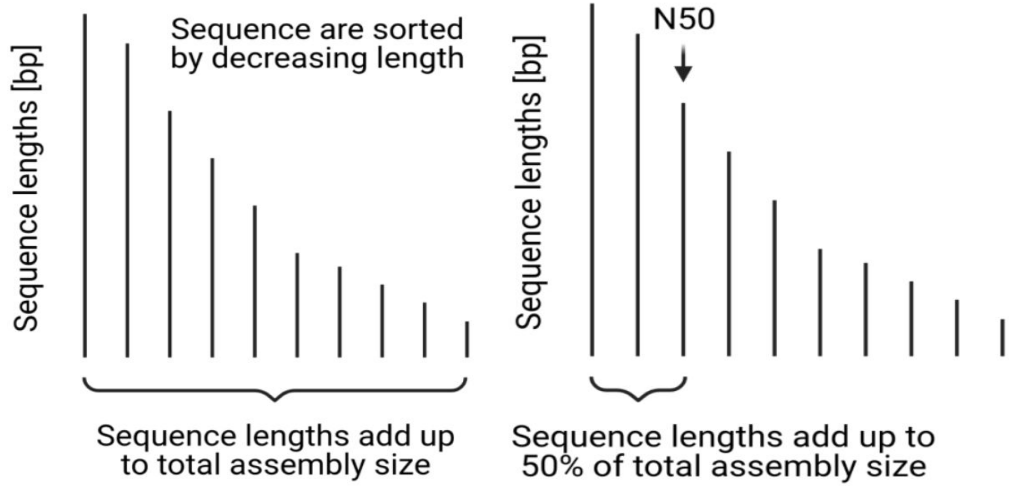
- Number of reads
- Total number of bases
- GC content
- Read length
- N50

→ Important for downstream analysis

Reads N50

- The N50 of a set of reads is the length of the shortest read among the longest reads that together make up 50% of the total bases sequenced.

1. Take all read lengths and sort them from longest to shortest;
2. Sum the total number of bases across all reads to get the total yield;
3. Starting from the longest read, cumulatively add read lengths until the sum reaches at least 50% of the total bases;
4. The length of the last read added is the N50.



Tools to Read Statistics

- FastQC/MultiQC: short reads
- NanoPlot, NanoComp: for ONT long reads
- Seqkit, Seqtk: generally for any sequence

    - Custom scripts:

⭐ FASTQ_stats3.py ⭐

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⭐ FASTQ_stats3.py ⭐

https://github.com/bpucker/PlantGenomicsGuide/

bpucker reference updated		9eac6ed · 2 days ago	23 Commits
Arabidopsis_thaliana_araport11.anno.txt	Req. for construct_anno.py	4 months ago
Arabidopsis_thaliana_araport11.pep.fasta	Req. for construct_anno.py	4 months ago
FASTQ_stats3.py	v0.42	2 days ago
LICENSE	Initial commit	5 months ago
README.md	Update README.md	last month
clean_fastq_headers.py	Update clean_fastq_headers.py	4 months ago
construct_anno.py	Create construct_anno.py	4 months ago
contig_stats3.py	reference updated	2 days ago
match_proteins.py	Create match_proteins.py	4 months ago

⭐ FASTQ_stats3.py ⭐

PlantGenomicsGuide / FASTQ_stats3.py

bpucker v0.42 — 9115e56 · 2 days ago — History

Code | Blame — 268 lines (214 loc) · 8.37 KB — Raw

```
### Boas Pucker ###
### pucker@uni-bonn.de ###

__version__ = "v0.42"


__usage__ = """
        Calculation of FASTQ statistics (""" + __version__ + """):

        python3 FASTQ_stats3.py
        --in <FULL_PATH_TO_FASTQ_FILE> |    --in_dir <FULL_PATH_TO_DIRECTORY>

        optional:
        --rfig <READ_LEN_HIST_FIGURE_FILE>
        --cutoff <READ_LEN_CUTOFF_FOR_PLOT_IN_KB>[100]
        --qfig <QUALITY_VS_READ_LEN_FIGURE_FILE>
        --lencut <UPPER_READ_LENGTH_CUTOFF_FOR_PLOT_IN_KB>[200]
        --qualcut <UPPER_QUAL_CUTOFF_FOR_PLOT>[40]
        --quality <ACTIVATES_QUALITY_ANALYSIS>

        bug reports and feature requests: pucker@uni-bonn.de
        """
```

⭐ FASTQ_stats3.py ⭐

wget https://raw.githubusercontent.com/bpucker/PlantGenomicsGuide/refs/heads/main/FASTQ_stats3.py


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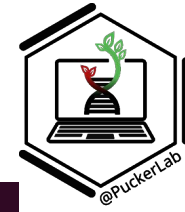


```
ubuntu@gossypiumhirsutum-ed98e:/vol/data/scripts$ wget https://raw.githubusercontent.com/bpucker/PlantGenomicsGuide/refs/heads/main/FASTQ_stats3.py
--2025-12-04 11:05:53--  https://raw.githubusercontent.com/bpucker/PlantGenomicsGuide/refs/heads/main/FASTQ_stats3.py
Resolving raw.githubusercontent.com (raw.githubusercontent.com)... 185.199.111.133, 185.199.108.133, 185.199.109.133, ...
Connecting to raw.githubusercontent.com (raw.githubusercontent.com)|185.199.111.133|:443... connected.
HTTP request sent, awaiting response... 200 OK
Length: 8569 (8.4K) [text/plain]
Saving to: ‘FASTQ_stats3.py’

FASTQ_stats3.py                                     100%[=================================================================================
====================>]   8.37K  --.-KB/s    in 0s

2025-12-04 11:05:53 (76.0 MB/s) - ‘FASTQ_stats3.py’ saved [8569/8569]

ubuntu@gossypiumhirsutum-ed98e:/vol/data/scripts$ ls
FASTQ_stats3.py  run_fastq_dump_parallel.sh
ubuntu@gossypiumhirsutum-ed98e:/vol/data/scripts$ python3 FASTQ_stats3.py -h
WARNING: matplotlib import failed - figure plotting not possible.

                Calculation of FASTQ statististics (v0.42):

                python3 FASTQ_stats3.py
                --in <FULL_PATH_TO_FASTQ_FILE> |        --in_dir <FULL_PATH_TO_DIRECTORY>

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```

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⭐ FASTQ_stats3.py ⭐

Dataset: ERR4760286

```
ubuntu@gossypiumhirsutum-ed98e:/vol/data/Final_dataset_deNBI/Read$ cd /vol/data/scripts/
ubuntu@gossypiumhirsutum-ed98e:/vol/data/scripts$ python3 FASTQ_stats3.py --in /vol/data/Final_dataset_deNBI/Read/ERR4760286_pass.fastq
WARNING: matplotlib import failed - figure plotting not possible.
/vol/data/Final_dataset_deNBI/Read/ERR4760286_pass.fastq
total number of nucleotides:    8.812031342 Gbp
N50: 123311
number of reads: 69689
average read length:    126447.95221627517
GC content:     0.37114296001331676
```

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HERRO correction

https://github.com/nanoporetech/dorado

- Haplotype-aware ERRor cOrrection

> 64 CPU cores

> 256GB GB RAM

> 32GB GPU VRAM

Input: FASTQ(.gz)

Output: FASTA

- Highly accurate
- Haplotype-aware
- Deep-learning tool
- Error correction of Nanopore Reads

HERRO correction


```
# Step 1: merge all FASTQs of different runs from the same species

cat \
/path/to/your/gzip/fastq/file/run1.mod.fastq.gz \
/path/to/your/gzip/fastq/file/run2.mod.fastq.gz \
/path/to/your/gzip/fastq/file/run3.mod.fastq.gz \
> /path/to/your/file/thaliana.fastq.gz &
```

```
# Step 2: CPU heavy part of HERRO

/vol/data/tools/dorado-1.3.0-linux-x64/bin/dorado \
correct \
/vol/data/your/folder/thaliana.fastq.gz \
--to-paf \
--threads 200 \
> /vol/data/your/folder/thaliana.overlaps.paf \
2> /vol/data/your/folder/thaliana.overlaps.doc.txt &
```

HERRO correction

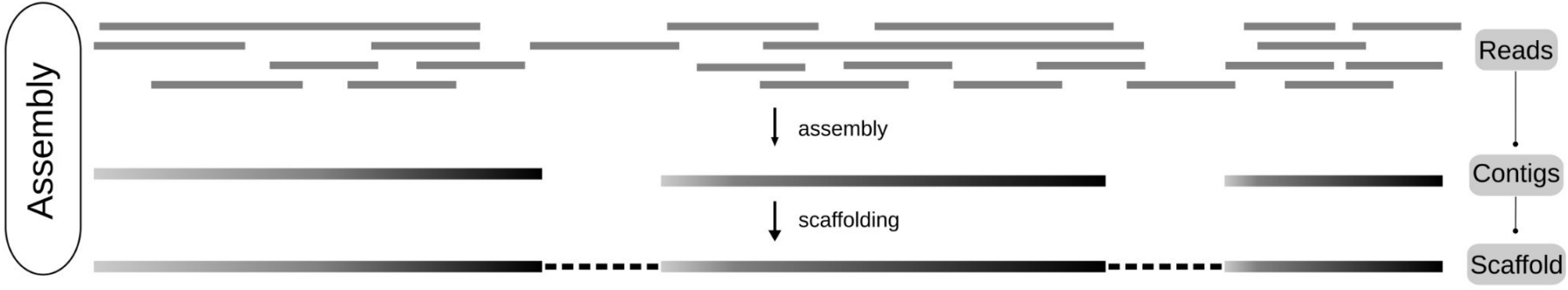
Step 3: Decompress the FASTQ file

```
gunzip /vol/data/herro/thaliana.fastq.gz
```

Step 4: GPU heavy part of HERRO

```
/vol/data/tools/dorado-1.3.0-linux-x64/bin/dorado \
correct \
/vol/data/herro/thaliana.fastq \
--from-paf /vol/data/herro/thaliana.overlaps.paf \
> /vol/data/herro/thaliana.corrected_reads.fasta \
2> /vol/data/herro/thaliana.corrected_reads.errors.txt &
```

Genome Assembly



- Computational process to piece together reads to form a complete representation of an organism's genome
- Reads are shorter than the chromosome - even long reads
- Genome = DNA in a cell
- Genome sequence = representation of DNA in the cell

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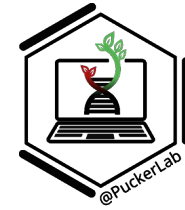
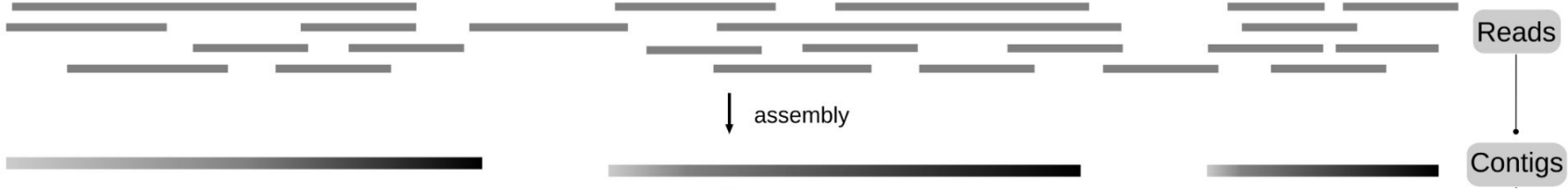
Long-read Assemblers





- Connecting reads - identify overlap between reads
- Reads -> Contigs (contiguous sequences)

Assemblers	Pros	Cons
Shasta	Specialized for ONT reads; Exceptional speed	Based on read quality, may produce fragmented or less contiguous sequences Smaller than expected genome sizes
NextDenovo	Superior error correction of raw reads (especially ONT); highly contiguous and accurate assemblies for ONT R9 data	Not optimized for recent, more accurate R10 data
Hifiasm	Leading assembler for diploid genomes using accurate reads (PacBio HiFi/ ONT R10 reads)	Does not work with ONT R9 data
Verkko	Works really well in hybrid approach - combining ONT reads with PacBio HiFi	Perform suboptimally on ONT-only moderate coverage reads

Genome Assembly (Hands on!)

Before, the assembly process -

A. Genome size estimation

- Read file - ERR4760286.fastq → ***Arabidopsis thaliana* Col-0**
- Estimated genome size? https://cvalues.science.kew.org/search/angiosperm

Genus	Species	Subspecies	DNA Amount 1C (pg)	Original Reference
Arabidopsis	thaliana	ecotype Columbia	0.16	Bennett et al.,2003

- 1C = amount of DNA in a haploid set of chromosomes
- 1 picogram (pg) = 978 megabases (Mb)
- Estimated genome size for *A. thaliana* Col = 0.16 pg X 978 **≈ 157 Mb**

(What if C-value database does not have your species of interest?)

Pucker et al 2021: 10.1186/s12864-021-07877-8

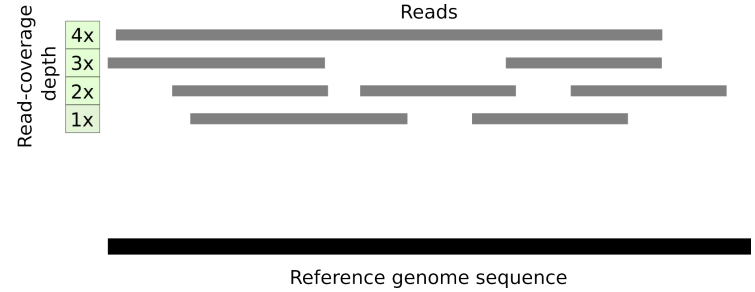
Before, the assembly process -

B. Selecting the right assembler

Coverage = $\frac{N \times L}{G}$,where N = number of reads

L = average read length

G = estimated genome size



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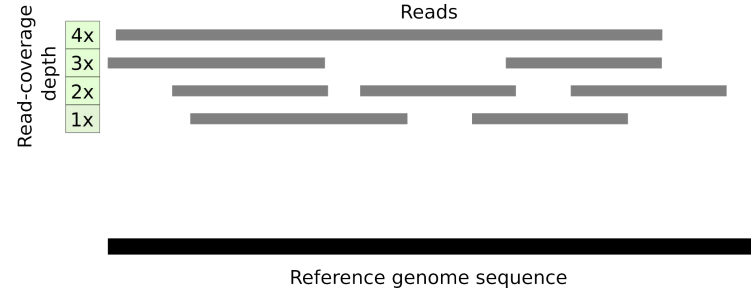
Genome Assembly (Hands-on!)

Before, the assembly process -

B. Selecting the right assembler

Coverage = ***56 x***



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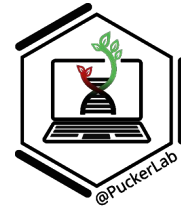
Genome Assembly (Hands on!)

	Shasta (DatasetA)	NextDenovo2 (DatasetB)
Documentation:	https://paoloshasta.github.io/shasta/	https://nextdenovo.readthedocs.io/en/latest/
Installation:		

Genome Assembly (Hands on!)



	Shasta (DatasetA)	NextDenovo2 (DatasetB)
Documentation:	https://paoloshasta.github.io/shasta/	https://nextdenovo.readthedocs.io/en/latest/
Installation:	wget https://github.com/paoloshasta/shasta/releases/download/0.14.0/shasta-Linux-X.Y.Z chmod ugo+x shasta-Linux-X.Y.Z ~~nano ~/.bashrc~~ ~~export PATH="/path/to/shasta_executable:$PATH"~~ ~~source ~/.bashrc~~ ./shasta-Linux–0.14.0 -h	sudo apt install pipx pipx ensurepath pipx install paralleltask pipx ensurepath pipx install paralleltask wget https://github.com/Nextomics/NextDenovo/releases/download/2.5.2/NextDenovo.tgz tar -xvzf NextDenovo.tgz && cd NextDenovo
Command:		

Genome Assembly (Hands on!)

	Shasta (DatasetA)	NextDenovo2 (DatasetB)
Documentation:	https://paoloshasta.github.io/shasta/	https://nextdenovo.readthedocs.io/en/latest/
Installation:	wget https://github.com/paoloshasta/shasta/releases/download/0.14.0/shasta-Linux-X.Y.Z chmod ugo+x shasta-Linux-X.Y.Z nano ~/.bashrc export PATH="/path/to/shasta_executable:$PATH" source ~/.bashrc	python3 -m venv mypython source mypython/bin/activate pip3 install paralletask wget https://github.com/Nextomics/NextDenovo/releases/download/2.5.2/NextDenovo.tgz tar -xvzf NextDenovo.tgz && cd NextDenovo
Command:	shasta-Linux-0.14.0 --config Nanopore-UL-May2022 --input Reads.fastq --AssemblyDirectory Shasta01/ --threads N >> shasta.log 2>&1 &	./nextDenovo run.cfg >>nextdenovo.log 2>&1 &

Calculating the right resources for running NextDenovo:

>run.cfg

```
[General]
job_type = local
job_prefix = nextDenovo
task = all
rewrite = yes
deltmp = yes
parallel_jobs = 4
input_type = raw
read_type = ont
input_fofn = input.fofn
workdir = 01_rundir
[correct_option]
read_cutoff = 1k
genome_size = 157m
sort_options = -m 20g -t 15
minimap2_options_raw = -t 8
pa_correction = 3
correction_options = -p 15

[assemble_option]
minimap2_options_cns = -t 8
nextgraph_options = -a 1
```

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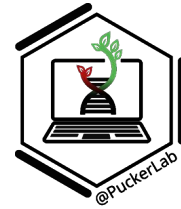
End of day 1



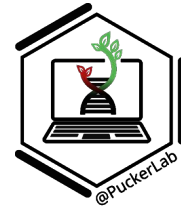
Today you learned:

- Accessing de.NBI VMs via SSH
- Working in a CLI environment
- Obtaining and evaluating sequencing reads
- Assembling a genome sequence

Tomorrow you will learn:

- Evaluating a genome assembly sequence
- Structural annotation of a genome assembly sequence

Start of day 2



Yesterday you learned:

- Accessing de.NBI VMs via SSH
- Working in a CLI environment
- Obtaining and evaluating sequencing reads
- Assembling a genome sequence

Today you will learn:

- Evaluating a genome assembly sequence
- Structural annotation of a genome assembly sequence

Genome assembly format

FASTA Format

```
>ctg000000 type:s:linear length:i:316896 node:i:15
cgccgtccttggtcgacgaatctctggaccttcggtcgcatgaagttcctccagacctggtcgatttattttcaactttga
>ctg000010 type:s:linear length:i:18291631 node:s:unknown
cctaaacctaaacCCTAAACCTAAACCTAAACCTAAACCCTAAACCCTAAACCCTAAACCCTAAACCCTAAACCCTAAACC
>ctg000020 type:s:linear length:i:18476100 node:s:unknown
TCCTGCTTCTTTCGGCTTCGGAGGGCTGTCTTTGGGCTTTCCGAAAAGGTATCACATGCCAAGTTTGGCCTCACGGTCTAA
>ctg000030 type:s:linear length:i:32605930 node:i:1836
tgTAGGATCAATCATTGCATTTTTCCATAGGATAGATAGGGCCGACAAGATCATCGGGGAAGAAGTCAAATCACATCCGAA
>ctg000040 type:s:linear length:i:11381128 node:i:703
cctaggcctaggcctaggcctgaaacctgaagcctaggcctaaggcctgagcctaggcctaggcctgagcctgaagcctga
>ctg000050 type:s:linear length:i:324797 node:s:unknown
aatacattttatttttttattgacaagtactcgtactcaaaaaagttaaaaatttttttattcatatttttttttgtagtatg
```

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Genome assembly format

Print contigs in the assembly

grep “>” assembly.fasta

Count number of contigs in the assembly

grep -c “>” assembly.fasta

```
>ctg000000 type:s:linear length:i:316896 node:i:15
cgccgtccttggtcgacgaatctctggaccttcggtcgcatgaagttcctccagacctggtcgatttattttcaactttga
>ctg000010 type:s:linear length:i:18291631 node:s:unknown
cctaaacctaaacCCTAAACCTAAACCTAAACCTAAACCCTAAACCCTAAACCCTAAACCCTAAACCCTAAACCCTAAACC
```

Shorten headers to avoid downstream analysis errors ⟶**clean_genomic_fasta.py**

available at : https://github.com/bpucker/GenomeAssembly

Genome assembly format

Shorten headers to avoid downstream analysis errors ⟶**clean_genomic_fasta.py**

available at : https://github.com/bpucker/GenomeAssembly

Commands:

wget https://raw.githubusercontent.com/bpucker/GenomeAssembly/refs/heads/main/clean_genomic_fasta.py

python3 clean_genomic_fasta.py

python3 clean_genomic_fasta.py --in genome.fasta --out cleaned_genome.fasta

All next steps using cleaned_genome.fasta !!

Post-Assembly evaluation

Parameters	DatasetA	DatasetB
No. of contigs		
Genome assembly size		
Minimum contig size		
Maximum contig size		
Contiguity (Contig N50)		
Contiguity (LAI)		
Completeness (BUSCO)		
Correctness (QV - Merqury)		

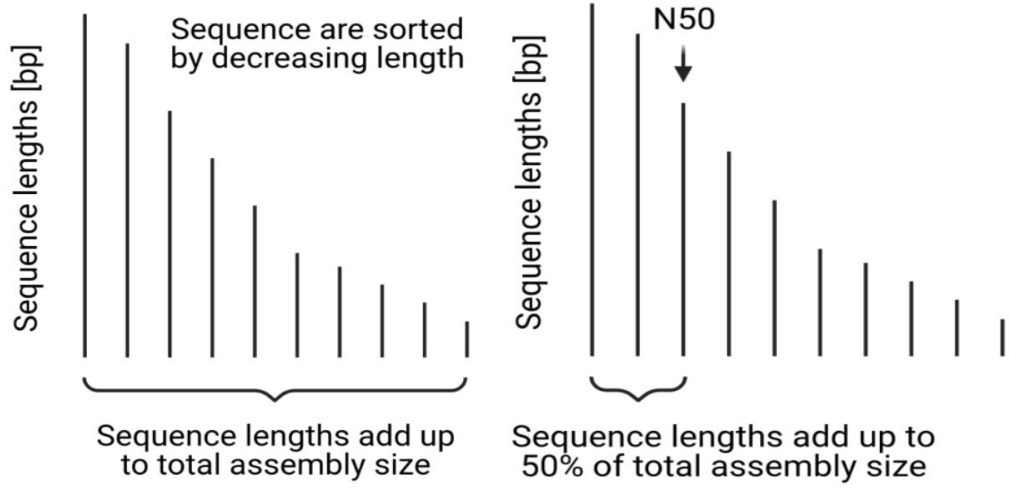
Assembly statistics

- Quantitative measures describing size, continuity, correctness, completeness of the assembled genome sequence

  - N50
  - Total assembly size
  - Number of contigs
  - GC content
  - Completeness
  - Error rate

- The length of the shortest sequence among the longest sequences that together make up at least 50% of the total assembly length

1. List all sequence lengths and sort them from longest to shortest;
2. Add up all sequence lengths to get the total assembly size;
3. Starting from the longest sequence, cumulatively add sequence lengths until the sum reaches at least 50% of the total assembly size;
4. The length of the last sequence added is the N50.



Tools for Assembly Statistics

- BUSCO
- Merqury
- LAI

    - Custom scripts:

⭐ contig_stats3.py ⭐

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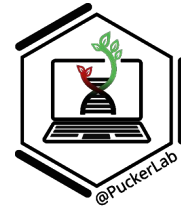
⭐ contig_stats3.py ⭐



https://github.com/bpucker/PlantGenomicsGuide/

bpucker reference updated		9eac6ed · 2 days ago	23 Commits
Arabidopsis_thaliana_araport11.anno.txt	Req. for construct_anno.py	4 months ago
Arabidopsis_thaliana_araport11.pep.fasta	Req. for construct_anno.py	4 months ago
FASTQ_stats3.py	v0.42	2 days ago
LICENSE	Initial commit	5 months ago
README.md	Update README.md	last month
clean_fastq_headers.py	Update clean_fastq_headers.py	4 months ago
construct_anno.py	Create construct_anno.py	4 months ago
contig_stats3.py	reference updated	2 days ago
match_proteins.py	Create match_proteins.py	4 months ago

⭐ contig_stats3.py ⭐

PlantGenomicsGuide / contig_stats3.py

bpucker reference updated — 9eac6ed · 2 days ago — History

Code | Blame — 361 lines (303 loc) · 11.6 KB

```
### Boas Pucker ###
### pucker@uni-bonn.de ###

## based on script contig_stats.py Pucker et al., 2016. doi:10.1371/journal.pone.0164321

import re, sys, os
from operator import itemgetter

# --- end of imports --- #

__version__ = "v3.1"

__citation__ = "de Oliveira, J. A. V. S.; Choudhary, N.; Meckoni, S. N.; Nowak, M. S.; Hagedorn, M.; Pucker, B. (2025). Cookbook for Plant Genome Sequences. doi: 10.20944/preprints202508.1176.v2"

__usage__ = """
                Calculation of assembly statistics (""" + __version__ + """):
                python3 contig_stats3.py
                --in <INPUT_FASTA_FILENAME>

                optional:
                --min <MIN_CONTIG_LENGTH> [1000]
                --out <FULL_PATH_TO_OUTPUT_DIRECTORY>
                --exp <EXPRESSION_FILE(normalized)>

                bug reports and feature requests: pucker@uni-bonn.de
                Please cite: \n""" + __citation__ + """
            """
```

⭐ contig_stats3.py ⭐

wget https://raw.githubusercontent.com/bpucker/PlantGenomicsGuide/refs/heads/main/contig_stats3.py

raw.githubusercontent.com/bpucker/PlantGenomicsGuide/refs/heads/main/contig_stats3.py

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                                bug reports and feature requests: pucker@uni-bonn.de
                                Please cite: \n""" + __citation__ + """
                        """
```

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⭐ contig_stats3.py ⭐

Dataset A

```
assembly name: DatasetA.genome.fasta_trimmed

number of contigs:      22
average contig length:  5510209.590909091
minimal contig length:  105320
maximal contig length:  16222222

total number of bases:  121224611
total number of bases without Ns:       121224611
GC content:     0.36077104013144656

N25:    15538466
N50:    14706422
N75:    11234264
N90:    9390472

E90N25: False
E90N50: False
E90N75: False
E90N90: False
```

Dataset B

```
assembly name: DatasetB.genome.fasta_trimmed

number of contigs:      13
average contig length:  10369230.23076923
minimal contig length:  262562
maximal contig length:  32605930

total number of bases:  134799993
total number of bases without Ns:       134799993
GC content:     0.3637223853565037

N25:    26122726
N50:    22402157
N75:    18291631
N90:    11381128

E90N25: False
E90N50: False
E90N75: False
E90N90: False
```

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⭐ contig_stats3.py ⭐

Dataset A

```
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N25:    15538466
N50:    14706422
N75:    11234264
N90:    9390472

E90N25: False
E90N50: False
E90N75: False
E90N90: False
```

Dataset B

```
assembly name: DatasetB.genome.fasta_trimmed

number of contigs:      13
average contig length:  10369230.23076923
minimal contig length:  262562
maximal contig length:  32605930

total number of bases:  134799993
total number of bases without Ns:       134799993
GC content:     0.3637223853565037

N25:    26122726
N50:    22402157
N75:    18291631
N90:    11381128

E90N25: False
E90N50: False
E90N75: False
E90N90: False
```

All next steps using trimmed_genome.fasta !!

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https://github.com/marbl/merqury
https://anaconda.org/bioconda/merqury

- Evaluates genome assembly quality without a reference by comparing k-mers from high-accuracy reads to those in the assembly, estimating metrics like base-level accuracy (QV)

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

UNIVERSITÄT BONN Merqury

Step 1: Calculate the right k size

```
bash /vol/data/tools/miniconda3/envs/merqury/bin/best_k.sh 134799993
```

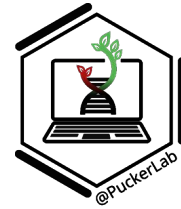
genome: 134799993

18.4853

Step 2: Count k-mers from raw reads using meryl

```
meryl k=18 count /vol/data/julie/deNBI/ERR4760286_pass.fastq \
output DatasetB.meryl >> /vol/data/julie/deNBI/merqury/DatasetB/meryl.log 2>&1 &
```

UNIVERSITÄT BONN Merqury



Step 3: Run Merqury to assess assembly quality

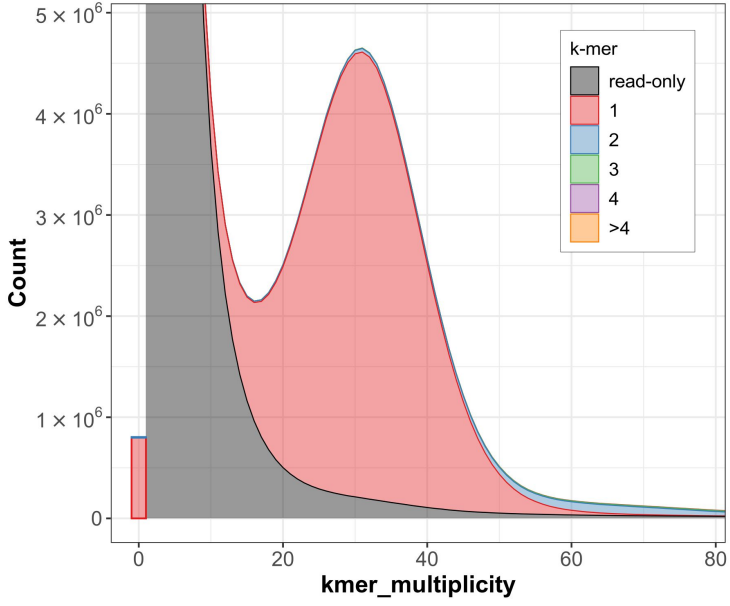
merqury.sh DatasetB.meryl /vol/data/julie/deNBI/DatasetB.genome.fasta output_DatasetB >> /vol/data/julie/deNBI/merqury/DatasetB/merqury.log 2>&1 &

Assembly of interest: **DatasetB.genome**
Total (present) k-mers uniquely found only in the assembly: **134799772**
QV: **40.6544**
Error rate: **8.6013e-05**

UNIVERSITÄT BONN Merqury

@PuckerLab

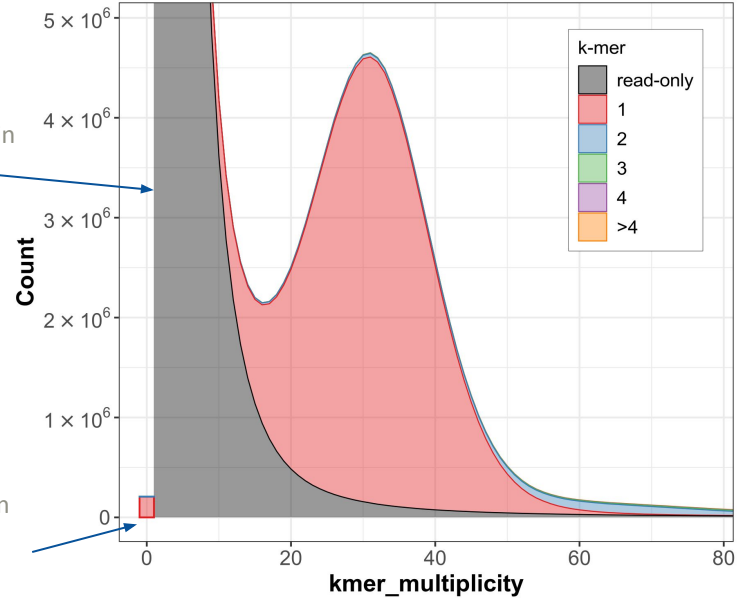
Dataset A QV: 34.3181



Dataset B QV: 40.6544

All k-mers found only in the reads

All k-mers found only in the assembly (error)

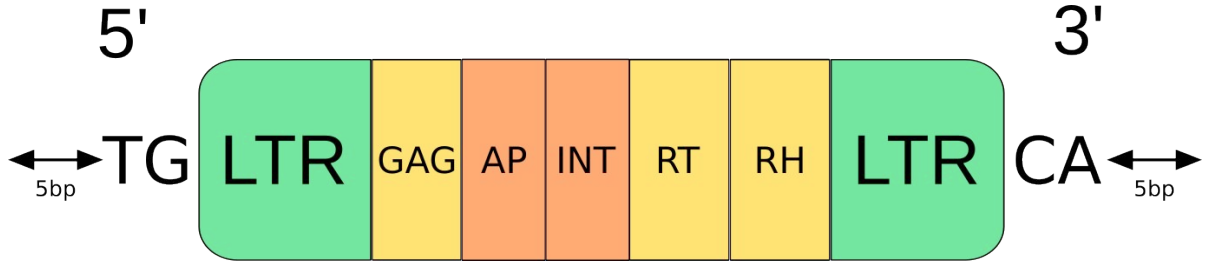


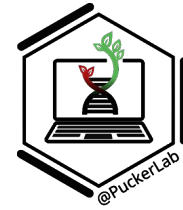
LAI - LTR Assembly Index

https://github.com/oushujun/LTR_retriever

- requires GenomeTools (https://github.com/genometools/genometools) for Step 1 and 2
- LTR_retriever can be used with conda

- The LTR Assembly Index (LAI) assesses assembly continuity by measuring intact LTR (Long-Terminal repeats) retrotransposons relative to total LTRs. Higher LAI (>10-15) indicates better assembly of repetitive regions






```
# Step 1: Index the genome using GenomeTools (gt)

/vol/data/tools/genometools/bin/gt suffixerator \
-db /vol/data/julie/deNBI/DatasetA.genome.fasta \
-indexname DatasetA.genome.fa \
-dna -tis -suf -lcp -des -ssp -sds &

# Step 2: Identify candidate LTR elements using similarity searches

/vol/data/tools/genometools/bin/gt ltrharvest \
-index DatasetA.genome.fa \
-minlenltr 100 -maxlenltr 7000 \
-mintsd 4 -maxtsd 6 \
-motif TGCA -motifmis 1 \
-similar 85 -vic 10 -seed 20 \
-seqids yes > DatasetA.genome.fa.harvest.scn &
```

Step 3: Detects additional LTR candidates using structural features

```
conda activate ltr_env

LTR_FINDER_parallel \
-seq /vol/data/julie/deNBI/DatasetA.genome.fasta \
-threads 10 -harvest_out -size 1000000 -time 300 2>&1 &
```

Step 4: Combine Results

```
cat DatasetA.genome.fa.harvest.scn DatasetA.genome.fasta.finder.combine.scn \
> DatasetA.genome.fa.rawLTR.scn
```

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Step 5: LTR_retriver calculates LAI and filters high confidence LTR-RTs

```
LTR_retriever \
-genome DatasetA.genome.fasta \
-inharvest /vol/data/julie/deNBI/LAI/DatasetA/DatasetA.genome.fa.rawLTR.scn \
-threads 10 &
```

To see LAI results

```
nano DatasetA.genome.fasta.out.LAI
```

Chr	From	To	Intact	Total	raw_LAI	**LAI**
whole_genome	1	121224611	0.0089	0.0981	9.08	**14.01**

0 to 10	draft quality
10-20	reference quality
> 20	gold quality

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BUSCO

- BUSCO = Benchmarking Universal Single-Copy Orthologs
- Universal genes -> expected to be in the genome
- Singe-Copy -> expected to be present only once per haplophase
- if all BUSCO genes are present in the assembly => good assembly
- some might be natively missing due to gene loss in evolution

UNIVERSITÄT BONN BUSCO

https://busco.ezlab.org/busco_userguide.html

- Installation with docker ⭐
- Installation with conda
- Manual installation - not recommended

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BUSCO

https://busco.ezlab.org/busco_userguide.html

- Installation with docker ⭐
- Installation with conda
- Manual installation - not recommended

- Tasks:
  1. Install Docker (https://docs.docker.com/engine/install/ubuntu/)
  2. install and run BUSCO on your genome assembly
     a. choose a lineage dataset
     b. choose the correct mode

- exemplary commands:

docker container name (will be automatically downloaded)

list datasets:

```
sudo docker run --rm -v /vol/data:/vol/data ezlabgva/busco:v6.0.0_cv1 busco --list-datasets
```

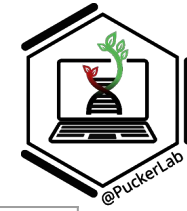
command that will be run in the docker container starts here

important for the docker container to access files

start the docker container
input file
mode and lineage-dataset
output file

```
sudo docker run --rm -v /vol/data:/vol/data ezlabgva/busco:v6.0.0_cv1 \
busco -i /vol/data/user/assembly.fasta \
-m genome --cpu 13 -l brassicales_odb12 \
--out_path /vol/data/user/BUSCO/ -o busco_run_id --opt-out-run-stats
```

Post-Assembly evaluation



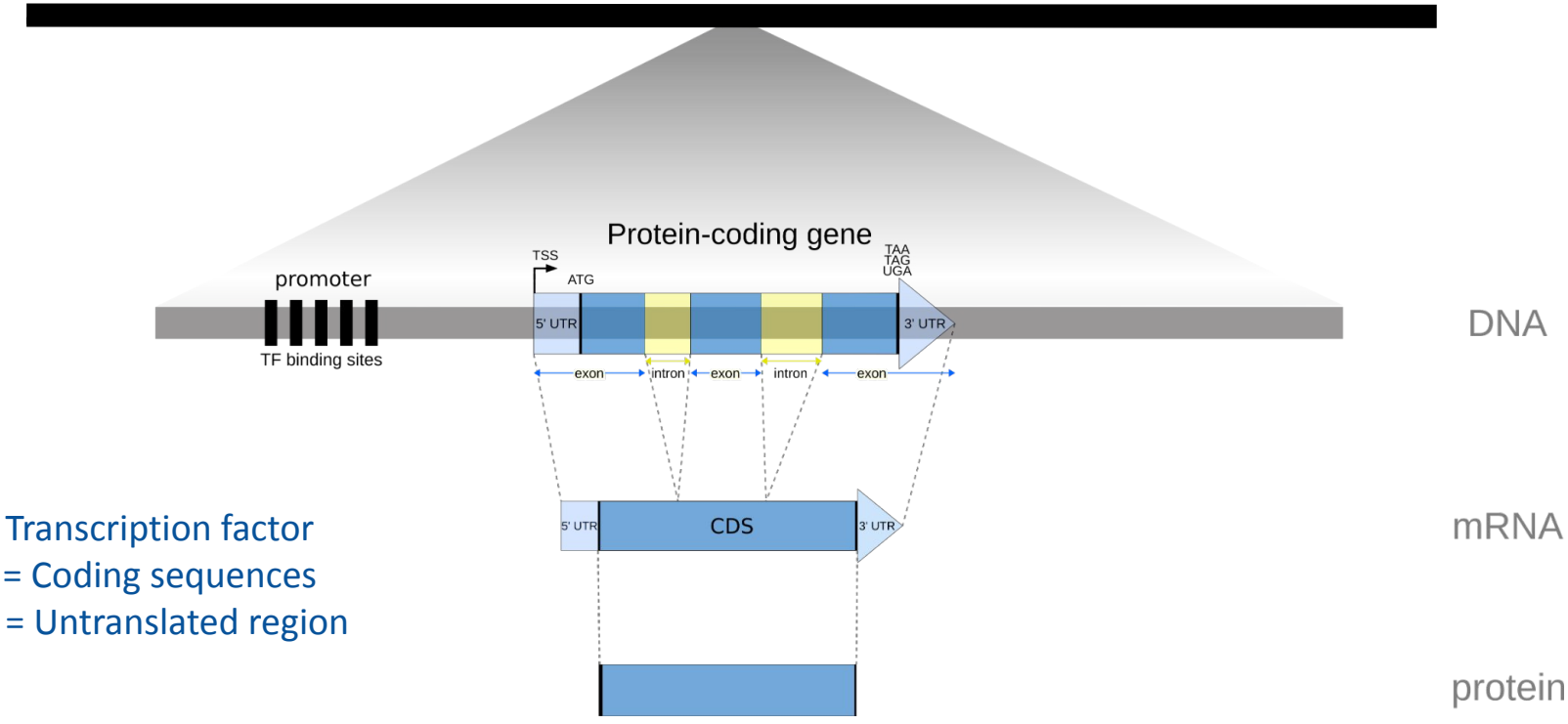
Parameters	DatasetA (Shasta)	DatasetB (NextDenovo)
No. of contigs		
Genome assembly size		
Minimum contig size		
Maximum contig size		
Contiguity (Contig N50)		
Contiguity (LAI)		
Completeness (BUSCO)		
Correctness (QV - Merqury)		

Structural annotation

- Process of finding genes in a genome sequence

TTTACAGTTTCTTTTGTATGTGTATTGTTTTTCCAACCGAAAAGTTTTGGATATTAAACGTTCAAATATAAAATAGTTGGAGTTTTGTTTGGGAATTTTCACTAGT
GTTACAAAAATGTGATTTAAGATGCGTTTTACTCGTTATATTTAGGTTTAGTCTTTGGTATAAGTATGTATTTTAAAAAAACGTAACGAGGTACATTTTTAAGTGT
GTTATTTACACTCGAAAACAAGCTAATTTAGAGGTGGTCGAGAGCAGATATCATGATTGTTTTTTTTAATGTATGTAATCCGTTCATTTGTGTCATCATTATTCAT
TATGATTTTCAGAAATGTATGTTTGTATCTTCTAATTTTTACCCACAAAATTCAATGTCCTACGCATATGATGGAATTTAACCAAATACAAGTAGCTAGTTAGTGA
CTAAACTATCAAACATATTAAAAACAATTATGAAGACTATTCGACAACTACGCTCACTATAAAAATTAATCAGAAGAGAAAAATGTAGATCTAAACTATCAAACAT
AAGTCCTAAAATATGTTAAAAGGCAAGGAGGGCCAACCATATCCATCTCTCGCCCACTTTTGCTAATATGTCACTCTCTCTCATATCCTAACCTTCCAATAACATT
CAAATCATTTATTATATCATTTTATTTTATCTGAATATACCATTTTTCATAACACAGAAATTTTAAAACCTAAACGAAAATCGAAACGAATCAGAACCGAAATCCG
TTGTGAGTCACAGTTGATTTGGTTCAACTGGATTGGGATAATATTTCAAGTGGAAAGAAATAAATAAAAGTTGTGGTGGAAAATTATTCCAATAAATCTCGTCGTG
TTTAATAAGAGATAAATATTTTTTCAAACTGTAACCTAAACAATAAATAAATTAAAAGAGAAATTAATGCAAATATTGCTATATTTTTGGGTTATTACTTAGAGAC
AGATAAGTGACCAAAGGGTTTCGATTTCTGGAAATAGTTTGAATAAAAAAGTAATTTTTATGTGATTATTGGCAAAGAAAAGTTTTAGTTTTTTTTAGTTTGTGAG
TCTAGGTGGTGGCTCAAGAACAGTTTGGCTCAGCTTGATTCTTCTCTTAGAAGTCTTGGTACTTGTCTTATCACCAAGAGATCTACTGATAGTGTTGCTTCTCTTC
ATAAGATGATCTGGAAATGATAAAGTTTTGATCTTTTTCAGATCCATTGTCTTTGGTGCGTGATCACCGAGCTAAAGATGTTTTGACGGCGCAAGGCATAGCGGTT
CTTGTGGGTTTTCGTTTTTTCCAAATCTGTATCATTCAATAATGTTTCTTTTATGTTGCAGGGGATGTGTCTAAATGTGTTGCGGATCCATTGGTGTTTGAGGATG
GTGAGTGTGAGAAAAGTTTTTCATCTTGTTCGGATCAAACAGGTCGCGTGGGCAAACGAAGGAAACGAGGCCGGGAAGAAAGCGTGAATCTTTTCCTGAAATCTAT
CTACTGGTTGGTTGCATGATCGCATAAGAGTAGTTGTTTCAAGCTTCTTTGTTAAAGTGCTTCAATTACCATGGAGATGGGGGATGAAGTATTTCTGGGACACACT
GGATACATCATCCGTGGAACGCACCTGAGTCCGTTCTTCAAGCTGCTGGTATCGAGCTTGGATCAAACTATCCTCTACCAATTGTAGGATTAGACGAAGCAAAAGC
GGAGATATGAGGATCAGATGGTTCCAAGCATTACTTCTTCTTTGATCAGACCTGAAGAAGACGAAGAGTCGTCTCTTAATTTGAGAAATTCAGTAGGAGATAGCAG
GCATAGTCCCCGAGTGGTCTCCAGGGTACTCAGAGCAGTTCCCTAGTGAAGAAAATGGTATTGGAGGAGGAAGTACAACGTCTAGCTACTTGCAGAATCACCATGA
GCACACTTGTCTAGCCAAGTAGTATCAGTTTTAAATTGAATTTAGCTGAAAAGTTTTGAACTGTTTTCAAATCCAAGTGTTTGCATTTACAGTAGACCACAGTAAA
TGTAAACCGAGTACATTTGGTACGGTTTAATGTAATTCCGGTTATGGGGCTGGAGAGAAACTATGTAGGAGTTTGTCTGATGTACATTTTTTATTTATCTCTGGTT

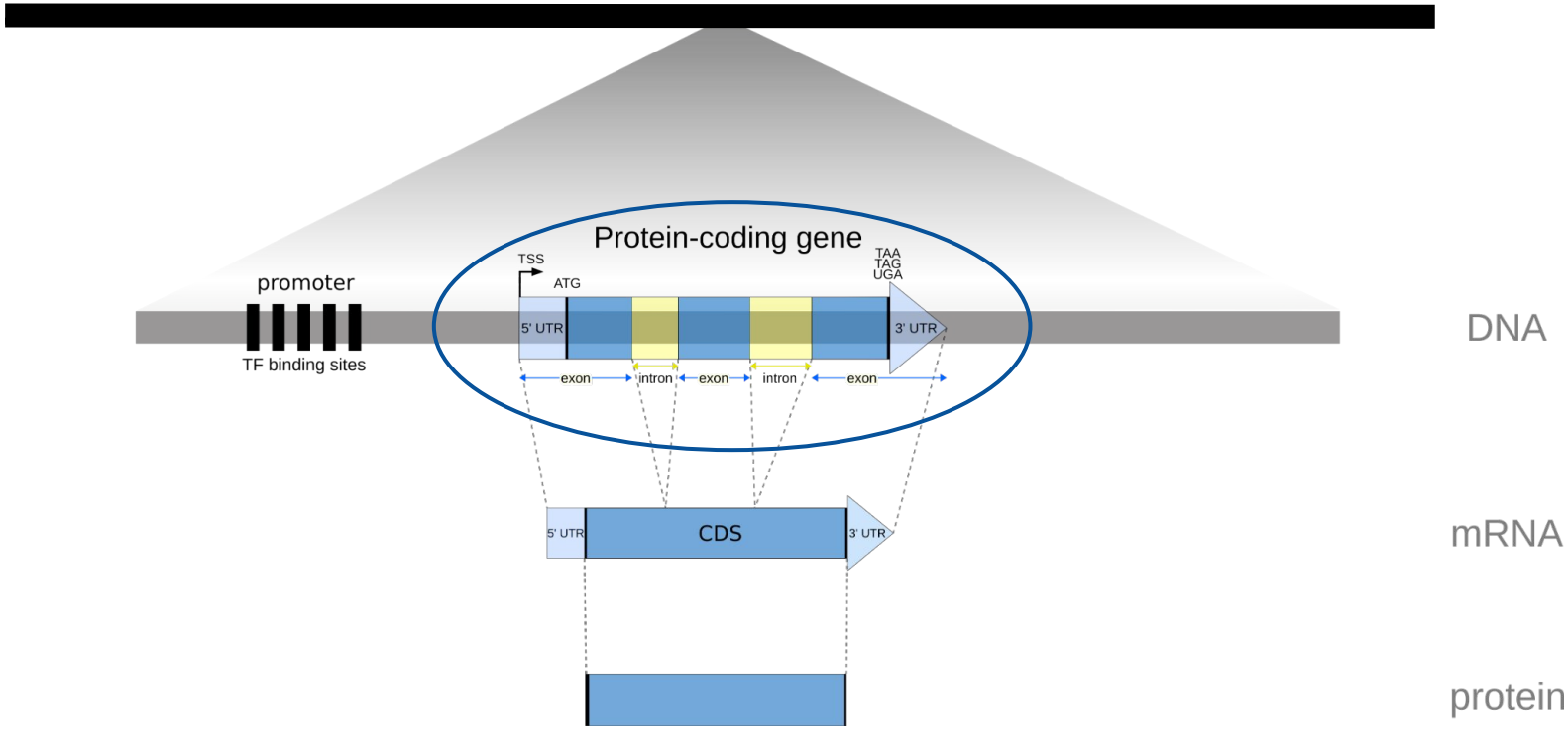
Plant gene structure



TF = Transcription factor
CDS = Coding sequences
UTR = Untranslated region

Oliveira et al., 2025: 10.20944/preprints202508.1176.v2

Structural annotation



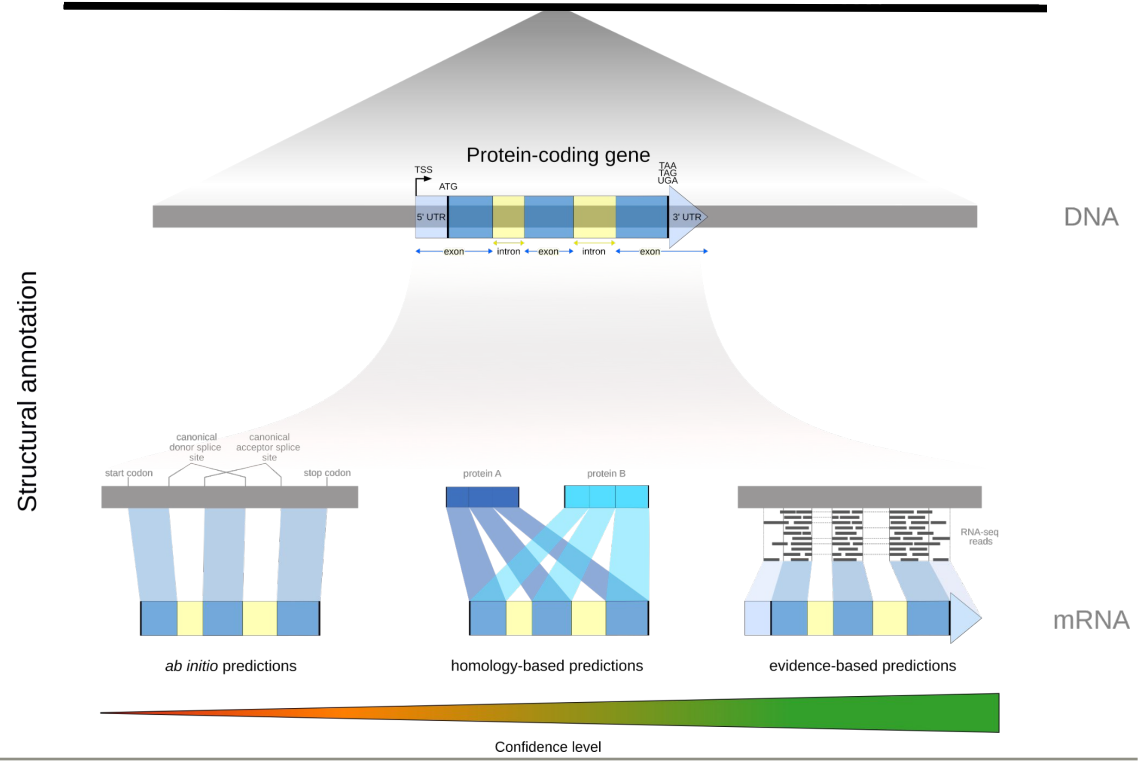
Oliveira et al., 2025: 10.20944/preprints202508.1176.v2

Structural annotation - Methods

(a) *ab initio* prediction:
uses intrinsic sequence features

(b) *homology-based*:
using gene sequences from closely related species

(c) *evidence-based*:
using transcriptomics and/or proteomic hints from the species itself



Oliveira et al., 2025: 10.20944/preprints202508.1176.v2

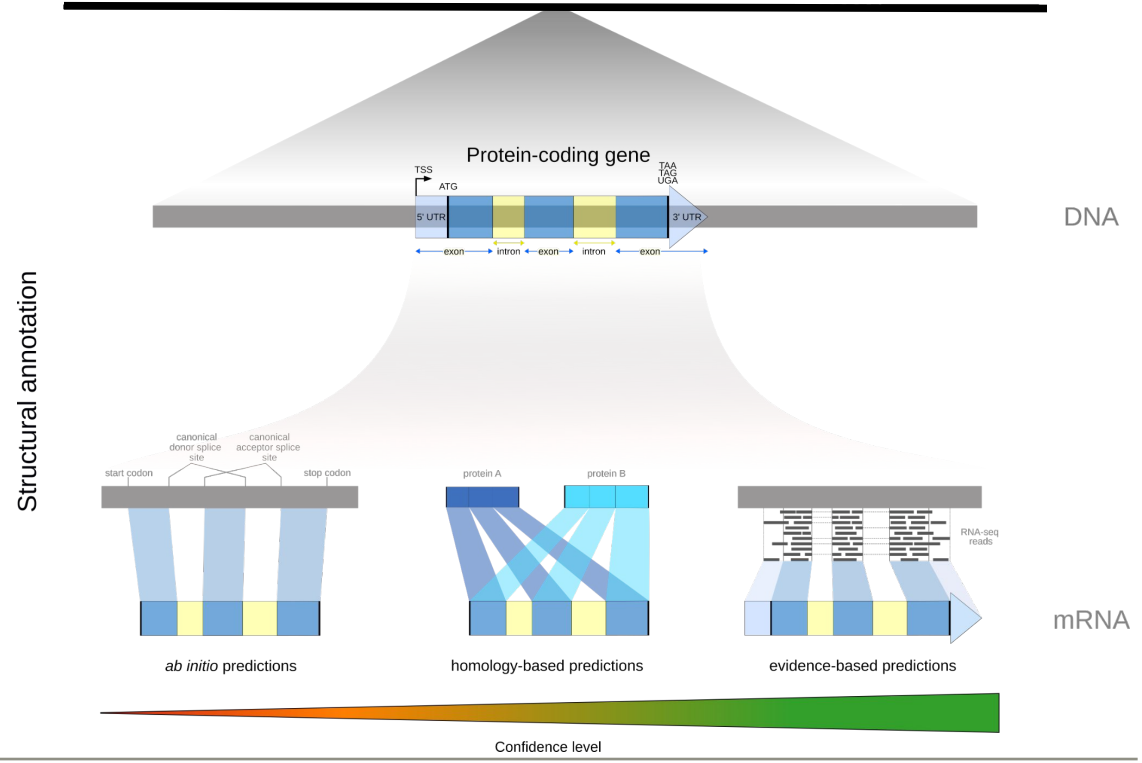
Structural annotation - Tools

(a) ***ab initio* prediction:**
Augustus, Helixer, Tiberius

(b) ***homology-based*:**
GeMoMa

(c) ***evidence-based*:**
BRAKER3

(d) ***Integrated pipelines:***
Helixer + GeMoMa + BRAKER3



Oliveira et al., 2025: 10.20944/preprints202508.1176.v2

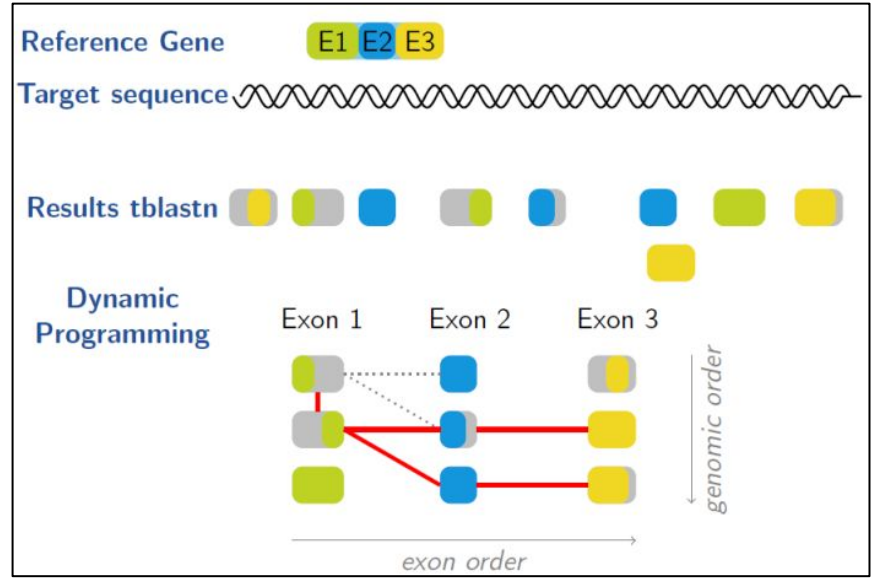
Gene annotation using GeMoMa

- GeMoMa → Gene Model Mapper
- Homology-based gene prediction

- Requirements for GeMoMa:

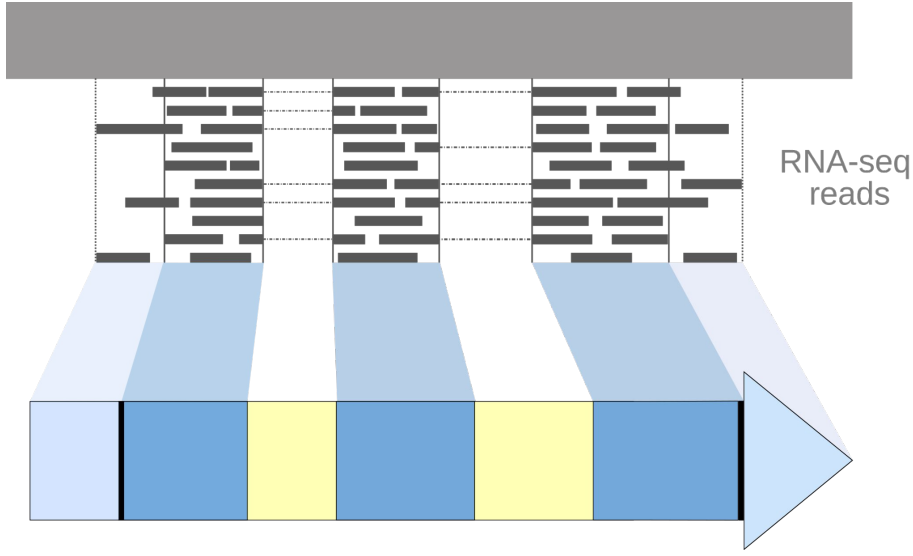
  (a) Protein-coding genes from closely-related reference genomes

  (b) RNA-seq evidence to refine borders of exons (optional)



https://www.jstacs.de/index.php/File:GeMoMa-schema.png

Structural annotation: RNA-seq hints



1. RNA-seq reads
2. read mapping of RNA-seq reads to the genome sequence assembly

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1. Downloading RNA-seq reads

- SRA = Sequence Read Archive
- downloading tools:
  1. prefetch (compressed file)
  2. fasterq-dump (un- compresses the files)
- December 2, 2025: **SRA Toolkit** Release 3.3.0 -> **GitHub** repository (apt version is outdated)

- Tasks:
  1. search for suitable two RNA-seq read files
     a. paired-end Illumina
     b. max. 6 Gb total size
  2. download them

1. Downloading RNA-seq reads

- SRA = Sequence Read Archive
- downloading tools:
  1. prefetch (compressed file)
  2. fasterq-dump (un- compresses the files)
- December 2, 2025: **SRA Toolkit** Release 3.3.0 -> **GitHub** repository (apt version is outdated)

```
/vol/data/tools/prefetch -p --max-size 250g --output-directory /vol/data/DNA_reads XXX
```

no. of threads (CPU)

SRA/ENA read ID

```
/vol/data/tools/fasterq-dump -e 15 /vol/data/DNA_reads/XXX --outdir /vol/data/DNA_reads/raw_reads
```

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2. Mapping RNA-seq reads

- RNA-seq reads alignment programs: HISAT2, STAR
- HISAT2 easily installable via apt
- samtools useful tool for SAM/BAM files (mapping files)

- Tasks:
  1. install HISAT2 (apt package manager recommended)
  2. map the RNA-seq reads to your genome assembly
  3. sort the mapping file

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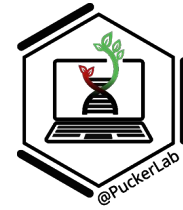
2. Mapping RNA-seq reads



1. Installation: sudo apt install hisat2 samtools

2. Indexing the genome assembly:

output index name (will create multiple files!)

```
hisat2-build -p 15 /vol/data/genome/assembly.fasta /vol/data/genome/assembly.fasta.hisat2.index
```

3. Starting the mapping:

```
hisat2 -p 15 -x  \
/vol/data/genome/assembly.fasta.hisat2.index -1 /vol/data/read_1.fq.gz -2 /vol/data/read_2.fq.gz \
| samtools sort - -O BAM -o /vol/data/assembly.fasta.RNA_reads.sorted.bam
```

input files

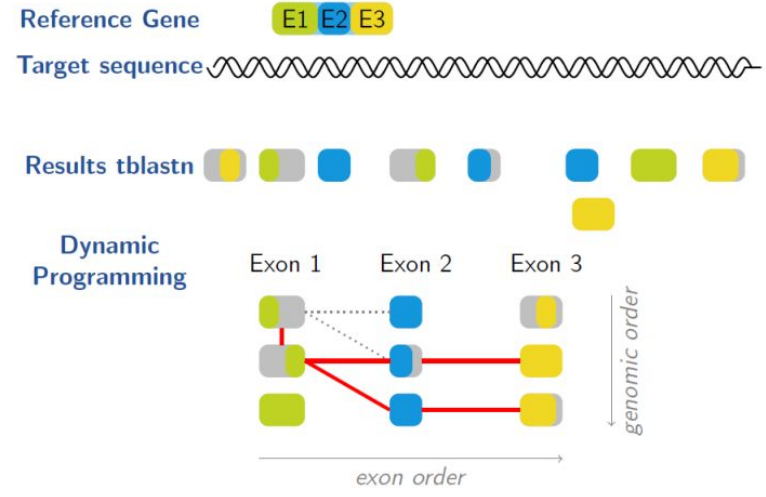
output file

very important

GeMoMa annotation

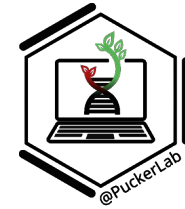
- Requirements for GeMoMa:

  (a) Protein-coding genes from closely-related reference genomes
  (b) RNA-seq evidence to refine borders of exons (optional) ✅



https://www.jstacs.de/index.php/File:GeMoMa-schema.png

GeMoMa annotation



- Requirements for GeMoMa:

  (a) Protein-coding genes from closely-related reference genomes

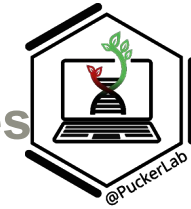
  Genome sequence (fasta format)

  Gene annotation (gff3 format)

GFF3 format

seqid	source	type	start	end	score	strand	phase	attributes
ctg1	GAF	gene	7031	7666	.	+	.	ID=gene001
ctg1	GeMoMa	mRNA	7031	7666	.	+	.	ID=gene001.1;Parent=gene001
ctg1	GeMoMa	CDS	7031	7097	.	+	0	Parent=gene001.1
ctg2	GeMoMa	CDS	7401	7666	.	+	2	Parent=gene001.1

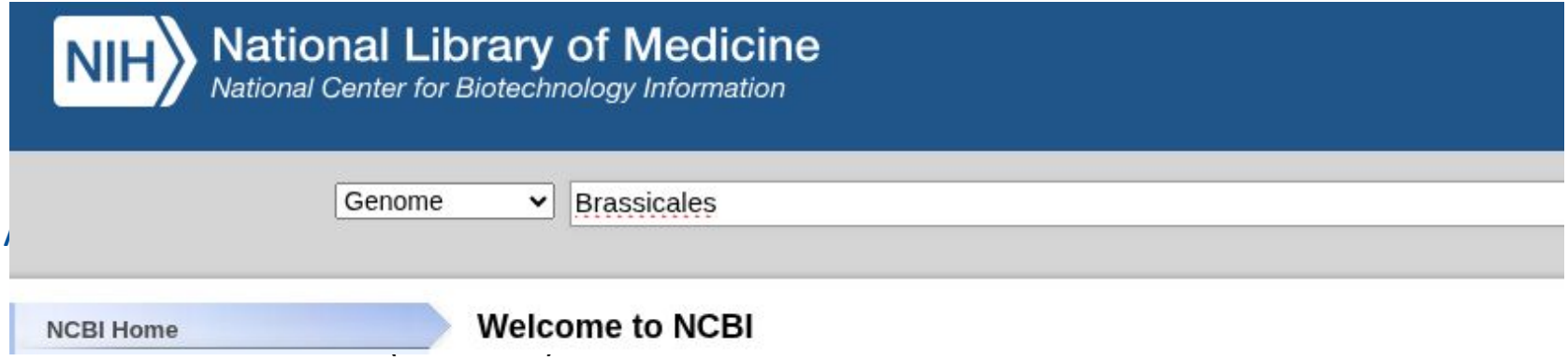
Getting protein hints from closely-related species



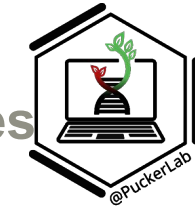
Tasks:

A. Search NCBI for nearly-complete annotated Brassicales genome sequences

- NCBI Genome (https://www.ncbi.nlm.nih.gov/)
- Search for Brassicales

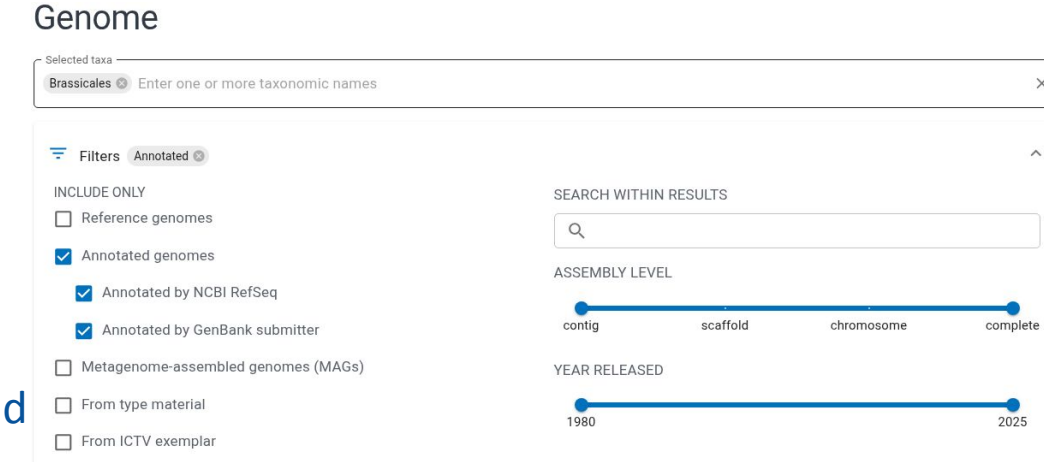


Getting protein hints from closely-related species



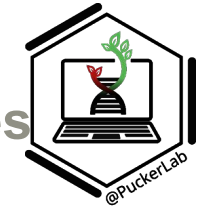
Tasks:

A. Search NCBI for nearly-complete annotated Brassicales genome sequences
- NCBI Genome
- Search for Brassicales
- Choose annotated from filters
- NCBI RefSeq assemblies recommended



A. Download any 4 using wget command (using FTP - file transfer protocol)
- Genome file (format ?)
- Gene annotation (format ?)

Getting protein hints from closely-related species



Tasks:

B. Download any 4 using wget command (using FTP - file transfer protocol)

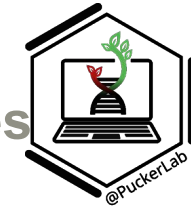
- Genome file (format ?)
- Gene annotation (format ?)

Genome assembly TAIR10.1 reference

Download datasets API FTP

NCBI RefSeq assembly	GCF_000001735.4
Submitted GenBank assembly	GCA_000001735.2

Getting protein hints from closely-related species



Tasks:

A. Download any 4 using wget command (using FTP - file transfer protocol)
- Genome file (format ?) (**_genomic.fna.gz**)
- Gene annotation (format ?) (**_genomic**.gff.gz)
- right-click on the link → copy link → In the VM,
  wget (paste copied link)

Running GeMoMa

1. Installing GeMoMa: https://www.jstacs.de/index.php/GeMoMa

   wget http://www.jstacs.de/download.php?which=GeMoMa

   mv download.php\?which\=GeMoMa GeMoMa

1. Installing requirements
   - mmseqs2: https://github.com/soedinglab/MMseqs2
     wget https://mmseqs.com/latest/mmseqs-linux-gpu.tar.gz; tar xvfz mmseqs-linux-gpu.tar.gz; export PATH=$(pwd)/mmseqs/bin/:$PATH
   - v15< java >v1.8: To check if available, java -version

     If java not found,
     sudo apt install openjdk-11-jdk **or** sudo update-alternatives --config java

1. Running GeMoMa pipeline

Running GeMoMa pipeline

Command:

```
java -jar /path/to/GeMoMa-1.9.jar CLI GeMoMaPipeline \
t=/path/to/genome.fasta \
i=SG1 a=/path/to/SG1.gff3.gz g=/path/to/SG1.genome.fasta.gz \
i=SG2 a=/path/to/SG2.gff3.gz g=/path/to/SG2.genome.fasta.gz \
i=SG3 a=/path/to/SG3.gff3.gz g=/path/to/SG3.genome.fasta.gz \
i=SG4 a=/path/to/SG4.gff3.gz g=/path/to/SG4.genome.fasta.gz \
r=MAPPED ERE.m=/path/to/assembly.fasta.RNA_reads.sorted.bam \
outdir=/path/to/Gemoma_output/ \
pc=true pgr=true p=true o=true \
GeMoMa.c=0.4 GeMoMa.Score=ReAlign Extractor.r=true \
GAF.f="start=='M' and stop=='*' and (isNaN(score) or score/aa>='0.75')" \
AnnotationFinalizer.r=NO threads=13 >> gemoma.log 2>&1 &
```

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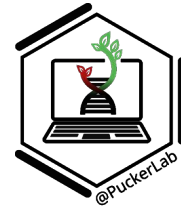
End of day 2



Today you learned:

- Evaluating a genome assembly sequence
- Structural annotation of a genome assembly sequence

Tomorrow you will learn:

- Evaluating your structural annotation
- Finalizing your structural annotation (Filtering)

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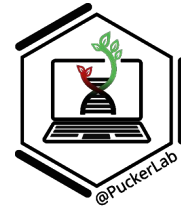
Availability of materials



Please find all course materials here:
https://github.com/bpucker/teaching/tree/master/deNBI2025

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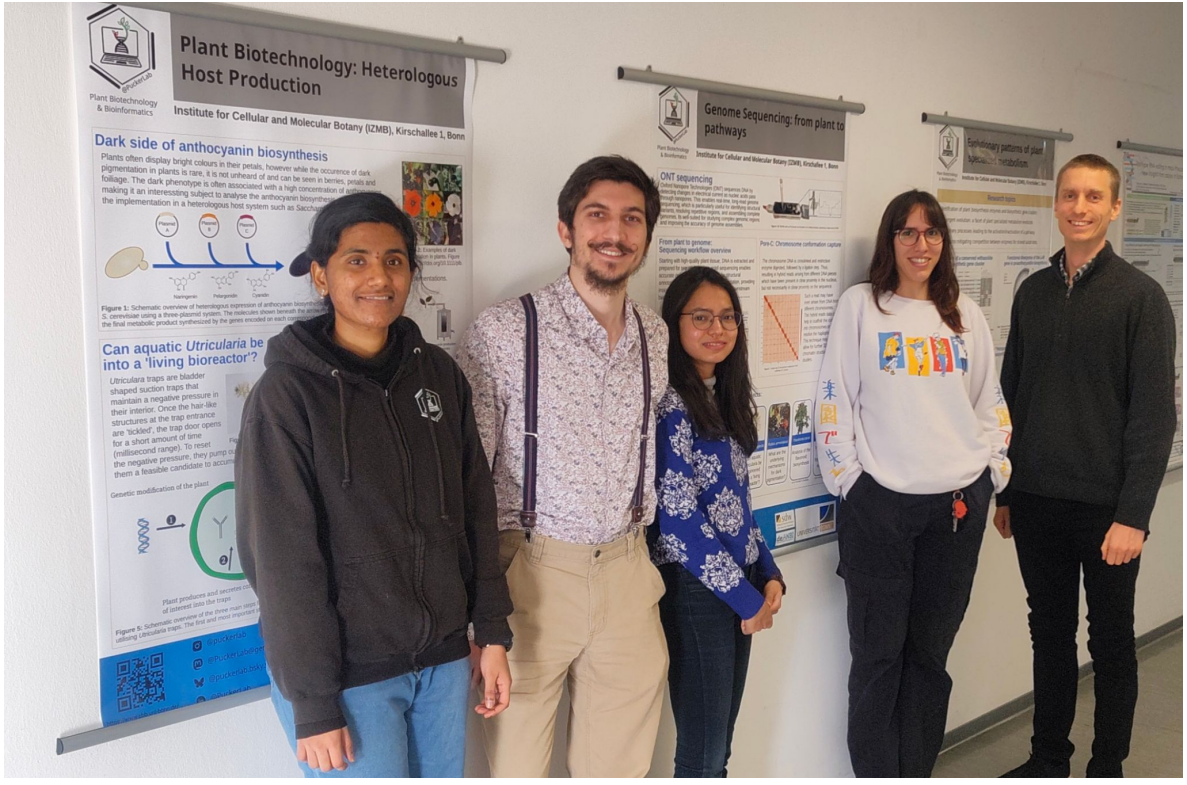
Acknowledgements



@PuckerLab

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Email: pucker@uni-bonn.de
Web: pbb.uni-bonn.de

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