Maldi-Tof Quick Guide for DNA or RNA

► Choose a matrix for your protein, keeping in mind the solubility of your DNA or RNA with the matrix. The most common matrices are 3-HPA. If your sample doesn't work with either of those, you are to provide your own matrix.

1. Weigh out 7mg of 3-HPA in a .5ml centrifuge tube. Dissolve using 50ul the resin beads in water and then add 50ul of Acetonitrile; vortex.

2. Figure out which standard you'll need to calibrate the instrument using an external calibration first. Do this by picking out a standard that will bracket your sample mw.

Standard	Amount of sample (pm/ul) needed on steel target	Dilution (sample to matrix)
Insul 1 6,800mw	150	1:1
Insul 2 10,987mw	200	none

3. First spot beads and water on the steel target. For each sample or standard place 2 spots of beads and water; let air dry. Make up the standard and sample using the appropriate dilution. Next place 1.0ul of matrix that you made up and spot 1.0 ul on the steel target. Then quickly spot 1.0ul of your samples and standards with the appropriate dilution you prepared. Dry the spots with warm air using the heat gun near the sink.

Use the sample plate sheet as a guide and write down where you put your sample.

4. After the sample has dried, place the steel target in the sample inlet chamber with the letters A-P (top to bottom) and spots on the plate facing you. On the computer screen, you'll see the "Automatic probe introduction" box, click on "probe in".

   DO NOT DO ANYTHING ON THE COMPUTER UNTIL THE PROBE HAS STOPPED!

   Wait until the "ready" light is green and go to the spot of interest.

5. In the "xacq control panel" you'll see "Par. Win" and this is where you choose your parameter file to run your DNA or RNA sample.

   a) SK6K-medium-neg

   Double click on the method you have highlighted and click "apply". The information is sent to the digitizer and then you can close the window. (The above methods are all positive ion mode).

6. Turn on the high voltage by going to the "XACQ 4.0.1" Box and click in the top left box next to "HV". Wait for the IS/1 and Lens voltage to come up to the proper value.

7. Start with your standard first to calibrate the instrument. Clear the previous spectrum in the "TOF real time display"

window. Start the laser in the “xacq control panel” by clicking on the “start/stop” button. You want to start with a high laser attentuation and go lower gradually.

8. Once you see the standard peaks, click on “calib” in the “TOF real time display window” (bottom right). For example, INSUL 1 6800 mw and 3400 doubly charged species, and for the HPA peak, 276 mw. You will have to “add peak” in the calib window and manually add these values while you pick the correct peaks and label them. Once all the peaks are picked, you must click on “calibrate” and “accept”, for the new calibration to be effective. All standard peaks should be properly labelled. Next, go to your sample spot and see your results! This is an external calibration.