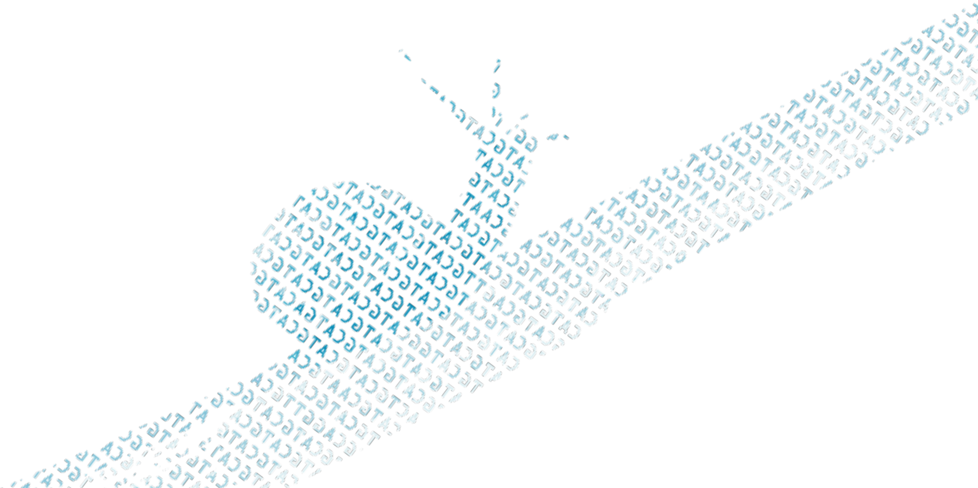
GDC

Genetic Diversity Centre Zurich

Genetic Diversity: Analysis

Sequence Databases

Monday 21. June 2021

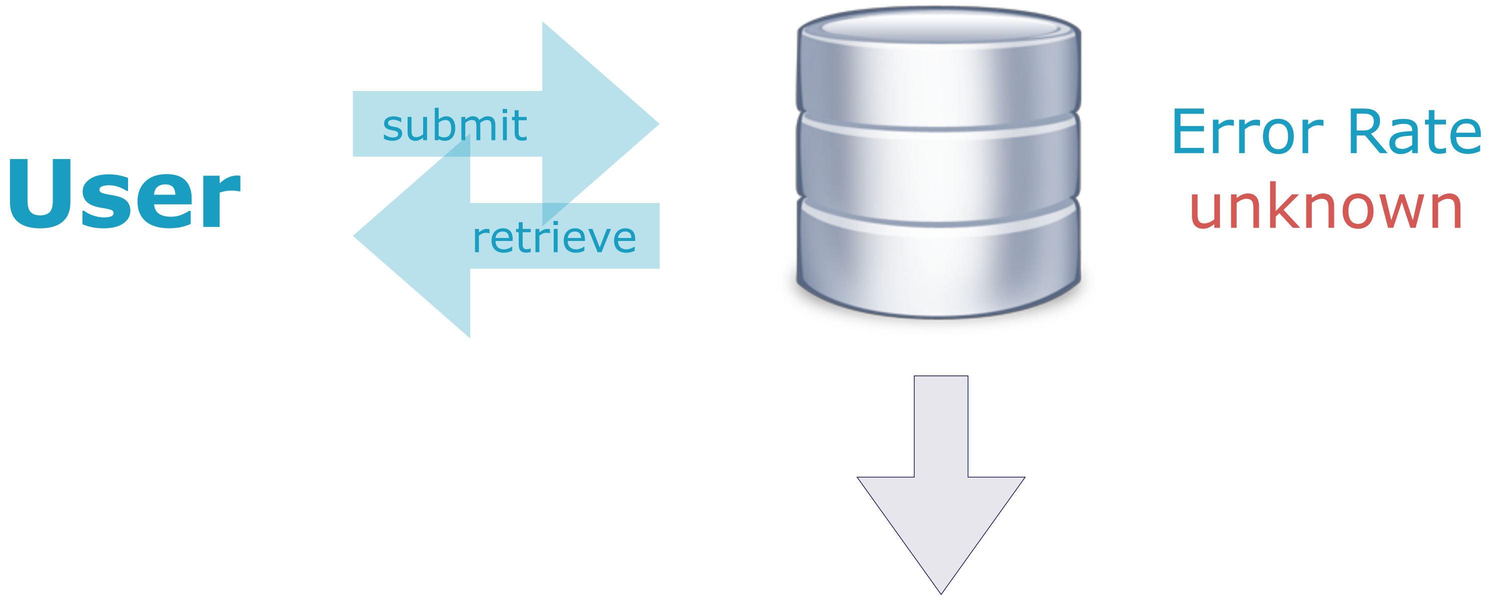


ETH

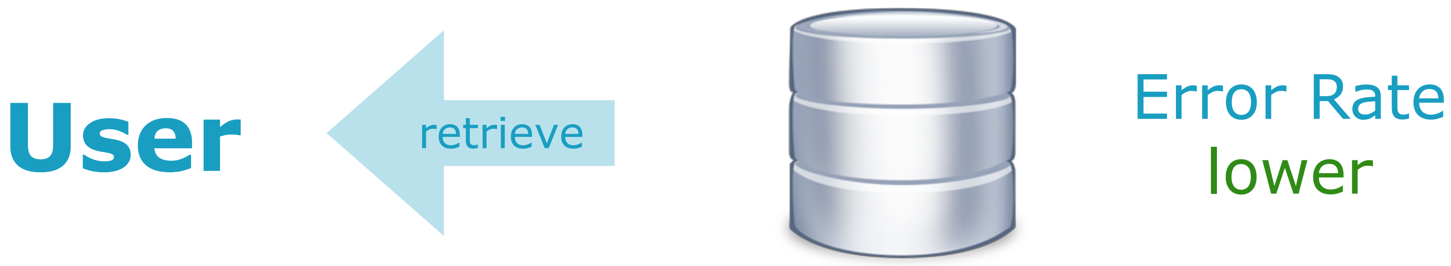
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

GDA21 ▷ Sequence Databases (Extra)

Primary Databases



Secondary Databases



GDA21 ▷ Sequence Databases (Extra)

Primary Databases

NCBI

National Center for Biotechnology

GenBank® : NIH genetic sequence database, an annotated collection of all publicly available DNA sequences. http://www.ncbi.nlm.nih.gov/genbank/

ENA

European Nucleotide Archive

ENA - The European Nucleotide Archive (ENA) captures and presents information relating to experimental workflows that are based around nucleotide sequencing. http://www.ebi.ac.uk/ena/

DDBJ

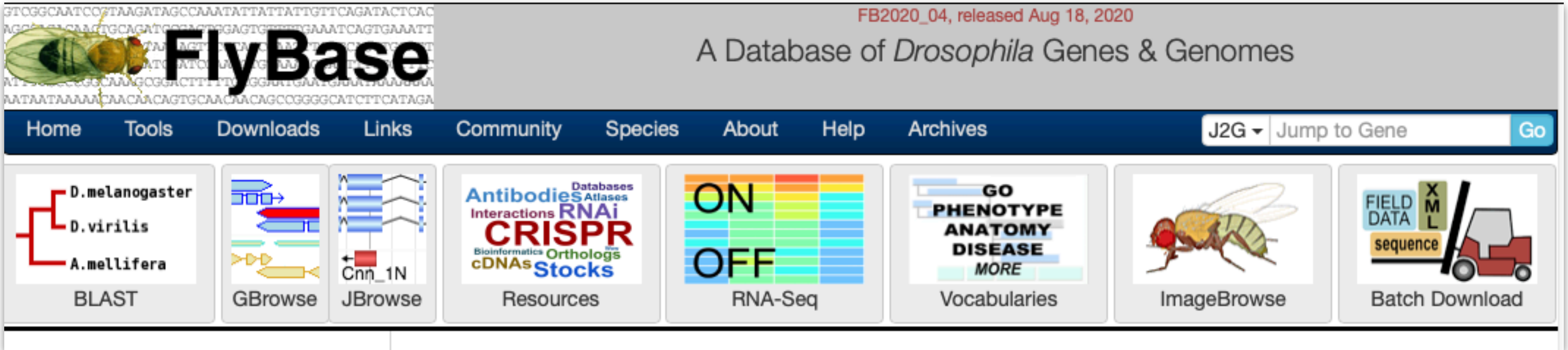
DNA Data Bank of Japan

DDBJ - DNA Data Bank of Japan was established 1986. http://www.ddbj.nig.ac.jp/

INSDC

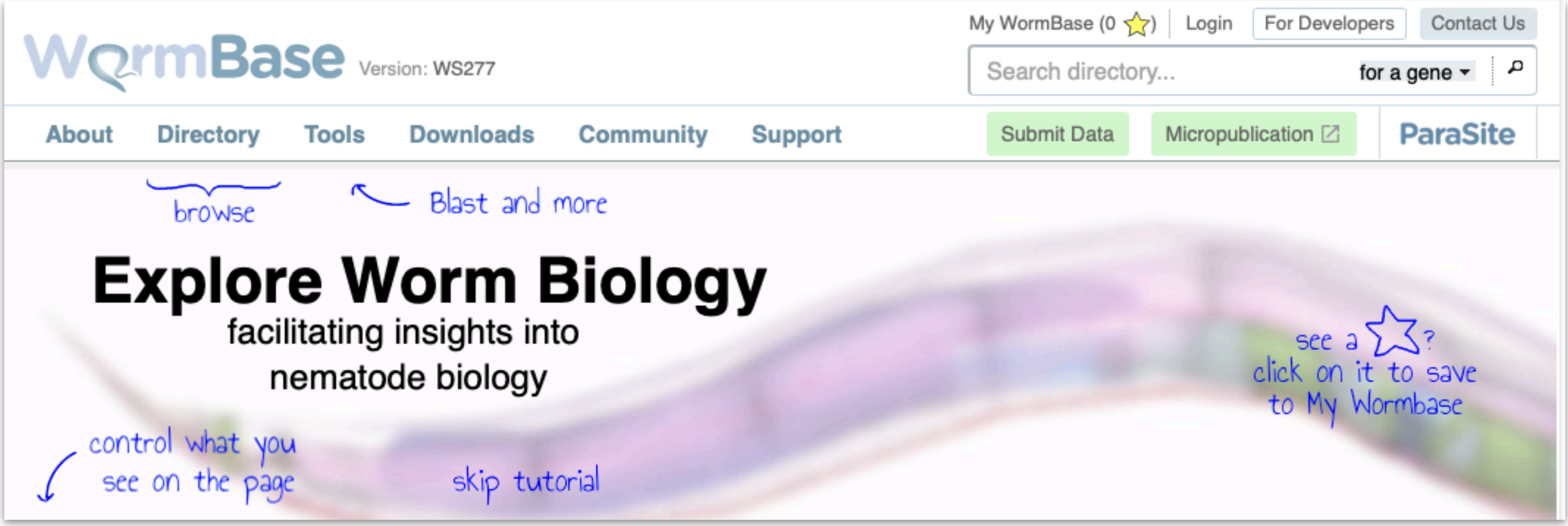
INSDC - The International Nucleotide Sequence Databases (INSD) have been developed and maintained collaboratively between DDBJ, ENA, and GenBank for over 18 years. http://insdc.org/

GDA21 ▷ Sequence Databases (Extra)



FB2020_04, released Aug 18, 2020

A Database of *Drosophila* Genes & Genomes



WormBase Version: WS277

Explore Worm Biology

facilitating insights into nematode biology

browse

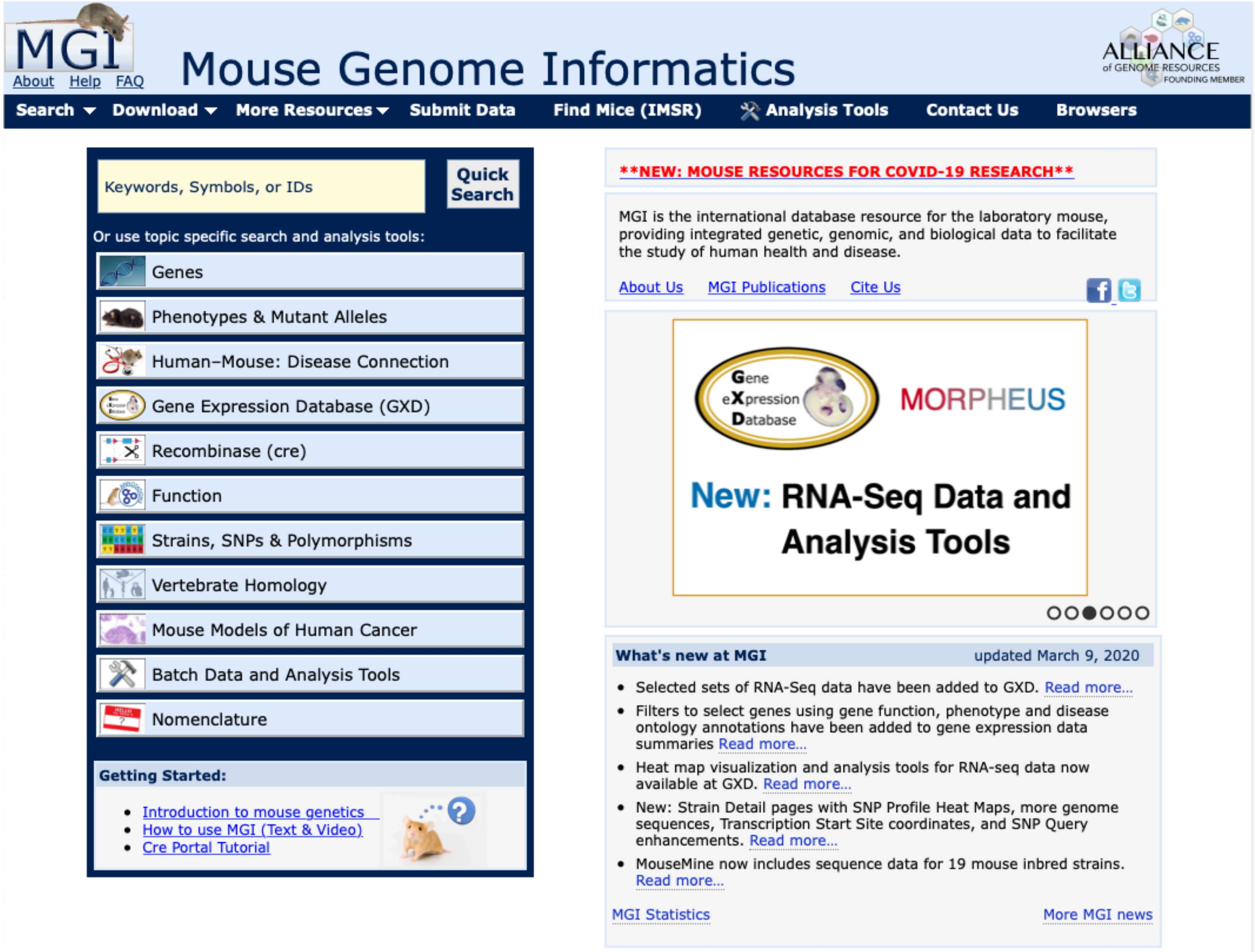
Blast and more

see a ☆? click on it to save to My Wormbase

control what you see on the page

skip tutorial

GDA21 ▷ Sequence Databases (Extra)



MGI

About Help FAQ

Mouse Genome Informatics

ALLIANCE of GENOME RESOURCES FOUNDING MEMBER

Search ▾ Download ▾ More Resources ▾ Submit Data Find Mice (IMSR) Analysis Tools Contact Us Browsers

Keywords, Symbols, or IDs

Quick Search

Or use topic specific search and analysis tools:

- Genes
- Phenotypes & Mutant Alleles
- Human–Mouse: Disease Connection
- Gene Expression Database (GXD)
- Recombinase (cre)
- Function
- Strains, SNPs & Polymorphisms
- Vertebrate Homology
- Mouse Models of Human Cancer
- Batch Data and Analysis Tools
- Nomenclature

Getting Started:

- Introduction to mouse genetics
- How to use MGI (Text & Video)
- Cre Portal Tutorial

****NEW: MOUSE RESOURCES FOR COVID-19 RESEARCH****

MGI is the international database resource for the laboratory mouse, providing integrated genetic, genomic, and biological data to facilitate the study of human health and disease.

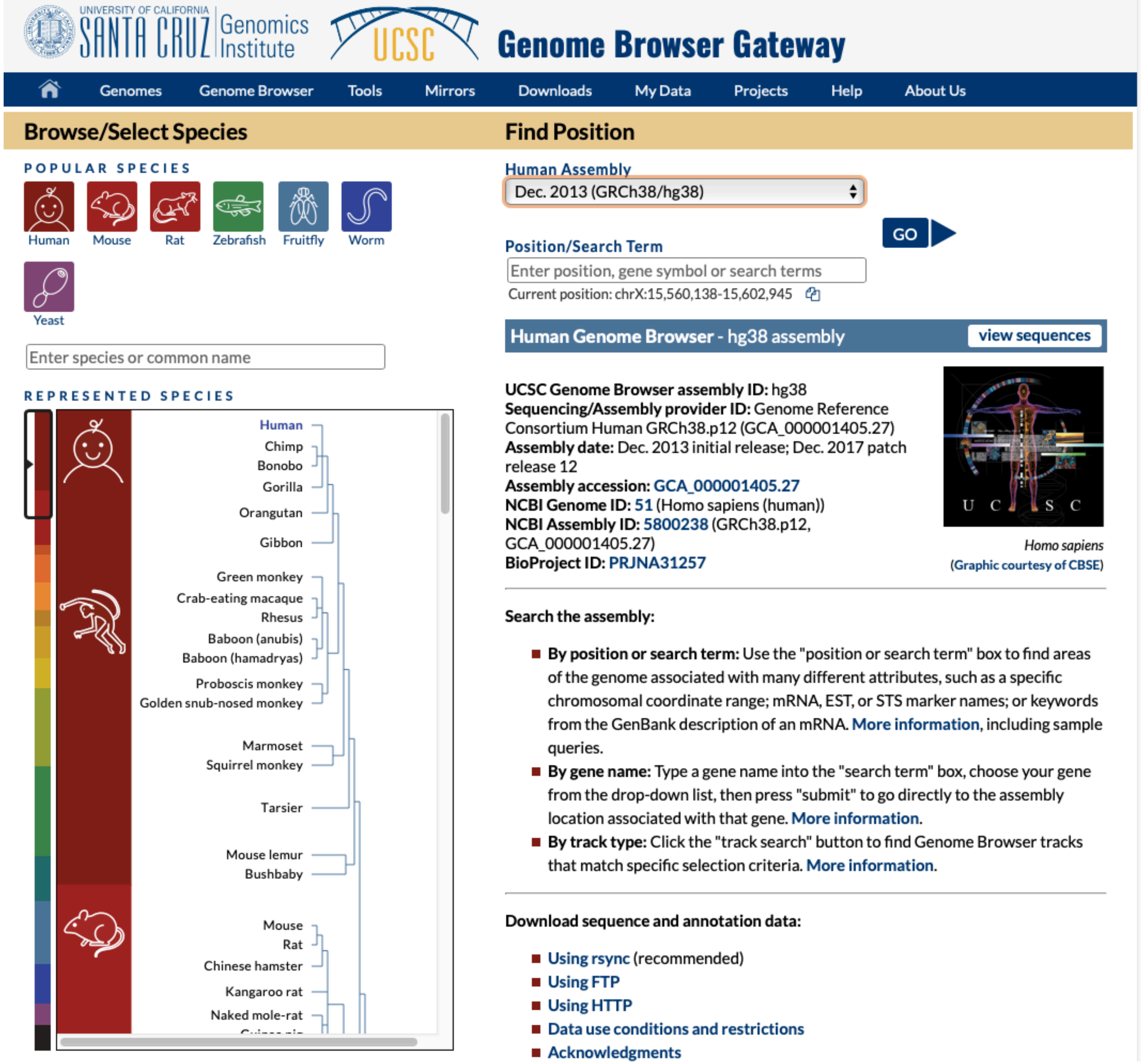
About Us MGI Publications Cite Us

○○●○○○

What's new at MGI updated March 9, 2020

- Selected sets of RNA-Seq data have been added to GXD. Read more...
- Filters to select genes using gene function, phenotype and disease ontology annotations have been added to gene expression data summaries Read more...
- Heat map visualization and analysis tools for RNA-seq data now available at GXD. Read more...
- New: Strain Detail pages with SNP Profile Heat Maps, more genome sequences, Transcription Start Site coordinates, and SNP Query enhancements. Read more...
- MouseMine now includes sequence data for 19 mouse inbred strains. Read more...

MGI Statistics More MGI news



UNIVERSITY OF CALIFORNIA SANTA CRUZ Genomics Institute UCSC **Genome Browser Gateway**

Genomes | Genome Browser | Tools | Mirrors | Downloads | My Data | Projects | Help | About Us

Browse/Select Species

POPULAR SPECIES

Human Mouse Rat Zebrafish Fruitfly Worm Yeast

Enter species or common name

REPRESENTED SPECIES

Human
Chimp
Bonobo
Gorilla
Orangutan
Gibbon
Green monkey
Crab-eating macaque
Rhesus
Baboon (anubis)
Baboon (hamadryas)
Proboscis monkey
Golden snub-nosed monkey
Marmoset
Squirrel monkey
Tarsier
Mouse lemur
Bushbaby
Mouse
Rat
Chinese hamster
Kangaroo rat
Naked mole-rat

Find Position

Human Assembly
Dec. 2013 (GRCh38/hg38)

Position/Search Term
Enter position, gene symbol or search terms
GO
Current position: chrX:15,560,138-15,602,945

Human Genome Browser - hg38 assembly view sequences

UCSC Genome Browser assembly ID: hg38
Sequencing/Assembly provider ID: Genome Reference Consortium Human GRCh38.p12 (GCA_000001405.27)
Assembly date: Dec. 2013 initial release; Dec. 2017 patch release 12
Assembly accession: GCA_000001405.27
NCBI Genome ID: 51 (Homo sapiens (human))
NCBI Assembly ID: 5800238 (GRCh38.p12, GCA_000001405.27)
BioProject ID: PRJNA31257

Homo sapiens
(Graphic courtesy of CBSE)

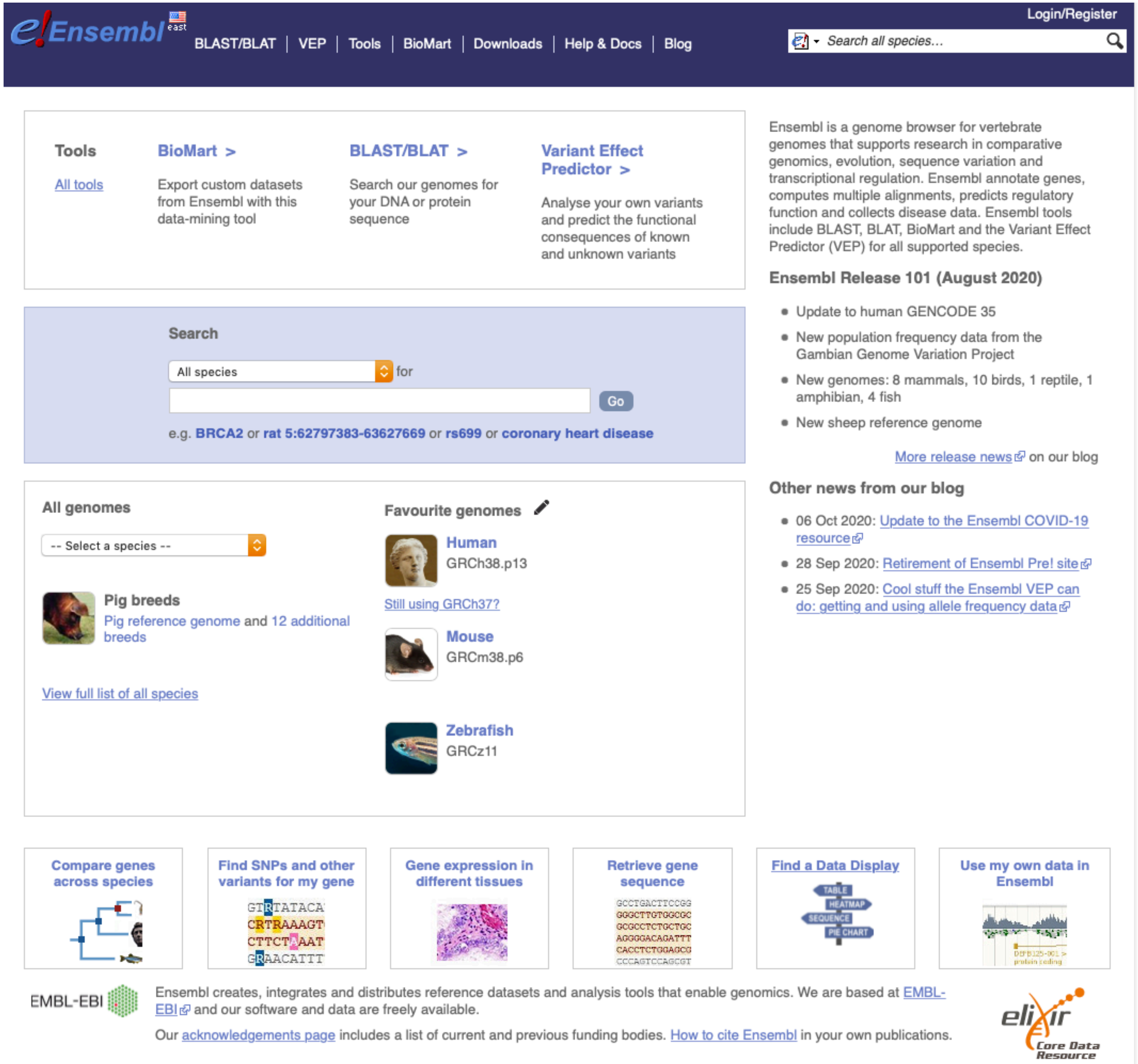
Search the assembly:

- **By position or search term:** Use the "position or search term" box to find areas of the genome associated with many different attributes, such as a specific chromosomal coordinate range; mRNA, EST, or STS marker names; or keywords from the GenBank description of an mRNA. More information, including sample queries.
- **By gene name:** Type a gene name into the "search term" box, choose your gene from the drop-down list, then press "submit" to go directly to the assembly location associated with that gene. More information.
- **By track type:** Click the "track search" button to find Genome Browser tracks that match specific selection criteria. More information.

Download sequence and annotation data:

- Using rsync (recommended)
- Using FTP
- Using HTTP
- Data use conditions and restrictions
- Acknowledgments

GDA21 ▷ Sequence Databases (Extra)



e! Ensembl east
BLAST/BLAT | VEP | Tools | BioMart | Downloads | Help & Docs | Blog
Login/Register
Search all species…

Tools

All tools

BioMart >

Export custom datasets from Ensembl with this data-mining tool

BLAST/BLAT >

Search our genomes for your DNA or protein sequence

Variant Effect Predictor >

Analyse your own variants and predict the functional consequences of known and unknown variants

Search

All species for

Go

e.g. **BRCA2** or **rat 5:62797383-63627669** or **rs699** or **coronary heart disease**

All genomes

-- Select a species --

Pig breeds

Pig reference genome and 12 additional breeds

View full list of all species

Favourite genomes

Human
GRCh38.p13

Still using GRCh37?

Mouse
GRCm38.p6

Zebrafish
GRCz11

Ensembl is a genome browser for vertebrate genomes that supports research in comparative genomics, evolution, sequence variation and transcriptional regulation. Ensembl annotate genes, computes multiple alignments, predicts regulatory function and collects disease data. Ensembl tools include BLAST, BLAT, BioMart and the Variant Effect Predictor (VEP) for all supported species.

Ensembl Release 101 (August 2020)

- Update to human GENCODE 35
- New population frequency data from the Gambian Genome Variation Project
- New genomes: 8 mammals, 10 birds, 1 reptile, 1 amphibian, 4 fish
- New sheep reference genome

More release news on our blog

Other news from our blog

- 06 Oct 2020: Update to the Ensembl COVID-19 resource
- 28 Sep 2020: Retirement of Ensembl Pre! site
- 25 Sep 2020: Cool stuff the Ensembl VEP can do: getting and using allele frequency data

Compare genes across species
Find SNPs and other variants for my gene
Gene expression in different tissues
Retrieve gene sequence
Find a Data Display
TABLE
HEATMAP
SEQUENCE
PIE CHART
Use my own data in Ensembl

EMBL-EBI

Ensembl creates, integrates and distributes reference datasets and analysis tools that enable genomics. We are based at EMBL-EBI and our software and data are freely available.

Our acknowledgements page includes a list of current and previous funding bodies. How to cite Ensembl in your own publications.

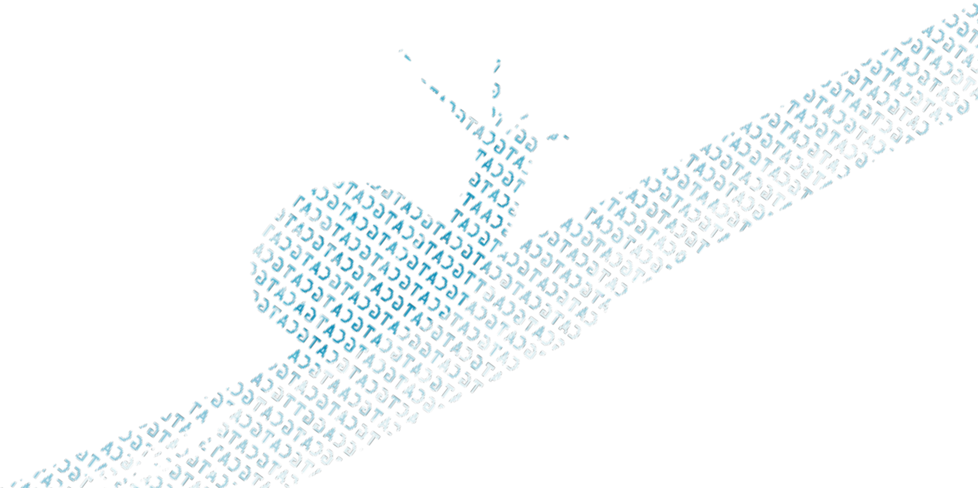
GDC

Genetic Diversity Centre Zurich

Genetic Diversity: Analysis

BLAST Searches

Monday 21. June 2021



ETH

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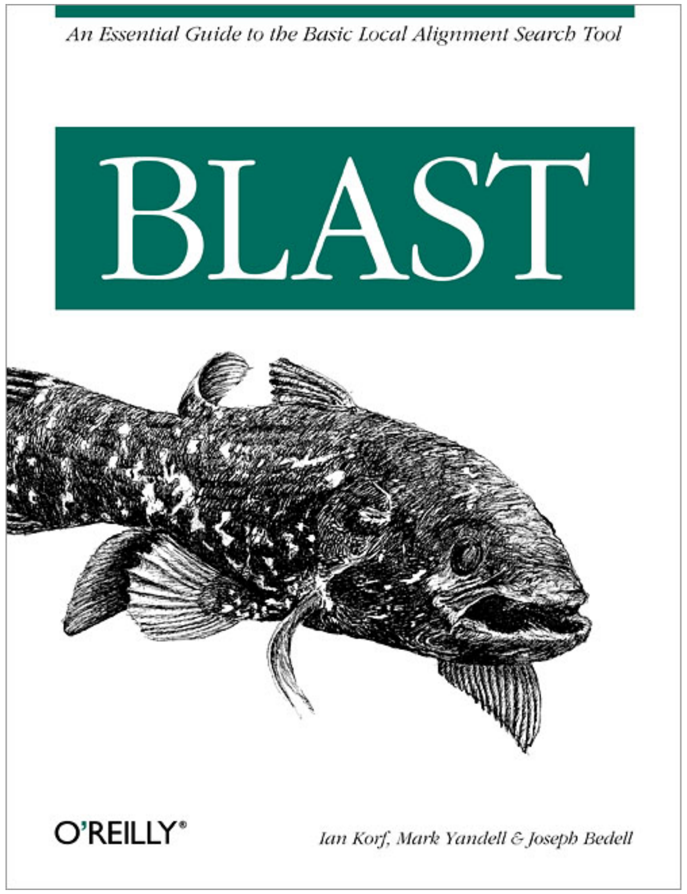
GDA21 ▷ BLAST Search (Extra)

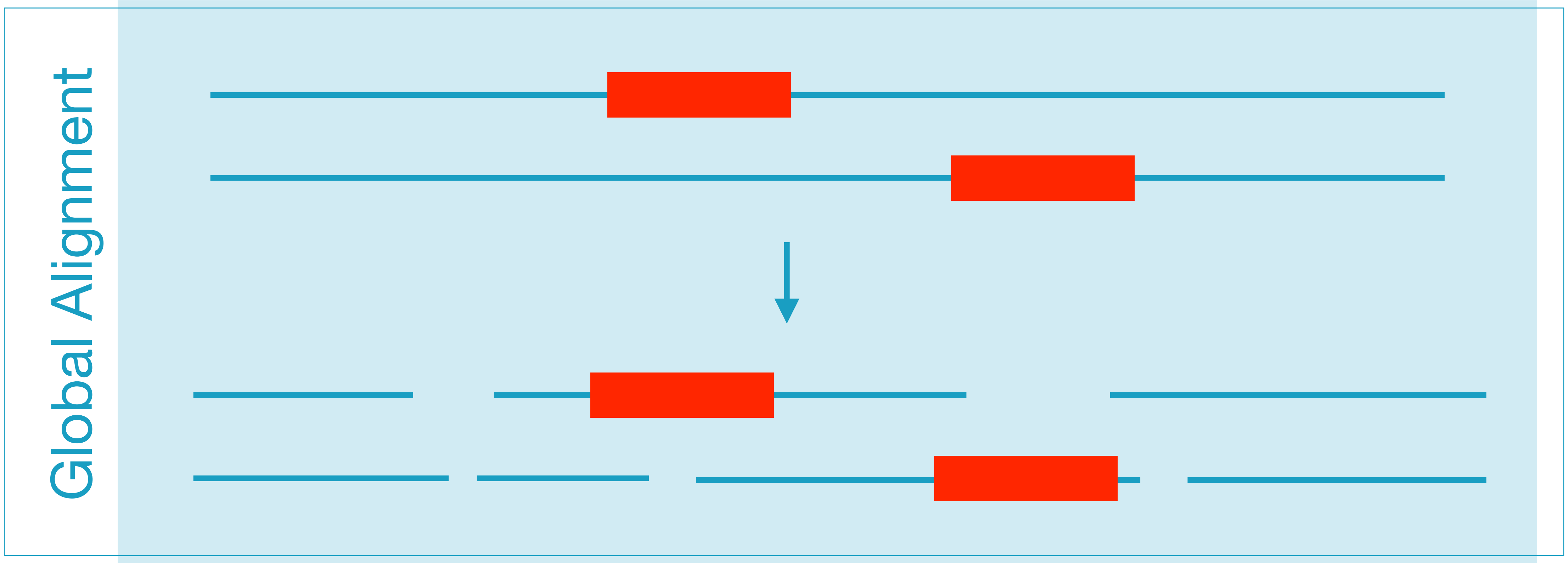
BLAST is the acronym for "**B**asic **L**ocal **A**lignment **S**earch **T**ool", which is a **local** alignment search tool first described by Altschul et al. (1990). NCBI started providing sequence alignment service to the public using BLAST in 1992, first through its blast email server (decommissioned in 2002) and later through the web (1997).

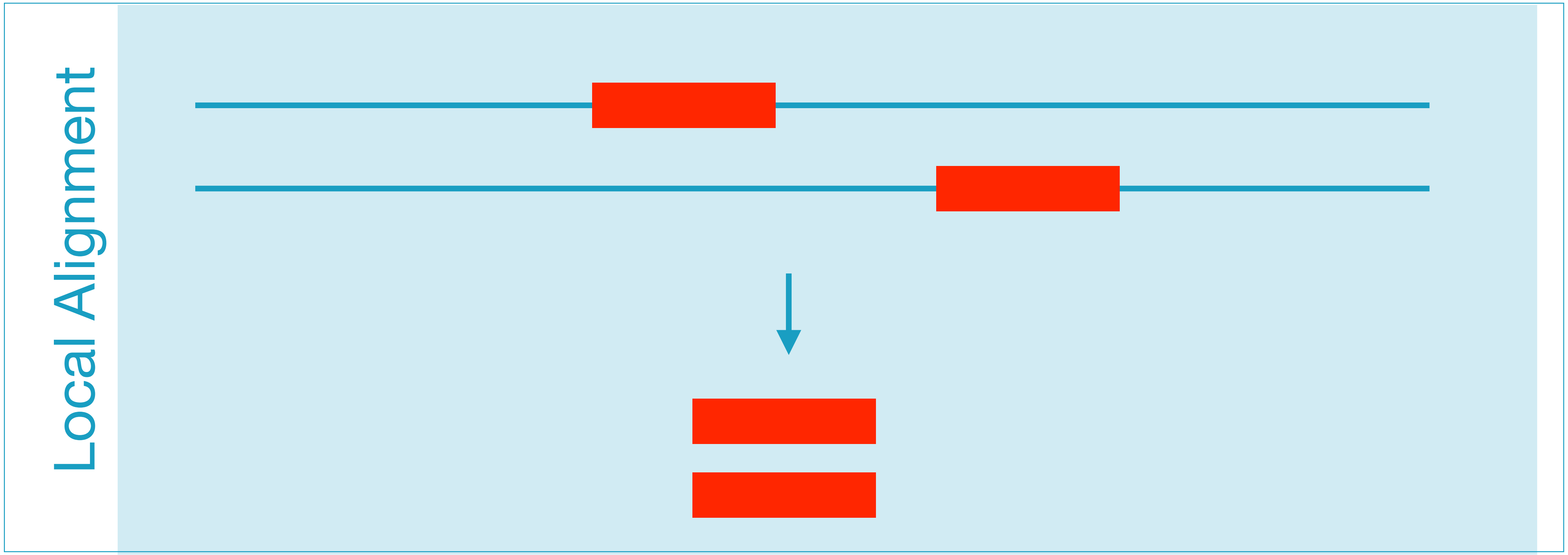

An Essential Guide to the Basic Local Alignment Search Tool
BLAST
O'REILLY®
Ian Korf, Mark Yandell & Joseph Bedell

BLAST
Authors: I Korf, M Yandell, J Bedell
Publisher: O'Reilly Media
Release Date: July 2003
Pages: 362

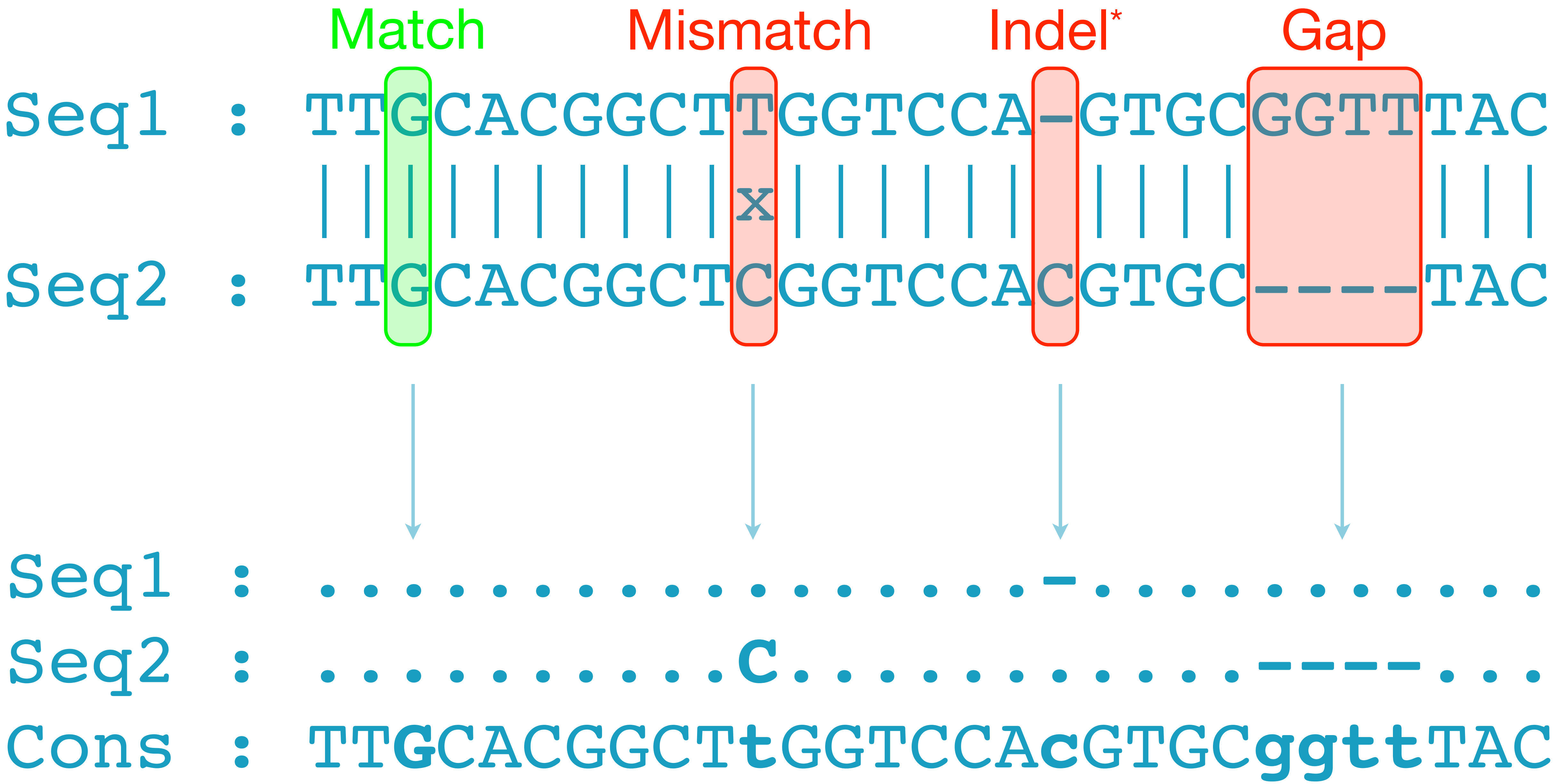
GDA21 ▷ BLAST Search (Extra)

GDC Genetic Diversity Centre Zurich





GDA21 ▷ BLAST Search (Extra)



```
Seq1 : TTGCACGGCTTGGTCCA-GTGCGGTTTAC
       ||||||||||x|||||| ||||    |||
Seq2 : TTGCACGGCTCGGTCCACGTGC----TAC

Seq1 : .................-...........
Seq2 : ..........C...........----...
Cons : TTGCACGGCTtGGTCCAcGTGCggttTAC
```

* indels: insertions & deletions

GDA21 ▷ BLAST Search (Extra)

BLAST finds the optimal alignment by using the **"word matching" algorithm**, in which BLAST does the search in several distinctive phases: 1) **generating overlapping words** from the input query, 2) scanning the database for **word matches** (hits), and 3) **extending word hits** to produce (local) alignments through multiple steps of extension.

During the first phase, BLAST breaks the input query into short overlapping segments (words/**seeds**). In the second phase BLAST takes those query words and **scans the target database** for initial matches. The nucleotide BLAST algorithm looks for any single exact word match. The protein BLAST algorithm uses a scoring threshold cutoff to identify matches. In addition, protein BLAST algorithm also requires two word hits within a certain distance in order to proceed to the next step.

GDA21 ▷ BLAST Search (Extra)

```
ATGCGGTCACGTCACG > query sequence
ATGCG      > word 1
 TGCGG     > word 2
  GCGGT    > word 3
   CGGTC   > word 4
    GGTCA  > word 5
     GTCAC > word 6
```

GDA21 ▷ BLAST Search (Extra)

Genetic
Diversity
Centre
Zurich

TCC

?

AAAAAAAAAAA

AGAGAGAGAGA

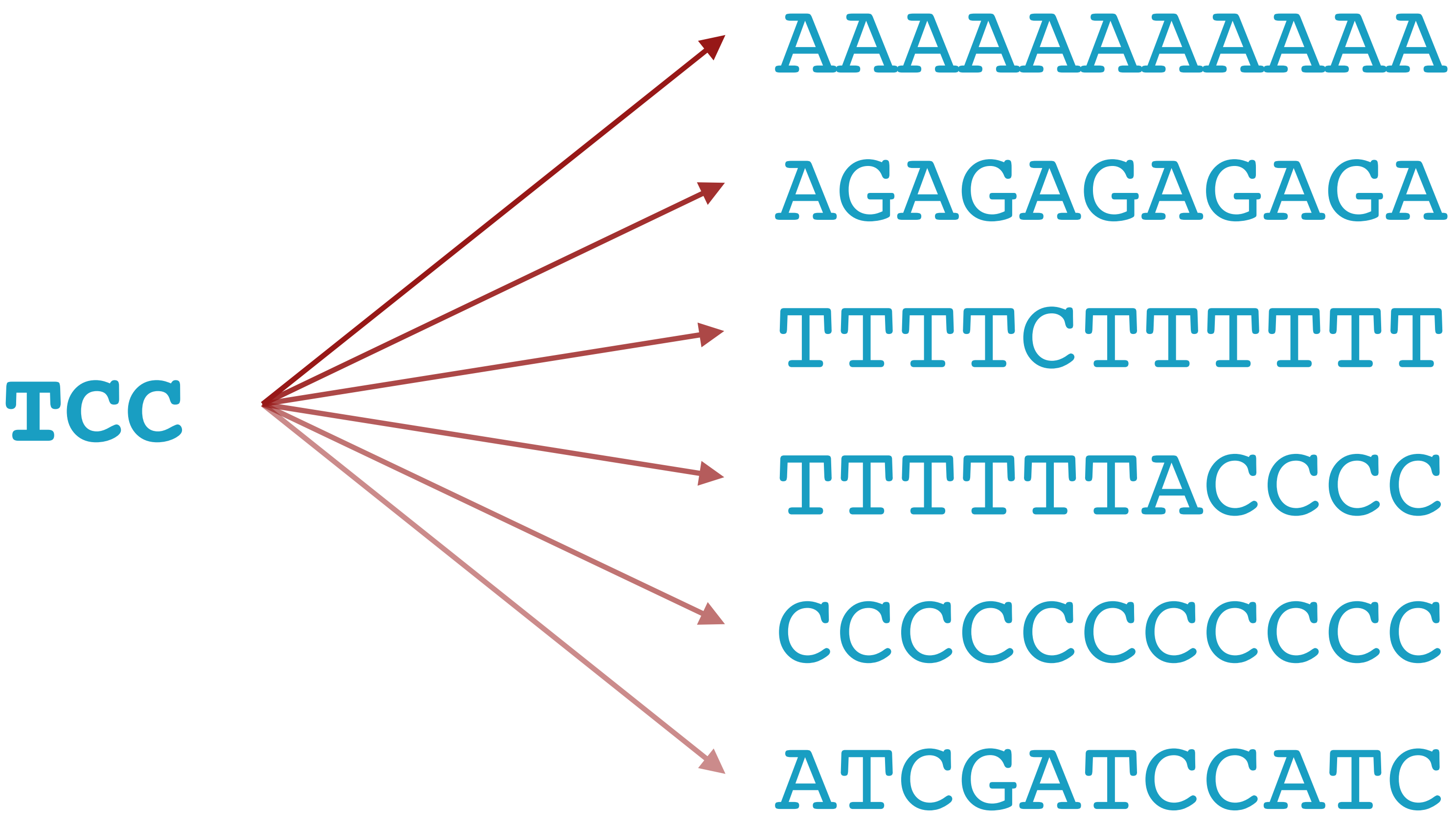
TTTTCTTTTTT

TTTTTTACCCC

CCCCCCCCCCC

ATCGATCCATC

GDA21 ▷ BLAST Search (Extra)



TCC

AAAAAAAAAAA

AGAGAGAGAGA

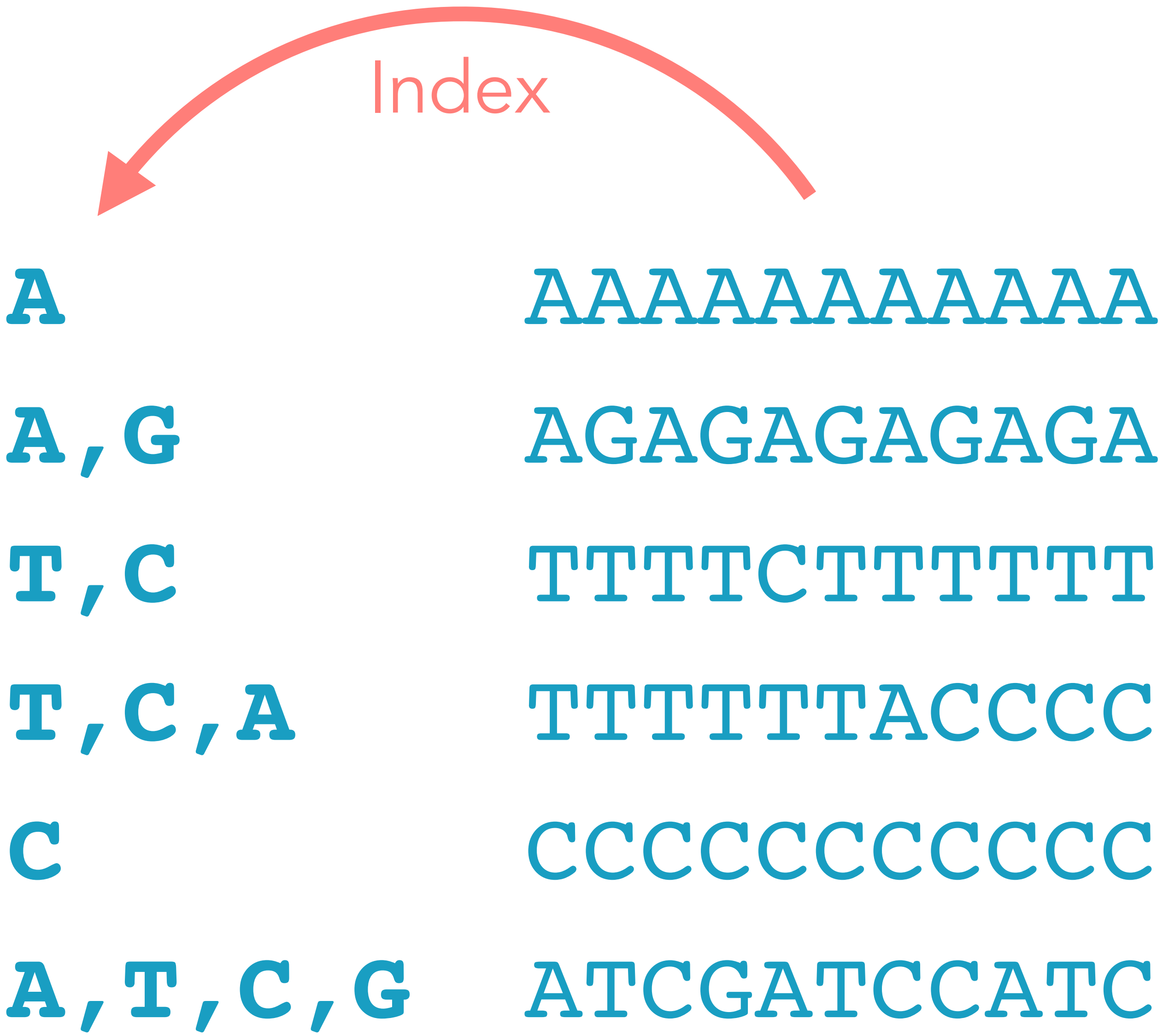
TTTTCTTTTTT

TTTTTTACCCC

CCCCCCCCCCC

ATCGATCCATC

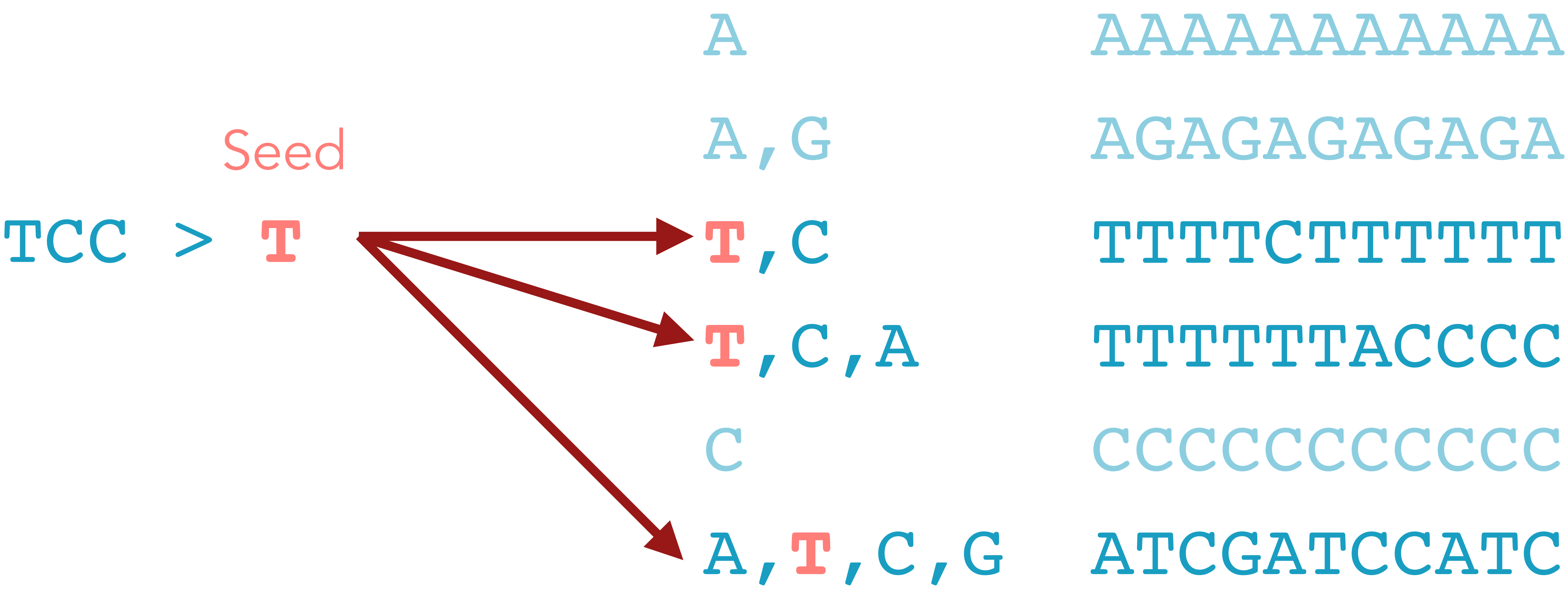
GDA21 ▷ BLAST Search (Extra)



Index

A	AAAAAAAAAAA
A,G	AGAGAGAGAGA
T,C	TTTTCTTTTTT
T,C,A	TTTTTTACCCC
C	CCCCCCCCCCC
A,T,C,G	ATCGATCCATC

GDA21 ▷ BLAST Search (Extra)



Seed

TCC > T

A AAAAAAAAAAA

A,G AGAGAGAGAGA

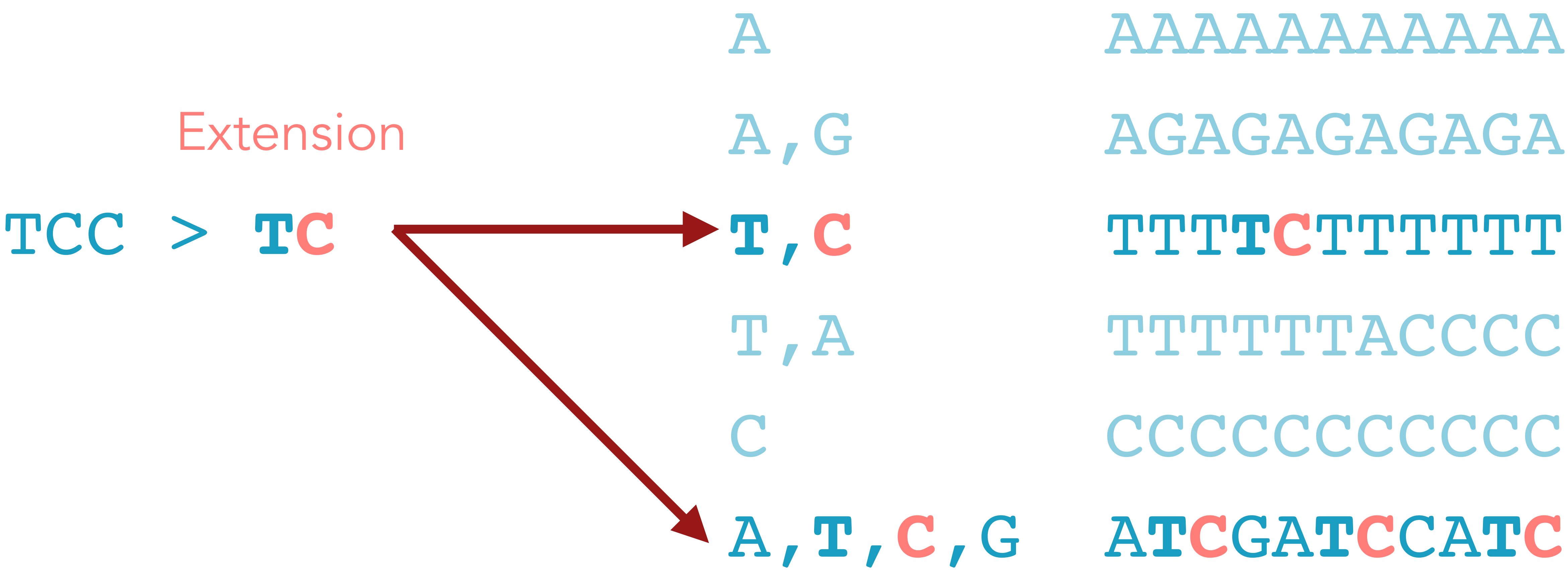
T,C TTTTCTTTTTT

T,C,A TTTTTTACCCC

C CCCCCCCCCCC

A,T,C,G ATCGATCCATC

BLAST Search (Extra)

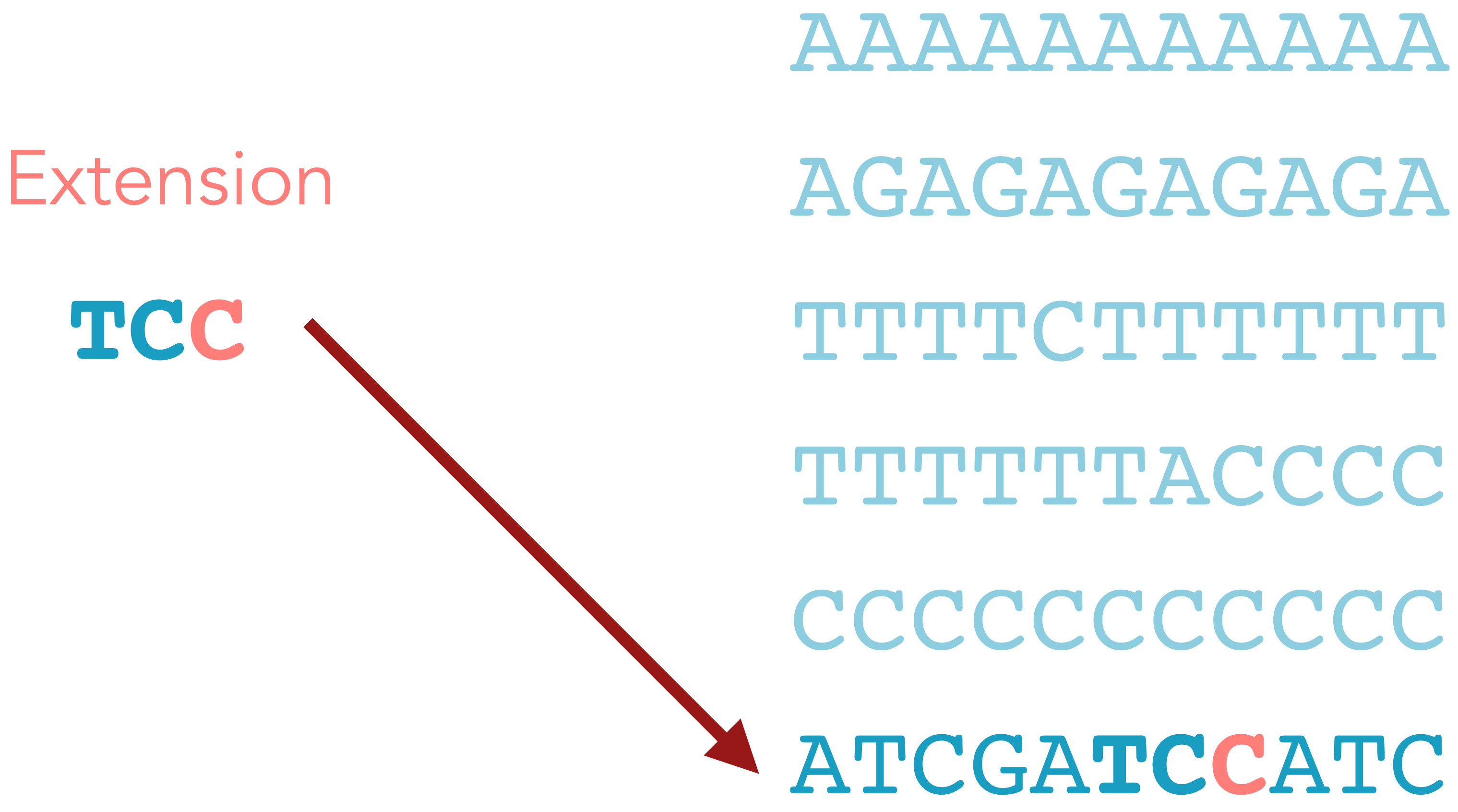


Extension

TCC > TC

A	AAAAAAAAAAA
A,G	AGAGAGAGAGA
T,C	TTT**T**CTTTTTT
T,A	TTTTTTACCCC
C	CCCCCCCCCCC
A,**T**,C,G	A**T**CGA**T**CCA**T**C

GDA21 ▷ BLAST Search (Extra)



Extension

TCC

AAAAAAAAAAA

AGAGAGAGAGA

TTTTCTTTTTT

TTTTTTACCCC

CCCCCCCCCCC

ATCGATCCATC

Global Alignment

```
  Query >   1 -----TCC--- 11
        Gaps >       ||| < Matches
Subject >   1 ATCGATCCATC 11
```

GDA21 ▷ BLAST Search (Extra)

Local Alignment

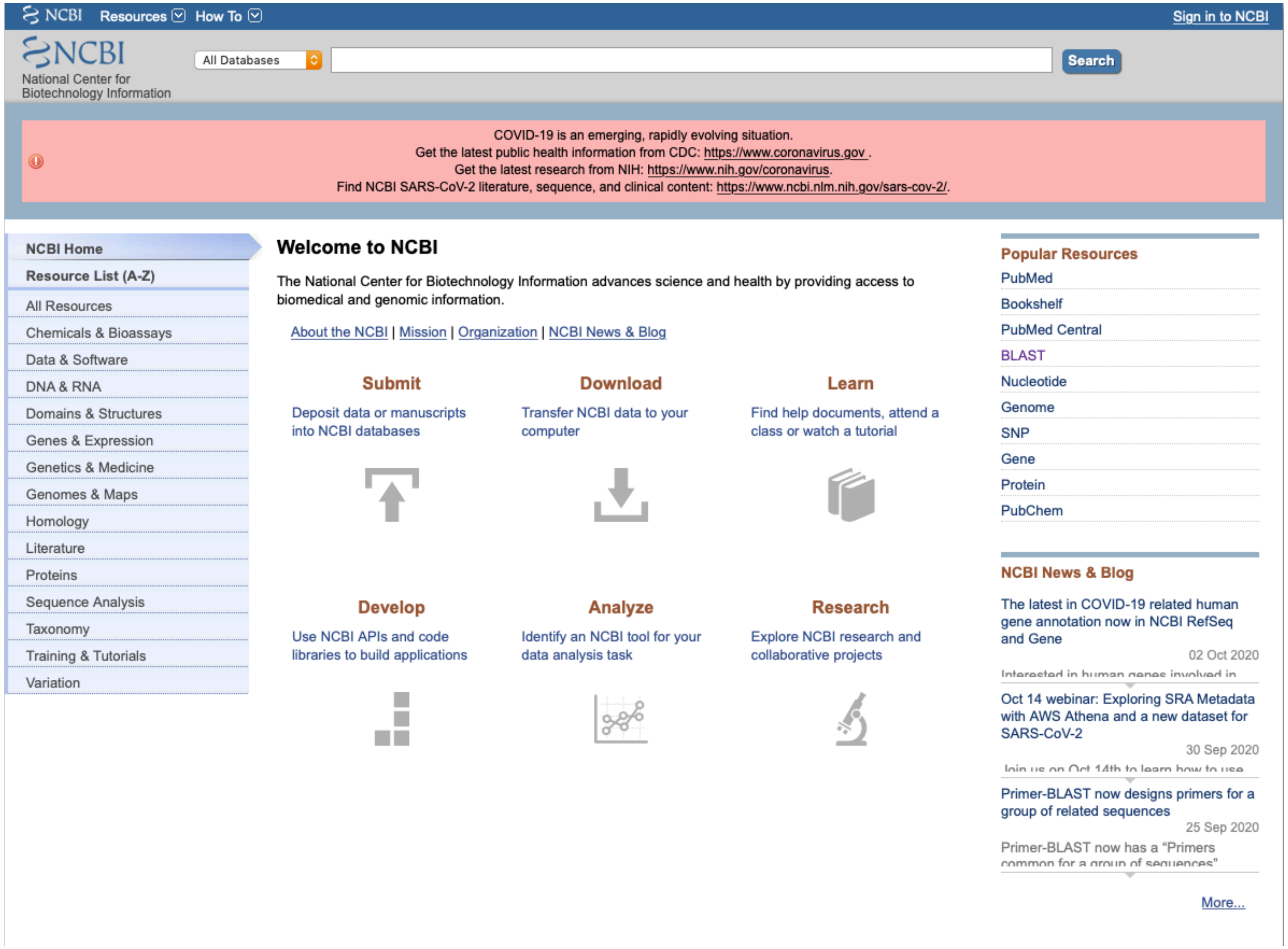
Identities **3/3** (100%)

```
Query   1 TCC 3
          |||
Subject 6 TCC 9
```

GDA21 ▷ BLAST Search (Extra)

National Center for Biotechnology Information

NCBI



COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: https://www.coronavirus.gov .
Get the latest research from NIH: https://www.nih.gov/coronavirus.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: https://www.ncbi.nlm.nih.gov/sars-cov-2/.

Welcome to NCBI

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Protein
PubChem

NCBI News & Blog

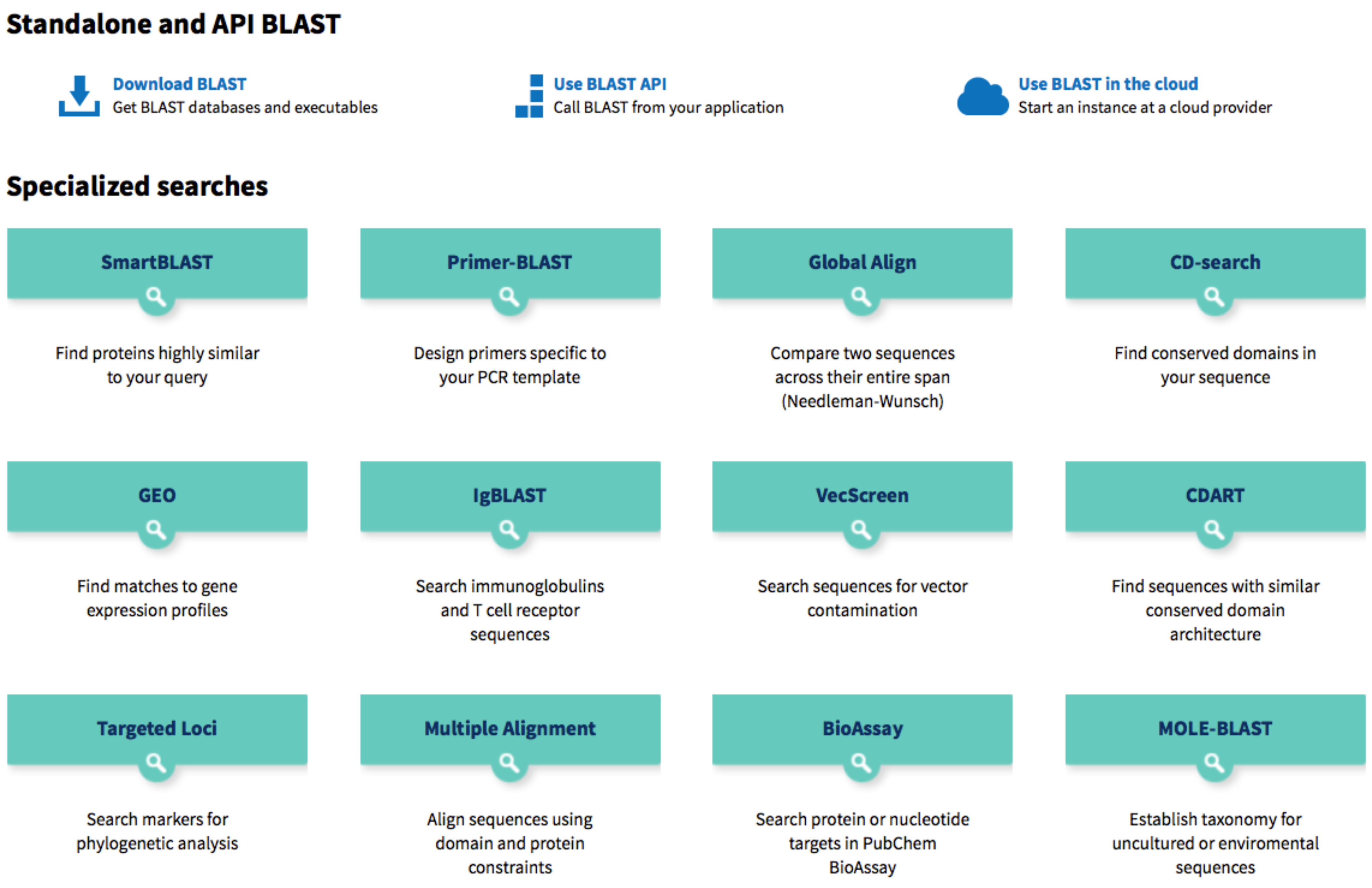
The latest in COVID-19 related human gene annotation now in NCBI RefSeq and Gene
02 Oct 2020
Interested in human genes involved in

Oct 14 webinar: Exploring SRA Metadata with AWS Athena and a new dataset for SARS-CoV-2
30 Sep 2020
Join us on Oct 14th to learn how to use

Primer-BLAST now designs primers for a group of related sequences
25 Sep 2020
Primer-BLAST now has a "Primers common for a group of sequences"

More...

GDA21 ▷ BLAST Search (Extra)



Standalone and API BLAST

Download BLAST
Get BLAST databases and executables

Use BLAST API
Call BLAST from your application

Use BLAST in the cloud
Start an instance at a cloud provider

Specialized searches

SmartBLAST
Find proteins highly similar to your query

Primer-BLAST
Design primers specific to your PCR template

Global Align
Compare two sequences across their entire span (Needleman-Wunsch)

CD-search
Find conserved domains in your sequence

GEO
Find matches to gene expression profiles

IgBLAST
Search immunoglobulins and T cell receptor sequences

VecScreen
Search sequences for vector contamination

CDART
Find sequences with similar conserved domain architecture

Targeted Loci
Search markers for phylogenetic analysis

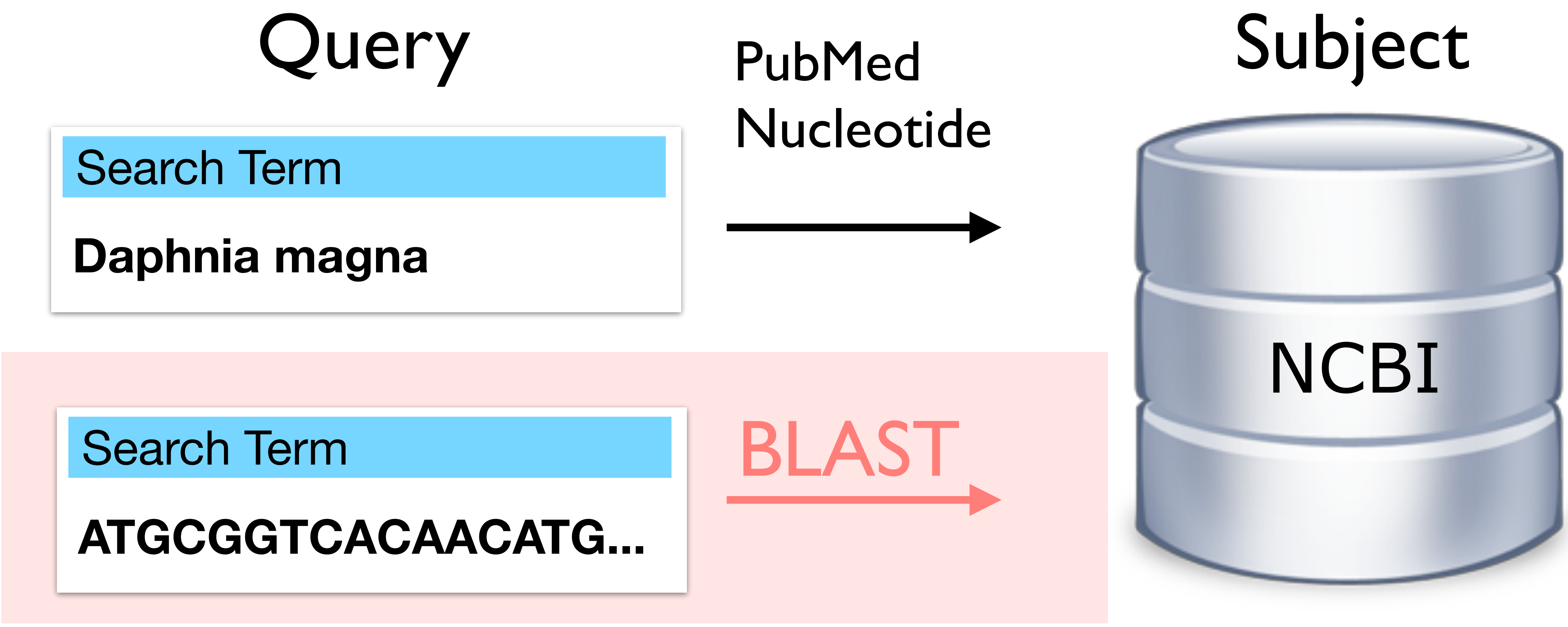
Multiple Alignment
Align sequences using domain and protein constraints

BioAssay
Search protein or nucleotide targets in PubChem BioAssay

MOLE-BLAST
Establish taxonomy for uncultured or enviromental sequences

GDA21 ▷ BLAST Search (Extra)

Genetic Diversity Centre Zurich



Query

Search Term

Daphnia magna

PubMed
Nucleotide

Search Term

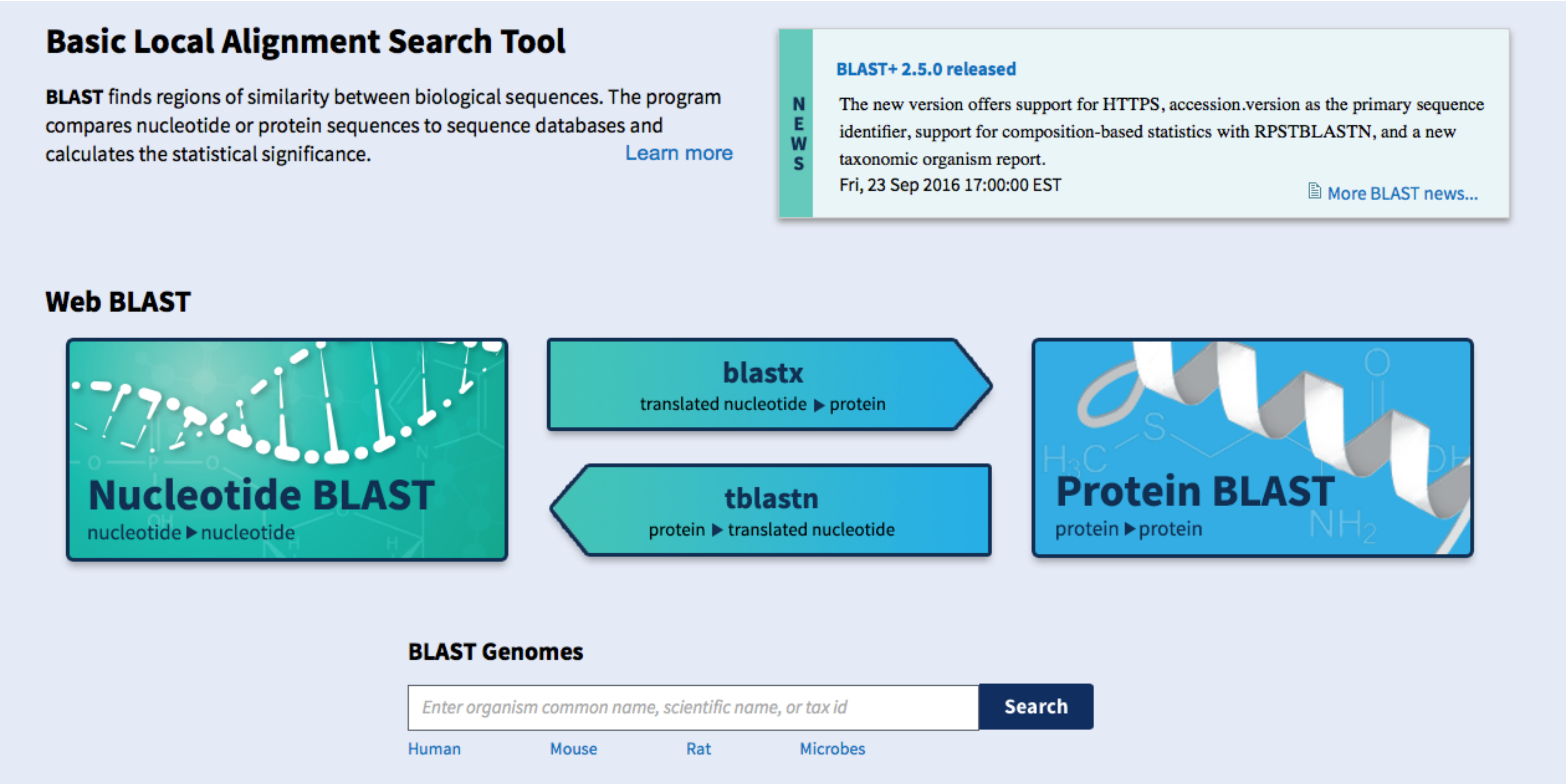
ATGCGGTCACAACATG...

BLAST

Subject

NCBI

GDA21 ▷ BLAST Search (Extra)



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

BLAST+ 2.5.0 released

The new version offers support for HTTPS, accession.version as the primary sequence identifier, support for composition-based statistics with RPSTBLASTN, and a new taxonomic organism report.

Fri, 23 Sep 2016 17:00:00 EST

More BLAST news...

Web BLAST

BLAST Genomes

Enter organism common name, scientific name, or tax id [Search]

Human Mouse Rat Microbes

GDA21 ▷ BLAST Search (Extra)

search	input		query		database
blastn	nt	→	nt	→	nt
blastp	pr	→	pr	→	pr
blastx	nt	→	pr (6)	→	pr
tblastn	pr	→	pr	→	pr (6)
tblastx	nt	→	pr (6)	→	pr (6)

blastn compares nucleotide queries to a nucleotide database

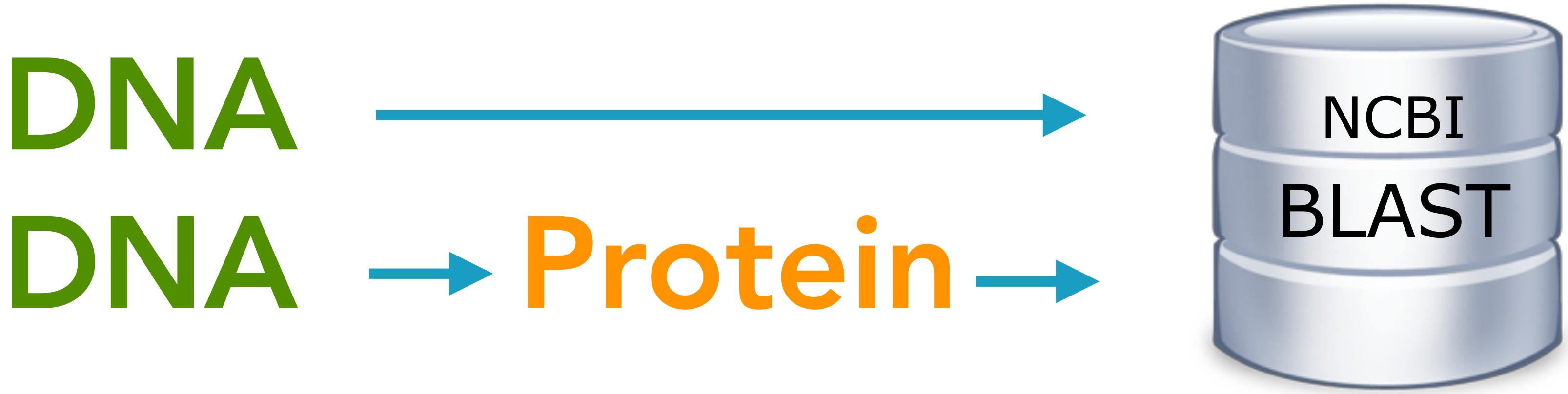
blastp compares protein queries to a protein database

blastx compares a nucleotide query translated in all six reading frames against a protein database

tblastn compares a protein query against a nucleotide sequence database dynamically translated in all six reading frames

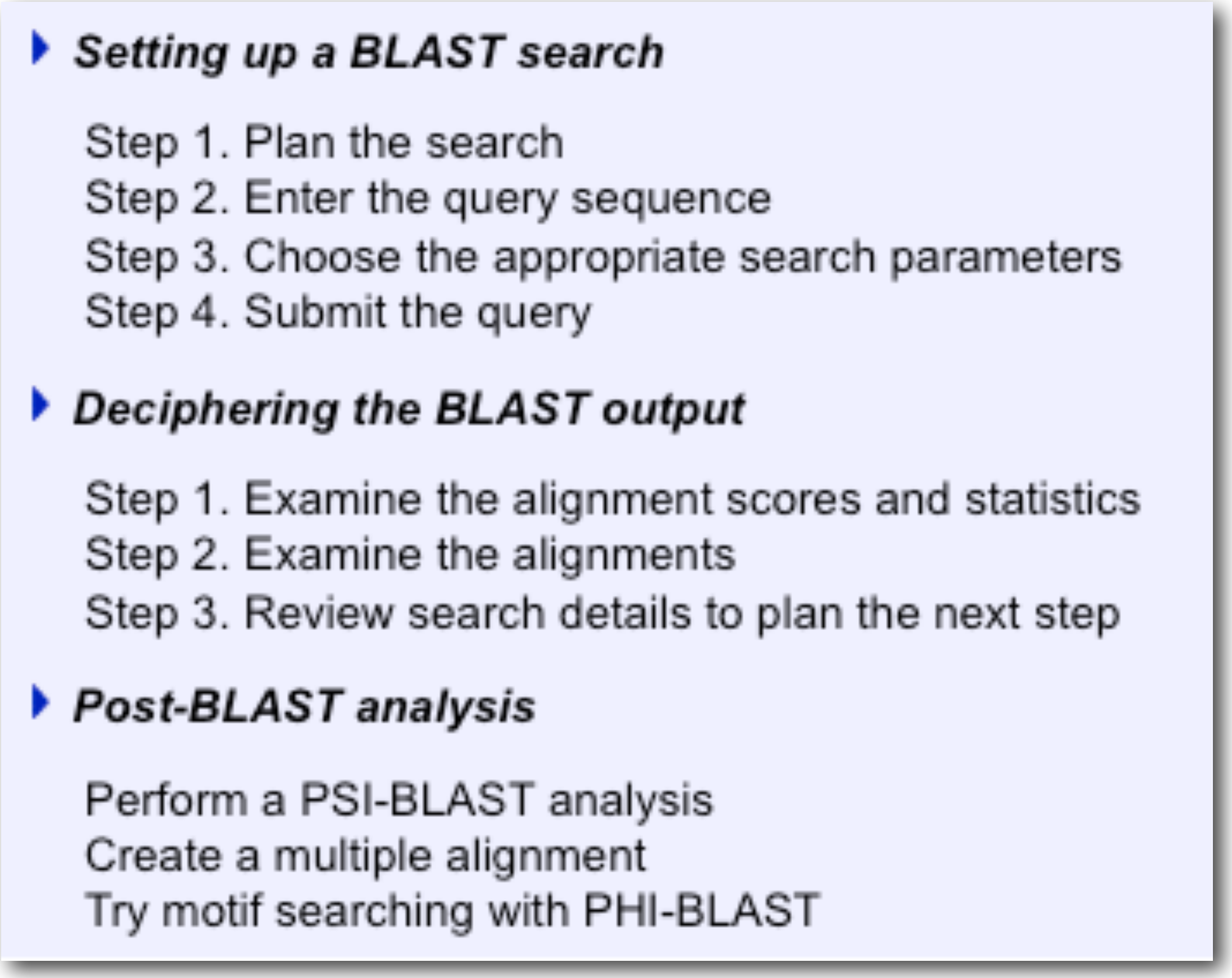
tblastx compares a nucleotide query in all six reading frames against a nucleotide sequence database in all six reading frames

GDA21 ▷ BLAST Search (Extra)



Nucleotide–nucleotide searches are beneficial because no information is lost in the alignment. When a codon is translated from nucleotides to amino acids, approximately 69% of the complexity is lost (4^3=64 possible nucleotide combinations mapped to 20 amino acids). In contrast, however, **the true physical relationship between two coding sequences is best captured in the translated view**. Matrices that take into account physical properties, such as PAM and BLOSUM, can be used to add power to the search. Additionally, in a nucleotide search, there are only four possible character states compared to 20 in an amino acid search. Thus the **probability of a match** due to chance versus a match due to common ancestry (identify in state versus identical by descent) is higher.

GDA21 ▷ BLAST Search (Extra)



- ***Setting up a BLAST search***

  Step 1. Plan the search
  Step 2. Enter the query sequence
  Step 3. Choose the appropriate search parameters
  Step 4. Submit the query

- ***Deciphering the BLAST output***

  Step 1. Examine the alignment scores and statistics
  Step 2. Examine the alignments
  Step 3. Review search details to plan the next step

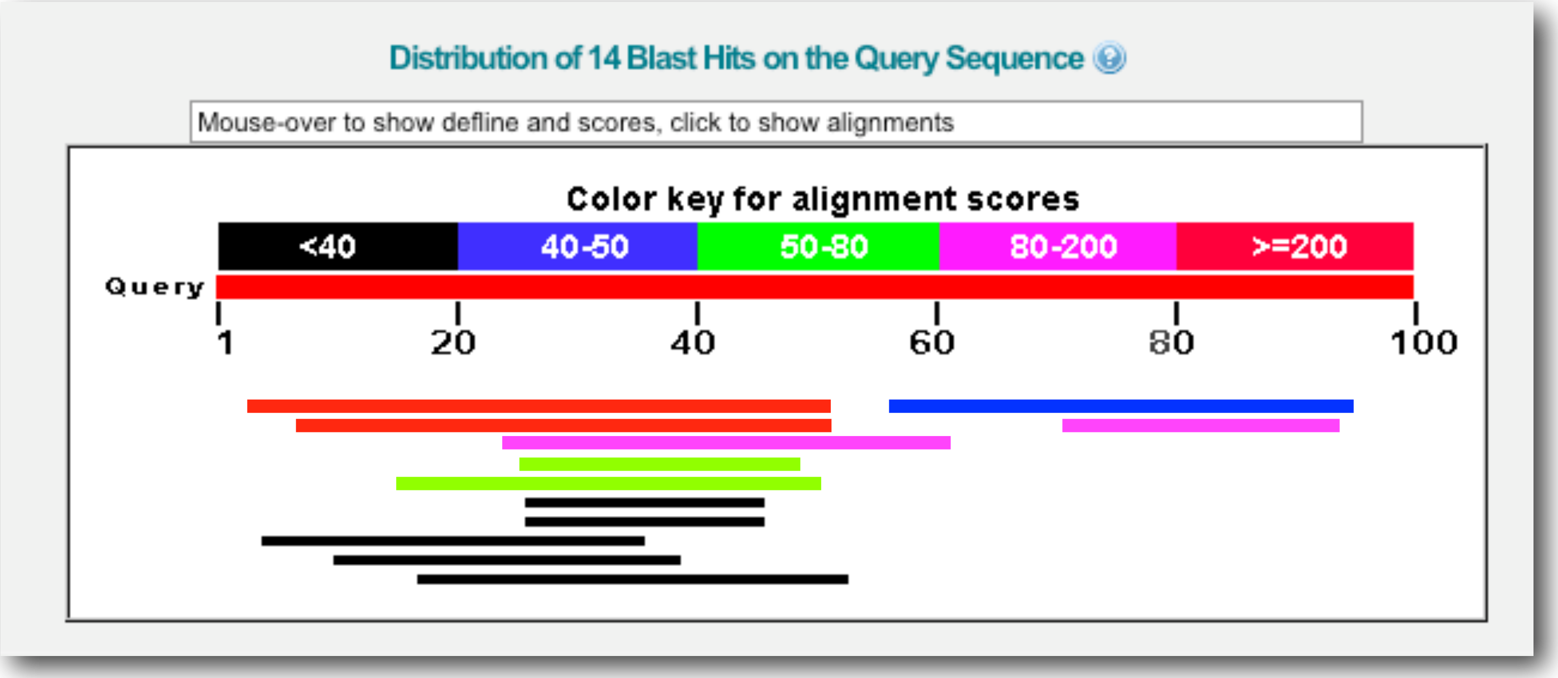
- ***Post-BLAST analysis***

  Perform a PSI-BLAST analysis
  Create a multiple alignment
  Try motif searching with PHI-BLAST

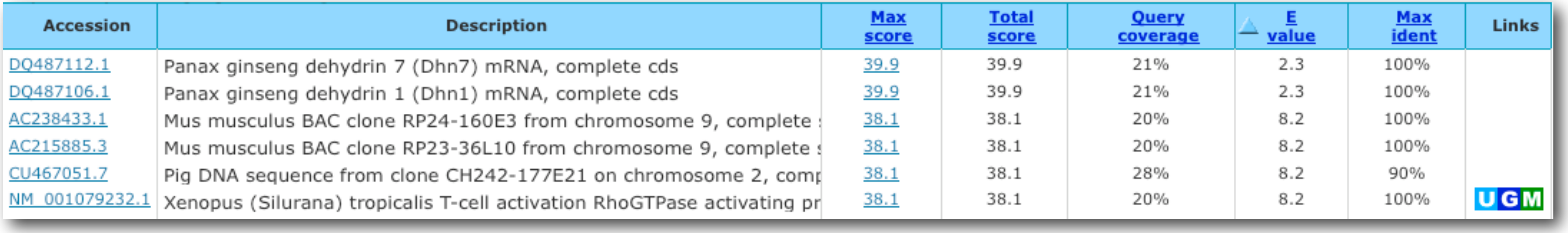
GDA21 ▷ BLAST Search (Extra)

Graphic Summary

Bit Score


Distribution of 14 Blast Hits on the Query Sequence
Mouse-over to show defline and scores, click to show alignments
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
20
40
60
80
100

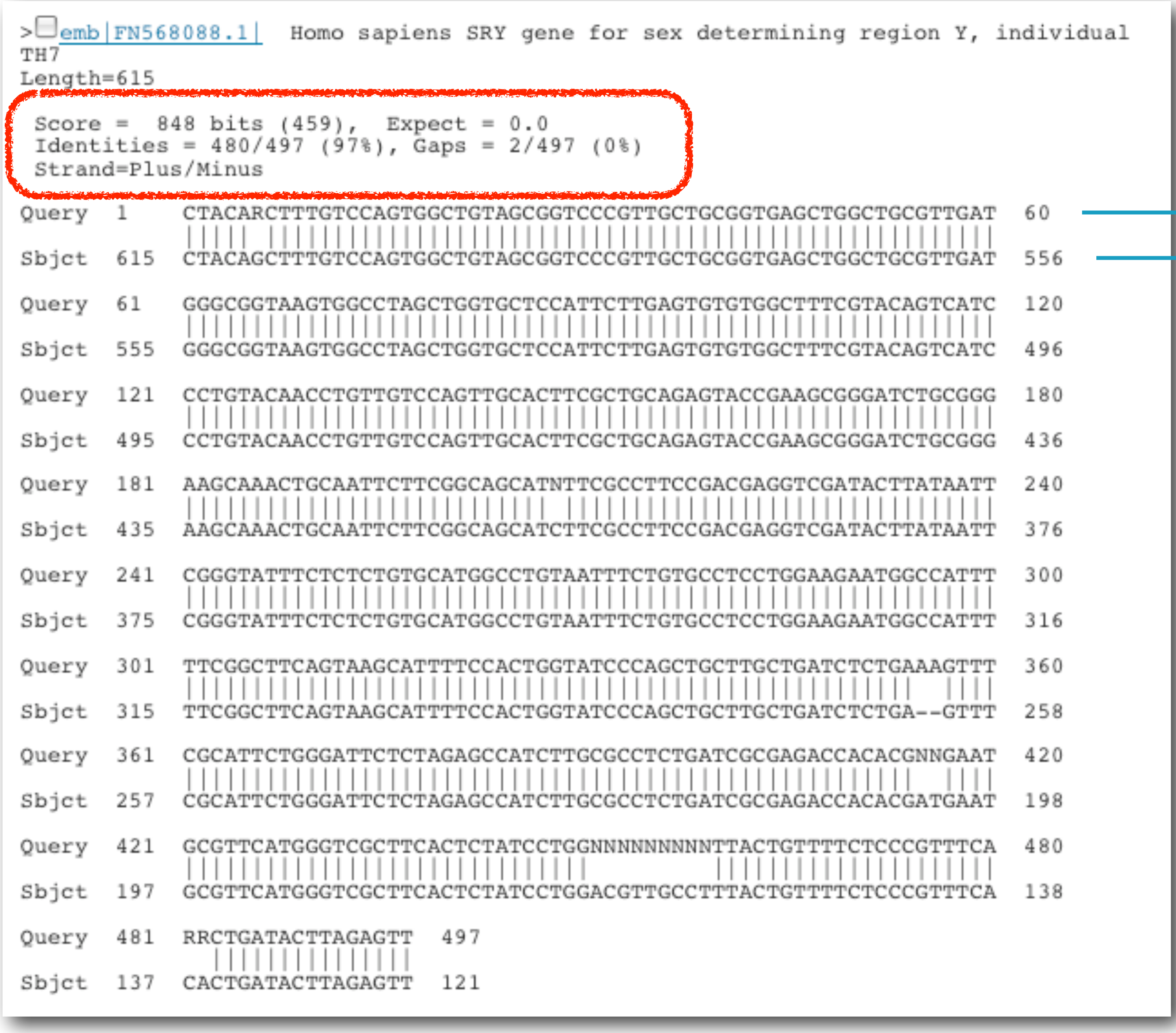
click here to see the nr entry (accession)



Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
DQ487112.1	Panax ginseng dehydrin 7 (Dhn7) mRNA, complete cds	39.9	39.9	21%	2.3	100%	
DQ487106.1	Panax ginseng dehydrin 1 (Dhn1) mRNA, complete cds	39.9	39.9	21%	2.3	100%	
AC238433.1	Mus musculus BAC clone RP24-160E3 from chromosome 9, complete	38.1	38.1	20%	8.2	100%	
AC215885.3	Mus musculus BAC clone RP23-36L10 from chromosome 9, complete	38.1	38.1	20%	8.2	100%	
CU467051.7	Pig DNA sequence from clone CH242-177E21 on chromosome 2, comp	38.1	38.1	28%	8.2	90%	
NM_001079232.1	Xenopus (Silurana) tropicalis T-cell activation RhoGTPase activating pr	38.1	38.1	20%	8.2	100%	UGM

click here to see the corresponding alignment

Nucleotide Alignment



```
>□emb|FN568088.1|  Homo sapiens SRY gene for sex determining region Y, individual
TH7
Length=615

 Score =  848 bits (459),  Expect = 0.0
 Identities = 480/497 (97%), Gaps = 2/497 (0%)
 Strand=Plus/Minus

Query  1    CTACARCTTTGTCCAGTGGCTGTAGCGGTCCCGTTGCTGCGGTGAGCTGGCTGCGTTGAT  60
            ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  615  CTACAGCTTTGTCCAGTGGCTGTAGCGGTCCCGTTGCTGCGGTGAGCTGGCTGCGTTGAT  556

Query  61   GGGCGGTAAGTGGCCTAGCTGGTGCTCCATTCTTGAGTGTGTGGCTTTCGTACAGTCATC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555  GGGCGGTAAGTGGCCTAGCTGGTGCTCCATTCTTGAGTGTGTGGCTTTCGTACAGTCATC  496

Query  121  CCTGTACAACCTGTTGTCCAGTTGCACTTCGCTGCAGAGTACCGAAGCGGGATCTGCGGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495  CCTGTACAACCTGTTGTCCAGTTGCACTTCGCTGCAGAGTACCGAAGCGGGATCTGCGGG  436

Query  181  AAGCAAACTGCAATTCTTCGGCAGCATNTTCGCCTTCCGACGAGGTCGATACTTATAATT  240
            ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  435  AAGCAAACTGCAATTCTTCGGCAGCATCTTCGCCTTCCGACGAGGTCGATACTTATAATT  376

Query  241  CGGGTATTTCTCTCTGTGCATGGCCTGTAATTTCTGTGCCTCCTGGAAGAATGGCCATTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375  CGGGTATTTCTCTCTGTGCATGGCCTGTAATTTCTGTGCCTCCTGGAAGAATGGCCATTT  316

Query  301  TTCGGCTTCAGTAAGCATTTTCCACTGGTATCCCAGCTGCTTGCTGATCTCTGAAAGTTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||  ||||
Sbjct  315  TTCGGCTTCAGTAAGCATTTTCCACTGGTATCCCAGCTGCTTGCTGATCTCTGA--GTTT  258

Query  361  CGCATTCTGGGATTCTCTAGAGCCATCTTGCGCCTCTGATCGCGAGACCACACGNNGAAT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||  ||||
Sbjct  257  CGCATTCTGGGATTCTCTAGAGCCATCTTGCGCCTCTGATCGCGAGACCACACGATGAAT  198

Query  421  GCGTTCATGGGTCGCTTCACTCTATCCTGGNNNNNNNNNTTACTGTTTTCTCCCGTTTCA  480
            ||||||||||||||||||||||||||||||         |||||||||||||||||||||
Sbjct  197  GCGTTCATGGGTCGCTTCACTCTATCCTGGACGTTGCCTTTACTGTTTTCTCCCGTTTCA  138

Query  481  RRCTGATACTTAGAGTT  497
              |||||||||||||||
Sbjct  137  CACTGATACTTAGAGTT  121
```

query sequence

database sequence

GDA21 ▷ BLAST Search (Extra)

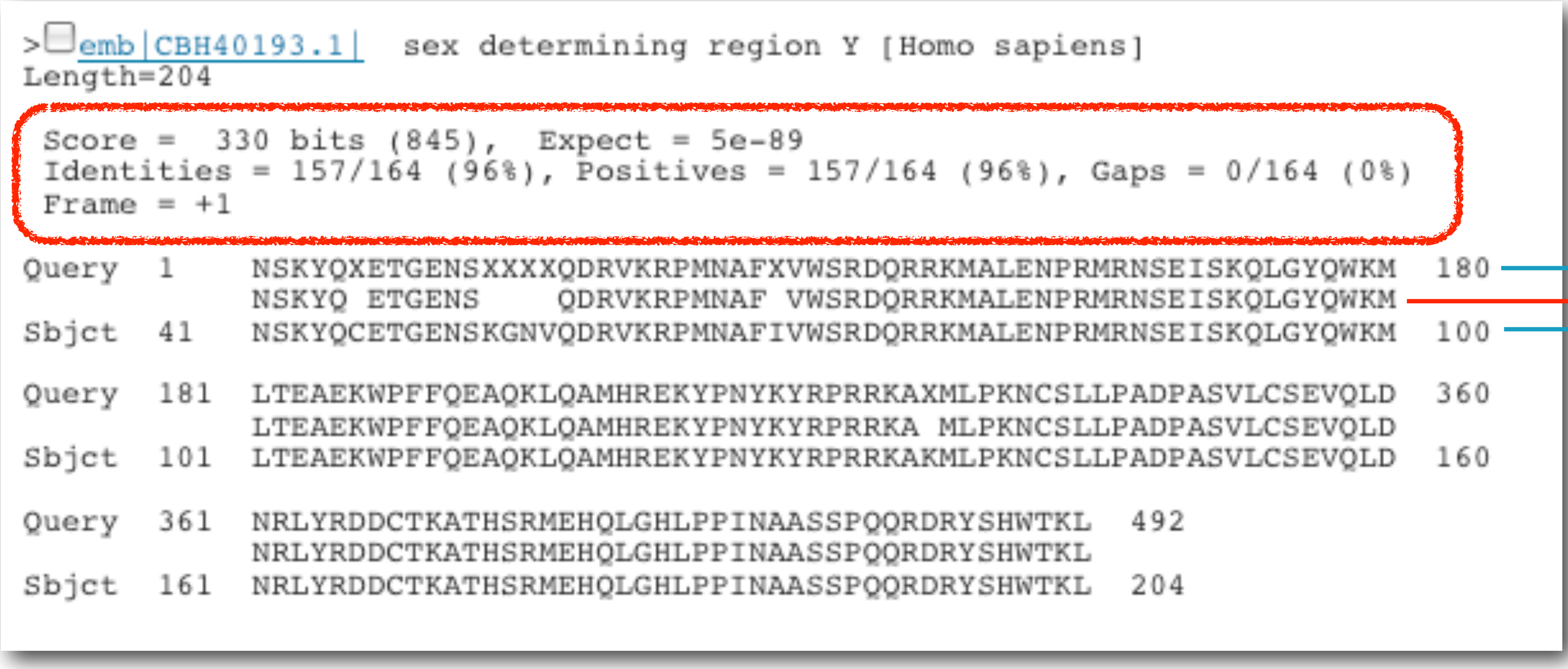
Alignment - The process of lining up two or more sequences to achieve **maximal levels of identity** (and conservation, in the case of amino acid sequences) for the purpose of assessing the **degree of similarity** and the **possibility of homology**.

Identity - The extent to which two (nucleotide or amino acid) sequences are invariant.

$$\boxed{PID = \frac{\#\,\text{of identical aa or nt}}{\#\,\text{of total aa or nt}} \times 100}$$

GDA21 ▷ BLAST Search (Extra)

Protein Alignment



```
>□emb|CBH40193.1|  sex determining region Y [Homo sapiens]
Length=204

 Score =  330 bits (845),  Expect = 5e-89
 Identities = 157/164 (96%), Positives = 157/164 (96%), Gaps = 0/164 (0%)
 Frame = +1

Query  1    NSKYQXETGENSXXXXQDRVKRPMNAFXVWSRDQRRKMALENPRMRNSEISKQLGYQWKM  180
            NSKYQ ETGENS    QDRVKRPMNAF VWSRDQRRKMALENPRMRNSEISKQLGYQWKM
Sbjct  41   NSKYQCETGENSKGNVQDRVKRPMNAFIVWSRDQRRKMALENPRMRNSEISKQLGYQWKM  100

Query  181  LTEAEKWPFFQEAQKLQAMHREKYPNYKYRPRRKAXMLPKNCSLLPADPASVLCSEVQLD  360
            LTEAEKWPFFQEAQKLQAMHREKYPNYKYRPRRKA MLPKNCSLLPADPASVLCSEVQLD
Sbjct  101  LTEAEKWPFFQEAQKLQAMHREKYPNYKYRPRRKAKMLPKNCSLLPADPASVLCSEVQLD  160

Query  361  NRLYRDDCTKATHSRMEHQLGHLPPINAASSPQQRDRYSHWTKL  492
            NRLYRDDCTKATHSRMEHQLGHLPPINAASSPQQRDRYSHWTKL
Sbjct  161  NRLYRDDCTKATHSRMEHQLGHLPPINAASSPQQRDRYSHWTKL  204
```

query sequence

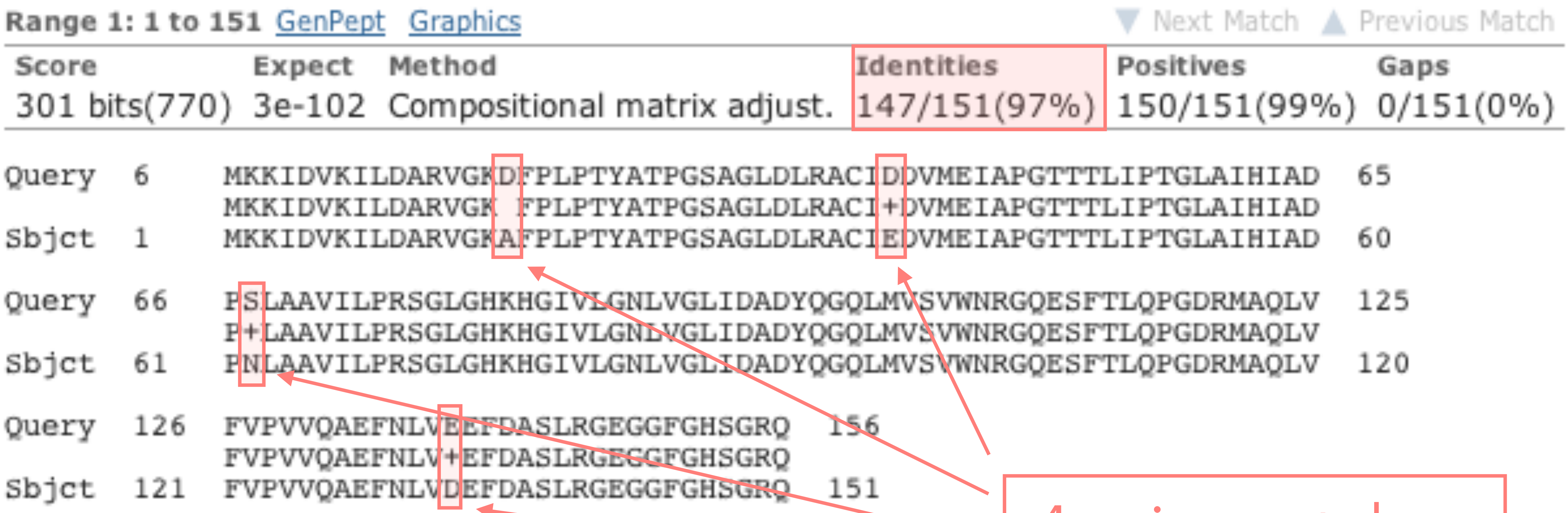
database sequence

matching sequence

GDA21 ▷ BLAST Search (Extra)

Protein Alignment - **Identities**

151 - 4 mis-matches = 147 identities



Range 1: 1 to 151 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
301 bits(770)	3e-102	Compositional matrix adjust.	147/151(97%)	150/151(99%)	0/151(0%)

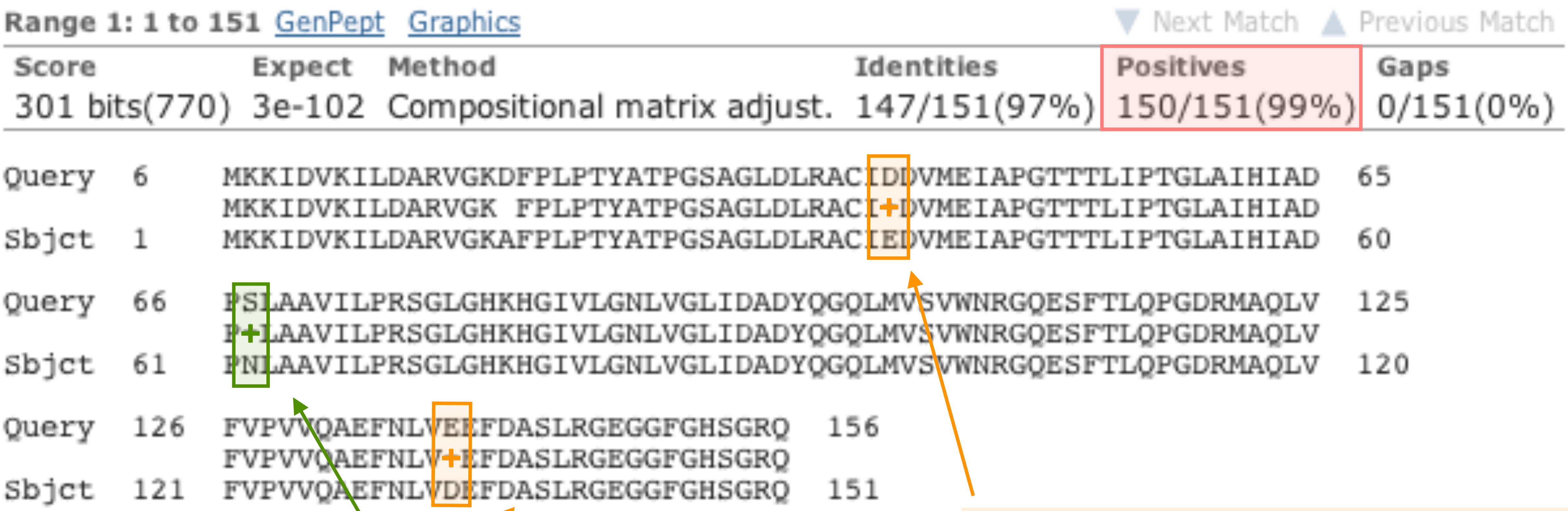
```
Query  6    MKKIDVKILDARVGKDFPLPTYATPGSAGLDLRACIDDVMEIAPGTTTLIPTGLAIHIAD  65
            MKKIDVKILDARVGK FPLPTYATPGSAGLDLRACI+DVMEIAPGTTTLIPTGLAIHIAD
Sbjct  1    MKKIDVKILDARVGKAFPLPTYATPGSAGLDLRACIEDVMEIAPGTTTLIPTGLAIHIAD  60

Query  66   PSLAAVILPRSGLGHKHGIVLGNLVGLIDADYQGQLMVSVWNRGQESFTLQPGDRMAQLV  125
            P+LAAVILPRSGLGHKHGIVLGNLVGLIDADYQGQLMVSVWNRGQESFTLQPGDRMAQLV
Sbjct  61   PNLAAVILPRSGLGHKHGIVLGNLVGLIDADYQGQLMVSVWNRGQESFTLQPGDRMAQLV  120

Query  126  FVPVVQAEFNLVEEFDASLRGEGGFGHSGRQ  156
            FVPVVQAEFNLV+EFDASLRGEGGFGHSGRQ
Sbjct  121  FVPVVQAEFNLVDEFDASLRGEGGFGHSGRQ  151
```

4 mis-matches

GDA21 ▷ BLAST Search (Extra)

Protein Alignment - **Positives**

147 identities + 3 similar = 150 positives



Range 1: 1 to 151 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
301 bits(770)	3e-102	Compositional matrix adjust.	147/151(97%)	150/151(99%)	0/151(0%)

```
Query  6    MKKIDVKILDARVGKDFPLPTYATPGSAGLDLRACIDDVMEIAPGTTTLIPTGLAIHIAD  65
            MKKIDVKILDARVGK FPLPTYATPGSAGLDLRACI+DVMEIAPGTTTLIPTGLAIHIAD
Sbjct  1    MKKIDVKILDARVGKAFPLPTYATPGSAGLDLRACIEDVMEIAPGTTTLIPTGLAIHIAD  60

Query  66   PSLAAVILPRSGLGHKHGIVLGNLVGLIDADYQGQLMVSVWNRGQESFTLQPGDRMAQLV  125
            P+LAAVILPRSGLGHKHGIVLGNLVGLIDADYQGQLMVSVWNRGQESFTLQPGDRMAQLV
Sbjct  61   PNLAAVILPRSGLGHKHGIVLGNLVGLIDADYQGQLMVSVWNRGQESFTLQPGDRMAQLV  120

Query  126  FVPVVQAEFNLVEEFDASLRGEGGFGHSGRQ  156
            FVPVVQAEFNLV+EFDASLRGEGGFGHSGRQ
Sbjct  121  FVPVVQAEFNLVDEFDASLRGEGGFGHSGRQ  151
```

Glutamate (E) - Aspartate (D)

Serine (S) - Asparagine (N)

GDA21 ▷ BLAST Search (Extra)

Alignment - The process of lining up two or more sequences to achieve **maximal levels of identity** (and conservation, in the case of amino acid sequences) for the purpose of assessing the **degree of similarity** and the **possibility of homology**.

Identity - The extent to which two (nucleotide or amino acid) sequences are invariant.

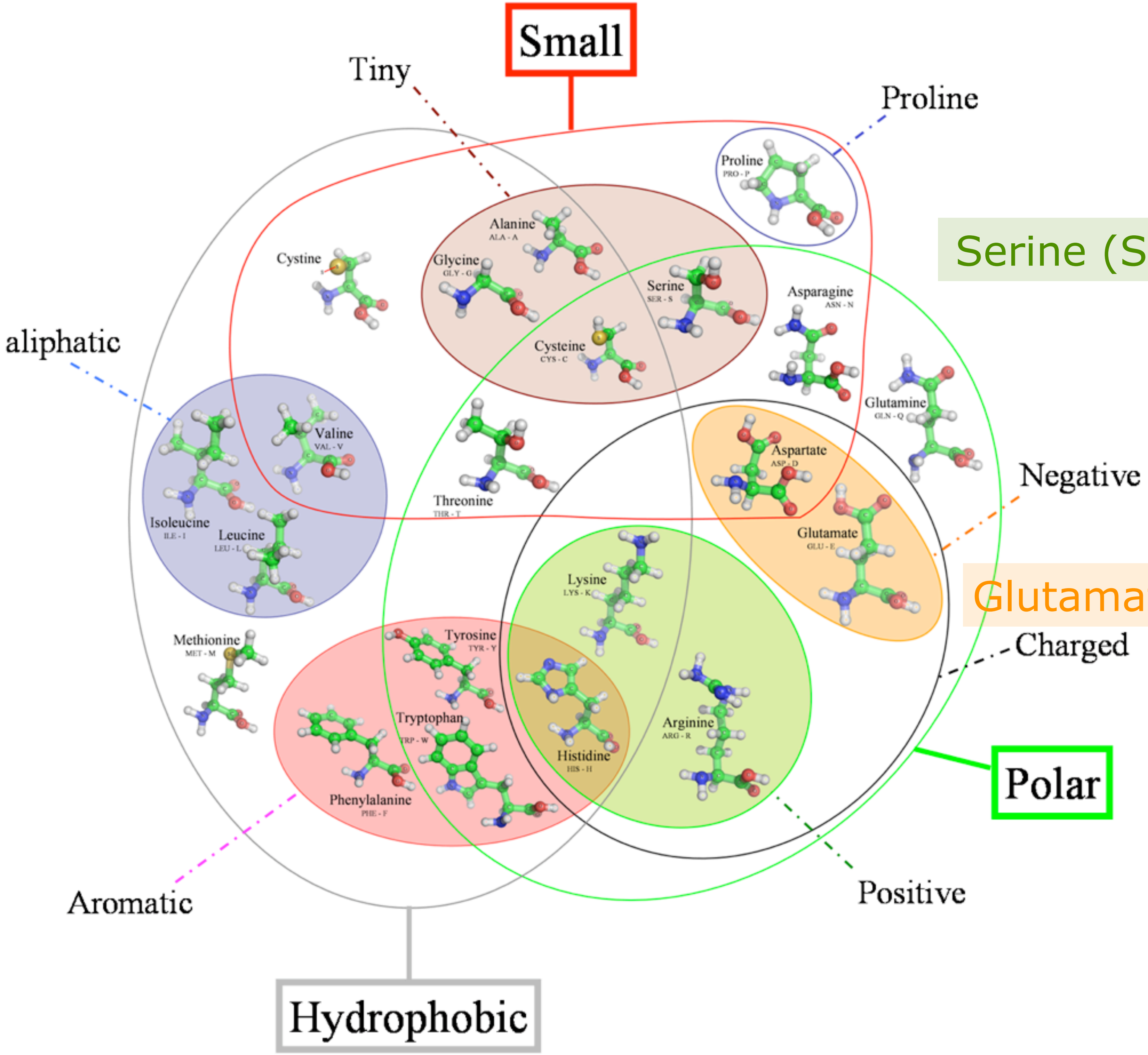
$$\boxed{PID = \frac{\#\text{ of identical aa or nt}}{\#\text{ of total aa or nt}} \times 100}$$

Similarity - The extent to which nucleotide or protein sequences are related. The extent of similarity between two sequences can be based on percent sequence identity and/or conservation. In BLAST similarity refers to a positive matrix score.

$$\boxed{\%Similarity = \frac{\#\text{ of identical aa or nt} + \#\text{ of similar aa or nt substitutions}}{\#\text{ of total aa or nt}} \times 100}$$

Homology - Similarity attributed to descent from a common ancestor.

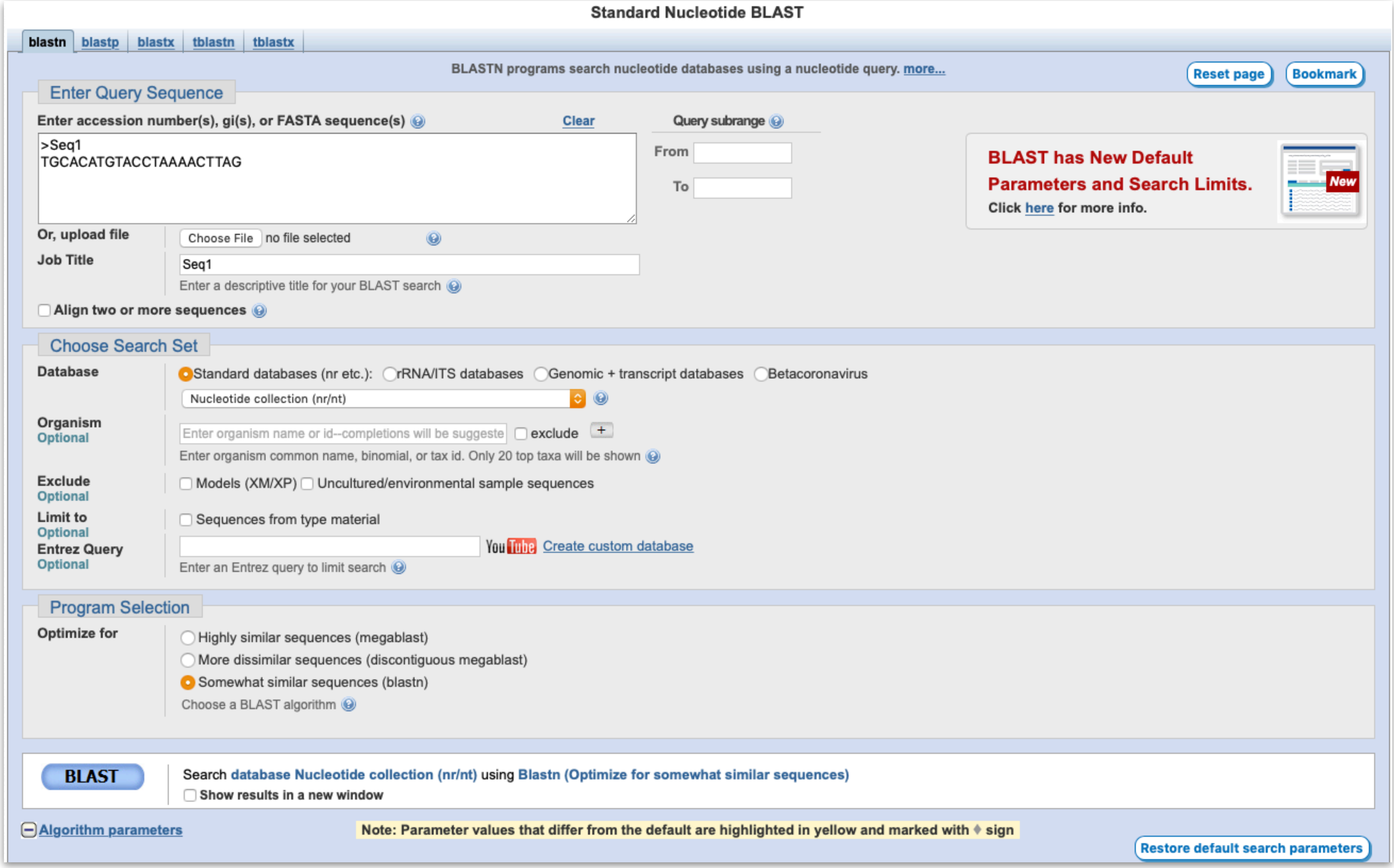
GDA21 ▷ BLAST Search (Extra)



Serine (S) - Asparagine (N)

Glutamate (E) - Aspartate (D)

GDA21 ▷ BLAST Search (Extra)



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) — Clear

```
>Seq1
TGCACATGTACCTAAAACTTAG
```

Query subrange

From

To

BLAST has New Default Parameters and Search Limits.
Click here for more info.

Or, upload file — Choose File no file selected

Job Title: Seq1
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 suggeste □ 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): YouTube Create custom database
Enter an Entrez query to limit search

Program Selection

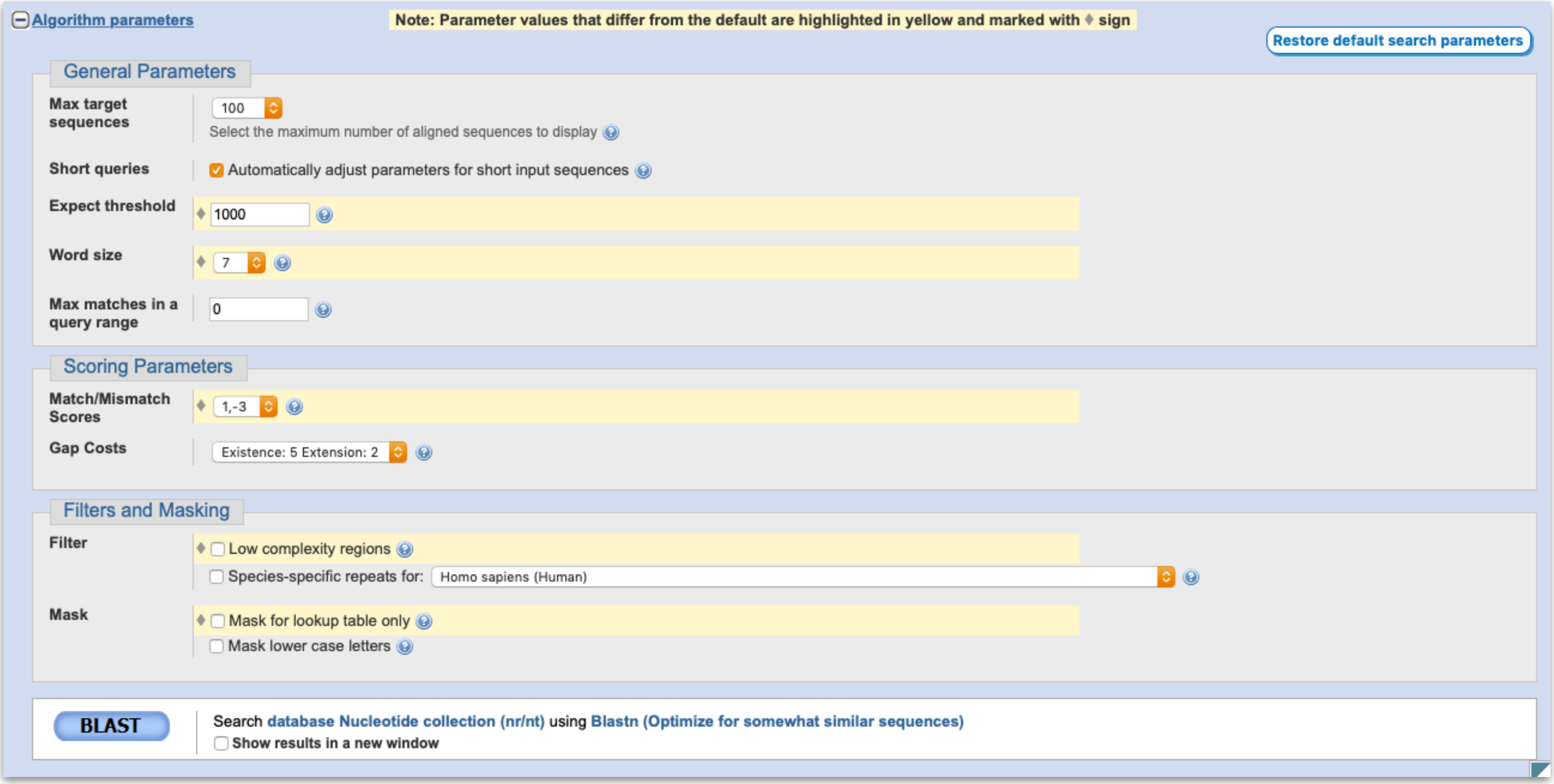
Optimize for:
○ Highly similar sequences (megablast)
○ More dissimilar sequences (discontiguous megablast)
● Somewhat similar sequences (blastn)
Choose a BLAST algorithm

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

⊖ Algorithm parameters

Note: Parameter values that differ from the default are highlighted in yellow and marked with ♦ sign

GDA21 ▷ BLAST Search (Extra)



Algorithm parameters

Note: Parameter values that differ from the default are highlighted in yellow and marked with ♦ sign

Restore default search parameters

General Parameters

Max target sequences: 100
Select the maximum number of aligned sequences to display

Short queries: ☑ Automatically adjust parameters for short input sequences

Expect threshold: ♦ 1000

Word size: ♦ 7

Max matches in a query range: 0

Scoring Parameters

Match/Mismatch Scores: ♦ 1,-3

Gap Costs: Existence: 5 Extension: 2

Filters and Masking

Filter: ♦ □ Low complexity regions
□ Species-specific repeats for: Homo sapiens (Human)

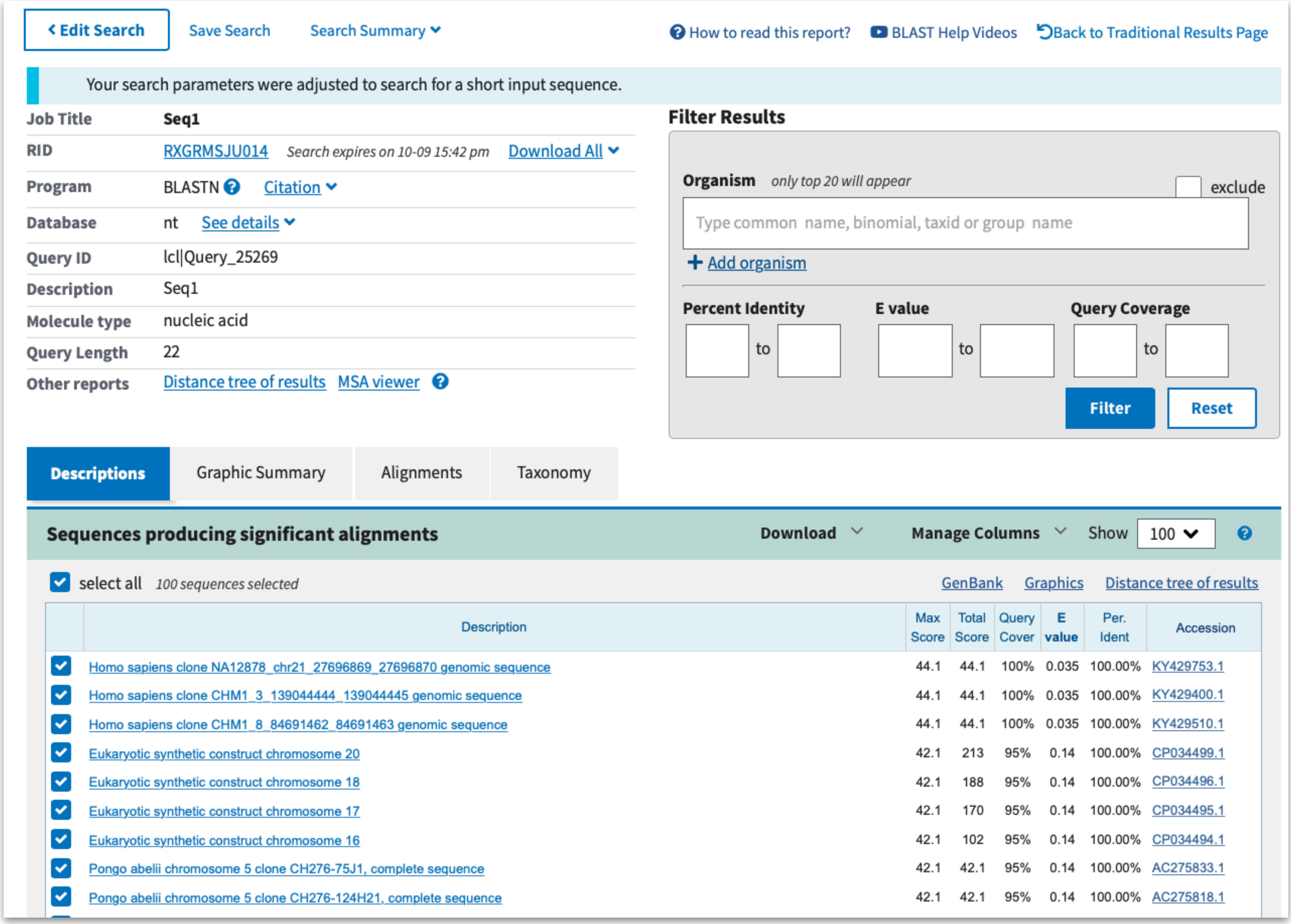
Mask: ♦ □ Mask for lookup table only
□ Mask lower case letters

BLAST

Search database Nucleotide collection (nr/nt) using Blastn (Optimize for somewhat similar sequences)

□ Show results in a new window

GDA21 ▷ BLAST Search (Extra)



Your search parameters were adjusted to search for a short input sequence.

Job Title	Seq1
RID	RXGRMSJU014 *Search expires on 10-09 15:42 pm* Download All ⌄
Program	BLASTN Citation ⌄
Database	nt See details ⌄
Query ID	lcl|Query_25269
Description	Seq1
Molecule type	nucleic acid
Query Length	22
Other reports	Distance tree of results MSA viewer

Filter Results

Organism *only top 20 will appear* □ exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity [ ] to [ ] E value [ ] to [ ] Query Coverage [ ] to [ ]

Sequences producing significant alignments

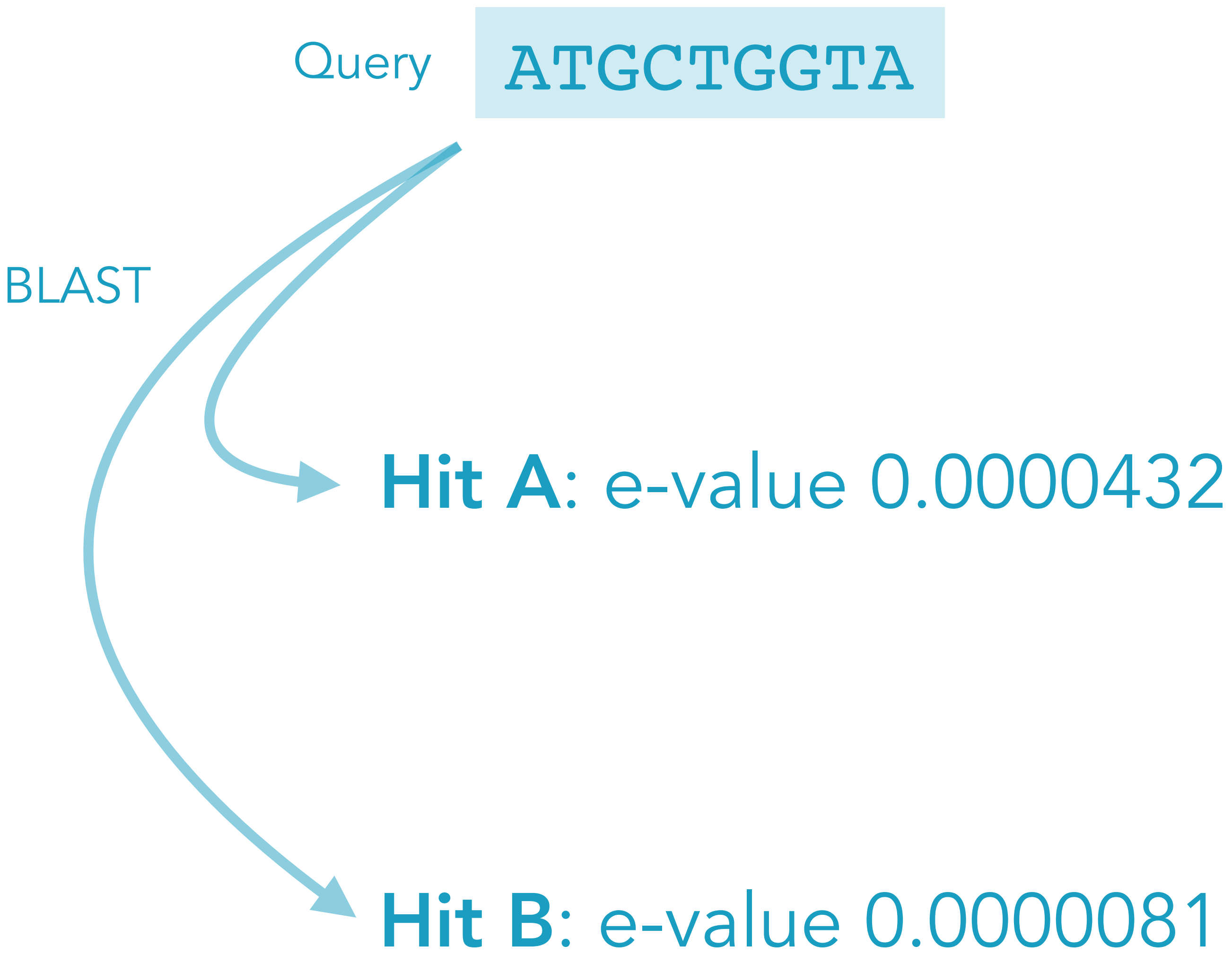
☑ select all *100 sequences selected*

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Homo sapiens clone NA12878_chr21_27696869_27696870 genomic sequence	44.1	44.1	100%	0.035	100.00%	KY429753.1
☑	Homo sapiens clone CHM1_3_139044444_139044445 genomic sequence	44.1	44.1	100%	0.035	100.00%	KY429400.1
☑	Homo sapiens clone CHM1_8_84691462_84691463 genomic sequence	44.1	44.1	100%	0.035	100.00%	KY429510.1
☑	Eukaryotic synthetic construct chromosome 20	42.1	213	95%	0.14	100.00%	CP034499.1
☑	Eukaryotic synthetic construct chromosome 18	42.1	188	95%	0.14	100.00%	CP034496.1
☑	Eukaryotic synthetic construct chromosome 17	42.1	170	95%	0.14	100.00%	CP034495.1
☑	Eukaryotic synthetic construct chromosome 16	42.1	102	95%	0.14	100.00%	CP034494.1
☑	Pongo abelii chromosome 5 clone CH276-75J1, complete sequence	42.1	42.1	95%	0.14	100.00%	AC275833.1
☑	Pongo abelii chromosome 5 clone CH276-124H21, complete sequence	42.1	42.1	95%	0.14	100.00%	AC275818.1

E value

Expectation value. The number of different alignments with scores equivalent to or better than S that are expected to occur in a database search by chance. **The lower the E value, the more significant the score.**

Sequence Databases (Extra)



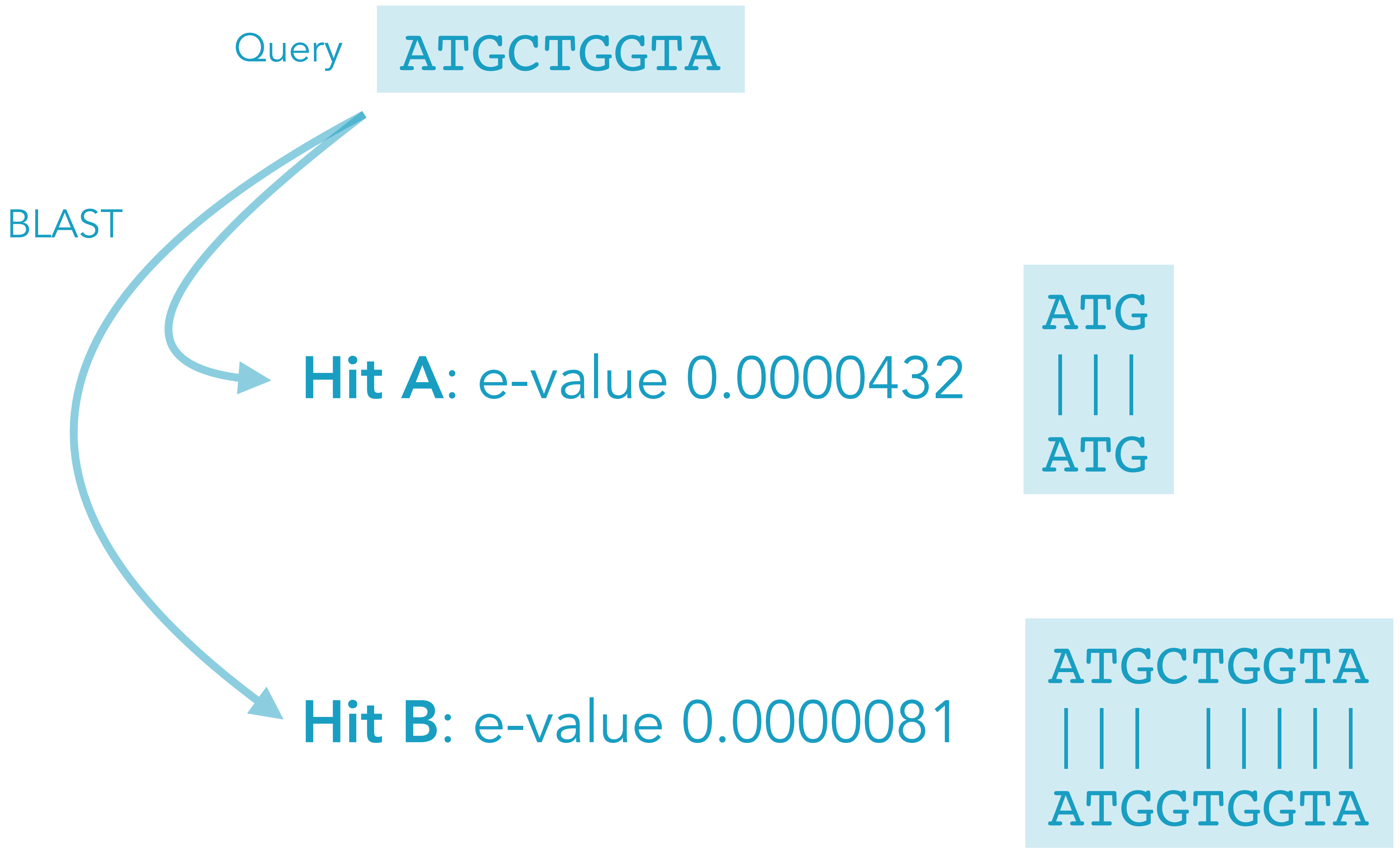
Query ATGCTGGTA

BLAST

Hit A: e-value 0.0000432

Hit B: e-value 0.0000081

GDA21 ▷ Sequence Databases (Extra)



Query

```
ATGCTGGTA
```

BLAST

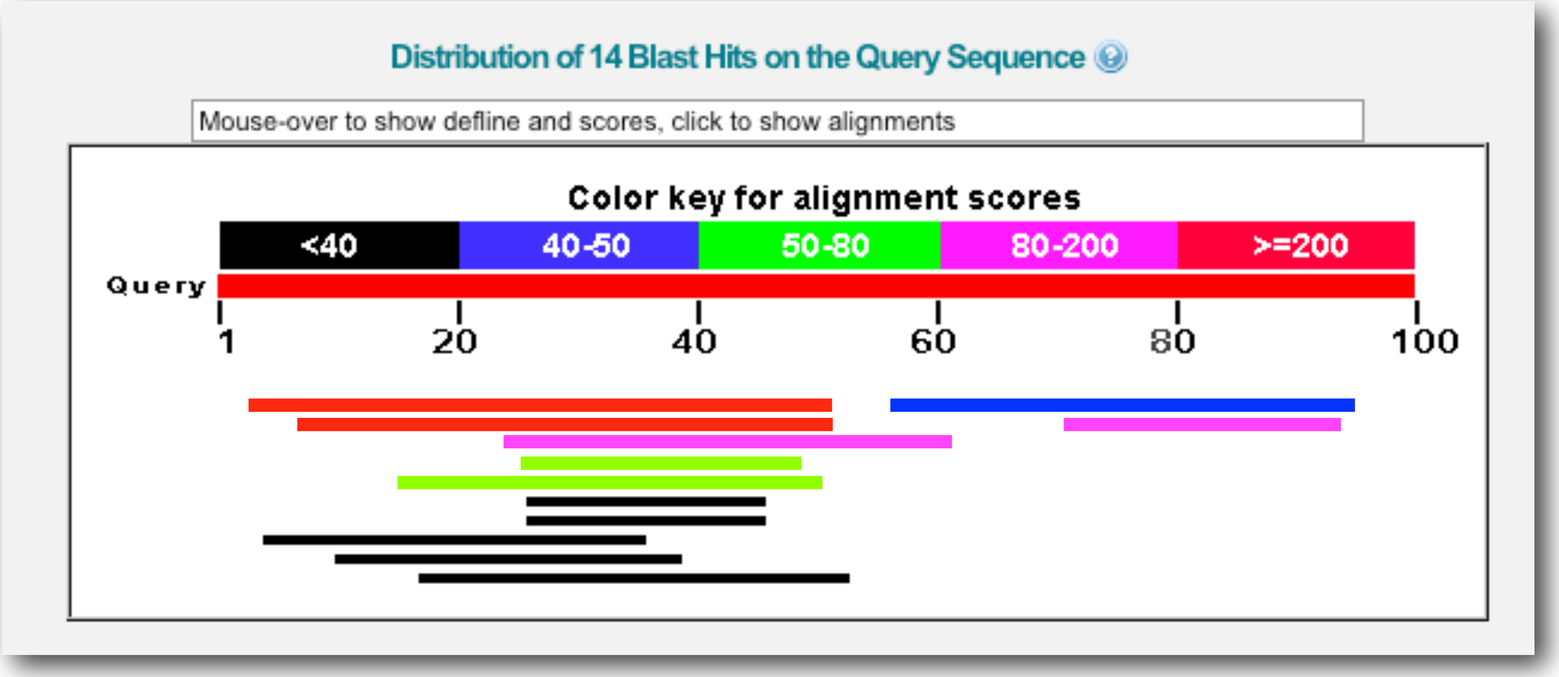
Hit A: e-value 0.0000432

```
ATG
|||
ATG
```

Hit B: e-value 0.0000081

```
ATGCTGGTA
||| |||||
ATGGTGGTA
```

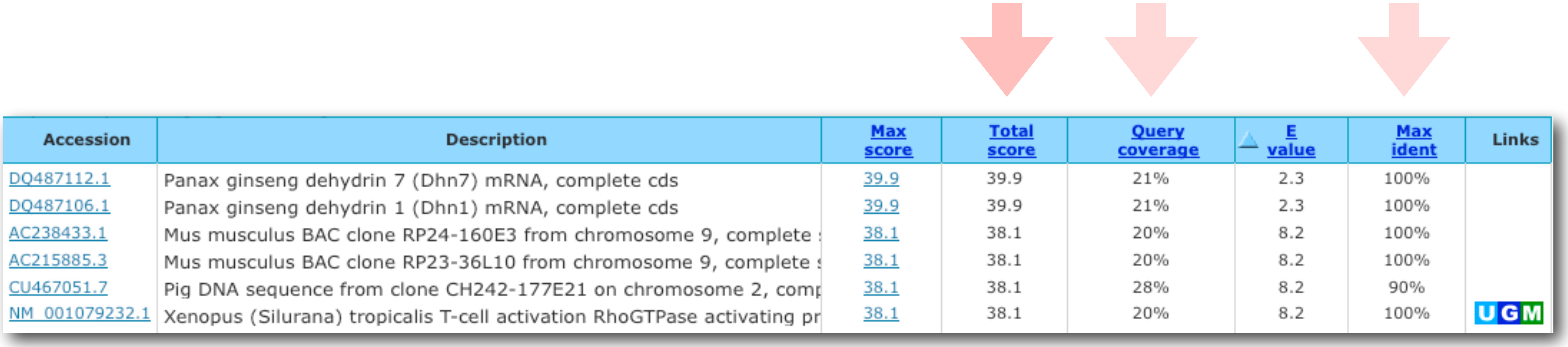
Graphic Summary



Distribution of 14 Blast Hits on the Query Sequence

Mouse-over to show defline and scores, click to show alignments

GDA21 ▷ Sequence Databases (Extra)



Accession	Description	Max score	Total score	Query coverage	▲ E value	Max ident	Links
DQ487112.1	Panax ginseng dehydrin 7 (Dhn7) mRNA, complete cds	39.9	39.9	21%	2.3	100%	
DQ487106.1	Panax ginseng dehydrin 1 (Dhn1) mRNA, complete cds	39.9	39.9	21%	2.3	100%	
AC238433.1	Mus musculus BAC clone RP24-160E3 from chromosome 9, complete :	38.1	38.1	20%	8.2	100%	
AC215885.3	Mus musculus BAC clone RP23-36L10 from chromosome 9, complete :	38.1	38.1	20%	8.2	100%	
CU467051.7	Pig DNA sequence from clone CH242-177E21 on chromosome 2, comp	38.1	38.1	28%	8.2	90%	
NM_001079232.1	Xenopus (Silurana) tropicalis T-cell activation RhoGTPase activating pr	38.1	38.1	20%	8.2	100%	UGM

GDA21 ▷ Sequence Databases (Extra)

E value

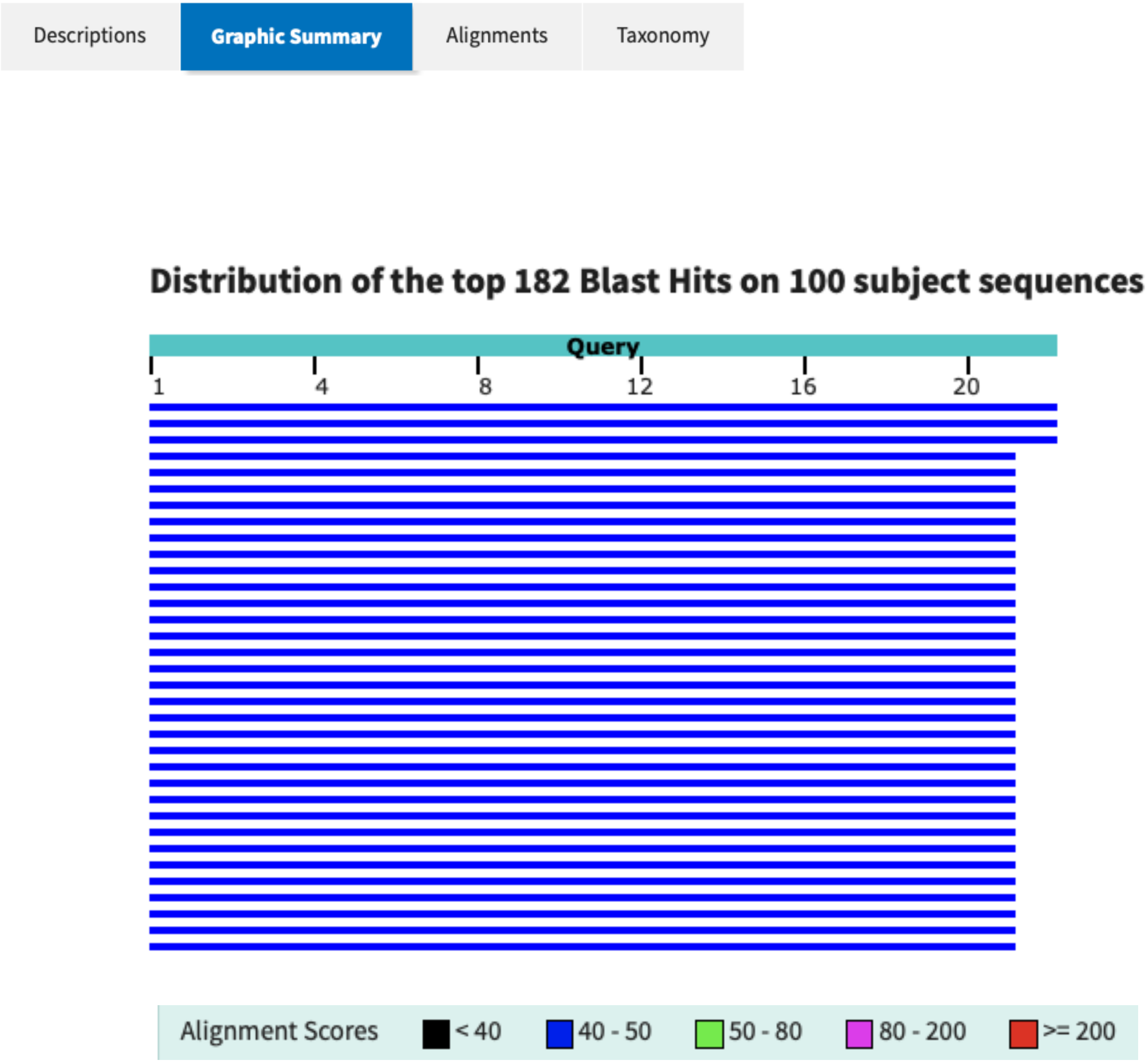
Expectation value. The number of different alignments with scores equivalent to or better than S that are expected to occur in a database search by chance. **The lower the E value, the more significant the score.**

Bit score

The value S' is derived from the raw alignment score S in which the statistical properties of the scoring system used have been taken into account. Because bit scores have been normalized with respect to the scoring system, they **can be used to compare alignment scores from different searches**.

GDA21 ▷ BLAST Search (Extra)

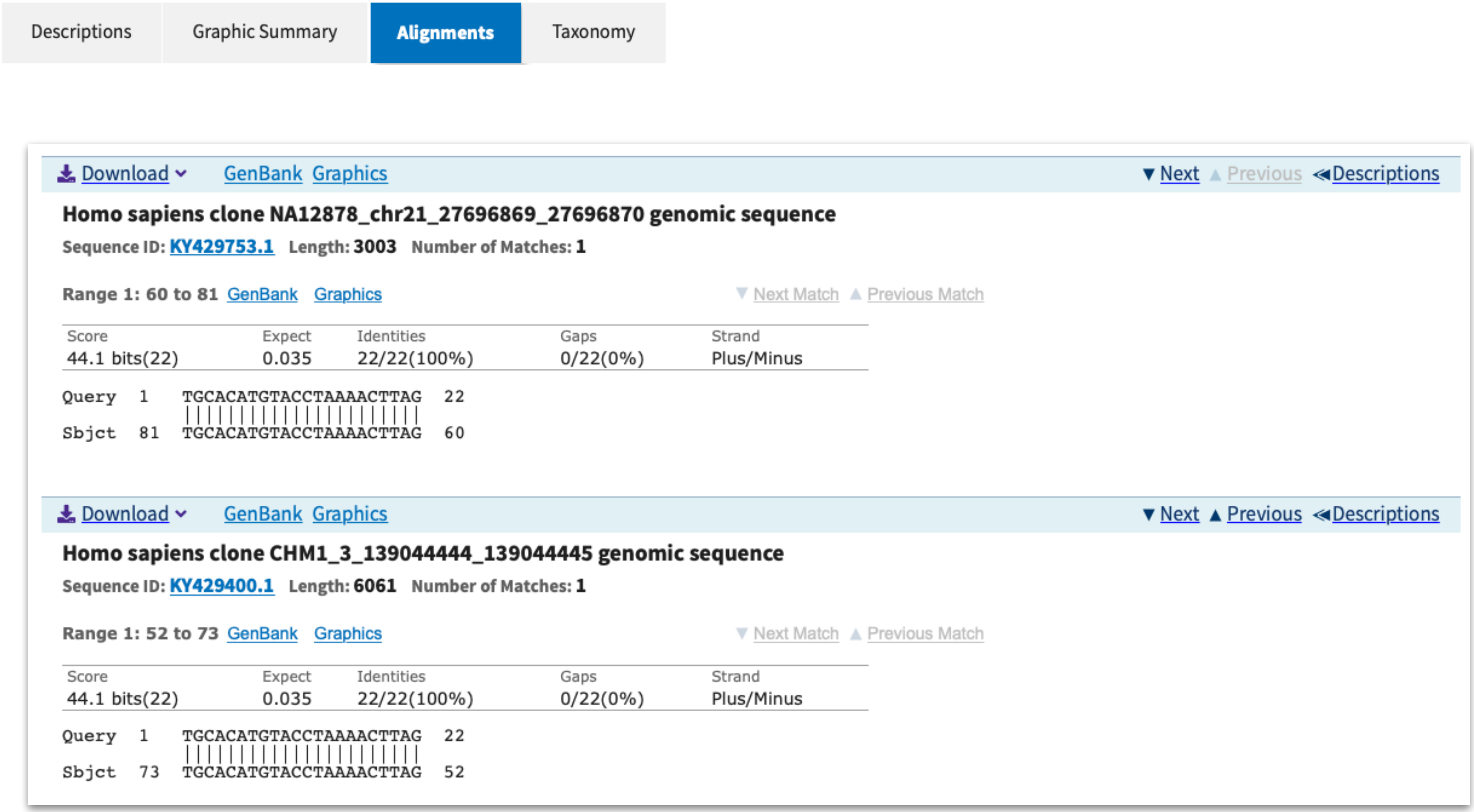
GDC
Genetic Diversity Centre Zurich



Distribution of the top 182 Blast Hits on 100 subject sequences

Alignment Scores ■ < 40 🟦 40 - 50 🟩 50 - 80 🟪 80 - 200 🟥 >= 200

GDA21 ▷ BLAST Search (Extra)



Descriptions
Graphic Summary
Alignments
Taxonomy

Download ⌄ GenBank Graphics ▼ Next ▲ Previous ◀Descriptions

Homo sapiens clone NA12878_chr21_27696869_27696870 genomic sequence

Sequence ID: KY429753.1 Length: 3003 Number of Matches: 1

Range 1: 60 to 81 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.035	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1   TGCACATGTACCTAAAACTTAG  22
           ||||||||||||||||||||||
Sbjct  81  TGCACATGTACCTAAAACTTAG  60
```

Download ⌄ GenBank Graphics ▼ Next ▲ Previous ◀Descriptions

Homo sapiens clone CHM1_3_139044444_139044445 genomic sequence

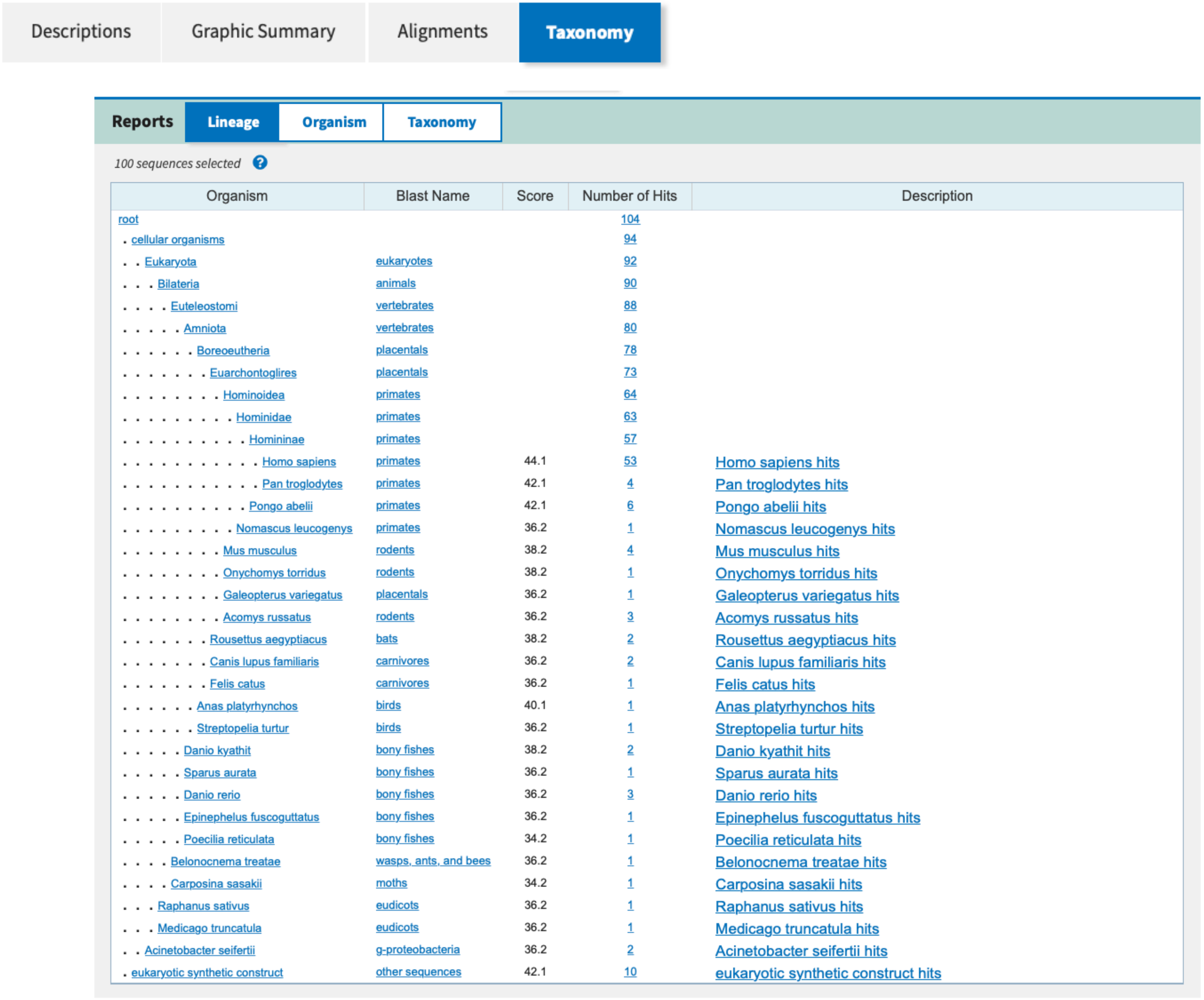
Sequence ID: KY429400.1 Length: 6061 Number of Matches: 1

Range 1: 52 to 73 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
44.1 bits(22)	0.035	22/22(100%)	0/22(0%)	Plus/Minus

```
Query  1   TGCACATGTACCTAAAACTTAG  22
           ||||||||||||||||||||||
Sbjct  73  TGCACATGTACCTAAAACTTAG  52
```

GDA21 ▷ BLAST Search (Extra)



100 sequences selected

Organism	Blast Name	Score	Number of Hits	Description
root			104	
. cellular organisms			94	
. . Eukaryota	eukaryotes		92	
. . . Bilateria	animals		90	
. . . . Euteleostomi	vertebrates		88	
. . . . . Amniota	vertebrates		80	
. . . . . . Boreoeutheria	placentals		78	
. . . . Euarchontoglires	placentals		73	
. . . . Hominoidea	primates		64	
. . . . Hominidae	primates		63	
. . . . Homininae	primates		57	
. . . . Homo sapiens	primates	44.1	53	Homo sapiens hits
. . . . Pan troglodytes	primates	42.1	4	Pan troglodytes hits
. . . . Pongo abelii	primates	42.1	6	Pongo abelii hits
. . . . Nomascus leucogenys	primates	36.2	1	Nomascus leucogenys hits
. . . . Mus musculus	rodents	38.2	4	Mus musculus hits
. . . . Onychomys torridus	rodents	38.2	1	Onychomys torridus hits
. . . . Galeopterus variegatus	placentals	36.2	1	Galeopterus variegatus hits
. . . . Acomys russatus	rodents	36.2	3	Acomys russatus hits
. . . . Rousettus aegyptiacus	bats	38.2	2	Rousettus aegyptiacus hits
. . . . Canis lupus familiaris	carnivores	36.2	2	Canis lupus familiaris hits
. . . . Felis catus	carnivores	36.2	1	Felis catus hits
. . . . . . Anas platyrhynchos	birds	40.1	1	Anas platyrhynchos hits
. . . . . . Streptopelia turtur	birds	36.2	1	Streptopelia turtur hits
. . . . . Danio kyathit	bony fishes	38.2	2	Danio kyathit hits
. . . . . Sparus aurata	bony fishes	36.2	1	Sparus aurata hits
. . . . . Danio rerio	bony fishes	36.2	3	Danio rerio hits
. . . . . Epinephelus fuscoguttatus	bony fishes	36.2	1	Epinephelus fuscoguttatus hits
. . . . . Poecilia reticulata	bony fishes	34.2	1	Poecilia reticulata hits
. . . . Belonocnema treatae	wasps, ants, and bees	36.2	1	Belonocnema treatae hits
. . . . Carposina sasakii	moths	34.2	1	Carposina sasakii hits
. . . Raphanus sativus	eudicots	36.2	1	Raphanus sativus hits
. . . Medicago truncatula	eudicots	36.2	1	Medicago truncatula hits
. . Acinetobacter seifertii	g-proteobacteria	36.2	2	Acinetobacter seifertii hits
. eukaryotic synthetic construct	other sequences	42.1	10	eukaryotic synthetic construct hits

GDA21 ▷ BLAST Search (Extra)

BLAST in Terminal

Blast on fasta file - for smaller references

```
blastn -db SUBJECT.fa -evalue 0.0001 -query QUERY.fa -outfmt 6 -out RES.blast
```

Index reference (subject) first and blast against index db

```
makeblastdb -dbtype nucl -in REF.fa -title "REF" -logfile REF.log
```

```
blastn -db REF -evalue 0.0001 -query Q.fa -outfmt 6 -out Q_dbREF.blast
```

GDA21 ▷ BLAST Search (Extra)

BLAST in R

Packages with blast functions:

```
blastSeq {hoardeR}
blastSequences {annotate}
rBLAST (GitHub)
```

Blast via system command:

```
system(command = "/path/to/blast/blastn -db REF -query Q.fa -outfmt 6 -evalue 1e-6)
```

```
system2(
  command = "/path/to/blast/blastn",
  args = c("-db REF -query Q.fa -outfmt 6 -evalue 10e-6))
```

GDA21 ▷ BLAST Search (Extra)

BLAT (BLAST-like alignment tool)

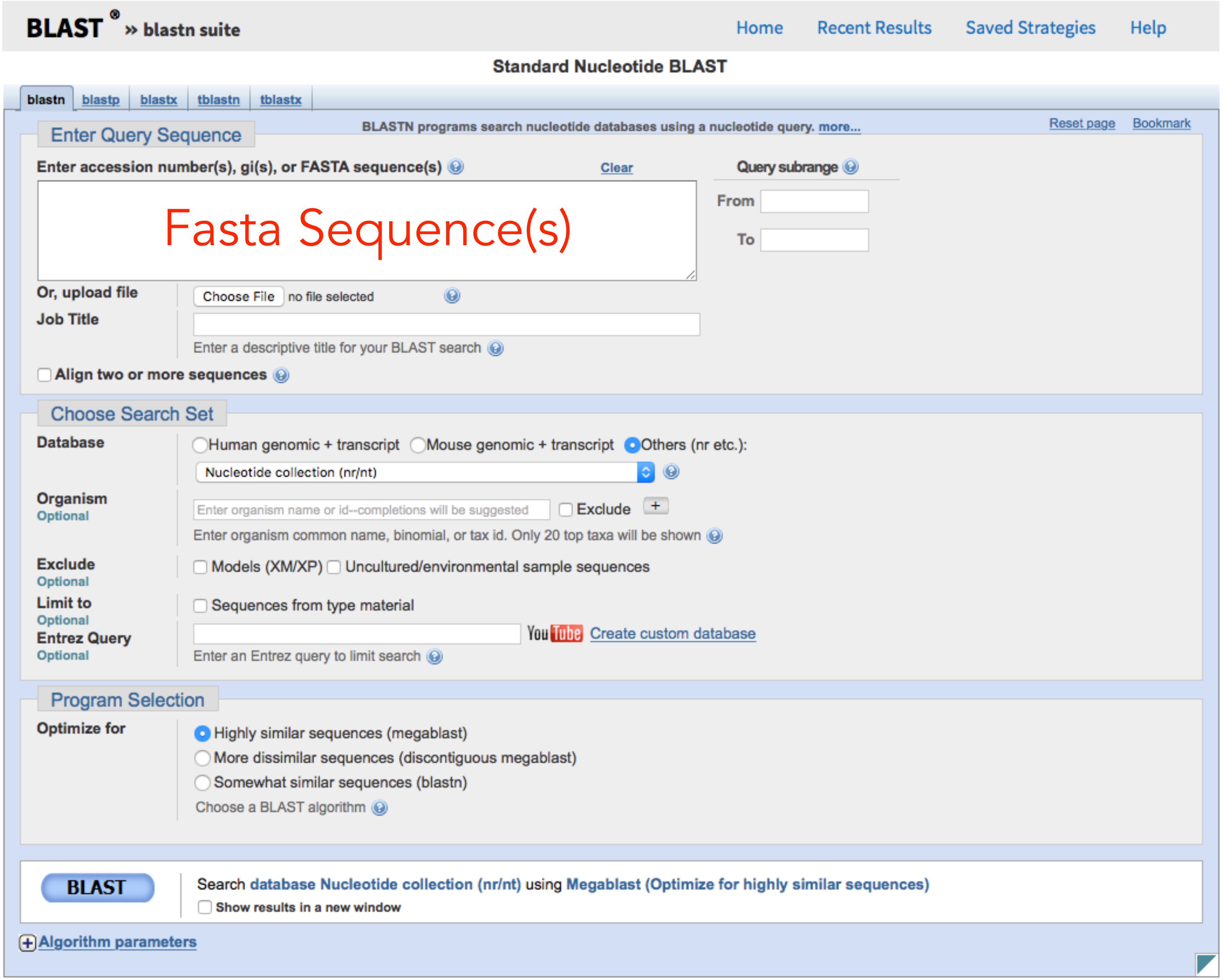
MegaBLAST (BLAT variant)

UBLAST (USEARCH BLAST alternative)

Self-Study Guide

GDA21 ▷ Sequence Databases (Extra)

GDC
Genetic Diversity Centre Zurich



BLAST® » blastn suite

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Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

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

Reset page | Bookmark

Enter Query Sequence

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

Fasta Sequence(s)

Query subrange
From
To

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: ○ 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
You Tube 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 Megablast (Optimize for highly similar sequences)

□ Show results in a new window

⊕ Algorithm parameters

GDA21 ▷ Sequence Databases (Extra)

```
>Dmag_B24_ORF0007_contigh23_2356_3466
ATGTGAACAAGTCTGAGAGATTCATCAACCGAATGTATATGAAAGCGGTGGTCCAGTCTG
ATCGGGCGGGCTTTCGATCAACAACAACAACAACAACAACAACAAGTACGATCGATCTAA
CTAGCTGACTAGCTGGACTGACTAGCTACTACGTACACGATCATATAATCGCGCGCGGCC
CCCTATATAGCTACGATGCATCGTATATAAATATTCTTATCTCCCTTA
```

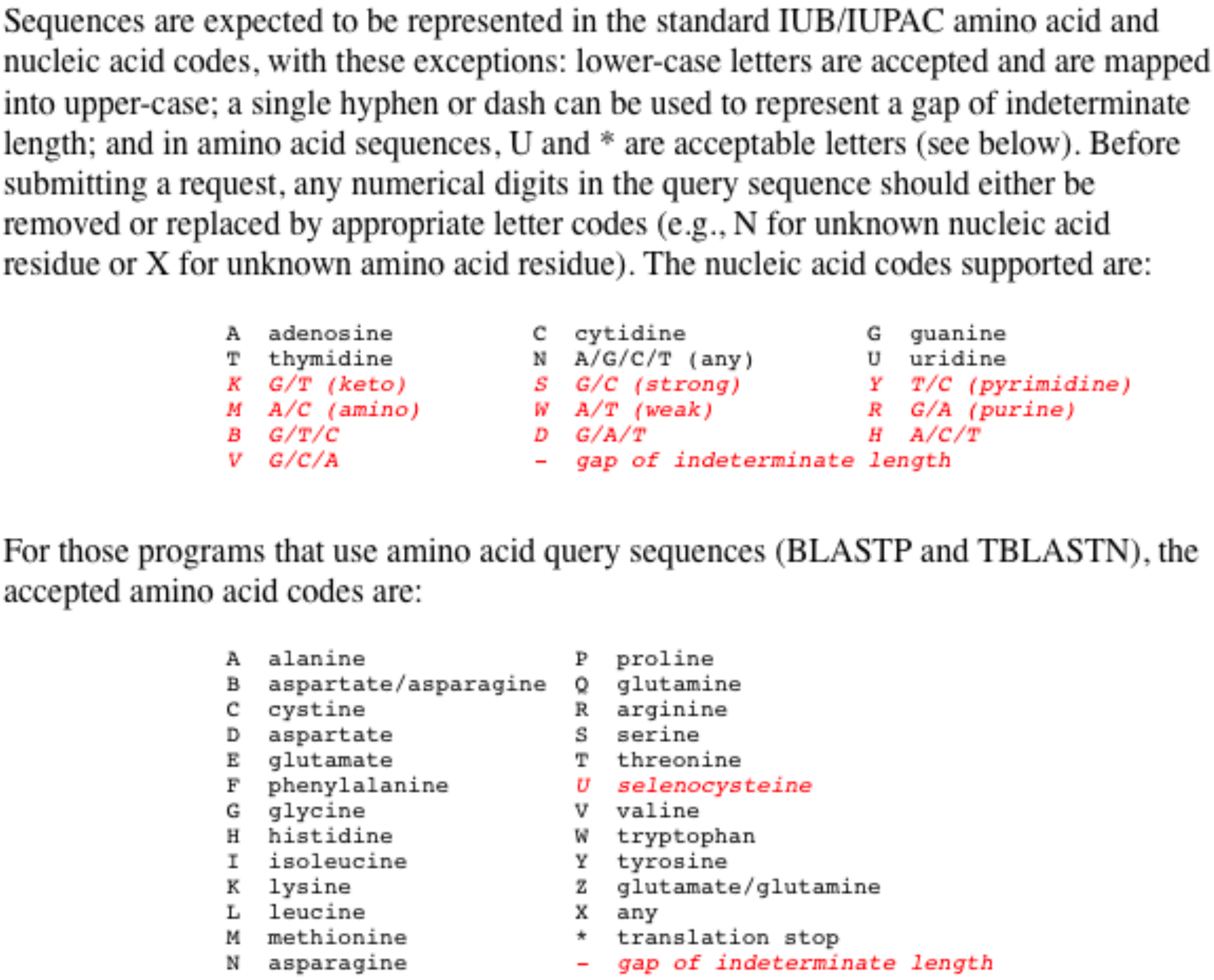
Fasta header

Sequence

```
NCBI fasta headers:
>gi|224922792|ref|NM_000860.4| Homo sapiens hydroxyprostaglandin
dehydrogenase 15-(NAD) (HPGD), transcript variant 1, mRNA

Your header:
>Code_Species_Location/Gene/Coordinates
```

GDA21 ▷ Sequence Databases (Extra)



Sequences are expected to be represented in the standard IUB/IUPAC amino acid and nucleic acid codes, with these exceptions: lower-case letters are accepted and are mapped into upper-case; a single hyphen or dash can be used to represent a gap of indeterminate length; and in amino acid sequences, U and * are acceptable letters (see below). Before submitting a request, any numerical digits in the query sequence should either be removed or replaced by appropriate letter codes (e.g., N for unknown nucleic acid residue or X for unknown amino acid residue). The nucleic acid codes supported are:

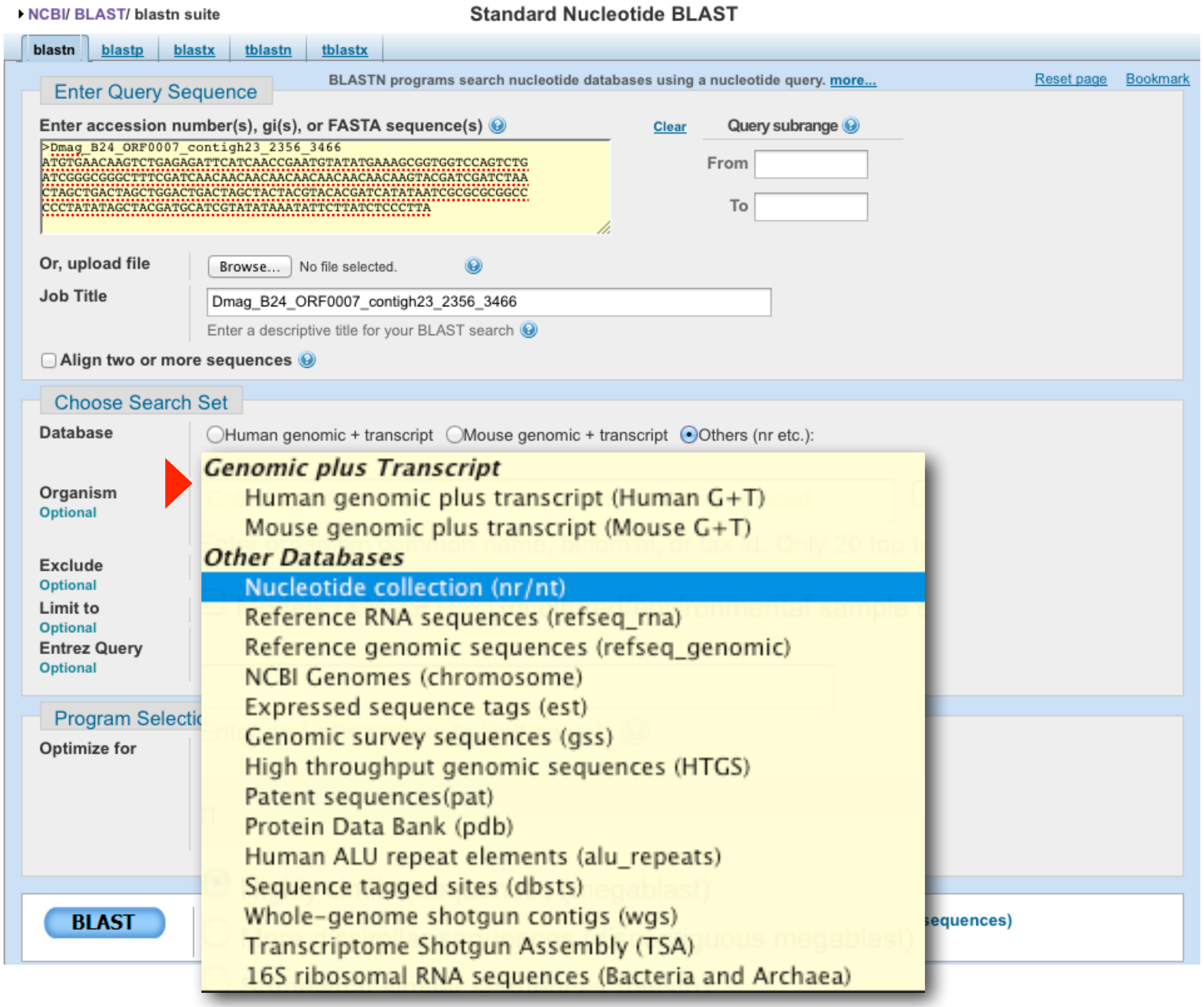
A	adenosine	C	cytidine	G	guanine
T	thymidine	N	A/G/C/T (any)	U	uridine
K	*G/T (keto)*	*S*	*G/C (strong)*	*Y*	*T/C (pyrimidine)*
M	*A/C (amino)*	*W*	*A/T (weak)*	*R*	*G/A (purine)*
B	*G/T/C*	*D*	*G/A/T*	*H*	*A/C/T*
V	*G/C/A*	*-*	*gap of indeterminate length*		

For those programs that use amino acid query sequences (BLASTP and TBLASTN), the accepted amino acid codes are:

A	alanine	P	proline
B	aspartate/asparagine	Q	glutamine
C	cystine	R	arginine
D	aspartate	S	serine
E	glutamate	T	threonine
F	phenylalanine	*U*	*selenocysteine*
G	glycine	V	valine
H	histidine	W	tryptophan
I	isoleucine	Y	tyrosine
K	lysine	Z	glutamate/glutamine
L	leucine	X	any
M	methionine	*	translation stop
N	asparagine	*-*	*gap of indeterminate length*

GDA21 ▷ Sequence Databases (Extra)

Choose Database (Subject)



NCBI/ BLAST/ blastn suite

Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

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

Reset page | Bookmark

Enter Query Sequence

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

```
>Dmag_B24_ORF0007_contigh23_2356_3466
ATGTGAACAAGTCTGAGAGATTCATCAACCGAATGTATATGAAAGCGGTGGTCCAGTCTG
ATCGGGCGGGCTTTCGATCAACAACAACAACAACAACAACAACAAGTACGATCGATCTAA
CTAGCTGACTAGCTGGACTGACTAGCTACTACGTACACGATCATATAATCGCGCGCGGCC
CCCTATATAGCTACGATGCATCGTATATAAATATTCTTATCTCCCTTA
```

Clear

Query subrange

From

To

Or, upload file: Browse... No file selected.

Job Title: Dmag_B24_ORF0007_contigh23_2356_3466

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.):

Organism
Optional

Exclude
Optional

Limit to
Optional

Entrez Query
Optional

Genomic plus Transcript

- Human genomic plus transcript (Human G+T)
- Mouse genomic plus transcript (Mouse G+T)

Other Databases

- Nucleotide collection (nr/nt)
- Reference RNA sequences (refseq_rna)
- Reference genomic sequences (refseq_genomic)
- NCBI Genomes (chromosome)
- Expressed sequence tags (est)
- Genomic survey sequences (gss)
- High throughput genomic sequences (HTGS)
- Patent sequences(pat)
- Protein Data Bank (pdb)
- Human ALU repeat elements (alu_repeats)
- Sequence tagged sites (dbsts)
- Whole-genome shotgun contigs (wgs)
- Transcriptome Shotgun Assembly (TSA)
- 16S ribosomal RNA sequences (Bacteria and Archaea)

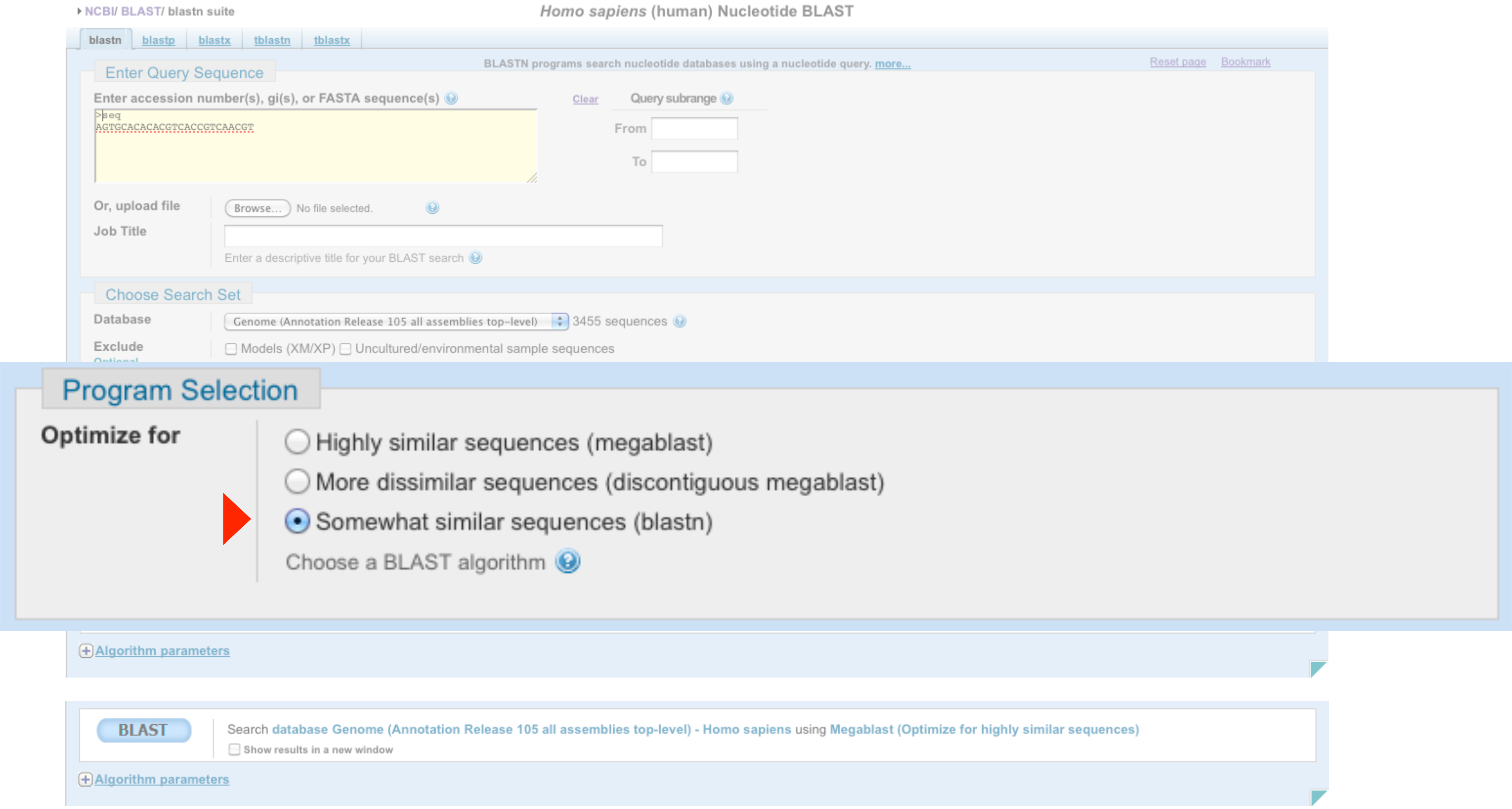
Program Selecti

Optimize for

BLAST

sequences)

GDA21 ▷ Sequence Databases (Extra)



▸ NCBI/ BLAST/ blastn suite

Homo sapiens (human) 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)

```
>seq
AGTGCACACACGTCACCGTCAACGT
```

Query subrange

From

To

Or, upload file No file selected.

Job Title

Enter a descriptive title for your BLAST search

Choose Search Set

Database: Genome (Annotation Release 105 all assemblies top-level) 3455 sequences

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

Program Selection

Optimize for

○ Highly similar sequences (megablast)

○ More dissimilar sequences (discontiguous megablast)

● Somewhat similar sequences (blastn)

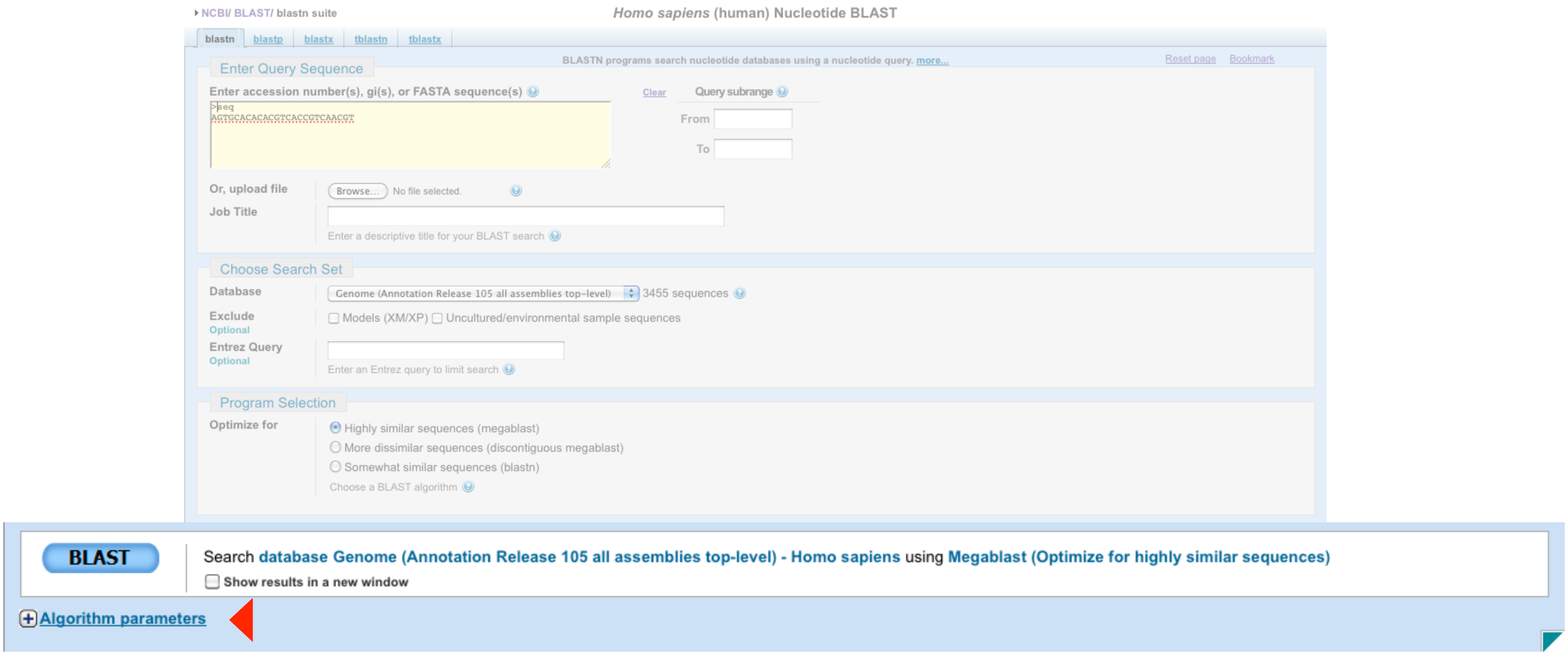
Choose a BLAST algorithm

Search database Genome (Annotation Release 105 all assemblies top-level) - Homo sapiens using Megablast (Optimize for highly similar sequences)

□ Show results in a new window

Megablast is intended for comparing a query to closely related sequences and works best if the target percent identity is 95% or more but is very fast.
Discontiguous megablast uses an initial seed that ignores some bases (allowing mismatches) and is intended for cross-species comparisons.
BlastN is slow, but allows a word-size down to seven bases.

Change Default Search Parameter



▸ NCBI/ BLAST/ blastn suite

Homo sapiens (human) 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)

```
>seq
AGTGCACACACGTCACCGTCAACGT
```

Query subrange

From

To

Or, upload file Browse... No file selected.

Job Title

Enter a descriptive title for your BLAST search

Choose Search Set

Database: Genome (Annotation Release 105 all assemblies top-level) 3455 sequences

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

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)

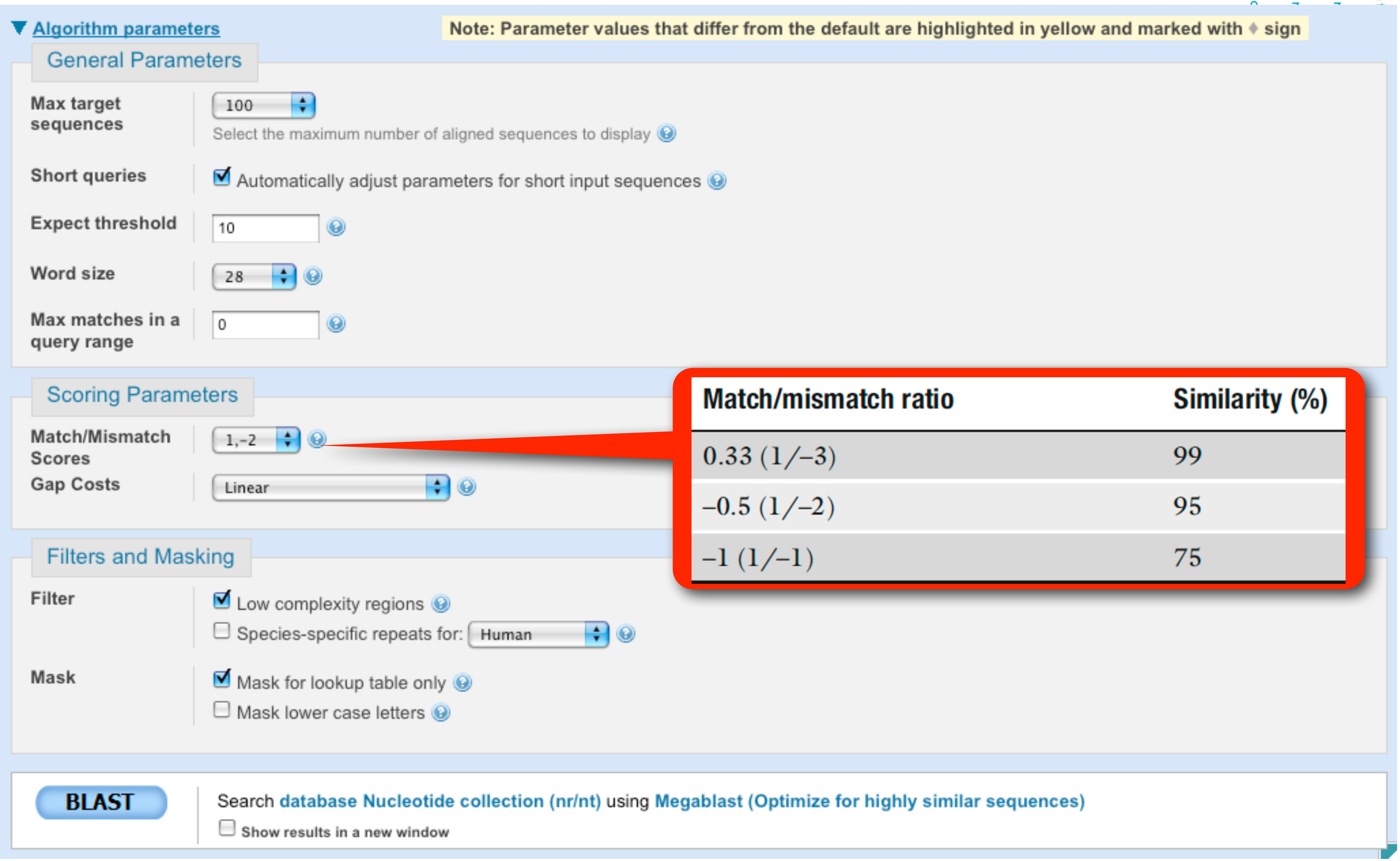
Choose a BLAST algorithm

Search database Genome (Annotation Release 105 all assemblies top-level) - Homo sapiens using Megablast (Optimize for highly similar sequences)

□ Show results in a new window

⊞ Algorithm parameters

GDA21 ▷ Sequence Databases (Extra)



▼ Algorithm parameters

Note: Parameter values that differ from the default are highlighted in yellow and marked with ♦ sign

General Parameters

Max target sequences: 100
Select the maximum number of aligned sequences to display

Short queries: ☑ Automatically adjust parameters for short input sequences

Expect threshold: 10

Word size: 28

Max matches in a query range: 0

Scoring Parameters

Match/Mismatch Scores: 1,-2

Gap Costs: Linear

Match/mismatch ratio	Similarity (%)
0.33 (1/–3)	99
–0.5 (1/–2)	95
–1 (1/–1)	75

Filters and Masking

Filter: ☑ Low complexity regions
☐ Species-specific repeats for: Human

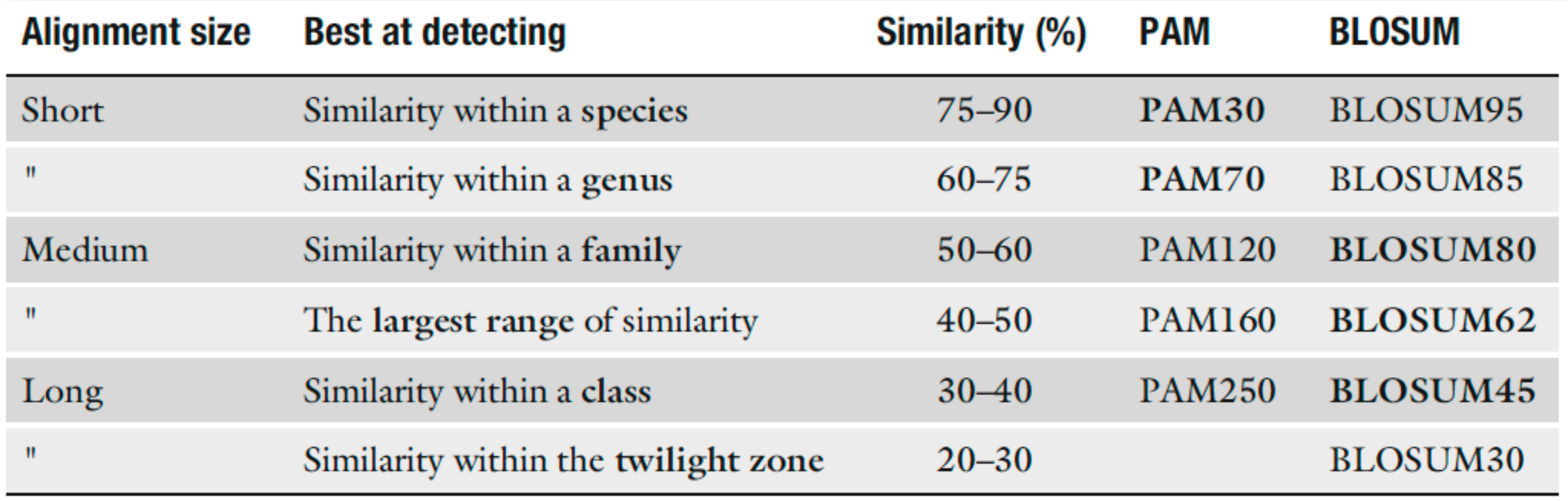
Mask: ☑ Mask for lookup table only
☐ Mask lower case letters

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

☐ Show results in a new window

GDA21 ▷ Sequence Databases (Extra)

When choosing a matrix, it is important to consider the alternatives. Do not simply choose the default setting without some initial consideration.



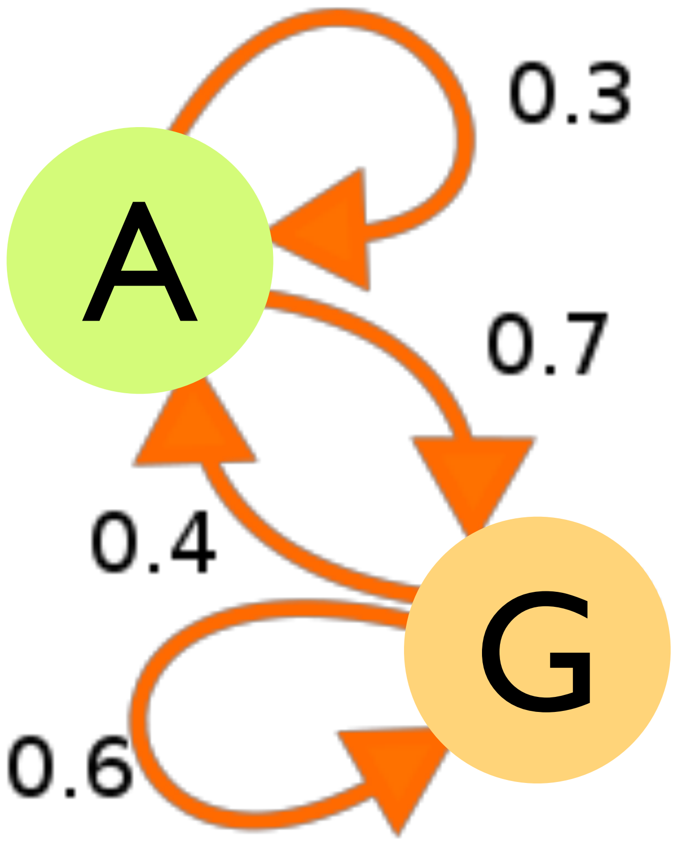
Alignment size	Best at detecting	Similarity (%)	PAM	BLOSUM
Short	Similarity within a species	75–90	**PAM30**	BLOSUM95
"	Similarity within a genus	60–75	**PAM70**	BLOSUM85
Medium	Similarity within a family	50–60	PAM120	**BLOSUM80**
"	The largest range of similarity	40–50	PAM160	**BLOSUM62**
Long	Similarity within a class	30–40	PAM250	**BLOSUM45**
"	Similarity within the twilight zone	20–30		BLOSUM30

The matrices highlighted in bold are available through NCBI’s BLAST web interface. **BLOSUM62** has been shown to provide the best results in BLAST searches overall due to its ability to detect large ranges of similarity. Nevertheless, the other matrices have their strengths. For example, if your goal is to only detect sequences of high similarity to infer homology within a species, the PAM30, BLOSUM90, and PAM70 matrices would provide the best results.

GDA21 ▷ Sequence Databases (Extra)

Percent **A**ccepted **M**utation (**PAM**) - A unit introduced by Margaret Dayhoff et al. (1978) to quantify the amount of evolutionary change in a protein sequence. 1.0 PAM unit, is the amount of evolution which will change, on average, 1% of amino acids in a protein sequence. A PAM(x) substitution matrix is a look-up table in which scores for each amino acid substitution have been calculated based on the frequency of that substitution in closely related proteins that have experienced a certain amount (x) of evolutionary divergence.

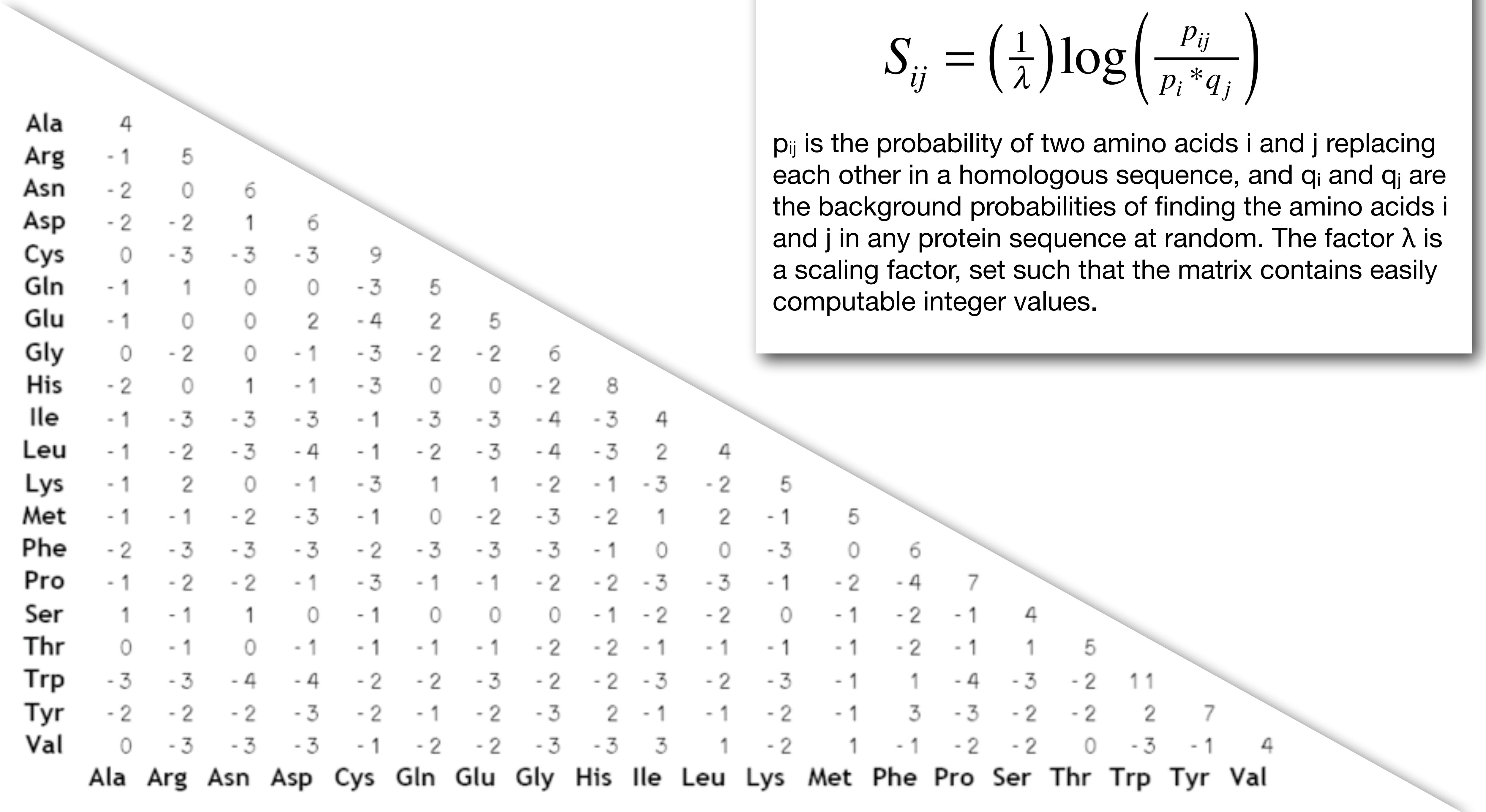
The PAM matrices imply a **Markov chain model** of protein mutation. The PAM matrices are normalized so that, for instance, the PAM1 matrix gives substitution probabilities for sequences that have experienced one point mutation for every hundred amino acids. The mutations may overlap so that the sequences reflected in the PAM250 matrix have experienced 250 mutation events for every 100 amino acids, yet only 80 out of every 100 amino acids have been affected.



A **Markov chain**, named for Andrey Markov, is a mathematical system that undergoes transitions from one state to another in a chainlike manner. It is a **random process** characterized as memoryless: the next state depends only on the current state and not on the entire past. This specific kind of "memorylessness" is called the Markov property. Markov chains have many applications as statistical models of real-world processes.

GDA21 ▷ Sequence Databases (Extra)

Blocks **Su**bstitution **M**atrix (**BLOSUM**). A substitution matrix in which scores for each position are derived from observations of the frequencies of substitutions in blocks of local alignments in related proteins. Each matrix is tailored to a particular evolutionary distance. In the BLOSUM62 matrix, for example, the alignment from which scores were derived was created using sequences sharing no more than 62% identity. Sequences more identical than 62% are represented by a single sequence in the alignment so as to avoid over-weighting closely related family members. (Henikoff and Henikoff 1992)



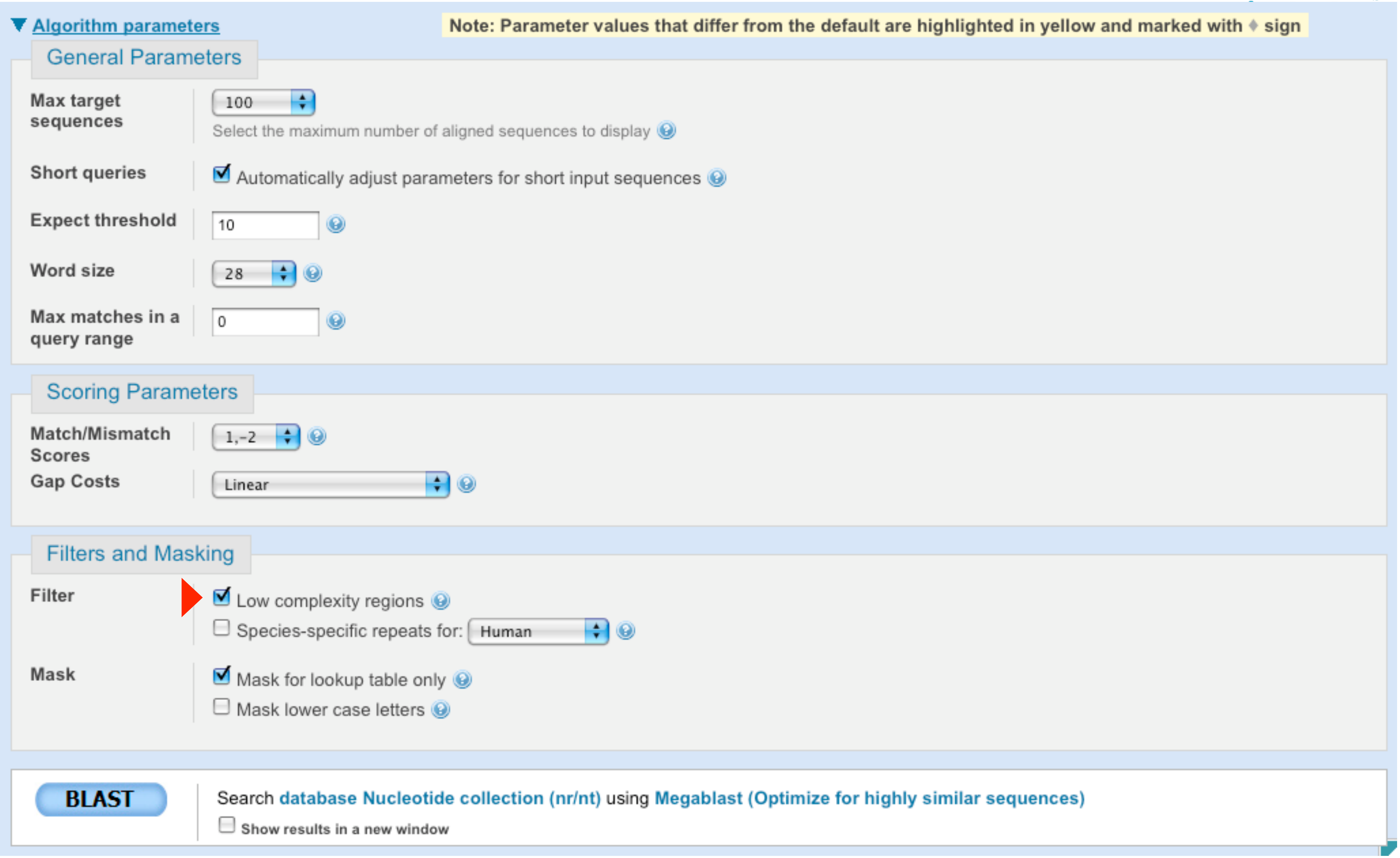
Ala	4																			
Arg	-1	5																		
Asn	-2	0	6																	
Asp	-2	-2	1	6																
Cys	0	-3	-3	-3	9															
Gln	-1	1	0	0	-3	5														
Glu	-1	0	0	2	-4	2	5													
Gly	0	-2	0	-1	-3	-2	-2	6												
His	-2	0	1	-1	-3	0	0	-2	8											
Ile	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
Leu	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
Lys	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
Met	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
Phe	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
Pro	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
Ser	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
Thr	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
Trp	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Tyr	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
Val	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val

The BLOSUM62 matrix

$$S_{ij} = \left(\frac{1}{\lambda}\right)\log\left(\frac{p_{ij}}{p_i * q_j}\right)$$

p_{ij} is the probability of two amino acids i and j replacing each other in a homologous sequence, and q_i and q_j are the background probabilities of finding the amino acids i and j in any protein sequence at random. The factor λ is a scaling factor, set such that the matrix contains easily computable integer values.

GDA21 ▷ Sequence Databases (Extra)



▼ Algorithm parameters

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Expect threshold: 10

Word size: 28

Max matches in a query range: 0

Scoring Parameters

Match/Mismatch Scores: 1,-2

Gap Costs: Linear

Filters and Masking

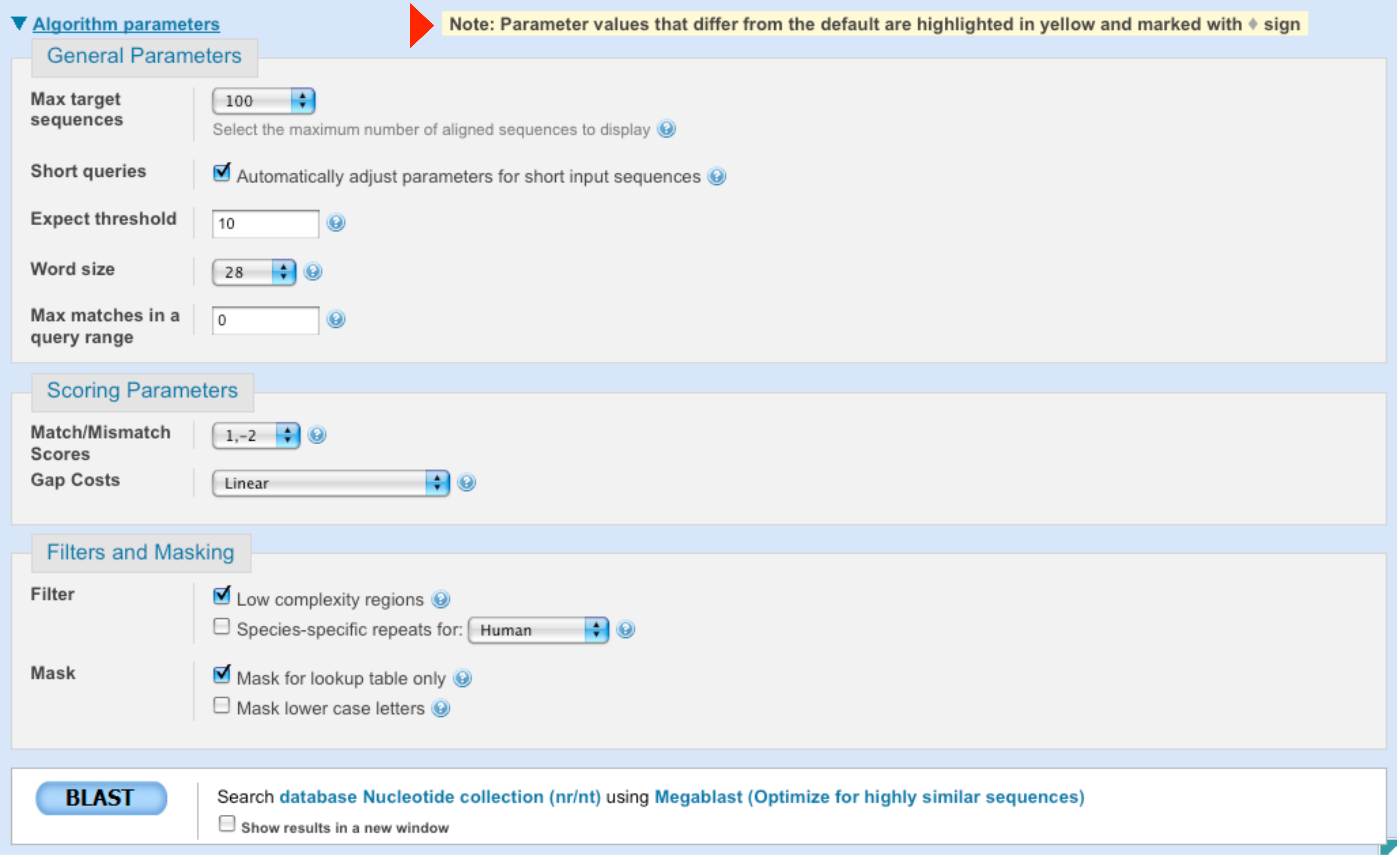
Filter:
☑ Low complexity regions
□ Species-specific repeats for: Human

Mask:
☑ Mask for lookup table only
□ Mask lower case letters

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

□ Show results in a new window

GDA21 ▷ Sequence Databases (Extra)



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BLAST

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