Nanopore Adaptive sampling: A powerful approach to decipher the genetic diversity of the NLRome/resistome in melon

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Nanopore Adaptive sampling: A powerful approach to decipher the genetic diversity of the NLRome/resistome in melon

Javier BELINCHON-MORENO

Nanopore Day, Paris, 21st March 2023

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INRAE

EPGV
Etude du Polymorphisme des Génomes Végétaux

université PARIS-SACLAY

GAFL
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EPGV – INRAE: plant genomic platform located at Genoscope, Evry - France

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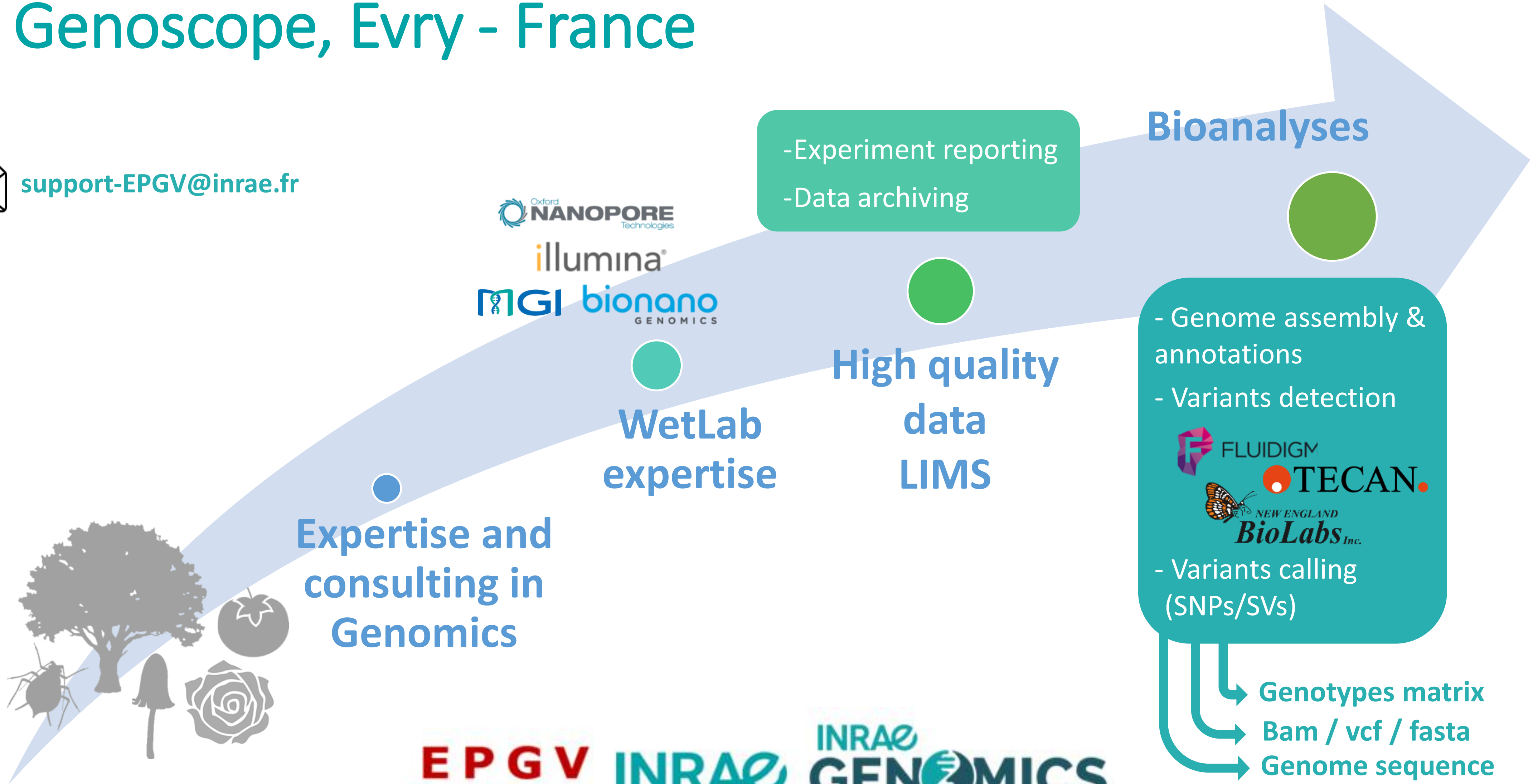


Expertise and consulting in Genomics

WetLab expertise

- -Experiment reporting
- -Data archiving

High quality data LIMS

Bioanalyses

- Genome assembly & annotations
- Variants detection
- Variants calling (SNPs/SVs)

Genotypes matrix

Bam / vcf / fasta

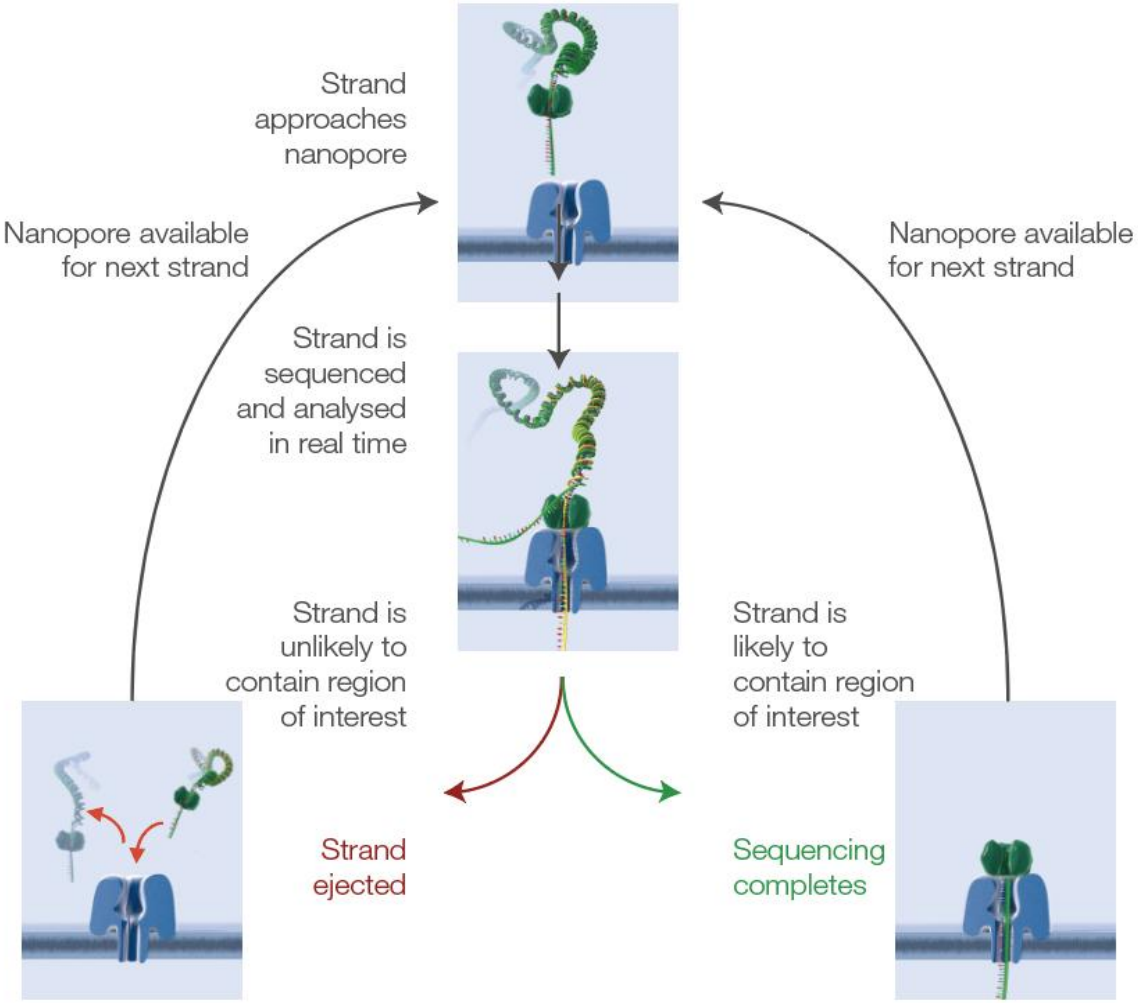
Genome sequence

EPGV

Etude du Polymorphisme des Génomes Végétaux

What is Nanopore adaptive sampling?

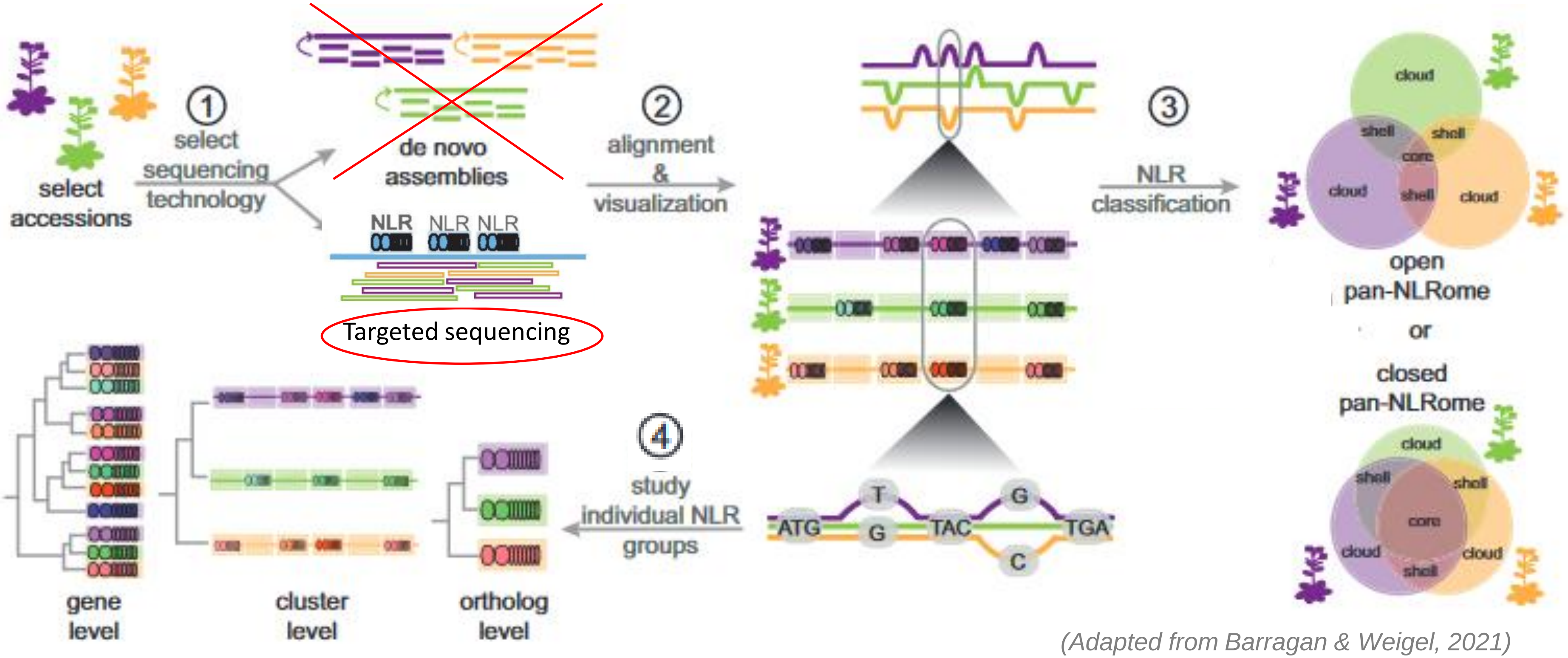
- Accept/reject molecules based on **small initial part** of sequence (~500 bp)
- **Enrich/deplete** specific regions of interest.
- Increase on-target data, reduce time-to-answer.
- **Real time** target selection: **easy** library preparation.



Our objective: Nanopore adaptive sampling to decipher the genetic diversity of the NLRome in melon

Reference genome not enough → Construction of **pan-NLRome**

High number of varieties (~150 varieties) → Need for **selective sequencing**



Targeted sequencing

(Adapted from Barragan & Weigel, 2021)

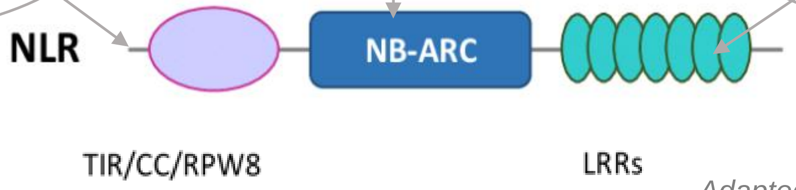
NLR resistance genes: a conserved structure

Nucleotide binding site (NB) -leucine rich repeat (LRR)

Intracellular immune receptor proteins

Major family of plant-resistance (R) genes

Broad range of **resistance**



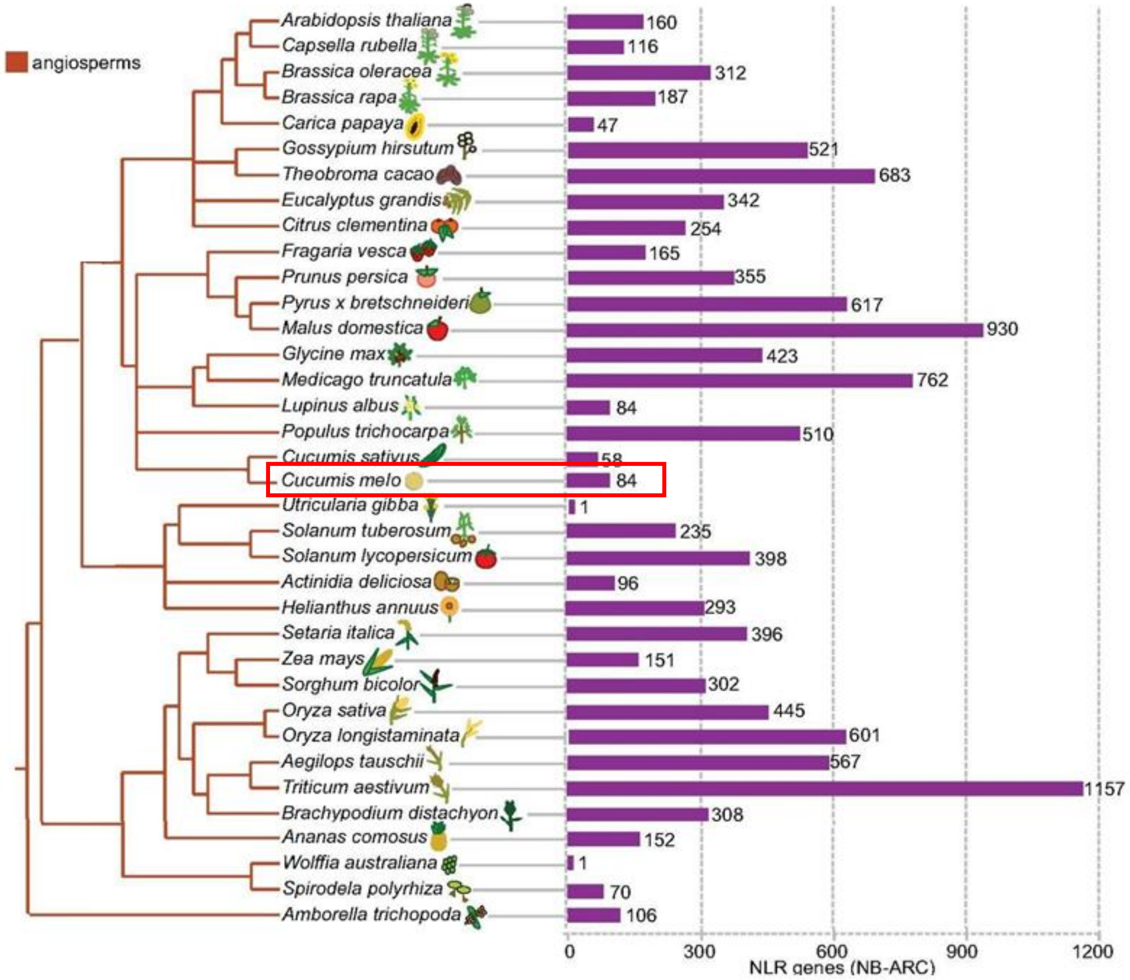
Signal-transducing ATPase domain.
Regulates the **ON/OFF state** of the NLR

Primary structural element of
signal transduction

Ligand binding platform with
auto-inhibitory function

Adapted from (Gottin et al., 2021)

Variable number of NLR genes between plant species



Adapted fom (Barragan & Weigel, 2021)

Usually grouped into **clusters**

High level of presence/absence polymorphisms (PAV)

Low frequency in the Cucurbitaceae family

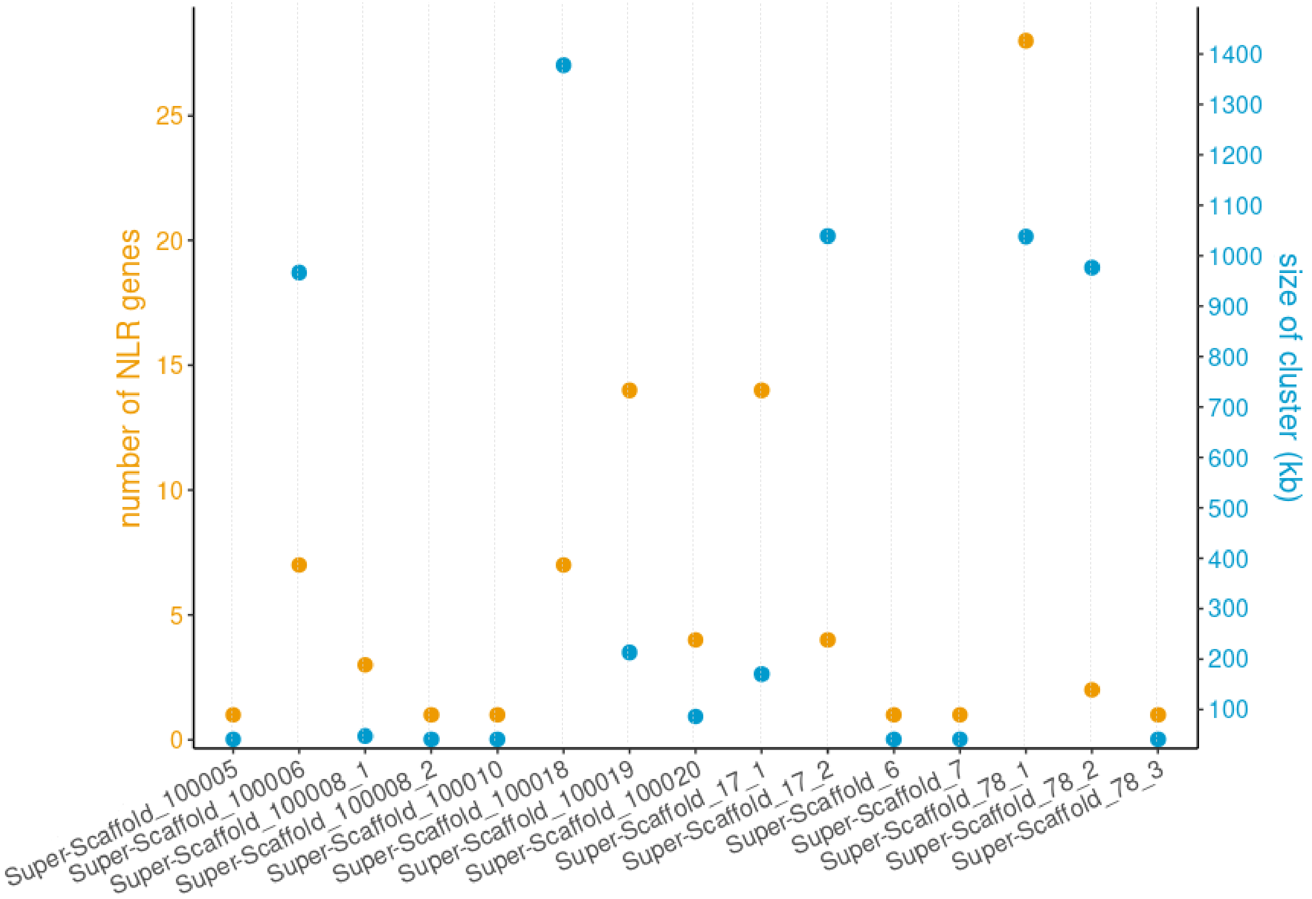
<u>*C.melo* variety Anso77</u>

84 NLR genes in 15 clusters

NLRGenomeSweeper *(Toda et al., 2020)*

Our target regions: 15 NLR-gene clusters

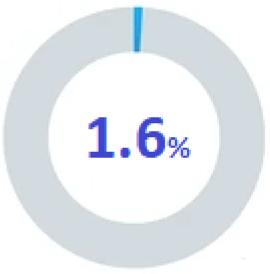
Reference genome → Variety **Anso77** (draft genome produced at INRAE-GAFL/EPGV)



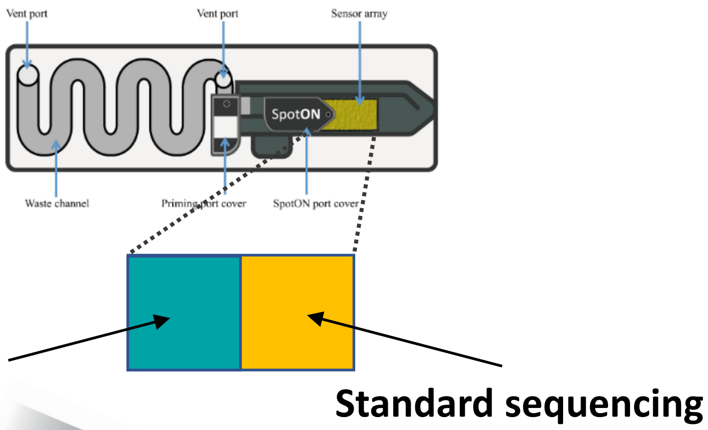
Reference genome (.fasta)
+
Target regions (.bed)

Clusters:
NLR genes + 20kb-extra flanking
Variable in size
Variable in number of genes

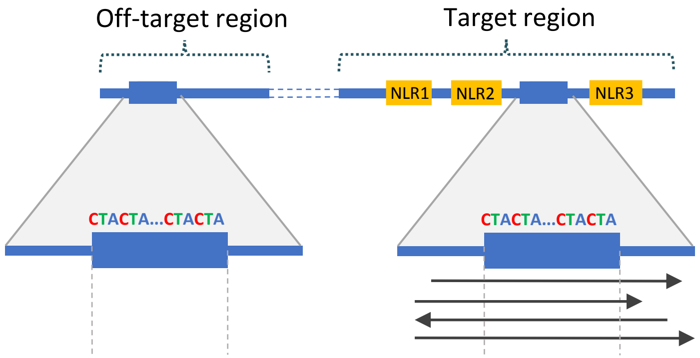
Genome length ~380Mb
Target regions length ~6.16Mb



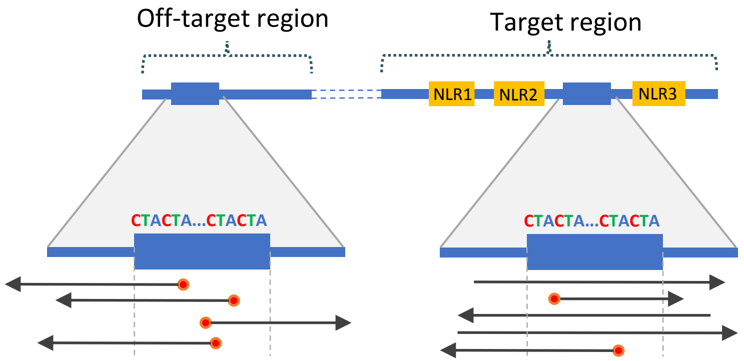
Our first experience: Variety Anso-77 (same as provided reference)



Masking repetitive elements >200kb



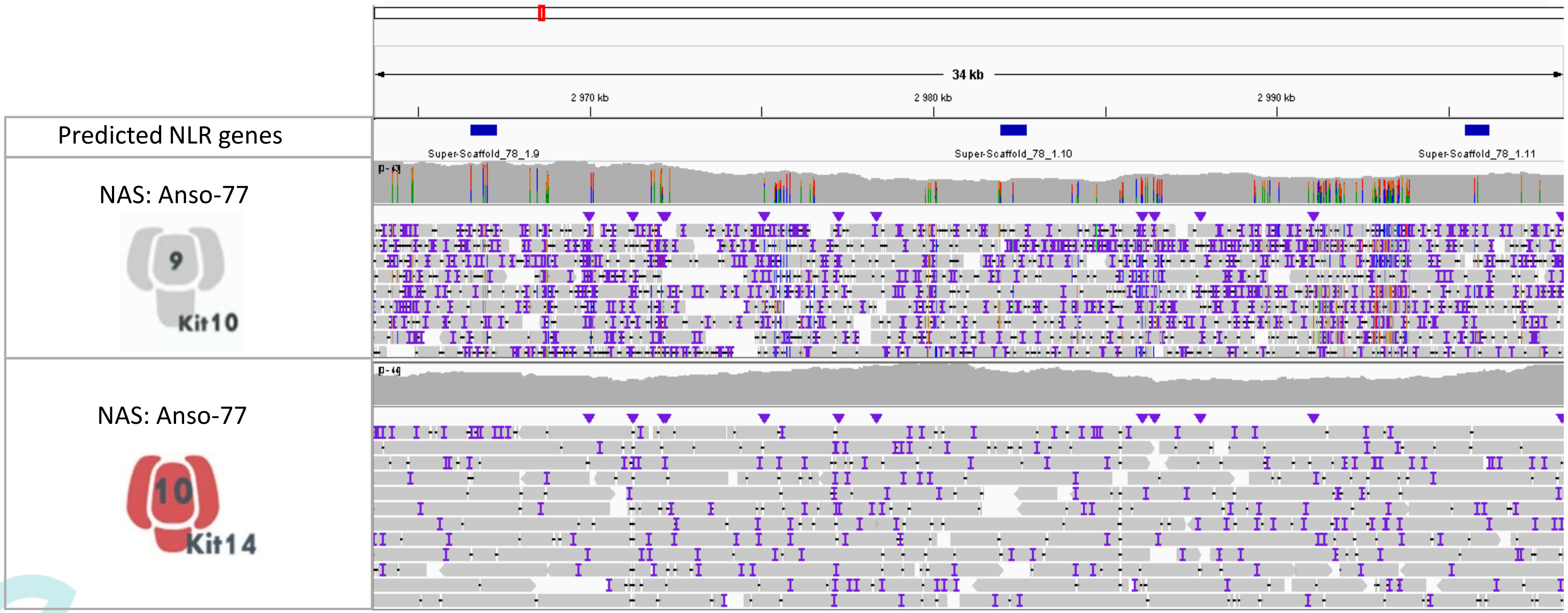
Not masking repetitive elements



Nanopore kit evolution: From kit10 to kit14

10 Kit14
R10.4.1
+
E8.2

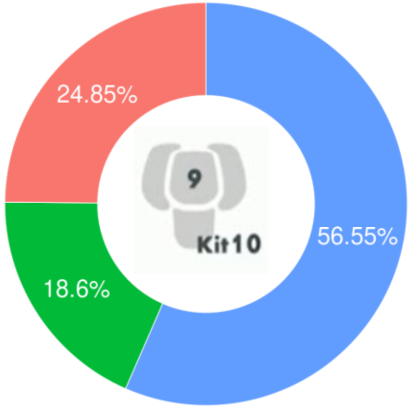
- **Increased accuracy**, improved sequencing **speed**
- **Reactive consumption reduced**, adaptors capture improved
- Duplex sequencing, **better throughput**

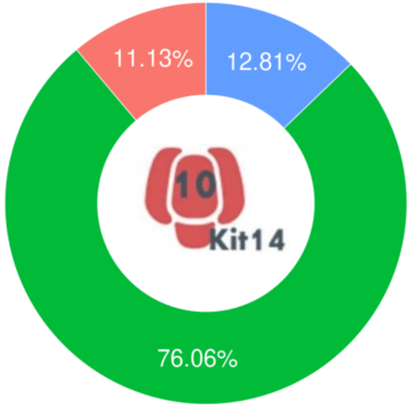

Predicted NLR genes

NAS: Anso-77

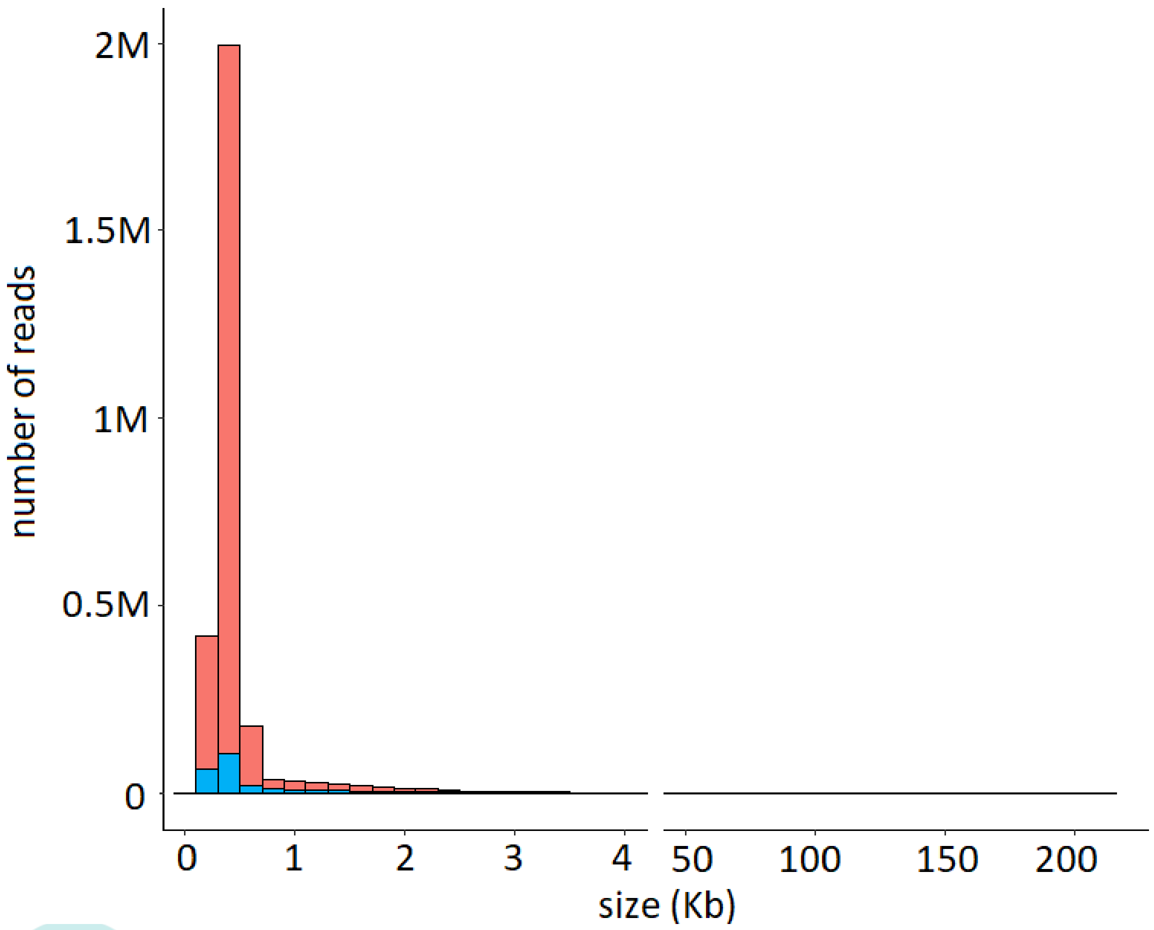
NAS: Anso-77

🟩 >Q30
🟦 Q20-Q30
🟥 <Q20

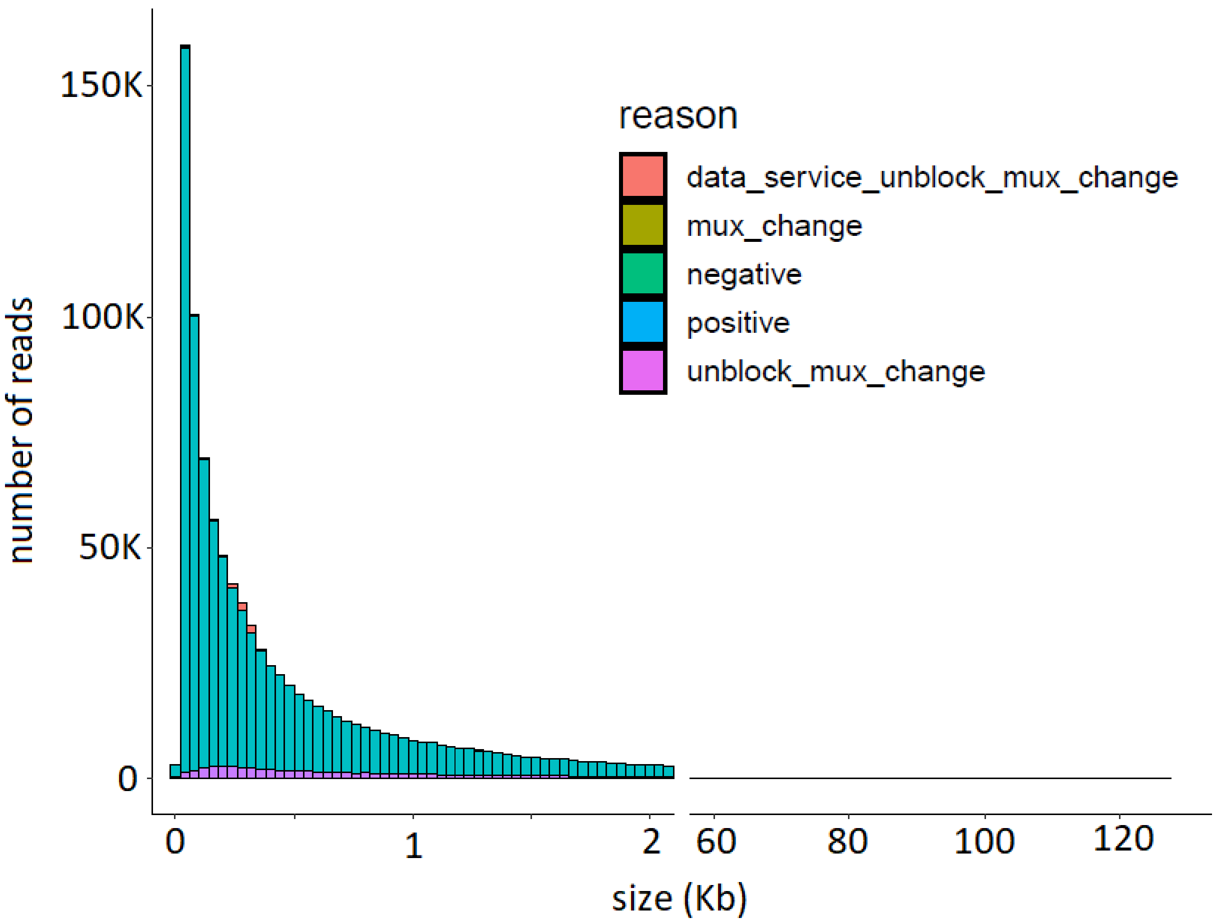




NAS: A characteristic distribution of read lengths

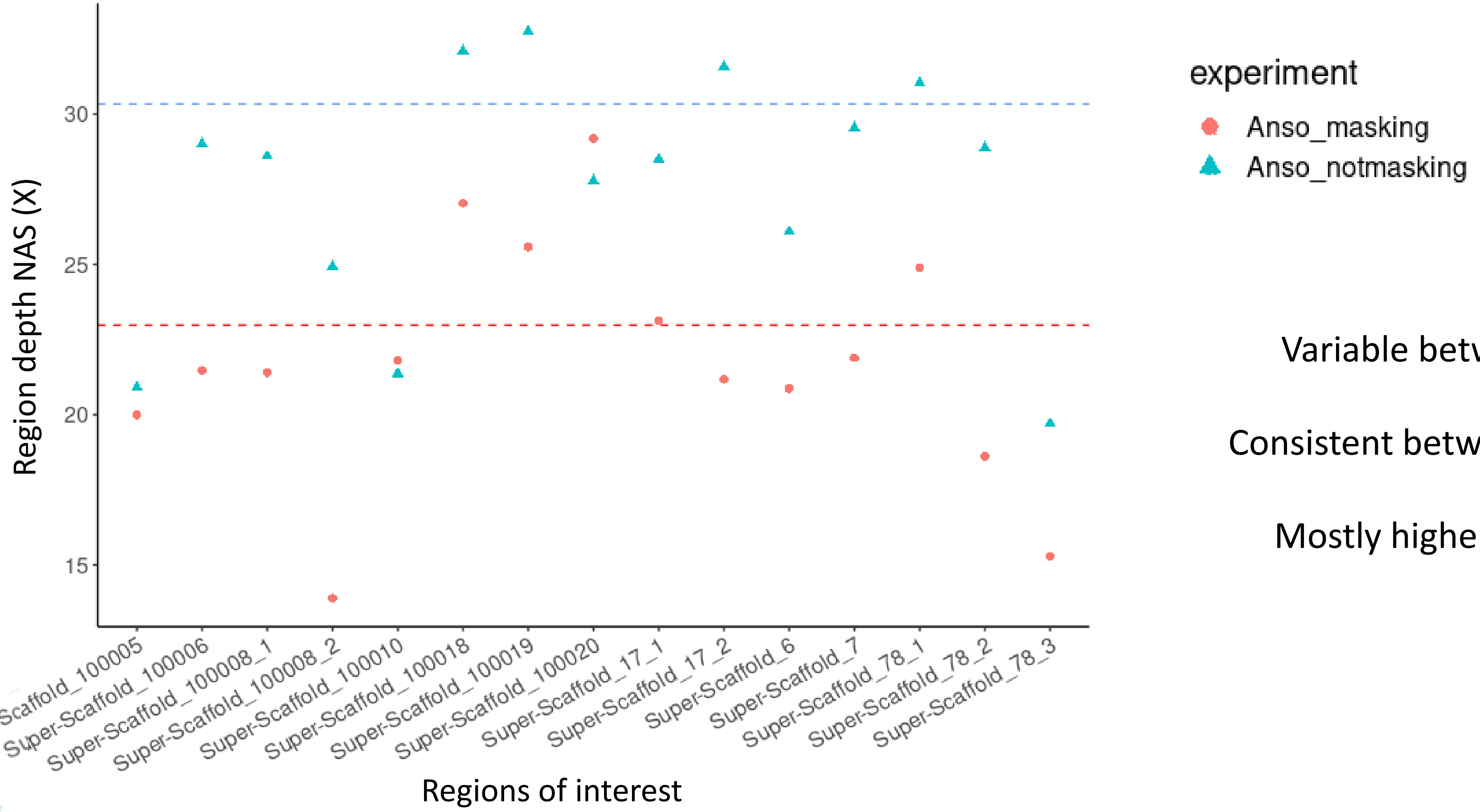


Nanopore adaptive sampling



Standard sequencing

Statistics: regions depth

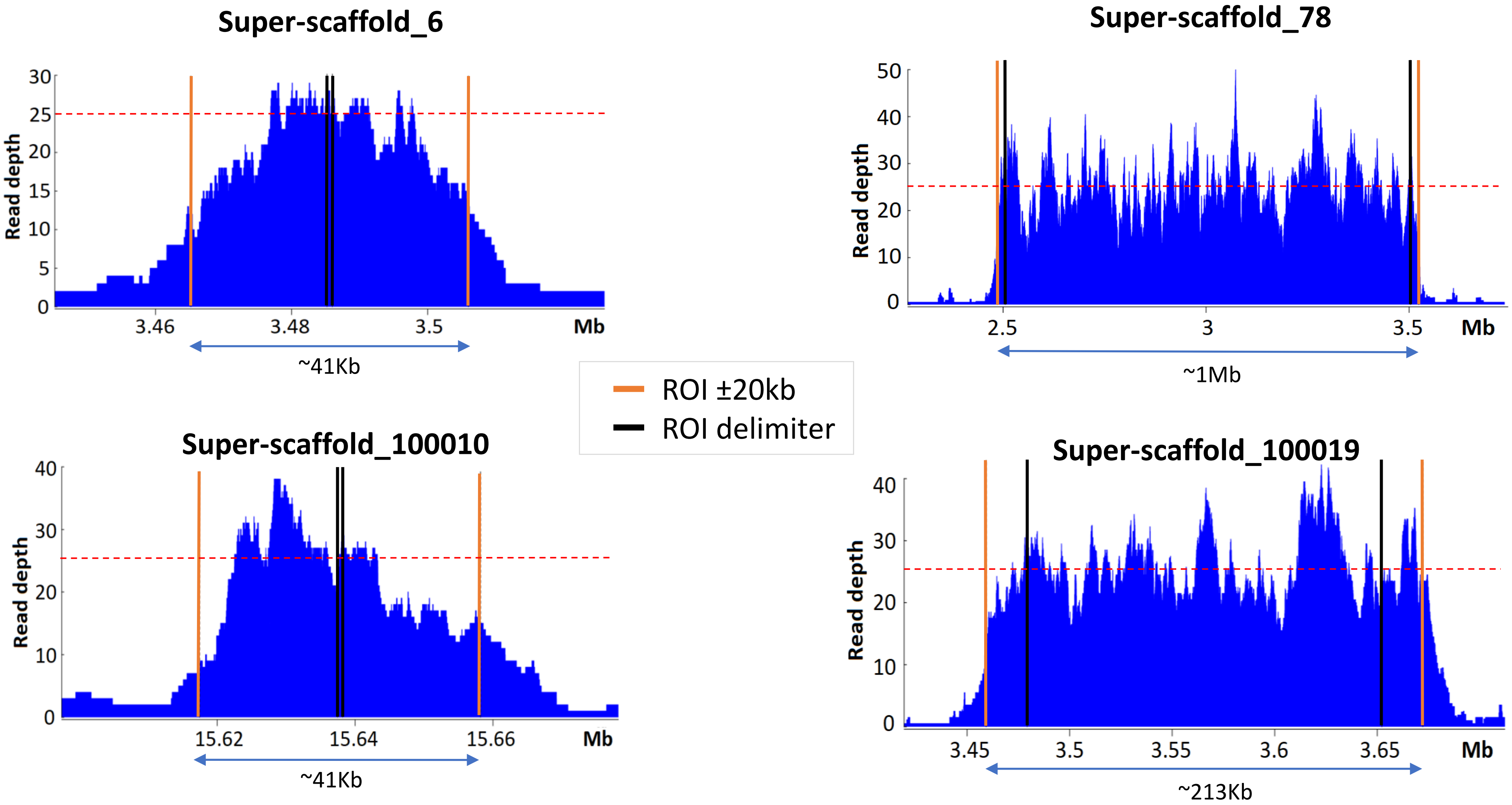


Variable between clusters

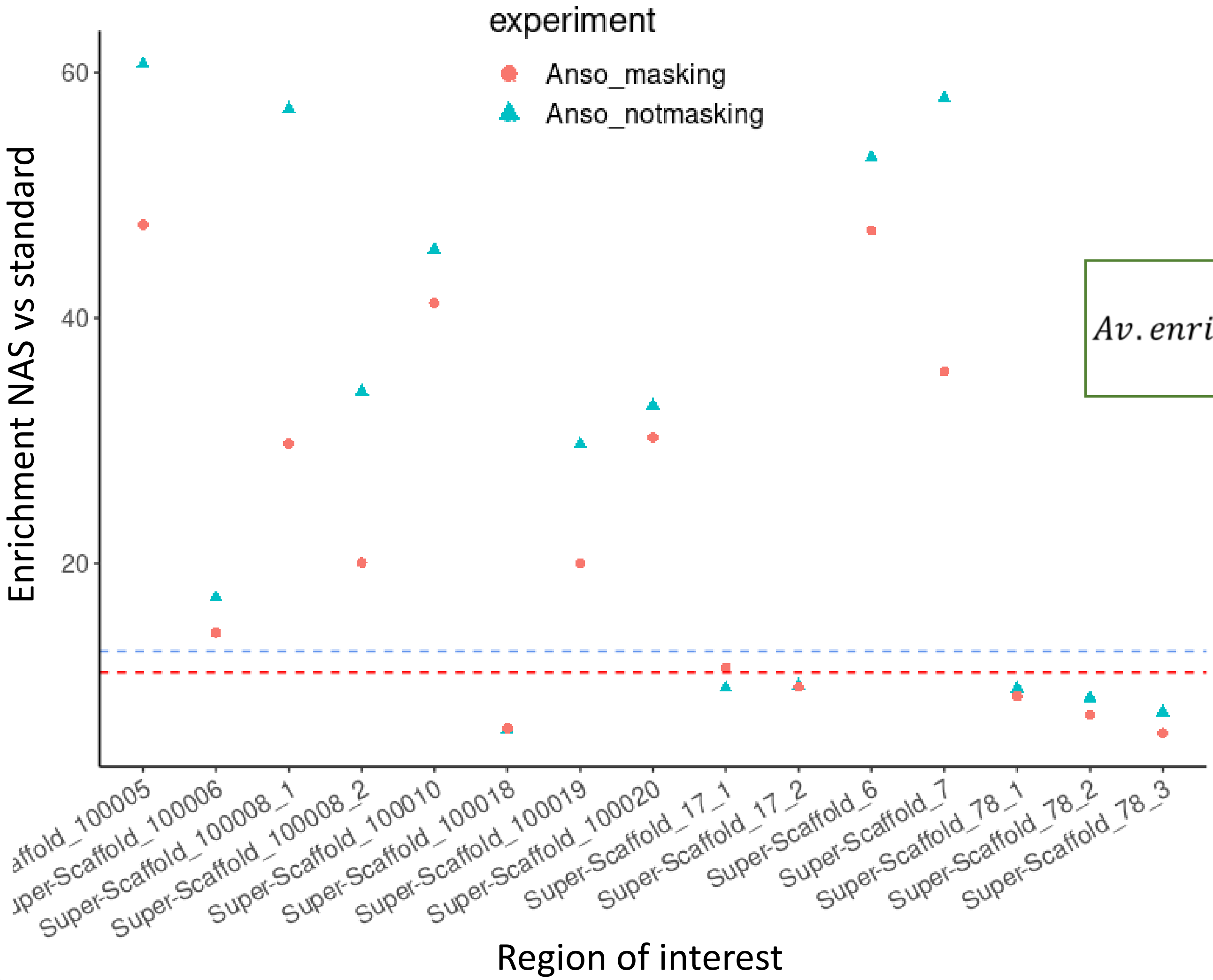
Consistent between experiences

Mostly higher not masking

Visual enrichment of our target regions



Good enrichment of our target regions



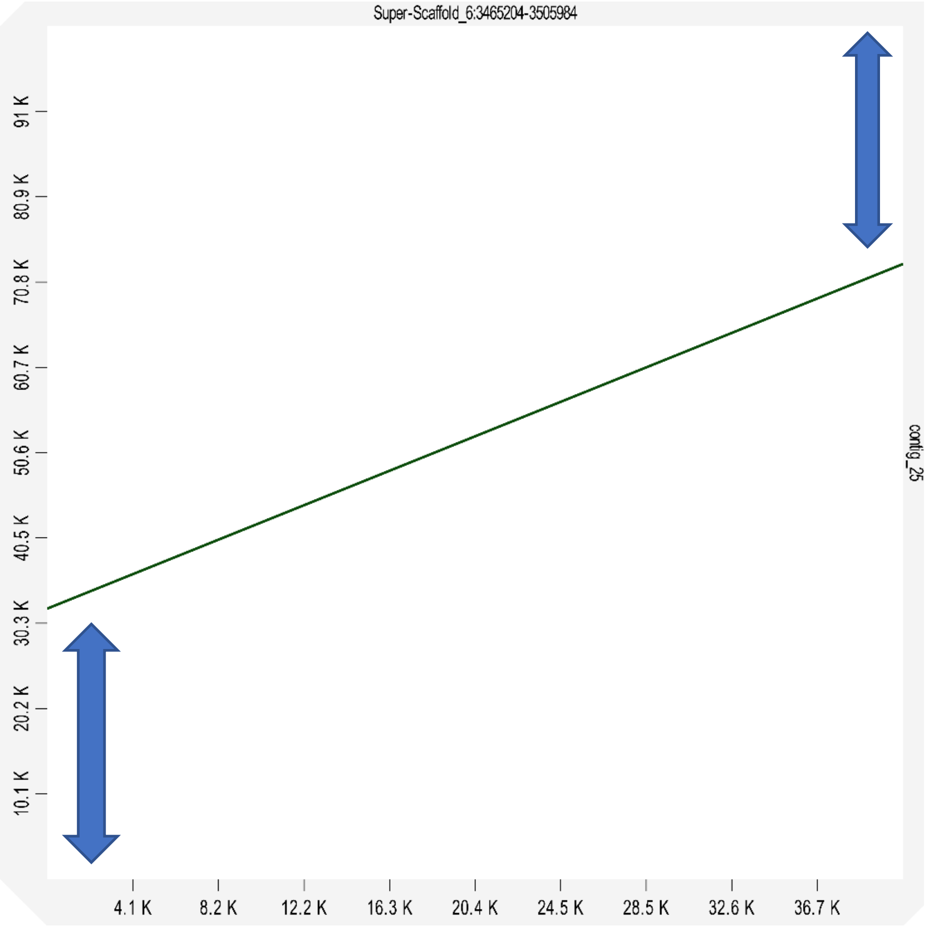
$$Enrichment_{region} = \frac{\frac{region\_cov\_NAS}{chrom\_cov\_NAS}}{\frac{region\_cov\_standard}{chrom\_cov\_standard}}$$

$$Av.enrichment = \sum_{region=1}^{N} \frac{enrichment_{region}(X) * region\_size(bp)}{total\_size\_regions(bp)}$$

- Variable between clusters
- Variable between experiences
- But consistent between experiences for clusters under 20X
- Average ~equal between experiences

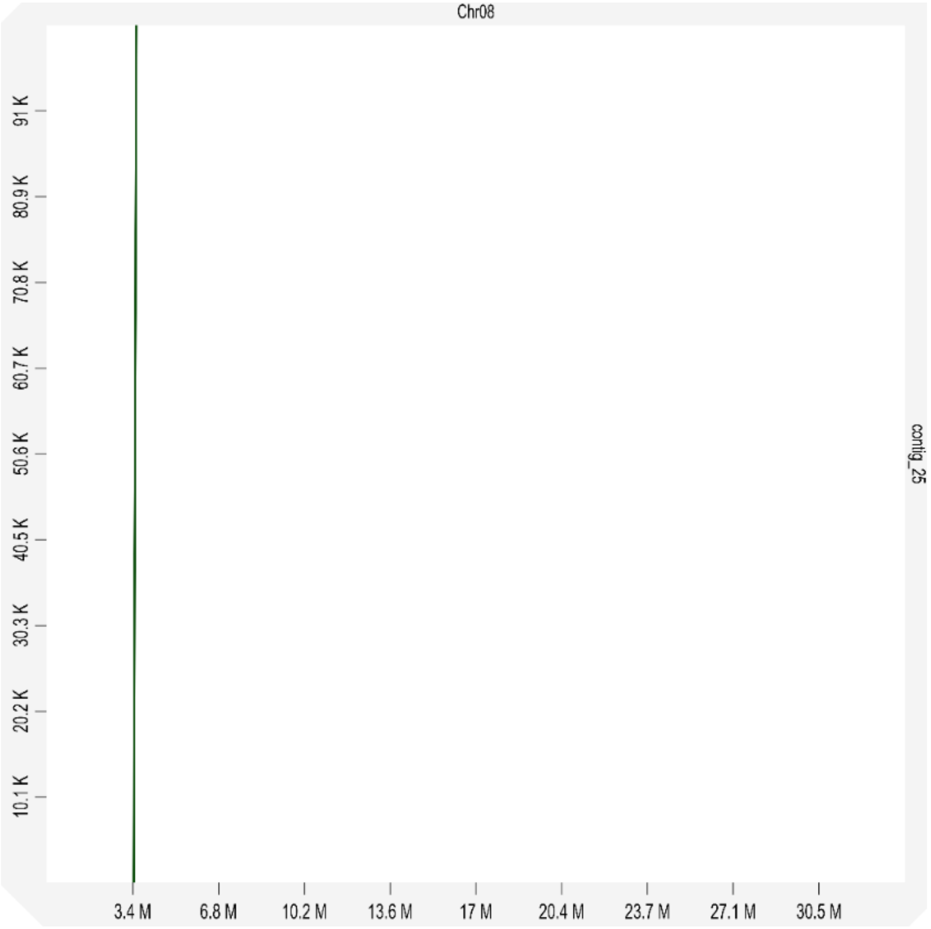
Genome assemblies of target regions: statistics

Anso-77 reference: one gene cluster



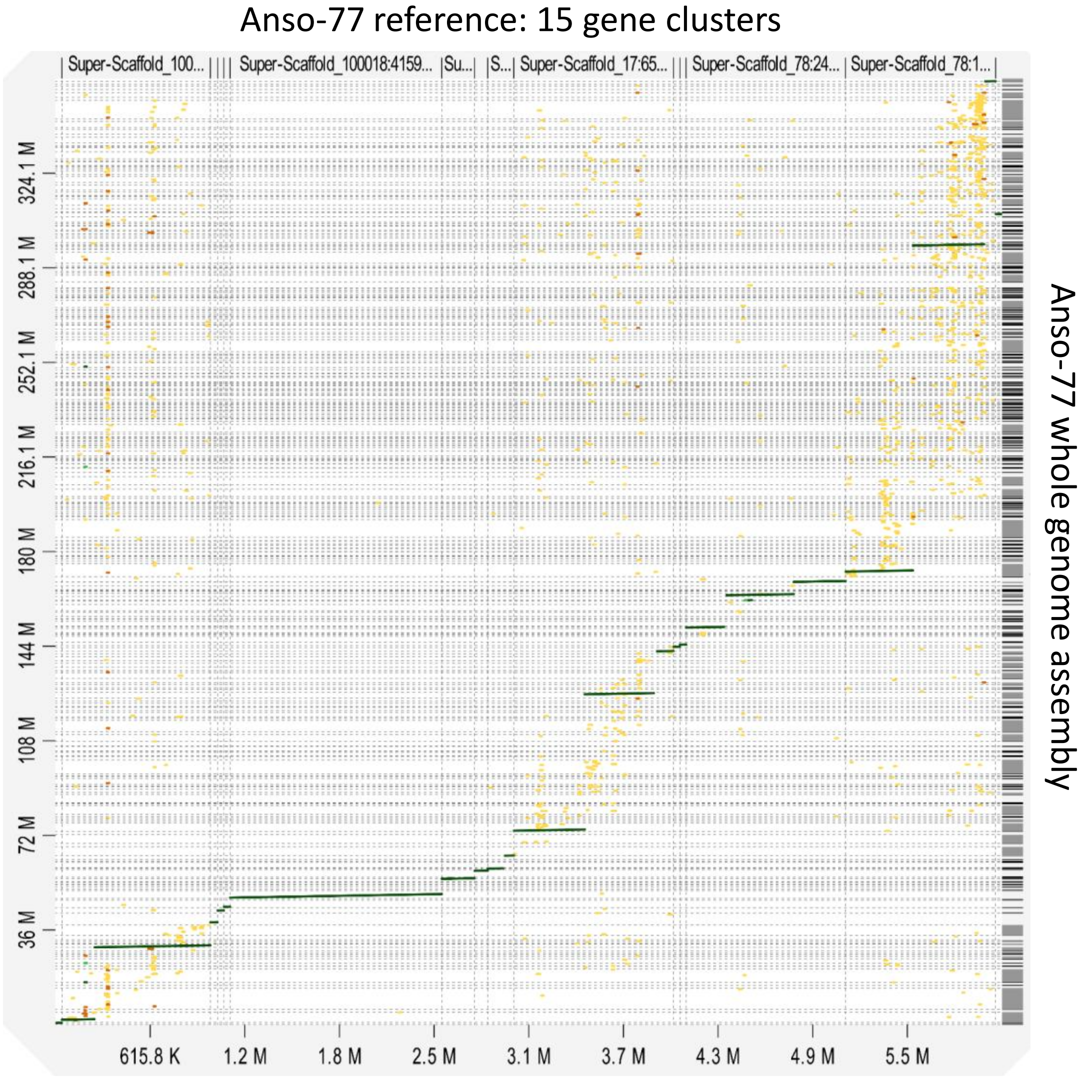
Anso-77 assembly

Anso-77 reference: complete chromosome



Anso-77 assembly

Genome assemblies: Good assembly of target regions

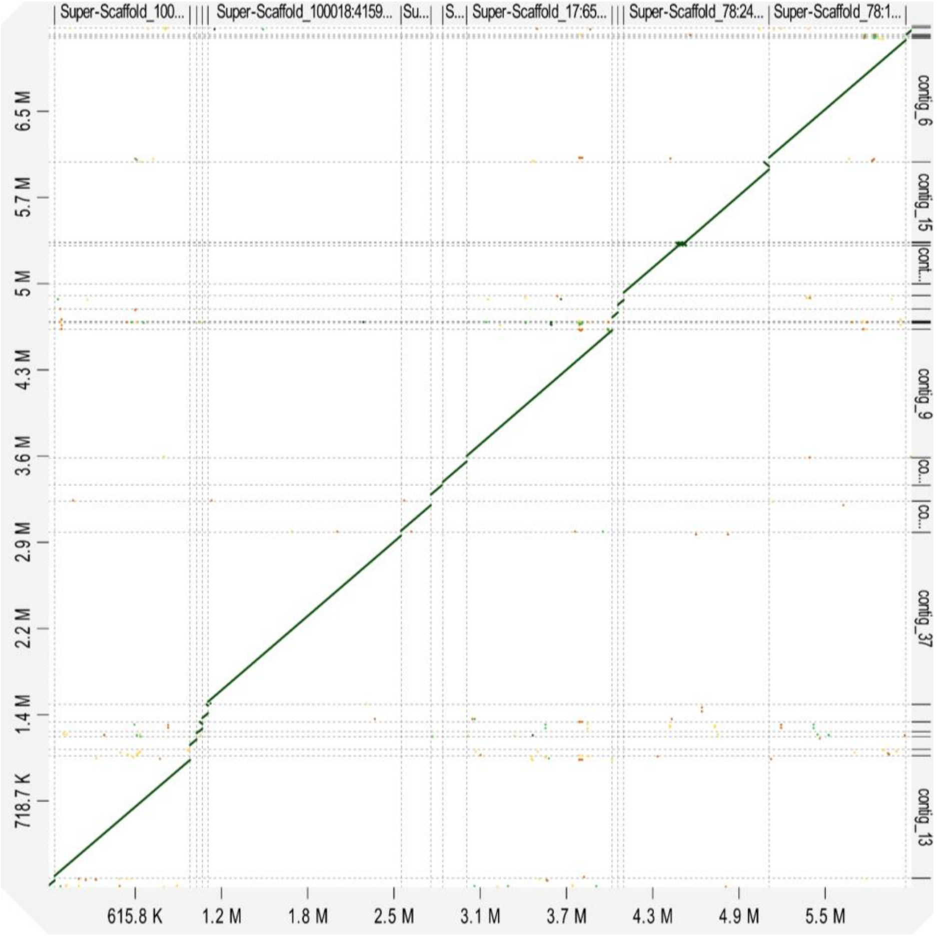


Assembly using WGS data:

- Target regions **fragmented** into multiple contigs
- Target regions **poorly covered** compared to adaptive sampling

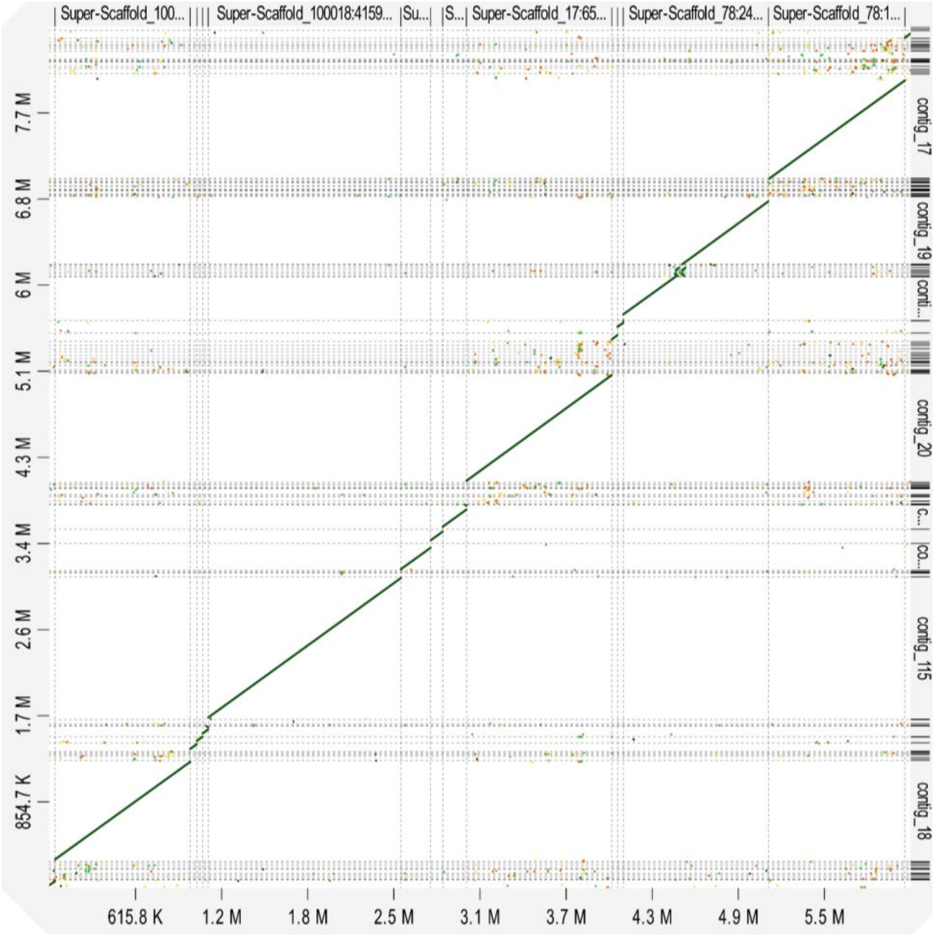
Genome assemblies: Good assembly of target regions

Anso-77 reference: 15 gene clusters



Anso-77 assembly **not** masking

Anso-77 reference: 15 gene clusters



Anso-77 assembly masking

Target regions fully assembled with a good depth

Genome assemblies: cluster Vat

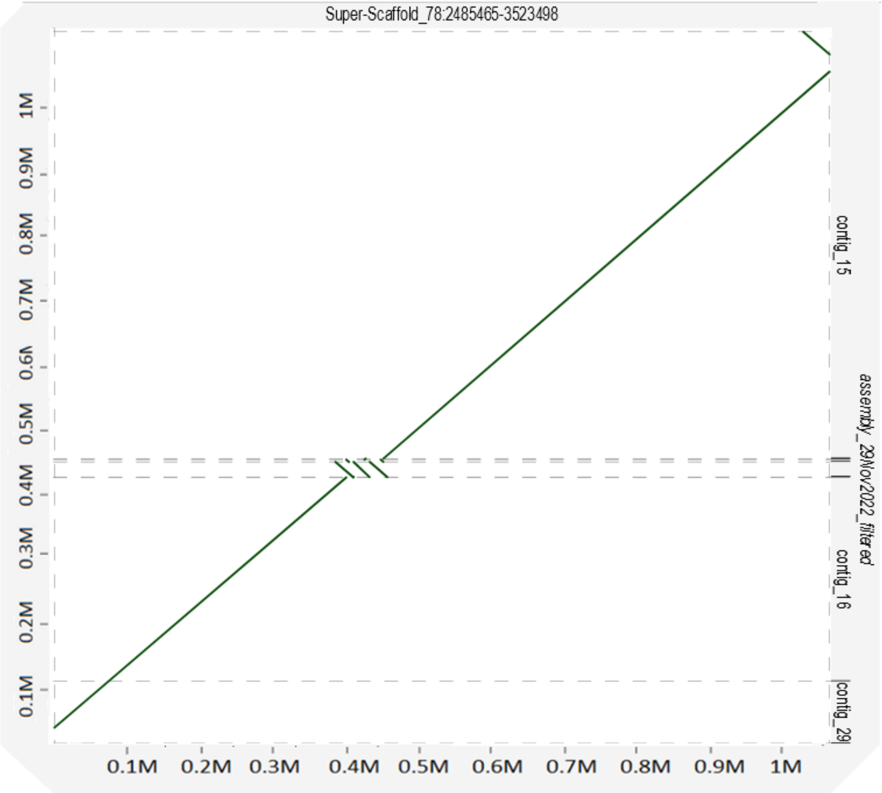
Cluster Vat: Most complex cluster of NLR genes in melon

28 NLR genes

1Mb length

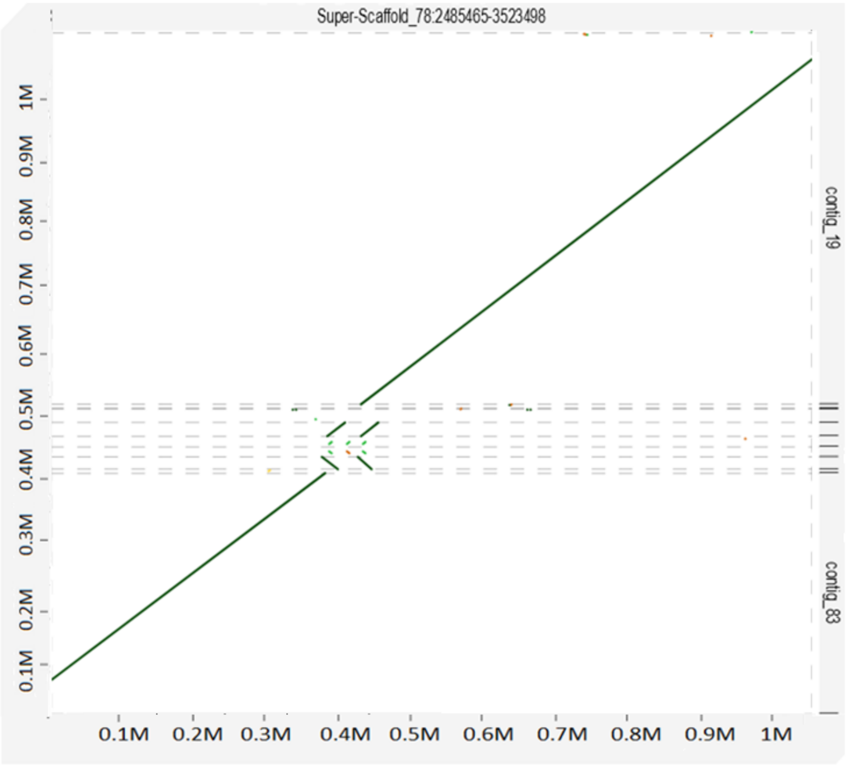
But also the most studied one (INRAE-GAFL)

Anso-77 reference: cluster Vat



Anso-77 assembly cluster Vat **not** masking

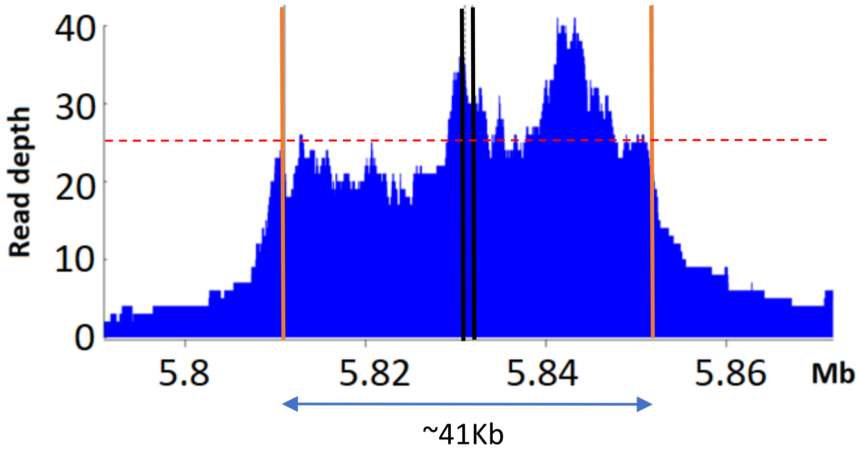
Anso-77 reference: cluster Vat



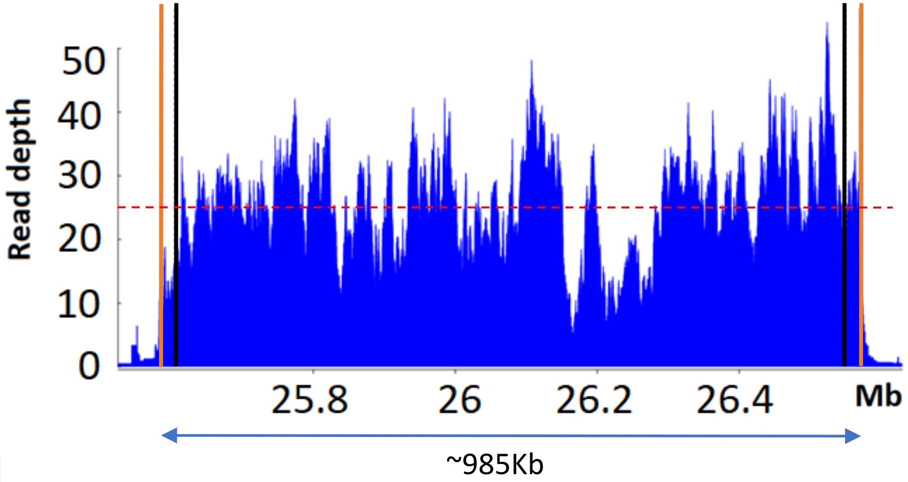
Anso-77 assembly cluster Vat masking

Preliminary results using a different variety: Doublon

Chr06

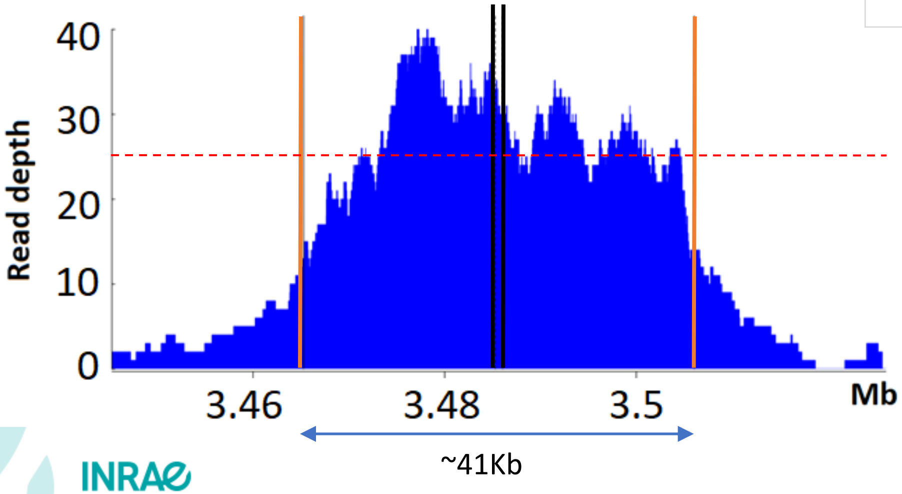


Chr05

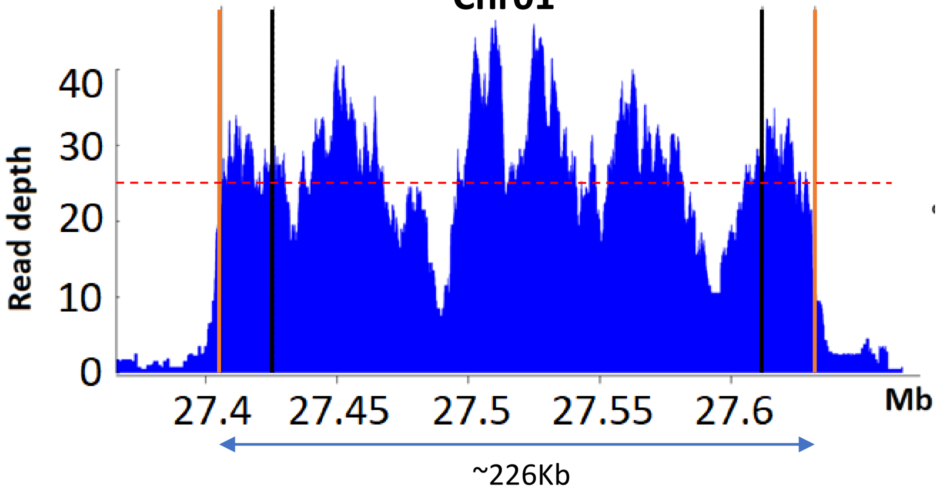


- ROI ±20kb
- ROI delimiter

Chr08



Chr01



Preliminary results using a different variety: Doublon

(Adapted from Chovelon et al., 2021)

Perspectives

Short-term perspectives:

1. Run on PromethION using a variety **genetically distant** from the provided reference: 'Canton'
2. **Multiplexing** on PromethION using **two barcodes**: Varieties 'Anso' + 'Doublon'

Middle-term perspectives:

1. **Increase the number of barcodes** on PromethION: Selective sequencing of 150 varieties.
2. Evaluate the ability to detect **structural variants** and **copy number variations** between varieties.

Long-term perspectives:

1. Perform a similar experience using a **diploid heterozygote** species with a **higher number of NLR genes** and **more complex clusters**: ***Vitis vinifera*.**

In conclusion

What Nanopore adaptive sampling offers?

- Nanopore adaptive sampling is a **simple**, **reliable**, **efficient** and **cost-saving** approach.
  - ✓ Standard DNA extraction (no need for HMW DNA)
  - ✓ Standard library preparation
  - ✓ No need of probes design/synthesis
  - ✓ No need of short-read polishing with Kit14
  - ✓ Real-time sequencing
  - ✓ Reduced off-target volume of data compared to WGS

What can we do?

1. Adaptive sampling allows to efficiently retrieve (**mapping** and **assembly**) our **15 ROI in melon**.
2. It will allow to **explore the genetic diversity** of melon **NLR genes** using a **large amount of varieties**.

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