

1. Follow this link – this link will show the sequencing reactions from the forward primer

<https://clims3.genewiz.com/links.aspx?oId=4p6LZ4Isjwk=&ref=00>

When prompted, login with:

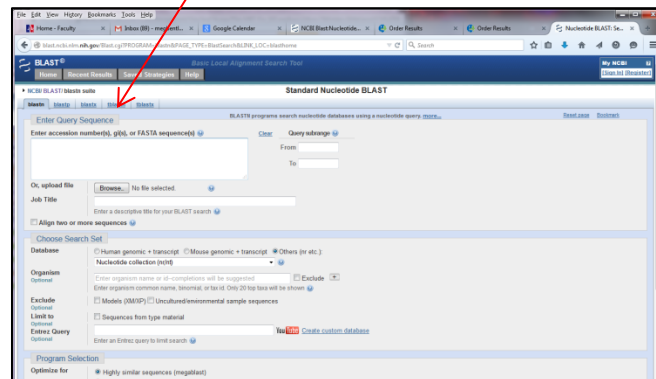
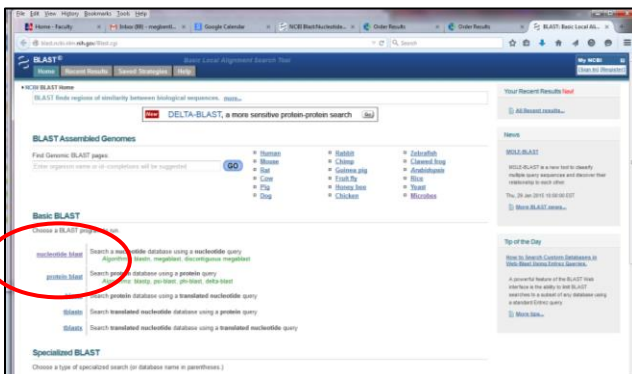
Username – meg@american.edu

Password – Monster1

2. Find your sequences that correspond to a MB# tube label. Once you find your sequences, select to “View” the SEQ File associated with your tubes. This will bring up a new window with the sequence of your 16S PCR product. The red circle in the screen shot below indicates the link to view the SEQ file for reaction MB05.

Number	Tube Label	QS	CRL	Failure Cause	Sample Name	SEQ File	Trace File	Seq File	Trace File	Download Seq Files	Download Trace Files	Repeat
1	MB01	37	902	Poor Quality	MB1-For_16S	View	View	Download	Download	☒	☒	
2	MB02	36	687	High Background	MB2-For_16S	View	View	Download	Download	☒	☒	
3	MB03	47	955		MB3-For_16S	View	View	Download	Download	☒	☒	
4	MB04	44	906		MB4-For_16S	View	View	Download	Download	☒	☒	
5	MB05	20	160	Poor Quality	MB5-For_16S	View	View	Download	Download	☒	☒	
6	MB06	12	22	Poor Quality	MB6-For_16S	View	View	Download	Download	☒	☒	
7	MB07	43	816		MB7-For_16S	View	View	Download	Download	☒	☒	
8	MB08	47	911		MB8-For_16S	View	View	Download	Download	☒	☒	
9	MB09	15	25	Poor Quality	MB9-For_16S	View	View	Download	Download	☒	☒	
10	MB10	38	924	Poor Quality	MB10-For_16S	View	View	Download	Download	☒	☒	

3. Copy and paste your sequences into your lab notebook at OWW
4. Navigate to BLAST at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>
5. Select “nucleotide blast” search and paste in your sequence into the box at the top of the screen and hit BLAST at the bottom of the page. You may have to wait as the website queries the database – this may take up to 30 seconds!



Your results will look something like the screen shot below! The series of red lines indicates a 'match' for your sequence. If you scroll down below the graphic summary, you will find descriptions of the matches. Select the highest match (which is at the top of the list) and follow this link. When you verify the genus species of your bacteria by 16S sequence analysis, then go back to the microbiology lab and confirm that your colony morphology, gram stain and bacterial cell shape are correct.

BLAST® Basic Local Alignment Search Tool

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NCBI BLAST/ blastn suite/ Formatting Results - EFTDAWK014

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Nucleotide Sequence (935 letters)

RID: EFTDAWK014 (Expires on 02-26 01:13 am)

Query ID: Id|Query_40079
 Description: None
 Molecule type: nucleic acid
 Query Length: 935

Database Name: nr
 Description: Nucleotide collection (nt)
 Program: BLASTN 2.2.30+ [Citation](#)

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

Graphic Summary

Distribution of 106 Blast Hits on the Query Sequence

Mouse-over to show details and scores, click to show alignments

Color key for alignment scores

Score Range	Color
<40	Black
40-60	Blue
60-80	Green
80-200	Yellow
>=200	Red

Query: 1 150 300 450 600 750 900

Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chryseobacterium jeikeense strain C-B10D 16S ribosomal RNA gene, partial sequence	1476	1476	99%	0.0	95%	KJ806496.1
Chryseobacterium jeikeense strain S-B5R 16S ribosomal RNA gene, partial sequence	1476	1476	99%	0.0	95%	KJ806472.1
Uncultured bacterium clone ncd255h11c1 16S ribosomal RNA gene, partial sequence	1472	1472	99%	0.0	95%	HM269821.1
Chryseobacterium sp. CH12 16S ribosomal RNA gene, partial sequence	1472	1472	99%	0.0	95%	EU598811.2
uncultured CFB group bacterium partial 16S rRNA gene, clone S15D-MN13	1465	1465	99%	0.0	94%	AJ583211.1
uncultured CFB group bacterium partial 16S rRNA gene, clone S15D-MN7	1465	1465	99%	0.0	94%	AJ583193.1
Chryseobacterium sp. C110 16S ribosomal RNA gene, partial sequence	1461	1461	99%	0.0	94%	DQ530078.1
Chryseobacterium sp. C109 16S ribosomal RNA gene, partial sequence	1461	1461	99%	0.0	94%	DQ530076.1
Chryseobacterium sp. C102 16S ribosomal RNA gene, partial sequence	1461	1461	99%	0.0	94%	DQ530067.1
Uncultured bacterium clone nby561b04c1 16S ribosomal RNA gene, partial sequence	1459	1459	99%	0.0	94%	HM836629.1
Uncultured bacterium clone nby58005c1 16S ribosomal RNA gene, partial sequence	1459	1459	99%	0.0	94%	HM840740.1