# USING NCBI to analyze DNA and protein Sequences for BoCAL and BobCAL Genes

# 1. Find the sequences you will be working with

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

NIH Nationa	I Library of Medicine ter for Biotechnology Information			Log in
All	Databases 🗸			Search
NCBI Home	Welcome to NCBI			Popular Resources
Resource List (A-Z)	The National Center for Biotechnol	oov Information advances science ar	nd health by providing access to	PubMed
All Resources	biomedical and genomic informatio	n.		Bookshelf
Chemicals & Bioassays	About the NCBI   Mission   Orga	nization   NCBI News & Blog		PubMed Central
Data & Software				BLAST
DNA & RNA	Submit	Download	Learn	Nucleotide
Domains & Structures	Deposit data or manuscripts	Transfer NCBI data to your	Find help documents, attend a	Genome
Genes & Expression	into NCBI databases	computer	class or watch a tutorial	SNP
Genetics & Medicine		_		Gene
Genomes & Maps				Protein
Homology				PubChem
Literature				
Proteins				NCBI News & Blog
Sequence Analysis	B1	Analysis	Descent	Introducing the New NCBI Datasets
Taxonomy	Develop	Analyze	Research	Genome Annotation Table
Training & Tutorials	Use NCBI APIs and code libraries to build applications	Identity an NCBI tool for your data analysis task	Explore NCBI research and collaborative projects	As part of our ongoing effort to moderniz
Variation		and you dow	consorante projecto	and improve vour experience, we are
		888	\$	Comparing Yeast Species Used in Beer Brewing and Bread Making 29 Sep 20

Select nucleotide from the dropdown menu and enter "BoCAL" or "BobCAL" in the dropdown menu

An official website of the United States government Here's now you know.							
Nucleo	otide V BoCAL	Search					
NCBI Home	Welcome to NCBI	Popular Resources					
Resource List (A-Z)	Resource List (A-Z) The National Center for Biotechnology Information advances science and health by providing access to PubMed						
All Resources	sources biomedical and genomic information. Bookshelf						
Chemicals & Bioassays	Chemicals & Bioassays About the NCBI   Mission   Organization   NCBI News & Blog PubMed Central						
Data & Software		BLAST					

# Results for BoCAL, make note of accession L36926

An official website	of the United States government Here's how you know.			
NIH Nation	onal Library of Medicine			Log in
Nucleotide	Nucleotide   BoCAL Create alert Advanced		Search	Help
Species Animals (2)	Summary - Sort by Default order -	Send to: 🗸	Filters: Manage Filters	
Plants (2) Customize Molecule types	See LOC106320120 (BOCAL) transcription factor CAULIFLOWER in the Gene database bocal reference sequences <u>Transcript(2)</u> Protein(2)		Results by taxon Top Organisms [Tree] Danio rerio (2)	
Customize	Items: 4		Brassica oleracea (2)	
Source databases INSDC (GenBank) (2) RefSeq (2) Customize	Brassica oleracea (clone pBS85) BoCAL mRNA, complete cds     756 bp linear mRNA     Accession: L36926.1 GI: 642590     Protein PubMed Taxonomy		Analyze these sequences Run BLAST	
Sequence Type Nucleotide (4)	GenBank FASTA Graphics		Find related data	
Sequence length Custom range Release date	Brassica oleracea var. capitata DNA binding protein (CAL) mRNA, partial cds     286 bp linear mRNA     Accession: AY514056.1 Gl: 45533881     Protein Taxonomy		Database: Select	<b>~</b>

# Results for BobCAL, make note of accession L36927

An official website of Nucleotide	f the United States government Here's how you know onal Library of Medicine I Center for Biotechnology Information Nucleotide BobCAL Create alert Advanced	Log in
Species Plants (6) Customize	Summary → 20 per page → Sort by Default order → Send to: →	Filters: Manage Filters
Molecule types genomic DNA/RNA (1) mRNA (5) Customize	See LOC106320120 (BOBCAL) transcription factor CAULIFLOWER in the Gene database bobcal reference sequences Transcript (2) Protein (2) Items: 6	Results by taxon Top Organisms [Tree] Brassica oleracea var. botrytis (5) Punica granatum (1)
Source databases INSDC (GenBank) (6) Customize Sequence Type Nucleotide (6)	Brassica oleracea (subspecies botrytis) BobCAL mRNA_complete cds  756 bp linear mRNA Accession: L36927.1 Gl: 642592 Protein PubMed Taxonomy GenBank FASTA Granbics	Analyze these sequences  Run BLAST
Sequence length Custom range Release date Custom range Revision date	Brassica oleracea var. botrytis DNA binding protein variant d (CAL) mRNA, complete cds, alternatively spliced 892 bp linear mRNA Accession: AY514055.1 Gi: 45533879 Protein Taxonomy Co. D. H. SADTA CO. Hit	Find related data  Database: Select  Find items Search details

You can click on the title (e.g. Brassica oleracea (subspecies botrytis) **BobCAL** mRNA, complete cds) in the results to see details including nucleotide and protein sequences, make note of the protein IDs for BoCAL and BobCAL (AAA64790 and AAA64791)

# 2. Compare two nucleotide sequences

Select BLAST under Popular Resources or go to <a href="https://blast.ncbi.nlm.nih.gov/Blast.cgi">https://blast.ncbi.nlm.nih.gov/Blast.cgi</a>

All	Databases 🗸			Search
NCBI Home	Welcome to NCBI			Popular Resources
Resource List (A-Z)	The National Center for Biotechnol	oov Information advances science an	d health by providing access to	PubMed
All Resources	biomedical and genomic informatio	n.		Bookshelf
Chemicals & Bioassays	About the NCBI   Mission   Organ	ization   NCBI News & Blog		PubMed Central
Data & Software				BLAST
DNA & RNA	Submit	Download	Learn	Nucleotide
Domains & Structures	Deposit data or manuscripts	Transfer NCBI data to your	Find help documents attend a	Genome
Genes & Expression	into NCBI databases	computer	class or watch a tutorial	SNP
Genetics & Medicine				Gene
Genomes & Maps				Protein
Homology	T T			PubChem
Literature				
Proteins				NCBI News & Blog
Sequence Analysis				Introducing the New NCBI Datas
Taxonomy	Develop	Analyze	Research	Genome Annotation Table
Training & Tutorials	Use NCBI APIs and code	Identify an NCBI tool for your	Explore NCBI research and	As part of our opnoing effort to m
Variation	indraries to build applications	udia analysis lask	conaborative projects	and improve your experience we
			1	Comparing Yeast Species Used i
	_=	380		Drewing and Dread Making
on nucleotide bla	ast			
Basic Local Align BLAST finds regions of sim program compares nucleor databases and calculates t	ment Search Tool nilarity between biological sequences tide or protein sequences to sequence the statistical significance. Le	The BLAST+ 2.1 Bearn more S Tue, 13 Jul	2.0 is here! ade some improvements to how BLAS d the amount of memory required by db. 2021 12:00:00 EST	ST multi-

check box "align two or more sequences" and enter L36926 in the first box and L36927 in the second box then click on BLAST

					Align Sequences Nucleotide BLAST
blastn bla	astp blastx	tblastn	tblastx		
-	•				BLASTN programs search nucleotide subjects using a nucleotide qu
Enter Query	Sequence		•		
Enter accession i	number(s), gi(s), or FA	STA sequence(s	) 😲 Clear	Query subrange 😗	
L36926				From	]
				То	1
Or upload file					]
	Choose File No	file chosen	8		
Job Title	L36926:Brassica	oleracea (clone p	BS85) BoCAL		
	Enter a descriptive ti	le for your BLAST s	earch 😗		
Aign two or m	ore sequences 😢				
Enter Subjec	t Sequence				
Enter accession I	number(s), gi(s), or FA	STA sequence(s	) 😯	Clear Subject subrar	nge 😮
L36927				From	
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Or, upload file	Choose File No	file chosen		0	
Program Sele	ection				
Optimize for	O Highly similar s	equences (megab	last)		
	More dissimilar	sequences (disco	ontiguous megab	last)	
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	010036 a DEAOT alg	viniil 😈			
	N.				
BLAST	Search nucleotid	e sequence using	Megablast (Op	timize for highly similar se	quences)
	Snow results in a	new window			

# Click on the Alignments tab in the results

BLAST <sup>®</sup> » bla	stn suite-2sequences » results for RID-N985A40211R		Home Recent Results Saved Strategies Help
< Edit Search	Save Search Search Summary 🗸	How to read this report?	BLAST Help Videos DBack to Traditional Results Page
Job Title	L36926:Brassica oleracea (clone pBS85) BoCAL	Filter Results	
RID	N985A40211R Search expires on 10-01 01:21 am Download All V	Percent Identity	E value Query Coverage
Program	Blast 2 sequences Citation ¥	to	to
Query ID	L36926.1 (nucleic acid)		
Query Descr	Brassica oleracea (clone pBS85) BoCAL mRNA, complete co	S	Filter Reset
Query Length	756		
Subject ID	L36927.1 (nucleic acid)		
Subject Descr	Brassica oleracea (subspecies botrytis) BobCAL mRNA, cc		
Subject Length	756		
Other reports	MSA viewer 😮		
Descriptions	Graphic Summary Alignments Dot Plot		
Sequences	producing significant alignments	Downloa	id ≚ New Select columns ≚ Show 100 ♥ 😯
select all	1 sequences selected		GenBank Graphics New MSA Viewer
	Description	Scientific Name	Max Total Query E Per. Acc. Score Score Cover value Ident Len Accession
Brassica ole	eracea (subspecies botrytis) BobCAL mRNA, complete cds Bra	ssica oleracea var. botrytis	1315 1315 100% 0.0 98.54% 756 <u>L36927.1</u>

The alignment of the two sequences is shown, the additional stop codon in the BobCAL (subjct sequence) has been highlighted in the results below

Brassica oleracea (subspecies botrytis) BobCAL mRNA, complete cds Sequence ID: <u>L36927.1</u> Length: 756 Number of Matches: 1

Range 1: 1 to 756 GenBank Graphics

Vext Match

Score 1315 b	oits(14	Expect (57) 0.0	Identities 745/756(99%)	Gaps 0/756(0%)	Strand Plus/Plus
Query	1	ATGGGAAGGGGTAGGGTT	GAAATGAAGAGGATAGAG	AACAAGATCAACCGACA	AGTGACG 60
Sbjct	1	ATGGGAAGGGGTAGGGTT	GAAATGAAGAGGATAGAG		AGTGACG 60
Query	61	TTTTCGAAAAGAAGAGCT	GGTCTTTTGAAGAAAGCC	CATGAGATCTCGATCCT	TTGTGAT 120
Sbjct	61	TTTTCGAAAAGAAGAGAGCT	GGTCTTTTGAAGAAAGCC	CATGAGATCTCGATTCT	TTGTGAT 120
Query	121	GCTGAGGTTTCCCTTATT	GTCTTCTCCCATAAGGGG	AAACTGTTCGAGTACTC	GTCTGAA 180
Sbjct	121	GCTGAGGTTTCCCTTATT	GTCTTCTCCCATAAGGGG	AAACTGTTCGAGTACTC	GTCTGAA 180
Query	181	TCTTGCATGGAGAAGGTA		TACTCTTACGCCGAGAA	ACAGCTA 240
Sbjct	181	TCTTGCATGGAGAAGGTA	CTAGAACGCTACGAGAGG	TACTCTTACGCCGAGAA	ACAGCTA 240
Query	241	AAAGTTCCAGACTCTCAC	GTCAATGCACAAACGAAC	TGGTCAGTGGAATATAG	CAGGCTT 300
Sbjct	241	AAAGC†ĊĊAGAĊ†Ċ†ĊAĊ	ĠŦĊĂĂŦĠĊĂĊĂĂĂĊĠĂĂĊ	tigi tidatigi aktatagi	icAggctt 300
Query	301	AAGGCTAAGATTGAGCTT	TTGGAGAGAGAAACCAAAGG	CATTATCTGGGCGAAGA	TTTAGAA 360
Sbjct	301	ÁAĠĠĊŦĂĂĠĂŦŦĠĂĠĊŦŦ	TGĠĠĂĠĂĠĠĂĂĊĊĂĂĂĠĠ	ĠĊĂŢŢĂŢĊŢĠĠĠAĠĂĂĠĂ	tŤŤÅĠÅÅ 360
Query	361	TCAATCAGCATAAAGGAG	CTACAGAATCTGGAGCAG	CAGCTTGACACTTCTCT	TAAACAT 420
Sbjct	361	ŤĊĂĂŤĊĂĠĊĂŤĂĂĂĠĠĂĠ	CTÁCÁGÁÁTCTGGÁGCÁG	CAGCTTGACACTTCTCT	TÁÁÁCÁT 420
Query	421	ATTCGCTCGAGAAAAAAT		СТСААССАССТССААА	AAAGGAG 480
Sbjct	421	ATTCGCTCCAGAAAAAAT	CAACTAATGCACTAGTCC	CTCAACCACCTCCAAAG	AAAGGAG 480
Query	481	AAAGAAATACTGGAGGAA	AACAGCATGCTTGCCAAA	ACAGATAAGGGAGAGGGG	GAGTATC 540
Sbjct	481	AAAGAAATACTGGAGGAA	AACAGCATGCTTGCCAAA	ACAGATAAAGGAGAGAGGA	GAGTATC 540
Query	541		CAATCAGAGCAGCAAAAC	CGCAGCCACCATGTAGC	TCCTCAG 600
Sbjct	541	CTAAGGACACATCAAAAC		CGCAGCCACCATGTAGC	TCCTCAG 600
Query	601				TGGCATG 660
Sbjct	601	CCGCAACCGCAGTTAAAT	CCTTACATGGCATCATCT	CCTTTCCTAAATATGGG	TGGCATG 660
Query	661		ACGGCGGTGAGGAGGAAC		TGAACCC 720
Sbjct	661	IACCAAGGAGAATATCCA		CGICTCGATCTGACTCI	IGAACCC 720
Query	721			756	
Sbjct	721	ATTTACAACTGCAACCTT	GGTTACTTTGCCGCATGA	/56	

# 3. Compare two protein Sequences: <u>https://blast.ncbi.nlm.nih.gov/Blast.cgi</u>

Click on Protein BLAST

Tue, 13 Jul 2021 12:00:00 EST        More BLAST news        Web BLAST        blastx       translated nucleotide ▶ protein        Nucleotide BLAST        thlastn	BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.	We have made some improvements to threads and the amount of memory re- makeblastdb.	how BLAST multi- quired by
Web BLAST		Tue, 13 Jul 2021 12:00:00 EST	More BLAST news
blastx translated nucleotide > protein Nucleotide BLAST			
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Nucleotide BLAST thists Protein BLAST	Web BLAST		
Nucleotide BLAST thiste IV Protein BLAST	Web BLAST	astx :leotide ▶ protein	
	Web BLAST	astx leotide ≽ protein	

check box "align two or more sequences", enter AAA64790 in the first box and AAA64791 in the second box then click on BLAST (same as for nucleotide BLAST)

blastn bl	astp blastx tblastn	tblastx	Align Sequences Protein BLAST
Enter Query	Anguence		BLASTP programs search protein subjects using a protein query. more
Enter accession n	umber(s), gi(s), or FASTA sequence(s) 😯 🤉	<u>Clear</u> Que	ery subrange 🕗
		From	
On unload file		То	
Job Title	Choose File No file chosen	0	
Ign two or mo	Enter a descriptive title for your BLAST search ore sequences ?	0	
Enter Subject	Sequence		
Enter accession n	umber(s), gi(s), or FASTA sequence(s) 😯	Clear	r Subject subrange ? From
		1	То
Or, upload file	Choose File No file chosen		0
Program Sele	ction		
Algorithm	blastp (protein-protein BLAST) Choose a BLAST algorithm ?		

In the results click on graphic summary, you can see that the subject protein (BobCAL AAA64791 in red) is much shorter than the Query protein (BoCAL AAA64790 in blue) because the translation stopped at the stop codon identified in the sequence above:

< Edit Search	Save Search	Search S	Summary 🗸		How to read this repo	ort? 🕒 I	BLAST Help \	/ideos 🖞	Back to Tradi	tional Result	s Page
Job Title	AAA64790:amino	acid feat	ure: K-box, bp 2	83480;	Filter Results						
RID	<u>N93N5W8Z11R</u>	Search expir	es on 10-01 00:04	am Download All	Percent Identity		E value		Ouerv Co	verage	
Program	Blast 2 sequence	s <u>Citati</u>	on 🗸		to		+	0		to	1
Query ID	<u>AAA64790.1</u> (am	ino acid)									
Query Descr	amino acid featu	re: K-box, ł	op 283480; am	nino acid feature					Filte	r Res	et
Query Length	251										
Subject ID	<u>AAA64791.1</u> (am	ino acid)									
Subject Descr	amino acid featu	re: MADS	box; codes for a	a putative DNA-b							
Subject Length	150										
Other reports	Multiple alignme	nt MSA v	iewer 😮								
Descriptions	Graphic Sumr	mary	Alignments	Dot Plot							
hover to see the	e title 🖡 click to show	alignments			Alignment Scores	< 40	40 - 50	50 - 80	80 - 200	>= 200	0
1 sequences sele	cted 🕜			Distribut	ion of the top 1 Blast	t Hits on y I 1 <sup>50</sup>	1 subject	sequence	<b>25</b> 50		

4. Align multiple Brassica sequences and make a phylogenetic tree: https://blast.ncbi.nlm.nih.gov/Blast.cgi

Click on nucleotide blast



check box "align two or more sequences" and enter AF241113 in the first box and the remaining 7 numbers (from page 7 section a in the Student Lab Handout) in the second box, then click on BLAST

blastn blastp blastx tblastn tblastx	Align Sequences Nucleotide BLAST
Enter Query Seguence	BLASTN programs search nucleotide subjects using a nucleotide query. more
Enter duery sequence Enter accession number(s), gi(s), or FASTA sequence(s) Clear Or, upload file Choose File No file chosen	Cuery subrange ?
Sob Title Enter a descriptive title for your BLAST search ? Image: Solution of more sequences ?	
Enter Subject Sequence	
Enter accession number(s), gi(s), or FASTA sequence(s) 🕑	Clear Subject subrange 🕜 From To
Or, upload file Choose File No file chosen	0
Program Selection	
Optimize for         Ighly similar sequences (megablast)           More dissimilar sequences (discontiguous megority)         Somewhat similar sequences (blastn)           Choose a BLAST algorithm ?         Choose a BLAST	jablast)

# In the results, click on "distance tree of results"

< Edit Search	Save Search Search Summary 🗸	How to read this report? 🔹 BLAST Help Videos 🏷 Back to Traditional Results Pa			
Job Title	AF241113:Brassica oleracea var. acephala isolate	Filter Results			
RID	N92E45HK11R Search expires on 09-30 23:44 pm Download All 🗸	Percent Identity E value Ouery Coverage			
Program	Blast 2 sequences Citation ~				
Query ID	AF241113.1 (nucleic acid)				
Query Descr	Brassica oleracea var. acephala isolate HRI/CGN 6210 cau	Filter Reset			
Query Length	1976				
Subject ID	AF241114.1 and 6 more subject(s) (nucleic acid)				
Subject Descr	See details 💙				
Subject Length	13903				
Descriptions	Graphic Summary Alignments				
Sequences producing significant alignments Download 🗡 🔤 Select columns 🐣 Show 100 🌱 💡					
select all	7 sequences selected	GenBank Graphic Distance tree of results Item MSA View			
	Description	Scientific Name Max Total Query E Per. Acc. Score Score Cover value Ident Len Accession			
Brassica ole	aracea var. oleracea isolate HRI/CGN 8707 cauliflower gene, partial cds	Brassica oleracea var. oleracea 3558 3558 99% 0.0 99.95% 1977 AF241149.			
Brassica ole	<u>aracea var. acephala isolate HRI/CGN 4036 cauliflower gene, partial cds</u>	Brassica oleracea var. viridis 3545 3545 99% 0.0 99.85% 1974 AF241114.			
Brassica ole	aracea var. oleracea isolate HRI/CGN 11125 cauliflower gene, partial cds	Brassica oleracea var. oleracea         3542         3542         99%         0.0         99.80%         1995         AF241140.			
Brassica ole	aracea var. italica isolate HRI/CGN 10674 cauliflower gene, partial sequence	Brassica oleracea var. italica 3525 3525 99% 0.0 99.65% 1995 <u>AF241130</u>			
		Mrannica oloracca var botodio 3636 3636 99% 0.0 99.66% 1072 AE3/1127			
Brassica ole	eracea var. botrytis isolate HRI/CGN 7369 cauliflower gene, partial sequence	Diassica oleradea var. bototia 2523 3523 55% 0.0 55.05% 1512 <u>M1241121</u> .			

You will see the following phylogenetic tree:

This tree was produced using BLAST pairwise alignments. more					
Reset Tree					
BLAST RI	0 <u>N92E45HK11R</u>	Query ID AF24111	Database n/a		
Tree method Fast Minimum Evolution 💙 🥹	Max Seq Difference 0.75	Sequence Label Sequence Title (if avail: 🗸 😡			
			Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download		
E Find:	▼ 🗖 all 🔸 🕧		🔀 Tools 🖌 🔀 Upload   🖓 🗸		
			Brassica oleracea var. Italica isolate HRICON 10074 cashflower gene, partial sequence     Brassica oleracea var. botrytis isolate HRICON 1567 cashflower gene, partial sequence     Brassica oleracea var. botrytis isolate HRICON 7,569 cashflower gene, partial sequence     Brassica oleracea var. botrytis isolate HRICON 4814 cashflower gene, partial sequence     Brassica oleracea var. botrytis isolate HRICON 4814 cashflower gene, partial sequence		
There is a direct a var. sceptials isolate TREPCENT 4210 califformer gene, partial edit      There is a direct a var. sceptials isolate TREPCENT 4210 califformer gene, partial edit      There is a direct a var. sceptials isolate TREPCENT 4210 califformer gene, partial edit					
Brassica oleracea var. oleracea var. oleracea isolate HRUCGN 8707 cauliflower gene, partial cds					