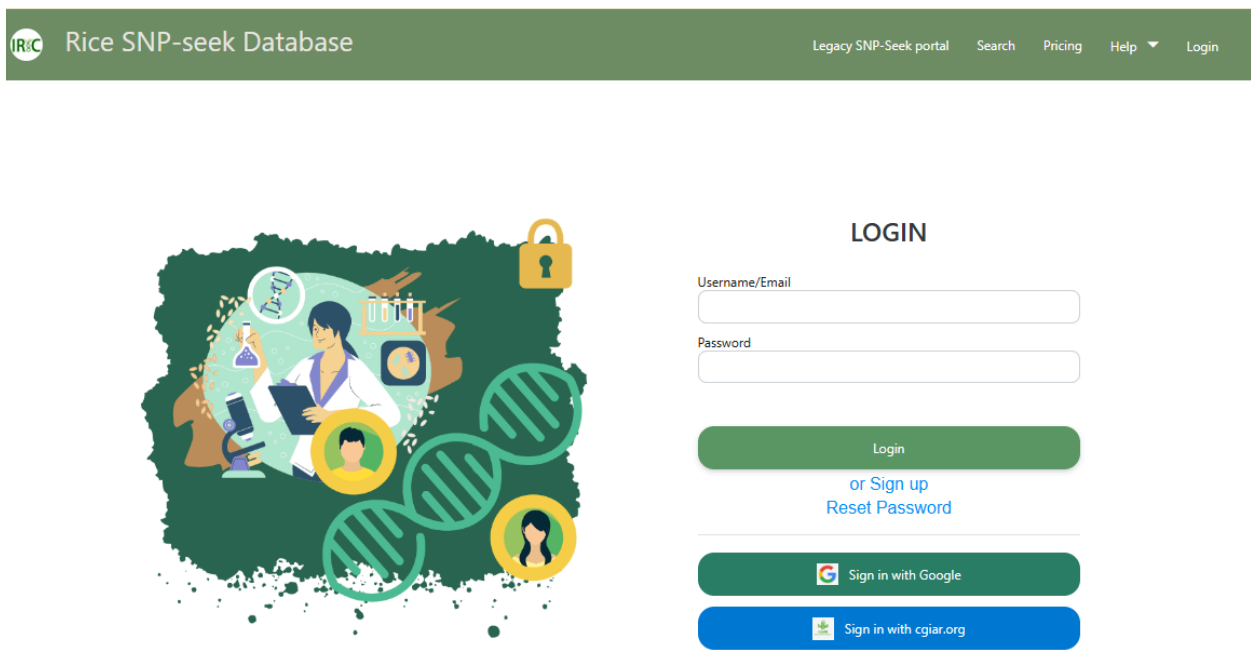


# Post-GWAS using RiceSNPSeek (using v3)

QTL of Interest: [PHS](#)

**Publication:** Lee JS, Chebotarov D, McNally KL, Pede V, Setiyono TD, Raquid R, Hyun WJ, Jeung JU, Kohli A, Mo Y. *Novel Sources of Pre-Harvest Sprouting Resistance for Japonica Rice Improvement*. *Plants (Basel)*. 2021 Aug 19;10(8):1709. doi: 10.3390/plants10081709. PMID: 34451754; PMCID: PMC8401653.

## 1.) Go to: <https://snp-seek.irri.org/>



Rice SNP-seek Database

Legacy SNP-Seek portal Search Pricing Help Login


### LOGIN


Username/Email

Password

Login

or Sign up  
[Reset Password](#)

 Sign in with Google

 Sign in with cgiar.org

- a. Login using your SNP-seek credentials. There are three ways to do this:
  - i. Username/email and Password
  - ii. Sign in using your Google account
  - iii. Sign in using [cgiar.org](https://cgiar.org) (if you are part of this domain)
- b. Sign up if you do not have an account in SNPSeek yet.

## 2.) Search Genotypes:

Note that our QTL/trait of interest is in this region: **Chr4:4662701-4670717**

You can begin by searching for SNPS that lie within this region using the 3k rice dataset. There are 4 SNP Sets available:

- **3K All**: 32 million full 3K RG SNPs Dataset

This SNP Set contains the full set of 32 million biallelic & multiallelic SNP.

Total SNPs: 32,064,217

Samples : 3024

- **3kbase**: 18 million base SNP

The Base SNP set of ~18 million SNPs was created from the ~29 million biallelic SNPs subset from the 32M full SNP set by removing SNPs with excess of heterozygous calls.

- **3K core**: 404k CoreSNP dataset

The Core SNP set was obtained from the filtered SNP set by applying two-step LD pruning procedure as follows:

- 1.) LD pruning with window size 10kb, step 1 SNP, R2 threshold 0.8
- 2.) LD pruning with window size 50 SNPs, step 1 SNP, R2 threshold 0.8

- **3k filtered**: 4.8million filtered SNP dataset

The filtered SNP set was obtained from the Base SNP set by applying the following filtering criteria:

- 1.) alternative allele frequency at least 0.01
- 2.) proportion of missing calls per SNP at most 0.2

You may use different SNP sets but for this hands-on, we will use the “**3Kbase**” SNP set.

In the Chromosome, put “**Chr4**”, in Start use “**4662701**” and end “**4670717**”.

Click “**Search**” to retrieve the positions within this region within the 3KRG accessions.

Rice SNP-seek Database

Search By Genotype

Include Advance Options

Dataset

Reference: Japonica nipponbare

Variety Set: 3k

Snp Set: 3kbase

Subpopulation:

Variety List:

Region

Chromosome: Chr4

(m. chr1)

Start: 4662701

End: 38502894 bp

Gene Locus:

(m. loc\_401g1010)

SNP List:

Locus List:

Options

Search Reset

### 3.) Query result

After querying the genotypes, you will have a table which displays the alleles for each 3KRG variety in a row, for all SNP positions within the specified region or gene. By default, the varieties are sorted by decreasing number of allele mismatches. The resulting table can be filtered using any of the column values, and sorted based on any column value by clicking the column header.

Result

Allele Frequency

Browse

Snp Effect

Haplotype

Download

Listname

Create List

Filters

Column:

Value:

Apply Filter

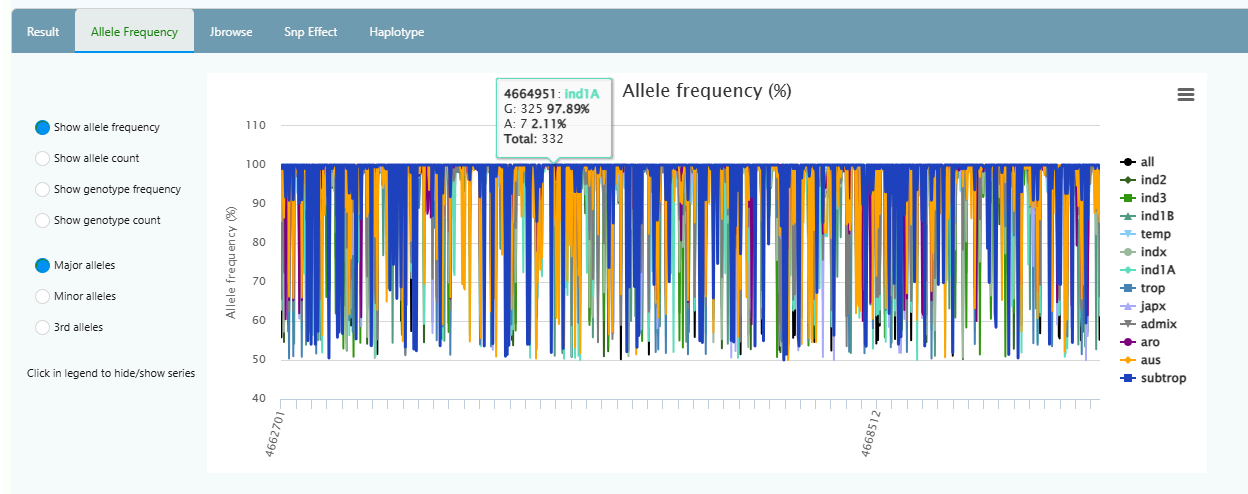
Clear

Allele color code: mismatch non-synonymous splice donor SNP splice acceptor SNP gap -

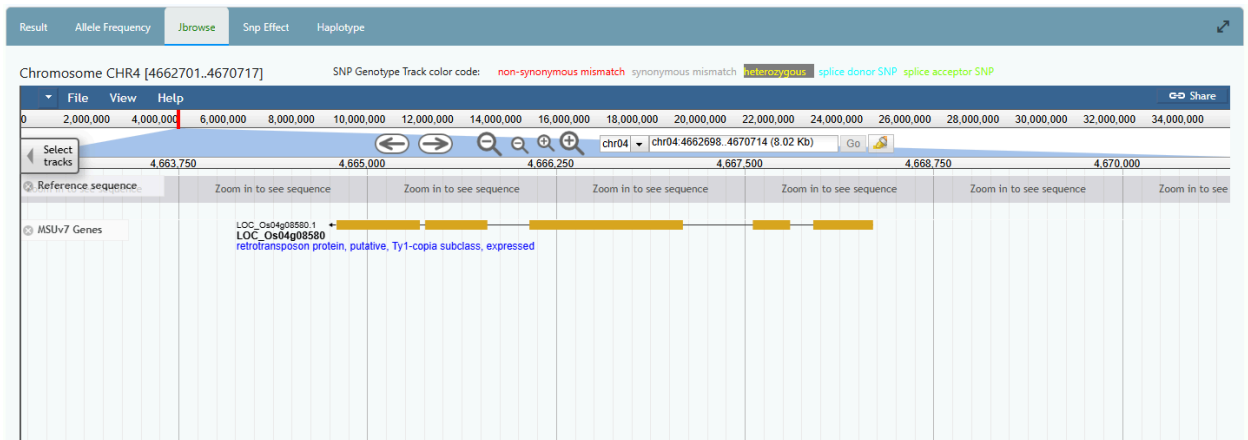
Japonica nipponbare positions	Assay	Accession	Variety 22	Assay	Accession	Subpopulation	Dataset	Mismatch	4662701	4662710	4662732	4662735	4662743	4662767	4662795	4662822	4662864	4662887	4662893	4662892	4662906	4662920	4662927
Japonica nipponbare								G	T	T	C	T	A	G	C	A	T	C	T	G	T	G	
NCS 766::IRGC 62478-1	IRIS 313-11645	IRGC 127664	NCS 7 IRIS 3 IRGC	3k	120.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
MELABOH::IRGC 66597-1	IRIS 313-8493	IRGC 128537	MELA IRIS 3 IRGC	3k	119.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
AUS 177::IRGC 29009-1	IRIS 313-9066	IRGC 125659	AUS 1 IRIS 3 IRGC	3k	118.5	A	T	T	G	T	T	G	C	A	T	C	T	G	T	G			
ZINVA KOLAMBA::IRGC 52402-1	IRIS 313-11453	IRGC 127916	ZINVA IRIS 3 IRGC	3k	118.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
KOLUBA::IRGC 30463-1	IRIS 313-11098	IRGC 134993	KOLU IRIS 3 IRGC	3k	118.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
LINANGAN::IRGC 47255-2	IRIS 313-11381	IRGC 132351	LINA IRIS 3 IRGC	3k	118.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
MR19	CX317	IRGC 135992	MR19 CX317 IRGC	temp	3k	118.5	A	T	T	G	T	T	G	C	A	T	C	T	G	T	G		
DA 7	IRIS 313-8703	IRGC 124432	DA 7 IRIS 3 IRGC	3k	117.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
FACAGRO 64::IRGC 82059-1	IRIS 313-10114	IRGC 125739	FACA IRIS 3 IRGC	3k	117.0	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
NACHIN ER 12::IRGC 14146-1	IRIS 313-8407		NACH IRIS 3	3k	117.0	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
DAW NOK KAEN::IRGC 65548-1	IRIS 313-11700	IRGC 127306	DAW IRIS 3 IRGC	3k	117.0	A	T	T	G	T	T	G	C	A	T	C	T	G	T	G			
NS 199::IRGC 68864-1	IRIS 313-11761	IRGC 127683	NS 15 IRIS 3 IRGC	3k	117.0	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
KHAO HAE::IRGC 29773-3	IRIS 313-11085	IRGC 132273	KHAC IRIS 3 IRGC	3k	116.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
CR 60-10::IRGC 15777-1	IRIS 313-9023	IRGC 125715	DAW IRIS 3 IRGC	3k	116.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			
DAWK PHU KHAO::IRGC 65808-1	IRIS 313-11705	IRGC 127303	ARC 1 IRIS 3 IRGC	3k	116.5	A	T	T	G	T	T	G	C	G	T	C	T	G	T	G			

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It will also report a graph of the allele frequencies where you can inspect the major and minor alleles per subpopulation for all SNP positions from the query.



In another panel, you can also view the region in a genome browser.



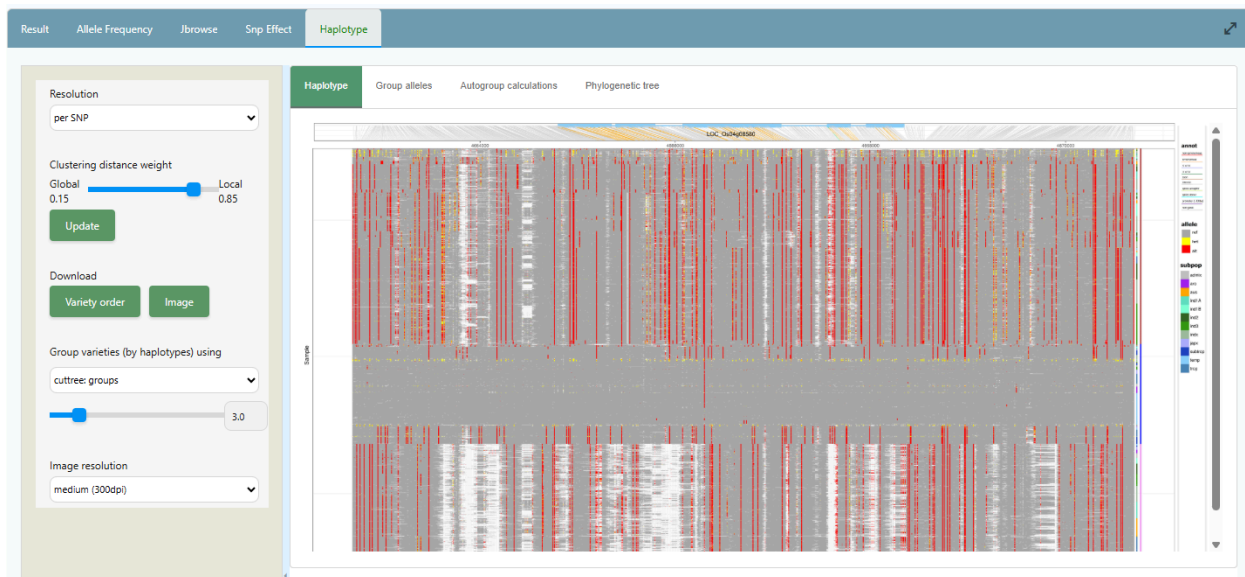
## 4.)SNP Effects

The Genotype query also returns a table containing the SNP effects detected on MSU gene models. This is calculated using the SNPEff program.

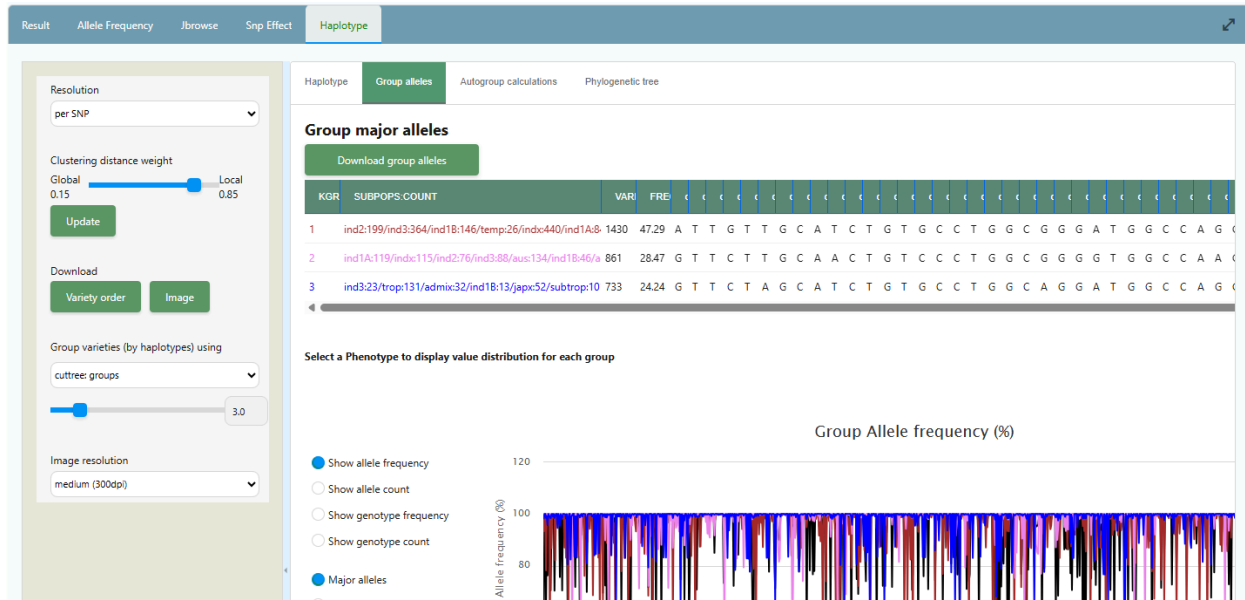
Result	Allele Frequency	Jbrowse	Snp Effect	Haplotype					
Download									
Snp Effect									
POSITION	"DELETERIOUSNESS"	Alt (ANN)	Effect (ANN)	Putative_impact (ANN)	Gene Name (ANN)	Gene ID (ANN)	Feature type (ANN)	Feature ID (ANN)	Transcript B
chr04-4662701	30	A	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		A	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662730	30	A	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		A	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662732	30	G	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		G	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662735	30	G	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		G	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662743	30	G	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		G	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662767	30	T	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		T	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662795	30	C	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		C	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662822	30	G	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		G	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
chr04-4662864	30	G	downstream_gene	MODIFIER	LOC_Os04g08580	LOC_Os04g08580	transcript	LOC_Os04g08580.1	Coding
		G	intergenic_region	MODIFIER	LOC_Os04g08570-LOC_Os04g08580	LOC_Os04g08570-LOC_Os04g08580			
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## 5.) Haplotype View

Another view for the result of the genotype query is the Haplotype View. This allows us to know whether our haplotypes make sense.



You should also see major grouping of alleles which contributes to the haplotype.



In this hands-on, you should see **3 major haplotype groups**.

## Group major alleles

Download group alleles

KGR	SUBPOPS.COUNT	VARI	FRE	Alleles
1	ind2:199/ind3:364/ind1B:146/temp:26/indx:440/ind1A:8	1430	47.29	A T T G T T G C A T C T G T G C C T G G C G G
2	ind1A:119/indx:115/ind2:76/ind3:88/aus:134/ind1B:46/a	861	28.47	G T T C T T G C A A C T G T C C C T G G C G G
3	ind3:23/trop:131/admix:32/ind1B:13/japx:52/subtrop:10	733	24.24	G T T C T A G C A T C T G T G C C T G G C A G

## 6.) Downloading the results table

Download the table in “Plink” format by clicking on the “Plink” button.

This will give you two files in a zipped folder: a ped and map file that by now you should be familiar with.

The first few lines of your downloaded .map file should look like this:

chr04	10404662701	0	4662701
chr04	10404662730	0	4662730
chr04	10404662732	0	4662732
chr04	10404662735	0	4662735
chr04	10404662743	0	4662743
chr04	10404662767	0	4662767
chr04	10404662795	0	4662795
chr04	10404662822	0	4662822
chr04	10404662864	0	4662864
chr04	10404662867	0	4662867
chr04	10404662873	0	4662873
chr04	10404662892	0	4662892
chr04	10404662906	0	4662906
chr04	10404662920	0	4662920
chr04	10404662927	0	4662927
chr04	10404662930	0	4662930

...

Likewise, the first few lines of your .ped file should be like this:

IRIS_313-11645	IRIS_313-11645	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-8493	IRIS_313-8493	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-9066	IRIS_313-9066	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11453	IRIS_313-11453	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11098	IRIS_313-11098	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11381	IRIS_313-11381	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
CX317	CX317	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-8703	IRIS_313-8703	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-10114	IRIS_313-10114	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-8407	IRIS_313-8407	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11700	IRIS_313-11700	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11761	IRIS_313-11761	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11085	IRIS_313-11085	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-9023	IRIS_313-9023	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11705	IRIS_313-11705	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-10863	IRIS_313-10863	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
B264	B264	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-10177	IRIS_313-10177	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11677	IRIS_313-11677	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11683	IRIS_313-11683	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11709	IRIS_313-11709	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11762	IRIS_313-11762	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11789	IRIS_313-11789	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-12259	IRIS_313-12259	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-11273	IRIS_313-11273	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
CX306	CX306	0	0	0	-9	A	A	T	T	T	T	C	C	T	T	T	T	G	G	C
IRIS_313-8356	IRIS_313-8356	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-10337	IRIS_313-10337	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-9160	IRIS_313-9160	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C
IRIS_313-10332	IRIS_313-10332	0	0	0	-9	A	A	T	T	T	T	G	G	T	T	T	T	G	G	C

## 7.) Add positions in my list

From the map file, remove the middle columns such that only the 1st column and the last columns remain (chr, position).

Go to “My lists”. Select “SNP” in List Type. Click on “Create” to make a new list.

Paste the chr-position table from the map file.

You may tick the box on “Verify if SNP is in Variant set”. However, in this hands-on, we will not do that since we know that these SNPs already came as a result of the genotype query.

Add SNP List

List name: PHS-CHR4

Set chromosome and SNP positions here

☐ Verify if SNP is inVariant set

☐ 3k-MH63v15  
☐ 3kall  
☐ 3kbase  
☐ 3kcore

☒ Autoconfirm (forced for >50 positions)

☐ SNPs have allele column (for allele filtering)

☐ SNPs have -log(p) column (from GWAS analysis)

Format: chromosome position

chr04	4670576
chr04	4670599
chr04	4670605
chr04	4670609
chr04	4670611
chr04	4670612
chr04	4670614
chr04	4670617
chr04	4670618
chr04	4670622
chr04	4670623
chr04	4670630
chr04	4670641
chr04	4670653
chr04	4670655
chr04	4670656
chr04	4670662
chr04	4670663
chr04	4670668
chr04	4670672
chr04	4670673
chr04	4670684
chr04	4670685
chr04	4670698
chr04	4670717

Save

Cancel

You will be able to see this new list on the left side of the panel.



My List

NOTE: Lists are not saved in the server and available only for this session. Click Download button to save your created lists for future reference. Click Upload button to recreate your list from previously downloaded MyList file.

List Type

List Type:
☐ Variety
☒ SNP
☐ Locus

SNP List

Select List

ANY:1KRICAv3
ANY:AG2-SNPList
ANY:PHS-CHR4

Set Operations: (enabled on multiple list selection, press Ctrl+click to select multiple items)

Create
Delete

Download / Upload

Download
Upload

Open Query

965 items in list

(GENOME, CONTIG, POSITION)
(chr04,4662701)
(chr04,4662730)
(chr04,4662732)
(chr04,4662735)
(chr04,4662743)
(chr04,4662767)
(chr04,4662795)
(chr04,4662822)
(chr04,4662864)
(chr04,4662867)
(chr04,4662873)
(chr04,4662892)
(chr04,4662906)

« < 1 / 20 > »

## 8.) Annotate SNP List

Next thing we want to do is to look for candidate genes within the QTL region in Nipponbare, go to “Search”-> “Gene Loci”.

Set “Japonica nipponbare” as reference genome.

Set “SNP Annotator (My SNP List)” in Search.

Set “All” to search all Gene models available.

Tick the box to “Include gene interactions (RiceNetv2, PRIN)” and “Gene Ontology” annotations. You may choose to add more to gather more annotations for this region.

**Rice SNP-seek Database**

Search By Gene Loci

Search ▼

Reference Genome  
Japonica nipponbare ▼

Search By  
SNP Annotator (My SNP List) ▼

Gene Model  
Maximum interacting loci

My SNP List ▼

My SNP List ANY:PHS-CHR4 ▼

+/- 0 basepairs

☒ Include gene interactions (RiceNetv2, PRIN) (slow)

☐ FGenesh++, PlantPromDB

☒ Gene Ontology

☐ PO, TO, Q-TARO

☐ Q-TARO QTLs

The result should be a table which contains the annotations that can be grouped by marker position, gene locus, or by QTL.

Search by SNP List positions: Contig ANY, SNP List ANY:PHS-CHR4 RESULT:964 markers

Search By Gene Name/Symbol/Function Result

Download: CSV Tab Create Gene List: List Name

Group by Marker/Position ☐ Gene locus ☐ QTL ☒ Include: ☒ gene model ☒ in promoter ☒ ricenet interaction ☒ prin interaction

CONTIG.POS,-LOGP	-LOGP	GENE MODELS	GO BIOLOGICAL PROCESS	GO CELLULAR COMPONENT	GO MOLECULAR FUNCTION
(chr04.4662730)		LOC_Os04g08580 chr04 [466479-	LOC_Os04g08580 chr04[4664796-		LOC_Os04g08580 chr04[4664796-4668349] -
(chr04.4662732)		4668349] -	4668349] -		GO:0003676 nucleic acid binding ()
(chr04.4662735)		retrotransposon protein, putative	GO:0015074 DNA integration ()		LOC_Os04g08580 chr04[4664796-4668349] - GO:0003677 D
(chr04.4662743)		copia subclass, expressed			
(chr04.4662767)					
(chr04.4662795)					
(chr04.4662822)					
(chr04.4662864)					
(chr04.4662867)					
(chr04.4662873)					

## 9.) Saving the Gene List

Set the List name as “PHS-genelist” and “click Create Gene List”.

Search by SNP List positions: Contig ANY, SNP List ANY:PHS-CHR4      RESULT:964 markers

Search By Gene Name/Symbol/Function Result

Download: CSV Tab      Create Gene List: List Name: PHS-genelist

Group by: Marker/Position ☐ Gene locus ☐ QTL ☒ Include: ☒ gene model ☒ in promoter ☒ ricenet interaction ☒ prin interaction

CONTIG.POS.-LOGP	-LOGP	GENE MODELS	GO BIOLOGICAL PROCESS	GO CELLULAR COMPONENT	GO MOLECULAR FUNCTION
(chr04.4662730) (chr04.4662732) (chr04.4662735) (chr04.4662743) (chr04.4662767) (chr04.4662795) (chr04.4662822)		LOC_Os04g08580 chr04 [4664791-4668349] - retrotransposon protein, putative copia subclass, expressed	LOC_Os04g08580 chr04[4664796-4668349] - GO:0015074 DNA integration ()		LOC_Os04g08580 chr04[4664796-4668349] - GO:0003676 nucleic acid binding () LOC_Os04g08580 chr04[4664796-4668349] - GO:0003677 D

Go to “My Lists” and you should see the gene locus/loci when you search under “Locus”.

Rice SNP-seek Database

My List

NOTE: Lists are not saved in the server and available only for this session. Click Download button to save your created lists for future reference. Click Upload button to recreate your list from previously downloaded MyList file.

List Type ☐ Variety ☐ SNP ☒ Locus

Locus List

Select List

- bacterial-blight-genes
- BLB-related
- BLB-related-region
- PHS-gene
- PHS-genelist**
- PHS-genes

Set Operations: (enabled on multiple list selection, press Ctrl+click to select multiple items)

Create Delete

Download / Upload

Download Upload

Open Query

1 items in list

NAME	CONTIG	START	STOP	STRAND	DESCRIPTION
LOC_OS04G08580	chr04	4664797	4668349	-1	

## 10.) Get Gene set/network

Using the gene list we generated, we will now do a gene enrichment analysis to know if there are other genes previously reported that are associated with our QTL of interest.

To do this, go to “Search”-> “Gene Loci.

Set as Reference genome: “Japonica Nipponbare”.

Set Search to: “Gene set/networks”.

Set Gene Models to: “All”.

Set the My Locus List to: “PHS-genegenes”.

Tick “Ricenetv2 direct neighbors” and “AgriGO”.

Click on “Gene set/enrichment”.

Search By Gene Loci

Search ▼

Reference Genome  
Japonica nipponbare ▼

Search By  
Gene set/networks ▼

Gene Model  
All ▼

Maximum interacting loci

Gene Set ▼

My Locus List PHS-geneist ▼

☒ Ricenetv2 direct neighbors GO

☐ String-DB prot-prot interaction GO

☐ CARMO functional Annotation GO

☐ Plant GSEA GO

☒ AgriGO GO

☐ IC4R Expression (RAP loci only) GO

☐ IC4R Coexpression (RAP loci only) GO

Gene set/enrichment

Unfortunately, this query will not return any result. Click the “Go” button beside the Ricenet option and it will give us this message:

*“No ROC analysis, because the valid query set size < 4.”*

In this case, our query size is very small. You can explore nearby regions and see if there are existing gene networks reported by increasing the upper and lower bound positions.

To know more about SNPSeek, visit the site: <https://snpseek.irri.org/>.