What's lurking in the soil?

A primer to using public DNA databases

GROW NEXT GEN

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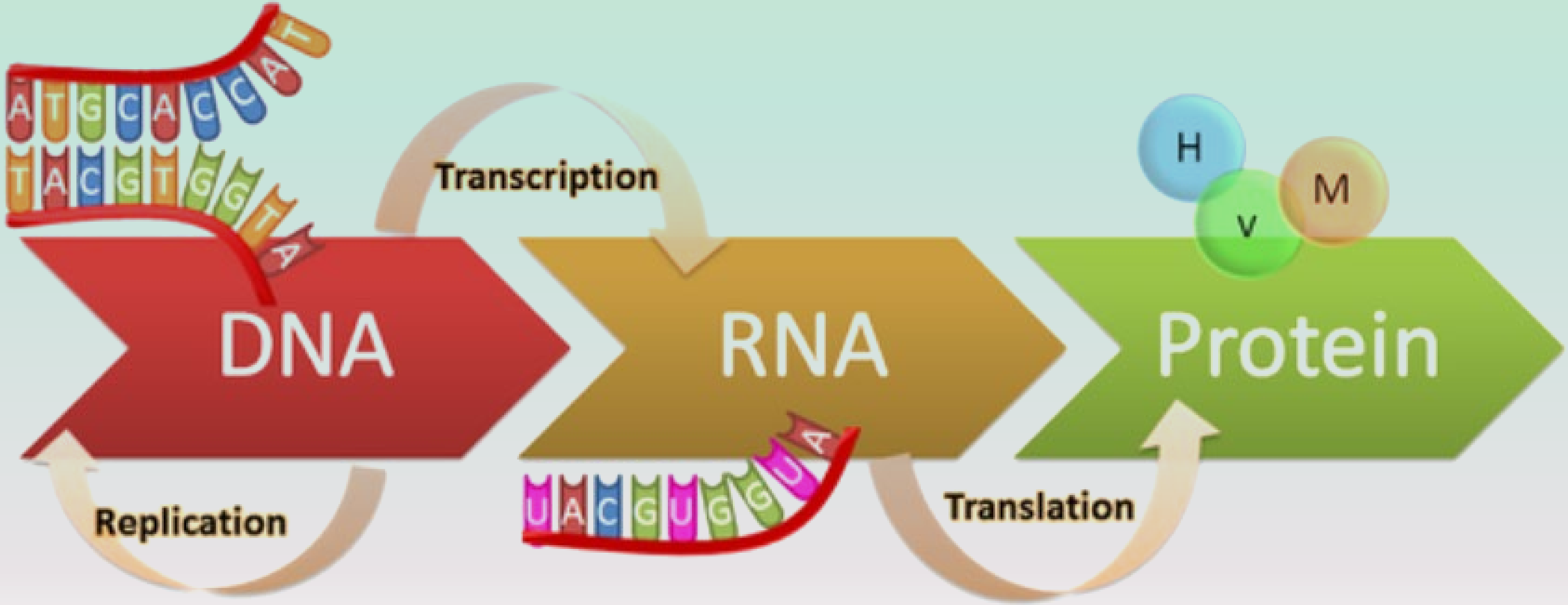
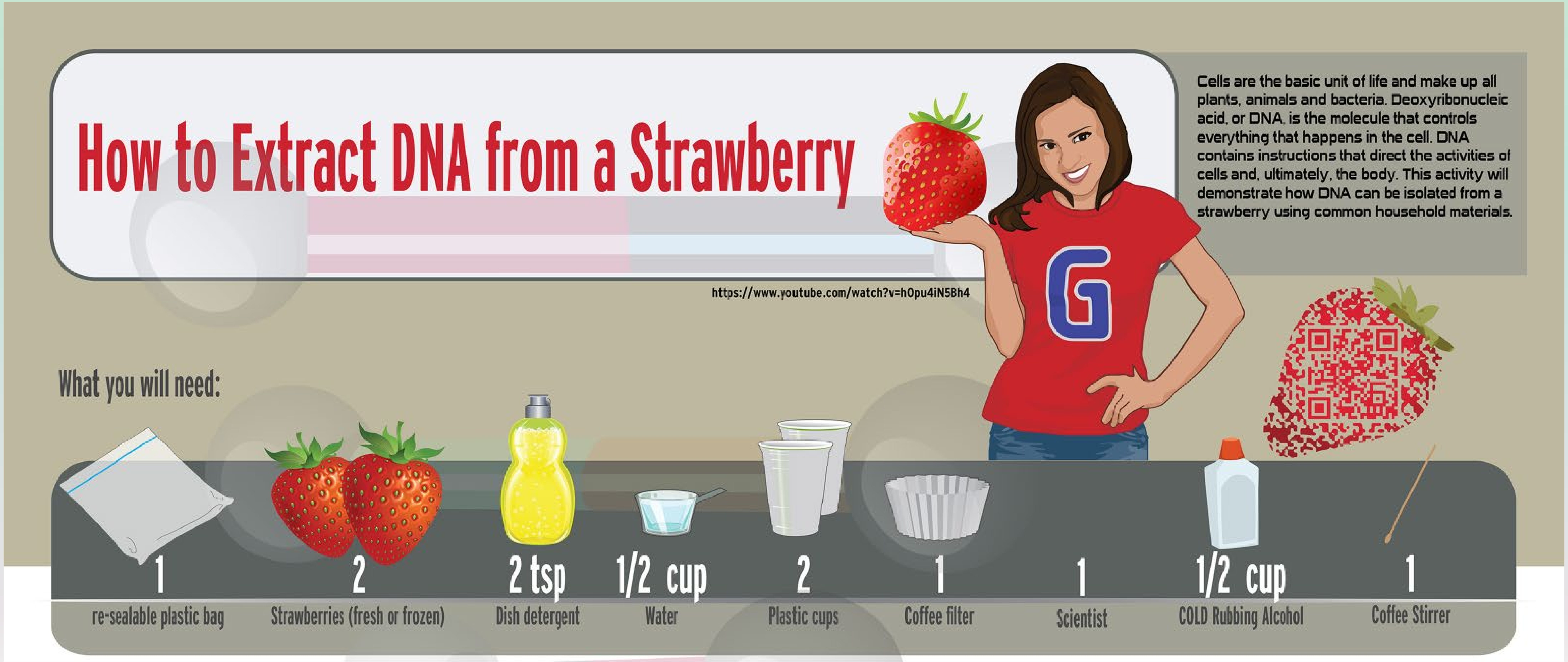
Central dogma of molecular biology



genius.com/Biology-genius-the-central-dogma-annotated

Extractions help students visualize DNA



How to Extract DNA from a Strawberry

Cells are the basic unit of life and make up all plants, animals and bacteria. Deoxyribonucleic acid, or DNA, is the molecule that controls everything that happens in the cell. DNA contains instructions that direct the activities of cells and, ultimately, the body. This activity will demonstrate how DNA can be isolated from a strawberry using common household materials.

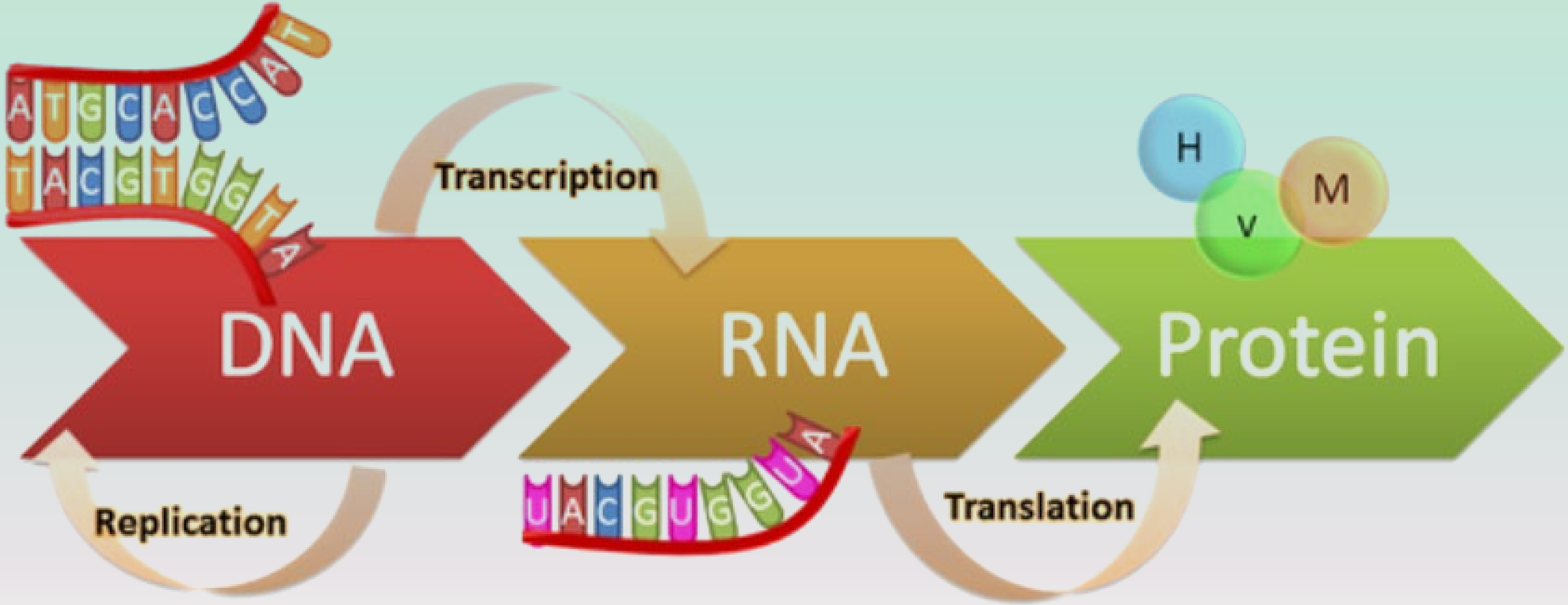
https://www.youtube.com/watch?v=h0pu4iN5Bh4

What you will need:

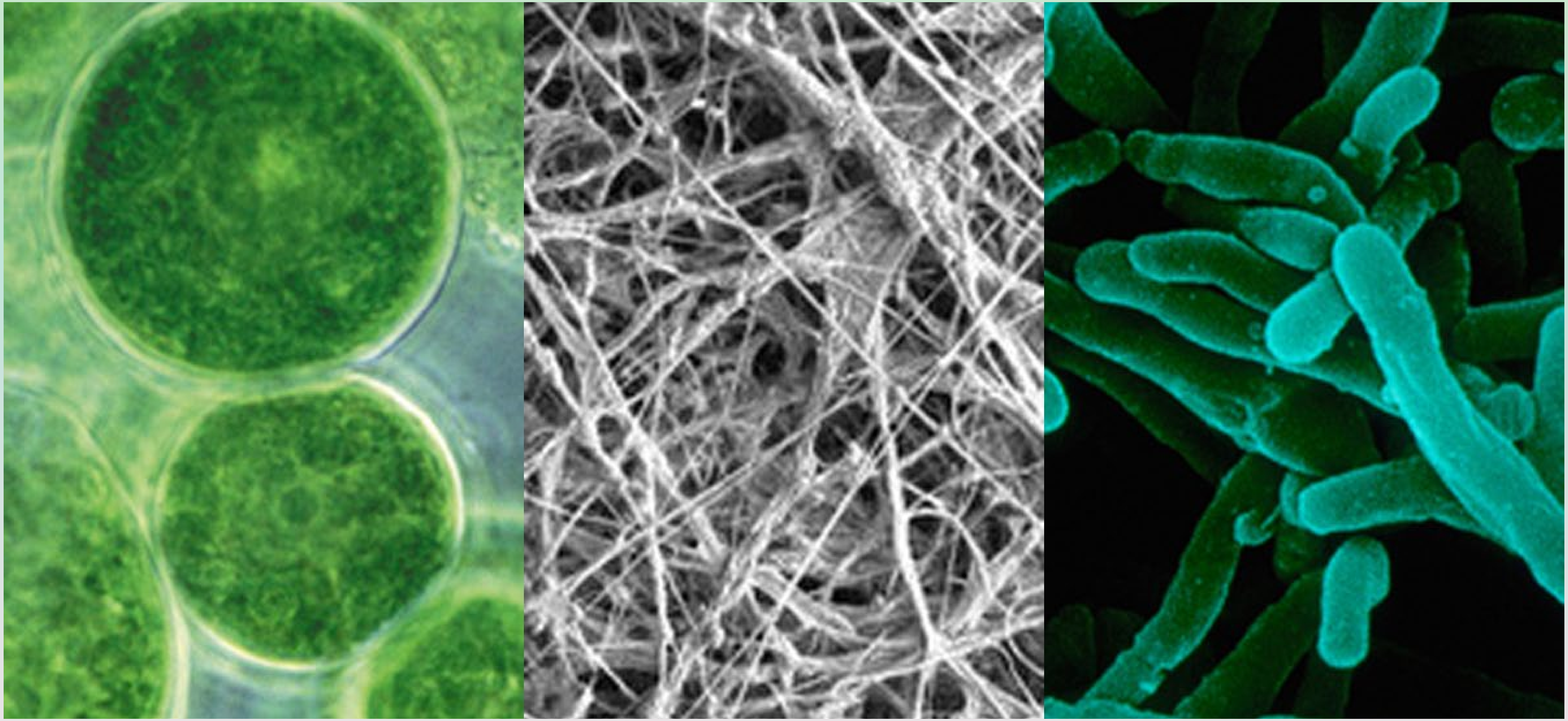
1	re-sealable plastic bag
2	Strawberries (fresh or frozen)
2 tsp	Dish detergent
1/2 cup	Water
2	Plastic cups
1	Coffee filter
1	Scientist
1/2 cup	COLD Rubbing Alcohol
1	Coffee Stirrer

genome.gov/Pages/Education/Modules/StrawberryExtractionInstructions.pdf

Central dogma of molecular biology

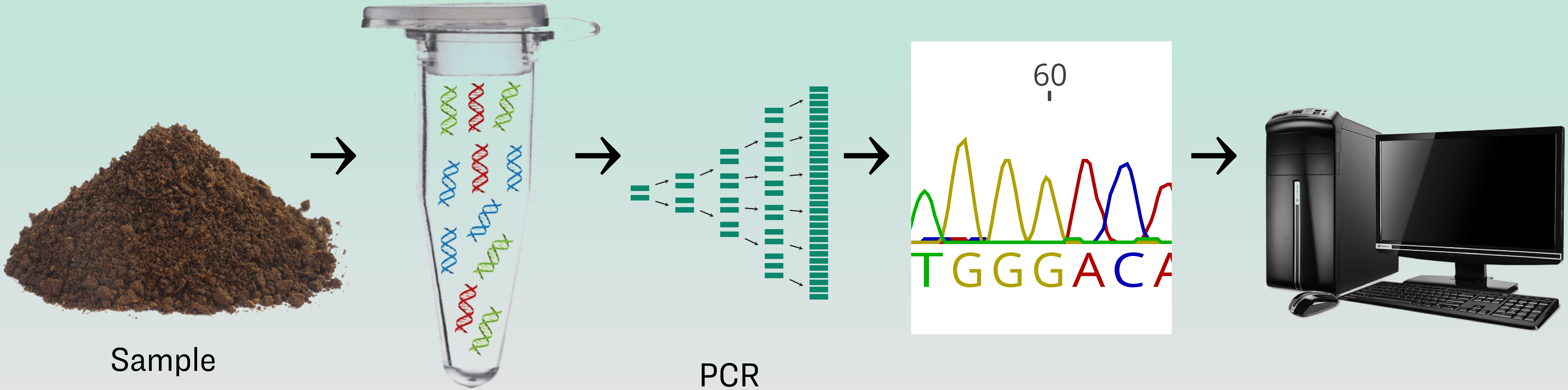


genius.com/Biology-genius-the-central-dogma-annotated



Soil is a complex matrix that includes many living things, both microscopic and visible.

The process: Sample to DNA analysis

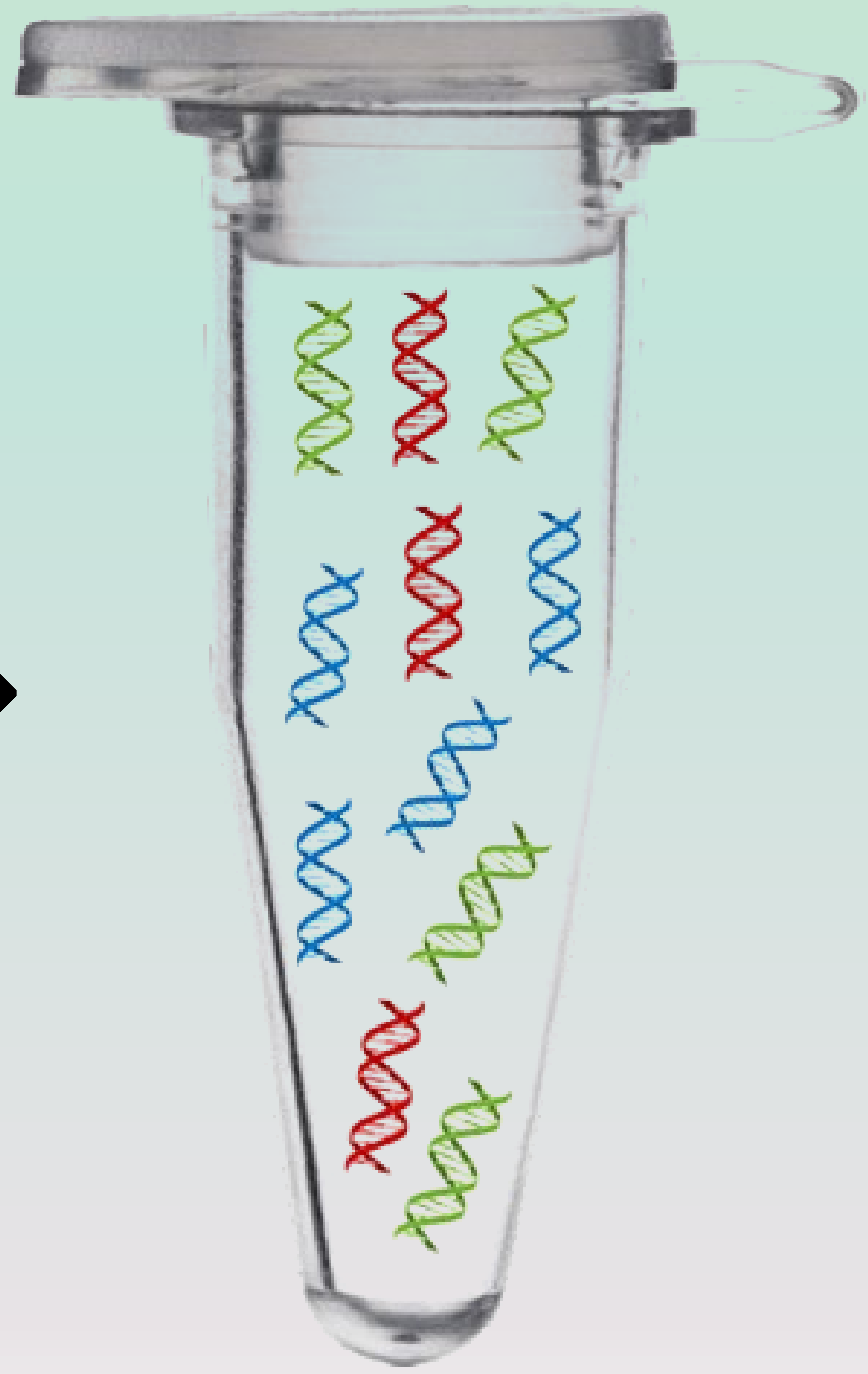


Sample collection → DNA extraction → PCR → Sequencing → Bioinformatics

The process: Sample to DNA analysis

Soil sample

→



DNA extraction

Sample collection

↓

DNA extraction

↓

PCR

↓

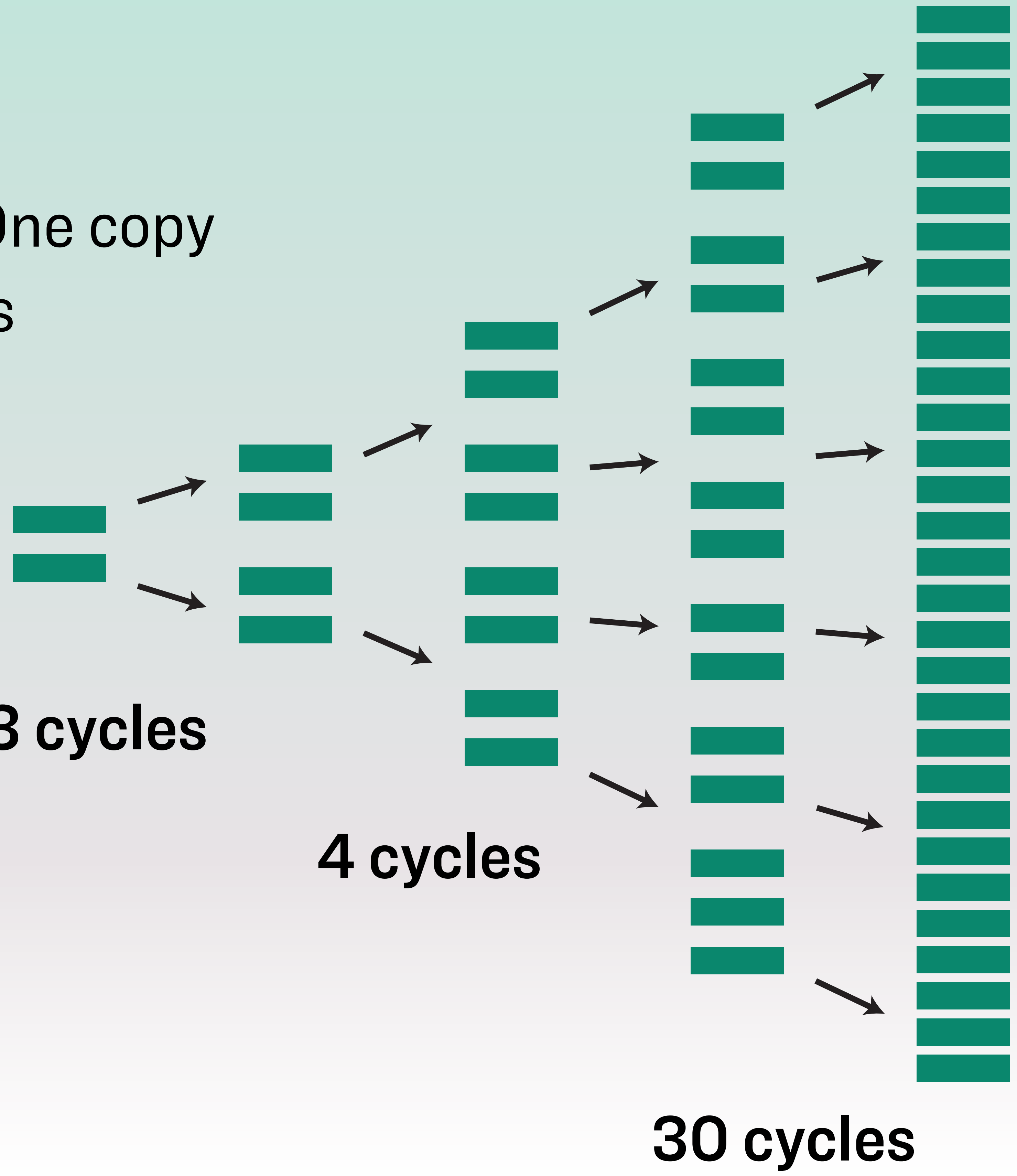
Sequencing

↓

Bioinformatics

The process: Sample to DNA analysis

Polymerase chain reaction (PCR): Cycling process to artificially make copies (amplify) specific pieces of DNA in the genome

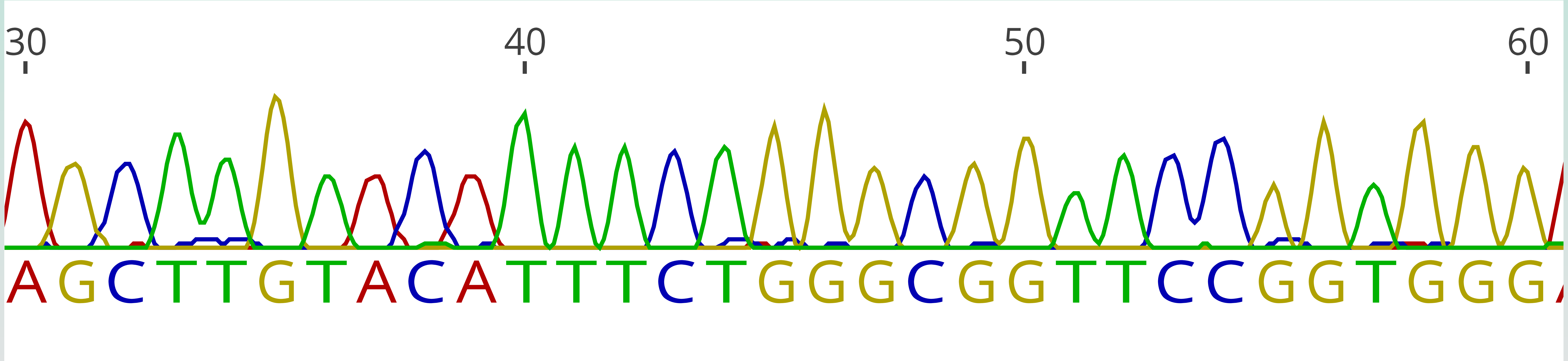


Result: One copy to billions

Sample collection
↓
DNA extraction
↓
PCR
↓
Sequencing
↓
Bioinformatics

The process: Sample to DNA analysis

DNA sequencing: Amplified PCR products are digitized into their nucleotide sequence



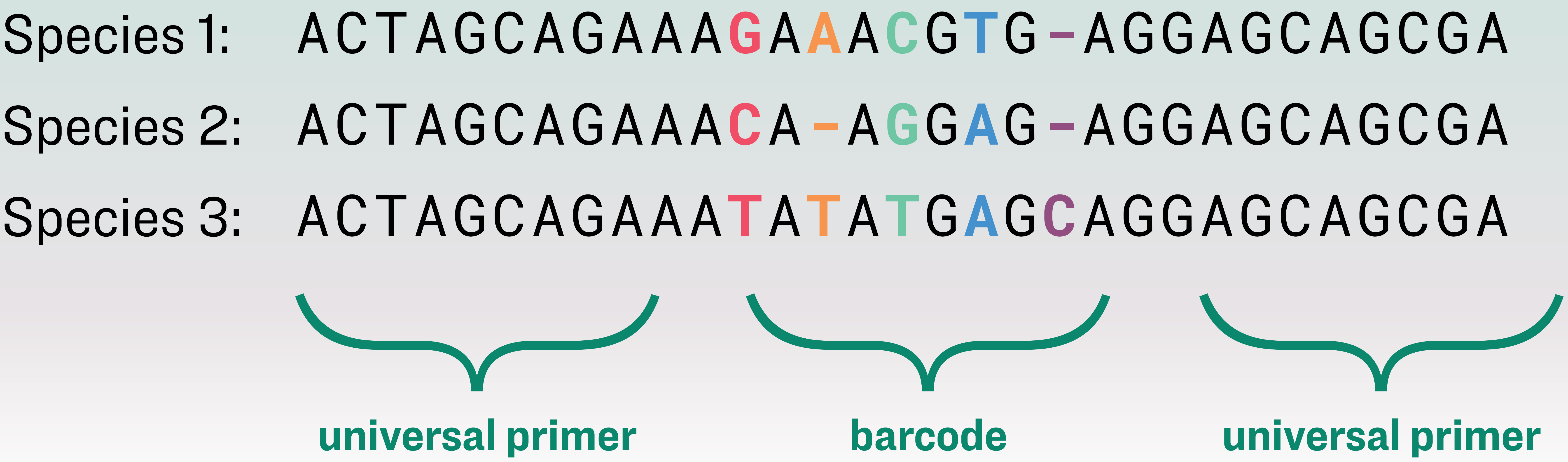
Sample collection
↓
DNA extraction
↓
PCR
↓
Sequencing
↓
Bioinformatics

What is amplified by PCR?

Barcodes: regions of DNA that differ between species.

Scientists use a set of **universal primers** in conserved regions that are upstream and downstream for PCR to amplify the barcode region. DNA sequencing and bioinformatics are then used to identify those differences.

DNA sequence alignment



Species 1: ACTAGCAGAAAGAAACGTG-AGGAGCAGCGA

Species 2: ACTAGCAGAAACA-AGGAG-AGGAGCAGCGA

Species 3: ACTAGCAGAAATATATGAGCAGGAGCAGCGA

Sample collection

↓

DNA extraction

↓

PCR

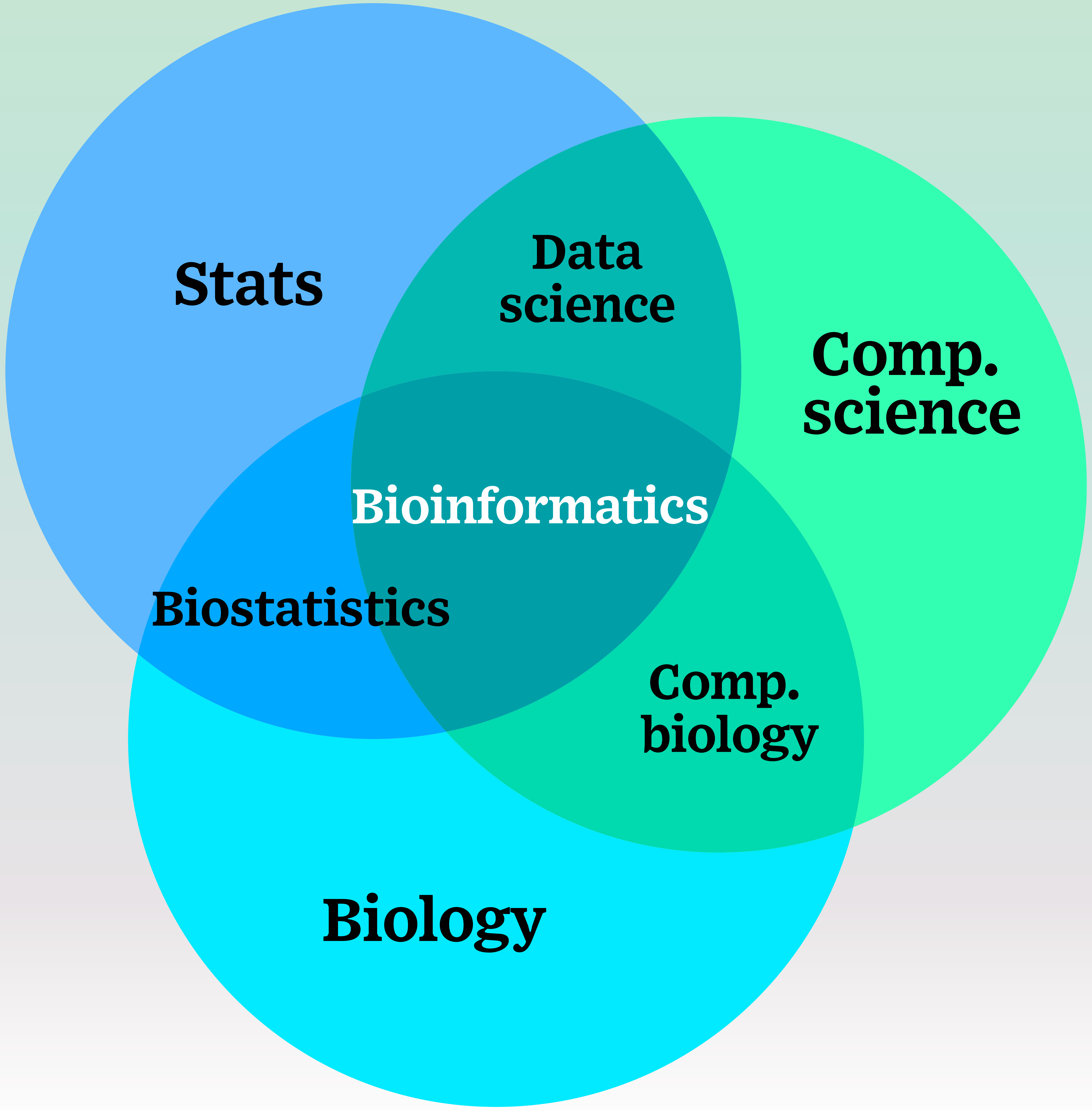
↓

Sequencing

↓

Bioinformatics

The process: Sample to DNA analysis



Sample collection

↓

DNA extraction

↓

PCR

↓

Sequencing

↓

Bioinformatics

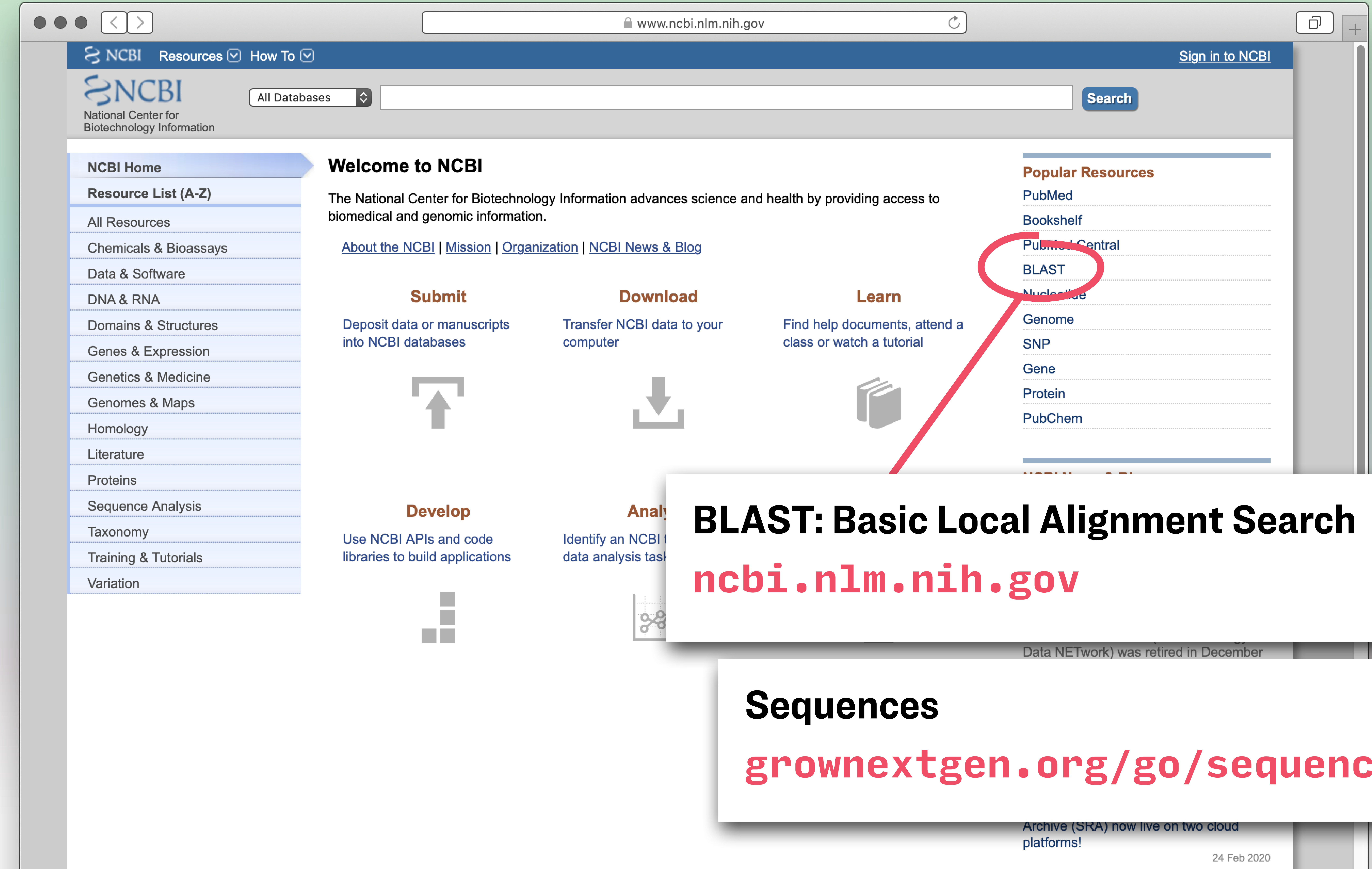
Applications of bioinformatics in food sustainability

1. Genome sequencing and analysis
2. Protein structure analysis
3. Gene discovery:
    - Insect and disease resistance
    - Improved nutritional quality
    - Drought resistance
4. Disease discovery and control

Farm story

Shadybrook Farm is having trouble with production of soybeans and has noticed increasing levels of disease, despite the use of fungicides. The farm manager elects for a soil test from a laboratory to identify potential disease-causing organisms. The laboratory produced a report that contained the top two most common DNA sequences from the soil.

Your job as a bioinformatician is to help the farm manager identify the species, using the given sequences of DNA, through the use of public databases and basic bioinformatic tools.



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Welcome to NCBI

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Identify an NCBI t
data analysis tas

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SNP
Gene
Protein
PubChem

Data NETwork) was retired in December

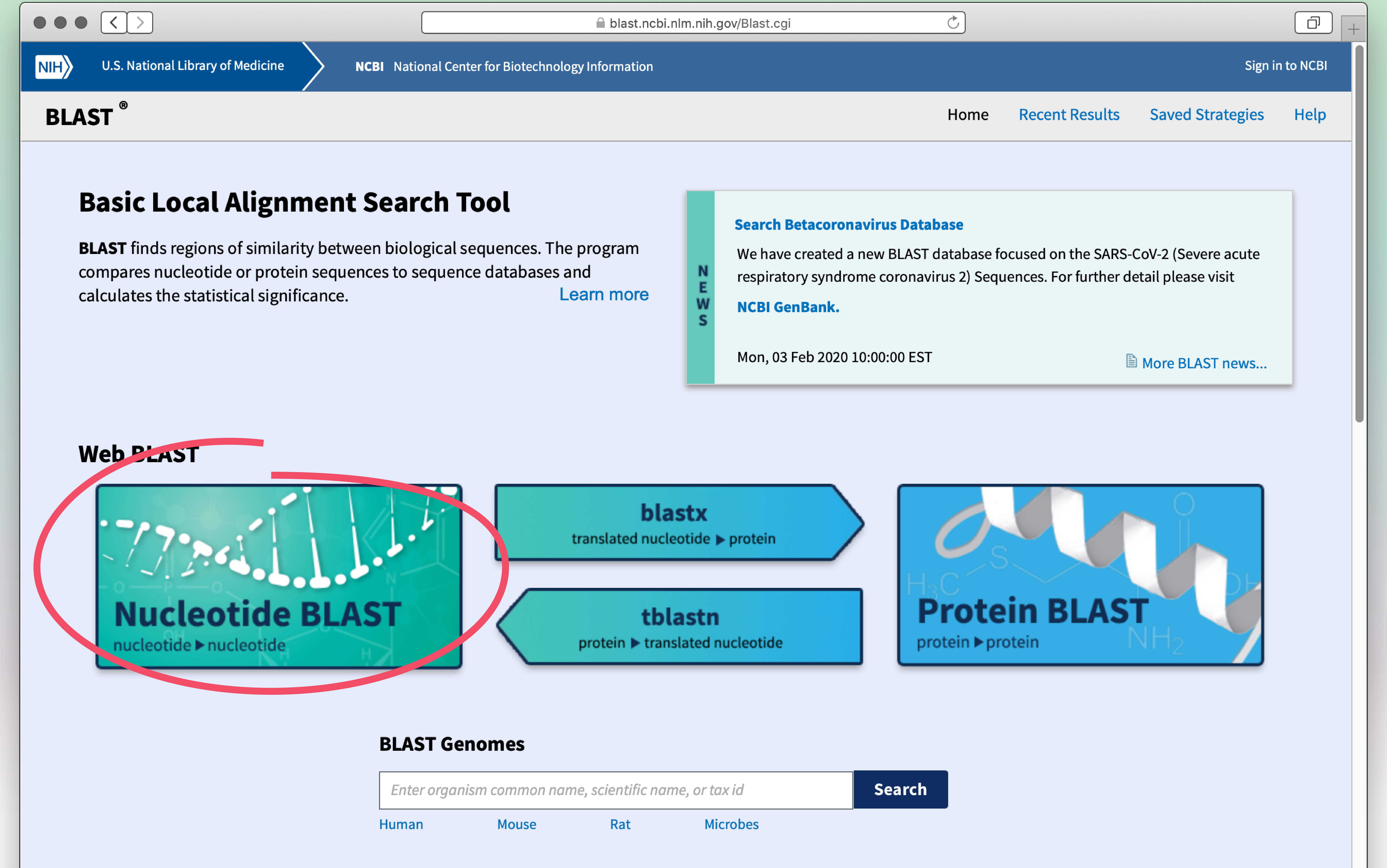
Archive (SRA) now live on two cloud platforms!
24 Feb 2020

BLAST: Basic Local Alignment Search

ncbi.nlm.nih.gov

Sequences

grownextgen.org/go/sequences



Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. Learn more

NEWS

Search Betacoronavirus Database

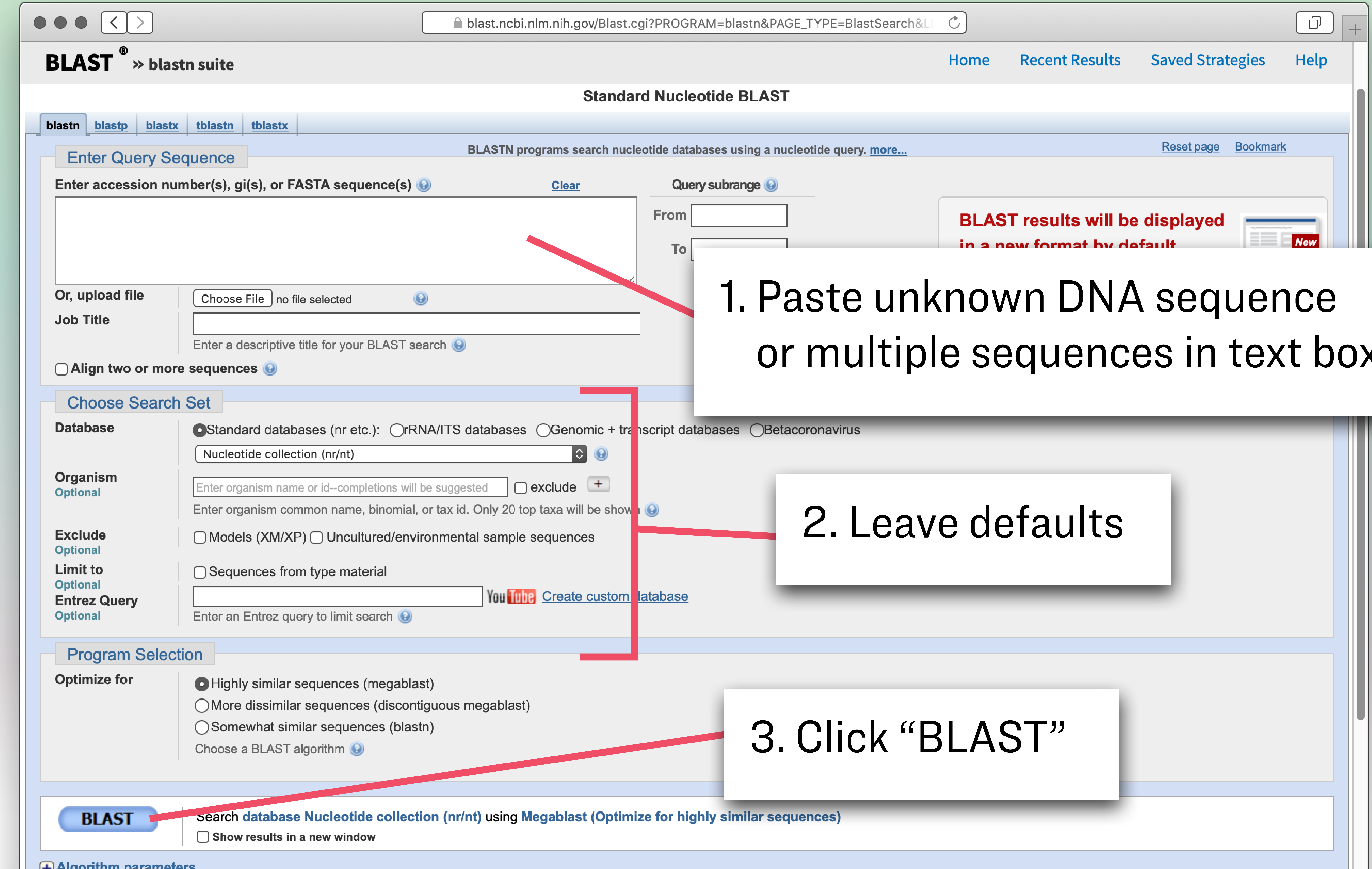
We have created a new BLAST database focused on the SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) Sequences. For further detail please visit **NCBI GenBank.**

Mon, 03 Feb 2020 10:00:00 EST

More BLAST news...

Web BLAST

BLAST Genomes



blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&L

BLAST® » blastn suite

Standard Nucleotide BLAST

BLASTN programs search nucleotide databases using a nucleotide query. more...

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

Query subrange

From

To

BLAST results will be displayed in a new format by default

Or, upload file: Choose File no file selected

Job Title

Enter a descriptive title for your BLAST search

☐ Align two or more sequences

Choose Search Set

Database: ● Standard databases (nr etc.): ○ rRNA/ITS databases ○ Genomic + transcript databases ○ Betacoronavirus

Nucleotide collection (nr/nt)

Organism Optional: Enter organism name or id--completions will be suggested ☐ exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude Optional: ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to Optional: ☐ Sequences from type material

Entrez Query Optional:

Enter an Entrez query to limit search

Program Selection

Optimize for:
● Highly similar sequences (megablast)
○ More dissimilar sequences (discontiguous megablast)
○ Somewhat similar sequences (blastn)

Choose a BLAST algorithm

Search database Nucleotide collection (nr/nt) using Megablast (Optimize for highly similar sequences)

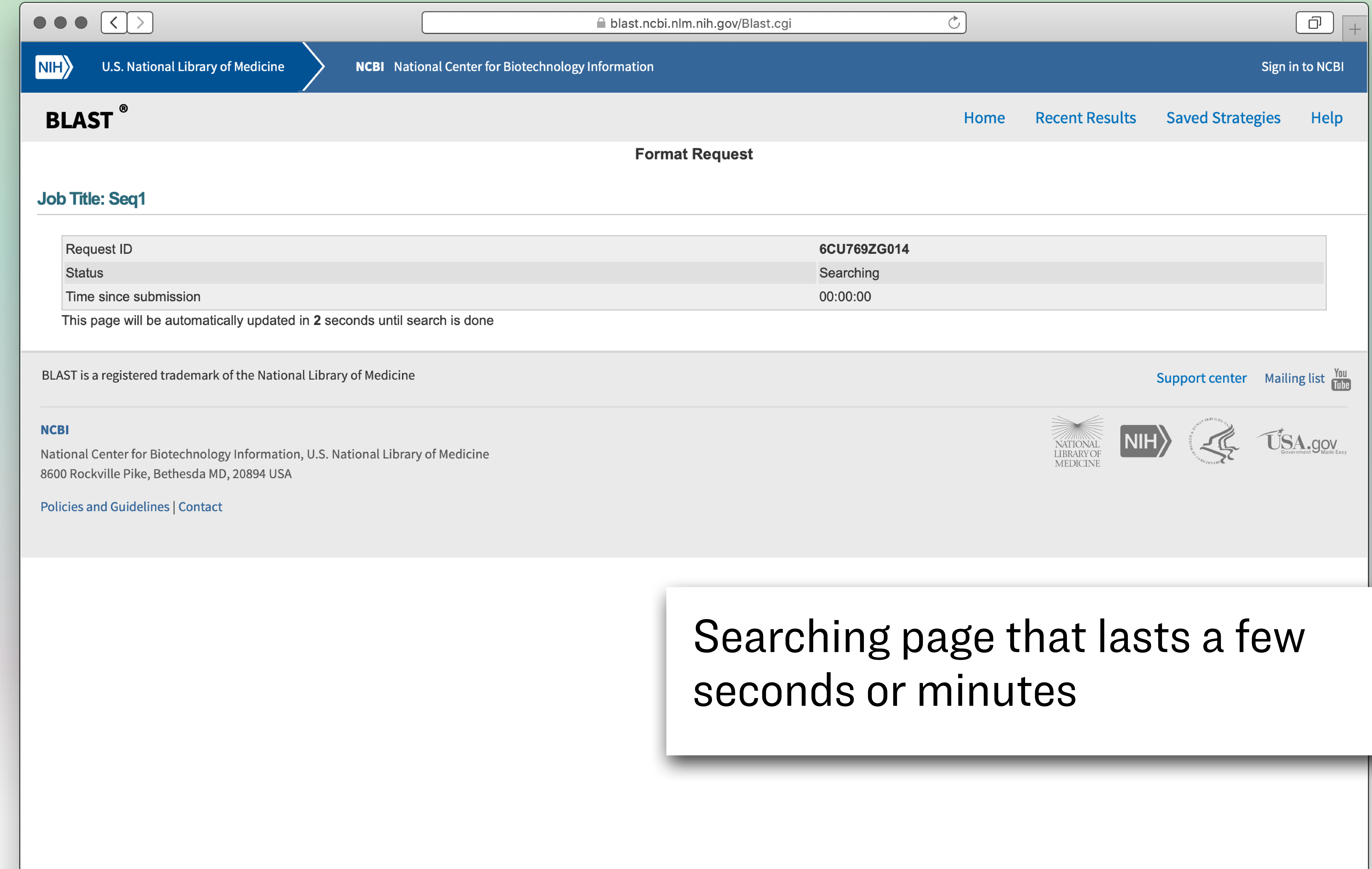
☐ Show results in a new window

Algorithm parameters

1. Paste unknown DNA sequence or multiple sequences in text box

2. Leave defaults

3. Click "BLAST"



Format Request

Job Title: Seq1

Request ID	**6CU769ZG014**
Status	Searching
Time since submission	00:00:00

This page will be automatically updated in **2** seconds until search is done

BLAST is a registered trademark of the National Library of Medicine

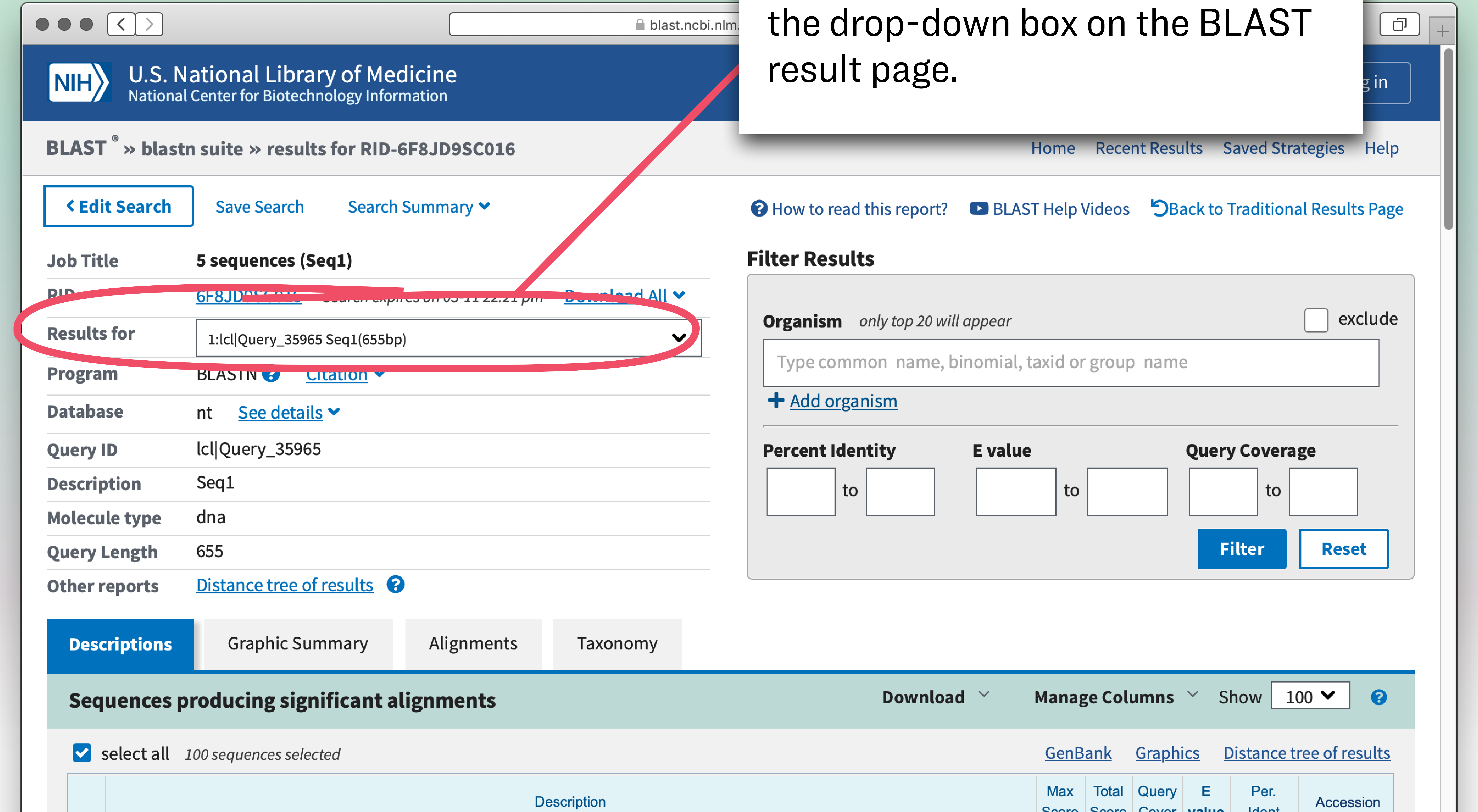
NCBI

National Center for Biotechnology Information, U.S. National Library of Medicine
8600 Rockville Pike, Bethesda MD, 20894 USA

Searching page that lasts a few seconds or minutes

BLAST results

Multiple sequence searches can be performed. Simply select from the drop-down box on the BLAST result page.



BLAST ® » blastn suite » results for RID-6F8JD9SC016

Job Title 5 sequences (Seq1)

RID 6F8JD9SC016 Search expires on 03-11 22:21 pm Download All

Results for 1:lcl|Query_35965 Seq1(655bp)

Program BLASTN Citation

Database nt See details

Query ID lcl|Query_35965

Description Seq1

Molecule type dna

Query Length 655

Other reports Distance tree of results

Filter Results

Organism *only top 20 will appear* ☐ exclude

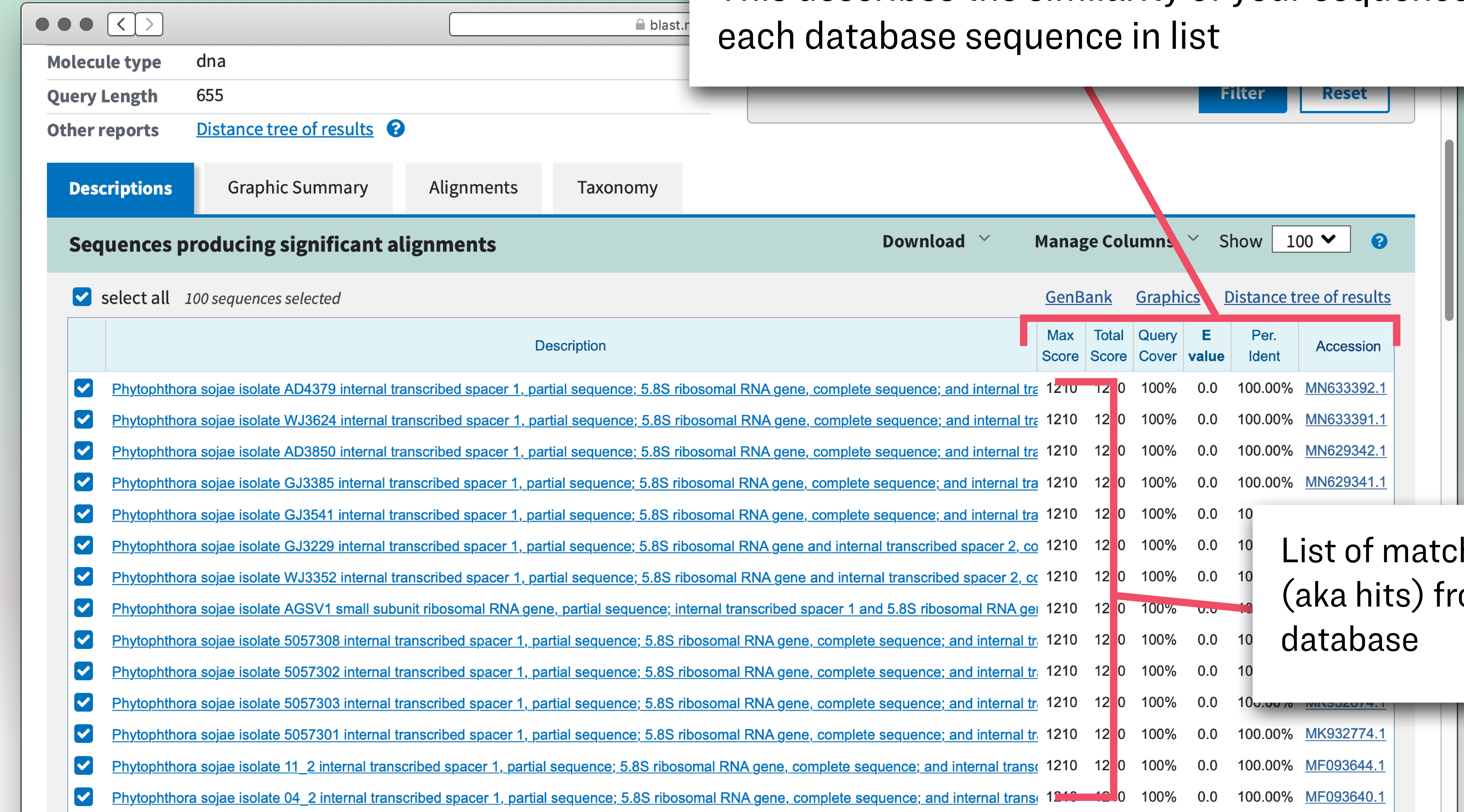
Type common name, binomial, taxid or group name

Sequences producing significant alignments

☑ select all *100 sequences selected*

BLAST results

This describes the similarity of your sequence to each database sequence in list



Molecule type dna
Query Length 655
Other reports Distance tree of results

Sequences producing significant alignments

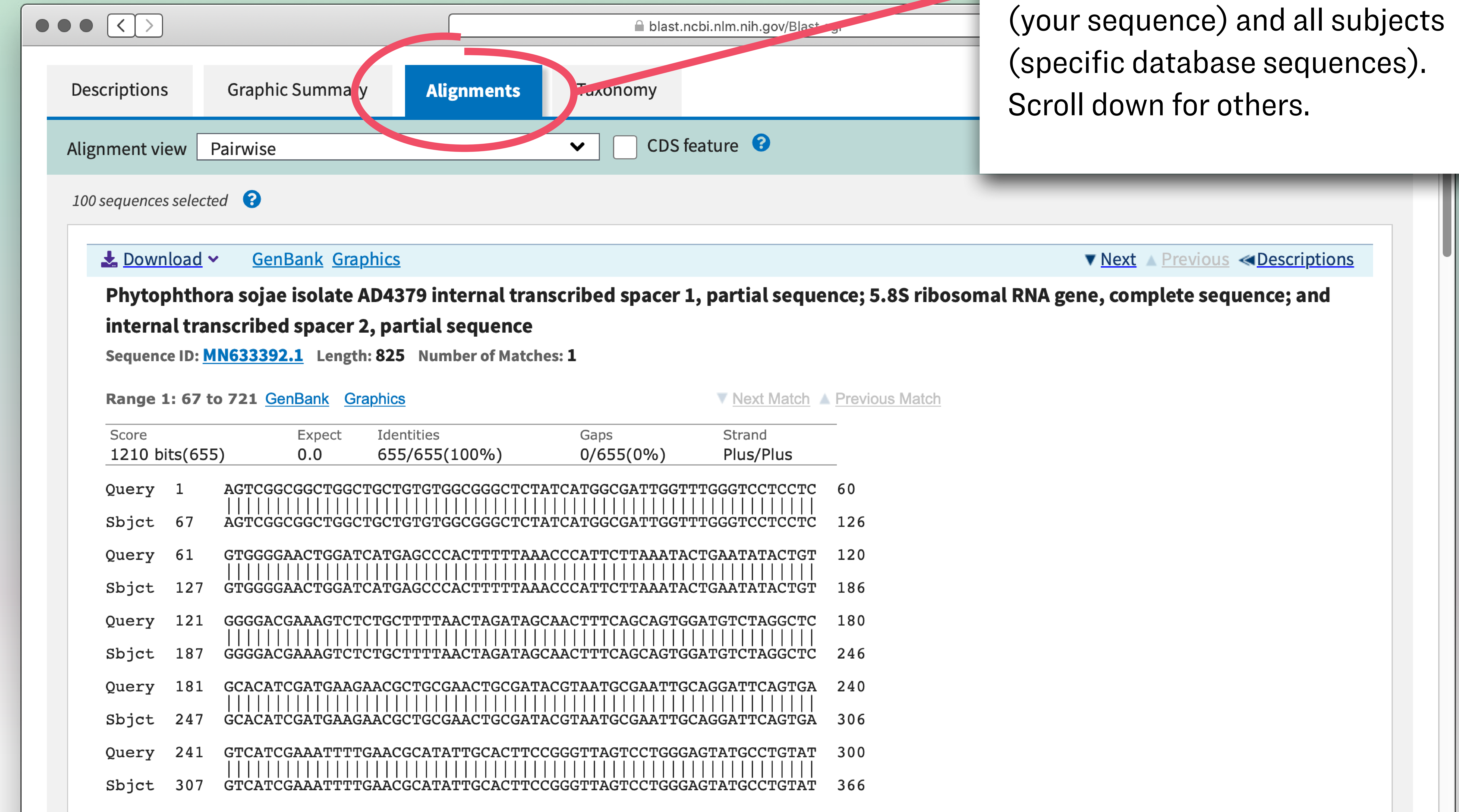
☑ select all *100 sequences selected*

☐	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Phytophthora sojae isolate AD4379 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	1210	12 0	100%	0.0	100.00%	MN633392.1
☑	Phytophthora sojae isolate WJ3624 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	1210	12 0	100%	0.0	100.00%	MN633391.1
☑	Phytophthora sojae isolate AD3850 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	1210	12 0	100%	0.0	100.00%	MN629342.1
☑	Phytophthora sojae isolate GJ3385 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	1210	12 0	100%	0.0	100.00%	MN629341.1
☑	Phytophthora sojae isolate GJ3541 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	1210	12 0	100%	0.0	10	
☑	Phytophthora sojae isolate GJ3229 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, co	1210	12 0	100%	0.0	10	
☑	Phytophthora sojae isolate WJ3352 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, co	1210	12 0	100%	0.0	10	
☑	Phytophthora sojae isolate AGSV1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gei	1210	12 0	100%	0.0	10	
☑	Phytophthora sojae isolate 5057308 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	1210	12 0	100%	0.0	10	
☑	Phytophthora sojae isolate 5057302 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	1210	12 0	100%	0.0	10	
☑	Phytophthora sojae isolate 5057303 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	1210	12 0	100%	0.0	100.00%	MK932874.1
☑	Phytophthora sojae isolate 5057301 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	1210	12 0	100%	0.0	100.00%	MK932774.1
☑	Phytophthora sojae isolate 11_2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transc	1210	12 0	100%	0.0	100.00%	MF093644.1
☑	Phytophthora sojae isolate 04_2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transc	1210	12 0	100%	0.0	100.00%	MF093640.1

List of matches (aka hits) from database

BLAST results: alignment

Alignment tab displays individual sequence alignments with query (your sequence) and all subjects (specific database sequences). Scroll down for others.



100 sequences selected

Phytophthora sojae isolate AD4379 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

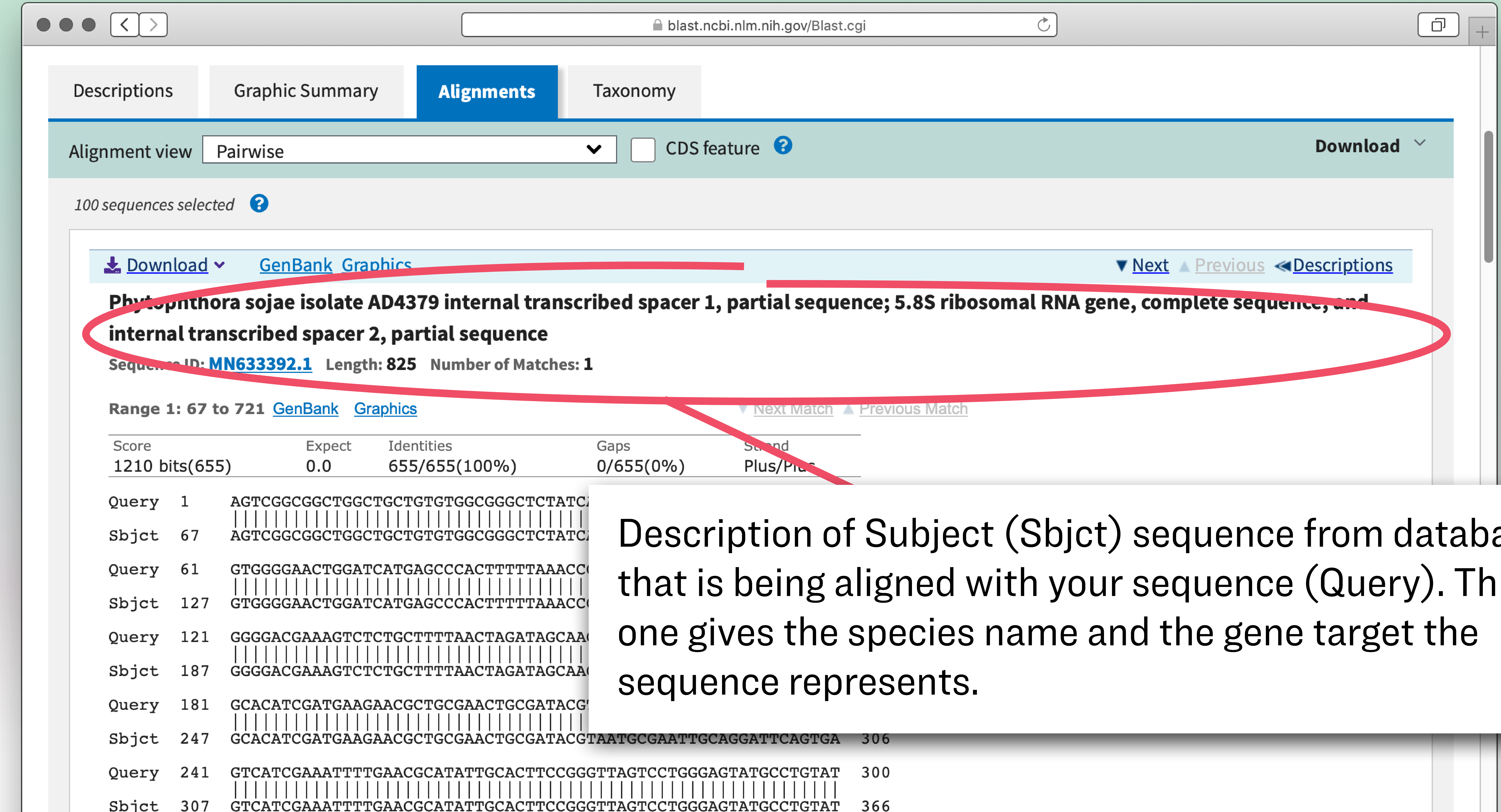
Sequence ID: MN633392.1 **Length:** 825 **Number of Matches:** 1

Range 1: 67 to 721 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
1210 bits(655)	0.0	655/655(100%)	0/655(0%)	Plus/Plus

```
Query  1    AGTCGGCGGCTGGCTGCTGTGTGGCGGGCTCTATCATGGCGATTGGTTTGGGTCCTCCTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   AGTCGGCGGCTGGCTGCTGTGTGGCGGGCTCTATCATGGCGATTGGTTTGGGTCCTCCTC  126

Query  61   GTGGGGAACTGGATCATGAGCCCACTTTTTAAACCCATTCTTAAATACTGAATATACTGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  GTGGGGAACTGGATCATGAGCCCACTTTTTAAACCCATTCTTAAATACTGAATATACTGT  186

Query  121  GGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  GGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTC  246

Query  181  GCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  GCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGA  306

Query  241  GTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  GTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTAT  366
```

Sequence alignment (100% match)



☐ CDS feature

100 sequences selected

Phytophthora sojae isolate AD4379 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence, and internal transcribed spacer 2, partial sequence

Sequence ID: MN633392.1 **Length:** 825 **Number of Matches:** 1

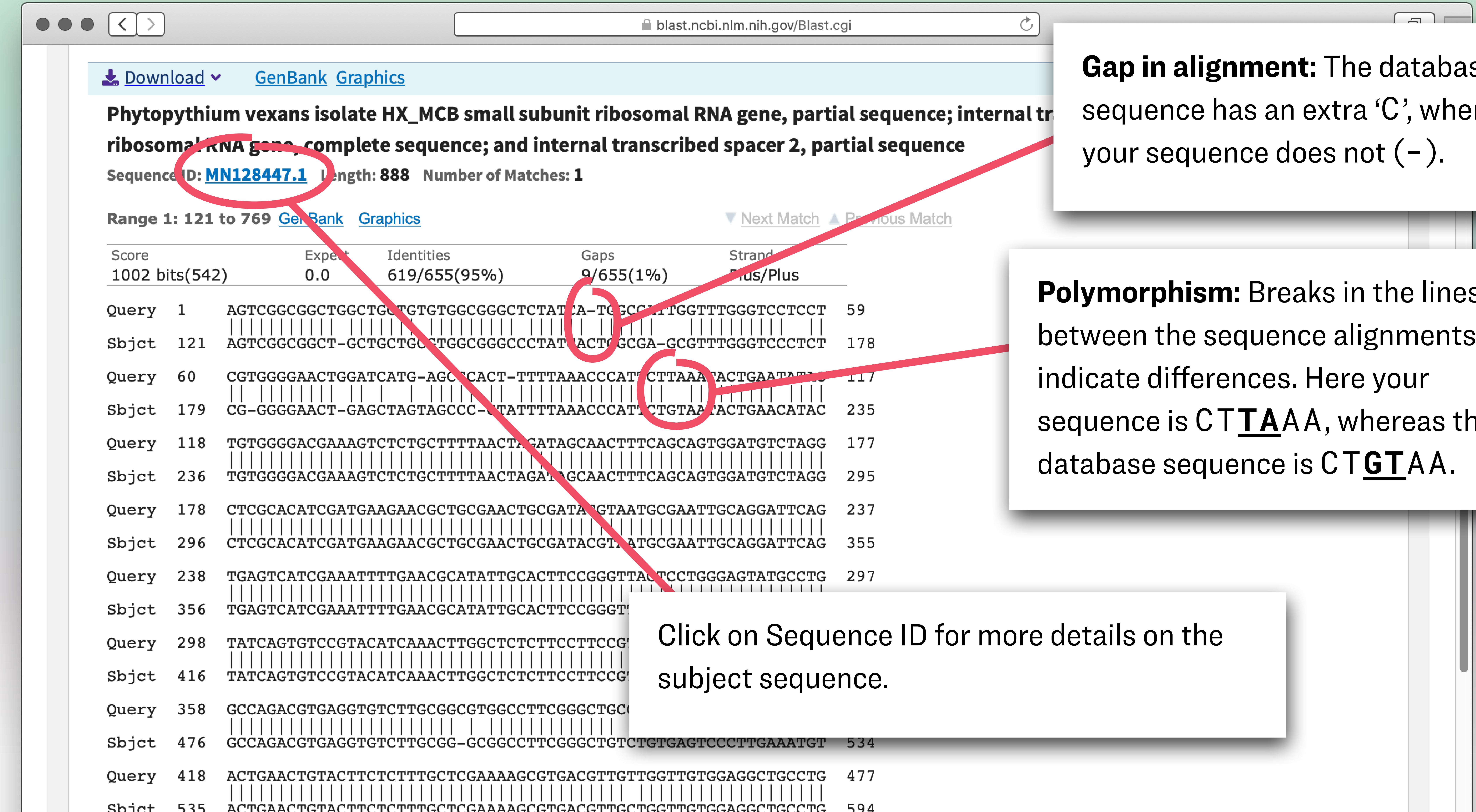
Range 1: 67 to 721

Score	Expect	Identities	Gaps	Strand
1210 bits(655)	0.0	655/655(100%)	0/655(0%)	Plus/Plus

```
Query  1    AGTCGGCGGCTGGCTGCTGTGTGGCGGGCTCTATC
            |||||||||||||||||||||||||||||||||||
Sbjct  67   AGTCGGCGGCTGGCTGCTGTGTGGCGGGCTCTATC

Query  61   GTGGGGAACTGGATCATGAGCCCACTTTTTAAACC
            |||||||||||||||||||||||||||||||||||
Sbjct  127  GTGGGGAACTGGATCATGAGCCCACTTTTTAAACC

Query  121  GGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAA
            |||||||||||||||||||||||||||||||||||
Sbjct  187  GGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAA

Query  181  GCACATCGATGAAGAACGCTGCGAACTGCGATACG
            |||||||||||||||||||||||||||||||||||
Sbjct  247  GCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGA  306

Query  241  GTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  GTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTAT  366
```

Description of Subject (Sbjct) sequence from database that is being aligned with your sequence (Query). This one gives the species name and the gene target the sequence represents.

Sequence alignment (95% match)



Phytopythium vexans isolate HX_MCB small subunit ribosomal RNA gene, partial sequence; internal tr
ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: MN128447.1 **Length:** 888 **Number of Matches:** 1

Range 1: 121 to 769 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1002 bits(542)	0.0	619/655(95%)	9/655(1%)	Plus/Plus

```
Query  1    AGTCGGCGGCTGGCTGCTGTGTGGCGGGCTCTATCA-TGGCCATTGGTTTGGGTCCTCCT  59
Sbjct  121  AGTCGGCGGCT-GCTGCTGCGTGGCGGGCCCTATCACTCGCGA-GCGTTTGGGTCCCTCT  178

Query  60   CGTGGGGAACTGGATCATG-AGCCCACT-TTTTAAACCCATTCTTAAATACTGAATATAC  117
Sbjct  179  CG-GGGGAACT-GAGCTAGTAGCCC-TTATTTTAAACCCATTCTGTAATACTGAACATAC  235

Query  118  TGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGG  177
Sbjct  236  TGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGG  295

Query  178  CTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAG  237
Sbjct  296  CTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAG  355

Query  238  TGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTACTCCTGGGAGTATGCCTG  297
Sbjct  356  TGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGT

Query  298  TATCAGTGTCCGTACATCAAACTTGGCTCTCTTCCTTCCG
Sbjct  416  TATCAGTGTCCGTACATCAAACTTGGCTCTCTTCCTTCCG

Query  358  GCCAGACGTGAGGTGTCTTGCGGCGTGGCCTTCGGGCTGC
Sbjct  476  GCCAGACGTGAGGTGTCTTGCGG-GCGGCCTTCGGGCTGTCTGTGAGTCCCTTGAAATGT  534

Query  418  ACTGAACTGTACTTCTCTTTGCTCGAAAAGCGTGACGTTGTTGGTTGTGGAGGCTGCCTG  477
Sbjct  535  ACTGAACTGTACTTCTCTTTGCTCGAAAAGCGTGACGTTGCTGGTTGTGGAGGCTGCCTG  594
```

Gap in alignment: The database sequence has an extra 'C', where your sequence does not (–).

Polymorphism: Breaks in the lines between the sequence alignments indicate differences. Here your sequence is CT**TA**AA, whereas the database sequence is CT**GT**AA.

Click on Sequence ID for more details on the subject sequence.

Farm story

Shadybrook Farm is having trouble with production of soybeans and has noticed increasing levels of disease, despite the use of fungicides. The farm manager elects for a soil test from a laboratory to identify potential disease-causing organisms. The laboratory produced a report that contained the top two most common DNA sequences from the soil.

Your job as a bioinformatician is to help the farm manager identify the species, using the given sequences of DNA, through the use of public databases and basic bioinformatic tools.

What did you find?
Enter your answers in the chat box.

Connecting the dots

- **Sequence 1:** *Phytophthora sojae:* oomycetes/fungus
- **Sequence 2:** *Heterodera glycines:* soybean cyst nematode
- **Sequence 3:** *Glycine max:* soybeans
- **Sequence 4:** *Bos taurus:* domestic cattle
- **Sequence 5:** *Odocoileus virginianus:* whitetail deer

Thank you!

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