A quick guide on *Boechera* Microsatellite Website (BMW)

Boechera Microsatellite Website is a portal that archives over 100,000 microsatellite allele calls from 4471 specimens (including 133 nomenclatural types). Here you can search and retrieve basic metadata using the Extrac# (a unique ID number annotated on every voucher specimen; e.g. JB111, CR1004, FW1222, …), taxon name (e.g. acruata, crandallii, …), or locality (e.g. Utah, Nevada, …). Searches based on taxon name can be limited to sexual diploids (i.e., just the epithet entered) or expanded to include all hybrids containing that genome. The output of a taxon query can be used to produce working state and county level distribution lists free of the misidentifications common in other on-line resources. The search by locality function allows investigators to quickly assemble checklists of confirmed *Boechera* identifications for any state, province, or county.

In addition to the basic search and display functions, we also include two novel algorithms: “Taxon Enquiry based on Similarity of Loci and Alleles” (TESLA) for sample identification, and “Parental Relationship Identification Using Subtraction” (PRIUS) for inferring the genomic constitution of hybrid individuals. See below for TESLA and PRIUS walk-through.

TESLA: Taxon Enquiry based on Similarity of Loci and Alleles

TESLA is a microsatellite-based species identification program. Once a user enters allelic information for their sample, TESLA compares this multi-locus genotype to every accession in the database, calculating an Allele Similarity score (AS) between two accessions. The AS score represents the proportion of alleles that are shared relative to the query, scaled by the amount of missing data. The output of a TESLA query consists of the specimens with the 100 highest AS scores listed in descending order. If “Search only sexual diploids” option is checked, TESLA will ignore any specimen in the database that has heterozygosity over 0.5.

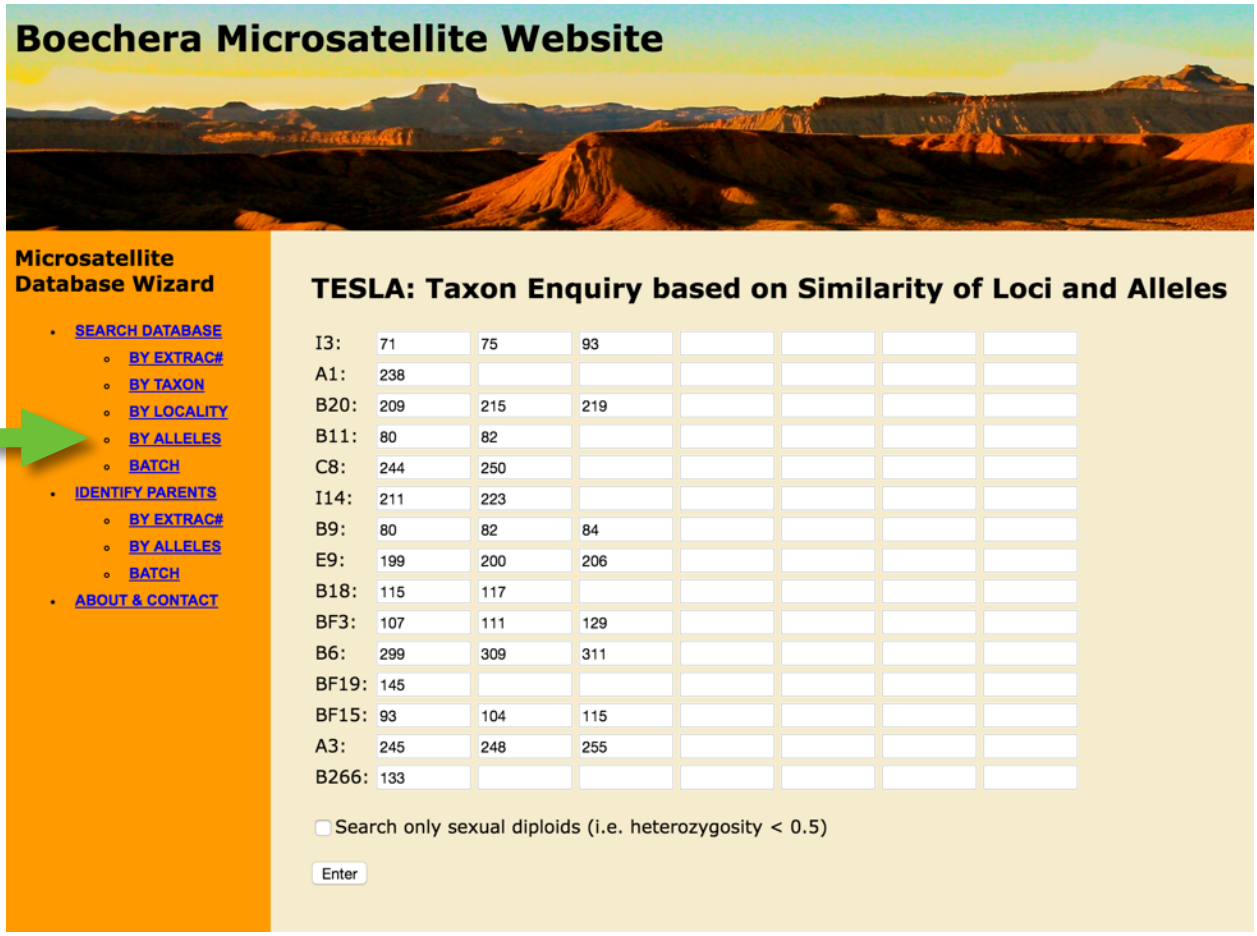
1. Run TESLA on samples aleady in the database

On the left panel, under “SEARCH DATABASE”, click “BY EXTRAC#” (green arrow), and you will enter the following page. You can then type in the Extrac# (a unique ID number annotated on every voucher specimen), and BMW will run TESLA to search the database for the closest multi-locus genotypes.

2. Run TESLA on your own samples

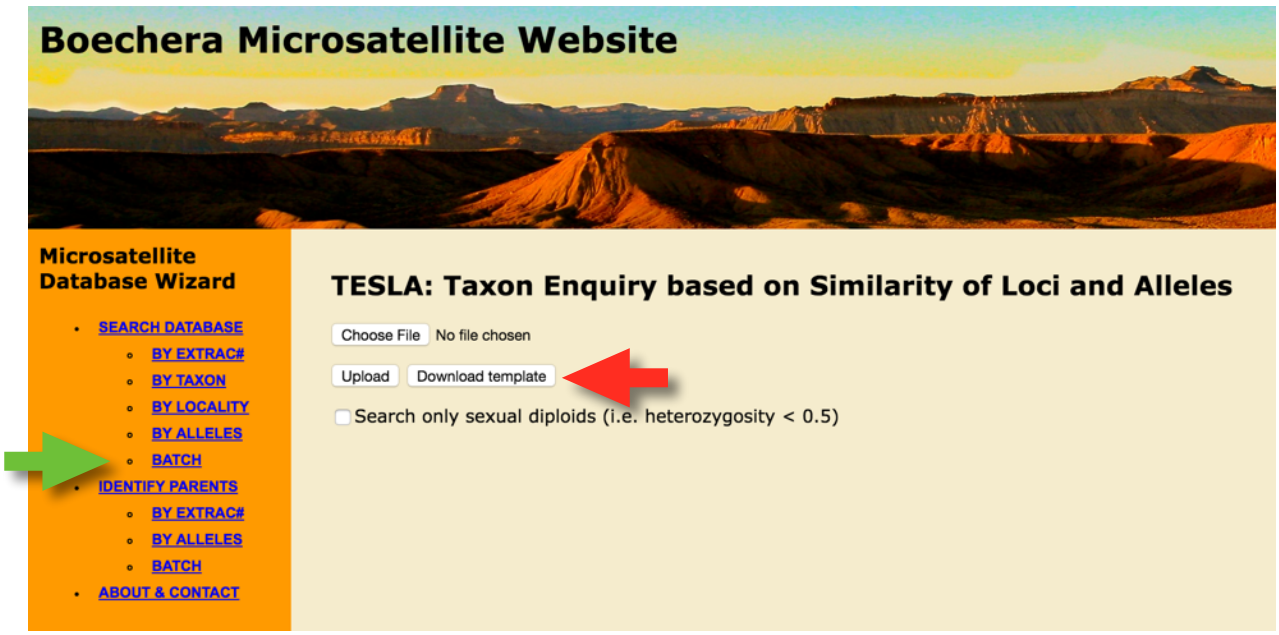
Option 1 - enter the microsatellite genotype manually

On the left panel, under “SEARCH DATABASE”, click “BY ALLELES” (green arrow), and you will see the following page. You would then need to enter the microsatellite alleles (as integers) for each of the 15 loci. Once complete, hit “Enter” to run TESLA on your own sample.



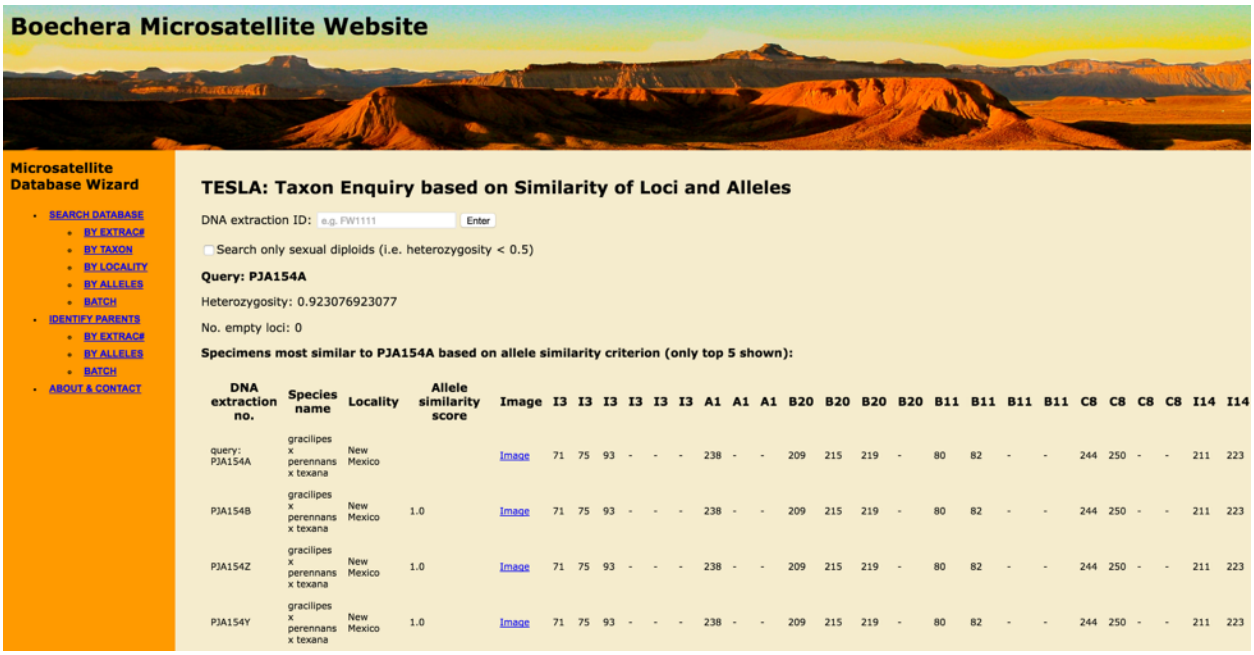
Option 2 - batch search by uploading a text file

If you have multiple samples and do not want to type them individually, you can use the batch function. Download the template first (red arrow), and use it to prepare your data sheet. It's very important to: (1) not delete or insert columns, and (2) save and upload it as a tab-delimited text file, not as an excel file.



3. Example TESLA output

Below shows the output from running TESLA on PJA154A. TESLA successully identified similar trigenomic hybrids (*Boechera perennans* x *B. texana* x *B. gracilipes*) in the database.



Boechera Microsatellite Website

Microsatellite Database Wizard

- SEARCH DATABASE
  - BY EXTRAC#
  - BY TAXON
  - BY LOCALITY
  - BY ALLELES
  - BATCH
- IDENTIFY PARENTS
  - BY EXTRAC#
  - BY ALLELES
  - BATCH
- ABOUT & CONTACT

TESLA: Taxon Enquiry based on Similarity of Loci and Alleles

DNA extraction ID: e.g. FW1111 Enter

Search only sexual diploids (i.e. heterozygosity < 0.5)

Query: PJA154A

Heterozygosity: 0.923076923077

No. empty loci: 0

Specimens most similar to PJA154A based on allele similarity criterion (only top 5 shown):

DNA extraction no.	Species name	Locality	Allele similarity score	Image	I3	I3	I3	I3	I3	I3	A1	A1	A1	B20	B20	B20	B20	B11	B11	B11	B11	C8	C8	C8	C8	I14	I14
query: PJA154A	gracilipes x perennans x texana	New Mexico		Image	71	75	93	-	-	-	238	-	-	209	215	219	-	80	82	-	-	244	250	-	-	211	223
PJA154B	gracilipes x perennans x texana	New Mexico	1.0	Image	71	75	93	-	-	-	238	-	-	209	215	219	-	80	82	-	-	244	250	-	-	211	223
PJA154Z	gracilipes x perennans x texana	New Mexico	1.0	Image	71	75	93	-	-	-	238	-	-	209	215	219	-	80	82	-	-	244	250	-	-	211	223
PJA154Y	gracilipes x perennans x texana	New Mexico	1.0	Image	71	75	93	-	-	-	238	-	-	209	215	219	-	80	82	-	-	244	250	-	-	211	223

PRIUS: Parental Relationship Identification Using Subtraction

The basic workflow of PRIUS is as follows: (i) identify the most similar sexual diploid specimens in the database based on AS score, (ii) subtract from the hybrid's multi-locus genotype the corresponding alleles from each top-scoring specimen, and (iii) repeat the previous steps using the subtracted genotype for one or two more iterations (see below). The end result is a list of diploid specimen combinations that best explain the hybrid's MLG. The corresponding species of these diploid specimens then form a working hypothesis of the putative parental species.

**Note that the PRIUS computation is demanding, and could take a while to finish; please be patient!

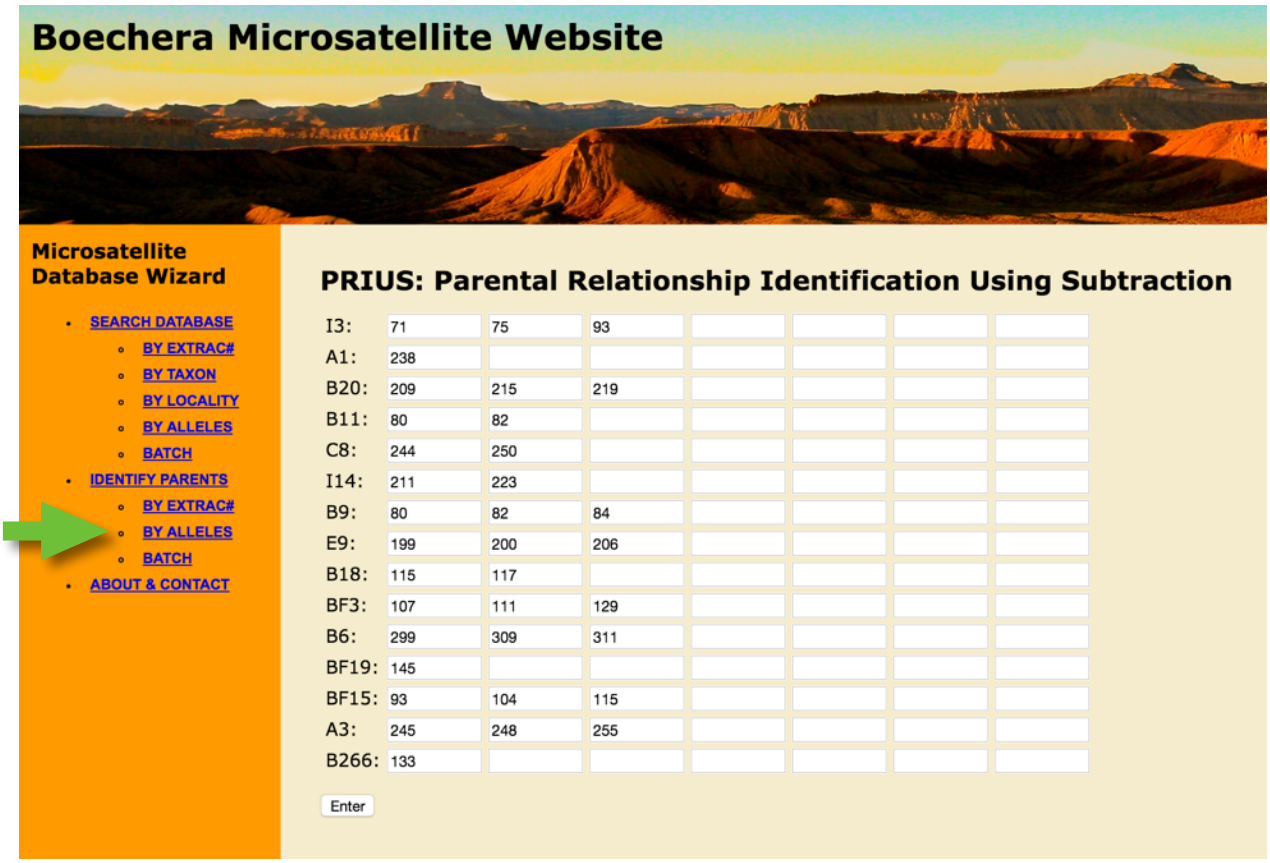
1. Run PRIUS on samples aleady in the database

On the left panel, under "IDENTIFY PARENTS", click "BY EXTRAC#" (green arrow), and you will see the following page. You can then type in the Extrac# (a unique ID number annotated on every voucher specimen), and BMW will run PRIUS to search the database for the putative parents.

2. Run PRIUS on your own samples

Option 1 - enter the microsatellite genotype manually

On the left panel, under “IDENTIFY PARENTS”, click “BY ALLELES” (green arrow), and you will see the following page. You would then need to enter the microsatellite alleles (as integers) for each of the 15 loci. Once complete, hit “Enter” to run PRIUS on your own sample.



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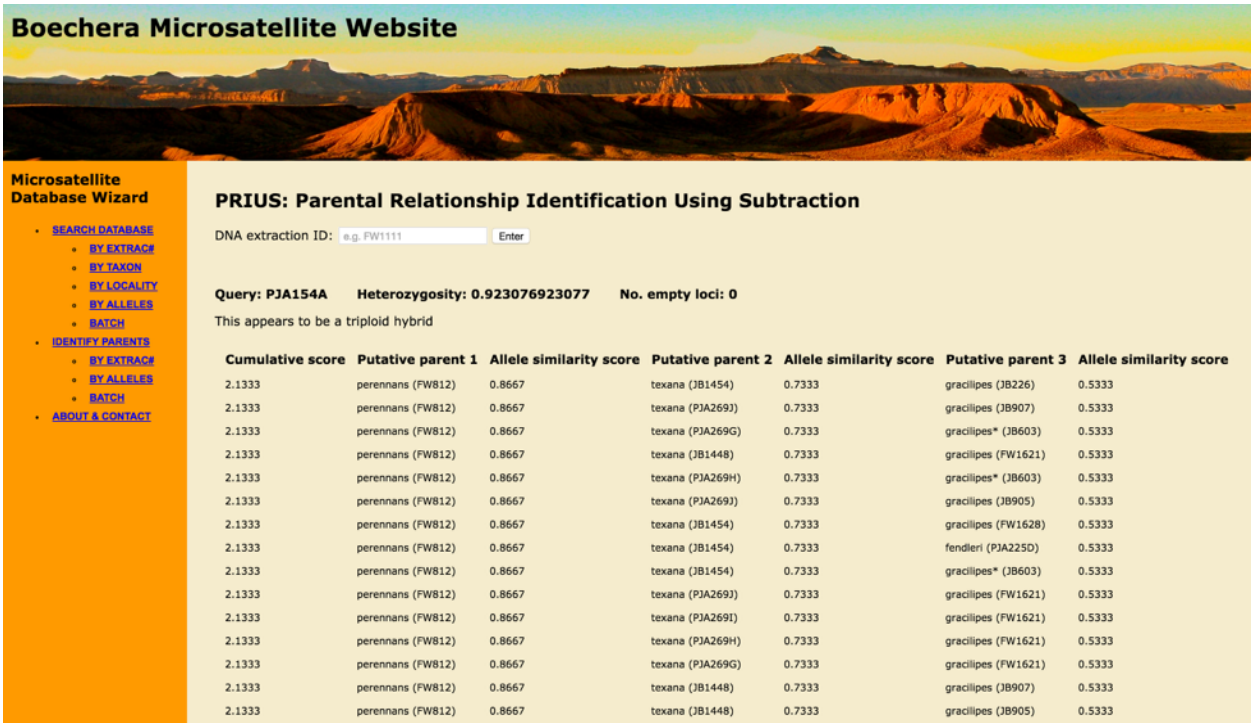
3. Example PRIUS output

Below shows the output from running PRIUS on PJA154A. PRIUS clearly identified this specimen as a trigenomic hybrid of *Boechera perennans*, *B. texana*, and *B. gracilipes*.



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PRIUS: Parental Relationship Identification Using Subtraction

DNA extraction ID: e.g. FW1111 Enter

Query: PJA154A **Heterozygosity: 0.923076923077** **No. empty loci: 0**

This appears to be a triploid hybrid

Cumulative score	Putative parent 1	Allele similarity score	Putative parent 2	Allele similarity score	Putative parent 3	Allele similarity score
2.1333	perennans (FW812)	0.8667	texana (JB1454)	0.7333	gracilipes (JB226)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269J)	0.7333	gracilipes (JB907)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269G)	0.7333	gracilipes* (JB603)	0.5333
2.1333	perennans (FW812)	0.8667	texana (JB1448)	0.7333	gracilipes (FW1621)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269H)	0.7333	gracilipes* (JB603)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269J)	0.7333	gracilipes (JB905)	0.5333
2.1333	perennans (FW812)	0.8667	texana (JB1454)	0.7333	gracilipes (FW1628)	0.5333
2.1333	perennans (FW812)	0.8667	texana (JB1454)	0.7333	fendleri (PJA225D)	0.5333
2.1333	perennans (FW812)	0.8667	texana (JB1454)	0.7333	gracilipes* (JB603)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269J)	0.7333	gracilipes (FW1621)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269I)	0.7333	gracilipes (FW1621)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269H)	0.7333	gracilipes (FW1621)	0.5333
2.1333	perennans (FW812)	0.8667	texana (PJA269G)	0.7333	gracilipes (FW1621)	0.5333
2.1333	perennans (FW812)	0.8667	texana (JB1448)	0.7333	gracilipes (JB907)	0.5333
2.1333	perennans (FW812)	0.8667	texana (JB1448)	0.7333	gracilipes (JB905)	0.5333