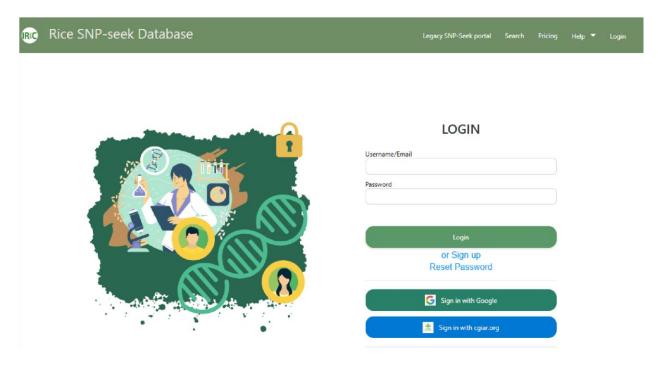
Local Genome Region Analysis using RiceSNPSeek (using v3)

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/



- 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 <u>cgiar.org</u> (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: <u>Chr4:4662701-4670717</u>
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.

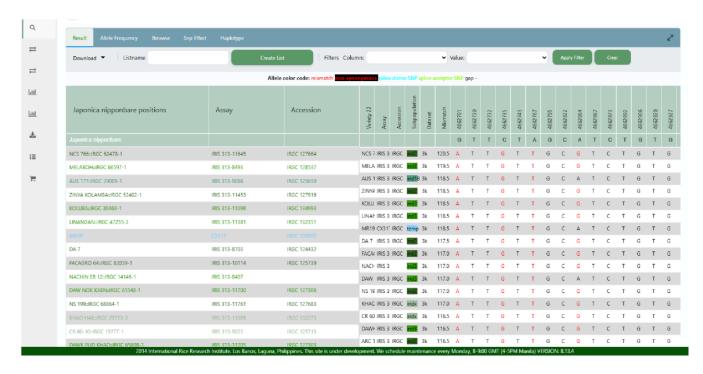
In the Chromosome, put "Chr4", in Start use "4662701" and end "4670717".

Click "Search" to retrieve the positions within this region within the 3KRG accessions.

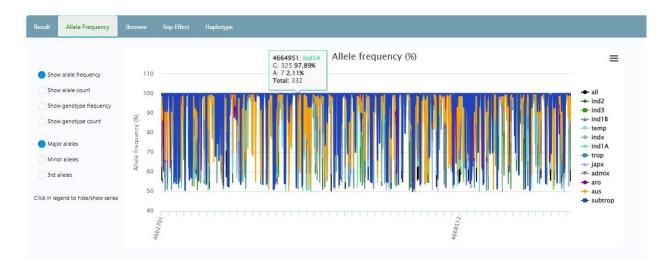


3.) Query result

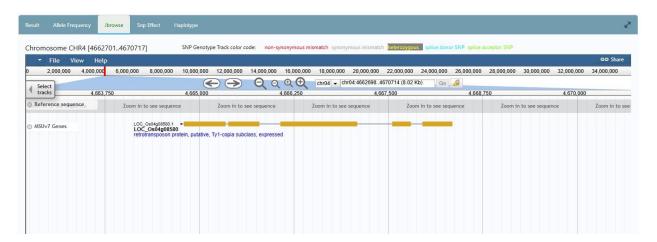
After querying the genotypes, you will have a table which displays the alleles for each 3KRG variety in a row, for all SNP positions within the specified region or gene. By default, the varieties are sorted by decreasing number of allele mismatches. The resulting table can be filtered using any of the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



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.



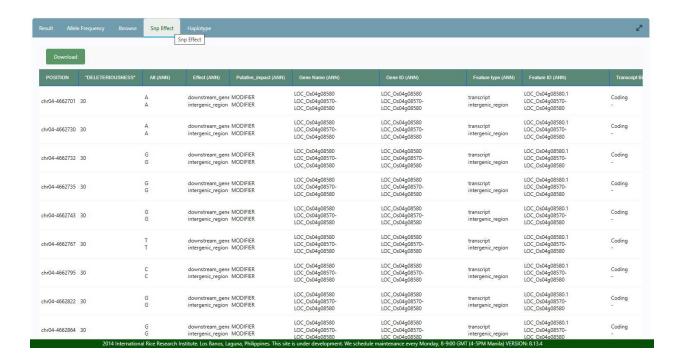
In another panel, you can also view the region in a genome browser.



4.) SNP Effects

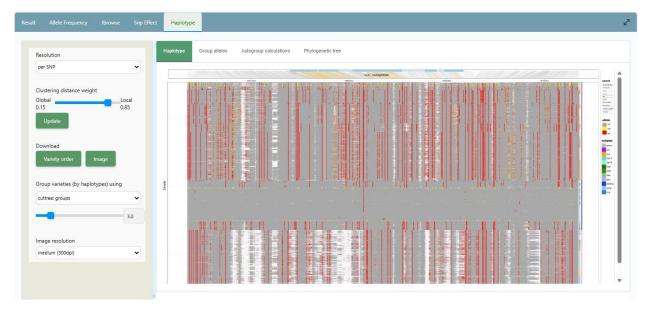
https://pcingola.github.io/SnpEff/snpeff/inputoutput/#eff-field-vcf-output-files

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



5.) Haplotype View

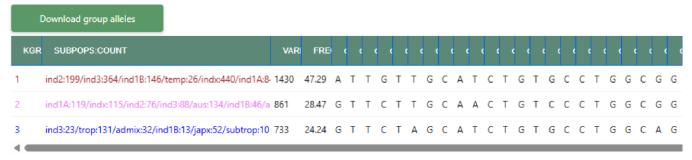
Another view for the result of the genotype query is the Haplotype View. This allows us to know whether our haplotypes make sense.



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



6.) Downloading the results table

FILE FORMATS:

- MAP https://zzz.bwh.harvard.edu/plink/data.shtml#map
- PED https://zzz.bwh.harvard.edu/plink/data.shtml#ped
- 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	C	A	A	T
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	C	G	G	T
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	C	A	A	T
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

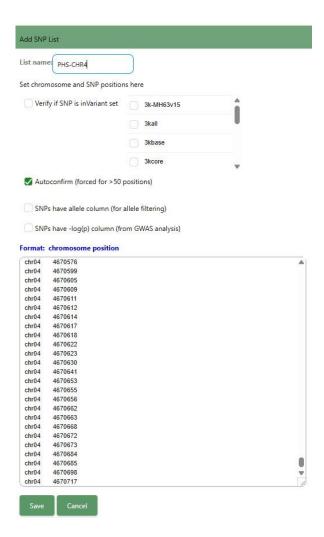
7.) Creating a SNP list

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

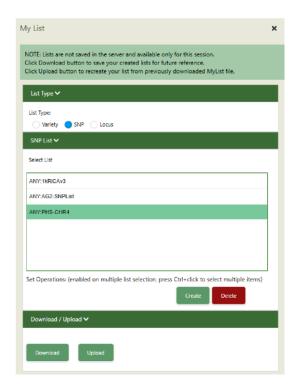
Go to "My lists". Select "SNP" in List Type. Click on "Create" to make a new list.

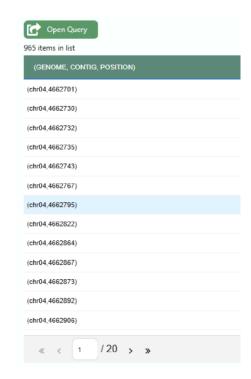
Paste the chr-position table from the map file.

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.



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





8.) Annotate SNP List

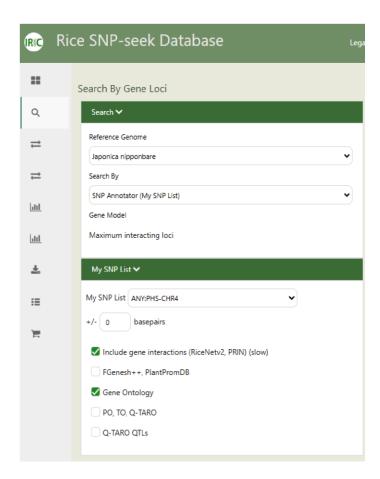
Next thing we want to do is to look for candidate genes within the QTL region in Nipponbare, go to "Search"-> "Gene Loci".

Set "Japonica nipponbare" as reference genome.

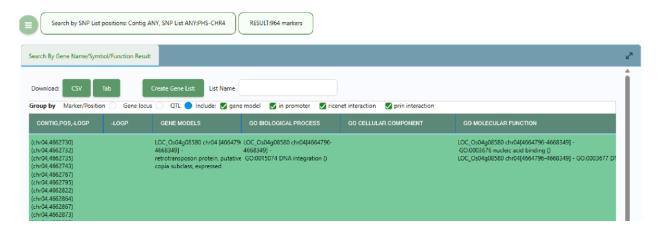
Set "SNP Annotator (My SNP List)" in Search.

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.



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

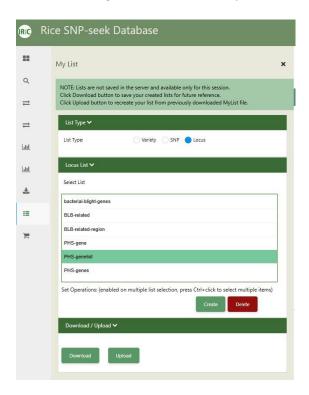


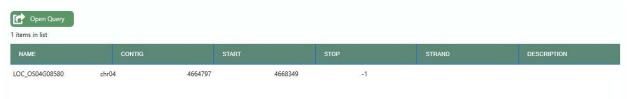
9.) Saving the Gene List

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



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





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".

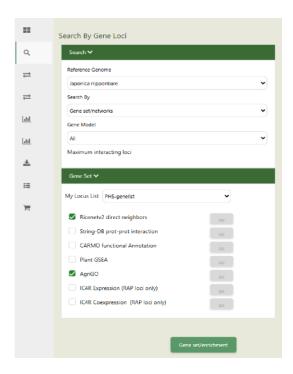
Set Search to: "Gene set/networks".

Set Gene Models to: "All".

Set the My Locus List to: "PHS-genegenes".

Tick "Ricenetv2 direct neighbors" and "AgriGO".

Click on "Gene set/enrichment".



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/.