Construction and characterization of a comprehensive ovine pangenome from 10 breeds provides new insights into their genetic diversity

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Projet cofinancé par le Fonds Européen de Développement Régional

> Context and objectives

Context:

⟶ Current ovine genome reference assembly
- Based on a **single Rambouillet sheep**
- **Does not capture all** genetic variations

⟶ **Structural variants**
- Genomic variations ≥ **50 nucleotides**
- Potential **impact** on both **complex** and **Mendelian phenotypic variations**
- **Difficult to detect**

⟶ Advances in **long-read sequencing** technologies
- Construct ***de novo* genome** assemblies

Objectives

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01

Construct an **ovine pangenome graph** using *de novo* assemblies from 10 French sheep breeds

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01 Construct an **ovine pangenome graph** using *de novo* assemblies from 10 French sheep breeds

02 Identify and study **structural variants** (SVs) & **non-reference sequences** (NRSs)

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01 Construct an **ovine pangenome graph** using *de novo* assemblies from 10 French sheep breeds

02 Identify and study **structural variants** (SVs) & **non-reference sequences** (NRSs)

03 Explore the **links between** these **SVs** & **NRSs** and **phenotypes of interest**

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> Strategy to study genetic variations from a sheep pangenome graph

Step 1

Whole genome sequencing

Step 2

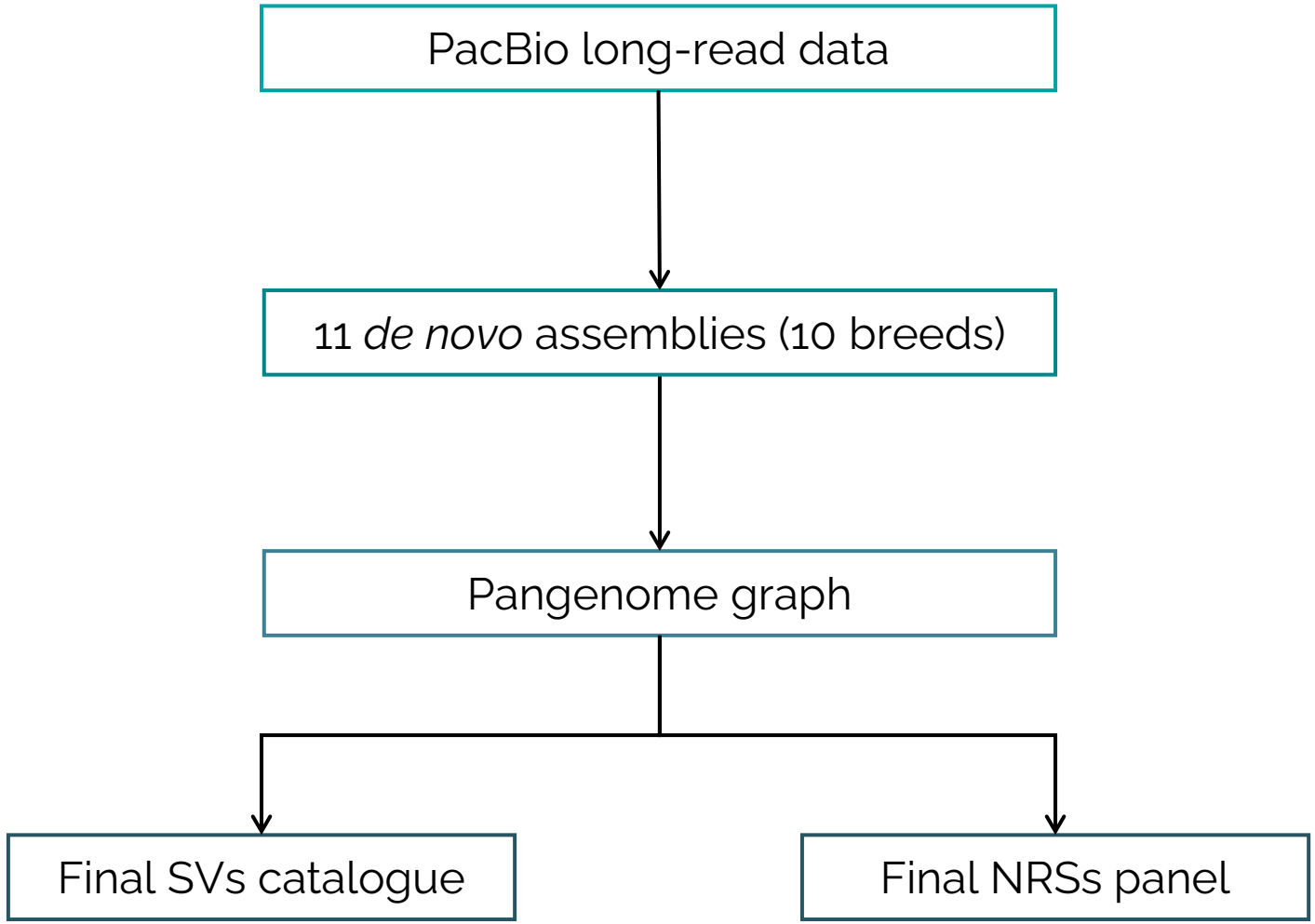
Construction of *de novo* genome assemblies

Step 3

Pangenome graph construction

Step 4

Identification & characterisation of SVs and NRSs



> Step 1: WGS data and phased & *de novo* genome assemblies

Whole genome sequence (WGS) data:

10 animals from 10 breeds
LR (PacBio CLR – 15-20kb & Hi-C and HiFi for phased assemblies)

Assembly construction:

9 *de novo* assemblies (9 breeds)
2 phased assemblies (Lacaune)


Basco Béarnais


Blanche du Massif Central


Corse


Ile de France


Lacaune


Manech Tête Noire


Noir Du Velay


Ouessant

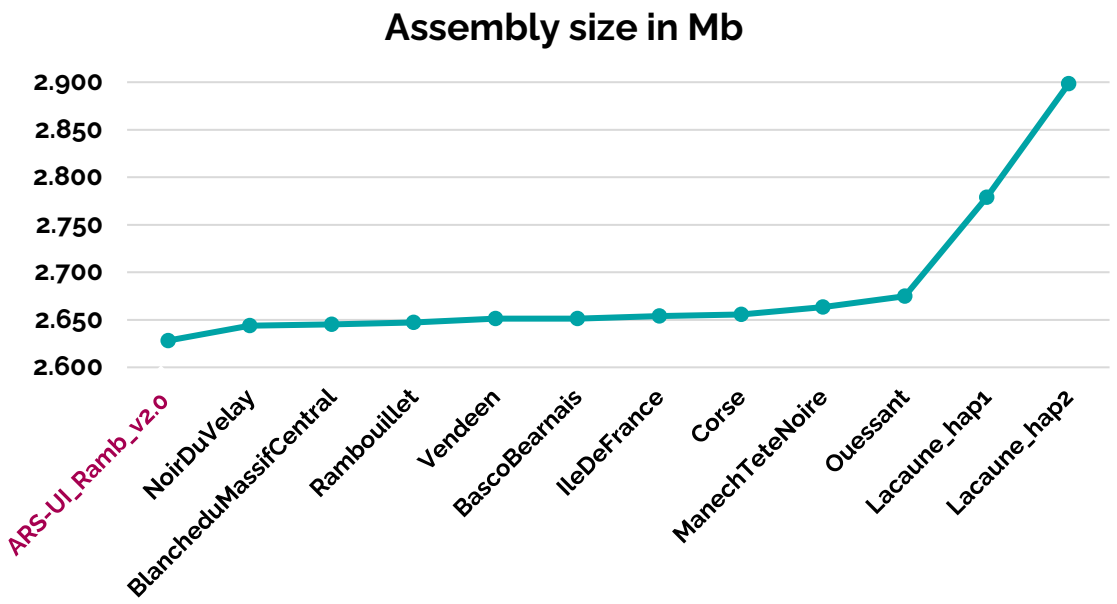

Rambouillet


Vendéen

> Step 2: Assembly metrics

3 main metrics used to assess assembly quality:

Assembly level: scaffolds



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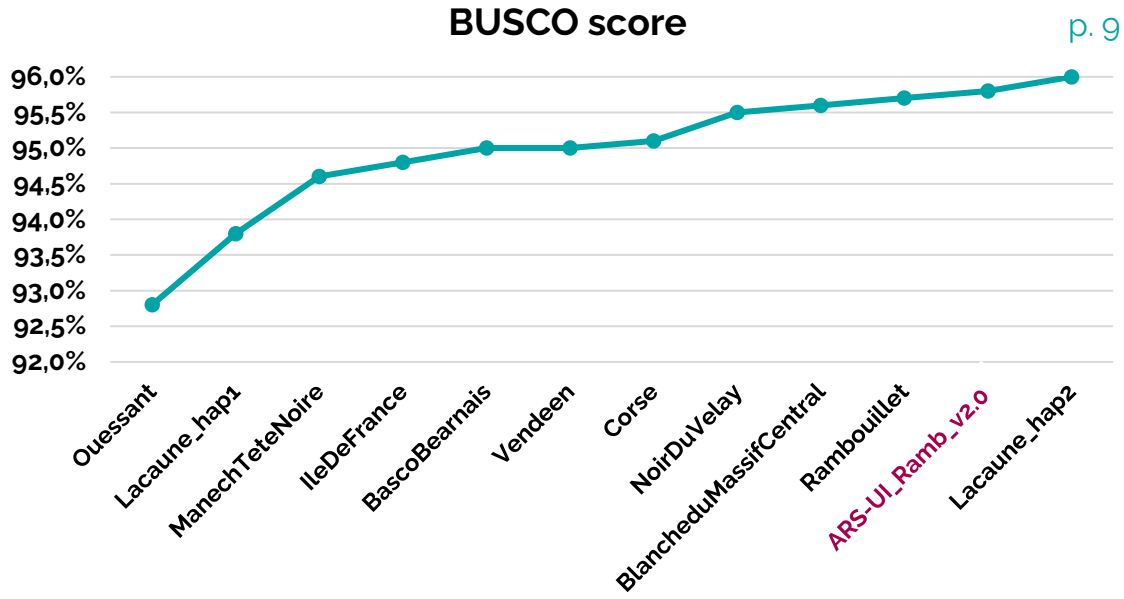
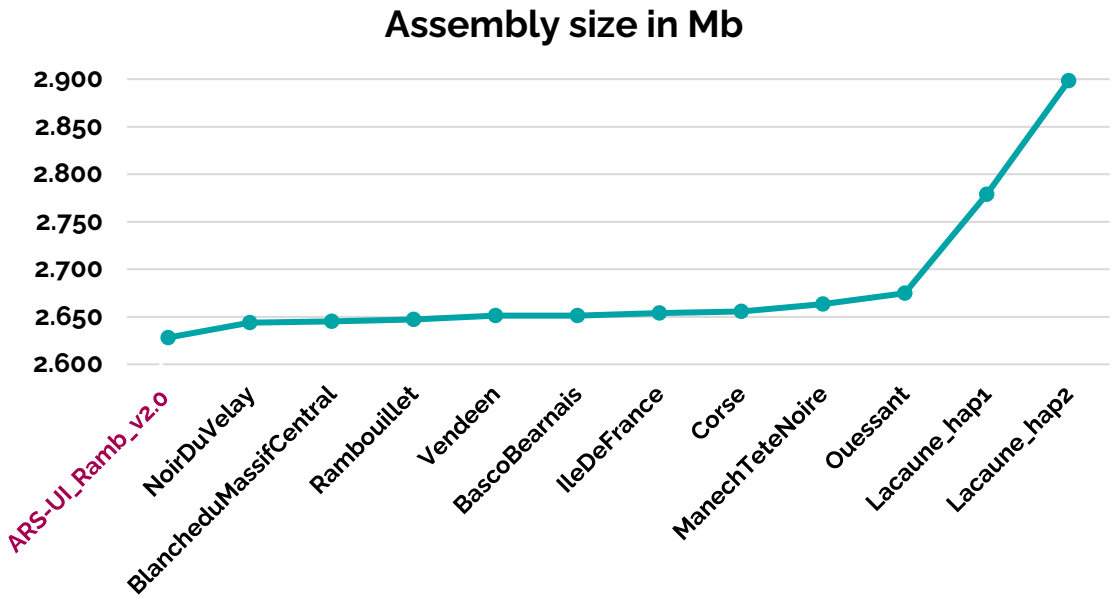
BUSCO: uses single copies of orthologs to estimate the completeness of genome annotation





> Step 2: Assembly metrics

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Assembly level: scaffolds

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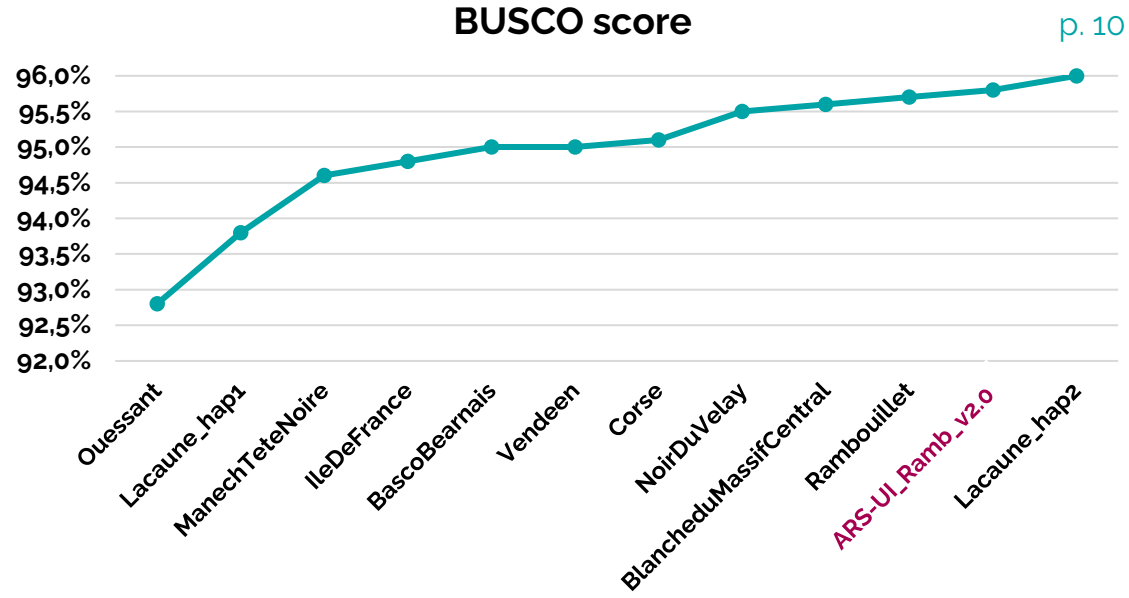
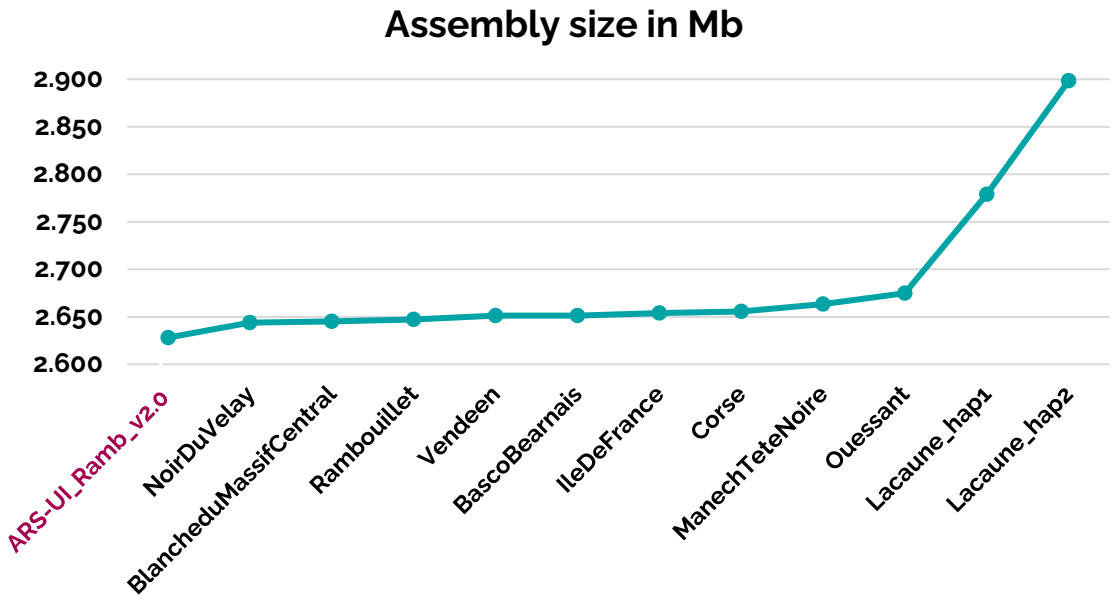
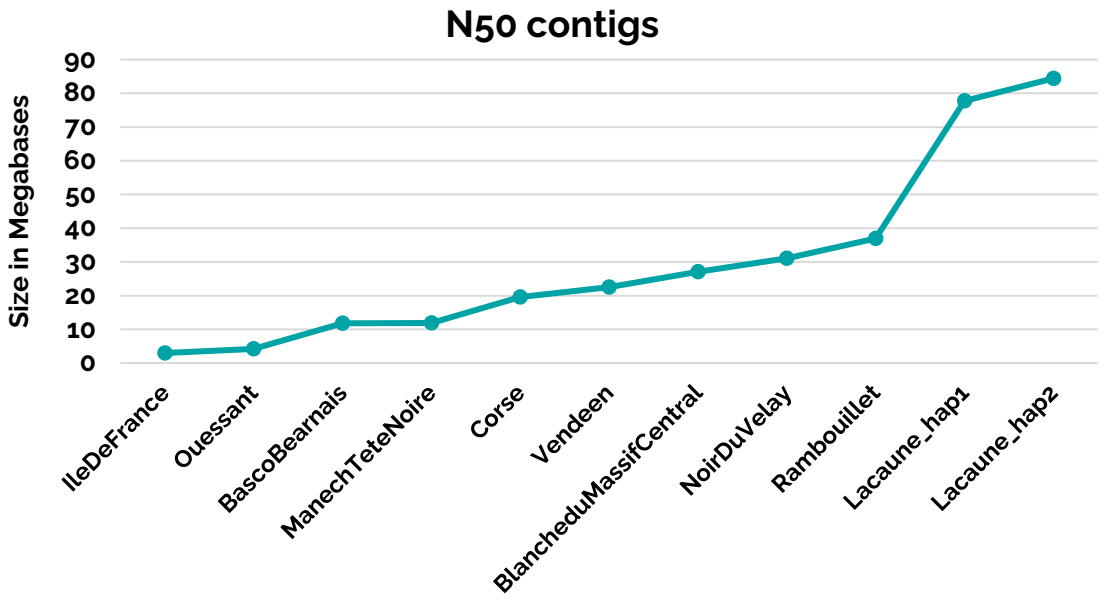
N50: size of the contig such as 50% of the genome is in contigs of greater size







> Step 3: Construction of pangenome graph

Tools to construct the pangenome graph:

- **Mash version 2.3:** phylogenetic distance
- **Minigraph (MG):** detects only SVs
- **Minigraph-Cactus (MGC):** detects both SNVs* and SVs
  produces 2 graphs
  (SVs / SVs+SNVs)

Genomes used to construct the pangenome graph:

- **Reference:** *GCF_016772045.1_ARS-UI_Ramb_v2.0*
- The autosomes of the 11 *de novo* assemblies

**SNVs = SNPs + InDels*

> Step 3: Construction of pangenome graph

Graph type	Autosomes		
	Minigraph	Minigraph-Cactus	
		Minigraph	Cactus
Graph length (*nt*)	2,577,492,938	2,577,492,938	2,631,736,745
Path length (*nt*)	2,576,522,092	2,577,156,663	-
Core	2,405,918,052	2,404,133,431	-
Flexible	170,604,040	173,023,232	-

Core genome: sequences common to all samples
Flexible genome: sequences present in a subset of the samples

Both tools produce a **structural variation pangenome graph** of the **same size**

The **cactus graph length** > MG **graph length**

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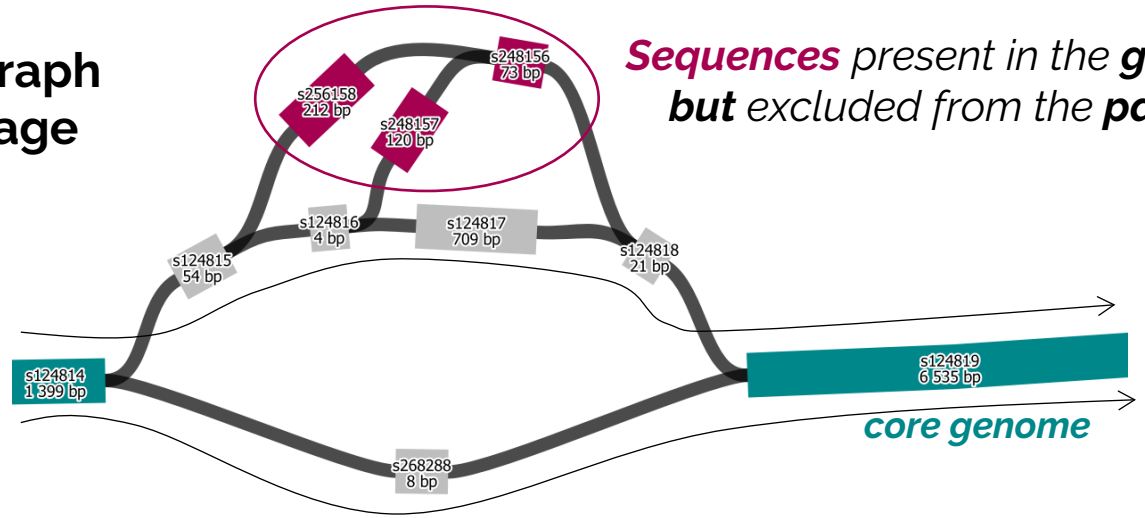
Example of a Minigraph bubble with Bandage



Sequences** present in the **graph** **but** excluded from the **path

→ **1 407 nodes** were **missing** from the MG **path**

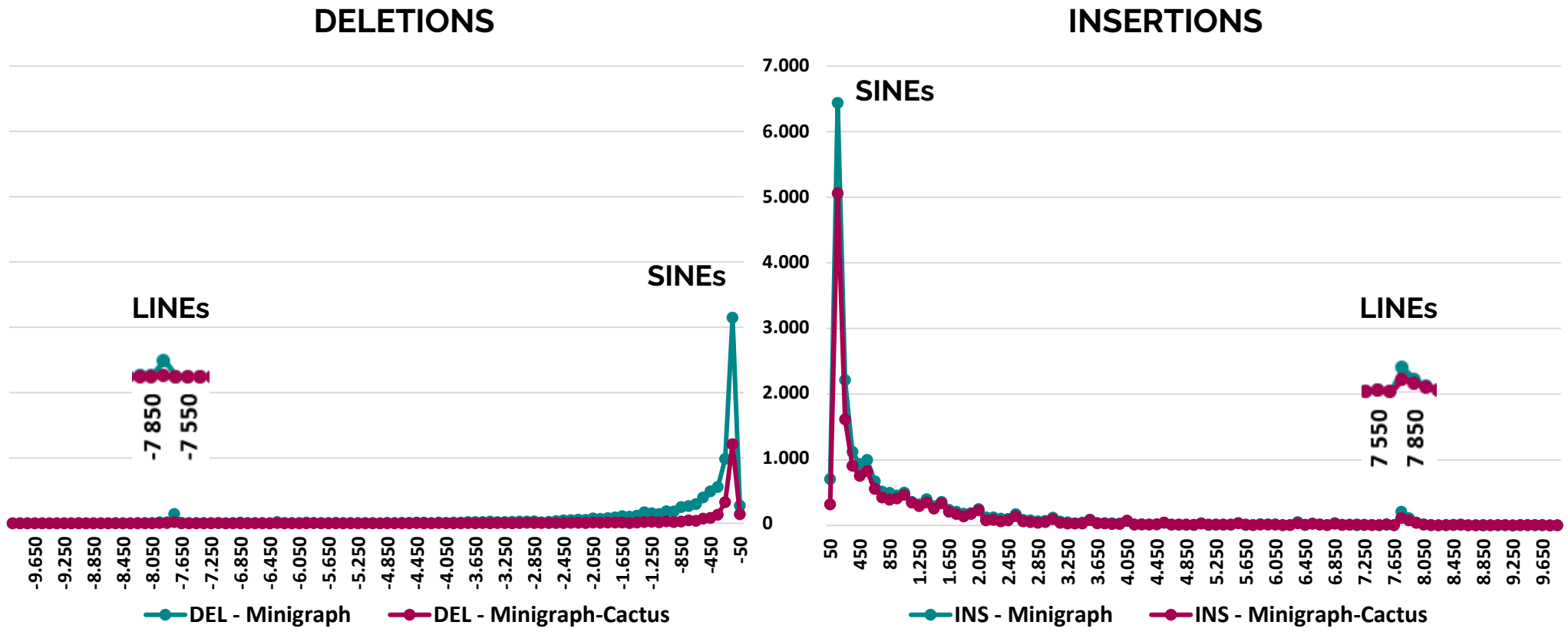
> Steps 4-5: Identification of SVs

	Autosomes	
	Minigraph	*Minigraph-Cactus*
Structural Variants (SVs)	**113,098**	**128,942**
Multi-allelic SVs	11,643	**80,811**
Bi-allelic SVs	**101,455**	**48,131**
Bi-allelic Insertions	**47,045**	**35,974**
Bi-allelic Deletions	**21,245**	**6,707**
Bi-allelic Other variants	33,165	5,450

The vast majority of SVs detected by MGC were **multi-allelic**

Fewer **deletions** were detected by both MG and MGC as compared to **insertions**

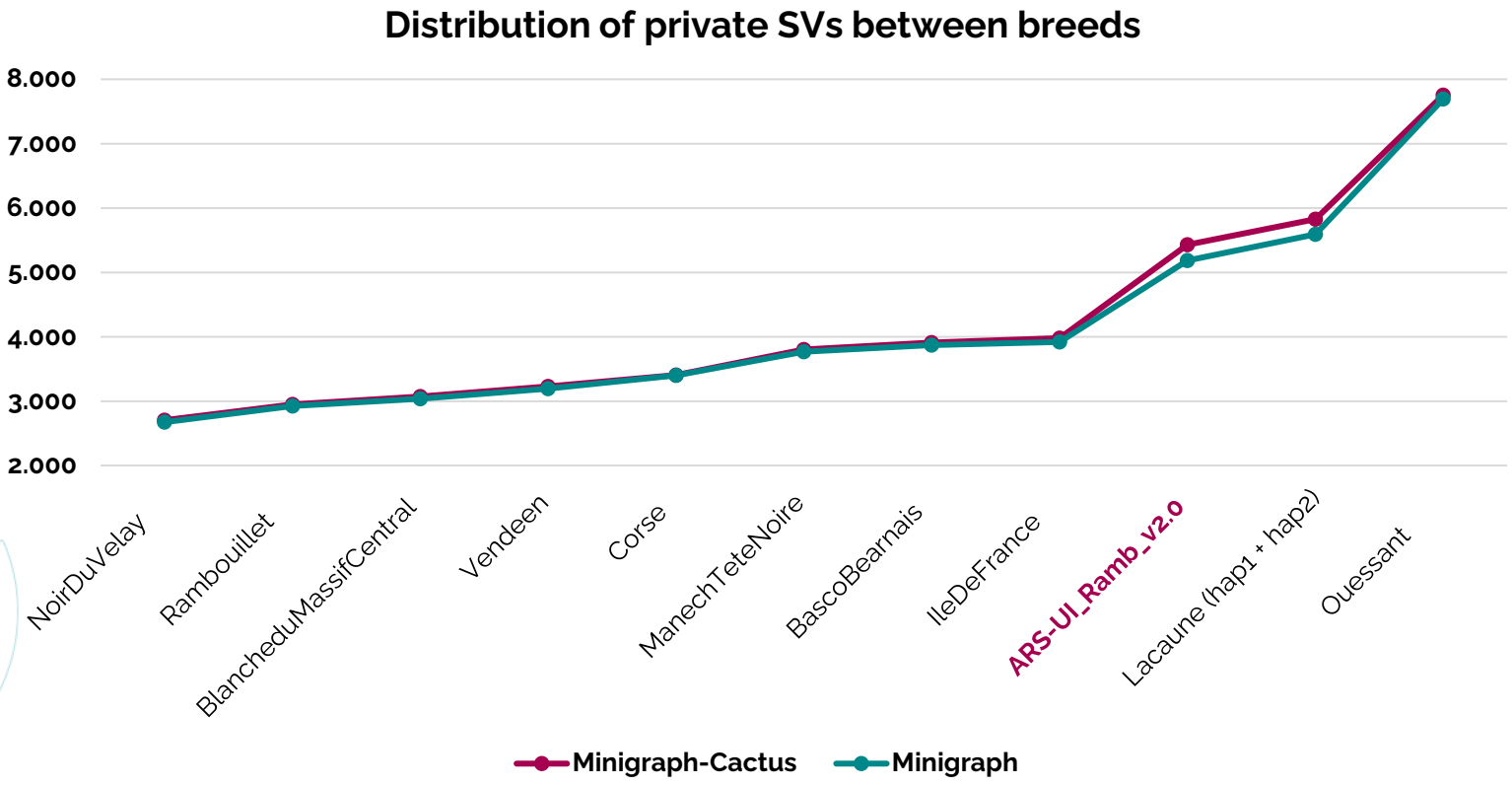
> Steps 4-5: Distribution of the length of deletions and insertions



The **vast majority** of INS and DEL were **small** in **size** (≤*1000 nt*)

2 main peaks corresponding to SVs due to **transposable elements** clearly detected by MG

> Steps 4-5: Identification of SVs

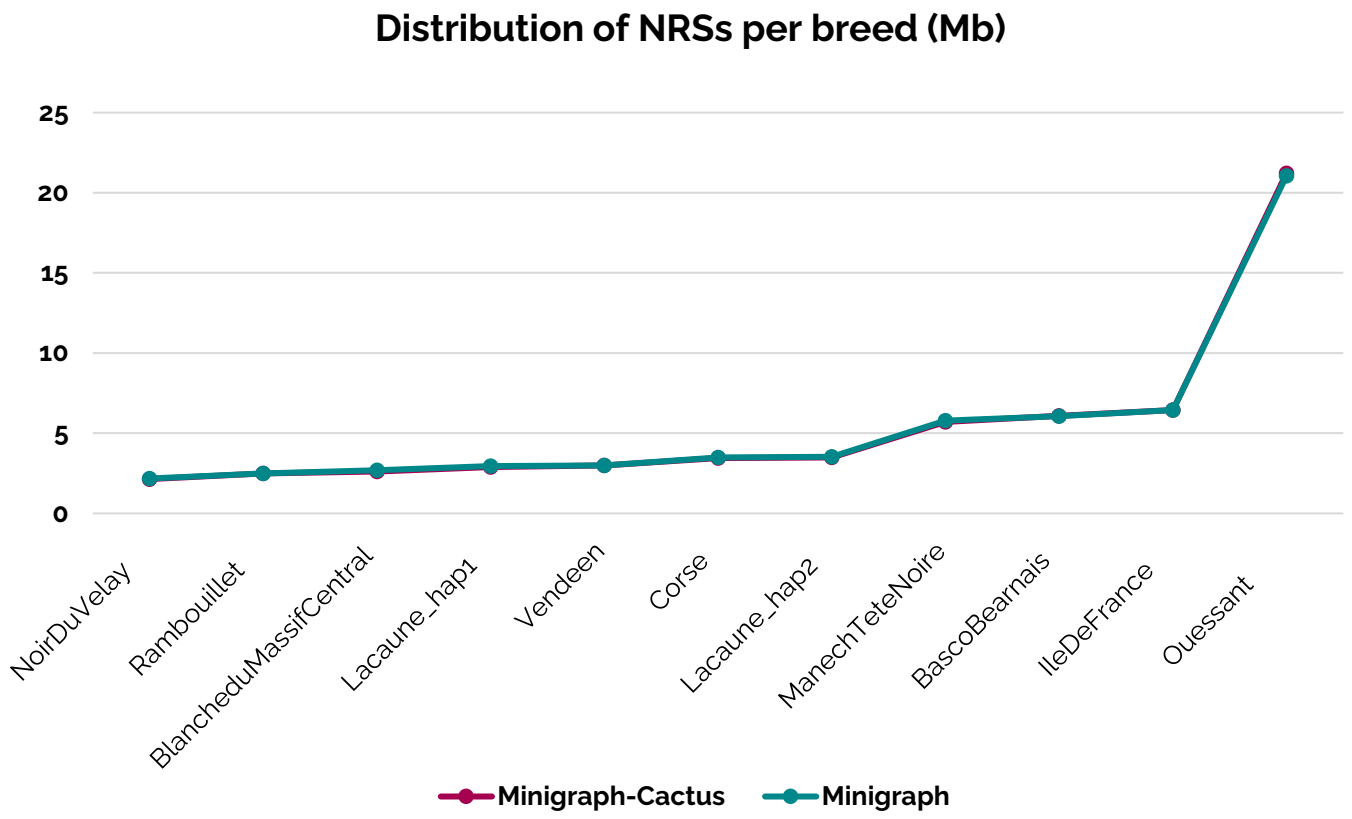


Ouessant breed provides a **large number** of **private SVs**

Lacaune haplotypes have **slightly** the **same number** of **SVs** than the **reference genome**

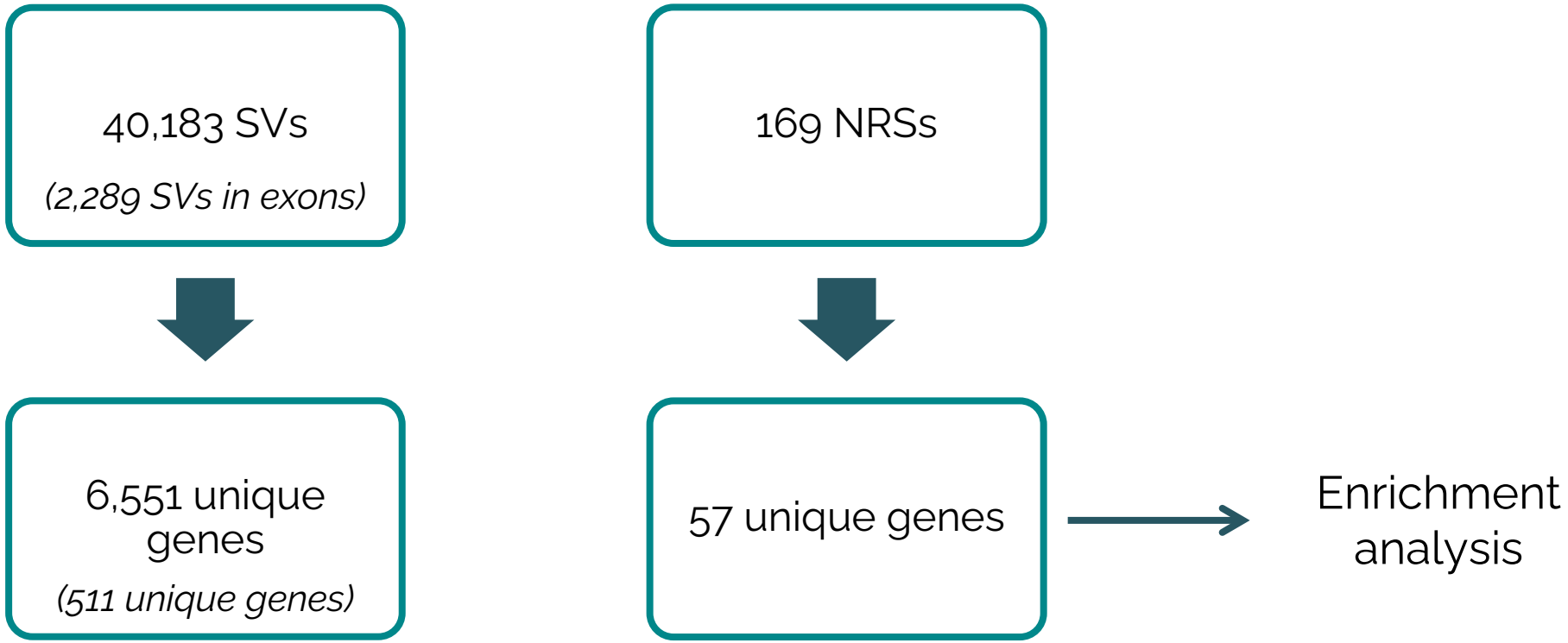
> Steps 4-5: Identification of NRSs from the 2 graphs

	Autosomes	
Pangenome graph	***Minigraph***	***Minigraph-Cactus***
Private NRSs (nt)	**59,700,898**	**59,593,434**



> Annotation of SVs and NRSs

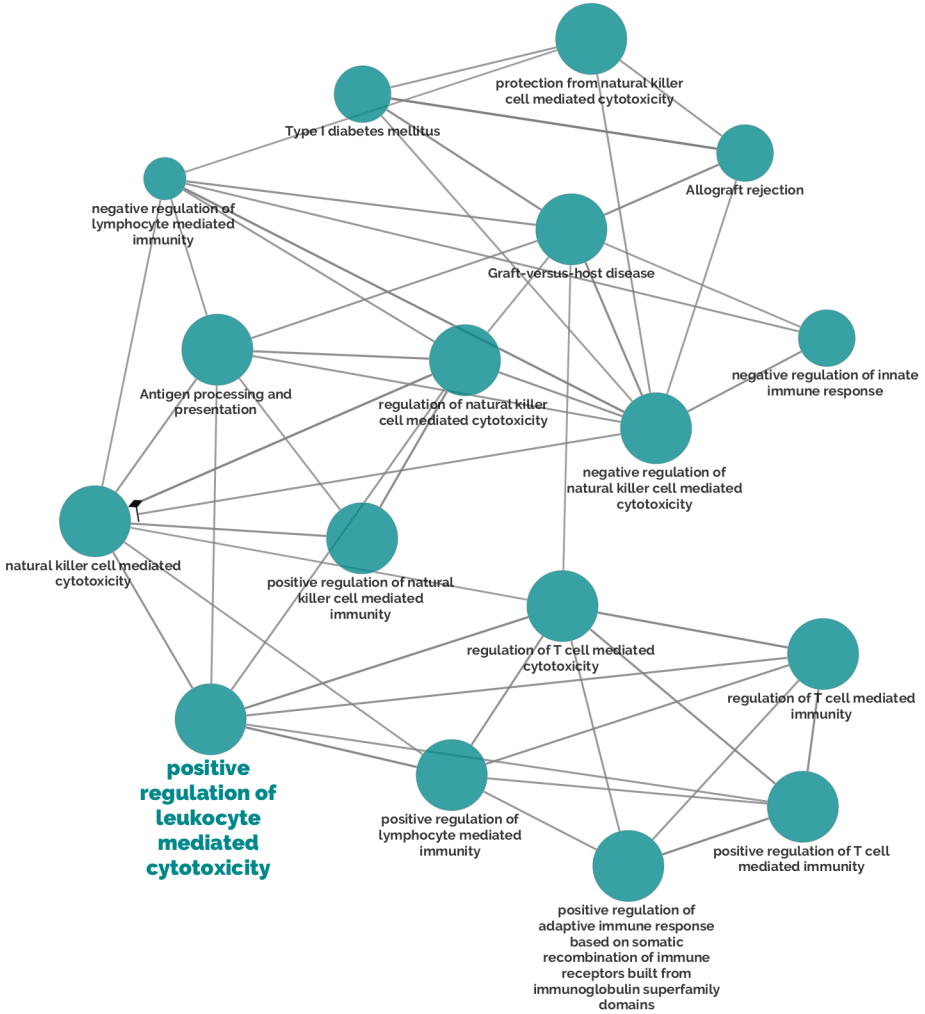
SVs that overlap/contain known sheep genes (exons/introns/regulating regions)

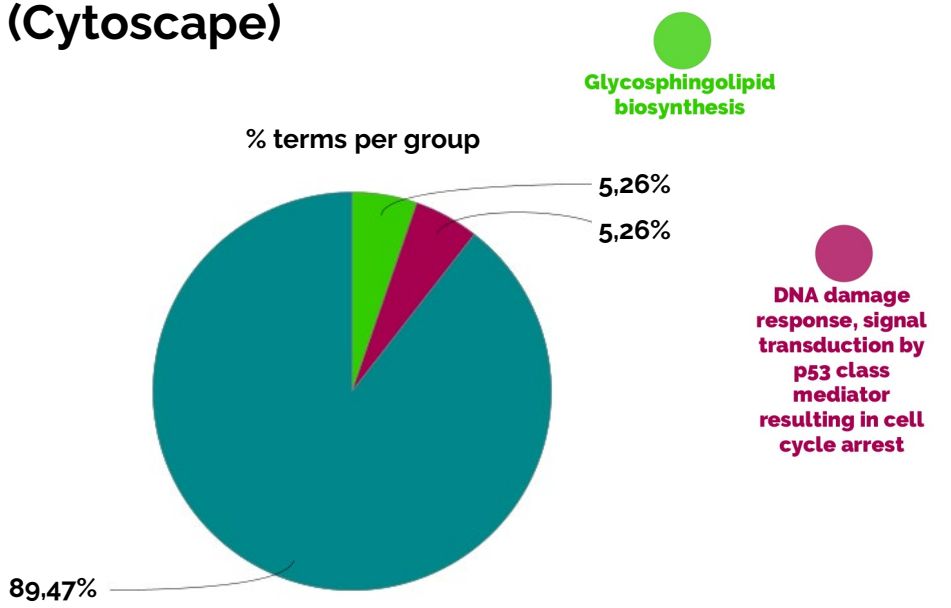


DB: https://ftp.ensembl.org/pub/release-112/gff3/ovis_aries_rambouillet/Ovis_aries_rambouillet.ARS-UI_Ramb_v2.0.112.chr.gff3.gz

> Steps 4-5: Preliminary results for the enrichment analysis

ClueGO (Cytoscape)





- Major Histocompatibility Complex
- Killer Cell Lectin Like Receptor
- Interleukin
- Immunoglobulin

Work is currently in progress to better characterize these results

> Steps 4-5: Repeated sequences in NRSs

		MINIGRAPH	MG - CACTUS
Total length		**104,040,480 bp**	**104,675,562 bp**
GC level		**42.20 %**	**42.21 %**
Bases masked		**47,585,037 bp (45.74 %)**	**47,803,284 bp (45.67 %)**
		% of sequences	**% of sequences**
LINEs		**30.61**	**30.56**
	LINE1	29.87	29.81
	LINE2	0.35	0.35
	L3/CR1	0.04	0.04
	RTE	0.35	0.36

Vast majority of masked sequences → **LINEs subclasses**

~58% of **NRSs** do not contain **repeated sequences**

> Conclusion

- **Assemblies:**
  - CLR LR reads → Construction of 9 *de novo* assemblies of good quality
  - Hi-C and HiFi LR reads of very good quality → Construction of 2 haplotype-resolved *de novo* assemblies

- **Pangenome:**
  - Both MG and MGC tools produce a pangenome of the same size, even if the characteristics differ slightly

- **Structural variants:**
  - MGC identifies more SVs than MG, but with a higher proportion of multi-allelic variants

- **Non-reference sequences:**
  - Approximately 60 Mb of non-reference sequences are identified with both tools and they provide the same distribution per breed

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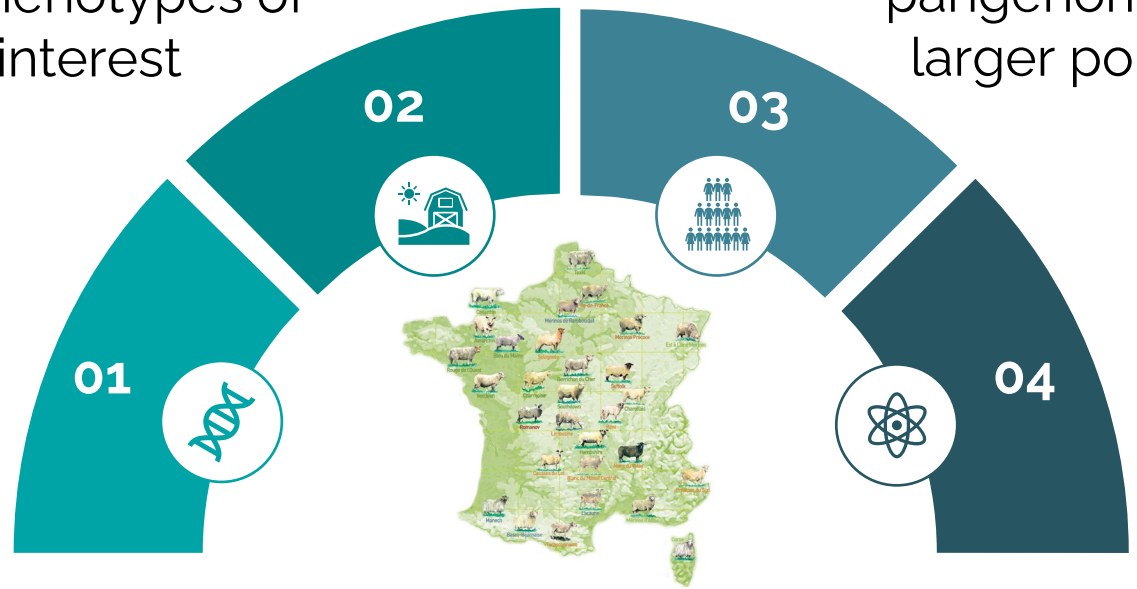
> Future work

01 Characterize SVs & NRSs and their gene content

02 Explore their effect on phenotypes of interest

03 Genotype SVs in the pangenome from a larger population

04 Perform gene prediction and protein homology analyses on NRSs



Same work is currently in progress in goat

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INRAE

Thanks for your attention

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