

- **Uploading your assignment as a PDF is mandatory. The system will only accept a single PDF file and won't accept anything else or more than one file.**
- [BLAST: Program Selection Guide](#)
  - Section 6.1 will be especially helpful.
- [BLAST: Frequently Asked Questions](#)
- You will have to add screenshots to your homework in this assignment. Remember the screenshot keyboard shortcuts. Feel free to use MS Powerpoint/GoogleSlides in your homework.

1) Use bl2seq to align **NM\_000558.3** to **NP\_000549.1**

- a) Which BLAST program did you choose? Why?
- b) What is the sequence type in the final alignment?
- c) What are the significance value and the total score of the alignment? Define them, and comment on the result you get.

2) Use `bl2seq` to align **NP\_000509.1** to **NP\_000549.1**

- a) Which BLAST program did you choose? Why?
- b) What is the sequence type in the final alignment?
- c) What are the significance value and the total score of the alignment? comment on the result you get.

3) Use [`Lalign`](#) to align **NM\_000558.3** to **AF230076.1**

- a) What type of pairwise alignment approach `Lalign` algorithm is based on?
- b) Try **local**, **global** and **global with no end-gap penalty** alignments separately. Compare the results. What kind of differences do you observe?
  - i) Do you see any differences at the termini of the alignment?
  - ii) Do you get any errors or do all methods work?

4) Theoretical:

- a) What are the differences between **local**, **global**, and **global with no end-gap penalty** alignment methods?
- b) Which alignment gives biologically more relevant information (amongst **local** and **global** alignment)? Explain your reasoning.

Nucleotide

Nucleotide

Advanced

Search



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FASTA

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# Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA

NCBI Reference Sequence: NM\_000558.3

This sequence has been updated. [See current version.](#)

[GenBank](#) [Graphics](#)

```
>NM_000558.3 Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA
ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGTCTCTCGCCGACAAGACCAACGTC
AAGGCCGCTGGGGTAAGGTGCGCGCGCACGCTGGCGAGTATGGTGGGAGGCCCTGGAGAGGATGTTCC
TGTCCTTCCCCACCACCAAGACCTACTTCCCGCACTTCGACCTGAGCCACGGCTCTGCCAGGTTAAGGG
CCACGGCAAGAAGGTGGCCGACGCGCTGACCAACGCCGTGGCGCACGTGGACGACATGCCAACGCGCTG
TCCGCGCTGAGCGACCTGCACGCGACAAGCTTGGGTGGACCGGTCAACTTCAAGCTCCTAAGCCACT
GCCTGCTGGTGACCTGGCCGCCACCTCCCCGCCGAGTTACCCCTGCGGTGCACGCCCTCCCTGGACAA
GTTCTGGCTTCTGTGAGCACCGTGTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTGGCCATGCTT
CTTGCCCTTGGGCTCCCCCAGCCCTCCTCCCTTCTGCACCGTACCCCGTGGTCTTTGAATAA
AGTCTGAGTGGGCGGC
```

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

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HBA1 hemoglobin subunit alpha 1 [Homo sapiens]

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FASTA

Send to:

Change region shown

## hemoglobin subunit alpha [Homo sapiens]

NCBI Reference Sequence: NP\_000549.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

```
>NP_000549.1 hemoglobin subunit alpha [Homo sapiens]
MVLSPADKTNVKAAMGKVGAGHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKVADALTNA
VAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
YR
```

### Analyze this sequence

Run BLAST

Identify Conserved Domains

Highlight Sequence Features

Find in this Sequence

Show in Genome Data Viewer

### Protein 3D Structure



THE CRYSTAL  
STRUCTURE OF HUMAN

# 1.bl2seq : BLASTX search protein subjects using a translated nucleotide query.

BLASTX search protein subjects using a translated nucleotide query. [more...](#)

### Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

>NM\_000558.3 Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA  
ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCT  
CCTGCCGACAAGACCAACGTC  
AAGCCGCCTGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCG

From   
To

Or, upload file  Dosya seçilmedi [?](#)

Genetic code

Job Title   
Enter a descriptive title for your BLAST search [?](#)

☒ Align two or more sequences [?](#)

### Enter Subject Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

>NP\_000549.1 hemoglobin subunit alpha [Homo sapiens]  
MVLSPADKTNVKAAWGKVGAAHAGEYGAEALERMFSLFPTTKTYFPHFDSLH  
GSAQVKGHGKKVADALTNA  
VAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVH

From   
To

Or, upload file  Dosya seçilmedi [?](#)

**New columns**  
**Description Ta**  
Click 'Select Column  
Columns'.



[< Edit Search](#)

[Save Search](#)   [Search Summary](#) ▾

[? How to read this report?](#)   [▶ BLAST Help Videos](#)   [↶ Back to Traditional Results Page](#)

Job Title	NM_000558.3 Homo sapiens hemoglobin, alpha
RID	<a href="#">STU06ZER114</a> <i>Search expires on 11-12 16:53 pm</i> <a href="#">Download All</a> ▾
Program	Blast 2 sequences <a href="#">Citation</a> ▾
Query ID	Icl Query_13615 (dna)
Query Descr	NM_000558.3 Homo sapiens hemoglobin, alpha 1 (HBA ...
Query Length	576
Subject ID	Icl Query_13617 (amino acid)
Subject Descr	NP_000549.1 hemoglobin subunit alpha [Homo sapiens]
Subject Length	142
Other reports	<a href="#">?</a>

Filter Results

Percent Identity	E value	Query Coverage
<input type="text"/> to <input type="text"/>	<input type="text"/> to <input type="text"/>	<input type="text"/> to <input type="text"/>
		<a href="#">Filter</a> <a href="#">Reset</a>

Other reports 

- Descriptions
- Graphic Summary
- Alignments
- Dot Plot

Sequences producing significant alignments

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Select columns 

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100 

☒ select all 1 sequences selected

[Graphics](#)

	Description 	Scientific Name 	Max Score 	Total Score 	Query Cover 	E value 	Per. Ident 	Acc. Len 	Accession
<input checked="" type="checkbox"/>	<a href="#">NP_000549.1 hemoglobin subunit alpha [Homo sapiens]</a>		286	286	73%	2e-105	100.00%	142	Query_18

how much of the query covered by targeted sequence

max score: highest alignment score  
total score: sum of all alignment scores  
no difference between total and max. score,  
this means there is no gaps between the alignment

Other reports 

Descriptions

Graphic Summary

Alignments

Dot Plot

## Sequences producing significant alignments

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**1 row**










Select columns 

Show

100 

☒ select all 1 sequences selected

[Graphics](#)

	Description 	Scientific Name 	Max Score 	Total Score 	Query Cover 	E value 	Per. Ident 	Acc. Len 	Accession 
<input checked="" type="checkbox"/>	NP_000549.1 hemoglobin subunit alpha [Homo sapiens]		286	286	73%	2e-105	100.00%	142	Query_180

E-values are just like p-values to evaluate the statistical similarity but BLAST uses E-value: significance value

how similar 2 sequences



# final alignment: protein sequence

Alignment view Pairwise ? Restore defaults

1 sequences selected ?

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**NP\_000549.1 hemoglobin subunit alpha [Homo sapiens]**  
Sequence ID: **Query\_180493** Length: **142** Number of Matches: **1**

Range 1: 1 to 142 [Graphics](#) ▼ Next Match ▲ Previous Match

	Score	Expect	Method	Identities	Positives	Gaps	Frame
	286 bits(732)	2e-105	Compositional matrix adjust.	142/142(100%)	142/142(100%)	0/142(0%)	+2
Query	38		MVLSPADKTNVKAANGKVGAGHAGEYGAEALERMF				
			LSFPTTKTYFPHFDLSHGSAQVKGHG				217
Sbjct	1		MVLSPADKTNVKAANGKVGAGHAGEYGAEALERMF				
			LSFPTTKTYFPHFDLSHGSAQVKGHG				60
Query	218		KKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP				
			KKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP				397
Sbjct	61		KKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP				
			KKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP				120
Query	398		AVHASLDKFLASVSTVLTSKYR				
			AVHASLDKFLASVSTVLTSKYR				463
Sbjct	121		AVHASLDKFLASVSTVLTSKYR				
			AVHASLDKFLASVSTVLTSKYR				142

graphical summary, it means the resemblances between the sequences are very high

Descriptions

**Graphic Summary**

Alignments

Dot Plot

 hover to see the title  click to show alignments


Alignment Scores

 < 40


 40 - 50

 50 - 80

 80 - 200

  $\geq 200$



1 sequences selected 

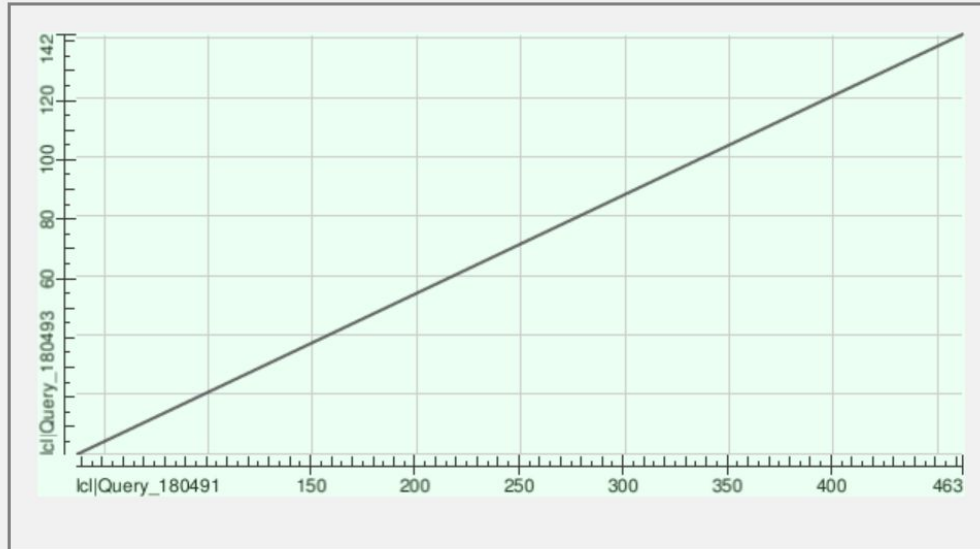
### Distribution of the top 1 Blast Hits on 1 subject sequences




# matched sequences as dot plot:

Descriptions Graphic Summary Alignments **Dot Plot**

Plot of lcl|Query\_180491 vs lcl|Query\_180493 ?





## 2.bl2seq: protein to protein

 NCBI Resources ☒ How To ☒

Protein

Advanced

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### hemoglobin subunit beta [Homo sapiens]

NCBI Reference Sequence: NP\_000509.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

```
>NP_000509.1 hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAMGNPKVKAHGKKVLG
AFSDGLAHLNLIKGTATLSEHCDKLVHDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVAN
ALAHKYH
```



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## hemoglobin subunit alpha [Homo sapiens]

NCBI Reference Sequence: NP\_000549.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

```
>NP_000549.1 hemoglobin subunit alpha [Homo sapiens]
MVLSPADKTNVKAAWGKVGAGHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKVADALTNA
VAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
YR
```

# Blastp was used : protein to protein

blastn

**blastp**

blastx

tblastn

tblastx

Align Sequences Protein BLAST

BLASTP programs search protein subjects using a protein query. [more...](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

PDAVMGNPKVKAHGKKVLG  
AFSDGLAHLNLKGTATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKE  
FTPPVQAAYQKVVGAVAN  
ALAHKYH

Or, upload file

Dosya Seç Dosya seçilmedi [?](#)

Job Title

NP\_000509.1 hemoglobin subunit beta [Homo...  
Enter a descriptive title for your BLAST search [?](#)

☒ Align two or more sequences [?](#)

Query subrange [?](#)

From

To

New columns added to the  
Description Table  
Click 'Select Columns' or 'Reset  
Columns'.

Enter Subject Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

>NP\_000549.1 hemoglobin subunit alpha [Homo sapiens]  
MVLSPADKTNVKAAWGKVGGAHAGEYGAELERMFLSFPTTKTYFPHFDLSH  
GSAQVKGHGKKVADALTNA  
VAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVH

Or, upload file

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Subject subrange [?](#)

From

To





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BLAST® » [blastp suite-2sequences](#) » results for RID-STVD02VC11N

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**Job Title** NP\_000509.1 hemoglobin subunit beta [Homo...

**RID** [STVD02VC11N](#) *Search expires on 11-12 17:17 pm*

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**Program** Blast 2 sequences [Citation](#) ▼

**Query ID** lcl|Query\_382385 (amino acid)

**Query Descr** NP\_000509.1 hemoglobin subunit beta [Homo sapiens]

**Query Length** 147

**Subject ID** lcl|Query\_382387 (amino acid)

**Subject Descr** NP\_000549.1 hemoglobin subunit alpha [Homo sapiens]

**Subject Length** 142

[Multiple alignment](#) [MSA viewer](#) [?](#)

## Filter Results

**Percent Identity**

to

**E value**

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Descriptions

Graphic Summary

Alignments

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Sequences producing significant alignments

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☒ select all   1 sequences selected

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[MSA Viewer](#)

	Description 	Scientific Name 	Max Score 	Total Score 	Query Cover 	E value 	Per. Ident 	Acc. Len 	Accession
<input checked="" type="checkbox"/>	<a href="#">NP_000549.1 hemoglobin subunit alpha [Homo sapiens]</a>		114	114	97%	2e-38	43.45%	142	Query_382387

how much of the query covered by targeted sequence

max score: highest alignment score  
total score: sum of all alignment scores  
no difference between total and max. score, this means there is no gaps between the alignment

Descriptions

Graphic Summary

Alignments

Dot Plot

## Sequences producing significant alignments

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?

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Graphics

Multiple alignment

New

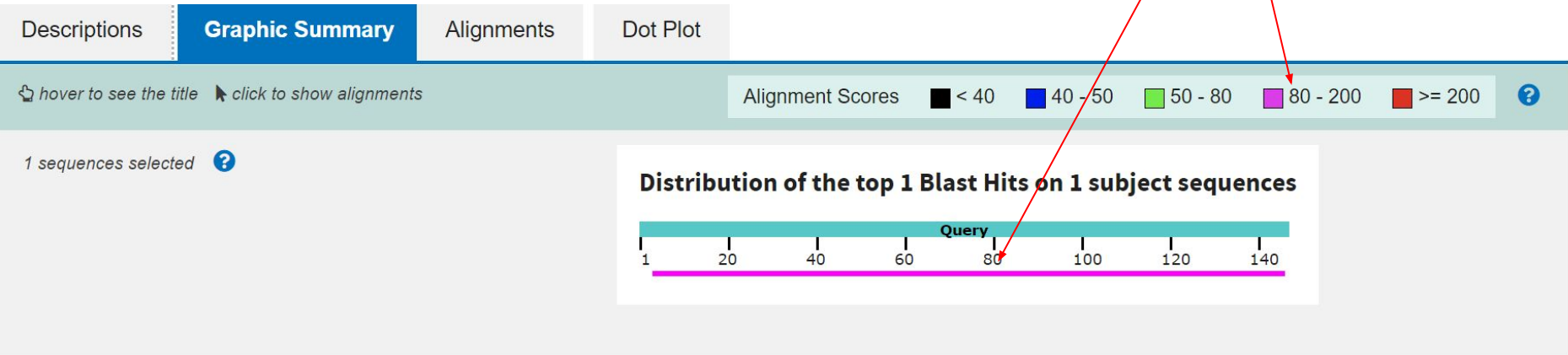
MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	<a href="#">NP_000549.1 hemoglobin subunit alpha [Homo sapiens]</a>		114	114	97%	2e-38	43.45%	142	Query_382387

E-values are just like p-values to evaluate the statistical similarity but BLAST uses E-value: significance value

how similar 2 sequences

high resemblances (but not higher as NM\_000558.3&NP\_000549.1 query)



# final alignment as protein seq.


1 sequences selected ?

[Download](#) [Graphics](#) [Next](#) [Previous](#) [Des](#)

**NP\_000549.1 hemoglobin subunit alpha [Homo sapiens]**  
Sequence ID: **Query\_382387** Length: **142** Number of Matches: **1**

**Range 1: 3 to 141** [Graphics](#) [Next Match](#) [Previous Match](#)

	Score	Expect	Method	Identities	Positives	Gaps
	114 bits(286)	2e-38	Compositional matrix adjust.	63/145(43%)	88/145(60%)	8/145(5%)
Query	4		LTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAMGNPKV	61		
			L+P +K+ V A WGKV + E G EAL R+ + +P T+ +F F DLS G+ +V			
Sbjct	3		LSPADKTNVKAAGWKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS-----HGSAQV	56		
Query	62		KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLCVLAHHFGK	121		
			K HGKKV A ++ +AH+D++ + LS+LH KL VDP NF+LL + L+ LA H			
Sbjct	57		KGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPA	116		
Query	122		EFTPPVQAAYQKVVAGVANALAHKY 146			
			EFTP V A+ K +A V+ L KY			
Sbjct	117		EFTPAVHASLDKFLASVSTVLTSKY 141			





dot plot: not straightly linear as NM\_000558.3&NP\_000549.1 query, high resemblance but 3 deletions / mismatches between two sub-sequences among query & target sequences

5 vs lcl|Query\_382387 ?






### 3. Lalign: William Pearson's lalign program

 **ExPASy**  
Bioinformatics Resource Portal

LALIGN

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**This web server will be shut down on 7 December 2021**

This EMBnet server has been functional for more than two decades but the offered services are no longer up-to-date. We ask you to use relevant other servers and thank you for your confidence you provided over the years.

In case you see issues with that, please contact [SIB's Core-IT group](#).

#### Find multiple matching subsegments in two sequences

This is William Pearson's *lalign* program. A manual page for this program is available [here](#). The lalign program implements the algorithm of Huang and Miller, published in Adv. Appl. Math. (1991) 12:337-357.

This program is part of the FASTA package of sequence analysis program.


**Usage:** Paste your two sequences in one of the supported [formats](#) into the sequence fields below and press the "Run lalign" button.  
[Make sure that both format buttons \(next to the sequence fields\) shows the correct formats](#)

Choose the alignment method: ☒ local (default) ☐ global ☐ global without end-gap penalty

Number of reported sub-alignments:  E-value threshold:  (default 10.0)

Scoring matrix:

Opening gap penalty :  (default -12) Extending gap penalty :  (default -2)

6°C Puslu 11:44 11.11.2021

Nucleotide

Nucleotide ▾

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FASTA ▾

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# Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA

NCBI Reference Sequence: NM\_000558.3

 This sequence has been updated. [See current version.](#)[GenBank](#) [Graphics](#)

```
>NM_000558.3 Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA
ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCACCATGGTGCTGTCTCCTGCCGACAAGACCAACGTC
AAGGCCGCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAGAGGATGTTCC
TGTCCTTCCCCACCAAGACCTACTTCCCGCACTTCGACCTGAGCCACGGCTCTGCCAGGTTAAGGG
CCACGGCAAGAAGGTGGCCGACGCGCTGACCAACGCCGTGGCGCACGTGGACGACATGCCAACGCGCTG
TCCGCCCTGAGCGACCTGCACGCGCACAAAGCTTCGGGTGGACCCGGTCAACTTCAAGCTCCTAAGCCACT
GCCTGCTGGTGACCTGGCCGCCACCTCCCCGCCGAGTTCACCCCTGCGGTGCACGCCTCCCTGGACAA
GTTCTTGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTGGCCATGCTT
CTTGCCCCCTTGGGCCTCCCCCAGCCCTCCTCCCCCTTCTGACCCGTACCCCGTGGTCTTTGAATAA
AGTCTGAGTGGGCGGC
```

**Analyze this sequence** ▴

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

**Recent activity** ▴[Turn Off](#) [Clear](#)Homo sapiens alpha-2-globin (HBA2) gene,  
complete cds  
Nucleotide



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FASTA ▾

Send to: ▾

Change region shown ▾

Customize view ▾

# Homo sapiens alpha-2-globin (HBA2) gene, complete cds

GenBank: AF230076.1

[GenBank](#) [Graphics](#)

>AF230076.1 Homo sapiens alpha-2-globin (HBA2) gene, complete cds  
CGCCCCGGCCGGGCGTGCCCCCGCGCCCCAAGCATAAACCTGGCGCGCTCGCGGCCGGCACTCTTCTGG  
TCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCTCTGCCGACAAGACCAACGTCAAGGCCGCCT  
GGGGTAAGGTGCGCGCGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAGAAGTGAGGCTCCCTCCCTG  
CTCCGACCCGGGCTCTCTGCCCCGCCGACCCACAGGCCACCCTCAACCGTCTCTGGCCCCGGACCAAAC  
CCCACCCCTCACTCTGCTTCTCCCCGAGGATGTTCTGTCTTCCCCACCAACAGACCTACTTCCCGC  
ACTTCGACCTGAGCCACGGCTCTGCCAGGTTAAGGGCCACGGCAAGAAGGTGGCCGACGCGCTGACCAA  
CGCCGTGGCGCACGTGGACGACATGCCCAACGCGCTGTCCGCCCTGAGCGACCTGCACGCGCACAAAGCTT  
CGGGTGGACCCGGTCAACTTCAAGGTGAGCGGCGGGCCGGGAGCGATCTGGGTGAGGGGGCGAGATGGCG  
CCTTCTCTCAGGGCAGAGGATCACGCGGGTTGCGGGAGGTGTAGCGCAGGCGGCGGCTGCGGGCCTGGG  
CCGCACTGACCTCTTCTCTGACAGCTCCTAAGCCACTGCCTGCTGGTGACCTGGCCGCCACCTCCC  
CGCCGAGTTACCCCTGCGGTGCACGCCTCCTGGACAAGTTCTGGCTTCTGTGAGCACCGTGCTGACC  
TCCAAATACCGTTAAGCTGGAGCCTCGGTAGCCGTTCTCTGCCCGCTGGGCTCCCAACGGGCCCTCC  
TCCCTCCTTGACCGGCCCTTCTGGTCTTTGAATAAAGTCTGAGTGGCGGCGAGCCTGTGTGTGCTCG  
GGTCTCTCTGTCCCGAATGTGCCAACAA

Analyze this sequence ▴

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Articles about the HBA2 gene ▴

Dual proteome-scale networks reveal cell-specific remodeling of the human inte [Cell. 2021]

Molecular and Hematological Analysis of Alpha- and Beta-Thal [Genet Test Mol Biomarkers. 2021]

Molecular Characterization and Hematological Aspects of Hb E-Myanmar [β; [Hemoglobin. 2020]

See all

local:

**Usage:** Paste your two sequences in one of the supported **formats** into the sequence fields below and press the "Run lalign" button.  
**Make sure that both format buttons (next to the sequence fields) shows the correct formats**

Choose the alignment method: ☒ local (default) ☐ global ☐ global without end-gap penalty

Number of reported sub-alignments:  E-value threshold:  (default 10.0)

Scoring matrix:

Opening gap penalty:  (default -12) Extending gap penalty:  (default -2)

First sequence title (optional):

Input sequence format:

1st Query sequence: or ID or AC or GI (see above for valid formats)

```
GCCTGCTGGTGACCTGGCCGCCACCTCCCCGCCGAGTTCACCCCTGCGGTGCACGCCT
CCCTGGACAA
GTTCTGGCTTCTGTGAGCACCCTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGT
GGCCATGCTT
CTTGCCCCCTGGGCCTCCCCCAGCCCCCTCTCCCTTCTGACCCGTACCCCGTGGT
CTTTGAATAA
AGTGTGAGTGGGGGGG
```

Second sequence title (optional):

Input sequence format:

2nd Query sequence: or ID or AC or GI (see above for valid formats)

```
CCCACCTCC
CGCCGAGTTCACCCCTGCGGTGCACGCCTCCCTGGACAAGTTCCTGGCTTCTGTGAGCAC
CGTGCTGACC
TCCAAATACCGTTAAGCTGGAGCCTCGGTAGCCGTTCTCTGCCCCTGGGCCTCCCAA
CGGGCCCTCC
TCCCTCCTTGACCCGGCCCTTCTGCTTTGAATAAAGTCTGAGTGGCGGCAGCCTG
TGTGTGCTG
```

# results:

```
# bin/lalign36 -E 10.0 -f -12 -g -2 10196.1.seq 10196.2.seq -J -K 3
LALIGN finds non-overlapping local alignments
version 36.3.5e Nov, 2012(preload8)
Please cite:
X. Huang and W. Miller (1991) Adv. Appl. Math. 12:373-381
```

```
*** Warning - unrecognized residue at 7:I - 73
*** Warning - unrecognized residue at 8:E - 69
*** Warning - unrecognized residue at 12:E - 69
*** Warning - unrecognized residue at 15:L - 76
*** Warning - unrecognized residue at 17:I - 73
*** Warning - unrecognized residue at 20:L - 76
```

Parameters not available for: +5/-4: -12/-2

```
Query: 10196.1.seq
1>>>unknown 611 bp - 607 nt
Library: 10196.2.seq
967 residues in 1 sequences
```

```
Statistics: (shuffled [500]) MLE statistics: Lambda= 0.0539; K=0.02506
statistics sampled from 1 (1) to 500 sequences
Threshold: E() < 10 score: 135
Algorithm: Smith-Waterman (SSE2, Michael Farrar 2006) (7.2 Nov 2010)
Parameters: +5/-4 matrix (5:-4), open/ext: -12/-2
Scan time: 0.110
```

Algorithm: Smith-Waterman

The E indicates the matching between the sequences, the score expands the threshold

```
>>unknown 982 bp (967 nt)
Waterman-Eggert score: 2157; 172.9 bits; E(1) < 5.1e-47
66.7% identity (66.7% similar) in 835 nt overlap (32-607:88-921)
```

```

          40      50      60      70      80      90
unknown ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCTCCTGCCGACAA
          .....
unknown ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCTCCTGCCGACAA
          90      100     110     120     130     140

          100     110     120     130     140     150
unknown GACCAACGTCAAGGCCGCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGA
          .....
unknown GACCAACGTCAAGGCCGCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGA
          150     160     170     180     190     200
```



results:

scores for locally  
optimal alignments

local similarities

matching  
sequences

```
>>unknown 982 bp (967 nt)
Waterman-Eggert score: 185; 19.7 bits; E(1) < 0.5
53.3% identity (53.3% similar) in 569 nt overlap (606-83:138-662)

      600      590      580      570      560
unknown CCGCCCACTCAGACTTTATTCAAAGACCACGGGGGTACGG-----GTGCAGGAAGGGGAG
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown CTGCCGACA-AGACCAACGTCAAGGCCGCTGGGGTAAGTTCGGCGCGCACGCTGGCGAG
      140      150      160      170      180      190

      550      540      530      520      510      500
unknown GAGGGGCTGGGGGGAGGCCCAAGGGGCAAGAAGCATGGC---CACCGAGGCTCCAGCTTA
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown TATGGT---GCGGAGGCCCTGG---AGAAGTGAGGCTCCCTCCCTGCTCCGACC--
      200      210      220      230      240

      490      480      470      460      450      440
unknown ACGGTATTTGAGGTCAGCACGGTGCTCACAGAAGCCAGG---AACTTGTCAGGGAGGC
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown -CGGGCTCCTC--GCCCCGCCGACC-CACAG--GCCACCCTCAACC-GTCTTGCCCCCG
      250      260      270      280      290

      430      420      410      400
unknown GTGC---ACCGCAGGGGTGAACCTCGGCGG-----GGAGGTGGGCCAGGGTCAC
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown GACCAAACCCACCCCTCA-CTCTGCTTCTCCCGCAGGATGTTCTGTCTTCCCCAC
      300      310      320      330      340      350

      390      380      370      360      350      340
unknown CAGCAGGCAGTGGCTTAGGAGCTTGAAGTTGACC---GGGTCCACCCGAAGCTT--GTGC
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown CACCAAGACCTACTTCCCGCACTTCGACCTGAGCCACGGCTCTGCC--AGGTTAAGGGC
      360      370      380      390      400      410

      330      320      310      300      290
unknown ----GCGTGCAGGTCGCTCAGG-GCGGAC-AGCGCGTTGG-GCATGTCGTCCACGTGCGC
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown CACGGCAAGAAGGTGGCCGACGCGCTGACCAACGCCGTGGCGCACGTGGACGACATGCC
      420      430      440      450      460      470

      280      270      260      250      240      230
unknown CACGGCGTTGGTCAGCGCGTCGGCCACCTTCTTGCCGTGGCCCTTAACCT--GGGCAGAG
      : : : : : : : : : : : : : : : : : : : : : : : : : : : :
unknown AACG-CGCTG-TCCGC-CCTGAGCGACCTGCACGC---GCAC--AAGCTTCGGGTGGAC
      480      490      500      510      520
```



gobal:

Choose the alignment method:	<input type="radio"/> local (default)	<input checked="" type="radio"/> global	<input type="radio"/> global without end-gap penalty
Number of reported sub-alignments:	3	E-value threshold:	10.0 (default 10.0)
Scoring matrix:	BLOSUM50 (default)		
Opening gap penalty:	-12 (default -12)	Extending gap penalty:	-2 (default -2)
First sequence title (optional):			
Input sequence format	Plain Text		
1st Query sequence: or ID or AC or GI (see above for valid formats)	<div>&gt;NM_000558.3 Homo sapiens hemoglobin, alpha 1 (HBA1), mRNA ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCTCTGCCGACAA GACCAACGTC AAGGCCGCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAG AGGATGTTCC TGTCTTCCCCACCACCAAGACCTACTTCCCGCACTTCGACCTGAGCCACGGCTCTGCC</div>		
Second sequence title (optional):			
Input sequence format	Plain Text		
2nd Query sequence: or ID or AC or GI (see above for valid formats)	<div>CGTGCTGACC TCCAAATACCGTTAAGCTGGAGCCTCGGTAGCCGTTCTCTCTGCCCGCTGGGCCTCCCAA CGGGCCCTCC TCCCCTCCTTGACCGGCCCTTCTGGTCTTTGAATAAAGTCTGAGTGGGCGGCAGCCTG TGTGTGCCTG GGTTCTCTCTGTCCCGAATGTGCCAACAA</div>		
<div>Run IalignClear Input</div>			

# result:

Readseq version 2.1.30 (12-May-2010) Readseq version 2.1.30 (12-May-2010)

## lalign output for UNKNOWN vs. UNKNOWN

[EMBnet-Server] Date: Thu Nov 11 8:51:18 2021

```
# bin/ggsearch36 -E 10.0 -f -12 -g -2 5804.1.seq 5804.2.seq
```

GGSEARCH performs a global/global database searches

version 36.3.5e Nov, 2012(preload8)

\*\*\* Warning - unrecognized residue at 7:I - 73

\*\*\* Warning - unrecognized residue at 8:E - 69

\*\*\* Warning - unrecognized residue at 12:E - 69

\*\*\* Warning - unrecognized residue at 15:L - 76

\*\*\* Warning - unrecognized residue at 17:I - 73

\*\*\* Warning - unrecognized residue at 20:L - 76

Parameters not available for: +5/-4: -12/-2

Query: 5804.1.seq

1>>>unknown 611 bp - 607 nt

Library: 5804.2.seq

967 residues in 1 sequences

Statistics: Unscaled normal statistics: mu= 50.0000 var=10.0000 Ztrim: 0

statistics sampled from 0 (0) to 0 sequences

Algorithm: Global/Global affine Needleman-Wunsch (SSE2, Michael Farrar 2010) (6.0 April 2007)

Parameters: +5/-4 matrix (5:-4), open/ext: -12/-2

Scan time: 0.000

!! No sequences with E() < 10

607 residues in 1 query sequences

967 residues in 1 library sequences

Tcomplib [36.3.5e Nov, 2012(preload8)] (4 proc in memory [0G])

start: Thu Nov 11 08:51:19 2021 done: Thu Nov 11 08:51:19 2021

Total Scan time: 0.000 Total Display time: 0.000

Function used was GGSEARCH [36.3.5e Nov, 2012(preload8)]

falling the  
recognize  
residues

Algorithm: Global/Global  
affine  
Needleman-Wunsch

statistics of the normal distribution

The small value of E indicates that  
in practice these two sequences do  
not match globally.

global  
with  
no  
end-gap  
penalty

Choose the alignment method:	<input type="radio"/> local (default)	<input type="radio"/> global	<input checked="" type="radio"/> global without end-gap penalty
Number of reported sub-alignments:	<input type="text" value="3"/>	E-value threshold:	<input type="text" value="10.0"/> (default 10.0)
Scoring matrix:	<input type="text" value="BLOSUM50 (default)"/>		
Opening gap penalty:	<input type="text" value="-12"/> (default -12)	Extending gap penalty:	<input type="text" value="-2"/> (default -2)
First sequence title (optional):	<input type="text"/>		
Input sequence format	<input type="text" value="Plain Text"/>		
1st Query sequence: or ID or AC or GI (see above for valid formats)	<div>CCCTGGACAA GTTCTTGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGT GGCCATGCTT CTTGCCCTTGGGCCTCCCCCAGCCCCCTCCTCCCTTCTGACCCGTACCCCGTGGT CTTTGAATAA AGTCTGAGTGGGCGGC</div>		
Second sequence title (optional):	<input type="text"/>		
Input sequence format	<input type="text" value="Plain Text"/>		
2nd Query sequence: or ID or AC or GI (see above for valid formats)	<div>CGTGCTGACC TCCAAATACCGTTAAGCTGGAGCCTCGGTAGCCGTTCTCTGCCCCTGGGCTCCCAA CGGGCCCTCC TCCCCTCCTTGACCGGCCCTTCTGGTCTTTGAATAAAGTCTGAGTGGGCGGCAGCCTG TGTGTGCTG GGTCTCTCTGTCCCGAATGTGCCAACAA</div>		
<div>Run lalignClear Input</div>			

# results:

```
# bin/glsearch36 -E 10.0 -f -12 -g -2 9779.1.seq 9779.2.seq
GLSEARCH performs a global-query/local-library search
version 36.3.5e Nov, 2012(preload8)
*** Warning - unrecognized residue at 7:I - 73
*** Warning - unrecognized residue at 8:E - 69
*** Warning - unrecognized residue at 12:E - 69
*** Warning - unrecognized residue at 15:L - 76
*** Warning - unrecognized residue at 17:I - 73
*** Warning - unrecognized residue at 20:L - 76
Parameters not available for: +5/-4: -12/-2
Query: 9779.1.seq
      1>>>unknown 611 bp - 607 nt
Library: 9779.2.seq
          967 residues in      1 sequences

Statistics: (shuffled [500]) Unscaled normal statistics: mu= 92.3571  var=33.1703 Ztrim: 0
statistics sampled from 1 (1) to 160 sequences
Algorithm: Global/Local affine Needleman-Wunsch (SSE2, Michael Farrar 2010) (6.0 April 2007)
Parameters: +5/-4 matrix (5:-4), open/ext: -12/-2
Scan time: 0.080

The best scores are:
unknown 982 bp ( 967) [f] 2144 679.1 0
unknown 982 bp ( 967) [r] 148 37.9 2.2e-22

>>unknown 982 bp (967 nt)
n-w opt: 2144 Z-score: 3612.3 bits: 679.1 E(1): 0
global/local score: 2144; 64.4% identity (65.7% similar) in 866 nt overlap (1-607:57-921)

          10      20      30
unknown      NMHMSARXXNSHXMGBXBNAXRHAHBAMRN
              .. :.  ..  .  ..  .
unknown SCGCCCCGCCGGGCGTGCCCCCGCGCCCAAGCATAAACCCCTGGCGCGCTCGCGGCCCGG
          30      40      50      60      70      80

          40      50      60      70      80      90
unknown AACTCTTCTGGTCCCCACAGACTCAGAGAGAACCACCATGGTGCTGTCTCCTGCCGACA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::
unknown CACTCTTCTGGTCCCCACAGACTCAGAGAGAACCACCATGGTGCTGTCTCCTGCCGACA
          90      100     110     120     130     140
```

Algorithm: Needleman-Wunsch

in global, there is error due to fail the global alignment, but local alignment and the global with no end-gap penalty are worked. Since global method is too restricted and comparing the sequences as a whole, the result of it gives no significant matching, this can be considered as error. But local alignment works better than global since it searches the local similarities, and finding the local similarities is more appropriate to align two sequences if the sequences are not very close. In the no end-gap penalty, the algorithm is not penalising the end gaps, and it can be used to identify the terminal domains better than the global one.

in no end-gap, a global query-local library search was performed. in local, non-overlapping local alignments were searched. in global, a global to global db search was done. global ones uses the same algorithm whereas the local one uses a different algorithm.



global to local /with no end gap penalty

```
      40          30          20          10
unkno- CA-----GAAGAGTTNYKTVDTDYNTNNVNCKNDSNNNYTSKDKN
      ::      :  ::::      .  :..      .  :.      .  :....
unknow CACCTCCCCGCCGAGTTCACCCCTGCGGTGCACGC-CTCCCTGGACAAGTTCCTGGCTTC
      720      730      740      750      760      770
unknow TGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTAGCCGTTCTCC
      780      790      800      810      820      830
```

```
      80      70      60      50
unknow GACAGCACCATGGTGGGTTCTCTCTGAGT-CTGTGGGGACC
      :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
unknow CA-AGTTCC----TGGCTTCTGT--GAGCACCGTGCTGACC
      770      780      790
```

local

there is no opportunity to jump some sequences and find the terminal domain, the alignment provides only the local matches between the local areas

since there is no end gap penalty, the algorithm can jump some sequences (to identify terminal domains better)

## 4. theoretical

**Local:** local alignment methods search only the best matching && high scoring regions among the sequence. it can start and end anywhere. it searches largest similar sub domains in the sequences.

**Global:** alignments match as many character as possible in the whole sequences. it requires 4 termini to process.

**Global with no end-gap penalty** uses the same algorithm as global alignment and try to match as many character as possible among the whole sequence but it requires 2 / 4 termini to process. it is also called semi-global alignment. it ignores end-gaps and therefore can find a more appropriate termini, but do the same thing with global alignment in general.

## 4. theoretical

the choice of the which alignment technique is used for the alignment is depend on the sequences. if the sequences are more similar, global alignment methods can be used but if there is less assumption about the sequences, the local alignment should be used firstly, and after that the global alignments can be proceeded. Since each gap and mismatch, etc. represent a evolutionary relatedness of divergence, it is irrelevant to do global alignment for the the unknown sequences. These differences in the sequences represents evolutionary distances between the organisms // sequences, therefore the selection process of local && global context-dependent and based on the assumptions about the sequences.

# references

<https://www.metagenomics.wiki/tools/blast/evaluate>

[https://bio.libretexts.org/Bookshelves/Cell\\_and\\_Molecular\\_Biology/Book%3A\\_Investigations\\_in\\_Molecular\\_Cell\\_Biology\\_\(O'Connor\)/09%3A\\_Protein\\_Conservation/9.07%3A\\_The\\_BLASTP\\_algorithm](https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/Book%3A_Investigations_in_Molecular_Cell_Biology_(O'Connor)/09%3A_Protein_Conservation/9.07%3A_The_BLASTP_algorithm)

[https://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/650/E\\_value.html](https://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/650/E_value.html)

<https://www.cs.purdue.edu/homes/ayg/TALKS/BLORE10/lecture4.pdf>

<https://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html#head3>