

1. Once the sample has finished running on the fluorescence detecting equipment, analyze the data using the MapMarker 1000[®] size standard.
2. Set analysis parameters for peak height threshold at 100 for all channels.
3. Analyze sample.
4. The user should see red peaks (MapMarker 1000[®]), blue peaks (forward fragments) and green peaks (reverse fragments). All peak heights called should be 100 or higher.

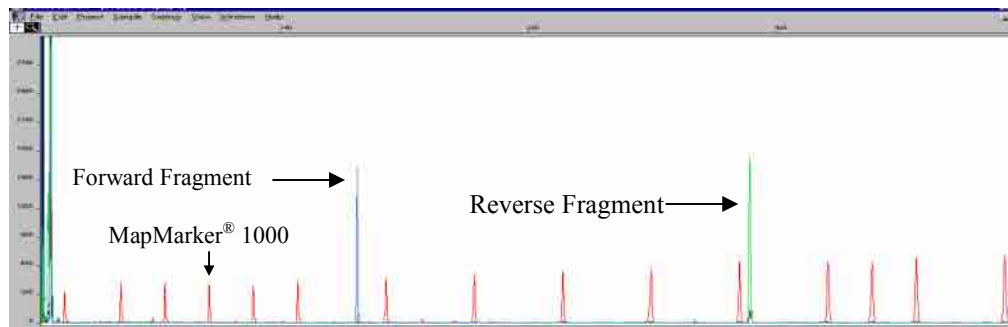


Figure 1. Hha I digest analyzed on ABI Prism 3100[®].

5. Highlight peaks for the forward fragments in each enzyme. Click view only selected rows.
6. Export this table as a file to be used later in FragSort 5 (<http://www.oardc.ohio-state.edu/trflpfragsort/downloads.php>).
7. This process may be repeated for the reverse fragments if desired.
8. Open the exported table file for the forward fragments and create 3 separate text tab delimited files. There will be one file for each of the different enzymes. Use the cut and paste functions to cut fragments for each enzyme and paste into a new document. Save these documents as tab delimited files. A helpful way to name the file, for example, is sample1_hha_fwd. This process needs to be repeated for each enzyme for forward and reverse (if desired). In the end, the user will have a total of three files for each sample (six files if reverse fragments are to be analyzed).

| | A | B | C | D | E | F | G | H | I | J | K |
|----|---|-------|-------|--------|------|-------|------|---|---|---|---|
| 1 | | 168,1 | 10.44 | 163.76 | 1736 | 14111 | 3916 | | | | |
| 2 | | | | | | | | | | | |
| 3 | | | | | | | | | | | |
| 4 | | | | | | | | | | | |
| 5 | | | | | | | | | | | |
| 6 | | | | | | | | | | | |
| 7 | | | | | | | | | | | |
| 8 | | | | | | | | | | | |
| 9 | | | | | | | | | | | |
| 10 | | | | | | | | | | | |

Figure 2. Genescan Data Table Exported To Excel.

9. Normalize the data. Visit the FragSort 5 website and click on Genescan Normalizer.
10. Once this page opens, enter an email address and browse to upload the file to be normalized. Note the examples of how the data MUST be formatted. If data is not in this format, it **WILL NOT** be analyzed.

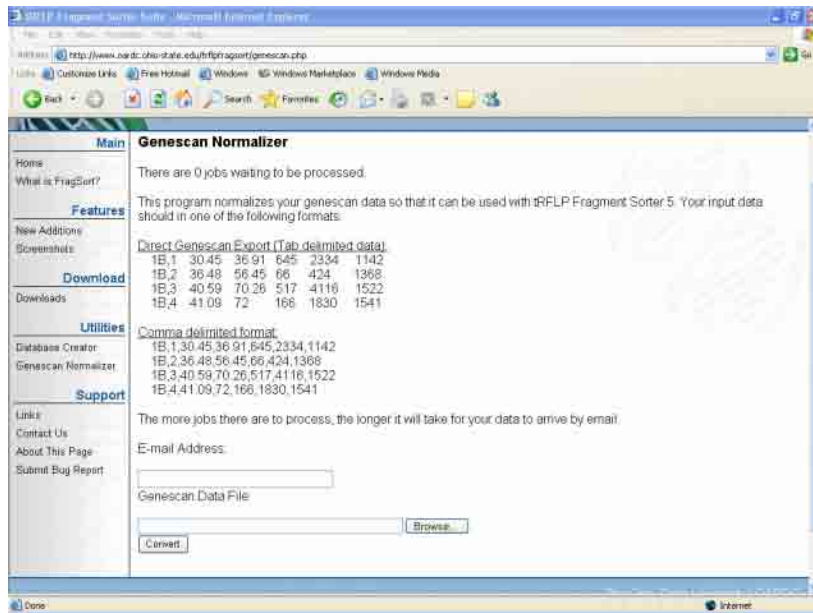


Figure 3. Genescan Normalizer Screen On FragSort 5 Website.

11. This process will need to be performed for all 3 enzymes for either the forward fragments, reverse fragments or both.
12. Once files have been uploaded and sent, a screen will give notice of a job pending. The normalized files will be emailed to the included email address within 2 to 3 minutes. Simply check email and download the attachments. Make sure to save files as .csv (comma delimited) files.

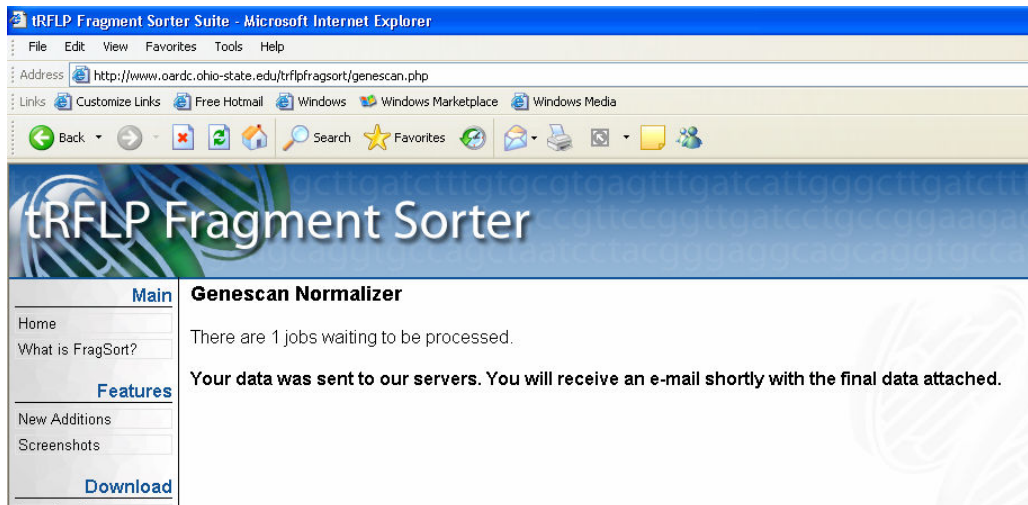


Figure 4. Genescan Normalizer Job Pending Screen.

13. Launch FragSort 5. Once the program has been launched, click in the upper left-hand corner on New Search.
14. Once New Search has been selected, the first screen seen is a search wizard to help through the process. Click Next.

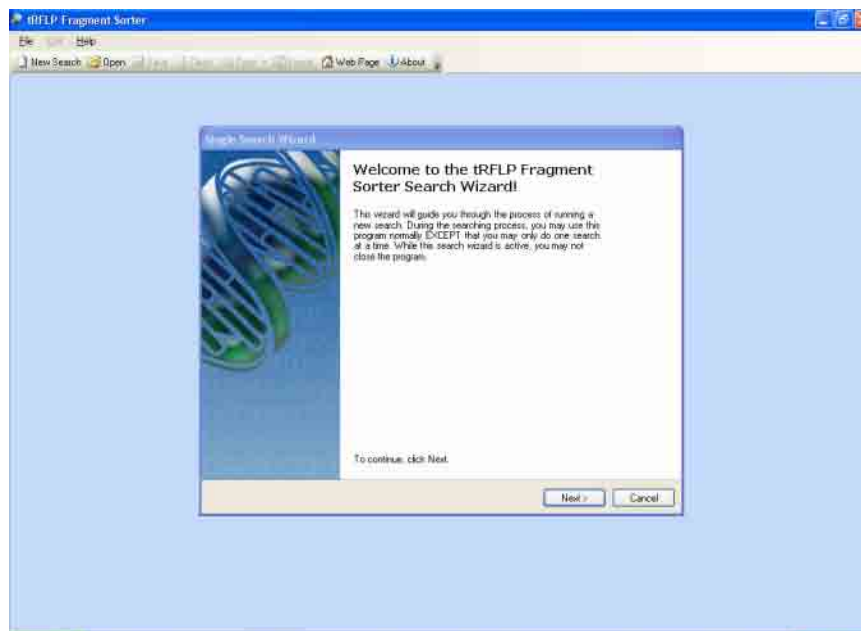


Figure 5. FragSort 5 Search Wizard Screen.

15. The next screen is for the Primer Database. FragSort 5 comes pre-loaded with 11F-907R forward and reverse. The 11F-907R forward choice is compatible with the forward primer provided in this kit. Click on 11F-907R forward (or other

choice) and click Next. A customized database may be assembled. Instructions on how to create a database are provided on the FragSort 5 website.

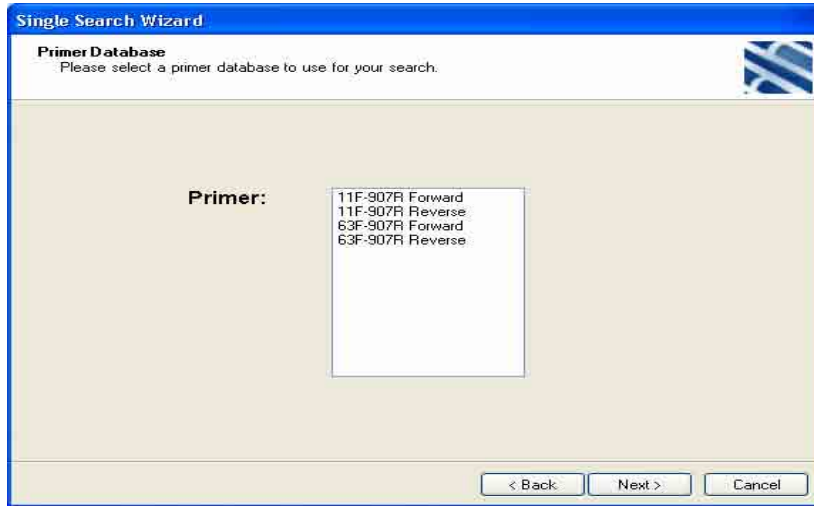


Figure 6. FragSort 5 Primer Database Choice Screen.

16. Once the primer database choice has been made, the program will ask for a description, such as a date or file name, and to check which enzymes are to be used. All 3 enzymes must be chosen. If other enzymes are ever used, a database for that enzyme will have to be created. Once again, directions for constructing a database are on the FragSort 5 website. Once the description and enzyme choices have been entered, click Next.

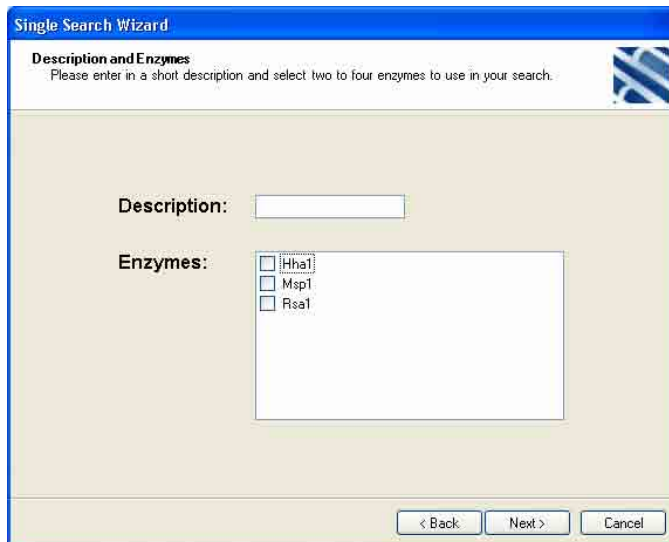


Figure 7. FragSort 5 Description and Enzyme Choice Screen.

17. The next page viewed will be asking for the input files. These will be the **Normalized** files that were emailed after using the Genescan Normalizer on the website. Simply click the square button with three dots to the right of each

enzyme and browse for that specific enzyme's file for either forward or reverse fragments. **REMEMBERTO USE EITHER ALL FORWARD OR ALL REVERSE** fragment files for each enzyme. In other words, a new search will have to be performed for whichever direction (forward or reverse) that was not chosen in the first search.

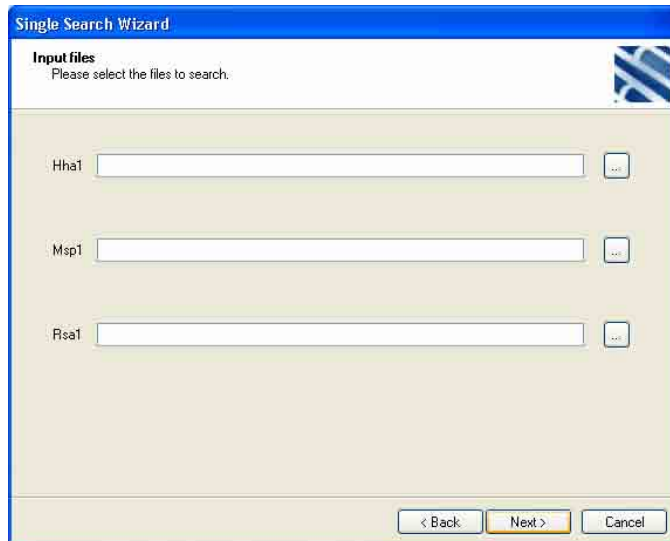


Figure 8. FragSort 5 Input Files Screen.

18. Once a file has been chosen for each enzyme, click Next.
19. The next screen will ask for the error calculation method and how many fragments to match.

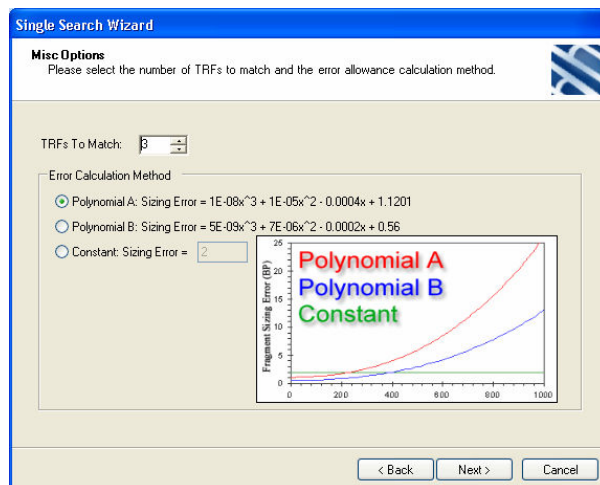


Figure 9. FragSort 5 Error Method Calculation and TRF to Match Choice Screen.

20. Start with the most stringent conditions. If no matches are made, lower the number of TRF matches or change error calculation method to see if a list is compiled. Once the choices have been made, click Next.
21. The next screen shows a log of what actions were performed and allows the log to be saved. Click Finish.

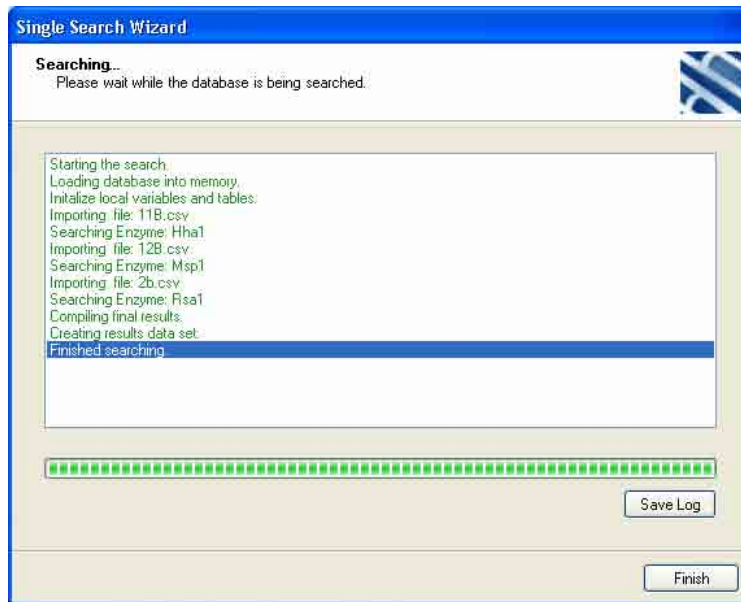


Figure 10. FragSort 5 Searching Database Screen.

22. The next screen lists all the organisms that match the fragment data entered. Each organism is also linked to an accession i.d. from NCBI. To access information on this organism, click on the accession i.d.

| Accession ID | Organism Name | HhaI TRF Size | HhaI TPF% Ar | HpaI TRF Size | HpaI TPF% Ar | PvuII TRF Size | PvuII TPF% Ar |
|--------------|------------------------------|---------------|--------------|---------------|--------------|----------------|---------------|
| AL596170 | Listeria innocua Clp11262 | 188 | 100 | 147 | 100 | 435 | 100 |
| AL596172 | Listeria innocua Clp11262 | 188 | 100 | 147 | 100 | 435 | 100 |
| AL596164 | Listeria innocua Clp11262 | 188 | 100 | 147 | 100 | 435 | 100 |
| AL596173 | Listeria innocua Clp11262 | 188 | 100 | 147 | 100 | 435 | 100 |
| AL596169 | Listeria innocua Clp11262 | 188 | 100 | 147 | 100 | 435 | 100 |
| AL596170 | Listeria innocua Clp11262 | 188 | 100 | 147 | 100 | 435 | 100 |
| AL591981 | Listeria monocytogenes E52-m | 188 | 100 | 147 | 100 | 435 | 100 |
| AL591974 | Listeria monocytogenes E52-m | 188 | 100 | 147 | 100 | 435 | 100 |
| AL591983 | Listeria monocytogenes E52-m | 188 | 100 | 147 | 100 | 435 | 100 |
| AL591982 | Listeria monocytogenes E52-m | 188 | 100 | 147 | 100 | 435 | 100 |
| AL591980 | Listeria monocytogenes E52-m | 188 | 100 | 147 | 100 | 435 | 100 |

Figure 11. FragSort 5 List of Possible Organisms in Sample.

23. To the left of the table, click to see a graphical representation of each fragments peak area percentage for each enzyme and also a search summary and more advanced data.
24. From this data, the researcher may determine a microbial profile for their sample.