

## Interpreting Your BLAST Results

1. I will assume you have performed a BLAST search, obtained the results of the search, and now need to analyze and interpret the results. Before I explain how I analyze and interpret the results of a search, you should remember that you need analyze and interpret your own results. A BLAST search is just a tool that you can use to help you determine relationships between sequences.
2. Here are the results from a search I performed using a cytochrome c oxidase I gene sequence from the eastern oyster (*Crassostrea virginica*).

The screenshot shows the NCBI BLAST interface. The search parameters are: Job Title: GS\_201066-501\_akm1\_cvlco1490A01.ab1, RID: GVUD6WY5016, Program: BLASTN, Database: nt, Query ID: lcl|Query\_19041, Description: GS\_201066-501\_akm1\_cvlco1490A01.ab1, Molecule type: dna, Query Length: 632. The 'Filter Results' section shows 'Organism' set to 'only top 20 will appear' and 'Exclude' checked. The 'Percent Identity' is set to 98% to 100%, 'E value' is 0.0 to 100.00%, and 'Query Coverage' is 98% to 100.00%. The 'Descriptions' tab is selected, and the table below shows 100 sequences selected. The table has columns: Description, Max Score, Total Score, Query Cover, E value, Per Ident, and Accession. The first few rows are:

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
Crassostrea virginica voucher LBDM 000213 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	1162	1162	100%	0.0	99.84%	FJ717610.1
Crassostrea virginica mitochondrion, complete genome	1162	1162	100%	0.0	99.84%	AY905542.2
Crassostrea virginica voucher LBDM 000212 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	1157	1157	100%	0.0	99.68%	FJ717609.1
Crassostrea virginica voucher 11ECMOL-0347 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	1146	1146	98%	0.0	99.84%	KF644145.1
Crassostrea virginica isolate Cv12 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	1146	1146	98%	0.0	99.84%	KF245600.1
Crassostrea virginica isolate VA_A cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	1146	1146	98%	0.0	99.84%	FJ743529.1
Crassostrea virginica isolate Cv1946_04 cytochrome oxidase subunit I gene, partial cds, mitochondrial	1146	1146	98%	0.0	99.84%	EU007484.1
Crassostrea virginica isolate 793 cytochrome c oxidase subunit 1 (cox1) gene, partial cds, mitochondrial	1142	1142	98%	0.0	99.68%	FJ693093.1
Crassostrea virginica voucher USNM IZ 1287256 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial	1140	1140	98%	0.0	99.68%	KU905769.1
Crassostrea virginica isolate Cv11 cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial	1140	1140	98%	0.0	99.68%	KF245699.1
Crassostrea virginica isolate 1033 cytochrome c oxidase subunit 1 (cox1) gene, partial cds, mitochondrial	1140	1140	98%	0.0	99.84%	FJ693067.1
Crassostrea virginica isolate 1027 cytochrome c oxidase subunit 1 (cox1) gene, partial cds, mitochondrial	1138	1138	98%	0.0	99.68%	FJ693062.1
Crassostrea virginica isolate 1004-NY cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	1136	1136	97%	0.0	100.00%	JX468917.1

- a. These results may look a little overwhelming but let's just concentrate on the bottom half of the results screen shown above.
- b. Make sure the "Description" tab is selected. Note: this should be the default for a search result.

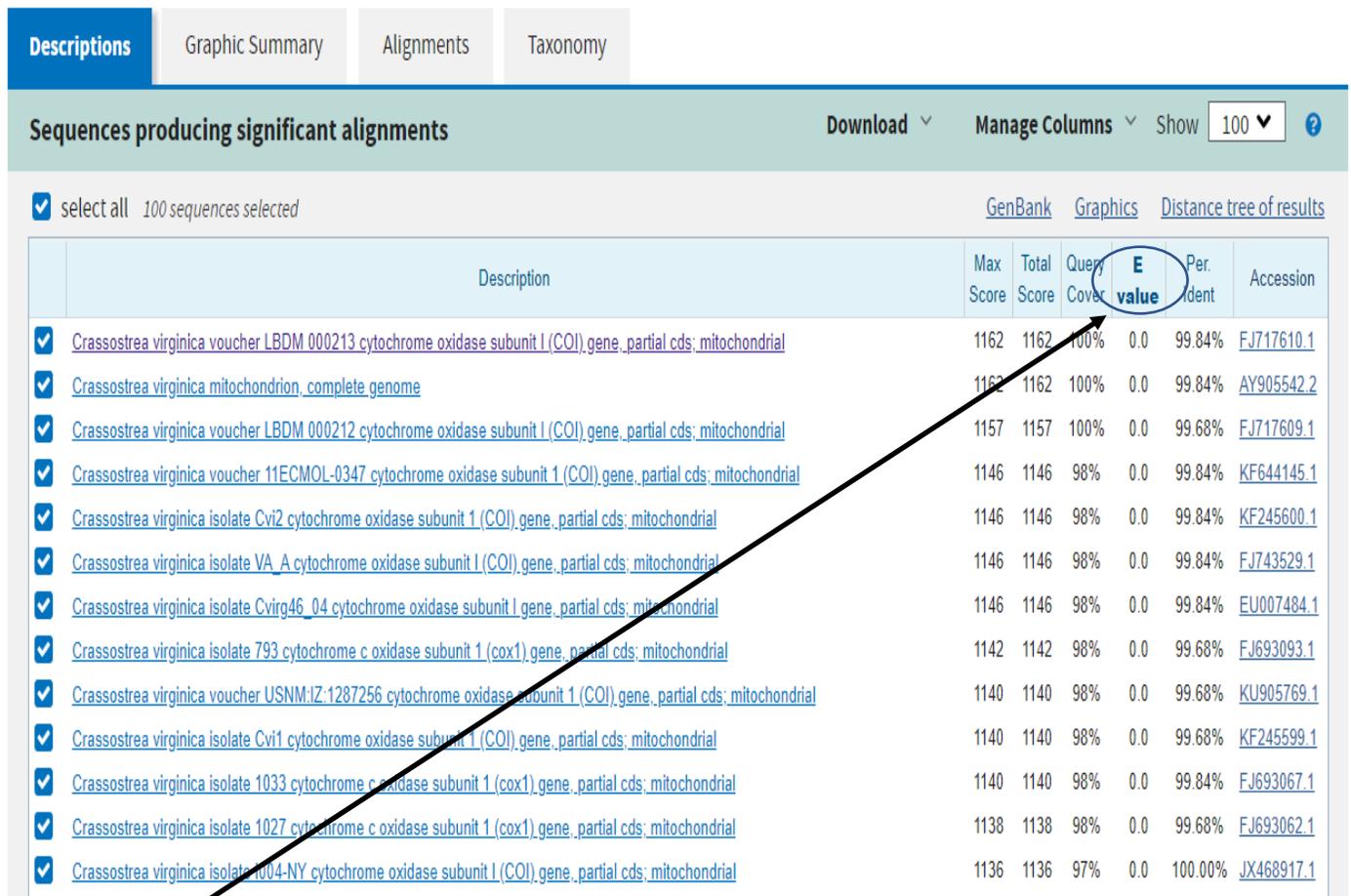
3. This screenshot shows the bottom half of the BLAST search from #2 above.

The screenshot displays the BLAST search results interface. At the top, there are tabs for 'Descriptions', 'Graphic Summary', 'Alignments', and 'Taxonomy'. Below these, the title 'Sequences producing significant alignments' is shown along with 'Download', 'Manage Columns', and a 'Show' dropdown set to '100'. A selection bar indicates '100 sequences selected'. The main table has columns: 'Description', 'Max Score', 'Total Score', 'Query Cover', 'E value', 'Per. Ident', and 'Accession'. The first row of the table is highlighted, and a large black arrow points from the 'Description' column header to the first row of the table.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<a href="#">Crassostrea virginica voucher LBDM 000213 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1162	1162	100%	0.0	99.84%	<a href="#">FJ717610.1</a>
<a href="#">Crassostrea virginica mitochondrion, complete genome</a>	1162	1162	100%	0.0	99.84%	<a href="#">AY905542.2</a>
<a href="#">Crassostrea virginica voucher LBDM 000212 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1157	1157	100%	0.0	99.68%	<a href="#">FJ717609.1</a>
<a href="#">Crassostrea virginica voucher 11ECMOL-0347 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">KF644145.1</a>
<a href="#">Crassostrea virginica isolate Cvi2 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">KF245600.1</a>
<a href="#">Crassostrea virginica isolate VA_A cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">FJ743529.1</a>
<a href="#">Crassostrea virginica isolate Cvirg46_04 cytochrome oxidase subunit 1 gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">EU007484.1</a>
<a href="#">Crassostrea virginica isolate 793 cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial</a>	1142	1142	98%	0.0	99.68%	<a href="#">FJ693093.1</a>
<a href="#">Crassostrea virginica voucher USNM:IZ:1287256 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1140	1140	98%	0.0	99.68%	<a href="#">KU905769.1</a>
<a href="#">Crassostrea virginica isolate Cvi1 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1140	1140	98%	0.0	99.68%	<a href="#">KF245599.1</a>
<a href="#">Crassostrea virginica isolate 1033 cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial</a>	1140	1140	98%	0.0	99.84%	<a href="#">FJ693067.1</a>
<a href="#">Crassostrea virginica isolate 1027 cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial</a>	1138	1138	98%	0.0	99.68%	<a href="#">FJ693062.1</a>
<a href="#">Crassostrea virginica isolate I004-NY cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1136	1136	97%	0.0	100.00%	<a href="#">JX468917.1</a>

- The sequences from the NCBI database that match your sequence are shown in the “Description” section on the left. Here you see some of the sequences (out of 100 total that were selected – there might be more than 100 sequences that match your sequence but the default to display is 100. If you want to change the number of sequences that are displayed as results, you need to do it before you perform the BLAST search).
- The description are hyperlinks that will open a new page and that shows a comparison of the described sequence to your search sequence.
- You can see that all of the sequences in this description are from *Crassostrea virginica*. This makes sense because the search sequence we used was from *Crassostrea virginica*.
- You can also see that the matches do not have the exact same names are but most of them are from the cytochrome c oxidase I gene. The names of the sequences vary based on how someone submits the name into database.
- There are a lot of matches to *Crassostrea virginica* but I would have obtained sequences other than *Crassostrea virginica* if I allowed more than 100 sequences to be displayed.
- These matches are not necessarily significant just because they are displayed. Just like with a “Google Search”, the BLAST program will try to find the best matches it can find.
- If you do not find matches, it could just mean that there are no sequences in the NCBI database that match. Remember, someone has to submit a sequence to the database before you can find it in a search.

4. How do you know if the match is relevant? That depends on your analysis and interpretation of the search results. Here is some information that will help you to decide whether the match is relevant.



The screenshot shows a BLAST search results page with the following table:

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica voucher LBDM 000213 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial</a>	1162	1162	100%	0.0	99.84%	<a href="#">FJ717610.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica mitochondrion, complete genome</a>	1162	1162	100%	0.0	99.84%	<a href="#">AY905542.2</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica voucher LBDM 000212 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial</a>	1157	1157	100%	0.0	99.68%	<a href="#">FJ717609.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica voucher 11ECMOL-0347 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">KF644145.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate Cvi2 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">KF245600.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate VA_A cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">FJ743529.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate Cvirg46_04 cytochrome oxidase subunit I gene, partial cds; mitochondrial</a>	1146	1146	98%	0.0	99.84%	<a href="#">EU007484.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate 793 cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial</a>	1142	1142	98%	0.0	99.68%	<a href="#">FJ693093.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica voucher USNM:IZ:1287256 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1140	1140	98%	0.0	99.68%	<a href="#">KU905769.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate Cvi1 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial</a>	1140	1140	98%	0.0	99.68%	<a href="#">KF245599.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate 1033 cytochrome oxidase subunit 1 (cox1) gene, partial cds; mitochondrial</a>	1140	1140	98%	0.0	99.84%	<a href="#">FJ693067.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate 1027 cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial</a>	1138	1138	98%	0.0	99.68%	<a href="#">FJ693062.1</a>
<input checked="" type="checkbox"/>	<a href="#">Crassostrea virginica isolate 1004-NY cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial</a>	1136	1136	97%	0.0	100.00%	<a href="#">JX468917.1</a>

- The “E-value” is the best value to examine to determine significance.
- In simple terms, the e-value is “the number of BLAST hits you expect to see by chance with the observed E-value or higher”.
- You might find the following videos from NCBI BLAST to be helpful in interpreting your results:  
<https://www.youtube.com/watch?v=nO0wJgZRZJs>  
<https://www.youtube.com/watch?v=Z7ek7UoP7Bg>
- In general, E-values of 0.1 and are often significant and even larger numbers can be significant.
- The E-values do not prove that the two sequences are related or unrelated, they help you to determine the relationship.
- As you can see above, the E-value in all of the matches shown here is 0.0. It is not really 0 but it is a smaller number than the BLAST program can write so it is shown as 0.0.
- This means there is almost no possibility that these matches are due to chance.