Go to the link below to download two text files (.txt) to your desktop

Folder: Pigweed FASTA Sequences
File 1: Unknown Pigweed
File 2: Pigweed Library

grownextgen.org/go/pigweed

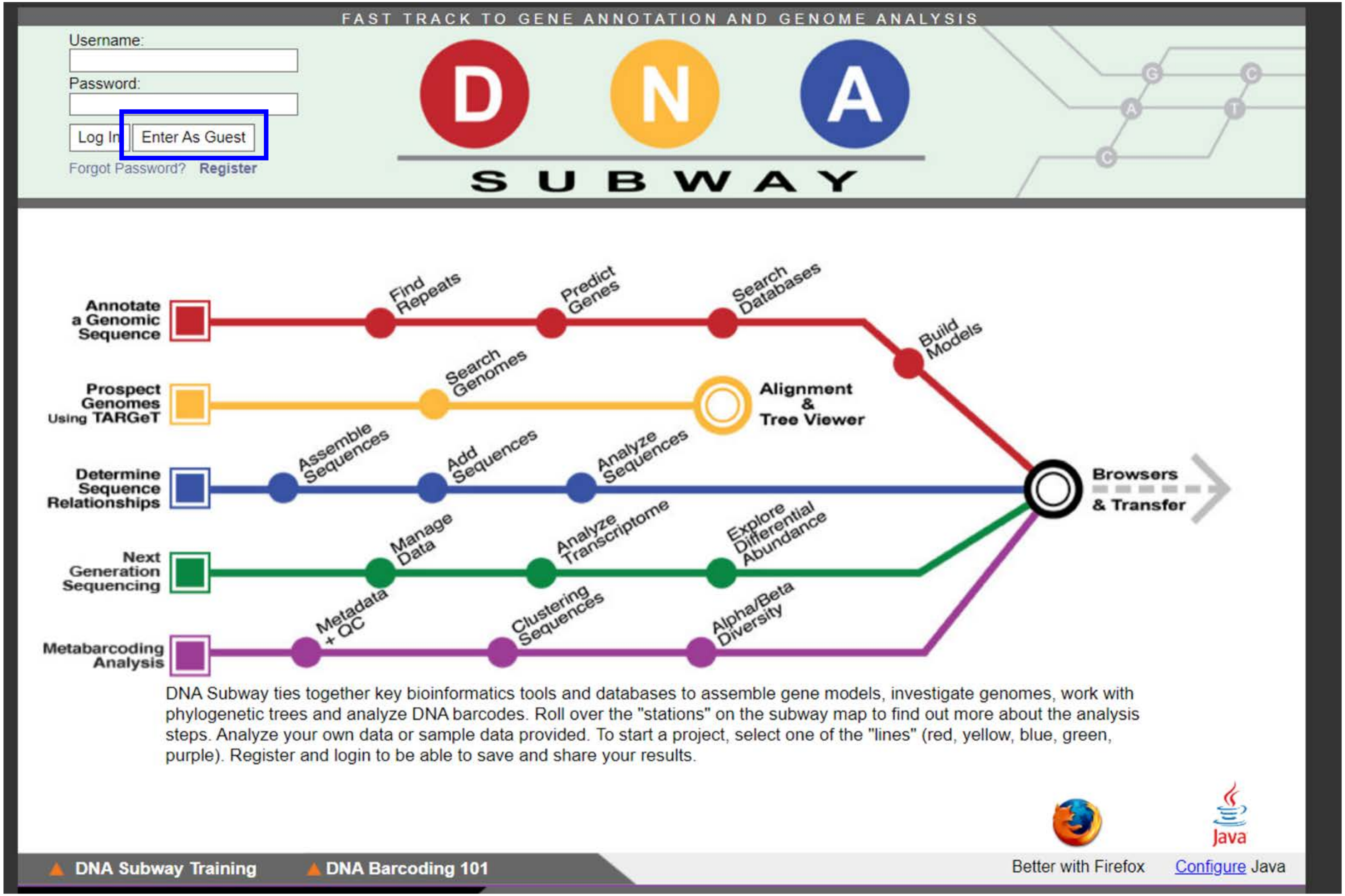
Superweeds!

Mutations that lead to herbicide resistance

with FASTA files & Library

```
>Smooth-Pigweed
ATGGTAATTCAATCCATTACCCACCTTTCACCAAAACTTGCATTGCCATCGCCATTGTCAGTTTCCACCAAGAACT
ACCCAGTAGCTGTAATGGGCAACATTTCTGAGCGGGAAGAACCCACTTCTGCTAAAAGGGTTGCTGTTGTTGGTGC
TGGAGTTAGTGGACTTGCTGCAGCATATAAGCTAAAATCCCATGGTTTGAATGTGACATTGTTTGAAGCTGATTCT
AGAGCTGGAGGCAAACTTAAAACTGTTAAAAAAGATGGTTTTATTTGGGATGAGGGGGCAAATACTATGACAGAAA
GTGAGGCAGAGGTCTCGAGTTTGATCGATGATCTTGGGCTTCGTGAGAAGCAACAGTTGCCAATTTCACAAAATAA
AAGGTACATAGCTAGAGATGGTCTTCCGGTGCTACTACCTTCAAATCCCGCTGCACTGCTCTCGAGCAATATCCTT
TCAGCAAAATCAAAGCTGCAAATTATGTTGGAACCATTTCTCTGGAGAAAACGCAATGCTACTGAGCTTTCTGATG
AGCATGTTCAGGAAAGCGTTGGTGAATTTTTTGAGCGACATTTTGGGAAAGAGTTTGTTGATTATGTTATTGACCC
TTTTGTTGCGGGTACATGTGGCGGAGATCCTCAATCGCTATCTG
```

https://dnasubway.cyverse.org/



DNA Subway ties together key bioinformatics tools and databases to assemble gene models, investigate genomes, work with phylogenetic trees and analyze DNA barcodes. Roll over the "stations" on the subway map to find out more about the analysis steps. Analyze your own data or sample data provided. To start a project, select one of the "lines" (red, yellow, blue, green, purple). Register and login to be able to save and share your results.

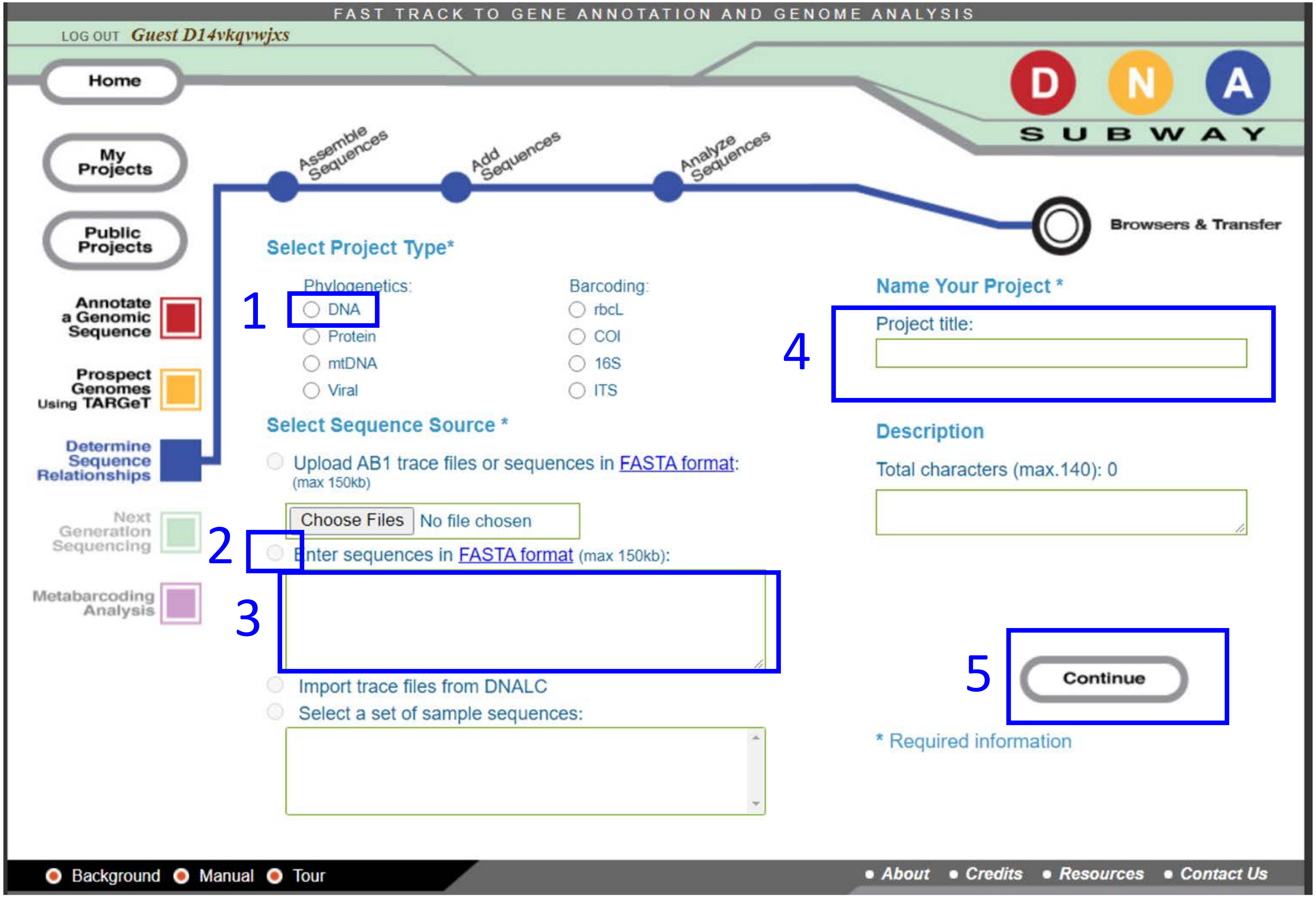
MY PROJECTS

You have no projects yet...

To view pre-computed public projects, click on the "Public Projects" button.

CLICK

To start a new project, click one of the colored squares.



1. Click DNA

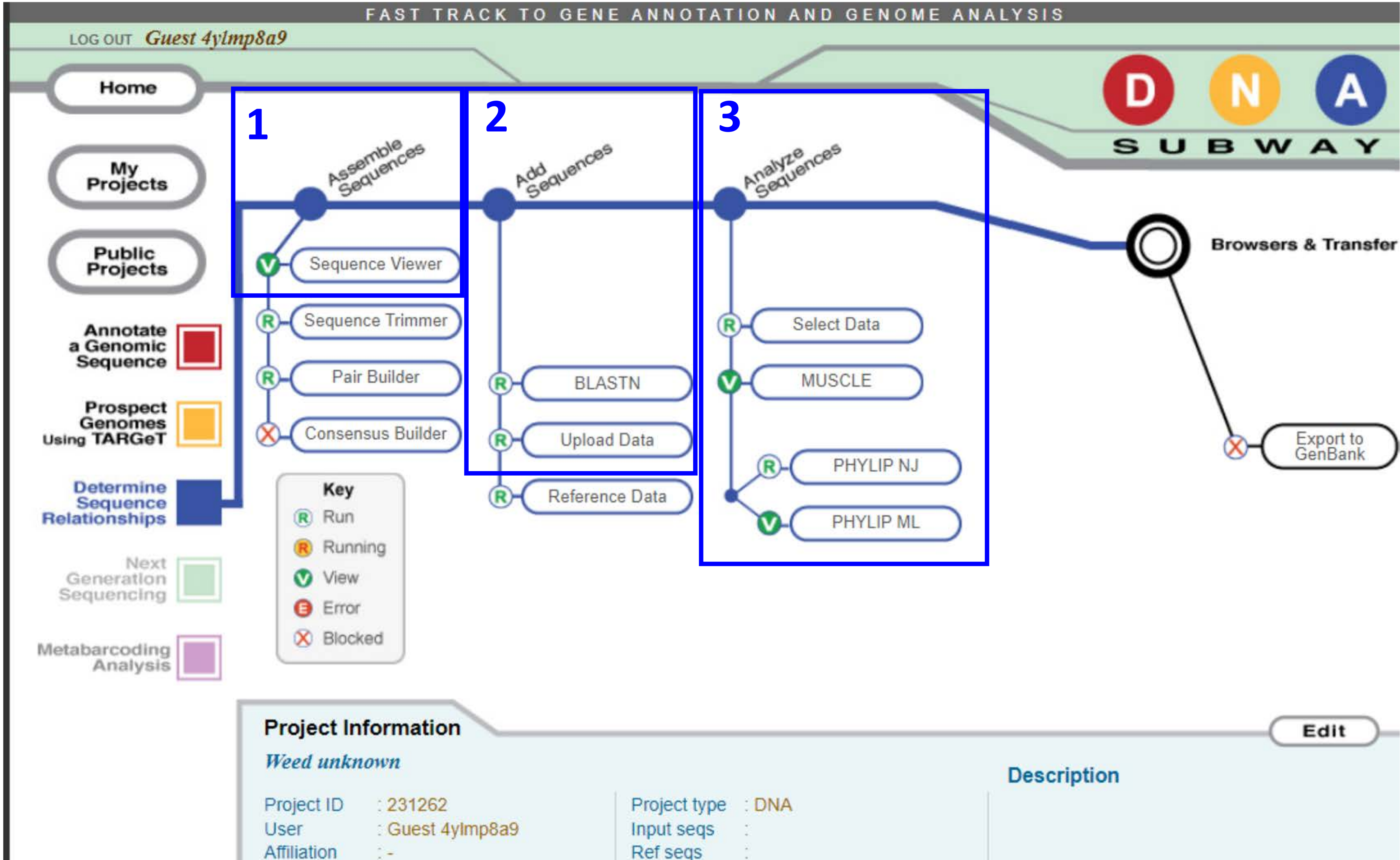
2. Click Enter sequences

3. Cut and paste unknown sequences

4. Name Project

5. Click Continue

3 Stops



Project Information

Weed unknown

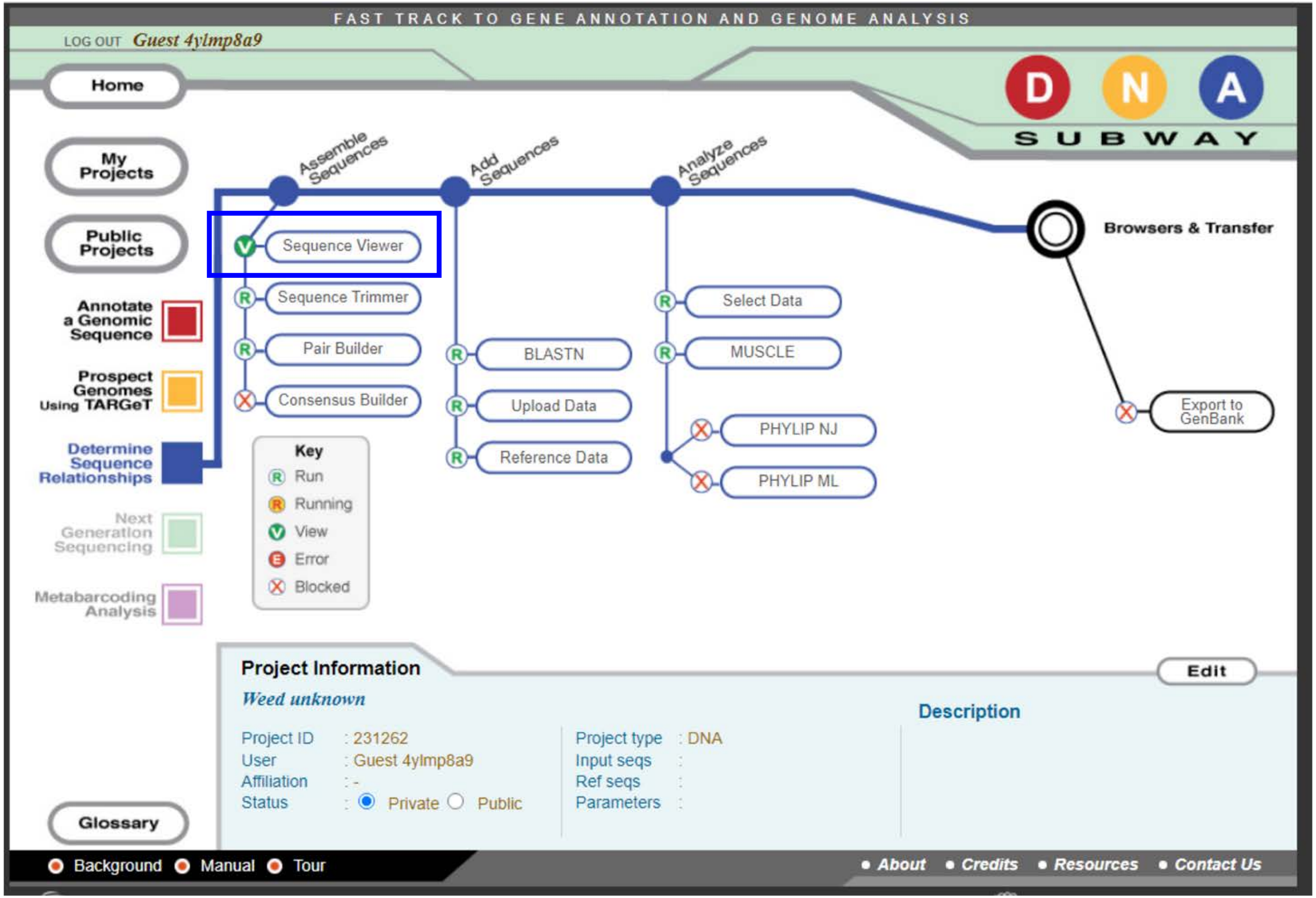
Project ID : 231262
User : Guest 4ylmp8a9
Affiliation : -

Project type : DNA
Input seqs :
Ref seqs :

Description

Stop #1 Assemble Sequences

Sequence Viewer



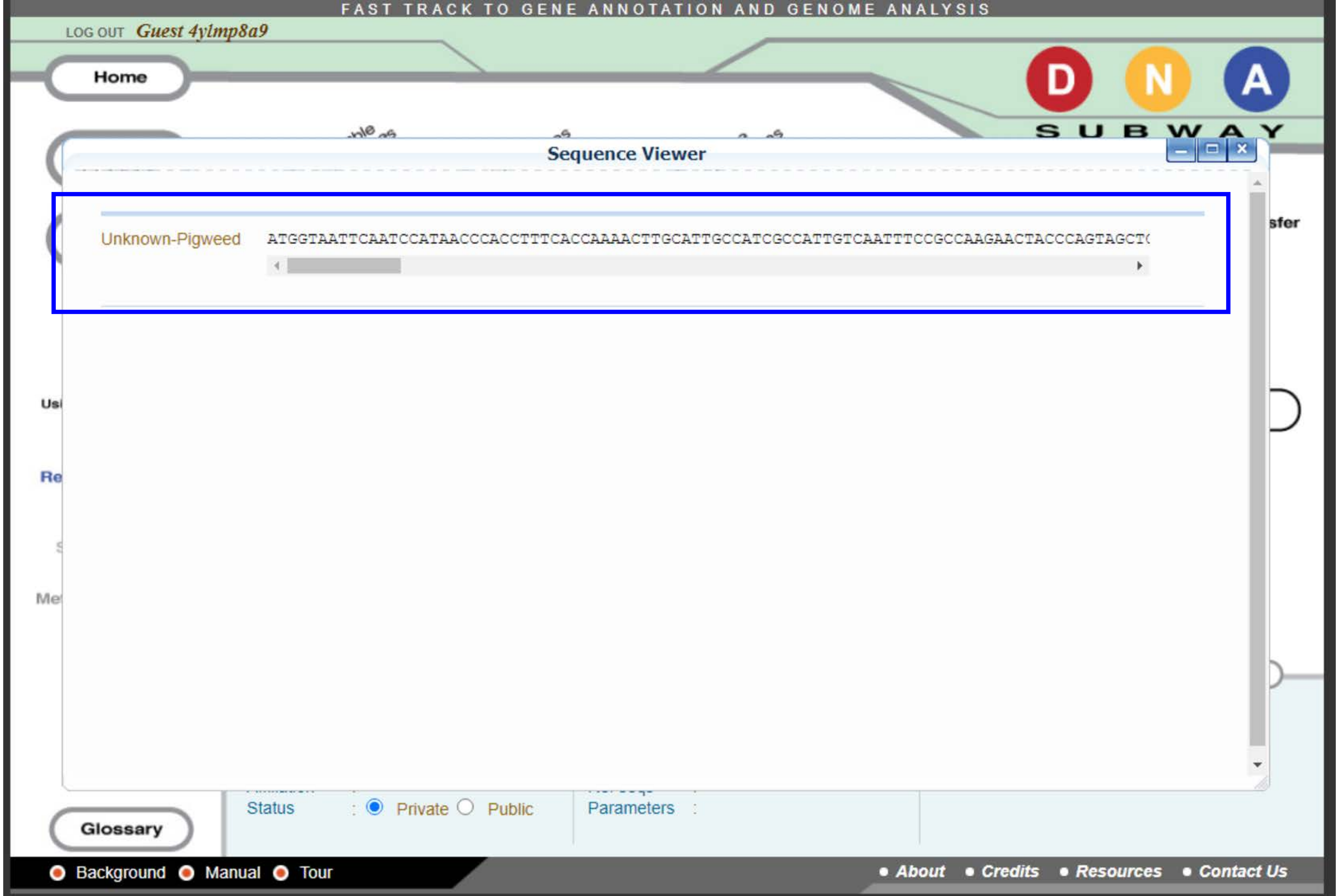
Project Information

Weed unknown

Project ID : 231262
User : Guest 4ylmp8a9
Affiliation : -
Status : ● Private ○ Public

Project type : DNA
Input seqs :
Ref seqs :
Parameters :

Description



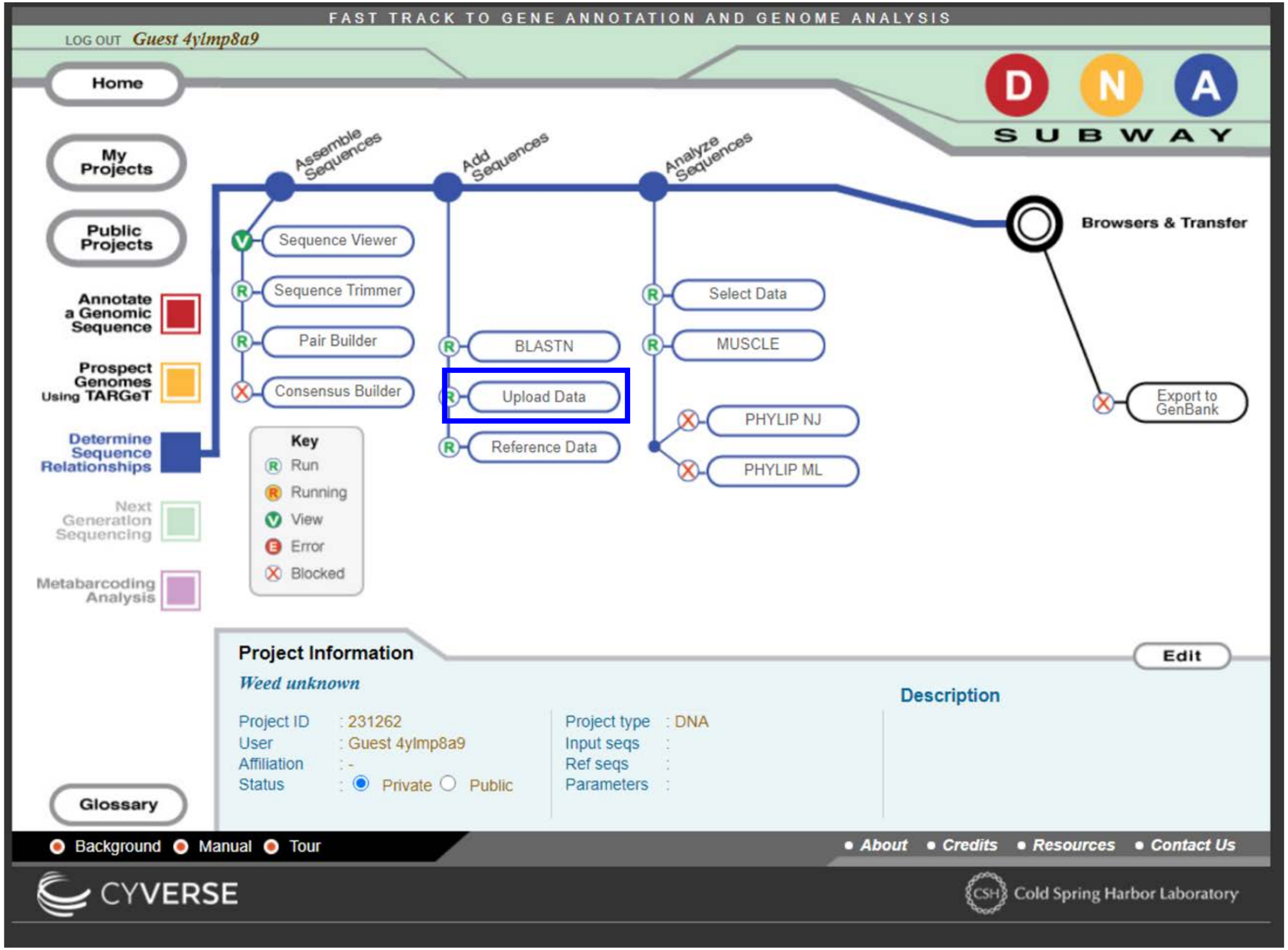
Unknown-Pigweed ATGGTAATTCAATCCATAACCCACCTTTCACCAAAACTTGCATTGCCATCGCCATTGTCAATTTCCGCCAAGAACTACCCAGTAGCT

Status : ● Private ○ Public

Parameters :

Stop #2 Add Sequences

Click Upload Data



Project Information

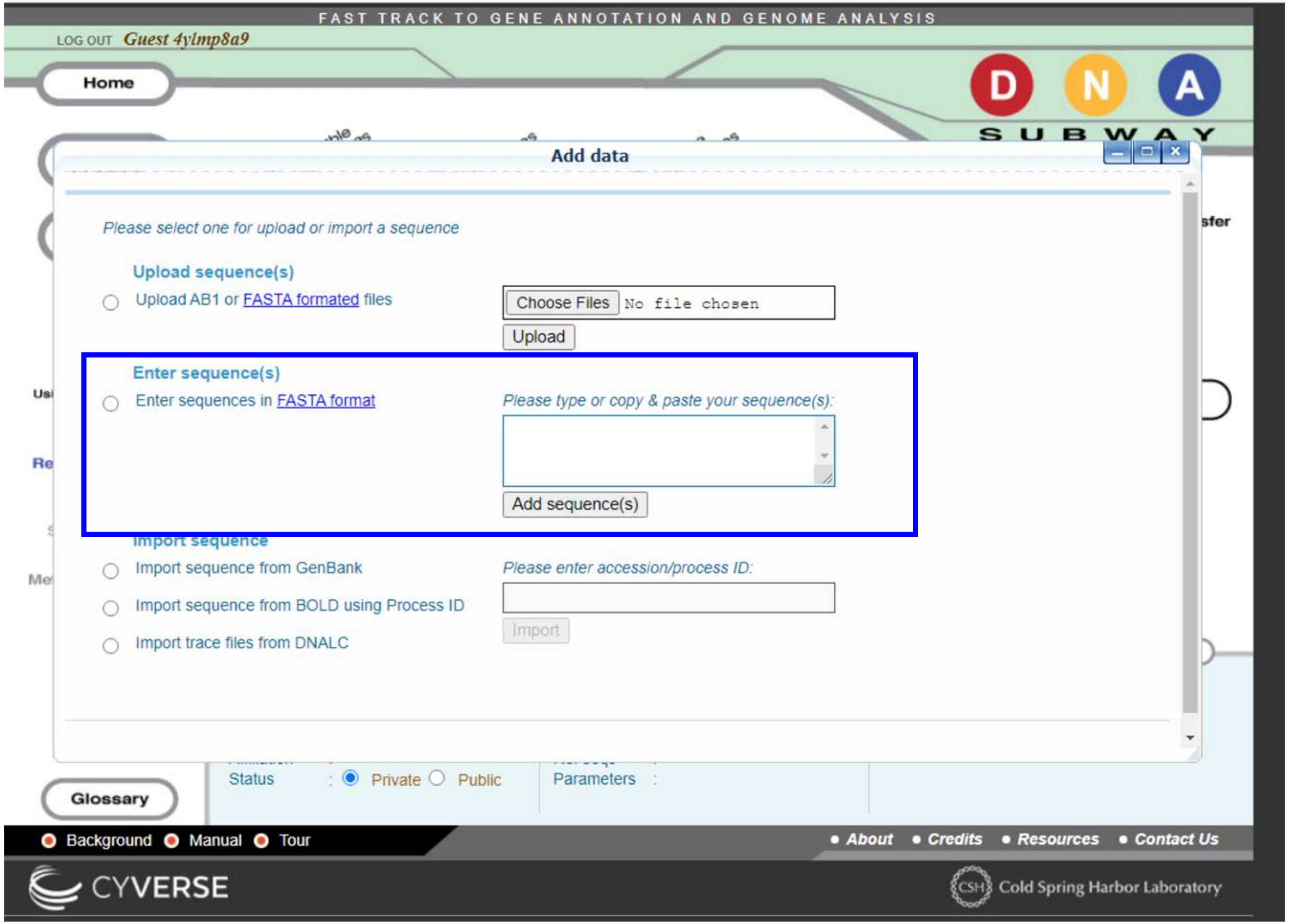
Weed unknown

Project ID : 231262
User : Guest 4ylmp8a9
Affiliation : -
Status : ● Private ○ Public

Project type : DNA
Input seqs :
Ref seqs :
Parameters :

Description

Upload Pigweed Library from Pigweed Library text file



Add data

Please select one for upload or import a sequence

Upload sequence(s)

○ Upload AB1 or FASTA formated files

Choose Files No file chosen

Upload

Enter sequence(s)

○ Enter sequences in FASTA format

Please type or copy & paste your sequence(s):

Add sequence(s)

Import sequence

○ Import sequence from GenBank

○ Import sequence from BOLD using Process ID

○ Import trace files from DNALC

Please enter accession/process ID:

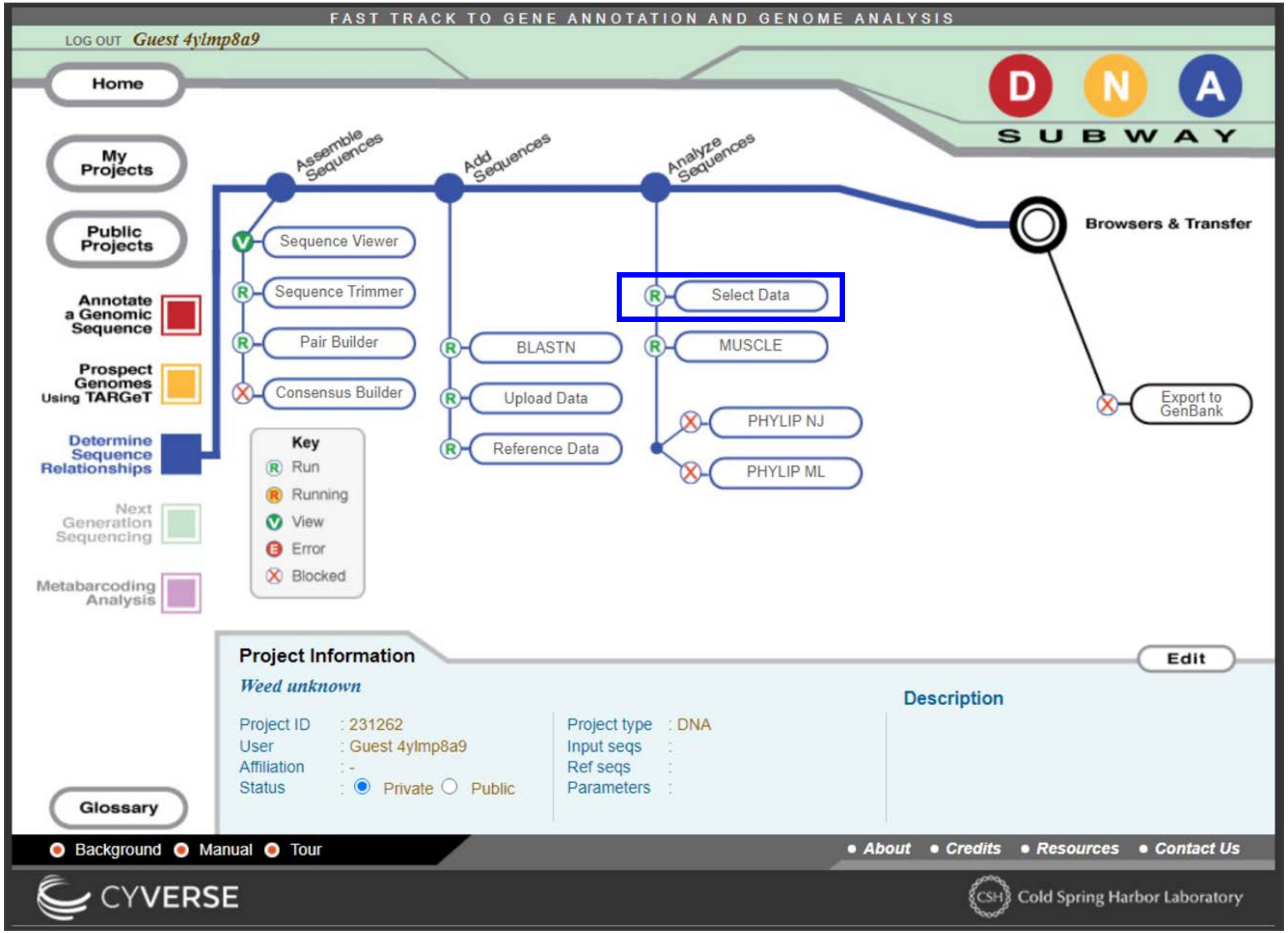
Import

Status : ● Private ○ Public

Parameters :

Stop #3 Analyze Sequences

Click Select Data



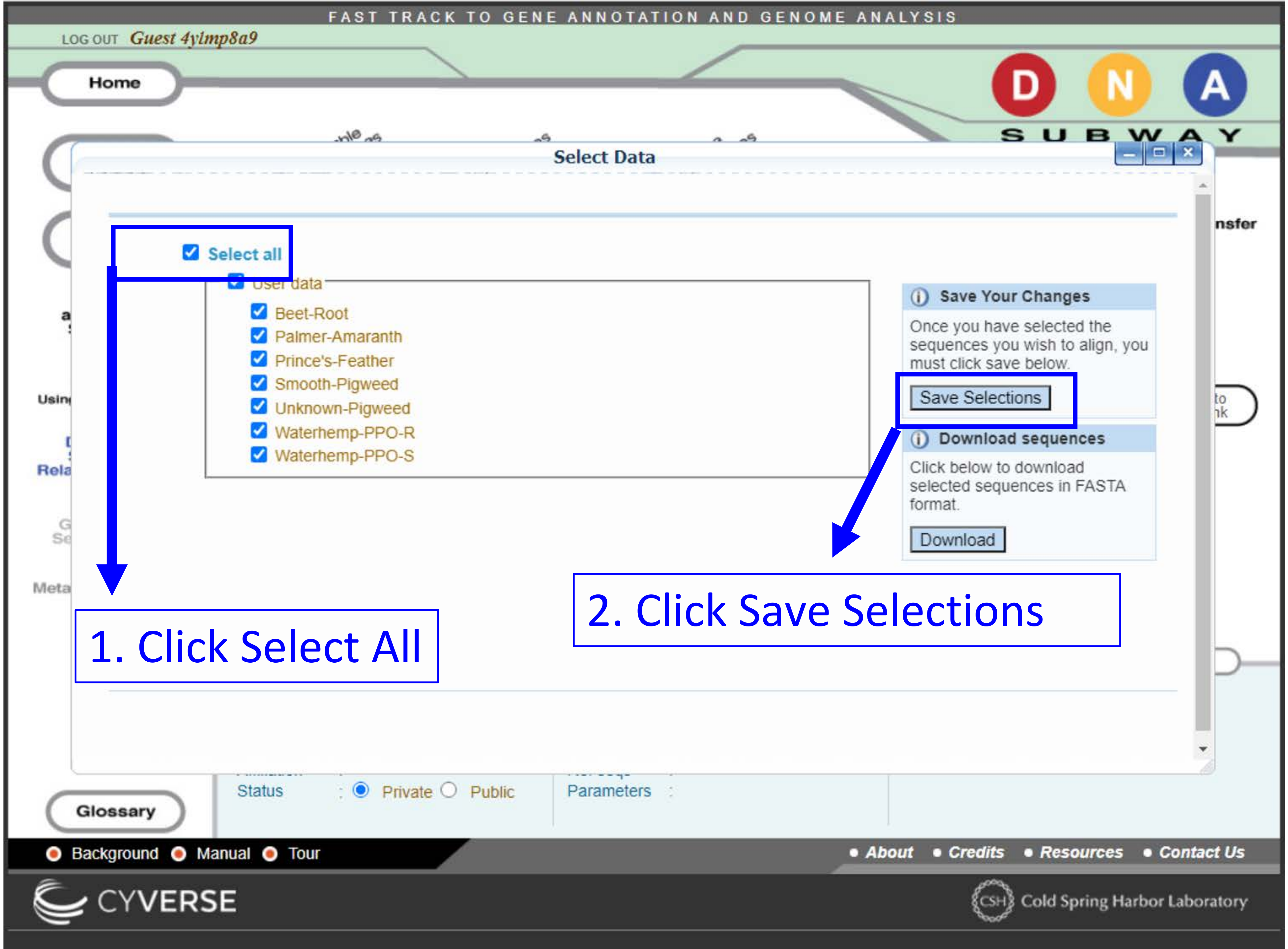
Project Information

Weed unknown

Project ID : 231262
User : Guest 4ylmp8a9
Affiliation : -
Status : ● Private ○ Public

Project type : DNA
Input seqs :
Ref seqs :
Parameters :

Description



Select Data

☑ Select all

☑ User data

☑ Beet-Root
☑ Palmer-Amaranth
☑ Prince's-Feather
☑ Smooth-Pigweed
☑ Unknown-Pigweed
☑ Waterhemp-PPO-R
☑ Waterhemp-PPO-S

Save Your Changes

Once you have selected the sequences you wish to align, you must click save below.

Save Selections

Download sequences

Click below to download selected sequences in FASTA format.

Download

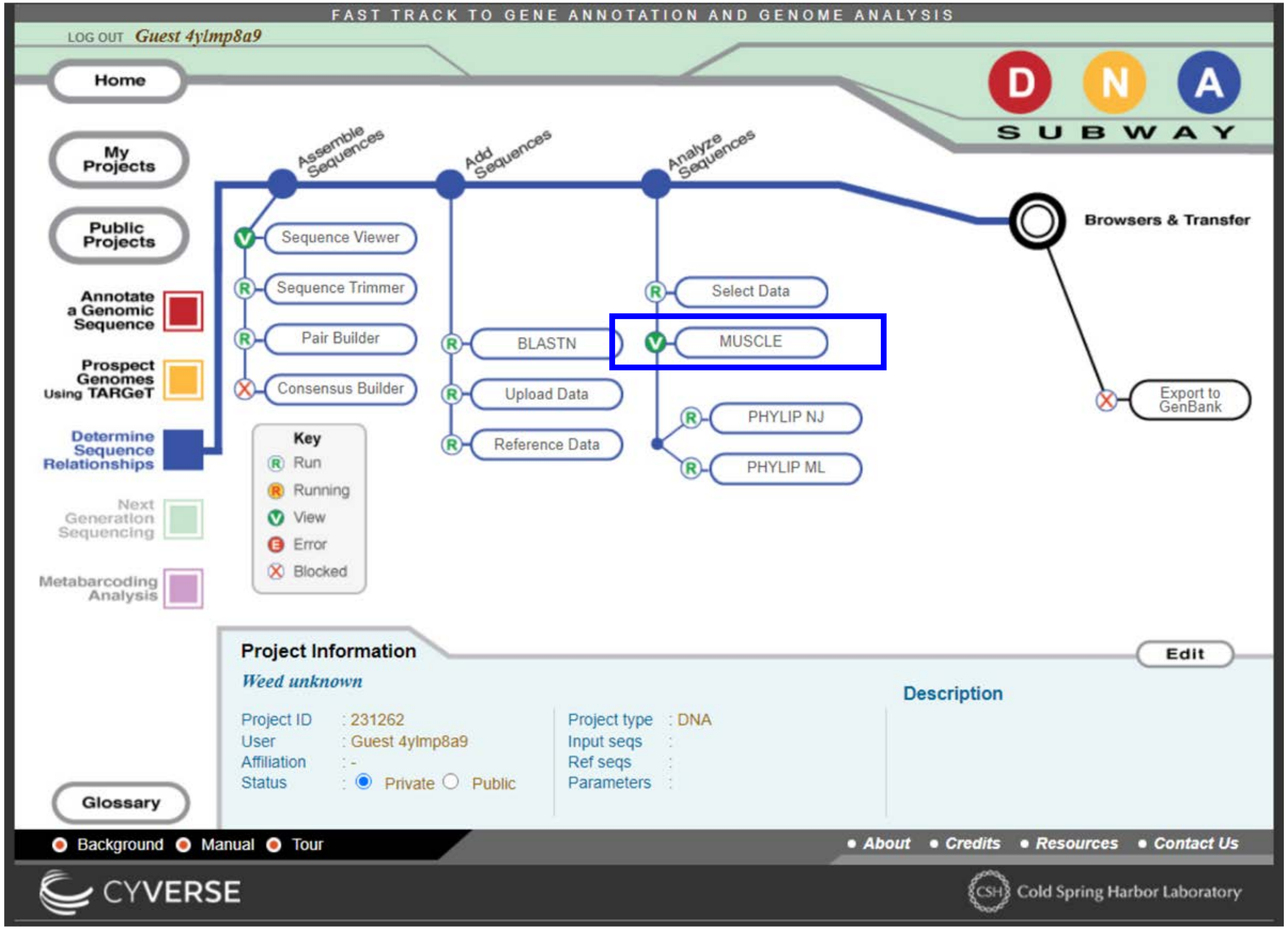
Status : ● Private ○ Public

Parameters :

1. Click Select All

2. Click Save Selections

Click MUSCLE (Multiple Sequence Comparison by Log-Expectation) to run algorithm, then click again to view



Project Information

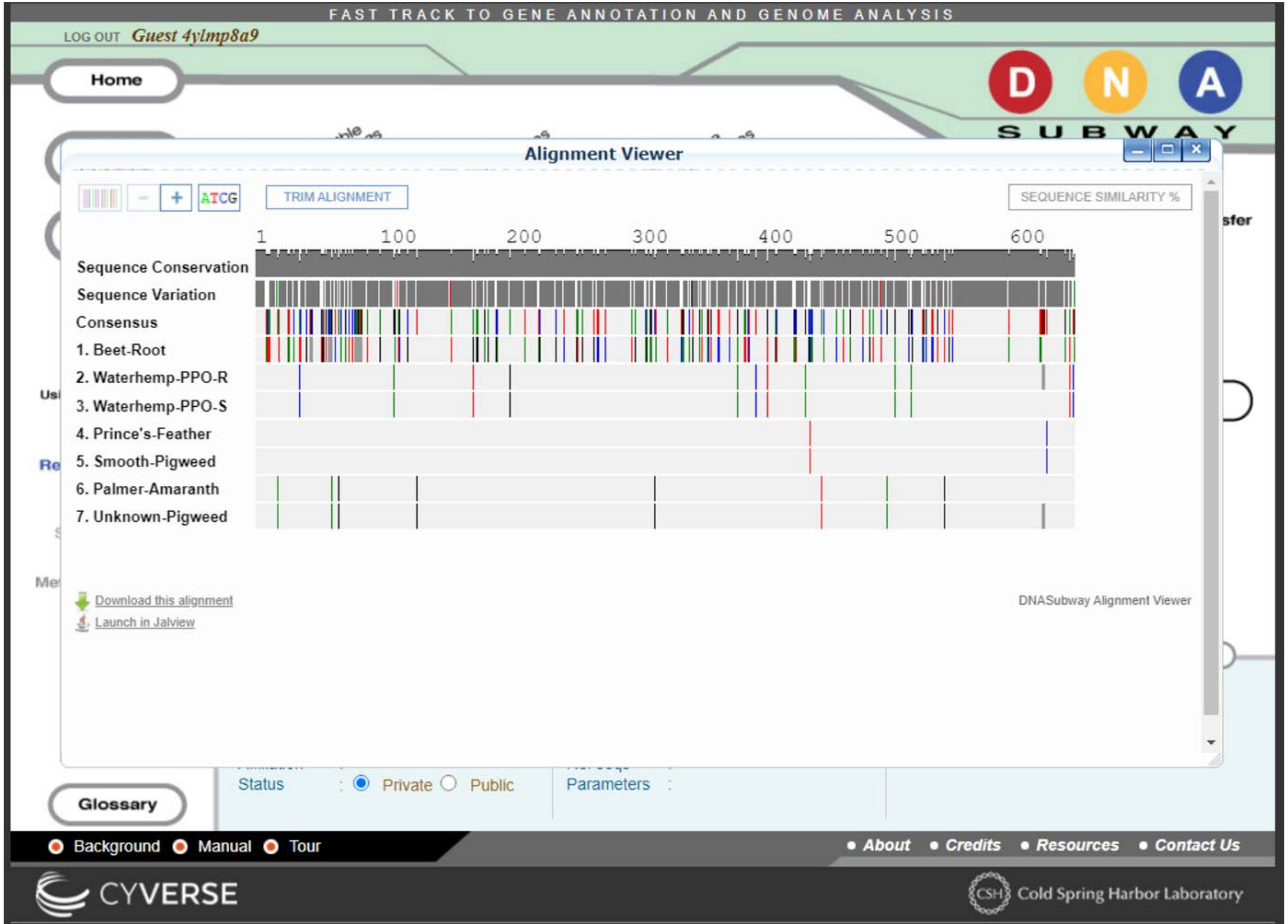
Weed unknown

Project ID : 231262
User : Guest 4ylmp8a9
Affiliation : -
Status : ● Private ○ Public

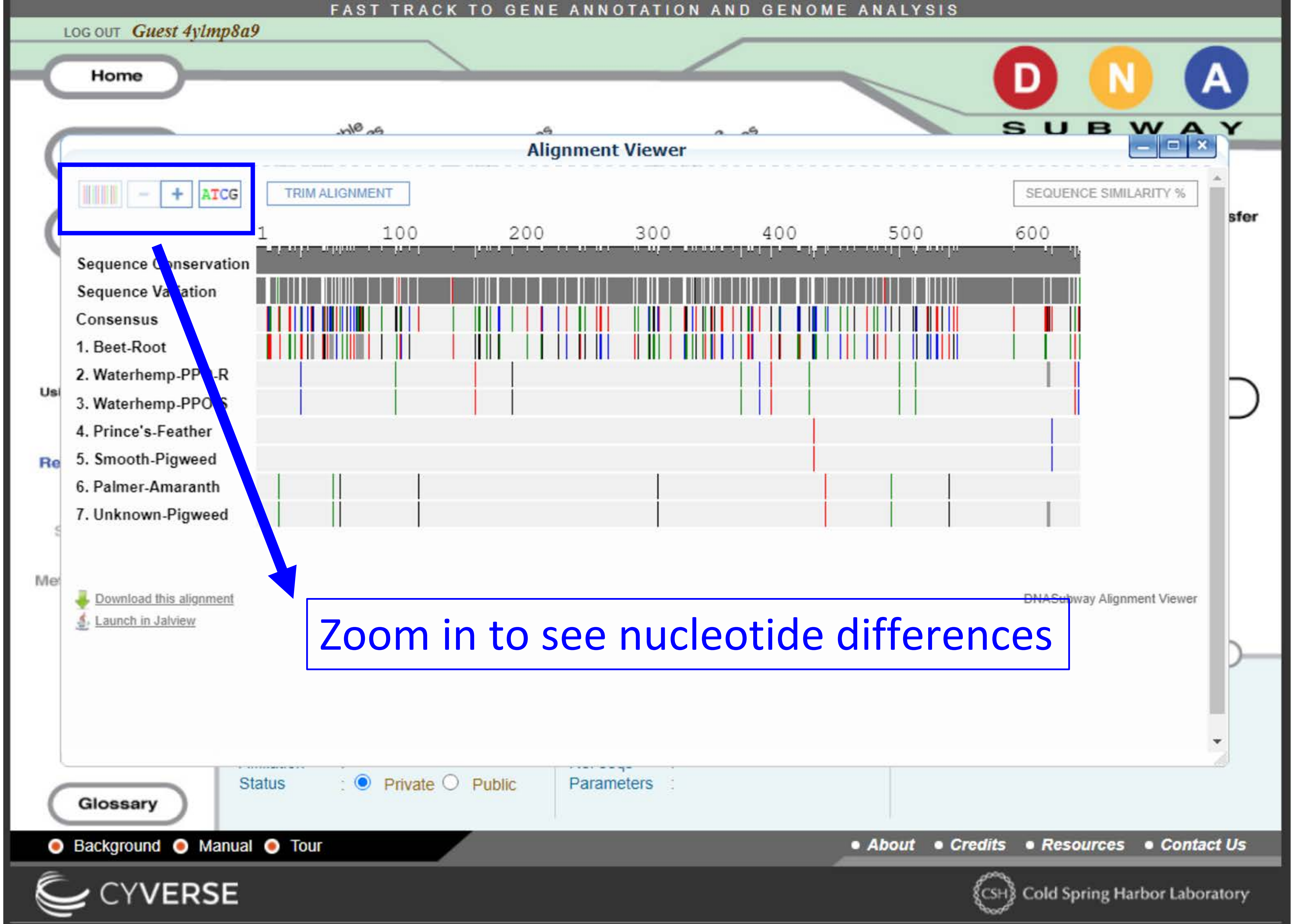
Project type : DNA
Input seqs :
Ref seqs :
Parameters :

Description

MUSCLE Results Window



Several Views of MUSCLE Alignment Analysis

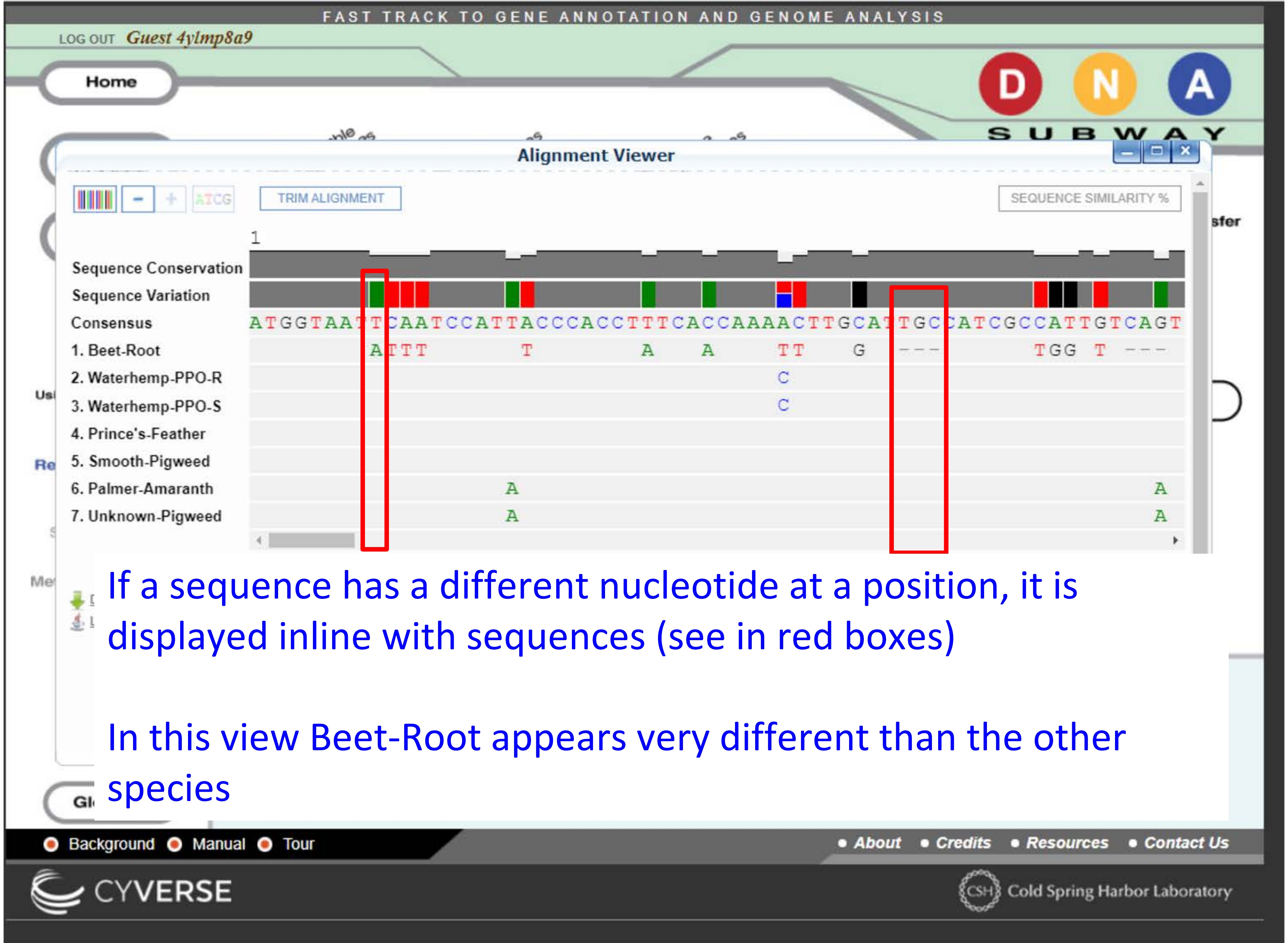


Sequence Conservation
Sequence Variation
Consensus
1. Beet-Root
2. Waterhemp-PPO-R
3. Waterhemp-PPO-S
4. Prince's-Feather
5. Smooth-Pigweed
6. Palmer-Amaranth
7. Unknown-Pigweed

Status : ● Private ○ Public

Parameters :

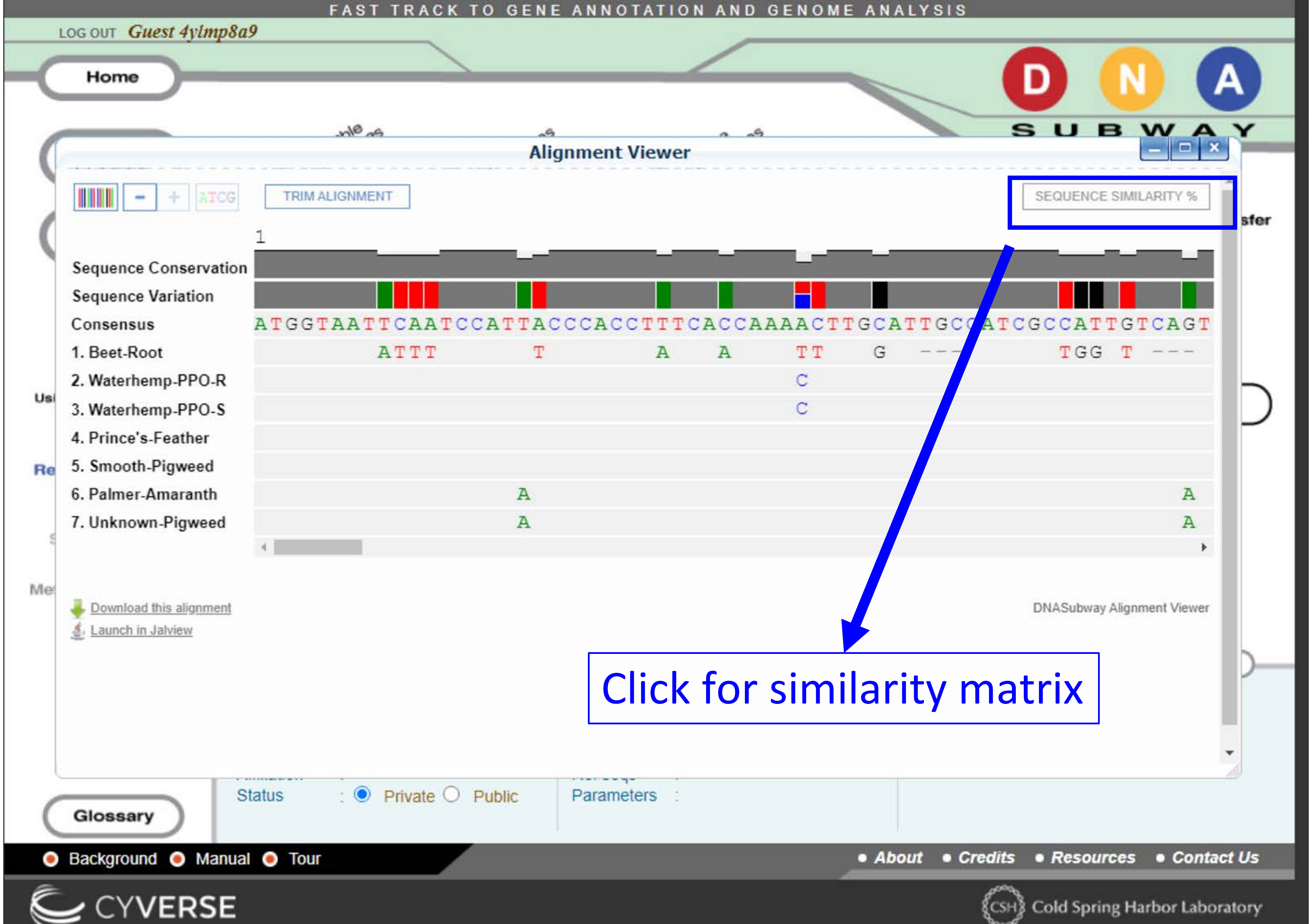
Zoom in to see nucleotide differences



```
                         1
Sequence Conservation
Sequence Variation
Consensus                ATGGTAATTCAATCCATTACCCACCTTTCACCAAAACTTGCATTGCCATCGCCATTGTCAGT
1. Beet-Root                     ATTT      T       A   A    TT   G  ---      TGG T ---
2. Waterhemp-PPO-R                                          C
3. Waterhemp-PPO-S                                          C
4. Prince's-Feather
5. Smooth-Pigweed
6. Palmer-Amaranth                        A                                          A
7. Unknown-Pigweed                        A                                          A
```

If a sequence has a different nucleotide at a position, it is displayed inline with sequences (see in red boxes)

In this view Beet-Root appears very different than the other species

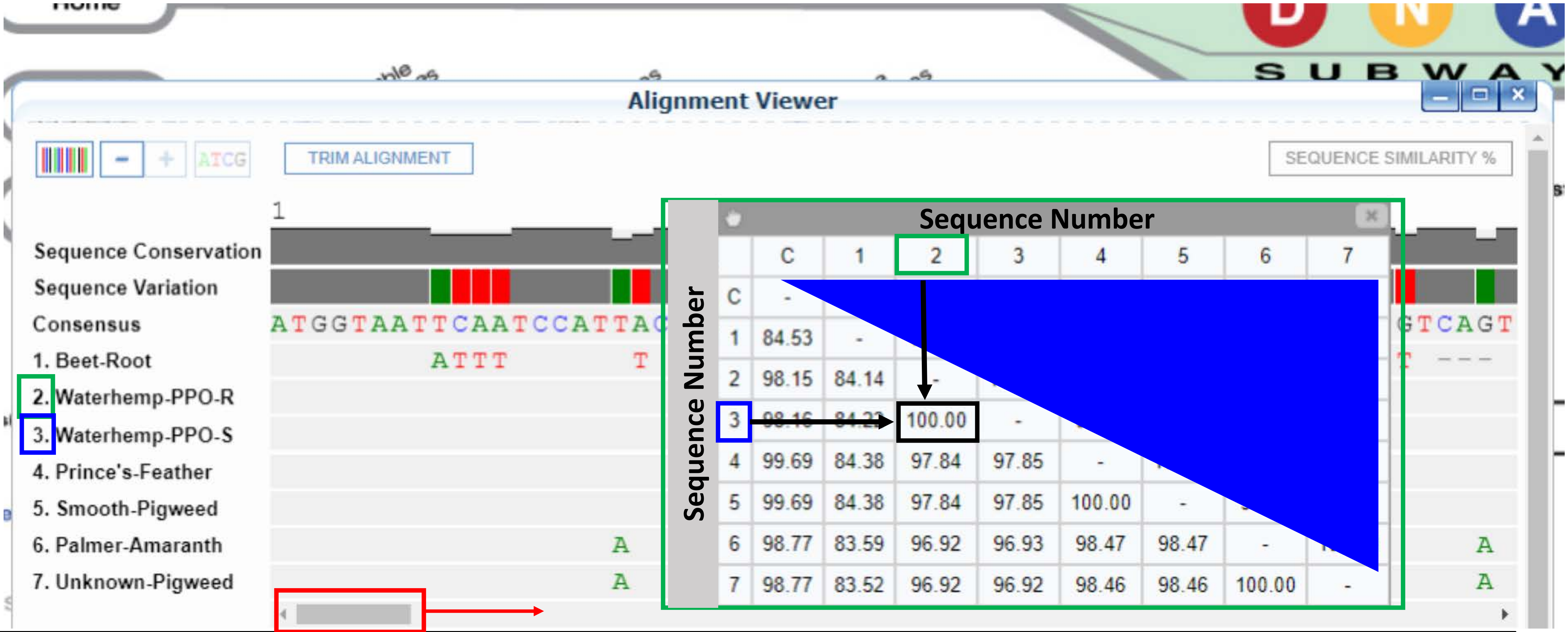


```
                        1
Sequence Conservation
Sequence Variation
Consensus               ATGGTAATTCAATCCATTACCCACCTTTCACCAAAACTTGCATTGCCCATCGCCATTGTCAGT
1. Beet-Root                    ATTT     T       A   A    TT   G  ---     TGG T ---
2. Waterhemp-PPO-R                                        C
3. Waterhemp-PPO-S                                        C
4. Prince's-Feather
5. Smooth-Pigweed
6. Palmer-Amaranth                   A                                           A
7. Unknown-Pigweed                   A                                           A
```

Status : ● Private ○ Public

Parameters :

Click for similarity matrix



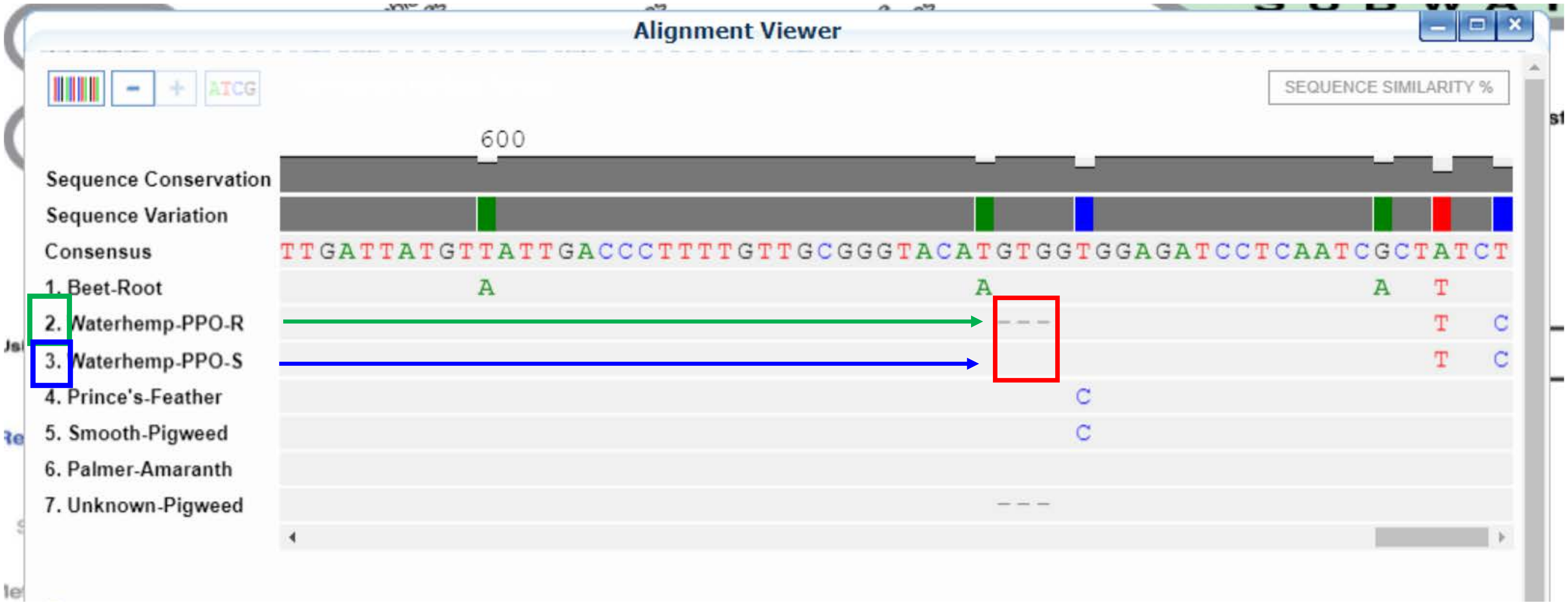
Sequence Conservation
Sequence Variation
Consensus ATGGTAATTCAATCCATTAC … GTCAGT
1. Beet-Root ATTT T … T ---
2. Waterhemp-PPO-R
3. Waterhemp-PPO-S
4. Prince's-Feather
5. Smooth-Pigweed
6. Palmer-Amaranth A … A
7. Unknown-Pigweed A … A

	C	1	2	3	4	5	6	7
C	-							
1	84.53	-						
2	98.15	84.14	-					
3	98.16	84.23	100.00	-				
4	99.69	84.38	97.84	97.85	-			
5	99.69	84.38	97.84	97.85	100.00	-		
6	98.77	83.59	96.92	96.93	98.47	98.47	-	
7	98.77	83.52	96.92	96.92	98.46	98.46	100.00	-

Table shows the pairwise comparisons between the different sequences in the alignment. The top diagonal and bottom diagonal give the same pairwise results (mirrored).

Waterhemp-PPO-R and Waterhemp-PPO-S appear to show **100% identity, HOWEVER!!**

Scroll through the Sequences!



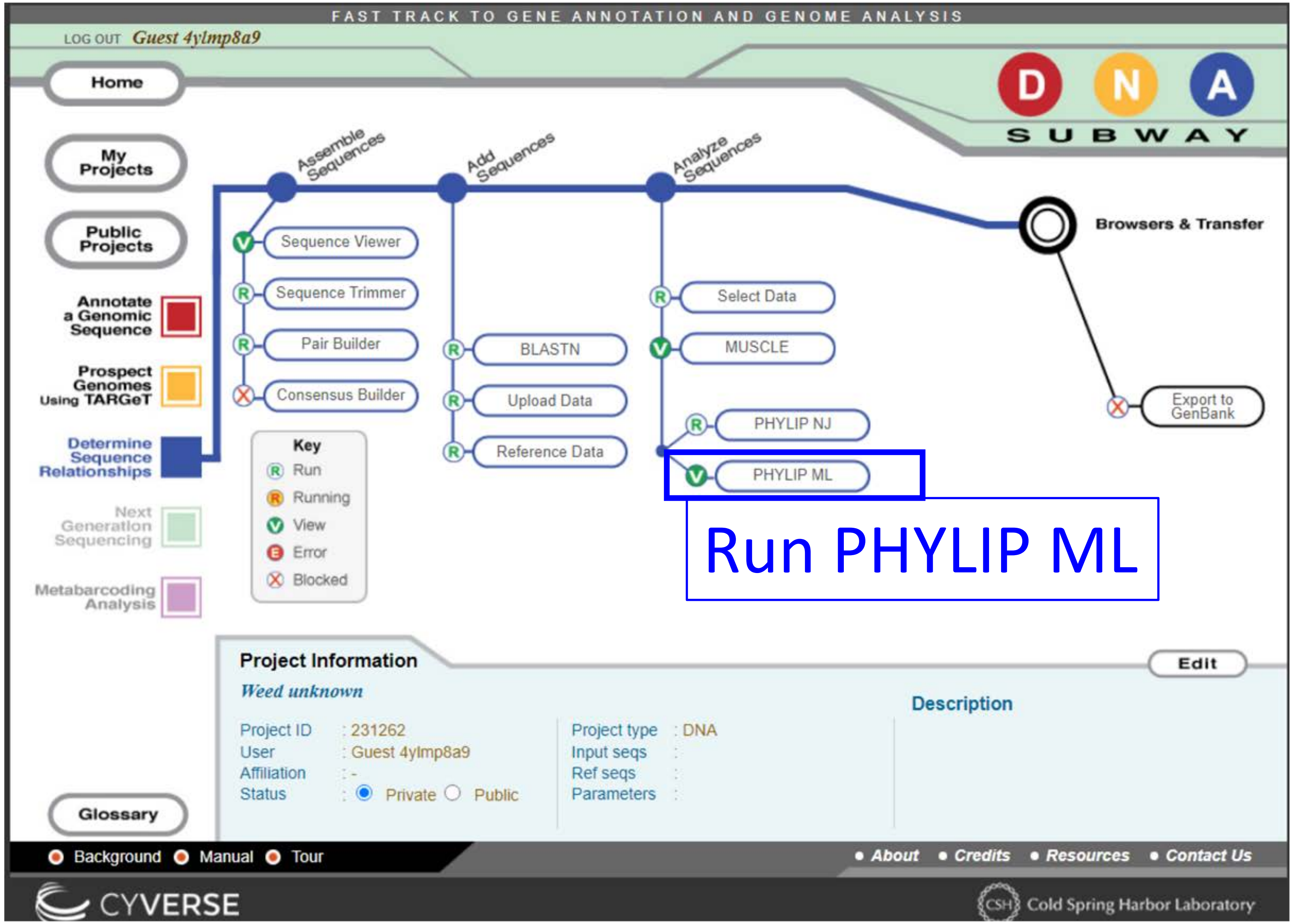
There's a 3-bp gap (- - -) between the PPO-Resistant (Sequence 2) and the PPO-Susceptible (Sequence 3) Waterhemp Sequences.

Is the unknown pigweed likely susceptible or resistant based off the library?

Gene Tree for Species Identification

PHYLIP = Phylogeny Inference Package

ML = Maximum Likelihood methods



Project Information

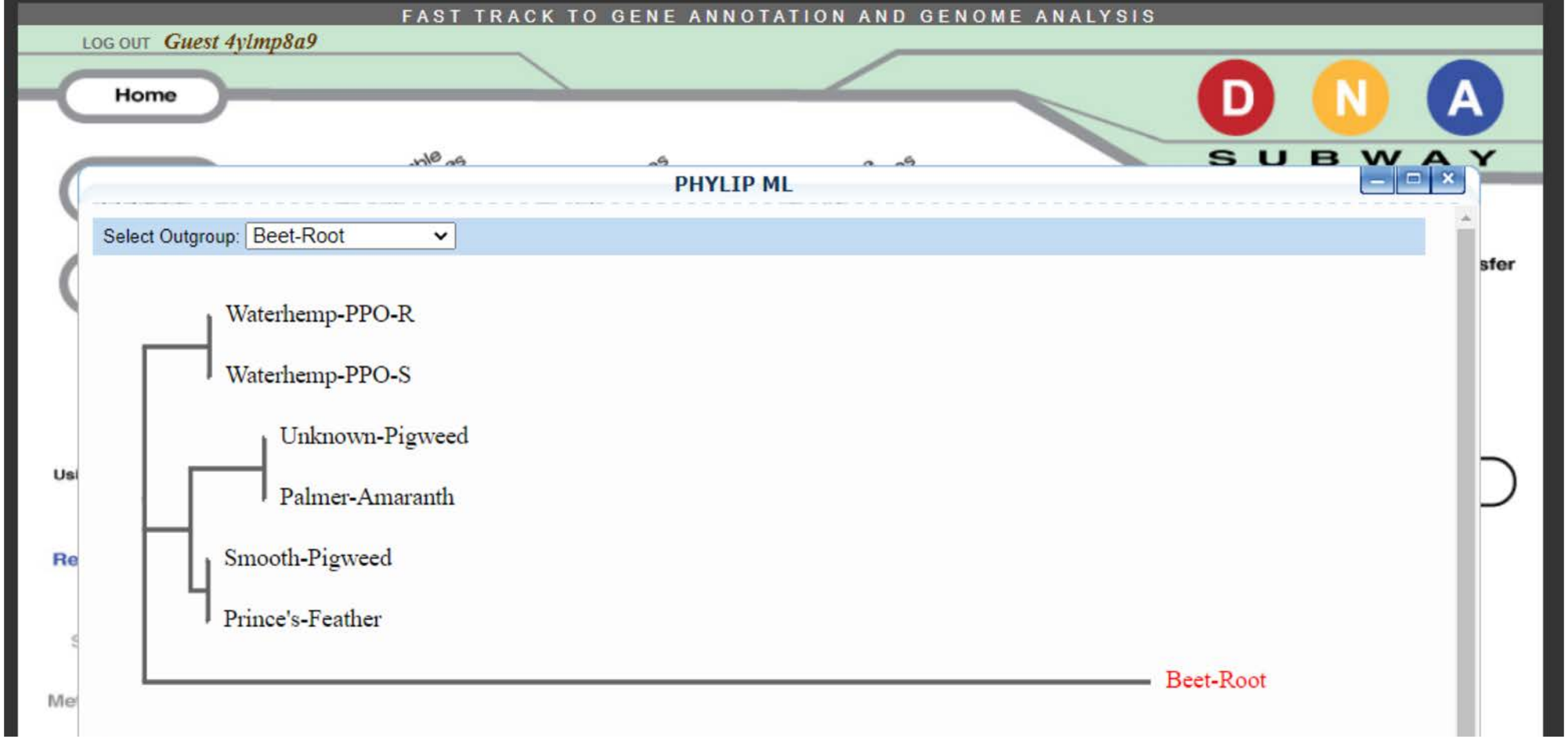
Weed unknown

Project ID : 231262
User : Guest 4ylmp8a9
Affiliation : -
Status : ● Private ○ Public

Project type : DNA
Input seqs :
Ref seqs :
Parameters :

Description

Run PHYLIP ML

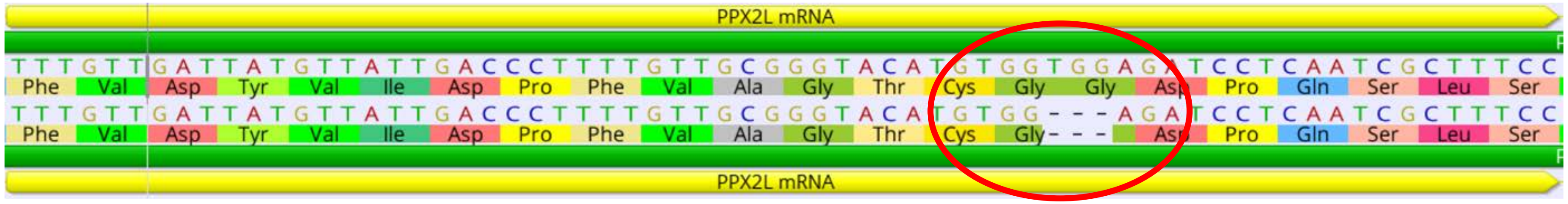


Select Outgroup: Beet-Root

Graphically shows the genetic distance between the sequences. The length of the line is determined by how many nucleotide differences there are among these sequences.

Our unknown has the **PPO-R genotype** AND is **Palmer Amaranth**!

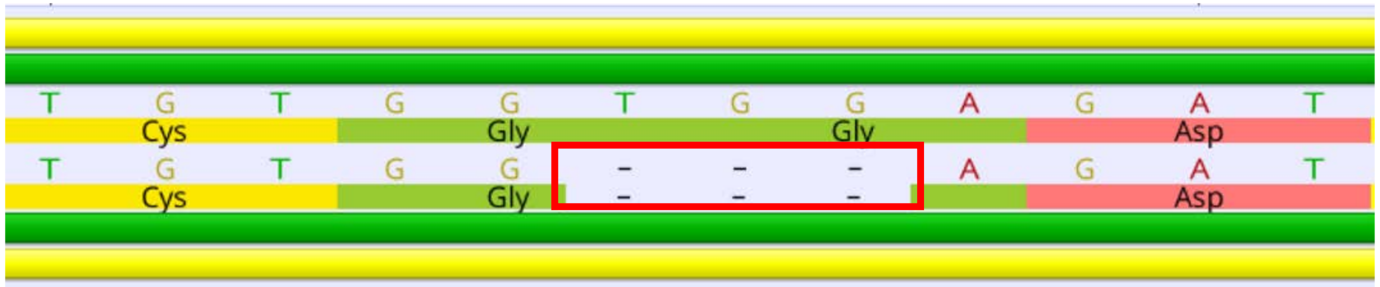
Genetic basis for herbicide resistance within the *PPX2L* gene



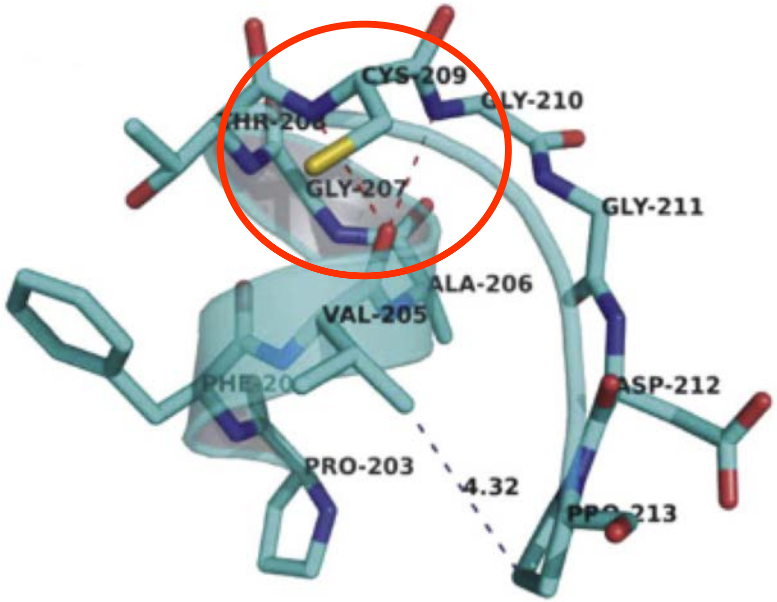
Missing Amino Acid in Mutant **ΔG210**

Wildtype Sequence

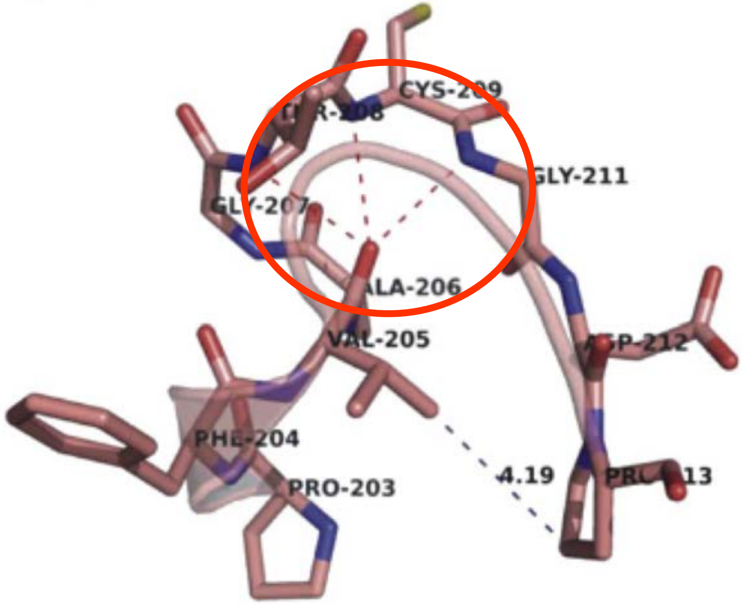
Mutant Sequence



Protein target of PPO-inhibiting herbicides (PPO molecule)



Wildtype Protein - Only space for the herbicide molecule



Mutant Protein – Large gap rendering the herbicide less effective

Structure = Function

Dayon Handbook of Pesticide Toxicology 2010

Final Thoughts on Bioinformatics

DNA sequence analysis using bioinformatics tools can answer many questions in biology and agriculture

1. Identify mutations associated with traits – (herbicide resistance)

1. Identify species – (weeds & pathogens)