

# Phylogenetic Analysis

Clyde Dapat



WHO Collaborating Centre  
for Reference and  
Research on Influenza  
**VIDRL**

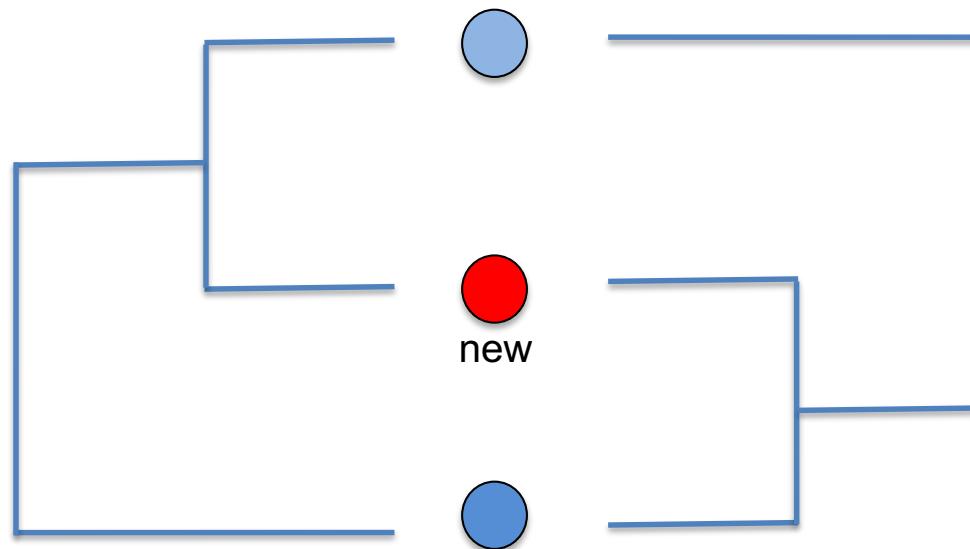


A joint venture between The University of Melbourne and The Royal Melbourne Hospital

# Objectives

- To gain a basic understanding of phylogenetics
- To know the different methods of creating a phylogenetic tree

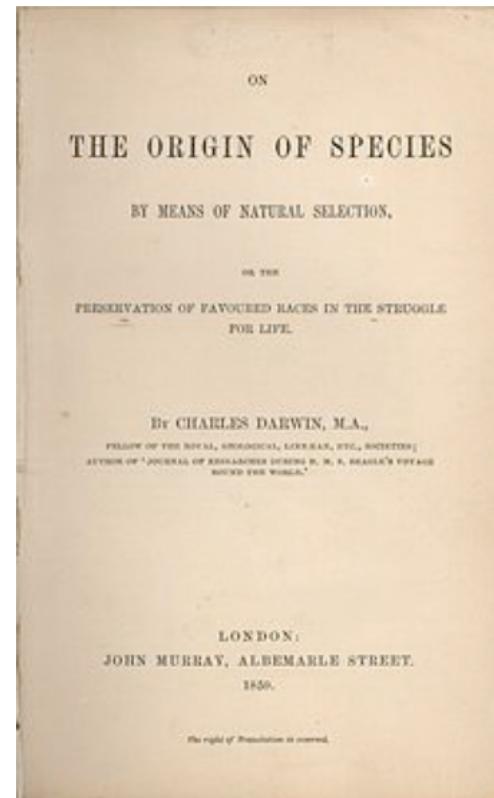
# Why do phylogenetics?



# Theory of Evolution: Darwin



Charles Darwin  
1809-1882  
[https://en.wikipedia.org/wiki/Charles\\_Darwin](https://en.wikipedia.org/wiki/Charles_Darwin)



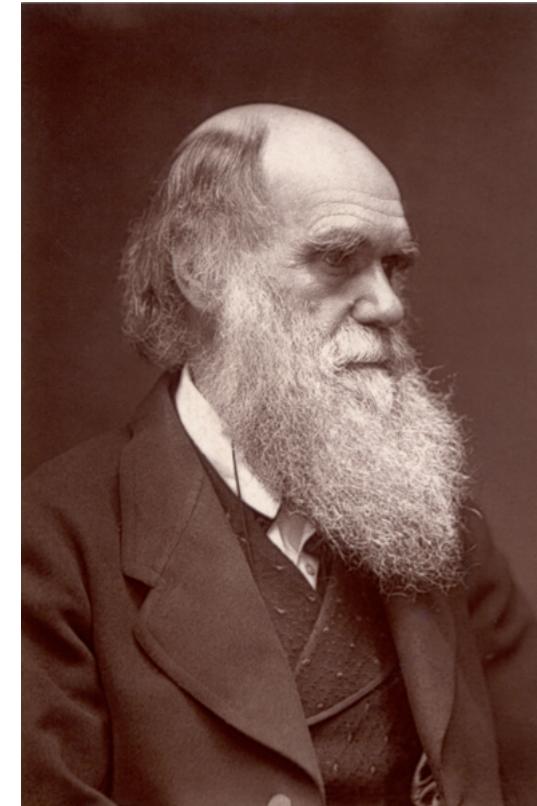
Origin of Species. First edition (1859)

The single most important idea in human history

# Natural Selection: Darwin's postulates

1. Individuals within species vary in their characteristics
2. Some of these variations are heritable
3. In each generation, more young are reproduced than can survive to reproduce
4. The survival and reproduction of individuals is not random: Individuals who survive and go on to reproduce are those with the most favorable variation. (They are naturally selected.)

Consequence: Populations change over time – they adapt to their environment



[commons.wikimedia.org](https://commons.wikimedia.org)

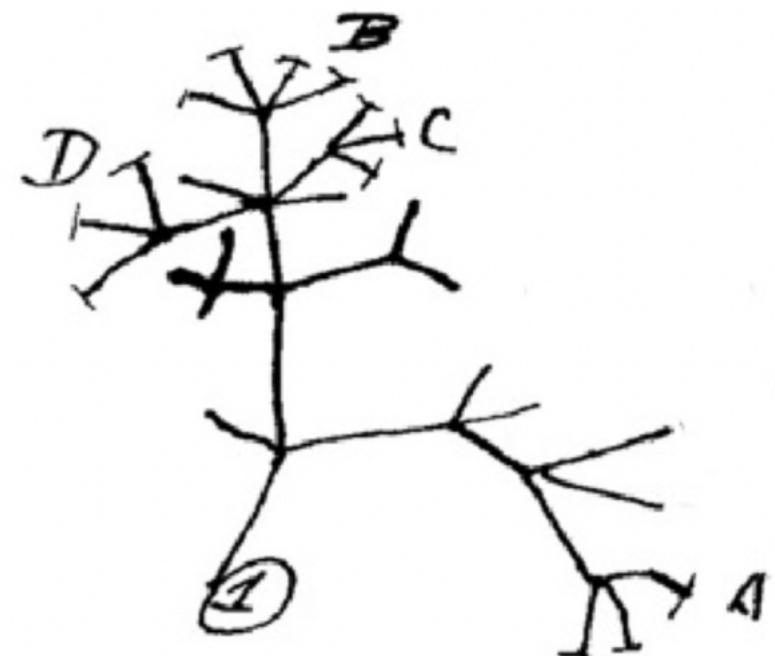
# Phylogenetics

Phylogenetics is the study of evolutionary relationships among biological entities – often species, individuals or genes (which may be referred to as taxa)

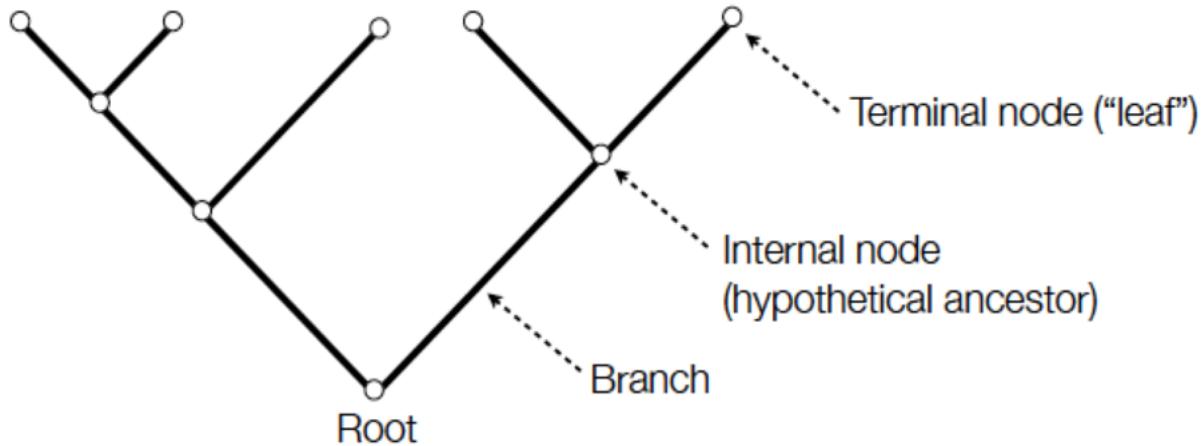
Use phylogenetic trees as models of evolution



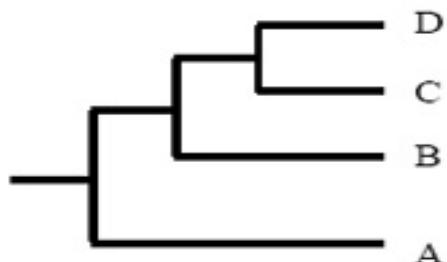
I think



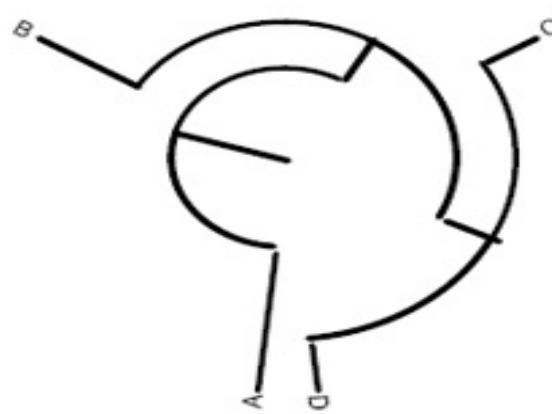
# Trees: terminology



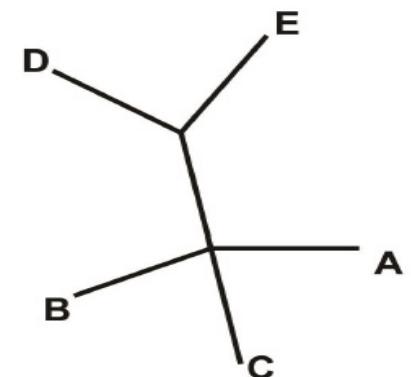
# Trees: types



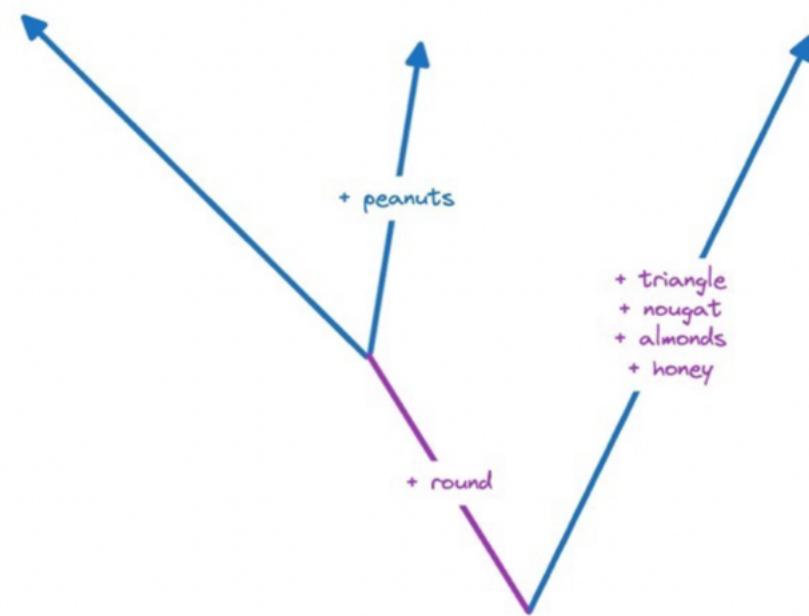
Traditional Tree



Circular/Radial  
Tree



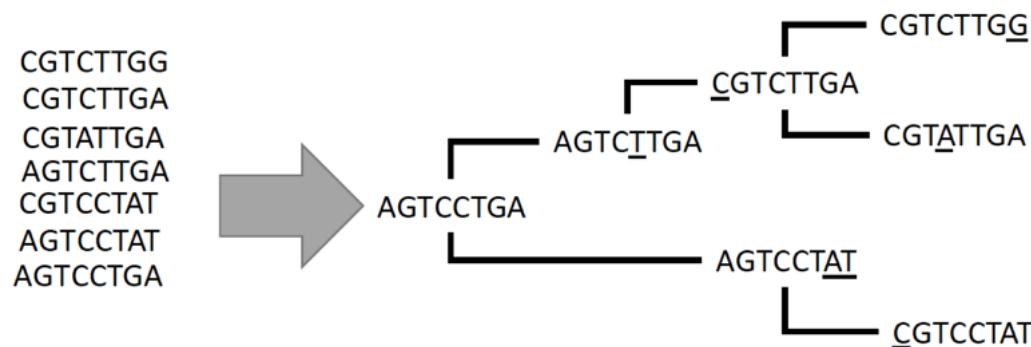
Unrooted Tree



# Assumptions in phylogenetics

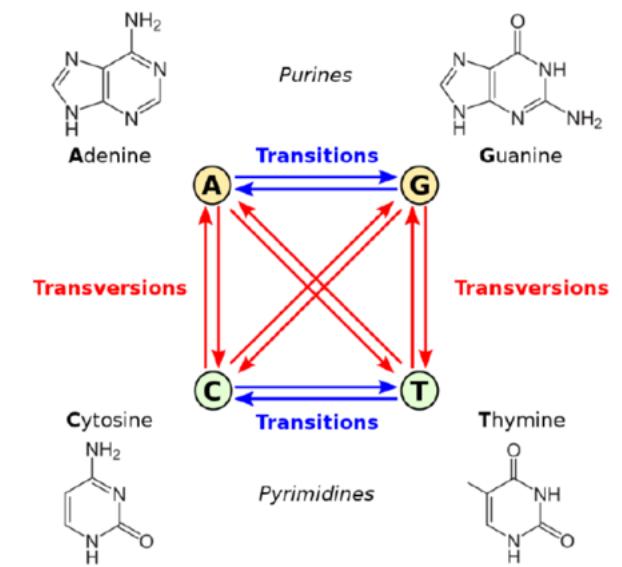
Several fundamental assumptions are made in phylogenetic methods

- Each aligned site represents a set of orthologous characters i.e. a gene in different species that evolved from a common ancestor by speciation
- Sites in alignment evolve independently
- Lineages evolve independently
- Relationships amongst sequences can be represented by a bifurcating (division into two branches) tree

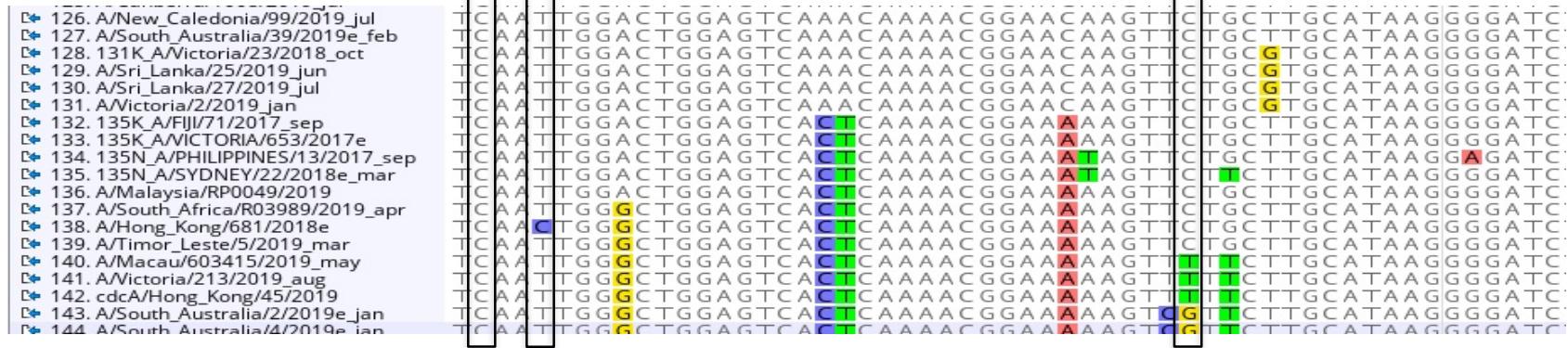


# Assumptions in phylogenetics

- Transitions are seen more often than transversions
- G/C → A/T is more commonly seen than A/T → G/C
- Each base → base mutation rate can be modeled separately
- However, **statistical assumptions like independence of sites** are made that are not always true



# Homologous and heterologous sites



## Homologous sites:

- Inherited from the common ancestor of all the species in the alignment
- Not informative for inferring information on the amount of evolution i.e. branch lengths, times, evolutionary rates
- Both type of sites are useful in phylogenetic studies as are insertions & deletions

## Heterologous sites:

- Informative for inferring information on the amount of evolution

# Popular phylogenetic methods for inferring trees

- Distance-based methods
  - Maximum likelihood
  - Bayesian inference
- Model based*

# Neighbour Joining method

Neighbor joining takes as input a distance matrix specifying the distance between each pair of taxa.

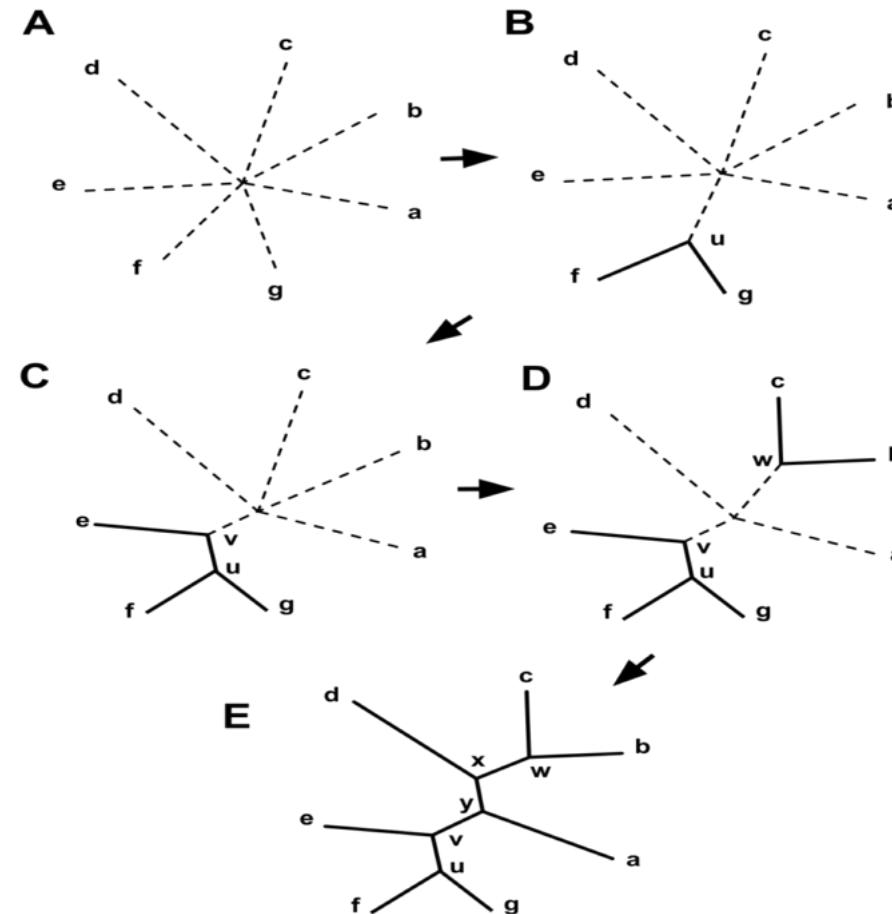
Seq A	ATC ATCG ATT TGCA . . . . .
Seq B	TTC CCT CGA TAT GCA . . . . .
Seq C	ATG ATGG ATAT GCA . . . . .
Seq D	TTCAAC GAATT TGCA . . . . .
Seq E	ATG ATGG ATT TGCA . . . . .
Seq F	TTCAAC GAAAT GCA . . . . .



Matrix: # Differences ▾ Decimal Places: 0 ▾ Style: Heatmap and Numbers ▾											
1_V1A_B/BRISBANE/60/20...	18	39	39	38	37	38	35	35	37	32	28
B/VICTORIA/502/2015e	18	41	41	40	39	40	37	37	39	34	30
B/Brisbane/22/2019_aug	39	41	0	1	10	21	24	24	26	21	29
B/New_Caledonia/10/2019...	39	41	0	1	10	21	24	24	26	21	29
B/Washington/02/2019_jan	38	40	1	1	9	20	23	23	25	20	28
V1A.2_B/Florida/39/2018_j...	37	39	10	10	9	17	22	22	24	19	27
B/Perth/1036/2019_jun	38	40	21	21	20	17	23	23	25	20	28
B/Canberra/38/2019_jun	35	37	24	24	23	22	23	0	2	5	25
B/Perth/32/2019_jun	35	37	24	24	23	22	23	0	2	5	25
B/Darwin/69/2019_jun	37	39	26	26	25	24	25	2	2	7	26
B/Malaysia/RP0467/2019_f...	32	34	21	21	20	19	20	5	5	7	22
B/Perth/18/2019_anr	28	30	29	29	28	27	28	25	25	26	22

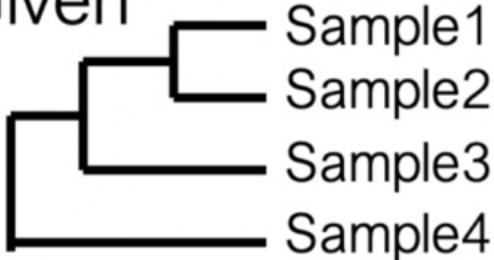
# Neighbour Joining method

Connects 2 taxa  
with smallest  
distance

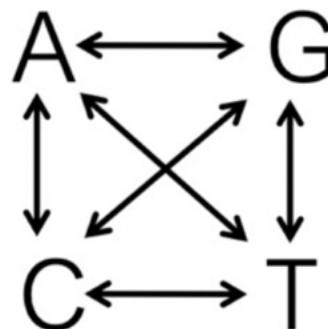


## Maximum likelihood

Given



+



Sample1

Sample2

Sample3

Sample4

Probability of?

CGTTAGTACACT

CGATAGTTCACT

CGTTAGTTTACC

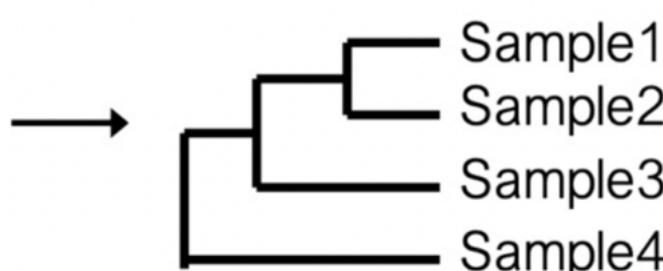
CATTGGTTTACT

## Bayesian inference

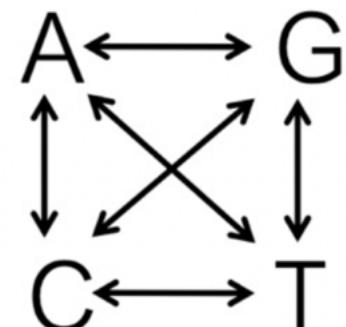
Given

Sample1 CGTTAGTACACT  
Sample2 CGATAGTTCACT  
Sample3 CGTTAGTTTACC  
Sample4 CATTGGTTTACT

Probability of?



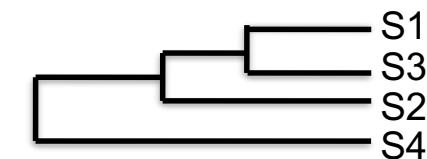
+



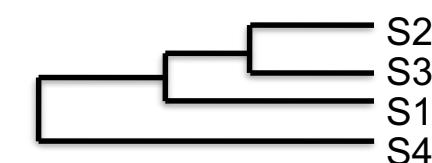
# Maximum Likelihood

- Maximum Likelihood is a statistical method of estimating the probability distributions to assign probabilities to a particular possible phylogenetic tree
- It allows for varying rates of evolution across both lineages & nucleotide sites

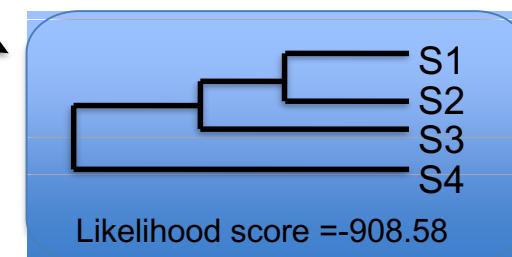
S1 CGTTAGTACACT  
S2 CGATAGTTCACT  
S3 CGTTAGTTTACC  
S4 CATTGGTTTACT



Likelihood score = -1203.83



Likelihood score = -1241.47



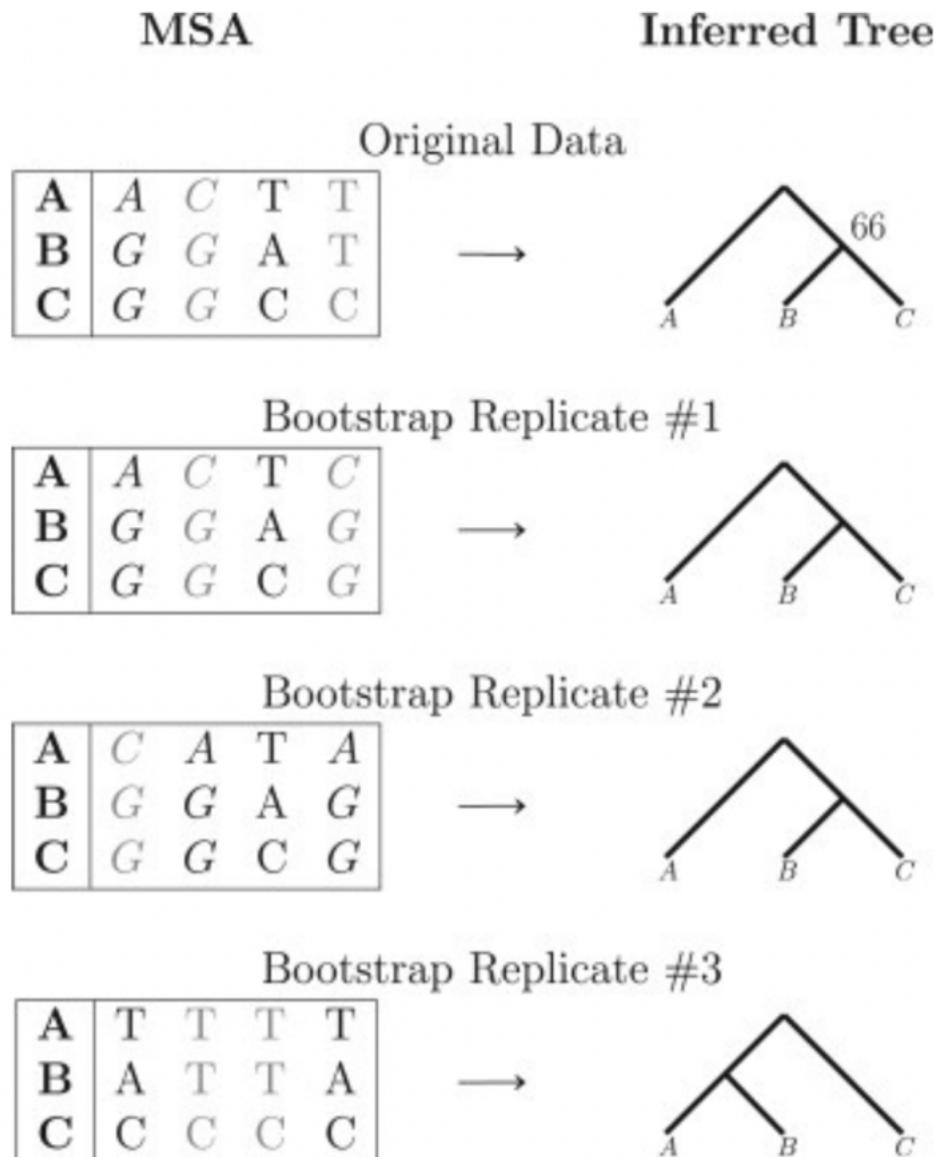
Likelihood score = -908.58

# Bootstrap

Felsenstein's application of the bootstrap method to evolutionary trees is one of the most cited scientific papers of all time.

The bootstrap method, which is based on resampling and replications, is used extensively to assess the robustness of phylogenetic inferences.

Felsenstein, Joseph. "Confidence limits on phylogenies: an approach using the bootstrap." *evolution* 39.4 (1985): 783-791.



# Which method?

	Pros	Cons
Clustering • Neighbour Joining	<ul style="list-style-type: none"><li>Fast</li><li>Excellent start tree</li></ul>	<ul style="list-style-type: none"><li>Only a single tree</li><li>Doesn't consider sequence evolution</li><li>Compress sequence information</li></ul>
Tree Searching • Maximum Likelihood	<ul style="list-style-type: none"><li>Evaluate all possible trees</li><li>Consider sequence evolution</li><li>Better estimates of branch lengths</li></ul>	<ul style="list-style-type: none"><li>Slow</li><li>Computationally intense</li><li>If you use the method incorrectly, you will get the incorrect answer (with high statistical support)</li></ul>

# Practical: Phylogenetic Analysis

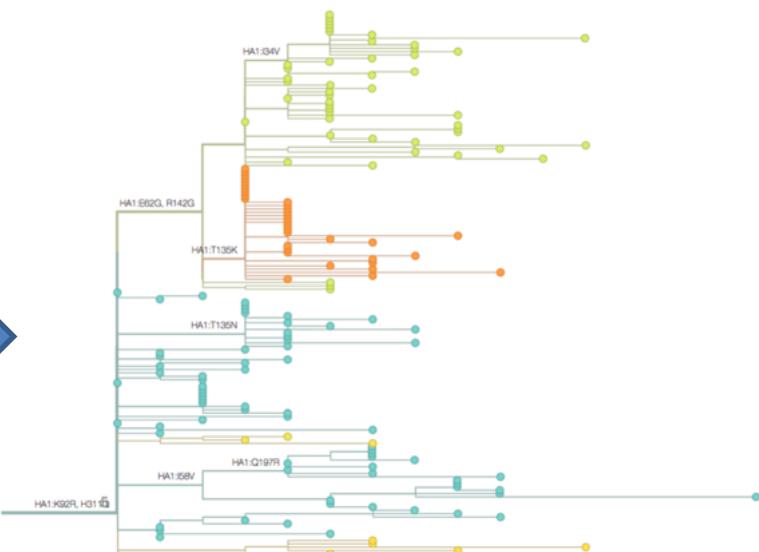


# Phylogenetic analysis

1. A/HongKong/VB17037915/2017  
 2. A/Hebei/27407/2017  
 3. A/Jiangsu/65/2016  
 4. A/Beijing/5156/2013  
 5. A/Chicken/Jiangsu/1848  
 6. A/Zhejiang/5/2017  
 7. A/Guangdong/17SF033/2017  
 8. A/Anhui/1883/2014  
 9. A/Guangxi/18910/2017  
 10. A/Beijing/9149/2017  
 11. A/Jiangsu/10/2013  
 12. A/Environment/Nanjing/29/2013  
 13. A/Anhui/3325/2015  
 14. A/Anhui/1876/2014  
 15. A/Environment/Inner Mongolia/2865  
 16. A/Chicken/Dongguan/1143  
 17. A/Environment/Henan/SC232/2013  
 18. A/Hebei/10/2017  
 19. A/Guangxi/5/2017  
 20. A/Beijing/40610/2015  
 21. A/Zhejiang/7807/2014  
 22. A/HongKong/5942/2013  
 23. A/Heinan/14905/2017  
 24. A/Fujian/56600/2016  
 25. A/Anhui/13423/2017  
 26. A/Environment/Inner Mongolia/2016  
 27. A/Zhejiang/15/2016  
 28. A/Yunnan/13500/2017  
 29. A/Environment/Shandong-Linyi/EV01/2015  
 30. A/Heinan/11159/2017  
 31. A/Zhejiang/33/2017  
 32. A/Jiangsu/6306/2014  
 33. A/Hebei/10/2017  
 34. A/Yunnan/13501/2017  
 35. A/Changsha/1/2013  
 36. A/HongKong/125/2017  
 37. A/Jiangxi/27569/2014  
 38. A/Environment/Shandong/SD309/2013  
 39. A/Heinan/33228/2015  
 40. A/Chicken/Anhui/749/2013  
 41. A/Beijing/14/2013  
 42. A/Fujian/1/2016  
 43. A/Taiwan/1/2017  
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 46. A/Fujian/8/2017  
 47. A/Environment/Guangdong/4740

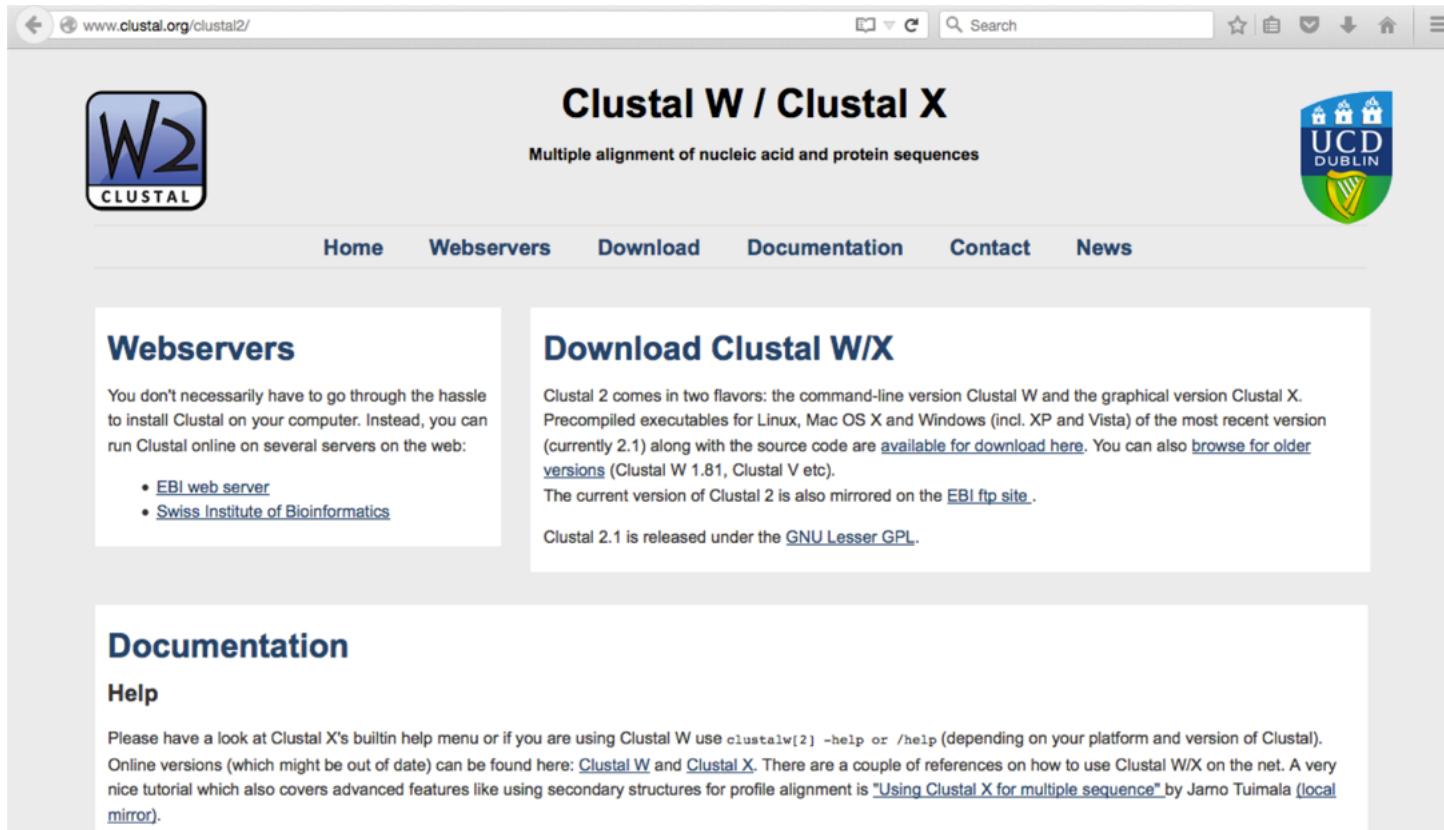
alignment

inference



tree

# Multiple sequence alignment



The screenshot shows the homepage of the Clustal W / Clustal X website at [www.clustal.org/clustal2/](http://www.clustal.org/clustal2/). The page features a header with the Clustal logo (W2 CLUSTAL), the title "Clustal W / Clustal X", and a subtitle "Multiple alignment of nucleic acid and protein sequences". It includes a search bar and navigation links for Home, Webservers, Download, Documentation, Contact, and News. A UCD Dublin logo is also present. The main content area is divided into sections: "Webservers" (listing EBI web server and Swiss Institute of Bioinformatics), "Download Clustal W/X" (describing the software's availability and mirroring), and "Documentation" (mentioning the built-in help menu and online versions). A note at the bottom of the documentation section refers to a local mirror of the site.

www.clustal.org/clustal2/

**Clustal W / Clustal X**

Multiple alignment of nucleic acid and protein sequences

UCD DUBLIN

Home Webservers Download Documentation Contact News

**Webservers**

You don't necessarily have to go through the hassle to install Clustal on your computer. Instead, you can run Clustal online on several servers on the web:

- [EBI web server](#)
- [Swiss Institute of Bioinformatics](#)

**Download Clustal W/X**

Clustal 2 comes in two flavors: the command-line version Clustal W and the graphical version Clustal X. Precompiled executables for Linux, Mac OS X and Windows (incl. XP and Vista) of the most recent version (currently 2.1) along with the source code are [available for download here](#). You can also [browse for older versions](#) (Clustal W 1.81, Clustal V etc.). The current version of Clustal 2 is also mirrored on the [EBI ftp site](#).

Clustal 2.1 is released under the [GNU Lesser GPL](#).

**Documentation**

**Help**

Please have a look at Clustal X's builtin help menu or if you are using Clustal W use `clustalw[2] -help` or `/help` (depending on your platform and version of Clustal). Online versions (which might be out of date) can be found here: [Clustal W](#) and [Clustal X](#). There are a couple of references on how to use Clustal W/X on the net. A very nice tutorial which also covers advanced features like using secondary structures for profile alignment is "[Using Clustal X for multiple sequence](#)" by Jarno Tuimala ([local mirror](#)).

# Multiple sequence alignment

The screenshot shows the MUSCLE web interface on the EMBL-EBI website. The URL in the browser bar is [www.ebi.ac.uk/Tools/msa/muscle/](http://www.ebi.ac.uk/Tools/msa/muscle/). The page title is "MUSCLE". The main navigation menu includes "Input form", "Web services", and "Help & Documentation". Below the menu, the breadcrumb navigation shows "Tools > Multiple Sequence Alignment > MUSCLE". The main content area is titled "Multiple Sequence Alignment". A brief description states: "MUSCLE stands for **M**Ultiple **S**equence **C**omparison by **L**og- **E**xpectation. MUSCLE is claimed to achieve both better average accuracy and better speed than [ClustalW2](#) or [T-Coffee](#), depending on the chosen options." The interface is divided into three steps: "STEP 1 - Enter your input sequences", "STEP 2 - Set your Parameters", and "STEP 3 - Submit your job".

www.ebi.ac.uk/Tools/msa/muscle/

EMBL-EBI

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# MUSCLE

Input form Web services Help & Documentation

Tools > Multiple Sequence Alignment > MUSCLE

## Multiple Sequence Alignment

MUSCLE stands for **M**Ultiple **S**equence **C**omparison by **L**og- **E**xpectation. MUSCLE is claimed to achieve both better average accuracy and better speed than [ClustalW2](#) or [T-Coffee](#), depending on the chosen options.

STEP 1 - Enter your input sequences

Enter or paste a set of sequences in any [supported](#) format:

Or upload a file: [Browse...](#) No file selected.

STEP 2 - Set your Parameters

OUTPUT FORMAT: ClustalW

*The default settings will fulfill the needs of most users and, for that reason, are not visible.*

[More options...](#) (Click here, if you want to view or change the default settings.)

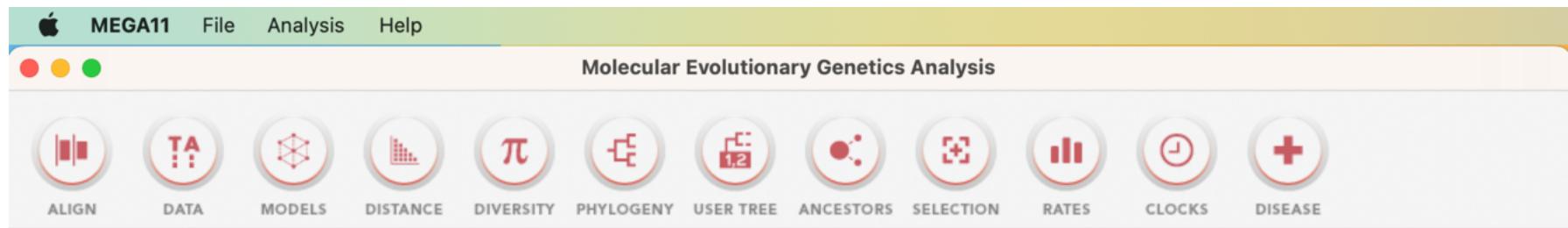
STEP 3 - Submit your job

Be notified by email (Tick this box if you want to be notified by email when the results are available)

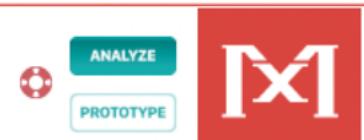
# MEGA

<https://www.megasoftware.net/>

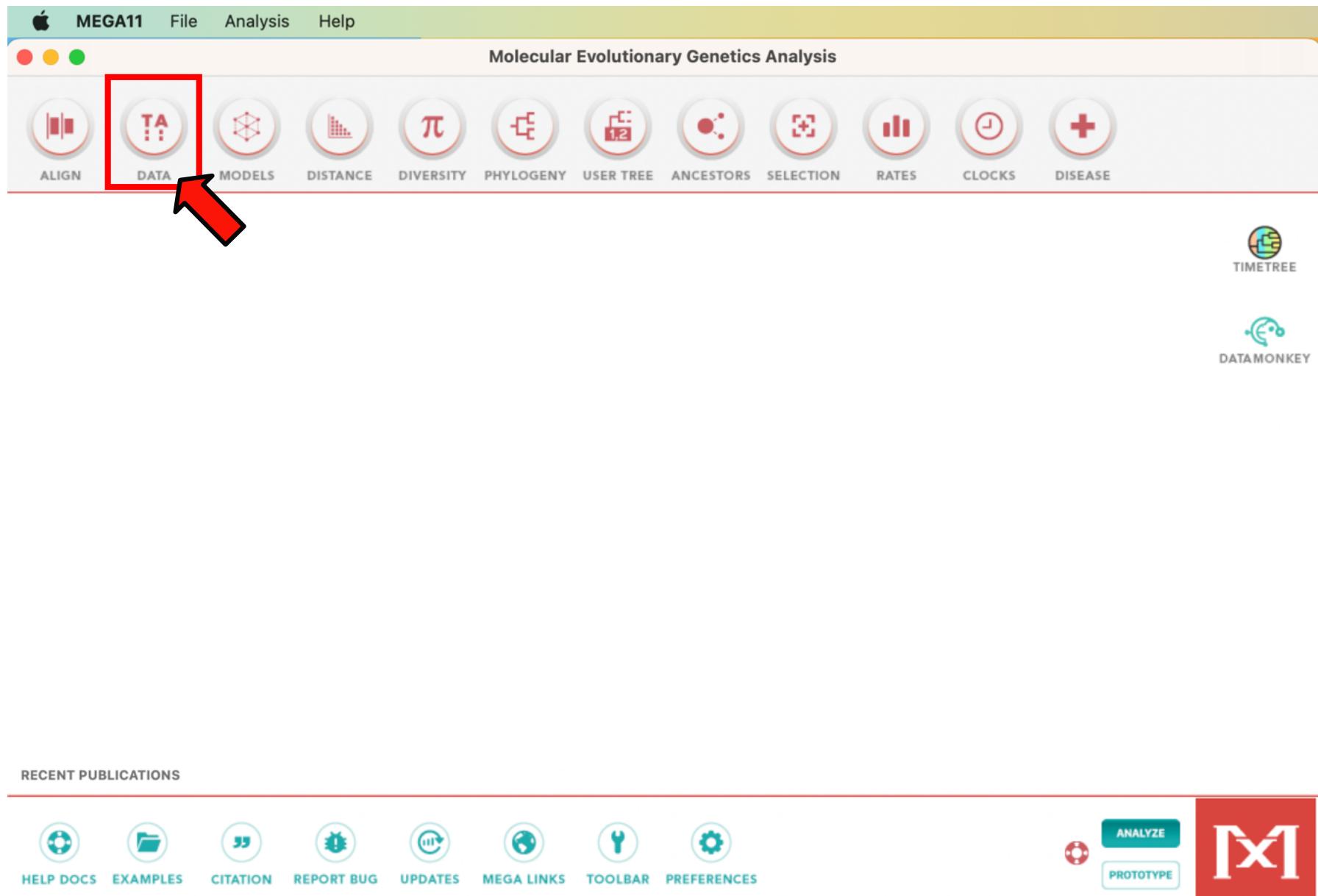
The screenshot shows the official website for MEGA (Molecular Evolutionary Genetics Analysis). The header features the MEGA logo with a red 'M' and blue 'E', followed by 'G', 'A', and 'ALGORITHM'. Below the logo is the text 'Molecular Evolutionary Genetics Analysis'. The navigation menu includes links for 'home', 'features', 'publications', 'manual', and 'feedback'. A decorative banner at the top displays various biological icons representing different species and analysis types. A prominent dark banner in the center contains a red circular icon with a white bell, a message about testing the macOS version, and a red 'X' button. Below this is a descriptive text block: 'Sophisticated and user-friendly software suite for analyzing DNA and protein sequence data from species and populations.' At the bottom of the main content area are three dropdown menus: 'macOS', 'Graphical (GUI)', and 'MEGA 7 (32-bit)'. To the right of these is a large green 'DOWNLOAD' button with a white circular arrow icon. The footer is divided into three columns: 'Sequence Analyses' (Phylogeny Inference, Model Selection, Dating and Clocks, Ancestral States, Selection and Tests, Sequence Alignment), 'Statistical Methods' (Maximum Likelihood, Distance Methods, Ordinary Least Squares, Maximum Parsimony, Composite Likelihood, Bayesian), and 'Powerful Visual Tools' (Alignment/Trace Editor, Tree Explorer, Data Explorers, Legend Generator, Gene Duplication Wizard, Timetree Wizard).



#### RECENT PUBLICATIONS



# Load FASTA file



MEGA11 File Analysis Help

Molecular Evolutionary Genetics Analysis

ALIGN  TA  USER TREE ANCESTORS SELECTION RATES CLOCKS DISEASE

Open a File/Session... ⌘ O

Concatenate Sequence Alignments

Explore Active Data F4

Export Data...

Save Data Session to File...

Select Genetic Code Table...

Select Genes and Domains...

Select Taxa and Groups...

TIMETREE

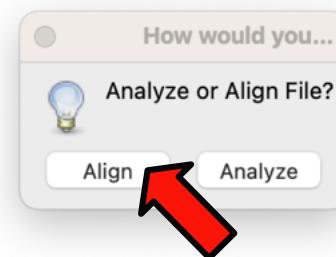
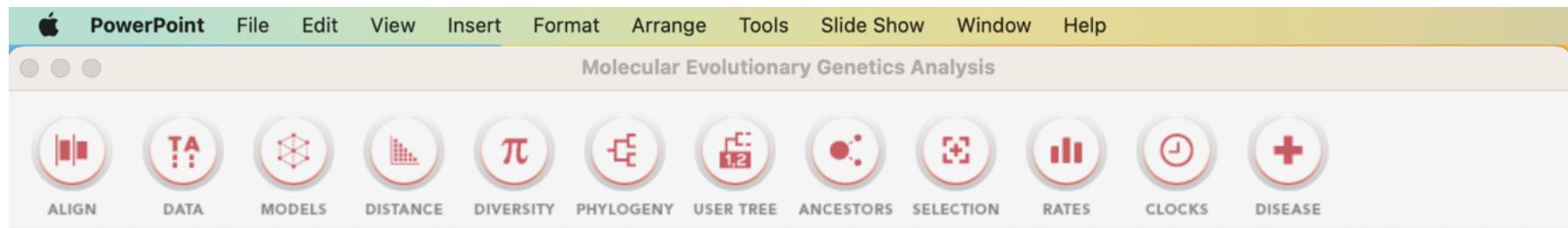
DATAMONKEY

RECENT PUBLICATIONS

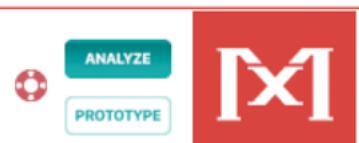
HELP DOCS EXAMPLES CITATION REPORT BUG UPDATES MEGA LINKS TOOLBAR PREFERENCES

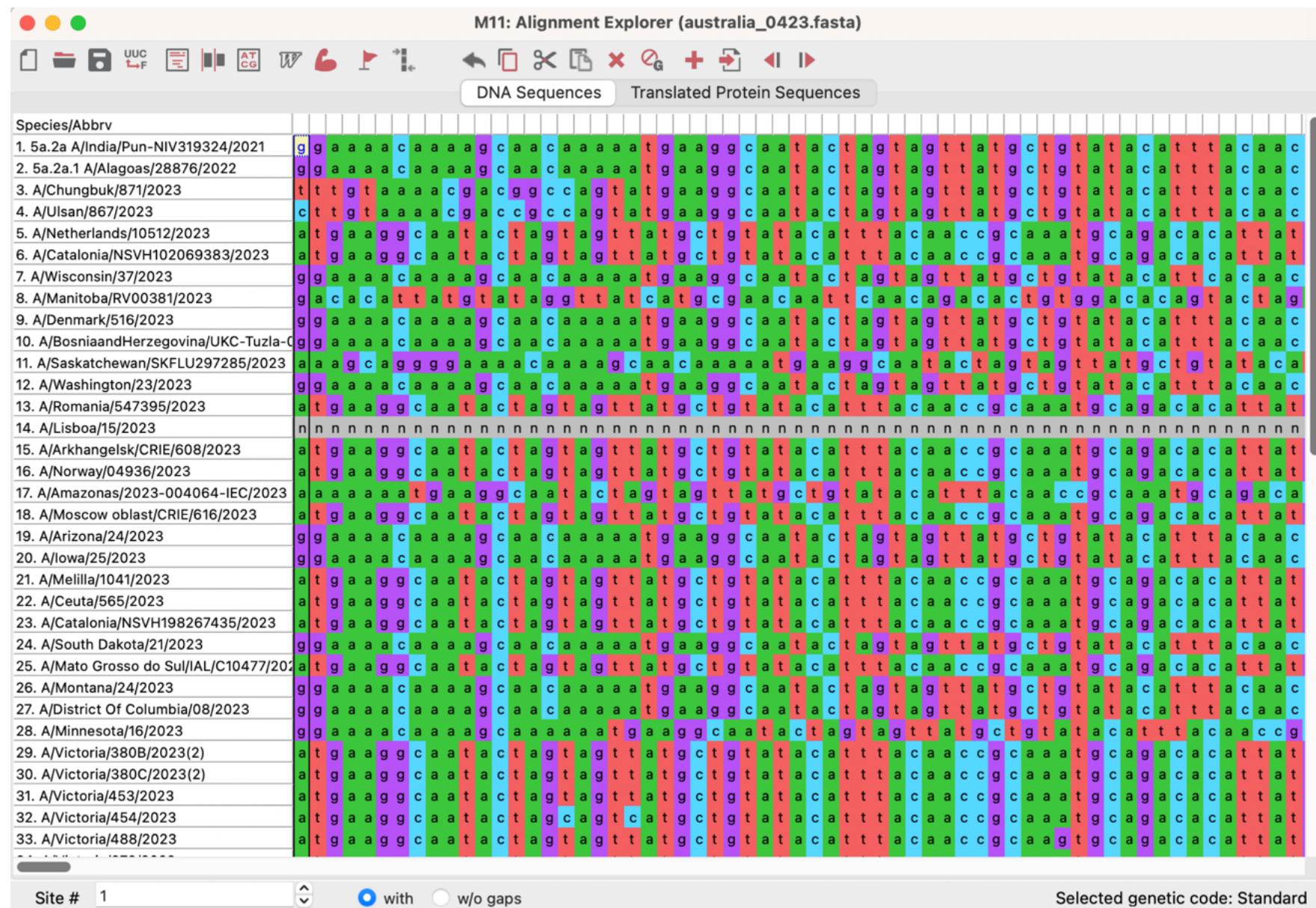
ANALYZE PROTOTYPE





#### RECENT PUBLICATIONS





MEGA11 Data Edit Search Alignment Web Sequencer Display Help

W Align by ClustalW  
W Align by ClustalW (Codons)  
**Align by MUSCLE**  
Align by MUSCLE (Codons)  
Mark/Unmark Site ⌘M  
Align Marked Sites ⌘L  
Unmark All Sites  
Delete Gap-Only Sites  
✓ Auto-Fill Gaps

australia\_0423.fasta)

Selected Protein Sequences

Species/Abbrv

1. 5a.2a A/India/Pun-NIV319324/2021  
2. 5a.2a.1 A/Alagoas/28876/2022  
3. A/Chungbuk/871/2023  
4. A/Ulsan/867/2023  
5. A/Netherlands/10512/2023  
6. A/Catalonia/NSVH102069383/2023  
7. A/Wisconsin/37/2023  
8. A/Manitoba/RV00381/2023  
9. A/Denmark/516/2023  
10. A/BosniaandHerzegovina/UKC-Tuzla-C  
11. A/Saskatchewan/SKFLU297285/2023  
12. A/Washington/23/2023  
13. A/Romania/547395/2023  
14. A/Lisboa/15/2023  
15. A/Arkhangelsk/CRIE/608/2023  
16. A/Norway/04936/2023  
17. A/Amazonas/2023-004064-IEC/2023  
18. A/Moscow oblast/CRIE/616/2023  
19. A/Arizona/24/2023  
20. A/Iowa/25/2023  
21. A/Melilla/1041/2023  
22. A/Ceuta/565/2023  
23. A/Catalonia/NSVH198267435/2023  
24. A/South Dakota/21/2023  
25. A/Mato Grosso do Sul/IAL/C10477/2023  
26. A/Montana/24/2023  
27. A/District Of Columbia/08/2023  
28. A/Minnesota/16/2023  
29. A/Victoria/380B/2023(2)  
30. A/Victoria/380C/2023(2)  
31. A/Victoria/453/2023  
32. A/Victoria/454/2023  
33. A/Victoria/488/2023

Site # 1 with w/o gaps Selected genetic code: Standard

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

M11: Alignment Explorer (australia\_0423.fasta)

DNA Sequences Translated Protein Sequences

Species/Abbrv

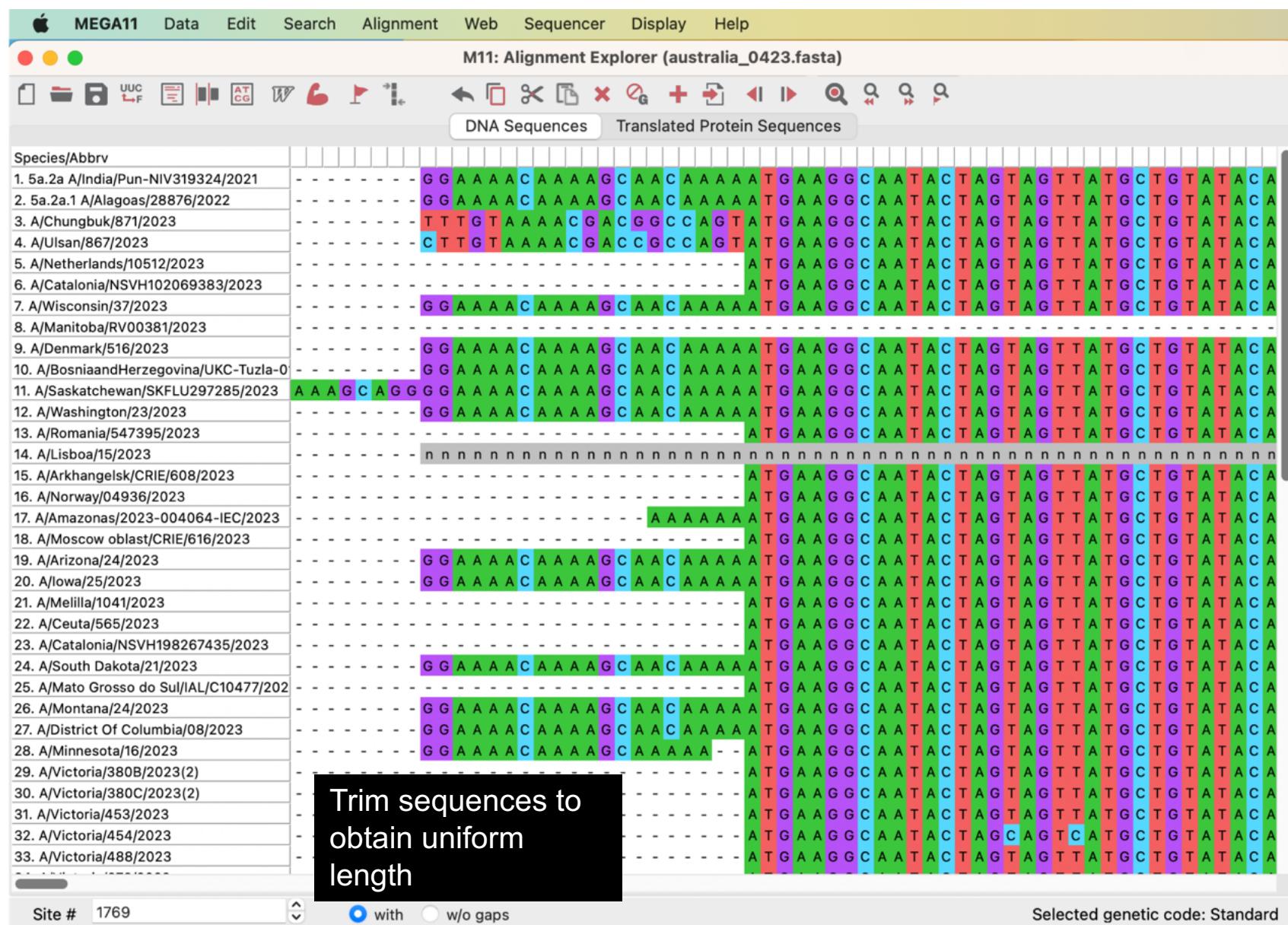
1. 5a.2a A/India/Pun-N  
 2. 5a.2a.1 A/Alagoas/2021  
 3. A/Chungbuk/871/2021  
 4. A/Ulsan/867/2023  
 5. A/Netherlands/1051/2023  
 6. A/Catalonia/NSVH1/2023  
 7. A/Wisconsin/37/2023  
 8. A/Manitoba/RV0038/2023  
 9. A/Denmark/516/2023  
 10. A/BosniaandHerzegovina/2023  
 11. A/Saskatchewan/SH/2023  
 12. A/Washington/23/2023  
 13. A/Romania/547395/2023  
 14. A/Lisboa/15/2023  
 15. A/Arkhangelsk/CRII/2023  
 16. A/Norway/04936/2023  
 17. A/Amazonas/2023-  
 18. A/Moscow oblast/CRII/2023  
 19. A/Arizona/24/2023  
 20. A/Iowa/25/2023  
 21. A/Melilla/1041/2023  
 22. A/Ceuta/565/2023  
 23. A/Catalonia/NSVH1/2023  
 24. A/South Dakota/21/2023  
 25. A/Mato Grosso do Sul/2023  
 26. A/Montana/24/2023  
 27. A/District Of Columbia/2023  
 28. A/Minnesota/16/2023  
 29. A/Victoria/380B/2023  
 30. A/Victoria/380C/2023  
 31. A/Victoria/453/2023  
 32. A/Victoria/454/2023  
 33. A/Victoria/488/2023

**MUSCLE Alignment Options**

Option	Setting
<b>GAP PENALTIES</b>	
Gap Open	<input checked="" type="checkbox"/> -400.00
Gap Extend	<input checked="" type="checkbox"/> 0.00
<b>MEMORY/ITERATIONS</b>	
Max Memory in MB	<input checked="" type="checkbox"/> 2048
Max Iterations	<input checked="" type="checkbox"/> 16
<b>ADVANCED OPTIONS</b>	
Cluster Method (Iterations 1,2)	<input checked="" type="checkbox"/> UPGMA
Cluster Method (Other Iterations)	<input checked="" type="checkbox"/> UPGMA
Min Diag Length (Lambda)	<input checked="" type="checkbox"/> 24

? Help    Reset    Cancel    OK

Site # 1    with    w/o gaps    Selected genetic code: Standard



M11: Alignment Explorer (australia\_0423.fasta)

DNA Sequences      Translated Protein Sequences

Species/Abbrv	Sequence 1	Sequence 2	Sequence 3	Sequence 4	Sequence 5	Sequence 6	Sequence 7	Sequence 8	Sequence 9	Sequence 10	Sequence 11	Sequence 12	Sequence 13	Sequence 14	Sequence 15	Sequence 16	Sequence 17	Sequence 18	Sequence 19	Sequence 20	Sequence 21	Sequence 22	Sequence 23	Sequence 24	Sequence 25	Sequence 26	Sequence 27	Sequence 28	Sequence 29	Sequence 30	Sequence 31	Sequence 32	Sequence 33				
1. 5a.2a A/India/Pun-NIV319324/2021	- G G A A A C A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
2. 5a.2a.1 A/Alagoas/28876/2022	- G G A A A C A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
3. A/Chungbuk/871/2023	- T T G T A A A A C G A C G G C C A G T	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
4. A/Ulsan/867/2023	- C T T G T A A A A C G A C C G C C A G T	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
5. A/Netherlands/10512/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
6. A/Catalonia/NSVH102069383/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
7. A/Wisconsin/37/2023	- G G A A A C A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
8. A/Manitoba/RV00381/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
9. A/Denmark/516/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
10. A/BosniaandHerzegovina/UKC-Tuzla-0	- G G A A A C A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
11. A/Saskatchewan/SKFLU297285/2023	A A A G C A G G G G A A A A C A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
12. A/Washington/23/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
13. A/Romania/547395/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
14. A/Lisboa/15/2023	- n	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
15. A/Arkhangelsk/CRIE/608/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
16. A/Norway/04936/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
17. A/Amazonas/2023-004064-IEC/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
18. A/Moscow oblast/CRIE/616/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
19. A/Arizona/24/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
20. A/Iowa/25/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
21. A/Melilla/1041/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
22. A/Ceuta/565/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
23. A/Catalonia/NSVH198267435/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
24. A/South Dakota/21/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
25. A/Mato Grosso do Sul/IAL/C10477/2022	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
26. A/Montana/24/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
27. A/District Of Columbia/08/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
28. A/Minnesota/16/2023	- G G A A A C A A A A A G C A A C A A A	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
29. A/Victoria/380B/2023(2)	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
30. A/Victoria/380C/2023(2)	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
31. A/Victoria/453/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
32. A/Victoria/454/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		
33. A/Victoria/488/2023	- - - - -	A T G A A G G C A A T A C T	T A G T A G T T A T G C T T G T A T A C A																																		

1. Go to at the beginning of the alignment
2. Select the positions to be deleted

Site # 28     with     w/o gaps      Selected genetic code: Standard

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

Species/Abbrv

- 1. 5a.2a A/India/Pun-NIV319
- 2. 5a.2a.1 A/Alagoas/28876/
- 3. A/Chungbuk/871/2023
- 4. A/Ulsan/867/2023
- 5. A/Netherlands/10512/2022
- 6. A/Catalonia/NSVH102069/
- 7. A/Wisconsin/37/2023
- 8. A/Manitoba/RV00381/2022
- 9. A/Denmark/516/2023
- 10. A/BosniaandHerzegovina/
- 11. A/Saskatchewan/SKFLU2/
- 12. A/Washington/23/2023
- 13. A/Romania/547395/2023
- 14. A/Lisboa/15/2023
- 15. A/Arkhangelsk/CRIE/608
- 16. A/Norway/04936/2023
- 17. A/Amazonas/2023-004064-IEC/2023
- 18. A/Moscow oblast/CRIE/616/2023
- 19. A/Arizona/24/2023
- 20. A/Iowa/25/2023
- 21. A/Melilla/1041/2023
- 22. A/Ceuta/565/2023
- 23. A/Catalonia/NSVH198267435/2023
- 24. A/South Dakota/21/2023
- 25. A/Mato Grosso do Sul/IAL/C10477/2022
- 26. A/Montana/24/2023
- 27. A/District Of Columbia/08/2023
- 28. A/Minnesota/16/2023
- 29. A/Victoria/380B/2023(2)
- 30. A/Victoria/380C/2023(2)
- 31. A/Victoria/453/2023
- 32. A/Victoria/454/2023
- 33. A/Victoria/488/2023

Undo ⌘ Z

Copy ⌘ C

Cut ⌘ X

Paste ⌘ V

Delete ⌘ D

Delete Gap ⌘ G

Insert Blank Sequence ⌘ N

Insert Sequence From File ⌘ I

Select Site(s)

Select Sequence(s)

Select All ⌘ A

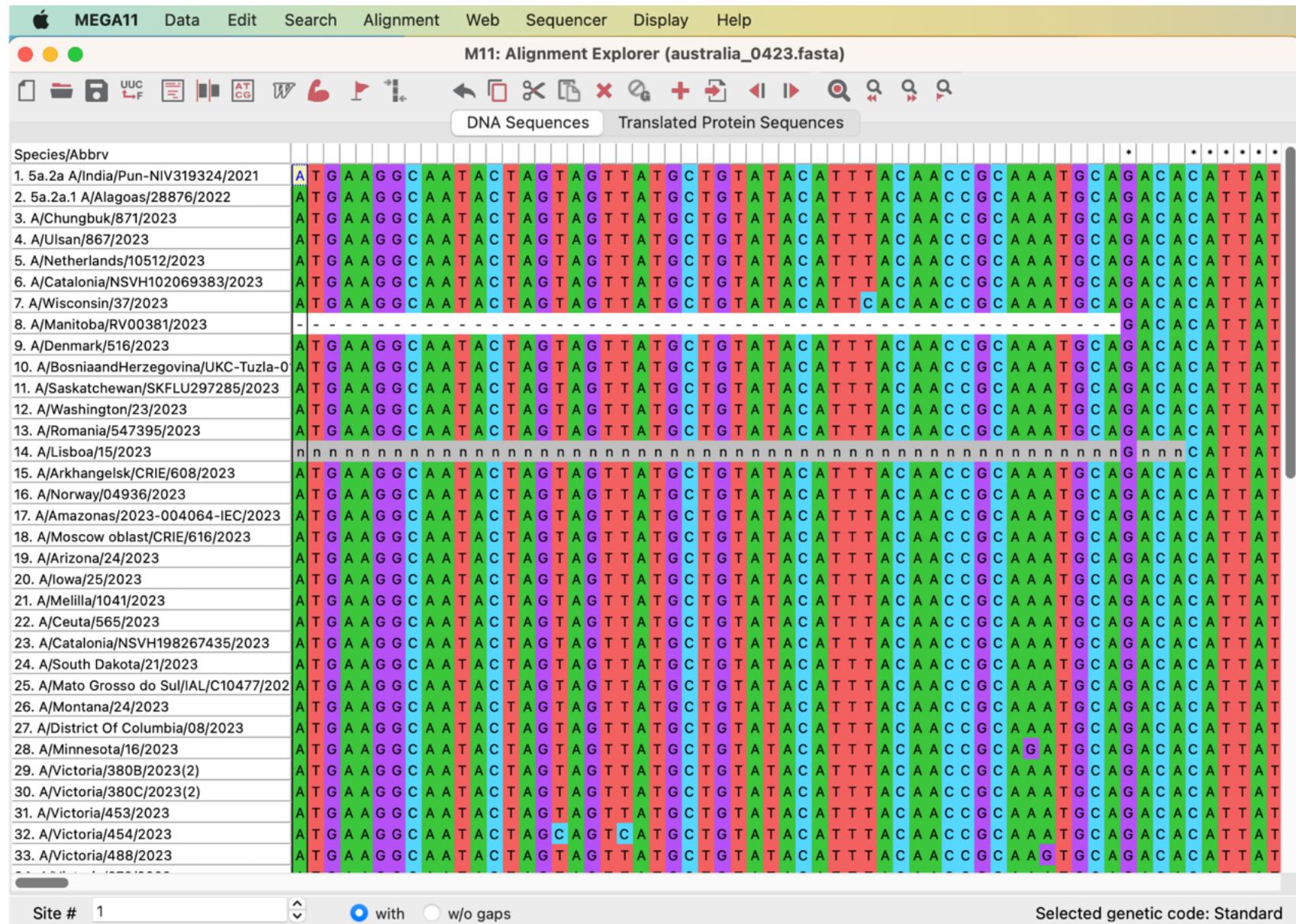
Allow Base Editing

Modify All Bases To Upper Case

Site # 28   with  w/o gaps Selected genetic code: Standard

Sequence Explorer (australia\_0423.fasta)

Sequences Translated Protein Sequences



MEGA11 Data Edit Search Alignment Web Sequencer Display Help

M11: Alignment Explorer (australia\_0423.mas)

DNA Sequences Translated Protein Sequences

Species/Abbrv	Sequence
41. A/Tasmania/30/2023	G T G T A G A A T A T G T A T T T A A
42. A/Brisbane/13/2023	G T G T A G A A T A T G T A T T T A A
43. A/South Australia/85/2023	G T G T A G A A T A T G T A T T T A A
44. A/Canberra/43/2023	G T G T A G A A T A T G T A T T T A A
45. A/South Australia/86/2023	G T G T A G A A T A T G T A T T T A A
46. A/South Australia/90/2023	G T G T A G A A T A T G T A T T T A A
47. A/Canberra/50/2023	G T G T A G A A T A T G T A T T T A A
48. A/Canberra/49/2023	G T G T A G A A T A T G T A T T T A A
49. A/South Australia/83/2023	G T G T A G A A T A T G T A T T T A A
50. A/Tasmania/25/2023	G T G T A G A A T A T G T A T T T A A
51. A/Victoria/380A/2023	G T G T A G A A T A T G T A T T T A A
52. A/Victoria/380B/2023	G T G T A G A A T A T G T A T T T A A
53. A/Canberra/47/2023	G T G T A G A A T A T G T A T T T A A
54. A/Victoria/382/2023	G T G T A G A A T A T G T A T T T A A
55. A/Victoria/473/2023	G T G T A G A A T A T G T A T T T A A
56. A/Brisbane/24/2023	G T G T A G A A T A T G T A T T T A A
57. A/Victoria/490/2023	G T G T A G A A T A T G T A T T T A A
58. A/Darwin/53/2023	G T G T A G A A T A T G T A T T T A A
59. A/Victoria/445/2023	G T G T A G A A T A T G T A T T T A A
60. A/Sydney/83/2023	G T G T A G A A T A T G T A T T T A A
61. A/Darwin/51/2023	G T G T A G A A T A T G T A T T T A A
62. A/Victoria/409/2023	G T G T A G A A T A T G T A T T T A A
63. A/Sydney/75/2023	G T G T A G A A T A T G T A T T T A A
64. A/Sydney/81/2023	G T G T A G A A T A T G T A T T T A A
65. A/Sydney/79/2023	G T G T A G A A T A T G T A T T T A A
66. A/Victoria/424/2023	G T G T A G A A T A T G T A T T T A A
67. A/Tasmania/53/2023	G T G T A G A A T A T G T A T T T A A
68. A/Tasmania/54/2023	G T G T A G A A T A T G T A T T T A A
69. A/Victoria/380C/2023	G T G T A G A A T A T G T A T T T A A
70. A/Victoria/540/2023	G T G T A G A A T A T G T A T T T A A
71. A/Victoria/537/2023	G T G T A G A A T A T G T A T T T A A
72. A/Victoria/380/2023	G T G T A G A A T A T G T A T T T A A
73. A/Sydney/101/2023	G T G T A G A A T A T G T A T T T A A

Site # 1741   with  w/o gaps Selected genetic code: Standard

1. Go to at the end of the alignment  
2. Select the positions to be deleted

MEGA11 Data Edit Search Alignment Web Sequencer Display Help

Species/Abbrv

- 41. A/Tasmania/30/2023
- 42. A/Brisbane/13/2023
- 43. A/South Australia/85/20
- 44. A/Canberra/43/2023
- 45. A/South Australia/86/20
- 46. A/South Australia/90/20
- 47. A/Canberra/50/2023
- 48. A/Canberra/49/2023
- 49. A/South Australia/83/20
- 50. A/Tasmania/25/2023
- 51. A/Victoria/380A/2023
- 52. A/Victoria/380B/2023
- 53. A/Canberra/47/2023
- 54. A/Victoria/382/2023
- 55. A/Victoria/473/2023
- 56. A/Brisbane/24/2023
- 57. A/Victoria/490/2023
- 58. A/Darwin/53/2023
- 59. A/Victoria/445/2023
- 60. A/Sydney/83/2023
- 61. A/Darwin/51/2023
- 62. A/Victoria/409/2023
- 63. A/Sydney/75/2023
- 64. A/Sydney/81/2023
- 65. A/Sydney/79/2023
- 66. A/Victoria/424/2023
- 67. A/Tasmania/53/2023
- 68. A/Tasmania/54/2023
- 69. A/Victoria/380C/2023
- 70. A/Victoria/540/2023
- 71. A/Victoria/537/2023
- 72. A/Victoria/380/2023
- 73. A/Sydney/101/2023

Sequence Explorer (australia\_0423.mas)

Clipboard

- Copy
- Cut
- Paste
- Delete
- Delete Gaps
- Insert Blank Sequence
- Insert Sequence From File
- Select Site(s)
- Select Sequence(s)
- Select All

Allow Base Editing

Modify All Bases To Upper Case

Site # 1741 with w/o gaps Selected genetic code: Standard

The screenshot shows the MEGA11 software interface. The main window displays a sequence alignment grid with 73 rows of sequences. The first few columns are highlighted in purple and green. A context menu is open over the first few columns, with the 'Delete' option highlighted. The menu also includes options for 'Copy', 'Cut', 'Paste', 'Delete Gaps', 'Insert Blank Sequence', 'Insert Sequence From File', 'Select Site(s)', 'Select Sequence(s)', and 'Select All'. At the bottom, there are buttons for 'Site #' (set to 1741), 'with' (radio button selected), 'w/o gaps', and 'Selected genetic code: Standard'. The top menu bar includes 'Edit', 'Search', 'Alignment', 'Web', 'Sequencer', 'Display', and 'Help'.

