

DNA sequence editing

Summer School:

„from fungal morphology to genotype”

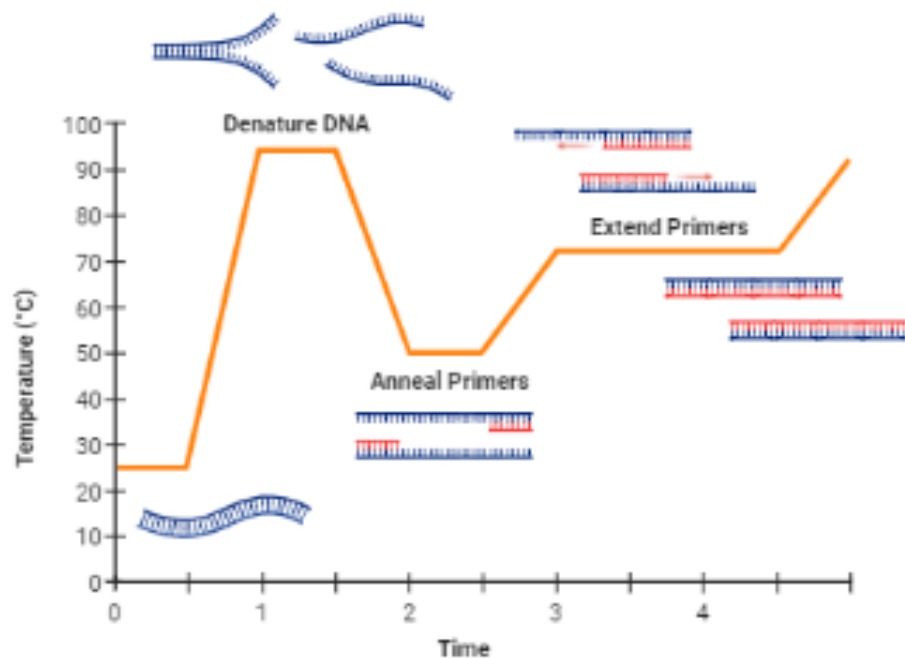
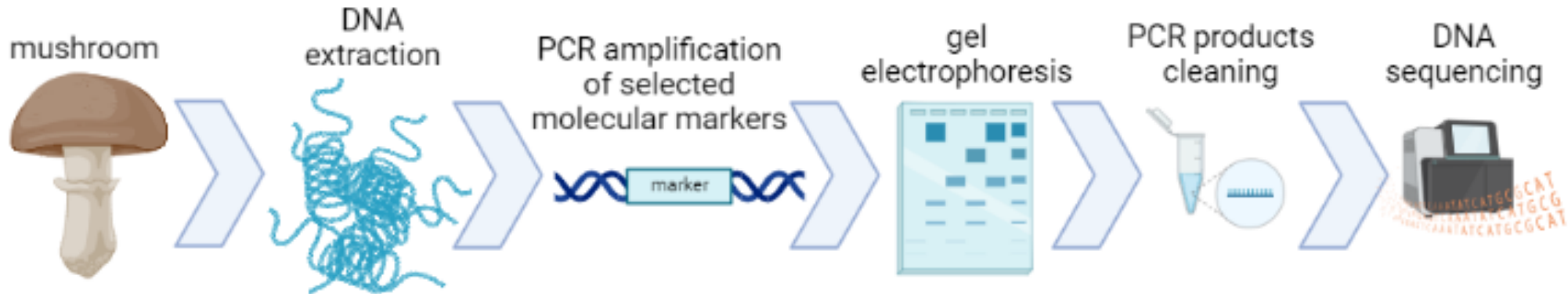


Julia Pawłowska

Univeristy of Warsaw

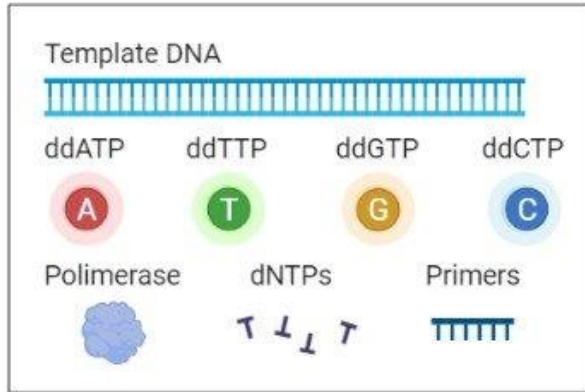
30.08.2024

General pipeline

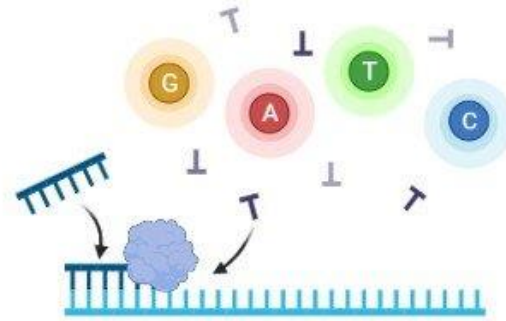


Sanger sequencing results

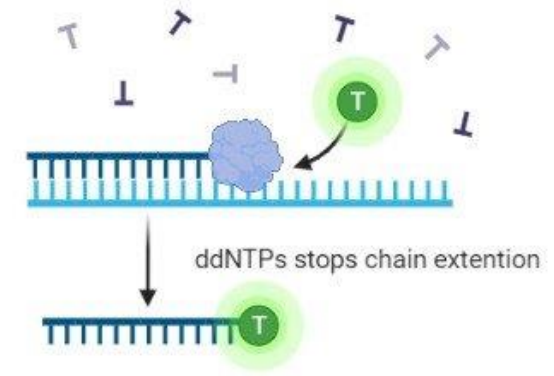
Reagents



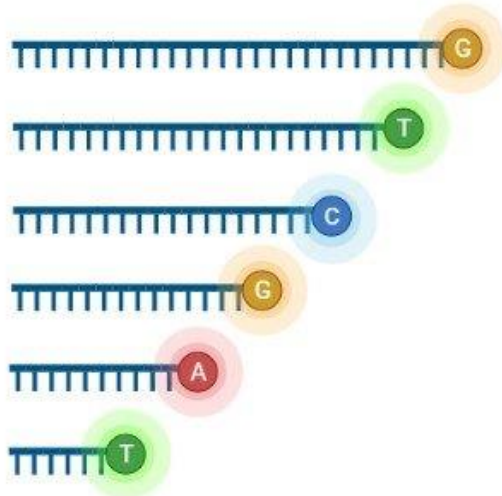
① Primer annealing and chain extension



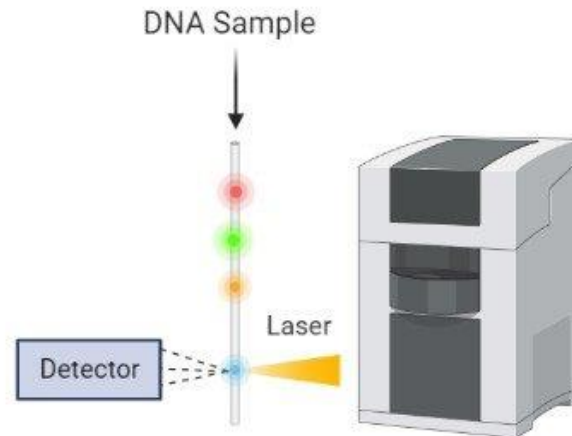
② ddNTP binding and chain termination



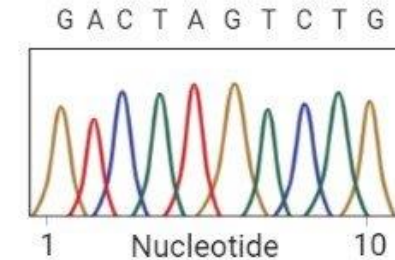
③ Fluorescently labelled DNA sample



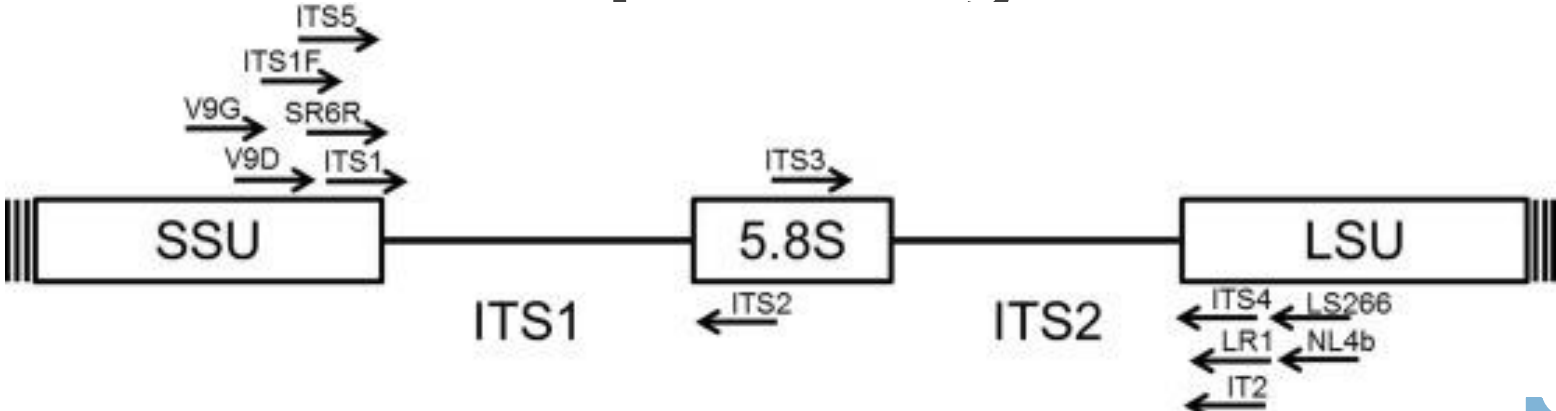
④ Capillary gel electrophoresis and fluorescence detection



⑤ Sequence analysis and reconstruction



Primers used for sequencing



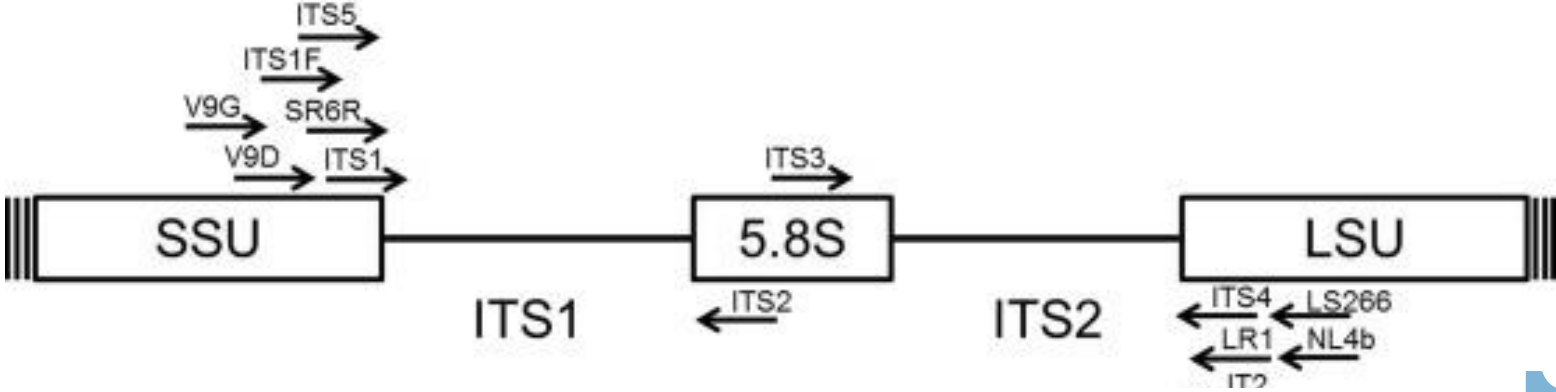
ITS1f - forward



ITS4 - reverse



Primers used for sequencing



ITS1f – forward



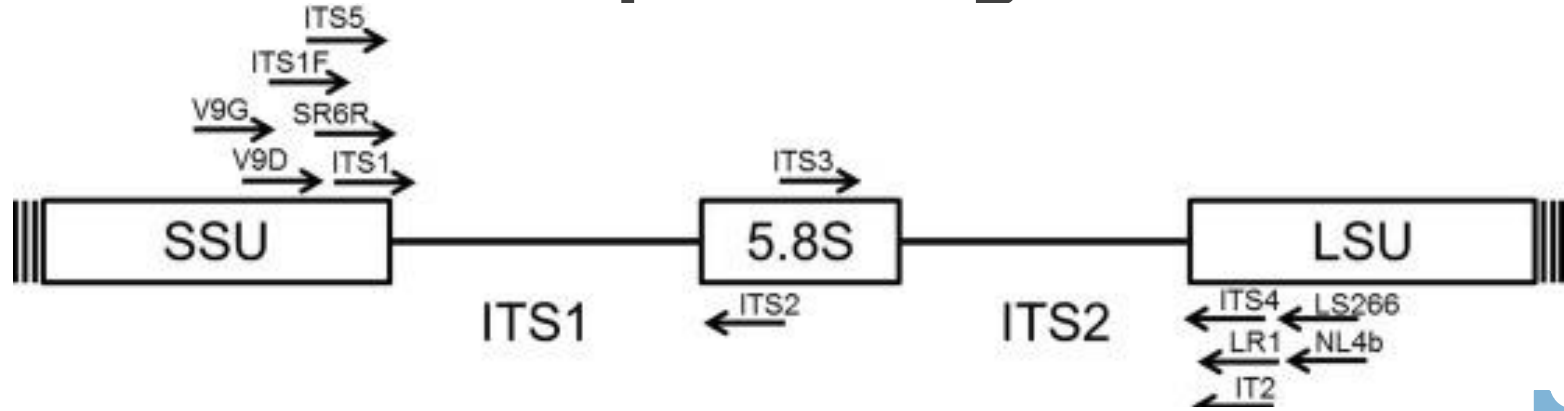
ITS4 – reverse



consensus



Primers used for sequencing



ITS1f – forward



ITS4 – reverse



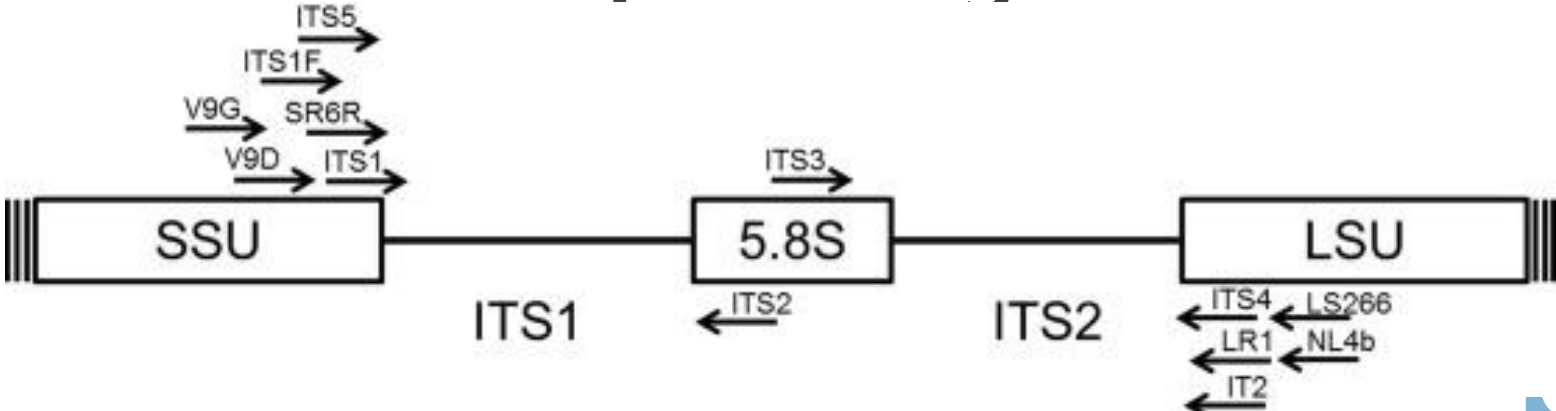
reverse reverse sequence



consensus



Primers used for sequencing



ITS1f – forward



ITS4 – reverse



reverse reverse sequence



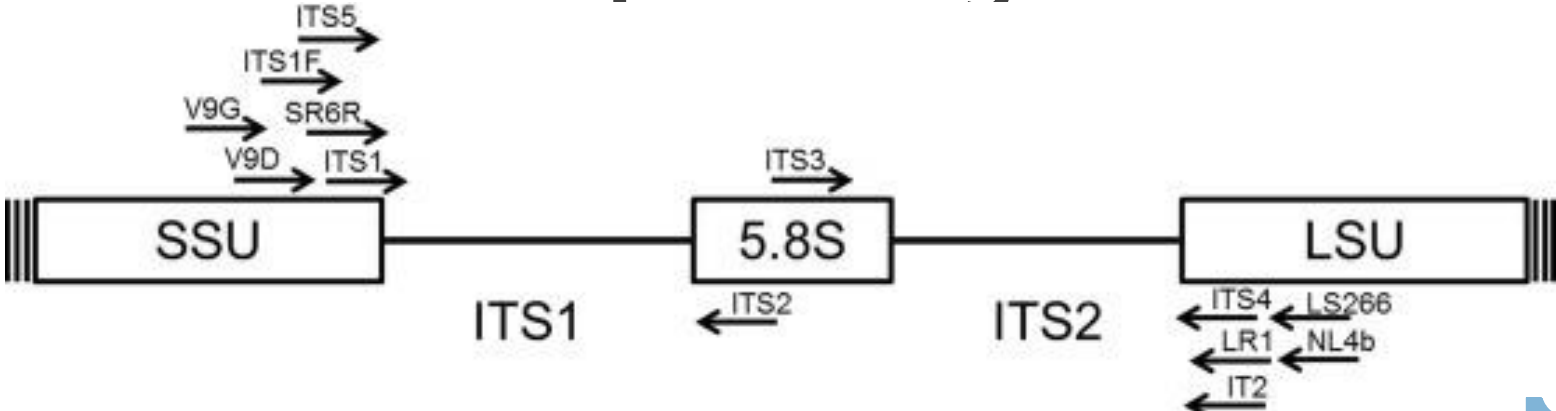
align it with forward one



consensus



Primers used for sequencing



ITS1f – forward



ITS4 – reverse



reverse reverse sequence



align it with forward one



consensus

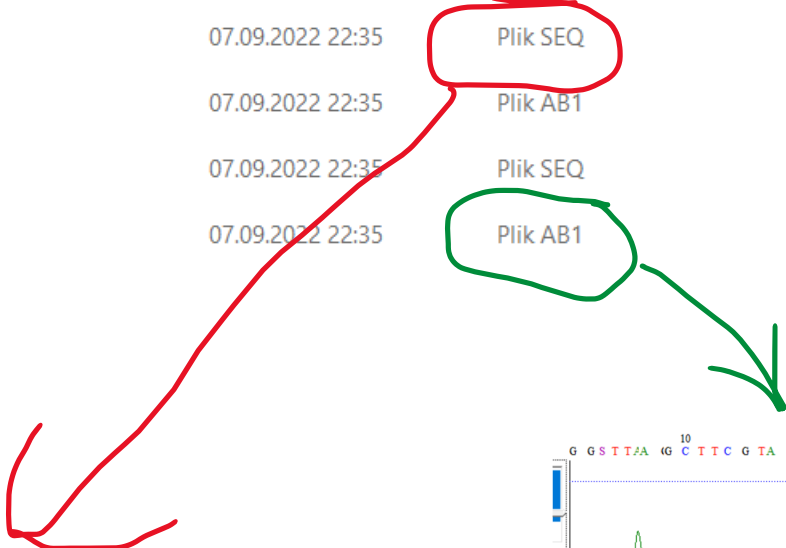


Download the test results

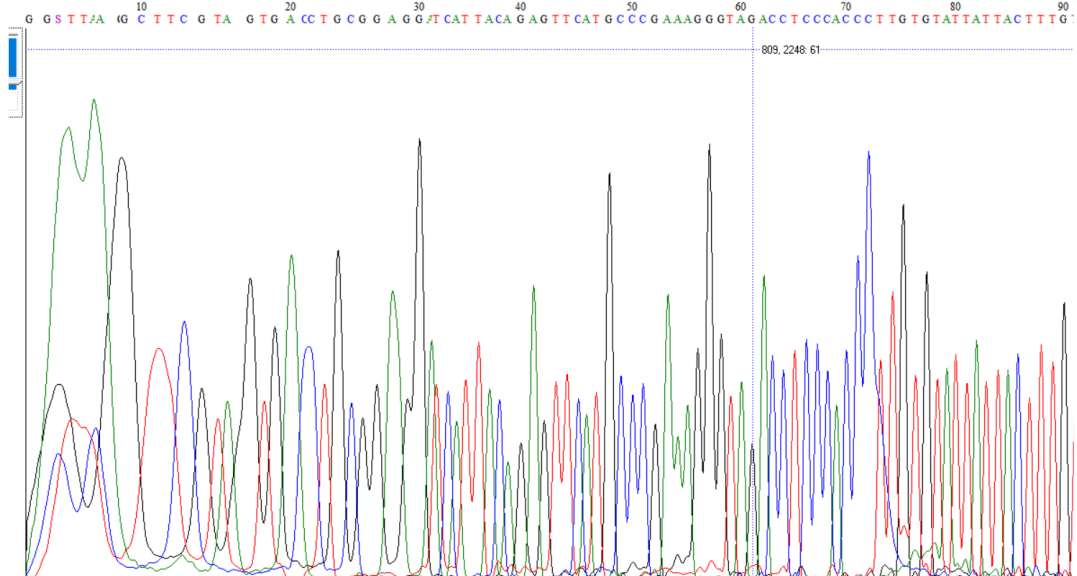
<https://ibe.biol.uw.edu.pl/wp-content/uploads/sites/22/2022/09/test-results.zip>



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
TGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
TGTTGAAAGTTTTAACTATTATATAGTACTCAGACGACAATAATAAAAAGAGTTTTGGTATCCTCTGGCGAGCATAACAAG
GCCCGAAGGCAGCTCGCCAAAGCAACAAAGTAATAATACACAAGGGTGGGAGGTCTACCCTTCGGGCATGAACTCTGTA
ATGATCCTTCCGCAGGTTACCTACGGAAACCTTGTTACGACTTTTACTTCTCTAAWTKGACCAAGAGA
```



How to see your results?

<https://dnasubway.cyverse.org>

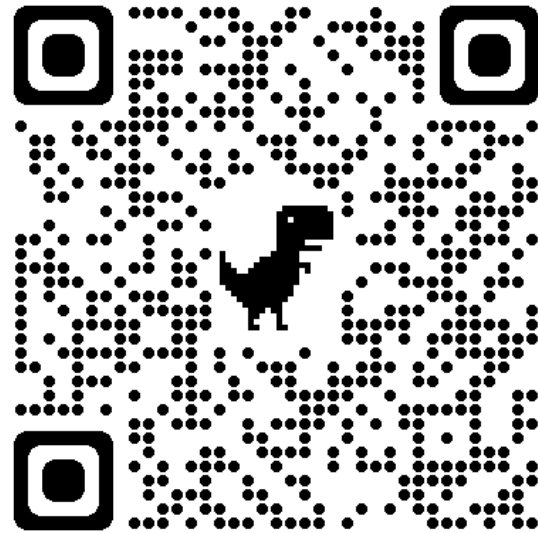
FAST TRACK TO GENE ANNOTATION AND GENOME ANALYSIS

Username:
Password:

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

D N A

S U B W A Y



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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Annotate a Genomic Sequence



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

Continue

* Required information



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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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- Running
- View
- Error
- Blocked

Project Information

test results

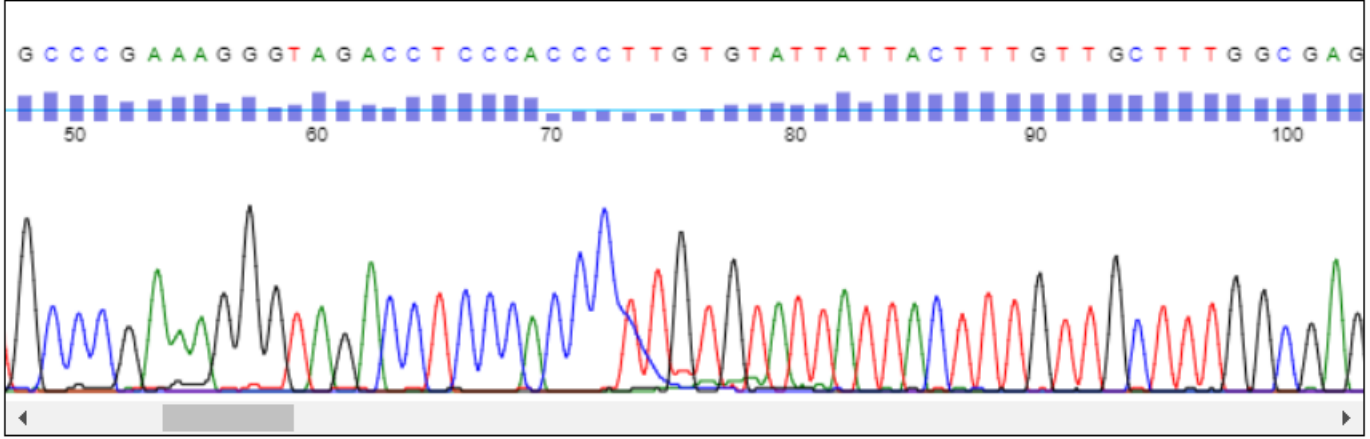
Project ID : 283911
 User : Julia Pawłowska
 Affiliation : -
 Status : Private Public

Input seqs :
 Ref seqs :
 Parameters :

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Sequence Viewer

- 6 ITS1f
- 6 ITS4



Reset

X:

Y:



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Project Information

test results

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

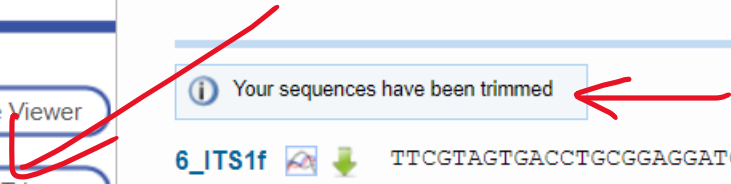
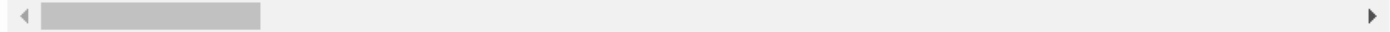
Ref seqs :
 Parameters :

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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Project Information

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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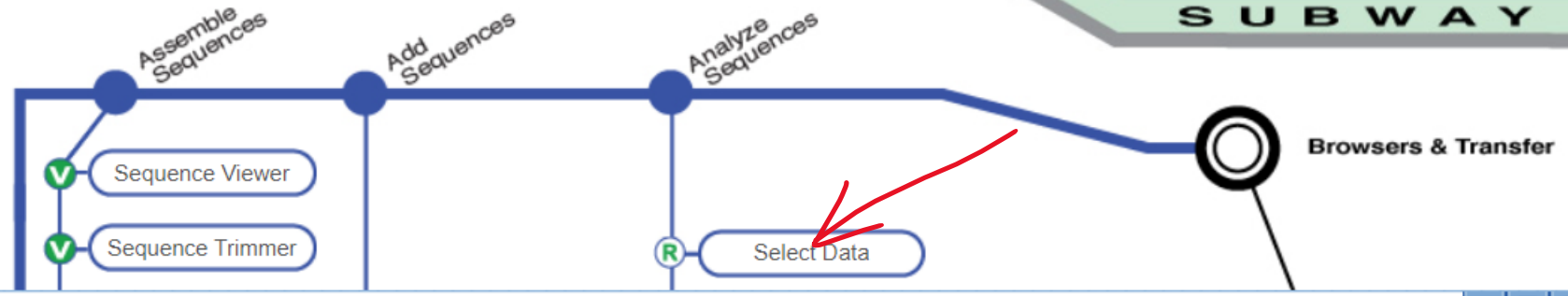
Prospect Genomes Using TARGET

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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.

[Save Selections](#)

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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Sequence Viewer

D N A

SUBWAY

Browsers & Transfer

selected-seq-283911 — Notatnik

Plik Edytuj Wyświetl

>consensus_6_ITS

TCTCTTGGTCMAWTTAGAGGAAGTAAAAGTCGTAACAAGGTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCACCCTTGTTGATTATTACTTTGTTGCTTTGGCGAGCTGCCTTCGGGCCTTGATGCTCGCCAGAGGATACCAAACTCTTTTGTGCTGAGTACTATATAATAGTTAAAACCTTCAACAACGGATCTCTTGGTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTCTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGCAATGGCCGGCTCTAAAATCAGTGGCGGCCGCTGGGTCTGAACGTAGTAATATCTCTCGTTACAGGTGCTCGGTGAGCTTCTGCCAAAACCAAAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAGCATTAAGCCGGAGGA

Sequencing

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must click save below.

Save Selections

i Download sequences

Click below to download selected sequences in FASTA format.

Download