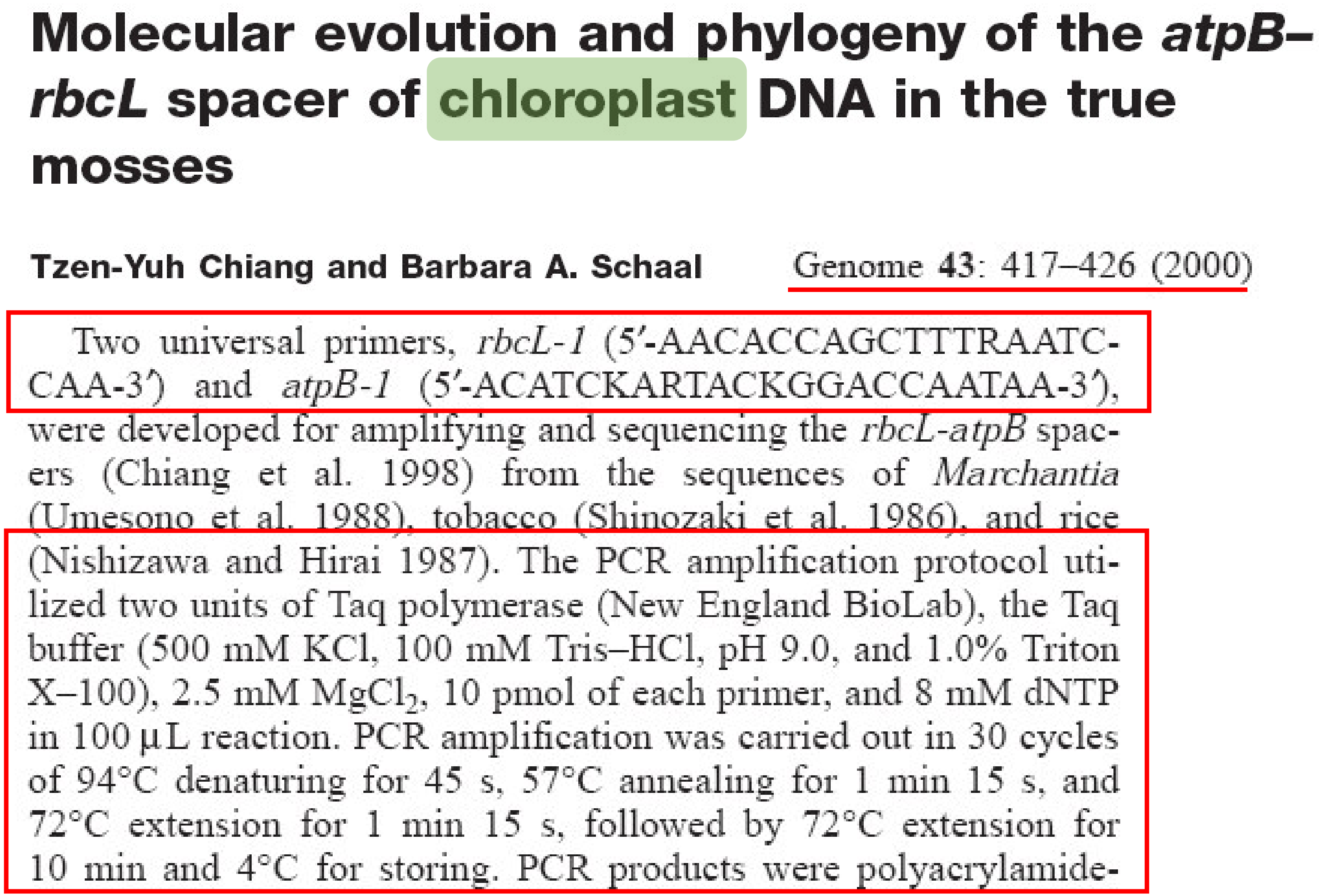
A primer for practical phylogenetic data gathering. Uconn EEB3899-007. Spring 2015

Session 3

Sequence manipulation.
Retrieving sequences from GenBank

Rafael Medina (rafael.medina.bry@gmail.com)
Yang Liu (yang.liu@uconn.edu)

*atp*B-*rbc*L



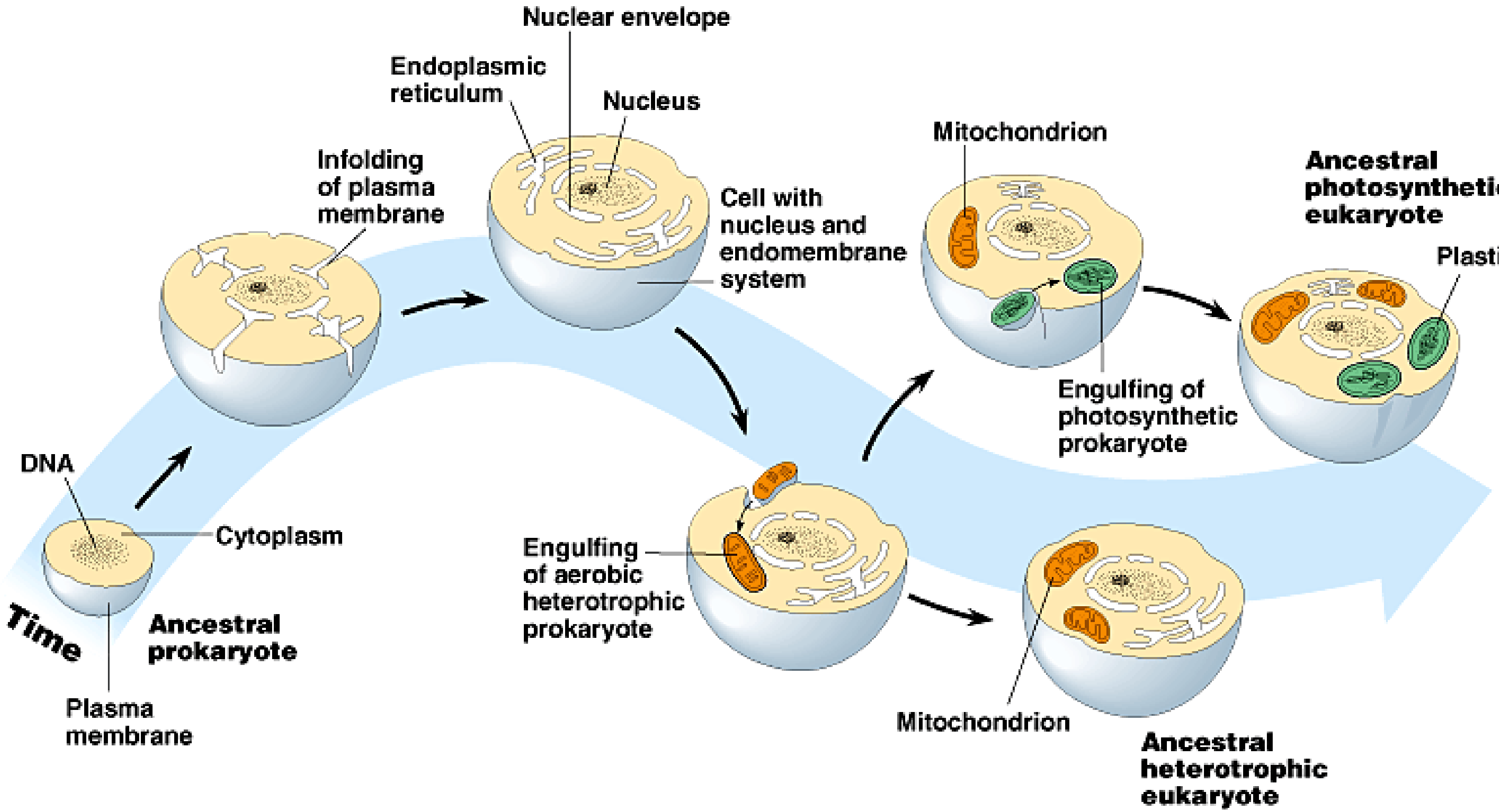
Molecular evolution and phylogeny of the *atpB–rbcL* spacer of chloroplast DNA in the true mosses

Tzen-Yuh Chiang and Barbara A. Schaal

Genome **43**: 417–426 (2000)

Two universal primers, *rbcL-1* (5′-AACACCAGCTTTRAATCCAA-3′) and *atpB-1* (5′-ACATCKARTACKGGACCAATAA-3′), were developed for amplifying and sequencing the *rbcL-atpB* spacers (Chiang et al. 1998) from the sequences of *Marchantia* (Umesono et al. 1988), tobacco (Shinozaki et al. 1986), and rice (Nishizawa and Hirai 1987). The PCR amplification protocol utilized two units of Taq polymerase (New England BioLab), the Taq buffer (500 mM KCl, 100 mM Tris–HCl, pH 9.0, and 1.0% Triton X–100), 2.5 mM $MgCl_2$, 10 pmol of each primer, and 8 mM dNTP in 100 µL reaction. PCR amplification was carried out in 30 cycles of 94°C denaturing for 45 s, 57°C annealing for 1 min 15 s, and 72°C extension for 1 min 15 s, followed by 72°C extension for 10 min and 4°C for storing. PCR products were polyacrylamide-

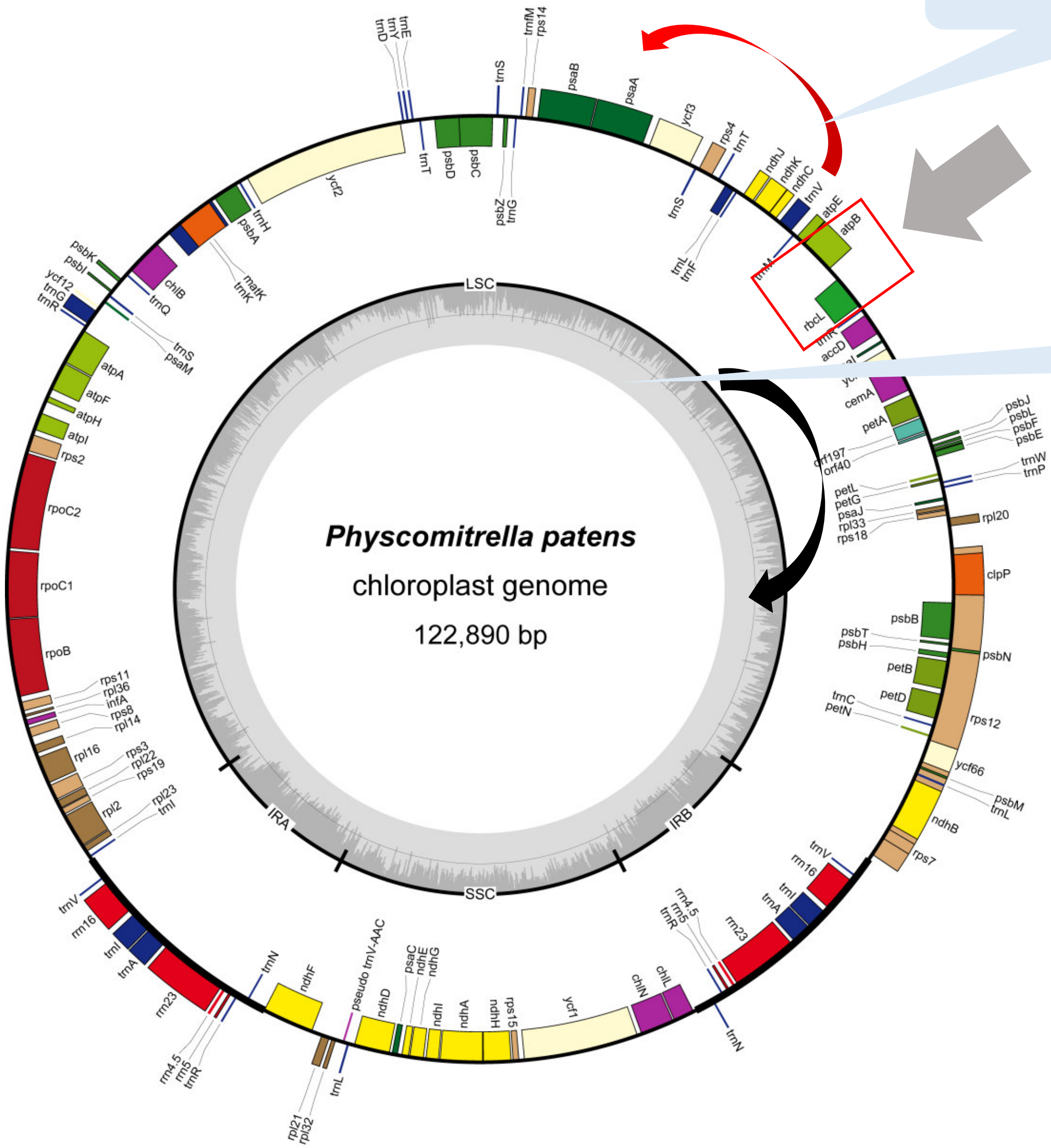
Origin of plant chloroplast genome


Nuclear envelope
Endoplasmic reticulum
Nucleus
Infolding of plasma membrane
Cell with nucleus and endomembrane system
Mitochondrion
Ancestral photosynthetic eukaryote
Plastid
Engulfing of photosynthetic prokaryote
DNA
Cytoplasm
Time
Ancestral prokaryote
Plasma membrane
Engulfing of aerobic heterotrophic prokaryote
Mitochondrion
Ancestral heterotrophic eukaryote

Physcomitrella (moss) chloroplast genome

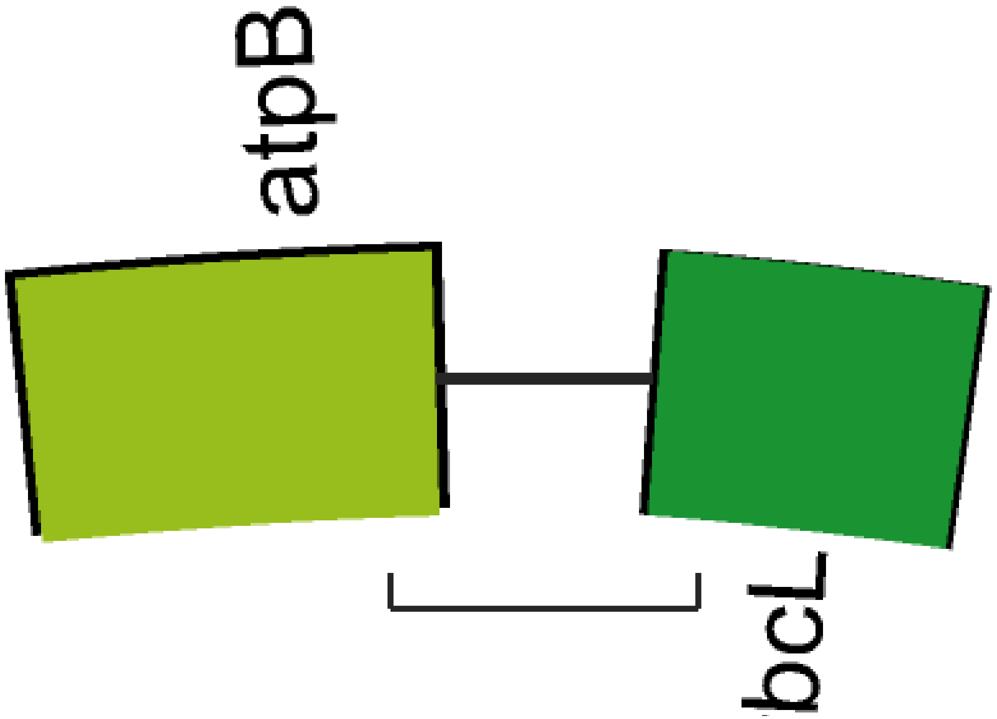
Outer genes: reverse

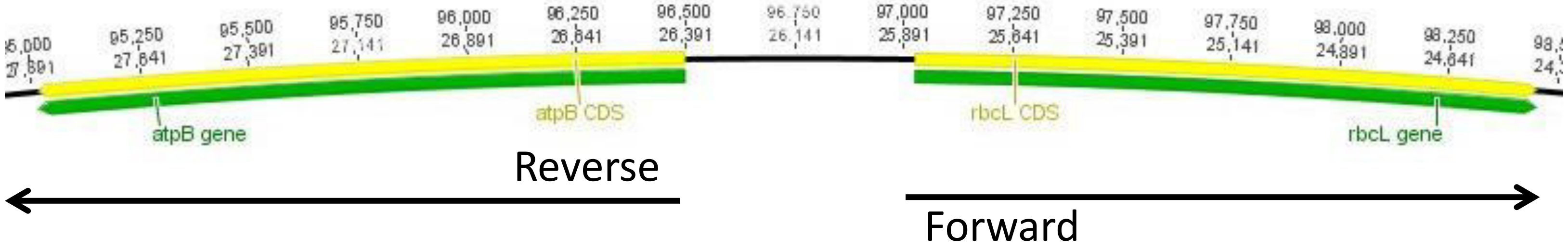
Inner genes: direct



Terasawa et al. *MBE* 2007

*atp*B-*rbc*L


atpB
bcL


5,000
27,891
95,250
27,641
95,500
27,391
95,750
27,141
96,000
26,891
96,250
26,641
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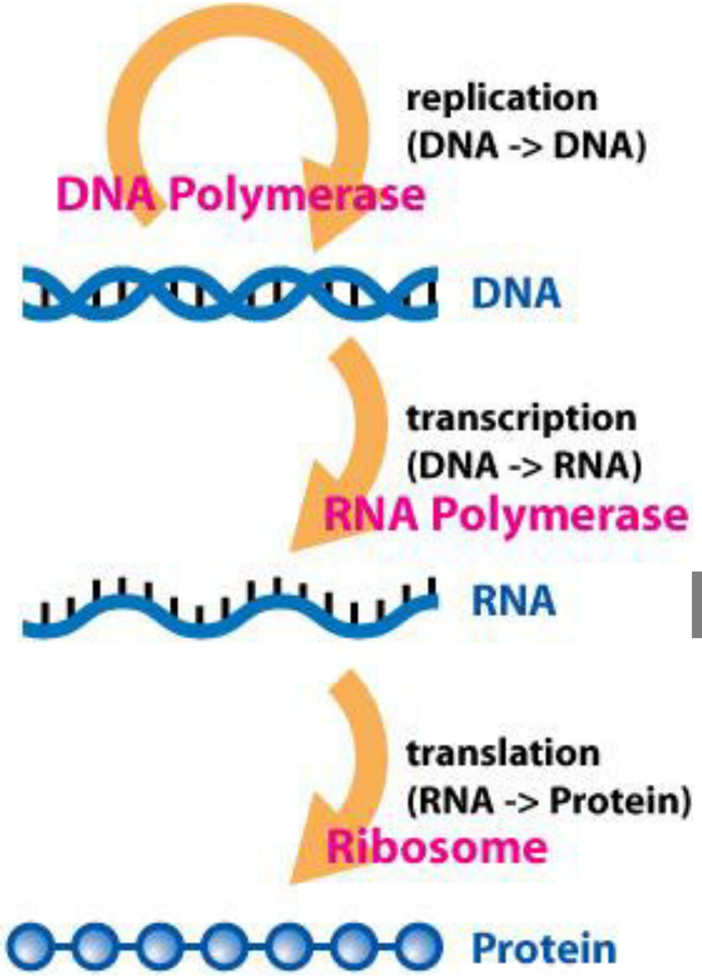
26,391
96,750
26,141
97,000
25,891
97,250
25,641
97,500
25,391
97,750
25,141
98,000
24,891
98,250
24,641
98,
24,
atpB gene
atpB CDS
rbcL CDS
rbcL gene
Reverse
Forward

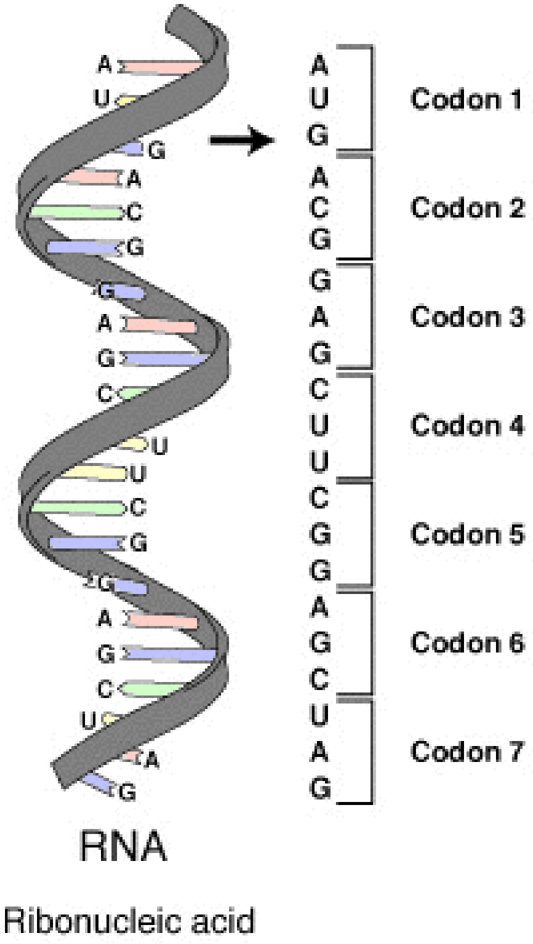
Central dogma

Start codons: ATG

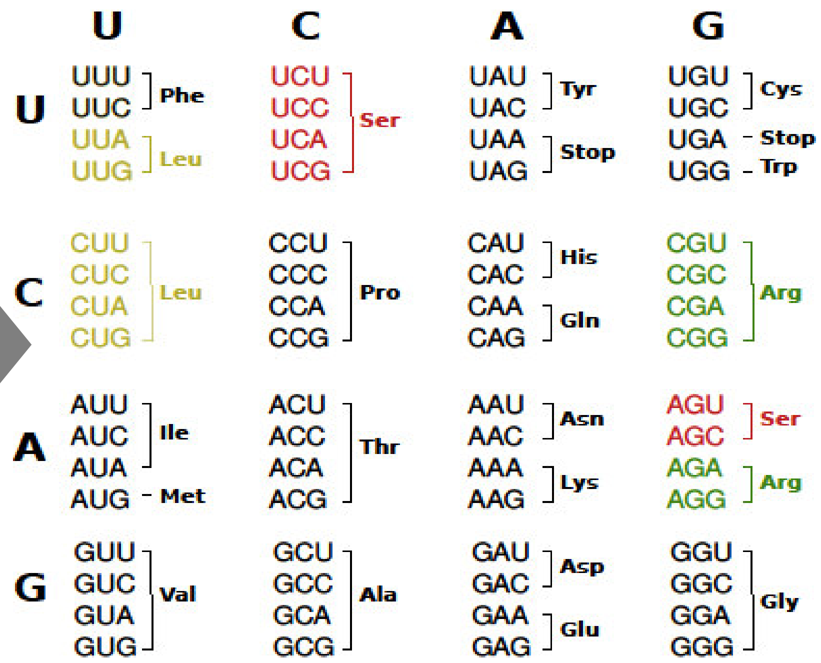
Stop codons: TAA, TGA, TAG



Dhorspool

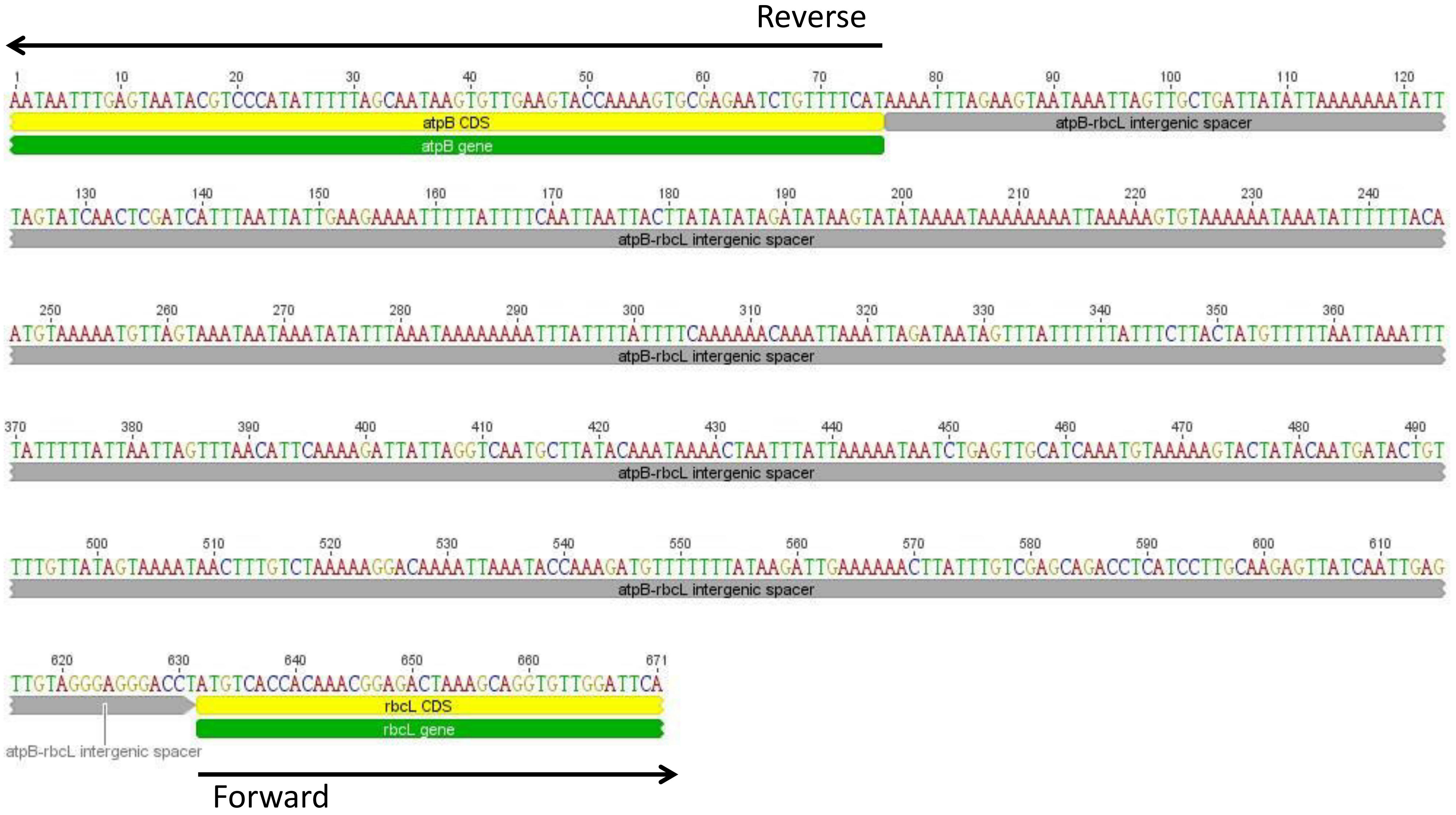


TransControl



	U	C	A	G
U	UUU UUC Phe UUA UUG Leu	UCU UCC UCA UCG Ser	UAU UAC Tyr UAA UAG Stop	UGU UGC Cys UGA - Stop UGG - Trp
C	CUU CUC CUA CUG Leu	CCU CCC CCA CCG Pro	CAU CAC His CAA CAG Gln	CGU CGC CGA CGG Arg
A	AUU AUC AUA Ile AUG - Met	ACU ACC ACA ACG Thr	AAU AAC Asn AAA AAG Lys	AGU AGC Ser AGA AGG Arg
G	GUU GUC GUA GUG Val	GCU GCC GCA GCG Ala	GAU GAC Asp GAA GAG Glu	GGU GGC GGA GGG Gly

*Plagiothecium latebricola atp*B-*rbc*L



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TTTGTTATAGTAAAATAACTTTGTCTAAAAAGGACAAAATTAAATACCAAAGATGTTTTTTTATAAGATTGAAAAAACTTATTTGTCGAGCAGACCTCATCCTTGCAAGAGTTATCAATTGAG
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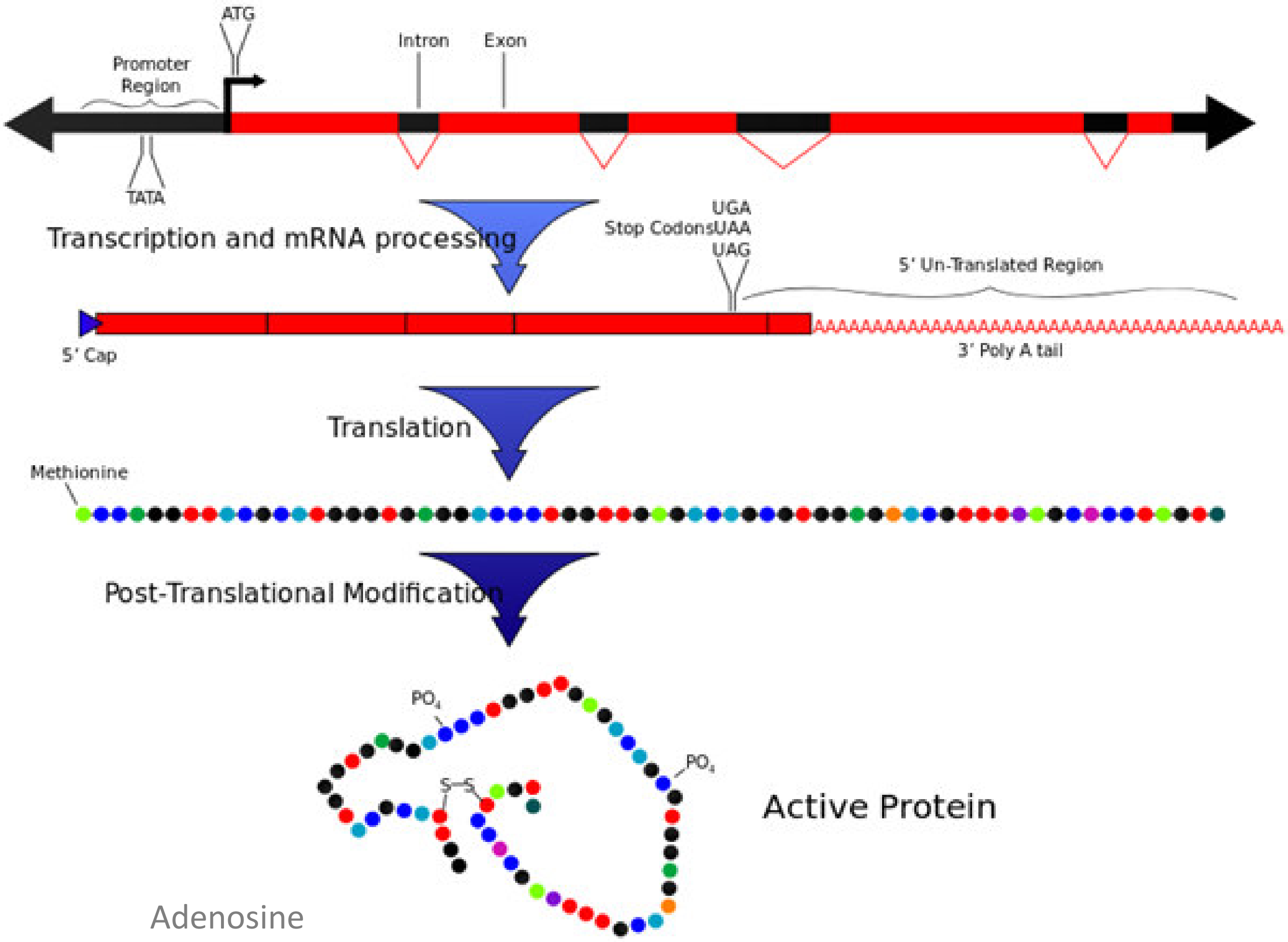
```

Transcription and translation a gene with intron



pre-mature mRNA

mature mRNA

protein

Adenosine

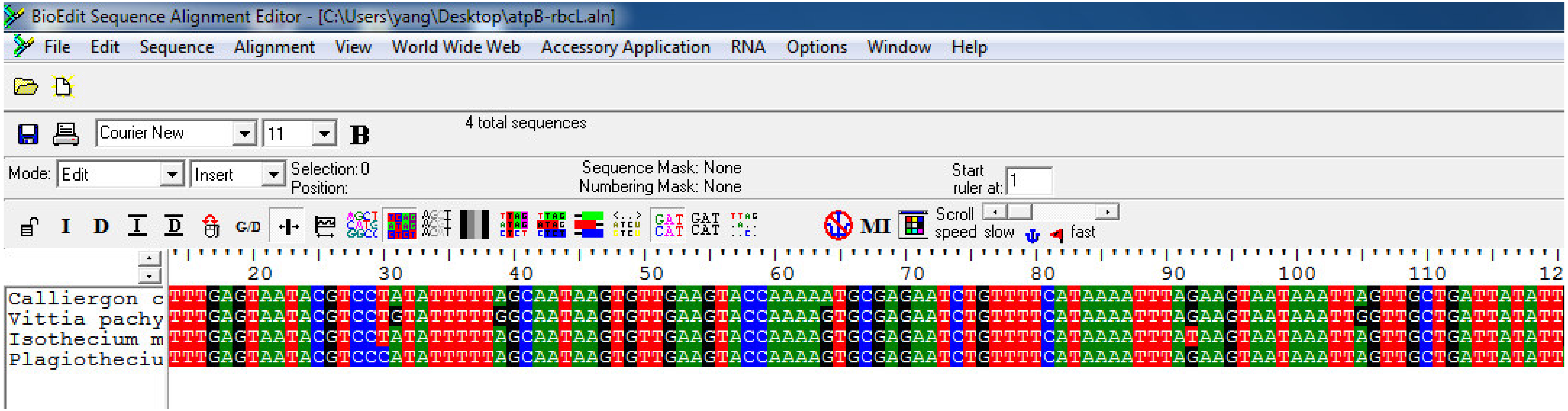
Organize sequences

- Use a text editor: WordPad, Notepad ++ (windows); TextEdit, TextWrangler (Mac)

- One file for one gene, always include GenBank **accession number** for a sequence

- Molecular programs: BioEdit v7.2, MEGA v5, PhyDe

BioEdit v7.2



BioEdit Sequence Alignment Editor - [C:\Users\yang\Desktop\atpB-rbcL.aln]

Courier New 11

4 total sequences

Mode: Edit Insert

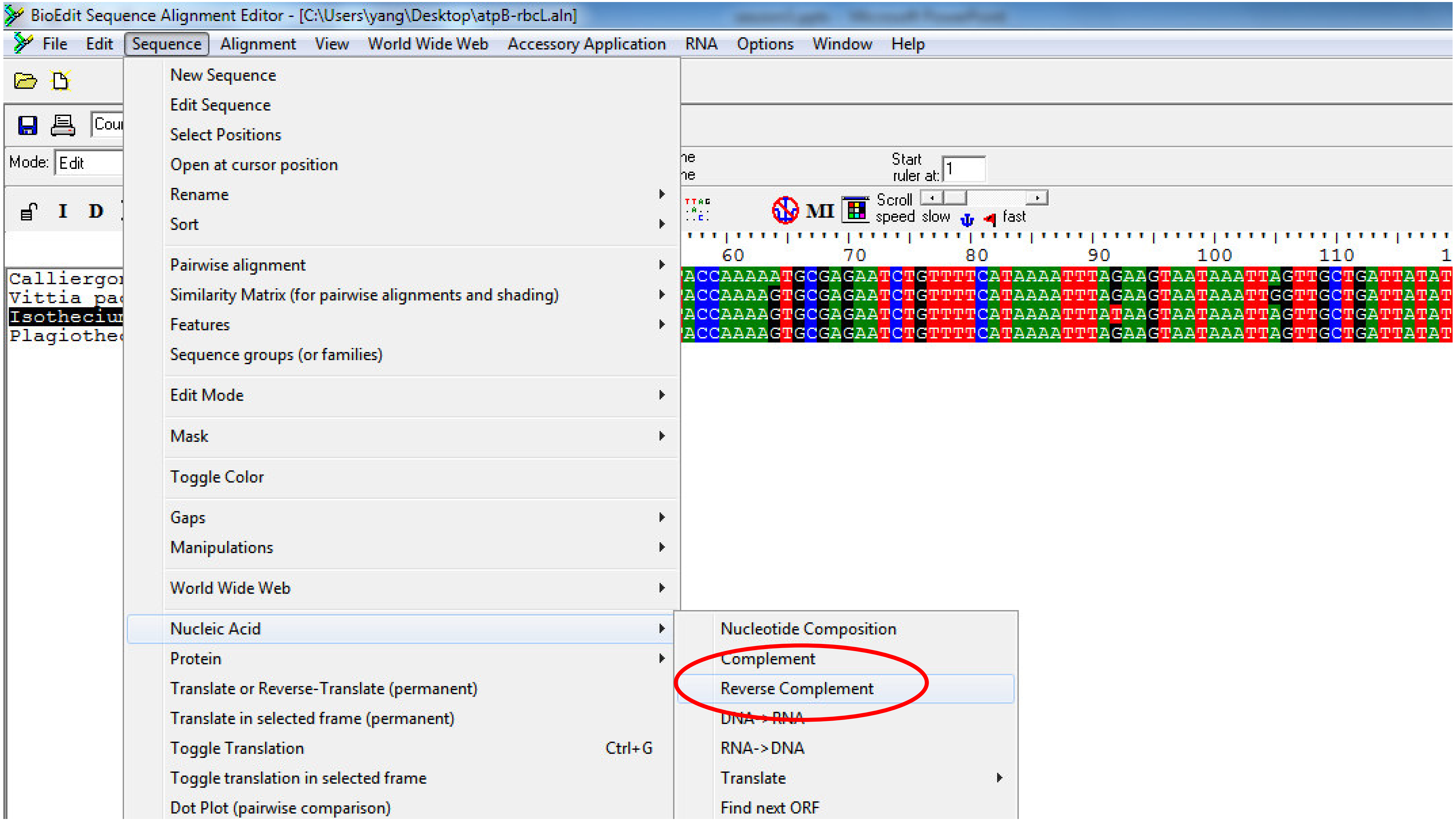
Selection: 0
Position:

Sequence Mask: None
Numbering Mask: None

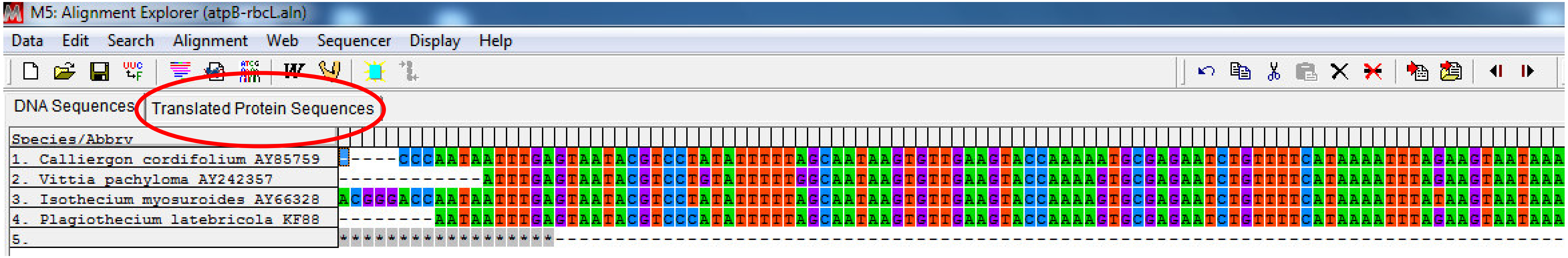
Start ruler at: 1

```
                 20        30        40        50        60        70        80        90        100       110       12
Calliergon c TTTGAGTAATACGTCCTATATTTTTAGCAATAAGTGTTGAAGTACCAAAAATGCGAGAATCTGTTTTCATAAAATTTAGAAGTAATAAATTAGTTGCTGATTATATT
Vittia pachy TTTGAGTAATACGTCCTGTATTTTTGGCAATAAGTGTTGAAGTACCAAAAGTGCGAGAATCTGTTTTCATAAAATTTAGAAGTAATAAATTGGTTGCTGATTATATT
Isothecium m TTTGAGTAATACGTCCTATATTTTTAGCAATAAGTGTTGAAGTACCAAAAGTGCGAGAATCTGTTTTCATAAAATTTATAAGTAATAAATTAGTTGCTGATTATATT
Plagiotheciu TTTGAGTAATACGTCCCATATTTTTAGCAATAAGTGTTGAAGTACCAAAAGTGCGAGAATCTGTTTTCATAAAATTTAGAAGTAATAAATTAGTTGCTGATTATATT
```

BioEdit v7.2


BioEdit Sequence Alignment Editor - [C:\Users\yang\Desktop\atpB-rbcL.aln]
File
Edit
Sequence
Alignment
View
World Wide Web
Accessory Application
RNA
Options
Window
Help
Mode: Edit
Start ruler at: 1
Scroll speed slow fast
New Sequence
Edit Sequence
Select Positions
Open at cursor position
Rename
Sort
Pairwise alignment
Similarity Matrix (for pairwise alignments and shading)
Features
Sequence groups (or families)
Edit Mode
Mask
Toggle Color
Gaps
Manipulations
World Wide Web
Nucleic Acid
Protein
Translate or Reverse-Translate (permanent)
Translate in selected frame (permanent)
Toggle Translation Ctrl+G
Toggle translation in selected frame
Dot Plot (pairwise comparison)
Nucleotide Composition
Complement
Reverse Complement
DNA->RNA
RNA->DNA
Translate
Find next ORF
Calliergo
Vittia pa
Isotheciu
Plagiothe
60
70
80
90
100
110

MEGA v5



M5: Alignment Explorer (atpB-rbcL.aln)
Data
Edit
Search
Alignment
Web
Sequencer
Display
Help
DNA Sequences
Translated Protein Sequences

```
Species/Abbrv
1. Calliergon cordifolium AY85759    -----CCCAATAATTTGAGTAATACGTCCTATATTTTTAGCAATAAGTGTTGAAGTACCAAAAATGCGAGAATCTGTTTTCATAAAATTTAGAAGTAATAAA
2. Vittia pachyloma AY242357         ------------ATTTGAGTAATACGTCCTGTATTTTTGGCAATAAGTGTTGAAGTACCAAAAGTGCGAGAATCTGTTTTCATAAAATTTAGAAGTAATAAA
3. Isothecium myosuroides AY66328    ACGGGACCAATAATTTGAGTAATACGTCCTATATTTTTAGCAATAAGTGTTGAAGTACCAAAAGTGCGAGAATCTGTTTTCATAAAATTTATAAGTAATAAA
4. Plagiothecium latebricola KF88    --------AATAATTTGAGTAATACGTCCCATATTTTTAGCAATAAGTGTTGAAGTACCAAAAGTGCGAGAATCTGTTTTCATAAAATTTAGAAGTAATAAA
5.                                   ******************------------------------------------------------------------------------------------
```

GenBank

http://www.ncbi.nlm.nih.gov/

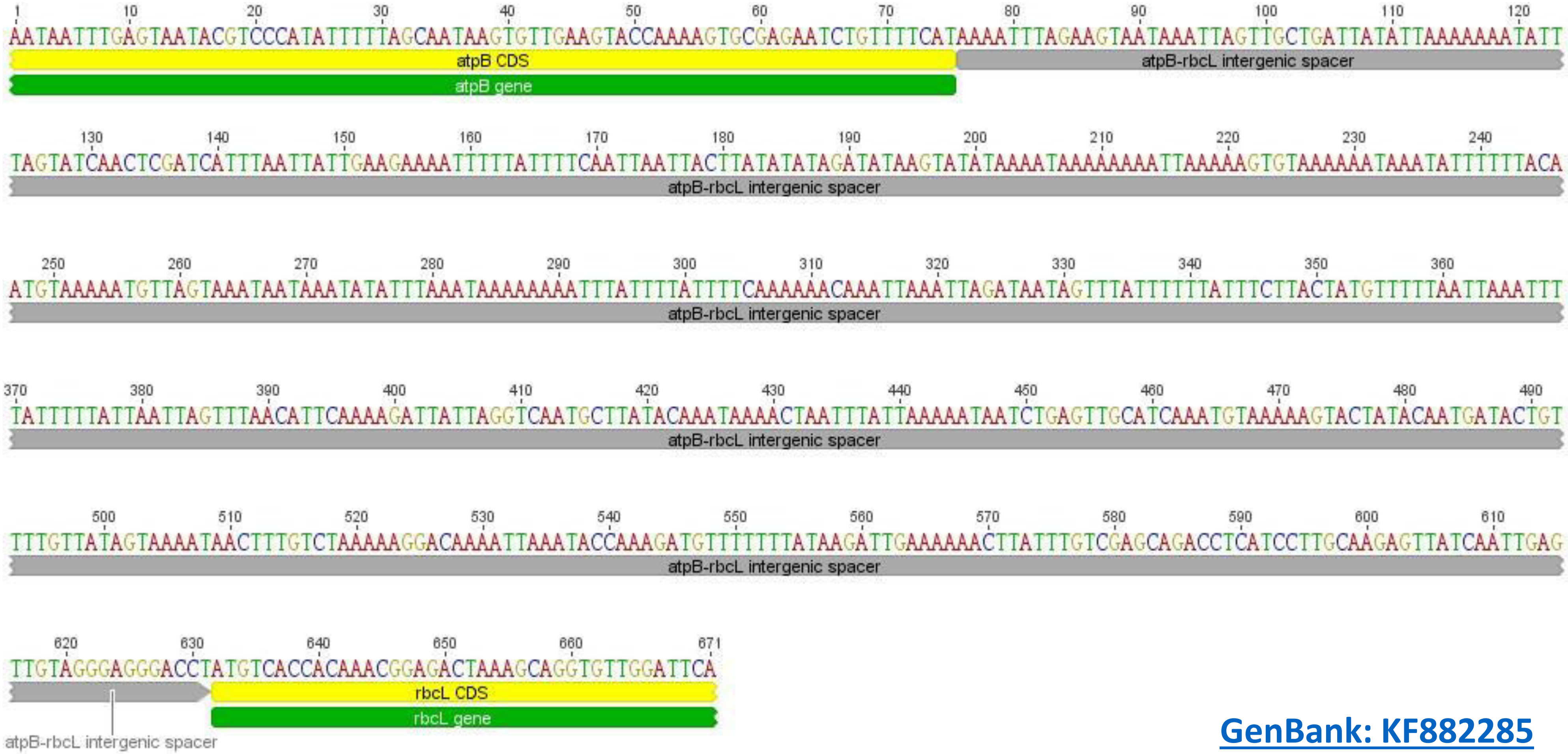
- GenBank: an open access sequence database collecting nucleotide sequences and their protein translations

- GenBank is found in 1982 by the National Center for Biotechnology Information (NCBI), which belongs to the National Institutes of Health (NIH)

- By August 2014, GenBank has 174 million loci, 165 billion bases, and for more than 300,000 organisms

- Doubling every 18 months

*Plagiothecium latebricola atp*B-*rbc*L



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TATTTTTATTAATTAGTTTAACATTCAAAAGATTATTAGGTCAATGCTTATACAAATAAAACTAATTTATTAAAAATAATCTGAGTTGCATCAAATGTAAAAAGTACTATACAATGATACTGT
TTTGTTATAGTAAAATAACTTTGTCTAAAAAGGACAAAATTAAATACCAAAGATGTTTTTTTATAAGATTGAAAAAACTTATTTGTCGAGCAGACCTCATCCTTGCAAGAGTTATCAATTGAG
TTGTAGGGAGGGACCTATGTCACCACAAACGGAGACTAAAGCAGGTGTTGGATTCA
```

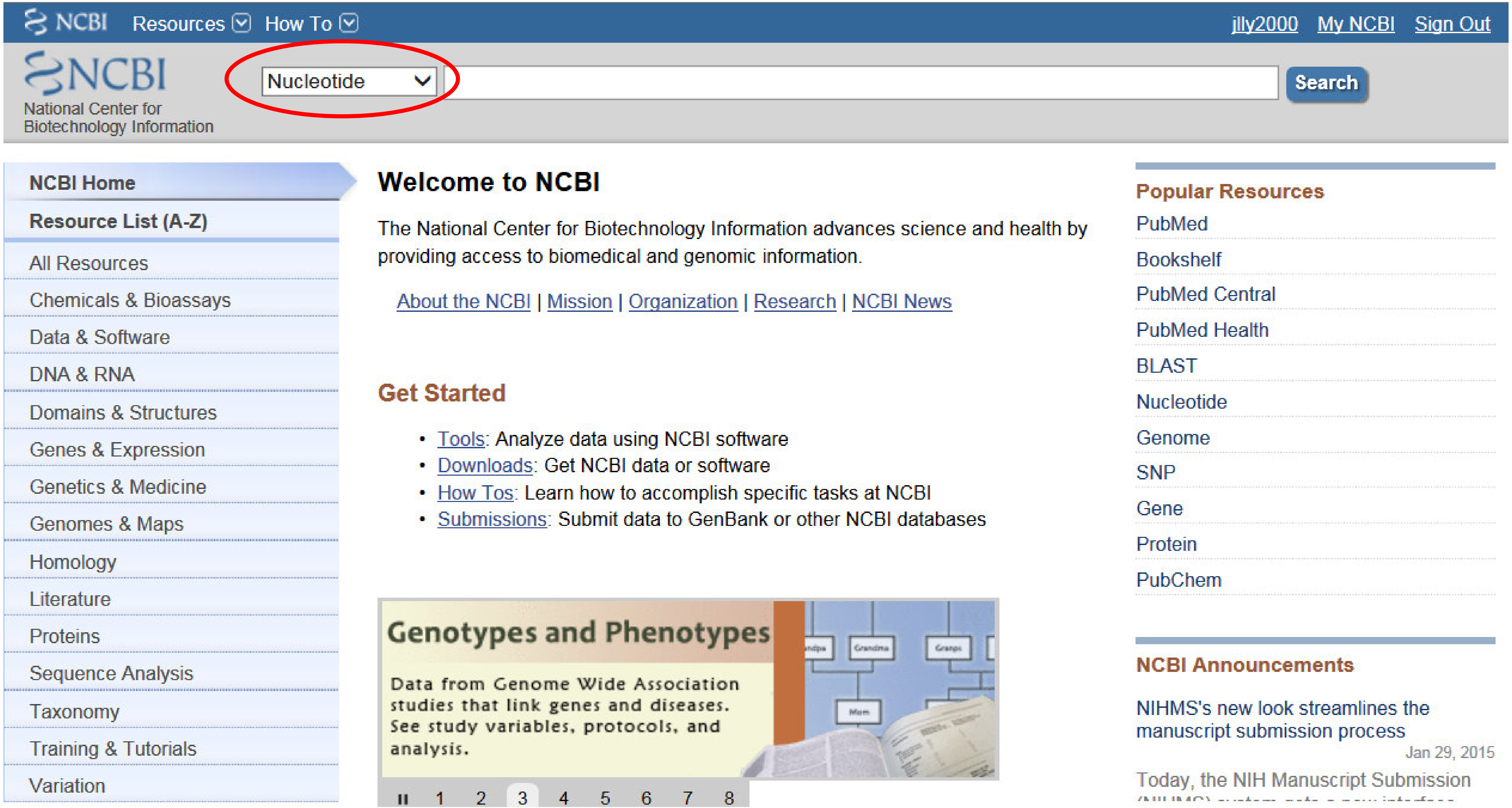
GenBank: KF882285

Retrieving sequences from GenBank

- ➢ Search by gene and organism name

- ➢ Search by a query sequence
  - Using BLAST (Basic Local Alignment Search Tool)

Acquiring sequences from GenBank: **Search by name**



Nucleotide

Welcome to NCBI

The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.

About the NCBI | Mission | Organization | Research | NCBI News

Get Started

- Tools: Analyze data using NCBI software
- Downloads: Get NCBI data or software
- How Tos: Learn how to accomplish specific tasks at NCBI
- Submissions: Submit data to GenBank or other NCBI databases

Genotypes and Phenotypes

Data from Genome Wide Association studies that link genes and diseases. See study variables, protocols, and analysis.

NCBI Announcements

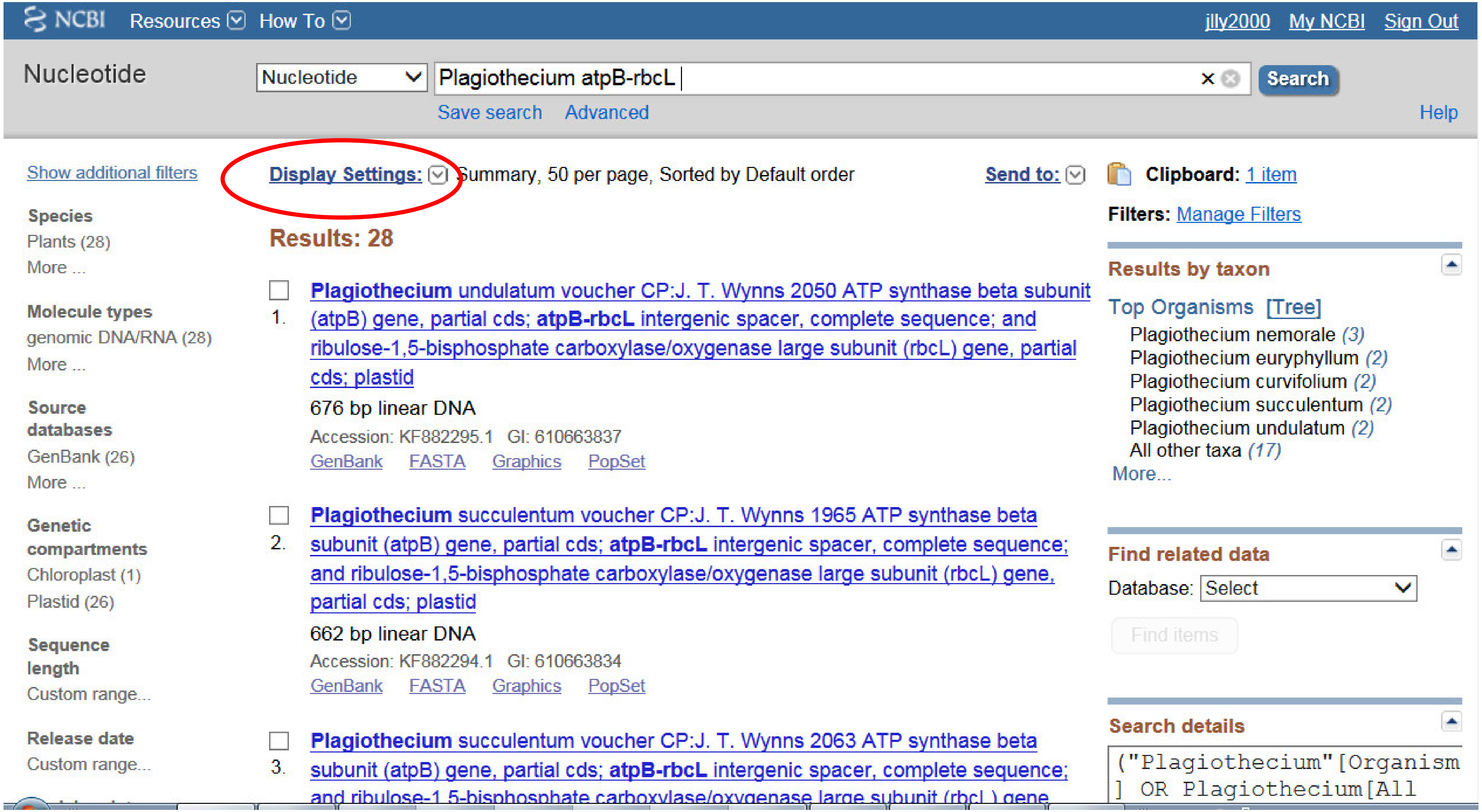
NIHMS's new look streamlines the manuscript submission process

Jan 29, 2015

Today, the NIH Manuscript Submission

(http://www.ncbi.nlm.nih.gov/)

Acquiring sequences from GenBank: **Search by name**



Plagiothecium atpB-rbcL

Display Settings: Summary, 50 per page, Sorted by Default order

Clipboard: 1 item

Filters: Manage Filters

Species
Plants (28)
More ...

Molecule types
genomic DNA/RNA (28)
More ...

Source databases
GenBank (26)
More ...

Genetic compartments
Chloroplast (1)
Plastid (26)

Sequence length
Custom range...

Release date
Custom range...

Results: 28

□ 1. **Plagiothecium** undulatum voucher CP:J. T. Wynns 2050 ATP synthase beta subunit (atpB) gene, partial cds; **atpB-rbcL** intergenic spacer, complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid
676 bp linear DNA
Accession: KF882295.1 GI: 610663837
GenBank FASTA Graphics PopSet

□ 2. **Plagiothecium** succulentum voucher CP:J. T. Wynns 1965 ATP synthase beta subunit (atpB) gene, partial cds; **atpB-rbcL** intergenic spacer, complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid
662 bp linear DNA
Accession: KF882294.1 GI: 610663834
GenBank FASTA Graphics PopSet

□ 3. **Plagiothecium** succulentum voucher CP:J. T. Wynns 2063 ATP synthase beta subunit (atpB) gene, partial cds; **atpB-rbcL** intergenic spacer, complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene

Results by taxon

Top Organisms [Tree]

Plagiothecium nemorale	(3)
Plagiothecium euryphyllum	(2)
Plagiothecium curvifolium	(2)
Plagiothecium succulentum	(2)
Plagiothecium undulatum	(2)
All other taxa	(17)

More...

Find related data

Database: Select

Find items

Search details

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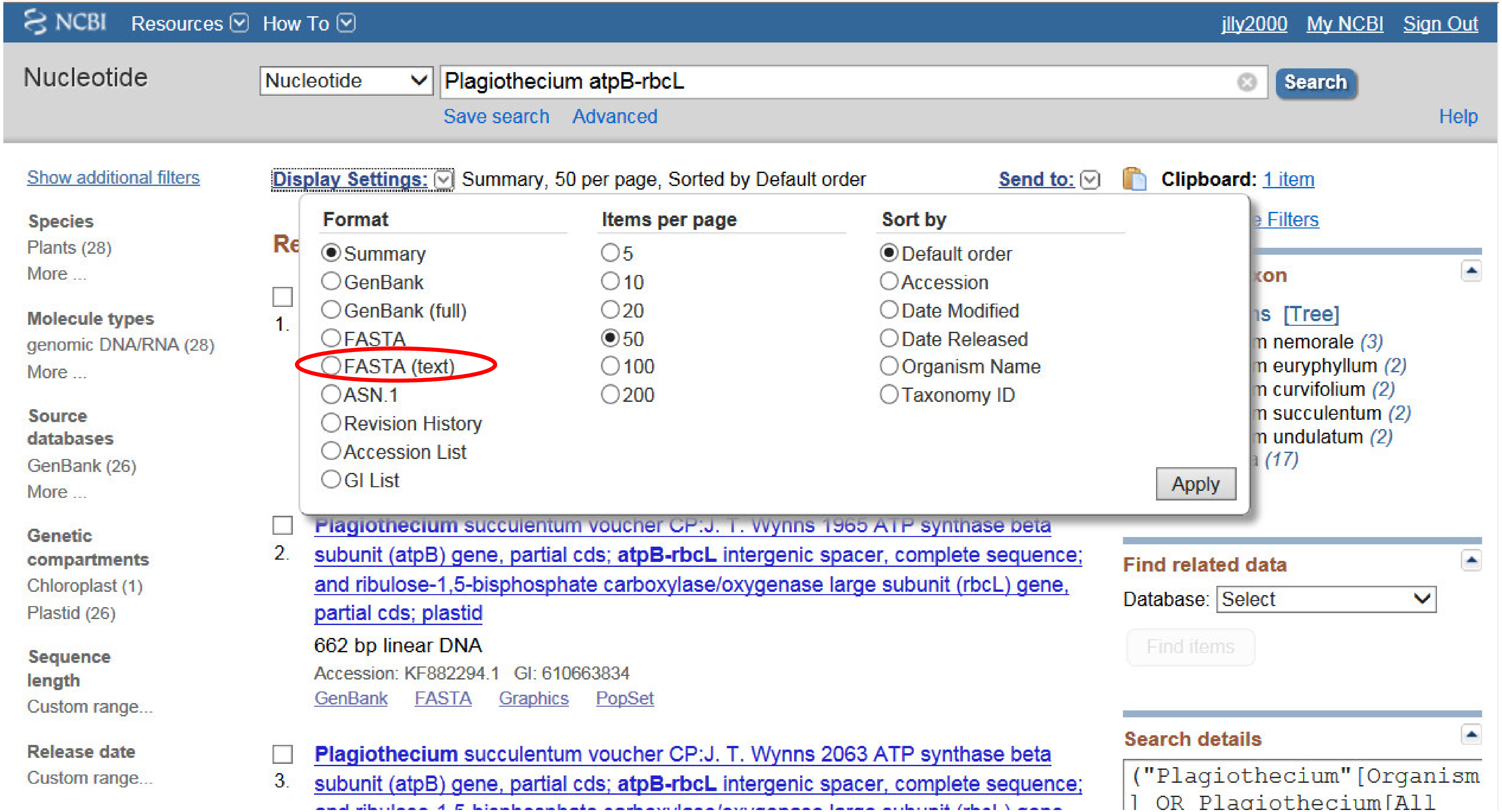
("Plagiothecium"[Organism
] OR Plagiothecium[All
```

Acquiring sequences from GenBank: **Search by name**



NCBI Resources How To jilly2000 My NCBI Sign Out

Nucleotide

Nucleotide Plagiothecium atpB-rbcL Search

Save search Advanced Help

Show additional filters

Species
Plants (28)
More ...

Molecule types
genomic DNA/RNA (28)
More ...

Source databases
GenBank (26)
More ...

Genetic compartments
Chloroplast (1)
Plastid (26)

Sequence length
Custom range...

Release date
Custom range...

Display Settings: Summary, 50 per page, Sorted by Default order

Send to: Clipboard: 1 item

Format
- ● Summary
- ○ GenBank
- ○ GenBank (full)
- ○ FASTA
- ○ [✓ FASTA (text)]
- ○ ASN.1
- ○ Revision History
- ○ Accession List
- ○ GI List

Items per page
- ○ 5
- ○ 10
- ○ 20
- ● 50
- ○ 100
- ○ 200

Sort by
- ● Default order
- ○ Accession
- ○ Date Modified
- ○ Date Released
- ○ Organism Name
- ○ Taxonomy ID

Apply

Re

□ 1.

□ 2. Plagiothecium succulentum voucher CP:J. T. Wynns 1965 ATP synthase beta subunit (atpB) gene, partial cds; atpB-rbcL intergenic spacer, complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid

662 bp linear DNA

Accession: KF882294.1 GI: 610663834

GenBank FASTA Graphics PopSet

□ 3. Plagiothecium succulentum voucher CP:J. T. Wynns 2063 ATP synthase beta subunit (atpB) gene, partial cds; atpB-rbcL intergenic spacer, complete sequence;

e Filters

xon

ns [Tree]
m nemorale (3)
m euryphyllum (2)
m curvifolium (2)
m succulentum (2)
m undulatum (2)
a (17)

Find related data

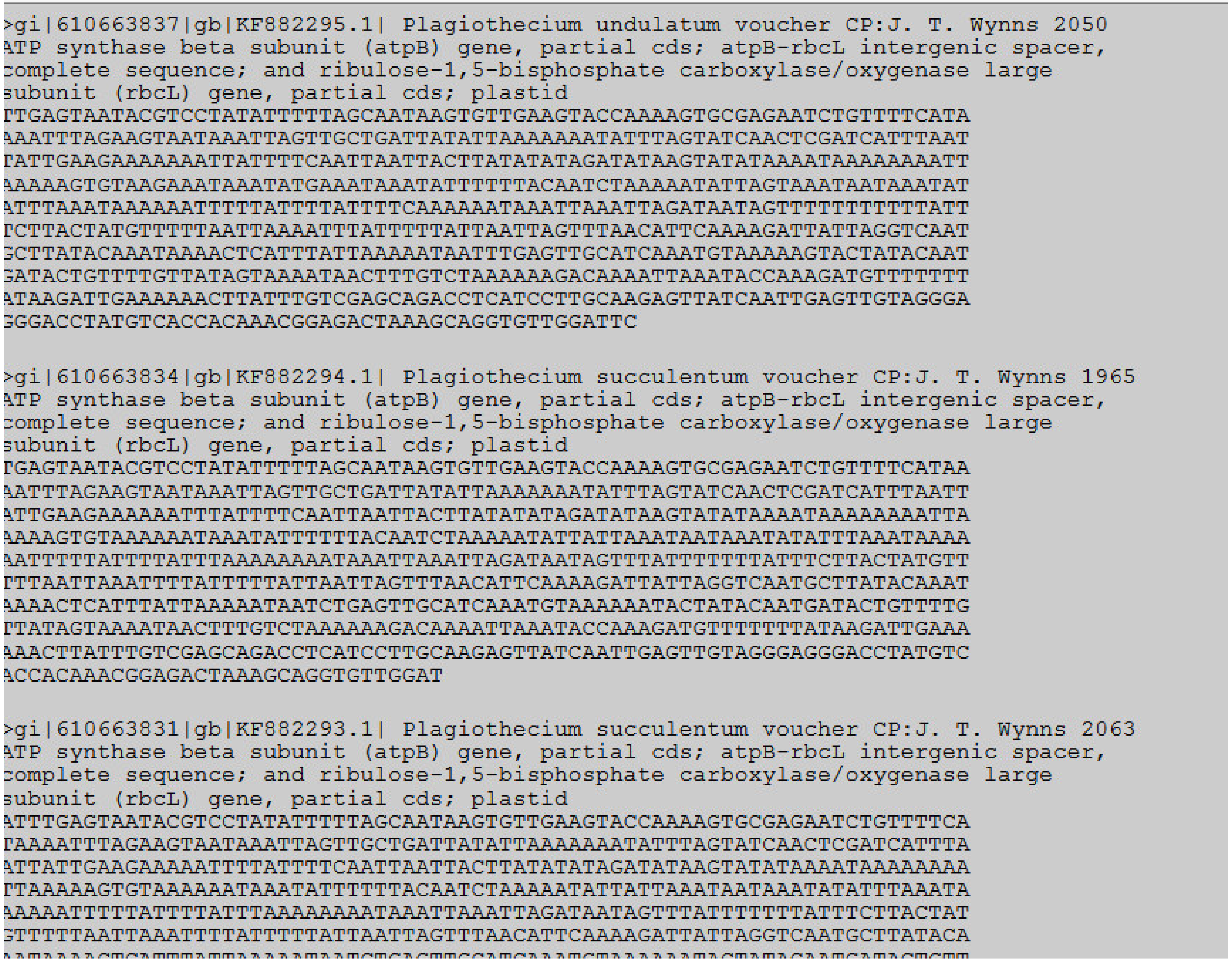
Database: Select

Find items

Search details

("Plagiothecium"[Organism] OR Plagiothecium[All

Acquiring sequences from GenBank: **Search by name**



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ATP synthase beta subunit (atpB) gene, partial cds; atpB-rbcL intergenic spacer,
complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large
subunit (rbcL) gene, partial cds; plastid
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ATP synthase beta subunit (atpB) gene, partial cds; atpB-rbcL intergenic spacer,
complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large
subunit (rbcL) gene, partial cds; plastid
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complete sequence; and ribulose-1,5-bisphosphate carboxylase/oxygenase large
subunit (rbcL) gene, partial cds; plastid
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AATAAAACTCATTTATTAAAAATAATCTGAGTTGCATCAAATGTAAAAAATACTATACAATGATACTGTT
```
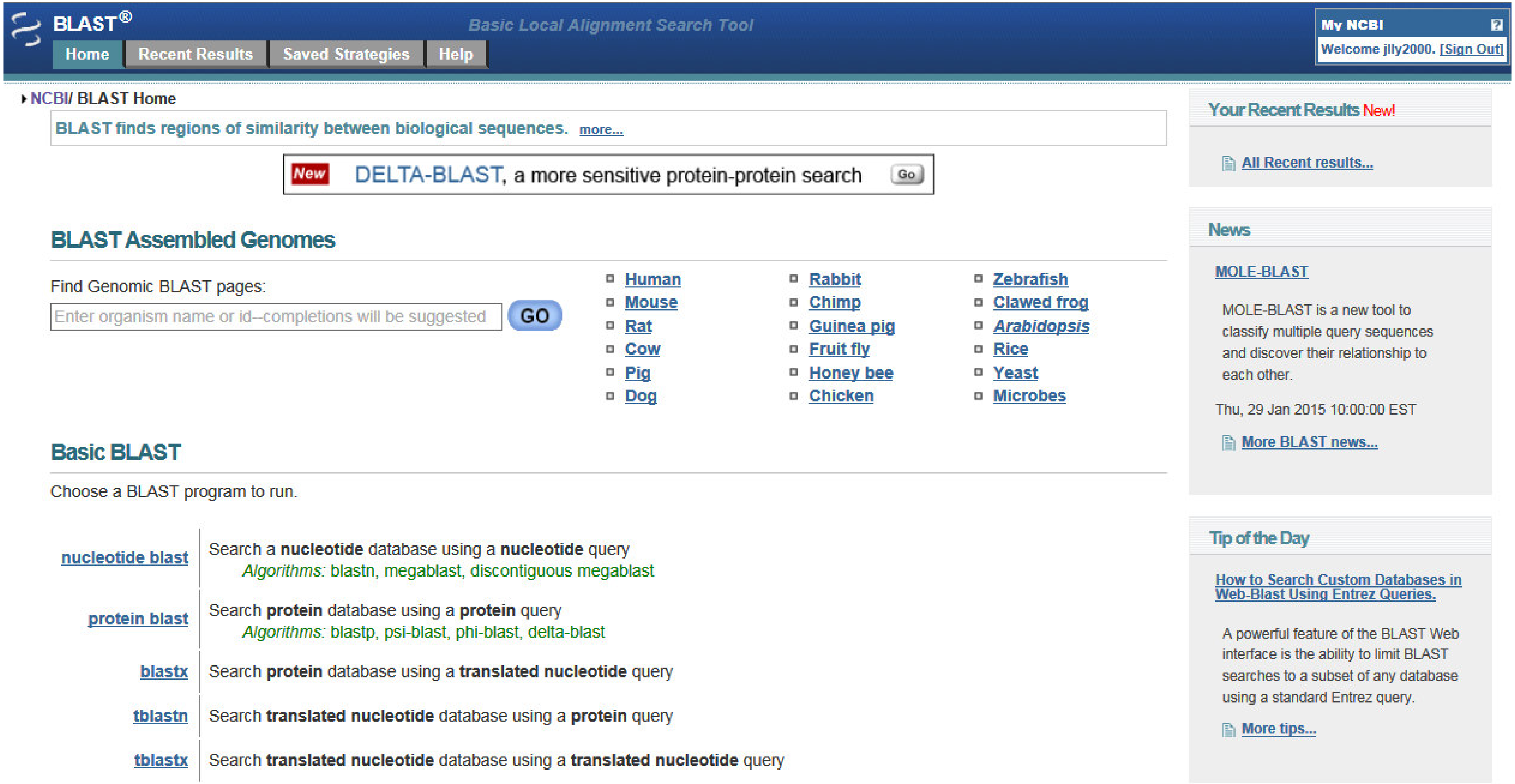

Retrieving sequences from GenBank

- ➢ Search by gene and organism name

- ➢ Search by a query sequence
  - Using BLAST (Basic Local Alignment Search Tool)

Acquiring sequences from GenBank: **BLAST**


BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
My NCBI
Welcome jlly2000. [Sign Out]
New
Go
GO

NCBI/ BLAST Home

BLAST finds regions of similarity between biological sequences. more...

New DELTA-BLAST, a more sensitive protein-protein search

BLAST Assembled Genomes

Find Genomic BLAST pages:

Enter organism name or id--completions will be suggested

- Human
- Mouse
- Rat
- Cow
- Pig
- Dog
- Rabbit
- Chimp
- Guinea pig
- Fruit fly
- Honey bee
- Chicken
- Zebrafish
- Clawed frog
- *Arabidopsis*
- Rice
- Yeast
- Microbes

Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast, delta-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

Your Recent Results New!

All Recent results...

News

MOLE-BLAST

MOLE-BLAST is a new tool to classify multiple query sequences and discover their relationship to each other.

Thu, 29 Jan 2015 10:00:00 EST

More BLAST news...

Tip of the Day

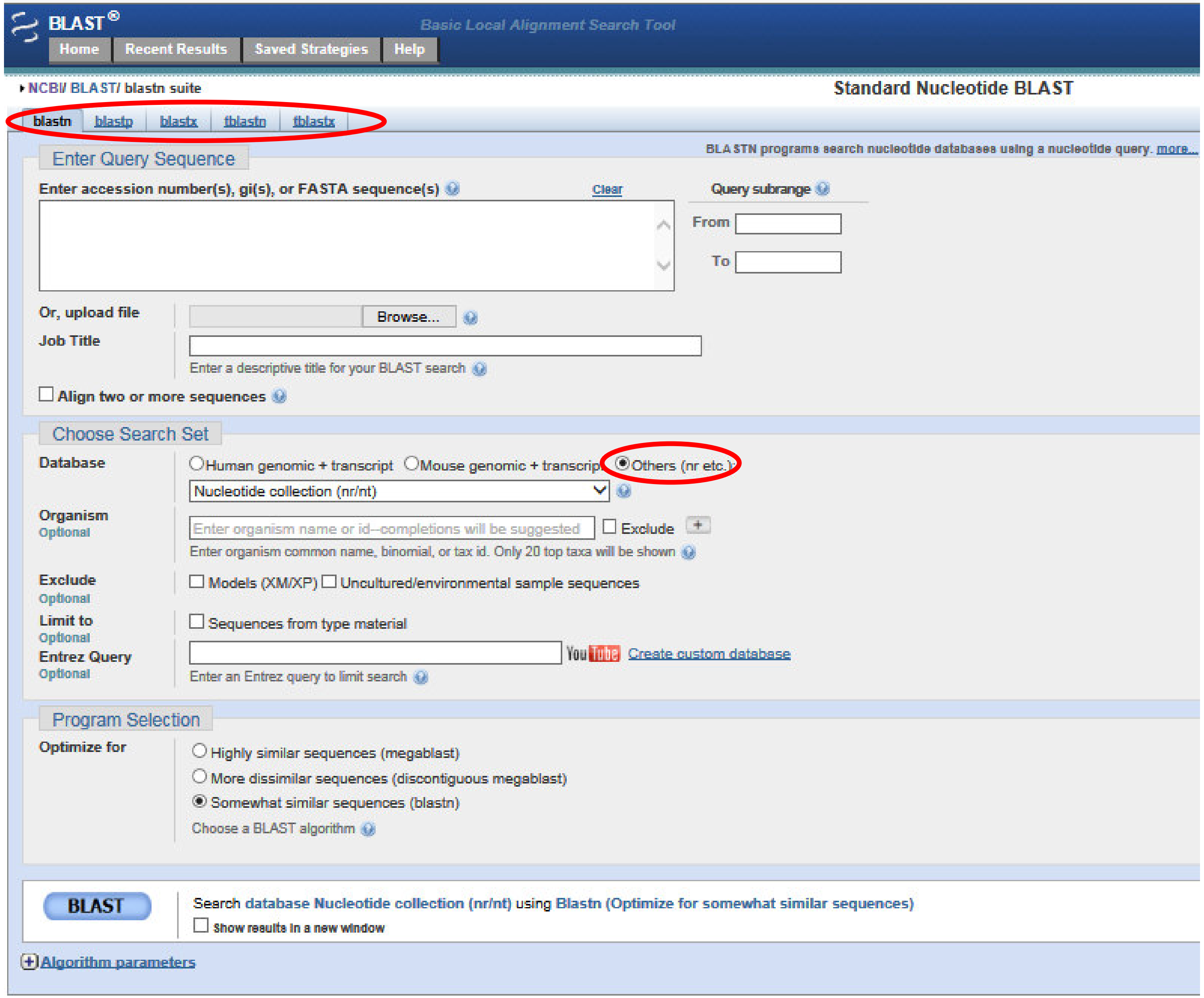
How to Search Custom Databases in Web-Blast Using Entrez Queries.

A powerful feature of the BLAST Web interface is the ability to limit BLAST searches to a subset of any database using a standard Entrez query.

More tips...

http://blast.ncbi.nlm.nih.gov/

Acquiring sequences from GenBank: **BLAST**



BLAST® Basic Local Alignment Search Tool

Home | Recent Results | Saved Strategies | Help

NCBI/ BLAST/ blastn suite

Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

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

Enter Query Sequence

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

Query subrange
From
To

Or, upload file Browse...

Job Title
Enter a descriptive title for your BLAST search

□ Align two or more sequences

Choose Search Set

Database ○ Human genomic + transcript ○ Mouse genomic + transcript ● Others (nr etc.):
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: Create custom database
Enter an Entrez query to limit search

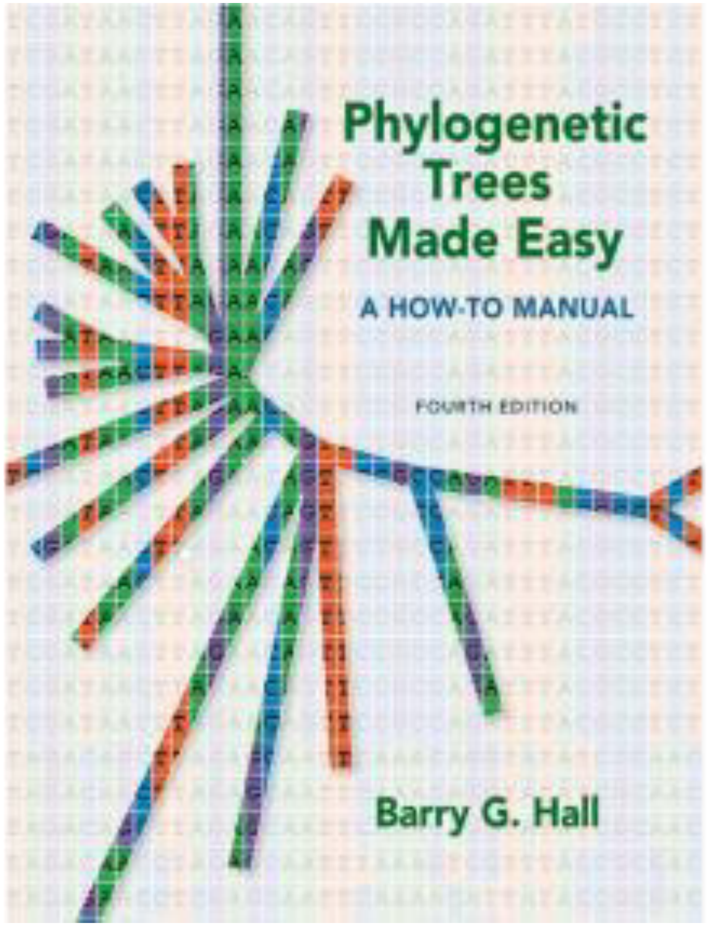
Program Selection

Optimize for
○ Highly similar sequences (megablast)
○ More dissimilar sequences (discontiguous megablast)
● Somewhat similar sequences (blastn)
Choose a BLAST algorithm

BLAST Search database Nucleotide collection (nr/nt) using Blastn (Optimize for somewhat similar sequences)
□ Show results in a new window

+ Algorithm parameters

- **blastn**
  Search nucleotide database by nucleotide query
- **blastp**
  Search protein database by protein query
- **blastx**
  Search protein database by translated nucleotide query
- **tblastn**
  Search translated nucleotide database by protein query
- **tblastx**
  Search translated nucleotide database by translated nucleotide query



Barry G. Hall
Phylogenetic Trees Made Easy: A How-To Manual
Sinauer Associates, Inc.; Fourth edition (April 30, 2011)

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