

To run bioinformatics for Case A of the Malaria case:

1. Open DNA files for Rosario and Milagros, then open the primer file pfmdr.txt.
2. Click on the primer file name in the Opened & processed window, then shift-click on the two DNA files to highlight them.
3. Use the Run button to run the PCR procedure.
4. Shift-click on the two PCR products in the O&P window, then use the Quick Load / Run button and select 'DNA gel -> Load well[s] and run gel in one step -> show light fragments on a dark background'.
5. Click anywhere in lane 1, then hover your mouse cursor over the gel fragments in that lane and verify that the three fragments have sizes of 182, 135, and 91 bp, respectively. Repeat for lane 2 and verify that those fragments have those same sizes.
6. Click on the 182 bp fragment in lane 1 so that it turns red, then click the 'Sequence' box on the gray horizontal bar below the gel. The sequence for that fragment will appear.
7. Click the yellow 'Analyze' button and select 'Export -> entire sequence -> adding to existing contents of Export field'. The sequence will appear in the Export field.
8. Click the 182 bp fragment in lane 2 so that it turns red, then use the Analyze button and select 'Export -> entire sequence -> adding to existing contents of Export field'. You should now have the 182 bp fragments for both Rosario and Milagros in the Export field.
9. Use the yellow 'Analyze' button and select 'Align / tree -> using MEGA software -> show alignment only'.
10. Scroll on the alignment window of MEGA to determine if there are any SNPs (single base pair differences) in the sequences. Repeat for the other pairs of fragments. Note that you can quickly get to any location by typing a number (in the box at lower left on the MEGA alignment window) and hitting the Enter/Return key of your keyboard.