

Comparing two sequences in BLAST:

1. Go to the BLAST main page: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

2. Click on Nucleotide BLAST:

The screenshot shows the BLAST main page from the National Library of Medicine. At the top, there is a navigation bar with the NIH logo and the text "National Library of Medicine National Center for Biotechnology Information". Below this, the "BLAST" logo is visible, along with links for "Home", "Recent Results", "Saved Strategies", and "Help". The main content area features a "Basic Local Alignment Search Tool" section with a description of BLAST and a "Learn more" link. To the right, there is a "NEWS" box announcing "BLAST+ 2.13.0 is here!" with a date of "Thu, 17 March 2022" and a link to "More BLAST news...". Below the news box, the "Web BLAST" section is displayed, featuring three main options: "Nucleotide BLAST" (circled in red), "blastx" (translated nucleotide to protein), and "Protein BLAST" (protein to protein). At the bottom, there is a "BLAST Genomes" section with a search input field and a "Search" button, and a list of organisms: Human, Mouse, Rat, and Microbes.

3. Make sure blastn tab is selected and click on “Align two or more sequences”

The screenshot shows the BLAST search interface. At the top, there are tabs for "blastn", "blastp", "blastx", "tblastn", and "tblastx". The "blastn" tab is selected and circled in red. Below the tabs, there is a section for "Enter Query Sequence" with a text input field, a "Clear" button, and a "Query subrange" section with "From" and "To" input fields. Below this, there is a section for "Or, upload file" with a "Choose File" button and "No file chosen" text. A "Job Title" input field is also present. Below the "Job Title" field, there is a checkbox labeled "Align two or more sequences" which is checked and circled in red. Below this, there is a section for "Enter Subject Sequence" with a text input field, a "Clear" button, and a "Subject subrange" section with "From" and "To" input fields. Below this, there is a section for "Or, upload file" with a "Choose File" button and "No file chosen" text. Below the "Or, upload file" section, there is a "Program Selection" section with radio buttons for "Optimize for": "Highly similar sequences (megablast)", "More dissimilar sequences (discontiguous megablast)", and "Somewhat similar sequences (blastn)". Below the radio buttons, there is a link to "Choose a BLAST algorithm". At the bottom, there is a "BLAST" button and a checkbox for "Show results in a new window". Below the "BLAST" button, there is a "+ Algorithm parameters" link.

4. Enter the Query sequence and Subject sequence in FASTA format i.e., there should be a > character followed by the name of the sequence and in the next line the sequence.

The image shows the BLAST suite web interface. At the top, it says "BLAST® » blastn suite". Below that are tabs for "blastn", "blastp", "blastx", "tblastn", and "tblastx", with "blastn" selected. The main area is divided into two sections: "Enter Query Sequence" and "Enter Subject Sequence".

In the "Enter Query Sequence" section, the input field contains a FASTA sequence starting with ">test" followed by a long string of nucleotide characters. A red circle highlights the ">" character. To the right, there are "Query subrange" fields for "From" and "To". Below the input field, there is a "Choose File" button and "No file chosen" text. The "Job Title" field contains "test". A checkbox labeled "Align two or more sequences" is checked.

In the "Enter Subject Sequence" section, the input field contains a FASTA sequence starting with ">test2" followed by a long string of nucleotide characters. A red circle highlights the ">" character. To the right, there are "Subject subrange" fields for "From" and "To". Below the input field, there is a "Choose File" button and "No file chosen" text.

5. Select "Highly similar sequences" and hit BLAST.

Program Selection

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

BLAST Search nucleotide sequence using Megablast (Optimize for highly similar sequences)
 Show results in a new window

+ Algorithm parameters

6. Results show the first sequence is 631 characters in length and matches 100% with the second sequence. However second sequence is the [reverse compliment](#) of the first sequence

Job Title **test**

RID [P6AS9JRS11N](#) Search expires on 11-04 10:44 am [Download All](#) ▾

Program Blast 2 sequences [Citation](#) ▾

Query ID Icl|Query_104021 (dna)

Query Descr test

Query Length 631

Subject ID Icl|Query_104023 (dna)

Subject Descr test2

Subject 632

Length

Other reports [MSA viewer](#) ?

Filter Results

Percent Identity to E value to Query Coverage to

[Filter](#) [Reset](#)

Descriptions **Alignments** Graphic Summary Dot Plot

Alignment view **Pairwise** CDS feature ? [Restore defaults](#) [Download](#) ▾

1 sequences selected ?

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test2
 Sequence ID: **Query_104023** Length: **632** Number of Matches: **1**

Range 1: 1 to 621 [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1147 bits(621)	0.0	621/621(100%)	0/621(0%)	Plus/Minus
Query 1	CCTGATTTGAGGTCAGAGGTCATAAAGCTGTCTCACAAACGAGACGGTTAGAAGCTCGCC	60		
Sbjct 621	CCTGATTTGAGGTCAGAGGTCATAAAGCTGTCTCACAAACGAGACGGTTAGAAGCTCGCC	562		
Query 61	AAAACGCTTCACGGTCACGGCGTAGACATTATCACACCGAGAGCCGATCCGCAAGGAATC	120		
Sbjct 561	AAAACGCTTCACGGTCACGGCGTAGACATTATCACACCGAGAGCCGATCCGCAAGGAATC	502		
Query 121	AAGCTAATACATTTAAGAGGAGCCGACCGAAACACGGCCGACAAGCCTCCAAGTCCAAGC	180		
Sbjct 501	AAGCTAATACATTTAAGAGGAGCCGACCGAAACACGGCCGACAAGCCTCCAAGTCCAAGC	442		
Query 181	CTACAAACCCGCAAAGGTTTGTAAAGTTGAAGATTTATGACACTCAAAACAGGCATGCTCC	240		
Sbjct 441	CTACAAACCCGCAAAGGTTTGTAAAGTTGAAGATTTATGACACTCAAAACAGGCATGCTCC	382		
Query 241	TCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCGCAA	300		
Sbjct 381	TCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCGCAA	322		