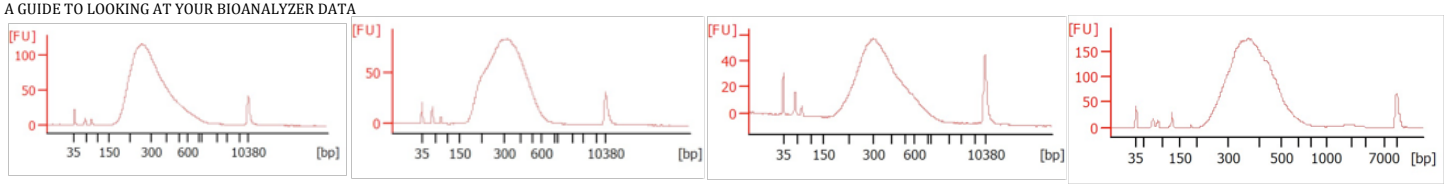
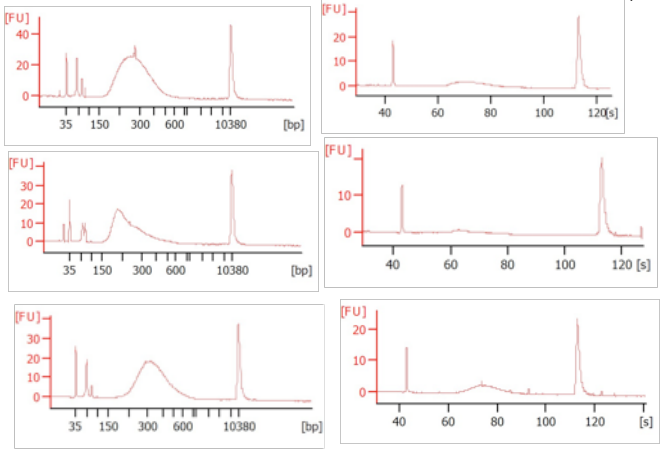
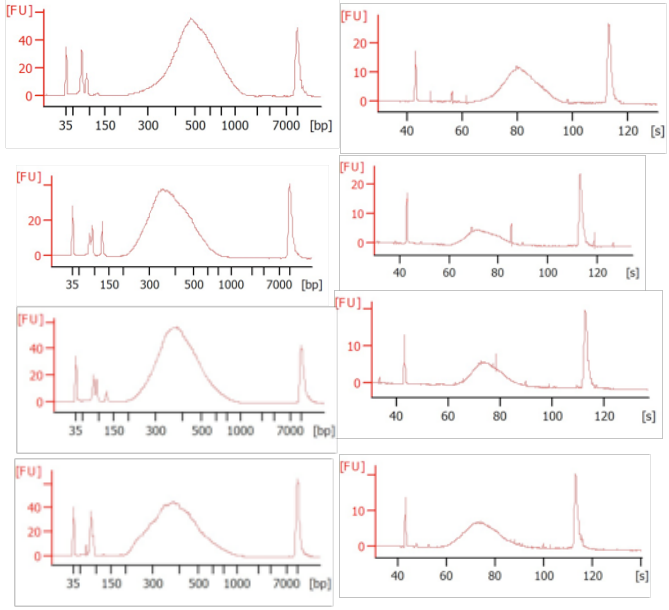
A GUIDE TO LOOKING AT YOUR BIOANALYZER DATA



Above are 4 examples of nice bioanalyzer curves that would be acceptable for NGS. There are peaks between 35 and 150bp that would indicate primer-dimer and adapters. However they are so small in proportion to the library that we would deem them acceptable to move forward with sequencing on the MiSeq. Note the differences in the scales of the Y axis. The bioanalyzer will give you molarity and quantitation but you can also just get a general feel for amounts just by looking at the Y axis scale. Lastly, the peaks at 35 and 10380 are the marker that you added to every well. You can see that in the second sample, the primer and adapter peaks are as high as the 35 peak just like in many of the ones below where we elected to further purify. But it is the size of these peaks in proportion to the size of the library peak that we are more concened with. We do not want equal molarity of adapter and library.

Below are some examples of samples that have an unacceptable amount of primer-dimer and adapter that will take up too much real estate on the flow cell in proportion to the library. To get rid of this, you want to put your sample through an additional purification with AMPure beads. However, caution should be taken as samples can be lost in this process as can be seen in the before and after electropherograms on the left. The small peaks are gone but so is the library. Thus, it is recommended that if you have enough, one should take half of their library, bring it up to 50 µl, and purify with 50µl of beads. If you have a large amount of library, you can try an even more stringent version of this purification whereby you would again take half of your sample (for safety in case you lose it) bring it up to 50µl, but only add 40µl of beads. This will eliminate more of those smaller peaks but also some of your library as you can see in the before and after electropherograms of the 4 samples on the right below. Again note the y axis to see how much library was lost. Note that the x axis on the right side electropherograms below are different. This is because the ladder failed in the run on the right. If this happens, you may be able to manually set the high and low using the marker peaks at 35 and 10,380 but that does not always work. Tech support may be able to help you salvage.





Finally, the electropherograms below are examples of overamplification. The second hump to the right is the result of fragments creating single stranded concatemers, whereby the reaction runs out of primers so they stick to each other. Thus, best to do maybe only 12 cycles from the beginning. They should sequence fine as they will be denatured before loading onto the NGS machine. However, one should use the center of the left peak for average size. if you want to get rid of them or verify that that is what they are simply set up another PCR reaction with fresh primers and indices and only do 2 cycles. The set of two on the right show a sample that has overamplification and. a rather large adapter peak that we wanted to get rid of, so a 50µl purification as describes above was performed and you can see that this eliminated the adapter peak but not the concatemers.