DNA sequence editing

Summer School:

„from fungal morphology to genotype”

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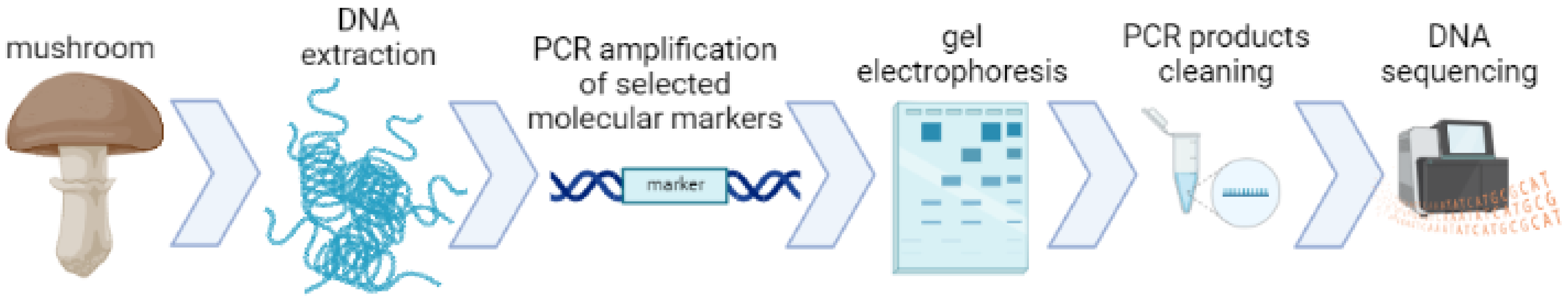
Univeristy of Warsaw

30.08.2024

General pipeline


mushroom

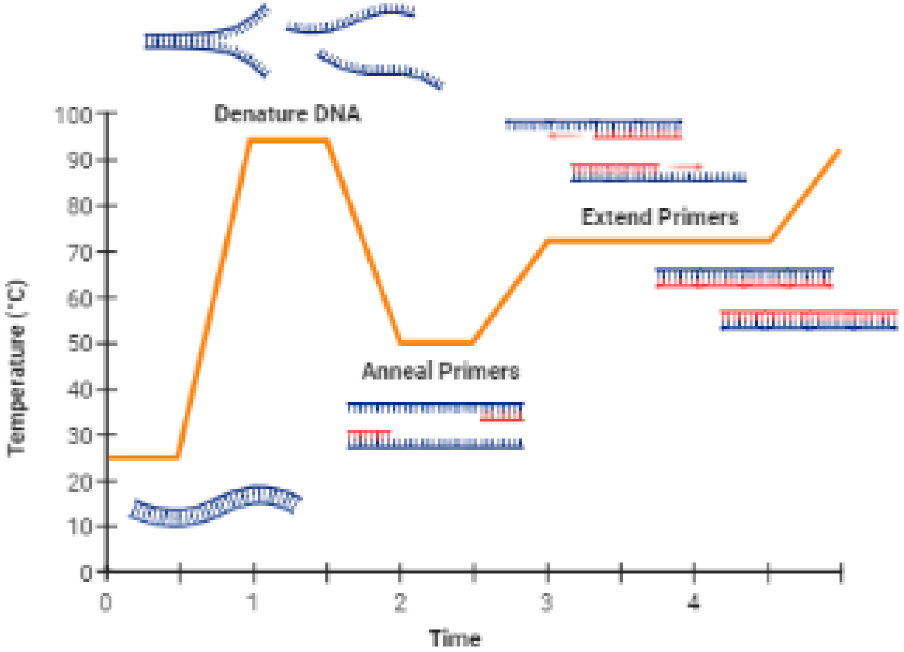
DNA extraction

PCR amplification of selected molecular markers

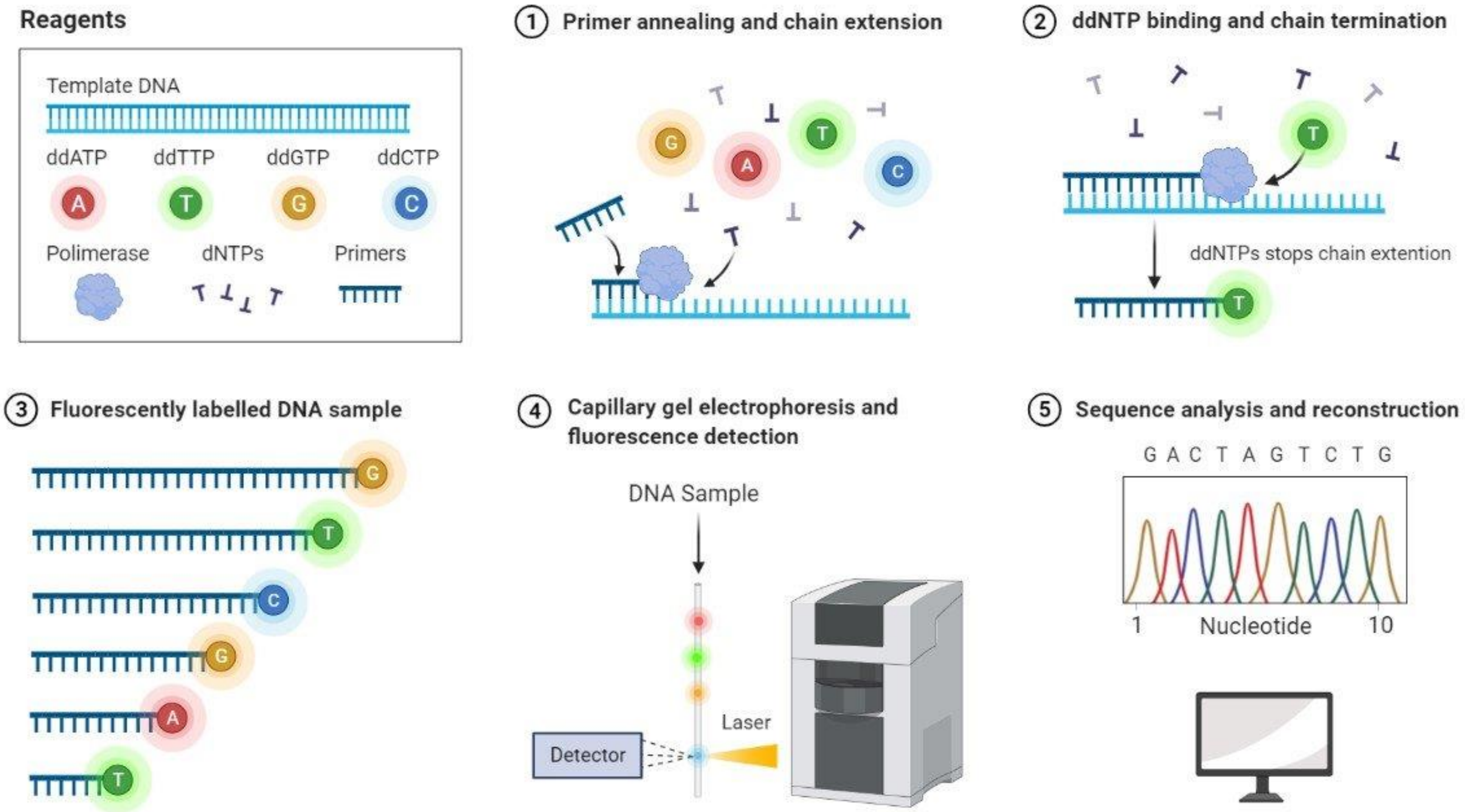
gel electrophoresis

PCR products cleaning

DNA sequencing

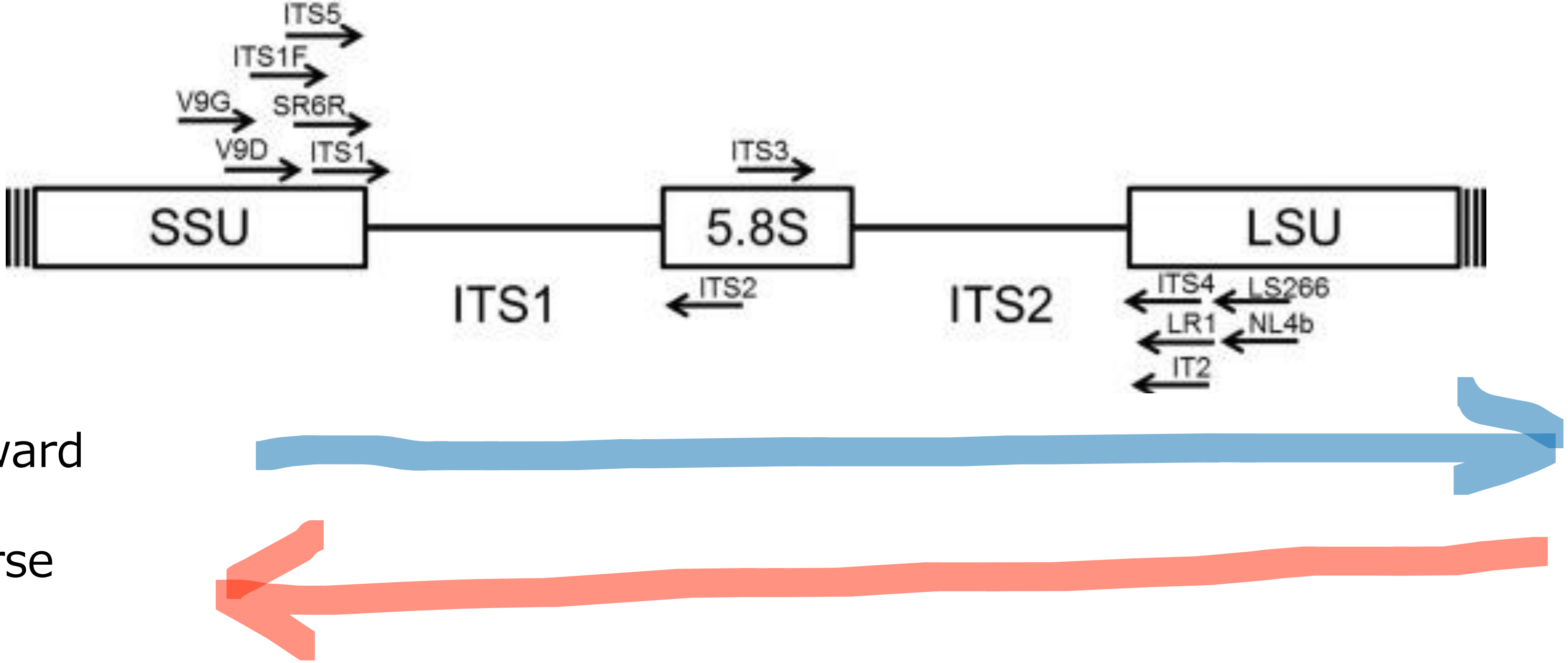


Sanger sequencing results



https://www.genscript.com/gsimages/news/sanger-sequencing-vs-next-generation-sequencing-1.jpg

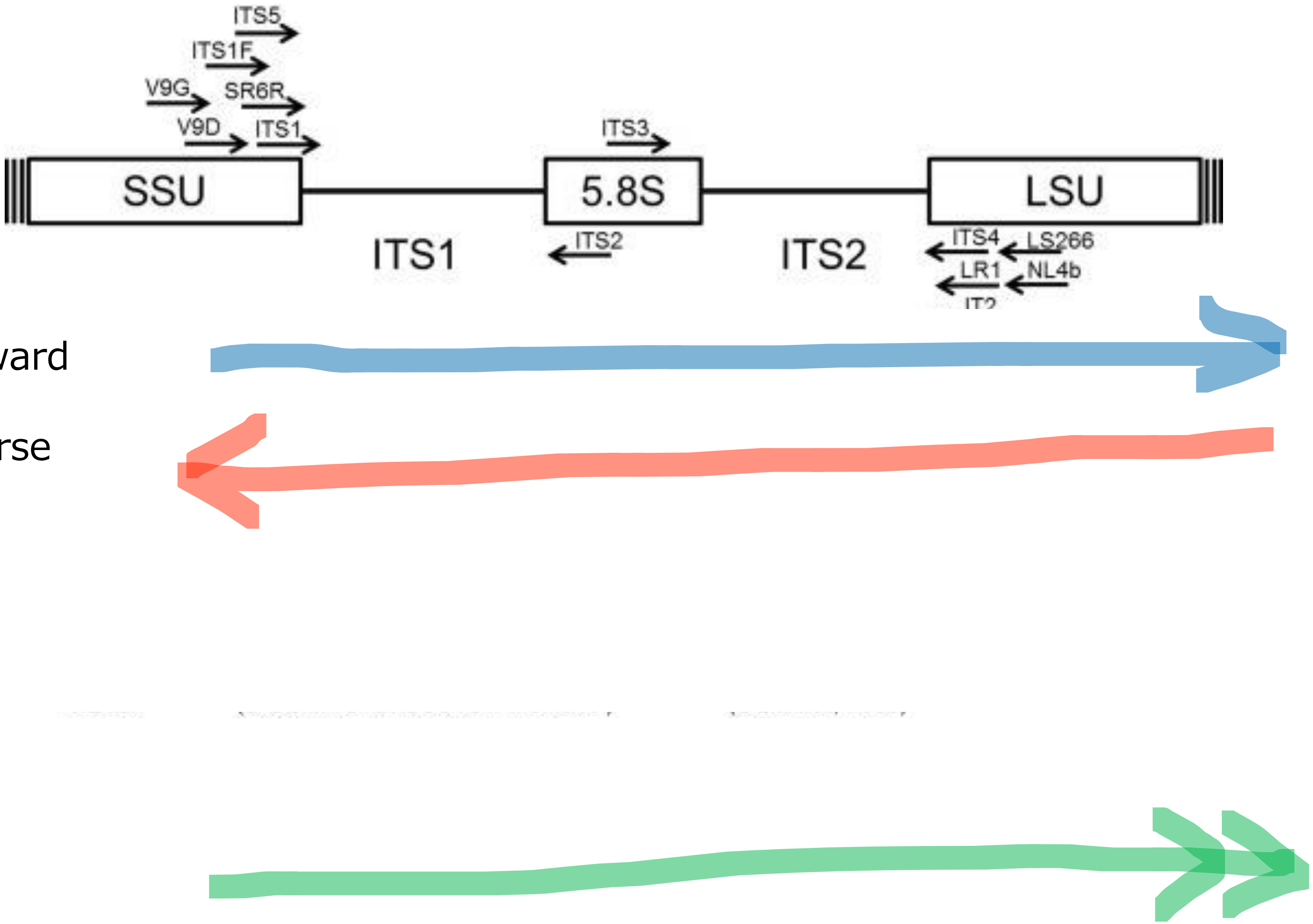
Primers used for sequencing



ITS1f – forward

ITS4 - reverse

Primers used for sequencing

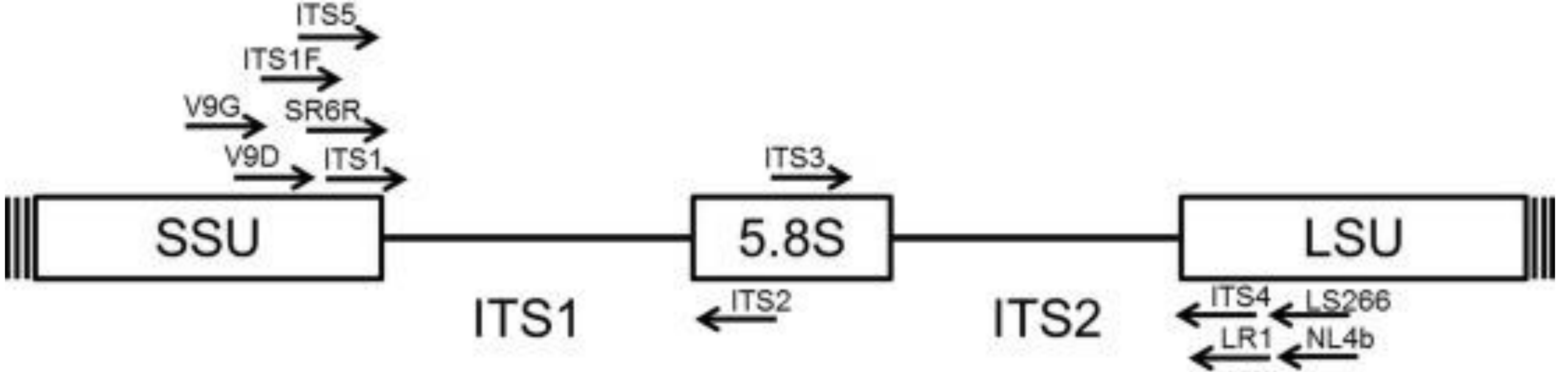


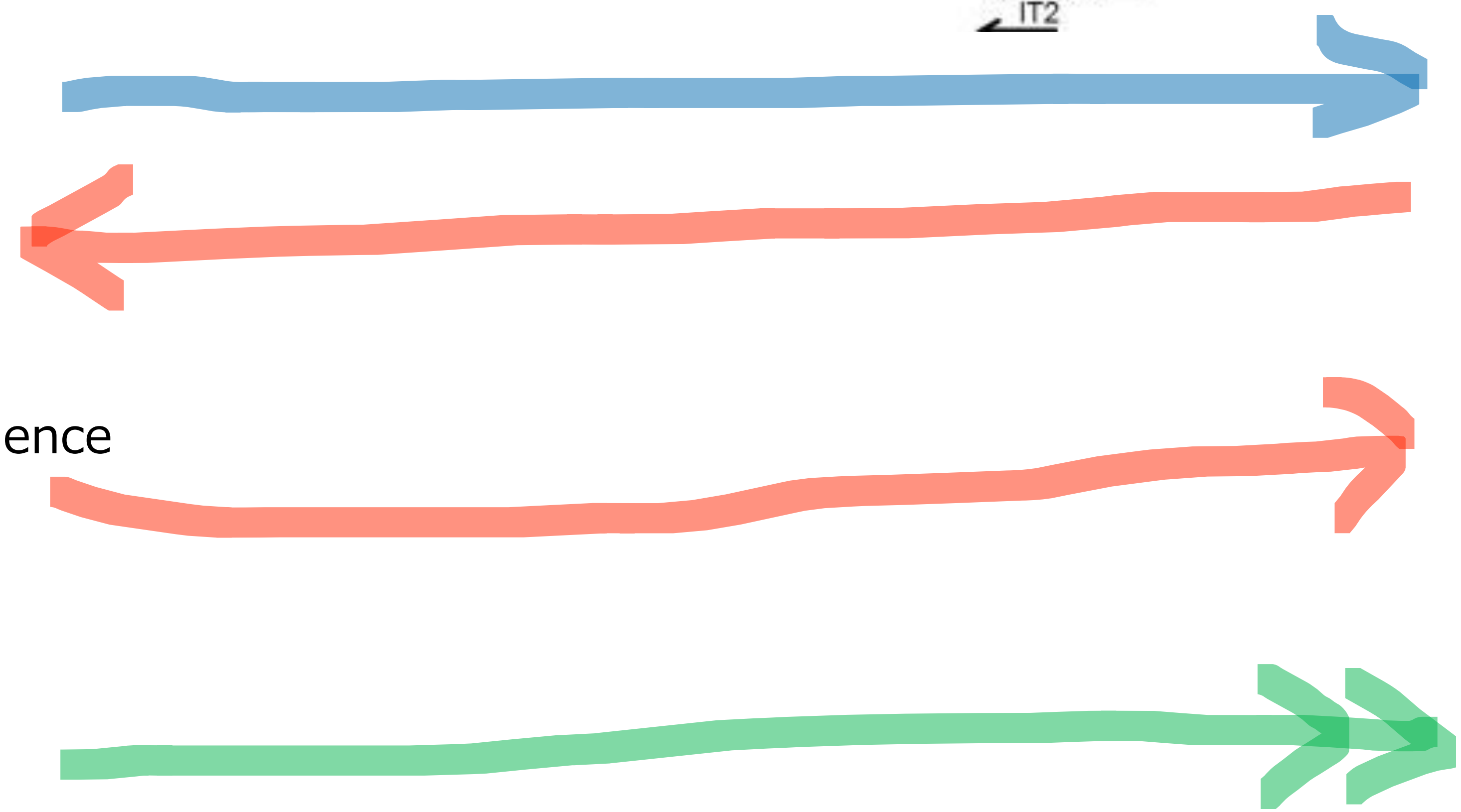
ITS1f – forward

ITS4 – reverse

consensus

Primers used for sequencing





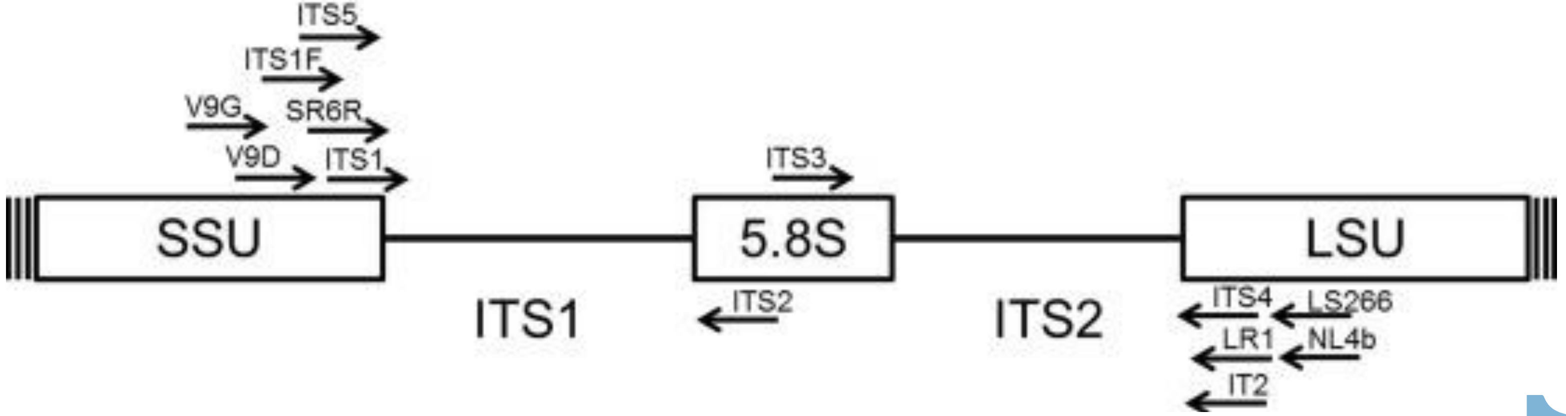
ITS1f – forward

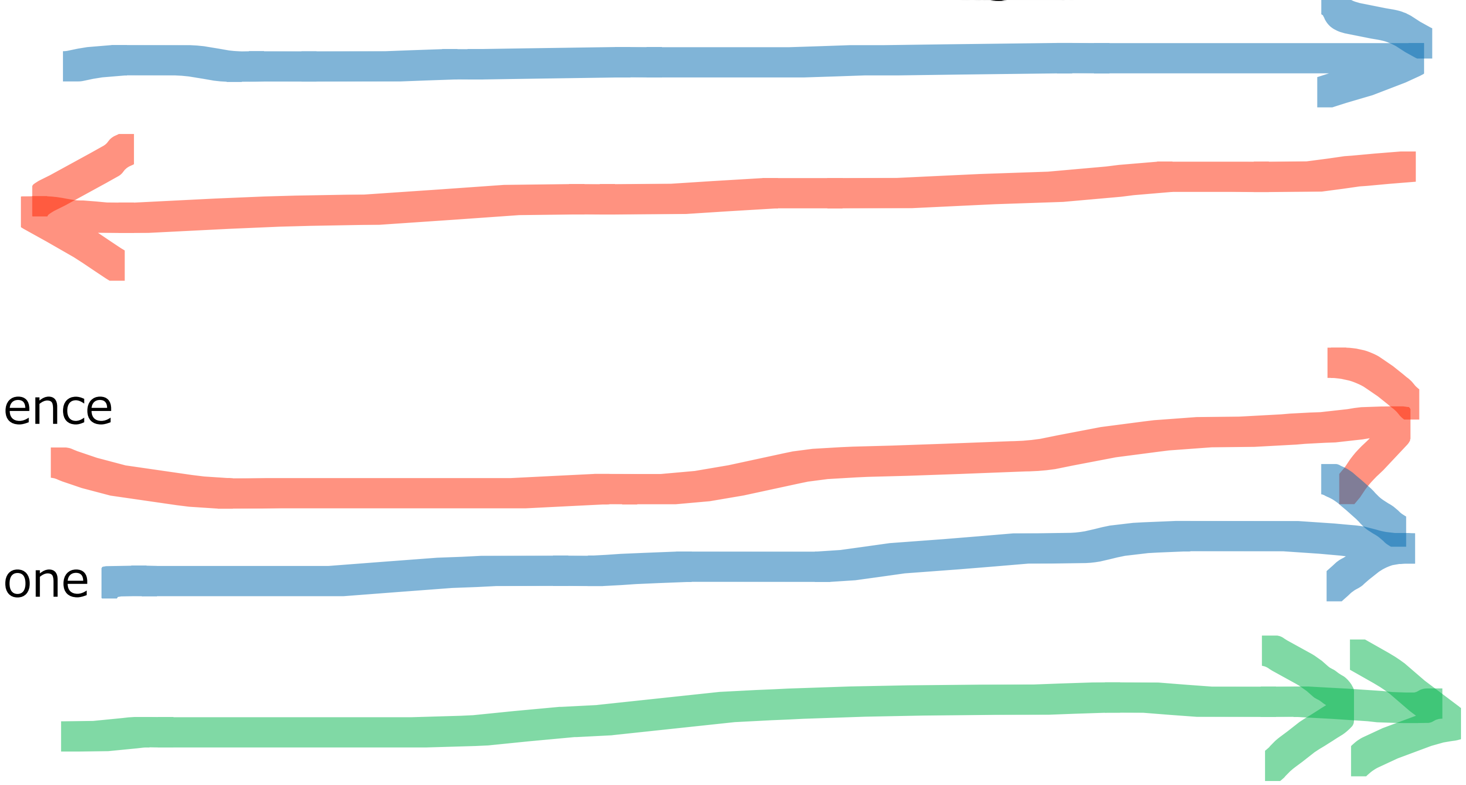
ITS4 – reverse

reverse reverse sequence

consensus

Primers used for sequencing





ITS1f – forward

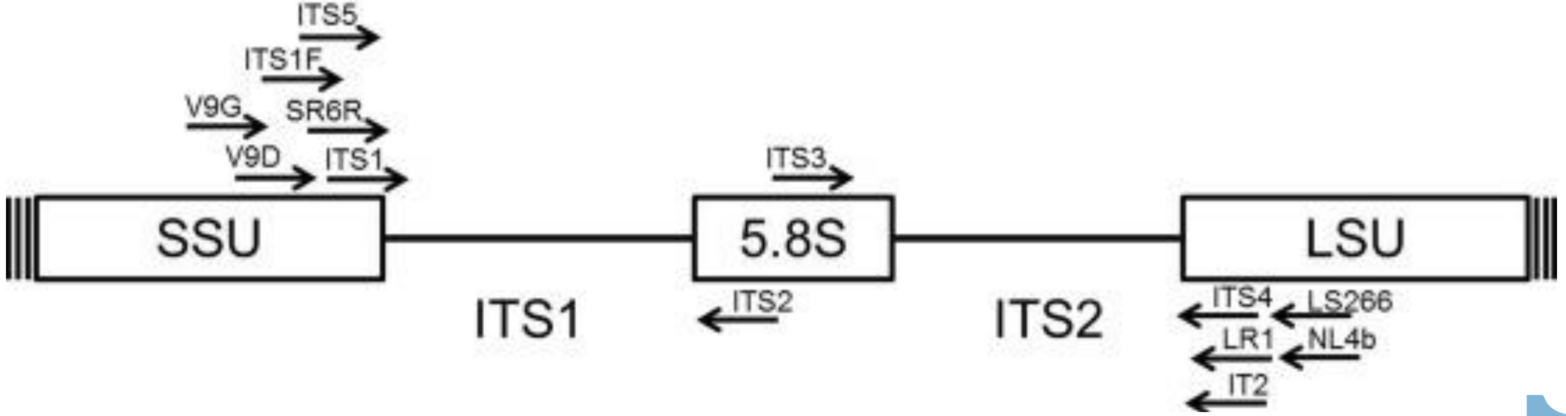
ITS4 – reverse

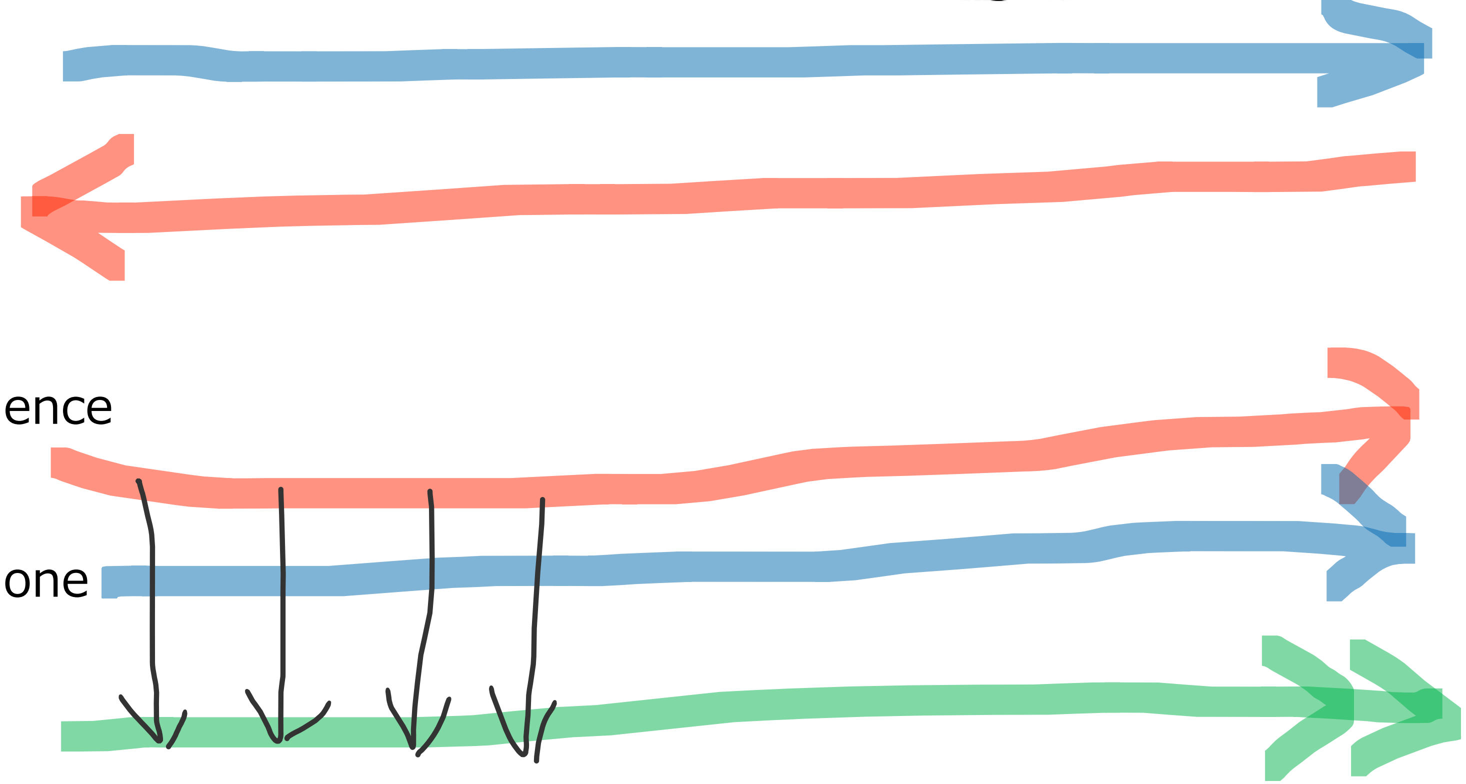
reverse reverse sequence

align it with forward one

consensus

Primers used for sequencing





ITS1f – forward

ITS4 – reverse

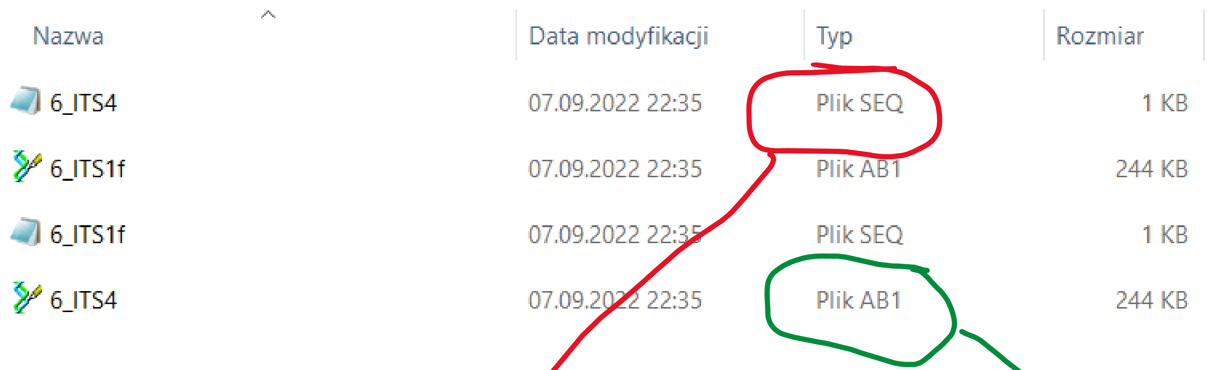
reverse reverse sequence

align it with forward one

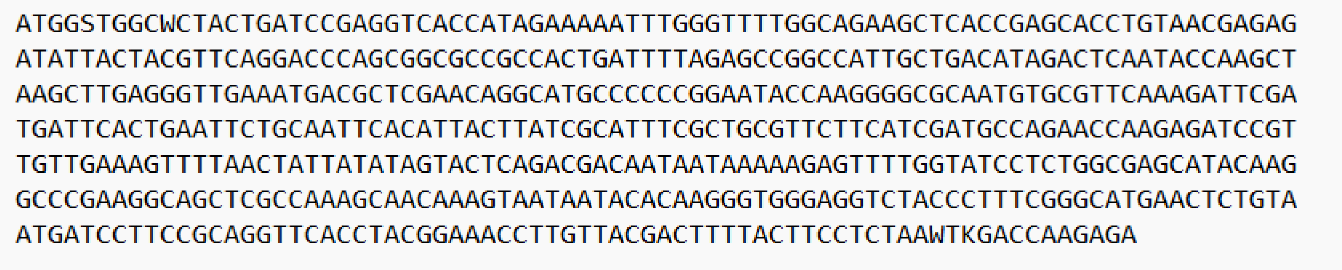
consensus

Download the test results

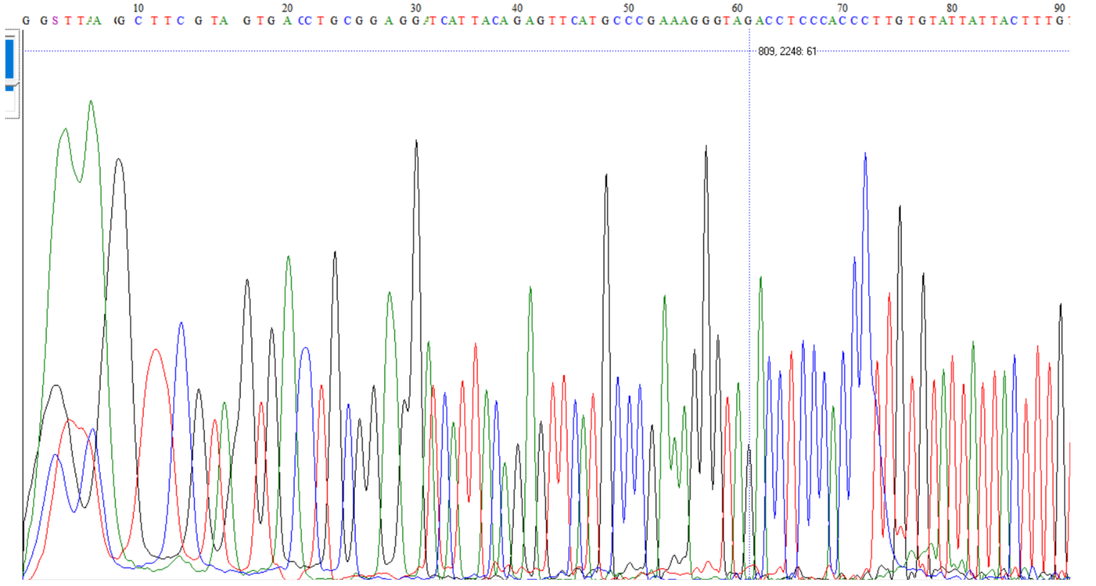
https://ibe.biol.uw.edu.pl/wp-content/uploads/sites/22/2022/09/test-results.zip



Nazwa	Data modyfikacji	Typ	Rozmiar
6_ITS4	07.09.2022 22:35	Plik SEQ	1 KB
6_ITS1f	07.09.2022 22:35	Plik AB1	244 KB
6_ITS1f	07.09.2022 22:35	Plik SEQ	1 KB
6_ITS4	07.09.2022 22:35	Plik AB1	244 KB

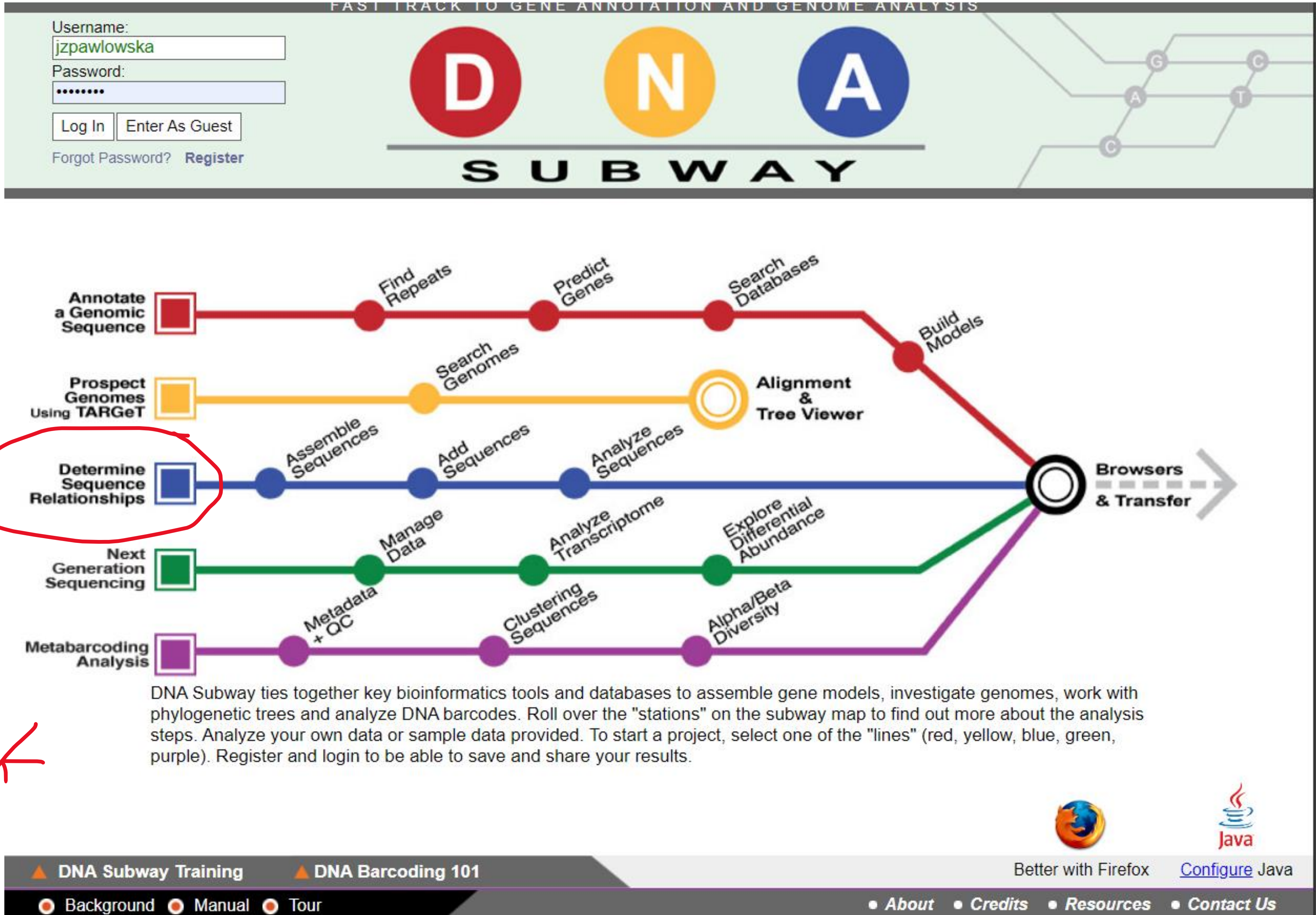


```
ATGGSTGGCWCTACTGATCCGAGGTCACCATAGAAAAATTTGGGTTTTGGCAGAAGCTCACCGAGCACCTGTAACGAGAG
ATATTACTACGTTCAGGACCCAGCGGCGCCGCCACTGATTTTAGAGCCGGCCATTGCTGACATAGACTCAATACCAAGCT
AAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGA
TGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
TGTTGAAAGTTTTAACTATTATATAGTACTCAGACGACAATAATAAAAAGAGTTTTGGTATCCTCTGGCGAGCATACAAG
GCCCGAAGGCAGCTCGCCAAAGCAACAAAGTAATAATACACAAGGGTGGGAGGTCTACCCTTTCGGGCATGAACTCTGTA
ATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCTCTAAWTKGACCAAGAGA
```



How to see your results?

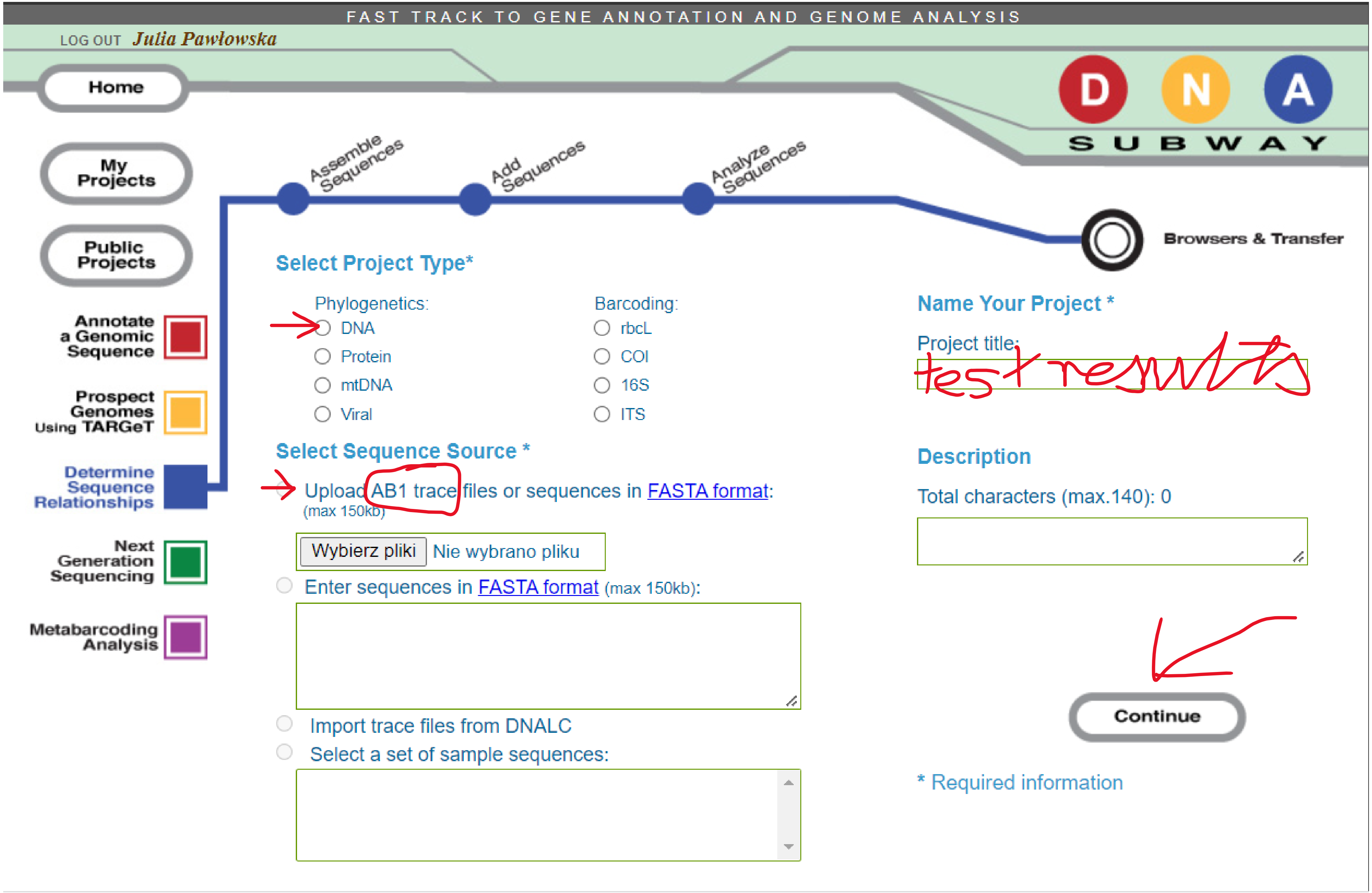
https://dnasubway.cyverse.org



Username: jzpawlowska
Password: ••••••••

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.

click



Select Project Type*

Phylogenetics:
○ DNA
○ Protein
○ mtDNA
○ Viral

Barcoding:
○ rbcL
○ COI
○ 16S
○ ITS

Select Sequence Source *

○ Upload AB1 trace files or sequences in FASTA format: (max 150kb)

Wybierz pliki Nie wybrano pliku

○ Enter sequences in FASTA format (max 150kb):

○ Import trace files from DNALC

○ Select a set of sample sequences:

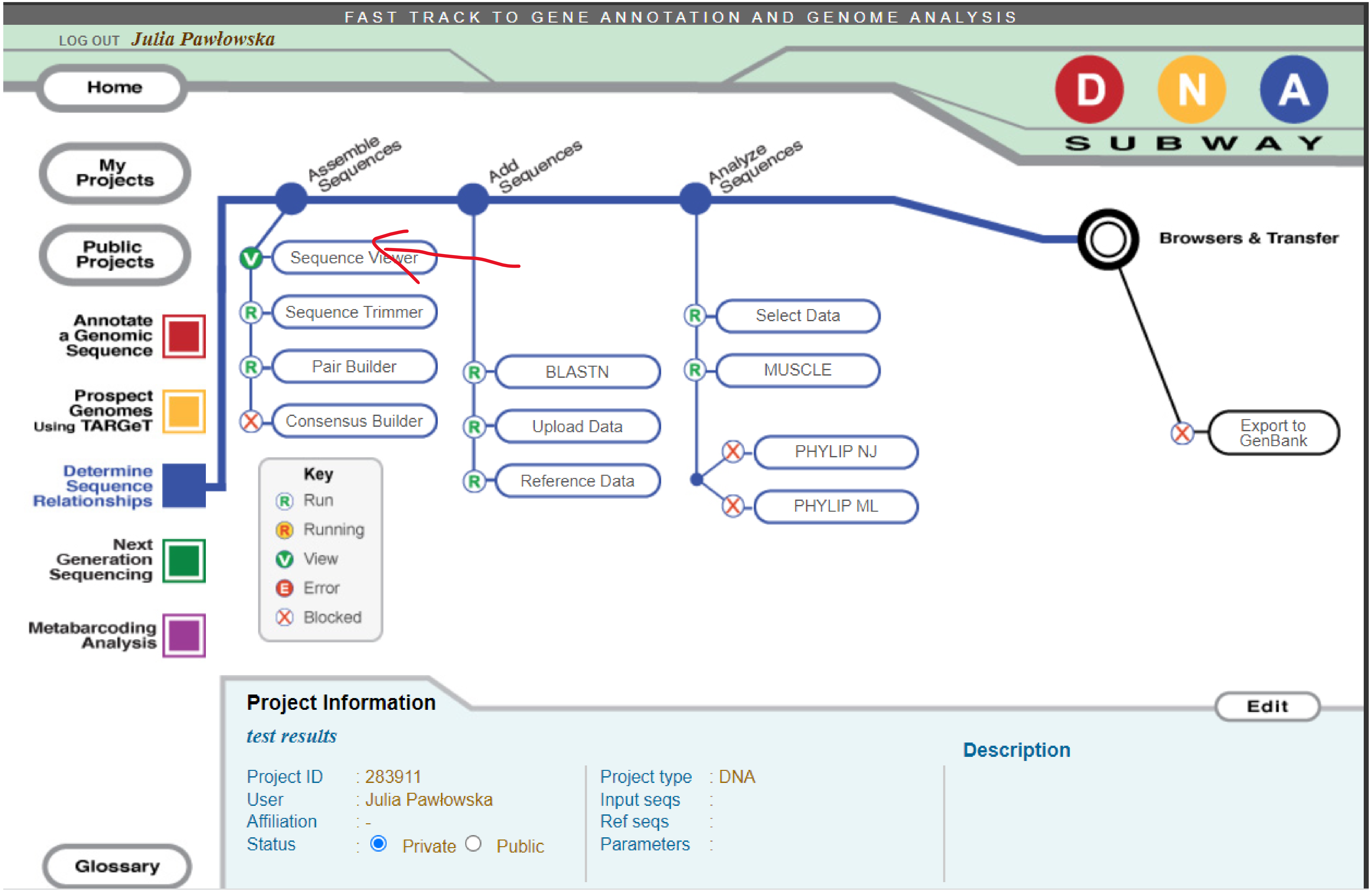
Name Your Project *

Project title: test results

Description

Total characters (max.140): 0

* Required information



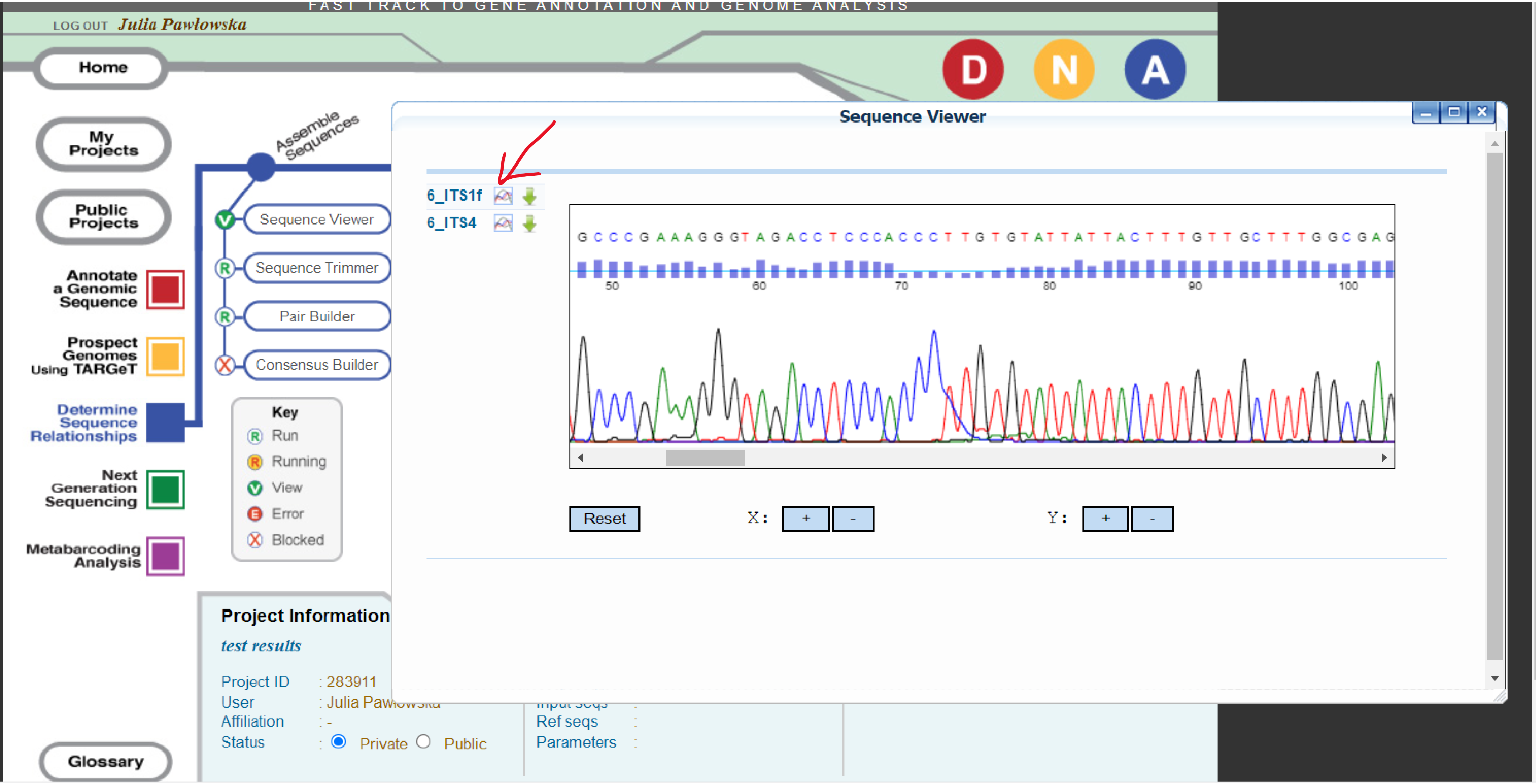
Project Information

test results

Project ID : 283911
User : Julia Pawłowska
Affiliation : -
Status : ● Private ○ Public

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

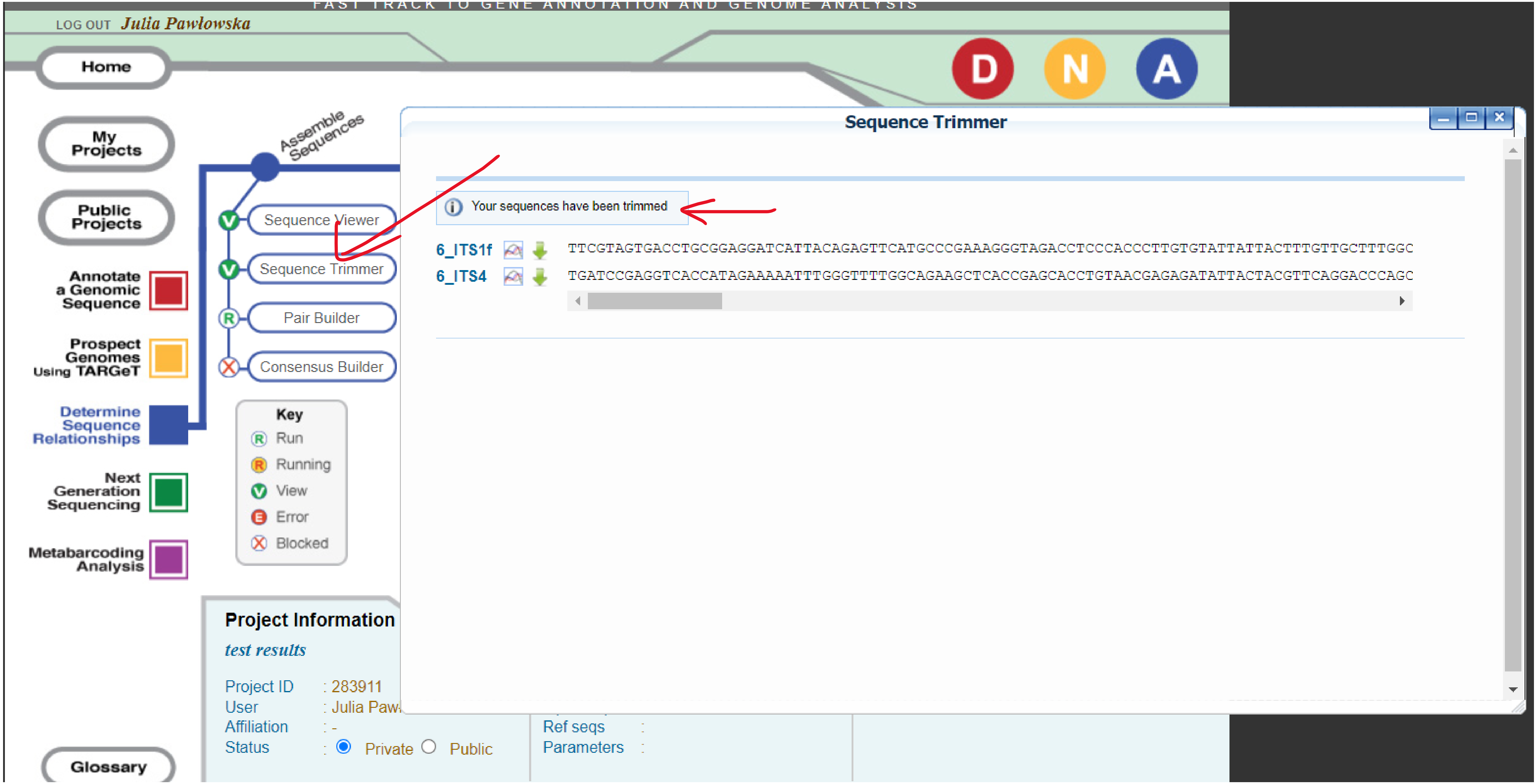
Description



test results

Project ID : 283911
User : Julia Pawlowska
Affiliation : -
Status : ● Private ○ Public

Input seqs :
Ref seqs :
Parameters :



Sequence Trimmer

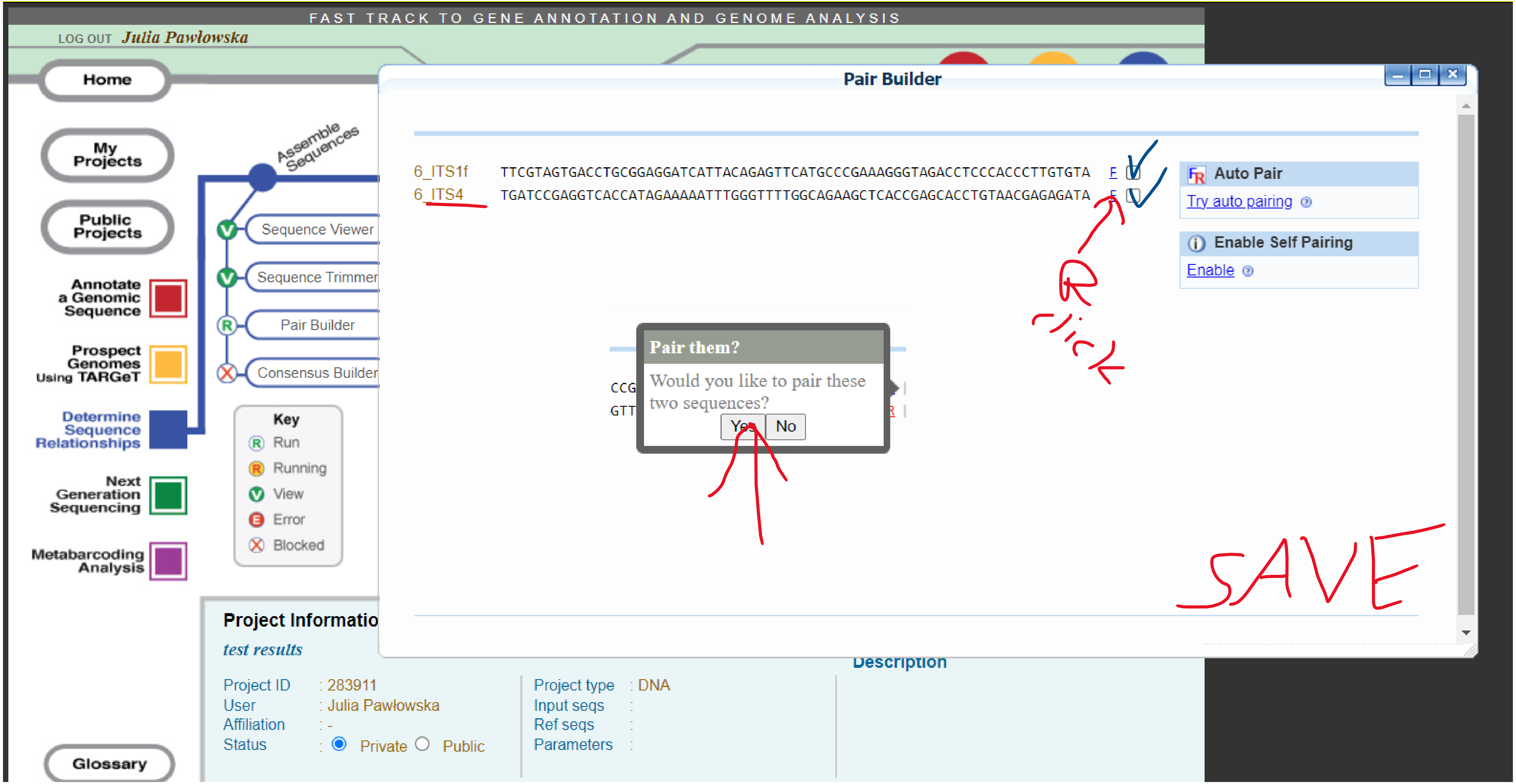
Your sequences have been trimmed

```
6_ITS1f   TTCGTAGTGACCTGCGGAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTTTGGC
6_ITS4    TGATCCGAGGTCACCATAGAAAAATTTGGGTTTTGGCAGAAGCTCACCGAGCACCTGTAACGAGAGATATTACTACGTTCAGGACCCAGC
```

Project Information

test results

Project ID : 283911
User : Julia Paw
Affiliation : -
Status : ● Private ○ Public
Ref seqs :
Parameters :



```
6_ITS1f    TTCGTAGTGACCTGCGGAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTA  F ☐
6_ITS4     TGATCCGAGGTCACCATAGAAAAATTTGGGTTTTGGCAGAAGCTCACCGAGCACCTGTAACGAGAGATA  F ☐
```

CCG
GTT

Pair them?

Would you like to pair these two sequences?

Yes No

click

SAVE

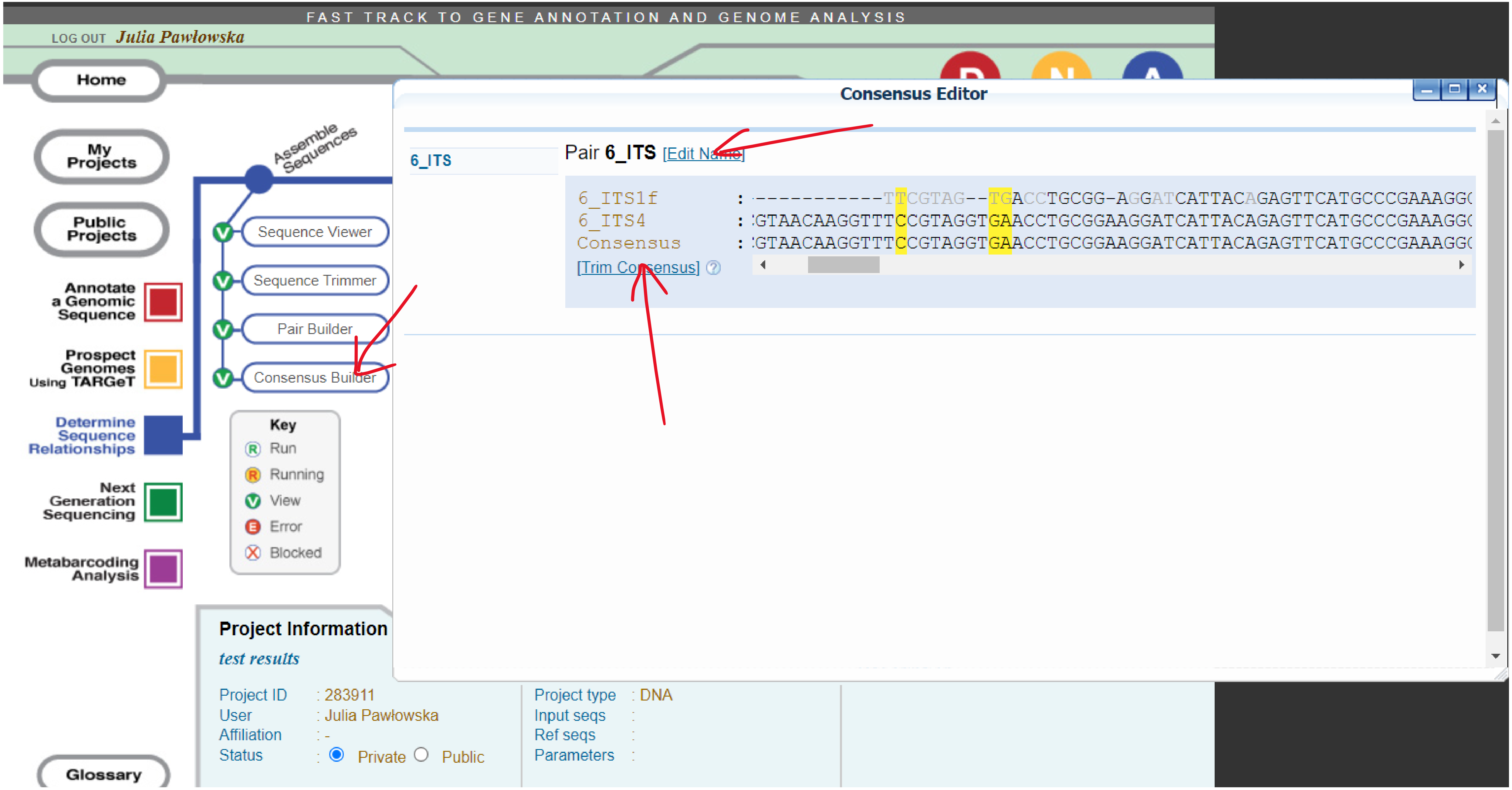
Project Information

test results

Project ID : 283911
User : Julia Pawłowska
Affiliation : -
Status : ● Private ○ Public

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

Description



Consensus Editor

6_ITS

Pair **6_ITS** [Edit Name]

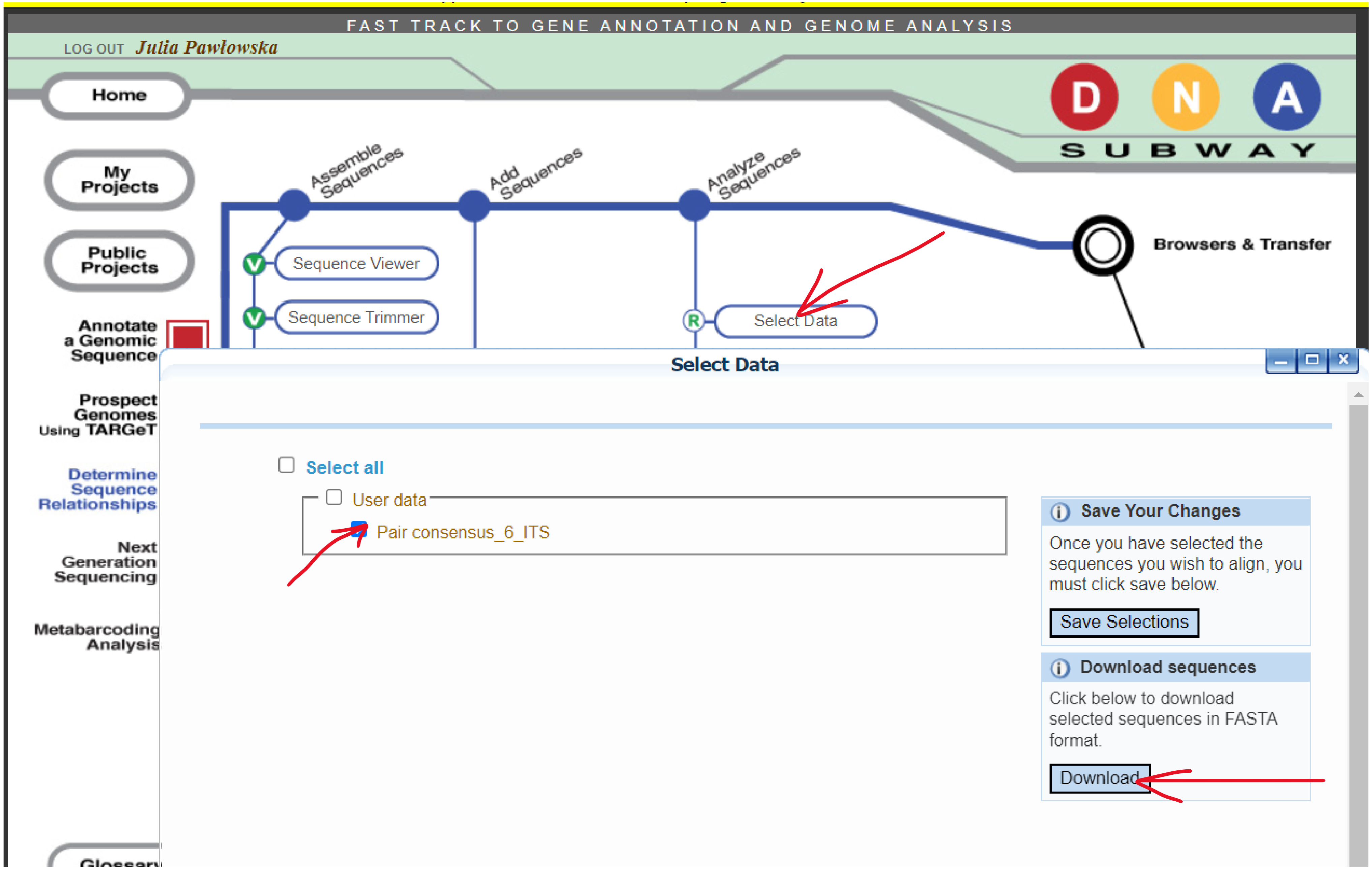
```
6_ITS1f     : -----------TTCGTAG--TGACCTGCGG-AGGATCATTACAGAGTTCATGCCCGAAAGG(
6_ITS4      : GTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGG(
Consensus   : GTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGG(
```

[Trim Consensus]

Project Information

test results

Project ID : 283911
User : Julia Pawłowska
Affiliation : -
Status : ● Private ○ Public
Project type : DNA
Input seqs :
Ref seqs :
Parameters :



Select Data

☐ Select all

☐ User data

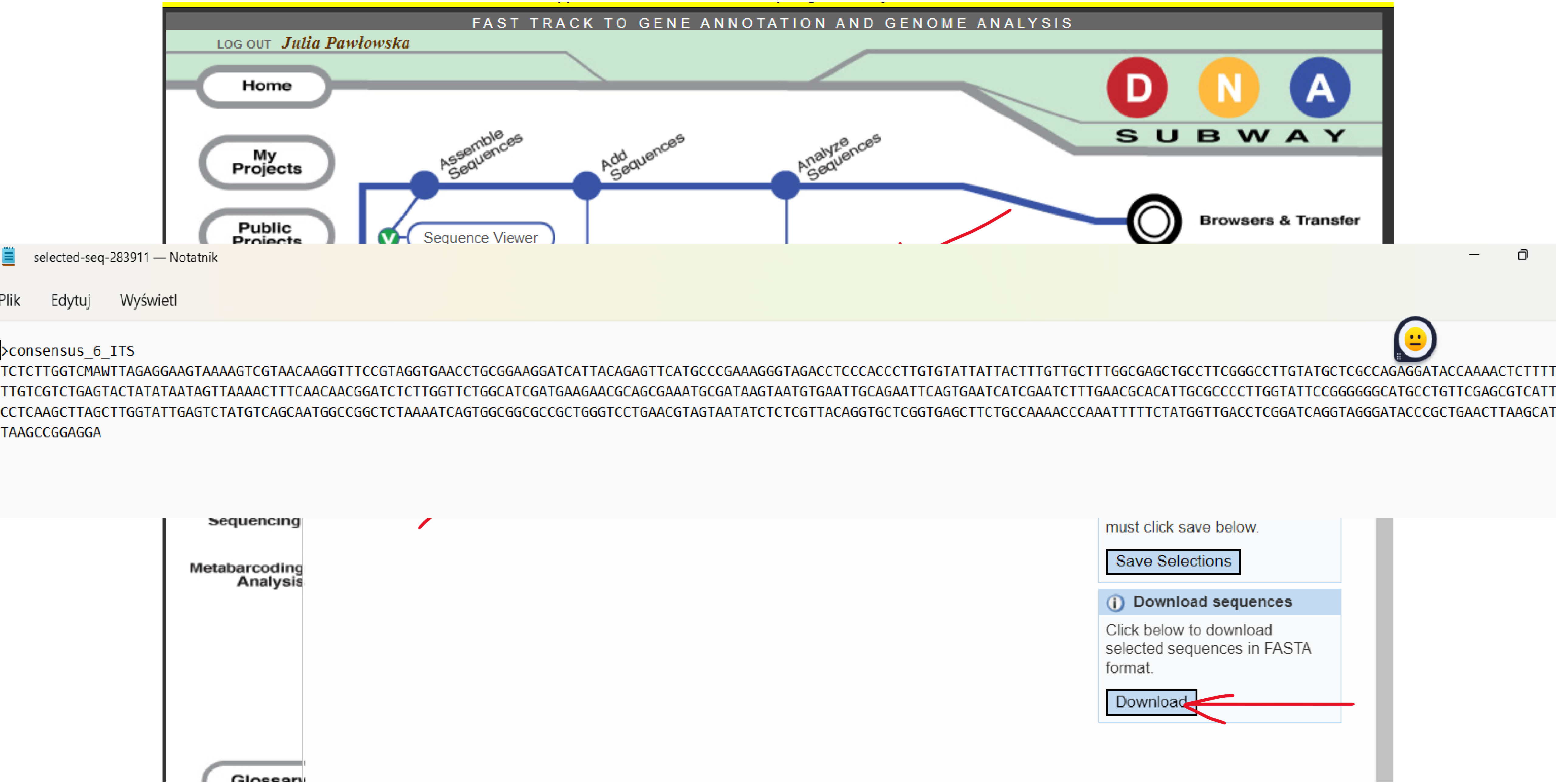
☑ Pair consensus_6_ITS

Save Your Changes

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

Download sequences

Click below to download selected sequences in FASTA format.



```
>consensus_6_ITS
TCTCTTGGTCMAWTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTTTGGCGAGCTGCCTTCGGGCCTTGTATGCTCGCCAGAGGATACCAAAACTCTTTT
TTGTCGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATT
CCTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGCAATGGCCGGCTCTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTGCTCGGTGAGCTTCTGCCAAAACCCAAATTTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCAT
TAAGCCGGAGGA
```

must click save below.

Click below to download selected sequences in FASTA format.