











## Analyse de variants génétiques (SNPs, indels)













## I- SNP calling and genotype assignation

## **II- SNP data analyses and visualization**



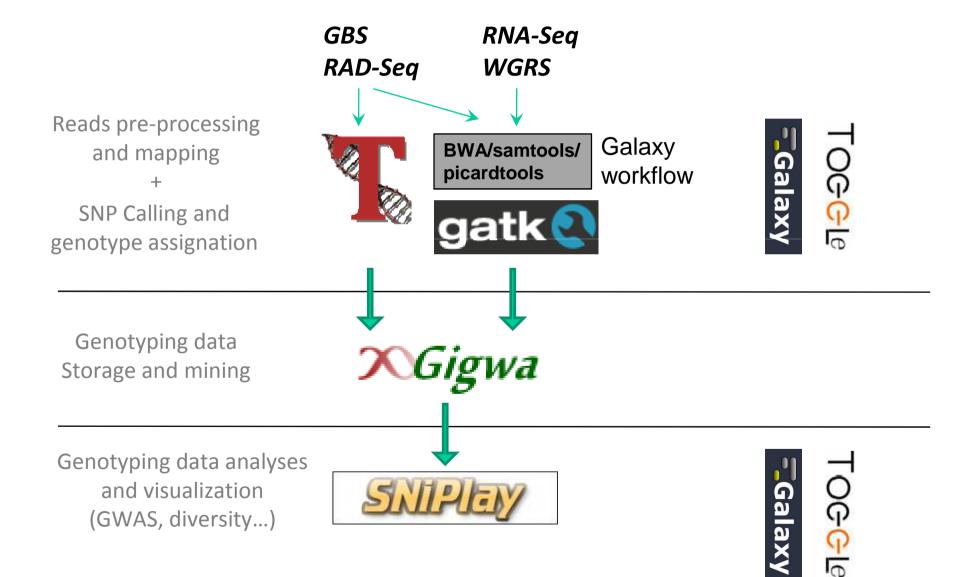
















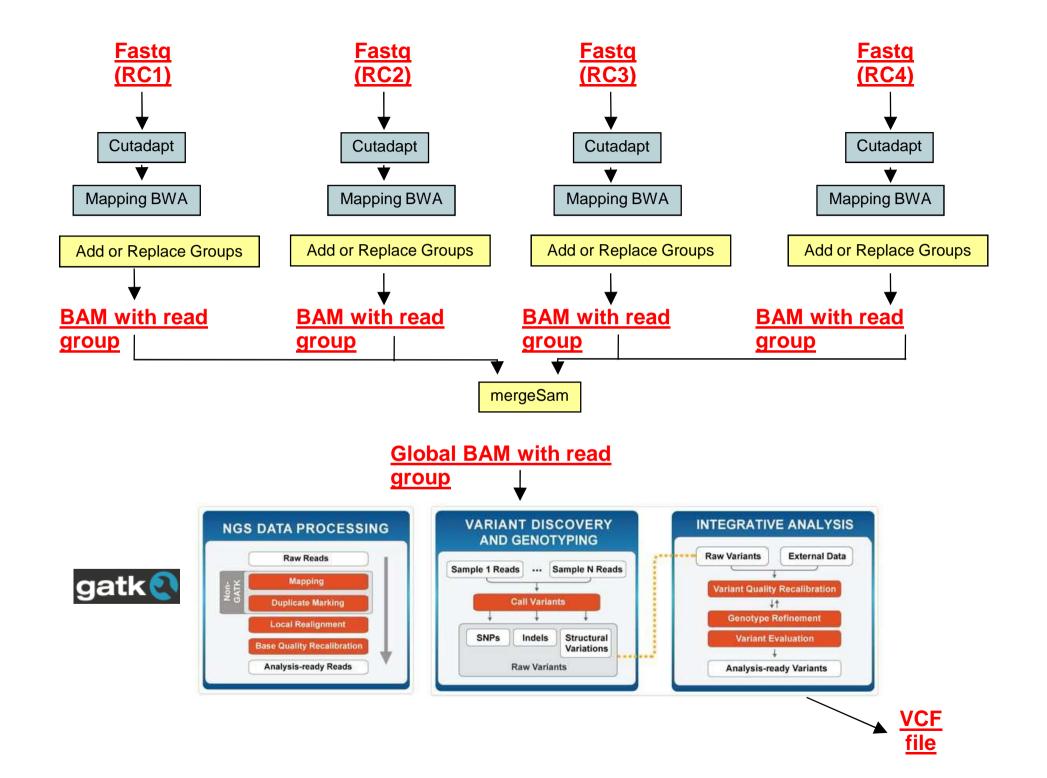








## I- SNP calling and genotype assignation















#### Format SAM/BAM

A header listing at least the different sequences in the reference and their length.

At least one line for each read. The different fields are separated by tabulation.

Read name

Bitwise flag

Reference sequence

Start position of the alignment on the reference

Mapping quality (255 means unknown quality)

**CIGAR String** 

Reference sequence and alignment start of the mate

Observed fragment length

Nucleotidic and quality sequence of the read

And optional fields













## **Format Pileup**

- Another format for variant calling (generated by samtools)
- Describe alignment row by row (not line by line like in SAM format)
- Used by softwares such as **Varscan** (varscan pileup2snp)
- Frequently used for rare variants, with a low frequency (e.g. viral pop)







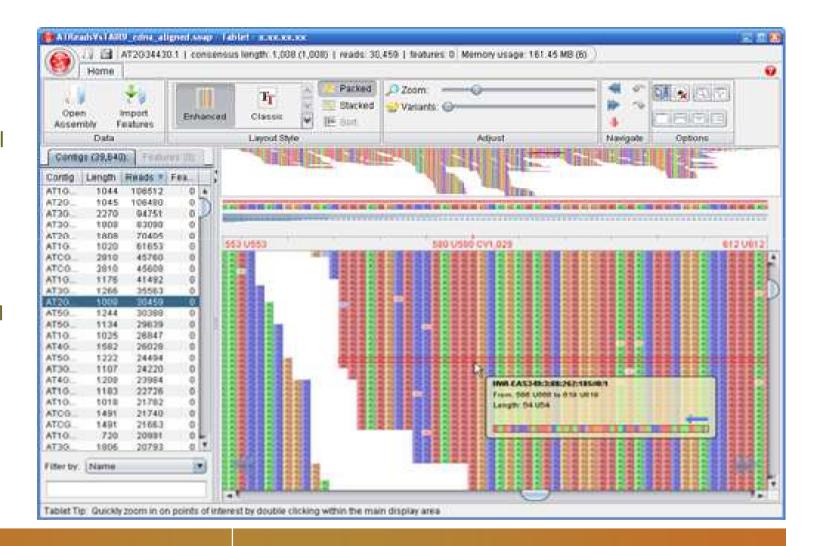






## **Tablet**

- Graphical tool to visualize assemblies
- Accept many formatsACE, SAM, BAM















## **GATK (Genome Analysis ToolKit)**



- Software package to analyse NGS data.
- Implemented to analyse human resequencing data, for medical purpose (1000 genomes, The Cancer Genome Atlas)
- Includes depth analyses, quality score recalibration, SNP/InDel detection
- Complementary with other packages: SamTools, PicardTools, VCFtools, BEDtools

#### PREPROCESS:

- \* Index human genome (Picard), we used HG18 from UCSC.
- \* Convert Illumina reads to Fastq format
- \* Convert Illumina 1.6 read quality scores to standard Sanger scores

#### FOR EACH SAMPLE:

- 1. Align samples to genome (BWA), generates SAI files.
- 2. Convert SAI to SAM (BWA)
- 3. Convert SAM to BAM binary format (SAM Tools)
- 4. Sort BAM (SAM Tools)
- 5. Index BAM (SAM Tools)
- 6. Identify target regions for realignment (Genome Analysis Toolkit)
- 7. Realign BAM to get better Indel calling (Genome Analysis Toolkit)
- 8. Reindex the realigned BAM (SAM Tools)
- 9. Call Indels (Genome Analysis Toolkit)
- 10. Call SNPs (Genome Analysis Toolkit)
- 11. View aligned reads in BAM/BAI (Integrated Genome Viewer)









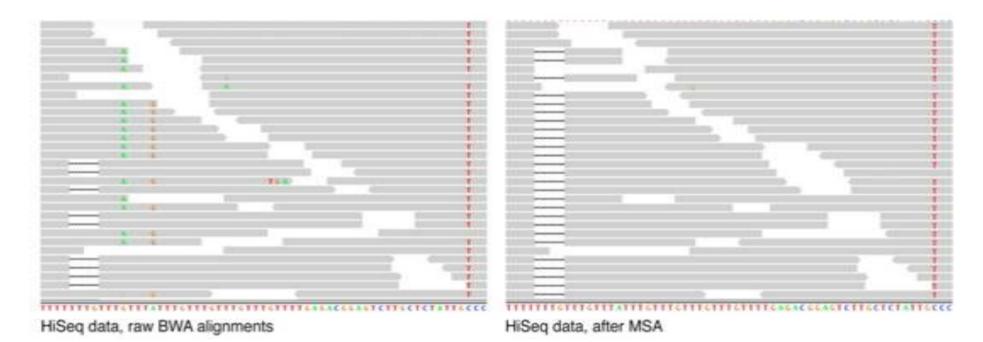




## **GATK (Genome Analysis ToolKit)**



• IndelRealigner module: realigns around indels in order to avoid false positive SNPs















## **Format VCF (Variant Call Format)**

## Advantages:

Variation description for each position + genotype assignations Indexed flat files.

Binary files also exist: BCF format

```
##fileformat=VCFv4.0
##fileDate=20110705
##reference=1000GenomesPilot-NCBI37
##phasing=partial
##INFO=<ID=NS, Number=1, Type=Integer, Description="Number of Samples With Data">
##INFO=<ID=OP, Number=1, Type=Integer, Description="Total Depth">
##INFO=<ID=AF, Number=., Type=Float, Description="Allele Frequency">
##INFC=<ID=AA, Number=1, Type=String, Description="Ancestral Allele">
##INFO=<ID=DB, Number=0, Type=Flag, Description="dbSNP membership, build 129">
##INFC=<ID=H2, Number=0, Type=Flag, Description="HapMap2 membership">
##FILTER=<ID=q10, Description="Quality below 10">
##FILTER=<ID=s50, Description="Less than 50% of samples have data">
##FCRMAT=<ID=GQ, Number=1, Type=Integer, Description="Genotype Quality">
##FCRMAT-<ID-GT, Number=1, Type-String, Description-"Genotype">
##FORMAT=<ID=DF, Number=1, Type=Integer, Description="Read Depth">
##FORMAT=<ID=HQ, Number=2, Type=Integer, Description="Haplotype Quality">
#CHROM POS
                                                                                                Samplel
                                                                                                               Sample2
                                                                                                                               Sample3
       4370 ra6057
                                                 NS=2;DP=13;AF=0.5;DB;H2
                                                                                    GT:GQ:DP:HQ 010:48:1:52,51 110:48:8:51,51 1/1:43:5:.,
      7330 .
                                     3 m10
                                              NS=5:DP=12:AF=0.017
                                                                                    GT:GO:DP:HO 010:46:3:58.50 011:3:5:65.3
                                                                                                                              0/0:41:3
2
      110696 rs6055
                           G.T
                                     67 PASS MS=2;De=10;AF=0.333,0.667;AA=T;DB GT:GQ:De:HQ 1/2:21:6:23,27 2/1:2:0:18,2
                                                                                                                              2/2:35:4
2
      130237 .
                                                 NS=2;DP=16;AA=T
                                                                                    GT:GQ:DP:HQ 010:54:7:56,60 010:48:4:56,51 0/0:61:2
       134567 microsat1 GTCT G,GTACT 50 PASS NS=2;DF=9;AA=G
                                                                                                               0/2:17:2
                                                                                                                               1/1:40:3
```













#### **Other GATK functionalities**

• Module DepthOfCoverage:

Allows to get sequencing depth for each gene, each position and each individual

Module ReadBackedPhasing:

Allows to set, if possible, associations between alleles (phase and haplotypes) when we are in an heterozygote situation.

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT SAMP1	SAMP2	
chr1	1		A	G	99	PASS	•	GT:GL:GQ	0/1:-100,0,-100:99	0/1:-100,0,-100:99
chr1	2		A	G	99	PASS		GT:GL:GQ:PQ	1 1:-100,0,-100:99:60	0 1:-100,0,-100:99:50
chr1	3		A	G	99	PASS		GT:GL:GQ:PQ	0 1:-100,0,-100:99:60	0 0:-100,0,-100:99:60
chr1	4	50.00	A	G	99	FAIL	(0.0)	GT:GL:GQ	<del>0/1</del> :-100,0,-100:99	0/1:-100,0,-100:99
chr1	5		A	G	99	PASS		GT:GL:GQ:PQ	0 1:-100,0,-100:99:70	1 0:-100,0,-100:99:60
chr1	6		A	G	99	PASS		GT:GL:GQ:PQ	0/1:-100,0,-100:99	1 1:-100,0,-100:99:70
chr1	7		A	G	99	PASS		GT:GL:GQ:PQ	0 1:-100,0,-100:99:80	0 1:-100,0,-100:99:70
chr1	8		A	G	99	PASS		GT:GL:GO:PO	0 1:-100,0,-100:99:90	0 1:-100,0,-100:99:80

The proper interpretation of these records is that SAMP1 has the following haplotypes at positions 1-5 of chromosome 1:

- 1. AGAAA
- 2. GGGAG

And two haplotypes at positions 6-8:

- 1. AAA
- 2. GGG

Et non AGG GGA







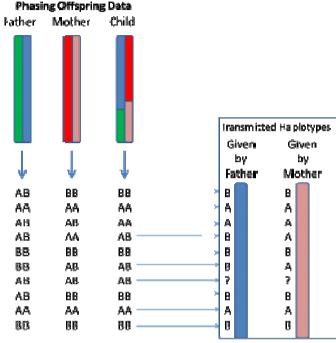






## **Haplotypes and phasing**

- **Haplotype**: Specific groups of genes or alleles that progeny inherited from one parent
- **Phasing**: Determination of haplotype phase. Process of statistical estimation of haplotypes from genotype data.
- Can be infered by statistics methods using nonambigous haplotypes present in the dataset (Gevalt, ShapeIT, Phase)
- Can be resolved using physical association of alleles within the reads
   (GATK ReadBackedPhasing, GATK HaplotypeCaller)







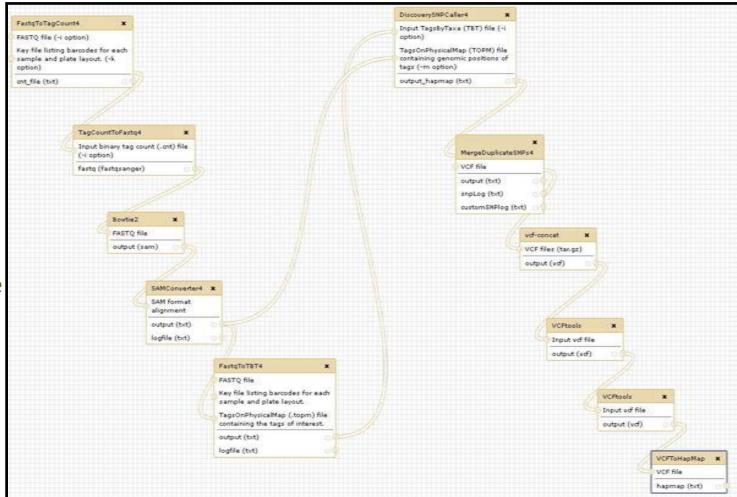












For GBS data

Tassel pipeline Version 5















## **II- SNP data analyses and visualization**













## Projet Gigwa, pour la gestion des données massives de variants (GBS, RADSeq, WGRS)

« With NGS arise serious computational challenges in terms of storage, search, sharing, analysis, and data visualization, that redefine some practices in data management. »

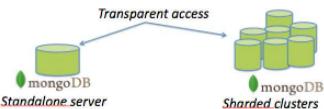
- Based on NoSQL technology



- Handles VCF files (Variant Call Format) and annotations
- Supports multiple variant types: SNPs, InDels, SSRs, SV
- Powerful genotyping queries
- Easily scalable with MongoDB sharding
- Transparent access



















Variant types	Sequences	Individ			Genotypes:	Апу		<b>‡</b>		Project	WGS			
INDEL MIXED	Chr01 Chr02	Yale_AFR298 Yale_AND696			This will return all variants whithout applying any filters									
SNP	Chr03 Chr04	Yale_G	5686						Export format:	BED		Exp		
Clear filters	Chr05 Chr06 Chr07 Chr08 Chr09 Chr10 Chr11 scaffold_12 scaffold_13 scaffold_14	Yale_G10474 Yale_G35346 Yale_G40001 Yale_MD23-24 Yale_SEA5 Yale_VAX1			Minimum read depth 1 (others will be treated as missing data)  Authorized missing data ratio: 100 %  Minor allelic frequency: from 0 % to 50 %  Position (bp): Min - Max 2 3									
Ciedi liitera	Scaliolo_14	-		-				Number of alleles						
	100 / 21694252	AND NOTE OF STREET				_		d	Al	nort				
ID		ce Start	Stop		Alleles									
	Chr01	65			T	12								
	Chr01	96		С	G	3								
	Chr01 Chr01	96 101		C G	G T	Q.					_			
	Chr01 Chr01 Chr01	96 101 112		C G C	G T G	2 2	httn://gi	gwa southgr	een fr/gi					
	Chr01 Chr01 Chr01 Chr01	96 101 112 114	125	C G C	G T G	0 0 0 0	http://gi	gwa.southgr	een.fr/gi	gwa/				
	Chr01 Chr01 Chr01	96 101 112 114 123	125	C G C C	G G T	Q Q Q Q	http://gi	gwa.southgr	een.fr/gig	gwa/				
	Chr01 Chr01 Chr01 Chr01	96 101 112 114	125	C C C ACC	G T G	2 2 2 2 2 2	http://gi	gwa.southgr	een.fr/gig	gwa/				
	Chr01 Chr01 Chr01 Chr01 Chr01	96 101 112 114 123 138	125	C C C ACC G	G T G AC A	Q Q Q Q	http://gi	gwa.southgr	een.fr/gig	gwa/				
	Chr01 Chr01 Chr01 Chr01 Chr01 Chr01	96 101 112 114 123 138 146	125	C C ACC G G	G T G AC A T		http://gi	gwa.southgr	een.fr/gig	gwa/				
	Chr01 Chr01 Chr01 Chr01 Chr01 Chr01 Chr01	96 101 112 114 123 138 146 147	125	C C ACC G G	G T G AC A T T		http://gi	gwa.southgr	een.fr/gig	gwa/				













MAF

28.1%

27,5%

28.1%

28.1%

28.1%

28.1%

28.1%

7.8%

4.2%

26.3%

MAF

amino acid change

missing data

0.0%

0.0%

0.0%

0.0%

0.096

0.0%

0.0%

0.0%

0.0%

30

missing data

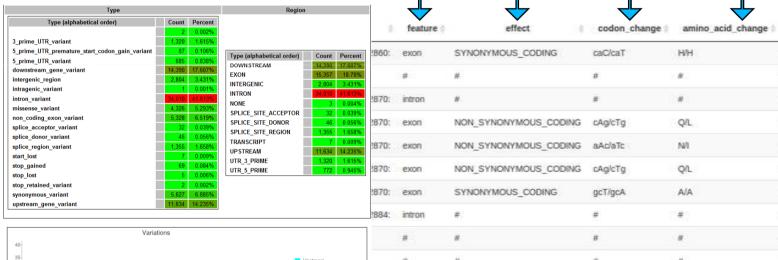
Next

## **SNP annotation using SnpEff**

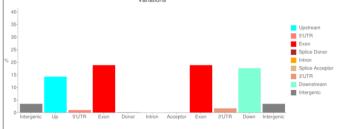
## **SnpEff**

Genetic variant annotation and effect prediction toolbox.

- It annotates and predicts the effects of variants on genes (amino acid changes...)
- Uses as input GFF annoation file and VCF



feature



Alexis Dereeper – Christine Tranchant

Formation Bio-informatique IRD Ouagadougou 2016

codon change

effect



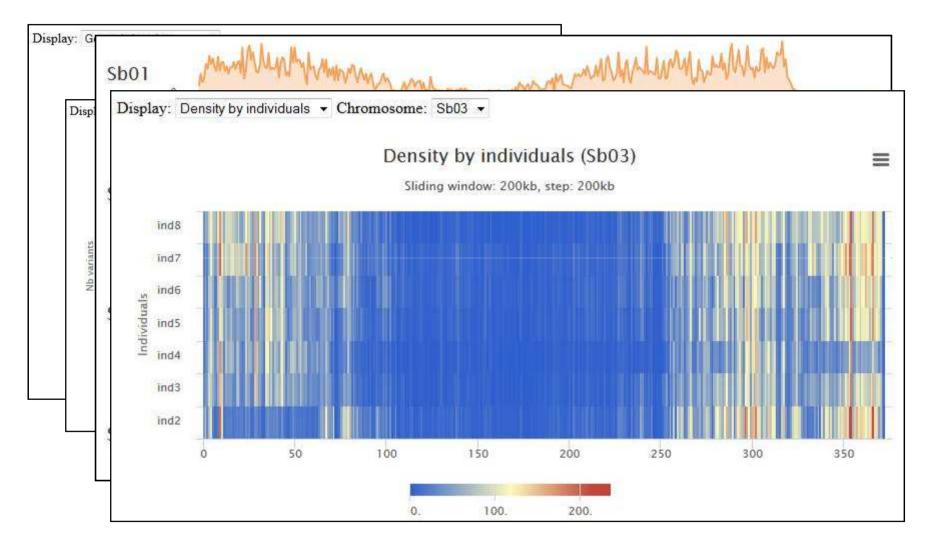














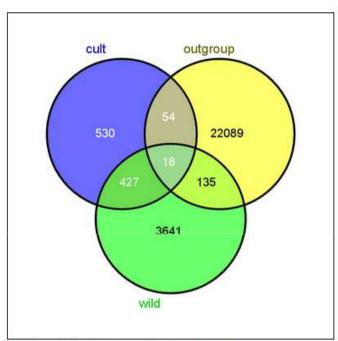












Specific and shared polymorphisms between groups

## **Comparison between individuals**

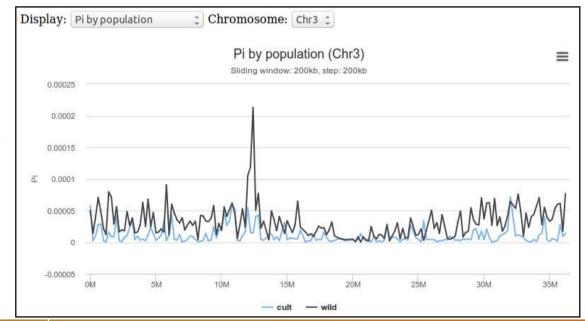
<u>Fst</u>: Fixation index: measure of population differentiation due to genetic structure.

<u>Pi</u>: Nucleotide diversity: Average number of nucleotide differences per site between any two DNA sequences chosen randomly from the sample population

Used to measure the degree of polymorphism within a population

+ 2186 polymorphisms inter-group

## **Diversity analysis**















SNP density by individuals can allow the detection of introgression event.

<u>Introgression</u> = Movement of a exogene region (gene flow) from one species into the gene pool of another by the repeated backcrossing of an interspecific hybrid with one of its parent species

Widely used in agronomy obtained but can occurs naturally







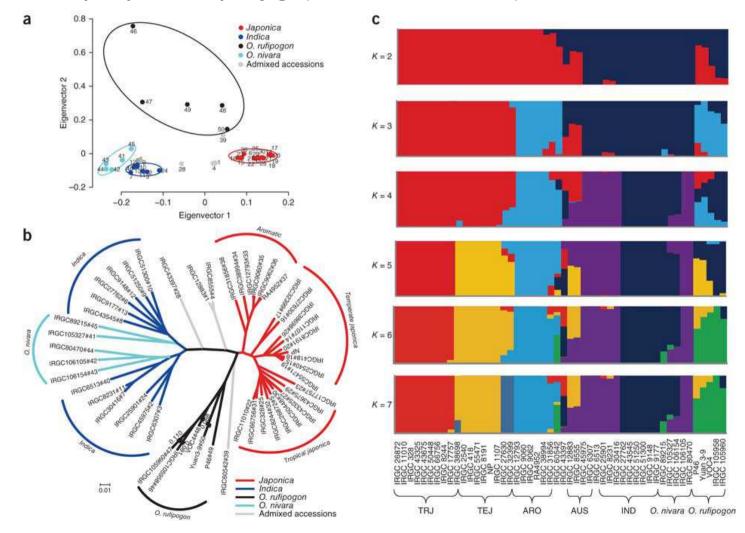






## **Population structure**

Ex: Riz asiatique après re-séquençage (Xun et al, Nature, 2011)









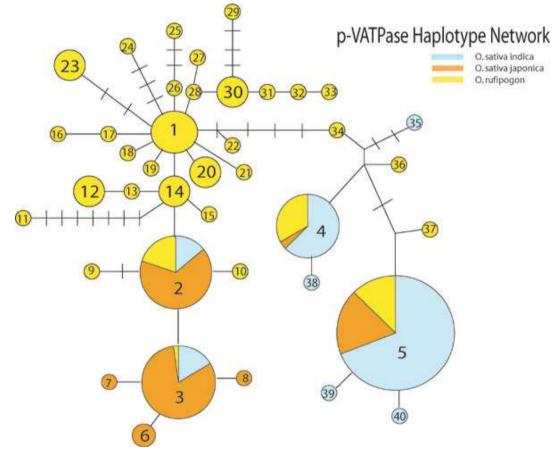






## Haplotype network

Exemple d'une région génomique chez le Riz









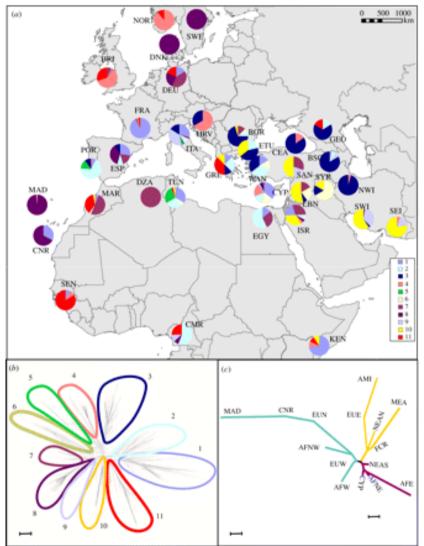






# Haplotype and geographical distribution

Différenciation génétique de la souris domestique (Bonhomme et al, 2010)







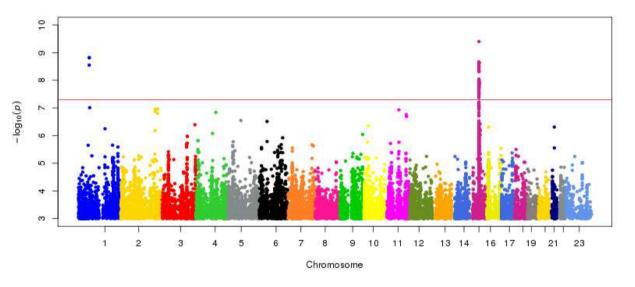








## **GWAS (Genome-Wide Association Studies)**



- Estimate association between a marker and a phenotypic character
- Manhattan plots: displays GWAS statistical tests (-log10 pvalue) along chromosomes
- TASSEL, MLMM sofwares
- False positives because of the studied structuration panel
- => correction using structure population et and kinship













#### **GWAS** issues

- Choice of genotypic panel: phenotypic diversity for target traits must be sufficient (core-collection, MAGIC lines, NAM...)
- Population structure induces high rates of false associations (false positives)
- Correction using structure population et and kinship. Mixed models:
  - 0 Q
  - K (widely used)
  - Q+K (widely used)
- **Density of markers** must be enough to provide a good genome cover. Density can be also highly variable.
- Linkage disequilibrium (LD) landscape: level of intra- and inter-chromosomal LD (number of loci in LD with loci from other chromosomes). Ideally, LD profile must be flat to avoid distorsion in association patterns.













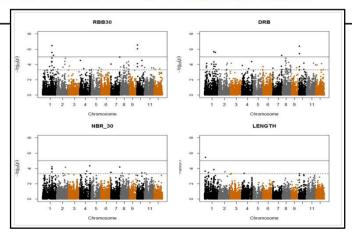
## Study of root characters using GWAS in Oryza sativa japonica. Influence of a correction using structure and kinship

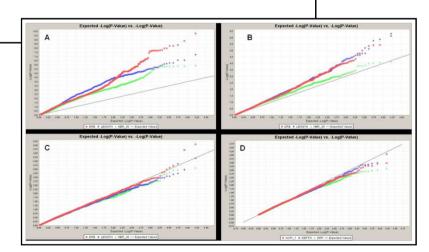


#### Genome-Wide Association Mapping of Root Traits in a Japonica Rice Panel

Brigitte Courtois , Alain Audebert, Audrey Dardou, Sandrine Roques, Thaura Ghneim- Herrera, Gaëtan Droc, Julien Frouin, Lauriane Rouan, Eric Gozé, Andrzej Kilian, Nourollah Ahmadi, Michael Dingkuhn

Published: November 5, 2013 • DOI: 10.1371/journal.pone.0078037









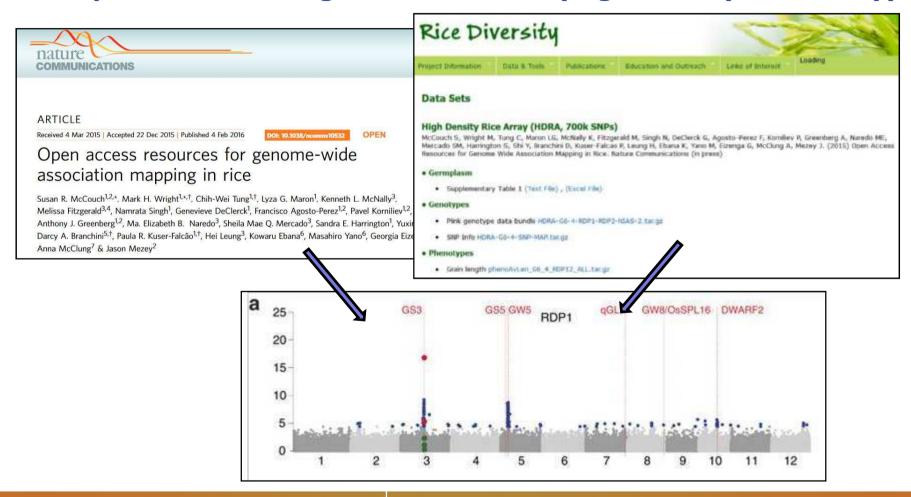








### **Exemple du Riz: 3000 genomes+ HDRA (High density Rice Array)**









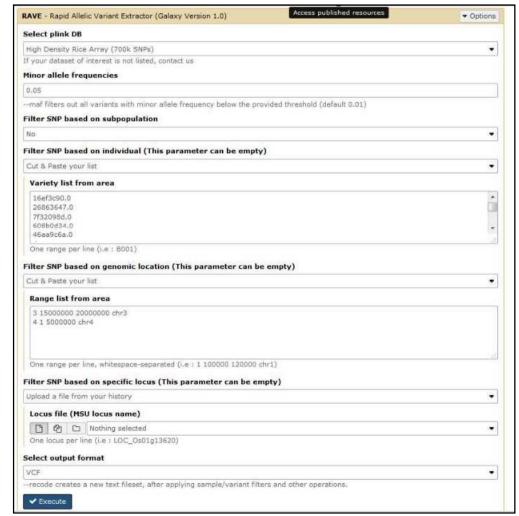






## Exemple du Riz: 3000 genomes+ HDRA (High density Rice Array)

Extraction rapide des variants après sélection d'une region / population donnée.









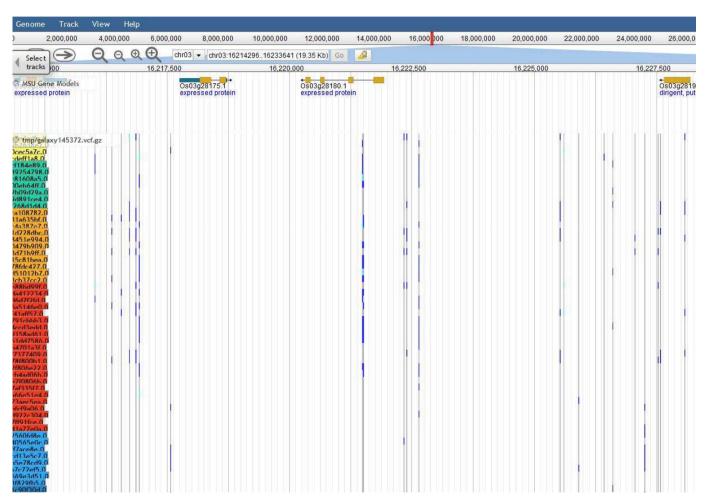






## **Exemple du Riz: 3000 genomes+ HDRA (High density Rice Array)**

Visualisation du contexte génomique dans un génome browser (plugin Jbrowse)











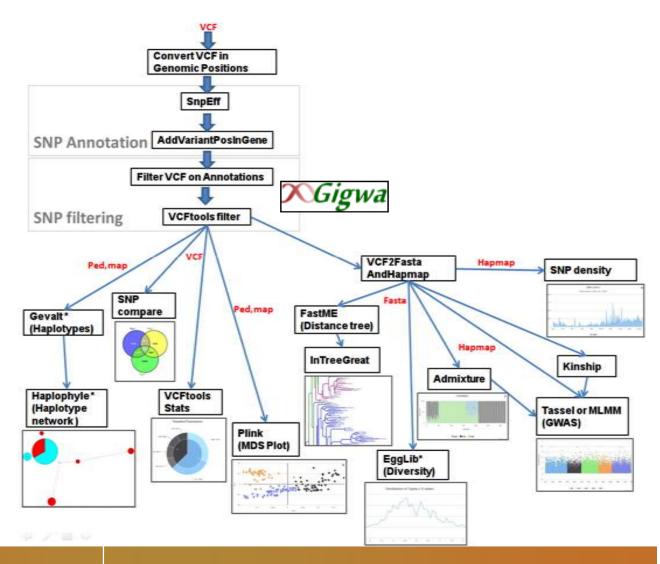






## SNiPlay: Web application for polymorphism analyses

http://sniplay.southgreen.fr















## **SNiPlay Site web**















## "Galaxy4Sniplay": SNiPlay sous Galaxy

