Lecture 07
molecular sequences

Course: Practical Bioinformatics (BIOL 4220)
Instructor: Michael Landis
Email: michael.landis@wustl.edu

Lecture 07 outline

Last time: shell scripts

This time: molecular sequences

Topics

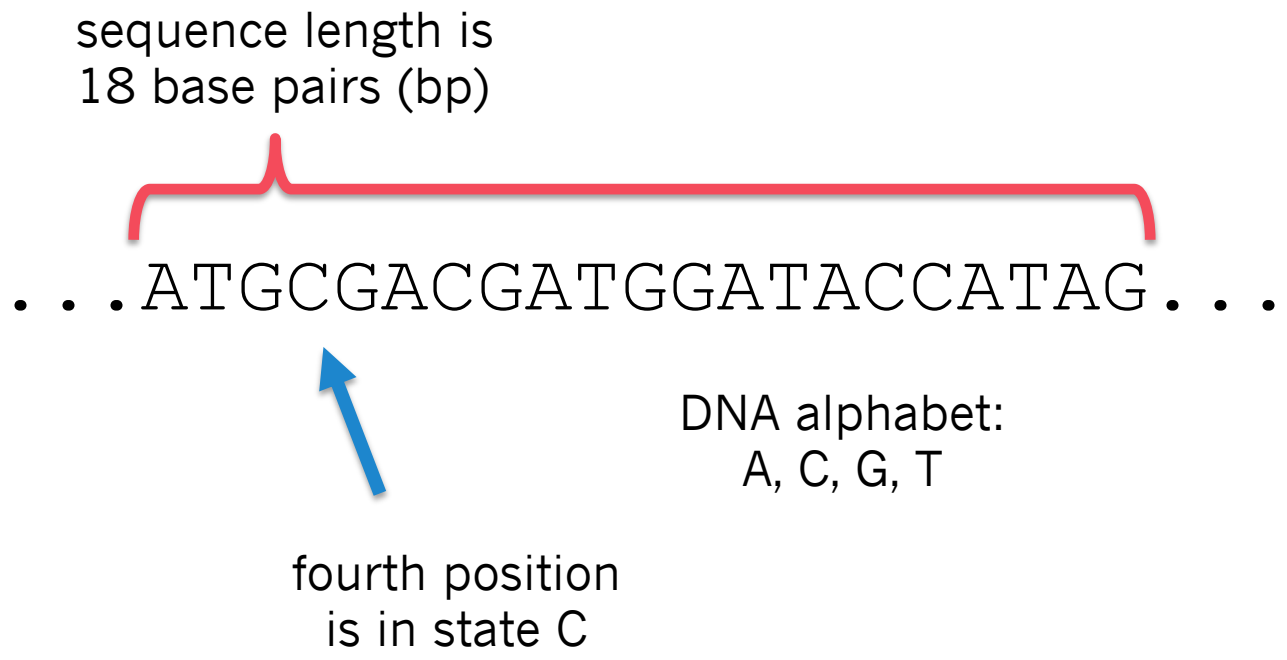
- sequence data
- GenBank
- BLAST

A ***molecular sequence*** is a string of characters from a molecular alphabet.

Examples: *DNA, RNA, amino acid sequences*

Sequences are key to understanding:

- disease mechanism
- gene expression
- developmental biology
- heredity and ancestry
- protein and cell function
- biodiversity patterns

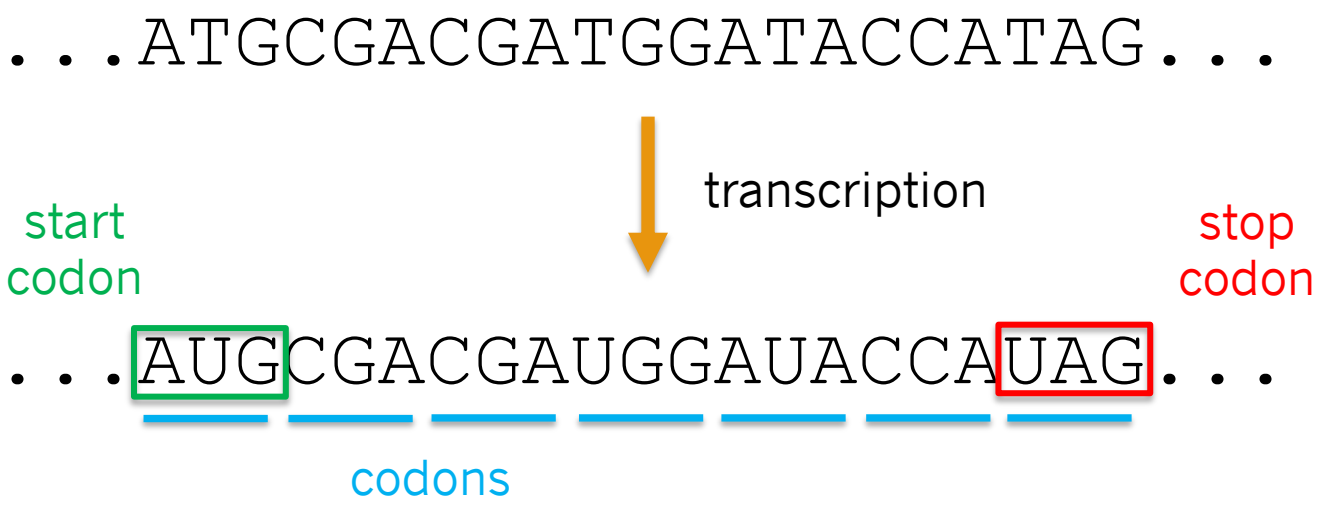

sequence length is
18 base pairs (bp)
...ATGCGACGATGGATACCATAG...
DNA alphabet:
A, C, G, T
fourth position
is in state C

...ATGCGACGATGGATACCATAG...

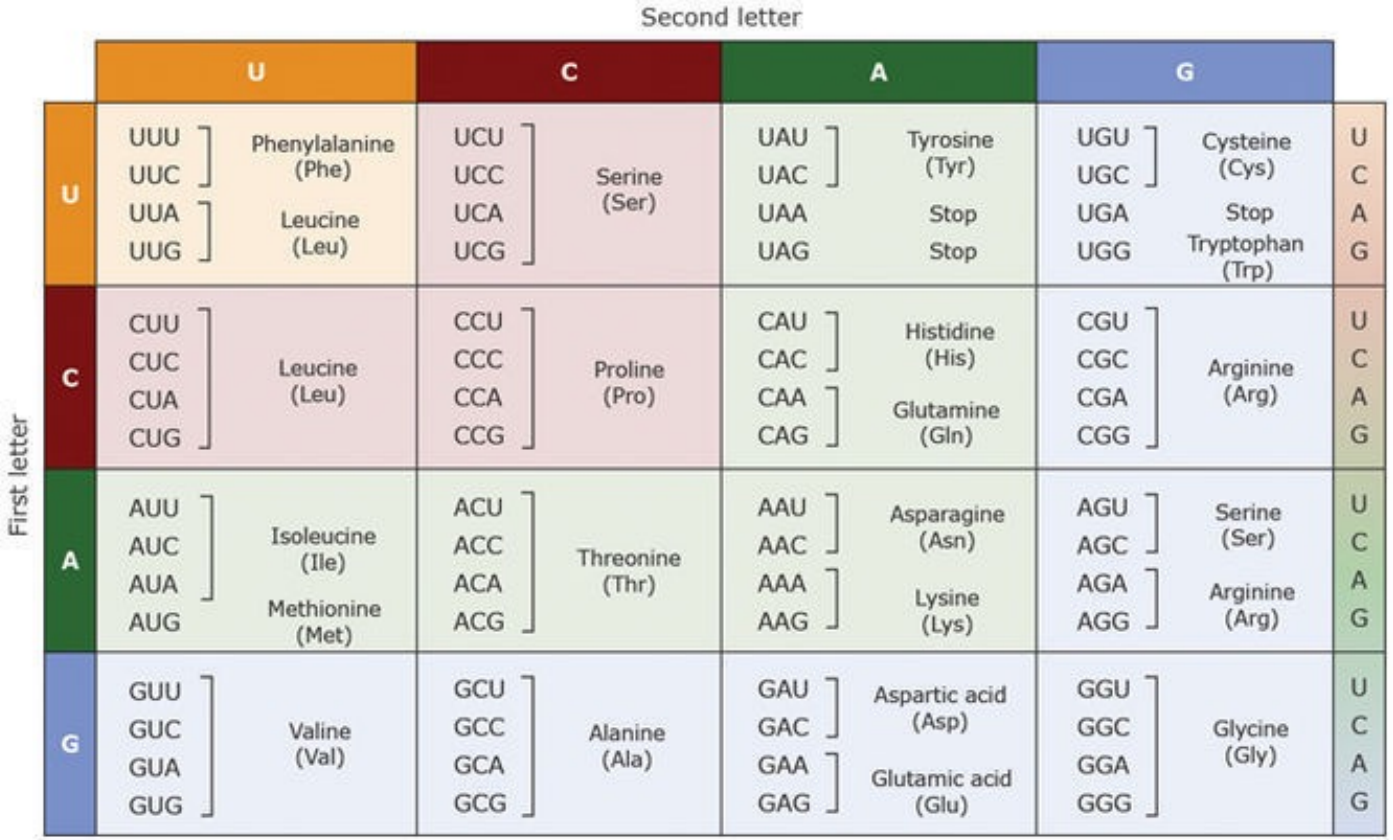
transcription

...AUGCGACGAUGGAUACCAUAG...

RNA alphabet:
A, C, G, U


...ATGCGACGATGGATACCATAG...
transcription
start codon
stop codon
...AUGCGACGAUGGAUACCAUAG...
codons

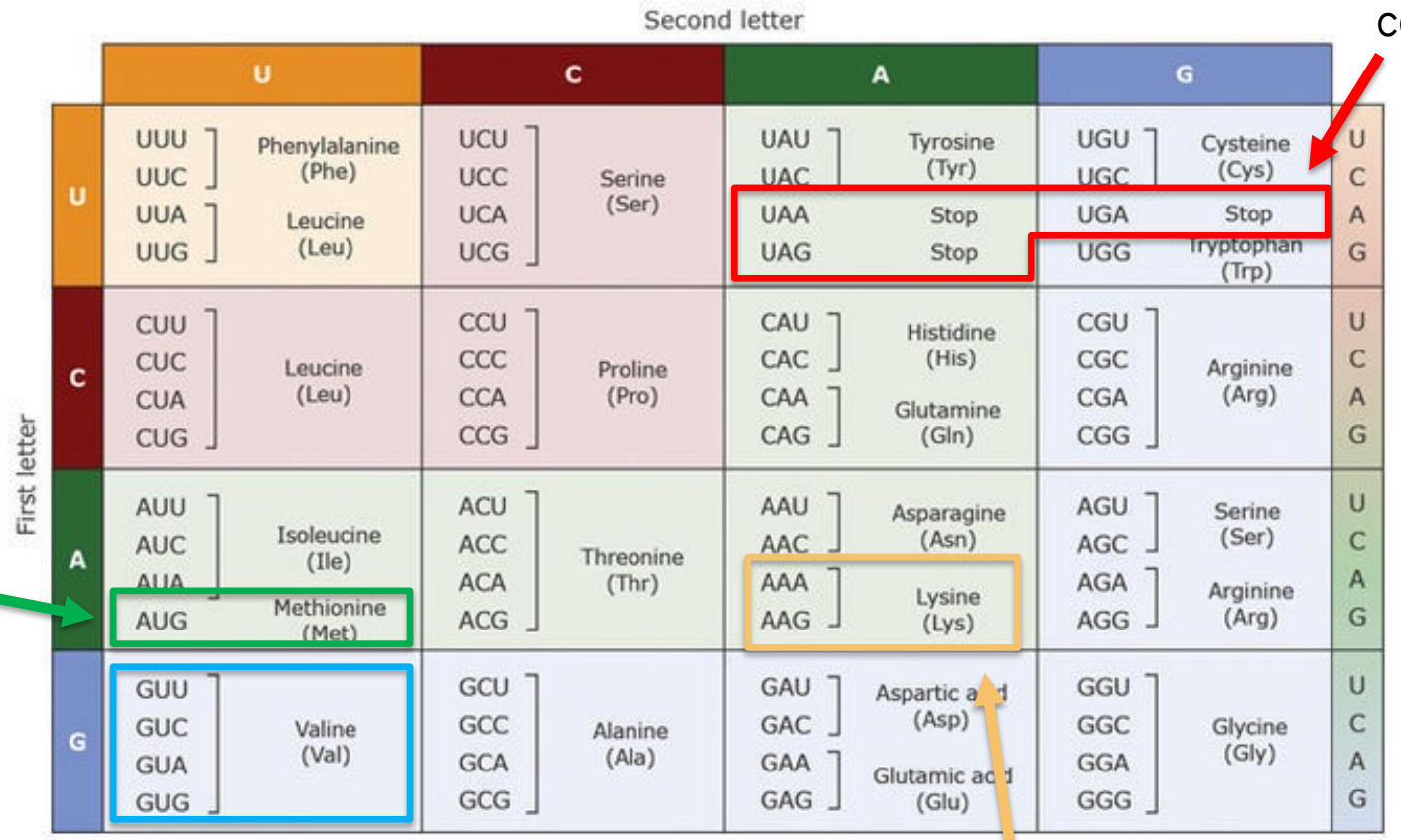
Standard genetic code



Standard genetic code

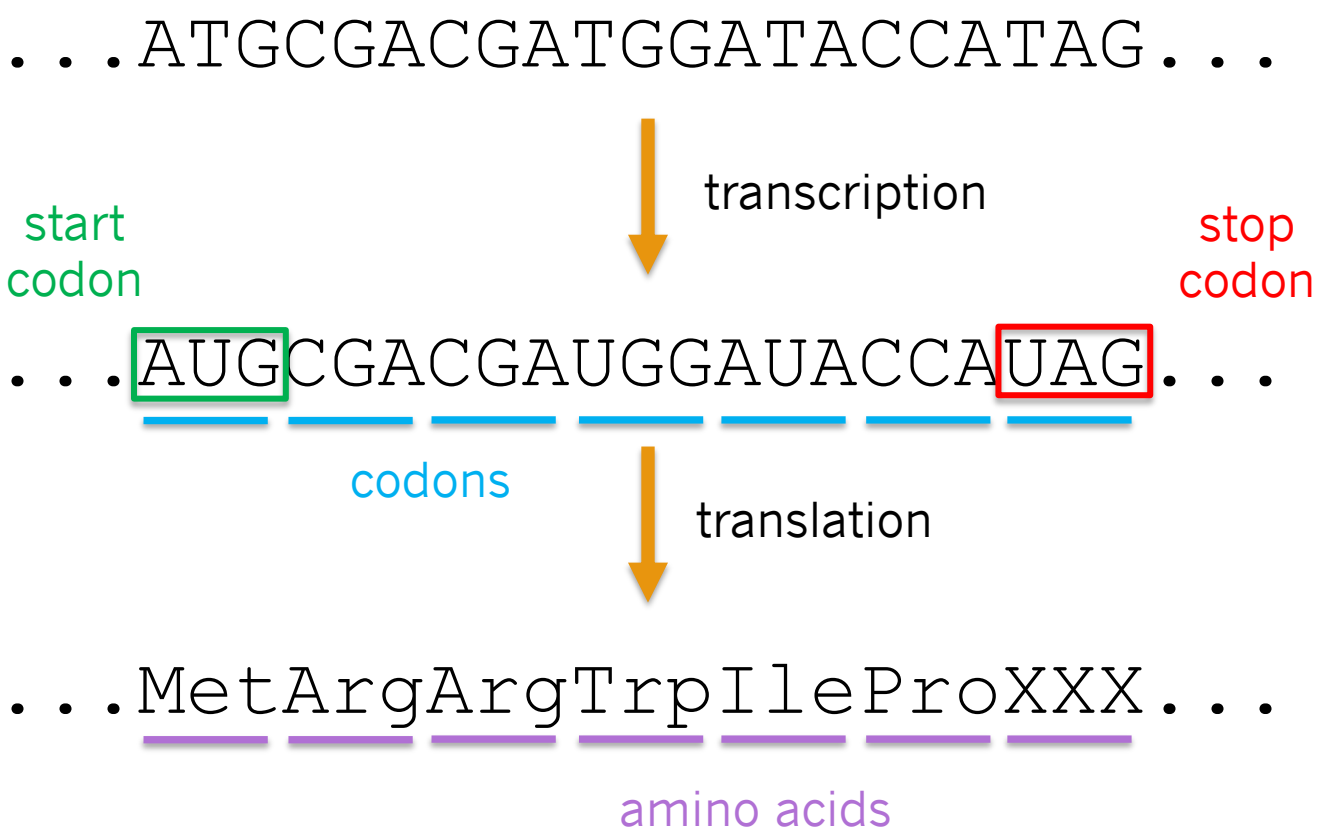
stop codons

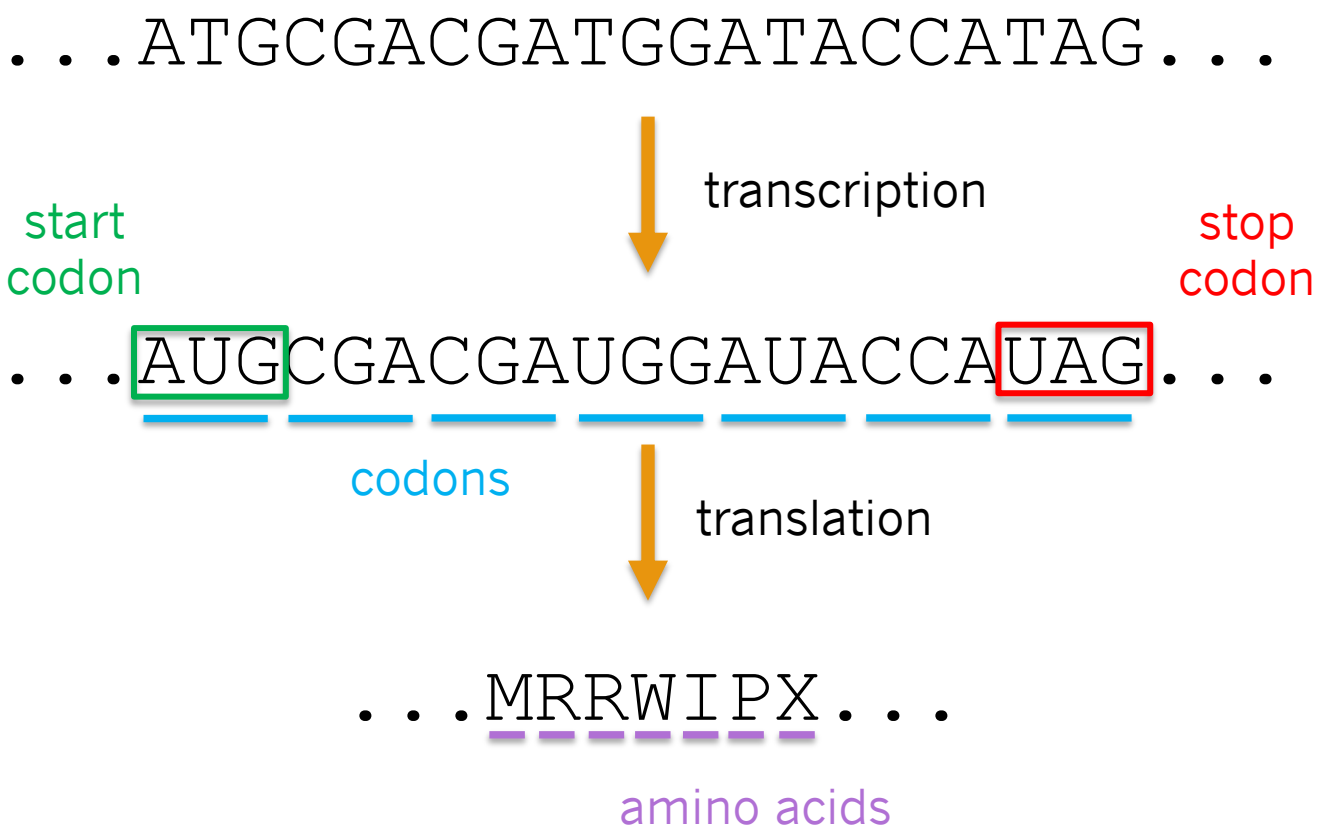
start codon



GUU, GUC, GUA, GUG all encode Valine

AAA and AAG encode Lysine


...ATGCGACGATGGATACCATAG...
transcription
start codon
stop codon
...AUGCGACGAUGGAUACCAUAG...
codons
translation
...MetArgArgTrpIleProXXX...
amino acids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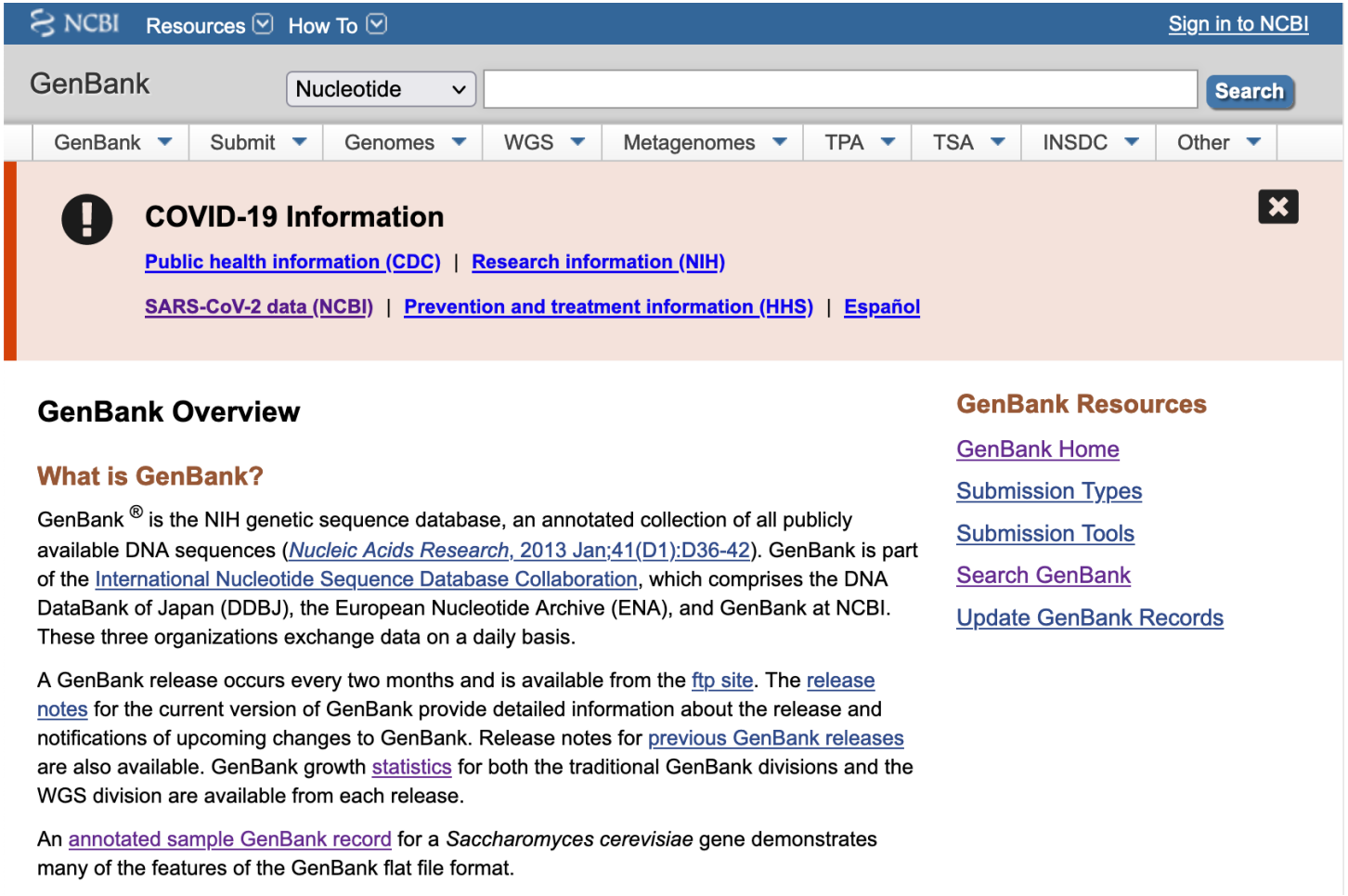
transcription
start codon
stop codon
...AUGCGACGAUGGAUACCAUAG...
codons
translation
...MRRWIPX...
amino acids

NCBI GenBank



https://www.ncbi.nlm.nih.gov/genbank/

GenBank sequences

GenBank publicly hosts (Aug ‘24)

- 251,998,350 sequences
- 3,675,462,701,077 bases

NCBI sequences are used extensively

- identifying anonymous sequences
- inferring gene function
- searching for drug targets
- expanding datasets
- met-analyses

Homo sapiens isolate C15542T cytochrome b (cytb) gene, partial cds; mitochondrial

GenBank: JN034136.1

FASTA Graphics PopSet

Go to:

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REFERENCE   1  (bases 1 to 1141)
  AUTHORS   Lingala,H., Kumaraswamy,T. and Penagaluru,P.
  TITLE     Novel variants in CytB mitochondrial gene in children with non
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1141)
  AUTHORS   Lingala,H., Kumaraswamy,T. and Penagaluru,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-MAY-2011) Molecular Biology, Research Scholar,
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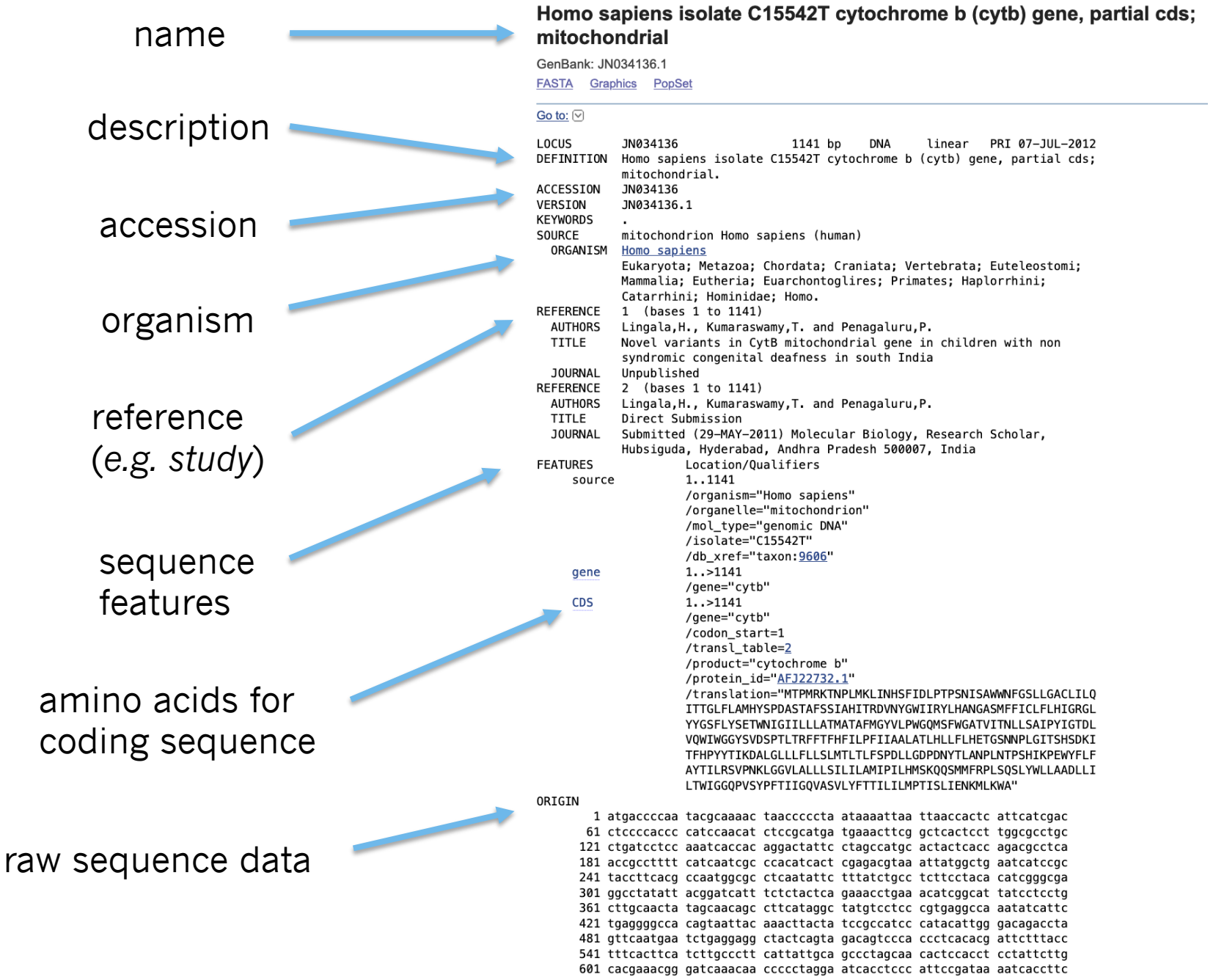
name
description
accession
organism
reference
(e.g. study)
sequence
features
amino acids for
coding sequence
raw sequence data
Homo sapiens isolate C15542T cytochrome b (cytb) gene, partial cds; mitochondrial
GenBank: JN034136.1
FASTA Graphics PopSet
Go to:
LOCUS JN034136 1141 bp DNA linear PRI 07-JUL-2012
DEFINITION Homo sapiens isolate C15542T cytochrome b (cytb) gene, partial cds; mitochondrial.
ACCESSION JN034136
VERSION JN034136.1
KEYWORDS .
SOURCE mitochondrion Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1141)
AUTHORS Lingala,H., Kumaraswamy,T. and Penagaluru,P.
TITLE Novel variants in CytB mitochondrial gene in children with non syndromic congenital deafness in south India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1141)
AUTHORS Lingala,H., Kumaraswamy,T. and Penagaluru,P.
TITLE Direct Submission
JOURNAL Submitted (29-MAY-2011) Molecular Biology, Research Scholar, Hubsiguda, Hyderabad, Andhra Pradesh 500007, India
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VQWIWGGYSVDSPTLTRFFTFHFILPFIIAALATLHLLFLHETGSNNPLGITSHSDKI
TFHPYYTIKDALGLLLFLLSLMTLTLFSPDLLGDPDNYTLANPLNTPSHIKPEWYFLF
AYTILRSVPNKLGGVLALLLSILILAMIPILHMSKQQSMMFRPLSQSLYWLLAADLLI
LTWIGGQPVSYPFTIIGQVASVLYFTTILILMPTISLIENKMLKWA"
ORIGIN
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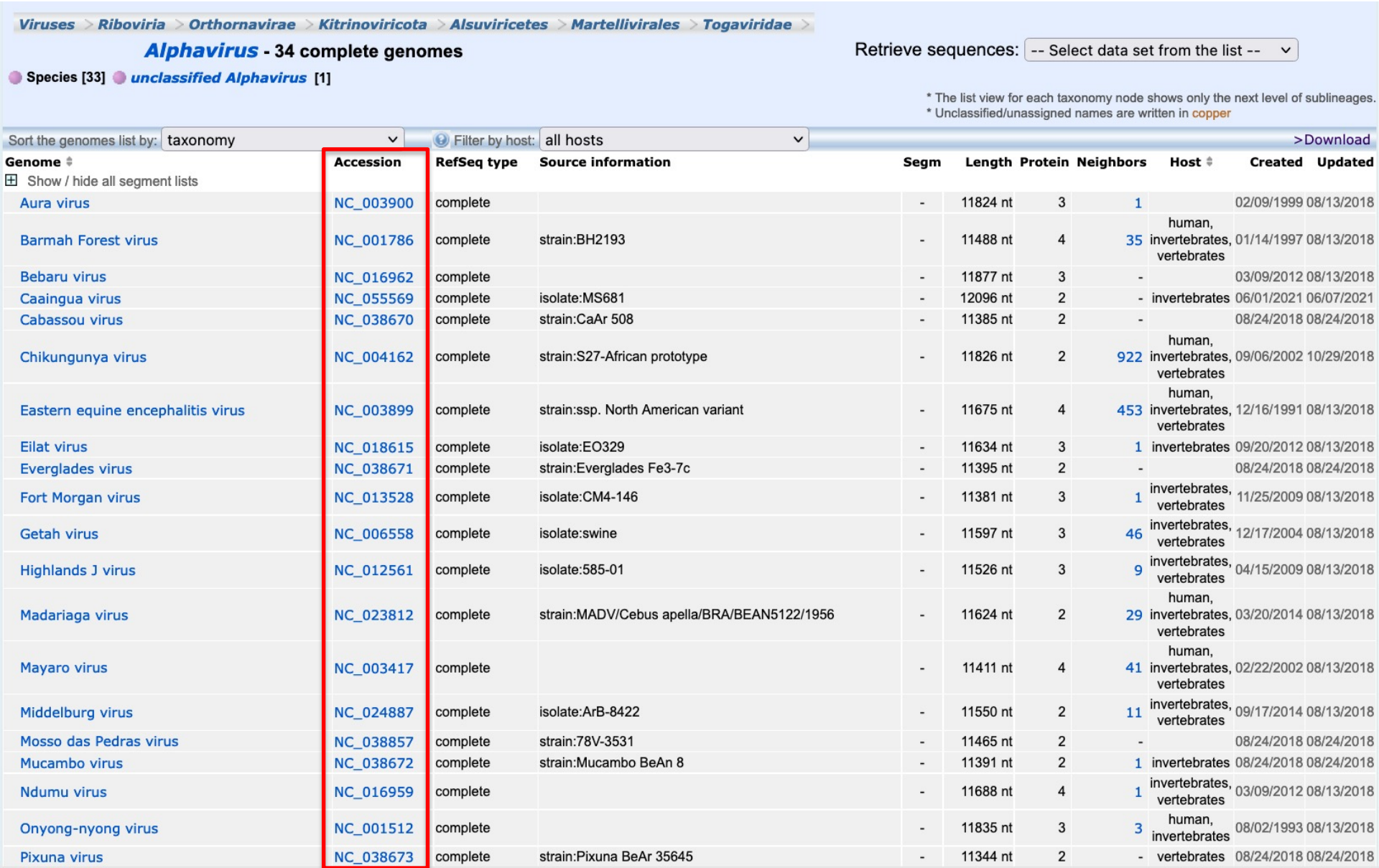
Genome collections



Viruses > Riboviria > Orthornavirae > Kitrinoviricota > Alsuviricetes > Martellivirales > Togaviridae >

Alphavirus - 34 complete genomes

Species [33] unclassified Alphavirus [1]

Retrieve sequences: -- Select data set from the list --

* The list view for each taxonomy node shows only the next level of sublineages.
* Unclassified/unassigned names are written in copper

Sort the genomes list by: taxonomy | Filter by host: all hosts | >Download

Genome	Accession	RefSeq type	Source information	Segm	Length	Protein	Neighbors	Host	Created	Updated
Show / hide all segment lists										
Aura virus	NC_003900	complete		-	11824 nt	3	1		02/09/1999	08/13/2018
Barmah Forest virus	NC_001786	complete	strain:BH2193	-	11488 nt	4	35	human, invertebrates, vertebrates	01/14/1997	08/13/2018
Bebaru virus	NC_016962	complete		-	11877 nt	3	-		03/09/2012	08/13/2018
Caaingua virus	NC_055569	complete	isolate:MS681	-	12096 nt	2	-	invertebrates	06/01/2021	06/07/2021
Cabassou virus	NC_038670	complete	strain:CaAr 508	-	11385 nt	2	-		08/24/2018	08/24/2018
Chikungunya virus	NC_004162	complete	strain:S27-African prototype	-	11826 nt	2	922	human, invertebrates, vertebrates	09/06/2002	10/29/2018
Eastern equine encephalitis virus	NC_003899	complete	strain:ssp. North American variant	-	11675 nt	4	453	human, invertebrates, vertebrates	12/16/1991	08/13/2018
Eilat virus	NC_018615	complete	isolate:EO329	-	11634 nt	3	1	invertebrates	09/20/2012	08/13/2018
Everglades virus	NC_038671	complete	strain:Everglades Fe3-7c	-	11395 nt	2	-		08/24/2018	08/24/2018
Fort Morgan virus	NC_013528	complete	isolate:CM4-146	-	11381 nt	3	1	invertebrates, vertebrates	11/25/2009	08/13/2018
Getah virus	NC_006558	complete	isolate:swine	-	11597 nt	3	46	invertebrates, vertebrates	12/17/2004	08/13/2018
Highlands J virus	NC_012561	complete	isolate:585-01	-	11526 nt	3	9	invertebrates, vertebrates	04/15/2009	08/13/2018
Madariaga virus	NC_023812	complete	strain:MADV/Cebus apella/BRA/BEAN5122/1956	-	11624 nt	2	29	human, invertebrates, vertebrates	03/20/2014	08/13/2018
Mayaro virus	NC_003417	complete		-	11411 nt	4	41	human, invertebrates, vertebrates	02/22/2002	08/13/2018
Middelburg virus	NC_024887	complete	isolate:ArB-8422	-	11550 nt	2	11	invertebrates, vertebrates	09/17/2014	08/13/2018
Mosso das Pedras virus	NC_038857	complete	strain:78V-3531	-	11465 nt	2	-		08/24/2018	08/24/2018
Mucambo virus	NC_038672	complete	strain:Mucambo BeAn 8	-	11391 nt	2	1	invertebrates	08/24/2018	08/24/2018
Ndumu virus	NC_016959	complete		-	11688 nt	4	1	invertebrates, vertebrates	03/09/2012	08/13/2018
Onyong-nyong virus	NC_001512	complete		-	11835 nt	3	3	human, invertebrates	08/02/1993	08/13/2018
Pixuna virus	NC_038673	complete	strain:Pixuna BeAr 35645	-	11344 nt	2	-	vertebrates	08/24/2018	08/24/2018

BLAST: Basic Local Alignment Search Tool

What if the identity of your sequence is unknown?

Matches a ***query sequence*** against any number of ***target*** sequences in a database (*e.g.* GenBank)

Simple, versatile, and fast

Used to
- identify species
- identify gene function
- locate domains in gene
- locate gene in genome
- expand datasets
- filter out noisy data
- perform meta-analysis

BLAST steps

1. Generate adjacent “words” from query sequence
2. Locate those words among subject sequences in database
3. Extend match between query and subject until score drops
4. Generate statistics for quality of match

Generate adjacent “words” from query sequence

1. Generate words from sequence above threshold (e.g. T=11)

Query Sequence:

```
>gi|16329320 (residues 412 to 594)
SGANFARQLRTHKRQRIARQATTETQADRTQQAVGRIIGSIGVVTTQTTG
RHQGILTSWVSQASFTPPGIMLAIPGEFDAYGLAGQNKAFVLNLLQEGRS
VRRHFDHQPLPKDGDNPFSRLEHYSTQNGCLILAEALAYLECLVQSWSNI
GDHVLVYATVQAGQVLQPNGITAIRHRKSGGQY
```

Fragmentation into words:

Selection of words scoring above threshold (for word SWV):

Substitution Matrix*

	R	G	I	K	F	S	T	W	V
R	5	0	-1	-1	-2	1	0	-3	0
G		6	-4	-2	-3	0	-2	-2	-3
I			4	-3	0	-2	-1	-3	3
K				5	-3	0	-1	-3	-2
F					6	-2	-2	1	-1
S						4	1	-3	-2
T							5	-2	0
W								11	-3
V									4

*A portion of the BLOSUM 62 matrix

SWV (4+11+4 = 19)
SWI (4+11+3 = 18)
TWV (1+11+4 = 16)
GWV (0+11+4 = 15)
KWV (0+11+4 = 15)
SWS (4+11-2 = 13)

Synonyms above threshold 11... (others not shown)

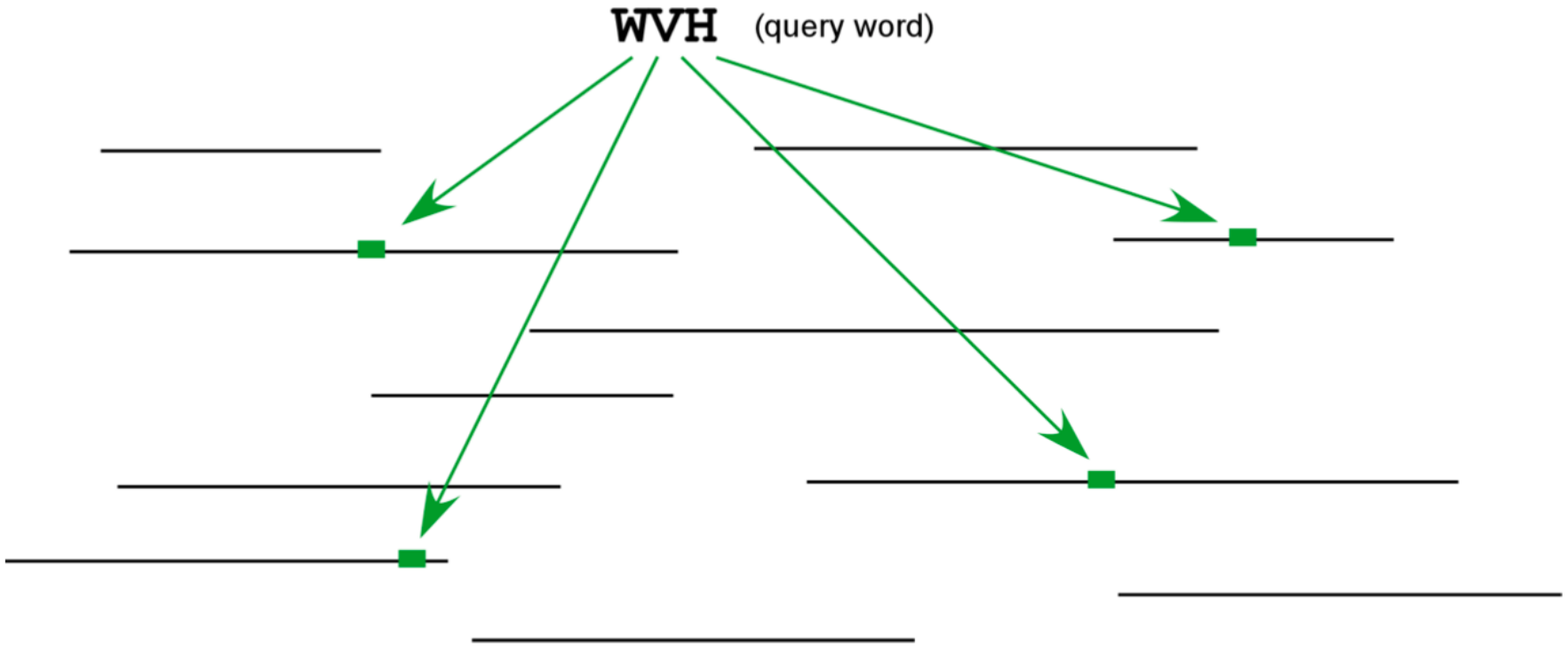
SFV (4+1+4 = 9)
SRV (4-3+4 = 5)

Synonyms below threshold 11... (others not shown)

expected costs of AA substitution, based on many sequence comparisons

from: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001014

Schematic of indexed matches



Result – instead of aligning these 3 amino acids to <u>everything</u>, they are aligned only with the tiny fraction of sequence regions that are good candidates for a valid alignment.

from: http://faculty.washington.edu/jht/GS559_2017/

Extension and scoring

	Match Score:	Total Score:
...QSVFEWVHLLPGA... ..WIY..	16	16
...QSVFEWVHLLPGA... ..WIY**Q**..	-3	13
...QSVFEWVHLLPGA... ..WIYQ**K**..	-2	11
...QSVFEWVHLLPGA... ..WIYQK**A**..	-1	10

cost of match, mismatch, and gap is assigned by program and user

from: http://faculty.washington.edu/jht/GS559_2017/

Generate statistics for quality of match

4. Generate alignment and calculate statistics

```
>ref|YP_002482587.1| flavin reductase domain protein FMN-binding [Cyanothece sp.
PCC 7425]
 gb|ACL44226.1| flavin reductase domain protein FMN-binding [Cyanothece sp. PCC
7425]
Length=585

 Score =  176 bits (446),  Expect = 1e-42,  Method: Compositional matrix adjust.
 Identities = 95/196 (48%), Positives = 125/196 (63%), Gaps = 16/196 (8%)

Query  1    SGANFARQLRTHKRQRIARQATTETQADRTQQAVGRIIGSIGVVTTQTTGRH--------  52
            +G++FA+ L+  K+QR  RQ+  E Q+DRT+QAVGRIIGS+ V+T +    H
Sbjct  393  AGSDFAQVLKKAKKQRSPRQSILEVQSDRTEQAVGRIIGSLCVLTAKQQQTHPHPEVEEP  452

Query  53   -----QGILTSWVSQASFTPPGIMLAIPGEFDAYGLAGQNKAFVLNLLQEGRSVRRHFDH  107
                  +L SWVSQASF PPG+ +A+  E  A GL     AFVLN+L+EG ++RRHF
Sbjct  453  QLEVPTAMLVSWVSQASFNPPGLTIALAKE-RAEGLDHSGDAFVLNVLKEGMNLRRHFSK  511

Query  108  QPLPKDGDNPFSRLEHYSTQNGCLILAEALAYLECLVQSWSNIGDHVLVYATVQAGQVLQ  167
               P  G++ F+ L     +NGC +L + LAYLEC VQS    GDH L+YATV  G+VLQ
Sbjct  512  SFAP--GEDRFAGLNIQWAENGCPVLQDCLAYLECTVQSRMECGDHWLIYATVNNGKVLQ  569

Query  168  PNGITAIRHRKSGGQY  183
            P G TA++HRKSG QY
Sbjct  570  PTGTTAVQHRKSGNQY  585
```

E-value: the number of expected random sequences
in database with better match than score

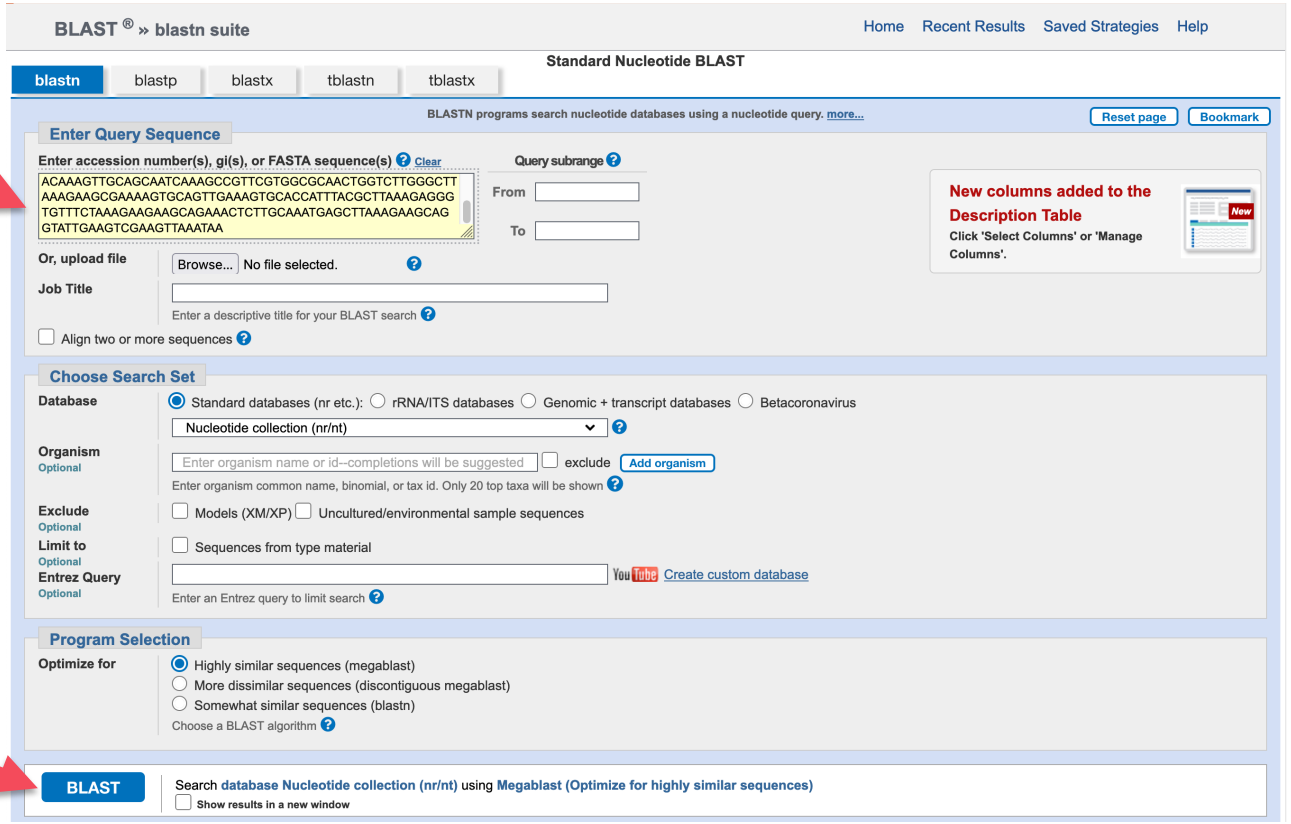
Higher score, lower E-value
Larger database, higher E-value

from: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001014

NCBI BLAST interface

Input your ***query sequence*** as text

Perform BLAST against ***subject sequences***



https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch

gene from Kerfeld and Scott (PLoS Biology 2011)

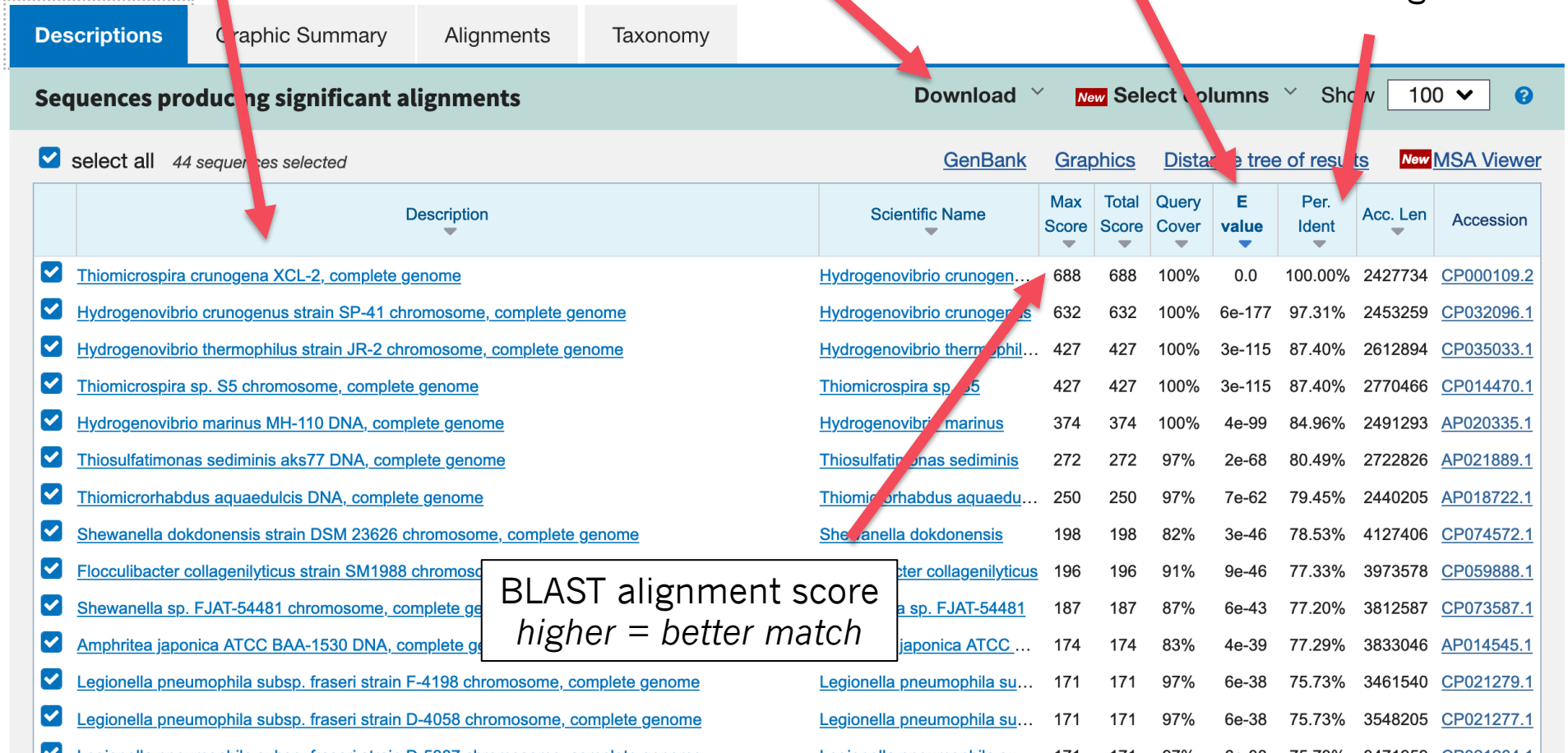
“BLAST hits”

Sequence name
e.g. species/gene ID

Download sequences

Expect value
lower = more significant

% identical characters in alignment



Descriptions | Graphic Summary | Alignments | Taxonomy

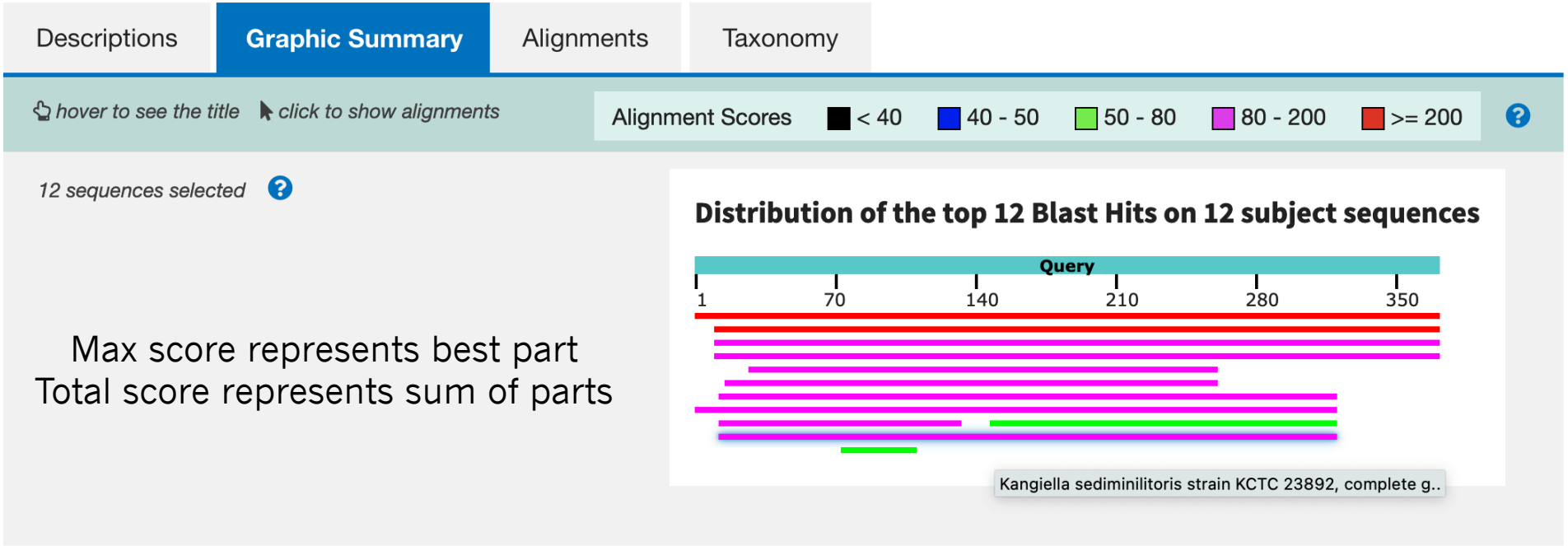
Sequences producing significant alignments Download | New Select columns | Show 100

select all *44 sequences selected* GenBank | Graphics | Distance tree of results | New MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Thiomicrospira crunogena XCL-2, complete genome	Hydrogenovibrio crunogen…	688	688	100%	0.0	100.00%	2427734	CP000109.2
Hydrogenovibrio crunogenus strain SP-41 chromosome, complete genome	Hydrogenovibrio crunogen…	632	632	100%	6e-177	97.31%	2453259	CP032096.1
Hydrogenovibrio thermophilus strain JR-2 chromosome, complete genome	Hydrogenovibrio thermophil…	427	427	100%	3e-115	87.40%	2612894	CP035033.1
Thiomicrospira sp. S5 chromosome, complete genome	Thiomicrospira sp. S5	427	427	100%	3e-115	87.40%	2770466	CP014470.1
Hydrogenovibrio marinus MH-110 DNA, complete genome	Hydrogenovibrio marinus	374	374	100%	4e-99	84.96%	2491293	AP020335.1
Thiosulfatimonas sediminis aks77 DNA, complete genome	Thiosulfatimonas sediminis	272	272	97%	2e-68	80.49%	2722826	AP021889.1
Thiomicrorhabdus aquaedulcis DNA, complete genome	Thiomicrorhabdus aquaedu…	250	250	97%	7e-62	79.45%	2440205	AP018722.1
Shewanella dokdonensis strain DSM 23626 chromosome, complete genome	Shewanella dokdonensis	198	198	82%	3e-46	78.53%	4127406	CP074572.1
Flocculibacter collagenilyticus strain SM1988 chromosc	cter collagenilyticus	196	196	91%	9e-46	77.33%	3973578	CP059888.1
Shewanella sp. FJAT-54481 chromosome, complete ge	a sp. FJAT-54481	187	187	87%	6e-43	77.20%	3812587	CP073587.1
Amphritea japonica ATCC BAA-1530 DNA, complete g	japonica ATCC …	174	174	83%	4e-39	77.29%	3833046	AP014545.1
Legionella pneumophila subsp. fraseri strain F-4198 chromosome, complete genome	Legionella pneumophila su…	171	171	97%	6e-38	75.73%	3461540	CP021279.1
Legionella pneumophila subsp. fraseri strain D-4058 chromosome, complete genome	Legionella pneumophila su…	171	171	97%	6e-38	75.73%	3548205	CP021277.1

BLAST alignment score
higher = better match

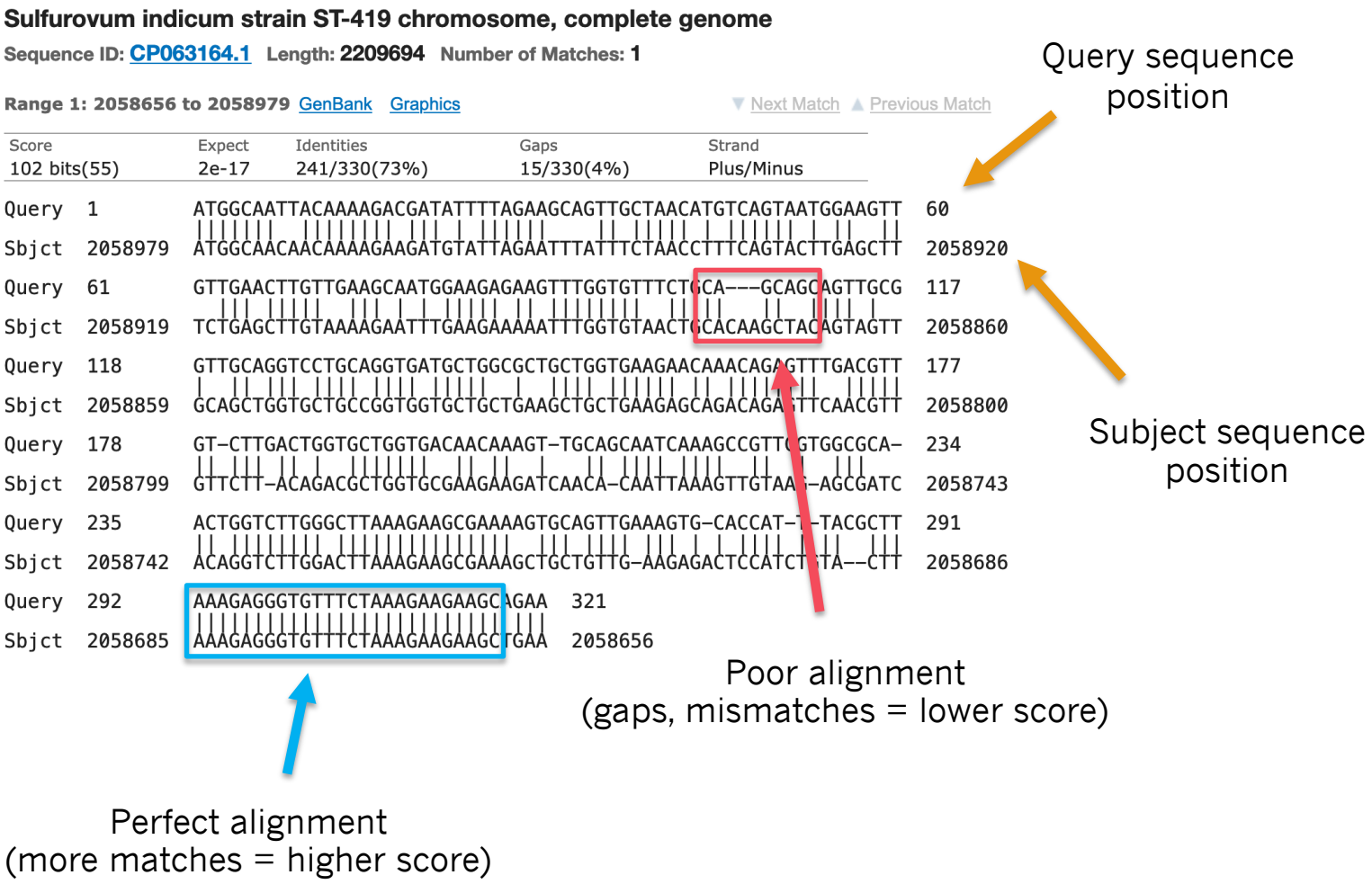
Graphic summary of BLAST scores



Max score represents best part
Total score represents sum of parts

Scores determined by alignment costs
for match/mismatch/indel reconciliation
(covered in a later lab)

BLAST generates a summary for each subject sequence



arrays

an ***array variable*** contains multiple values
that are individually accessed by index

```
#!/bin/sh
# populate array
LETTERS[0]="A"
LETTERS[1]="B"
LETTERS[2]="C"
# INCORRECT: access array, show element 0
echo $LETTERS
# INCORRECT: access array, add "[1]" text
echo $LETTERS[1]
# CORRECT: access element 1
echo ${LETTERS[1]}
```

array1.sh

```
$ ./array1.sh
A
A[1]
B
```

running *array1.sh*

```
#!/bin/sh
# create array
NUMBERS=(1 2 3 4)
echo ${NUMBERS[2]} # 3
echo ${NUMBERS[9]} # empty (no entry)
# assign new array values
NUMBERS[2]="three"
NUMBERS[9]="ten"
echo ${NUMBERS[2]} # "three"
echo ${NUMBERS[9]} # "ten" (new entry)
```

array2.sh

```
$ ./array2.sh
3

three
ten
```

running *array2.sh*

more with arrays

```
#!/bin/sh
# arg1 is directory
DIR=$1
# make array with `ls` command subst.
FILES=( $(ls ${DIR}) )
# print individual array elements
echo "Individual elements"
echo "${FILES[0]}"
echo "${FILES[1]}"
# print entire array
echo "Entire array"
echo "${FILES[@]}"
```

array3.sh

```
$ ls tmp
file1.txt file2.txt file3.txt
$ ./array3.sh tmp
Individual elements
file1.txt
file2.txt
Entire array
file1.txt file2.txt file3.txt
```

running *array3.sh*

```
#!/bin/sh
# all args are stored in $@ array
for i in "$@"; do
  if [[ -f ${i} ]]; then
    echo "Copying ${i} to ${i}.bak"
    cp ${i} ${i}.bak
  fi
done
```

```
$ ls tmp
file1.txt file2.txt file3.txt
$ ./array4.sh tmp/*.txt
Copying tmp/file1.txt to tmp/file1.txt.bak
Copying tmp/file2.txt to tmp/file2.txt.bak
Copying tmp/file3.txt to tmp/file3.txt.bak
$ ls tmp
file1.txt  file1.txt.bak  file2.txt
file2.txt.bak  file3.txt  file3.txt.bak
```

running *array4.sh*

for-loops with files

two approaches to loop over lines in a file;
set the ***input field separator*** (IFS) to split by line

```
#!/bin/bash
FILE=$1               # arg1: input file
SAVE_IFS=${IFS}       # restore IFS when done
IFS=$'\n'             # set '\n' for IFS
LINES=$(cat ${FILE}) # extract text from FILE
for LINE in ${LINES} # for each line (split by IFS)
do
    echo ${LINE}      # print LINE to stdout
done
IFS=${SAVE_IFS}       # done! restore IFS
```

forloop1.sh is simpler, but less portable

```
$ ./forloop1.sh file.txt
Knock, knock!
Who's there?
```

running *forloop1.sh*

```
#!/bin/bash
FILE=$1           # arg1: input file
# while : loop until stop condition (EOF)
# IFS= : IFS set to null ('\n')
# read : split input by IFS + advance file position
# -r : do not treat '\' as special char
# LINE : store each token in LINE
while IFS= read -r LINE
do                # begin code block
    echo ${LINE}  # print each LINE
done < ${FILE}    # input redirect to while/done
```

forloop2.sh is more complex, but more portable

```
$ ./forloop2.sh file.txt
Knock, knock!
Who's there?
```

running *forloop2.sh*

xargs

xargs (e**x**tended **arg**uments) converts input into arguments for another command

```
$ # find all .txt files
$ find data -name "*.txt"
data/file1.txt
data/docs/file2.txt
data/secrets/password.txt
$ # word count of `find` output
$ find data -name "*.txt" | wc
      3 3 61
$ # word count against each found file
$ find data -name "*.txt" | xargs wc
0 0 0 data/file1.txt
0 0 0 data/docs/file2.txt
0 0 0 data/secrets/password.txt
0 0 0 total
```

```
$ # argument file
$ cat files.txt
example/file1.txt
example/file2.txt
example/file3.txt
$ # target files to remove
$ ls example/
file1.txt file2.txt file3.txt
$ # use argument file lines for rm
$ xargs -a files.txt rm
$ # files deleted
$ ls example/
```

```
$ ls
a.txt b.txt
$ echo "a.txt a.txt.tmp b.txt b.txt.tmp" | xargs cp
cp: target 'b.txt.tmp' is not a directory
$ echo "a.txt a.txt.tmp b.txt b.txt.tmp" | xargs -n2
a.txt a.txt.tmp
b.txt b.txt.tmp
$ echo "a.txt a.txt.tmp b.txt b.txt.tmp" | xargs -n2 cp
$ ls
a.txt a.txt.tmp b.txt b.txt.tmp
```

Overview for Lab 07