

# ➤ Overview of the genetic variability of genes FUT1, FUT2 and FUT3 in various pig populations for the HoloOligo project

# ➤ HoloOligo Project

- Goal :
  - Look for functional links between Milk Oligosaccharides (MOs) structures and the offspring microbiota and immune system in pigs and rabbits
- 4 year project
- 5 INRAE teams, 1 CEA team, 2 experimental units
- Multilevel approach
  - *In silico* (data management and discovery)
  - *In vitro* (animal experiments)
  - *In vivo* (intestinal immune cells and anaerobic bacterial strains cultivations)



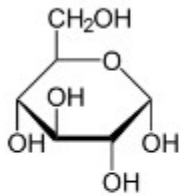
## ➤ Scientific context

- Milk provides essential nutrients and bio-active components to newborns
- MOs :
  - prevent epithelial adhesion by pathogens
  - promote specific bacteria growth
  - modulate epithelial and mucosal barrier
  - exert an antimicrobial activity and modulate host immune responses

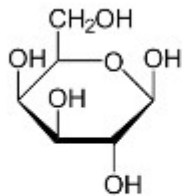


# ➤ What are oligosaccharides ?

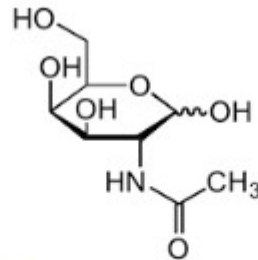
- Sugar polymers of varying complexity
- 5 sugars



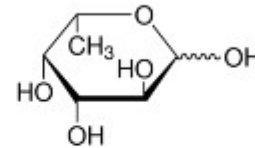
● Glc  
Glucose



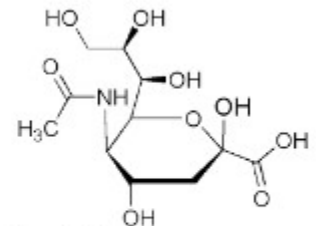
● Gal  
Galactose



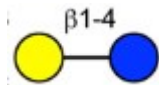
■ GlcNAc  
N-acetyl-glucosamine



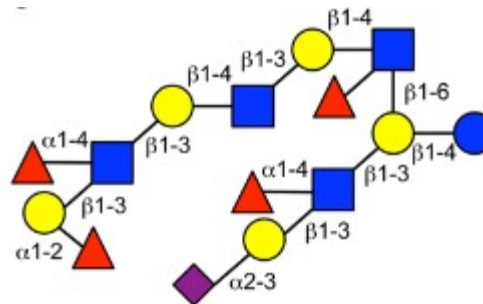
▼ Fuc  
Fucose



◆ Neu5Ac  
N-acetylneuraminic acid



lactose



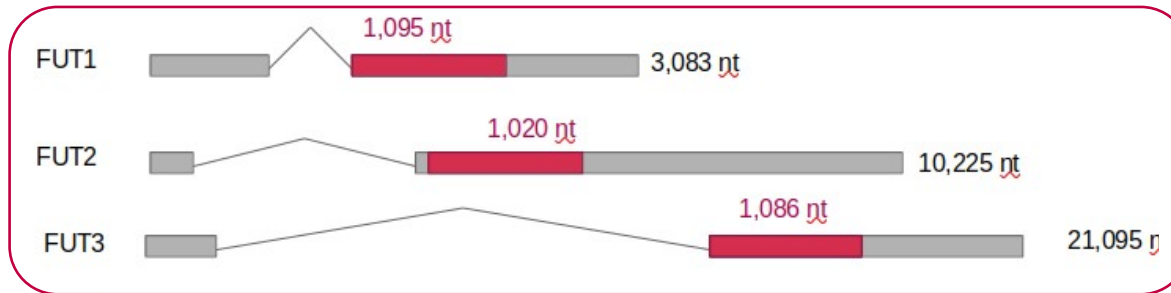
# ➤ Genes of interest

- MO structures are conditioned by glycosyltransferases, including fucosyltransferases



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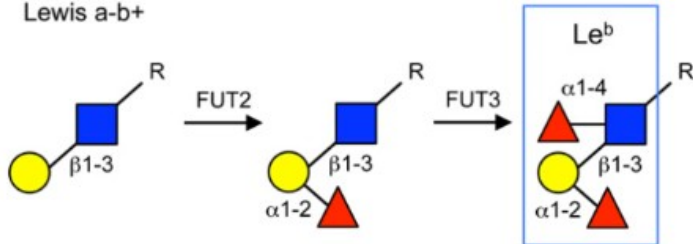


For humans :  
Lewis group a/b : FUT2, FUT3  
Lewis group x/y : FUT1, FUT3

# ➤ Lewis groups and HMOs

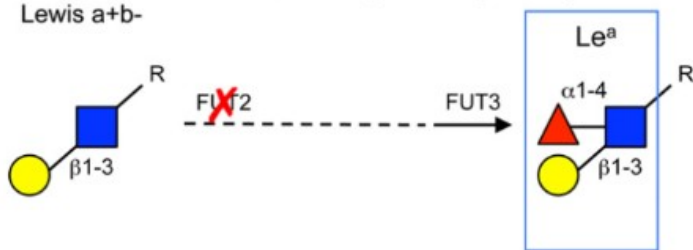
## Group 1: Secretor, Lewis-positive (Se+Le+)

Lewis a-b+



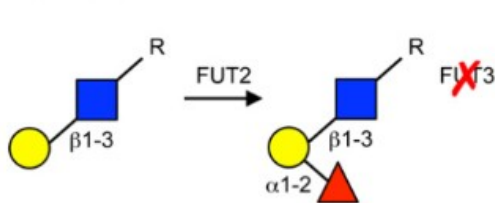
## Group 2: Nonsecretor, Lewis-positive (Se-Le+)

Lewis a+b-



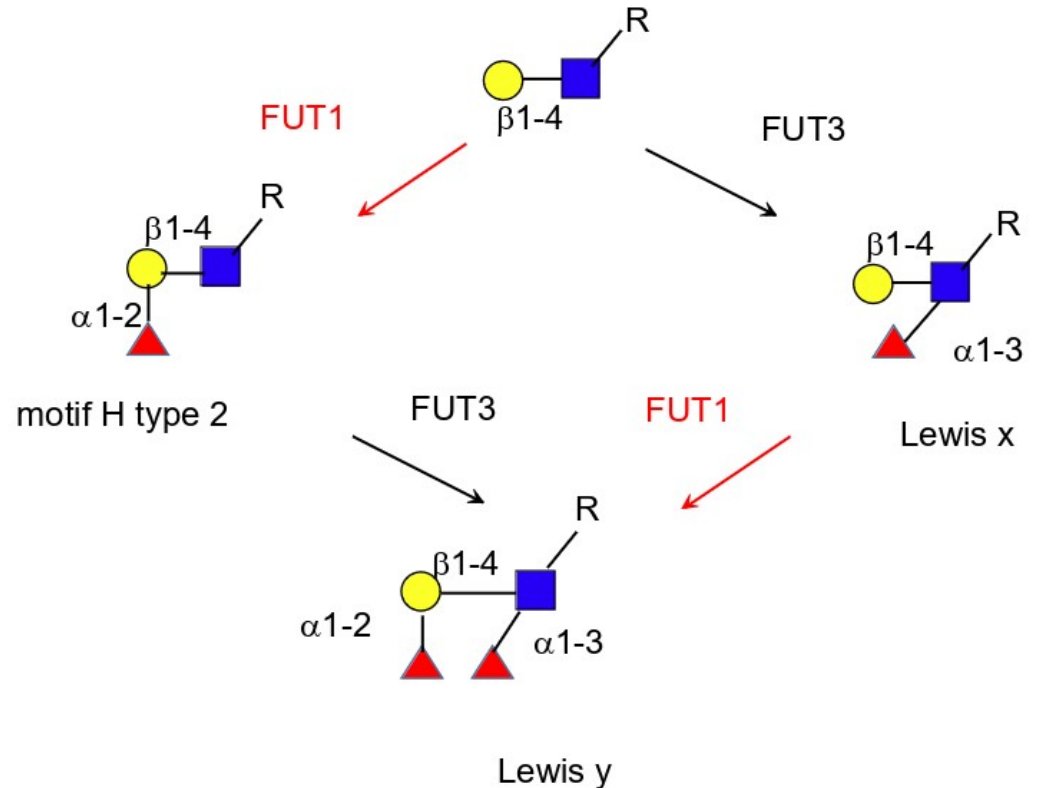
## Group 3: Secretor, Lewis-negative (Se+Le-)

Lewis a-b-



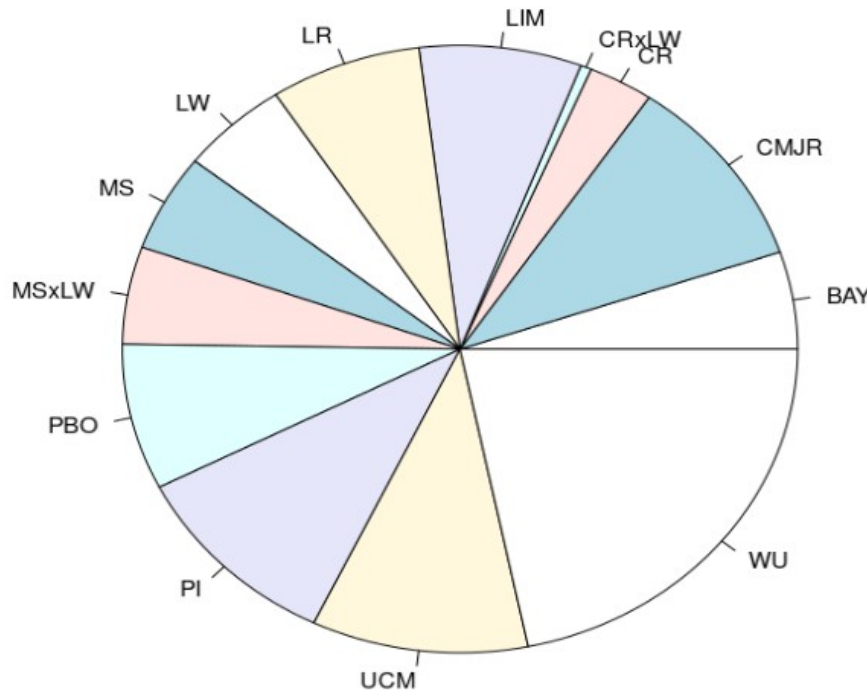
## Group 4: Nonsecretor, Lewis-negative (Se-Le-)

Lewis a-b-



## > Our goal

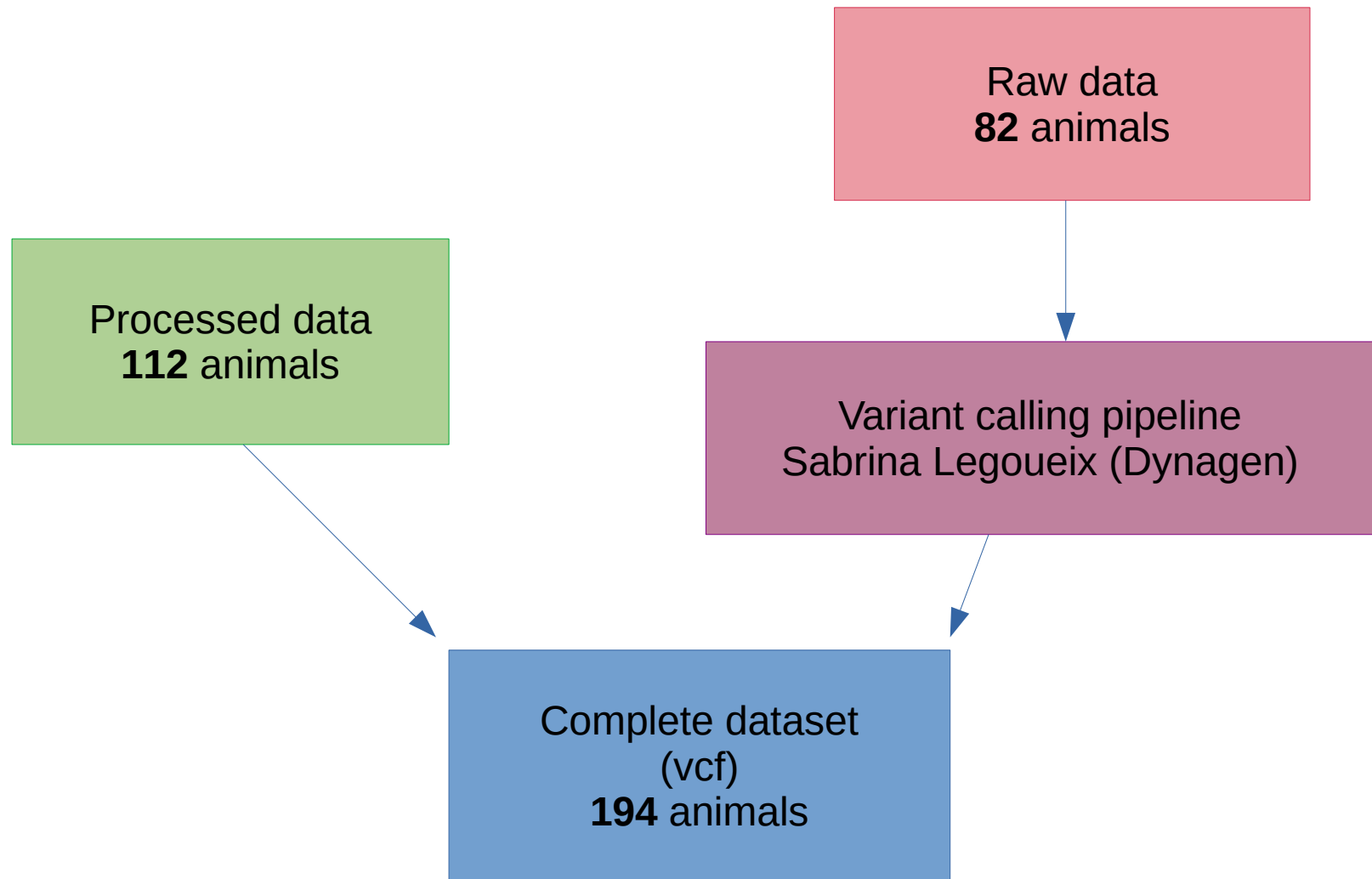
- Examine variants and haplotype diversity of FUT1, FUT2 and FUT3 across multiple pig population
  - Use available data : 194 animals



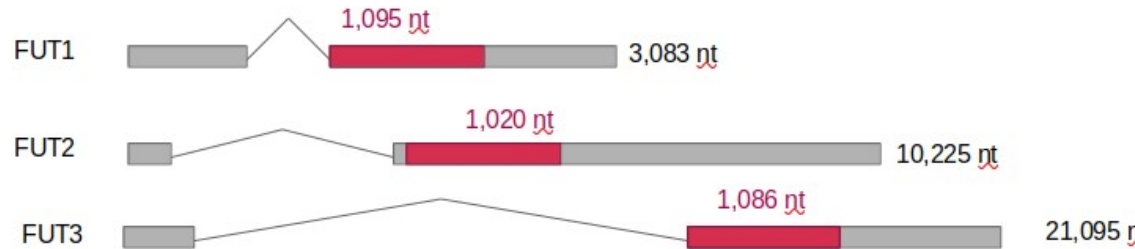
|       |    |
|-------|----|
| BAY   | 10 |
| CMJR  | 20 |
| CR    | 6  |
| CRxLW | 1  |
| LIM   | 15 |
| LR    | 14 |
| LW    | 10 |
| MS    | 10 |
| MSxLW | 10 |
| PBO   | 15 |
| PI    | 20 |
| UCM   | 20 |
| WU    | 42 |



# > Available



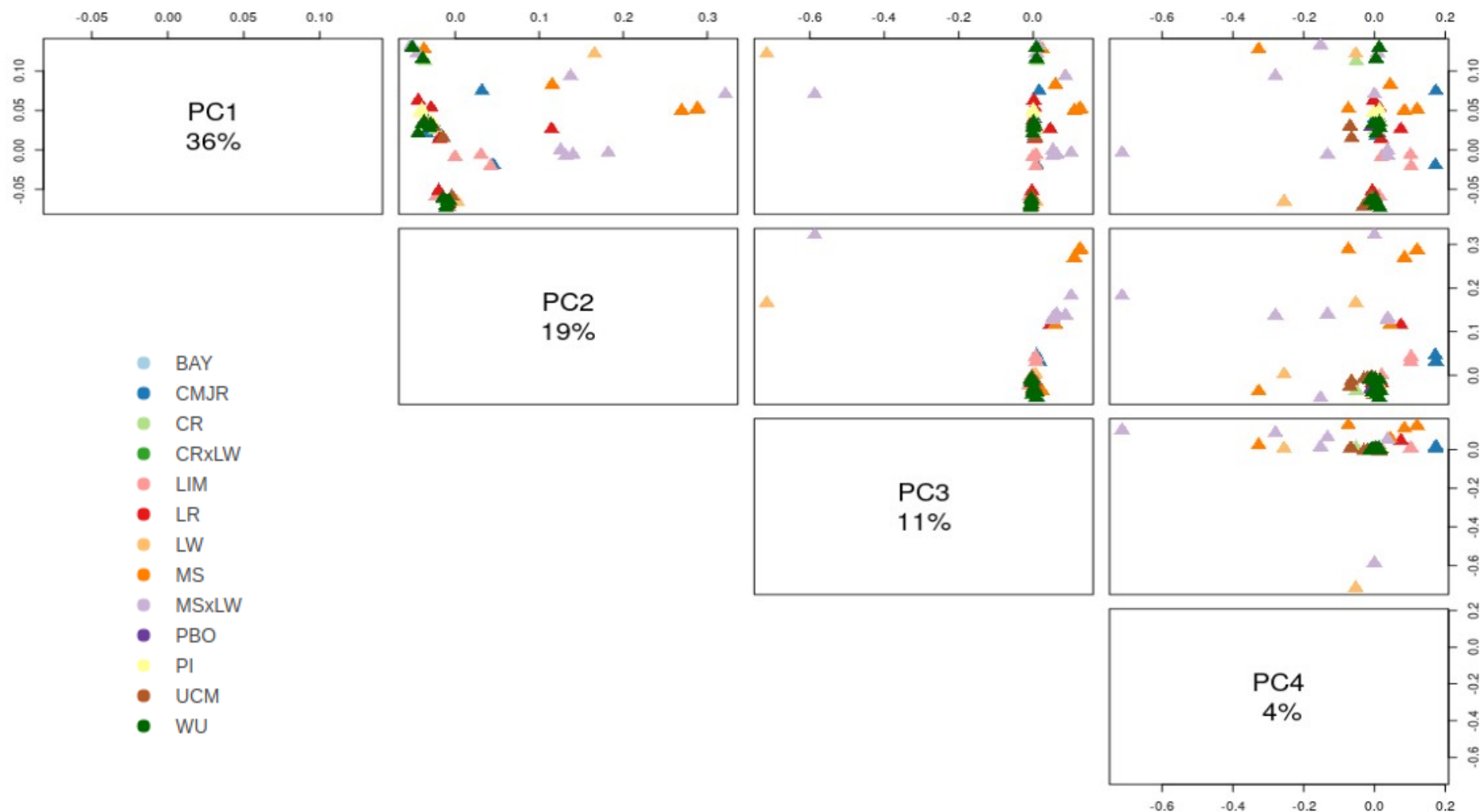
# > FUT1, FUT2 and FUT3 variants



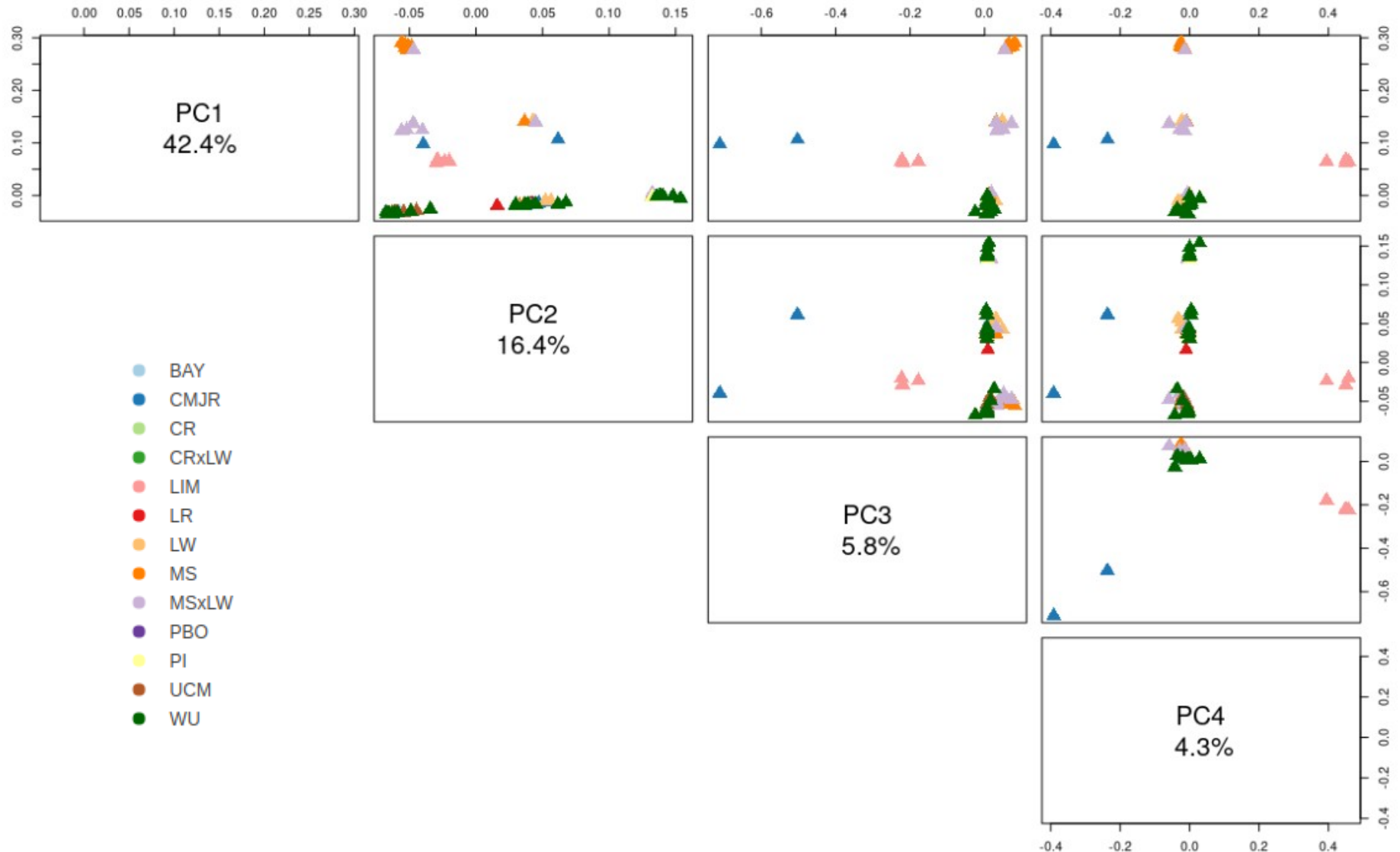
- Annotation with VEP
- Filters:
  - Quality  $\geq 30$
  - Biallelic only

| Variant consequence           | FUT1      | FUT2       | FUT3       |
|-------------------------------|-----------|------------|------------|
| Frameshift variant            | 2         | 0          | 2          |
| Missense variant              | 7         | 4          | 2          |
| Synonymous variant            | 3         | 3          | 4          |
| 5' UTR variant                | 1         | 0          | 0          |
| 3' UTR variant                | 6         | 45         | 2          |
| Intron variant                | 3         | 91         | 277        |
| Upstream gene variant         | 9         | 36         | 19         |
| Downstream gene variant       | 12        | 7          | 9          |
| Splice donor 5th base variant | 0         | 0          | 1          |
| Stop gained                   | 0         | 1          | 0          |
| <b>TOTAL</b>                  | <b>43</b> | <b>187</b> | <b>316</b> |

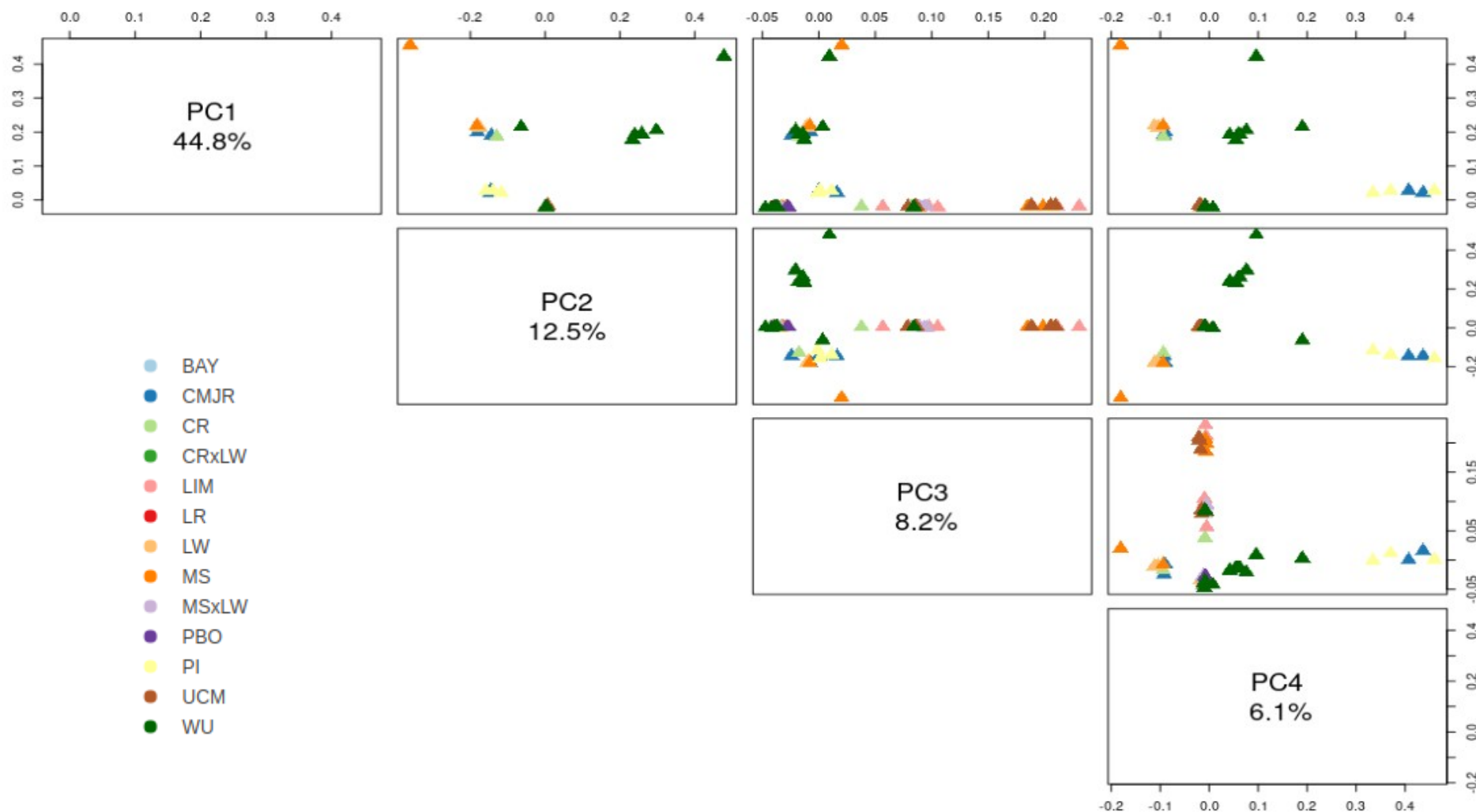
# ➤ PCA - FUT1



# ➤ PCA - FUT2



# ➤ PCA - FUT3



INRAE

FUT1, FUT2 and FUT3 variability  
03/04/2023

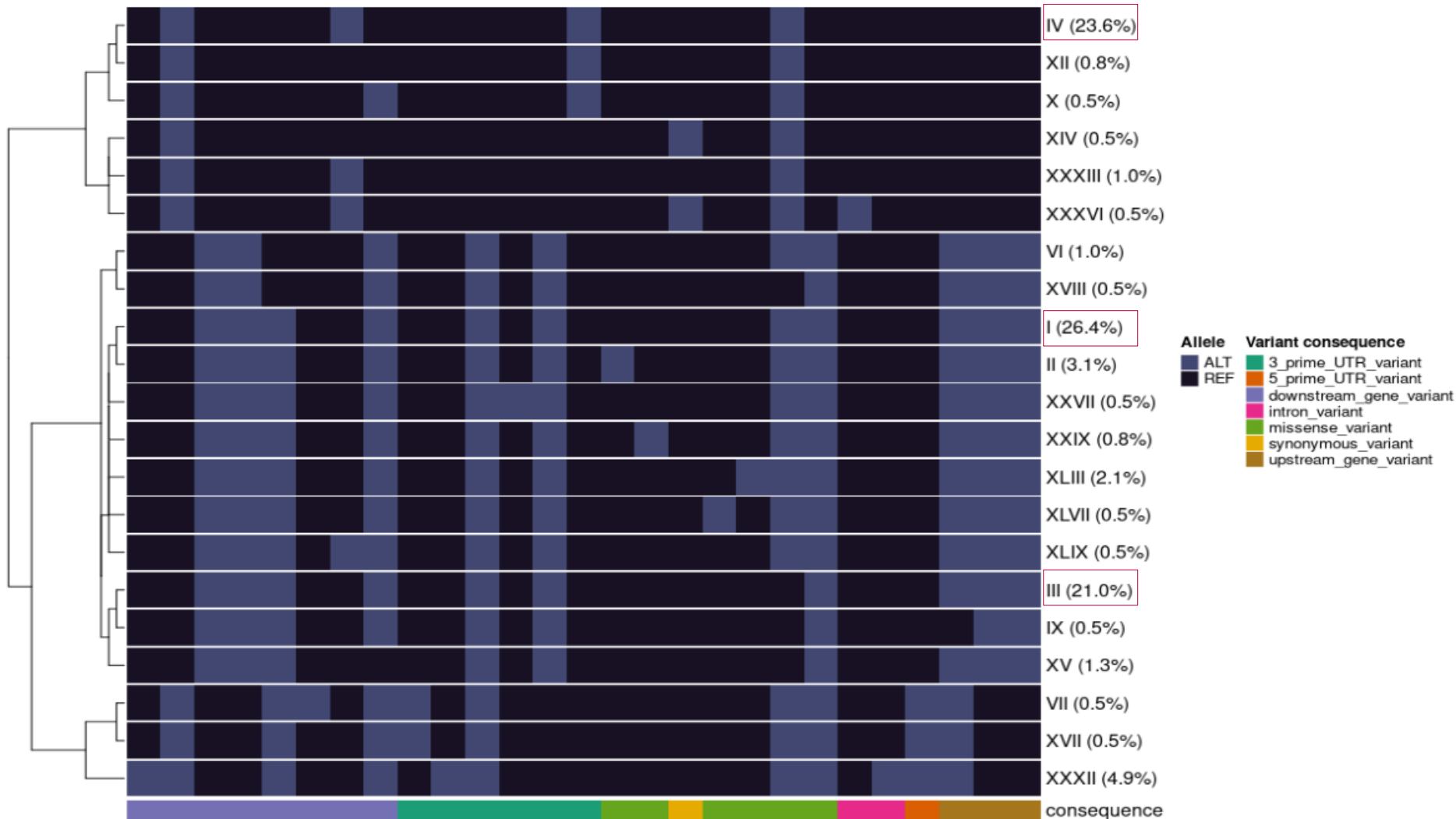
## ➤ PCA conclusions

- First 3 principal components explain at least 60% of the observed variance
- Meishan and MSxLW crossbreeds seem to cluster together for FUT1 and FUT2, but not for FUT
- Next step: haplotype distribution
  - In overall population ?
  - Among subpopulations ?



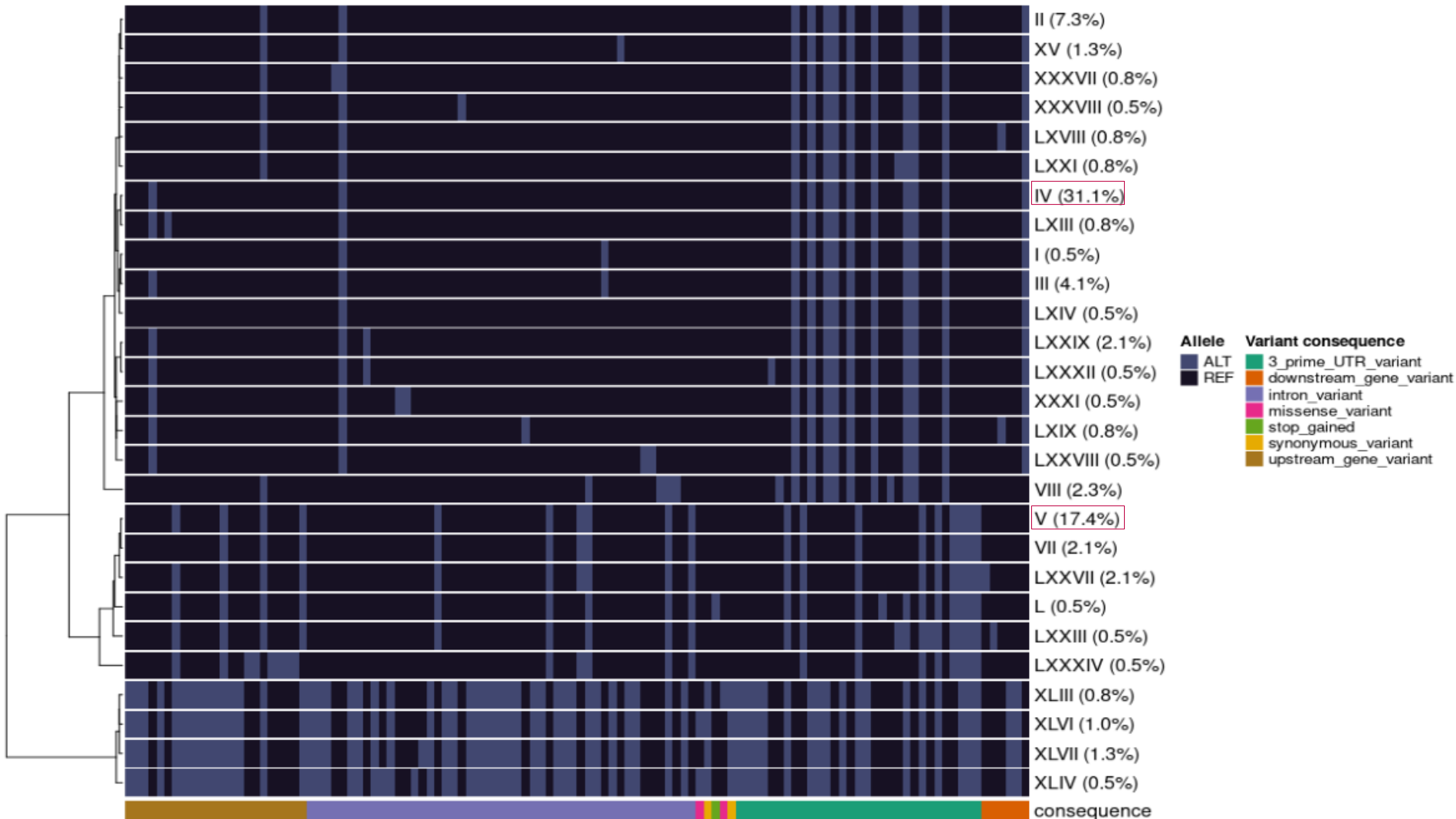
# ➤ Majority haplotypes - FUT1

- 55 haplotypes in total, 25 observed at least twice



## ➤ Majority haplotypes - FUT2

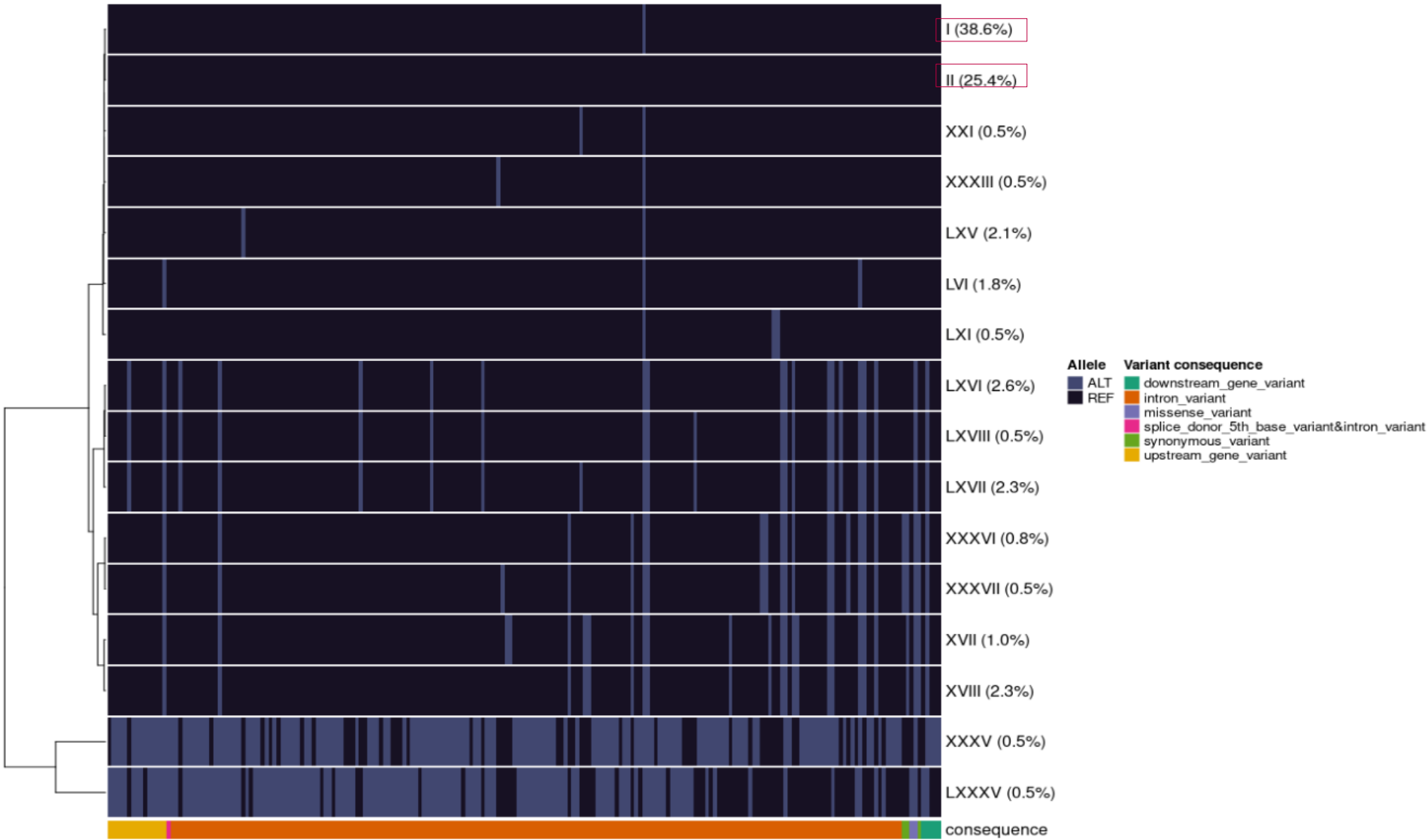
- 97 haplotypes in total, 27 observed at least twice





# ➤ Majority haplotypes - FUT3

- 91 haplotypes in total, 16 observed at least twice



# ➤ Majority haplotypes in overall population

- FUT1:
  - I (26,4%)
  - III (21%)
  - IV (23,6%)
  - Total : 71%
- FUT2:
  - IV (31,1%)
  - V (17,4%)
  - Total : 48,5%
- FUT3
  - I (38,6%)
  - II (25,4%)
  - Total : 64%



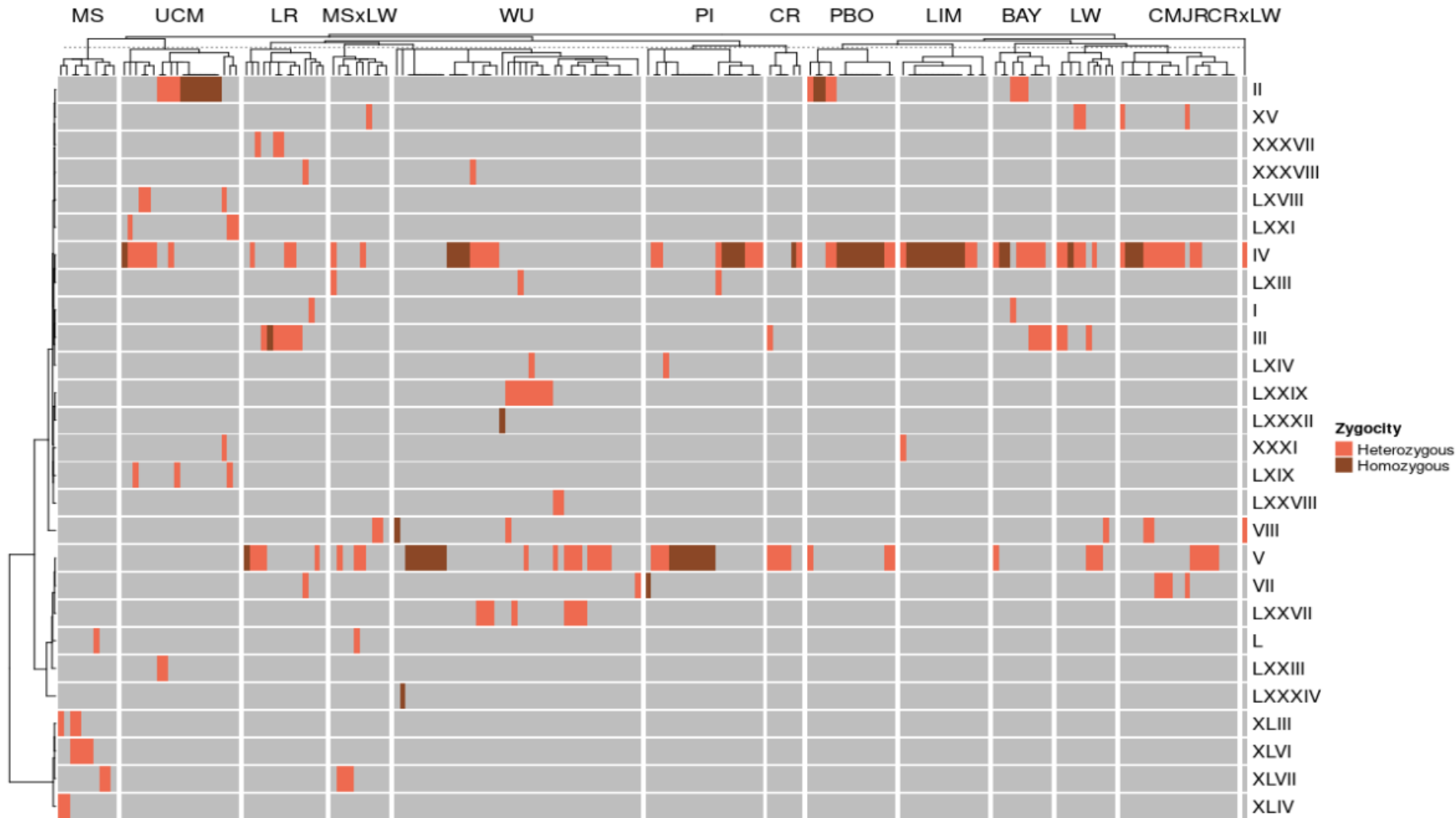
# ➤ Haplotype distribution across populations - FUT1



INRAE

FUT1, FUT2 and FUT3 variability  
03/04/2023

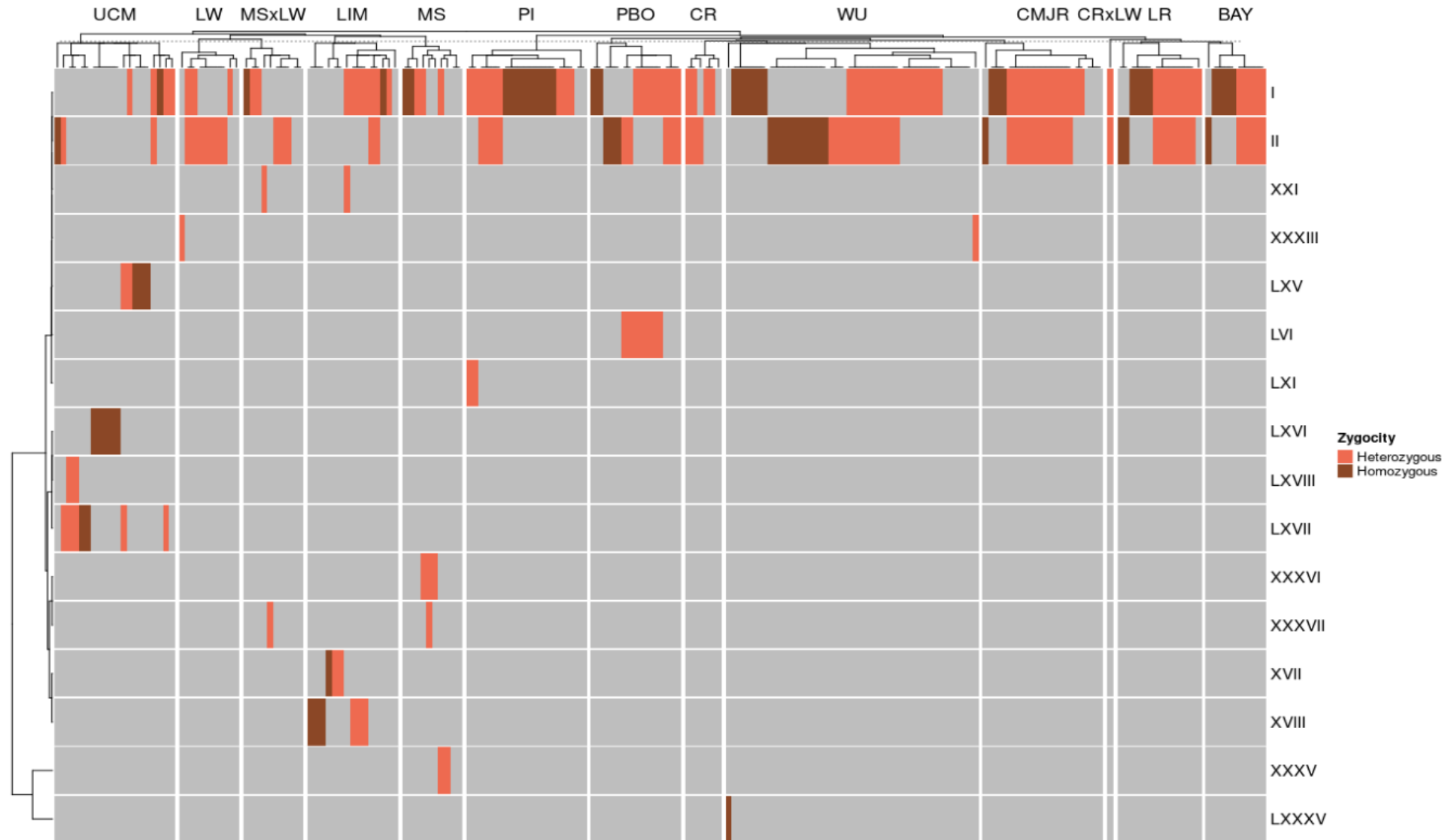
# ➤ Haplotype distribution across populations - FUT2



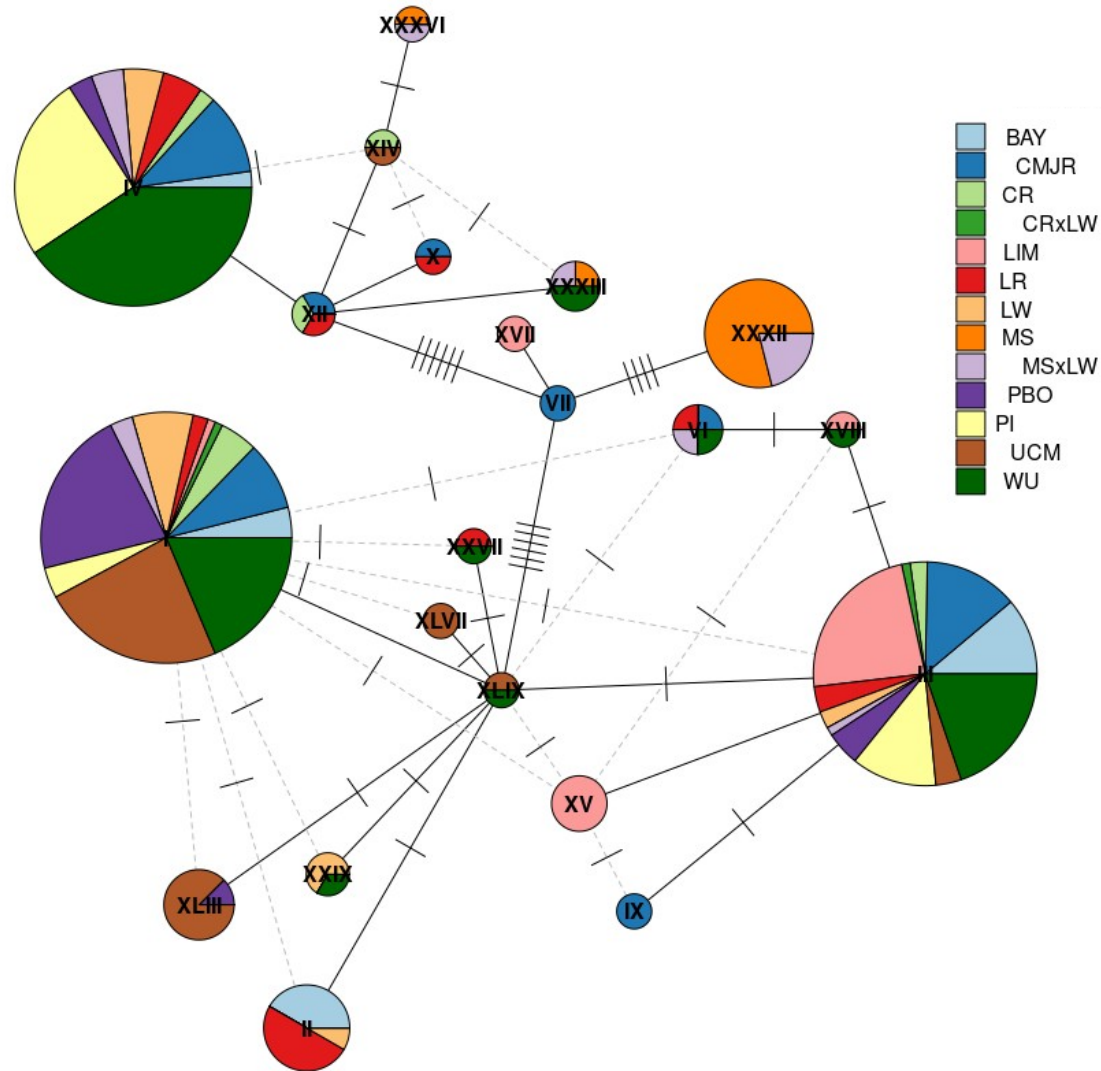
INRAE

FUT1, FUT2 and FUT3 variability  
03/04/2023

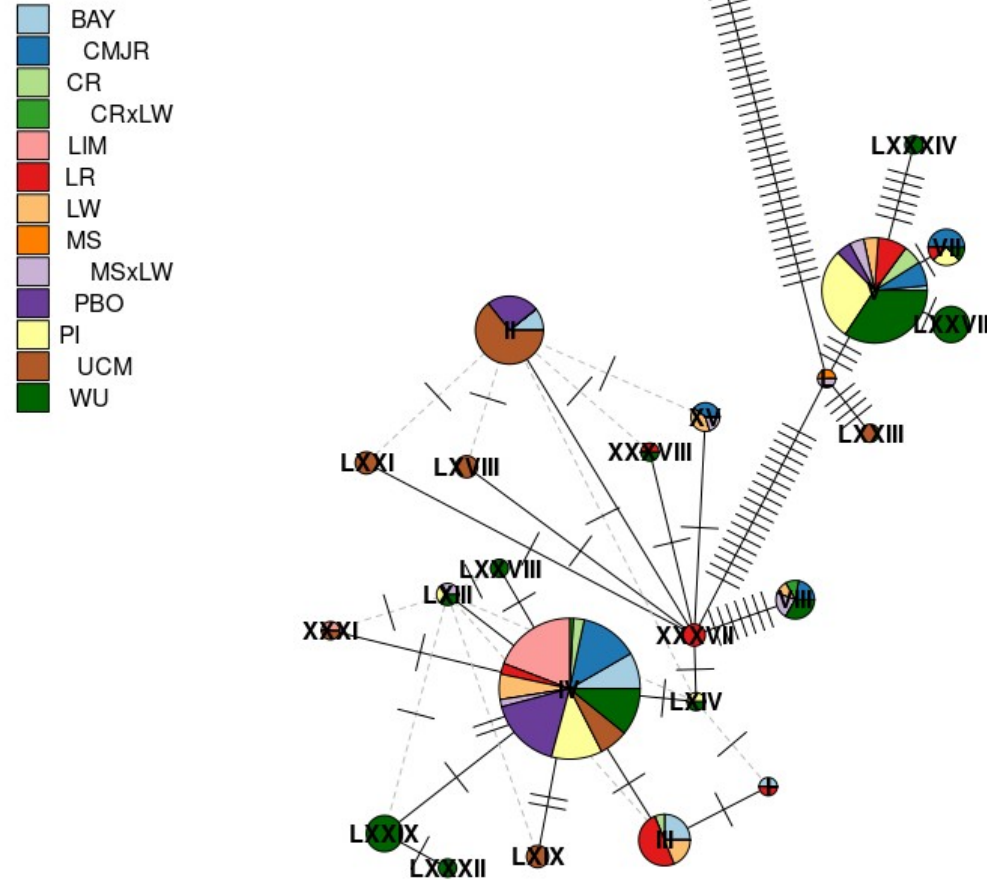
# ➤ Haplotype distribution across populations - FUT3



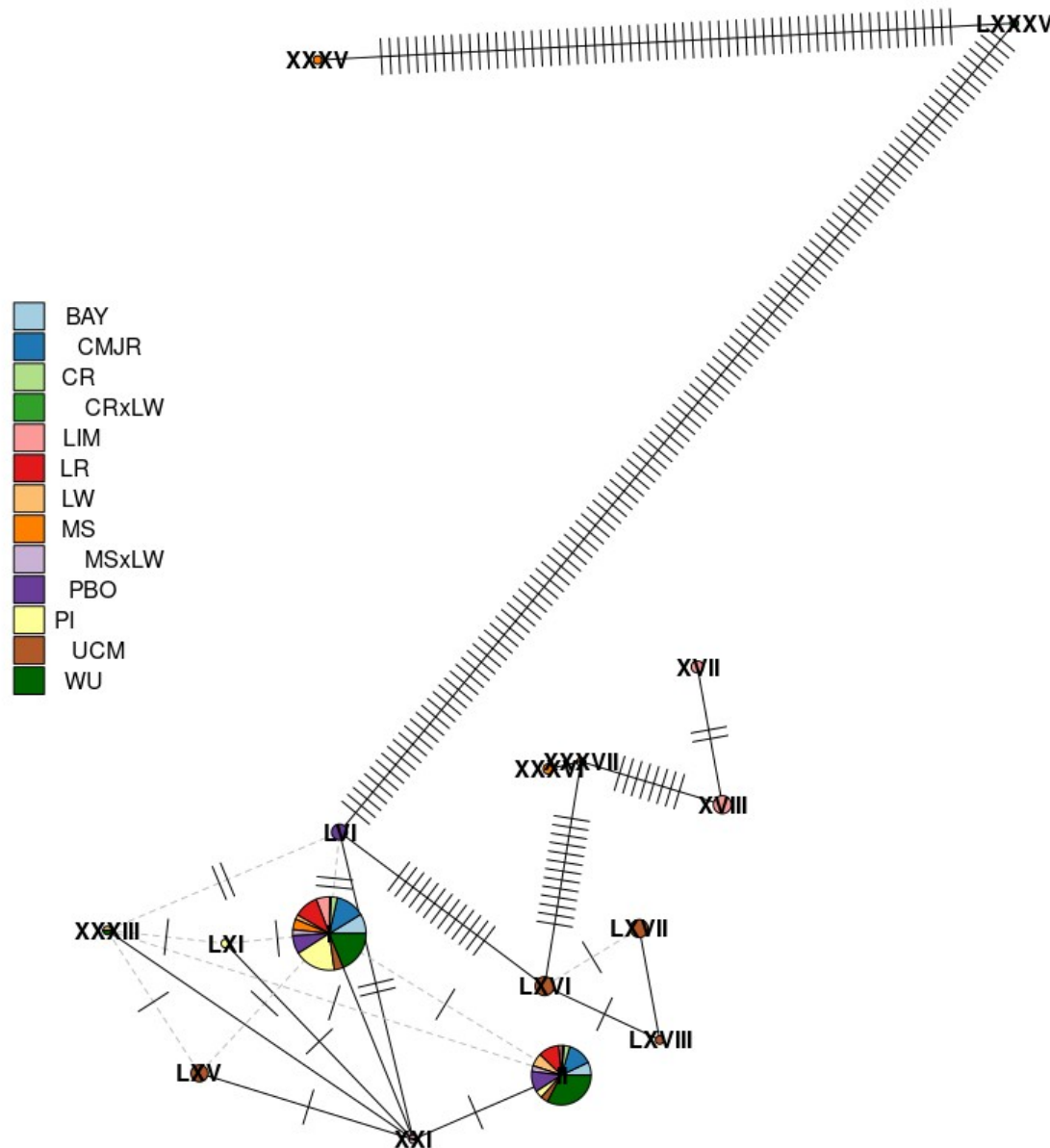
## ➤ Haplotype distribution across populations - FUT1



## ➤ Haplotype distribution across populations - FUT2

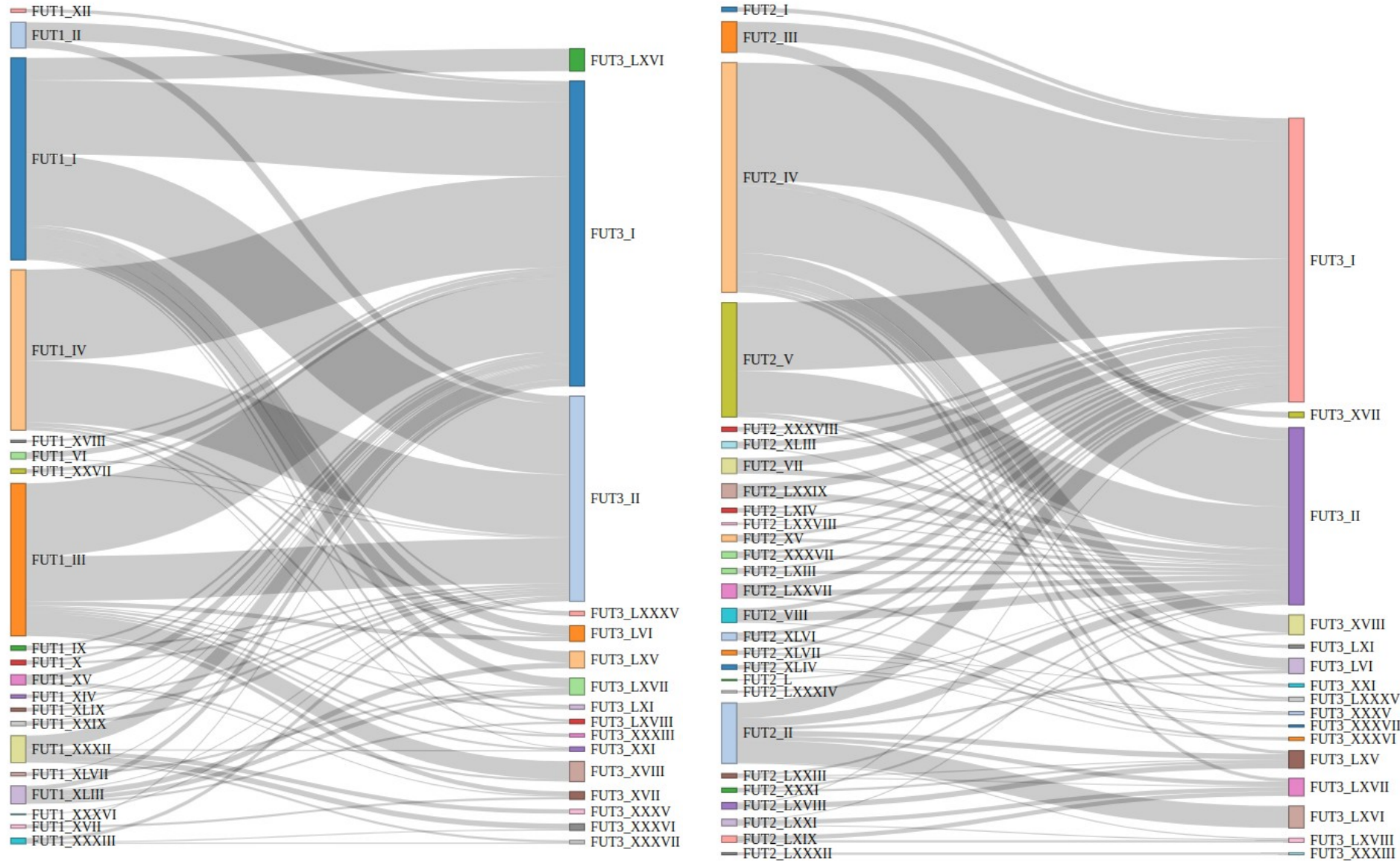


# ➤ Haplotype distribution across populations - FUT3





# ➤ Haplotype combinations



FUT1, FUT2 and FUT3 variability

03/04/2023

## ➤ Conclusions

- No strong evidence of inactive FUT1, FUT2 or FUT3
- FUT3 highly conserved
- Meishan population distinct for FUT1 and FUT2, less for FUT3

