

Computational Practical 11: Genomics Surveillance of AMR Analysis of resistance in genomes

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13.1 Using genomics to investigate antibiotics resistance

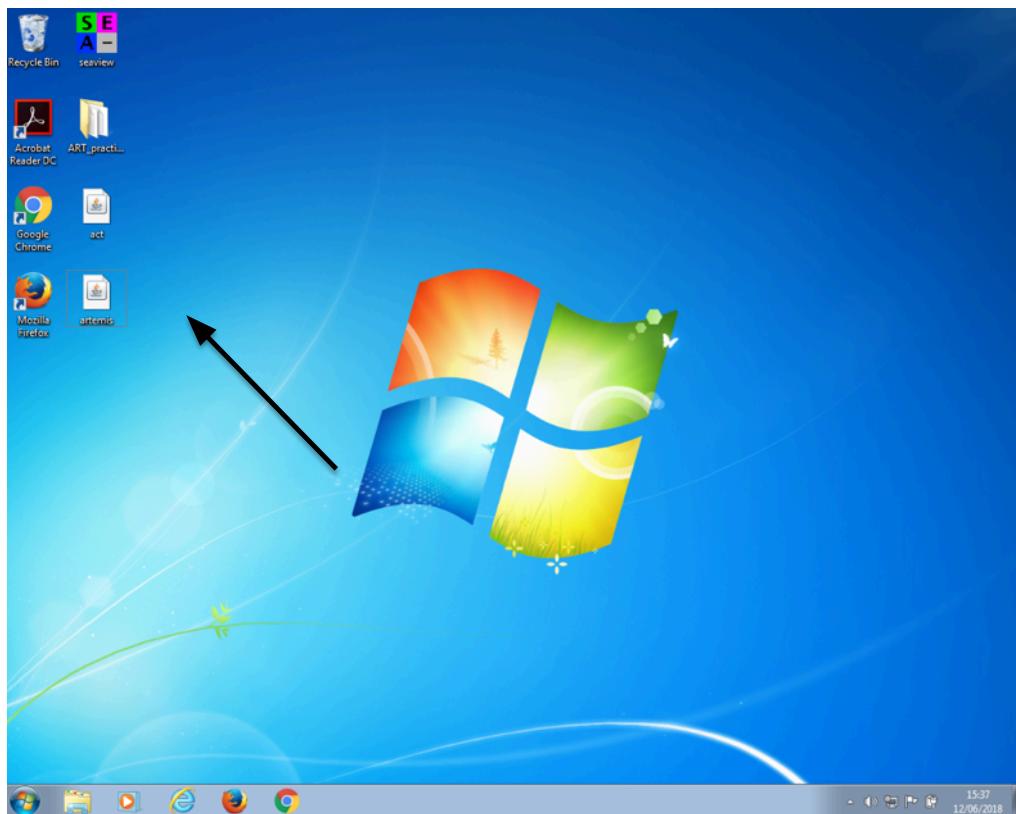
13.2 Overview

As has been outlined in previous lectures and practical's genomics is an incredibly powerful tool to understand the molecular epidemiology, transmission and evolution of bacterial pathogens.

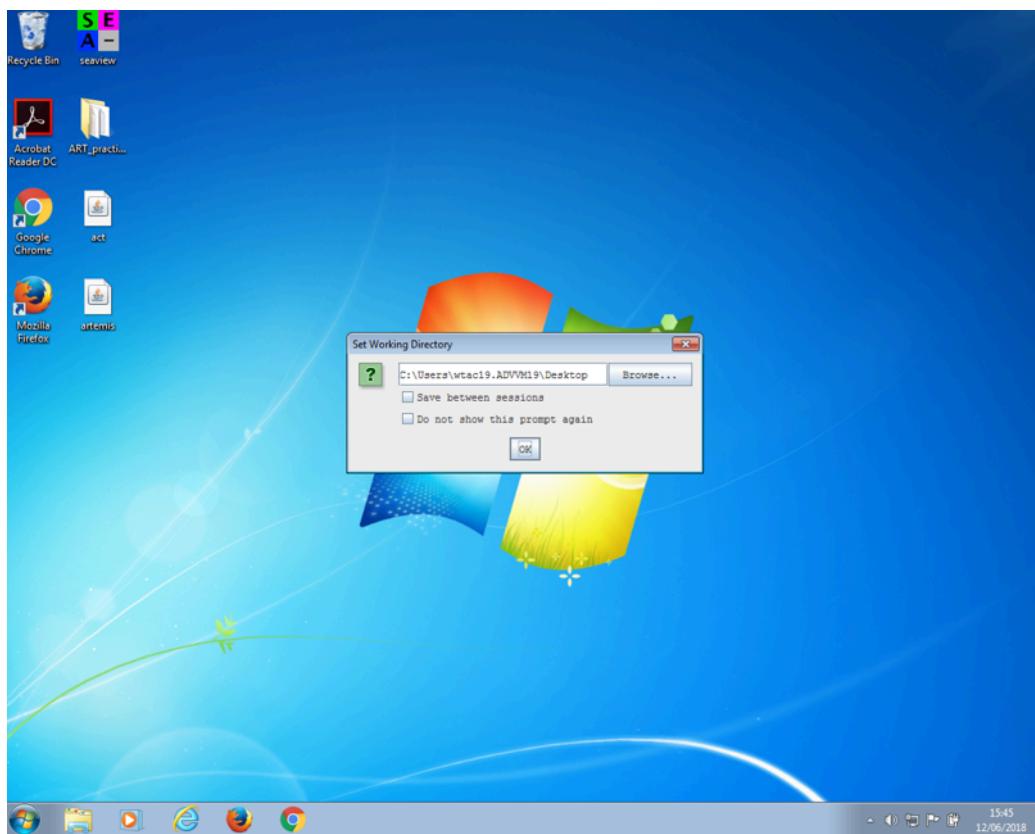
In the previous practical you investigate resistance using online tools that identify the presence of genes and resistance conferring mutations, we are now going to zoom down to individual bacterial genome which can help us further understand antibiotic resistance and extract data for further analysis.

13.3 Part I: Getting going with Artemis

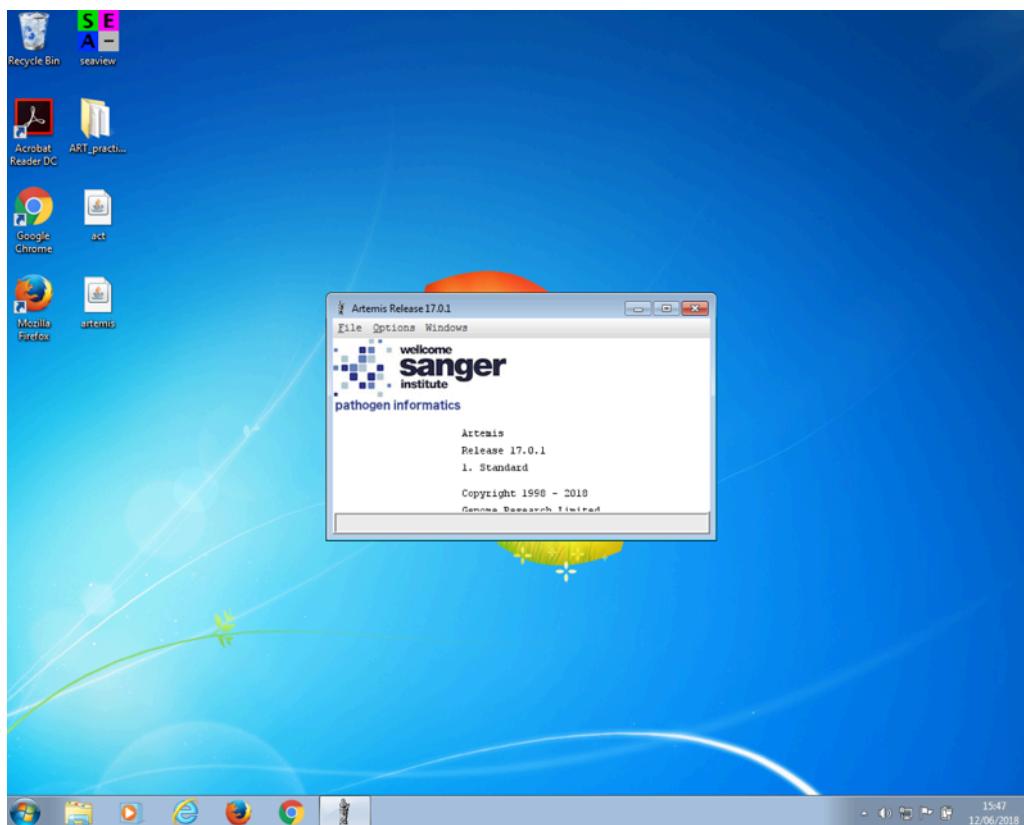
- Artemis is a genome browser and annotation software developed at the Wellcome Sanger Institute (<http://www.sanger.ac.uk/science/tools/artemis>). It is freely available to download for PCs and Mac.
- The practical is designed to give you a basic understanding of Artemis software and to get a better idea of genome structure and content. It is not expected for you to master this software in a single session.
- **Important note – Artemis has a huge number of features for many different tasks – we are just concentrating on the basics – so don't worry about most of the what is there.**
- If you have any questions during the practical please ask!



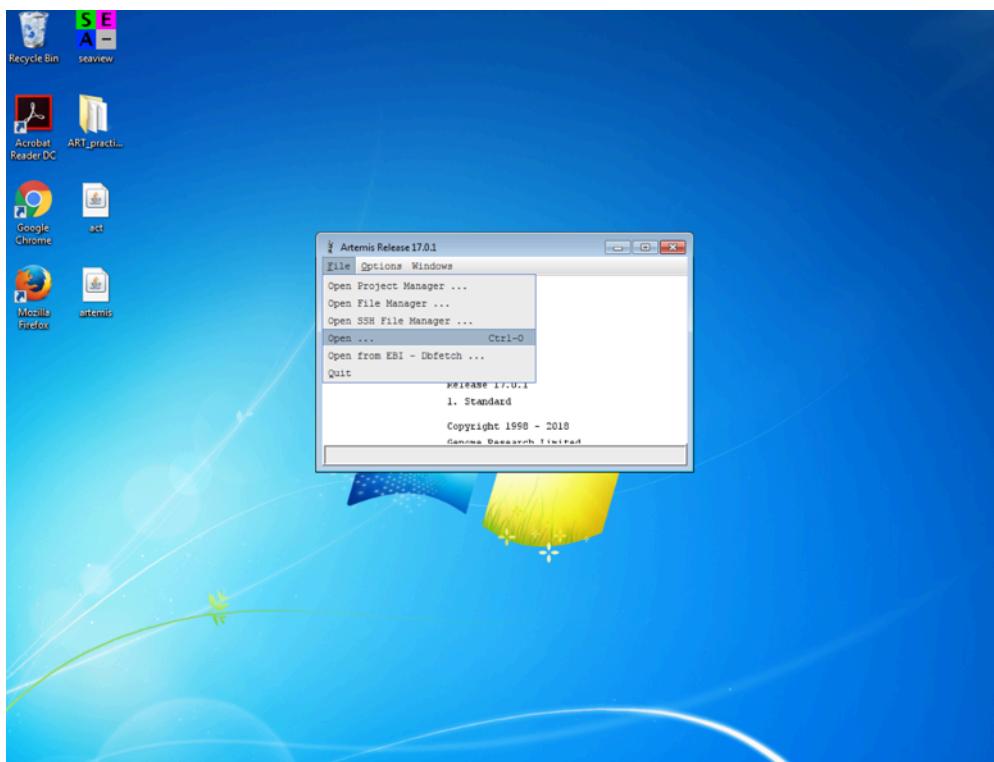
Step 1: Your screen should look like this. Now click on the ‘artemis’ icon as indicated. **NOTE:** if you cannot locate the ‘artemis’ icon on your desktop, open a new terminal window and type in ‘art’. This will also open Artemis tool.



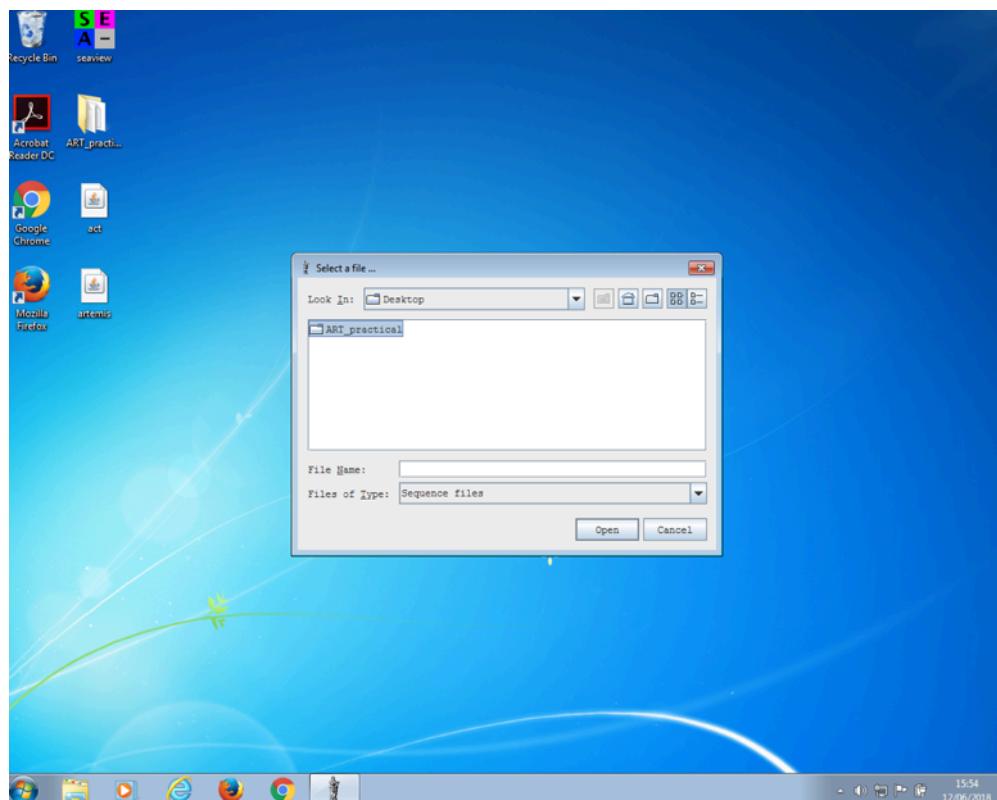
Step 2: The 'Set Working directory box should appear as shown above. Click 'OK'.



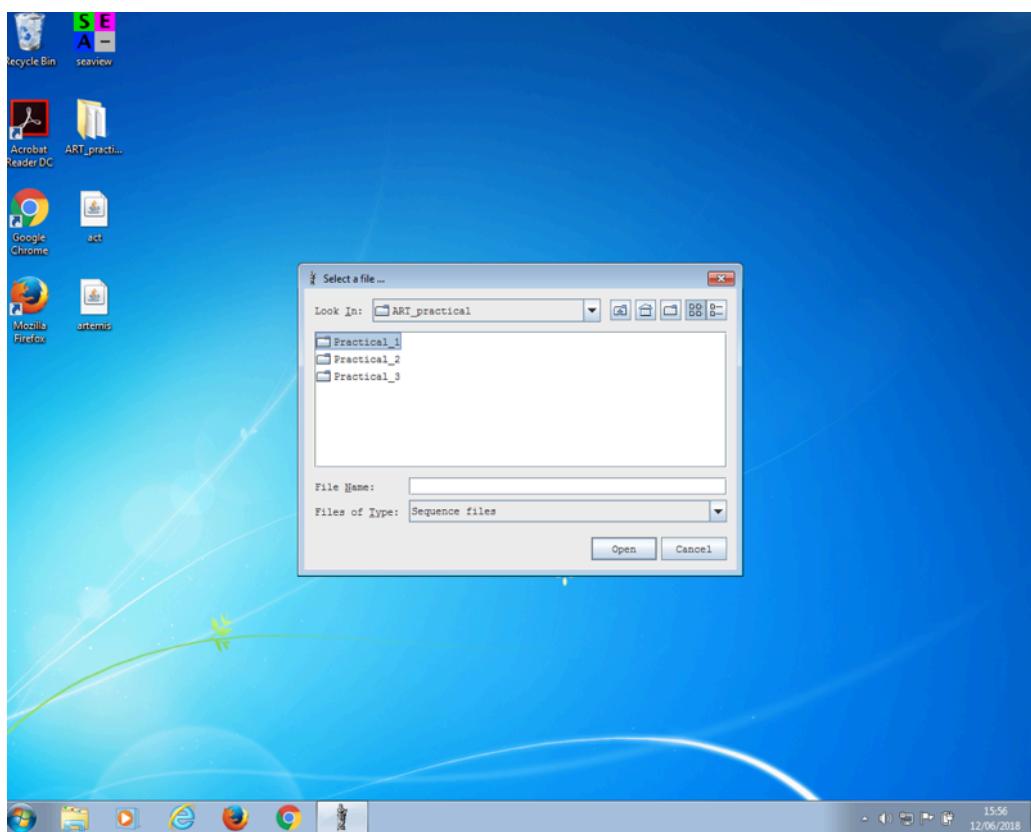
Step 3: The Artemis window should appear like this. Now click 'File'.



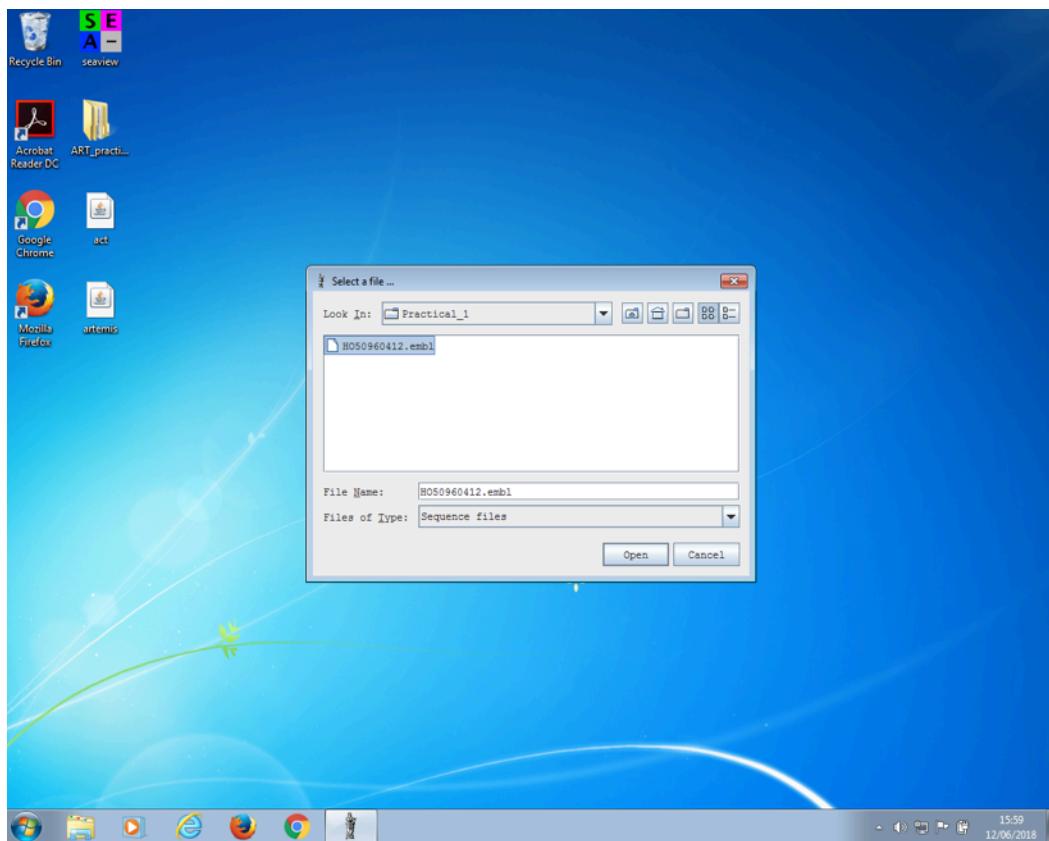
Step 4: Now select 'Open ...'.



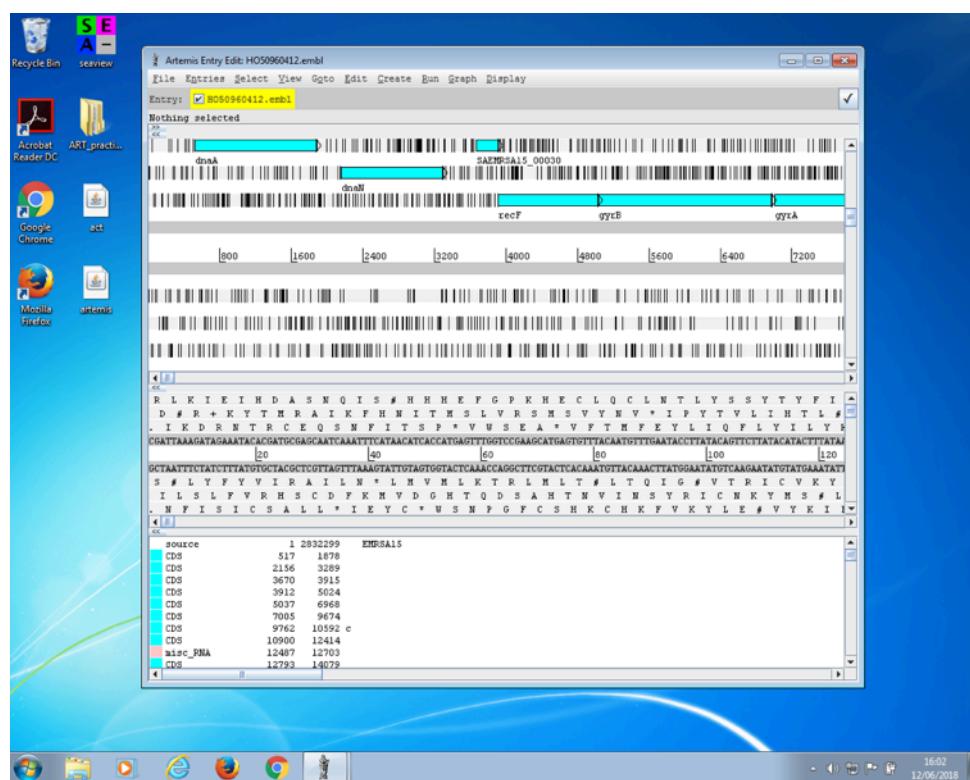
Step 5: Your window should now look like this – navigate to ~/course/ directory



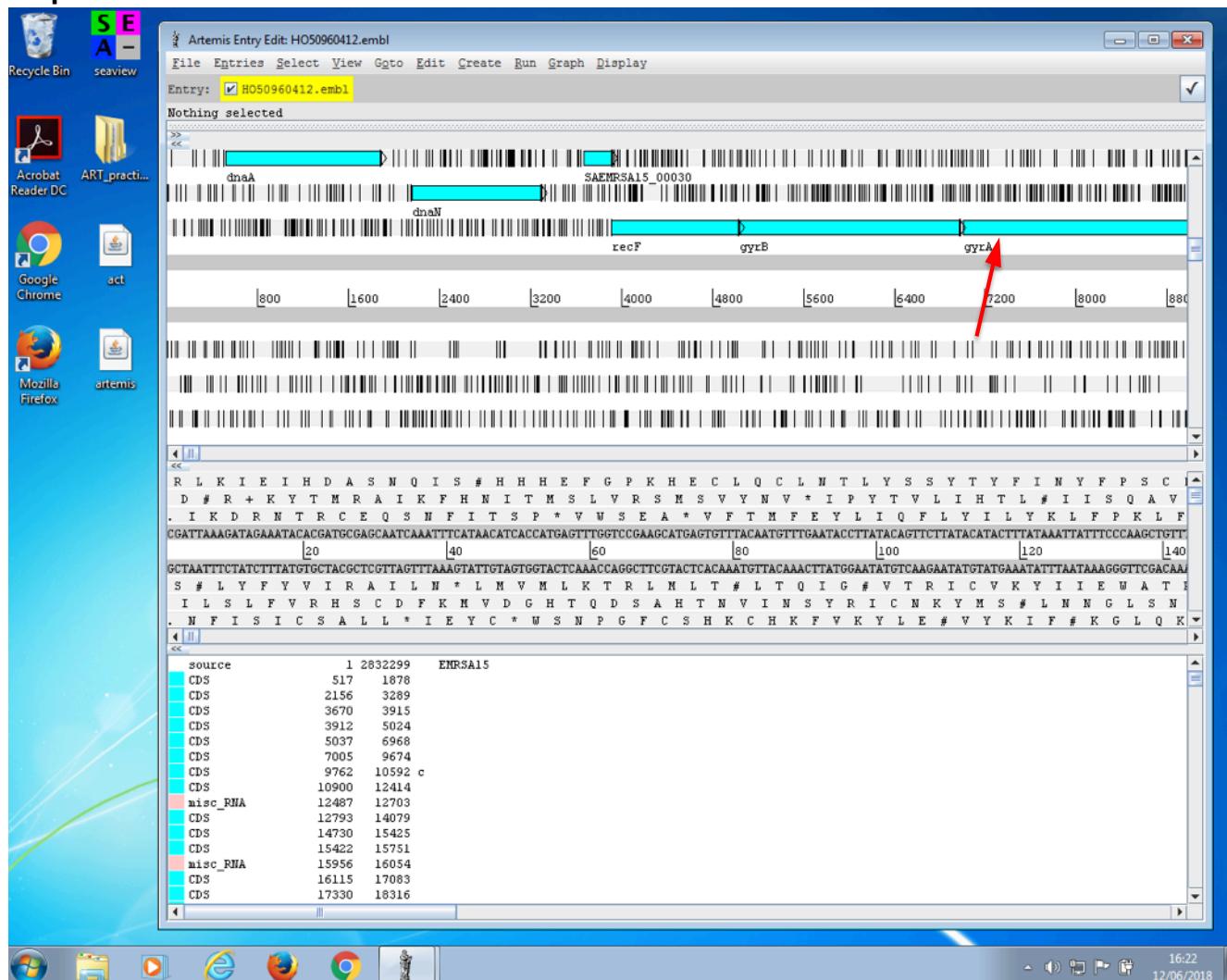
Step 6: Now select the ‘cp13’ folder and click ‘Open’.



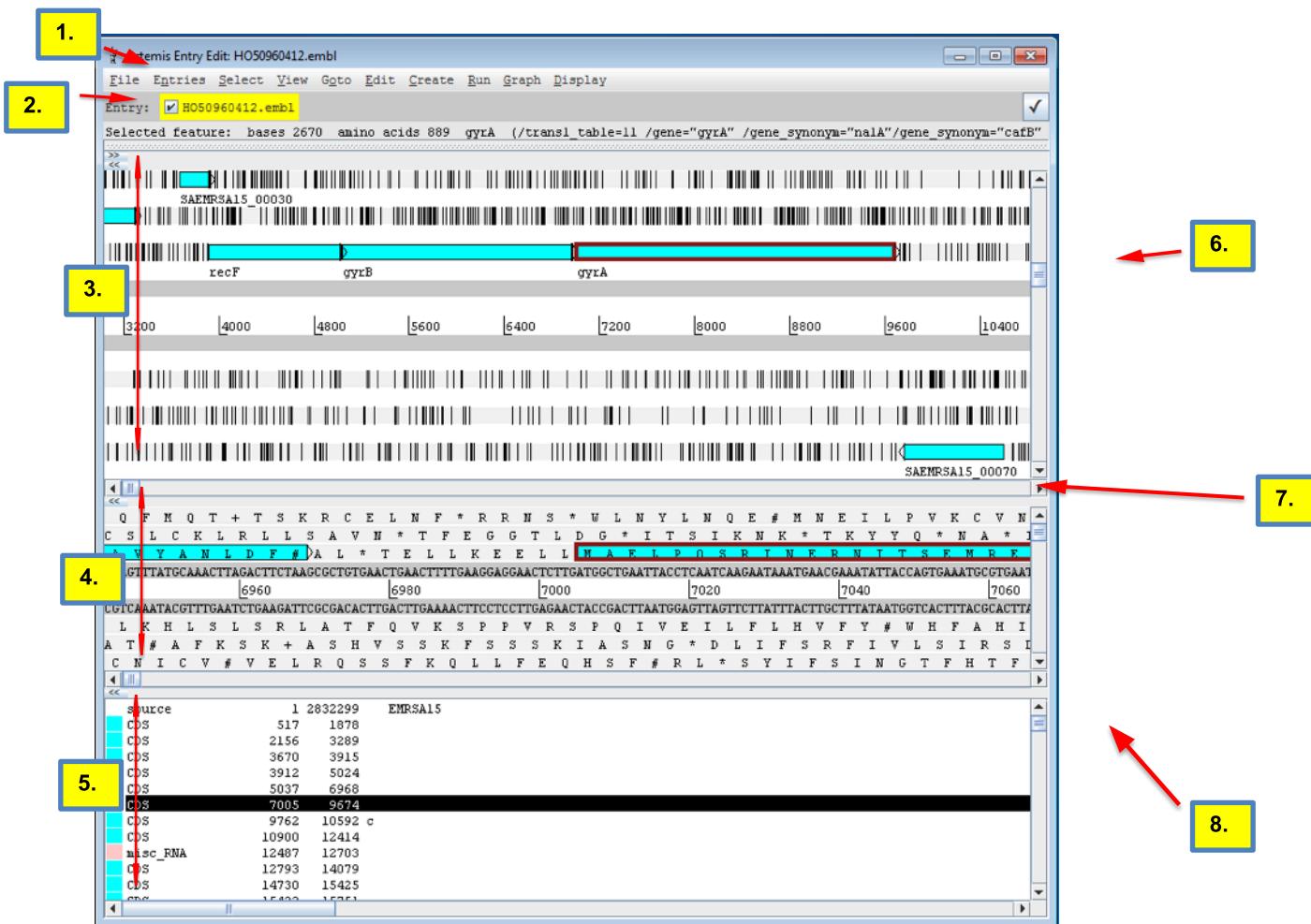
Step 7: Now select the file named: ‘HO50960412.embl’ and click ‘open’.



Step 8: Your window should now look like this.



Step 9: Now double click on the gene labelled 'gyrA'. The window should move and the *gyrA* gene should be highlighted in red. The next page explains what all the different parts of the Artemis window are for.

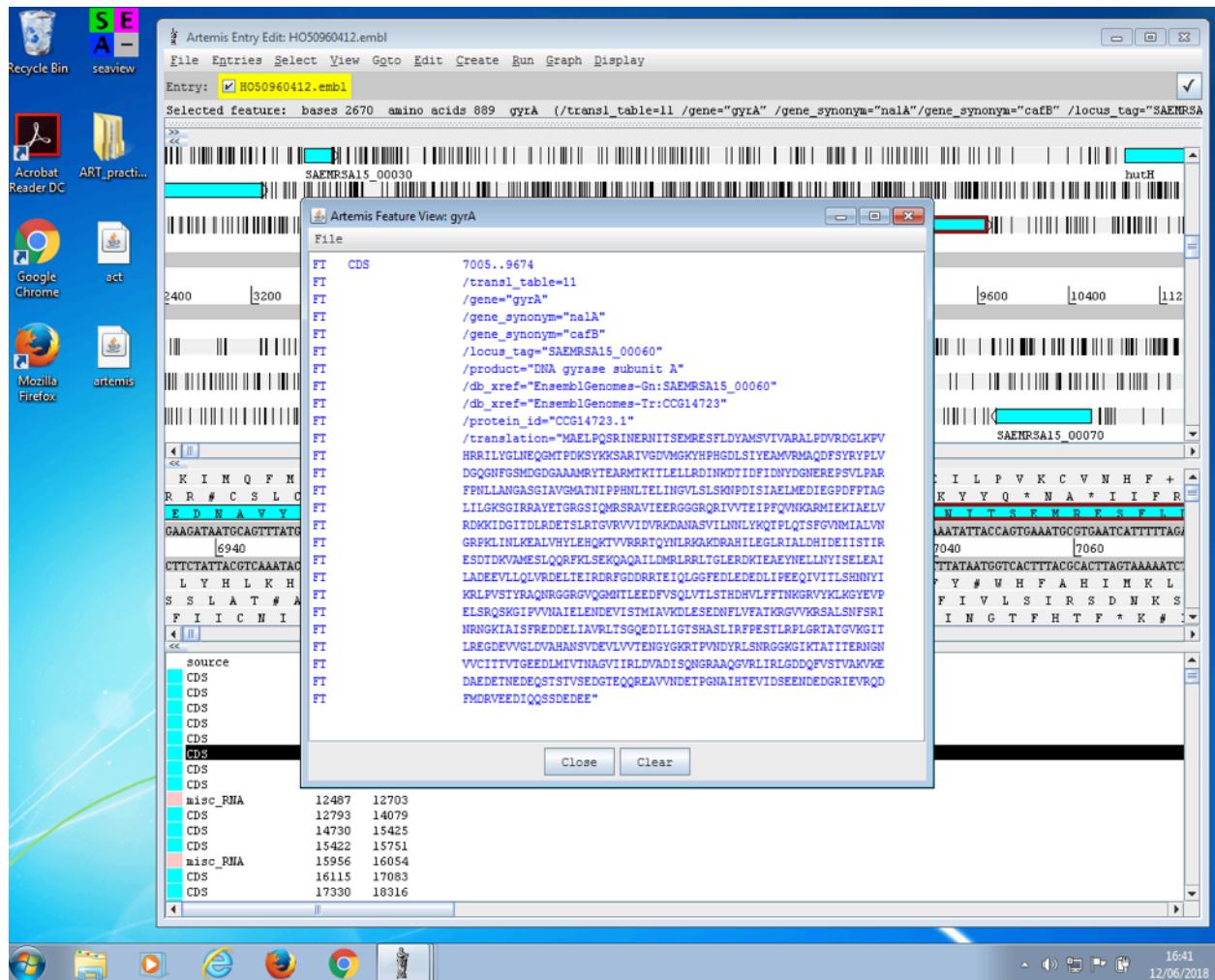


1. **Drop-down menus:** There's lots in there so don't worry about all the details right now.
2. **Entry (top line):** shows which entries are currently loaded with the default entry highlighted in yellow (this is the entry into which newly created features are created). Selected feature: the details of a selected feature are shown here; in this case gene **gyrA** (blue box surrounded by thick red line).
3. This is the main **sequence view panel**. The central 2 grey lines represent the forward (top) and reverse (bottom) DNA strands. Above and below those are the 3 forward and 3 reverse reading frames. Stop codons are marked on the reading frames as black vertical bars. Genes and other annotated features (eg. Pfam and Prosite matches) are displayed as coloured boxes. We often refer to predicted genes as coding sequences or CDSs.
4. This panel has a similar layout to the main panel but is zoomed in to show nucleotides and amino acids. Double click on a CDS in the main view to see the zoomed view of the start of that CDS. Note that both this and the main panel can be scrolled left and right (7, below) zoomed in and out (6, below).
5. **Feature panel:** This panel contains details of the various features, listed in the order that they occur on the DNA. Any selected features are highlighted. The list can be scrolled (8, below).
6. **Sliders for zooming view panels.**
7. **Sliders for scrolling along the DNA.**

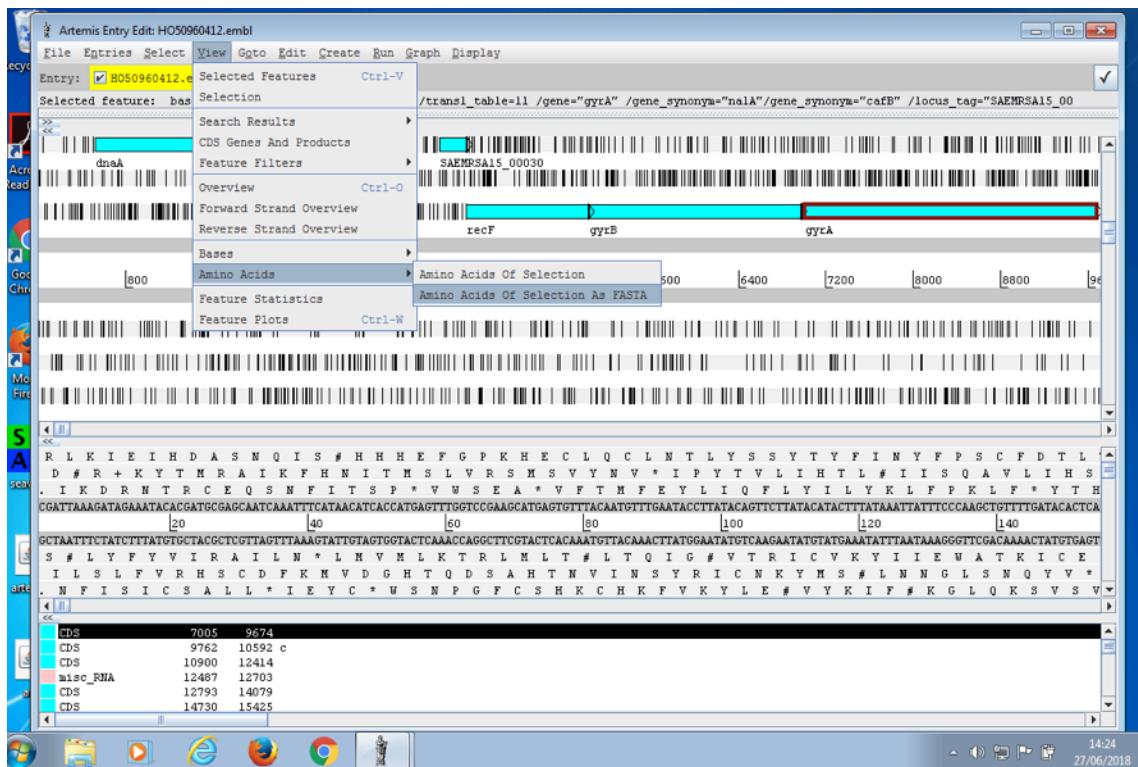
9. Slider for scrolling feature list.

Step 10: Try zooming the view in and out (no. 6 in figure above) and moving genome location using the slider for scrolling the DNA (no. 7 in figure above).

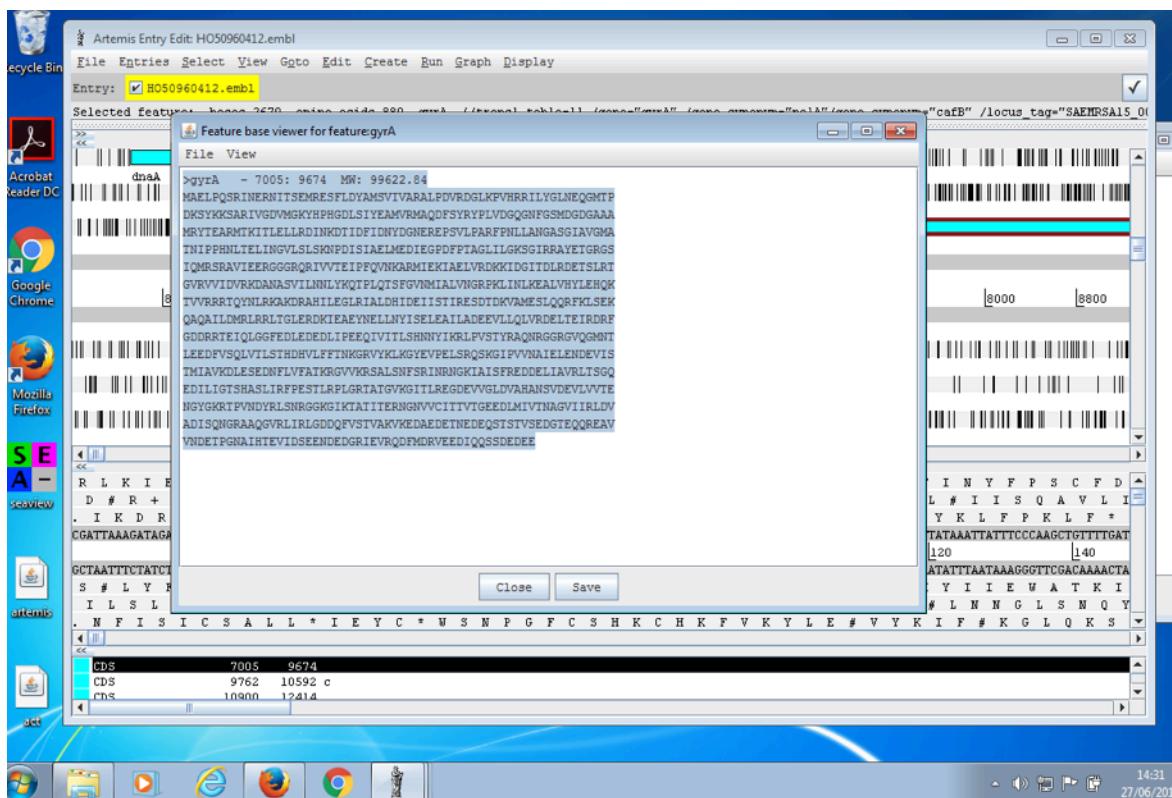
Step 11: Now scroll back to the start of the genome and click again on the *gyrA* gene. Now on the drop-down menu (No.1 in the figure above) and select ‘View’ and ‘Selected features’ (a short cut for this is ctrl – v). This brings up all the information that is stored in the entry about this gene or feature. In this case you can see that the product of *gyrA* is ‘DNA gyrase subunit A’.



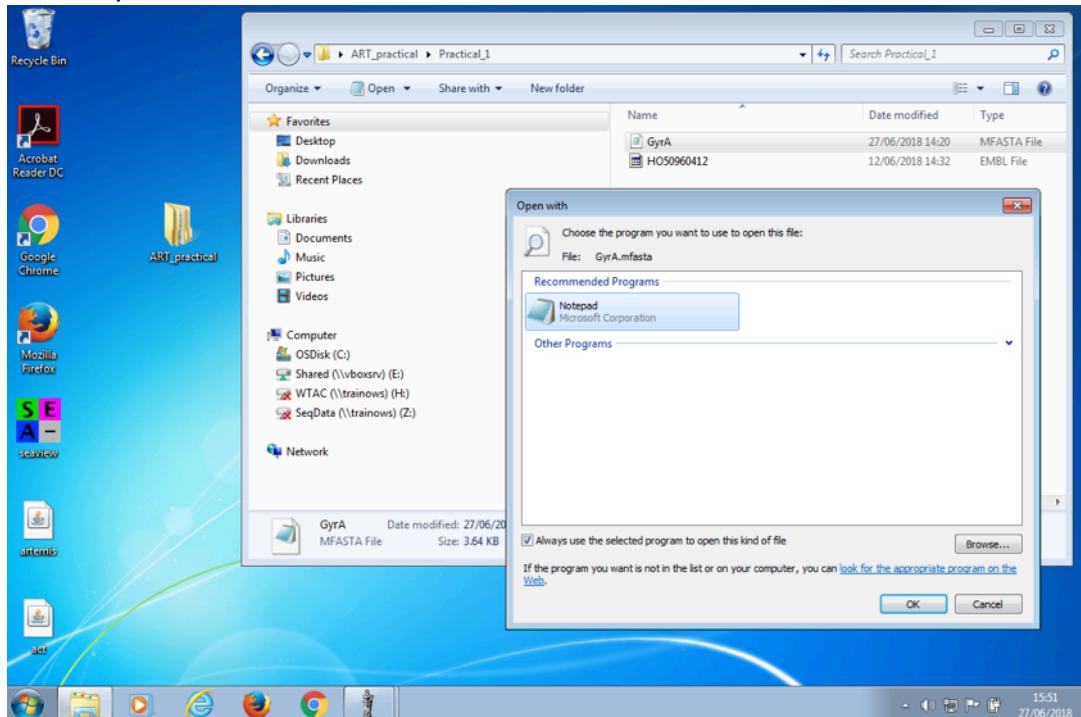
Step 12: You might have noticed this is one of the genes that we looked at when we were tracking the origin of the EMRSA-15 (ST22) MRSA clone. If you remember a point mutation in *gyrA* that generates the Ser84Leu substitution can mediate resistance to fluoroquinolone antibiotics such as ciprofloxacin. We are now going to investigate if this isolate is likely resistant by seeing if this gene contains the substitution necessary for resistance.



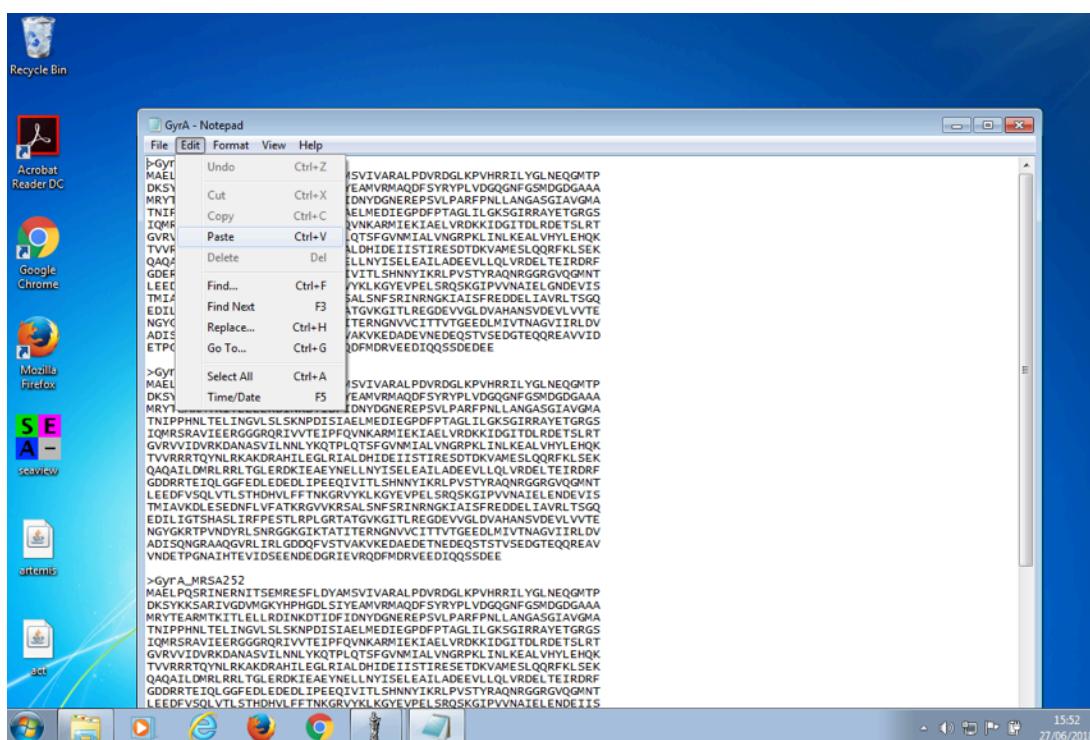
Step 13: Now making sure that *gyrA* is still selected. Click on the ‘View’ window and select ‘Amino Acids’ and then select ‘Amino Acids of Selection As Fasta’



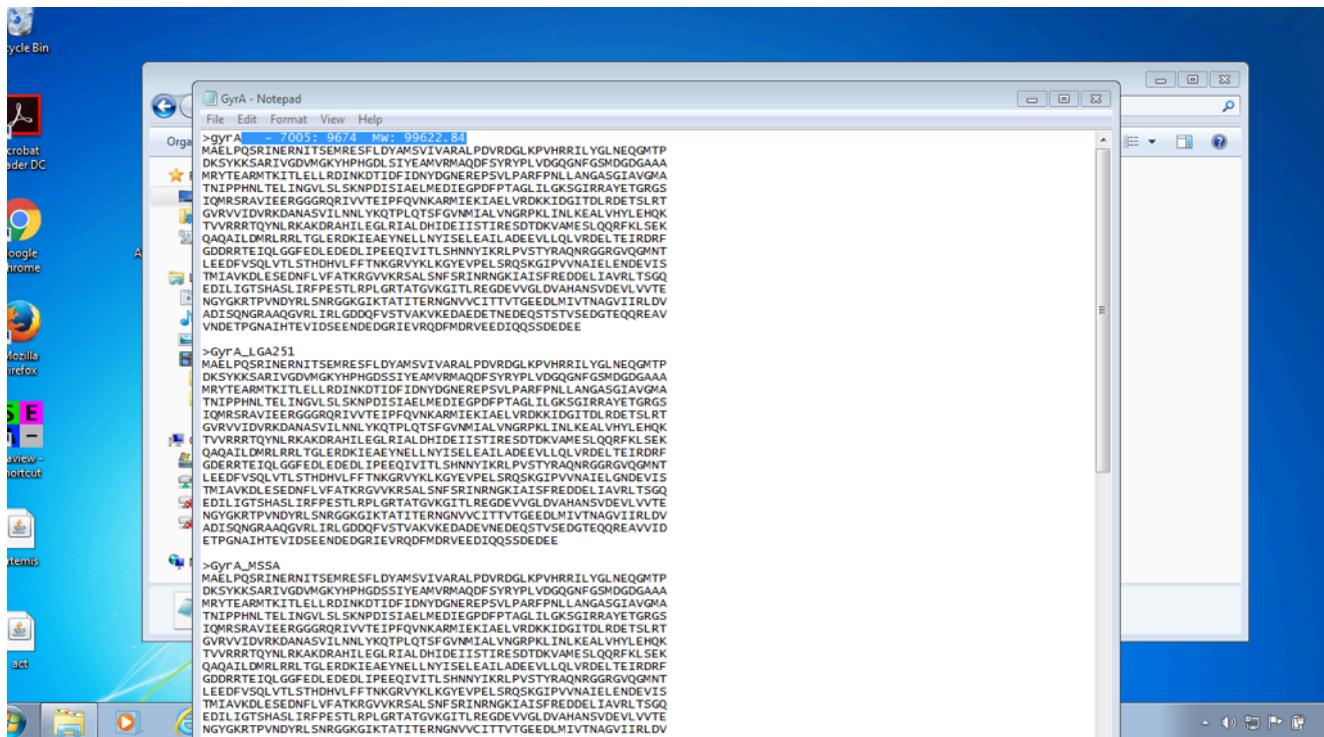
Step 14: A window will now open with the GyrA amino acid sequence. Now press **ctrl + A** to select all the whole sequence and then press **ctrl + c** to copy the amino acid sequence.



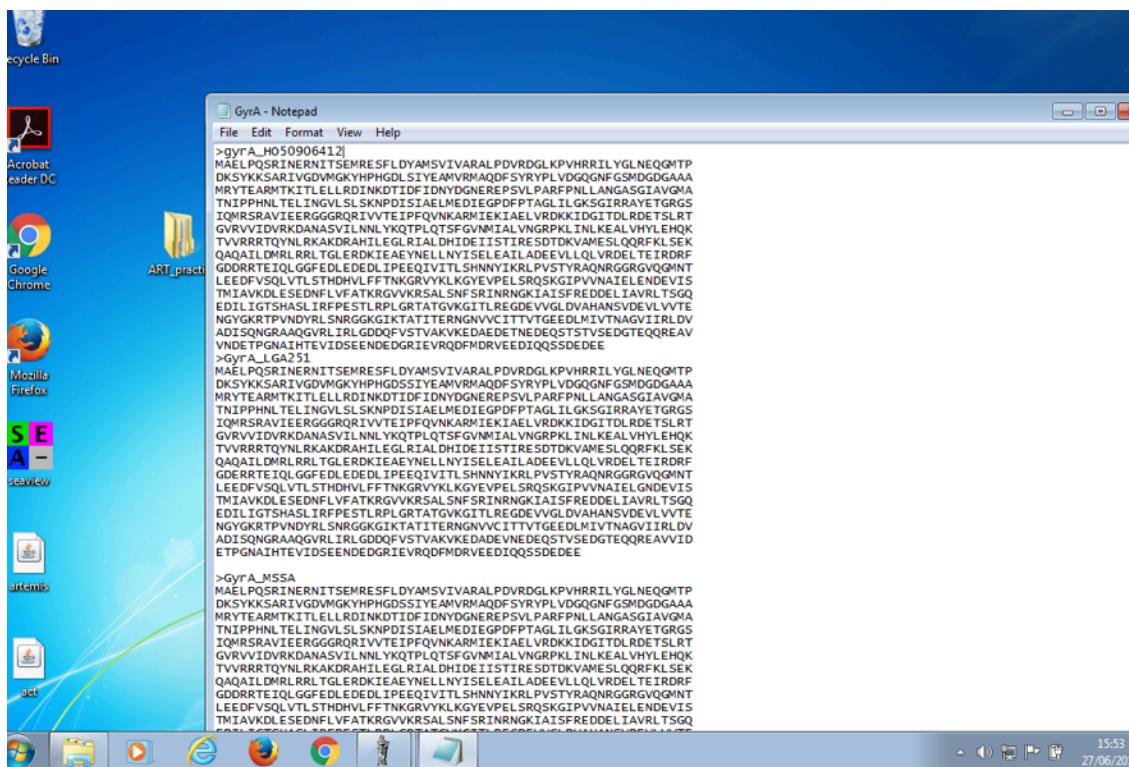
Step 15: Minimise the Artemis window and navigate to `~/course/` directory. Now open the 'cp13' folder. Inside this file you will find a file called 'GyrA.mfasta' – right click on this and select 'Open with Text Editor'.



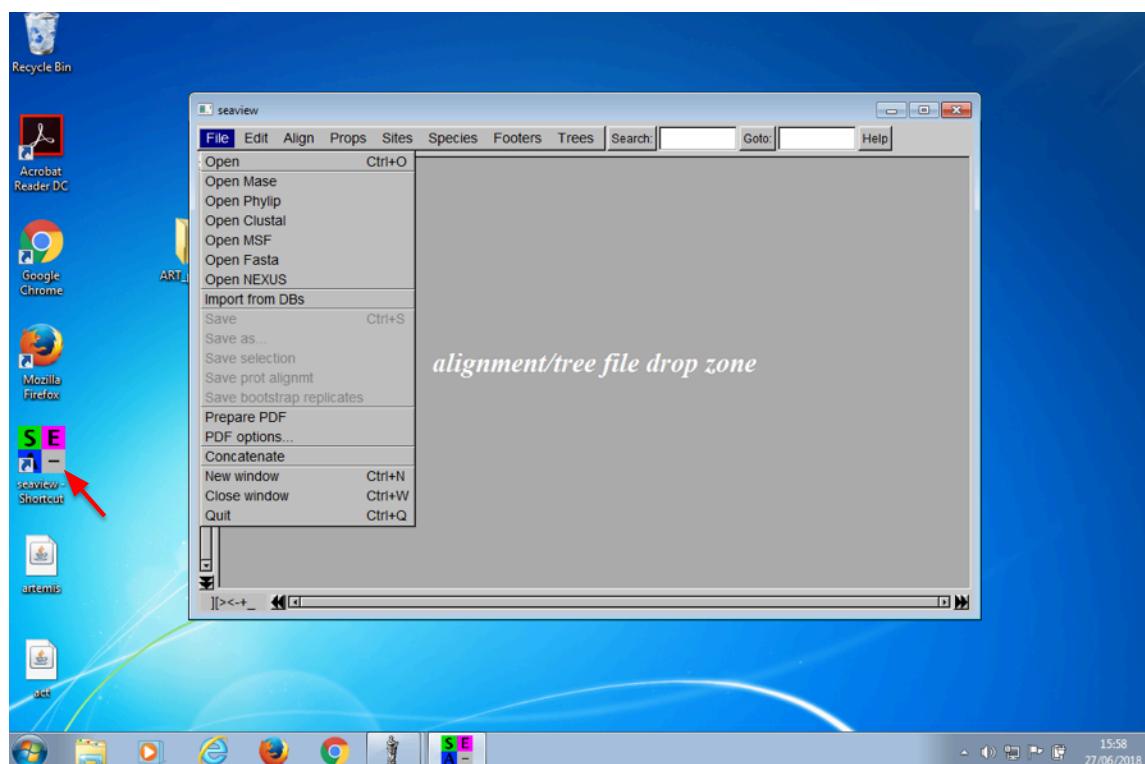
Step 16: The file should now open. The file contains the amino acid sequences of GyrA from three other *S. aureus* isolates. Making sure the cursor is at the top, select the ‘edit’ menu and then click ‘paste’. This will now copy the GyrA sequence you copied from the HO50960412 sequence into the file.



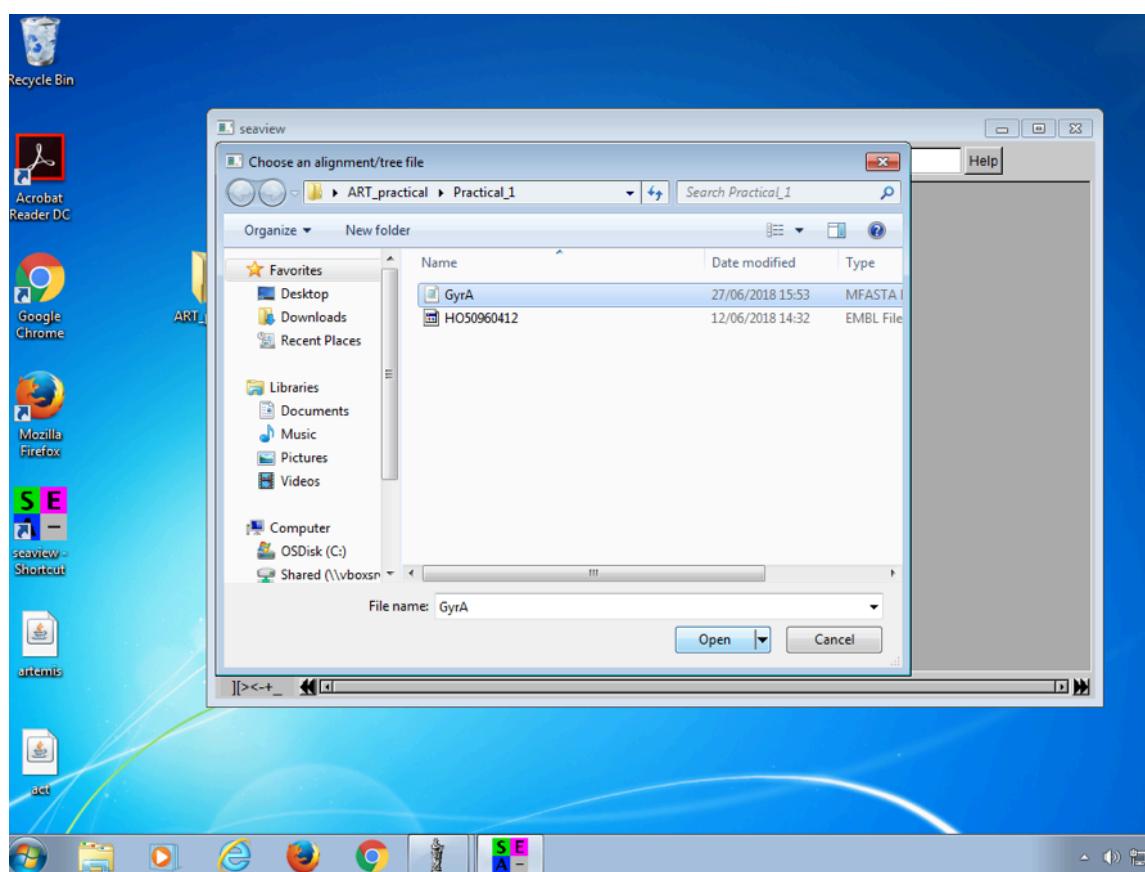
Step 17: Now move the cursor to highlight the text shown at the top of the sequence you just pasted into the file. Once you have highlighted this text – select the ‘edit’ menu and then click ‘delete’ to delete this text.



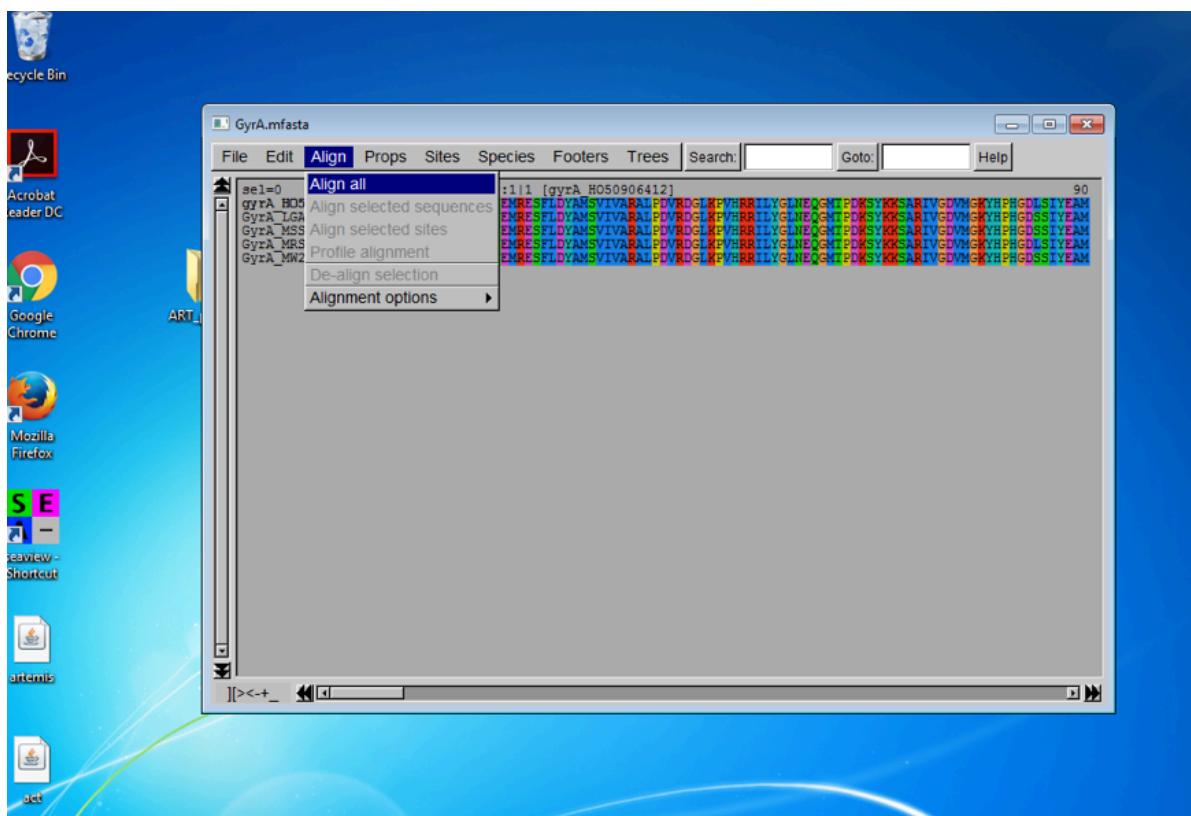
Step 18: Now type ‘HO50960412’ – where you deleted the text. Then select ‘File’ from the menu and click ‘Save’.



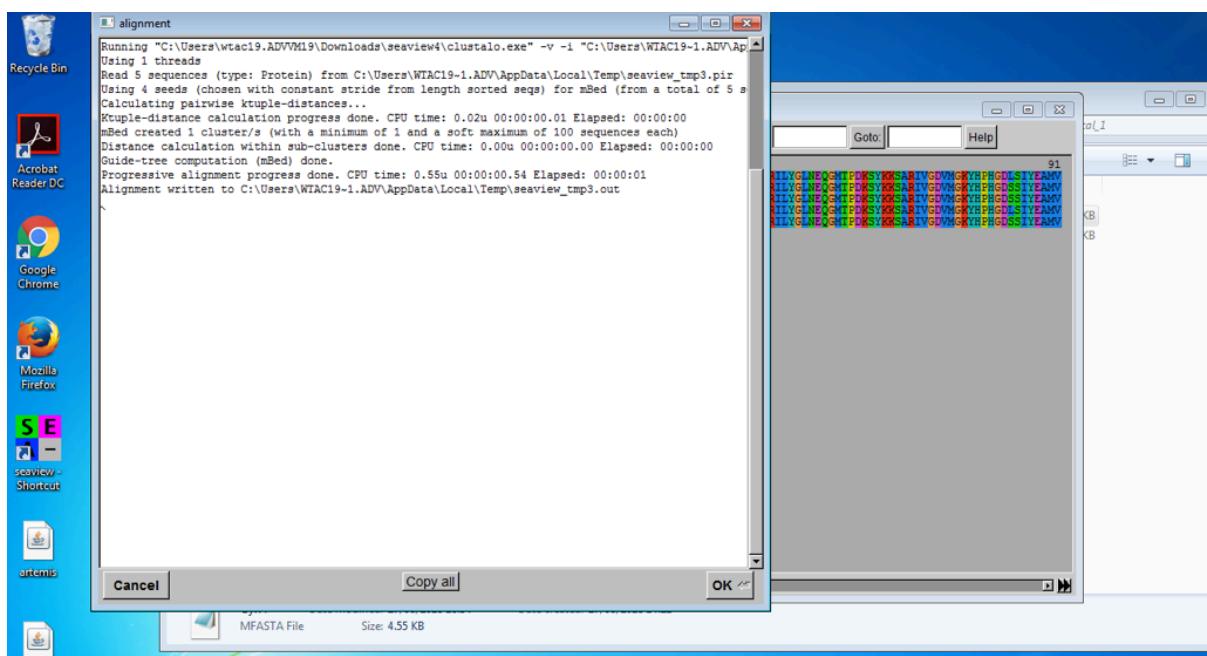
Step 19: Now got to the desktop and select the icon ‘Seaview’ and double click to open the program. Once the window opens select ‘File’ and then click ‘open’. **NOTE: if you cannot locate ‘Seaview’ icon on your desktop, open up a new terminal window and type in ‘seaview’. This will also open Seaview tool too.**



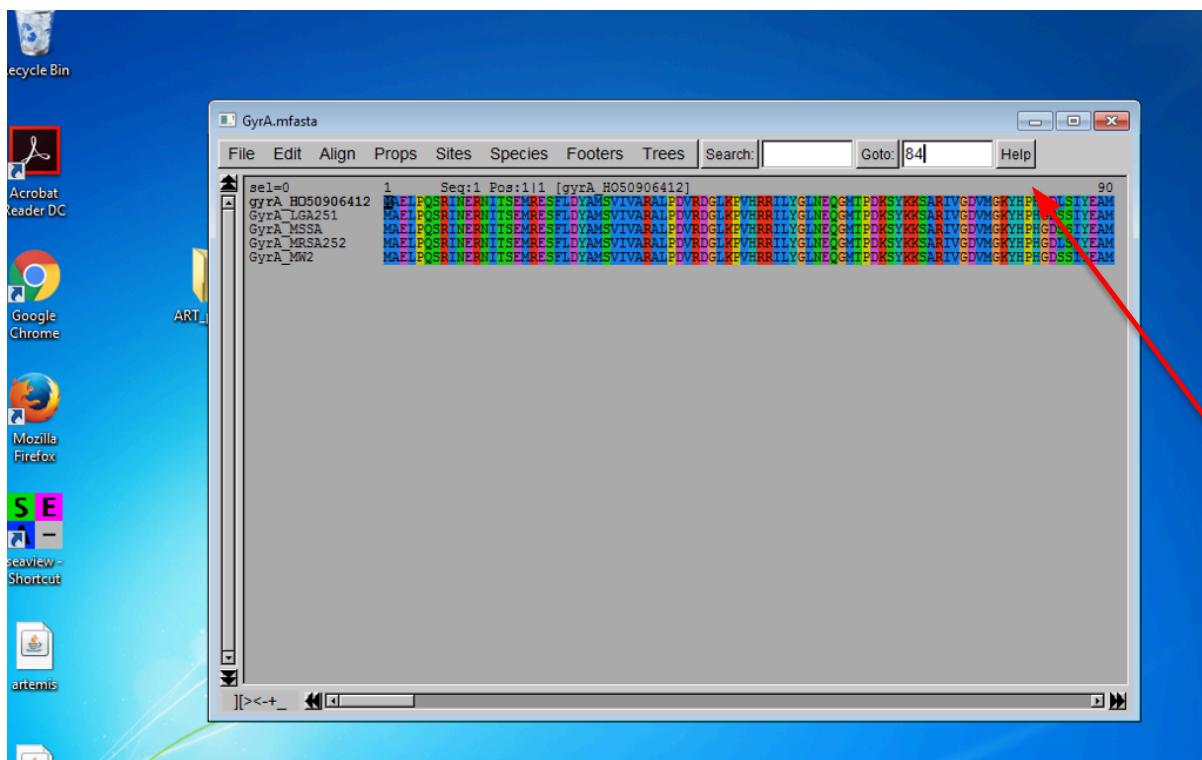
Step 20: Now navigate to `~/course/cp13` directory. Then click on the file '`GyrA.mfasta`' you just saved and click 'open'.



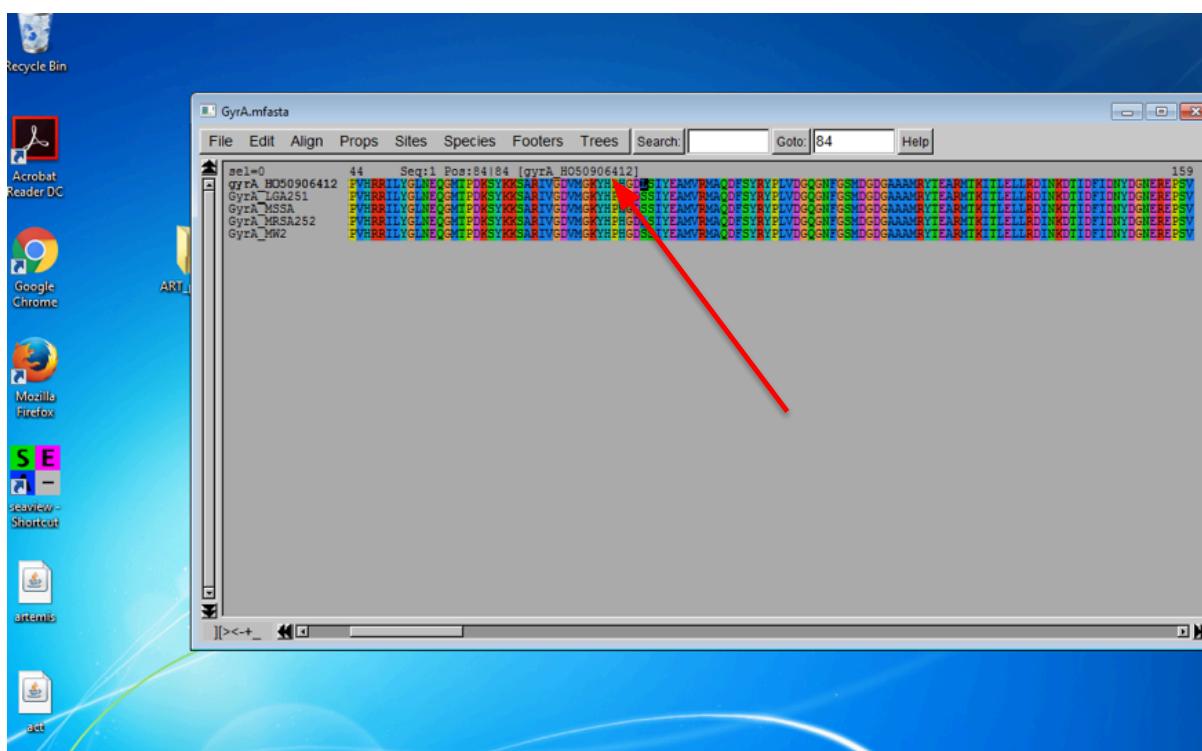
Step 21: Now select the 'Align' menu and click 'Align all' in the drop down menu. This will run a program that will align your sequences.



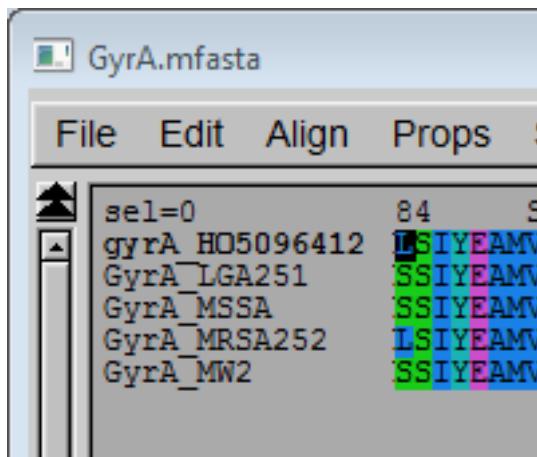
Step 22: The program will run and you will see a screen like this. Click 'okay'.



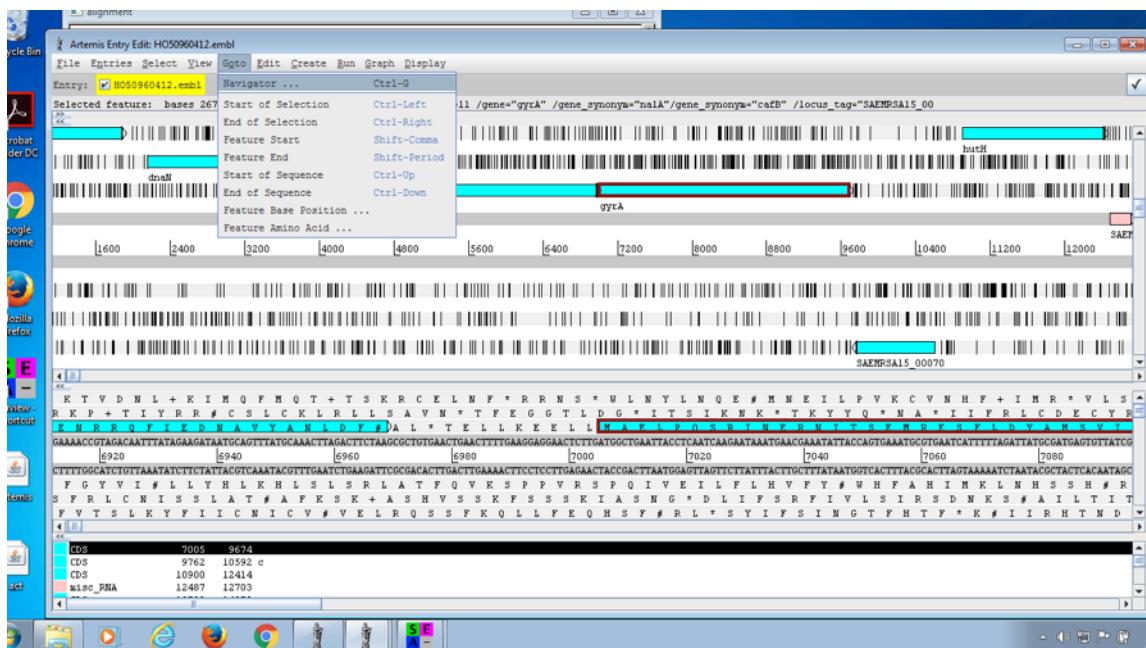
Step 23: You can now use the scroll bar to move up and down the alignment. This view allows you to compare differences between the sequences. Now click on the 'Goto' box at the top (see arrow) and type '84' and click the 'Goto' button. The program will now take you to amino acid position 84 in the sequence.



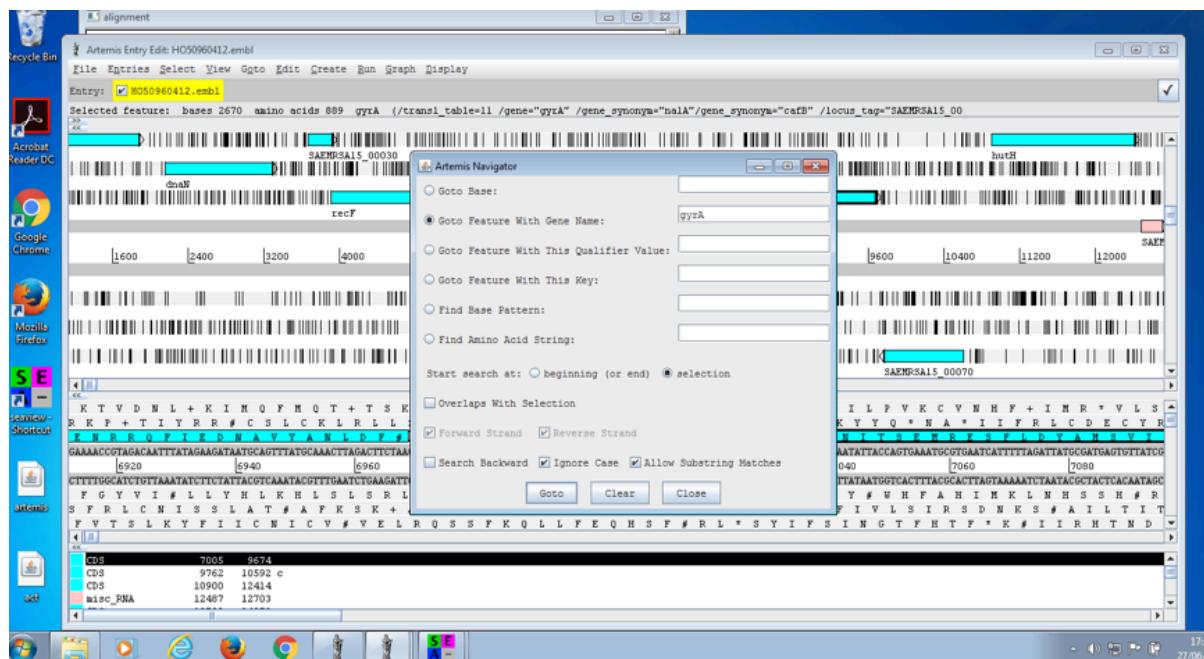
Step 24: You should now have a view like this, with the black cursor highlighting position 84.



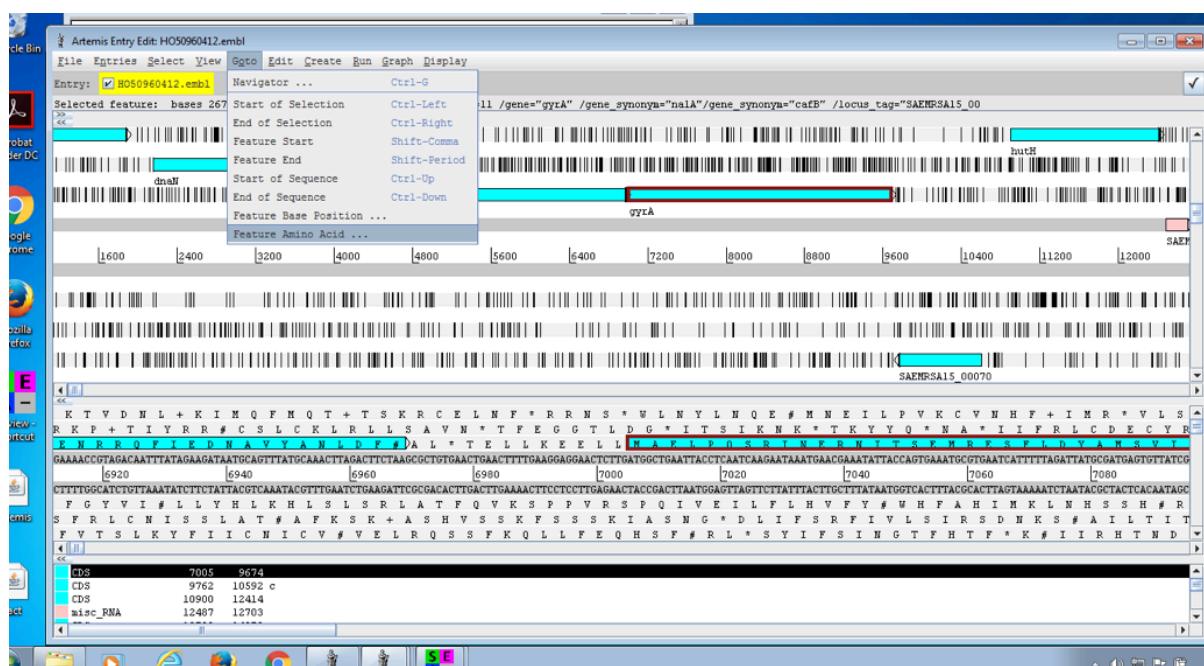
Step 25: Above is a zoomed in view. What you can see if that two of the isolates (MRSA252 and the one we have been working with HO5096412) both have a L (L = Leucine = Leu). While the other three isolates (MSSA, MW2 and LGA251) have an S (S = Serine = Ser). If you remember back to the previous practical – a Serine to Leucine (Ser84Leu) substitution at this position mediates resistance to ciprofloxacin (a fluoroquinolone). As you can see our isolate HO5096412 has the Ser84Leu substitution and so is resistant. This is what we would expect from an EMRSA-15 isolate. The other isolate with the substitution MRSA252 is from another hospital associated lineage of MRSA known as EMRSA-16, in which fluoroquinolone resistance is also common.



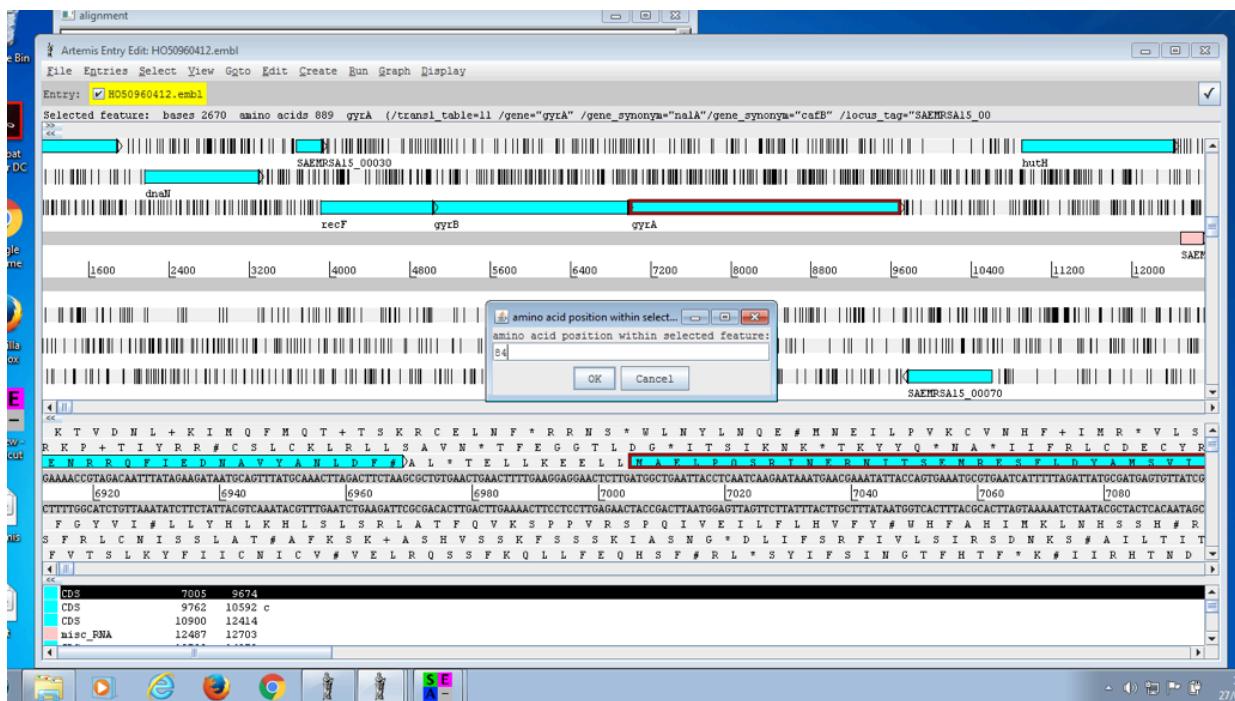
Step 26: If you go back to the Artemis window. There is another way to see if a gene or protein has a mutation or substitution at a particular position in the gene or amino acid sequence. Select the 'Goto' menu at the top of the Artemis window and then select and click on 'Navigate'.



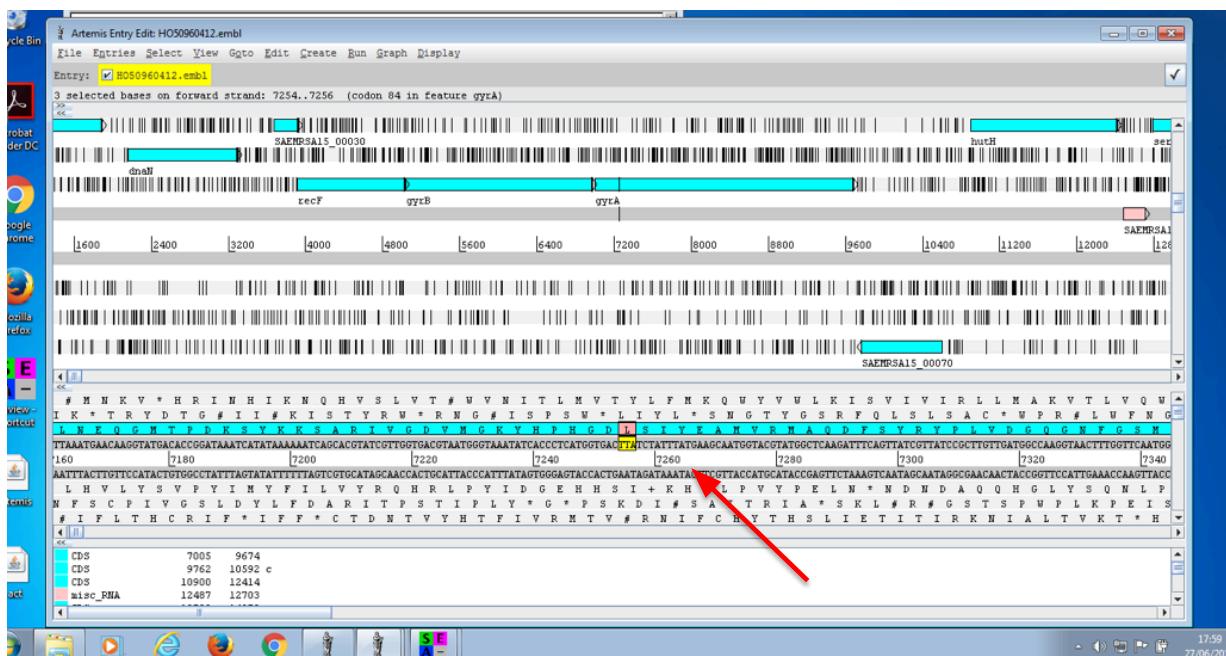
Step 27: Click the 'Goto feature with Gene Name' and then type 'gyrA'. If you now click the 'Goto' button at the bottom of the box this will select and take you to the *gyrA* gene again.



Step 28: If you now go to the 'Goto' menu again and select 'Feature Amino Acid' from the drop-down menu.



Step 29: In the box that appears type '84' – this will take you to amino acid position 84 in the *gyrA* gene.



Step 30: You can see here that amino acid 84 is highlighted and you can see that position 84 as we know is L = Leucine.

Step 31: Two different amino acid substitutions are known to mediate fluoroquinolone resistance in *S. aureus* ST22 isolates. The second being a Serine (Ser / S) to Phenylalanine (Phe / F) substitution at amino acid position 80 (Ser80Phe) in GrlA. Using what you just learned, can you now go back to the Artemis window and find out if strain HO5906412 also has this substitution?

Step 32: Now using what you have learned you can look for some more genes involved in resistance namely the beta-lactamase gene: *blaZ* and the *mecA* the gene that you targeted in your PCRs. Using the ‘Goto’ command search for these two genes. What is strange about the *blaZ* gene? What other genes are close by? When you find the *mecA* gene, what other genes are close by? Does this tell you anything? In the next practical we are going to look more closely at this region.