

DNA sequence editing

Summer School:

„from fungal morphology to genotype”

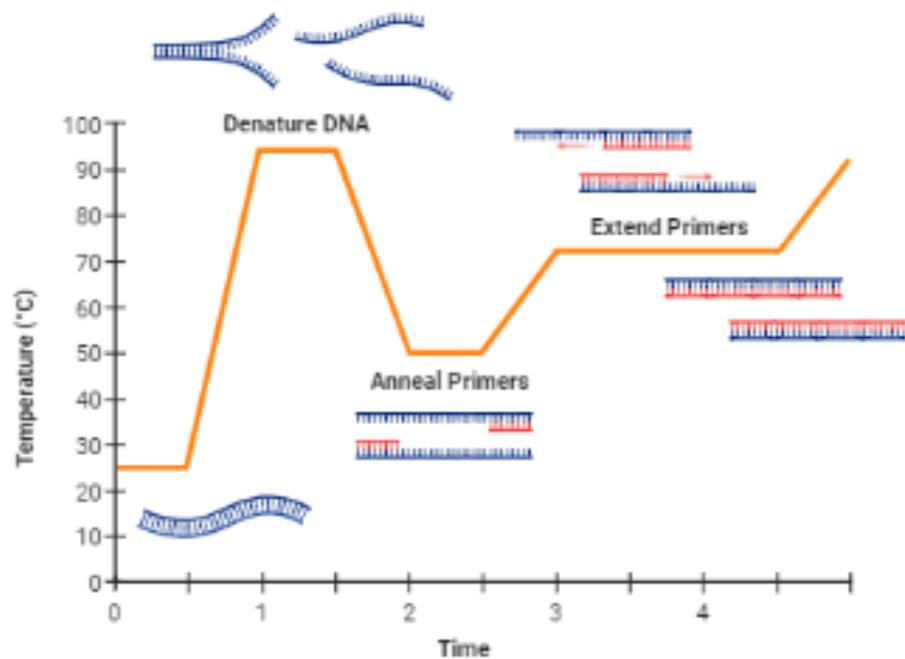
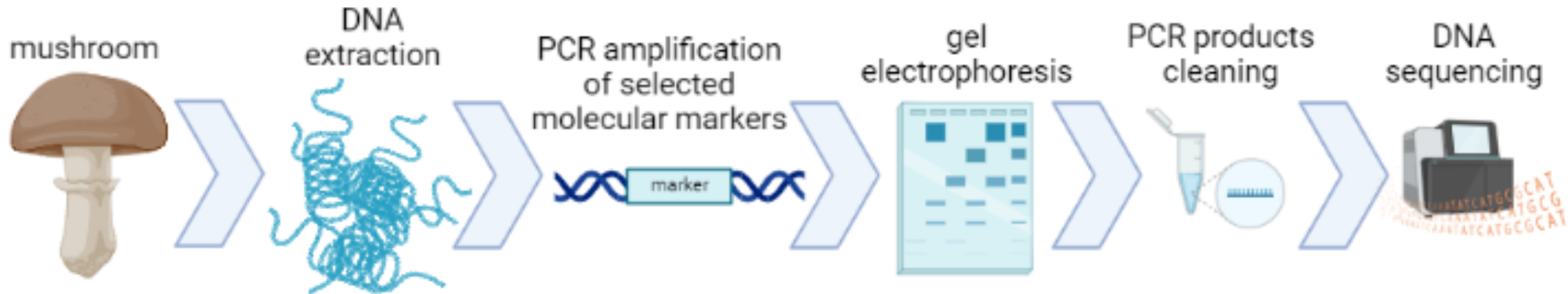


Julia Pawłowska

Univeristy of Warsaw

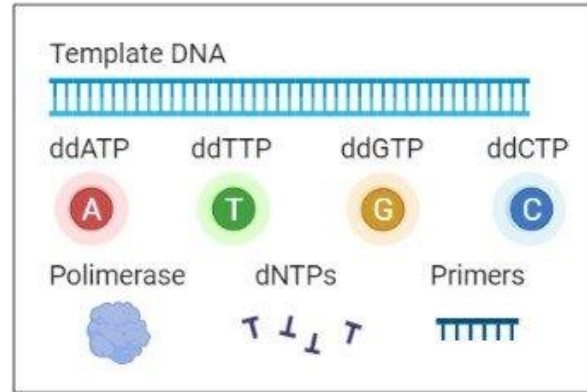
25.08.2023

General pipeline

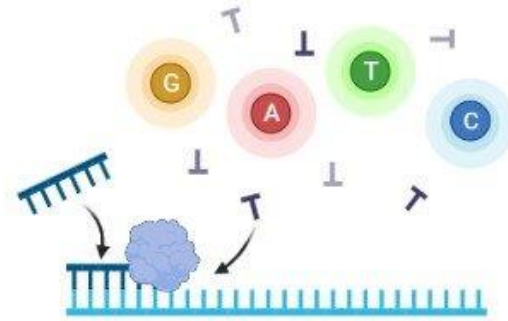


Sanger sequencing results

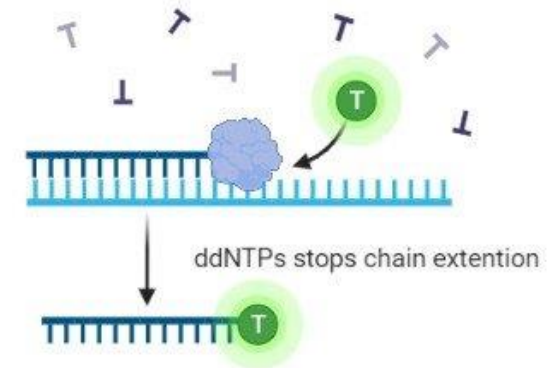
Reagents



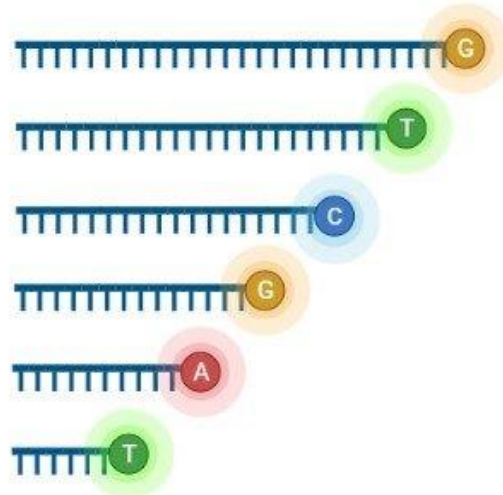
① Primer annealing and chain extension



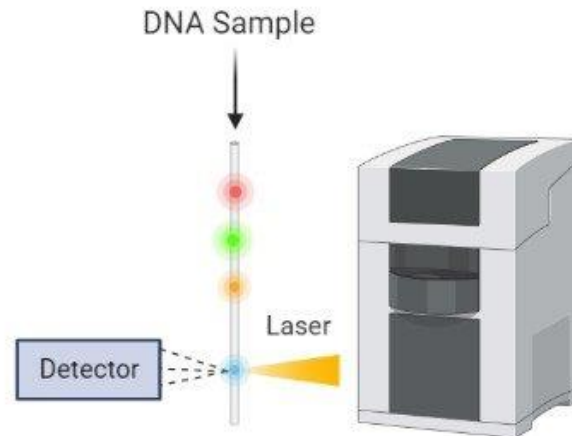
② ddNTP binding and chain termination



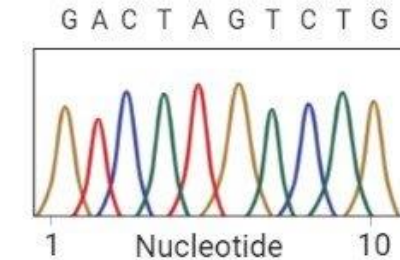
③ Fluorescently labelled DNA sample



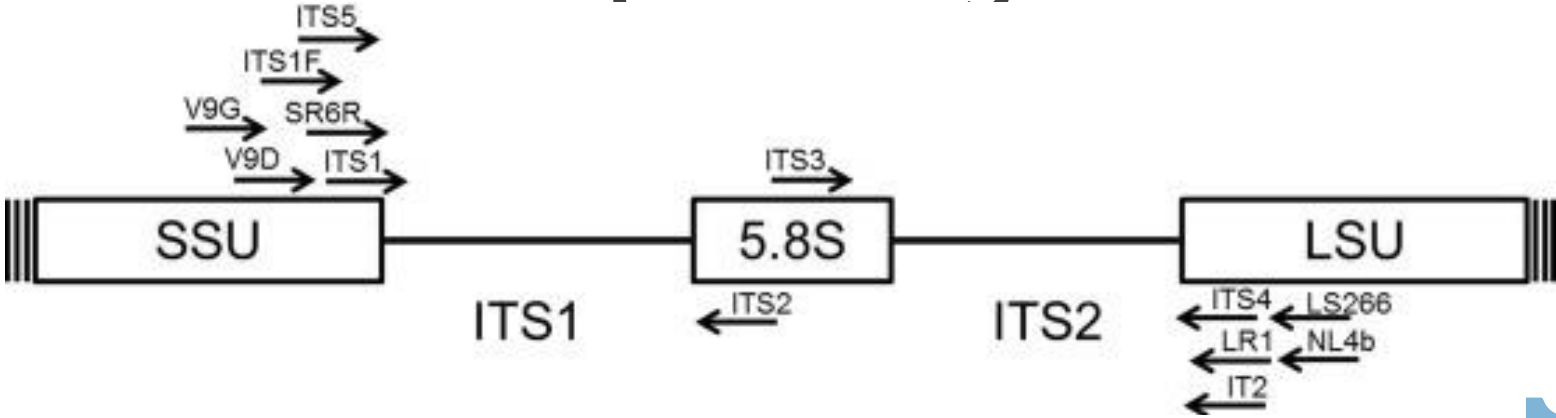
④ Capillary gel electrophoresis and fluorescence detection



⑤ Sequence analysis and reconstruction



Primers used for sequencing



ITS1f - forward



ITS4 - reverse



Primers used for sequencing



ITS1f – forward



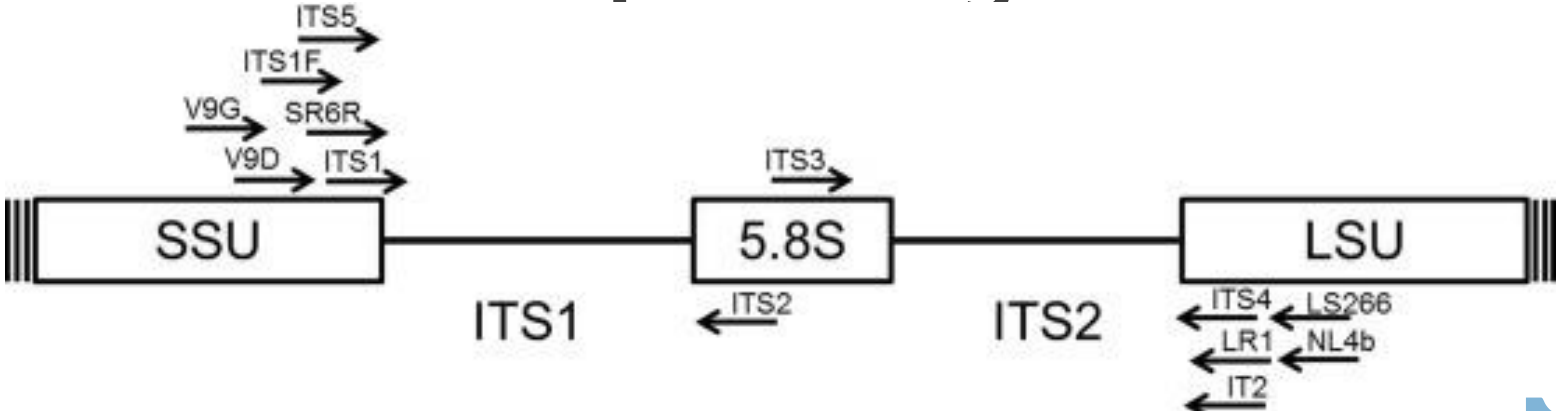
ITS4 – reverse



consensus



Primers used for sequencing



ITS1f – forward



ITS4 – reverse



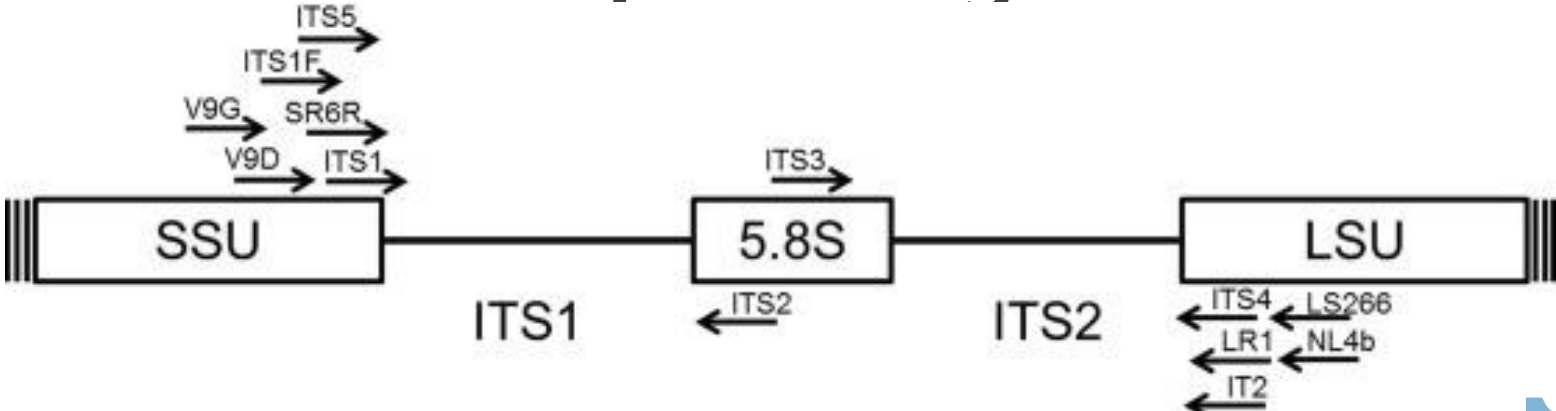
reverse reverse sequence



consensus



Primers used for sequencing



ITS1f – forward



ITS4 – reverse



reverse reverse sequence



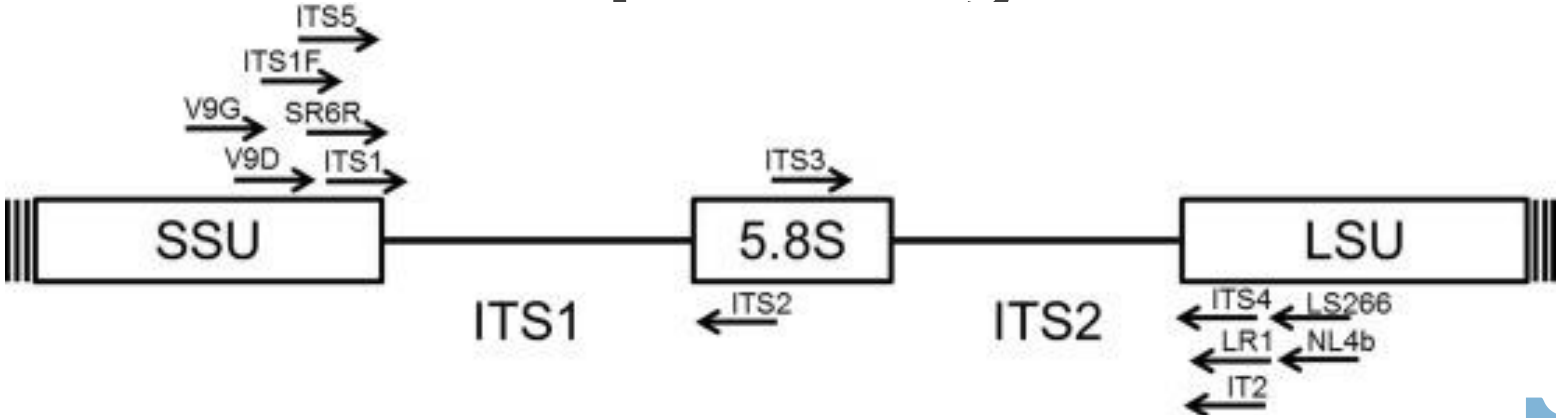
align it with forward one



consensus



Primers used for sequencing



ITS1f – forward



ITS4 – reverse



reverse reverse sequence



align it with forward one



consensus

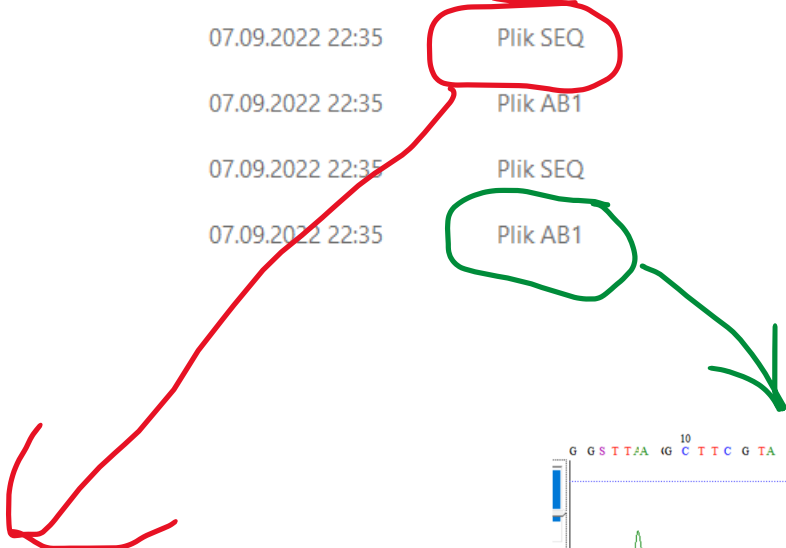


Download the test results

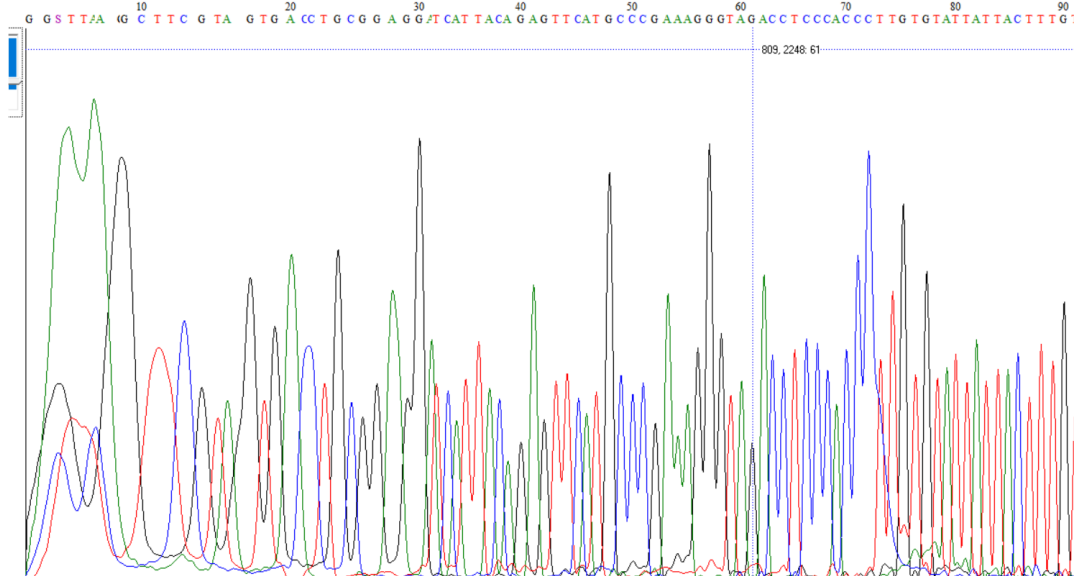
<https://ibe.biol.uw.edu.pl/wp-content/uploads/sites/22/2022/09/test-results.zip>



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
ATATTACTACGTTCCAGGACCCAGCGGCCGCCACTGATTTTAGAGCCGGCCATTGCTGACATAGACTCAATACCAAGCT
AAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGA
TGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
TGTTGAAAGTTTTAACTATTATATAGTACTCAGACGACAATAATAAAAAGAGTTTTGGTATCCTCTGGCGAGCATAACAAG
GCCCGAAGGCAGCTCGCCAAAGCAACAAAGTAATAATACACAAGGGTGGGAGGTCTACCCTTTCGGGCATGAACTCTGTA
ATGATCCTTCCGCAGGTTACCTACGGAAACCTTGTTACGACTTTTACTTCTCTAAWTKGACCAAGAGA
```



How to see your results?

<https://dnasubway.cyverse.org>

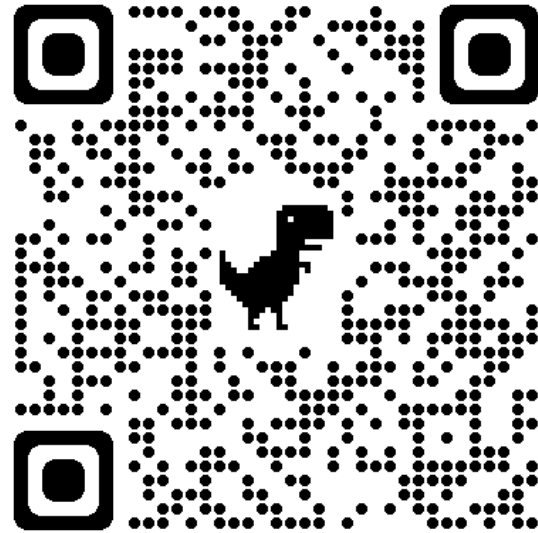
FAST TRACK TO GENE ANNOTATION AND GENOME ANALYSIS

Username:
Password:

[Forgot Password?](#) [Register](#)

D N A

SUBWAY



click



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.



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Prospect Genomes Using TARGET



Determine Sequence Relationships



Next Generation Sequencing



Metabarcoding Analysis



Assemble Sequences

Add Sequences

Analyze Sequences



Browsers & Transfer

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:

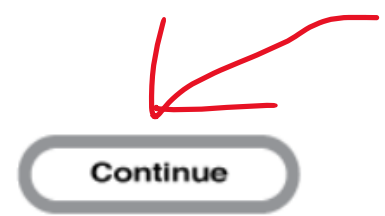
Name Your Project *

Project title:

test results

Description

Total characters (max.140): 0



* Required information



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Assemble Sequences Add Sequences Analyze Sequences

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- R Sequence Trimmer
- R Pair Builder
- X Consensus Builder

- R BLASTN
- R Upload Data
- R Reference Data

- R Select Data
- R MUSCLE
- X PHYLIP NJ
- X PHYLIP ML

Browsers & Transfer

- X Export to GenBank

Key

- R Run
- R Running
- V View
- E Error
- X Blocked

Project Information

test results

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

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

Description

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Key

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test results

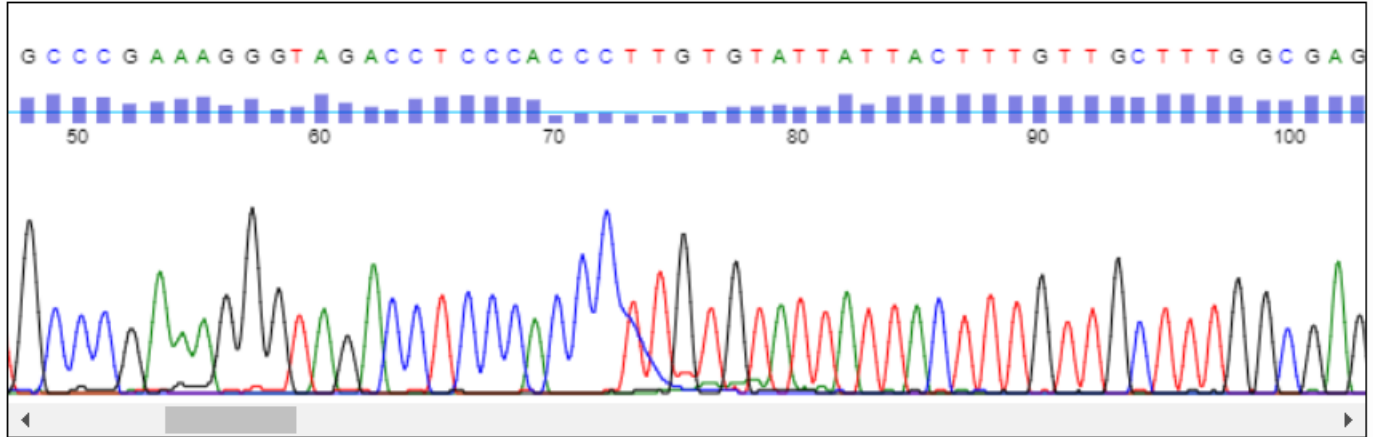
Project ID : 283911
 User : Julia Pawłowska
 Affiliation : -
 Status : Private Public

Input seqs :
 Ref seqs :
 Parameters :

Sequence Viewer

6 ITS1f

6 ITS4



Reset

X: + -

Y: + -



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Assemble Sequences






Sequence Viewer

Sequence Trimmer

Pair Builder

Consensus Builder

Key

-  Run
-  Running
-  View
-  Error
-  Blocked


Project Information

test results

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

Ref seqs :
 Parameters :

Sequence Trimmer

 Your sequences have been trimmed

6_ITS1f

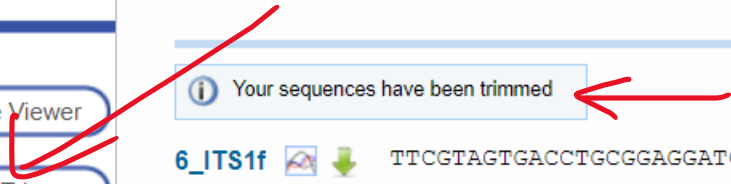
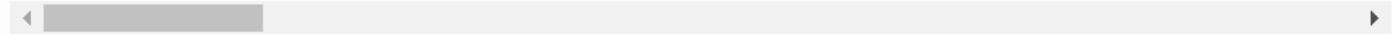


TTCGTAGTGACCTGCGGAGGATCATTACAGAGTTCATGCCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTTTGGC

6_ITS4



TGATCCGAGGTCACCATAGAAAAATTTGGGTTTTGGCAGAAGCTCACCGAGCACCTGTAACGAGAGATATTACTACGTTCAGGACCCAGC



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Project Information

test results

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

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

Pair Builder

6 ITS1f	TTCGTAGTGACCTGCGGAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTA	E	<input checked="" type="checkbox"/>
6 ITS4	TGATCCGAGGTCACCATAGAAAAATTTGGGTTTTGGCAGAAGCTCACCGAGCACCTGTAACGAGAGATA	E	<input checked="" type="checkbox"/>

Auto Pair

[Try auto pairing](#)

Enable Self Pairing

[Enable](#)

Pair them?

Would you like to pair these two sequences?

Yes No

Click

SAVE

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- Ⓡ Running
- Ⓥ View
- ⓔ Error
- ⓧ Blocked

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test results

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

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

Consensus Editor

6_ITS

Pair **6_ITS** [\[Edit Name\]](#)

```

6_ITS1f      : -----TTCGTAG--TGACCTGCGG-AGGATCATTACAGAGTTCATGCCCGAAAGGC
6_ITS4      : :GTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGC
Consensus    : :GTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGC

```

[\[Trim Consensus\]](#) ?



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Annotate a Genomic Sequence

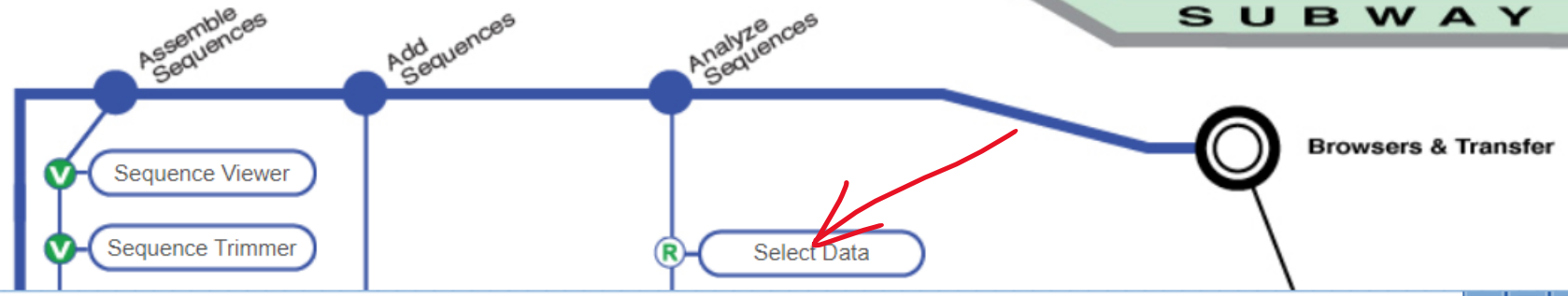
Prospect Genomes Using TARGET

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Select Data

Select all

User data

Pair consensus_6_ITS

i Save Your Changes

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

Save Selections

i Download sequences

Click below to download selected sequences in FASTA format.

Download

FAST TRACK TO GENE ANNOTATION AND GENOME ANALYSIS

LOG OUT *Julia Pawłowska*

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SUBWAY

Browsers & Transfer

selected-seq-283911 — Notatnik

Plik Edytuj Wyświetl

>consensus_6_ITS

TCTCTTGGTCMAWTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCACCCTTGTGTATTATTACTTTGTTGCTTTGGCGAGCTGCCTTCGGGCCTTGATGCTCGCCAGAGGATACCAAACTCTTTTGTGCTGCTGAGTACTATATAATAGTTAAAACCTTCAACAACGGATCTCTTGGTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTCTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGCAATGGCCGGCTCTAAAATCAGTGGCGGCCGCTGGGTCTGAACGTAGTAATATCTCTCGTTACAGGTGCTCGGTGAGCTTCTGCCAAAACCAAAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAAGCATAGCCGGAGGA

Sequencing

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must click save below.

Save Selections

i Download sequences

Click below to download selected sequences in FASTA format.

Download