

The complete annotation guide can be found on [PhagesDB.org](http://PhagesDB.org)  
 Use this document for a quick start, but refer liberally to the complete annotation guide often.

## Part 1: Creating a Draft Annotation

### Setting Key Preferences

1. From the **File** menu, select **Preferences** to open the preference window.
2. Click on the **Local Settings** tab, then on the **Colors** sub-tab.
3. In the LEFT box, change the display colors for tRNAs, tmRNAs, and ORFs by clicking on the appropriate boxes. The default is black, but we strongly recommend the colors shown in **Figure 1**.
4. Still under the **Local Settings** tab, click on the **Codons** sub-tab.
5. If the box labeled "Use TTG start codons" is not checked, then check it.
6. Click the **Apply** button on the right to save changes, then **OK** to exit.

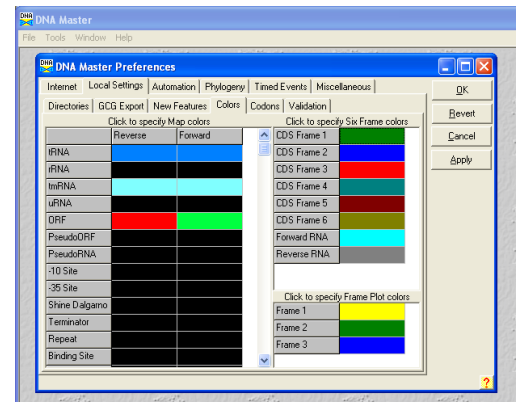


Figure 1: Setting Display Colors

### Importing a DNA Sequence

1. Verify that your DNA sequence, in fasta format, is saved in a known location (or you may download a fasta file from phagesdb.org).  
**NOTE:** If using Virtual Box or another emulator to run Windows, you should copy the fasta file to the virtual machine desktop before proceeding.
2. From the **File** menu, select **Open → FastA Multiple Sequence File** as shown in **Figure 2**.
3. Browse to your genome's fasta-formatted file, then click **Open**.
4. A window like the one shown in **Figure 3** should appear. Click on the button in the lower right hand corner that looks like a piece of paper with the upper right corner folded over, then select "Create Sequence from this entry only" and a new window titled "Extracted from YourPhage.fasta" will open within DNA Master.

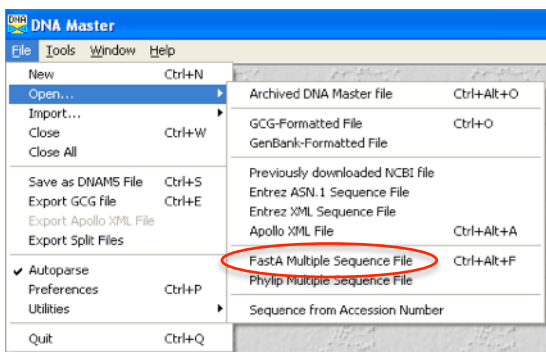


Figure 2: Opening a FastA File

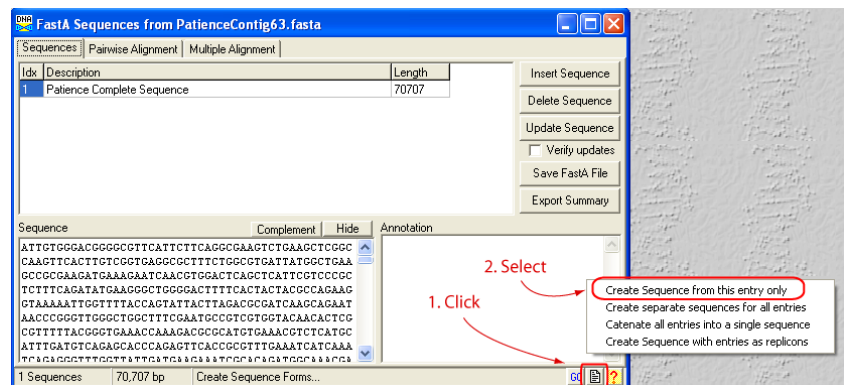


Figure 3: Importing the Sequence into DNA Master

### Auto-Annotating a Genome

1. Make sure the "Extracted from YourPhage.fasta" window is open and selected, then go to the **Genome** menu at the top of the program, and select **Annotation → Auto-Annotate**.

**NOTE:** Many options are available in the Auto-Annotate window that opens. Our standard choices for each are shown in **Figure 4**.

**NOTE:** Be prepared to wait **30-90 minutes** if you choose to “Perform BLAST search on nr database” because BLAST searches take time. If you’d rather move on quickly, then un-check that BLAST box. You will still be able to BLAST all genes—and store the results in DNA Master—later.

2. After selecting your desired options, click **Annotate**.
3. When DNA Master asks if you want to “Erase features and annotate genome?”, click **Yes**.
4. Wait while DNA Master annotates your genome. This should be a fairly quick process (<5 minutes without BLASTing), and you can check the status in the lower-left corner of the Auto-Annotate window.
5. When Auto-Annotation is complete, the Auto-Annotate window will close, and you’ll be returned to the main window for your genome. Congratulations, you’ve created a draft genome annotation!

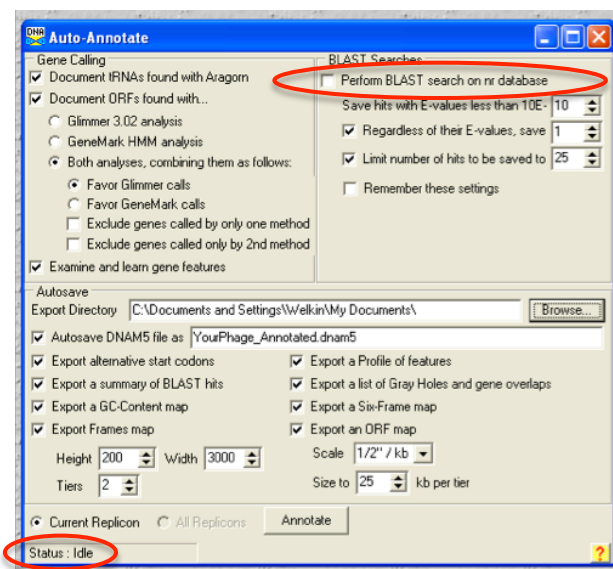


Figure 4: Auto-Annotation Options; BLAST Search Unchecked

## Part 2: Refining Your Positional Annotation

### Features (Genes)

1. Click on the **Features** tab at the top of your genome’s window (the red box in **Figure 5**). Here you can see a list of all features (genes) in your current annotation with their left and right coordinates. If you’ve set up your color preferences properly, forward genes should be in green and reverse genes in red. At the bottom of this window a map of your features is displayed, also color-coded, along with the number of features and total sequence length.
2. The currently selected feature will be identified by a black triangle (the blue box in **Figure 5**). Data for this selected feature will be shown in the right pane. The right pane has several tabs of its own, the default being **Description** (see the green box in **Figure 5**). By changing tabs, you can see this gene’s DNA sequence, amino acid product, any saved BLAST information, and many other statistics. Please explore!

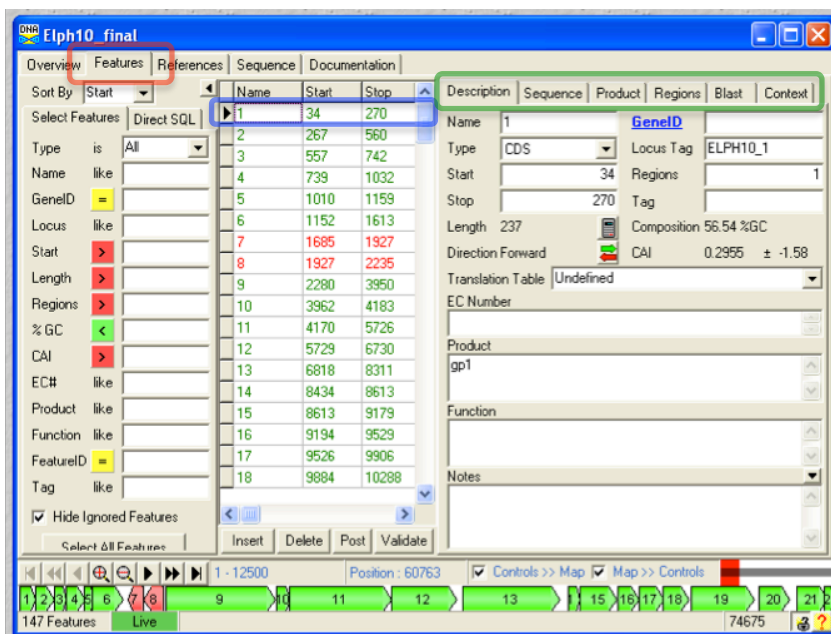


Figure 5: Looking at Auto-Annotated Features

### Frames View

3. From the main DNA Master menu, select **DNA → Frames**. A new window titled “ORF Analysis...” will open where the top three rows represent the forwards reading frames, the bottom three rows the reverse reading frames. To see your current genes displayed in this view, click the ORFs button in the lower right corner of this Frames window (enlarged portion of **Figure 6**). You should now see green and/or red regions highlighted that represent currently called genes. (You may want to zoom in for better resolution by using the buttons near the bottom left of this window.) All potential start codons are shown as vertical lines that are half the height of a given frame, while stop codons are shown as vertical lines that are the full height of a given frame.

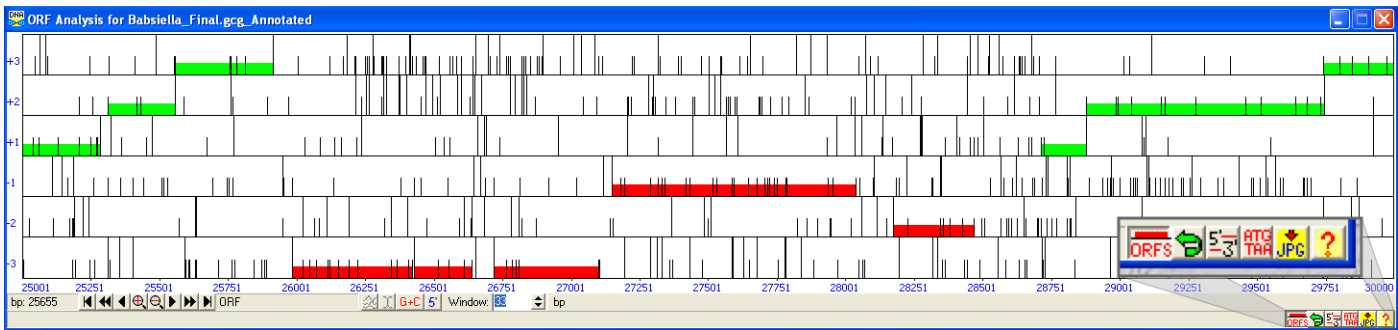


Figure 6: The Frames Window

### Comparing Potential Start Codons

- From the Frames window, select an ORF by clicking within it (DNA Master will draw a line showing your selection).
- Click the button labeled **5'-3'** in the bottom right corner (enlarged portion of **Figure 6**), and a new window called "Choose ORF Start" will open. In this window will be a list of **each possible start codon** for the selected ORF, along with each Shine-Dalgarno score, upstream sequence, start position, and resulting ORF length.

## Part 3: Functional Annotation

### BLASTing Predicted Proteins

- A powerful feature of DNA Master is the ability to BLAST all gene products from an annotation, then store the results in the archived file so that they can later be accessed as needed, even without an internet connection. If a feature has stored BLAST results, you can view them by going to the **Blast** tab for that feature (green box, **Figure 5**).
- If you did not BLAST during auto-annotation, you can do so at any time by going to the **Blast** tab for any feature (green box, **Figure 5**), selecting **Blast ALL genes**, modifying settings if desired, then clicking **Blast All**. BLASTing a complete phage genome annotation takes 30+ minutes. Be patient.
- Once finished, "Genome BLAST Complete" should display, and BLAST data for each feature are in the **Blast** tab.

## Part 4: Important Features

### Suggested Annotation Layout

The figure below shows how you might arrange windows in DNA Master to work on an annotation. The **Frames Window** (on top of the figure) has all 6 reading frames clearly delineated, and allows you to see currently called genes, other choices for start codons, as well as all other potential ORFs in the genome. The **Main Window** (bottom left in the figure) can be used to view precise coordinates, DNA or amino acid sequence, BLAST results (shown in figure), and more. It is also where you will change start positions, add notes, or add/delete genes. The **Choose ORF Start Window** (bottom right in the figure) allows you to see all potential start codons for a given reading frame along with the associated Shine-Dalgarno scores, start positions, and resulting ORF lengths.

Using all of DNA Master's capabilities in concert facilitates fast and accurate genome annotation.

The screenshot shows the DNA Master software interface. The main window, titled 'ORF Analysis for Extracted from FastA Library Patiencece.fasta', displays a genomic map with ORFs highlighted in green. A 'Choose ORF start' dialog box is open, showing a table of ORF data and a BLAST hit for gp19.

#	Score	Space	Upstream of the Start	Codon	Start	Start	ORF
1	441	8	CGACAATTTAGGACTTCGATAA	GTC	12413	822	
2	364	6	CCCCGGCAACCAAGGACATCTCT	GTC	12449	786	
3	399	7	TTCTTTCCCGTGGAGCAAGCGA	ATG	12485	750	
4	378	7	GTGGAGCAAGCGAATGCACCCG	ATG	12494	741	
5	345	9	GCGAATGGACCCGATGTTTTCT	GTC	12503	732	
6	345	9	GGAAGGCAAGGACCGCTGCG	ATC	12791	444	
7	273	7	CGCTGCTCTGAAAATCAAGCT	GTC	12878	357	
8	378	7	CGCCCCGAGATTTCCGAAGAC	ATG	12977	258	
9	399	7	TTTCGAAGACATGGAAGTCGAT	TTG	12989	246	
10	273	8	CGGAACCGTTACTGTTCCGGCT	GTC	13019	216	
11	294	7	GGCTCTCGTGAGAAGTCCCC	GTC	13208	27	

BLAST Hit:  
 Accession NP\_818557  
 GI 29566934  
 Length 280  
 Max Score 132  
 Date 7/18/2011

High-Scoring Pairs (HSP)  
 HSP Data | Alignment  
 Bit Score 55.5 Positives 91  
 Score 132 Identities 48  
 E-Value 2.5E-6 Similarity 50.6 %  
 Length 190 Gaps 22  
 % Aligned 64.3 %  
 Query 90 - 267  
 Target 93 - 272

## Creating a Spreadsheet of Current Gene Calls

1. Make sure your main window is open and selected. From the top menu, select **Genome** → **Profile**.
2. Click **OK**, then save in the format you want.
3. Open the file you generated with Excel or a similar spreadsheet program.

## Creating an ORF Map of Current Gene Calls

1. Make sure your main window is open and selected. From the top menu, select **DNA** → **Export Map**.
2. Modify any options you'd like to, then click **Draw**.
3. A Windows Meta File (.wmf) image is generated, and can be opened with most image-viewing programs.