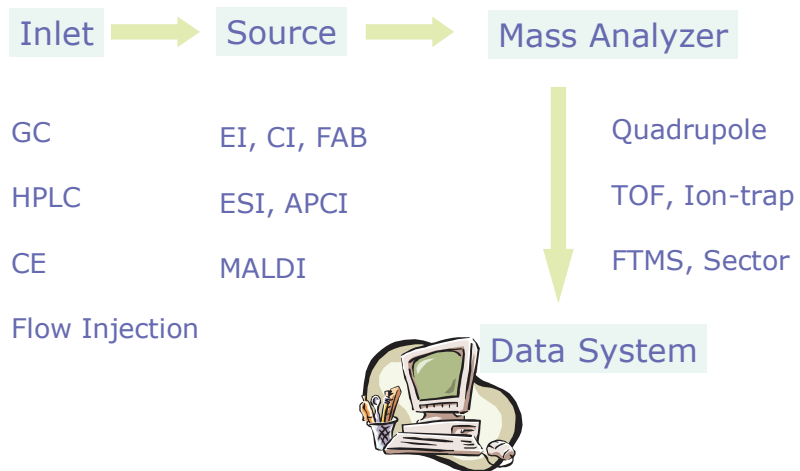


Applications of MALDI-TOF and TOF/TOF

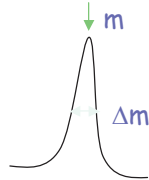
Mass Spectrometry: Components



Modern Mass Spectrometry: Terms



Resolution: $m/\Delta m$



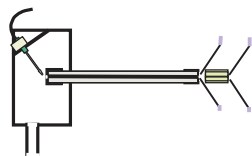
Tandem Mass Spectrometry (in Space and in Time)

MS1 → MS2 → MS3 MS/MS
select fragment analysis

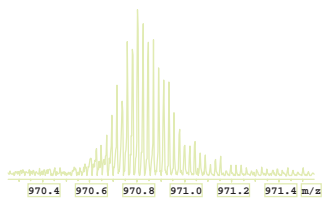
MS1 → MS2 → MS3 → MS4 → MS5 MS/MS/MS
select fragment select fragment analysis

Bruker Daltonics

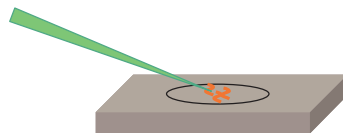
Modern Mass Spectrometry: Sources



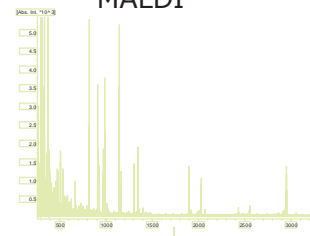
Electrospray



Multiply Charged



MALDI



Singly Charged + Matrix

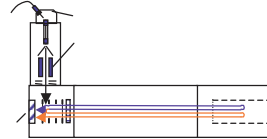
Bruker Daltonics

Modern Mass Spectrometry: Analyzers

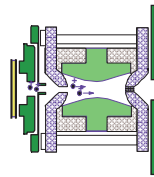


Quadrupole
+ Cheap, Robust

- Low Resolution



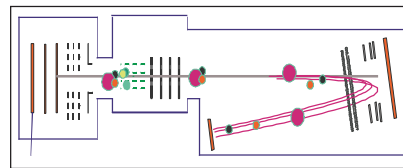
Quadrupole TOF



Quadrupole Ion Trap

+ Moderate, Robust

+ MSⁿ



TOF/TOF

+ Higher Resolution

+ MS/MS capability

+ Sensitivity

Bruker Daltonics



Proteomics Analysis with MALDI-TOF/TOF

Most Sensitive, versatile, accurate means

Bruker Daltonics

Gel Based Quantitation and ID

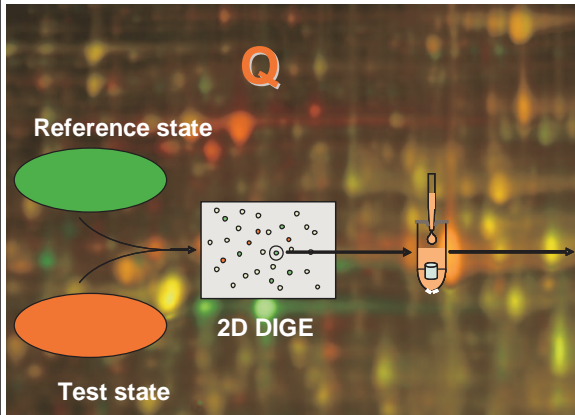


Physiology,
staining

Protein
separation,
quantitation

Gel
processing

Mass
spectrometry



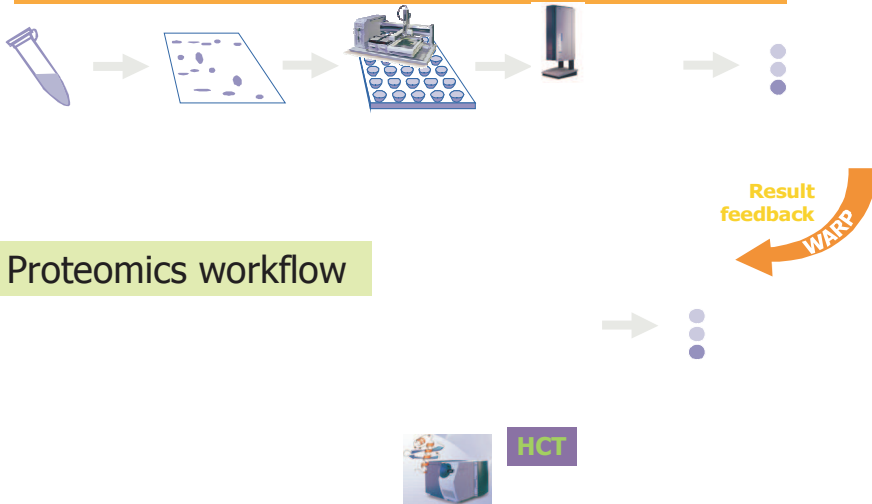
1. Quantitation of protein spot
2. Pick and Digest
3. MS analysis of "relevant" proteins

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2D Gel based Proteiner workflow

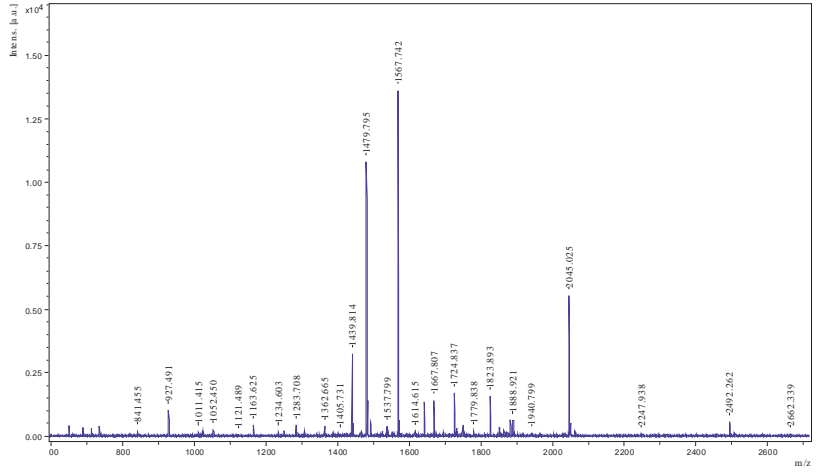


PROTEINER workflow using WARP result feedback



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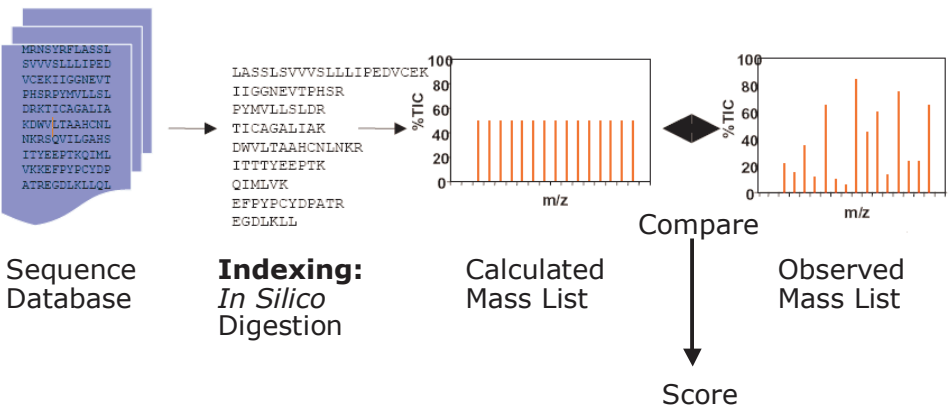
Tryptic digest peptide mass fingerprint of BSA



17.04.07

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Database Search of Protein Mass Fingerprint



17.04.07

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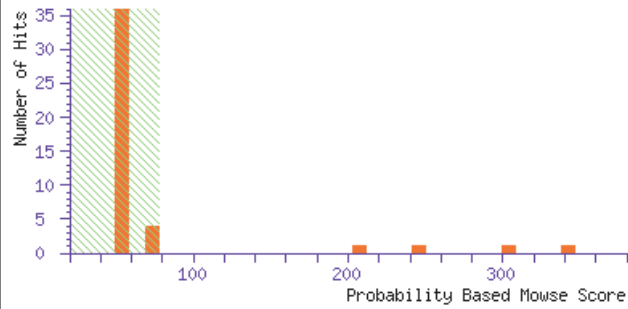
Annotated MS spectrum of BSA tryptic digest



Top Score : 342 for Mixture 1, AAN17824 + BAE23748

Probability Based Mowse Score

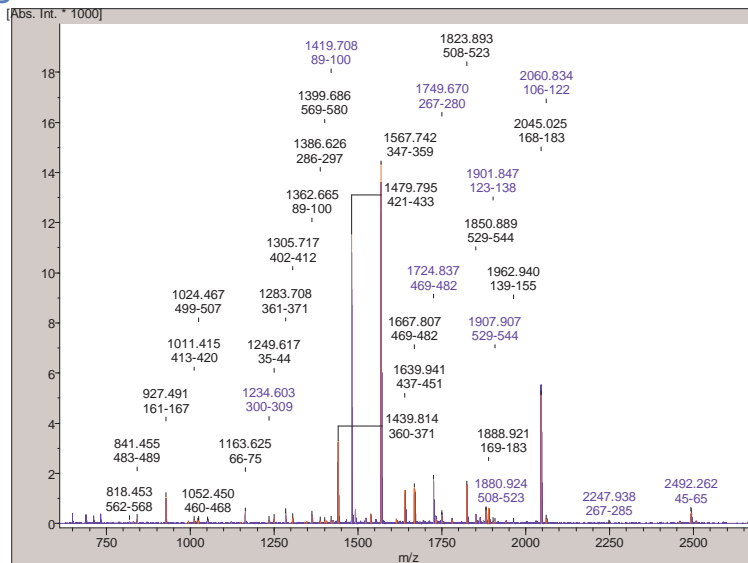
Protein score is $-10 \cdot \log(P)$, where P is the probability that the observed m Protein scores greater than 78 are significant ($p < 0.05$).



17.04.07

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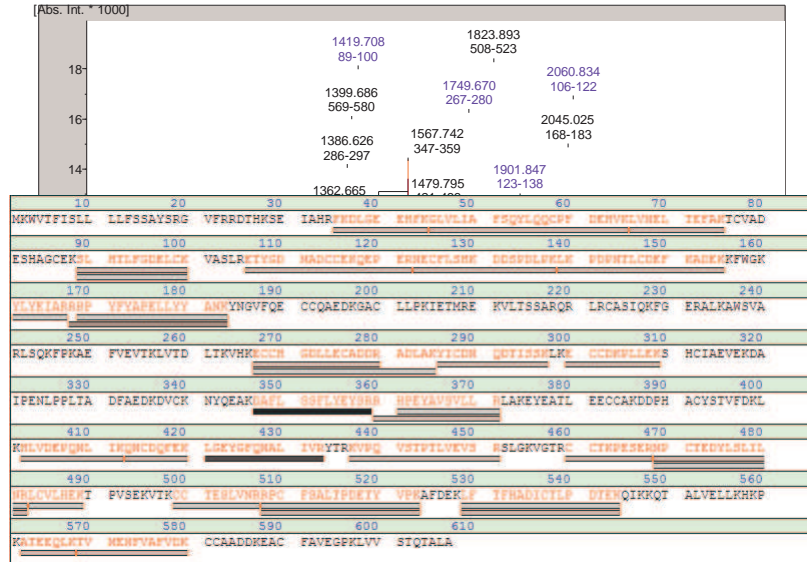
Annotated MS spectrum of BSA tryptic digest



17.04.07

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Annotated MS spectrum of BSA tryptic digest



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MS/MS Fragment Ions from Various Methods

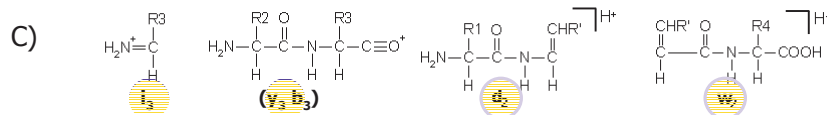
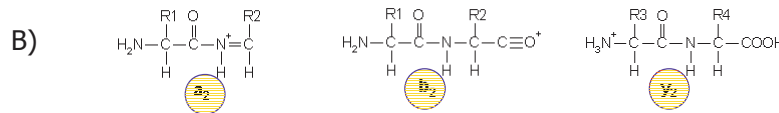
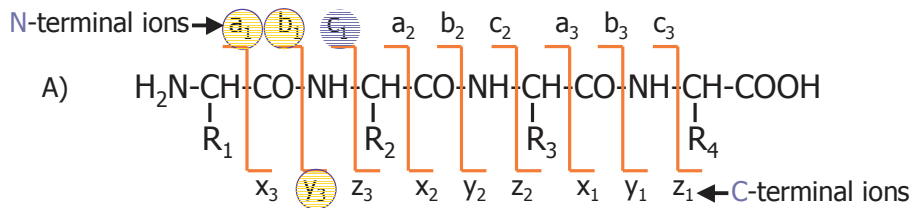


LID, PSD

ISD

low energy CID

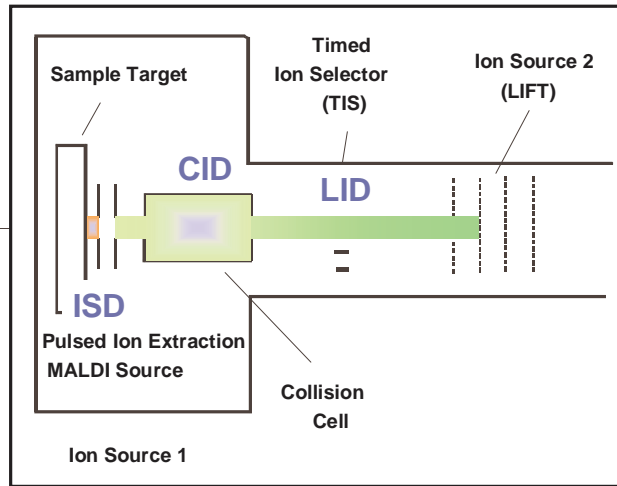
high energy CID



Ehlmann et al. (1988) JTMSP 86:137

Bruker Daltonics

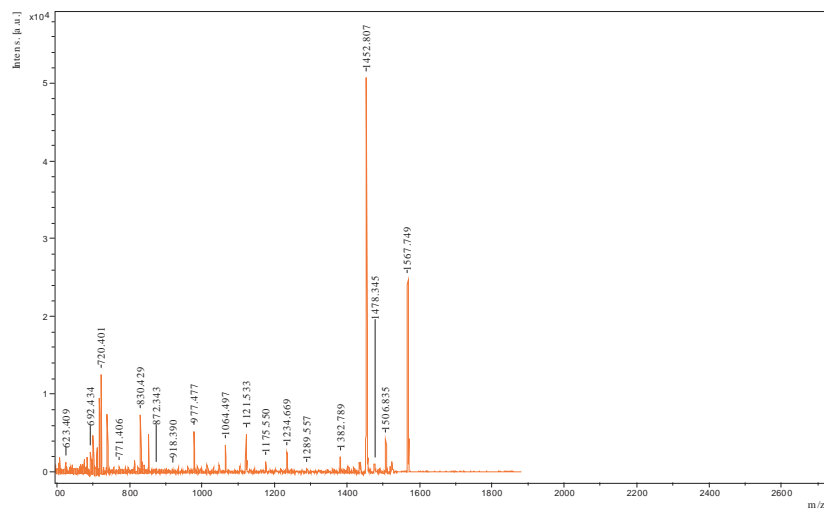
Recent Innovations in MALDI-TOF/TOF: In-source decay of intact proteins



17.04.07

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TOF/TOF spectrum of BSA m/z 1567.7



17.04.07

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In Source Decay

Sequencing of intact protein with MALDI-TOF

Bruker Daltonics

Protein Characterization with In-source decay



Pro

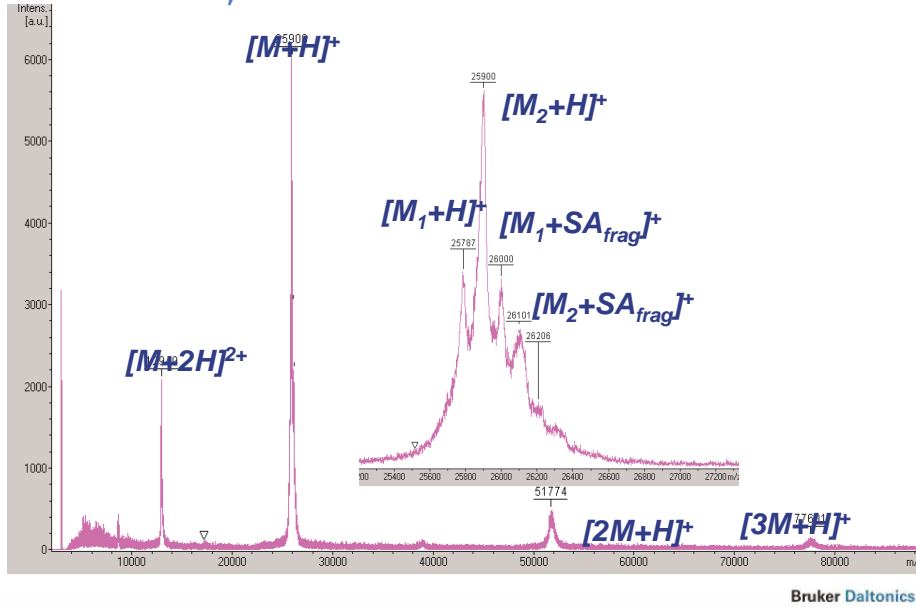
- Simple
- Accurate
- Fast
- Sequencing of N-terminal blocked proteins
- No digestion required

Con

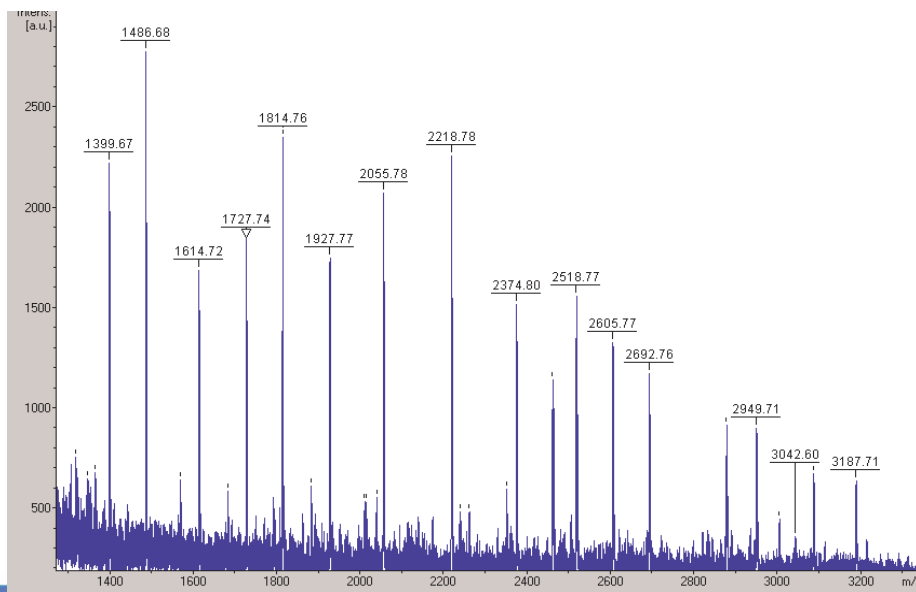
- Requires high concentration of samples (5-20pmol)
- Pure Protein

Bruker Daltonics

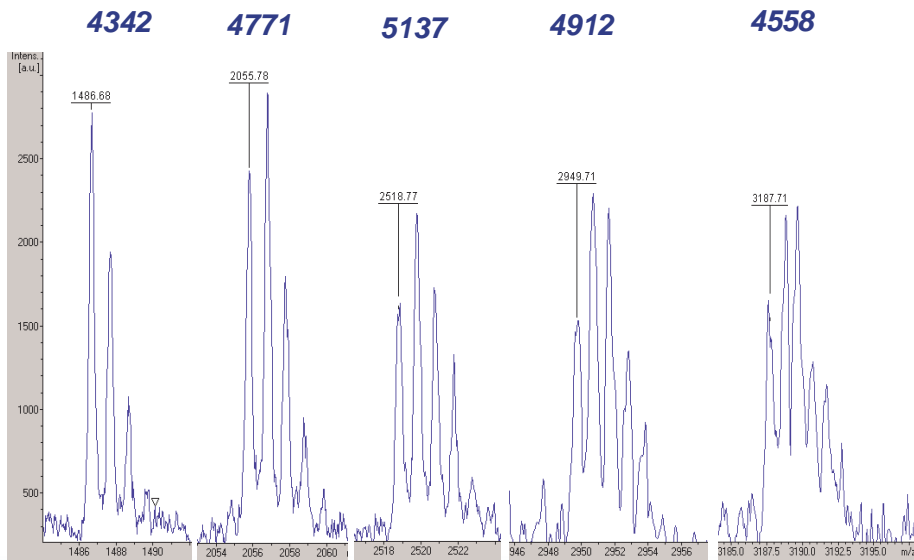
RCM, intact protein, linear mode, external calibration, Matrix: SA



Ref-ISD of RCM, matrix: Sinapinic acid



Ref-ISD of RCM, matrix: Sinapinic acid, Resolution



Ref-ISD of RCM, matrix: Sinapinic acid. DeNovo Sequencing and Blast search



Screenshot of the BioTools software interface. The top panel displays a mass spectrum plot with intensity (Abs. Int. * 1000) versus m/z. The x-axis ranges from 1200 to 3000 m/z. The y-axis ranges from 0 to 8 intensity units. Several peaks are labeled with their m/z values: 1388.673, 1814.716, 1727.136, 1807.774, 2066.793, 2216.777, 2461.767, 2692.757, 2374.759, and 2835.772.

The bottom panel shows the MS/MS analysis results. The 'MSMS Analysis' section displays the following data:

MSMS fragments	MSMS Analysis
32	Above Threshold: 16, Assigned: 0, Not Assigned: 16
0.100000	Threshold: 500.000000, MH mono (exp): 1000.000000 (calc)
	Uncertain:
	(1399.6795)QLSLQYRSGSS(2692.757)

The 'Check Low Masses' section includes options for 'by ions', 'Distances', 'Create Tag', 'Search Tag', 'Filters', and 'Copy/Mascot SeqQuery'. The 'Errors' table shows the following data:

Errors	Acids	Start Mass	Sequence
0.5839	12	1399.672824	SLQSLYRSGSS
0.5839	12	1399.672824	SKLSLQYRSGSS
0.5839	12	1399.672824	SKLSLQYRSGSS
0.5839	12	1399.672824	SKLSLQYRSGSS
0.5475	12	1399.672824	QLSLQYRSGSS
0.5475	12	1399.672824	QLSLQYRSGSS
0.5475	12	1399.672824	QLSLQYRSGSS
0.5475	12	1399.672824	QLSLQYRSGSS
0.5475	12	1399.672824	QLSLQYRSGSS

The 'MS Blast homology search' section includes options for 'Copy/MS Blast', 'Open MS Blast', and 'Typic Frag'. The 'RapDeNovo Sequencing' section is also visible.

Ref-ISD of RCM, Blast search result



Summary:

Sequences producing High-scoring Segment Pairs: High Total
Score Score

```

/:swissnewIP00772|EIAA1_PIG Elastase 1 precursor (EC 3.4.2... 72 72 ✓
/:swissnewIQ28153|EIAA1_BOVIN Elastase 1 precursor (EC 3.4... 64 64 ✓
/:sptreml1Q6ISM6|Q6ISM6 Elastase 1, pancreatic. >/:sptre... 64 64 ✓
/:swissnewIQ28151|EIAA1_HUMAN Elastase 1 precursor (EC 3.4... 64 64 ✓
/:sptreml1Q5HLF0|Q5HLF0_HUMAN Elastase 1, pancreatic, is... 64 64 ✓
    
```

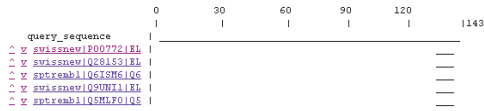
```

/:sptreml1Q9W702|Q9W702_PAROL Elastase 1. >/:sptreml1Q9... 60 60 □
    
```

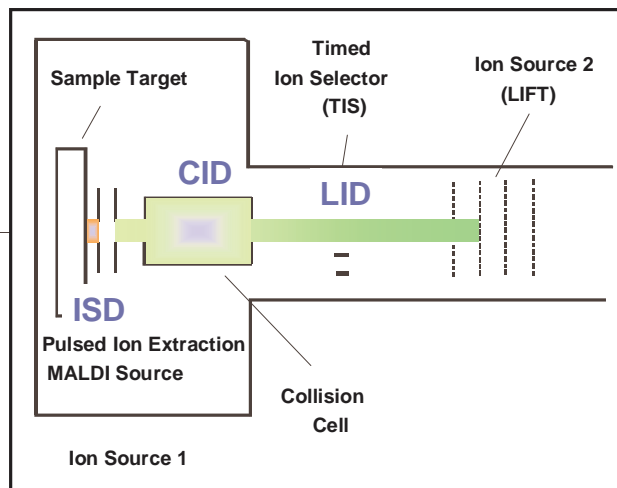
```

/:sptreml1Q76IG3|Q76IG3_SALSA Serine proteinase. >/:sptr... 58 58 □
/:sptreml1Q9W701|Q9W701_PAROL Elastase 2 (Fragment). >/:... 52 52 □
/:sptreml1Q8JMF9|Q8JMF9_SEHAB Glycoprotein (Fragment). >... 50 50 □
/:sptreml1Q9PFW1|Q9PFW1_CHICK Elastase 2 (Fragment). >/:... 46 46 □
    
```

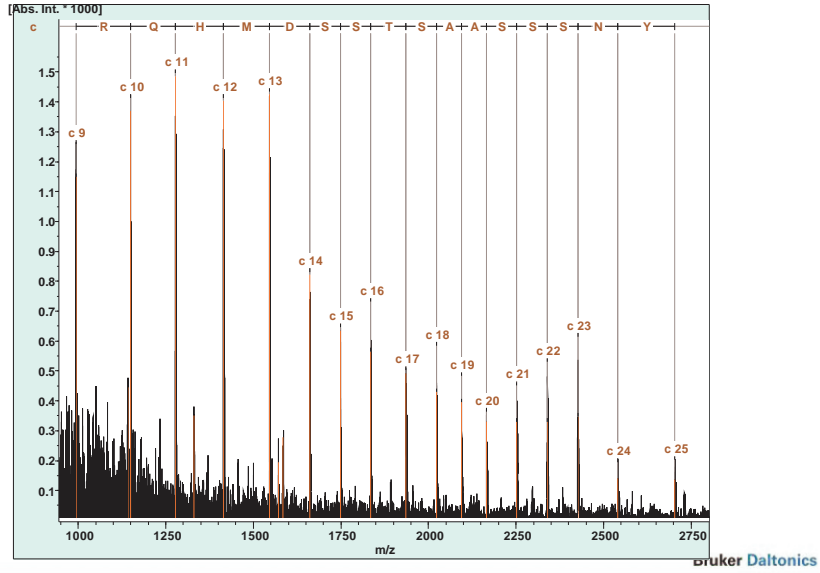
Query sequence and locally-aligned MSPs from database sequences



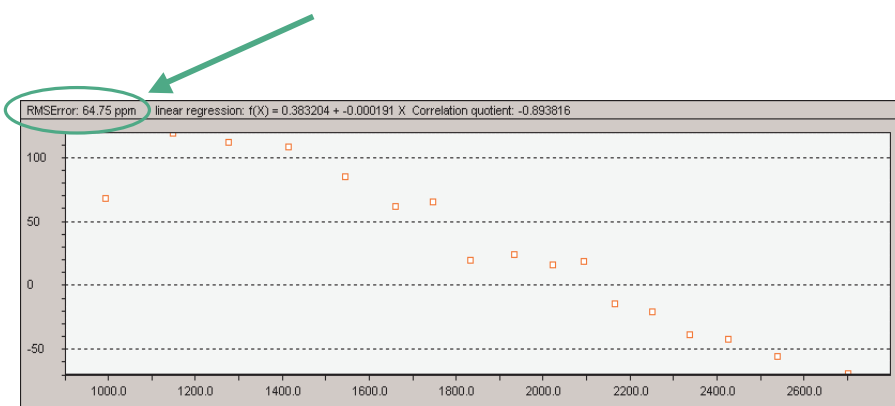
Recent Innovations in MALDI-TOF/TOF: In-source decay of intact proteins



RNase B, oxidized



RNase B, ISD error distribution (avg. masses)



Increasing the sequence coverage



RNAse B without reduction

KETAAAKFER QHMDSSTSAAS SSSNYCNQMM KSRNLT KDRC KPVNTFVHES
LADVQAVCSQ KNVACKNGQT NCYQSYSTMS ITDCRETGSS KYPNCAYKTT
QANKHIIIVAC EGNPYVPVHF DASV 12% seq. cov.

RNAse B after reduction (DTT, pH 8, 57° C 10')

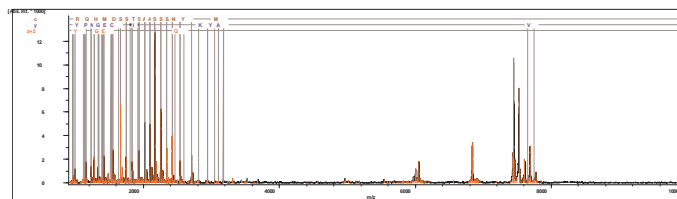
KETAAAKFER QHMDSSTSAAS SSSNYCNQMM KSRNLT KDRC KPVNTFVHES
LADVQAVCSQ KNVACKNGQT NCYQSYSTMS ITDCRETGSS KYPNCAYKTT
QANKHIIIVAC EGNPYVPVHF DASV 84% seq. cov.

Bruker Daltonics

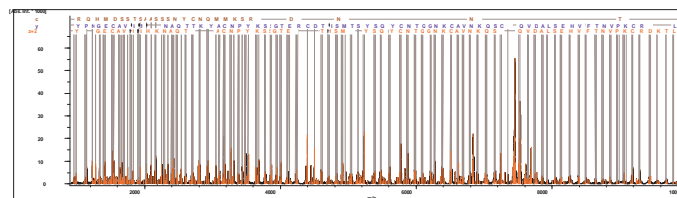
Increasing the sequence coverage



RNAse B without reduction

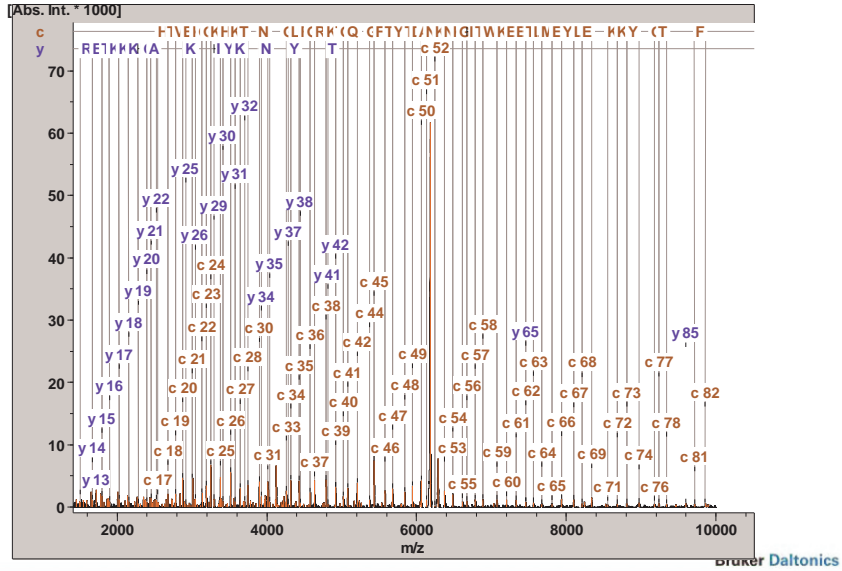


RNAse B after reduction (DTT, pH 8, 57° C 10')

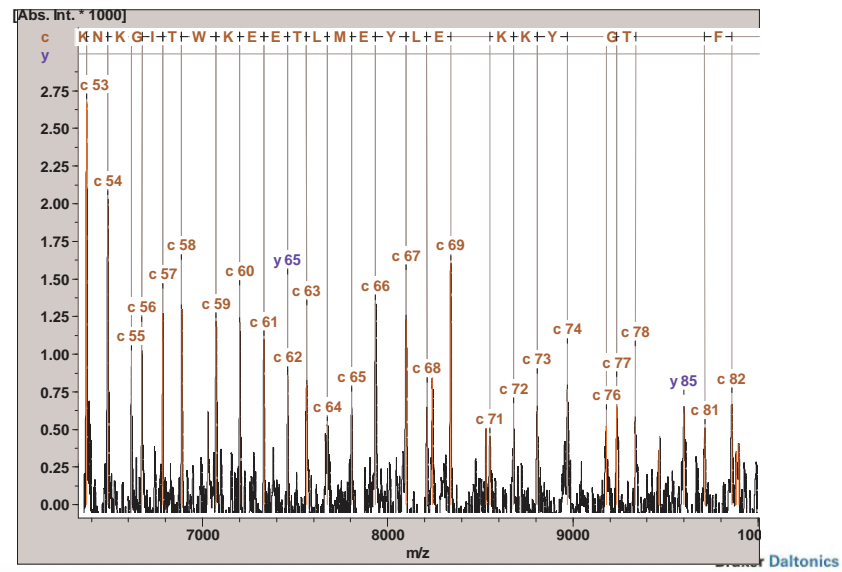


Bruker Daltonics

Cytochrome C (12.5 kDa), ISD, autoflex TOF/TOF



Cytochrome C (12.5 kDa), ISD, autoflex TOF/TOF



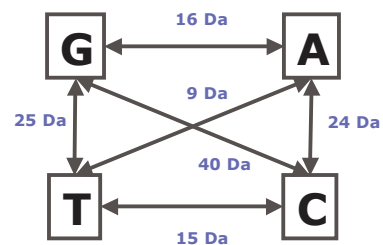
Analysis of Oligonucleotides

- Single nucleotide polymorphism, Genotyping
- QC of primer production

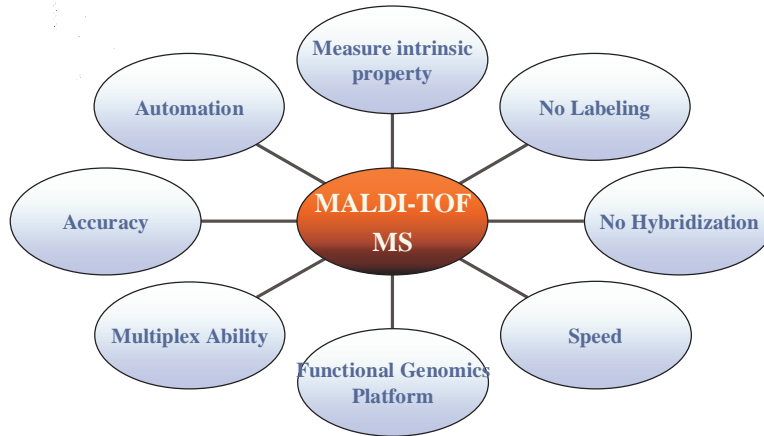
MASS of BASES



Simply measure the molecular weight!



Why MALDI-TOF MS based Genotyping ?



Bruker Daltonics

Gene vs Proteins



Functional Genomics

MALDI-TOF Mass Spectrometry

Proteomics



Differential Protein Patterns



Genomics

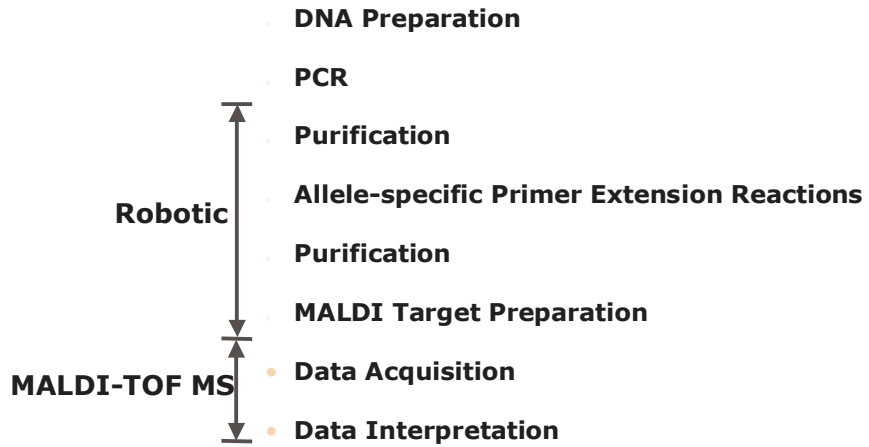


Underlying Genetic Polymorphisms

"The Missing Link"

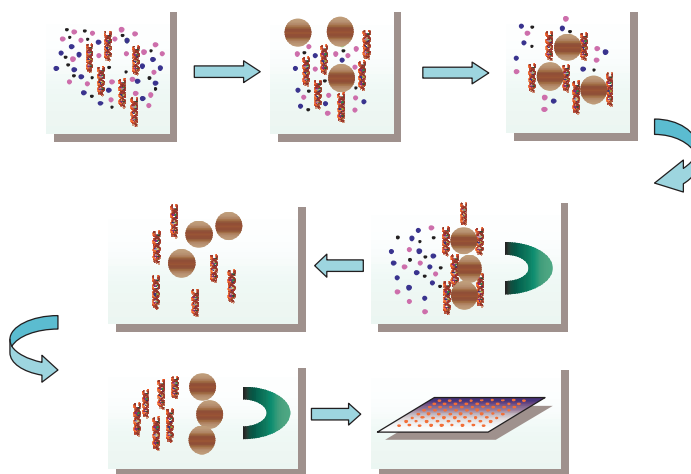
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Workflow of the SNP Genotyping Procedure



Bruker Daltonics

genopure: Magnetic Bead Purification System



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Allele-specific Primer Extension: PAI 1 4G/5G Polymorphism

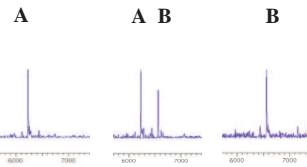


Template: 5' agagtcgggacacgtggg a 3'
 5' agagtcgggacacgtgggga 3'

Primer: 5' agagtcgggacacgtggg 3' 5933.8 Da

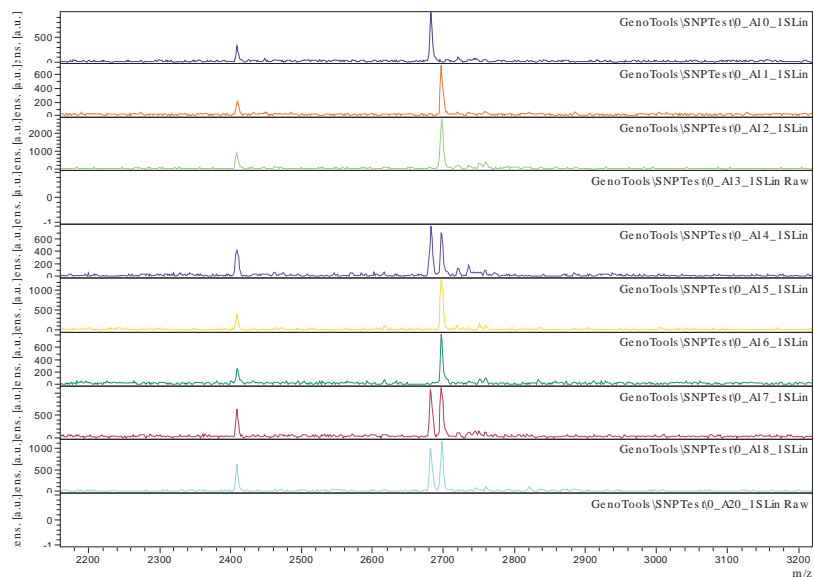
Extension: + ddATP
 + dGTP

Product A: 5' agagtcgggacacgtggg a 3' 6231.0 Da
Product B: 5' agagtcgggacacgtggg ga 3' 6560.2 Da



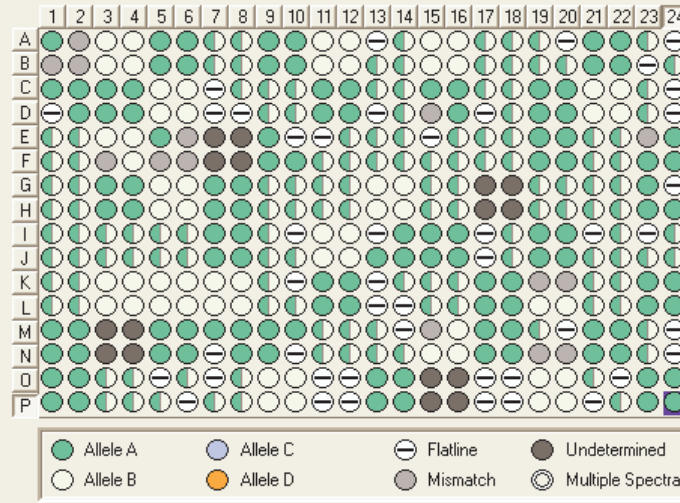
Bruker Daltonics

Single Nucleotide Polymorphism with MALDI-TOF: CETP



Bruker Daltonics

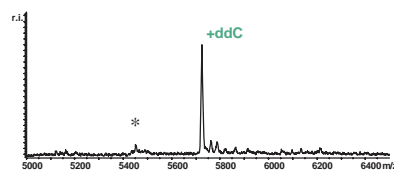
SNP test summary results: CETP



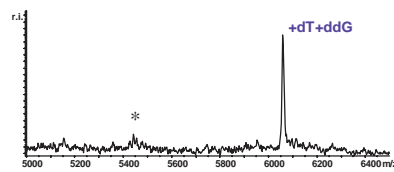
CCTAATC = 2409.55
CCTAATCC = 2682.73
CCTAATCT = 2697.74

Bruker Daltonics

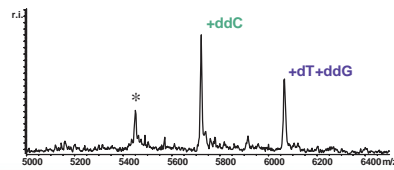
Factor 7-Polymorphism Arg353Gln



Arg/Arg



Gln/Gln



Arg/Gln

Template (sense)

... ctacc **g/a** gggcac ...

cccgtg ... Primer (antisense)

c cccgtg ... Extension (Arg)

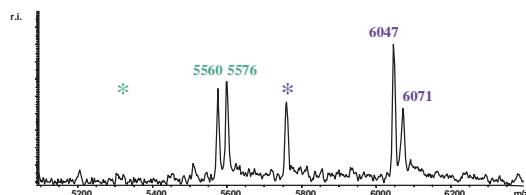
g t cccgtg ... Extension (Gln)

Bruker Daltonics

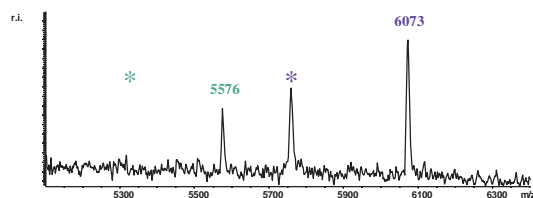
ColA1A double analysis



analysis from both directions with primers $\cdot +ddA/ddC$ and $\cdot +ddG/ddT$
internal calibration with primer \cdot



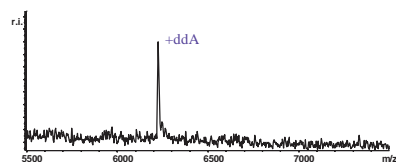
G/T heterozygous



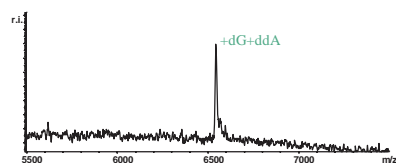
G/G homozygous

Bruker Daltonics

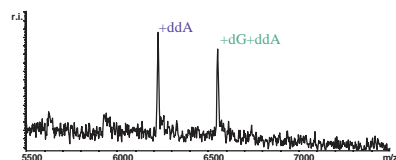
PAI 4G/5G Polymorphism



4G/4G homozygous



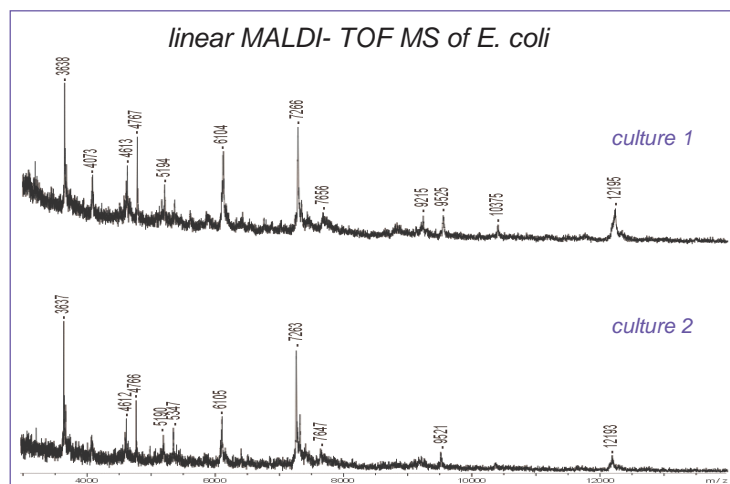
5G/5G homozygous



4G/5G heterozygous

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Direct MALDI-TOF-MS analysis of
microorganisms (bacteria)

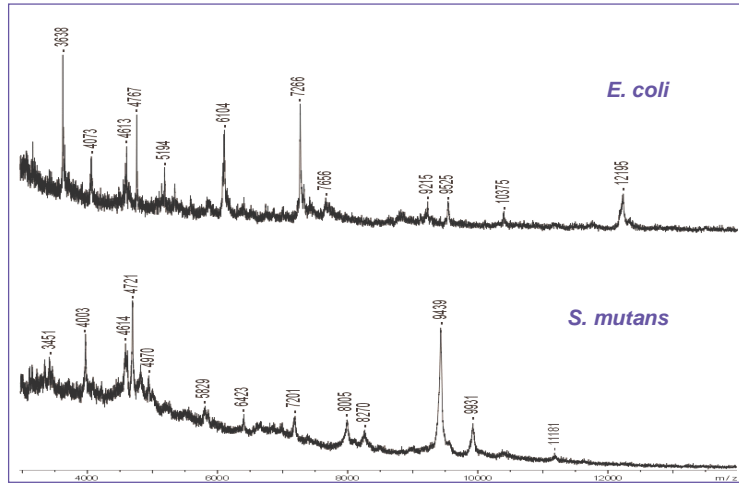


⇒ same species result in comparable spectra

MALDI-TOF-MS analysis of microorganisms (cont.)



Bacterial Typing



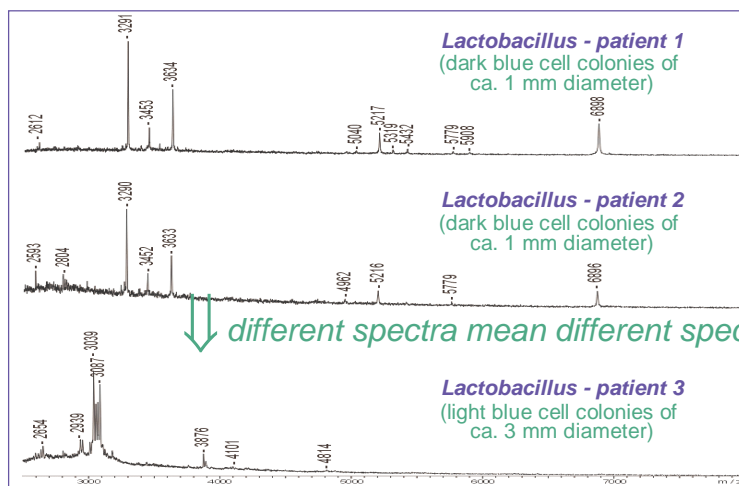
⇒ different species result in different spectra

Bruker Daltonics

MALDI-TOF-MS analysis of microorganisms (cont.)



Bacterial Typing



↓ different spectra mean different species

Bruker Daltonics

Applications of MALDI-TOF and TOF/TOF



Peptide MW measurement	1-4 kDa using CHCA or DHB
Proteins	Up to 100 kDa using Sinapinic acid or DHB
Oligosaccharides	2-3 kDa using DHB
Small molecules	<1000 Da, must contain chromophore
Oligonucleotide	40 kDa, using 3-HPA + Ammonium Citrate
Soluble proteins from microorganisms	•Various matrices are used.
Polymers	<ul style="list-style-type: none"> •In general 20 kDa, up to 100 kDa will require skills •PD ~ 1.5 max •Various matrices depending on solubility and structure of analytes

Bruker Daltonics

Detergents and their effect on MALDI



<http://prowl.rockefeller.edu/contam/deterg.htm>

Detergent	Class	Detergent	Class
n-octyl-glucoside	1	dodecylpoly(ethyleneglycolether) ₉ (Thesit)	4
n-dodecyl-glucoside	1	isotridecylpoly(ethyleneglycol ether) ₈	4
octanoyl-N-methylglucamide	1	CHAPS	4
decanoyl-n-methylglucamide	1	CHAPSO	4
n-dodecyl-beta-D-maltoside	2	n-dodecyl-N,N-dimethyl-	
octylphenolpoly(ethyleneglycolether) ₁₀ (Triton X-100)	3	3-ammonio-1-propanesulfonate	4
octylphenolpoly(ethyleneglycolether) ₇ (Triton X-114)	3	sodium dodecylsulfate (SDS)	4
polyethylene glycol (PEG 2000)	3		

1. no bad effect: may improve results in mixtures
2. little effect
3. spectrum quality & signal intensity reduced
4. spectrum suppressed: detergent must be removed

Bruker Daltonics