## Before you get to the instrument

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| ☐ | 1. Calibration is on plate |
| ☐ | 2. Calibration covers the detection range to be used |
| ☐ | 3. Blank is on plate |
| ☐ | 4. Samples are on plate |
| ☐ | 5. At least one dilution for each sample/matrix combination is also present on plate |
| ☐ | 6. All samples and their dilutions are no more than 2 spots away from the calibration (in any direction) |

## When you get to the instrument

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| ☐ | 7. Load the plate |
| ☐ | 8. Start the LSU MSF software and follow on screen instruction to start the Bruker software |
| ☐ | 9. Load your method. If this is the first time you load this method, save it outside the Standard Method folder and proceed further. If this is not the first time and you executed the steps below already, got to point #17 |
| ☐ | 10. Wait for instrument to complete loading sequence. Then, click on sample carrier tab and set the mode to “Off” |
| ☐ | 11. Make sure that “Use Target Profile” and “Use Focus Adjustment” are selected |
| ☐ | 12. Click on “Generate Now” and wait for the instrument to finish operations |
| ☐ | 13. Click on the Laser Tab |
| ☐ | 14. From the application drop down menu, select “Imaging 20 µm” |
| ☐ | 15. Click on the “detection Tab” and set the mass range appropriately for your experiment |
| ☐ | 16. Make sure the Sample rate is set to “5.00” |
| ☐ | 17. Using the target outline, click on the calibration spot and select a suitable area to acquire spectra |
| ☐ | 18. Set the energy slider to 25 % (standard value for polyalanine and CHCA calibration) |
| ☐ | 19. Set the number of shots to 1000 |
| ☐ | 20. Click on the calibration tab and select “Polyalanine Na adduct Positive” (or the version with the CHCA peaks, if appropriate) |

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| ☐ | 21. Click on start and observe the signal. |
| ☐ | 22. If you see the polyalanine peaks and they have an intensity between 1 and 8*10^4, proceed to step #26 |
| ☐ | 23. If you did not see a good signal (or just baseline), click on the “Sample carrier” tab and the press “Adjust now” to refocus the laser |
| ☐ | 24. After the procedure is done, select a new area on the calibration spot and repeat step #21 and #22. Remember to set the energy to 25%. |
| ☐ | 25. If you see the expected signal, proceed further. If not, stop the experiment and contact the facility. |
| ☐ | 26. Select the calibration tab (if it is not already selected) and click on “Automatic Assign”. If the peaks are assigned correctly, you will see several peaks assigned and the standard deviation values at the bottom should populate with a before and now value. For reference, a correct polyalanine calibration should give you a “now” value of < 5 ppm |
| ☐ | 27. Click on the peak selection cursor  and select one of the peaks assigned |
| ☐ | 28. Click on the information button  and check the resolution of the peak and its S/N ratio. For reference, peaks in the 1200-1400 range should have an S/N >100 and a resolution between 15,000 and 20,000. If you cannot achieve this |
| ☐ | 29. (optional) Save the calibration |
| ☐ | 30. If you reach this point, the instrument is calibrated correctly, and it is calibrated to specs. Remember that different matrixes require different energies. For example, if you use DCTB, start at an energy of 10% and increase it by increments of 1%. All spectra should have an intensity as close as possible to the one of the calibration spectrum. |