Combine with General Plate Maintenance and Prep

- 1. Wash MALDI plate:
  - Rinse the plate thoroughly with 100% methanol. Wipe the plate dry with a Kimwipe. Repeat.
  - Rinse the plate thoroughly with 100% acetone. Wipe the plate dry with a Kimwipe. Repeat.
- 2. Handle plate on the sides with Kimwipe to prevent contamination
- 3. Cover plate with a plastic lid to protect from dust until use.
- 4. Prepare a worksheet (A-H/1-12) indicating sample placement on the MALDI plate. For optimum measurement, place sample in 2 wells each, also include 2 standard wells and 2 blanks.

## Preparation of Samples (Sample dependent\*)

# Metallic Ions:

1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.

Options for matrix solutions (depending on specific sample):

- 10 mg Universal Matrix solution (comprised of DBH/CHCA/SA) dissolved in 1 mL of 50% ACN /50% HPLC water
- 10 mg dihydrobenzoic acid (DHB) dissolved in 1 mL of 50% ACN /50% HPLC water.
- 10 mg  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) dissolved in 1 mL of 50% ACN/50% HPLC water.
- 10 mg sinapinic acid (SA) dissolved in 1 mL of 50% ACN /50% HPLC water
- Binary matrices: 10 mg each matrix material (a total of 20 mg matrix) dissolved in 1 mL of 50% ACN /50% HPLC water
- Tertiary matrices: 10 mg matrix (a total of 30 mg matrix) dissolved in 1 mL of 50% ACN /50% HPLC water
- 2. To load the sample, spot 1 µL of the matrix mixture onto a stainless-steel plate on sampling locations
- 3. Load 2  $\mu$ L od sample onto each spot.
- 4. Allow sample plate to dry

# Polymers::

1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.

Options for matrix solutions (depending on specific sample):

- 10 mg Universal Matrix solution (comprised of DBH/CHCA/SA) dissolved in 1 mL of 0.1% TFAin/50% ACN/50% HPLC water.
- 10 mg dihydrobenzoic acid (DHB) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
- 10 mg α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in 1 mL of 0.1%TFAin/50% ACN/50% HPLC water.
- 10 mg sinapinic acid (SA) dissolved in 1 mL of 0.1% TFAin/50% ACN/50% HPLC water.
- Binary matrices: 10 mg each matrix material (a total of 20 mg matrix) dissolved in 1 mL of 0.1% TFAin/50% ACN/50% HPLC water.
- Tertiary matrices: 10 mg matrix (a total of 30 mg matrix) dissolved in 1 mL of 0.1% TFAin/50% ACN/50% HPLC water
- 2. Dissolve 10 mg sodium trifloroacetate in 1 ml of 50% ACN/0.1% TFA in distilled water.

- 3. Dissolve 10 mg solid sample in 1.0 mL of 0.5% TFA in HPLC water (or solvent that best dissolves material)
- 4. To load the sample, spot 1 µL of the matrix mixture onto a stainless-steel plate on sampling locations
- 5. After the spots dry, spot  $1 \mu L$  NaTFA solution on top of the spots.
- 6. Load  $2 \mu L$  polymer sample onto each spot.
- 7. Allow sample plate to dry

## Biological:

1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.

Options for matrix solutions (depending on specific sample):

- 35 mg of 3-hydroxypicolinic acid (3-HPA) in 500 microL 70/30 ACN/water
- 35 mg 2',4',6'-Trihydroxyacetophenone (THAP) in 500 microL 70/30 ACN/water
- 2. Dissolve 25 mg Dibasic ammonium citrate in 500 microL 50/50 ACN/water
- 3. To load the sample, spot 1 µL of the matrix mixture onto a stainless-steel plate on sampling locations
- 4. After the spots dry, spot 1 µL Dibasic ammonium citrate solution on top of the spots.
- 5. Load 2  $\mu$ L sample onto each spot.
- 6. Allow sample plate to dry

### Small Molecules:

1. Prepare Matrix Solution: Prepare the Matrix Solution just prior to use. Solution shelf life maximum is 2 hours at room temperature.

Options for matrix solutions (depending on specific sample):

- 35 mg 2',4',6'-Trihydroxyacetophenone (THAP) in 500 microL 70/30 ACN/water
- 2. Dissolve 10 mg α-Cyclodextrin in 1 mL water
- 3. Mix 120 microL of aqueous  $\alpha$ -Cyclodextrin with 20 microL of THAP solution.
- 4. To load the sample, spot 1  $\mu$ L of the matrix mixture solution (CD+THAP) onto a stainless-steel plate on sampling locations
- 5. Then load 2  $\mu L$  sample onto each spot.
- 6. Allow sample plate to dry

### Sample Analysis Procedure (General)

1. Open Biotech Launchpad software and open Maldi -MS icon



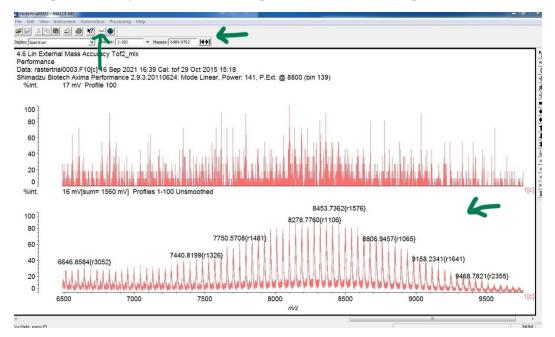
- 2. In the Acquisition Window go to Exp. Tech. tab and press "Open Door" to open Maldi Door
- 3. Take out previous sample plate if there by holding plates on sides.
- 4. Insert sample plate by holding plate on the sides and not touching samples.
- 5. Once placing the plate inside, press "Close door" <u>Allow vacuum pressure to equilibrate before</u> <u>preforming experiments.</u>
- 6. Set tuning mode to Linear, Linear\_neg, Reflectron, Reflectron\_neg, Reflectron\_HiRes, or Reflectron\_HiRes\_neg. (Depending on what mode works best for your specific sample)
- 7. Set the mass range of interest.
- 8. Set instrument in "Operate" mode

Firing Exp. Tech. Auto Quality Storage Slide Raster Tuning	-
Tuning mode: Linear	
Mode: Standby Operate	
Mass Range: 200-10000	
Max Laser Rep Rate: 50.0	
CID: Enable	
11	
K K	
Pump Open Door Close door	Vent

- 9. In the Acquisition window go to Firing tab
- 10. "Profiles" should always be set to 200
- 11. Set "Power" to at least 90. Adjust Power accordingly Higher power may increase quality of data.
- 12. Set "**Shots**" accordingly. Less shots take less time but more shots with result in better quality data.
- 13. Check "Pulsed Extraction optimized at (Da):" and enter in Mass m/z value predicted.
- 14. To select all wells hit blue check mark. To unselect all wells hit blue x.
  - For automatic or manual mode:
    - a. For automatic sampling choose which well(s) to analyze by clicking circles in yellow box and click the red arrows below. Make sure yellow button with x across is not selected.
    - b. For manual sampling click the small yellow box with the x and move location using black arrows and dragging window. Or right click yellow box and enter in well location of choice.
- 15. When ready to analyze samples hit "FIRE". Name file.

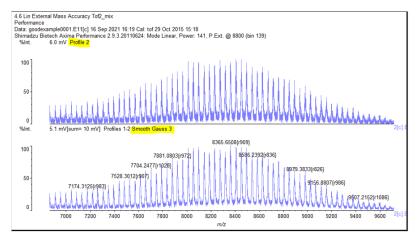
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Power: 141	₫)_	- <u>ie&gt;i&gt;ie †</u> 1	141 Hold 888	
Profiles: 200	per sample	n		
Shots: 5	accumulated per profile	<mark>ه د</mark>	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	
Ion Gate (Da): Of	f On Blank 700.0			
□ Low Mass Zoom**	up to mass (Da): 525.0	<u>+</u>		
✓ Pulsed Extraction	optimised at (Da): 8800			· · · · · · · · · · · · · · · · · · ·
Syspend	Abort		11 <u></u>	
Resume	Store FIRE Clear d	ata		
	es to File:			Browse

- 16. Set mass range of interest. Or also use the mouse to close up on range of interest.
- 17. To stop the run early hit Abort. Or hit Suspend and then Resume to pause the run.



The spectra at the top and bottom of the screen are for the same sample but are types difference of spectra. The difference between the two spectra is that the one on top is of a profile. In this case below profile number 2 is the last profile captured. The spectra at the bottom is the processed spectra after processing parameters have been applied.

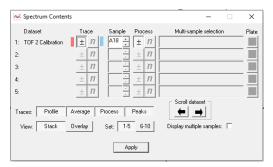
The spectra on the top states a specific profile, "Profile 2" highlighted in yellow. The spectra on the bottom states" Smooth Gauss 3" highlighted in yellow which means the smoothing method is Gaussian and a smoothing filter width of 3 is applied.



If you want change which type of spectrum you see you can do this under View < Display Options.

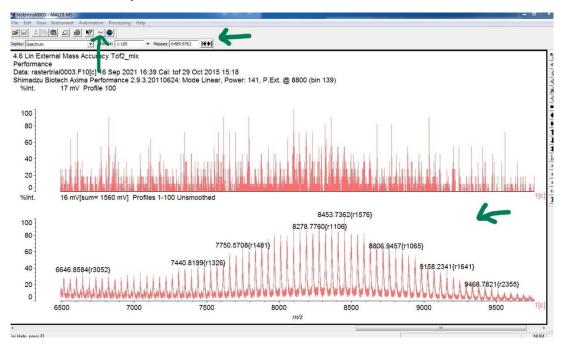
🔣 TOF 2 Ca File Edit	libration for Reflectron- Pulsed Extr View Instrument Automation	action at 15000Da_0001 - MALDI-MS Processing Help
	<ul> <li>✓ Toolbar</li> <li>✓ Status Bar</li> </ul>	Display: Spectrum   Profiles: 1-100  Masses: 700-4000
Data: Shim	Dasic Parameters	for Reflectron- Pulsed Extraction at 15000Da_0001.A18[c] a Performance 2.9.3.20110624; Mode Reflectron, Power; 7
	Options	, · · · · · · · · · · · · · · · · · · ·
%lı	Instrument Status	Profile 500
10	Database Viewer Tile Manager Events Logged	
	0	

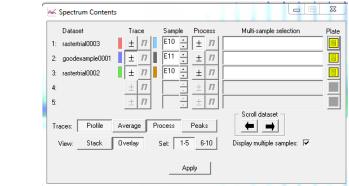
Then click on the type of spectra (profile, average, process, and/or peaks) to see or hide and hit apply for the changes to take effect.



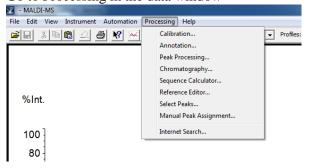
### Data Analysis Procedure

- 1. Press Spectrum Contents button in Data window
- 2. Select which data to look at in Spectrum Contents window.
- 3. Scroll through data in Spectrum Contents window with black arrows.
- 4. In the Spectrum Contents window, choose to Profile or Average data/ Process or mark Peaks/ view Stack or Overlay





5. Go to Processing in the data window



6. In the Processing tab, go to Select Peaks for full list of peaks in selected peak range

Select Peaks		<u> </u>
± Dataset: 1: goodexample0001		
Automatic Automatic Masses: 4000 6000 Peaks: 25 ÷ List peaks Manual Mass: 5000.0000 t↓1 Edit Mass List Insert	Mass 400817 404876 404972 405038 405130 405168 409565 413722 413875 413875 413875 4138258 422500	A 11
Delete > Selected mass -	4226.44	Ψ F

7. In the Processing tab, go to peak processing more accurately identify peaks qualities

Ī	Peak Processing		
	Dataset: 1: goodexample0001	Trace: ± 11 Sample: E11 ↔	
	Peak Cleanup Peak Picking Peak	Filtering	1
	Scenario:	Isotopically resolved peaks	ſ
l	Isotopically Resolved Peal	ks Settings	
	Peak width:	1 + chans + +	I.
l	Smooth data		
l	Subtract baseline		
l	Peak detection method:	Threshold - 25% Centroid 💌	I.
	Threshold 25% Centroid P	eak Detection Settings	L.
	Double Threshold:		I.
	Threshold type:		I.
	Threshold offset:	0.500 mV 11	V
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	Apply to Tiles:	Apply to Samples:	
		<u>C</u> lose <u>Apply</u>	
	💚 Iso.		Ľ.

8. To understand sequence, use sequence calculator in Processing tab. Enter in N-Terminus and C-Terminus (the end groups), and the cation.

File Edit View Settings
•
Itte A A A A A A A A A A A A A A A A A A
Nuterminus V Averane V 5000
Ceterminus:     Fragmentation: Singly charged
Generate Peak Markers
Length: 0

### **Database Look Up**

1. In Biotech Launchpad click on the compound database



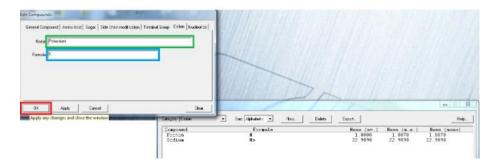
2. Approximate masses can be looked up in general or by category.

ategory: All 🗨 Sort: Alphabetic	▼ New Delete	Export		Help
Cat Compound	Formula	Mass (av.)	Mass (m.a.)	Mass (mon
Gener ACTH_fragmenti_xvii:ACTH_xvi		2093.4484	2093.0817	2092.0789
Gener ACTH_fragmentxviii_xxxix:ACT	C112 H165 N27 O36	2465.7087	2465.1937	2464.1911
Prot Acetamidomethyl:Acm	C3 H6 N O	72.0870	72.0449	72.0449
Prot Acetyl:Ac	C2 H3 O	43.0453	43.0184	43.0184
Prot Adamantyloxy:Adao	C10 H13 O	149.2134	149.0966	149.0966
Amino Alanine:Ala:A	C3 H5 N O	71.0790	71.0371	71.0371
Gener Aldolase:Ald	C1733 H2773 N489 O525 S11	39211.8752	39210.9227	39187.2250
C- Amide	H2 N	16.0227	16.0187	16.0187
Gener Angiotensin i:Angi	C62 H89 N17 O14	1296.4987	1295.6769	1295.6775
Gener Angiotensin ii:Angii	C50 H71 N13 O12	1046.1972	1045.5340	1045.5345
Gener Angiotensin iii:Angiii	C46 H66 N12 O9	931.1085	930.5071	930.5076
Gener Anthranilic_acid:Anth	C7 H7 N O2	137.1384	137.0476	137.0477
Amino Arginine:Arg:R	C6 H12 N4 O	156.1881	156.1010	156.1011
Amino Asparagine:Asn:N	C4 H6 N2 O2	114.1041	114.0429	114.0429
Amino Aspartic_Acid:Asp:D	C4 H5 N O3	115.0887	115.0269	115.0269
Gener Azothiothymine:Att	C6 H5 N3 O S	167.1900	167.0153	167.0153
Prot Benzoyl:Bz	C7 H5 O	105.1164	105.0340	105.0340
Prot Benzyl:Bzl	C7 H7	91.1331	91.0547	91.0548
Prot Benzyloxy:Bz10	C7 H7 O	107.1324	107.0496	107.0497
Prot Benzyloxycarbonyl:BcZ	C8 H7 O2	135.1427	135.0445	135.0446
Prot Benzyloxymethyl:Bom	C8 H9 O	121.1594	121.0653	121.0653
Gener Bovine_insulin:Ins	C254 H377 N65 O75 S6	5733.5815	5732.6062	5729.6009
Gener Bovine_serum_albumin:Bsa	C2935 H4582 N780 O899 S39	66430.0694	66427.4319	66386.5910
Gener Bradykinin:Brad	C50 H73 N15 O11	1060.2273	1059.5609	1059.5614
Conon Bradukinin fragmenti mii Bra	C35 H52 N10 09	756 8620	756 3915	756 3919

3. To add Cations or another category, choose "Cation" category in the dropdown menu and add "New"

ategory: Cation	Soit Alphabelic	New. Delete Export.	Heit
Compound	Formula	Creates a new compound entry 19 (50 .)	Ness (a.a.) Ness (aono)
Proton	H	1.0080	1.0078 1.0078
Sodium	Na	22.9898	22.9898 22.9898

5. Type out the name and formula of the cation to add and click okay



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