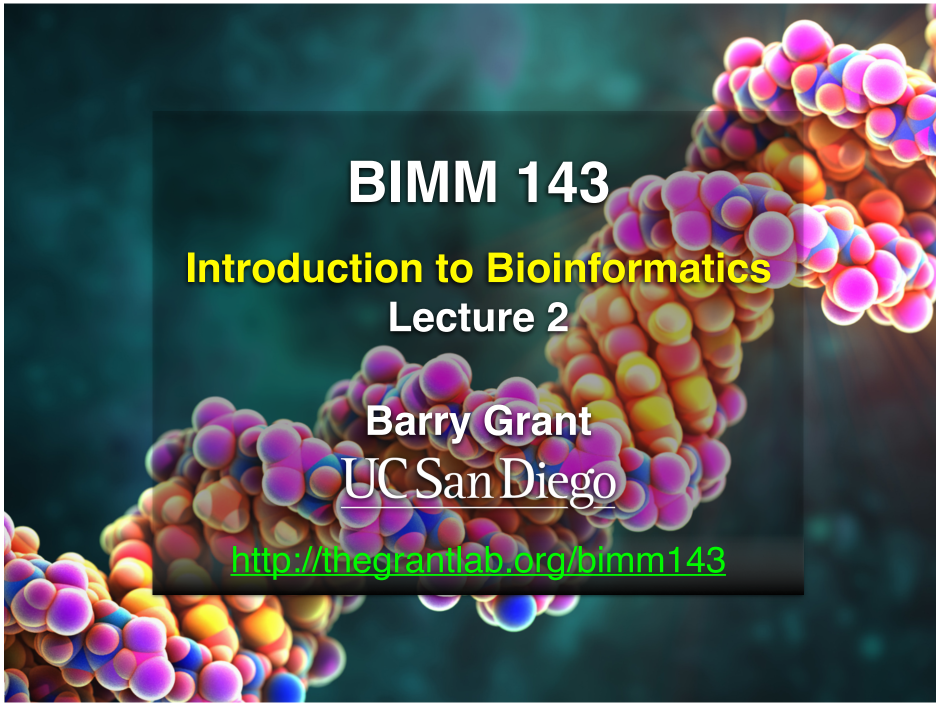


BIMM 143

Introduction to Bioinformatics
Lecture 2

Barry Grant

UC San Diego

http://thegrantlab.org/bimm143

Recap From Last Time:

- Bioinformatics is computer aided biology.
  - Deals with the collection, archiving, organization, and interpretation of a wide range of biological data.
- The NCBI and EBI are major online bioinformatics service providers.
- Introduced via **hands-on session** the BLAST, Entrez, GENE, OMIM, UniProt, Muscle and PDB bioinformatics tools and databases.
  - Muddy point assessment (see results)
- There are a large number of bioinformatics databases (see handout!).
- Also covered: Course structure; Supporting course website, Ethics code, and Introductions…

Today’s Menu

Classifying Databases	Primary, secondary and composite Bioinformatics databases
Using Databases	**Vignette** demonstrating how major Bioinformatics databases intersect
Major Biomolecular Formats	How nucleotide and protein sequence and structure data are represented
Alignment Foundations	**Introducing the *why* and *how* of comparing sequences**
Alignment Algorithms	**Hands-on** exploration of alignment algorithms and applications

Primary, secondary & composite databases

Bioinformatics databases can be usefully classified into *primary*, *secondary* and *composite* according to their data source.

- **Primary databases** (or *archival databases*) consist of data derived experimentally.
  - **GenBank**: NCBI’s primary nucleotide sequence database.
  - **PDB:** Protein X-ray crystal and NMR structures.
- **Secondary databases** (or *derived databases*) contain information derived from a primary database.
  - **RefSeq**: non redundant set of curated reference sequences primarily from GenBank
  - **PFAM**: protein sequence families primarily from UniProt and PDB
- **Composite databases** (or *metadatabases*) join a variety of different primary and secondary database sources.
  - **OMIM**: catalog of human genes, genetic disorders and related literature
  - **GENE**: molecular data and literature related to genes with extensive links to other databases.

DATABASE VIGNETTE

You have just come out a seminar about gastric cancer and one of your co-workers asks:

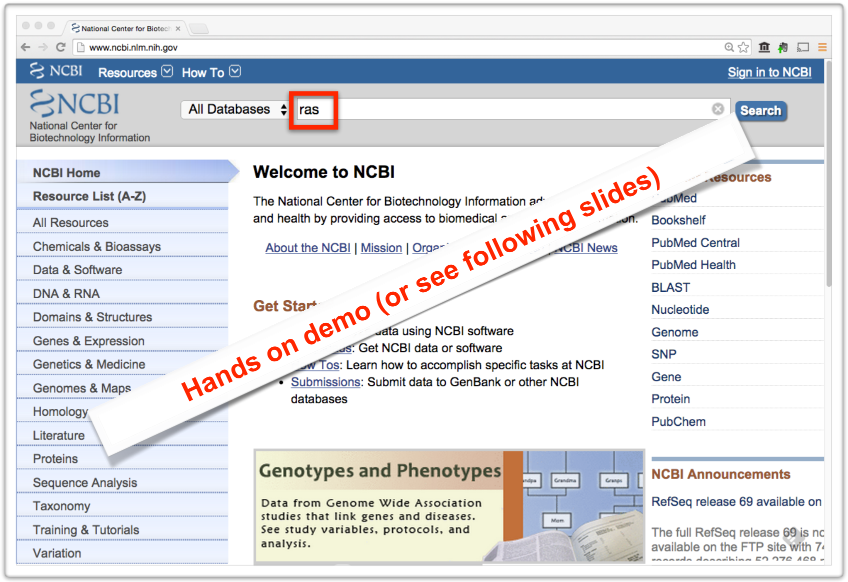
"What do you know about that 'Kras' gene the speaker kept taking about?"

You have some recollection about hearing of 'Ras' before. How would you find out more?

- Google?
- Library?
- **Bioinformatics databases at NCBI and EBI!**

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

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



National Center for Biotech ×

www.ncbi.nlm.nih.gov

NCBI Resources How To Sign in to NCBI

NCBI National Center for Biotechnology Information

All Databases ras Search

Hands on demo (or see following slides)

Welcome to NCBI

The National Center for Biotechnology Information ad… and health by providing access to biomedical …on.

About the NCBI | Mission | Orga… …NCBI News

Get Start…

…data using NCBI software

…s: Get NCBI data or software

…w Tos: Learn how to accomplish specific tasks at NCBI

- Submissions: Submit data to GenBank or other NCBI databases

Genotypes and Phenotypes

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

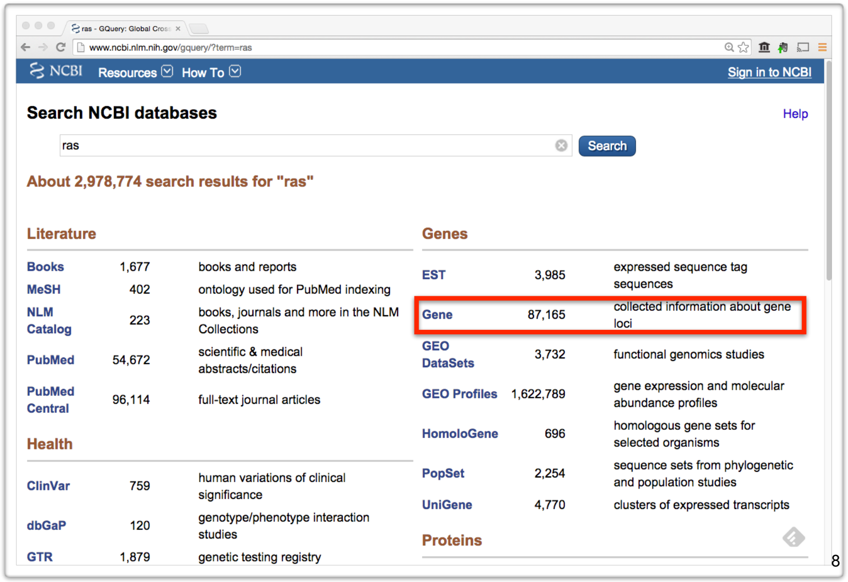
NCBI Announcements

RefSeq release 69 available on

The full RefSeq release 69 is n… available on the FTP site with 7…

Example Vignette Questions:

- What chromosome location and what genes are in the vicinity of a given query gene? NCBI **GENE**
- What can you find out about molecular functions, biological processes, and prominent cellular locations? EBI **GO**
- What amino acid positions in the protein are responsible for ligand binding? EBI **UniProt**
- What variants of this gene are associated with gastric cancer and other human diseases? NCBI **OMIN**
- What is known about the protein family, its species distribution, number in humans and residue-wise conservation? EBI **PFAM**
- Are high resolution protein structures available to examine the details of these mutations? How might we explain their potential molecular effects? RCSB **PDB**



ras - GQuery: Global Cross ×

www.ncbi.nlm.nih.gov/gquery/?term=ras

NCBI Resources How To Sign in to NCBI

Search NCBI databases Help

ras Search

About 2,978,774 search results for "ras"

Literature

Books	1,677	books and reports
MeSH	402	ontology used for PubMed indexing
NLM Catalog	223	books, journals and more in the NLM Collections
PubMed	54,672	scientific & medical abstracts/citations
PubMed Central	96,114	full-text journal articles

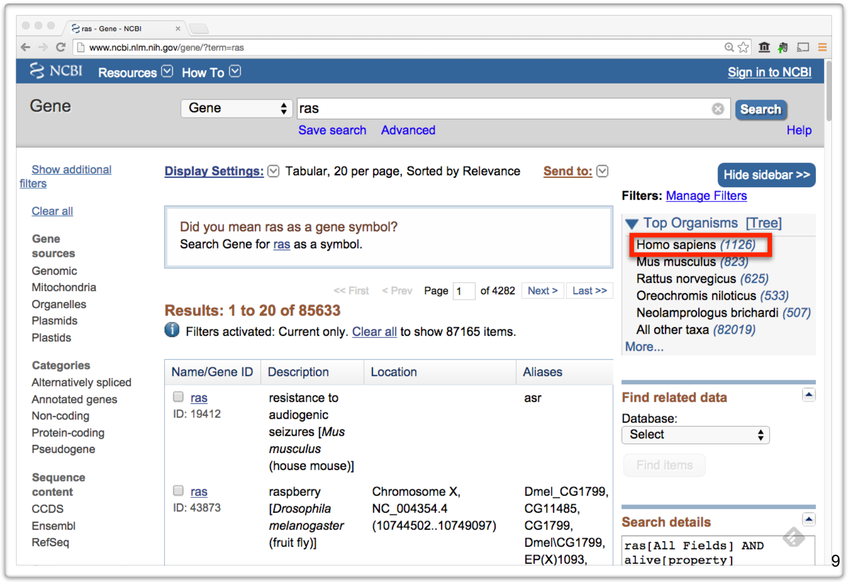
Health

ClinVar	759	human variations of clinical significance
dbGaP	120	genotype/phenotype interaction studies
GTR	1,879	genetic testing registry

Genes

EST	3,985	expressed sequence tag sequences
Gene	87,165	collected information about gene loci
GEO DataSets	3,732	functional genomics studies
GEO Profiles	1,622,789	gene expression and molecular abundance profiles
HomoloGene	696	homologous gene sets for selected organisms
PopSet	2,254	sequence sets from phylogenetic and population studies
UniGene	4,770	clusters of expressed transcripts

Proteins



Gene

Gene: ras

Save search Advanced Help

Show additional filters

Clear all

Gene sources
Genomic
Mitochondria
Organelles
Plasmids
Plastids

Categories
Alternatively spliced
Annotated genes
Non-coding
Protein-coding
Pseudogene

Sequence content
CCDS
Ensembl
RefSeq

Display Settings: Tabular, 20 per page, Sorted by Relevance **Send to:**

Did you mean ras as a gene symbol?
Search Gene for ras as a symbol.

<< First < Prev Page 1 of 4282 Next > Last >>

Results: 1 to 20 of 85633

Filters activated: Current only. Clear all to show 87165 items.

Name/Gene ID	Description	Location	Aliases
□ ras ID: 19412	resistance to audiogenic seizures [*Mus musculus* (house mouse)]		asr
□ ras ID: 43873	raspberry [*Drosophila melanogaster* (fruit fly)]	Chromosome X, NC_004354.4 (10744502..10749097)	Dmel_CG1799, CG11485, CG1799, Dmel\CG1799, EP(X)1093,

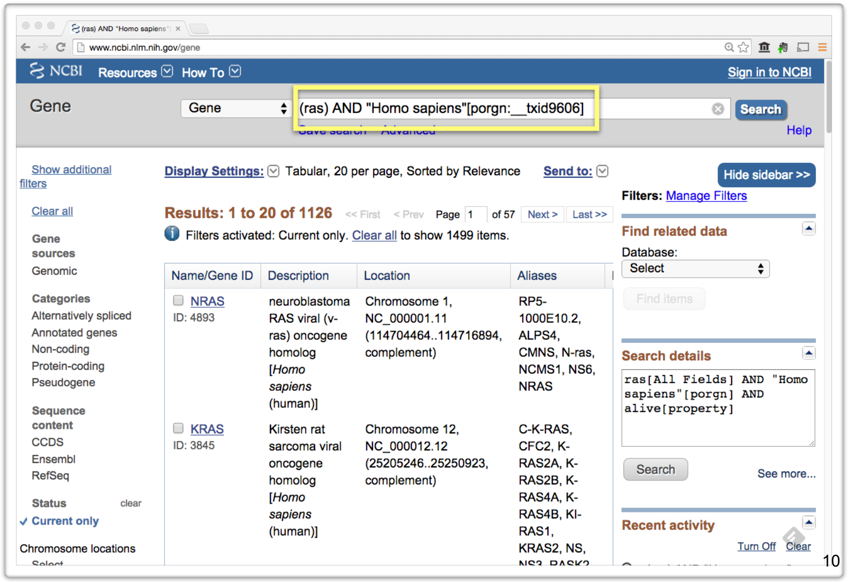
Filters: Manage Filters

Top Organisms [Tree]
Homo sapiens *(1126)*
Mus musculus *(823)*
Rattus norvegicus *(625)*
Oreochromis niloticus *(533)*
Neolamprologus brichardi *(507)*
All other taxa *(82019)*
More...

Find related data
Database: Select
Find items

Search details

```
ras[All Fields] AND
alive[property]
```



Gene

Gene: (ras) AND "Homo sapiens"[porgn:__txid9606]

Save search Advanced Help

Show additional filters

Clear all

Gene sources
Genomic

Categories
Alternatively spliced
Annotated genes
Non-coding
Protein-coding
Pseudogene

Sequence content
CCDS
Ensembl
RefSeq

Status clear
✓ **Current only**

Chromosome locations
Select

Display Settings: Tabular, 20 per page, Sorted by Relevance **Send to:**

Results: 1 to 20 of 1126 << First < Prev Page 1 of 57 Next > Last >>

Filters activated: Current only. Clear all to show 1499 items.

Name/Gene ID	Description	Location	Aliases
□ NRAS ID: 4893	neuroblastoma RAS viral (v-ras) oncogene homolog [*Homo sapiens* (human)]	Chromosome 1, NC_000001.11 (114704464..114716894, complement)	RP5-1000E10.2, ALPS4, CMNS, N-ras, NCMS1, NS6, NRAS
□ KRAS ID: 3845	Kirsten rat sarcoma viral oncogene homolog [*Homo sapiens* (human)]	Chromosome 12, NC_000012.12 (25205246..25250923, complement)	C-K-RAS, CFC2, K-RAS2A, K-RAS2B, K-RAS4A, K-RAS4B, KI-RAS1, KRAS2, NS, NS3, RASK2

Filters: Manage Filters

Find related data
Database: Select
Find items

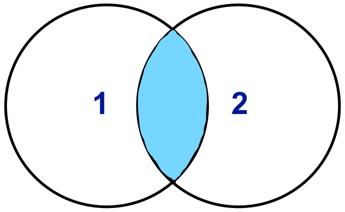
Search details

```
ras[All Fields] AND "Homo
sapiens"[porgn] AND
alive[property]
```

Search See more...

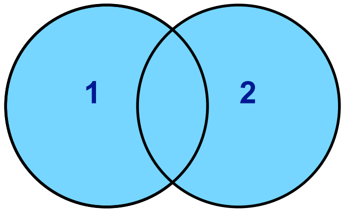
Recent activity
Turn Off Clear

1 AND 2



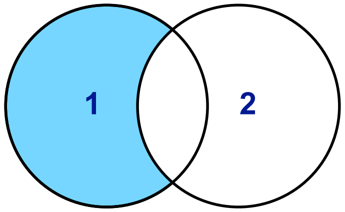
ras AND disease (1185 results)

1 OR 2

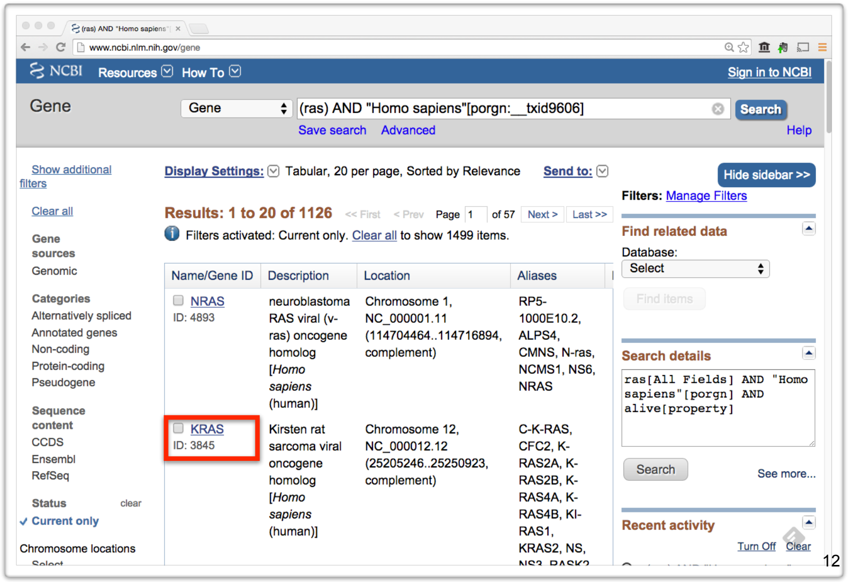


ras OR disease (134,872 results)

1 NOT 2



ras NOT disease (84,448 results)



Gene

Gene: (ras) AND "Homo sapiens"[porgn:__txid9606]

Save search Advanced Help

Show additional filters

Clear all

Gene sources
Genomic

Categories
Alternatively spliced
Annotated genes
Non-coding
Protein-coding
Pseudogene

Sequence content
CCDS
Ensembl
RefSeq

Status clear
✓ **Current only**

Chromosome locations
Select

Display Settings: Tabular, 20 per page, Sorted by Relevance **Send to:**

Results: 1 to 20 of 1126 << First < Prev Page 1 of 57 Next > Last >>

Filters activated: Current only. Clear all to show 1499 items.

Name/Gene ID	Description	Location	Aliases
□ NRAS ID: 4893	neuroblastoma RAS viral (v-ras) oncogene homolog [*Homo sapiens* (human)]	Chromosome 1, NC_000001.11 (114704464..114716894, complement)	RP5-1000E10.2, ALPS4, CMNS, N-ras, NCMS1, NS6, NRAS
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Filters: Manage Filters

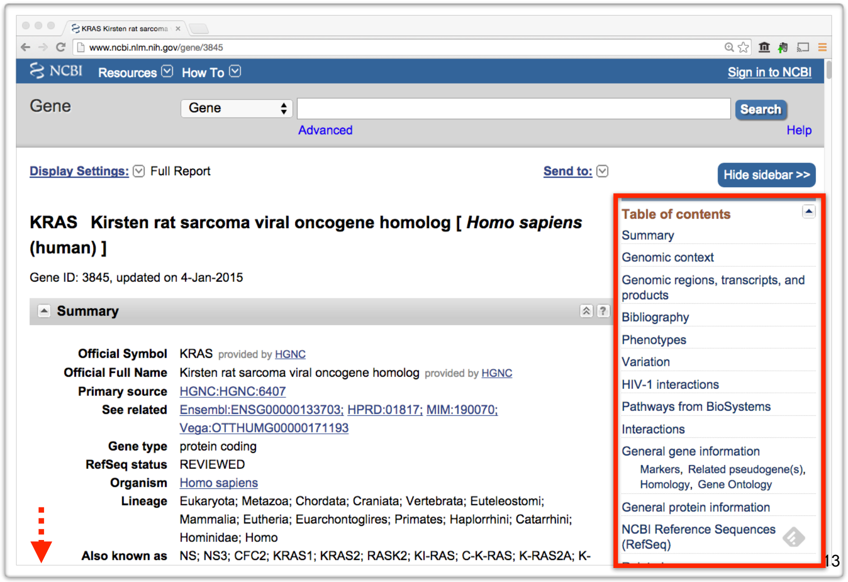
Find related data
Database: Select
Find items

Search details

```
ras[All Fields] AND "Homo
sapiens"[porgn] AND
alive[property]
```

Search See more...

Recent activity
Turn Off Clear



KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845

Display Settings: Full Report

KRAS Kirsten rat sarcoma viral oncogene homolog [*Homo sapiens* (human)]

Gene ID: 3845, updated on 4-Jan-2015

Summary

Official Symbol	KRAS provided by HGNC
Official Full Name	Kirsten rat sarcoma viral oncogene homolog provided by HGNC
Primary source	HGNC:HGNC:6407
See related	Ensembl:ENSG00000133703; HPRD:01817; MIM:190070; Vega:OTTHUMG00000171193
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	NS; NS3; CFC2; KRAS1; KRAS2; RASK2; KI-RAS; C-K-RAS; K-RAS2A; K-

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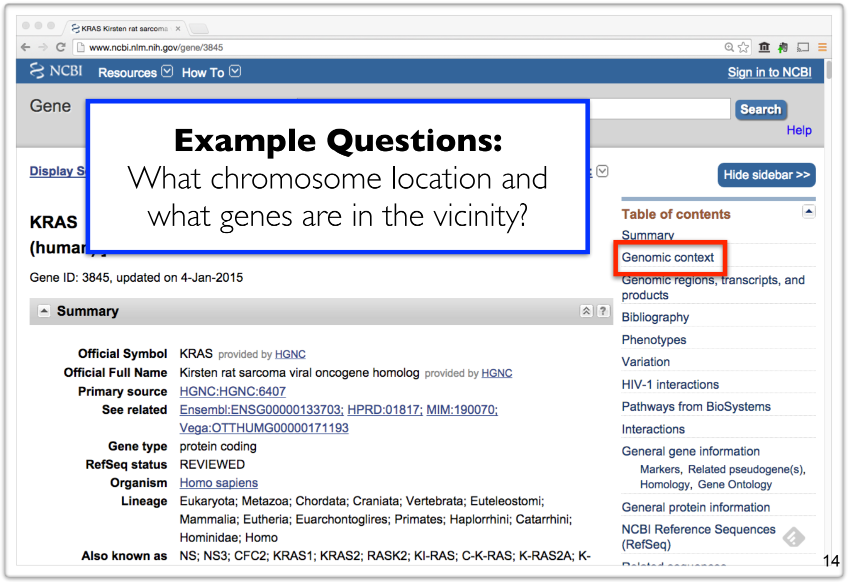
Variation
HIV-1 interactions
Pathways from BioSystems
Interactions
General gene information
Markers, Related pseudogene(s), Homology, Gene Ontology
General protein information
NCBI Reference Sequences (RefSeq)



Example Questions:

What chromosome location and what genes are in the vicinity?

KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845

KRAS (human)]

Gene ID: 3845, updated on 4-Jan-2015

Summary

Official Symbol	KRAS provided by HGNC
Official Full Name	Kirsten rat sarcoma viral oncogene homolog provided by HGNC
Primary source	HGNC:HGNC:6407
See related	Ensembl:ENSG00000133703; HPRD:01817; MIM:190070; Vega:OTTHUMG00000171193
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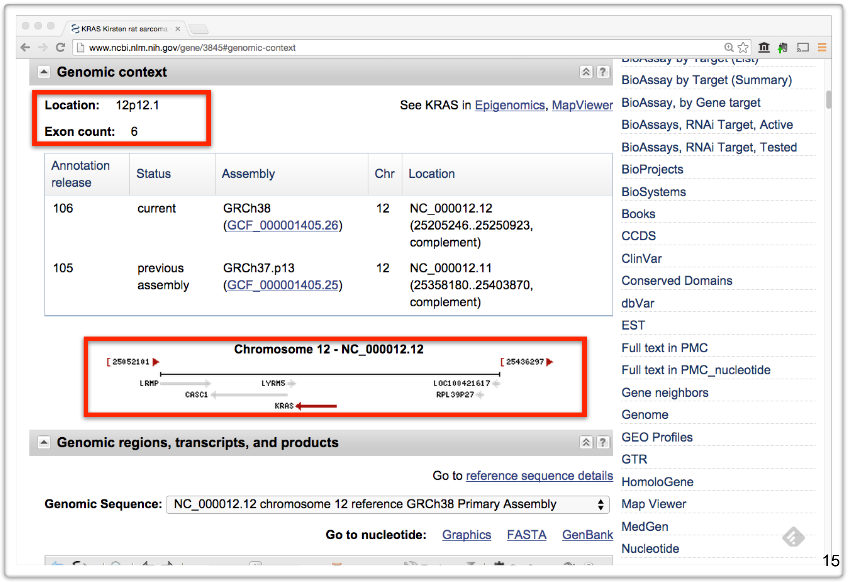


KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845#genomic-context

Genomic context

Location: 12p12.1

Exon count: 6

See KRAS in Epigenomics, MapViewer

Annotation release	Status	Assembly	Chr	Location
106	current	GRCh38 (GCF_000001405.26)	12	NC_000012.12 (25205246..25250923, complement)
105	previous assembly	GRCh37.p13 (GCF_000001405.25)	12	NC_000012.11 (25358180..25403870, complement)

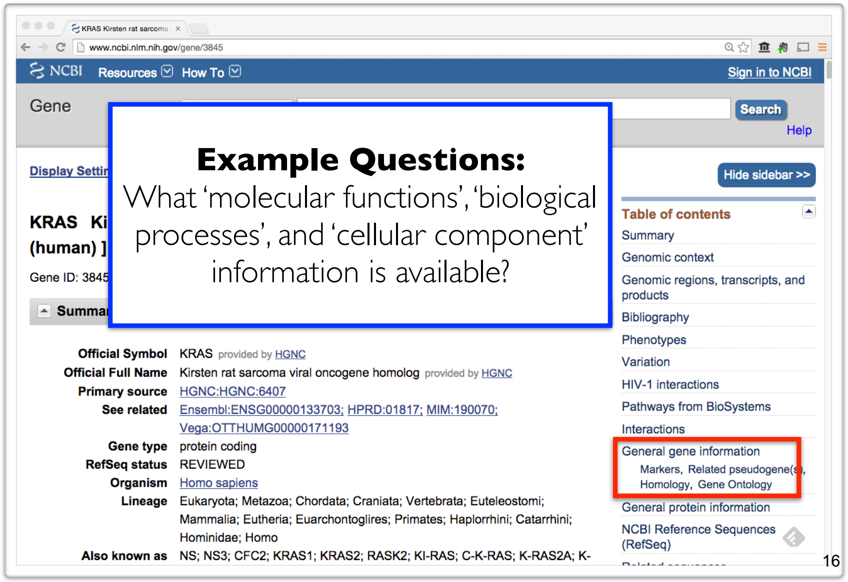
Genomic regions, transcripts, and products

Go to reference sequence details

Genomic Sequence: NC_000012.12 chromosome 12 reference GRCh38 Primary Assembly

Go to nucleotide: Graphics FASTA GenBank

BioAssay by Target (List)
BioAssay by Target (Summary)
BioAssay, by Gene target
BioAssays, RNAi Target, Active
BioAssays, RNAi Target, Tested
BioProjects
BioSystems
Books
CCDS
ClinVar
Conserved Domains
dbVar
EST
Full text in PMC
Full text in PMC_nucleotide
Gene neighbors
Genome
GEO Profiles
GTR
HomoloGene
Map Viewer
MedGen
Nucleotide



Example Questions:

What 'molecular functions', 'biological processes', and 'cellular component' information is available?

KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845

KRAS Ki (human)]

Gene ID: 3845

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Official Full Name	Kirsten rat sarcoma viral oncogene homolog provided by HGNC
Primary source	HGNC:HGNC:6407
See related	Ensembl:ENSG00000133703; HPRD:01817; MIM:190070; Vega:OTTHUMG00000171193
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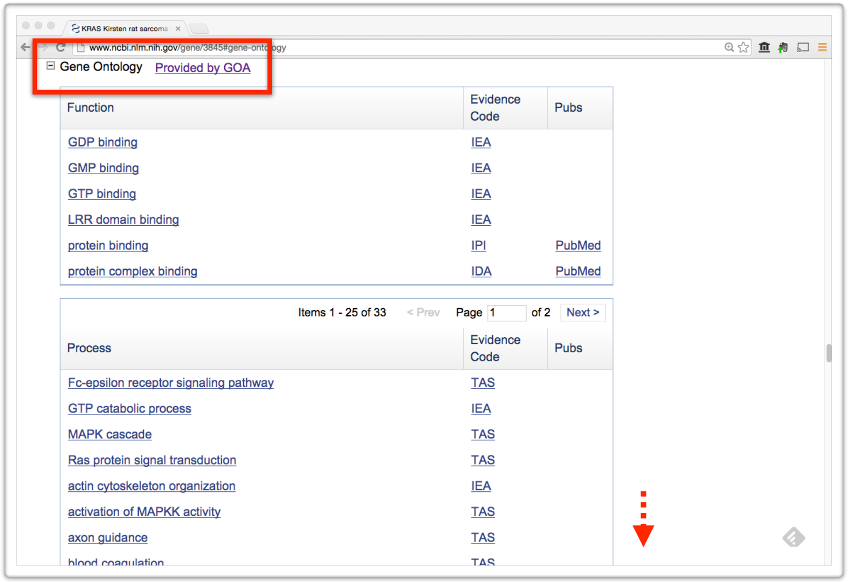
NCBI Reference Sequences (RefSeq)



KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845#gene-ontology

⊟ Gene Ontology Provided by GOA

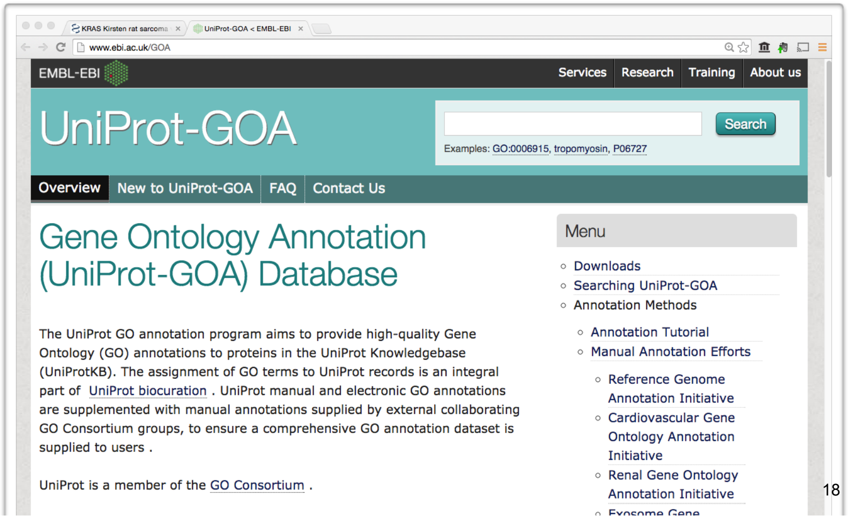
Function	Evidence Code	Pubs
GDP binding	IEA	
GMP binding	IEA	
GTP binding	IEA	
LRR domain binding	IEA	
protein binding	IPI	PubMed
protein complex binding	IDA	PubMed

Items 1 - 25 of 33 < Prev Page 1 of 2 Next >

Process	Evidence Code	Pubs
Fc-epsilon receptor signaling pathway	TAS	
GTP catabolic process	IEA	
MAPK cascade	TAS	
Ras protein signal transduction	TAS	
actin cytoskeleton organization	IEA	
activation of MAPKK activity	TAS	
axon guidance	TAS	
blood coagulation	TAS	

GO: Gene Ontology

GO provides a controlled vocabulary of terms for describing gene product characteristics and gene product annotation data



KRAS Kirsten rat sarcoma × UniProt-GOA < EMBL-EBI ×

www.ebi.ac.uk/GOA

UniProt-GOA

Examples: GO:0006915, tropomyosin, P06727

Gene Ontology Annotation (UniProt-GOA) Database

The UniProt GO annotation program aims to provide high-quality Gene Ontology (GO) annotations to proteins in the UniProt Knowledgebase (UniProtKB). The assignment of GO terms to UniProt records is an integral part of UniProt biocuration . UniProt manual and electronic GO annotations are supplemented with manual annotations supplied by external collaborating GO Consortium groups, to ensure a comprehensive GO annotation dataset is supplied to users .

UniProt is a member of the GO Consortium .

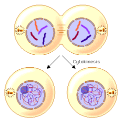
- Downloads
- Searching UniProt-GOA
- Annotation Methods
  - Annotation Tutorial
  - Manual Annotation Efforts
    - Reference Genome Annotation Initiative
    - Cardiovascular Gene Ontology Annotation Initiative
    - Renal Gene Ontology Annotation Initiative
    - Exosome Gene

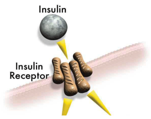
Why do we need Ontologies?

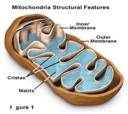
- Annotation is essential for capturing the understanding and knowledge associated with a sequence or other molecular entity
- Annotation is traditionally recorded as “free text”, which is easy to read by humans, but has a number of disadvantages, including:
  - Difficult for computers to parse
  - Quality varies from database to database
  - Terminology used varies from annotator to annotator
- Ontologies are annotations using standard vocabularies that try to address these issues
- GO is integrated with UniProt and many other databases including a number at NCBI

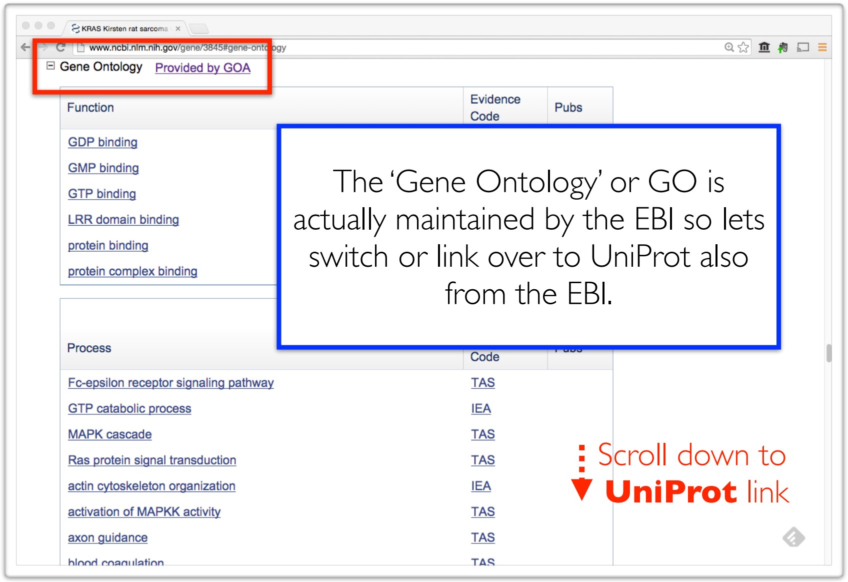
GO Ontologies

- There are three ontologies in GO:
  - **Biological Process**
    A commonly recognized series of events
    e.g. cell division, mitosis,
  - **Molecular Function**
    An elemental activity, task or job
    e.g. kinase activity, insulin binding
  - **Cellular Component**
    Where a gene product is located
    e.g. mitochondrion, mitochondrial membrane









KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845#gene-ontology

⊟ Gene Ontology Provided by GOA

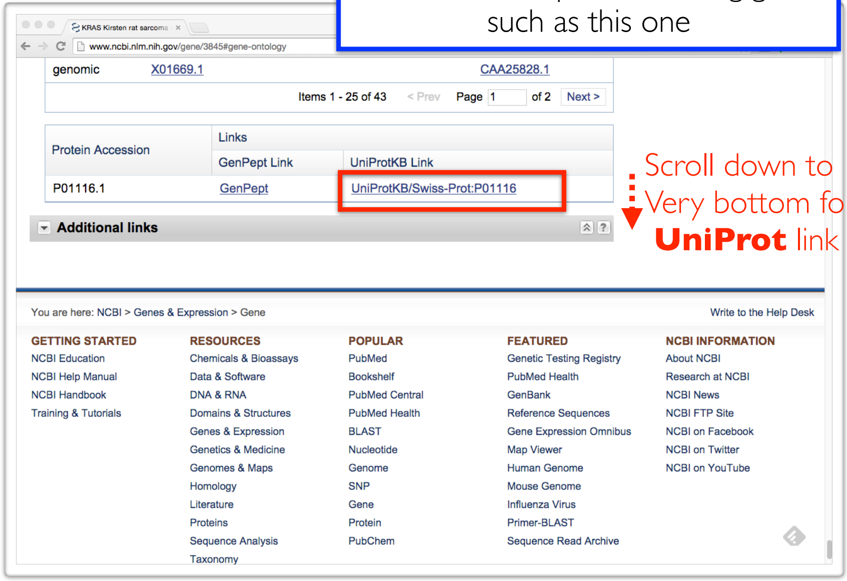
Function	Evidence Code	Pubs
GDP binding		
GMP binding		
GTP binding		
LRR domain binding		
protein binding		
protein complex binding		

Process	Evidence Code	Pubs
Fc-epsilon receptor signaling pathway	TAS	
GTP catabolic process	IEA	
MAPK cascade	TAS	
Ras protein signal transduction	TAS	
actin cytoskeleton organization	IEA	
activation of MAPKK activity	TAS	
axon guidance	TAS	
blood coagulation	TAS	

The 'Gene Ontology' or GO is actually maintained by the EBI so lets switch or link over to UniProt also from the EBI.

Scroll down to **UniProt** link

UniProt will detail much more information for protein coding genes such as this one



KRAS Kirsten rat sarcoma

www.ncbi.nlm.nih.gov/gene/3845#gene-ontology

genomic	X01669.1	CAA25828.1

Items 1 - 25 of 43 < Prev Page 1 of 2 Next >

Protein Accession	Links	
	GenPept Link	UniProtKB Link
P01116.1	GenPept	UniProtKB/Swiss-Prot:P01116

Additional links

Scroll down to Very bottom for **UniProt** link

You are here: NCBI > Genes & Expression > Gene

Write to the Help Desk

GETTING STARTED
NCBI Education
NCBI Help Manual
NCBI Handbook
Training & Tutorials

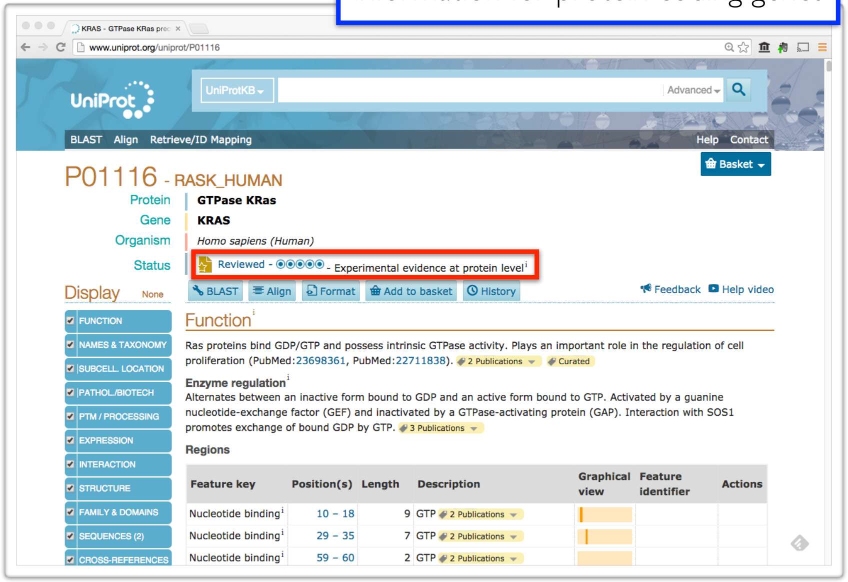
RESOURCES
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy

POPULAR
PubMed
Bookshelf
PubMed Central
PubMed Health
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem

FEATURED
Genetic Testing Registry
PubMed Health
GenBank
Reference Sequences
Gene Expression Omnibus
Map Viewer
Human Genome
Mouse Genome
Influenza Virus
Primer-BLAST
Sequence Read Archive

NCBI INFORMATION
About NCBI
Research at NCBI
NCBI News
NCBI FTP Site
NCBI on Facebook
NCBI on Twitter
NCBI on YouTube

UniProt will detail much more information for protein coding genes



KRAS - GTPase KRas prec

www.uniprot.org/uniprot/P01116

UniProt

UniProtKB Advanced

BLAST Align Retrieve/ID Mapping Help Contact

Basket

P01116 - RASK_HUMAN

Protein | GTPase KRas
Gene | KRAS
Organism | *Homo sapiens (Human)*
Status | Reviewed - ◉◉◉◉◉ - Experimental evidence at protein level[i]

Display None

☑ FUNCTION
☑ NAMES & TAXONOMY
☑ SUBCELL. LOCATION
☑ PATHOL./BIOTECH
☑ PTM / PROCESSING
☑ EXPRESSION
☑ INTERACTION
☑ STRUCTURE
☑ FAMILY & DOMAINS
☑ SEQUENCES (2)
☑ CROSS-REFERENCES

BLAST Align Format Add to basket History Feedback Help video

Function[i]

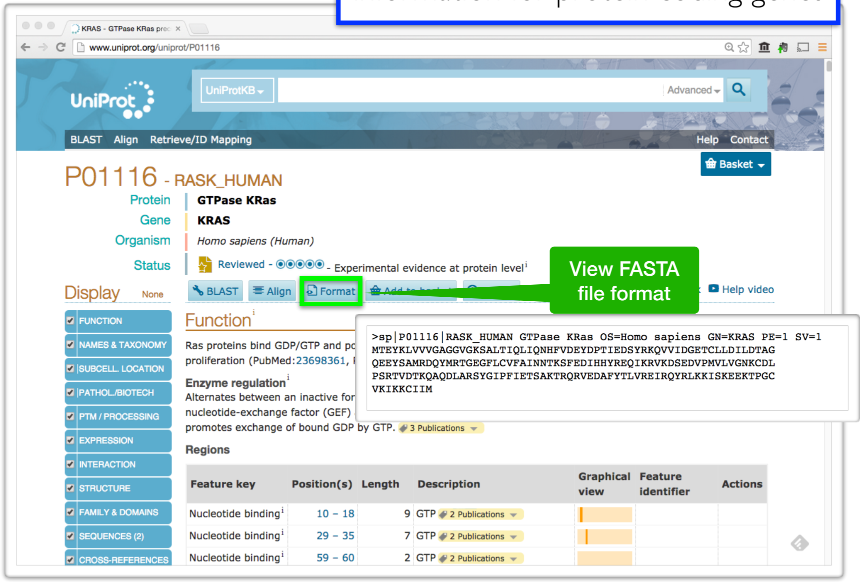
Ras proteins bind GDP/GTP and possess intrinsic GTPase activity. Plays an important role in the regulation of cell proliferation (PubMed:23698361, PubMed:22711838). 2 Publications Curated

Enzyme regulation[i]
Alternates between an inactive form bound to GDP and an active form bound to GTP. Activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP). Interaction with SOS1 promotes exchange of bound GDP by GTP. 3 Publications

Regions

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Nucleotide binding[i]	10 – 18	9	GTP 2 Publications			
Nucleotide binding[i]	29 – 35	7	GTP 2 Publications			
Nucleotide binding[i]	59 – 60	2	GTP 2 Publications			

UniProt will detail much more information for protein coding genes



KRAS - GTPase KRas prec

www.uniprot.org/uniprot/P01116

UniProt

UniProtKB Advanced

BLAST Align Retrieve/ID Mapping Help Contact

Basket

P01116 - RASK_HUMAN

Protein | GTPase KRas
Gene | KRAS
Organism | *Homo sapiens (Human)*
Status | Reviewed - ◉◉◉◉◉ - Experimental evidence at protein level[i]

Display None

☑ FUNCTION
☑ NAMES & TAXONOMY
☑ SUBCELL. LOCATION
☑ PATHOL./BIOTECH
☑ PTM / PROCESSING
☑ EXPRESSION
☑ INTERACTION
☑ STRUCTURE
☑ FAMILY & DOMAINS
☑ SEQUENCES (2)
☑ CROSS-REFERENCES

BLAST Align Format Help video

View FASTA file format

```
>sp|P01116|RASK_HUMAN GTPase KRas OS=Homo sapiens GN=KRAS PE=1 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCIIM
```

Function[i]

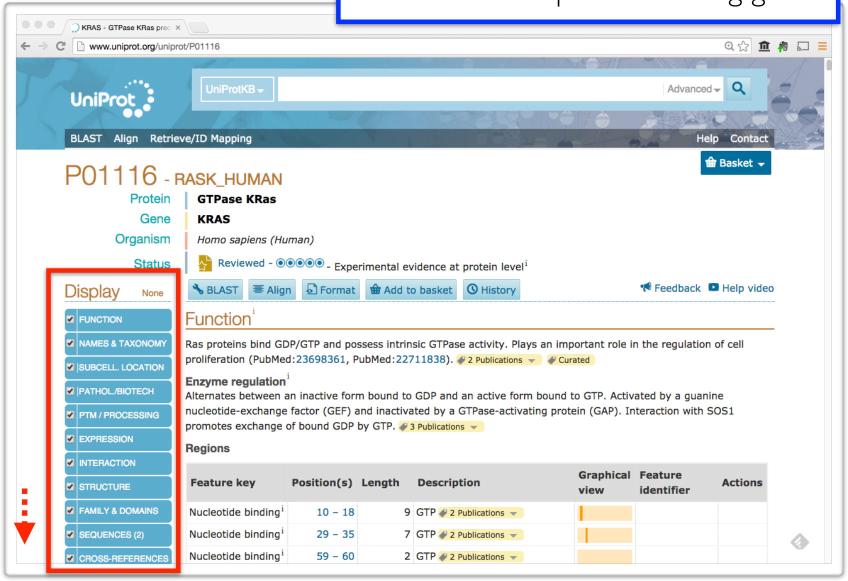
Ras proteins bind GDP/GTP and pc
proliferation (PubMed:23698361, I

Enzyme regulation[i]
Alternates between an inactive for
nucleotide-exchange factor (GEF)
promotes exchange of bound GDP by GTP. 3 Publications

Regions

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Nucleotide binding[i]	10 – 18	9	GTP 2 Publications			
Nucleotide binding[i]	29 – 35	7	GTP 2 Publications			
Nucleotide binding[i]	59 – 60	2	GTP 2 Publications			

UniProt will detail much more information for protein coding genes



P01116 - RASK_HUMAN

Protein	GTPase KRas
Gene	KRAS
Organism	*Homo sapiens (Human)*
Status	Reviewed - ●●●●● - Experimental evidence at protein level[i]

Function[i]

Ras proteins bind GDP/GTP and possess intrinsic GTPase activity. Plays an important role in the regulation of cell proliferation (PubMed:23698361, PubMed:22711838). 2 Publications Curated

Enzyme regulation[i]
Alternates between an inactive form bound to GDP and an active form bound to GTP. Activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP). Interaction with SOS1 promotes exchange of bound GDP by GTP. 3 Publications

Regions

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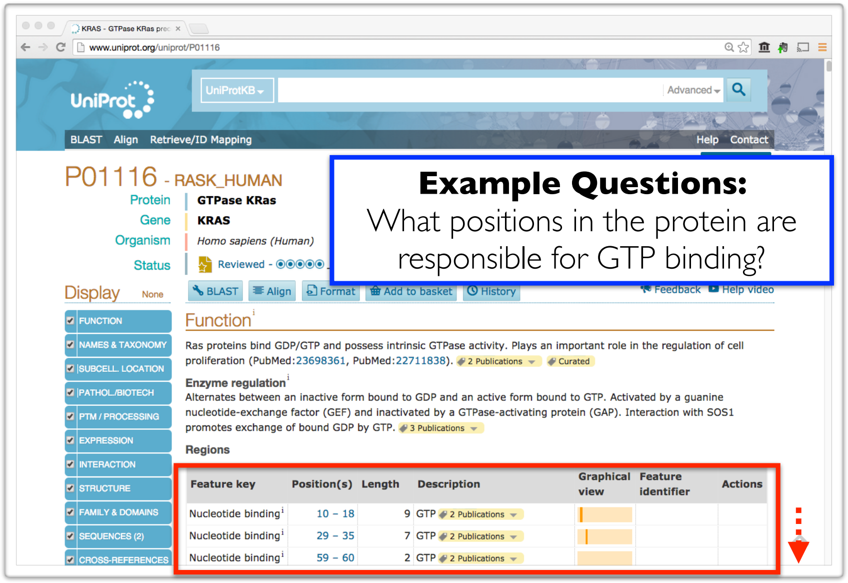

Example Questions:
What positions in the protein are responsible for GTP binding?

P01116 - RASK_HUMAN

Protein	GTPase KRas
Gene	KRAS
Organism	*Homo sapiens (Human)*
Status	Reviewed - ●●●●●

Function[i]

Ras proteins bind GDP/GTP and possess intrinsic GTPase activity. Plays an important role in the regulation of cell proliferation (PubMed:23698361, PubMed:22711838). 2 Publications Curated

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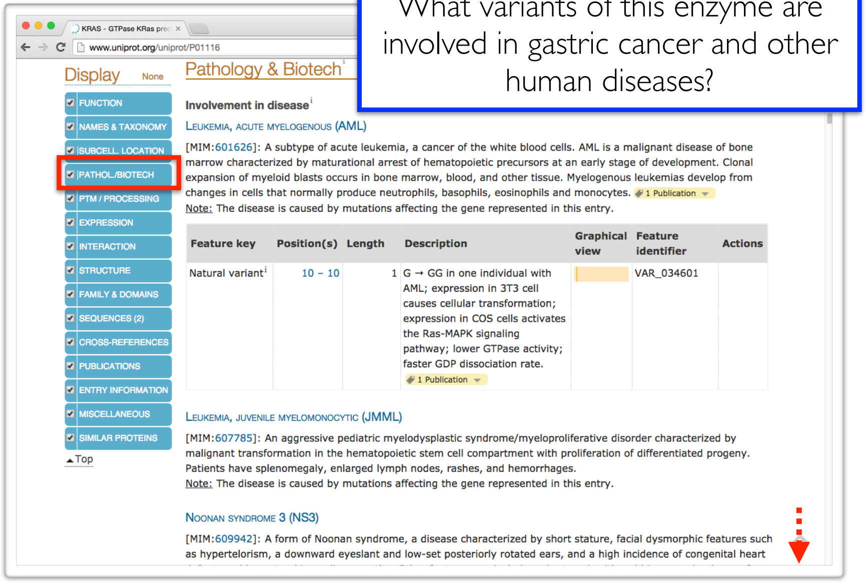
Nucleotide binding[i]	59 – 60	2	GTP 2 Publications			



Example Questions:
What variants of this enzyme are involved in gastric cancer and other human diseases?

Pathology & Biotech[i]

Involvement in disease[i]

LEUKEMIA, ACUTE MYELOGENOUS (AML)

[MIM:601626]: A subtype of acute leukemia, a cancer of the white blood cells. AML is a malignant disease of bone marrow characterized by maturational arrest of hematopoietic precursors at an early stage of development. Clonal expansion of myeloid blasts occurs in bone marrow, blood, and other tissue. Myelogenous leukemias develop from changes in cells that normally produce neutrophils, basophils, eosinophils and monocytes. 1 Publication
Note: The disease is caused by mutations affecting the gene represented in this entry.

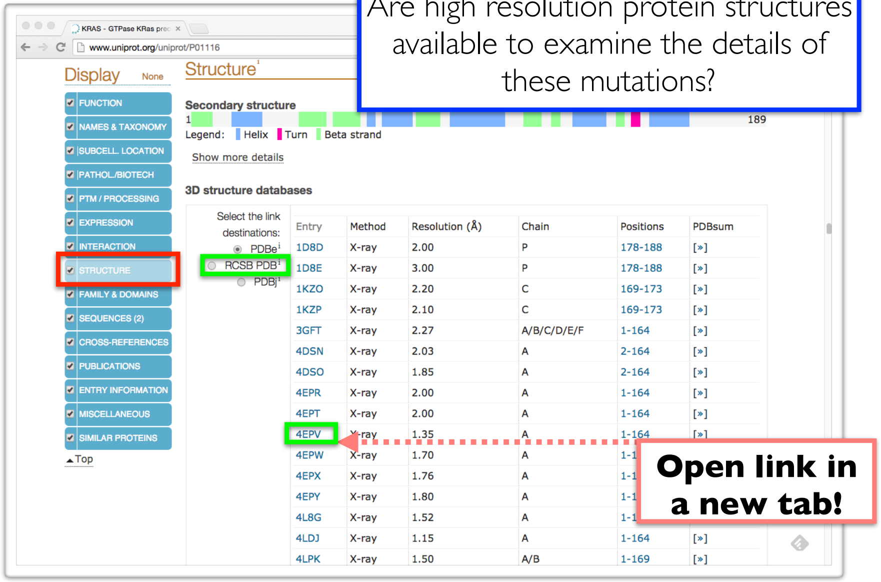
Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Natural variant[i]	10 – 10	1	G → GG in one individual with AML; expression in 3T3 cell causes cellular transformation; expression in COS cells activates the Ras-MAPK signaling pathway; lower GTPase activity; faster GDP dissociation rate. 1 Publication		VAR_034601	

LEUKEMIA, JUVENILE MYELOMONOCYTIC (JMML)

[MIM:607785]: An aggressive pediatric myelodysplastic syndrome/myeloproliferative disorder characterized by malignant transformation in the hematopoietic stem cell compartment with proliferation of differentiated progeny. Patients have splenomegaly, enlarged lymph nodes, rashes, and hemorrhages.
Note: The disease is caused by mutations affecting the gene represented in this entry.

NOONAN SYNDROME 3 (NS3)

[MIM:609942]: A form of Noonan syndrome, a disease characterized by short stature, facial dysmorphic features such as hypertelorism, a downward eyeslant and low-set posteriorly rotated ears, and a high incidence of congenital heart



Example Questions:
Are high resolution protein structures available to examine the details of these mutations?

Structure[i]

Secondary structure

Legend: Helix Turn Beta strand

Show more details

3D structure databases

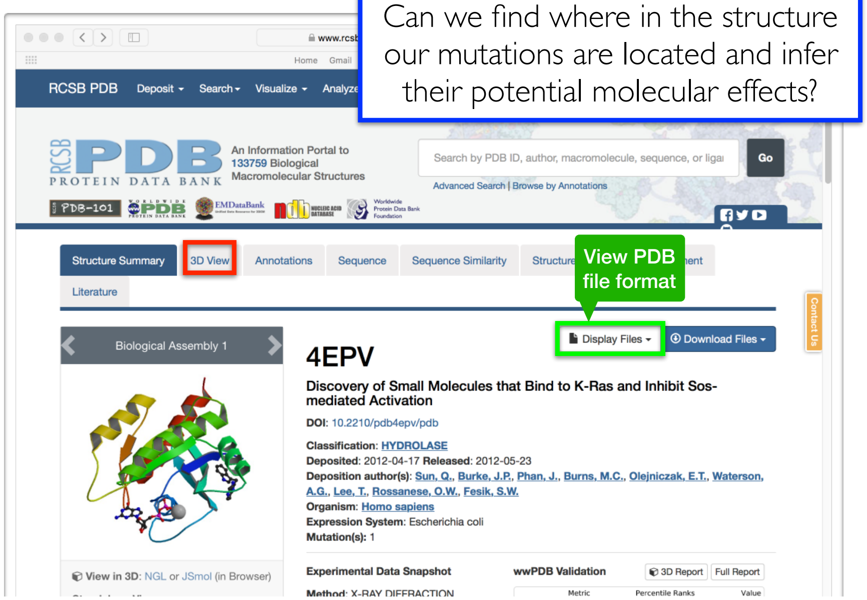
Select the link destinations:
● PDBe[i]
○ RCSB PDB[i]
○ PDBj[i]

Entry	Method	Resolution (Å)	Chain	Positions	PDBsum
1D8D	X-ray	2.00	P	178-188	[»]
1D8E	X-ray	3.00	P	178-188	[»]
1KZO	X-ray	2.20	C	169-173	[»]
1KZP	X-ray	2.10	C	169-173	[»]
3GFT	X-ray	2.27	A/B/C/D/E/F	1-164	[»]
4DSN	X-ray	2.03	A	2-164	[»]
4DSO	X-ray	1.85	A	2-164	[»]
4EPR	X-ray	2.00	A	1-164	[»]
4EPT	X-ray	2.00	A	1-164	[»]
4EPV	X-ray	1.35	A	1-164	[»]
4EPW	X-ray	1.70	A	1-1	
4EPX	X-ray	1.76	A	1-1	
4EPY	X-ray	1.80	A	1-1	
4L8G	X-ray	1.52	A	1-1	
4LDJ	X-ray	1.15	A	1-164	[»]
4LPK	X-ray	1.50	A/B	1-169	[»]

Open link in a new tab!

Lets view the 3D structure:

Can we find where in the structure our mutations are located and infer their potential molecular effects?



An Information Portal to 133759 Biological Macromolecular Structures

Search by PDB ID, author, macromolecule, sequence, or ligai

View PDB file format

4EPV

Discovery of Small Molecules that Bind to K-Ras and Inhibit Sos-mediated Activation

DOI: 10.2210/pdb4epv/pdb

Classification: HYDROLASE
Deposited: 2012-04-17 **Released:** 2012-05-23
Deposition author(s): Sun, Q., Burke, J.P., Phan, J., Burns, M.C., Olejniczak, E.T., Waterson, A.G., Lee, T., Rossanese, O.W., Fesik, S.W.
Organism: Homo sapiens
Expression System: Escherichia coli
Mutation(s): 1

View in 3D: NGL or JSmol (in Browser)

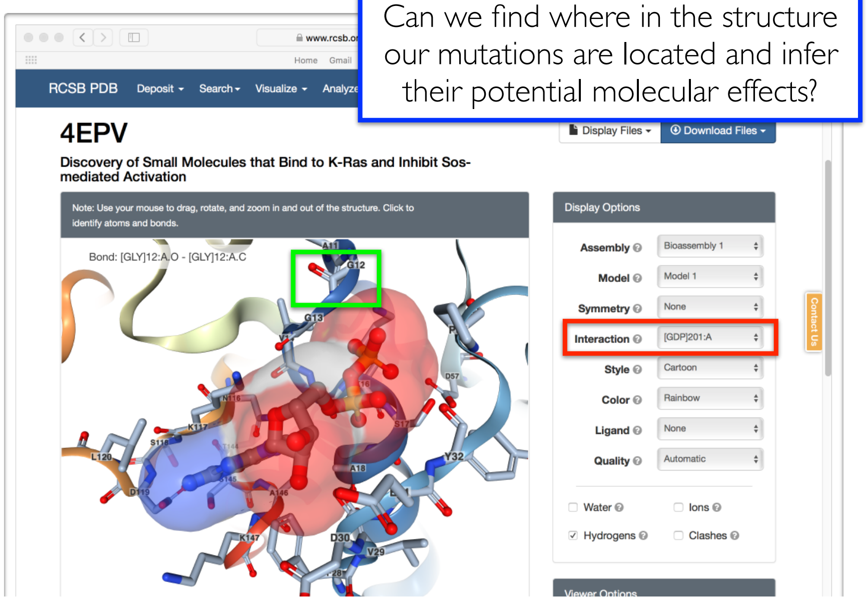
Experimental Data Snapshot

Method: X-RAY DIFFRACTION

wwPDB Validation

Lets view the 3D structure:

Can we find where in the structure our mutations are located and infer their potential molecular effects?



4EPV

Discovery of Small Molecules that Bind to K-Ras and Inhibit Sos-mediated Activation

Note: Use your mouse to drag, rotate, and zoom in and out of the structure. Click to identify atoms and bonds.

Bond: [GLY]12:A.O - [GLY]12:A.C

Display Options

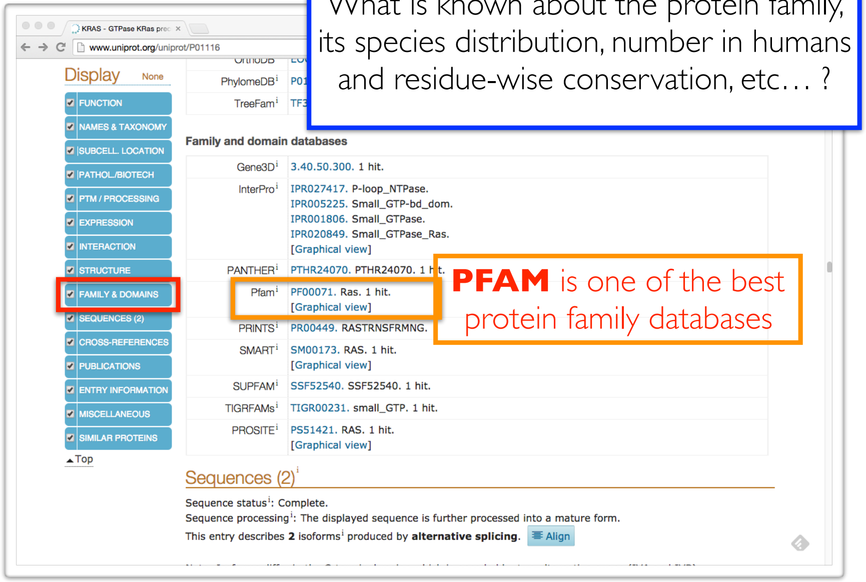
Assembly	Bioassembly 1
Model	Model 1
Symmetry	None
Interaction	[GDP]201:A
Style	Cartoon
Color	Rainbow
Ligand	None
Quality	Automatic

☐ Water ☐ Ions
☑ Hydrogens ☐ Clashes

Viewer Options

Back to UniProt:

What is known about the protein family, its species distribution, number in humans and residue-wise conservation, etc… ?



OrthoDB	LO
PhylomeDB	P01
TreeFam	TF3

Family and domain databases

Gene3D	3.40.50.300. 1 hit.
InterPro	IPR027417. P-loop_NTPase. IPR005225. Small_GTP-bd_dom. IPR001806. Small_GTPase. IPR020849. Small_GTPase_Ras. [Graphical view]
PANTHER	PTHR24070. PTHR24070. 1 hit.
Pfam	PF00071. Ras. 1 hit. [Graphical view]
PRINTS	PR00449. RASTRNSFRMNG.
SMART	SM00173. RAS. 1 hit. [Graphical view]
SUPFAM	SSF52540. SSF52540. 1 hit.
TIGRFAMs	TIGR00231. small_GTP. 1 hit.
PROSITE	PS51421. RAS. 1 hit. [Graphical view]

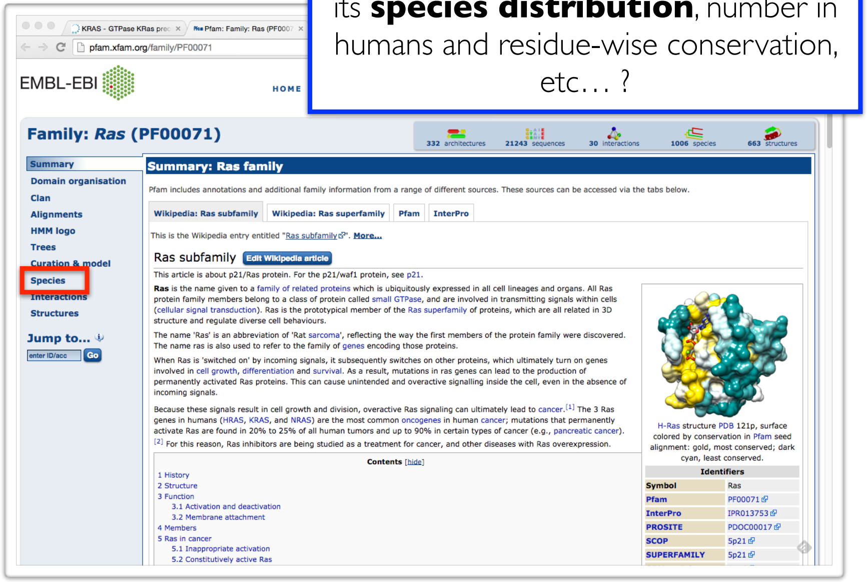
PFAM is one of the best protein family databases

Sequences (2)

Sequence status: Complete.
Sequence processing: The displayed sequence is further processed into a mature form.
This entry describes **2** isoforms produced by **alternative splicing**.

Example Questions:

What is known about the protein family, its **species distribution**, number in humans and residue-wise conservation, etc… ?



Family: *Ras* (PF00071)

Summary: Ras family

Pfam includes annotations and additional family information from a range of different sources. These sources can be accessed via the tabs below.

This is the Wikipedia entry entitled "Ras subfamily". More...

Ras subfamily

This article is about p21/Ras protein. For the p21/waf1 protein, see p21.

Ras is the name given to a family of related proteins which is ubiquitously expressed in all cell lineages and organs. All Ras protein family members belong to a class of protein called small GTPase, and are involved in transmitting signals within cells (cellular signal transduction). Ras is the prototypical member of the Ras superfamily of proteins, which are all related in 3D structure and regulate diverse cell behaviours.

The name 'Ras' is an abbreviation of 'Rat sarcoma', reflecting the way the first members of the protein family were discovered. The name ras is also used to refer to the family of genes encoding those proteins.

When Ras is 'switched on' by incoming signals, it subsequently switches on other proteins, which ultimately turn on genes involved in cell growth, differentiation and survival. As a result, mutations in ras genes can lead to the production of permanently activated Ras proteins. This can cause unintended and overactive signalling inside the cell, even in the absence of incoming signals.

Because these signals result in cell growth and division, overactive Ras signaling can ultimately lead to cancer.[1] The 3 Ras genes in humans (HRAS, KRAS, and NRAS) are the most common oncogenes in human cancer; mutations that permanently activate Ras are found in 20% to 25% of all human tumors and up to 90% in certain types of cancer (e.g., pancreatic cancer).[2] For this reason, Ras inhibitors are being studied as a treatment for cancer, and other diseases with Ras overexpression.

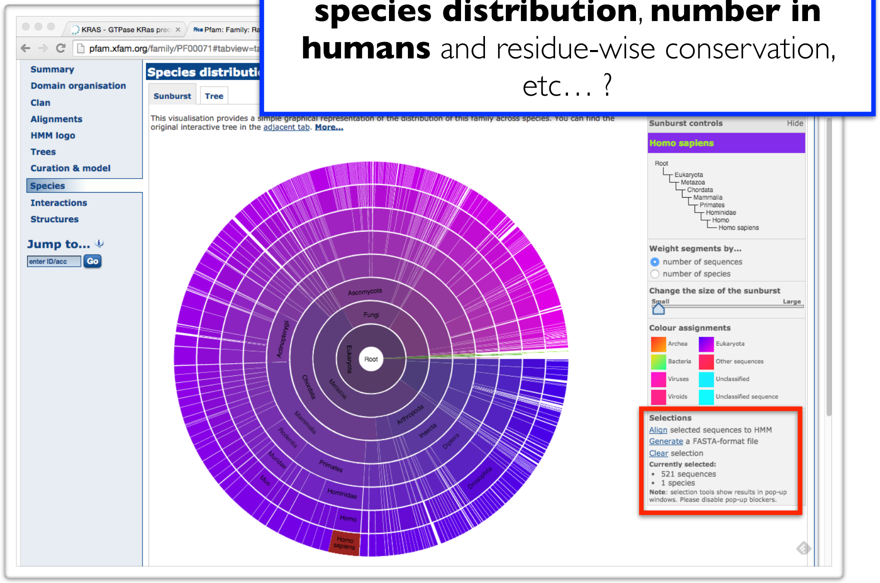
Contents [hide]

1 History
2 Structure
3 Function
3.1 Activation and deactivation
3.2 Membrane attachment
4 Members
5 Ras in cancer
5.1 Inappropriate activation
5.2 Constitutively active Ras

H-Ras structure PDB 121p, surface colored by conservation in Pfam seed alignment: gold, most conserved; dark cyan, least conserved.

Identifiers	
Symbol	Ras
Pfam	PF00071
InterPro	IPR013753
PROSITE	PDOC00017
SCOP	5p21
SUPERFAMILY	5p21

Example Questions:
What is known about the protein family, its **species distribution**, **number in humans** and residue-wise conservation, etc… ?



KRAS - GTPase KRas prec × | Pfam: Family: Ra

pfam.xfam.org/family/PF00071#tabview=ta

- Summary
- Domain organisation
- Clan
- Alignments
- HMM logo
- Trees
- Curation & model
- Species
- Interactions
- Structures

Jump to… enter ID/acc Go

Species distributi

Sunburst | Tree

This visualisation provides a simple graphical representation of the distribution of this family across species. You can find the original interactive tree in the adjacent tab. More…

Sunburst controls Hide

Homo sapiens

```
Root
└ Eukaryota
  └ Metazoa
    └ Chordata
      └ Mammalia
        └ Primates
          └ Hominidae
            └ Homo
              └ Homo sapiens
```

Weight segments by…
● number of sequences
○ number of species

Change the size of the sunburst
Small — Large

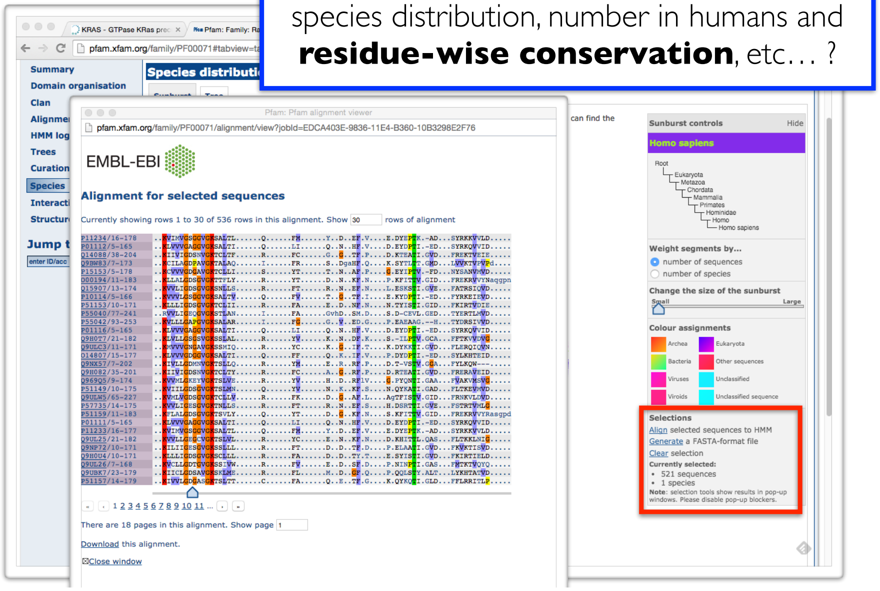
Colour assignments

🟥	Archea	🟪	Eukaryota
🟩	Bacteria	🟥	Other sequences
🟥	Viruses	🟦	Unclassified
🟥	Viroids	🟦	Unclassified sequence

Selections
Align selected sequences to HMM
Generate a FASTA-format file
Clear selection
Currently selected:
- 521 sequences
- 1 species

Note: selection tools show results in pop-up windows. Please disable pop-up blockers.

Example Questions:
What is known about the protein family, its species distribution, number in humans and **residue-wise conservation**, etc… ?



KRAS - GTPase KRas prec × | Pfam: Family: Ra

pfam.xfam.org/family/PF00071#tabview=ta

Pfam: Pfam alignment viewer

pfam.xfam.org/family/PF00071/alignment/view?jobId=EDCA403E-9836-11E4-B360-10B3298E2F76

EMBL-EBI

Alignment for selected sequences

Currently showing rows 1 to 30 of 536 rows in this alignment. Show 30 rows of alignment

```
P11234/16-178  ..KVIMVGSGGVGKSALTL......Q......FM......Y..D..EF.V....E.DYEPTK.-AD...SYRKKVVLD.....
P01112/5-165   ..KLVVVGAGGVGKSALTI......Q......LI......Q..N..HF.V....D.EYDPTI.-ED...SYRKQVVID.....
Q14088/38-204  ..KIIVIGDSNVGKTCLTF......R......FC......G..G..TF.P....D.KTEATI.GVD...FREKTVEIE.....
Q9BW83/7-173   ..KCILAGDPAVGKTALAQ......I......FR......S..DgaHF.Q....K.SYTLTT.GMD...LVVKTVPVPd....
P15153/5-178   ..KCVVVGDGAVGKTCLLI......S......YT......T..N..AF.P....G.EYIPTV.-FD...NYSANVMVD.....
O00194/11-183  ..KLLALGDSGVGKTTFLY......R......YT......D..N..KF.N....P.KFITTV.GID...FREKRVVYNaqgpn
Q15907/13-174  ..KVVLIGDSGVGKSNLLS......R......FT......R..N..EF.N....L.ESKSTI.GVE...FATRSIQVD.....
P10114/5-166   ..KVVVLGSGGVGKSALTV......Q......FV......T..G..TF.I....E.KYDPTI.-ED...FYRKEIEVD.....
P51153/10-171  ..KLLLIGDSGVGKTCLII......R......FA......E..D..NF.N....N.TYISTI.GID...FKIRTVDIE.....
P55040/77-241  ..RVVLIGEQGVGKSTLAN......I......FA......GvhD..SM.D....S.D-CEVL.GED...TYERTLMVD.....
P55042/93-253  ..KVLLLGAPGVGKSALAR......I......FG......G..V..ED.G....P.EAEAAG.--H...TYDRSIVVD.....
P01116/5-165   ..KLVVVGAGGVGKSALTI......Q......LI......Q..N..HF.V....D.EYDPTI.-ED...SYRKQVVID.....
Q9H0T7/21-182  ..KLVLLGSGSVGKSSLAL......R......YV......K..N..DF.K....S.-ILPTV.GCA...FFTKVVDVG.....
Q9ULC3/11-171  ..KMVVVGNGAVGKSSMIQ......R......YC......K..G..IF.T....K.DYKKTI.GVD...FLERQIQVN.....
O14807/15-177  ..KLVVVGDGGVGKSALTI......Q......FF......Q..K..IF.V....P.DYDPTI.-ED...SYLKHTEID.....
Q9NX57/7-202   ..KIVLLGDMNVGKTSLLQ......R......YM......E..R..RF.P....D.T-VSTV.GGA...FYLKQW---.....
Q9H082/35-201  ..KIIVIGDSNVGKTCLTY......R......FC......A..G..RF.P....D.RTEATI.GVD...FRERAVEID.....
Q969Q5/9-174   ..KVVMLGKEYVGKTSLVE......R......YV......H..D..RFlV....G.PYQNTI.GAA...FVAKVMSVG.....
P51149/10-175  ..KVIILGDSGVGKTSLMN......Q......YV......N..K..KF.S....N.QYKATI.GAD...FLTKEVMVD.....
Q9ULW5/65-227  ..KVMLVGDSGVGKTCLLV......R......FK......D..G..AF.L....AgTFISTV.GID...FRNKVLDVD.....
P57735/14-175  ..KVVLIGESGVGKTNLLS......R......FT......R..N..EF.S....H.DSRTTI.GVE...FSTRTVMLG.....
P51159/11-183  ..KFLALGDSGVGKTSVLY......Q......YT......D..G..KF.N....S.KFITTV.GID...FREKRVVYRasgpd
P01111/5-165   ..KLVVVGAGGVGKSALTI......Q......LI......Q..N..HF.V....D.EYDPTI.-ED...SYRKQVVID.....
P11233/16-177  ..KVIMVGSGGVGKSALTL......Q......FM......Y..D..EF.V....E.DYEPTK.-AD...SYRKKVVLD.....
Q9UL25/21-182  ..KVVLLGEGCVGKTSLVL......R......YC......E..N..KF.N....D.KHITTL.QAS...FLTKKLNIG.....
Q9NP72/10-171  ..KILIIGESGVGKSSLLL......R......FT......D..D..TF.D....P.ELAATI.GVD...FKVKTISVD.....
Q9H0U4/10-171  ..KLLLIGDSGVGKSCLLL......R......FA......D..D..TY.T....E.SYISTI.GVD...FKIRTIELD.....
Q9UL26/7-168   ..KVCLLGDTGVGKSSIVW......R......FV......E..D..SF.D....P.NINPTI.GAS...FMTKTVQYQ.....
Q9UBK7/23-179  ..KIICLGDSAVGKSKLME......R......FL......M..D..GF.Q....P.QQLSTY.ALT...LYKHTATVD.....
P51157/14-179  ..KIVVLGDGASGKTSLTT......C......FA......Q..E..TF.G....K.QYKQTI.GLD...FFLRRITLP.....
```

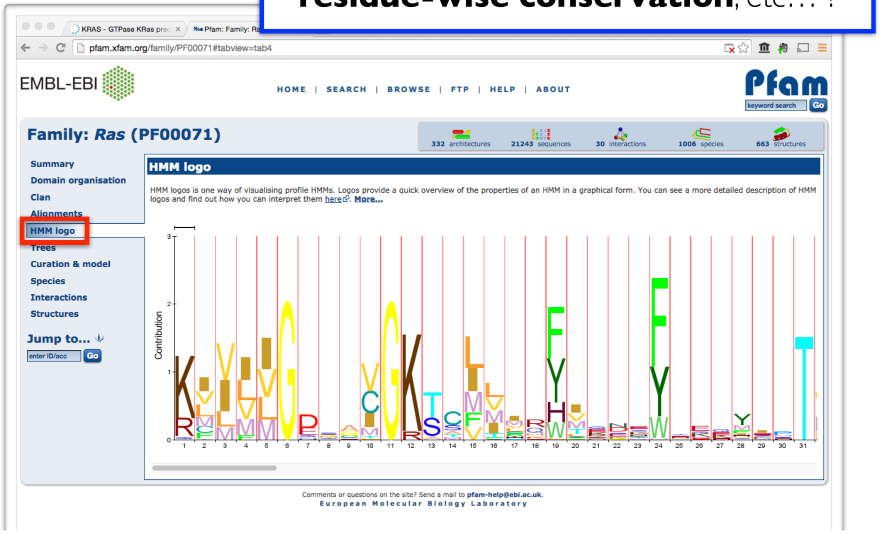
« ‹ 1 2 3 4 5 6 7 8 9 10 11 … › »

There are 18 pages in this alignment. Show page 1

Download this alignment.

☒Close window

Example Questions:
What is known about the protein family, its species distribution, number in humans and **residue-wise conservation**, etc… ?



KRAS - GTPase KRas prec × | Pfam: Family: Ra

pfam.xfam.org/family/PF00071#tabview=tab4

EMBL-EBI

HOME | SEARCH | BROWSE | FTP | HELP | ABOUT

Pfam keyword search Go

Family: *Ras* (PF00071)

332 architectures | 21243 sequences | 30 interactions | 1006 species | 663 structures

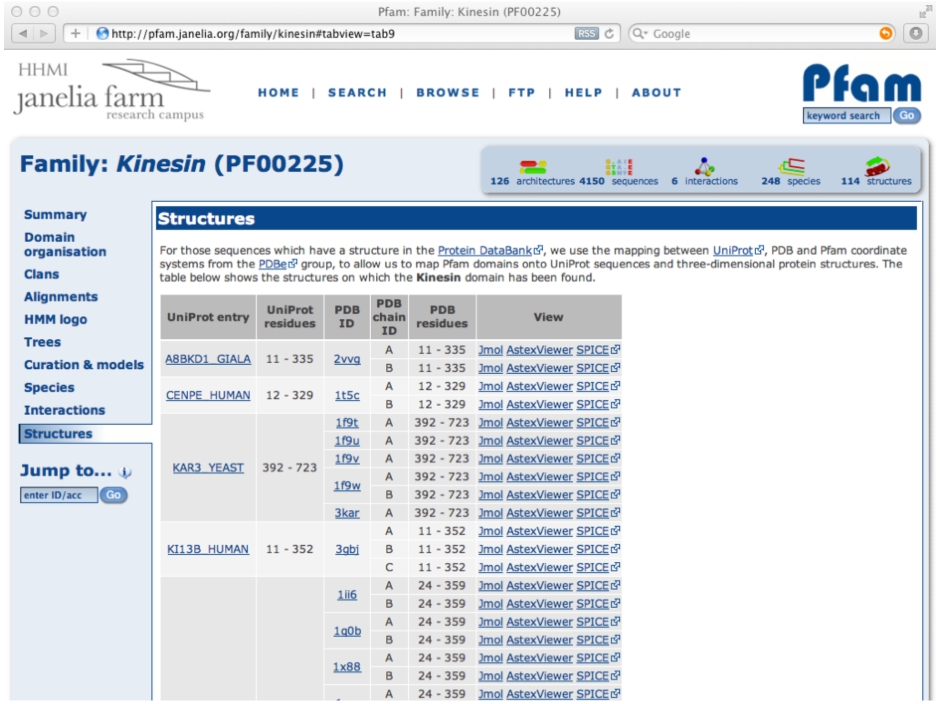
- Summary
- Domain organisation
- Clan
- Alignments
- HMM logo
- Trees
- Curation & model
- Species
- Interactions
- Structures

Jump to… enter ID/acc Go

HMM logo

HMM logos is one way of visualising profile HMMs. Logos provide a quick overview of the properties of an HMM in a graphical form. You can see a more detailed description of HMM logos and find out how you can interpret them here. More…

Comments or questions on the site? Send a mail to pfam-help@ebi.ac.uk.
European Molecular Biology Laboratory



Pfam: Family: Kinesin (PF00225)

http://pfam.janelia.org/family/kinesin#tabview=tab9

HHMI janelia farm research campus

HOME | SEARCH | BROWSE | FTP | HELP | ABOUT

Pfam keyword search Go

Family: *Kinesin* (PF00225)

126 architectures | 4150 sequences | 6 interactions | 248 species | 114 structures

- Summary
- Domain organisation
- Clans
- Alignments
- HMM logo
- Trees
- Curation & models
- Species
- Interactions
- Structures

Jump to… enter ID/acc Go

Structures

For those sequences which have a structure in the Protein DataBank, we use the mapping between UniProt, PDB and Pfam coordinate systems from the PDBe group, to allow us to map Pfam domains onto UniProt sequences and three-dimensional protein structures. The table below shows the structures on which the **Kinesin** domain has been found.

UniProt entry	UniProt residues	PDB ID	PDB chain ID	PDB residues	View
A8BKD1_GIALA	11 - 335	2vvq	A	11 - 335	Jmol AstexViewer SPICE
			B	11 - 335	Jmol AstexViewer SPICE
CENPE_HUMAN	12 - 329	1t5c	A	12 - 329	Jmol AstexViewer SPICE
			B	12 - 329	Jmol AstexViewer SPICE
KAR3_YEAST	392 - 723	1f9t	A	392 - 723	Jmol AstexViewer SPICE
		1f9u	A	392 - 723	Jmol AstexViewer SPICE
		1f9v	A	392 - 723	Jmol AstexViewer SPICE
		1f9w	A	392 - 723	Jmol AstexViewer SPICE
			B	392 - 723	Jmol AstexViewer SPICE
		3kar	A	392 - 723	Jmol AstexViewer SPICE
KI13B_HUMAN	11 - 352	3gbj	A	11 - 352	Jmol AstexViewer SPICE
			B	11 - 352	Jmol AstexViewer SPICE
			C	11 - 352	Jmol AstexViewer SPICE
		1ii6	A	24 - 359	Jmol AstexViewer SPICE
			B	24 - 359	Jmol AstexViewer SPICE
		1q0b	A	24 - 359	Jmol AstexViewer SPICE
			B	24 - 359	Jmol AstexViewer SPICE
		1x88	A	24 - 359	Jmol AstexViewer SPICE
			B	24 - 359	Jmol AstexViewer SPICE
			A	24 - 359	Jmol AstexViewer SPICE

Recap: Major NCBI and EBI databases

- What chromosome location and what genes are in the vicinity of a given query gene? NCBI **GENE**
- What can you find out about molecular functions, biological processes, and prominent cellular locations? EBI **GO**
- What amino acid positions in the protein are responsible for ligand binding? EBI **UniProt**
- What variants of this gene are associated with gastric cancer and other human diseases? NCBI **OMIN**
- What is known about the protein family, its species distribution, number in humans and residue-wise conservation? EBI **PFAM**
- Are high resolution protein structures available to examine the details of these mutations? How might we explain their potential molecular effects? RCSB **PDB**

Today's Menu

Classifying Databases	Primary, secondary and composite Bioinformatics databases
Using Databases	**Vignette** demonstrating how major Bioinformatics databases intersect
Major Biomolecular Formats	How nucleotide and protein sequence and structure data are represented
Alignment Foundations	**Introducing the *why* and *how* of comparing sequences**
Alignment Algorithms	**Hands-on** exploration of alignment algorithms and applications

ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

ALIGNMENT FOUNDATIONS

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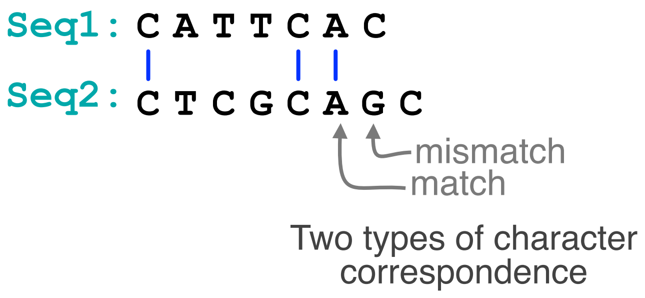
Basic Idea: Display one sequence above another with spaces (termed gaps) inserted in both to reveal similarity of nucleotides or amino acids.

```
Seq1: C A T T C A C

Seq2: C T C G C A G C
```

[Screencast Material]

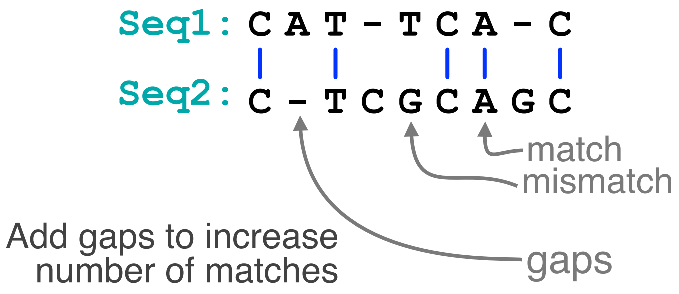
Basic Idea: Display one sequence above another with spaces (termed gaps) inserted in both to reveal similarity of nucleotides or amino acids.



```
Seq1: C A T T C A C
      |       | |
Seq2: C T C G C A G C
```

Two types of character correspondence

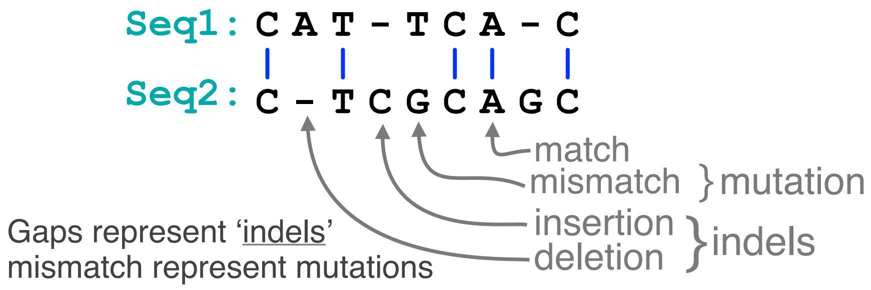
Basic Idea: Display one sequence above another with spaces (termed gaps) inserted in both to reveal similarity of nucleotides or amino acids.



```
Seq1: C A T - T C A - C
      |   |     | |   |
Seq2: C - T C G C A G C
```

Add gaps to increase number of matches

Basic Idea: Display one sequence above another with spaces (termed gaps) inserted in both to reveal similarity of nucleotides or amino acids.



```
Seq1: C A T - T C A - C
      |   |     | |   |
Seq2: C - T C G C A G C
```

Gaps represent ‘indels’
mismatch represent mutations

Why compare biological sequences?

- To obtain functional or mechanistic insight about a sequence by inference from another potentially better characterized sequence
- To find whether two (or more) genes or proteins are evolutionarily related
- To find structurally or functionally similar regions within sequences (e.g. catalytic sites, binding sites for other molecules, etc.)
- Many practical bioinformatics applications…

Practical applications include...

- Similarity searching of databases
  - – Protein structure prediction, annotation, etc...
- Assembly of sequence reads into a longer construct such as a genomic sequence
- Mapping sequencing reads to a known genome
  - – "Resequencing", looking for differences from reference genome - SNPs, indels (insertions or deletions)
  - – Mapping transcription factor binding sites via ChIP-Seq (chromatin immuno-precipitation sequencing)
  - – Pretty much all next-gen sequencing data analysis

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N.B. Pairwise sequence alignment is arguably the most fundamental operation of bioinformatics!

ALIGNMENT FOUNDATIONS

- Why…
  - Why compare biological sequences?
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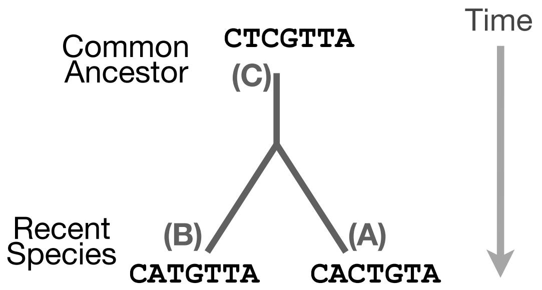
Sequence changes during evolution

There are three major types of sequence change that can occur during evolution.

- Mutations/Substitutions
- Deletions
- Insertions

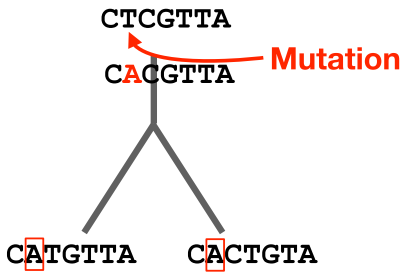


Mutations, deletions and insertions

There are three major types of sequence change that can occur during evolution.

- Mutations/Substitutions
- Deletions
- Insertions

CTCGTTA → CACGTTA



Likely occurred prior to speciation

Mutations, deletions and insertions

There are three major types of sequence change that can occur during evolution.

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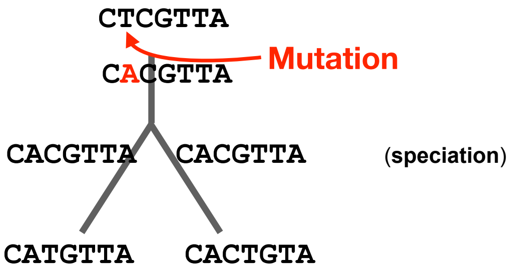
CTCGTTA → CACGTTA



Mutations, deletions and insertions

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- Mutations/Substitutions
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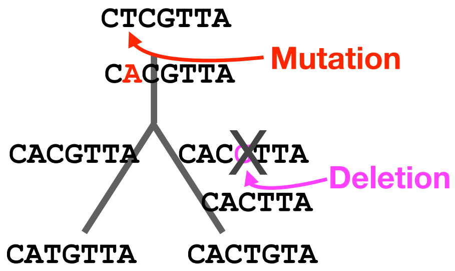
CTCGTTA → CACGTTA

CACGTTA → CACTTA



Mutations, deletions and insertions

There are three major types of sequence change that can occur during evolution.

- Mutations/Substitutions
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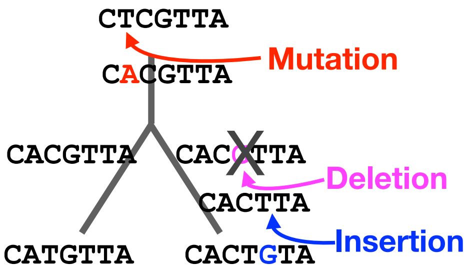
- Insertions

CTCGTTA → CACGTTA

CACGTTA → CACTTA

CACTTA → CACTGTA



Mutations, deletions and insertions

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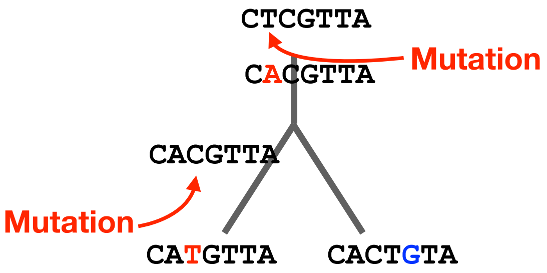
- Insertions

CTCGTTA → CACGTTA

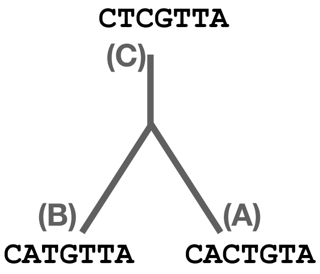
CACGTTA → CATGTTA

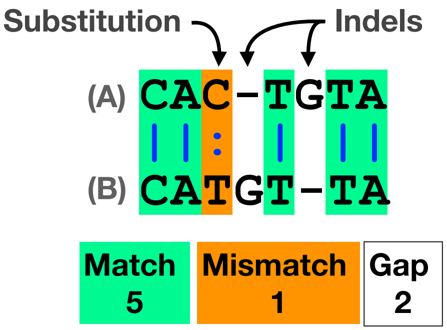


Alignment view

Alignments are great tools to visualize sequence similarity and evolutionary changes in homologous sequences.

- Mutations represent mutations/substitutions
- Gaps represent insertions and deletions (indels)

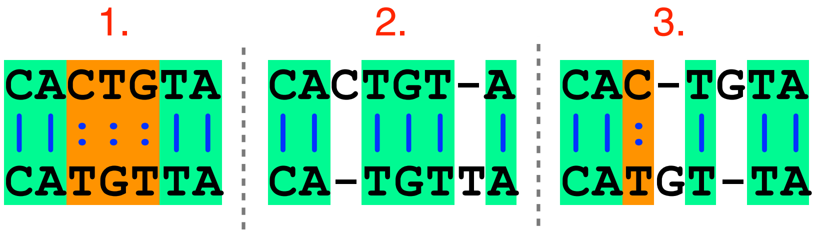




Alternative alignments

- Unfortunately, finding the correct alignment is difficult if we do not know the evolutionary history of the two sequences

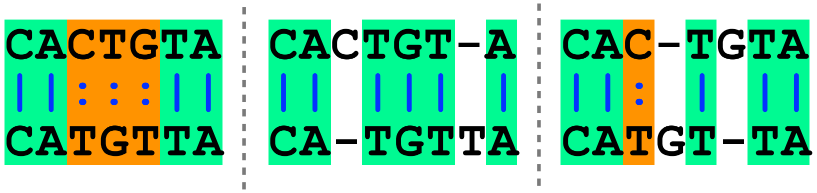
Q. Which of these 3 possible alignments is best?



Alternative alignments

- One way to judge alignments is to compare their number of matches, insertions, deletions and mutations

🟢 4 matches	🟢 6 matches	🟢 5 matches
🟠 3 mismatches	🟠 0 mismatches	🟠 1 mismatches
○ 0 gaps	○ 2 gaps	○ 2 gaps

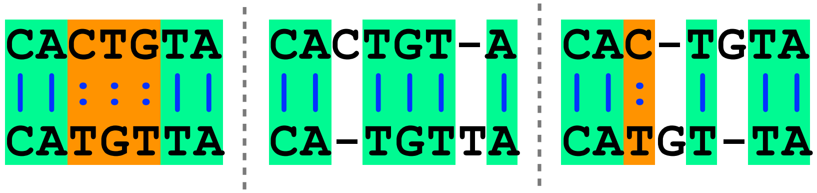


```
CACTGTA   CACTGT-A   CAC-TGTA
||:::||   || ||| |   ||: | ||
CATGTTA   CA-TGTTA   CATGT-TA
```

Scoring alignments

- We can assign a score for each match (+3), mismatch (+1) and indel (-1) to identify the optimal alignment for this scoring scheme

🟢 4 (+3)	🟢 6 (+3)	🟢 5 (+3)
🟠 3 (+1)	🟠 0 (+1)	🟠 1 (+1)
○ 0 (-1) = 15	○ 2 (-1) = 16	○ 2 (-1) = 14

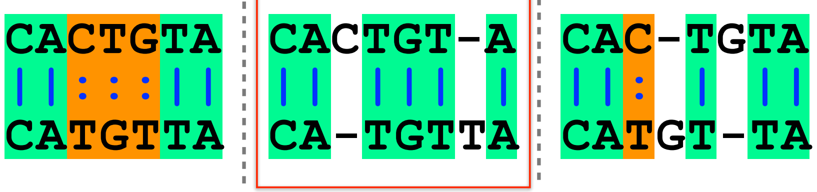


```
CACTGTA   CACTGT-A   CAC-TGTA
||:::||   || ||| |   ||: | ||
CATGTTA   CA-TGTTA   CATGT-TA
```

Optimal alignments

- Biologists often prefer parsimonious alignments, where the number of postulated sequence changes is minimized.

🟢 4 matches	🟢 6 matches	🟢 5 matches
🟠 3 mismatches	🟠 0 mismatches	🟠 1 mismatches
○ 0 gaps	○ 2 gaps	○ 2 gaps



```
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||:::||   || ||| |   ||: | ||
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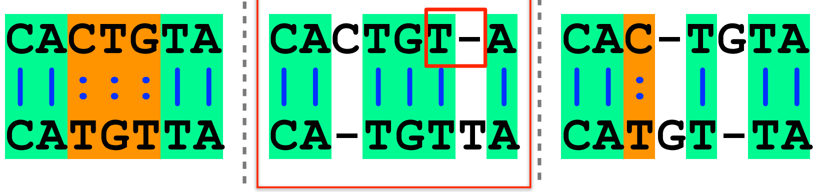

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CACTGTA   CACTGT-A   CAC-TGTA
||:::||   || ||| |   ||: | ||
CATGTTA   CA-TGTTA   CATGT-TA
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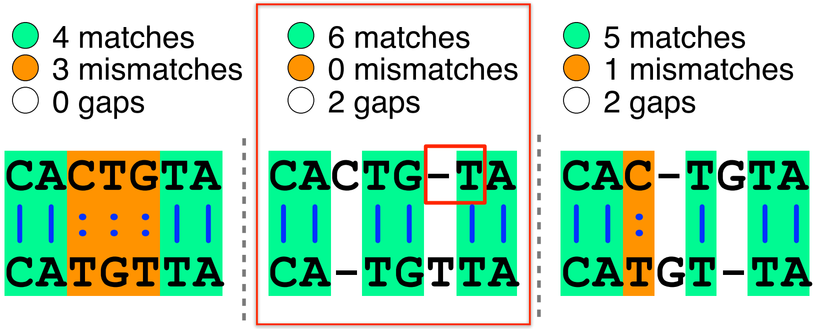

Optimal alignments

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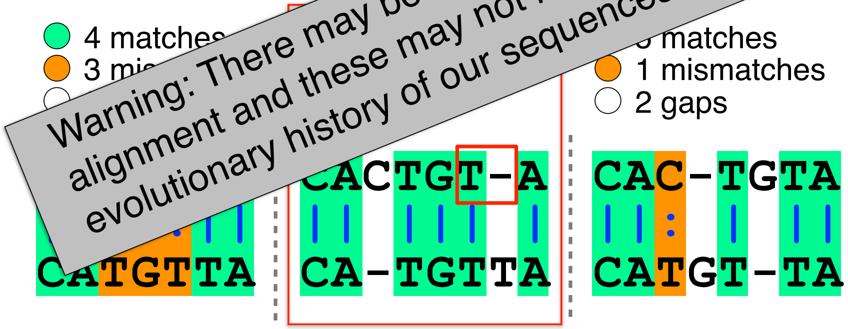

Warning: There may be more than one optimal alignment and these may not reflect the true evolutionary history of our sequences!

ALIGNMENT FOUNDATIONS

- **Why…**
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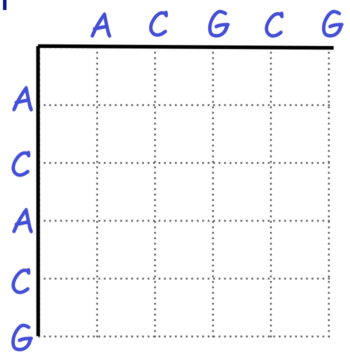
How do we compute the optimal alignment between two sequences?

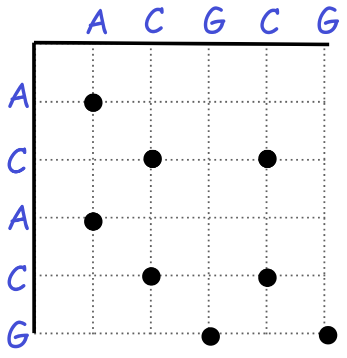
Dot plots: simple graphical approach

- Place one sequence on the vertical axis of a 2D grid (or matrix) and the other on the horizontal



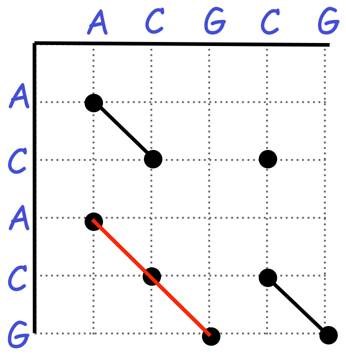
Dot plots: simple graphical approach

- Now simply put dots where the horizontal and vertical sequence values match



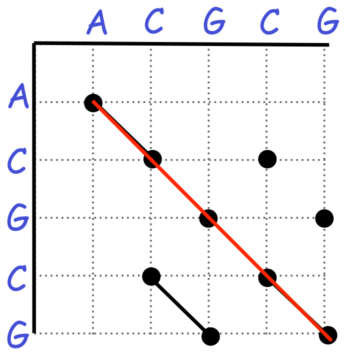
Dot plots: simple graphical approach

- Diagonal runs of dots indicate matched segments of sequence



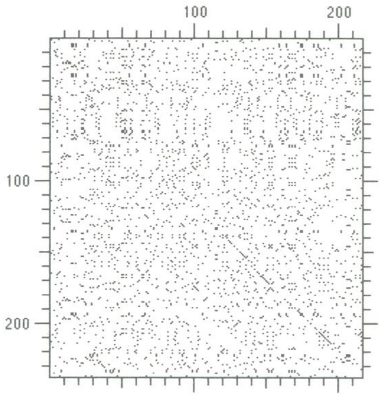
Dot plots: simple graphical approach

Q. What would the dot matrix of a two identical sequences look like?



Dot plots: simple graphical approach

- Dot matrices for long sequences can be noisy



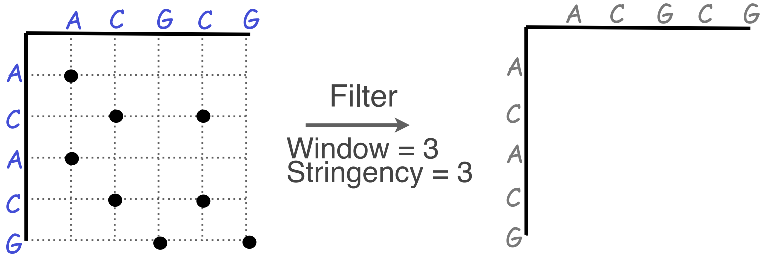
Dot plots: window size and match stringency

Solution: use a window and a threshold

– compare character by character within a window

– require certain fraction of matches within window in order to display it with a dot.

- You have to choose window size and stringency



Filter

Window = 3
Stringency = 3

Dot plots: window size and match stringency

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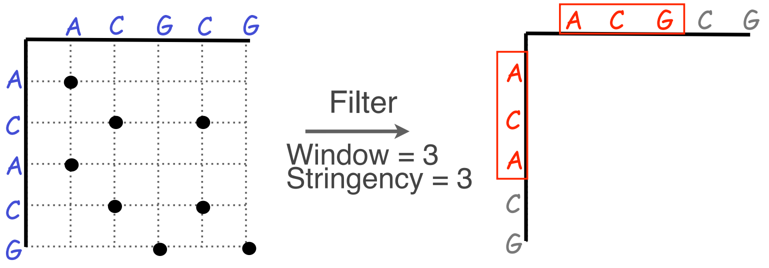

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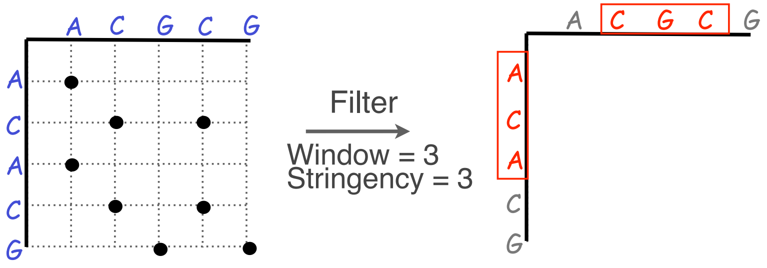

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Window = 3
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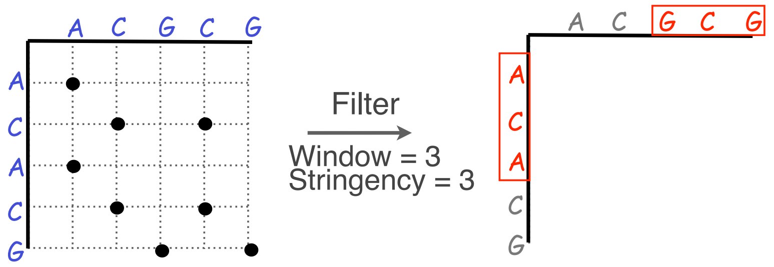

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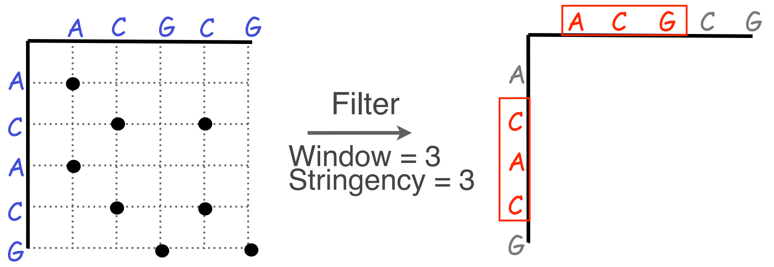

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Window = 3
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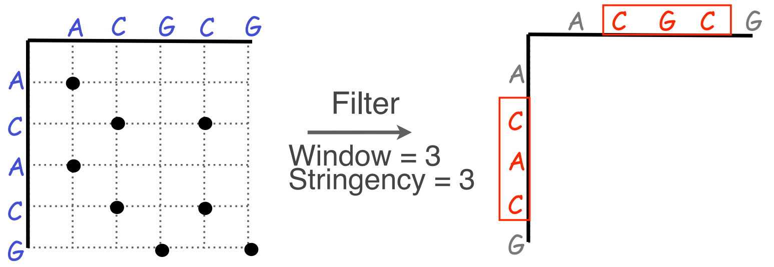

Filter

Window = 3
Stringency = 3

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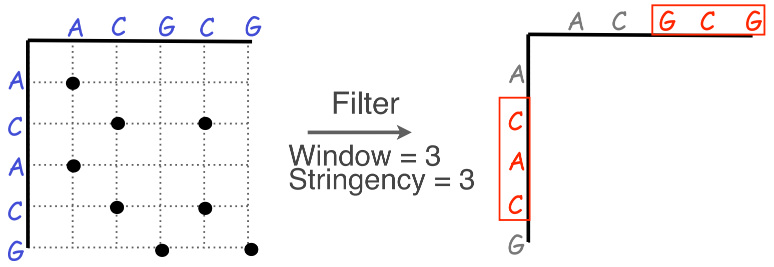

Filter

Window = 3
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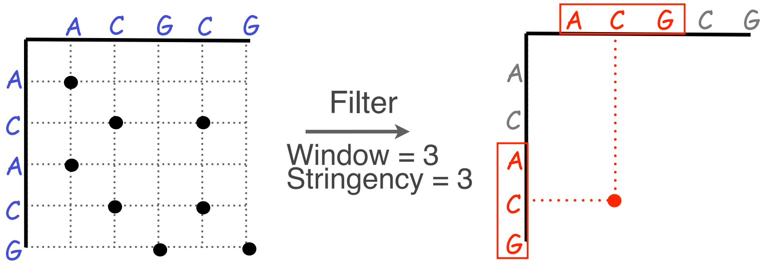

Filter

Window = 3
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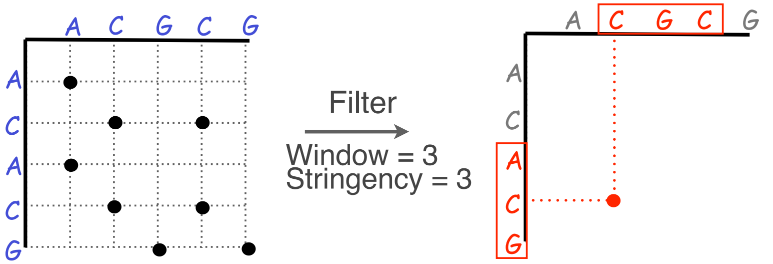

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Window = 3
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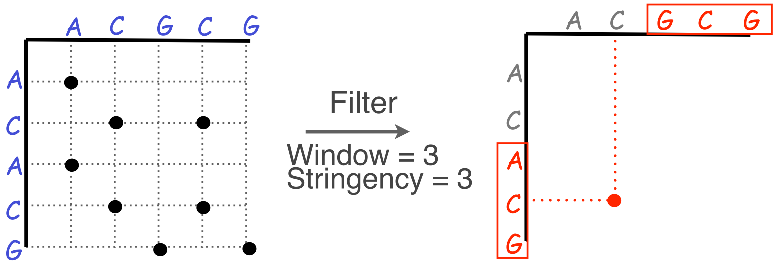

Filter

Window = 3
Stringency = 3

Dot plots: window size and match stringency

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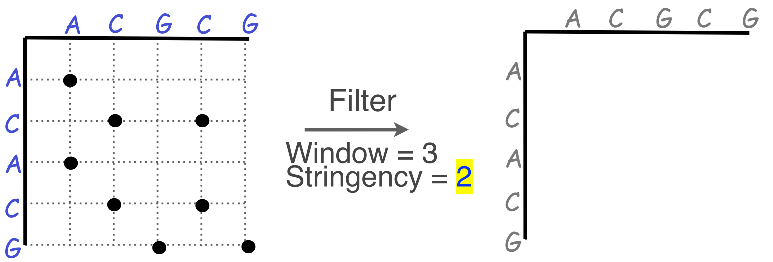


Filter

Window = 3
Stringency = 2

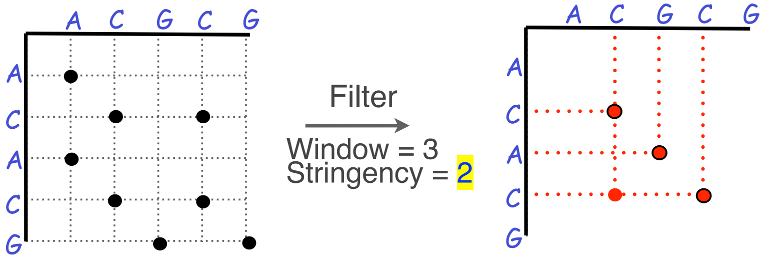
Dot plots: window size and match stringency

Solution: use a window and a threshold

– compare character by character within a window

– require certain fraction of matches within window in order to display it with a dot.

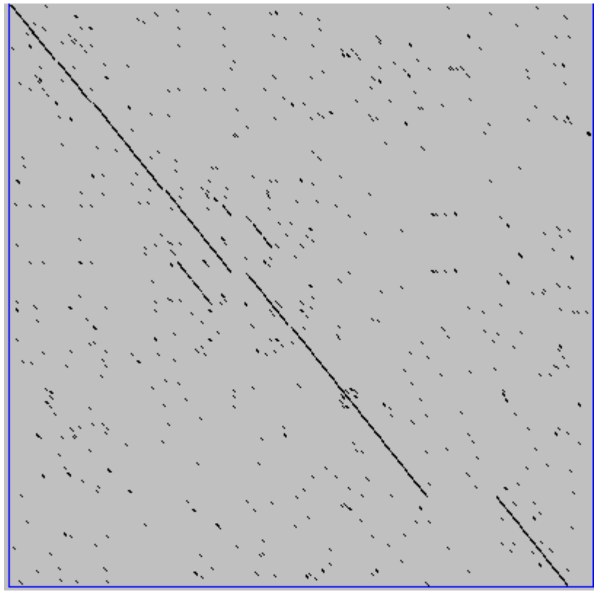
- You have to choose window size and stringency



Filter

Window = 3
Stringency = 2

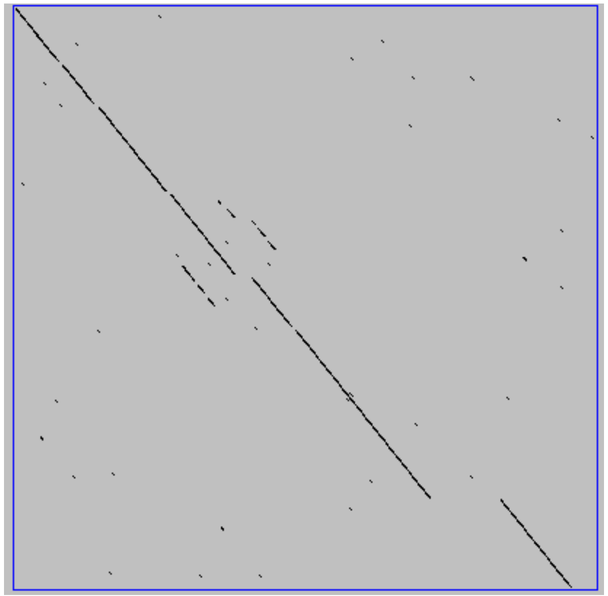
Window size = 5 bases



A dot plot simply puts a dot where two sequences match. In this example, dots are placed in the plot if 5 bases in a row match perfectly. Requiring a 5 base perfect match is a **heuristic** – only look at regions that have a certain degree of identity.

Do you expect evolutionarily related sequences to have more word matches (matches in a row over a certain length) than random or unrelated sequences?

Window size = 7 bases



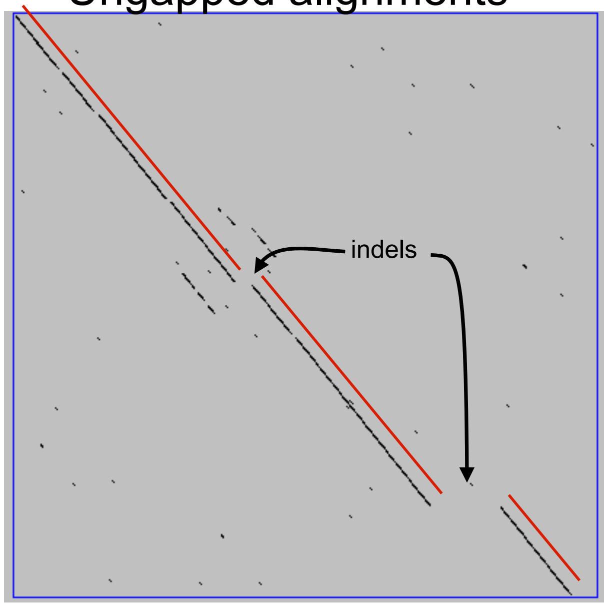
This is a dot plot of the same sequence pair. Now 7 bases in a row must match for a dot to be place. Noise is reduced.

Using windows of a certain length is very similar to using words (kmers) of N characters in the heuristic alignment search tools

Bigger window (kmer) fewer matches to consider

Web site used: http://www.vivo.colostate.edu/molkit/dnadot/

Ungapped alignments



Only **diagonals** can be followed.

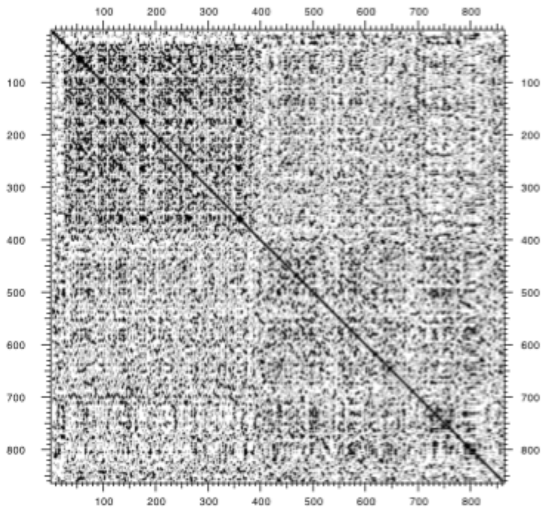
Downward or rightward paths represent **insertion** or **deletions** (gaps in one sequence or the other).

Web site used: http://www.vivo.colostate.edu/molkit/dnadot/

Uses for dot matrices

- Visually assessing the similarity of two protein or two nucleic acid sequences
- Finding local repeat sequences within a larger sequence by comparing a sequence to itself
  - Repeats appear as a set of diagonal runs stacked vertically and/or horizontally

Repeats

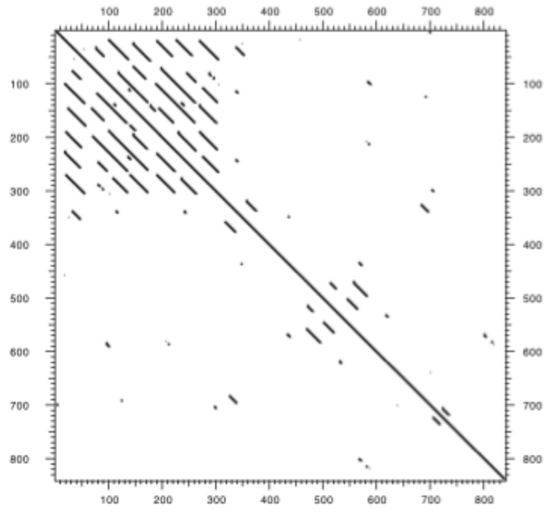


Human LDL receptor protein sequence (Genbank P01130)

W = 1
S = 1

(Figure from Mount, “Bioinformatics sequence and genome analysis”)

Repeats



Human LDL receptor protein sequence (Genbank P01130)

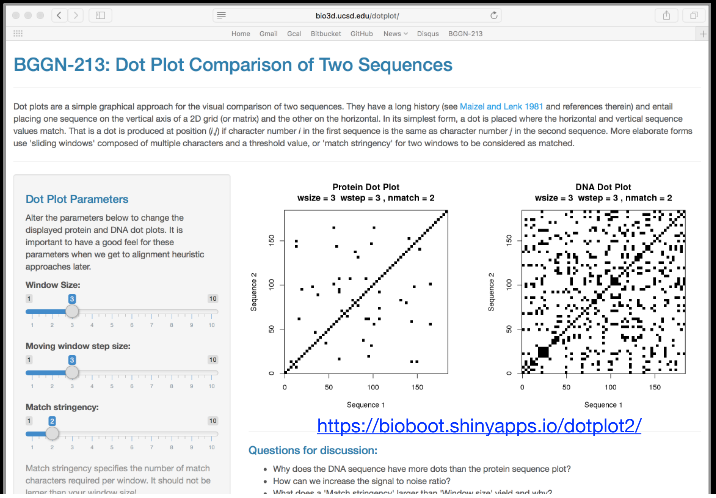
W = 23
S = 7

(Figure from Mount, “Bioinformatics sequence and genome analysis”)

Your Turn!

Exploration of dot plot parameters (hands-on worksheet **Section 1**)

http://bio3d.ucsd.edu/dotplot/   https://bioboot.shinyapps.io/dotplot/



BGGN-213: Dot Plot Comparison of Two Sequences

Dot plots are a simple graphical approach for the visual comparison of two sequences. They have a long history (see Maizel and Lenk 1981 and references therein) and entail placing one sequence on the vertical axis of a 2D grid (or matrix) and the other on the horizontal. In its simplest form, a dot is placed where the horizontal and vertical sequence values match. That is a dot is produced at position (*i*,*j*) if character number *i* in the first sequence is the same as character number *j* in the second sequence. More elaborate forms use 'sliding windows' composed of multiple characters and a threshold value, or 'match stringency' for two windows to be considered as matched.

Dot Plot Parameters

Alter the parameters below to change the displayed protein and DNA dot plots. It is important to have a good feel for these parameters when we get to alignment heuristic approaches later.

Window Size: 1 3 10

Moving window step size: 1 3 10

Match stringency: 1 2 10

Match stringency specifies the number of match characters required per window. It should not be larger than your window size!

https://bioboot.shinyapps.io/dotplot2/

Questions for discussion:

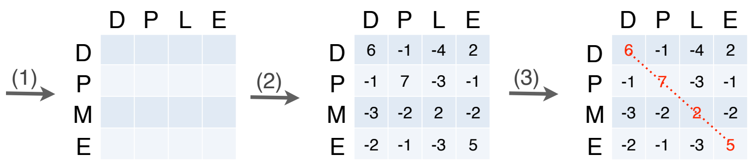
- Why does the DNA sequence have more dots than the protein sequence plot?
- How can we increase the signal to noise ratio?
- What does a 'Match stringency' larger than 'Window size' yield and why?

ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

The Dynamic Programming Algorithm

- The dynamic programming algorithm can be thought of an extension to the dot plot approach
  - One sequence is placed down the side of a grid and another across the top
  - Instead of placing a dot in the grid, we compute a score for each position
  - Finding the optimal alignment corresponds to finding the path through the grid with the best possible score



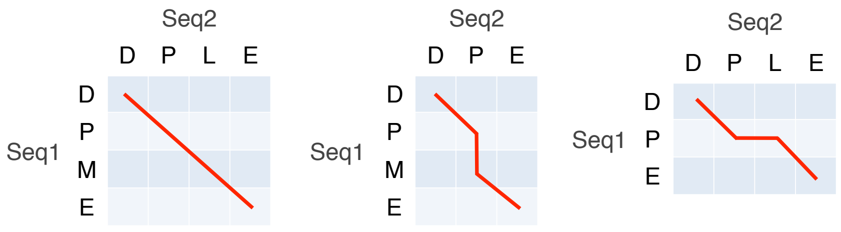
	D	P	L	E
D				
P				
M				
E				

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." J. Mol. Biol. 48:443-453.

Different paths represent different alignments

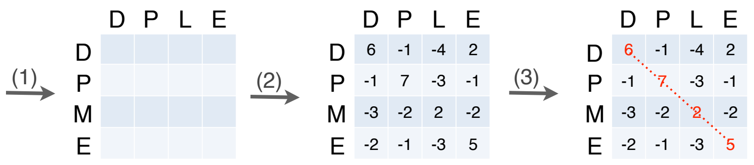


```
Seq1: D P L E      Seq1: D P M E      Seq1: D P - E
      | | : |            | |   |            | |   |
Seq2: D P M E      Seq2: D P - E      Seq2: D P L E
```

Matches are represented by diagonal paths & indels with horizontal or vertical path segments

Algorithm of Needleman and Wunsch

- The Needleman–Wunsch approach to global sequence alignment has three basic steps:
  (1) setting up a 2D-grid (or alignment matrix),
  (2) scoring the matrix, and
  (3) identifying the optimal path through the matrix



	D	P	L	E
D				
P				
M				
E				

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." J. Mol. Biol. 48:443-453.

Scoring the alignment matrix

- Start by filling in the first row and column – these are all indels (gaps).
  - Each step you take you will add the gap penalty to the score ($S_{i,j}$) accumulated in the previous cell

Scores: match = +1, mismatch = -1, gap = -2

Sequence 2

	j	-	D	P	L	E
i	-	0	-2	-4	-6	-8
Sequence 1	D	-2				
	P	-4				
	M	-6				
	E	-8				

Scoring the alignment matrix

- Start by filling in the first row and column – these are all indels (gaps).
  - Each step you take you will add the gap penalty to the score ($S_{i,j}$) accumulated in the previous cell

Scores: match = +1, mismatch = -1, gap = -2

Sequence 2

	j	-	D	P	L	E
i	-	0 ↓	-2	-4	-6	-8
Sequence 1	D	-2 ↓				
	P	-4 ↓				
	M	-6 ↓				
	E	-8				

$$S_{i+4} = (-2) + (-2) + (-2) + (-2)$$

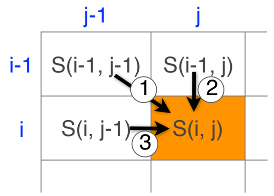
```
Seq1: DPME
Seq2: ----
```

Scoring the alignment matrix

- Then go to the empty corner cell (upper left). It has filled in values in up, left and diagonal directions
  - Now can ask which of the three directions gives the highest score?
  - keep track of this score and direction

Scores: match = +1, mismatch = -1, gap = -2

	-	j D	P	L	E
-	0	-2	-4	-6	-8
i D	-2	?			
P	-4				
M	-6				
E	-8				



	j-1	j
i-1	S(i-1, j-1)	S(i-1, j)
i	S(i, j-1)	S(i, j)

Scoring the alignment matrix

- Then go to the empty corner cell (upper left). It has filled in values in up, left and diagonal directions
  - Now can ask which of the three directions gives the highest score?
  - keep track of this score and direction

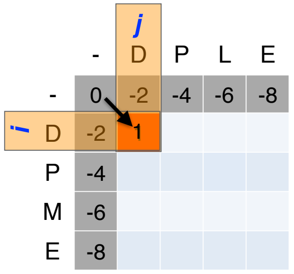
Scores: match = +1, mismatch = -1, gap = -2

	-	j D	P	L	E
-	0	-2	-4	-6	-8
i D	-2	?			
P	-4				
M	-6				
E	-8				

$$S(i, j) = \text{Max}\begin{cases} S(i-1, j-1) + \text{(mis)match} & \searrow \; ① \\ S(i-1, j) + \text{gap penalty} & \downarrow \; ② \\ S(i, j-1) + \text{gap penalty} & \rightarrow \; ③ \end{cases}$$

Scoring the alignment matrix

- Then go to the empty corner cell (upper left). It has filled in values in up, left and diagonal directions
  - Now can ask which direction gives the highest score
  - keep track of direction and score



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1			
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2

↘ ① (0)+(+1) = +1 <= (D-D) match!

↓ ② (-2)+(-2) = -4

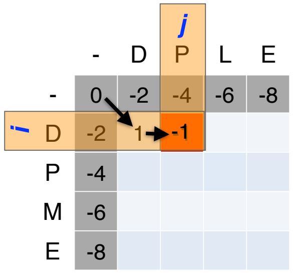
→ ③ (-2)+(-2) = -4

Alignment

```
D
D
```

Scoring the alignment matrix

- At each step, the score in the current cell is determine by the scores in the neighboring cells
  - The maximal score and the direction that gave that score is stored (we will use these later to determine the optimal alignment)



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1		
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2

↘ ① (-2)+(-1) = -3 <= (D-P) mismatch!

↓ ② (-4)+(-2) = -6

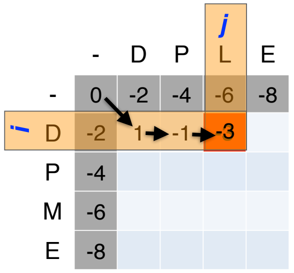
→ ③ (1)+(-2) = -1

Alignment

```
D-
DP
```

Scoring the alignment matrix

- We will continue to store the alignment score ($S_{i,j}$) for all possible alignments in the alignment matrix.



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2

↘ ① (-4)+(-1) = -5 <= (D-L) mismatch

↓ ② (-6)+(-2) = -8

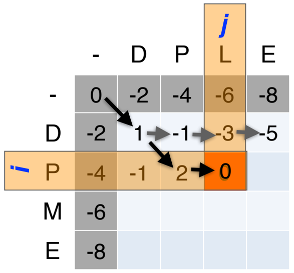
→ ③ (-1)+(-2) = -3

Alignment

```
D--
DPL
```

Scoring the alignment matrix

- For the highlighted cell, the corresponding score ($S_{i,j}$) refers to the score of the optimal alignment of the first i characters from sequence1, and the first j characters from sequence2.



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	-5
P	-4	-1	2	0	
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, indel = -2

↘ ① (-1)+(-1) = -2

↓ ② (-3)+(-2) = -5

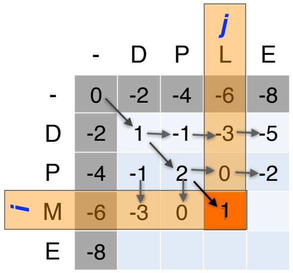
→ ③ (2)+(-2) = 0

Alignment

```
DP-
DPL
```

Scoring the alignment matrix

- At each step, the score in the current cell is determine by the scores in the neighboring cells
  - The maximal score and the direction that gave that score is stored



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	-5
P	-4	-1	2	0	-2
M	-6	-3	0	1	
E	-8				

Scores: match = +1, mismatch = -1, indel = -2

↘ ① (2)+(-1) = 0 <= mismatch

↓ ② (0)+(-2) = -2

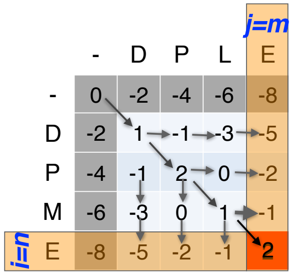
→ ③ (0)+(-2) = -2

Alignment

```
DPM
DPL
```

Scoring the alignment matrix

- The score of the best alignment of the entire sequences corresponds to $S_{n,m}$
  - (where n and m are the length of the sequences)



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	-5
P	-4	-1	2	0	-2
M	-6	-3	0	1	-1
E	-8	-5	-2	-1	2

Scores: match = +1, mismatch = -1, indel = -2

↘ ① (+1)+(+1) = +2

↓ ② (-1)+(-2) = -3

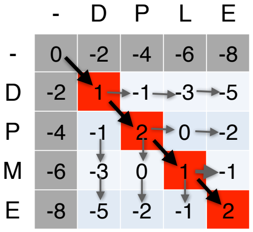
→ ③ (-1)+(-2) = -3

Alignment

```
DPME
DPLE
```

Scoring the alignment matrix

- To find the best alignment, we retrace the arrows starting from the bottom right cell
  - N.B. The optimal alignment score and alignment are dependent on the chosen scoring system



	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	-5
P	-4	-1	2	0	-2
M	-6	-3	0	1	-1
E	-8	-5	-2	-1	2

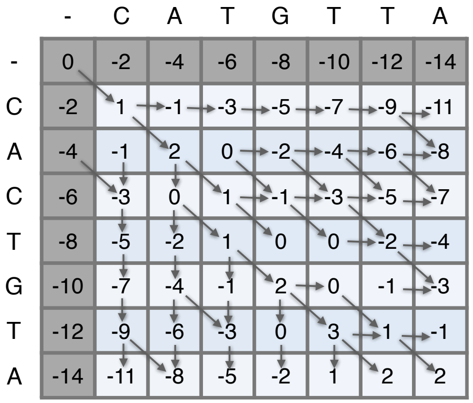
Scores: match = +1, mismatch = -1, indel = -2

Alignment

```
DPME
DPLE
```

Questions:

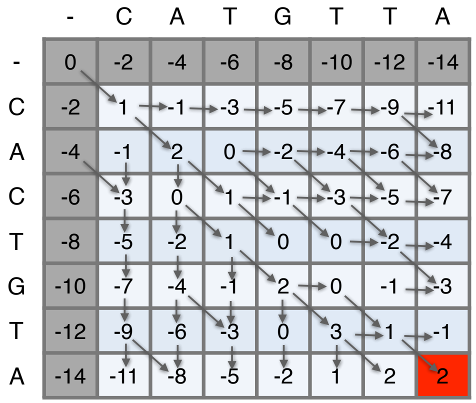
- What is the optimal score for the alignment of these sequences and how do we find the optimal alignment?



	-	C	A	T	G	T	T	A
-	0	-2	-4	-6	-8	-10	-12	-14
C	-2	1	-1	-3	-5	-7	-9	-11
A	-4	-1	2	0	-2	-4	-6	-8
C	-6	-3	0	1	-1	-3	-5	-7
T	-8	-5	-2	1	0	0	-2	-4
G	-10	-7	-4	-1	2	0	-1	-3
T	-12	-9	-6	-3	0	3	1	-1
A	-14	-11	-8	-5	-2	1	2	2

Questions:

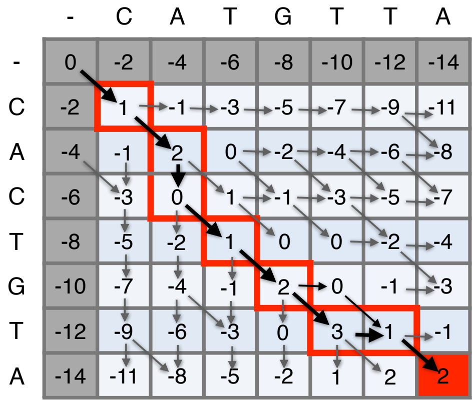
- What is the optimal score for the alignment of these sequences and how do we find the optimal alignment?



	-	C	A	T	G	T	T	A
-	0	-2	-4	-6	-8	-10	-12	-14
C	-2	1	-1	-3	-5	-7	-9	-11
A	-4	-1	2	0	-2	-4	-6	-8
C	-6	-3	0	1	-1	-3	-5	-7
T	-8	-5	-2	1	0	0	-2	-4
G	-10	-7	-4	-1	2	0	-1	-3
T	-12	-9	-6	-3	0	3	1	-1
A	-14	-11	-8	-5	-2	1	2	2

Questions:

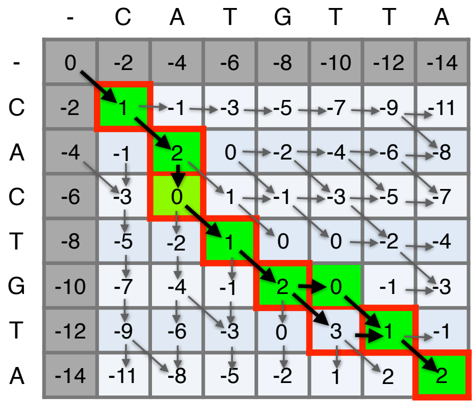
- To find the best alignment we retrace the arrows starting from the bottom right cell



	-	C	A	T	G	T	T	A
-	0	-2	-4	-6	-8	-10	-12	-14
C	-2	1	-1	-3	-5	-7	-9	-11
A	-4	-1	2	0	-2	-4	-6	-8
C	-6	-3	0	1	-1	-3	-5	-7
T	-8	-5	-2	1	0	0	-2	-4
G	-10	-7	-4	-1	2	0	-1	-3
T	-12	-9	-6	-3	0	3	1	-1
A	-14	-11	-8	-5	-2	1	2	2

More than one alignment possible

- Sometimes more than one alignment can result in the same optimal score



	-	C	A	T	G	T	T	A
-	0	-2	-4	-6	-8	-10	-12	-14
C	-2	1	-1	-3	-5	-7	-9	-11
A	-4	-1	2	0	-2	-4	-6	-8
C	-6	-3	0	1	-1	-3	-5	-7
T	-8	-5	-2	1	0	0	-2	-4
G	-10	-7	-4	-1	2	0	-1	-3
T	-12	-9	-6	-3	0	3	1	-1
A	-14	-11	-8	-5	-2	1	2	2

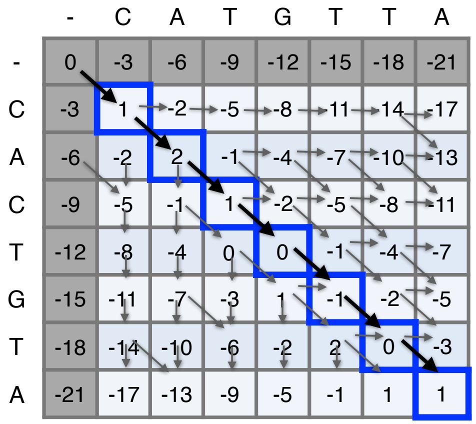
Alignment

```
CACTGT-A
CA-TGTTA

CACTG-TA
CA-TGTTA
```

The alignment and score are dependent on the scoring system

- Here we increase the gap penalty from -2 to -3



	-	C	A	T	G	T	T	A
-	0	-3	-6	-9	-12	-15	-18	-21
C	-3	1	-2	-5	-8	-11	-14	-17
A	-6	-2	2	-1	-4	-7	-10	-13
C	-9	-5	-1	1	-2	-5	-8	-11
T	-12	-8	-4	0	0	-1	-4	-7
G	-15	-11	-7	-3	1	-1	-2	-5
T	-18	-14	-10	-6	-2	2	0	-3
A	-21	-17	-13	-9	-5	-1	1	1

Alignment

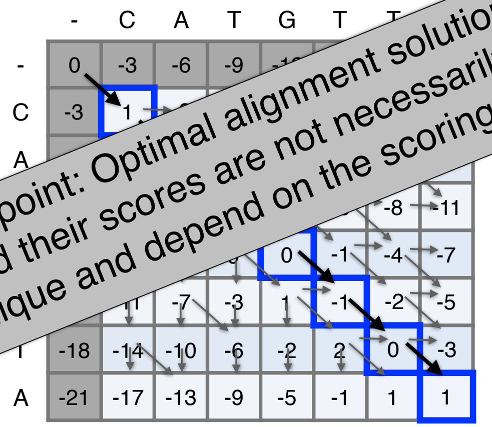
```
CACTGT-A
CA-TGTTA

CACTG-TA
CA-TGTTA

CACTGTA
CATGTTA
```

The alignment and score are dependent on the scoring system

- Here we increase the gap penalty from -2 -3



Key point: Optimal alignment solutions and their scores are not necessarily unique and depend on the scoring system!

Alignment

```
CACTGT-A
CA-TGTTA

CACTG-TA
CA-TGTTA
-----------
CACTGTA
CATGTTA
```

NW DYNAMIC PROGRAMMING

Match: +2
Mismatch: -1
Gap: -2

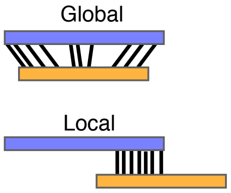
		A	G	T	T	C
	0					
A						
T						
T						
G						
C						

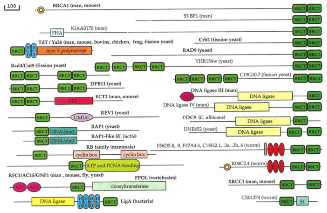
ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

Global vs local alignments

- Needleman-Wunsch is a global alignment algorithm
  - Resulting alignment spans the complete sequences end to end
  - This is appropriate for closely related sequences that are similar in length
- For many practical applications we require local alignments
  - Local alignments highlight sub-regions (e.g. protein domains) in the two sequences that align well





Local alignment: Definition

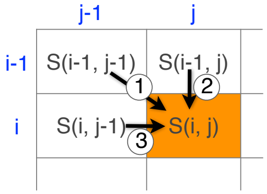
- Smith & Waterman proposed simply that a local alignment of two sequences allow arbitrary-length segments of each sequence to be aligned, with no penalty for the unaligned portions of the sequences. Otherwise, the score for a local alignment is calculated the same way as that for a global alignment

Smith, T.F. & Waterman, M.S. (1981) “Identification of common molecular subsequences.” J. Mol. Biol. 147:195-197.

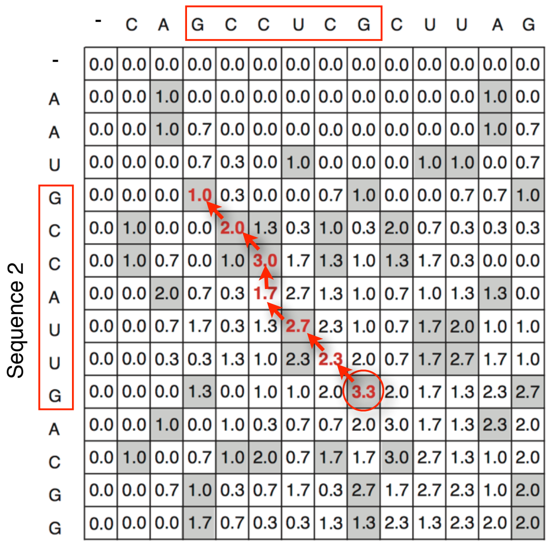
The Smith-Waterman algorithm

- Three main modifications to Needleman-Wunsch:
  - – Allow a node to start at 0
  - – The score for a particular cell cannot be negative
    - if all other score options produce a negative value, then a zero must be inserted in the cell
  - – Record the highest- scoring node, and trace back from there

$$S(i, j) = \text{Max} \begin{cases} S(i-1, j-1) + \text{(mis)match} & \searrow \textcircled{1} \\ S(i-1, j) - \text{gap penalty} & \downarrow \textcircled{2} \\ S(i, j-1) - \text{gap penalty} & \rightarrow \textcircled{3} \\ 0 & \textcircled{4} \end{cases}$$



Sequence 1



	-	C	A	G	C	C	U	C	G	C	U	U	A	G
-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
A	0.0	0.0	1.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.7
U	0.0	0.0	0.0	0.7	0.3	0.0	1.0	0.0	0.0	0.0	1.0	1.0	0.0	0.7
G	0.0	0.0	0.0	1.0	0.3	0.0	0.0	0.7	1.0	0.0	0.0	0.7	0.7	1.0
C	0.0	1.0	0.0	0.0	2.0	1.3	0.3	1.0	0.3	2.0	0.7	0.3	0.3	0.3
C	0.0	1.0	0.7	0.0	1.0	3.0	1.7	1.3	1.0	1.3	1.7	0.3	0.0	0.0
A	0.0	0.0	2.0	0.7	0.3	1.7	2.7	1.3	1.0	0.7	1.0	1.3	1.3	0.0
U	0.0	0.0	0.7	1.7	0.3	1.3	2.7	2.3	1.0	0.7	1.7	2.0	1.0	1.0
U	0.0	0.0	0.3	0.3	1.3	1.0	2.3	2.3	2.0	0.7	1.7	2.7	1.7	1.0
G	0.0	0.0	0.0	1.3	0.0	1.0	1.0	2.0	3.3	2.0	1.7	1.3	2.3	2.7
A	0.0	0.0	1.0	0.0	1.0	0.3	0.7	0.7	2.0	3.0	1.7	1.3	2.3	2.0
C	0.0	1.0	0.0	0.7	1.0	2.0	0.7	1.7	1.7	3.0	2.7	1.3	1.0	2.0
G	0.0	0.0	0.7	1.0	0.3	0.7	1.7	0.3	2.7	1.7	2.7	2.3	1.0	2.0
G	0.0	0.0	0.0	1.7	0.7	0.3	0.3	1.3	1.3	2.3	1.3	2.3	2.0	2.0

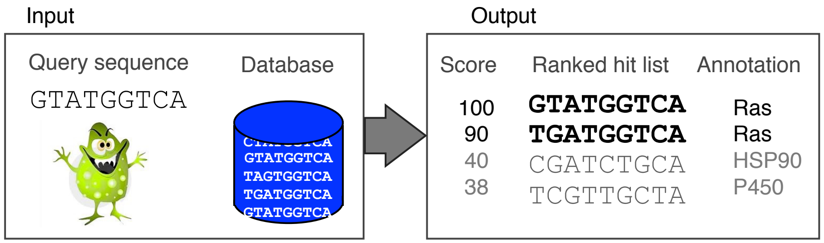
Sequence 2

Local alignment

GCC-UCG
GCCAUUG

Local alignments can be used for database searching

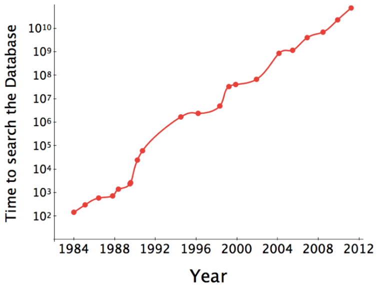
- Goal: Given a query sequence (Q) and a sequence database (D), find a list of sequences from D that are most similar to Q
  - – Input: Q, D and scoring scheme
  - – Output: Ranked list of hits



Score	Ranked hit list	Annotation
100	**GTATGGTCA**	Ras
90	**TGATGGTCA**	Ras
40	CGATCTGCA	HSP90
38	TCGTTGCTA	P450

The database search problem

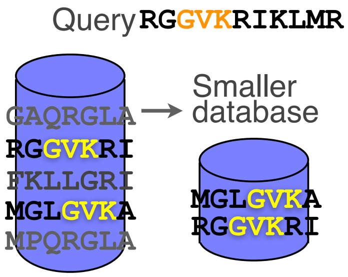
- Due to the rapid growth of sequence databases, search algorithms have to be both efficient and sensitive
  - Time to search with SW is proportional to m x n (m is length of query, n is length of database), too slow for large databases!



To reduce search time heuristic algorithms, such as BLAST, first remove database sequences without a strong local similarity to the query sequence in a quick initial scan.

The database search problem

- Due to the rapid growth of sequence databases, search algorithms have to be both efficient and sensitive
  - Time to search with SW is proportional to m x n (m is length of query, n is length of database), too slow for large databases!



To reduce search time heuristic algorithms, such as BLAST, first remove database sequences without a strong local similarity to the query sequence in a quick initial scan.

ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

Rapid, heuristic versions of Smith–Waterman: BLAST

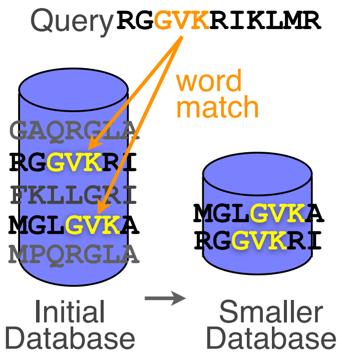
- BLAST (Basic Local Alignment Search Tool) is a simplified form of Smith-Waterman (SW) alignment that is popular because it is fast and easily accessible
  - BLAST is a heuristic approximation to SW - It examines only part of the search space
  - BLAST saves time by restricting the search by scanning database sequences for likely matches before performing more rigorous alignments
  - Sacrifices some sensitivity in exchange for speed
  - In contrast to SW, BLAST is not guaranteed to find optimal alignments

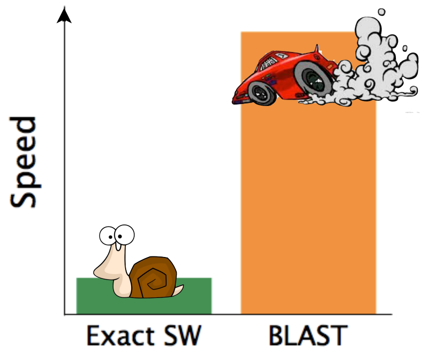
Rapid, heuristic versions of Smith–Waterman: BLAST

- BLAST (Basic Local Alignment Search To simplified form of Smith-Waterman that is popular because it is f ble
  - BLAST finds regions gical sequences
  - BLAST ne search by scanning ely matches before performing ents
  - me sensitivity in exchange for speed
  - ntrast to SW, BLAST is not guaranteed to find optimal alignments

“The central idea of the BLAST algorithm is to confine attention to sequence pairs that contain an initial word pair match”
Altschul et al. (1990)

- BLAST uses this pre-screening heuristic approximation resulting in an an approach that is about 50 times faster than the Smith-Waterman





How BLAST works

- Four basic phases
  - Phase 1: compile a list of query word pairs (w=3)

```
                  RGGVKRI    Query sequence
                  RGG
                   GGV
generate list       GVK
of  w=3 words        VKR
for  query            KRI
```

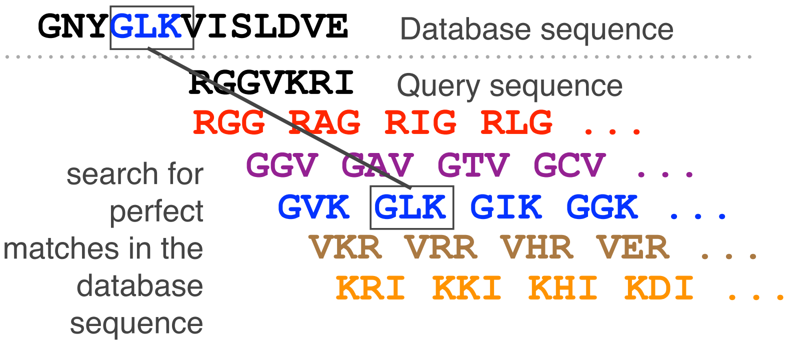
Blast

- Phase 2: expand word pairs to include those similar to query (defined as those above a similarity threshold to original word, i.e. match scores in substitution matrix)

```
                  RGGVKRI    Query sequence
                  RGG  RAG  RIG  RLG  . . .
                   GGV  GAV  GTV  GCV  . . .
extend list of      GVK  GAK  GIK  GGK  . . .
words similar        VKR  VRR  VHR  VER  . . .
to query              KRI  KKI  KHI  KDI  . . .
```

Blast

– Phase 3: a database is scanned to find sequence entries that match the compiled word list



GNYGLKVISLDVE Database sequence

RGGVKRI Query sequence

RGG RAG RIG RLG . . .

GGV GAV GTV GCV . . .

GVK GLK GIK GGK . . .

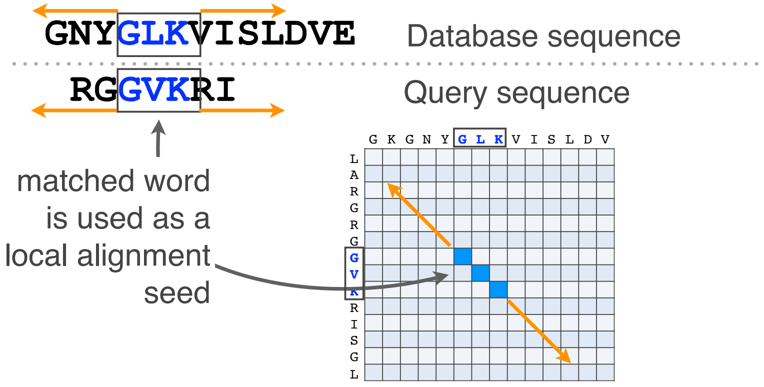
VKR VRR VHR VER . . .

KRI KKI KHI KDI . . .

search for perfect matches in the database sequence

Blast

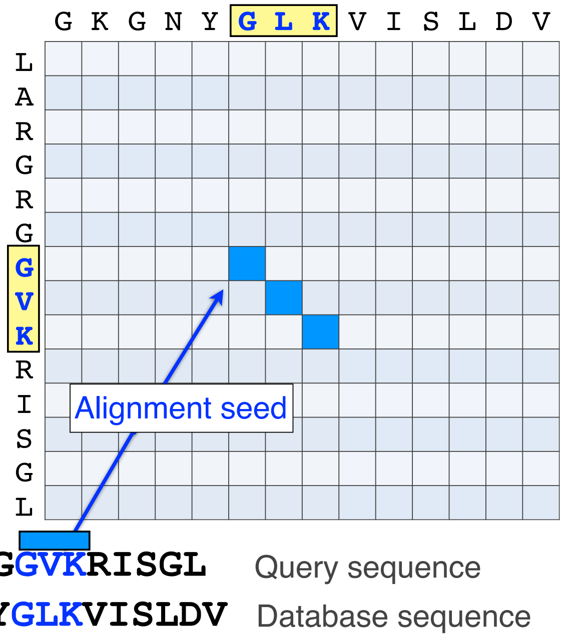
– Phase 4: the initial database hits are extended in both directions using dynamic programing



GNYGLKVISLDVE Database sequence

RGGVKRI Query sequence

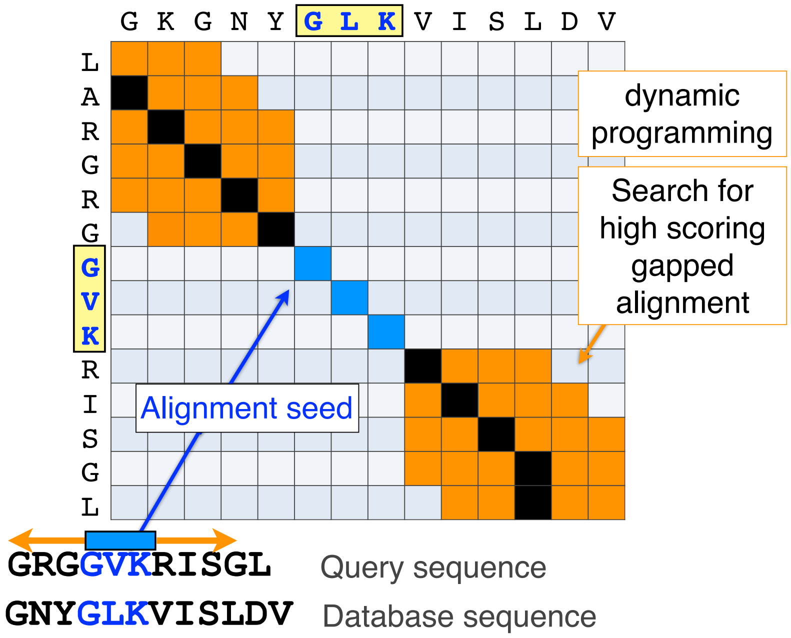
matched word is used as a local alignment seed



Alignment seed

GRGGVKRISGL Query sequence

GNYGLKVISLDV Database sequence



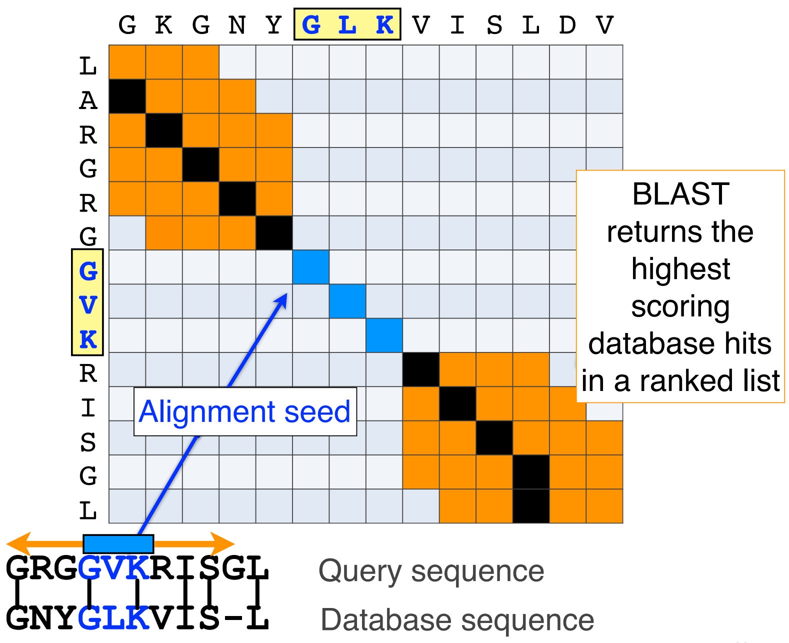
dynamic programming

Search for high scoring gapped alignment

Alignment seed

GRGGVKRISGL Query sequence

GNYGLKVISLDV Database sequence



BLAST returns the highest scoring database hits in a ranked list

Alignment seed

```
GRGGVKRISGL
| |  | |||
GNYGLKVIS-L
```

Query sequence

Database sequence

BLAST output

- BLAST returns the highest scoring database hits in a ranked list along with details about the target sequence and alignment statistics

Description	Max score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo sapiens]	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	100%	0	98%	AAA20133.1
Kinesin-14 heavy chain [Danio rerio]	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	48.2	40%	0.03	32%	ELK35081.1
mKIAA4102 protein [Mus musculus]	42.7	38%	3.02	24%	EHH28205.1

Statistical significance of results

- An important feature of BLAST is the computation of statistical significance for each hit. This is described by the E value (expect value)

Description	Max score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo sapiens]	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	100%	0	98%	AAA20133.1
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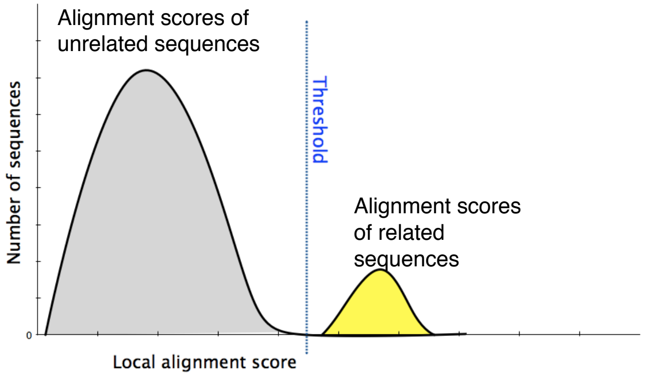
hypothetical protein EGK_18589	48.2	40%	0.03	32%	ELK35081.1
mKIAA4102 protein [Mus musculus]	42.7	38%	3.02	24%	EHH28205.1

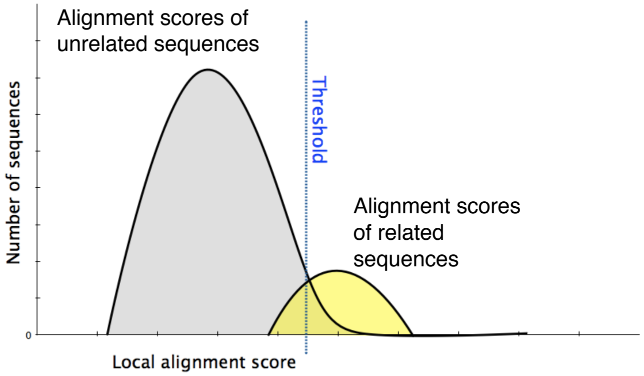
BLAST scores and E-values

- The E value is the expected number of hits that are as good or better than the observed local alignment score (with this score or better) if the query and database are random with respect to each other
  - i.e. the number of alignments expected to occur by chance with equivalent or better scores
- Typically, only hits with E value below a significance threshold are reported
  - This is equivalent to selecting alignments with score above a certain score threshold

- Ideally, a threshold separates all query related sequences (yellow) from all unrelated sequences (gray)



- Unfortunately, often both score distributions overlap
  - The E value describes the expected number of hits with a score above the threshold if the query and database are unrelated



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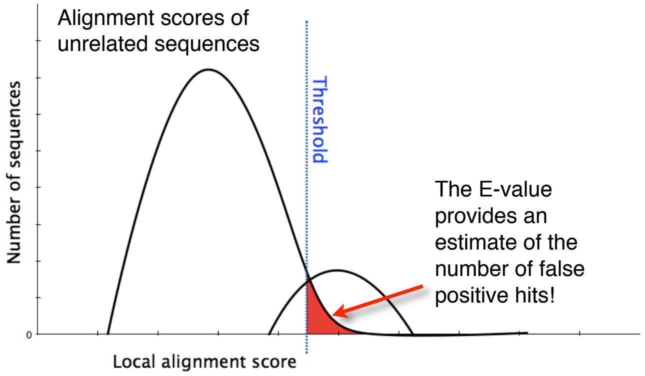
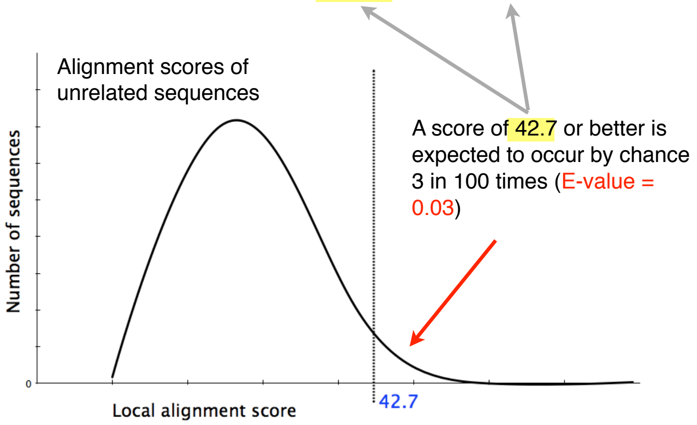


The E-value provides an estimate of the number of false positive hits!

Description	Max score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo sapiens]	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	100%	0	98%	AAA20133.1
Kinesin-14 heavy chain [Danio rerio]	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	42.7	40%	**0.03**	32%	ELK35081.1



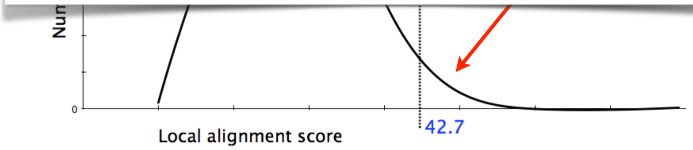
A score of 42.7 or better is expected to occur by chance 3 in 100 times (E-value = 0.03)

Description	Max score	Total score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo	677	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	676	100%	0	98%	AAA20133.1

In general *E* values < 0.005 are usually significant.

To find out more about *E* values see: "*The Statistics of Sequence Similarity Scores*" available in the help section of the NCBI BLAST site:

http://www.ncbi.nlm.nih.gov/blast/tutorial/Altschul-1.html



Your Turn!

Hands-on worksheet **Sections 6**

- Please do answer the last lab review question (**Q19**).
- We encourage discussion and exploration!

Summary

- Sequence alignment is a fundamental operation underlying much of bioinformatics.
- Even when optimal solutions can be obtained they are not necessarily unique or reflective of the biologically correct alignment.
- Dynamic programming is a classic approach for solving the pairwise alignment problem.
- Reviewed global and local alignment approaches and their major application areas.
- Heuristic approaches are often necessary for large database searches and many genomic applications.

FOR NEXT CLASS…

Check out the online:

☑ **Reading**: Sean Eddy's "What is dynamic programming?"

☑ **Homework**: (1) **Quiz**, (2) **Alignment Exercise**.

Homework Grading

Both (1) quiz questions and (2) alignment exercise carry equal weights (*i.e.* 50% each).

(Homework 2) Assessment Criteria	Points	
Setup labeled alignment matrix	1	
Include initial column and row for GAPs	1	
All alignment matrix elements scored (i.e. filled in)	1	
Evidence for correct use of scoring scheme	1	
Direction arrows drawn between all cells	1	
Evidence of multiple arrows to a given cell if appropriate	1	D
Correct optimal score position in matrix used	1	C
Correct optimal score obtained for given scoring scheme	1	B
Traceback path(s) clearly highlighted	1	A
Correct *alignment(s)* yielding optimal score listed	1	A+

REFERENCE SLIDES...

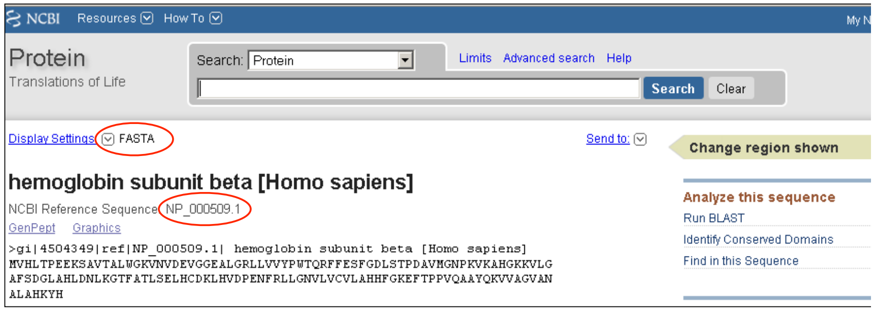
Additional reference slides for the motivated student

Practical database searching with BLAST

- There are four basic components to a traditional BLAST search
  - (1) Choose the sequence (query)
  - (2) Select the BLAST program
  - (3) Choose the database to search
  - (4) Choose optional parameters
- Then click “BLAST”

Step 1: Choose your sequence

- Sequence can be input in FASTA format or as accession number



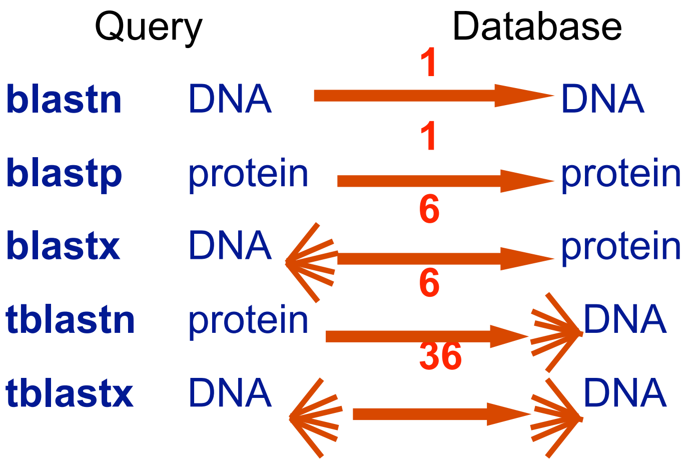
Display Settings: FASTA

hemoglobin subunit beta [Homo sapiens]

NCBI Reference Sequence: NP_000509.1

```
>gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLG
AFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVAN
ALAHKYH
```

Step 2: Choose the BLAST program



Query Database

blastn DNA 1 DNA

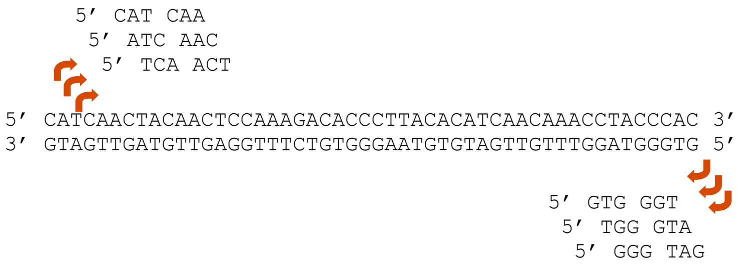
blastp protein 1 protein

blastx DNA 6 protein

tblastn protein 6 DNA

tblastx DNA 36 DNA

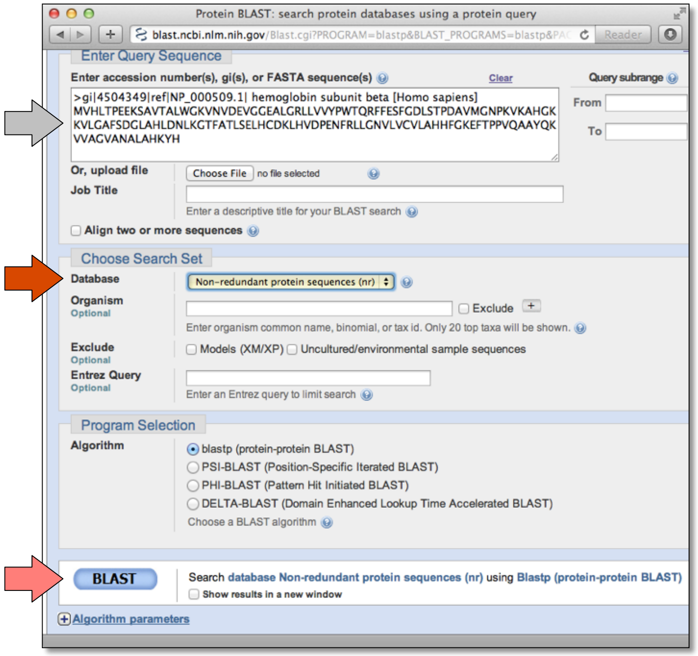
DNA potentially encodes six proteins



```
           5′ CAT CAA
            5′ ATC AAC
             5′ TCA ACT

5′ CATCAACTACAACTCCAAAGACACCCTTACACATCAACAAACCTACCCAC 3′
3′ GTAGTTGATGTTGAGGTTTCTGTGGGAATGTGTAGTTGTTTGGATGGGTG 5′

                                            5′ GTG GGT
                                             5′ TGG GTA
                                              5′ GGG TAG
```



Protein BLAST: search protein databases using a protein query

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PA

Enter Query Sequence

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

```
>gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGK
KVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQK
VVAGVANALAHKYH
```

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: Non-redundant protein sequences (nr)

Organism (Optional): □ 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

Entrez Query (Optional): Enter an Entrez query to limit search

Program Selection

Algorithm:
- ● blastp (protein-protein BLAST)
- ○ PSI-BLAST (Position-Specific Iterated BLAST)
- ○ PHI-BLAST (Pattern Hit Initiated BLAST)
- ○ DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

BLAST — Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)

□ Show results in a new window

Algorithm parameters

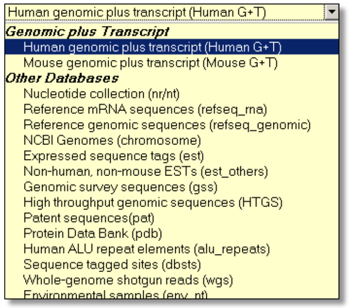
Step 3: Choose the database

nr = non-redundant (most general database)

dbest = database of expressed sequence tags

dbsts = database of sequence tag sites

gss = genomic survey sequences

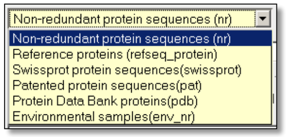


Human genomic plus transcript (Human G+T)

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

Other Databases
- Nucleotide collection (nr/nt)
- Reference mRNA sequences (refseq_rna)
- Reference genomic sequences (refseq_genomic)
- NCBI Genomes (chromosome)
- Expressed sequence tags (est)
- Non-human, non-mouse ESTs (est_others)
- 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 reads (wgs)
- Environmental samples (env_nt)

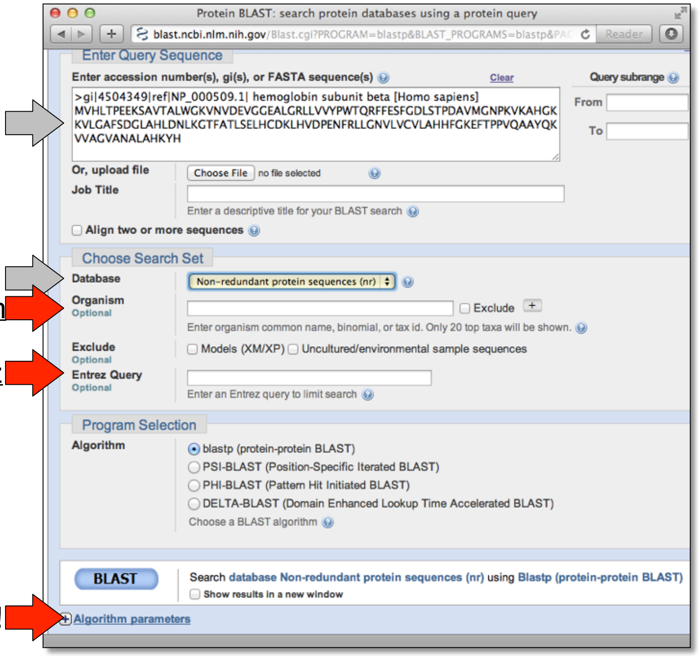
nucleotide databases



Non-redundant protein sequences (nr)

- Non-redundant protein sequences (nr)
- Reference proteins (refseq_protein)
- Swissprot protein sequences(swissprot)
- Patented protein sequences(pat)
- Protein Data Bank proteins(pdb)
- Environmental samples(env_nr)

protein databases



Protein BLAST: search protein databases using a protein query

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PA

Enter Query Sequence

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

```
>gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGK
KVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQK
VVAGVANALAHKYH
```

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: Non-redundant protein sequences (nr)

Organism (Optional): — □ 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

Entrez Query (Optional): — Enter an Entrez query to limit search

Program Selection

Algorithm:
- ● blastp (protein-protein BLAST)
- ○ PSI-BLAST (Position-Specific Iterated BLAST)
- ○ PHI-BLAST (Pattern Hit Initiated BLAST)
- ○ DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

BLAST — Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
□ Show results in a new window

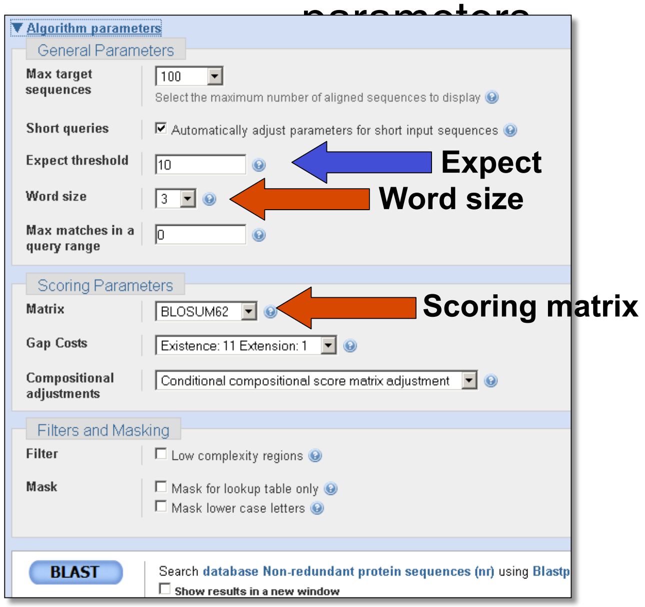
+ Algorithm parameters

Organism

Entrez

Settings!

Step 4a: Select optional search parameters



▼ Algorithm 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: 10

Word size: 3

Max matches in a query range: 0

Scoring Parameters

Matrix: BLOSUM62

Gap Costs: Existence: 11 Extension: 1

Compositional adjustments: Conditional compositional score matrix adjustment

Filters and Masking

Filter: □ Low complexity regions

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

BLAST — Search database Non-redundant protein sequences (nr) using Blastp
□ Show results in a new window

Expect

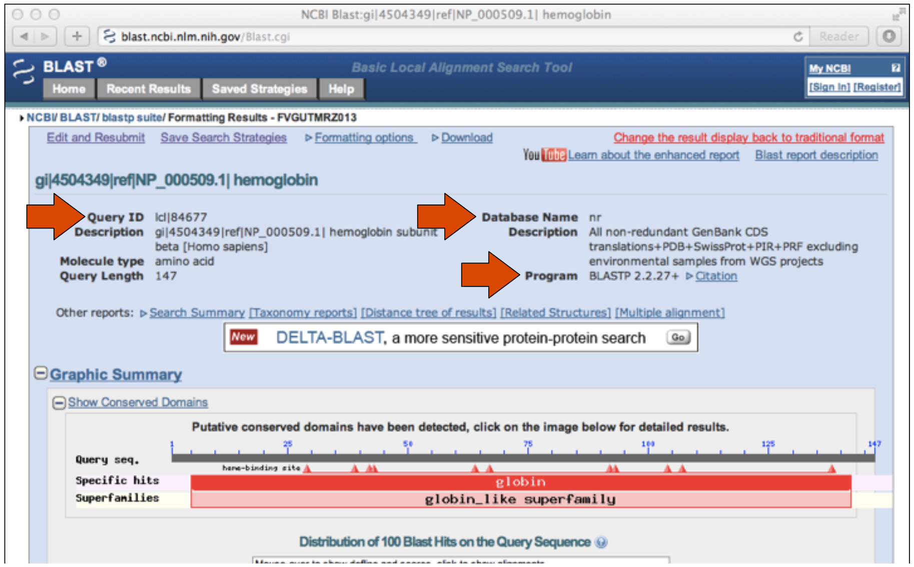
Word size

Scoring matrix

Step 4: Optional parameters

- You can...
  - choose the organism to search
  - change the substitution matrix
  - change the expect (E) value
  - change the word size
  - change the output format

Results page



NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

NCBI/ BLAST/ blastp suite/ Formatting Results - FVGUTMRZ013

Edit and Resubmit — Save Search Strategies — ▷ Formatting options — ▷ Download — Change the result display back to traditional format

YouTube Learn about the enhanced report — Blast report description

gi|4504349|ref|NP_000509.1| hemoglobin

Query ID: lcl|84677
Description: gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
Molecule type: amino acid
Query Length: 147

Database Name: nr
Description: All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects
Program: BLASTP 2.2.27+ ▷ Citation

Other reports: ▷ Search Summary [Taxonomy reports] [Distance tree of results] [Related Structures] [Multiple alignment]

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

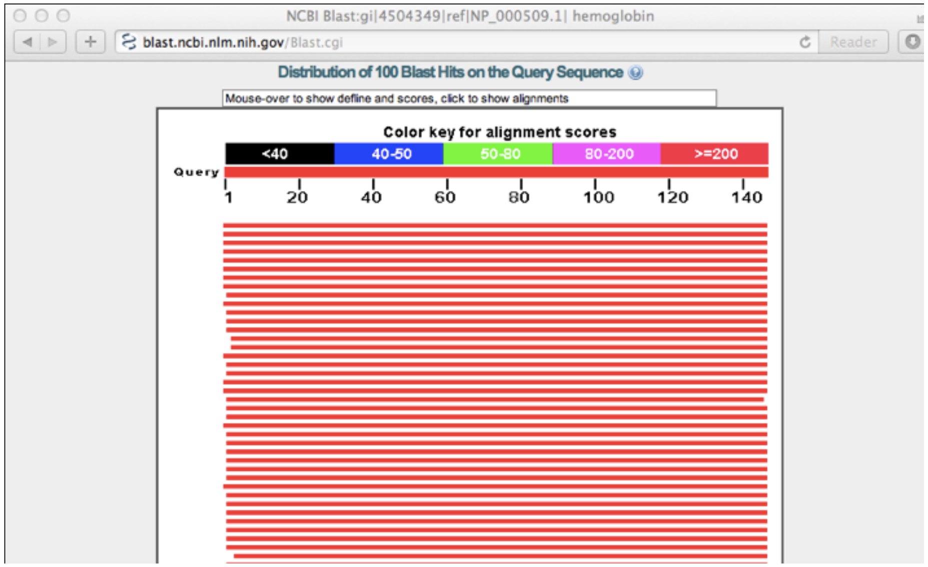
⊟ Graphic Summary

⊟ Show Conserved Domains

Putative conserved domains have been detected, click on the image below for detailed results.

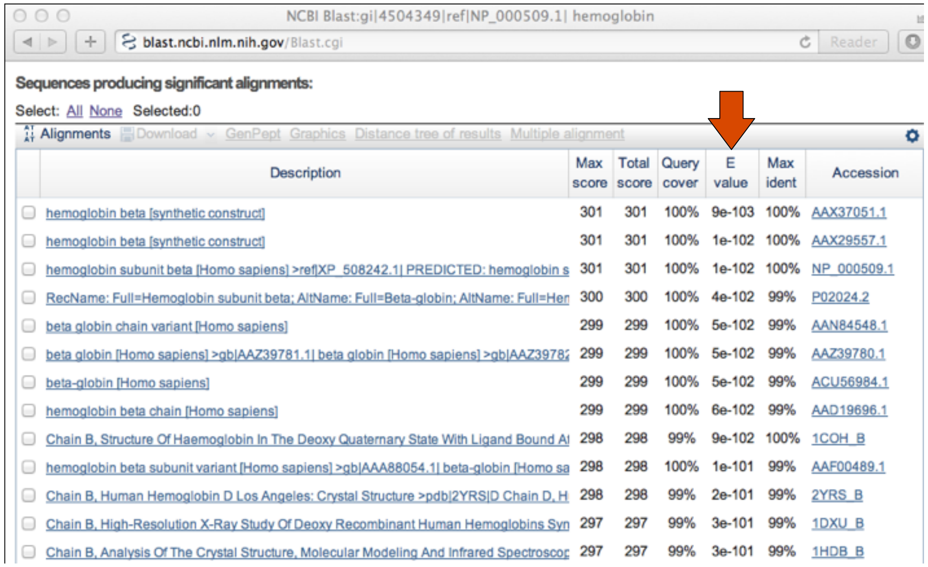
Distribution of 100 Blast Hits on the Query Sequence

Further down the results page...



NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin
blast.ncbi.nlm.nih.gov/Blast.cgi
Reader
Distribution of 100 Blast Hits on the Query Sequence
Mouse-over to show define 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
120
140

Further down the results page...



NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin
blast.ncbi.nlm.nih.gov/Blast.cgi
Reader

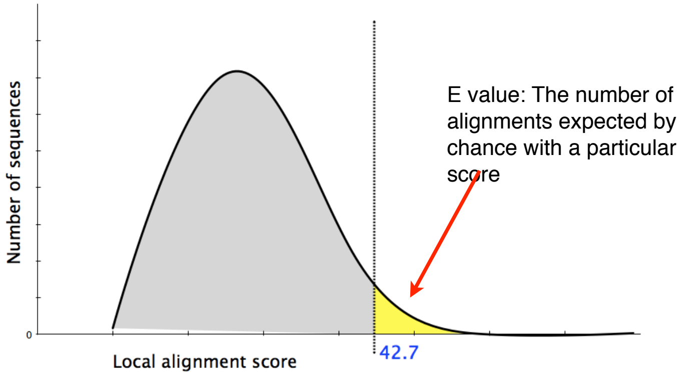
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
□	hemoglobin beta [synthetic construct]	301	301	100%	9e-103	100%	AAX37051.1
□	hemoglobin beta [synthetic construct]	301	301	100%	1e-102	100%	AAX29557.1
□	hemoglobin subunit beta [Homo sapiens] >ref|XP_508242.1| PREDICTED: hemoglobin s	301	301	100%	1e-102	100%	NP_000509.1
□	RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Hen	300	300	100%	4e-102	99%	P02024.2
□	beta globin chain variant [Homo sapiens]	299	299	100%	5e-102	99%	AAN84548.1
□	beta globin [Homo sapiens] >gb|AAZ39781.1| beta globin [Homo sapiens] >gb|AAZ3978:	299	299	100%	5e-102	99%	AAZ39780.1
□	beta-globin [Homo sapiens]	299	299	100%	5e-102	99%	ACU56984.1
□	hemoglobin beta chain [Homo sapiens]	299	299	100%	6e-102	99%	AAD19696.1
□	Chain B, Structure Of Haemoglobin In The Deoxy Quaternary State With Ligand Bound At	298	298	99%	9e-102	100%	1COH_B
□	hemoglobin beta subunit variant [Homo sapiens] >gb|AAA88054.1| beta-globin [Homo sa	298	298	100%	1e-101	99%	AAF00489.1
□	Chain B, Human Hemoglobin D Los Angeles: Crystal Structure >pdb|2YRS|D Chain D, H	298	298	99%	2e-101	99%	2YRS_B
□	Chain B, High-Resolution X-Ray Study Of Deoxy Recombinant Human Hemoglobins Syn	297	297	99%	3e-101	99%	1DXU_B
□	Chain B, Analysis Of The Crystal Structure, Molecular Modeling And Infrared Spectroscop	297	297	99%	3e-101	99%	1HDB_B

Description	Max score	Total score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo	677	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	676	100%	0	98%	AAA20133.1
Kinesin-14 heavy chain [Danio rerio]	595	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	48.2	52	40%	0.03	32%	ELK35081.1
mKIAA4102 protein [Mus musculus]	42.7	42.7	38%	3.02	24%	EHH28205.1

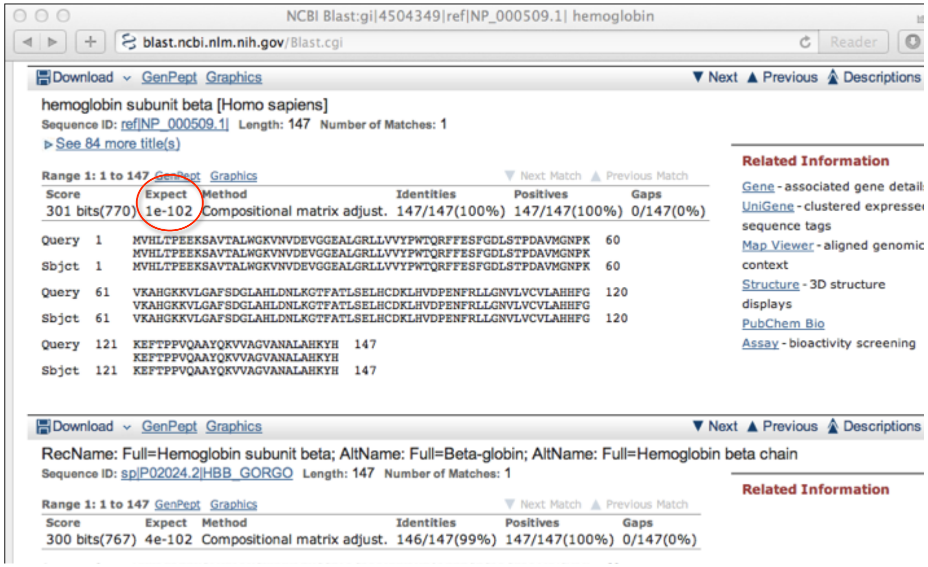


E value: The number of alignments expected by chance with a particular score

E values in BLAST

- Each alignment gets a score determined from the alignment and doesn't take into account the full length of the query, target or database
- The E value is what you want to look at
- E value = Expect
  - How often do I expect an alignment with this score give the length of my query and the size of the database
  - $E = Kmne^{-\lambda s}$
    - K and λ are scaling factors
    - S is the score
    - m – length of query, n – length of database
  - E corrects for multiple comparisons, i.e., query compared to many sequences – proportional to length of database and query for a given S (score)

Further down the results page...



NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

hemoglobin subunit beta [Homo sapiens]

Sequence ID: ref|NP_000509.1| Length: 147 Number of Matches: 1

▷ See 84 more title(s)

Range 1: 1 to 147 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
301 bits(770)	1e-102	Compositional matrix adjust.	147/147(100%)	147/147(100%)	0/147(0%)

```
Query  1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
            MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK
Sbjct  1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60

Query  61   VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG  120
            VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG
Sbjct  61   VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG  120

Query  121  KEFTPPVQAAYQKVVAGVANALAHKYH  147
            KEFTPPVQAAYQKVVAGVANALAHKYH
Sbjct  121  KEFTPPVQAAYQKVVAGVANALAHKYH  147
```

Related Information

Gene - associated gene detail

UniGene - clustered expresse sequence tags

Map Viewer - aligned genomic context

Structure - 3D structure displays

PubChem Bio

Assay - bioactivity screening

RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Hemoglobin beta chain

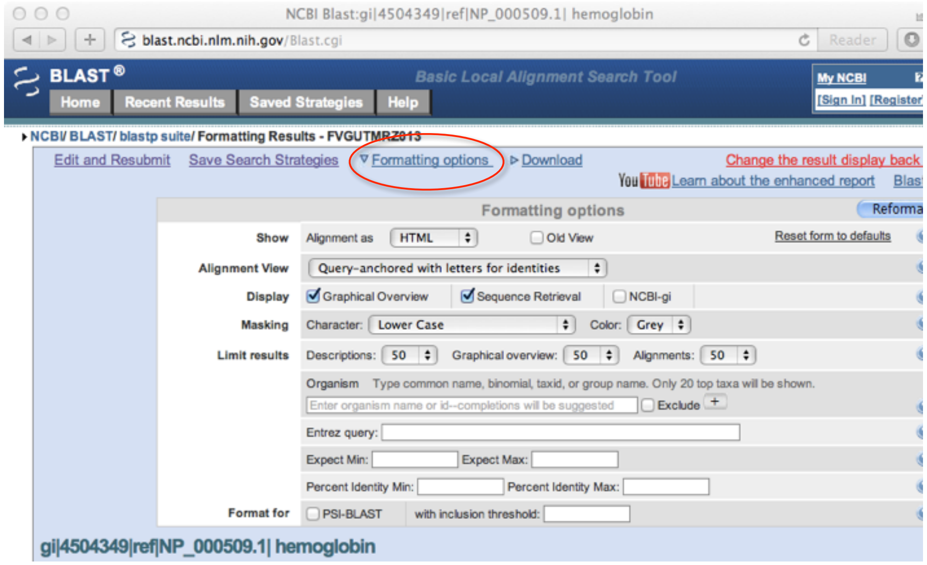
Sequence ID: sp|P02024.2|HBB_GORGO Length: 147 Number of Matches: 1

Related Information

Range 1: 1 to 147 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
300 bits(767)	4e-102	Compositional matrix adjust.	146/147(99%)	147/147(100%)	0/147(0%)

Different output formats are available



NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

▸ NCBI/ BLAST/ blastp suite/ Formatting Results - FVGUTMRZ013

Formatting options

Show: Alignment as HTML ⇕ □ Old View

Alignment View: Query-anchored with letters for identities ⇕

Display: ☑ Graphical Overview ☑ Sequence Retrieval □ NCBI-gi

Masking: Character: Lower Case ⇕ Color: Grey ⇕

Limit results: Descriptions: 50 ⇕ Graphical overview: 50 ⇕ Alignments: 50 ⇕

Organism Type common name, binomial, taxid, or group name. Only 20 top taxa will be shown.

Enter organism name or id--completions will be suggested □ Exclude +

Entrez query:

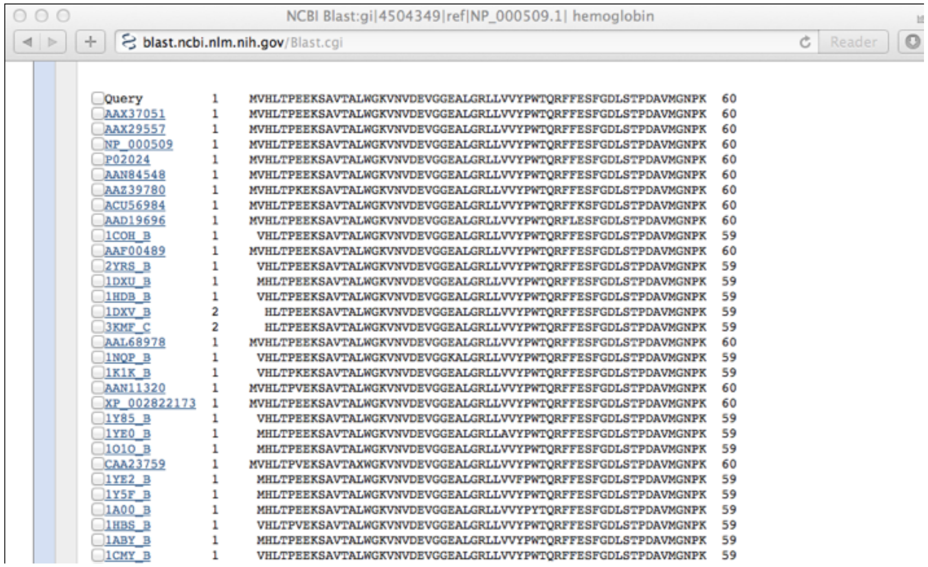
Expect Min: Expect Max:

Percent Identity Min: Percent Identity Max:

Format for: □ PSI-BLAST with inclusion threshold:

gi|4504349|ref|NP_000509.1| hemoglobin

E.g. Query anchored alignments

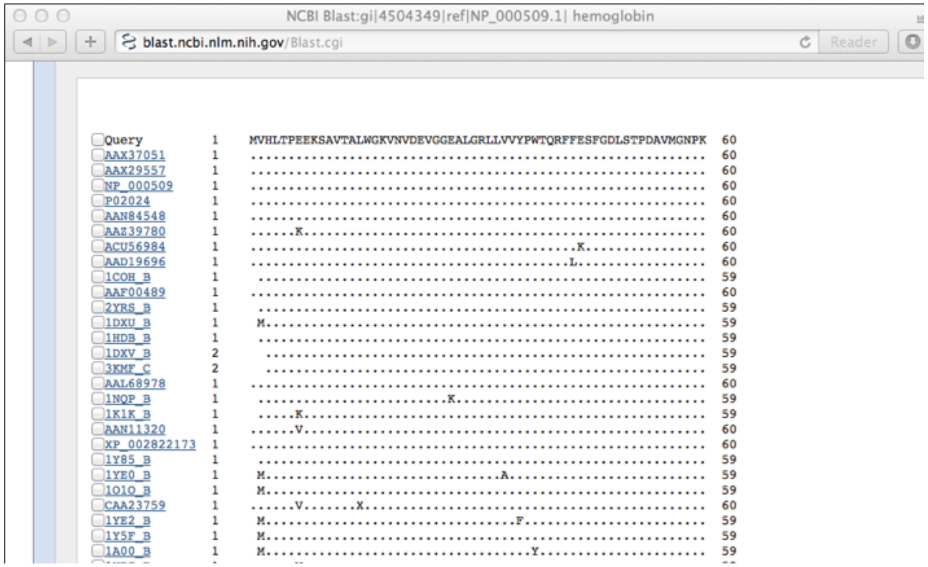


NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

```
□Query         1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□AAX37051      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□AAX29557      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□NP_000509     1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□P02024        1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□AAN84548      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□AAZ39780      1    MVHLTPKEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□ACU56984      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFKSFGDLSTPDAVMGNPK  60
□AAD19696      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFLESFGDLSTPDAVMGNPK  60
□1COH_B        1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□AAF00489      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□2YRS_B        1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1DXU_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1HDB_B        1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1DXV_B        2      HLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□3KMF_C        2      HLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□AAL68978      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□1NQP_B        1     VHLTPEEKSAVTALWGKVNVDEVGGKALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1K1K_B        1     VHLTPKEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□AAN11320      1    MVHLTPVEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□XP_002822173  1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□1Y85_B        1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1YE0_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLAVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1O1O_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□CAA23759      1    MVHLTPVEKSAVTAXWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□1YE2_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVFPWTQRFFESFGDLSTPDAVMGNPK  59
□1Y5F_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1A00_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPYTQRFFESFGDLSTPDAVMGNPK  59
□1HBS_B        1     VHLTPVEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1ABY_B        1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
□1CMY_B        1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
```

... and alignments with dots for identities



NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

```
□Query         1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
□AAX37051      1    ............................................................  60
□AAX29557      1    ............................................................  60
□NP_000509     1    ............................................................  60
□P02024        1    ............................................................  60
□AAN84548      1    ............................................................  60
□AAZ39780      1    ......K.....................................................  60
□ACU56984      1    ...........................................K................  60
□AAD19696      1    ..........................................L.................  60
□1COH_B        1     ...........................................................  59
□AAF00489      1    ............................................................  60
□2YRS_B        1     ...........................................................  59
□1DXU_B        1     M..........................................................  59
□1HDB_B        1     ...........................................................  59
□1DXV_B        2      ..........................................................  59
□3KMF_C        2      ..........................................................  59
□AAL68978      1    ............................................................  60
□1NQP_B        1     .........................K.................................  59
□1K1K_B        1     .....K.....................................................  59
□AAN11320      1    ......V.....................................................  60
□XP_002822173  1    ............................................................  60
□1Y85_B        1     ...........................................................  59
□1YE0_B        1     M...............................A..........................  59
□1O1O_B        1     M..........................................................  59
□CAA23759      1    ......V.......X.............................................  60
□1YE2_B        1     M.................................F........................  59
□1Y5F_B        1     M..........................................................  59
□1A00_B        1     M...................................Y......................  59
```

Common problems

- Selecting the wrong version of BLAST
- Selecting the wrong database
- Too many hits returned
- Too few hits returned
- Unclear about the significance of a particular result - are these sequences homologous?

How to handle too many results

- Focus on the question you are trying to answer
  - select “refseq” database to eliminate redundant matches from “nr”
  - Limit hits by organism
  - Use just a portion of the query sequence, when appropriate
  - Adjust the expect value; lowering E will reduce the number of matches returned

How to handle too few results

- Many genes and proteins have no significant database matches
  - remove Entrez limits
  - raise E-value threshold
  - search different databases
  - try scoring matrices with lower BLOSUM values (or higher PAM values)
  - use a search algorithm that is more sensitive than BLAST (e.g. PSI-BLAST or HMMer)