This tutorial supplements the manuscript submitted to Plant Journal November 13, 2007:

Toward systems genetic analyses in barley: Integration of phenotypic, expression and genotype data into GeneNetwork

Arnis Druka, Ilze Druka, Arthur G. Centeno, Hongqiang Li, Zhaohui Sun, William T. B. Thomas, Nicola Bonar, Brian J. Steffenson, Steven E. Ullrich, Andris Kleinhofs, Roger P. Wise, Timothy J. Close, Elena Potokina, Zewei Luo, Carola Wagner, Günther F. Schweizer, David F. Marshall, Michael J. Kearsey, Robert W. Williams and Robbie Waugh

The tutorial demonstrates how to:

1) query GeneNetwork for barley related information;
2) map barley mRNA abundance and higher-order traits;
3) associate different traits using correlation analysis;
4) enter your own data;
5) map genes and Mendelian (binary) traits.

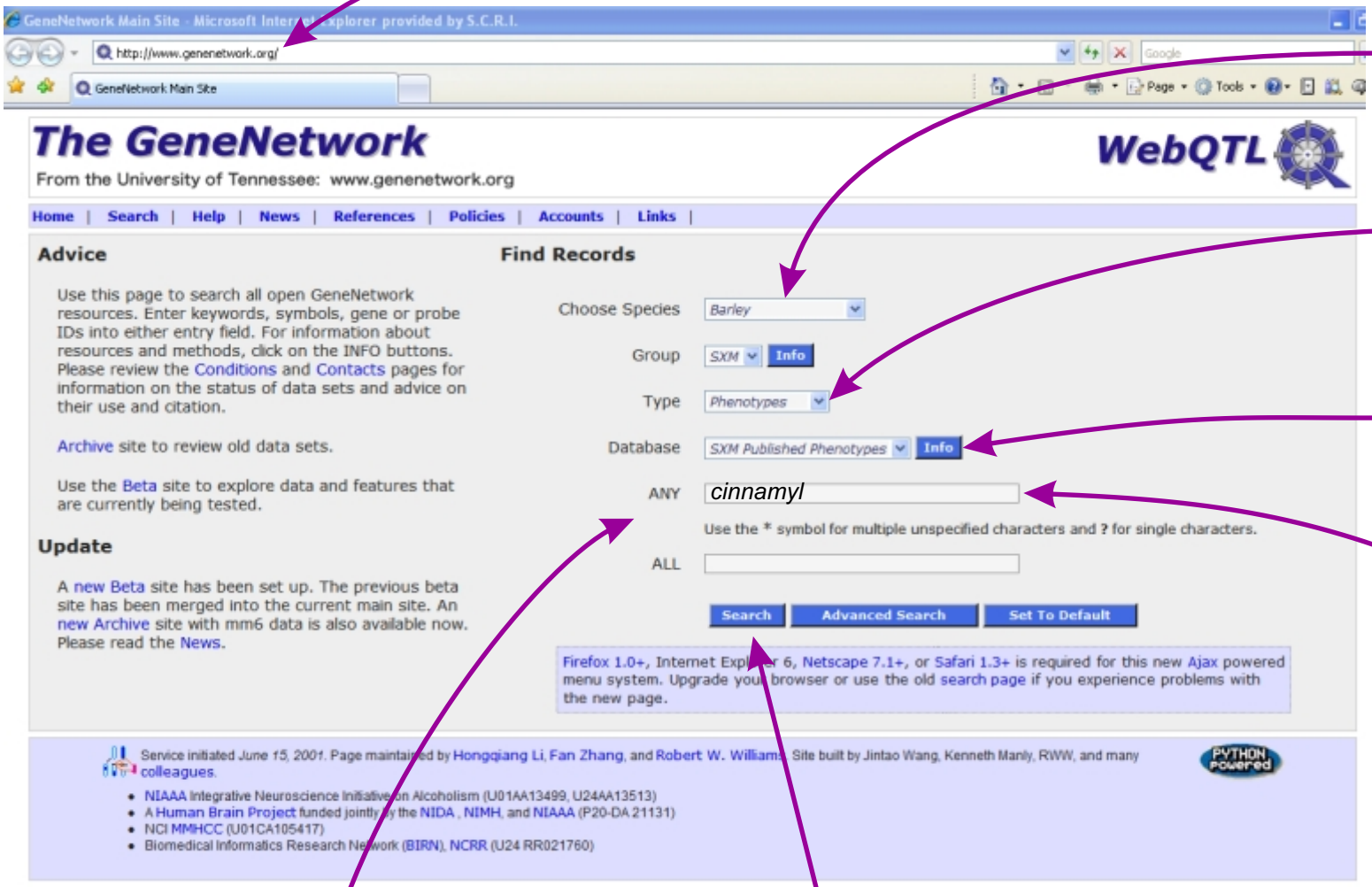
As an example, one of the lignin pathway genes, cinnamyl alcohol dehydrogenase (CAD) will be used throughout the tutorial.

Please open the Bookmark panel in the Acrobat Reader to navigate through the tutorial.

http://barleygenetics.net/GN_barley_tutorial.html

How to query GeneNetwork for barley related information?

1) go to the www.genenetwork.org



2) select 'Barley'

3) select the data set type
'Phenotypes' - to query higher order traits;
'Embryo mRNA' or 'Leaf' mRNA - to query genes.

4) select the database.
INFO file describes available options

5) write or copy&paste your query in the ANY and/or ALL field. Queries containing multiple strings should be space separated.

To look up the contents (all entries) of the 'Phenotypes' database type * .

For the mRNA databases query string can contain Barley1 GeneChip probe set ID (eg Contig20_at), or part of the gene annotation text (eg histone) or multiple space delimited strings.

Type 'cinnamyl' and click the 'Search' button

Difference between the fields ANY and ALL
ANY will find all the entries that match to any entered space separated string. For example,' cinnamyl dehydrogenase' typed in ANY will identify 254 records. If 'cinnamyl dehydrogenase' is queried from the ALL field, only five records are retrieved. ALL looks for the records where all query strings are present.

There are two analysis options to choose:

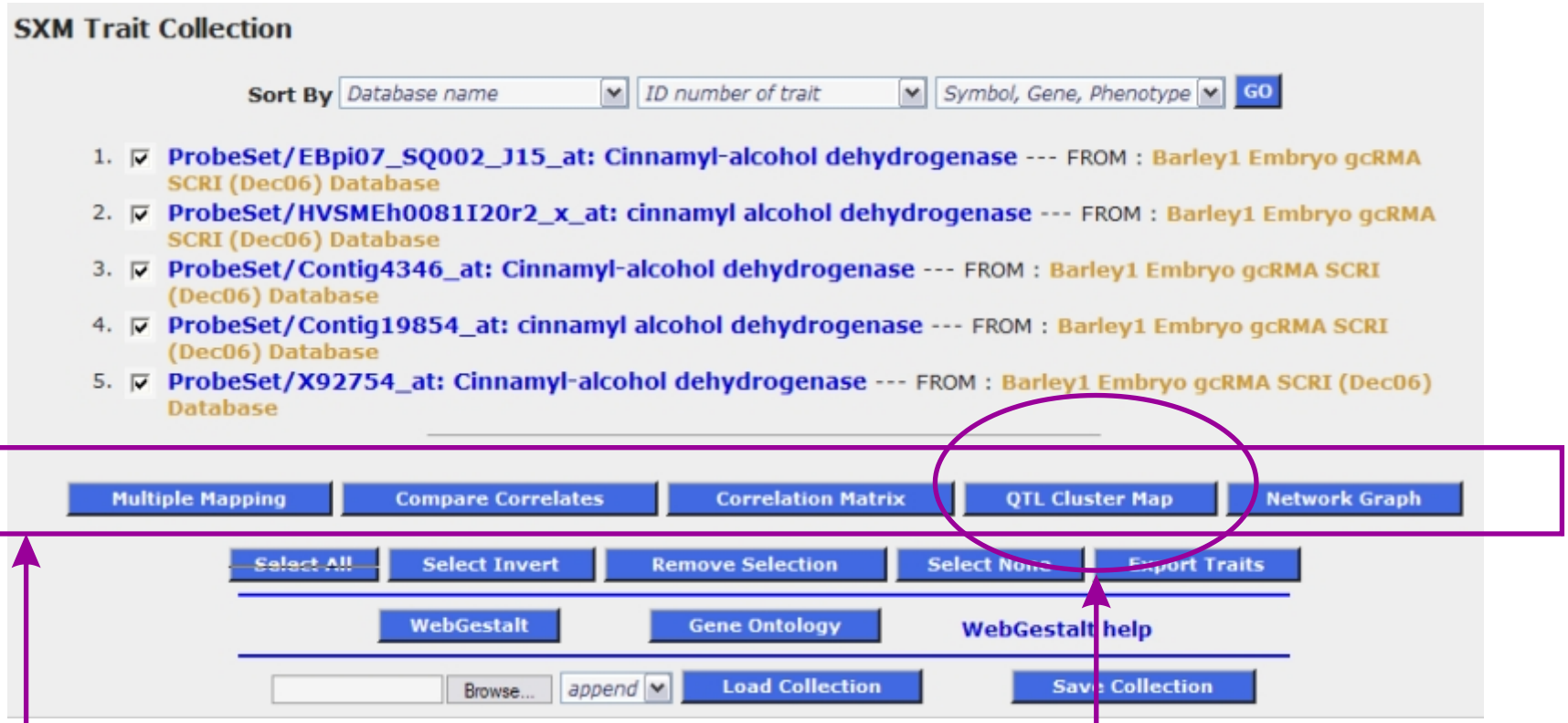
1) Multiple trait analysis

By comparing side by side QTL profiles of these 5 probe sets it may be possible to evaluate their performance (or see if there is a QTL at all) and may be determine what they actually represent - a single gene or different members of the family ?
1) click 'Select All';
2) then click 'Add to Collection'.
It will open 'SXM Trait Collection' window, which has several functions for multiple trait analysis.

2) Single trait analysis

If the best probe set for the query is known, or intention is to inspect the returned results individually, clicking directly on the record will open the 'Trait Data and Analysis form'.

Collection *allows to create combined list of traits from several independent searches (mRNA abundance and higher order traits), so that they can be analyzed together.*

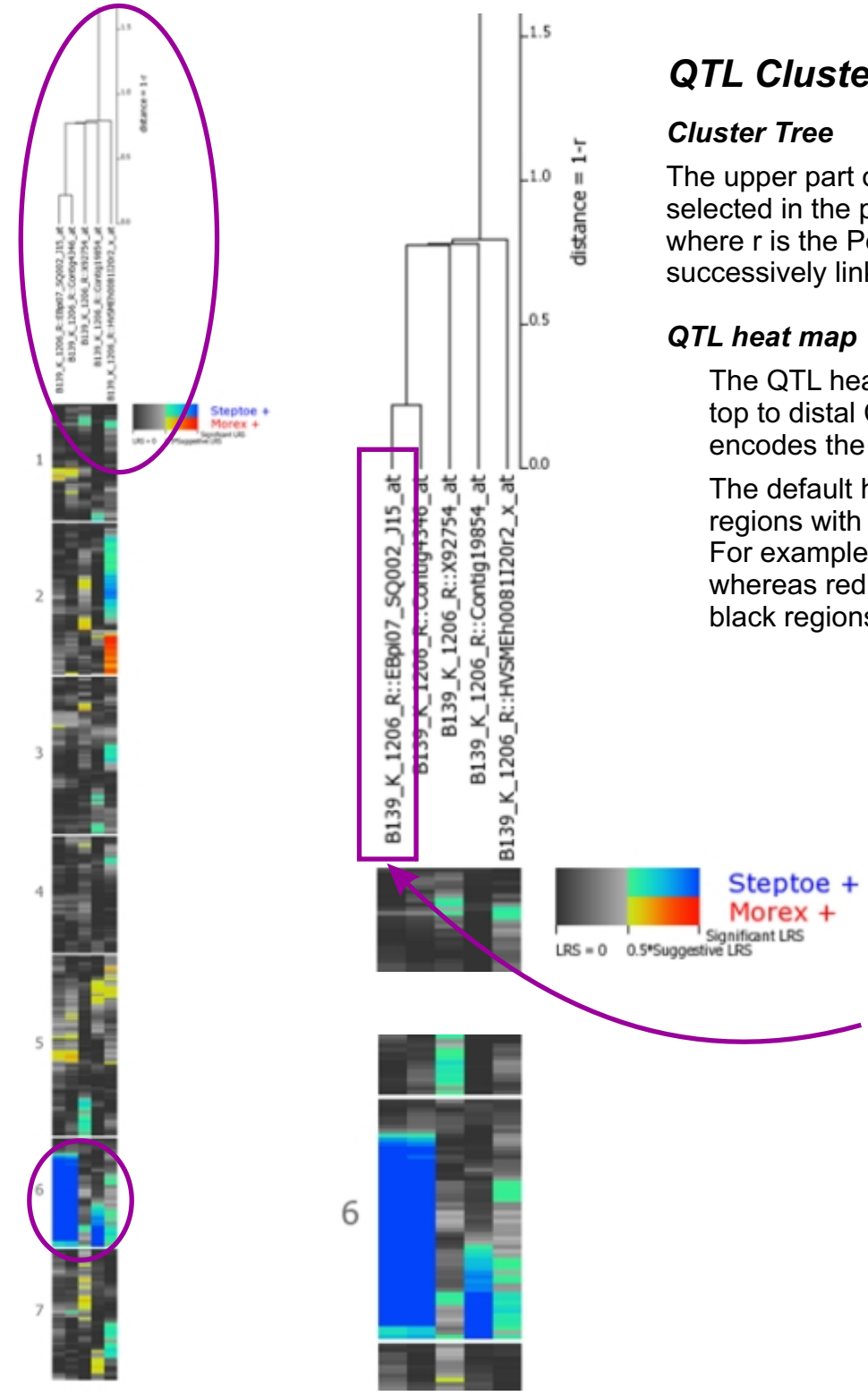


These functions are for multiple trait analysis

A quick way to obtain an overview on the QTLs for multiple traits is to use' QTL Cluster Map' function:

1) click 'Select All';

2) click 'QTL Cluster Map' .



QTL Cluster Map

Cluster Tree

The upper part of the QTL Cluster otput is a hierarchical cluster tree of the set of traits that were selected in the previous window. To generate this plot, distances between pairs of traits using (1 - r) where r is the Pearson product-moment correlation were computed. The hierarchy is assembled by successively linking traits and groups of traits.

QTL heat map

The QTL heat map of all members of the Cluster Tree, extending from proximal Chr 1H at the top to distal Chr 7H at the bottom is generated below the tree. Each vertical column or stripe encodes the genome-wide p- value computed on the basis of 1000 permutations.

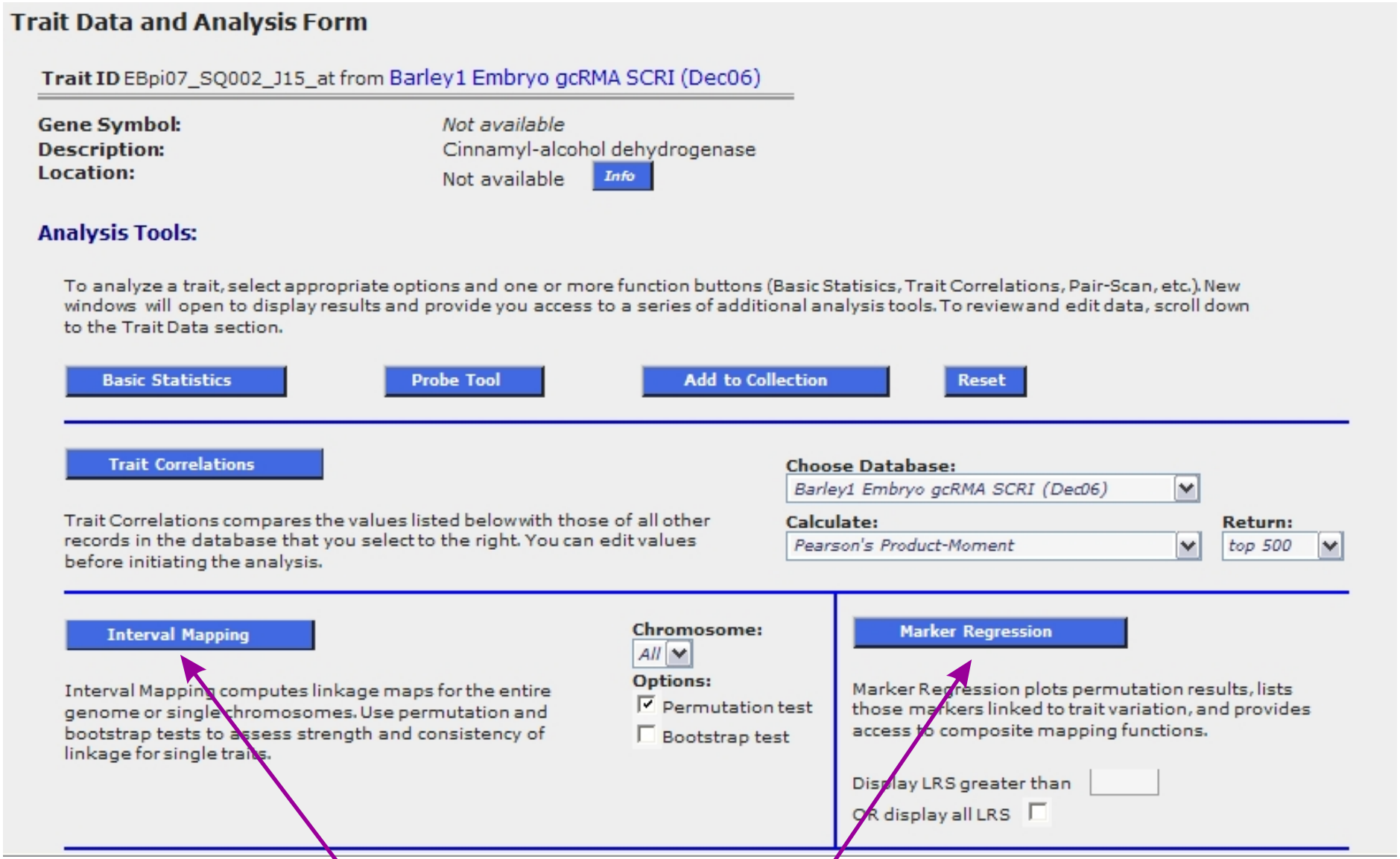
The default heat map is "Grey + Blue + Red" in which more intense colors mark chromosomal regions with comparatively high linkage statistics and the spectrum encodes the allelic effect. For example, blue regions are those in which Steptoe is associated with higher trait values, whereas red regions are those in which Morex is associated with higher trait values. Grey and black regions have insignificant linkage to trait variance.

Strong QTL on chromosome 6H is supported by two of the probe sets, while others either have single, weaker QTLs at different locations or have none at all. Therefore, the first two can be prioritized for further investigation.

However, thorough, independent, bioinformatics-based analysis or even lab work may be required to determine the cause of differential performance of the probe-sets and to decide which one is the best representative of the CAD gene.

Here, it is assumed that the first one is the best.

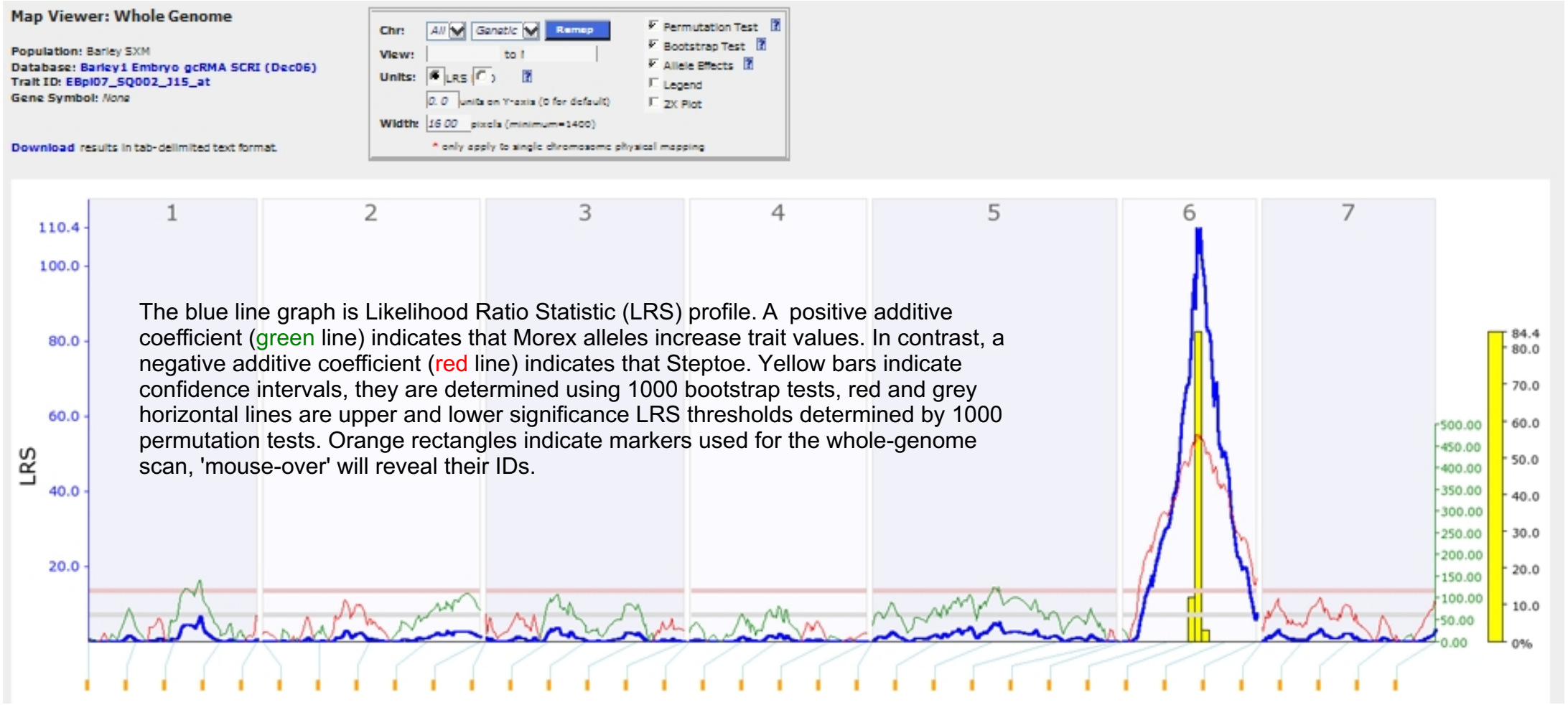
To proceed, click the trait ID, it will open the 'Trait Data and Analysis Form' .



These are the functions to map QTLs more precisely

Click 'Interval Mapping' button

Profile of the genome-wide QTL scan obtained using the 'Interval Mapping' function.



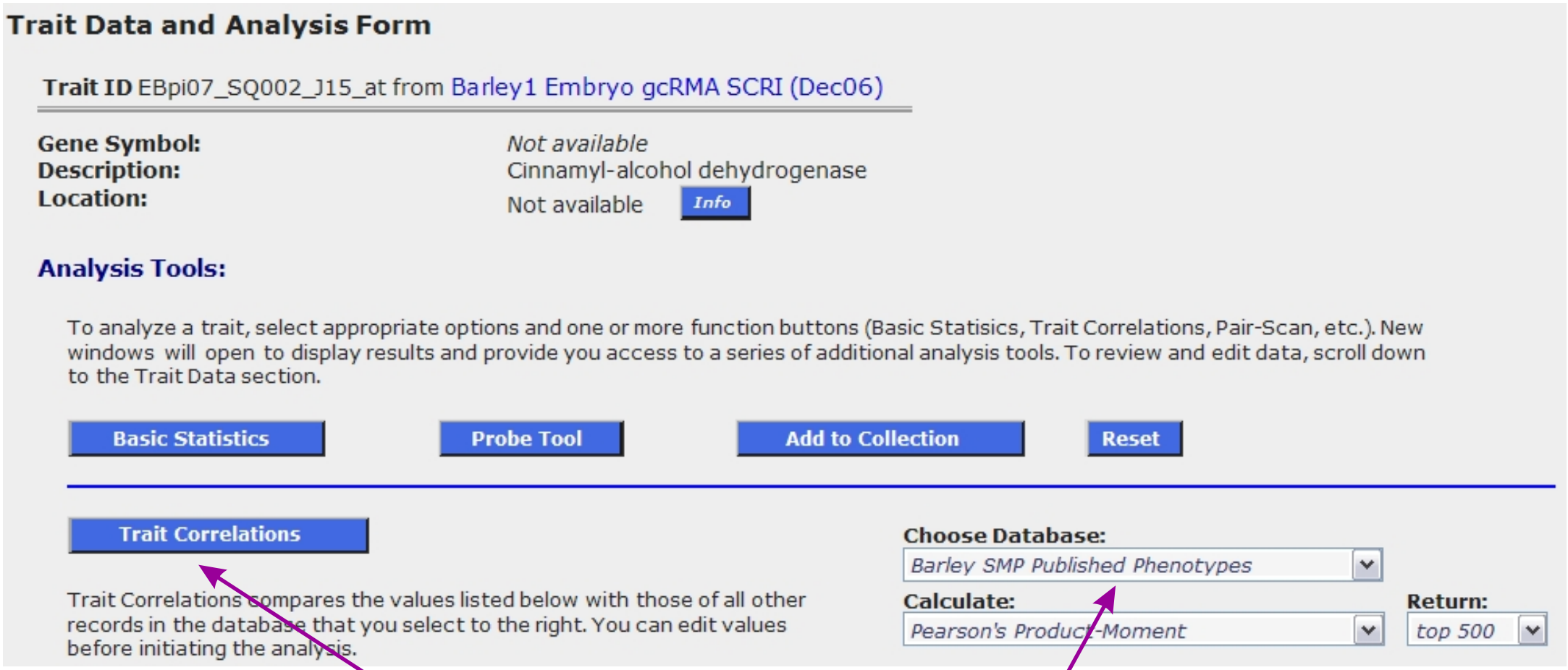
This graph reveals the following information about the CAD gene:
1) number of significant eQTLs (single in this case);
2) location of the eQTL (chromosome 6H). Scanning only chromosome 6H will reveal more precise location of the QTL peak;
3) allelic effect (Steptoe);
4) significance level (LRS=110, high).

Based on 1) and 4), it can be concluded that the CAD gene itself also maps to the same location on chromosome 6H.

Next:
1) minimize this window;
2) go back to the 'Trait Data and Analysis form'

Trait Correlations

Which higher order traits correlate to CAD mRNA abundance? Can any suggestive associations be revealed?



1) select 'Barley SXM Published Phenotypes'

2) click 'Trait Correlations'

Results of the correlation analysis

Correlation Table

Values of Record EBpi07_SQ002_J15_at in the Barley1 Embryo gcRMA SCRI (Dec06) database were compared to all 211 records in the Barley SMP Published Phenotypes database. The top 211 correlations ranked by the Pearson's product-moment correlation are displayed. You can resort this list using the small arrowheads in the top row.

Clicking on the record ID will open the published phenotype data for that publication. Click on the correlation to see a scatter plot of the trait data.

Multiple Mapping | QTL Cluster Map | Download Table

Select All | Invert | Clear | Add to Collection

☑ Display strain names in correlation plot
☑ Display fit line in correlation plot

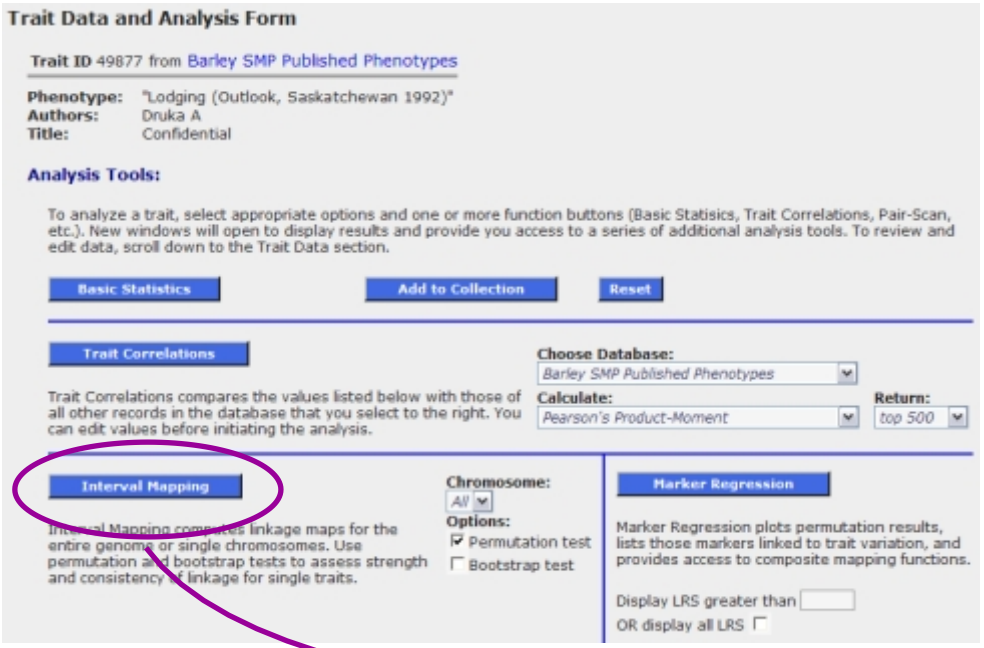
	Record ID	Phenotype	Authors	Year	Correlation	N Cases	p Value
1 ☐	49890	Emergence of the second leaf - single leaf frequency (SCRI glasshouse 2004)	*Druka A*	2006	-0.4046	139	5.59e-07
2 ☐	49951	Grain width F4 (Okayama 2006)	*Druka A*	2006	-0.3497	139	2.06e-05
3 ☐	49877	"Lodging (Outlook, Saskatchewan 1992)"	*Druka A*	2006	0.3443	139	2.83e-05
4 ☐	49784	"Yield - MT/ha (Aberdeen, Idaho 1991)"	*Druka A*	2006	-0.3223	139	9.72e-05
5 ☐	49932	Grain width F7 (SCRI 2003)	*Druka A*	2006	0.3189	139	0.00012
6 ☐	49768	Thousand grain weight (SCRI 2005)	*Druka A*	2006	0.3162	139	0.00013

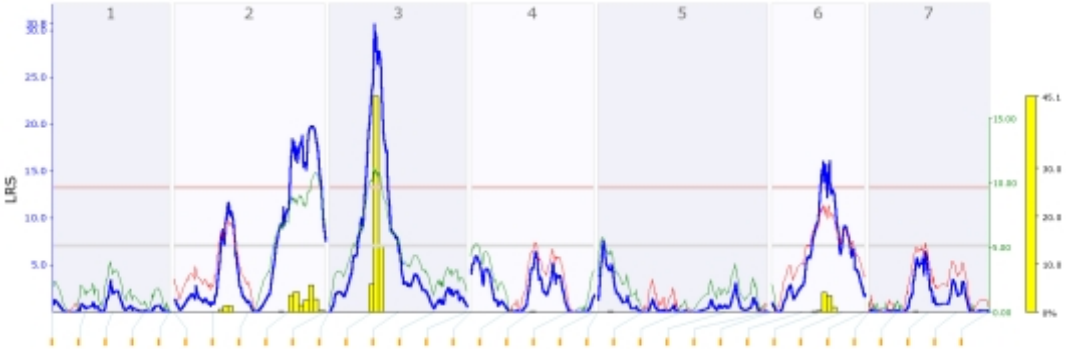
In this particular example, one of the top correlates Lodging seems to be suggestive CAD response trait. One can interpret that variation in the CAD mRNA may cause differential enzyme level and consecutively variation in the lignin composition (CAD is lignin biosynthetic gene). Lignins, as a constituents of the plant secondary cell walls can determine mechanical properties of the plant organs. Conceivably, lodging may depend on the cell wall mechanical properties determined by the lignins.

Correlation between lodging trait values and CAD mRNA is significant, but not very strong. This suggests that other factors (environmental or genetic) can determine lodging. Mapping of the lodging trait may provide clues on what they are.

Click on the Lodging Record ID.

Interval Mapping of the higher order traits





Mapping of the lodging trait provides the following information:

1) lodging locus on chr 6H coincides with that of CAD mRNA abundance;

2) they both have the same allelic effects;

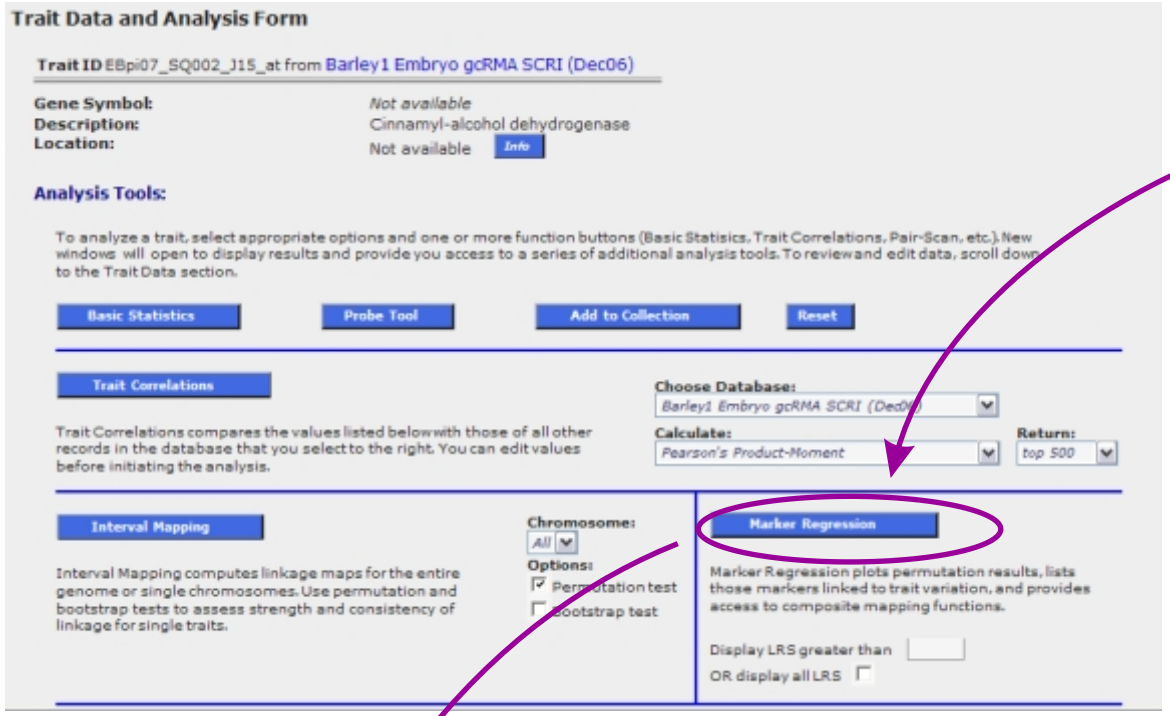
3) major lodging QTL is located on chromosome 3H, and there are at least two additional loci on chromosome 2H.

The CAD mRNA has a strong QTL on chromosome 6H. What additional loci can be revealed if major CAD mRNA QTL is considered as a background and only residual variation used for mapping?

The **Composite Interval Mapping** function of the GeneNetwork can do this.

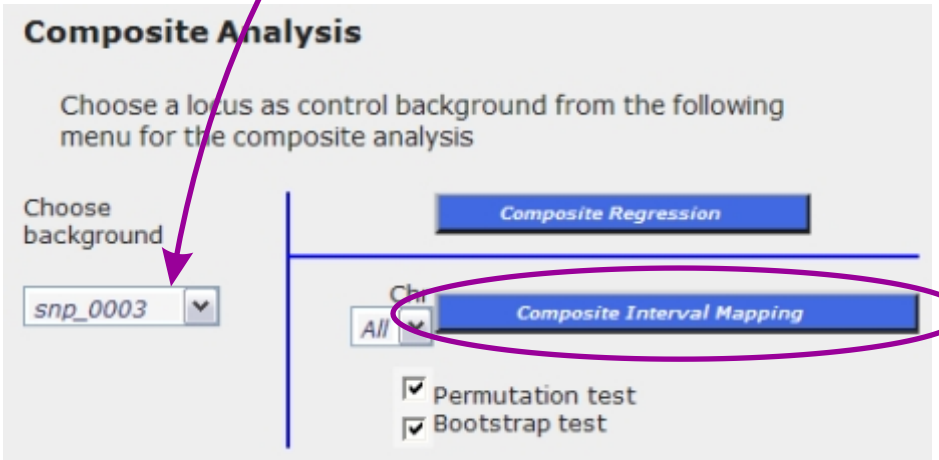
Open the CAD Trait Data and Analysis Form that was minimized before.

Composite Interval Mapping



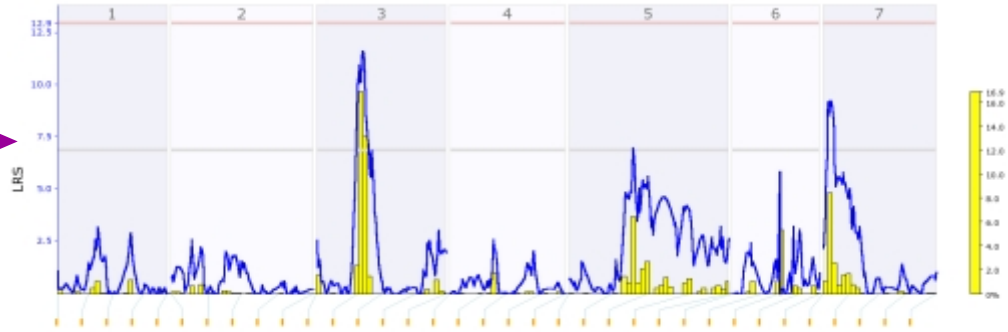
Click 'Marker Regression' button.
This will automatically identify the best associated with CAD mRNA abundance marker.

Part of the screen that comes up after hitting 'Marker Regression' button

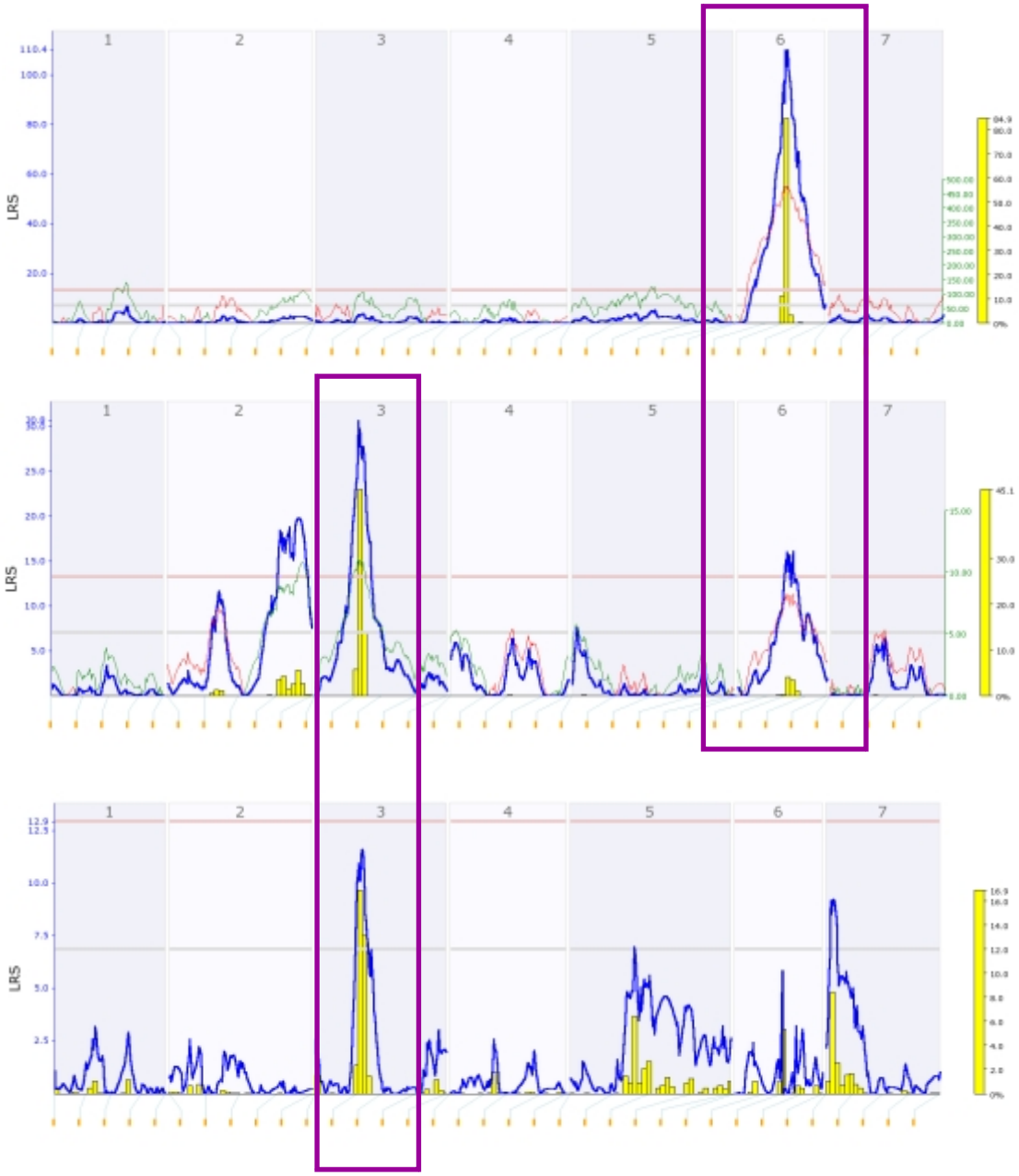


Conclusion

The mRNA accumulation of the CAD gene also seems to be controlled by the locus on chromosome 3H



Summary of the mapping results



CAD mRNA cisQTL

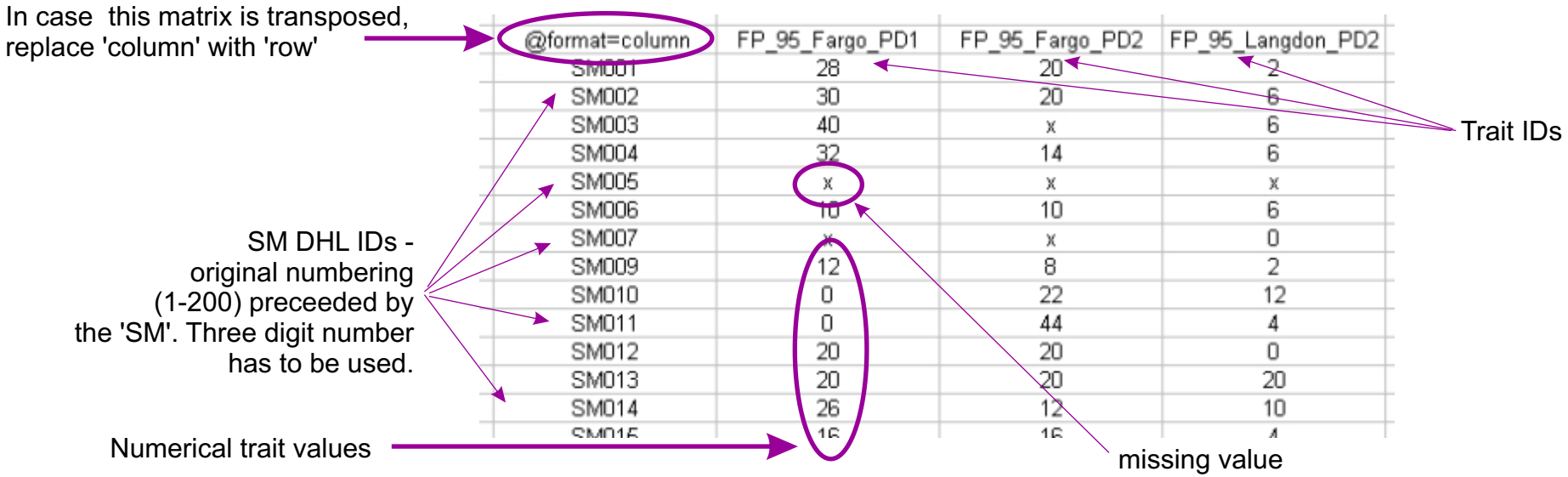
lodging

CAD mRNA transQTL

Identification of the overlapping QTLs for different traits may suggest a common regulatory mechanism

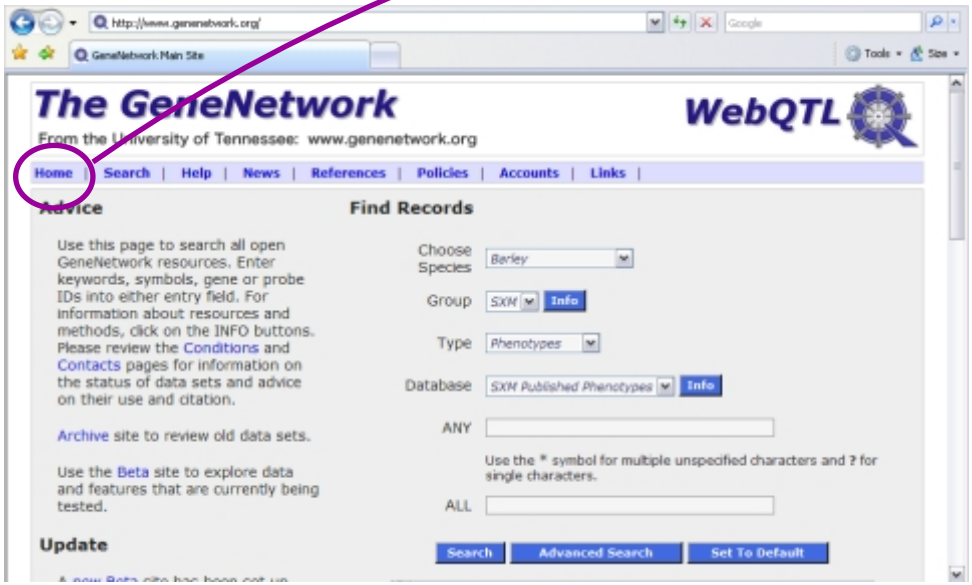
How to enter your own data set into the GeneNetwork?

Format of the file to be entered into the GeneNetwork for analysis



@format=column	FP_95_Fargo_PD1	FP_95_Fargo_PD2	FP_95_Langdon_PD2
SM001	28	20	2
SM002	30	20	6
SM003	40	x	6
SM004	32	14	6
SM005	x	x	x
SM006	10	10	6
SM007	x	x	0
SM009	12	8	2
SM010	0	22	12
SM011	0	44	4
SM012	20	20	0
SM013	20	20	20
SM014	26	12	10
SM015	16	16	4

How to enter your own data set into the GeneNetwork?



Batch Submission
Select SXM
Type in or browse to your data file

Describes the format of the data file

Mapping genes or Mendelian (binary) traits using GeneNetwork

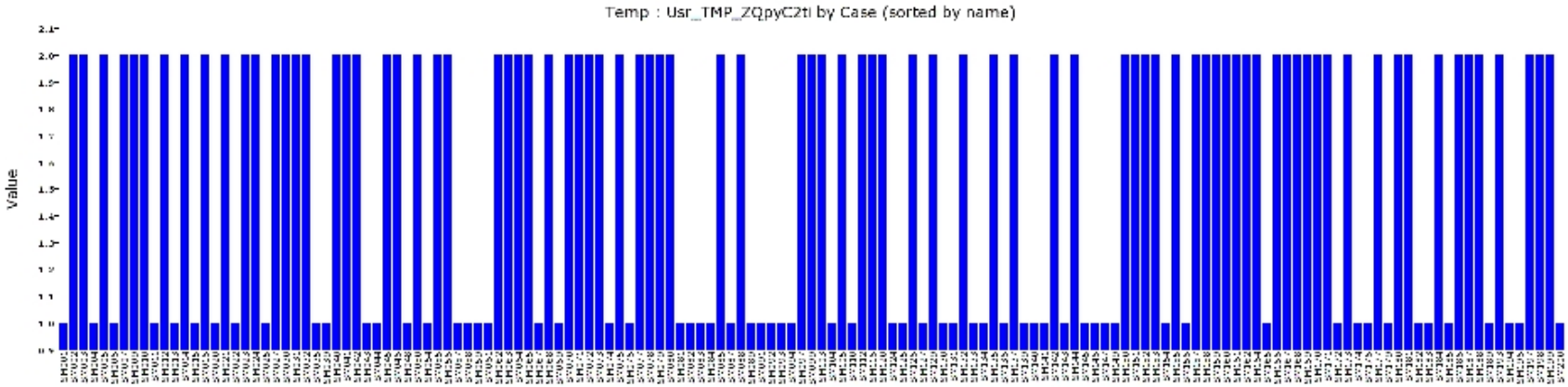
1) convert genotype scores or trait values to binary numerical values, eg 1,2; -1,1 or 0,1;

2) format as shown in the previous page;

3) enter the data into the GeneNetwork;

4) submit to the 'Marker Regression' to identify map position.

Output generated by using 'Basic Statistics' function with genotype scores (Steptoe '1' and Morex '2') of one of the markers.



Marker Regression Report

The following loci in the SXM data set have associations with the above trait data.

LRS	Chromosome	Locus	Additive Effect
162.371*	2	snp_1061	-0.410
192.512*	2	snp_1054	-0.429
214.643*	2	MWG557	-0.442
235.056*	2	snp_1272	-0.447
235.856*	2	CDO537	-0.450
302.873*	2	ABG316C	-0.469
334.595*	2	snp_0474	-0.475
376.050*	2	glad_259	-0.480
376.050*	2	P40FA	-0.480
334.152*	2	Hvex1	-0.473
302.409*	2	snp_1121	-0.465
237.836*	2	snp_0545	-0.444
168.552*	2	snp_0265	-0.411
158.332*	2	snp_0407	-0.404
148.992*	2	bBE54D	-0.398
132.498*	2	snp_0650	-0.386
111.777*	2	snp_1449	-0.365
105.611*	2	snp_1435	-0.358

High LRS clearly indicates co-segregating markers.