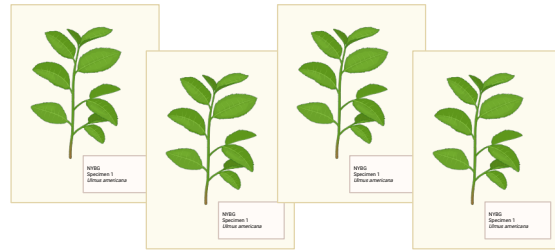


Step 1a) Sample collection

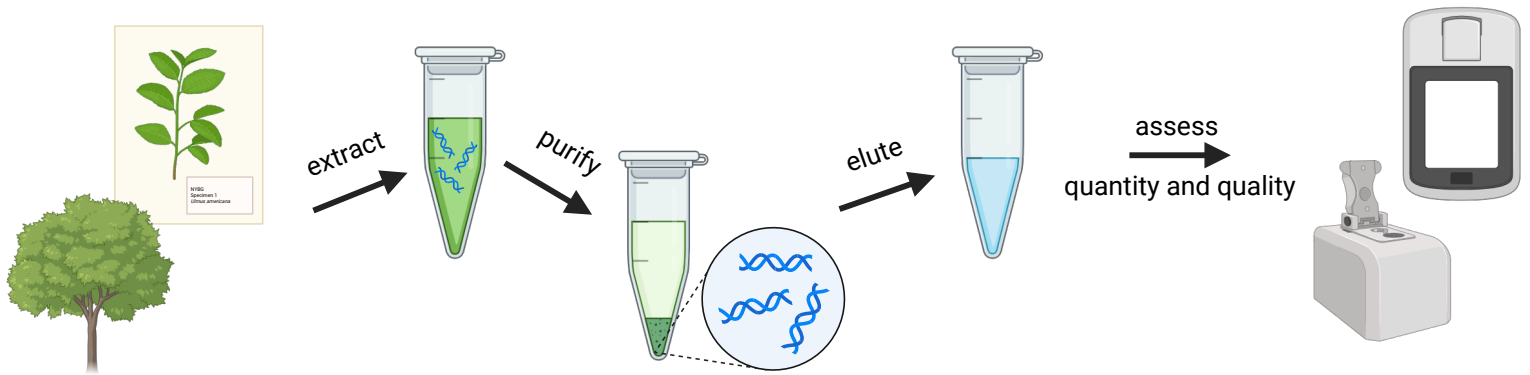
Extant trees



Herbarium specimens

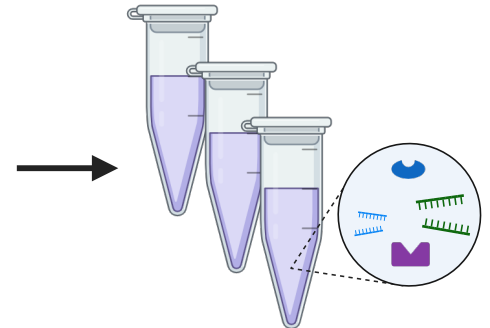


Step 2a) DNA extraction with modified CTAB protocol

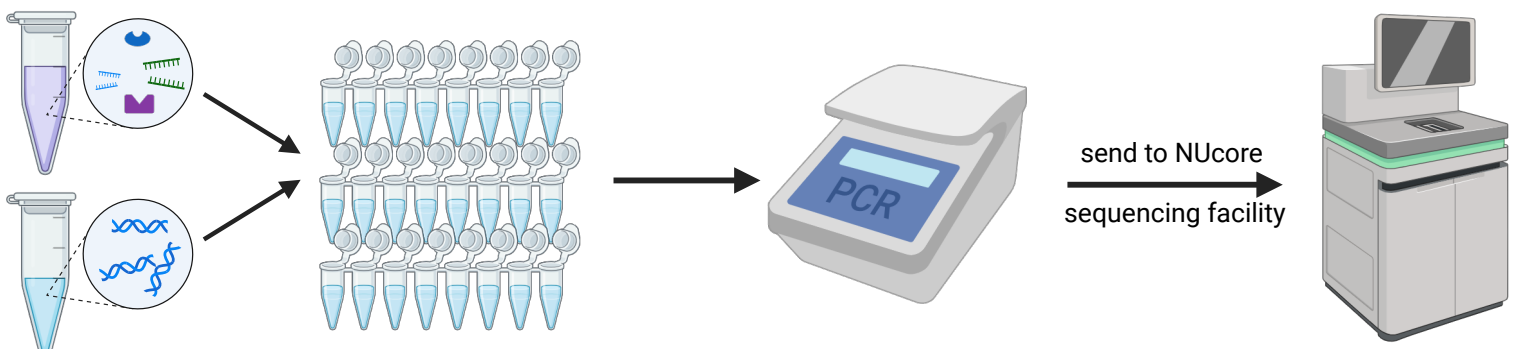


Step 3a) Create custom amplicon panel

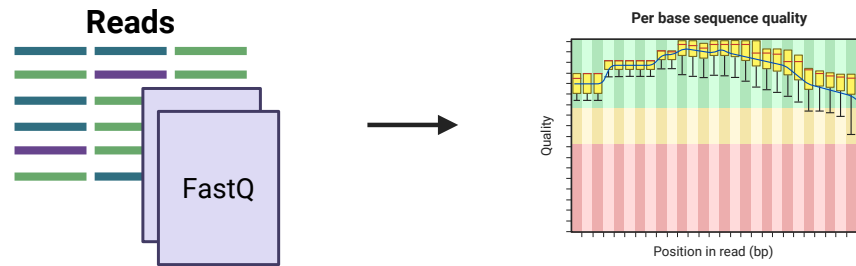
```
>elm gene 1
ATGCATTGGCGGCTACTAGCTGCTAAGC
GCGCGTACTAGCTGCTAAGCATTAGCAT
>elm gene 2
CGTACTAGCTGCTAAGCATTAGCATCTA
AGCATTAGCATCTATACTAGCTGCTAAG
>disease gene 1
AAGCATTAGCATCTAATGCATTGGCGCG
TACTAGCTGCTAAGCGTACTAGCTGTAG
```



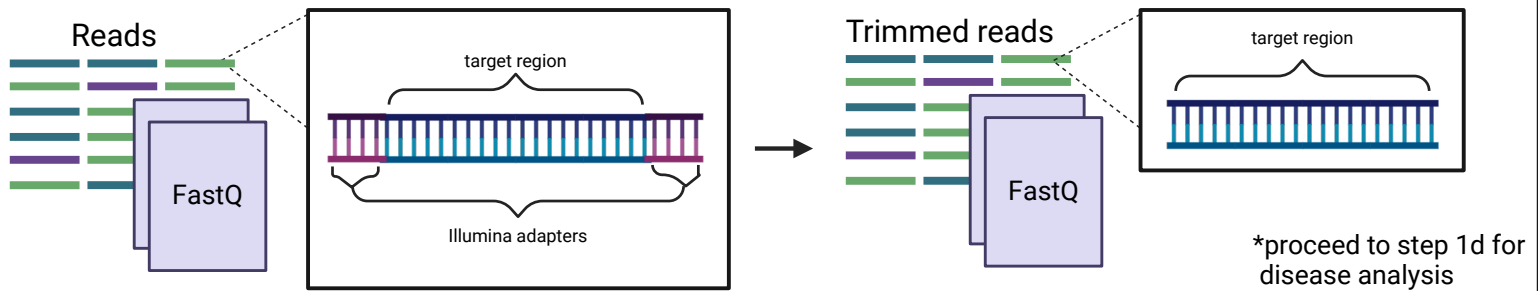
Step 4a) Library preparation and sequencing



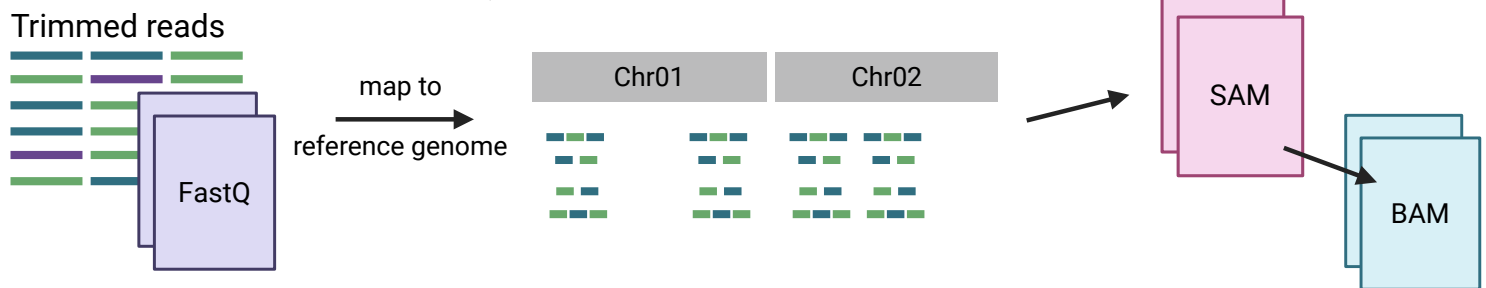
Step 1b) Quality control with FastQC and MultiQC



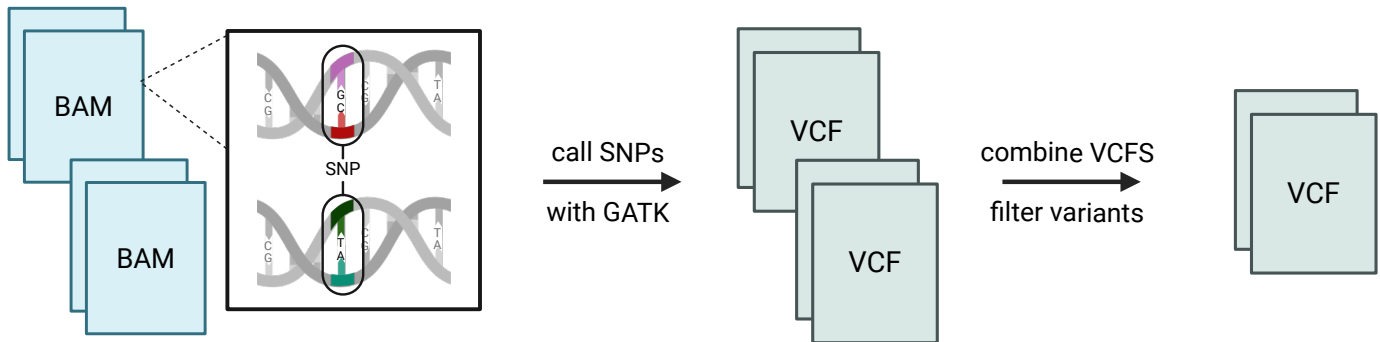
Step 2b) Trimming with Trimmomatic



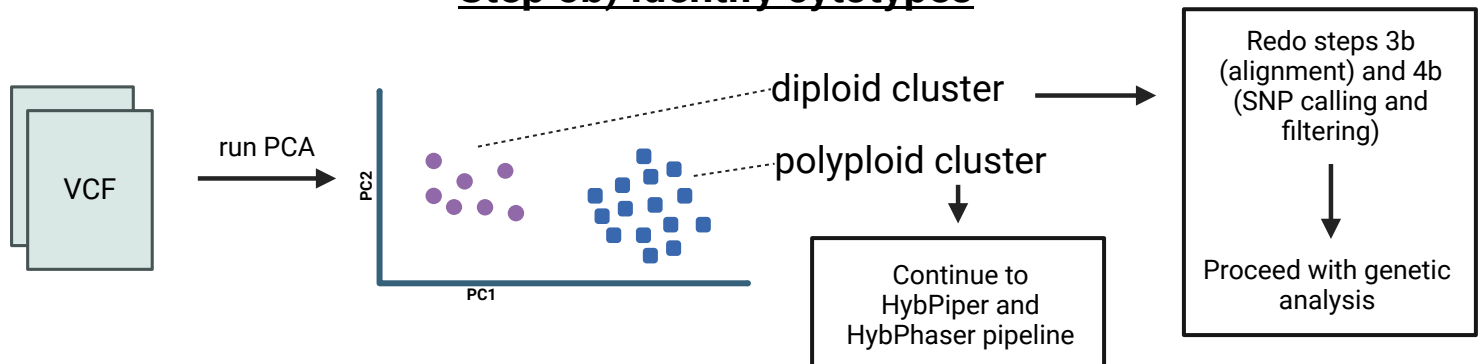
Step 3b) Align with to reference with BWA



Step 4b) Call SNPs with GATK

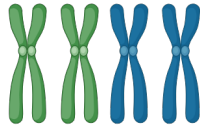


Step 5b) Identify cytotypes



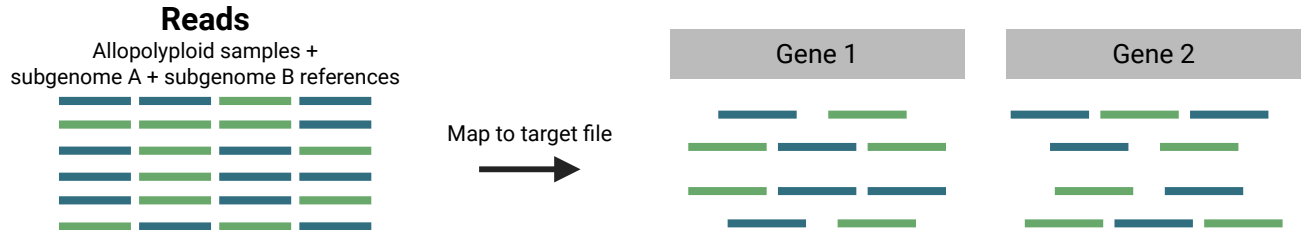
Step 0) Allopolyploid subgenome identification

Subgenome A:
diploid *U. americana*



Subgenome B:
unknown (likely *U. rubra*,
used *U. glabra* as reference)

Step 1) Assemble allopolyploid reads with HybPiper



Step 2) Generate subgenome consensus sequences with HybPhaser I

Subgenome A

Gene 1

A T G W C G T C N N N A G C G C A

Gene 2

T A C G G C T A D G A G C T R C T

Subgenome B

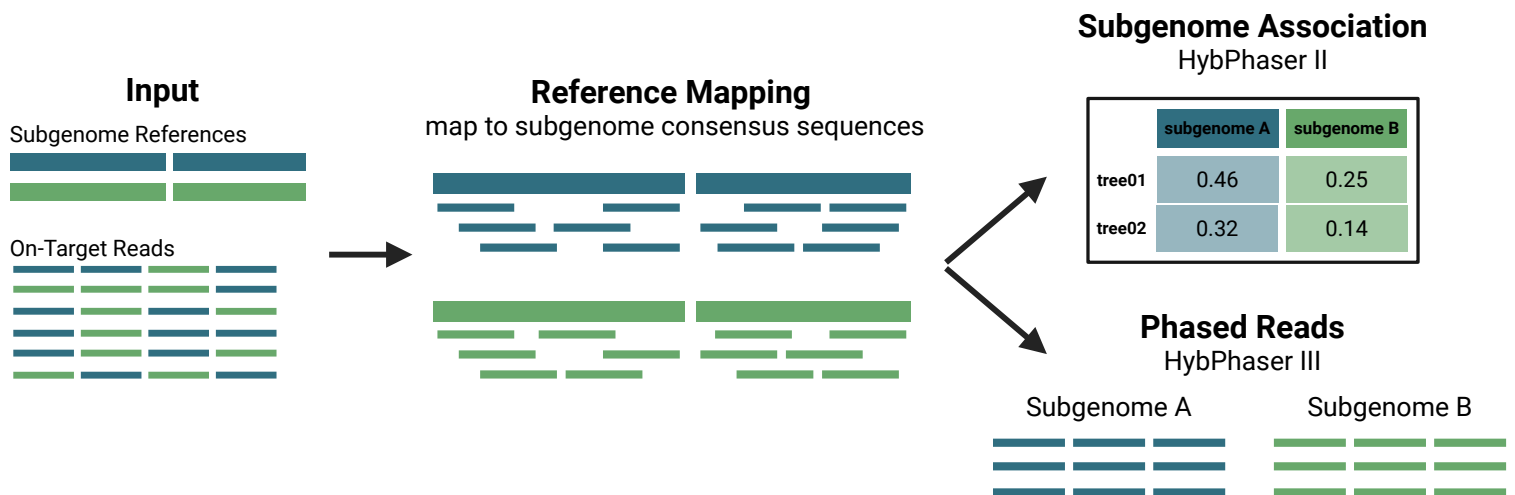
Gene 1

A T G T C G K C A N N A G C G C A

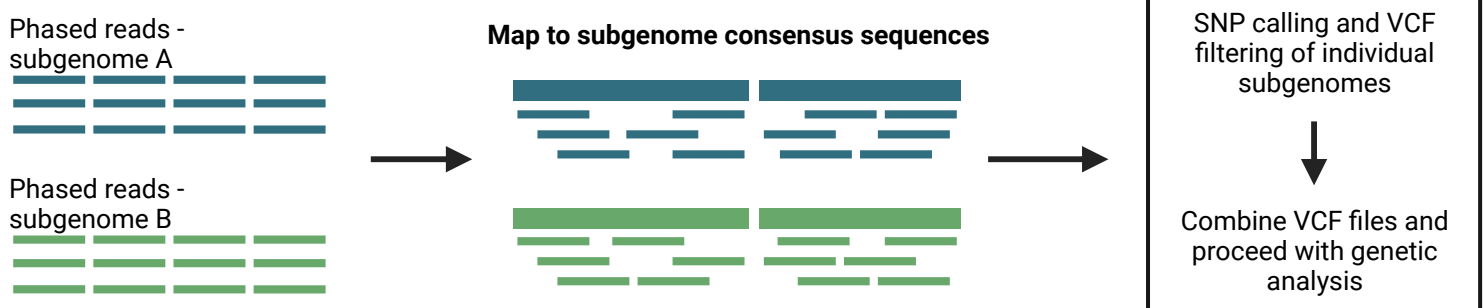
Gene 2

T A C S G C T A A G M G C T A C T

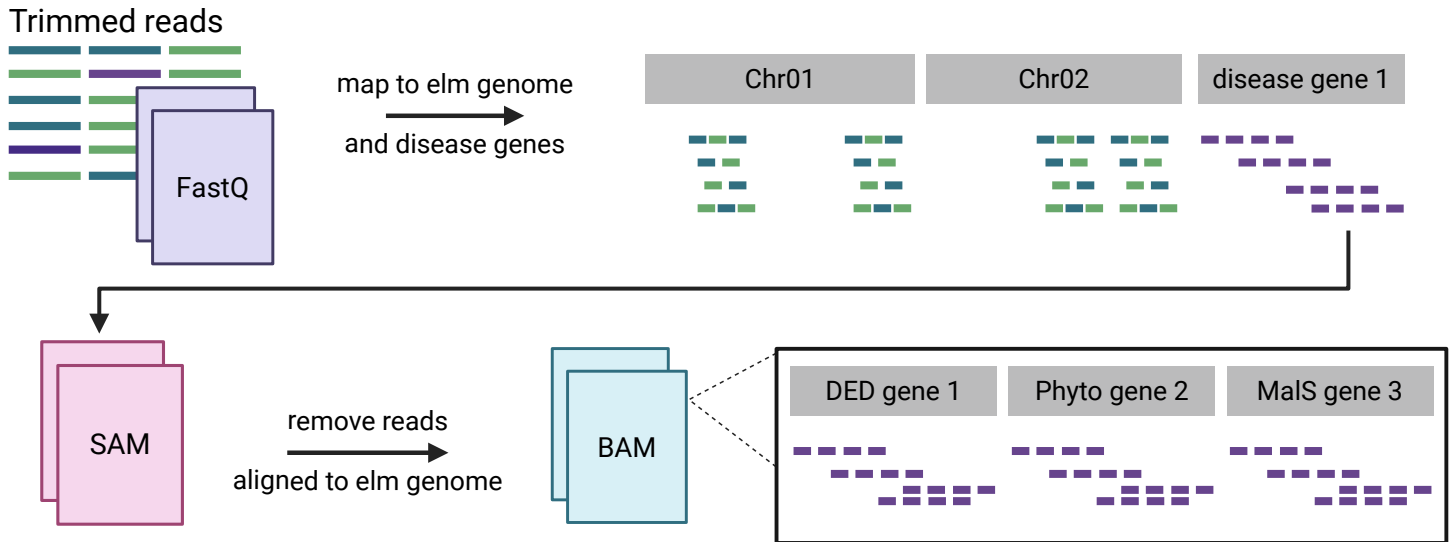
Step 3) Subgenome Association and Phasing with HybPhaser II & III



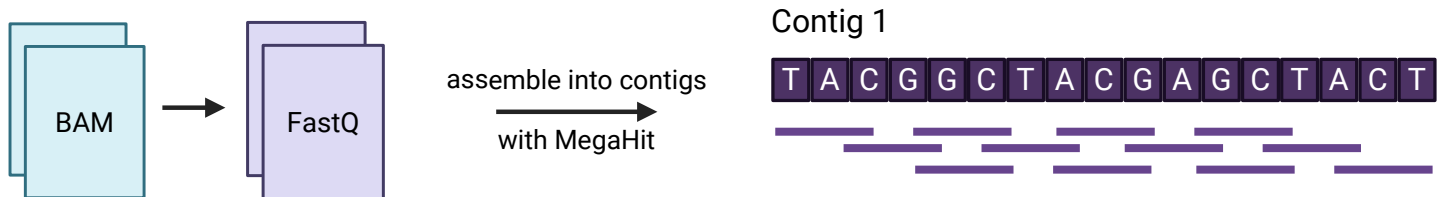
Step 4) Assemble phased reads with HybPiper



Step 1d) Align to reference with BWA



Step 2d) Assemble reads into contigs with MegaHit

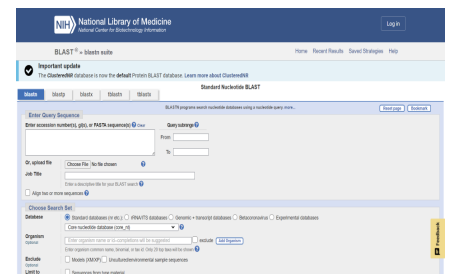


Step 3d) Compare contigs to reference database

Contig 1
T A C G G C T A C G A G C T A C T

Contig 2
A T G T C G T C A A T A G C G C A

Blast against
NCBI Nucleotide Database



Step 4d) Identify low and high confidence infections

Low confidence
An individual who has 1 or more Blast hits matching the **family** of the causal agent.

High confidence
An individual who has 1 or more Blast hits matching the **genus** of the causal agent.

tree01	DED	Phyto	MalS
Low confidence	4	0	0
High confidence	1	0	0
tree02	DED	Phyto	MalS
Low confidence	0	2	0
High confidence	0	0	0

