

Comparison of methods:  
Genotyping-by-sequencing and exome capture  
applied in genomic selection of Norway spruce  
(*Picea abies*)

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# Outline

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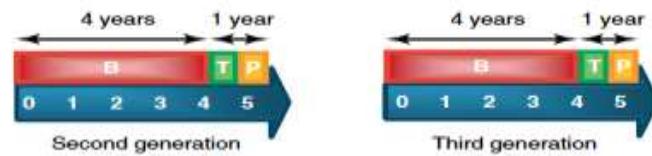
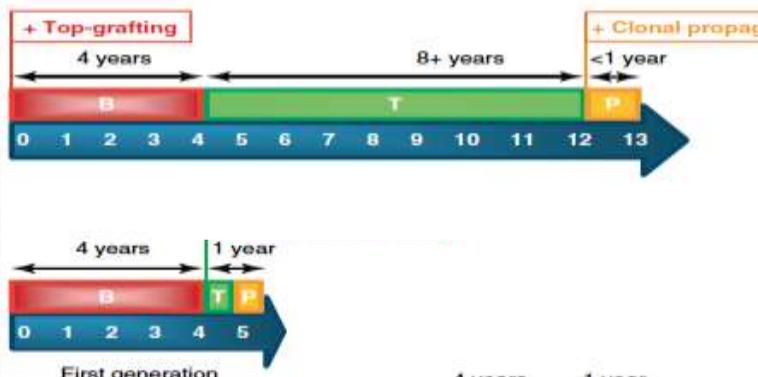
- Background
- Project aims
- Materials and Methods
- Results
- Summary

# Molecular breeding

Traditional breeding



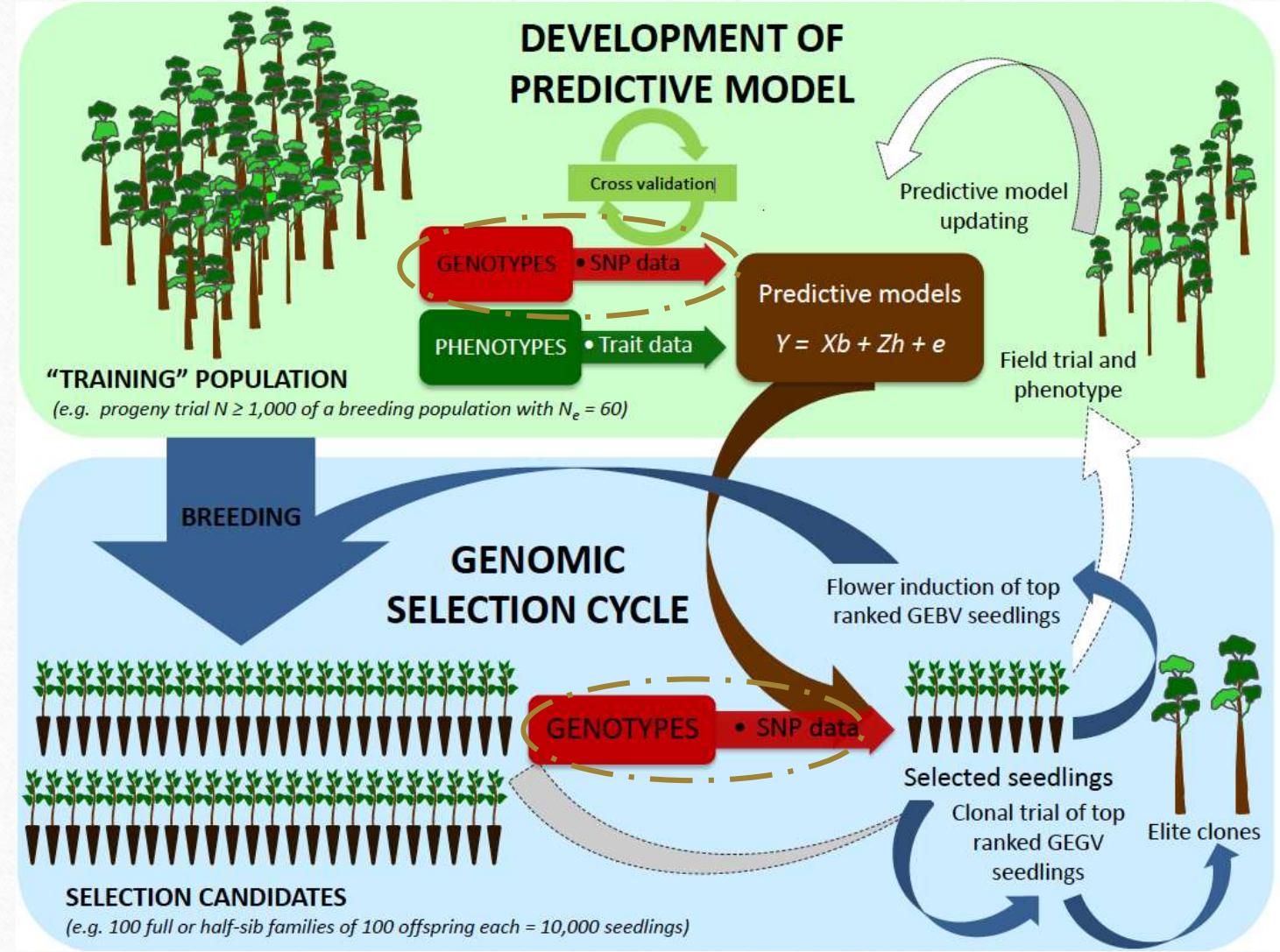
Molecular breeding



B: Breeding  
T: Testing  
P: Propagate

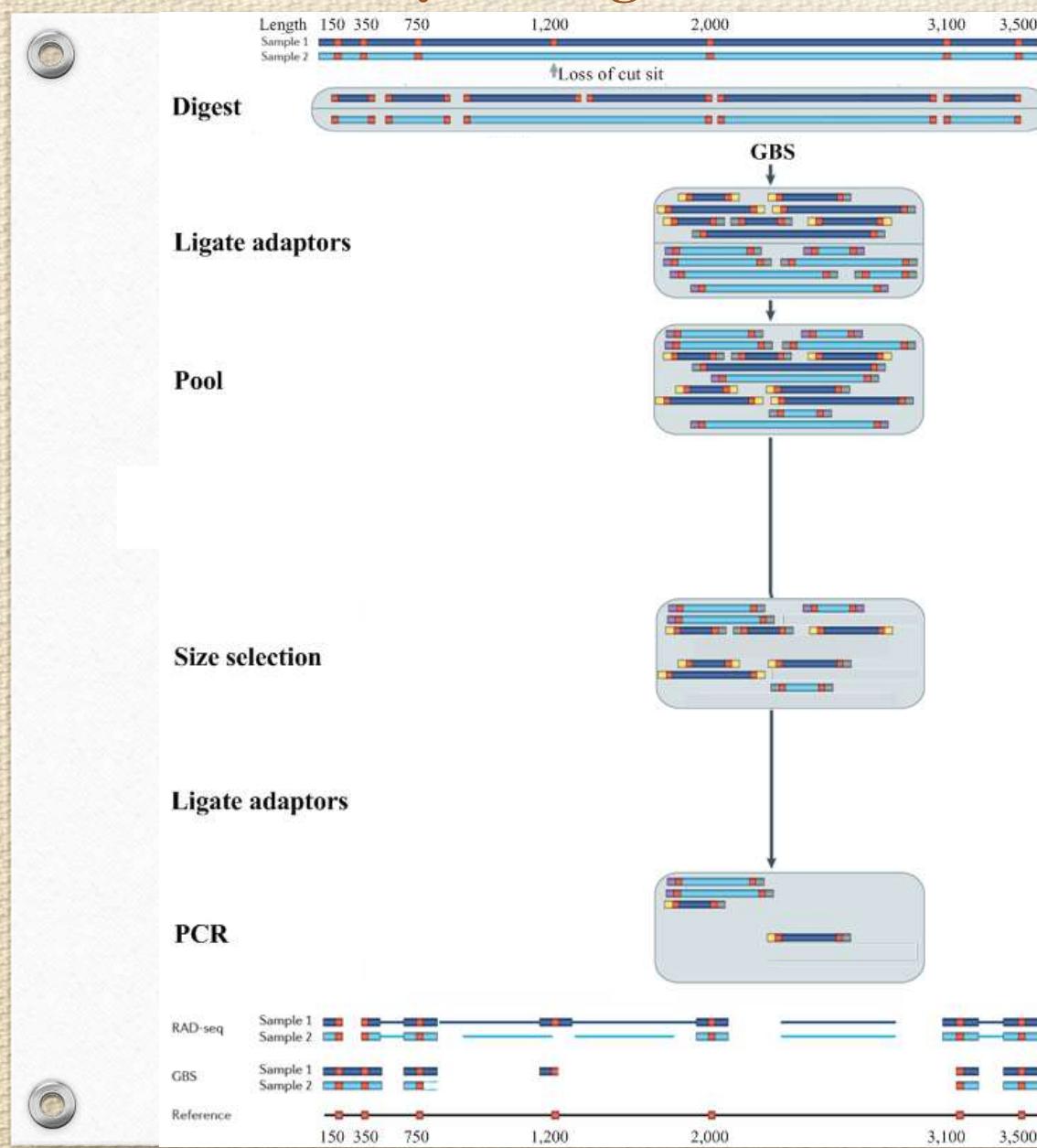
# Genomic selection

Background

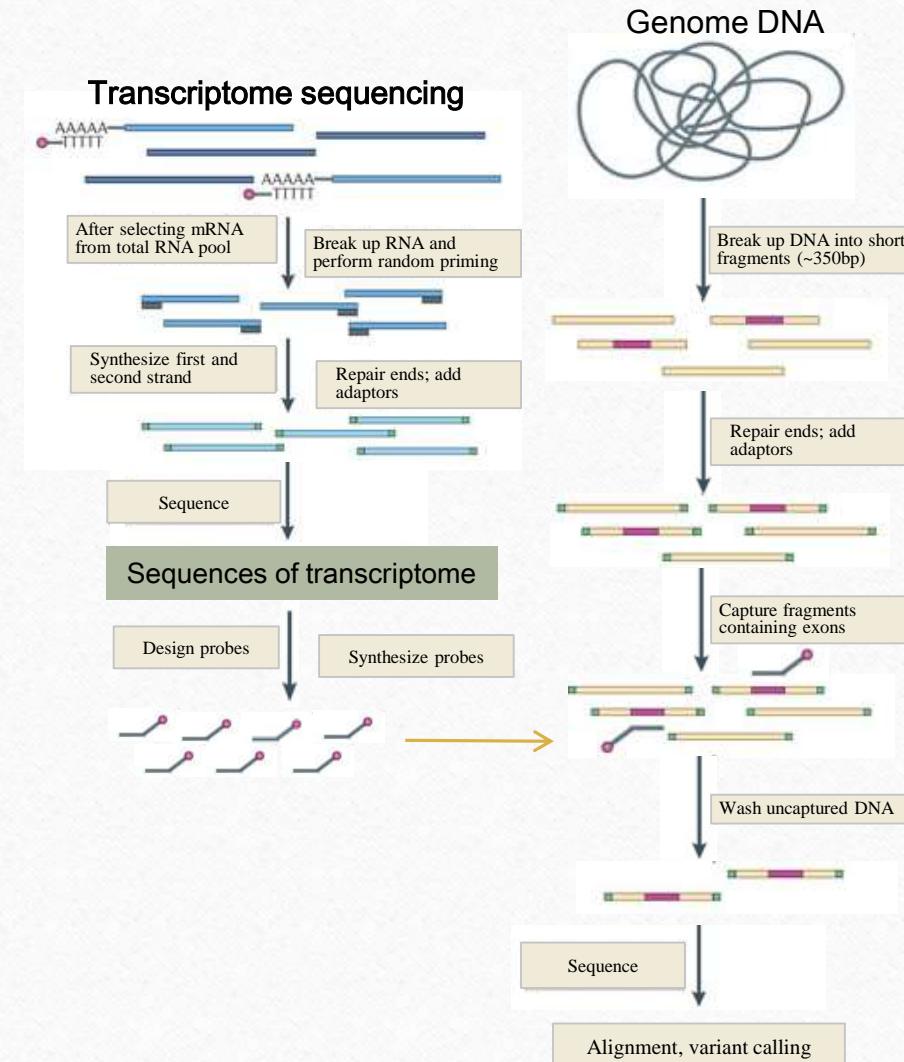


# Restriction enzymes digestion Genotyping-By-Sequencing

Background



## Target enrichment Exome capture



# Disadvantage

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## GBS

- A large percentage of uncalled genotypes (missing)
- Under-calling of heterozygous genotypes (false homozygous genotypes)

# Project aims

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- Optimize SNP-calling
  - Estimate the rate of false genotypes and the rate of discordant genotypes in replicated samples
- Evaluate the potential of GBS and EC in genomic selection of Norway spruce

# Materials

## I Genotyping

- 376 samples from 24 ful-sib families (31 parental individuals)
- ~100 endospurms and 5 mother trees
- 5 (GBS) or 21 (EC) individuals sequenced repeatedly (2-8×)

## II Phenotyping

4 growth and wood quality traits:

- Tree height
- Wood density (Pilodyn and Hitman)
- Wood stiffness (Modulus of elasticity - MOE)



# Methods

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## ➤ Genotyping-by-sequencing (GBS)

- *PstI* & 230 - 430bp restriction fragments
- ~ 20,000 restriction fragments

## ➤ Exome capture (EC)

- 40,019 probes located in **24,919** scaffolds

# Results

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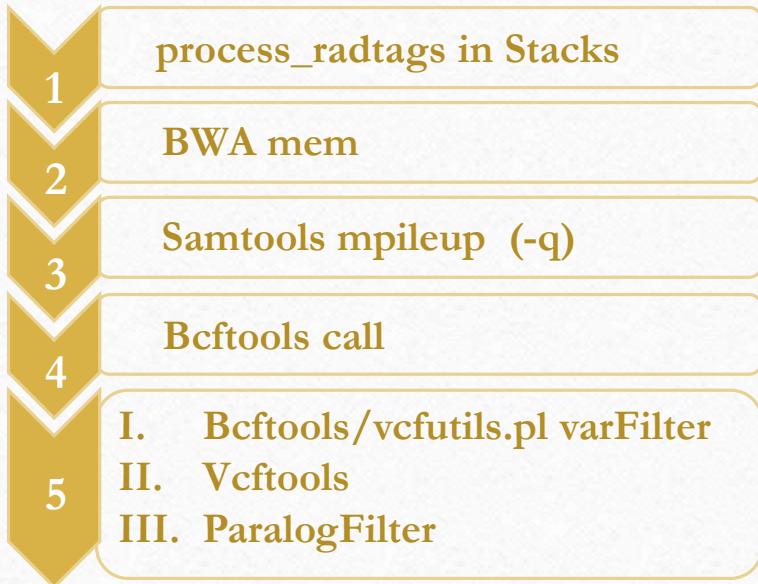
1. Optimization of SNP-calling
2. Comparison of GBS- and EC-SNP data

# Results

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1. Optimization of SNP-calling
2. Comparison of GBS and EC data

# GBS-SNP-calling pipeline



- Barcode identification and quality trimming of raw illumina reads
- Align sample reads to reference genome
- Align all samples together
- Call SNPs and indels
- Filter SNPs

## bcftools/vcfutils.pl varFilter

```

-Q INT      minimum RMS mapping quality for SNPs [10]
-d INT      minimum read depth [2]
-D INT      maximum read depth [10000000]
-a INT      minimum number of alternate bases [2]
-w INT      SNP within INT bp around a gap to be filtered [3]
-W INT      window size for filtering adjacent gaps [10]
-1 FLOAT   min P-value for strand bias (given PV4) [0.0001]
-2 FLOAT   min P-value for baseQ bias [1e-100]
-3 FLOAT   min P-value for mapQ bias [0]
-4 FLOAT   min P-value for end distance bias [0.0001]
-e FLOAT   min P-value for HWE (plus F<0) [0.0001]
  
```

## vcftools

```

SNP-site-filtering options
--max-missing  FLOAT   Minimum calling rate
--max-meanDP  INT     Maximum mean read depth
--maf          FLOAT   Minimum allele frequency
Genotype-filtering options
--minQ         INT     Minimum base quality
--minGQ        INT     Minimum genotype quality
--minDP        INT     Minimum read depth
  
```

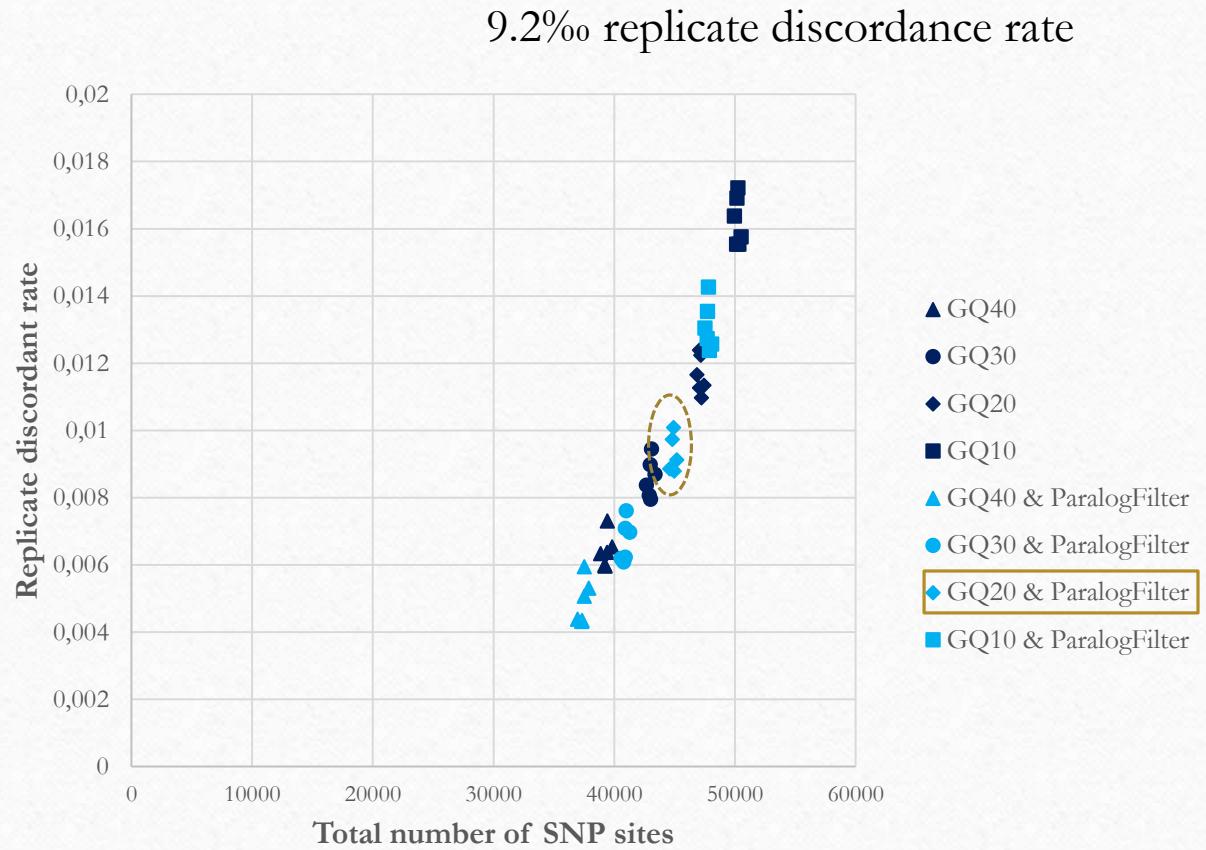
## ParalogFilter

```

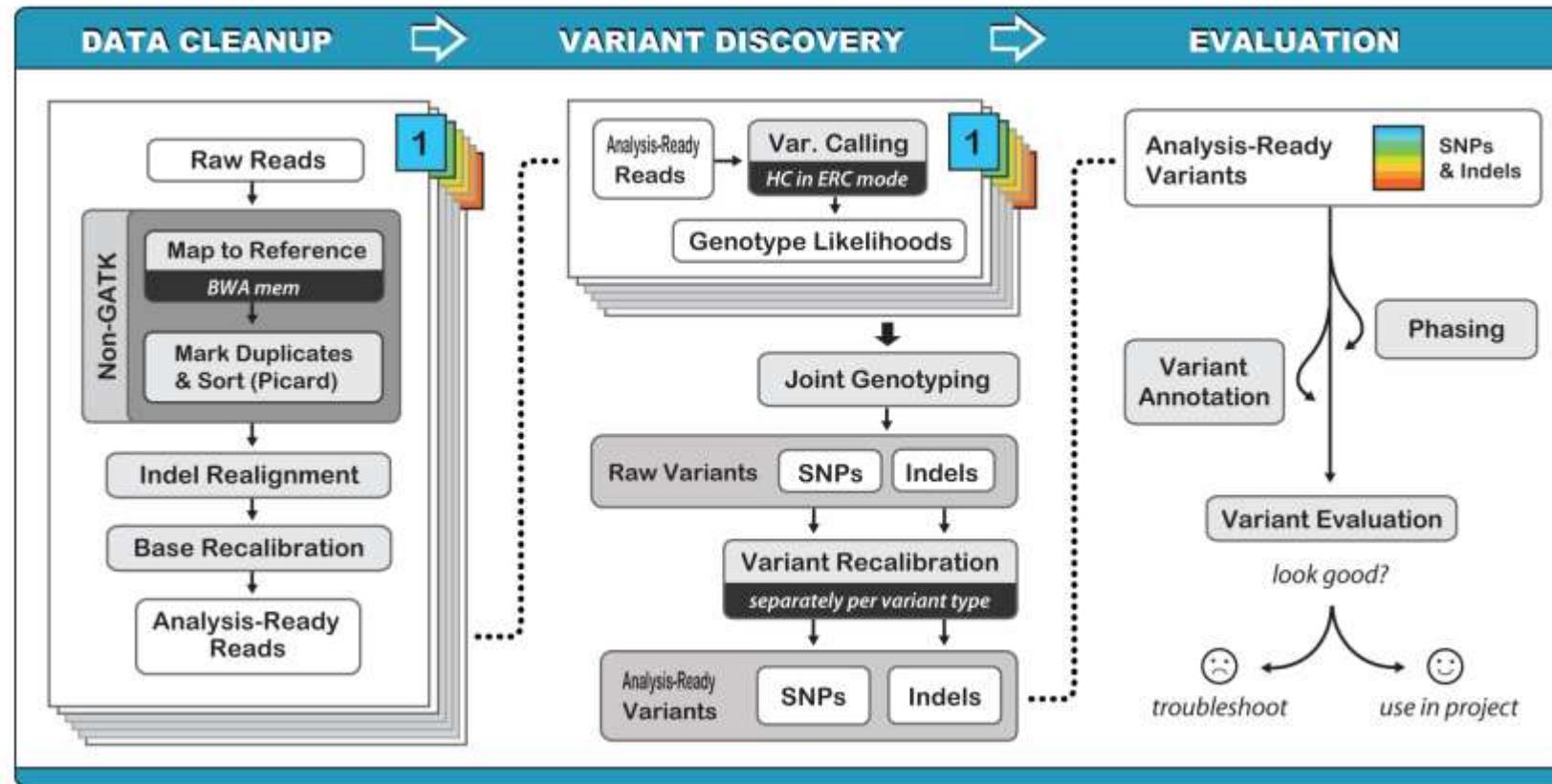
options
-F FLOAT   Minimum heterozygous rate based on genotypes of haplotypes
  
```

## Optimization of GBS-SNP calling

- GQ (genotype quality)
- ParalogFilter



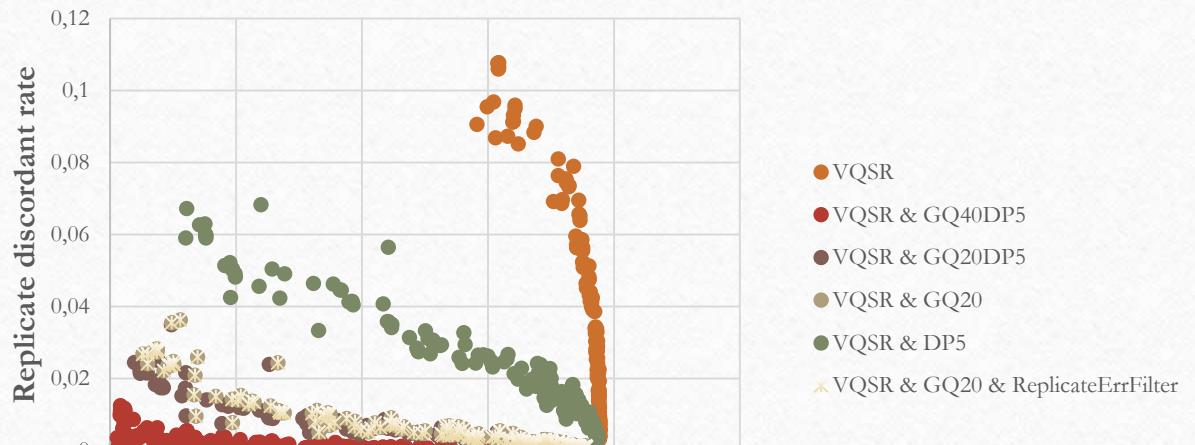
# EC-SNP-calling pipeline



- **BWA mem**
- **Picard**
- **samtools**
- **GATK**

## Optimization of EC-SNP calling

- GQ (genotype quality)
- minDP (minimum read depth)
- ReplicateErrFilter



Filtering strategy	Mean replicate discordance rate	No. of SNPs with <0.5 missing rate
VQSR	4%	204726
VQSR & minDP=5	2.5%	204265
VQSR & minGQ=20	0.95%	194886
VQSR & minDP=5 & minGQ=20	0.85%	192635
VQSR & minDP=5 & minGQ=40	0.28%	106714
VQSR & minGQ=20 & ReplicateErrFilter	0.80%	187496

# Results

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1. Optimization of GBS-SNP-calling
2. Comparison of GBS and EC data

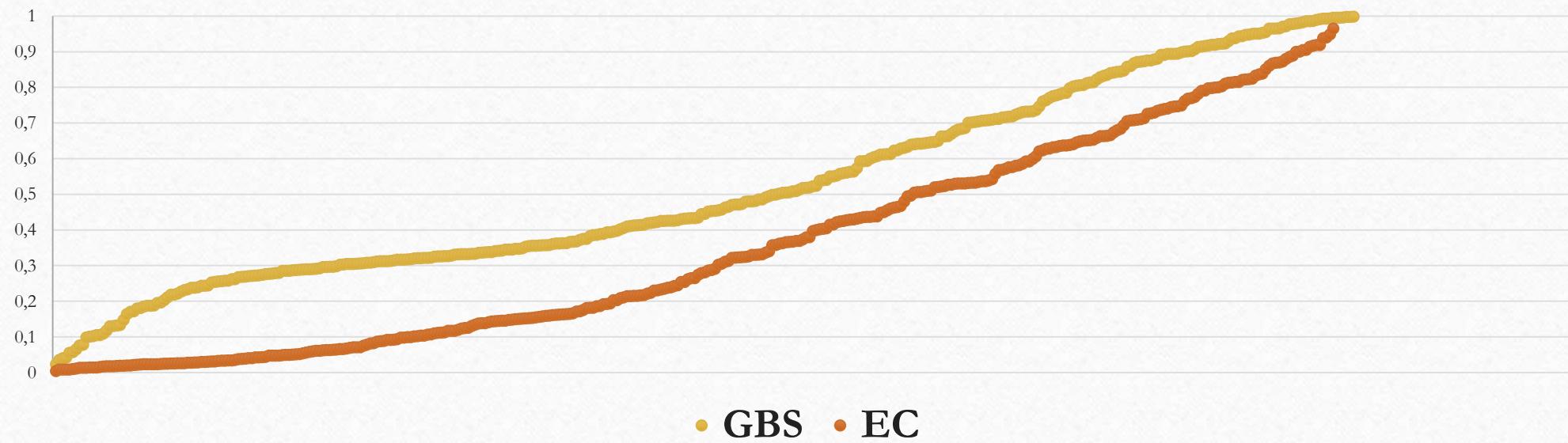
## Comparison of GBS and EC SNP data

# 1 - Mapping results

	GBS (Median ± SD)	EC (Median ± SD)
Total No.	1,879,903 ± 5,104,604 reads	4,897,552 ± 2,597,783 reads
Mapped reads	1,313,744 ± 4,064,215 reads	4,821,132 ± 2,584,842 reads
properly_paired	1,156,043 ± 3,569,836 reads	NA
MQ0 reads	94,272 ± 249,546 reads	504,494 ± 253,716 reads
Mean covered bases (5×)	2,037,017 ± 1,104,354 bp	19,249,840 ± 6,473,049 bp
Mean coverage depth (5×)	×58 ± 72	×16 ± 5

## 2 – Coverage bias

Sample missing rate **GBS > EC**



## 3 - Replicate discordance

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After optimization of SNP-calling

- GBS ~9.2% replicate discordance rate
- EC ~ 8 % replicate discordance rate

## Comparison of GBS and EC SNP data

# 4 - SNP data

### Individual Filter

- Maximum missing rate: 90%

### SNP site Filter

- Minimum calling rate: 50%
- Minimum allele frequency: 0.05

### Genotype Filter

- Minimum read depth: 5
- Minimum genotype quality: 20

	GBS	EC
Sample No.	334	367
SNP No.	19,603	105,209
Overlapped SNP No.		572
Scaffold No.	6,002	21,547
Overlapped scaffold No.		2,279
Mean SNP No. per scaffold	$3 \pm 3$	$5 \pm 4$
SNP density (per 1000bp)	~10	~5
Proportion of missing	27.3%	26.5%

# 5 – SNP annotation

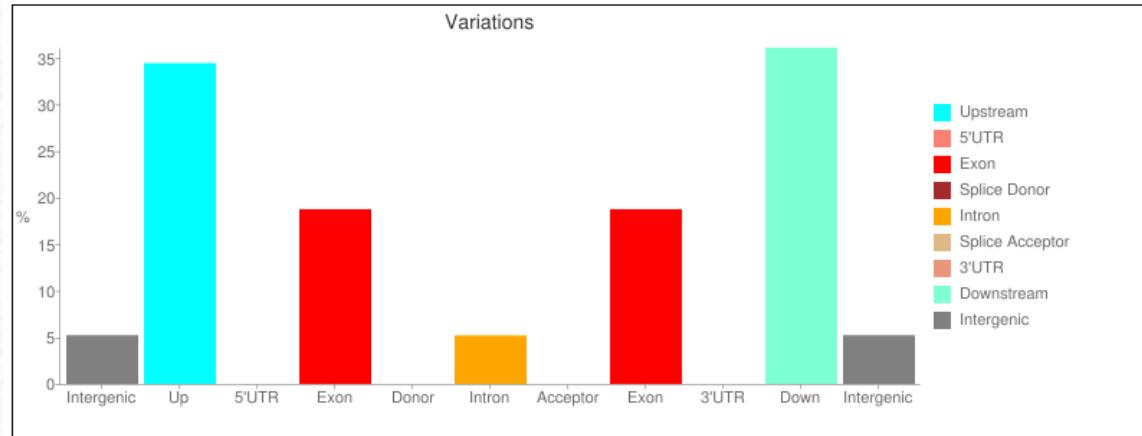
## Comparison of GBS and EC SNP data

GBS

### Number of effects by functional class

Type (alphabetical order)	Count	Percent
MISSENSE	1,683	34.014%
NONSENSE	48	0.97%
SILENT	3,217	65.016%

Missense / Silent ratio: 0.5232

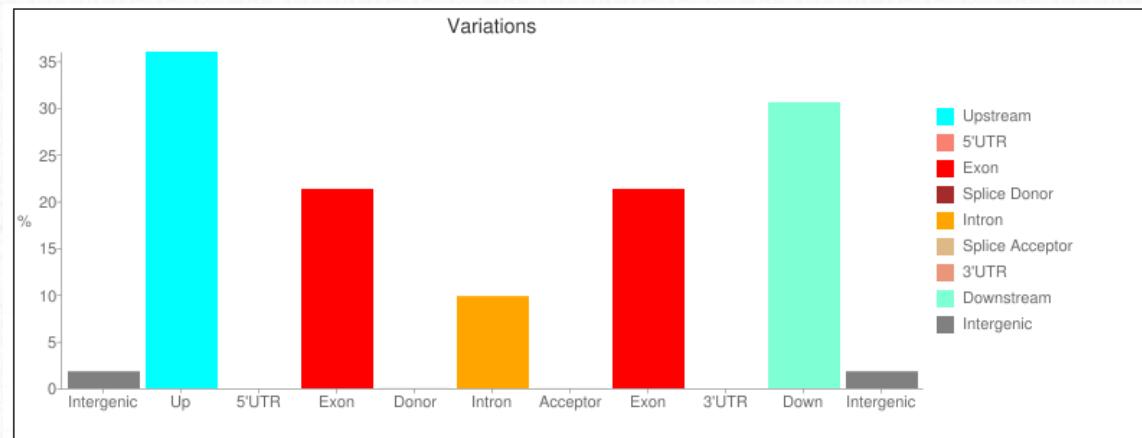


EC

### Number of effects by functional class

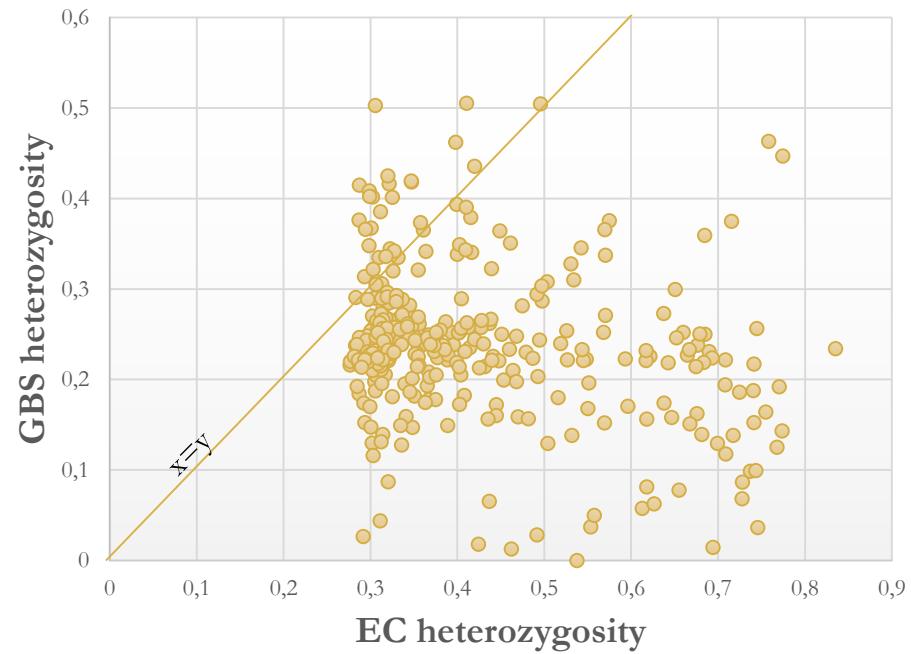
Type (alphabetical order)	Count	Percent
MISSENSE	29,835	60.793%
NONSENSE	968	1.972%
SILENT	18,273	37.234%

Missense / Silent ratio: 1.6327



## Comparison of GBS and EC SNP data

# 6 - Proportion of heterozygou genotypes



**EC > GBS**

**Under-calling of *heterozygous* genotypes  
(false homozygous genotypes):  
~8%**

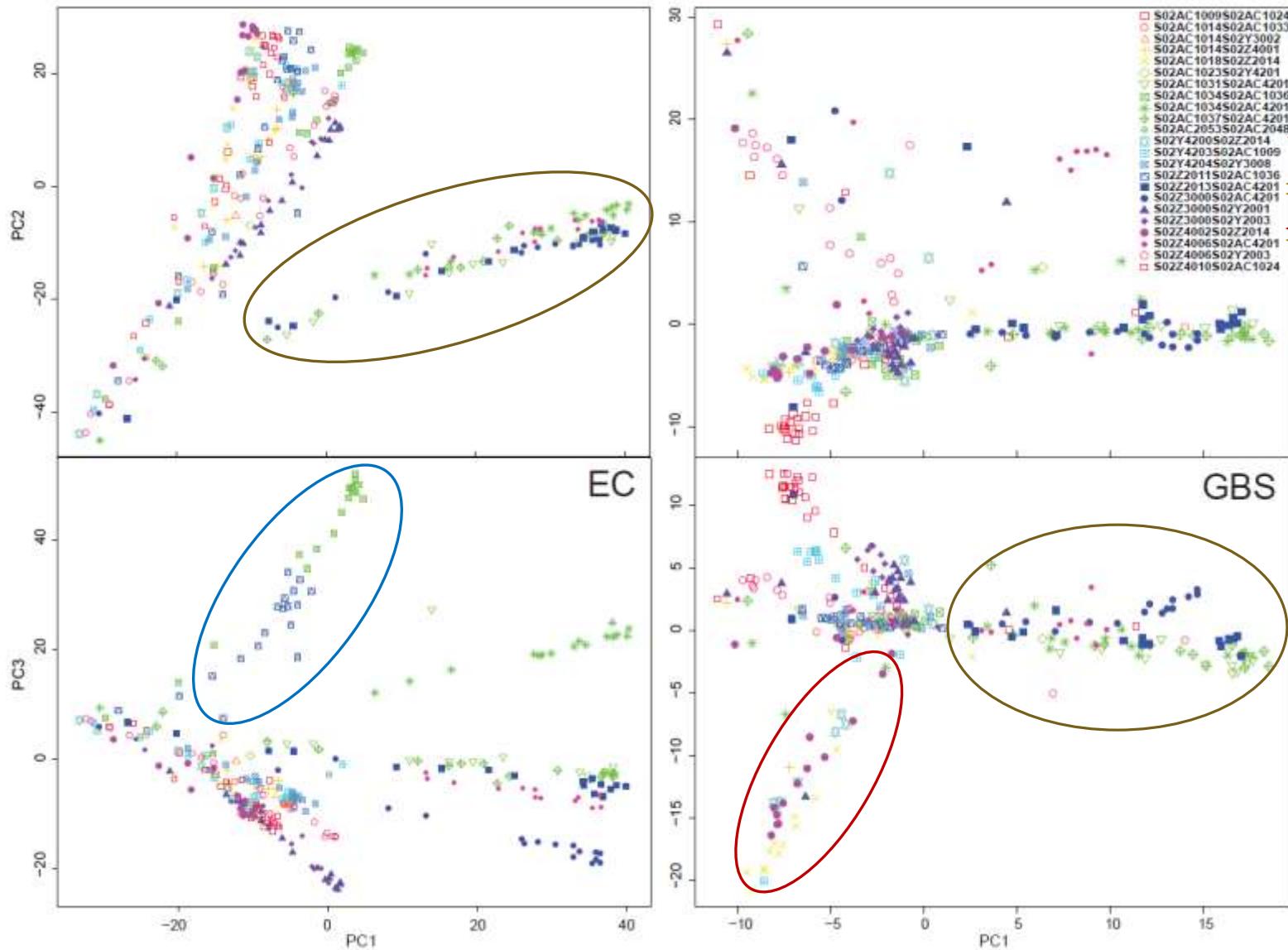
## 7 – Relatedness analyses

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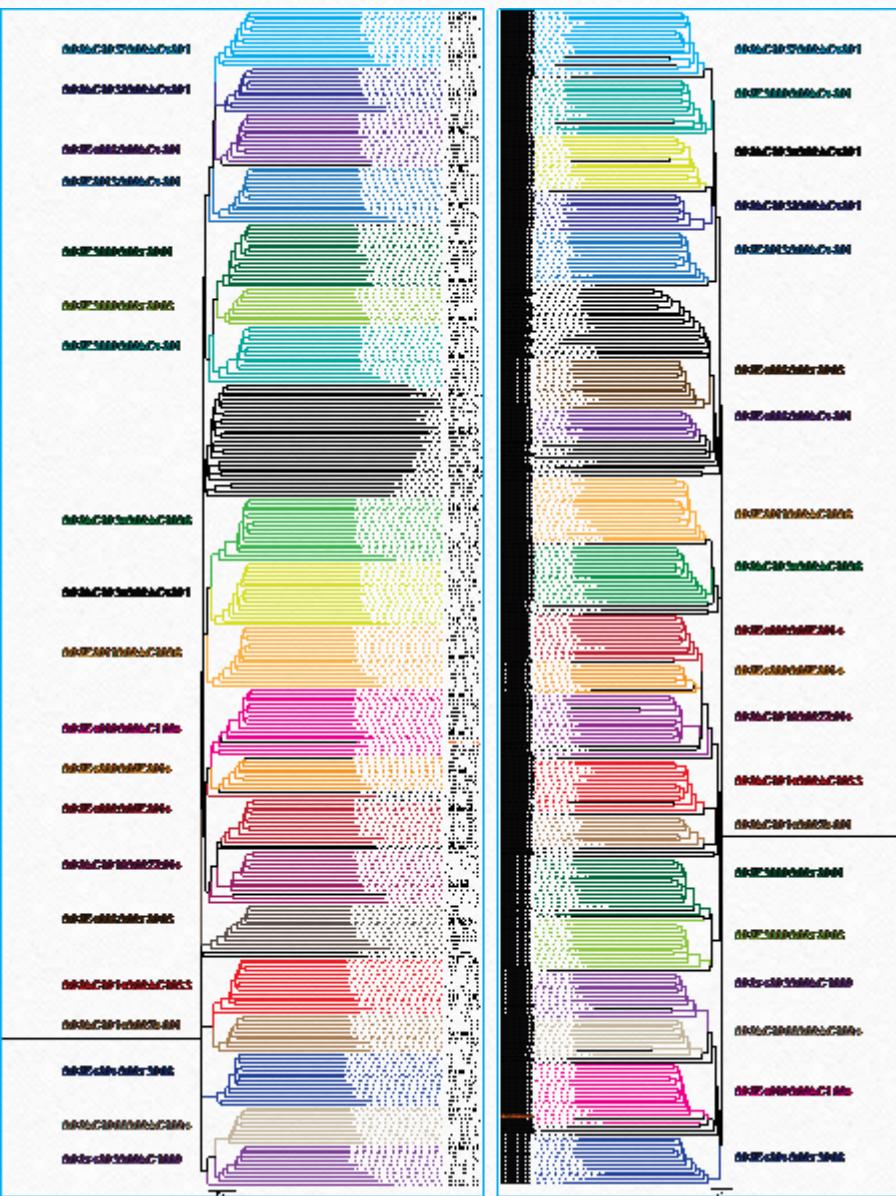
- PCA
- Genetic distance
- Kinship ( under EM and LD kNNi imputation )

## 7 – Relatedness analyses

### PCA analysis



EC-NJtree



GBS-NJtree

Genetic distance

## 7 – Relatedness analyses

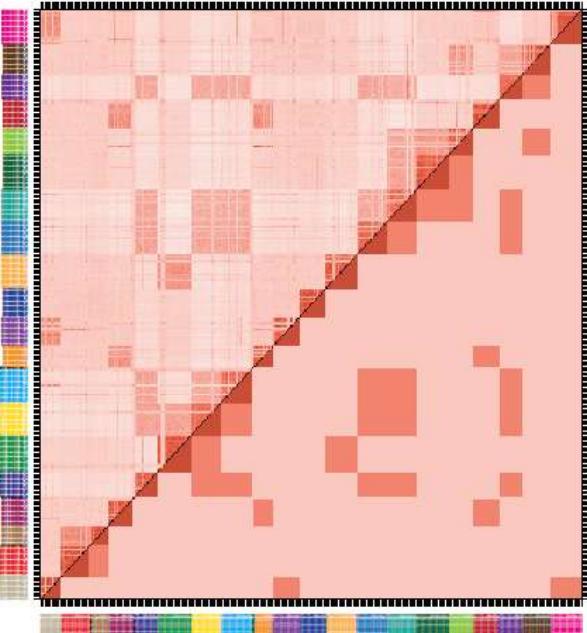
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- PCA
- Genetic distance
- Kinship ( under EM and LD kNNi imputation )

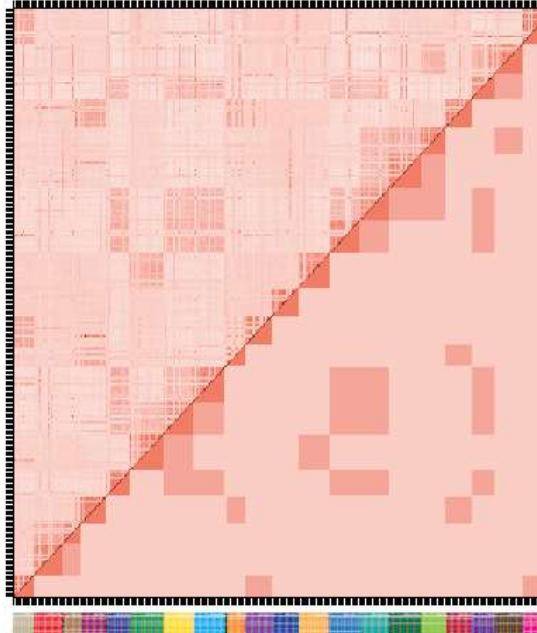
# Kinship

Pedigree & genomic relationship based on EC-SNPs

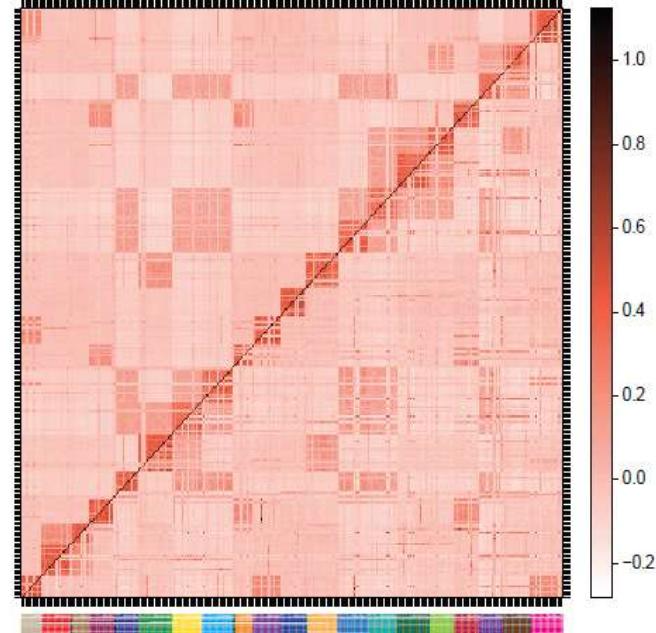
S02Z4010S02AC1024  
S02Z4006S02Y2003  
S02Z4006S02AC4201  
S02Z4002S02Z2014  
S02Z3000S02Y2003  
S02Z3000S02Y2001  
S02Z3000S02AC4201  
S02Z2013S02AC4201  
S02Z2011S02AC1036  
S02Z4204S02Y3008  
S02Y4203S02AC1009  
S02Z4200S02Z2014  
S02AC1037S02AC4201  
S02AC1034S02AC4201  
S02AC1034S02AC1036  
S02AC1031S02AC4201  
S02AC1018S02Z2014  
S02Z4002S02Z2014  
S02AC1014S02AC1033  
S02AC1014S02Z24001  
S02AC1018S02Z2014  
S02AC1031S02AC4201  
S02AC1034S02AC4201  
S02Z4204S02Y3008  
S02Z2011S02AC1036  
S02Z3000S02Y2001  
S02Z4006S02AC4201  
S02Z4010S02AC1024



Pedigree & genomic relationship based on GBS-SNPs



Genomic relationship based on EC- & GBS-SNPs



Imputation method: EM (Endelman and Jannink 2012)

The RMS error < 0.01

7 – Relatedness analyses

# Mean genomic relationship within and between family based on the EC-SNP data

Imputation method: **EM** (Endelman and Jannink 2012) with RMS error < 0.01 & **LD-kNNi** (Money et al., 2015)

SO2AC1009	SO2AC10145	SO2AC10145	SO2AC10185	SO2AC10315	SO2AC10345	SO2AC10345	SO2AC10375	SO2Y420050	SO2Y420350	SO2Z201150	SO2Z201350	SO2Z300050	SO2Z300050	SO2Z400250	SO2Z400650	SO2Z400650	SO2Z401050			
SO2AC1024	02AC1033	0224001	0222014	02AC4201	02AC1036	02AC4201	02AC4201	2Z2014	2AC1009	2Y3008	2AC1036	2AC4201	2Y2001	2Y2003	2Z2014	2AC4201	2Y2003	2AC1024		
0.296 ± 0.173	-0.015 ± 0.020	-0.015 ± 0.018	-0.008 ± 0.029	-0.068 ± 0.026	-0.029 ± 0.025	-0.079 ± 0.028	-0.039 ± 0.054	-0.005 ± 0.025	0.110 ± 0.018	-0.010 ± 0.019	-0.011 ± 0.025	-0.049 ± 0.045	-0.064 ± 0.044	-0.030 ± 0.015	-0.025 ± 0.020	-0.012 ± 0.025	-0.075 ± 0.020	-0.017 ± 0.025	0.135 ± 0.089	SO2AC1009 EM
0.288 ± 0.168	-0.013 ± 0.013	-0.012 ± 0.009	-0.004 ± 0.024	-0.069 ± 0.027	-0.030 ± 0.019	-0.082 ± 0.030	-0.040 ± 0.061	-0.003 ± 0.012	0.113 ± 0.017	-0.006 ± 0.012	-0.011 ± 0.014	-0.050 ± 0.045	-0.070 ± 0.038	-0.030 ± 0.013	-0.022 ± 0.017	-0.011 ± 0.013	-0.076 ± 0.025	-0.012 ± 0.013	0.142 ± 0.086	SO2AC1024 LD kNNi
0.186 ± 0.053	0.188 ± 0.057	-0.022 ± 0.017	-0.051 ± 0.017	-0.027 ± 0.018	-0.063 ± 0.025	-0.042 ± 0.040	-0.015 ± 0.022	0.010 ± 0.053	-0.002 ± 0.013	-0.018 ± 0.019	-0.045 ± 0.029	-0.065 ± 0.022	-0.024 ± 0.013	-0.019 ± 0.014	-0.013 ± 0.022	-0.058 ± 0.014	-0.008 ± 0.017	-0.019 ± 0.019	SO2AC1014	
0.187 ± 0.053	0.180 ± 0.068	-0.017 ± 0.010	-0.050 ± 0.020	-0.027 ± 0.017	-0.065 ± 0.027	-0.041 ± 0.036	-0.012 ± 0.014	0.013 ± 0.049	0.001 ± 0.009	-0.016 ± 0.014	-0.045 ± 0.030	-0.065 ± 0.027	-0.022 ± 0.010	-0.016 ± 0.012	-0.012 ± 0.016	-0.058 ± 0.019	-0.003 ± 0.008	-0.018 ± 0.017	SO2AC1033	
0.094 ± 0.003	-0.019 ± 0.013	-0.057 ± 0.010	-0.026 ± 0.014	-0.069 ± 0.016	-0.046 ± 0.025	-0.012 ± 0.010	-0.005 ± 0.016	0.003 ± 0.010	-0.006 ± 0.015	-0.046 ± 0.013	-0.065 ± 0.017	-0.010 ± 0.011	-0.010 ± 0.011	-0.008 ± 0.011	-0.009 ± 0.012	-0.014 ± 0.010	SO2AC1014			
0.015 ± 0.011	-0.055 ± 0.014	-0.026 ± 0.016	-0.070 ± 0.021	-0.044 ± 0.027	-0.009 ± 0.009	-0.004 ± 0.013	-0.006 ± 0.008	0.004 ± 0.017	-0.045 ± 0.018	-0.065 ± 0.022	-0.009 ± 0.007	0.008 ± 0.011	-0.008 ± 0.011	-0.067 ± 0.013	-0.002 ± 0.008	-0.013 ± 0.009	SO224001			
0.301 ± 0.143	-0.062 ± 0.021	-0.030 ± 0.021	-0.085 ± 0.036	-0.052 ± 0.029	0.118 ± 0.075	-0.003 ± 0.028	-0.010 ± 0.016	-0.020 ± 0.023	-0.055 ± 0.012	-0.077 ± 0.034	-0.036 ± 0.020	-0.020 ± 0.064	0.147 ± 0.073	-0.062 ± 0.025	-0.009 ± 0.031	0.015 ± 0.060	SO2AC1018			
0.297 ± 0.138	-0.064 ± 0.020	-0.033 ± 0.019	-0.074 ± 0.033	-0.053 ± 0.028	0.139 ± 0.083	-0.002 ± 0.013	-0.006 ± 0.010	-0.020 ± 0.016	-0.059 ± 0.010	-0.082 ± 0.034	-0.036 ± 0.016	-0.015 ± 0.064	0.159 ± 0.072	-0.067 ± 0.025	-0.008 ± 0.020	0.015 ± 0.055	SO222014			
0.068 ± 0.004	-0.053 ± 0.062	0.114 ± 0.037	0.097 ± 0.060	-0.053 ± 0.023	-0.055 ± 0.019	-0.051 ± 0.012	-0.050 ± 0.036	0.103 ± 0.058	0.088 ± 0.046	-0.072 ± 0.012	-0.067 ± 0.024	-0.071 ± 0.017	0.105 ± 0.040	-0.064 ± 0.025	-0.066 ± 0.016	SO2AC1031				
0.047 ± 0.064	0.112 ± 0.042	0.097 ± 0.067	-0.049 ± 0.035	-0.051 ± 0.023	-0.049 ± 0.015	-0.050 ± 0.036	0.109 ± 0.060	0.085 ± 0.046	-0.067 ± 0.017	-0.058 ± 0.028	-0.069 ± 0.021	0.105 ± 0.041	-0.053 ± 0.031	-0.066 ± 0.021	SO2AC4201					
0.068 ± 0.004	0.105 ± 0.057	-0.055 ± 0.013	-0.025 ± 0.020	-0.015 ± 0.022	-0.024 ± 0.015	0.135 ± 0.075	-0.069 ± 0.015	-0.090 ± 0.033	-0.040 ± 0.019	-0.037 ± 0.020	-0.030 ± 0.022	-0.067 ± 0.022	-0.028 ± 0.025	-0.022 ± 0.023	SO2AC1034					
0.068 ± 0.004	0.102 ± 0.060	-0.052 ± 0.014	-0.024 ± 0.017	-0.015 ± 0.017	-0.023 ± 0.016	0.138 ± 0.080	-0.067 ± 0.015	-0.088 ± 0.039	-0.042 ± 0.023	-0.037 ± 0.024	-0.031 ± 0.018	-0.085 ± 0.030	-0.026 ± 0.021	-0.024 ± 0.019	SO2AC1036					
0.076 ± 0.059	-0.069 ± 0.027	-0.065 ± 0.014	-0.066 ± 0.017	-0.068 ± 0.019	0.084 ± 0.051	0.069 ± 0.044	-0.084 ± 0.017	-0.088 ± 0.030	-0.079 ± 0.026	0.091 ± 0.032	-0.073 ± 0.031	0.073 ± 0.031	-0.073 ± 0.023	SO2AC1034						
0.076 ± 0.061	-0.067 ± 0.034	-0.063 ± 0.027	-0.065 ± 0.020	-0.063 ± 0.030	0.085 ± 0.054	0.070 ± 0.040	-0.084 ± 0.022	-0.077 ± 0.037	-0.081 ± 0.027	0.093 ± 0.033	-0.066 ± 0.039	-0.076 ± 0.026	SO2AC4201							
-0.047 ± 0.034	-0.036 ± 0.035	-0.046 ± 0.024	-0.041 ± 0.036	0.079 ± 0.062	0.065 ± 0.061	-0.060 ± 0.022	-0.058 ± 0.028	-0.056 ± 0.030	0.084 ± 0.059	-0.050 ± 0.036	-0.044 ± 0.056	-0.050 ± 0.036	-0.041 ± 0.057	SO2AC1037						
-0.045 ± 0.032	-0.034 ± 0.031	-0.044 ± 0.025	-0.043 ± 0.032	0.078 ± 0.065	0.065 ± 0.059	-0.056 ± 0.024	-0.051 ± 0.030	-0.055 ± 0.028	0.086 ± 0.059	-0.042 ± 0.035	-0.041 ± 0.057	SO2AC4201								
0.017 ± 0.049	-0.004 ± 0.016	-0.011 ± 0.020	-0.054 ± 0.035	-0.072 ± 0.037	-0.025 ± 0.023	-0.005 ± 0.098	0.143 ± 0.078	-0.061 ± 0.029	-0.007 ± 0.030	0.006 ± 0.034	SO2Y42005									
0.015 ± 0.042	-0.001 ± 0.008	-0.011 ± 0.010	-0.051 ± 0.034	-0.070 ± 0.041	-0.025 ± 0.020	-0.001 ± 0.094	0.139 ± 0.086	-0.059 ± 0.031	-0.008 ± 0.016	0.004 ± 0.023	0.022014									
0.002 ± 0.017	0.001 ± 0.024	-0.039 ± 0.042	-0.054 ± 0.037	-0.018 ± 0.018	-0.012 ± 0.027	-0.005 ± 0.025	-0.056 ± 0.018	SO2Y42035												
0.004 ± 0.010	0.000 ± 0.015	-0.036 ± 0.041	-0.054 ± 0.032	-0.010 ± 0.024	-0.004 ± 0.014	-0.054 ± 0.022	0.001 ± 0.012	0.014 ± 0.023												
0.010 ± 0.016	-0.042 ± 0.022	-0.061 ± 0.020	-0.000 ± 0.021	-0.008 ± 0.016	-0.003 ± 0.022	-0.055 ± 0.011	-0.001 ± 0.018	0.005 ± 0.015	SO2Y42045											
-0.008 ± 0.011	-0.042 ± 0.022	-0.060 ± 0.023	-0.000 ± 0.016	-0.005 ± 0.012	0.004 ± 0.015	-0.055 ± 0.013	0.002 ± 0.009	0.007 ± 0.010	02Y3008											
-0.050 ± 0.034	-0.074 ± 0.033	-0.034 ± 0.015	-0.027 ± 0.021	-0.015 ± 0.022	-0.068 ± 0.026	-0.015 ± 0.024	-0.018 ± 0.019	SO2Z20115												
-0.052 ± 0.030	-0.076 ± 0.034	-0.034 ± 0.013	-0.026 ± 0.017	-0.015 ± 0.015	-0.071 ± 0.030	-0.015 ± 0.015	-0.018 ± 0.014	02AC1036												
0.072 ± 0.056	-0.065 ± 0.020	-0.058 ± 0.026	-0.059 ± 0.031	-0.077 ± 0.053	-0.065 ± 0.020	-0.059 ± 0.031	0.097 ± 0.053	-0.044 ± 0.031	-0.054 ± 0.029	SO2Z20135										
0.073 ± 0.055	-0.062 ± 0.023	-0.053 ± 0.029	-0.059 ± 0.029	-0.071 ± 0.054	-0.062 ± 0.023	-0.059 ± 0.031	0.101 ± 0.054	-0.040 ± 0.029	-0.055 ± 0.031	02AC4201										
0.082 ± 0.055	0.082 ± 0.063	0.065 ± 0.077	-0.082 ± 0.037	0.086 ± 0.060	-0.082 ± 0.044	-0.056 ± 0.044	-0.070 ± 0.029	SO2Z30005												
0.097 ± 0.055	0.062 ± 0.072	-0.083 ± 0.038	-0.089 ± 0.038	0.084 ± 0.045	-0.050 ± 0.041	-0.072 ± 0.029	SO2AC4201													
0.124 ± 0.072	-0.022 ± 0.044	-0.086 ± 0.009	-0.022 ± 0.014	-0.025 ± 0.013	-0.013 ± 0.013	-0.013 ± 0.013	-0.021 ± 0.013	SO2Z30005												
0.121 ± 0.077	-0.025 ± 0.042	-0.084 ± 0.013	-0.023 ± 0.013	-0.027 ± 0.013	-0.013 ± 0.013	-0.013 ± 0.013	-0.024 ± 0.010	02Y2001												
0.015 ± 0.053	-0.075 ± 0.022	-0.095 ± 0.022	-0.024 ± 0.013	-0.013 ± 0.013	-0.013 ± 0.013	-0.016 ± 0.018	SO2Z30005													
-0.013 ± 0.056	-0.064 ± 0.027	0.083 ± 0.078	-0.013 ± 0.014	-0.013 ± 0.014	-0.013 ± 0.014	-0.015 ± 0.014	-0.015 ± 0.014	02Y2003												
0.284 ± 0.148	-0.071 ± 0.019	-0.012 ± 0.026	-0.017 ± 0.017	0.011 ± 0.034	SO2Z40025															
0.284 ± 0.143	-0.074 ± 0.022	-0.013 ± 0.017	-0.017 ± 0.017	0.011 ± 0.034	0222014															
0.093 ± 0.049	-0.071 ± 0.017	-0.013 ± 0.017	-0.017 ± 0.017	0.011 ± 0.034	SO2Z40065															
0.094 ± 0.053	-0.074 ± 0.018	-0.013 ± 0.018	-0.017 ± 0.018	0.011 ± 0.034	02AC4201															
0.079 ± 0.049	-0.076 ± 0.019	-0.013 ± 0.019	-0.017 ± 0.019	0.011 ± 0.034	SO2Z40065															
0.079 ± 0.048	-0.077 ± 0.019	-0.013 ± 0.019	-0.017 ± 0.019	0.011 ± 0.034	02Y2003															
0.273 ± 0.386	SO2Z40105																			
0.273 ± 0.362	02AC1024																			

# Mean genomic relationship within and between family based on the EC- and GBS-SNP data

Imputation method: EM (Endelman and Jannink 2012) with RMS error < 0.01

502AC10095 02AC1024	502AC101450 2AC1033	502AC10145 0224001	502AC101850 ZZ2014	502AC10315 02AC4201	502AC10345 02AC1036	502AC10345 02AC4201	502AC10375 ZZ2014	502Y420050 ZAC1009	502Y420350 ZY3008	502Y420450 ZAC1036	5022201150 ZAC4201	5022201350 ZAC4201	5022300050 ZY2001	5022300050 ZY2003	5022400250 ZAC4201	5022400650 ZY2003	50224010502 AC1024	
0.296 ± 0.177 -0.015 ± 0.020	-0.015 ± 0.010 -0.020 ± 0.045	-0.015 ± 0.010 -0.013 ± 0.057	-0.008 ± 0.029 -0.029 ± 0.019	-0.068 ± 0.026 -0.047 ± 0.075	-0.028 ± 0.023 -0.012 ± 0.057	-0.039 ± 0.064 -0.060 ± 0.061	-0.005 ± 0.025 -0.007 ± 0.088	0.139 ± 0.098 0.124 ± 0.108	-0.010 ± 0.019 -0.010 ± 0.033	-0.011 ± 0.025 -0.028 ± 0.123	-0.049 ± 0.045 -0.066 ± 0.063	-0.064 ± 0.044 -0.038 ± 0.052	-0.030 ± 0.018 -0.040 ± 0.025	-0.025 ± 0.022 -0.019 ± 0.095	-0.012 ± 0.025 -0.043 ± 0.102	-0.017 ± 0.025 -0.033 ± 0.121	0.135 ± 0.089 0.119 ± 0.121	02AC1009 EC 02AC1024 GBS
0.257 ± 0.212 0.136 ± 0.136	-0.020 ± 0.045 0.183 ± 0.057	-0.013 ± 0.057 0.022 ± 0.017	-0.013 ± 0.043 -0.027 ± 0.018	-0.029 ± 0.052 -0.018 ± 0.012	-0.027 ± 0.076 -0.018 ± 0.091	-0.012 ± 0.075 -0.012 ± 0.022	-0.015 ± 0.025 -0.007 ± 0.083	0.010 ± 0.053 0.012 ± 0.013	-0.013 ± 0.013 -0.018 ± 0.019	-0.045 ± 0.029 -0.045 ± 0.022	-0.065 ± 0.022 -0.043 ± 0.068	-0.024 ± 0.013 -0.021 ± 0.061	-0.019 ± 0.014 -0.013 ± 0.022	-0.058 ± 0.014 -0.051 ± 0.022	-0.008 ± 0.017 -0.019 ± 0.019	-0.019 ± 0.017 -0.019 ± 0.019	0.019 ± 0.019 0.019 ± 0.019	02AC1014
0.268 ± 0.204 0.194 ± 0.059	-0.019 ± 0.133 -0.019 ± 0.013	-0.013 ± 0.043 -0.057 ± 0.010	-0.026 ± 0.014 -0.026 ± 0.014	-0.069 ± 0.016 -0.046 ± 0.026	-0.012 ± 0.010 -0.005 ± 0.016	-0.005 ± 0.026 -0.003 ± 0.058	-0.005 ± 0.030 -0.000 ± 0.000	0.003 ± 0.043 0.003 ± 0.034	-0.003 ± 0.016 -0.003 ± 0.010	-0.046 ± 0.013 -0.055 ± 0.017	-0.010 ± 0.011 -0.062 ± 0.068	-0.004 ± 0.075 -0.008 ± 0.075	-0.008 ± 0.030 -0.023 ± 0.109	-0.028 ± 0.139 -0.010 ± 0.101	-0.016 ± 0.071 -0.016 ± 0.071	-0.016 ± 0.058 -0.028 ± 0.046	0.019 ± 0.019 0.019 ± 0.019	02AC1033
0.214 ± 0.150 0.301 ± 0.141	0.007 ± 0.067 -0.062 ± 0.021	-0.049 ± 0.049 -0.030 ± 0.021	-0.007 ± 0.036 -0.035 ± 0.021	-0.047 ± 0.075 -0.065 ± 0.036	-0.057 ± 0.067 -0.052 ± 0.029	-0.003 ± 0.058 0.138 ± 0.075	-0.005 ± 0.030 -0.003 ± 0.028	0.010 ± 0.015 -0.010 ± 0.023	-0.020 ± 0.023 -0.055 ± 0.032	-0.077 ± 0.034 -0.060 ± 0.048	-0.016 ± 0.034 -0.075 ± 0.043	-0.020 ± 0.072 -0.020 ± 0.072	-0.036 ± 0.025 -0.147 ± 0.073	-0.062 ± 0.023 -0.020 ± 0.023	-0.009 ± 0.011 -0.015 ± 0.060	0.015 ± 0.023 0.015 ± 0.023	02AC1014	
0.377 ± 0.179 0.217 ± 0.222	-0.050 ± 0.030 -0.037 ± 0.026	-0.049 ± 0.049 -0.043 ± 0.034	-0.027 ± 0.078 -0.065 ± 0.105	-0.047 ± 0.062 -0.086 ± 0.105	-0.055 ± 0.067 -0.078 ± 0.044	-0.003 ± 0.058 -0.042 ± 0.044	-0.053 ± 0.023 -0.034 ± 0.048	-0.043 ± 0.036 -0.081 ± 0.035	-0.058 ± 0.058 -0.088 ± 0.046	-0.050 ± 0.036 -0.072 ± 0.012	-0.015 ± 0.017 -0.067 ± 0.024	-0.010 ± 0.011 -0.071 ± 0.011	-0.030 ± 0.013 -0.035 ± 0.040	-0.064 ± 0.025 -0.064 ± 0.016	-0.066 ± 0.016 -0.066 ± 0.016	0.016 ± 0.016 0.016 ± 0.016	02AC4201	
0.394 ± 0.167 0.216 ± 0.175	0.109 ± 0.057 0.065 ± 0.075	-0.055 ± 0.033 -0.051 ± 0.042	-0.025 ± 0.020 -0.021 ± 0.030	-0.016 ± 0.022 -0.013 ± 0.030	-0.024 ± 0.024 -0.010 ± 0.025	-0.024 ± 0.135 0.110 ± 0.078	-0.035 ± 0.075 -0.069 ± 0.025	-0.050 ± 0.050 -0.050 ± 0.030	-0.069 ± 0.030 -0.040 ± 0.019	-0.050 ± 0.037 -0.040 ± 0.019	-0.015 ± 0.017 -0.037 ± 0.020	-0.010 ± 0.011 -0.030 ± 0.023	-0.028 ± 0.023 -0.027 ± 0.023	-0.028 ± 0.023 -0.027 ± 0.023	-0.022 ± 0.023 -0.022 ± 0.023	0.012 ± 0.043 0.012 ± 0.043	02AC1034	
0.328 ± 0.191 0.371 ± 0.189	0.076 ± 0.059 0.059 ± 0.100	-0.069 ± 0.027 -0.011 ± 0.057	-0.065 ± 0.024 -0.056 ± 0.050	-0.066 ± 0.017 -0.037 ± 0.038	-0.068 ± 0.029 -0.048 ± 0.053	-0.029 ± 0.051 0.061 ± 0.096	-0.084 ± 0.029 -0.089 ± 0.044	-0.084 ± 0.021 0.069 ± 0.044	-0.084 ± 0.016 -0.089 ± 0.020	-0.079 ± 0.020 -0.070 ± 0.041	-0.055 ± 0.061 -0.055 ± 0.061	-0.040 ± 0.071 -0.049 ± 0.091	-0.044 ± 0.092 -0.044 ± 0.092	-0.058 ± 0.073 -0.058 ± 0.073	0.02AC4201			
0.199 ± 0.144 0.236 ± 0.199	-0.047 ± 0.034 -0.035 ± 0.055	-0.016 ± 0.035 -0.051 ± 0.028	-0.046 ± 0.024 -0.044 ± 0.040	-0.041 ± 0.036 -0.052 ± 0.030	-0.075 ± 0.062 -0.043 ± 0.036	-0.065 ± 0.061 -0.068 ± 0.091	-0.060 ± 0.022 -0.062 ± 0.067	-0.058 ± 0.028 -0.069 ± 0.032	-0.056 ± 0.045 -0.060 ± 0.045	-0.055 ± 0.040 -0.057 ± 0.126	-0.054 ± 0.036 -0.066 ± 0.099	-0.041 ± 0.057 -0.037 ± 0.027	-0.050 ± 0.036 -0.050 ± 0.036	-0.041 ± 0.057 -0.041 ± 0.057	0.02AC1037 0.02AC4201			
0.225 ± 0.165 0.141 ± 0.209	0.017 ± 0.049 0.032 ± 0.082	-0.004 ± 0.016 -0.008 ± 0.031	-0.011 ± 0.020 -0.015 ± 0.020	-0.005 ± 0.035 -0.017 ± 0.060	-0.072 ± 0.037 -0.052 ± 0.050	-0.037 ± 0.037 -0.023 ± 0.033	-0.005 ± 0.037 -0.033 ± 0.033	-0.005 ± 0.036 -0.033 ± 0.024	-0.045 ± 0.037 -0.052 ± 0.032	-0.072 ± 0.037 -0.060 ± 0.045	-0.067 ± 0.027 -0.057 ± 0.126	-0.067 ± 0.027 -0.066 ± 0.027	-0.067 ± 0.030 -0.067 ± 0.030	-0.066 ± 0.030 -0.066 ± 0.030	0.02AC1037 0.02AC4201			
0.195 ± 0.174 0.234 ± 0.231	0.082 ± 0.037 0.004 ± 0.023	0.001 ± 0.024 -0.001 ± 0.024	-0.001 ± 0.024 -0.037 ± 0.054	-0.039 ± 0.041 -0.074 ± 0.033	-0.054 ± 0.037 -0.074 ± 0.033	-0.016 ± 0.015 -0.027 ± 0.029	-0.022 ± 0.029 -0.022 ± 0.029	-0.028 ± 0.019 -0.038 ± 0.019	-0.012 ± 0.026 -0.027 ± 0.026	-0.046 ± 0.071 -0.046 ± 0.071	-0.032 ± 0.021 -0.032 ± 0.021	-0.007 ± 0.028 -0.007 ± 0.028	-0.007 ± 0.028 -0.007 ± 0.028	0.02AC1009 0.02AC1036				
0.164 ± 0.130 0.240 ± 0.211	-0.010 ± 0.018 -0.005 ± 0.021	-0.043 ± 0.022 -0.087 ± 0.032	-0.061 ± 0.029 -0.048 ± 0.050	-0.000 ± 0.020 -0.007 ± 0.066	-0.084 ± 0.018 0.000 ± 0.034	-0.003 ± 0.022 0.001 ± 0.028	-0.088 ± 0.022 -0.041 ± 0.055	-0.003 ± 0.022 -0.008 ± 0.051	-0.011 ± 0.011 -0.012 ± 0.056	-0.001 ± 0.016 -0.008 ± 0.029	-0.001 ± 0.011 -0.008 ± 0.029	-0.001 ± 0.011 -0.008 ± 0.029	-0.005 ± 0.011 -0.005 ± 0.011	0.02AC4205 0.02Y3008				
0.295 ± 0.144 0.257 ± 0.142	-0.050 ± 0.054 -0.048 ± 0.028	-0.074 ± 0.054 -0.061 ± 0.038	-0.062 ± 0.022 -0.022 ± 0.027	-0.029 ± 0.024 -0.029 ± 0.024	-0.012 ± 0.019 -0.012 ± 0.019	-0.039 ± 0.018 -0.039 ± 0.018	-0.039 ± 0.018 -0.039 ± 0.018	-0.055 ± 0.011 -0.055 ± 0.011	-0.001 ± 0.016 -0.001 ± 0.016	-0.005 ± 0.011 -0.005 ± 0.011	-0.001 ± 0.016 -0.001 ± 0.016	-0.001 ± 0.016 -0.001 ± 0.016	-0.005 ± 0.011 -0.005 ± 0.011	0.02AC1036 0.02AC1035				
0.236 ± 0.134 0.221 ± 0.191	0.072 ± 0.056 0.063 ± 0.084	-0.072 ± 0.056 -0.058 ± 0.060	-0.065 ± 0.030 -0.062 ± 0.042	-0.058 ± 0.030 -0.058 ± 0.030	-0.059 ± 0.030 -0.059 ± 0.030	-0.042 ± 0.021 -0.042 ± 0.021	-0.015 ± 0.022 -0.015 ± 0.022	-0.068 ± 0.026 -0.068 ± 0.026	-0.016 ± 0.024 -0.016 ± 0.024	-0.018 ± 0.019 -0.018 ± 0.019	-0.023 ± 0.040 -0.023 ± 0.040	-0.015 ± 0.027 -0.015 ± 0.027	-0.015 ± 0.027 -0.015 ± 0.027	0.02AC1036 0.02AC1035				
0.217 ± 0.129 0.187 ± 0.156	0.092 ± 0.063 0.058 ± 0.089	0.065 ± 0.049 0.070 ± 0.067	-0.072 ± 0.057 -0.075 ± 0.057	-0.082 ± 0.037 -0.082 ± 0.037	-0.086 ± 0.049 0.070 ± 0.067	-0.086 ± 0.049 -0.075 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.056 ± 0.018 -0.060 ± 0.018	-0.000 ± 0.018 -0.000 ± 0.018	-0.000 ± 0.018 -0.000 ± 0.018	-0.000 ± 0.018 -0.000 ± 0.018	0.02AC1009 0.02AC4201				
0.351 ± 0.082 0.274 ± 0.196	0.124 ± 0.072 0.111 ± 0.095	-0.022 ± 0.044 -0.021 ± 0.069	-0.088 ± 0.044 -0.043 ± 0.118	-0.086 ± 0.044 -0.082 ± 0.057	-0.086 ± 0.044 -0.086 ± 0.044	-0.077 ± 0.037 -0.077 ± 0.037	-0.086 ± 0.044 -0.086 ± 0.044	-0.086 ± 0.044 -0.086 ± 0.044	-0.072 ± 0.014 -0.072 ± 0.014	-0.023 ± 0.013 -0.023 ± 0.013	0.02AC1009 0.02Y2001							
0.206 ± 0.167 0.249 ± 0.166	0.015 ± 0.055 0.058 ± 0.089	-0.075 ± 0.022 -0.075 ± 0.027	-0.075 ± 0.022 -0.071 ± 0.057	-0.115 ± 0.066 0.115 ± 0.066	-0.023 ± 0.020 -0.023 ± 0.020	-0.046 ± 0.071 -0.046 ± 0.071	-0.020 ± 0.057 -0.020 ± 0.057	-0.057 ± 0.086 -0.057 ± 0.086	-0.057 ± 0.086 -0.057 ± 0.086	-0.057 ± 0.086 -0.057 ± 0.086	0.02AC1009 0.02AC4201							
0.284 ± 0.148 0.255 ± 0.161	-0.071 ± 0.019 0.048 ± 0.077	-0.012 ± 0.026 -0.017 ± 0.070	-0.012 ± 0.026 -0.012 ± 0.041	0.02AC1009 0.02AC4201														
0.321 ± 0.038 0.181 ± 0.170	0.093 ± 0.049 0.099 ± 0.110	-0.065 ± 0.037 -0.048 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.077 ± 0.049 -0.077 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.086 ± 0.049 -0.086 ± 0.049	-0.056 ± 0.044 -0.056 ± 0.044	-0.044 ± 0.044 -0.044 ± 0.044	-0.044 ± 0.044 -0.044 ± 0.044	-0.044 ± 0.044 -0.044 ± 0.044	0.02AC1009 0.02AC4201				
0.236 ± 0.146 0.261 ± 0.148	-0.020 ± 0.098 0.020 ± 0.098	-0.000 ± 0.026 -0.020 ± 0.026	0.02AC1009 0.02AC4201															
0.273 ± 0.166 0.278 ± 0.256	0.037 ± 0.055 0.027 ± 0.055	-0.075 ± 0.027 -0.075 ± 0.027	-0.075 ± 0.027 -0.075 ± 0.027	-0.115 ± 0.066 0.115 ± 0.066	-0.023 ± 0.020 -0.023 ± 0.020	-0.046 ± 0.071 -0.046 ± 0.071												

# GBS Summary & Next work

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- Coverage bias
  - ~ 8% underestimation of herterozygous genotypes
  - Relatively high proportion of genetic variances in non-coding regions
  - The number of covered bases is less than predicted
  - Obvious difference between GBS- and EC- G matrixes
- 
- ✓ Develop predictive model and evaluate predictive ability
  - ✓ Estimate the impact of error genotypes in Genomic prediction
  - ✓ ... ...

# Acknowledgement

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**Forest Genetic Group in UPSC**



*Boosting business with science*



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**Thanks for your attention!**

