## **USER GUIDE**

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## 1. Overview

The Gene4Breed web-tool has been designed to facilitate the data integration and its application to plant breeding programs, considering data structure and standardization as a preliminary step to improve data management. Gene4Breed holds diverse variety of data types, ranging from genotypic (SNP and other types of markers) to phenotypic data (morphological and biochemical markers), and allows querying between such data from a minor crop species with not abundant public data repositories.

Data are stored in Gene4Breed in a technology-independent manner, so that new technologies can be accommodated in the database as they appear, without modification of the underlying schema.

Plant breeders and researchers can access data in Gene4Breed databases via a lightweight Python framework Web interface.

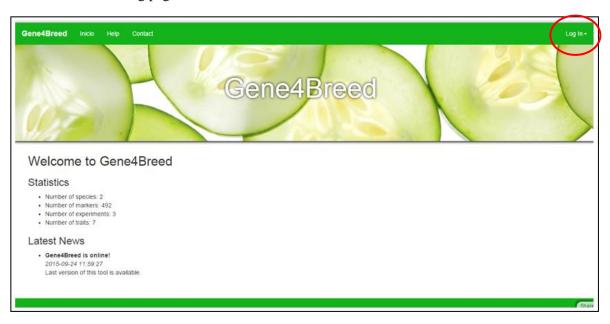
Gene4Breed specializes in storage of markers, traits, plant lines, genotypes and phenotypes, allowing plant breeders and researchers to run some preliminary analysis to take decisions in their day by day work.

The final goal of Gene4Breed is to help in the characterization of the genetic basis of variation in plants and linking them to observable agronomic traits (phenotype) to significantly increase the efficiency of selection in plant breeding for developing highly adapted plant material.

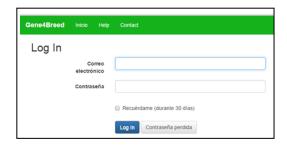
# 2. Accessing the Gene4Breed Database

The Gene4Breed Database can be accessed at <a href="http://jordiquilis.cat/gene4breed/">http://jordiquilis.cat/gene4breed/</a>

This is the welcoming page:



User can  $\log$  in the <u>Log in</u> field on the top right (red circle) and the following dialogue box will appear:

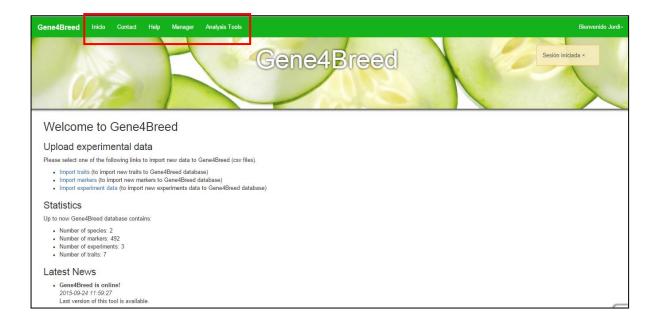


User will be required to fill email and password information and then click on "Log in"

## 3. Main Menu

This is the Main/Home Menu. The Gene4Breed home page is divided into two frames.

The upper frame appears in all the Gene4Breed interfaces. User can browse the web-based tool by clicking the buttons of each section (highlighted in the red box), which are **Home**, **Contact**, **Help**, **Manager** and **Analysis Tools**. This buttons have further submenus.



In the central frame there are three sections. **Upload experimental data** (to import new data to the database), **Statistics** (general information about the data included in the database) and **Latest News**.

In the **Upload experimental data section,** user can choose various importing options from here. Importing data to traits, markers and experiments can be access by clicking the respective links.

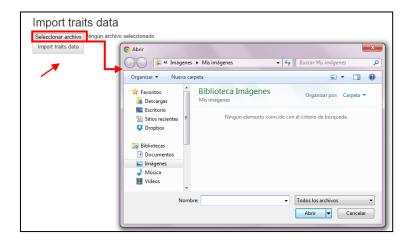
## 4. Upload Experimental Data

On **Upload Experimental Data**, user can select from three options available for importing new data to Gene4Breed: • "Import traits" • "Import markers" • "Import experiment data".

All the upload experimental data have the same structure. User could follow the import traits system described below as an example.

### - Import traits

Click on **Import traits** to bulk new traits originally in CSV format into the database.



Then choose the input CSV file containing the traits data and press the "Import traits data" button. If the data is correctly imported, a message will be displayed confirming this situation. If there is some error importing the data, you would need more information about the error displayed in the form.

## **Templates for data importation**

The uploaded file has to follow the following formats depending on the table where is imported.

Header to each column is important to head the database structure, that's why the header columns must be named exactly as it is shown in the template.

The order of the columns is not mandatory.

An example of each template is shown here.

#### A) Traits

In this case, the header columns must be named: **traits.id**, **traits.species**, **traits.name**, **traits.acronym**, **traits.genetic\_control**, **traits.dominance**, **traits.related\_markers**, **traits.description**, **traits.project**, as it is shown in the template (highlighted in red box)

Į	Λ	<u>D</u>		<u> </u>	<u> </u>	F		н	
	traits.id	traits.species	traits.name	traits.acronym	traits.genetic _control	traits.dominance	traits.related_ markers	traits.description	traits.project
	1	Cucumber	PM	PM	Oligogenic	Dominant	Pmh1 Pml1 Pmh2 Pml2	Oidium Resistance Gene	UM001
	2	Cucumber	CVYV	CVYV	Monogenic	Dominant	Cs_0354 CVYVSNP1 CVYVSNP2	Cucumber vein yellowing virus	UM002
	3	Cucumber	Blue-Leaf	Blue-Leaf	Polygenic	Recessive	-	Blue leaf	UM003
	4	Cucumber	CYSDV	CYSDV	Oligogenic	Recessive	-	Cucurbit yellow stunting disorder virus	UM004
	5	Cucumber	Compact Plant	СР	Polygenic	Recessive	-	Compact Plant	UM005

### B) Markers

In this case, the header columns must be named: markers.id\_markers, markers. Species, markers. Chromosome, markers. name1, markers. name2, markers. chr\_position, markers.marker\_sequence, markers.marker\_type, markers.variant\_type, markers.related\_traits, markers.project, as it is shown in the template (highlighted in red box)

A B		С	D	Е	F	G	Н	I	J	K	
markers.id_ markers	markers. species	markers. chromosome	markers. name1	markers. name2	markers. chr_position	markers.marker_sequence	markers.mar ker_type	markers. variant_type	markers.related _traits	markers.project	
1	Cucumber	Chr1	Cs 0001		2601022	TATTGGTCATCTGATTTGTTCTATTCACCTATTTAATATCAAGCA	KΔSPΔr	SNP			
2	Cucumber	Chr1	Cs_0049		5365857	ATTTTCCCTCTTGAACTGTATTAGTATAACCAAGCGCTTCATTA	KASPAr	SNP		UM001	
3	Cucumber	Chr1	Cs_0050		6076637	CGGTTGGTTTAGCTCCGCTCACCTCATCAATCCTAGAGTCTTTA	KASPAr	SNP		UM001	
4	Cucumber	Chr1	Cs_0051		6781627	TTTGTTCAAGTCCAGGATAGAACCTTTTCAATTGATGAAGAGA	KASPAr	SNP		UM001	
5	Cucumber	Chr1	Cs_0002		7675951	AAGATATTGTTTACTTTAAACGTAAATTTAATGTGATTAAGTTT	KASPAr	SNP		UM001	
6	Cucumber	Chr1	Cs_0052		7979834	AATCCACACCCCAGCCTTAGGTAGTCCTCCTTCCACAAAATTC	KASPAr	SNP		UM001	
7	Cucumber	Chr1	Cs_0053		8303304	CACTGGTTGTTTTTGCCAATGGCAAGATTAGGGTTTCGTCATT	KASPAr	SNP		UM001	
8	Cucumber	Chr1	Cs_0054		8678846	TGCCACTAAATCATTATGTGTCTCTCTCTAAAGGAATGAGAGC	KASPAr	SNP		UM001	
9	Cucumber	Chr1	Cs_0055		9145004	GCACTCTCCTTCCGAGCATACTATTAATGGTGAAAAATTCGCA	KASPAr	SNP		UM001	
10	Cucumber	Chr1	Cs_0056		9525867	GCCTCTCCCGACGCCTCAGTTTGTGCATCGGGAGAGGCCCCTA	KASPAr	SNP		UM001	
11	Cucumber	Chr1	Cs_0057		9888873	ACTGTTTTCAAGTGCAAAACAAAAATAGTTAGAAACACGACC	KASPAr	SNP		UM001	
12	Cucumber	Chr1	Cs_0058		10172339	TTAAAATTAGTTGGAATAATGATTAAATGATCCATAAATAGTA	KASPAr	SNP		UM001	
13	Cucumber	Chr1	Cs_0059		10513529	ATTAAAAGCCTAAGTGCTTTATGCTTTAAAATAACAAAATATG	KASPAr	SNP		UM001	
14	Cucumber	Chr1	Cs_0060		10743532	CCAAGATTTTGCTTGTCTATGGTCATGATTAAAAAATTCAAGA	KASPAr	SNP		UM001	
15	Cucumber	Chr1	Cs_0061		10949000	AAAAGTTCAAAAGATAATTCATTTACAAAGTGTAGCAAACCA	KASPAr	SNP		UM001	
16	Cucumber	Chr1	Cs_0003		11278261	GACATCCTAAGGAAAGTAAATTTCTACCTTTGGTATTGTTTGT	KASPAr	SNP		UM001	
17	Cucumber	Chr1	Cs_0062		11518666	TGGTAAAGGAAATACAAATGGATAGTAACACCGACGTATTTA	KASPAr	SNP		UM001	
18	Cucumber	Chr1	Cs_0063		11826376	TCTTATCCAATTCCTTTTCTTCTTTCCTTCGCCGGCGATGCCTTT	KASPAr	SNP		UM001	
19	Cucumber	Chr1	Cs_0064		12101615	ATACAAAAGTGATATTGGCGCGGCTCAATCAGAGATCGTACT	KASPAr	SNP		UM001	
20	Cucumber	Chr1	Cs_0065		12430196	AATAAGGAAGAAAGAAAGCACAAAAGTTGGGGCTTTGCT	KASPAr	SNP		UM001	
21	Cucumber	Chr1	Cs_0066		12729237	GATTTAAATCACCCTCTACAACTTTCATAAACCATATATGATAT	KASPAr	SNP		UM001	

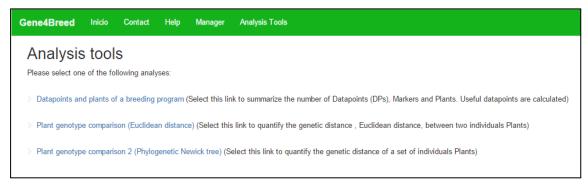
### C) Experiments

In this case, the header columns must be named: experiments.name, experiments.experiment\_date, experiments.users, species.name, plant\_lines.species\_type, plant\_lines.generation, plant\_lines.treatment\_num, plant\_lines.plant\_num, plant\_lines.material\_code, plant\_lines.plot\_nr, plant\_lines.p1, plant\_lines.p2, plant\_lines.pedigree, plant\_lines.declared \_resistance, plant\_lines.lab\_code, Pmh1, Pml2, Cs\_0002, Cs\_0004, ... as it is shown in the template (highlighted in red box)

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A	В	С	D	E	F	G	Н		J	K	L	M	N	0	Р	Q	R	S	Т	U	V	W
experiments. name	experiments. experiment_ date	experiments. users	species.name	plant_lines. species _type	plant_lines. generation	plant_lines. treatment_ num	plant_lines. plant_num	plant_lines. material _code	plant_lines. plot_nr	plant_lines.p1	plant_lines.p2	plant_lines. pedigree	plant_lines. declared _resistance	plant_lines.lab _code	Pmh1	Pml1	PmI2	Cs_000 2	Cs_000 4	Cs_000 5	Cs_000 7	Cs_0008
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	1	2	FRADERA	1-10	M001	AUUZ	-	Fmn	MCRF15-5176	А	А		· ·	· ·	U	A	U
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	1	3	PRADERA	T-10	A001	A002	-	Pmh	MCAP15-5177	A	A	В	C	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	1	4	PRADERA	T-10	A001	A002	-	Pmh	MCAP15-5178	A	Α	В	C	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	2	1	TARAY	T-11	A001	A003	-	Pmh	MCAP15-5191	A	Α	Α	C	G	С	H	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	2	2	TARAY	T-11	A001	A003	-	Pmh	MCAP15-5192	A	A	A	С	G	С	H	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	3	1	BENITO	T-12	A001	A004	-	Pmh	MCAP15-5204	В	Н	В	С	H	H	G	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	3	2	BENITO	T-12	A001	A004	-	Pmh	MCAP15-5205	В	H	nd	C	H	H	G	H
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	3	3	BENITO	T-12	A001	A004	-	Pmh	MCAP15-5206	В	H	В	C	H	H	G	H
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	3	4	BENITO	T-12	A001	A004	-	Pmh	MCAP15-5207	В	Н	В	C	H	H	G	H
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	4	1	MITRE	T-13	A001	A005	-	Pmh	MCAP15-5221	H	A	nd	C	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	4	2	MITRE	T-13	A001	A005	-	Pmh	MCAP15-5222	H	A	В	C	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	4	3	MITRE	T-13	A001	A005	-	Pmh	MCAP15-5223	н	Α	В	nd	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	4	4	MITRE	T-13	A001	A005	-	Pmh	MCAP15-5224	Н	Α	nd	C	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	5	1	ENCINA	T-14	A001	A007	-	Pmh	MCAP15-5238	H	Α	Н	C	G	С	A	H
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	5	2	ENCINA	T-14	A001	A007	-	Pmh	MCAP15-5239	н	A	H	C	G	С	Α	H
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	6	1	HOL14001	T-15	A001	-	-	Pmh	MCAP15-5253	н	Α	В	С	G	С	Α	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	6	2	HOL14001	T-15	A001	-	-	Pmh	MCAP15-5254	н	Α	В	С	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	7	1	Hipower	T-16	A001	A009	-	Pmh	MCAP15-5270	н	н	н	С	G	С	Α	Н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	7	2	Hipower	T-16	A001	A009	-	Pmh	MCAP15-5271	H	H	H	С	G	С	Α	H
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	7	3	Hipower	T-16	A001	A009	-	Pmh	MCAP15-5272	nd	н	Н	С	G	С	Α	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	7	4	Hipower	T-16	A001	A009	-	Pmh	MCAP15-5273	н	н	nd	С	G	С	Α	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	8	1	Kasja	T-17	A003	A009	-	Pmh	MCAP15-5290	A	Α	Α	С	H	A	Α	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	8	2	Kasja	T-17	A003	A009	-	Pmh	MCAP15-5291	A	A	A	С	н	A	A	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	8	3	Kasja	T-17	A003	A009		Pmh	MCAP15-5292	Α	Α	Α	С	н	A	A	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	F1	8	4	Kasja	T-17	A003	A009	-	Pmh	MCAP15-5293	Α	Α	Α	С	н	A	Α	н
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	LP	9	1	CS 1102-142	1824	CS 1102-142	-	Manglar	Pmh	MCAP15-5440	Α	Α	В	С	G	С	Α	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	LP	9	2	CS 1102-142	1824	CS 1102-142		Manglar	Pmh	MCAP15-5441	Α	Α	В	С	G	С	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	LP	10	1	CS 1102-144	1825	CS 1102-144		Manglar	Pmh	MCAP15-5454	A	A	В	C	G	C	A	G
MCAP15	25/07/2015	Jordi Quilis	Cucumber	HOL	LP	10	2	CS 1102-144	1825	CS 1102-144	-	Manglar	Pmh	MCAP15-5455	A	Α	В	С	G	С	A	G
MCAP1E	25/07/2015	Jordi Ouilie	Cucumbor	HOI	1.0	11	- 1	CC 1221 40	1026	CC 1221 40		Manglar	Pmh	MCAD1E EAEO						_	۸	G

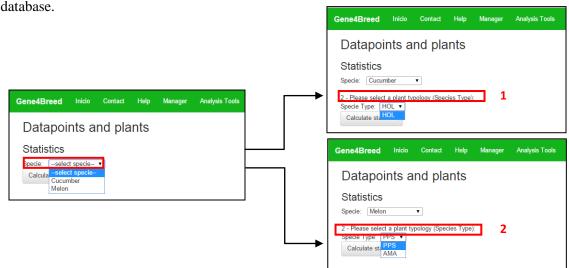
## 5. Analysis Tools

When you click **Analysis Tools**, three options are available on the link menu in the Statistics panel: • "**Datapoints and plants**" • "**Plant genotype comparison**" • "**Plant genotype comparison** 2 (**Biopython**)"



### - Datapoints and Plants of a Breeding Program

Click **Datapoints and Plants** to consult statistical parameters of data points and plants into the



**Species** and **Species type** options are available on the drop-down menu. User can choose first **Species** available in Gene4Breed and then Plant typology (**Species Type**) and press the "<u>Calculate Statistics</u>" button. In the screenshot example, 2 species can be selected, cucumber and melon. From this first selection, different Species types can be selected in the following level, cucumber (red box 1) or melon typologies (red box 2).

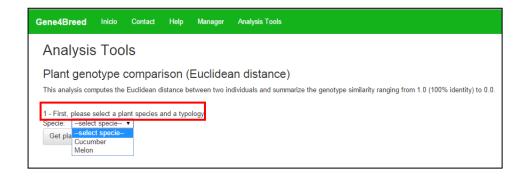
The database would show a list with Total DPs, number of markers, number of plants, number of useful DPs and percentage of useful DPs.



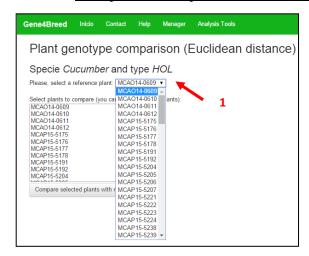
## - Plant Genotype Comparison (Euclidean distance)

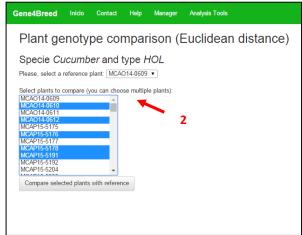
Click **Plant Genotype Comparison** to consult statistical parameters of plant genotype comparison quantifying the genetic distance (Euclidean distance) between two individuals into the database.

Species and Species type (typology) options are available on the drop-down menu. User can choose first Species (melon or cucumber) and then Plant typology (HOL, PPS or AMA) and press the "Get Plants" button.

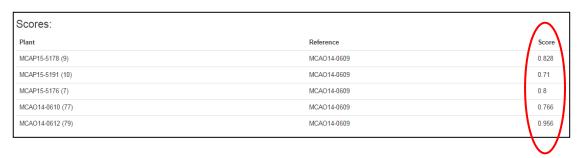


The database displays first a list of all the plants available for use as Reference plant (1) and then a list of all plants to be compared with (2). Use the ctrl command to select multiple plants. Press the "Compare selected plants with reference" button.





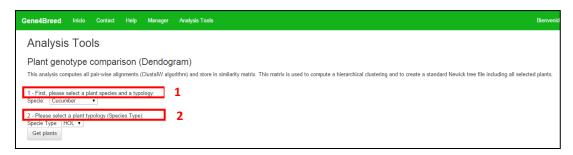
This analysis computes the Euclidean distance between two individuals (reference and selected plant) and summarize the genotype similarity ranging from 1.0 (100% identity) to 0.0. User could obtain a list with Scores (highlighted in red circle).



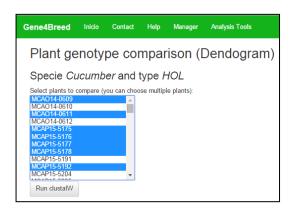
## - Plant Genotype Comparison 2 (Dendogram)

Click **Plant Genotype Comparison 2** to consult statistical parameters of plant genotype comparison obtaining genotyped clusters representation (dendogram).

Species and Species type (typology) options are available on the drop-down menu. User can choose first Species (e.g. melon or cucumber) and then Plant typology (e.g. HOL, PPS or AMA) and press the "Get Plants" button.



The database would display a list of all the plants available for comparison between them. Use the ctrl command to select multiple plants, and then press the "Run Clustalw" button.

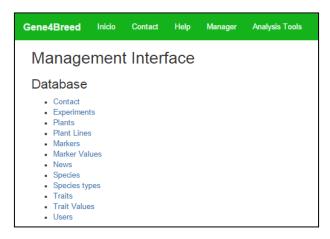


This analysis computes all pair-wise alignments (ClustalW algorithm) and stores a similarity matrix. This matrix is used to compute a hierarchical clustering and to create a standard Newick tree file including all selected plants. User can obtain a genotyped cluster or dendogram.

## 6- Manager

As a user of Gene4Breed in the role of manager of the application, user would need to add, remove or update any data in the data base.

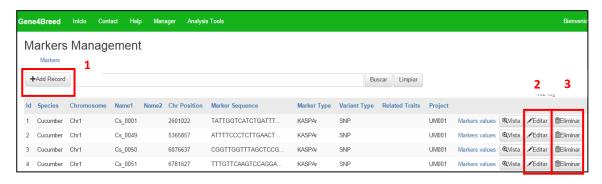
When you click **Manager**, twelve links are available on the list of database entities of the Manager panel: • "Contact" • "Experiments"• "Plants"• "Plant Lines"• "Markers"• "Marker Values"• "News"• "Species"• "Species types"• "Traits"• "Trait Values"• "Users".



All the management database entities have the same structure. User could follow the markers management described below as an example.

## - Markers management

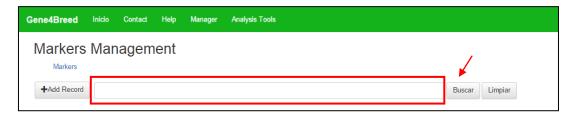
Click **Markers** on Management Interface and then a list for all the markers in the system will be displayed.



User would have the option to **Add** a new one (box 1), to **Edit** an existing one (box 2) or to **Remove** (box 3) a selected marker. After the changes, the database would change accordingly.

#### **Basic Search**

User could also do **Basic Searches** simply entering the terms in the search box (highlighted in red box) and click on the "Search" button:



The search engine will search for the term ranking the entries by "Id". Searches are case-insensitive, so a search for "kaspar", "KASPAr", or "KASPAR" will return the same entries.

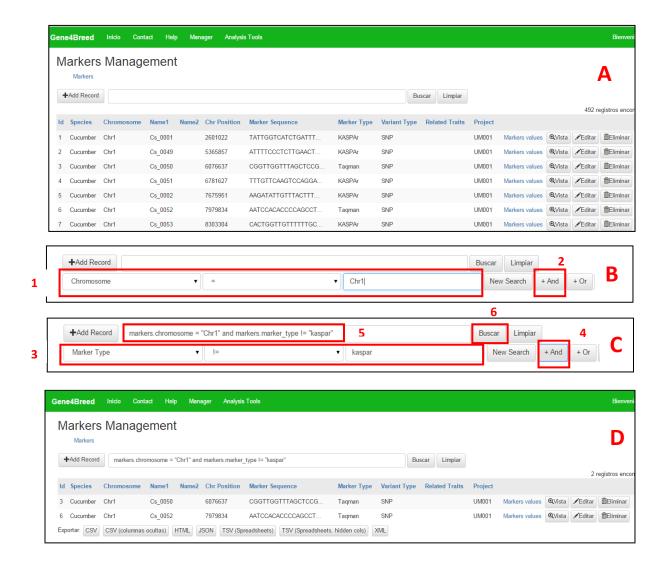
### **Search with Operators**

User could also do a **Search with Operators** adding a determinate operator before specific terms.

- Logical operators that user could use are:
- **AND** operator will returned entries containing both terms (this Boolean operator narrows the search).
- **OR** operator will returned entries containing any of the terms, or both (this Boolean operator broadens the search).
- Comparison operators that user can use are (depending on the type of data):
  - "="(equal) operator will ensure that the term appear in the entries returned
  - "!=" (not equal) operator will exclude terms that contain it.
  - "<"(less than) operator will returned entries lower than the term
  - ">" (greater than) operator will returned entries higher than the term
  - "<=" (less than or equal to) operator will returned entries lower or equal than the term
  - ">="(greater than or equal to) operator will returned entries higher or equal than the term
  - "Starts with" operator will returned entries that start with the term
  - "Contains" operator will returned entries that contains the term
  - "in" operator will returned entries that are in the term
  - "not in" operator will returned entries that not are in with the term

<u>Example 1:</u> To obtain all the markers with typology different to KASPAr mapped on a determinate chromosome.

Thus, user could enter the term "Chromosome = Chr1" in the search box (Panel B, box 1), and click the "+And" button (Panel B, box 2). Then user could enter the term "Marker Type != KASPAr" in the search box (Panel C, box 3), and click the "+And" button (Panel C, box 4). In the search box will appear: markers chromosome="Chr1" and markers.marker\_type !="kaspar" (Panel C, box 5). Click the "Search" button (Panel C, box 6). The returned entries will contain the terms "Ch1" as chromosome but not contain the term "KASPAr" as marker\_type (Panel A vs result view of Panel D).

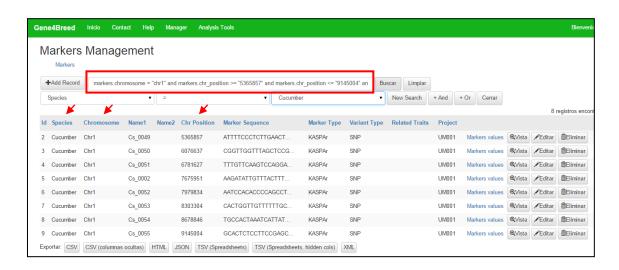


The resulting entries are displayed in a formatted table as is shown above. Obtained data can be also exported using CSV and other formats (this is explained in the **Exporting data section**).

<u>Example 2:</u> To obtain all the markers scattered between two specific positions on a determinate chromosome species.

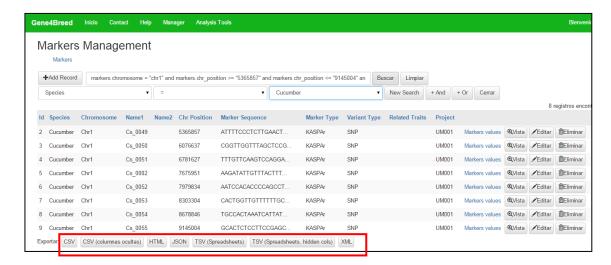
Thus, user could enter the term "Chromosome = Chr1" in the search box, click on the "+And" button, enter the term "chr\_position=>5365857" in the search box, click on the "+And" button, enter the term "chr\_position<=9145004" in the search box, click on the "+And" button, enter the term "Species=cucumber" in the search box. In the search box will appear: markers chromosome="Chr1" and markers chr\_position>="5365857" and markers chr\_position<="9145004" and markers species="cucumber" (highlighter in red box). Click the "Search" button. The returned entries will contain the terms "Ch1" as chromosome and "cucumber" as species and show all the markers located between the two positions.

The resulting entries are displayed in a formatted table as is shown below. Obtained data can be also exported using CSV and other formats (this is explained in the **Exporting data section**).



### **Exporting data section**

User could **Export data** resulting from a search simply clicking the button of the <u>specific</u> <u>format</u>, which are at the bottom of the result view (box red):



The different formats for exporting data which are available in the database are: **CSV** (Comma Separated Values), **HTML** (HyperText Markup Language), **JSON** (JavaScript Object Notation), **TSV** (Tab Separated Values) and **XML** (eXtensible Markup Language)

Following the *Example 2* of the Search Section, user could exported the obtained data using CSV format by clicking "CSV" button (Box red, Panel A). User will be obtained a <u>rows.csv</u> <u>archive</u> (Result view: Panel B).

