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Proteomik dengan *High-Resolution Mass Spectrometry (HRMS)* untuk Analisis Halal

Tri Joko Raharjo

KOMPAS.com NEWS EKONOMI BOLA TEKNO SAINS ENTERTAINMENT OTOMOTIF LIFESTYLE PROPERTI TRAVEL EDUKASI KOLOM IMAGES TV VIK

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Dalam Sehari, 1.200 Anjing Dikonsumsi di Kota Solo

Kompas.com - 21/02/2018, 13:22 WIB

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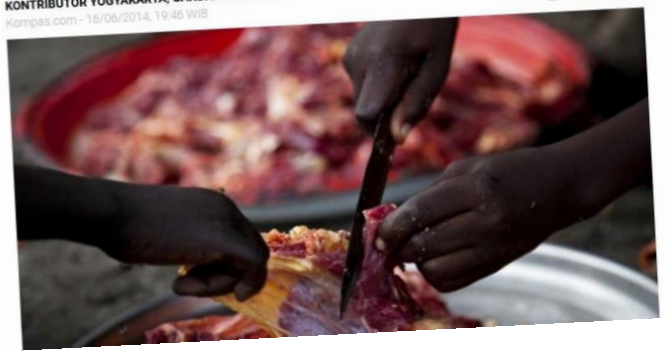
Kurang Pengawasan, Sebagian Daging di DIY Tercampur Daging Babi

KONTRIBUTOR YOGYAKARTA, GANDANG SAJARWO

Kompas.com - 16/06/2014, 19:46 WIB



The Daily Telegraph

Horse meat in burgers fo

DAILY Mirror

FREE Ladybird BOOKS

D FRAUD

CNN Indonesia Home Nasional Internasional Ekonomi Olahraga Teknologi Hiburan Gaya Hi

Home > Gaya Hidup > Berita Kesehatan

BPOM Perintahkan Produsen Tarik Viostin DS dan Enzyplex

Rahman Indra, CNN Indonesia | Rabu, 31/01/2018 16:37 WIB

Bagikan :

Keberadaan komponen non halal pada makanan dan bahan obat?

- Motivasi ekonomi: RpRpRp
  - Penggantian atau penambahan daging dengan daging lain dalam rangka menurunkan biaya produksi dan mendapatkan untuk lebih banyak
  - Harga daging babi << daging sapi
- Ketidakcukupan suplai bahan yang halal
  - Gelatin yang dari material halal ???
- Apa konsekwensinya?
  - Terjadi pelanggaran hukun (pemalsuan)
  - Keselamatan dan keamanan konsumen (alergi dll)
  - Masalah etika, budaya dan agama

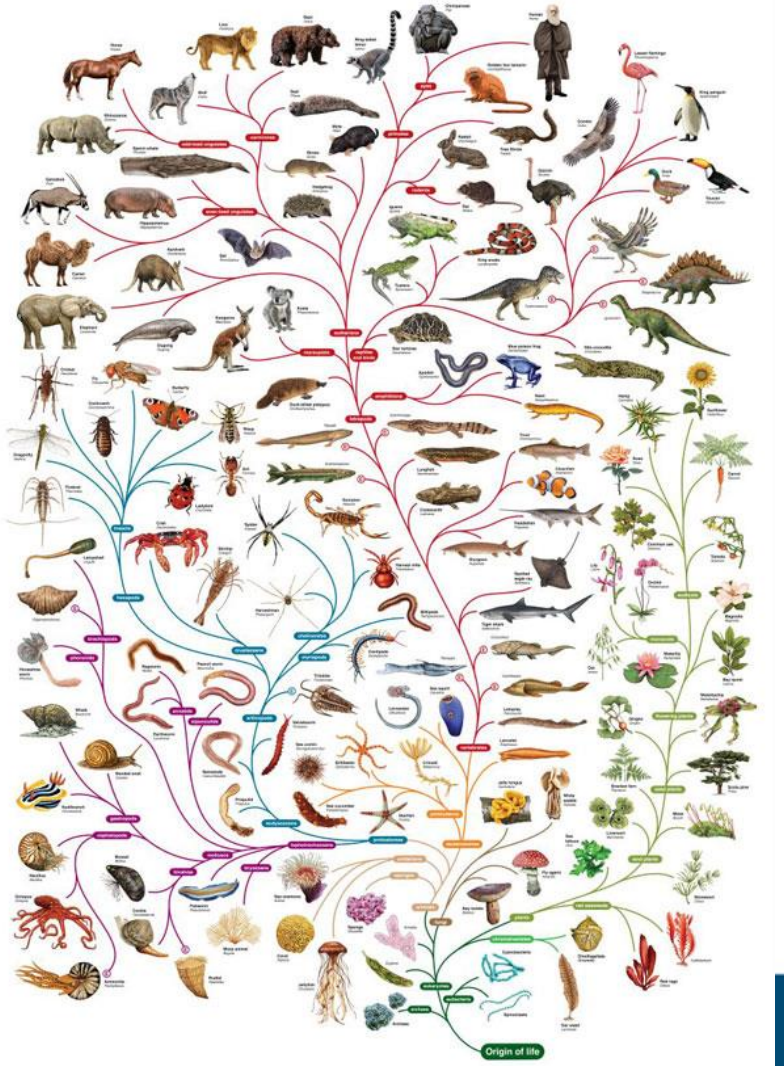
Metode analisis halal

- Two-dimensional polyacrylamide gel electrophoresis and western-blot analysis
- PCR dan Real-Time PCR
- Enzyme-linked immunosorbent assay (ELISA)

Beberapa kekurangan

- **Sebagian besar kualitatif**
- **Tidak selalau bisa digunakan (PCR untuk gelatin?)**
- **Data tidak dapat dapat diekplorasi lagi untuk data mining**

Bottom-up proteomics sebagai dasar pengembangan analisis berbasis MS



- Semua makhluk hidup mempunyai hubungan kekerabatan secara genetic
- Filogeni menunjukkan diversitas dan hubungan kekerabatan.
- Filogeni dibuat dengan menggunakan **urutan DNA.**
- **Hubungan antara gen dengan spesies (variabilitas) menjadi sentral dalam analisis halal**

Evolusi molekuler dari DNA ke protein

Gly Ala Ile Leu asp Arg

-GGAGCCATATTAGATAGA-

-GGAGCAATTTTTGATAGA-

Gly Ala Ile Phe asp Arg

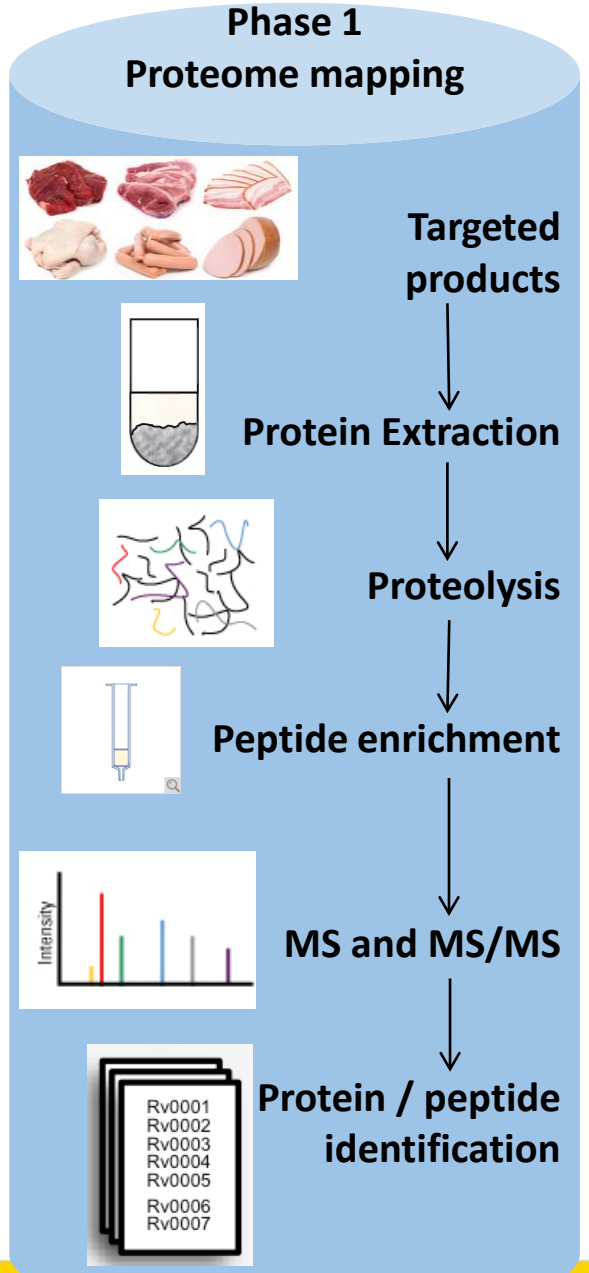
- 3 perbedaan nukeotida DNA pada tiga kodon hanya menyebabkan perbedaan satu asam amino
- 2 penggantian nucleotida dikatakan sinonim dan satu penggantian lain disebut non-sinonim

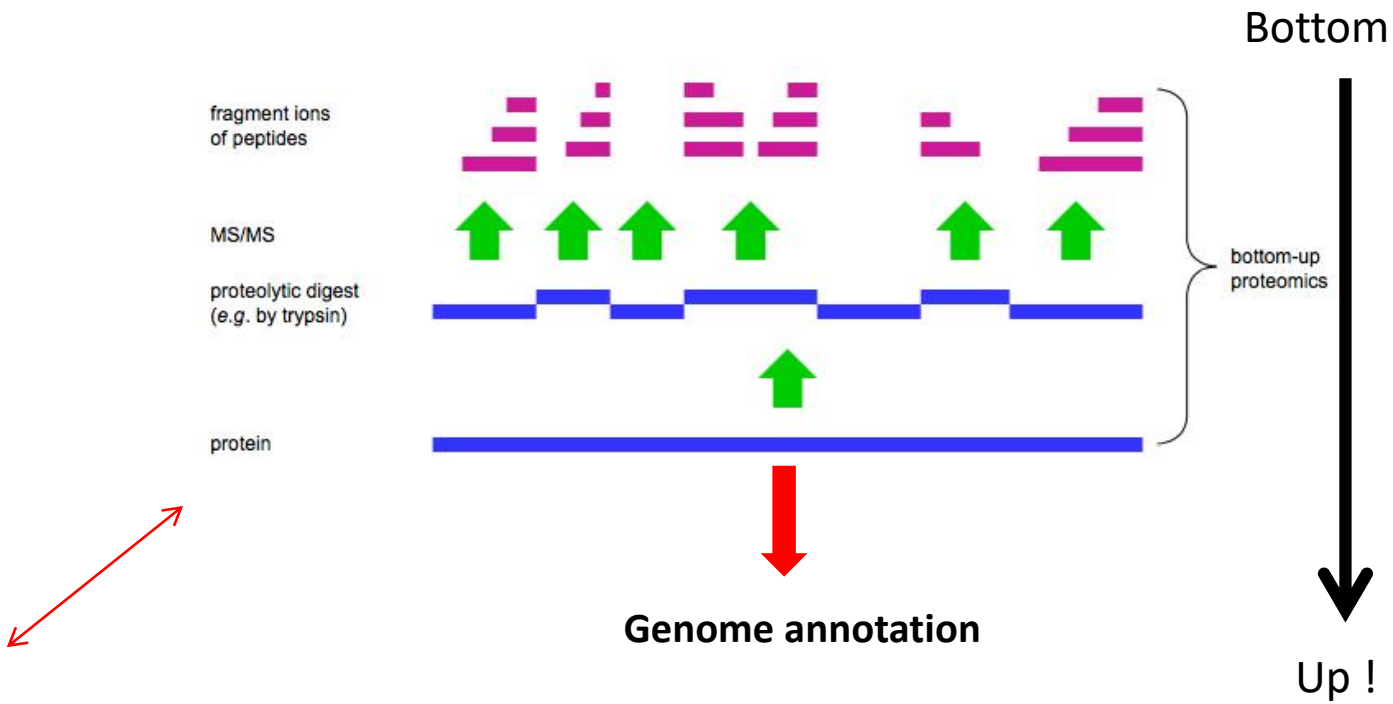
Terdapat lebih satu kodon untuk setiap asam amino.

↓

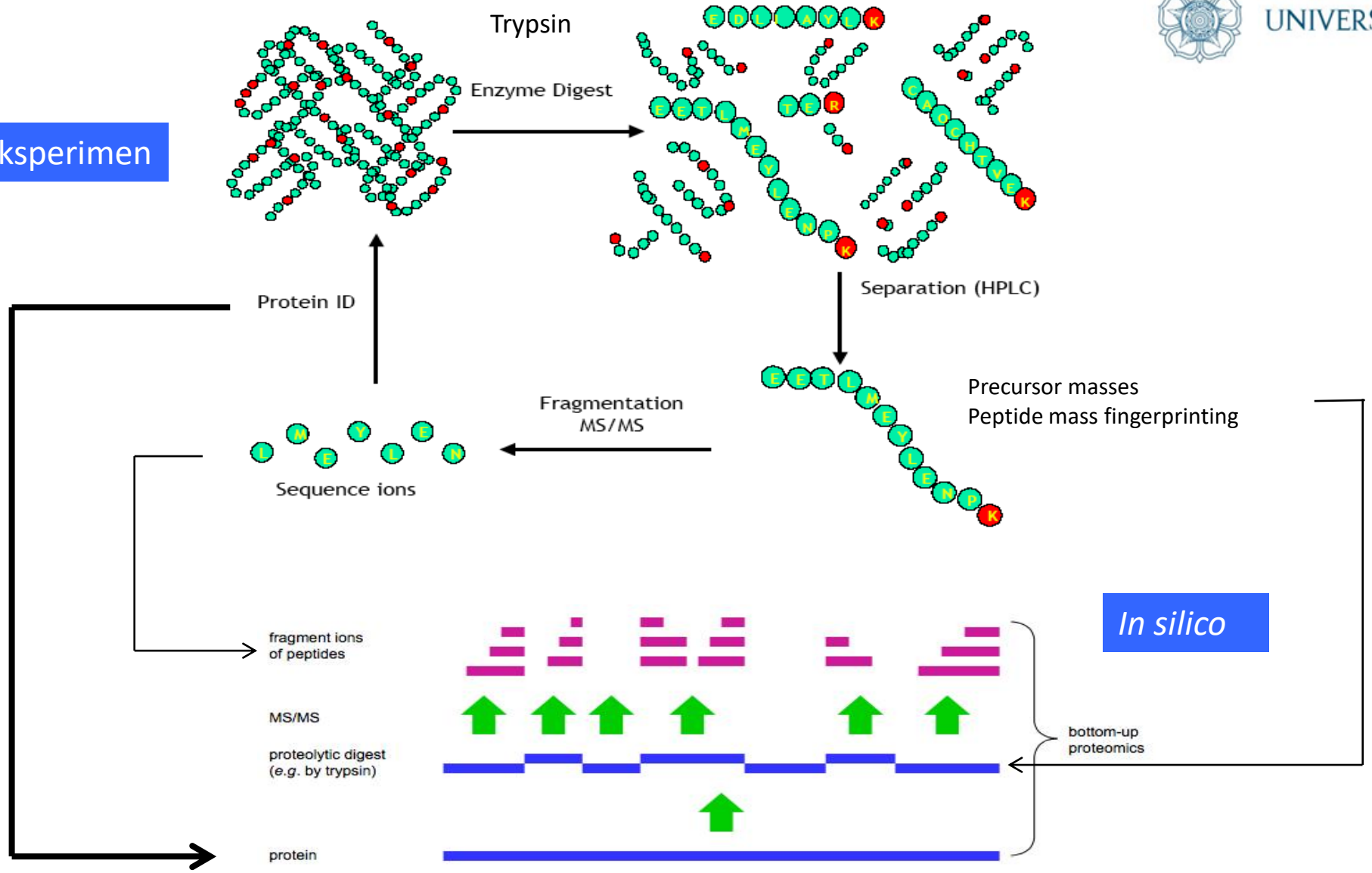
Menemukan urutan protein spesifik spesies menjadi semakin kompleks disbanding urutan DNA, tetapi lebih menjanjikan untuk pembeda

Bagaimana *bottom-up proteomics* digunakan untuk identifikasi asal species suatu bahan?





Eksperimen



In silico

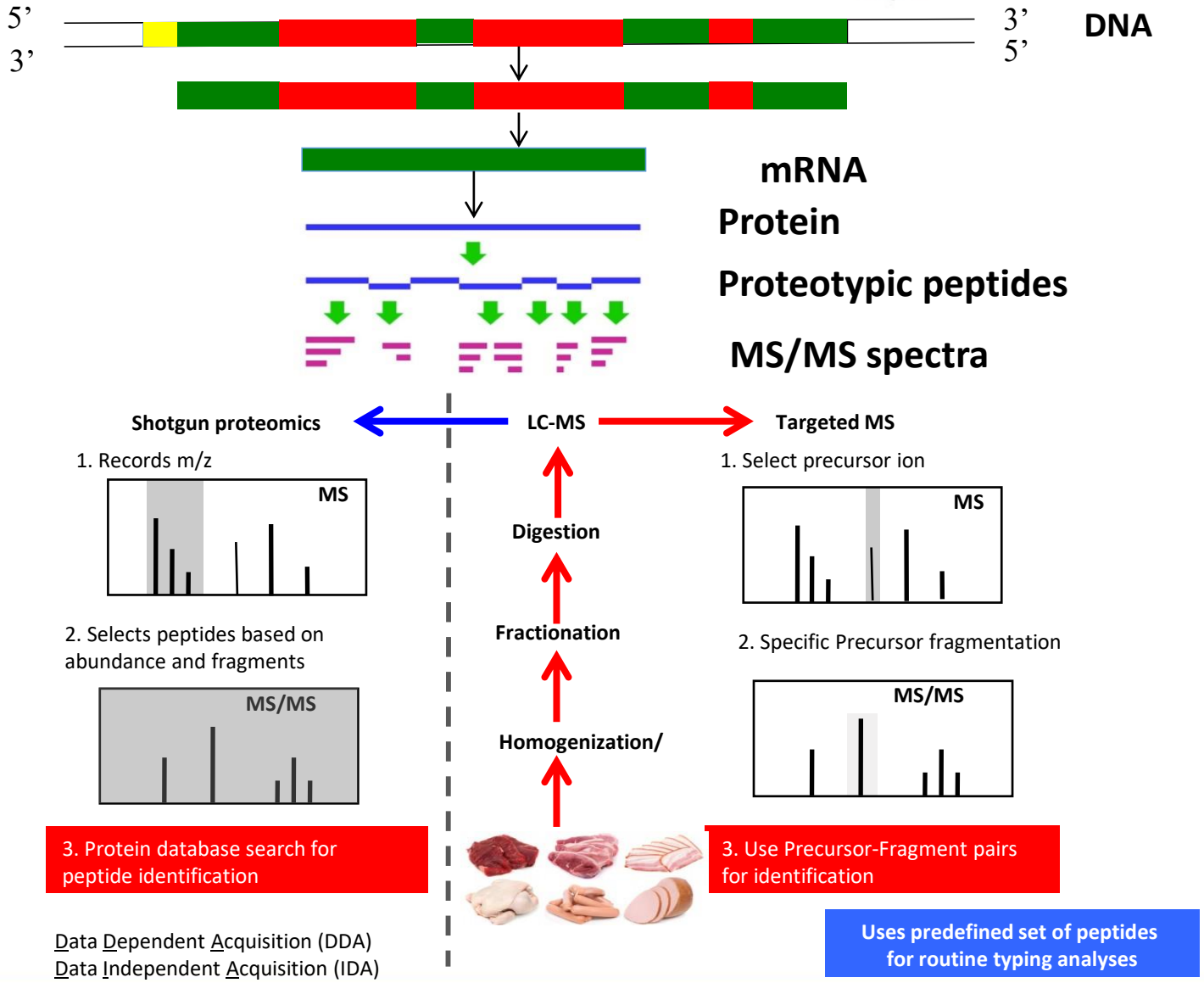
Define Set of Proteins

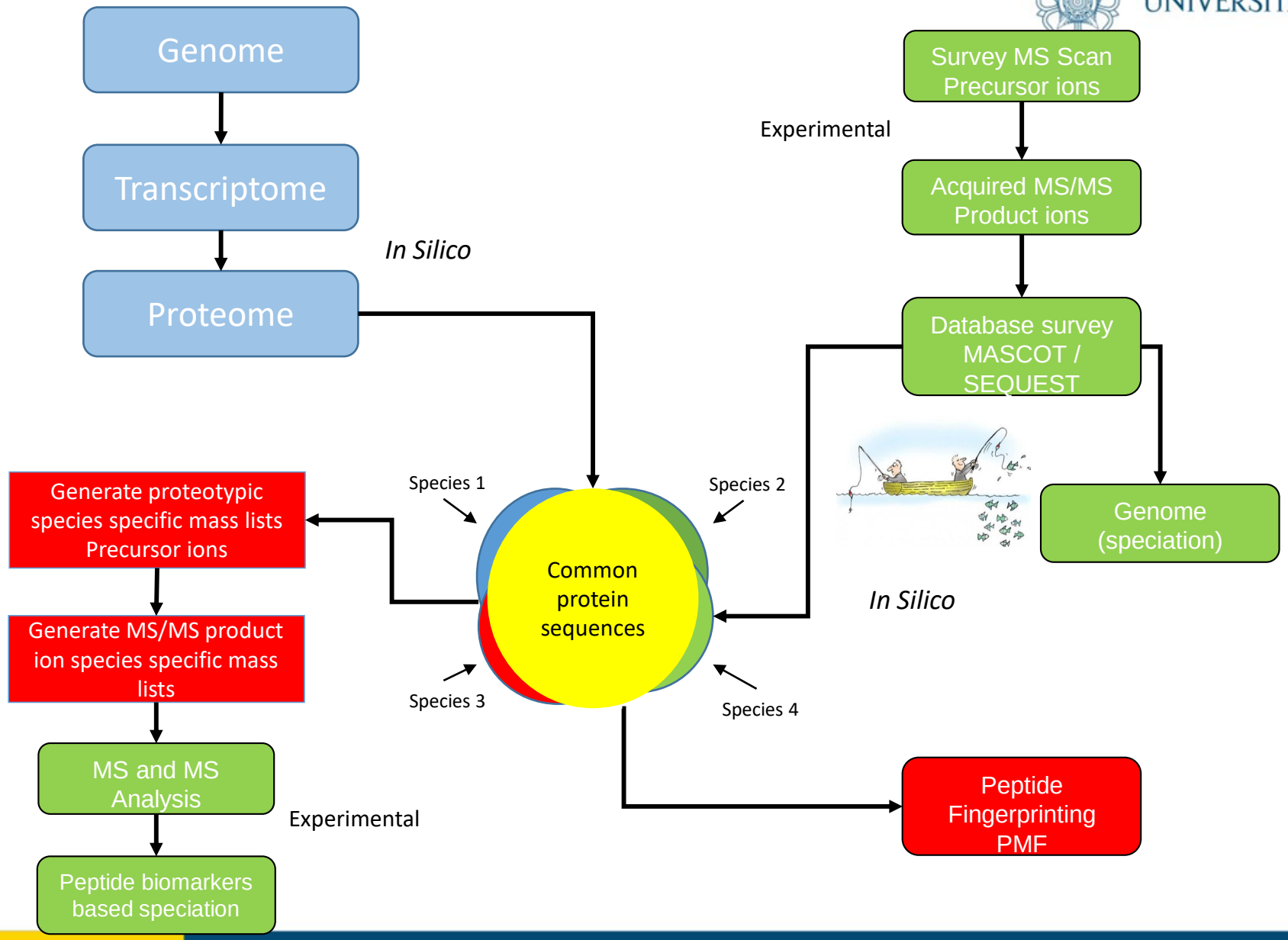
Select Peptides

Select precursor and product ions

Validate precursor and product ions

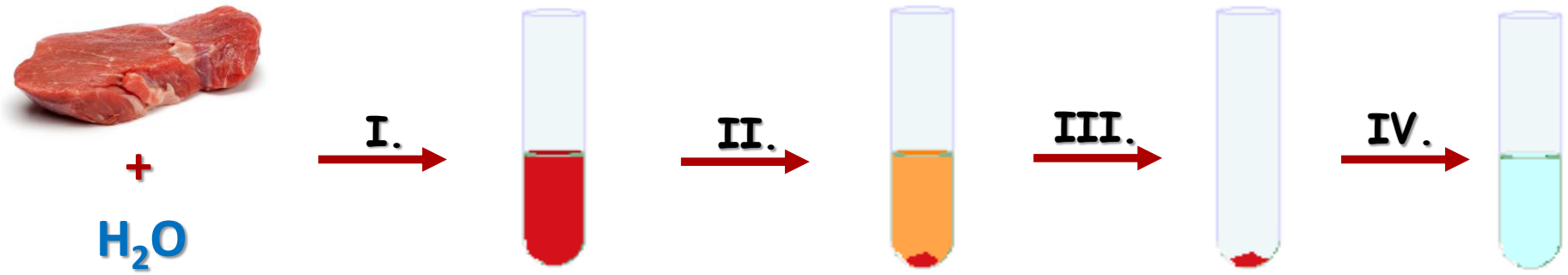
Protein Detection and Quantitation




Genome
Transcriptome
Proteome
In Silico
Survey MS Scan Precursor ions
Experimental
Acquired MS/MS Product ions
Database survey MASCOT / SEQUEST
Genome (speciation)
In Silico
Species 1
Species 2
Common protein sequences
Species 3
Species 4
Generate proteotypic species specific mass lists Precursor ions
Generate MS/MS product ion species specific mass lists
MS and MS Analysis
Experimental
Peptide biomarkers based speciation
Peptide Fingerprinting PMF

Preparasi sampel pada *Bottom-up proteomics*

1. Sampel (daging) dicampur air (1:5) is homogenisasi, sonikasi
2. Protein dalam suspensi diendapkan dengan aseton (1:1)
3. Aseton dipisahkan menghasilkan pellet protein
4. Pelet protein dilarutkan dalam ammonium bicarbonate (pH 8.5).
5. Denaturasi protein pada 120˚C
6. Reduksi protein dengan Dithiothreitol (DTT) dan alkilasi dengan Iodoacetamide IAA
7. Gidesti dengan **trypsin** pada 40 C selama 24jam. Trypsin memotong ujung C: **Lys (K) & Arg (R)**



LC-MS

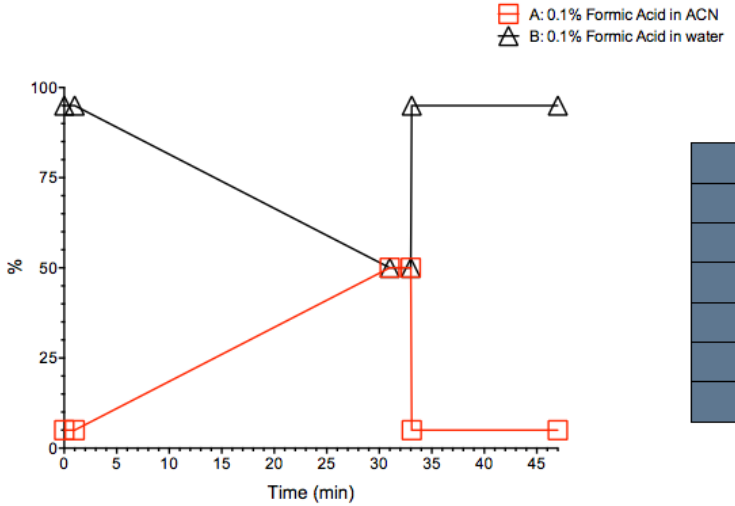
QqQ
LIT or QIT

Thermo Scientific™ UltiMate ™ 3000 LC system and Thermo Scientific™ TSQ Quantiva MS

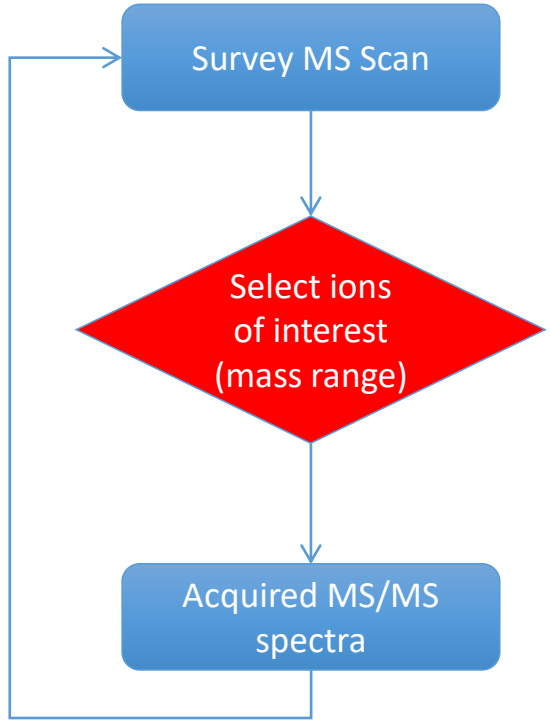
Hybrid Q-Orbitrap

UltiMate 3000 LC system and Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap MS

Injection volume: 2µL
Flow rate: 75µL/min



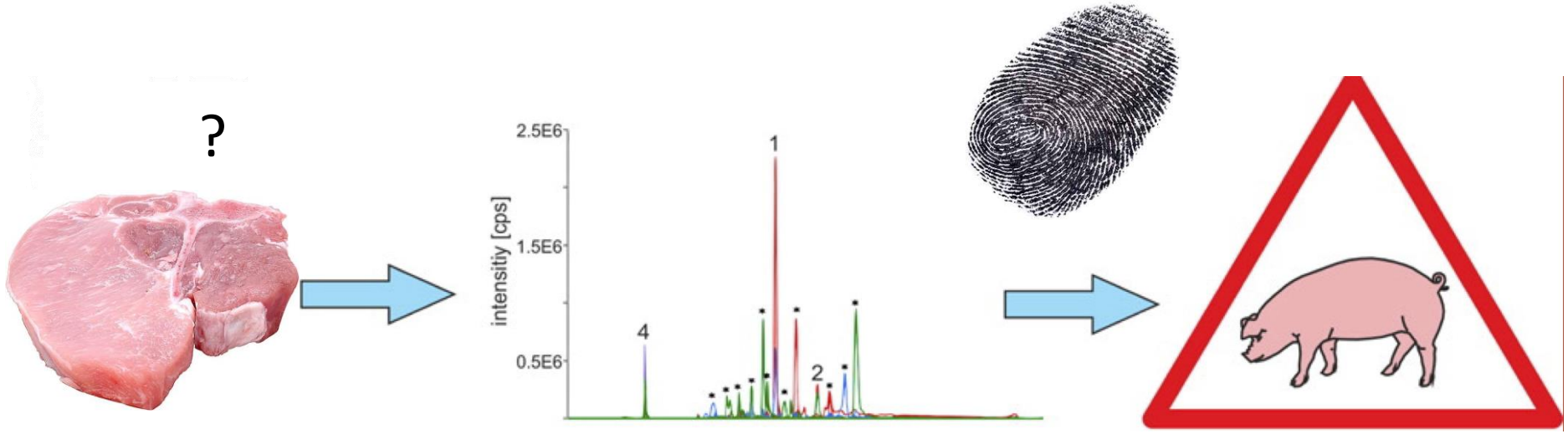
Time		A	B
0		5	95
1		5	95
31		50	50
33		50	50
33.1		5	95
47		5	95



Please note that these conditions can be easily adapted onto a 2 mm column for ease of use.

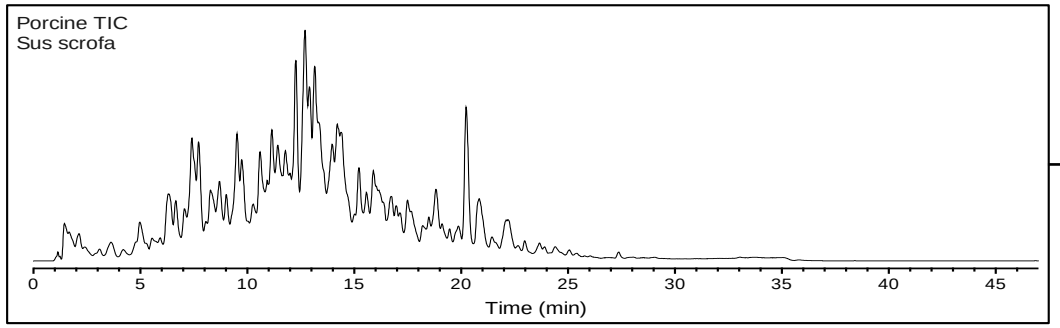
Otentifikasi daging dengan *mass spectrometry*

- Bargen *et al.* (2013), Journal of Agricultural and Food Chemistry, 61:11986-11994



Peptide biomarkers

Pendekatan Peptide Fingerprinting



Generate a mass list (MS and MS/MS data)

Database search (MASCOT)

MS and MS/MS matching

Peptide fingerprinting

Beef
17 specific peptides

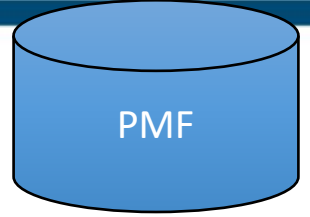
Horse
23 specific peptides

Using a relatively high abundance threshold …

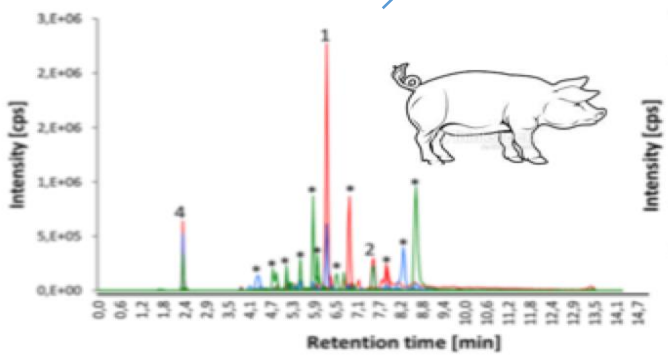
7 429 common unique peptides identified

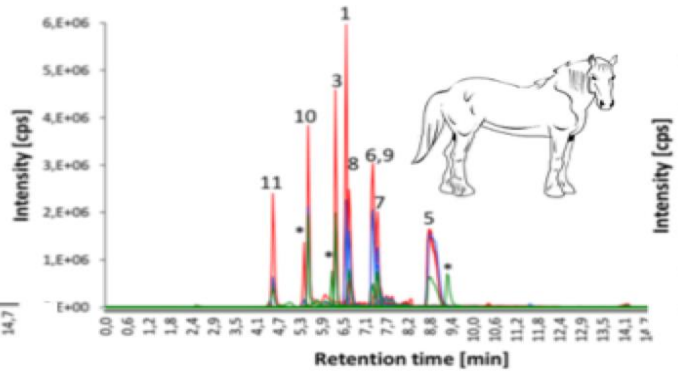
Pork
14 specific peptides

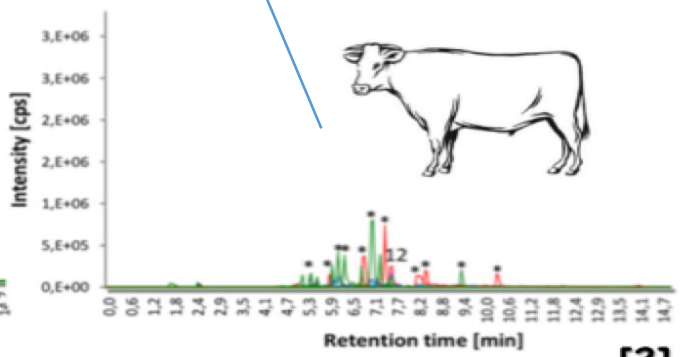
Lamb
9 specific peptides



Bargen *et al.* (2013), Journal of Agricultural and Food Chemistry, 61:11986-11994







[2]

marker	species	protein	biomarker peptide sequence
1	pig/horse	troponin T/unknown	YDIINLR
2	pig	myosin-4	TLAFLFAER
3	horse	myosin-2	EFEIGNLQSK
4	pig	myosin-1 and myosin-4	SALAHAVQSSR
5	horse	myoglobin	YLEFISDAIIHVLHSK
6	horse	myosin-1 and myosin-2	VVETMQTMLDAEIR
*	Unspecific signals		

marker	species	protein	biomarker peptide sequence
7	horse	pyruvate kinase	IYVDDGLISLQVK
8	horse	hemoglobin	FLSSVSTVLTSK
9	horse	myoglobin	HGTVVLTALGGILK
10	horse	myoglobin	VEADIAGHGQEVLIR
11	horse	myosin-1	LVNDLTGQR
12	cattle	myosin-1	TLALLFSGPASGEAEGGPK

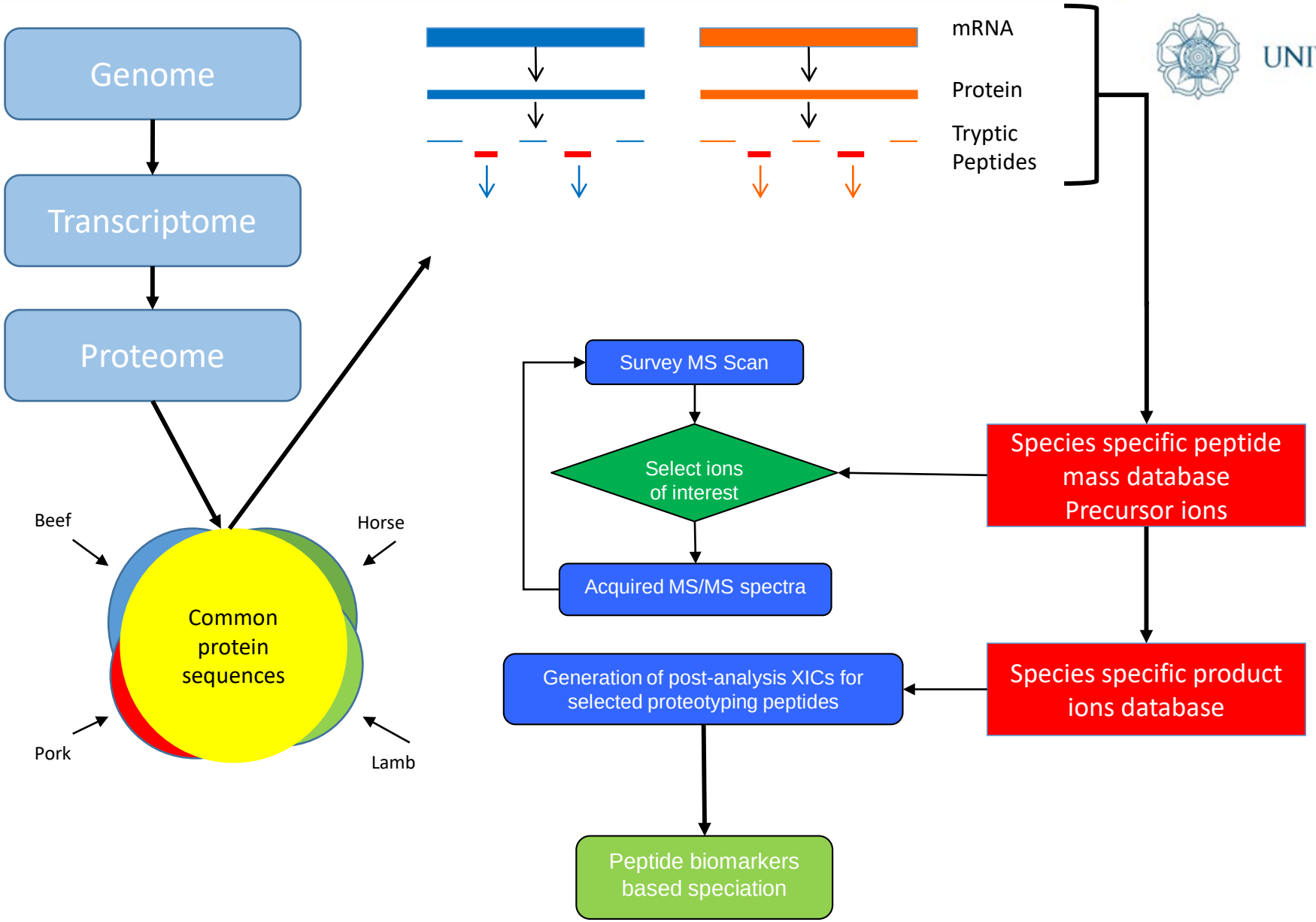
Several peaks used were not adequately identified

Kelemahan PMF

- Tergantung pada data MS dan MS/MS
- Tergantung setting parameter dalam bioinformatik
- Memerlukan ekspert proteomic dan bioinformatik

Sulit diaplikasikan untuk analisis rutin

Apakah ada alternatif lain?

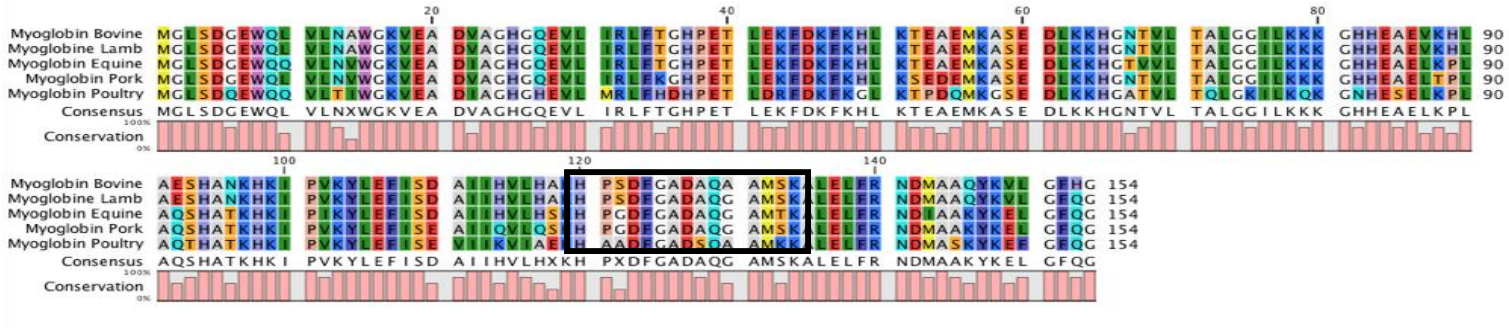

Genome
Transcriptome
Proteome
mRNA
Protein
Tryptic Peptides
Beef
Horse
Pork
Lamb
Common protein sequences
Survey MS Scan
Select ions of interest
Acquired MS/MS spectra
Species specific peptide mass database Precursor ions
Species specific product ions database
Generation of post-analysis XICs for selected proteotyping peptides
Peptide biomarkers based speciation

Targeted bioinformatics analysis

Daging: Myoglobin

- Extraction of gene information
- *In silico* sequence alignment
- *In silico* protein translation and alignment
- Sequence analysis and *In silico* tryptic digestion
- Generate proteotypic mass lists

Myoglobin adalah protein pembawa oksigen di jaringan otot
Keberadaannya melimpah di otot/daging vertebrata termasuk mamalia

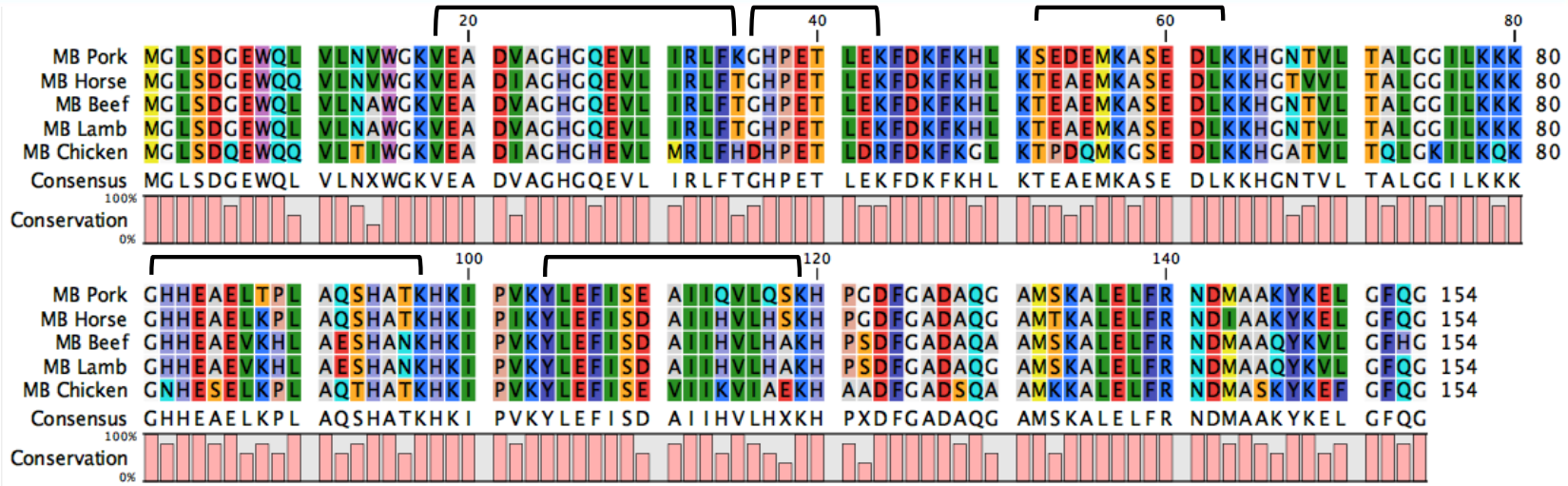


Proteotypic peptides can be identified (120-134)

120 134

Beef	YLEFISDA IIHVLHAKHP SDFGADAQAA MSKALELFR
Horse	YLEFISDA IIHVLHSKHP GDFGADAQGA MTKALELFR
Pork	YLEFISEA IIQVLQSKHP GDFGADAQGA MSKALELFR
Lamb	YLEFISDA IIHVLHAKHP SDFGADAQGA MSKALELFR

	Proteotypic **peptides**	**Mass (m/z)**
Beef	HPSDFGADAQAAMSK	x_1
Horse	HPGDFGADAQGAMTK	x_2
Pork	HPGDFGADAQGAMSK	x_3
Lamb	HPSDFGADAQGAMSK	x_4



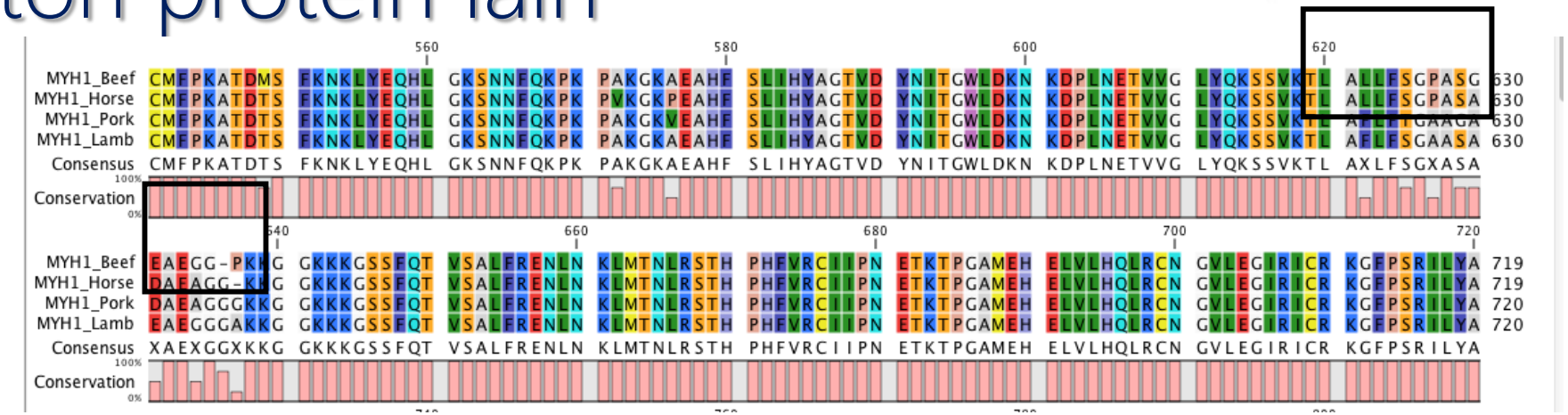
Specific pork myoglobin tryptic peptides

^{18}VEADVAGHGQEVLIRLFK^{35}
^{36}GHPETLEK^{43}
^{52}SEDEMKASEDLK^{63}
^{81}GHHEAELTPLAQSHATK^{97}
^{104}YLEFISEAIIQVLQSK^{119}

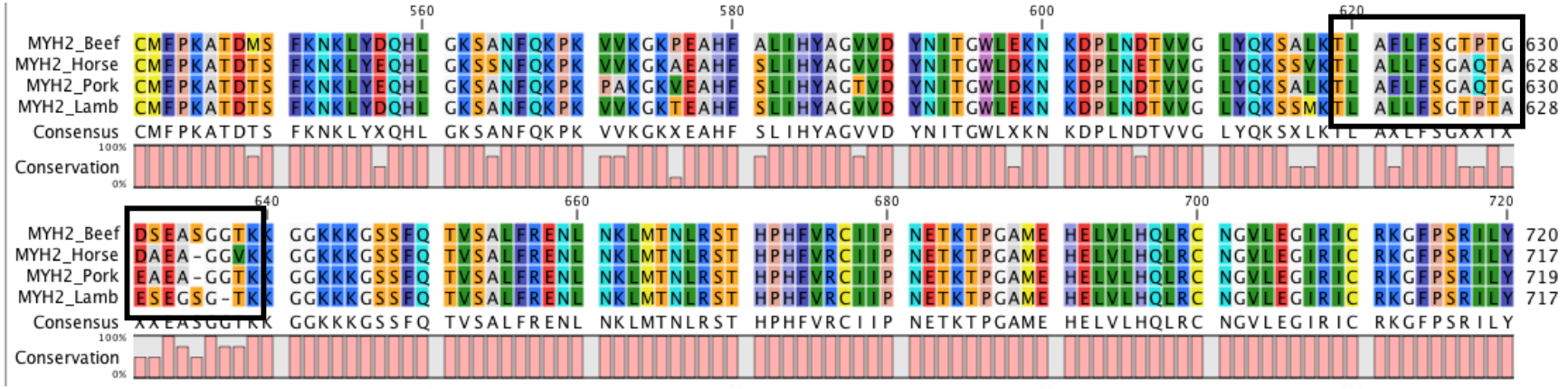
Specific MS and MS/MS data

Contoh protein lain

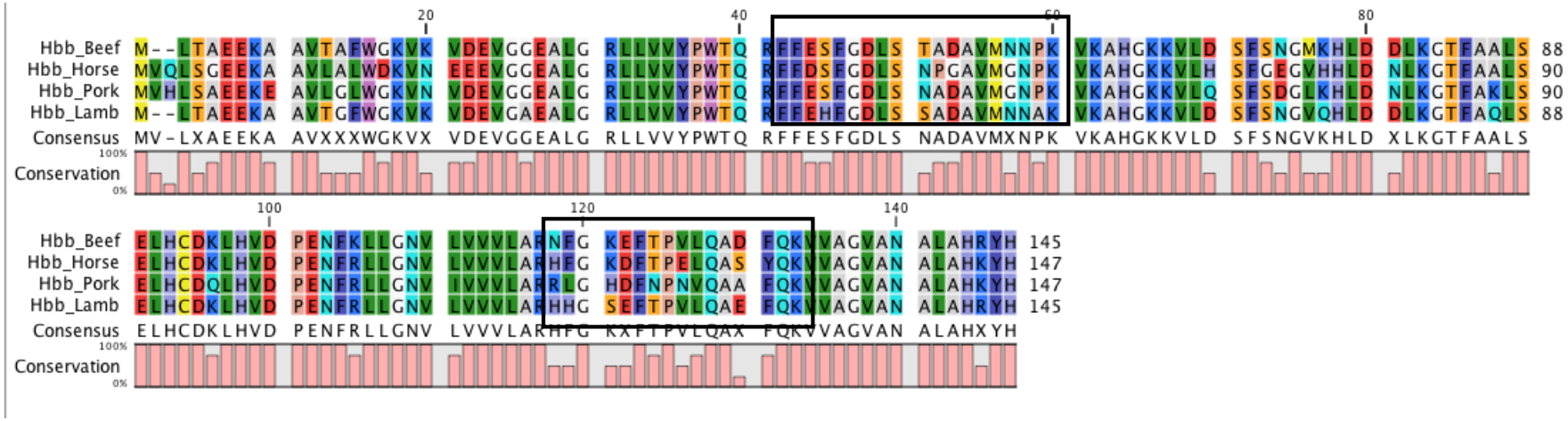
Myosin-1 (MYH1)



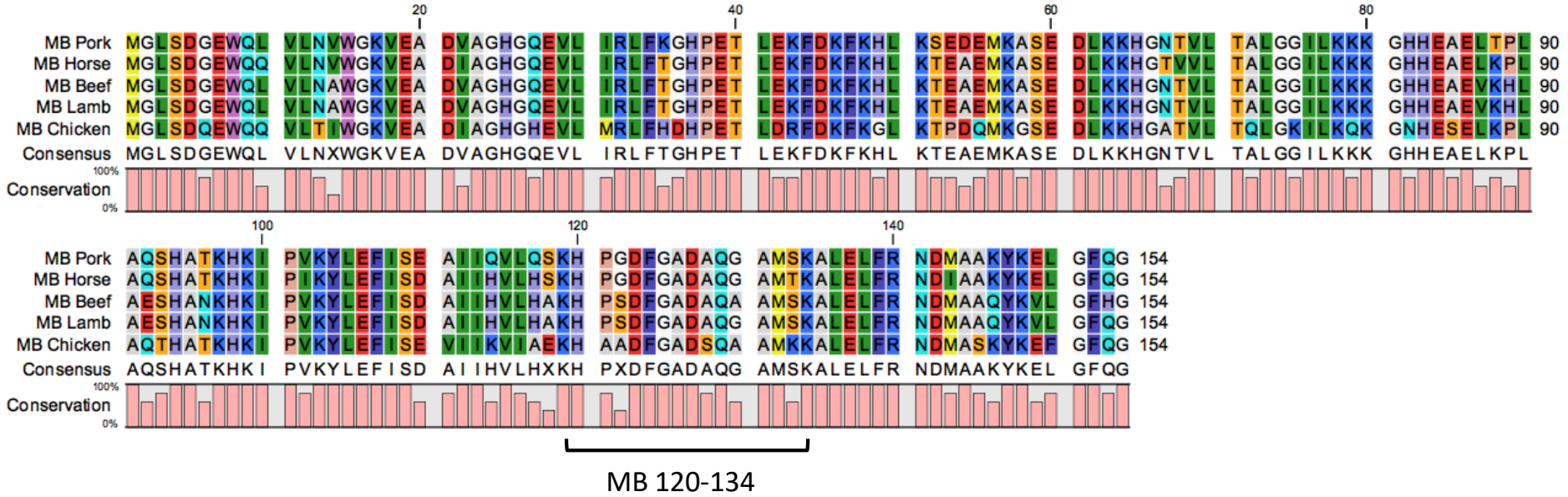
Myosin-2 (MYH2)



β Hemoglobin (Hbb)

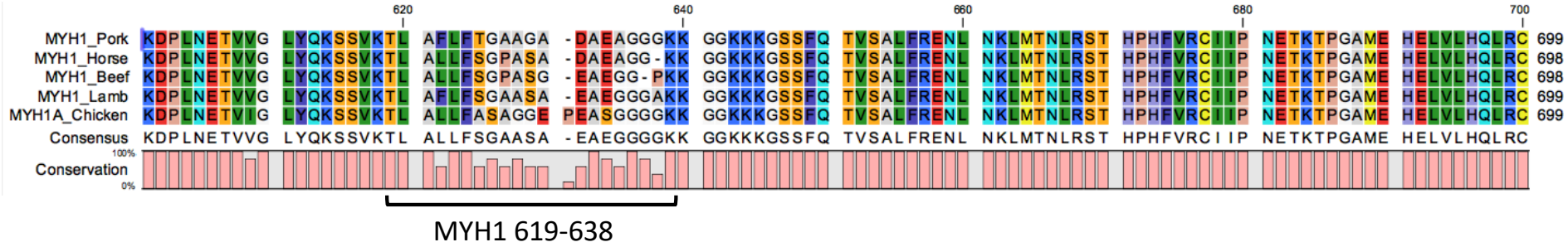


Myoglobin (MB)



Species	Tryptic Peptide sequence (biomarker)	Theoritical mass (z=2)
Pork	HPGDFGADAQGAMSK	744.8304
Horse	HPGDFGADAQGAMTK	751.8383
Beef	HPSDFGADAQAAMSK	766.8435
Lamb	HPSDFGADAQGAMSK	759.8357
Chicken	HAADFGADSQAAMKK HAADFGADSQAAMK	774.3672 710.3197

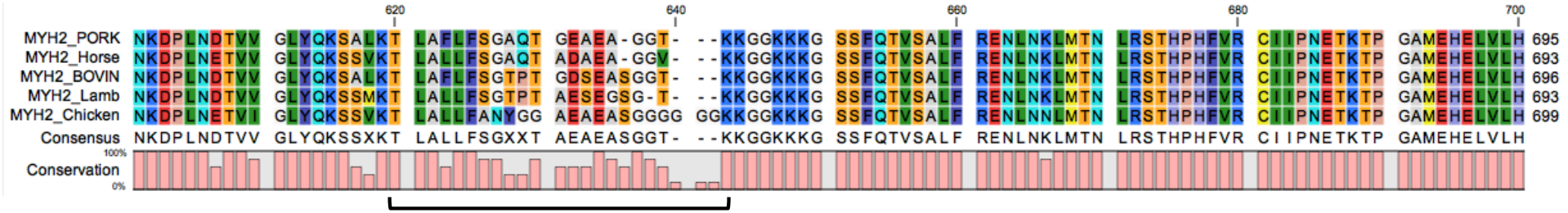
Myosin-1 (MYH1)



MYH1 619-638

Species	Tryptic Peptide sequence (biomarker)	Theoritical mass (z=2)
Pork	TLAFLFTGAAGADAEAGGGK	912.9600
Horse	TLALLFSGPASADAEAGGK	888.4623
Beef	TLALLFSGPASGEAEGGPK	901.4702
Lamb	TLAFLFSGAASAEAEGGGAK	927.9652
Chicken	TLALLFASAGGEPEASGGGGK	945.4838

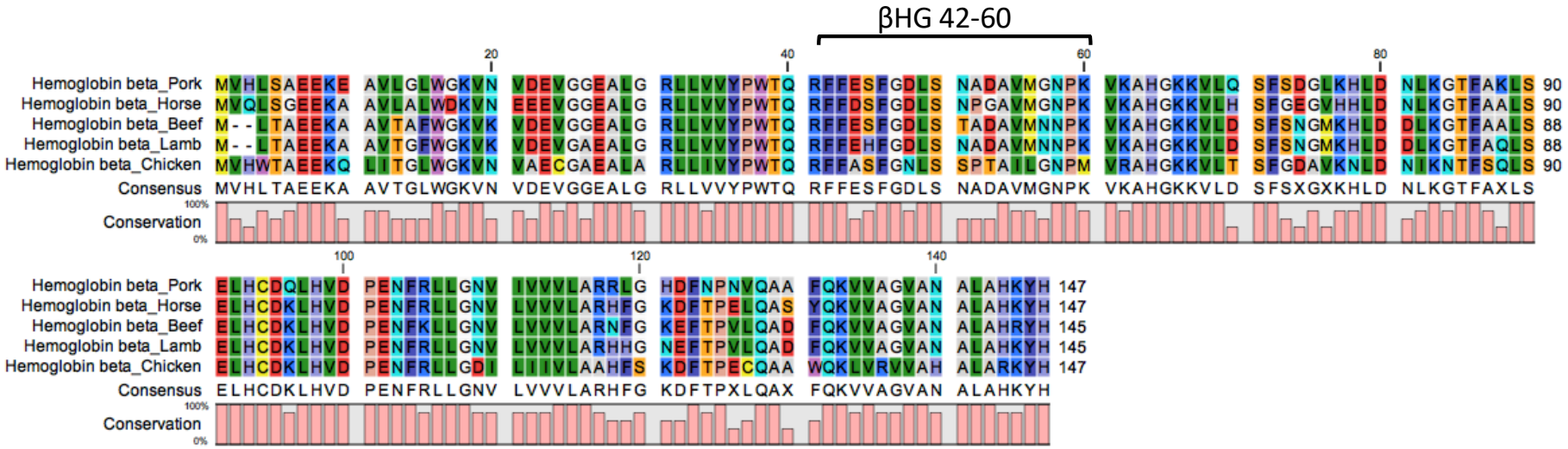
Myosin-2 (MYH2)



MYH2 619-638

Species	Tryptic Peptide sequence (biomarker)	Theoritical mass (z=2)
Pork	TLAFLFSGAQTGEAEAGGTK	978.4891
Horse	TLALLFSGAQTADAEAGGVK	960.5073
Beef	TLAFLFSGTPTGDSEASGGTK	1022.4971
Lamb	TLALLFSGTPTAESEGSGTK	984.0020
Chicken	TLALLFANYGGAEAEASGGGGGGK	1084.5346

β-Hemoglobin



Species	Tryptic Peptide sequence (biomarker)	Theoritical mass (z=2)
Pork	FFESFGDLSNADAVMGNPK	1023.4673
Horse	FFDSFGDLSNPGAVMGNPK	1000.4646
Beef	FFESFGDLSTADAVMNNPK	1045.4804
Lamb	FFEHFGDLSNADAVMNNPK	1076.9915
Chicken	FFASFGNLSSPTAILGNPMVR	1113.5724

Analisis Bioinformatik

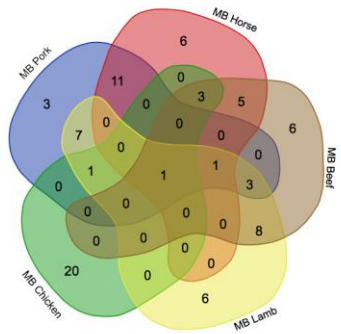
Pork
Horse
Beef
Lamb
Chicken

In silico MS^2 fragmentation prediction

Venn diagrams

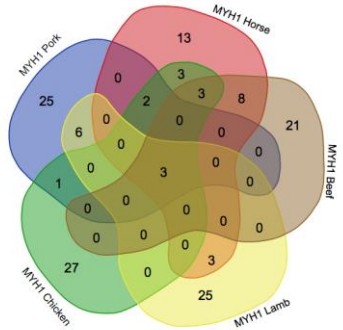
Myoglobin

^{120}HPGDFGADAQGAMSK^{140}
^{120}HPGDFGADAQGAMTK^{140}
^{120}HPSDFGADAQAAMSK^{140}
^{120}HPSDFGADAQGAMSK^{140}
^{120}HAADFGADSQAAMKK^{140}



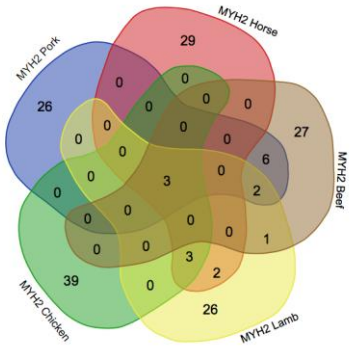
Myosin-1

^{619}TLAFLFTGAAGADAEAGGGK^{638}
^{619}TLALLFSGPASADAEAGGK^{637}
^{619}TLALLFSGPASGEAEGGPK^{637}
^{619}TLAFLFSGAASAEAEGGGAK^{638}
^{618}TLALLFASAGGEPEASGGGGK^{638}



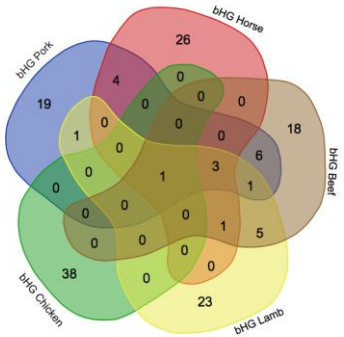
Myosin-2

^{619}TLAFLFSGAQTGEAEAGGTK^{638}
^{617}TLALLFSGAQTADAEAGGVK^{636}
^{619}TLAFLFSGTPTGDSEASGGTK^{639}
^{617}TLALLFSGTPTAESEGSGTK^{636}
^{618}TLALLFANYGGAEAEASGGGGGGK^{642}



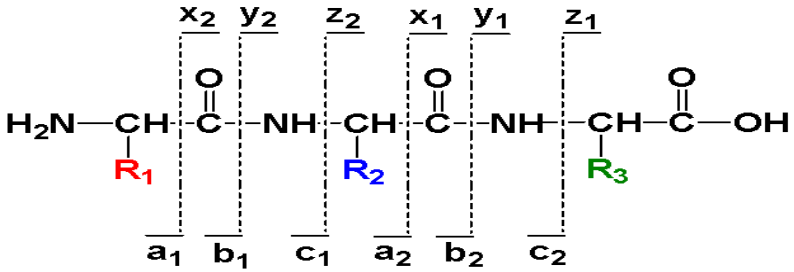
β-Haemoglobin

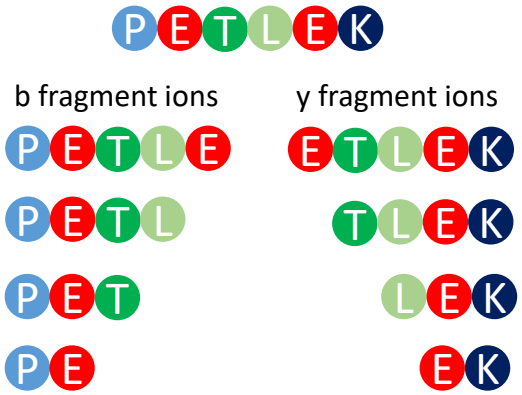
^{42}FFESFGDLSNADAVMGNPK^{60}
^{42}FFDSFGDLSNPGAVMGNPK^{60}
^{40}FFESFGDLSTADAVMNNPK^{58}
^{40}FFEHFGDLSNADAVMNNPK^{58}
^{42}FFASFGNLSSPTAILGNPMVR^{62}



→ Otentifikasi dapat dilakukan pada MS^1 dan MS^2 untuk meningkatkan spesifisitas

Peptida target dicobakan untuk MS^2 dimana dihasilkan fragmen unik **b dan y.**

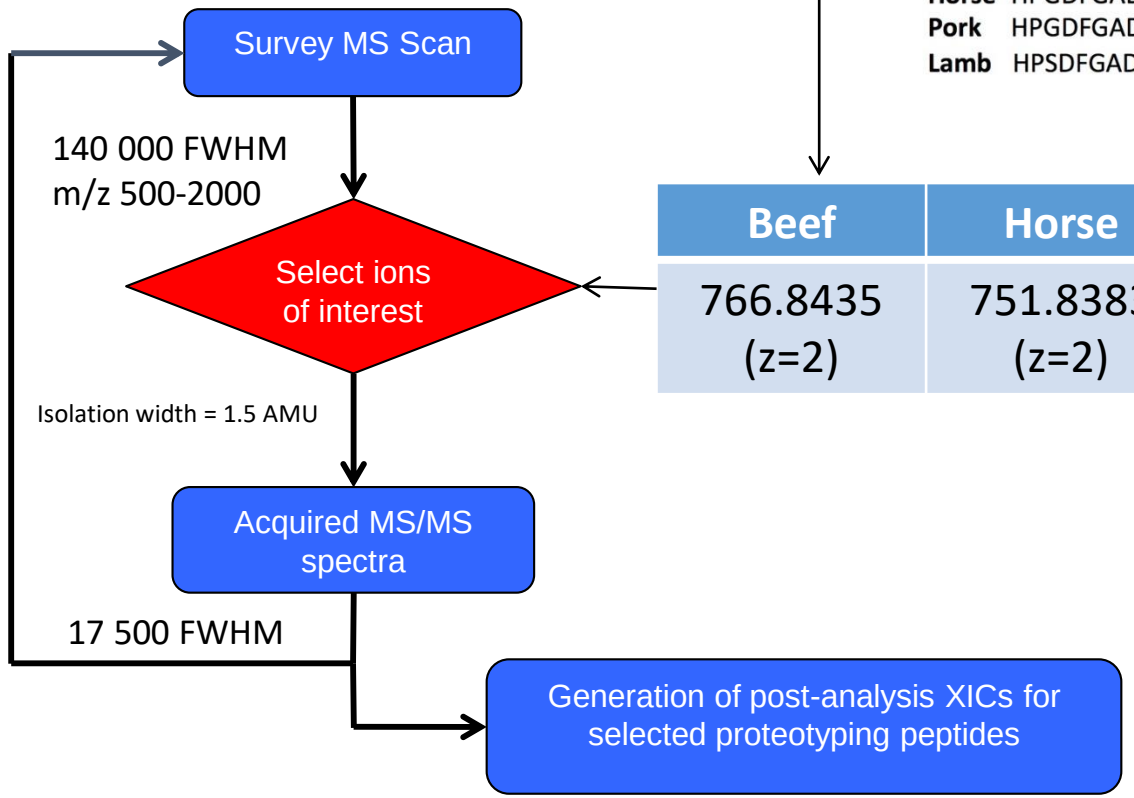




Species	Myoglobin	Myosin-1	Myosin-2	β- Hemoglobin
Pork	→[a]1254.5419	704.3210	[a]433.2405	831.4029
	1342.5481	775.3581	562.2831	902.4400
	→[a]1351.5947	794.4447	633.3202	[a]1016.483
		[a]832.3795	762.3628	[a]1103.515
		851.4662	[a]819.3843	1144.4946
		[a]903.4167	908.4876	1215.5317
		922.5033	[a]920.4320	[a]1216.5990
		974.4538	1036.5462	1330.5586
		993.5404	1048.4905	[a]1331.6260
		[a]1031.4752	1119.5277	1401.5957
		1050.5619	1137.5939	1500.6642
		1121.5990	[a]1176.5491	1535.7159
		[a]1132.5229	1194.6154	1622.7479
		1236.6259	[a]1263.5811	1631.7046
		[a]1279.5913	1323.6579	1688.7261
		1307.6630	1394.6951	1751.7905
		[a]1392.6754	[a]1410.6496	1802.7690
		1436.7056	1523.7336	1898.8589
		1507.7427	1523.7377	1899.8218
		1539.7438	1594.7748	
		1564.7642	1651.7962	
		1610.7809	1670.8020	
		1621.7857	1708.8177	
		1678.8071	1741.8392	
		1723.8650	1809.8654	
			1854.9232	
Horse	248.1605	461.2354	246.1812	410.1710
	[a]379.2010	532.2726	303.2027	497.2031
	450.2381	647.2995	360.2241	644.2715
	→[a]1268.5576	[a]805.3686	431.2613	701.2930
	1356.5637	[a]973.4585	560.3039	816.3199
	→[a]1365.6103	[a]1030.4800	[a]631.3410	[a]870.4502
		[a]1117.5120	[a]746.3679	929.4040
		1129.6252	[a]817.4050	[a]984.4931
		[a]1264.5804	874.5033	1016.4360
		[a]1377.6645	[a]918.4527	[a]1071.5252
		1490.7485	1002.5619	1130.4789
		1561.7857	1046.5113	[a]1184.6092
		1674.8697	1103.6095	1227.5317
			[a]1117.5484	1284.5531
			[a]1174.5699	[a]1299.6362
			1174.6467	1355.5903
			[a]1261.6019	[a]1356.6576
			1289.6736	1454.6587
			1360.7107	1503.7260
			[a]1408.6703	1585.6992
			1489.7533	1590.7581
			1521.7544	1642.7206
			1560.7904	1705.7850
			1617.8119	1756.7635
			1634.8384	1852.8534
			1674.8333	1853.8163
			1705.8755	
			1773.9018	
			1818.9596	

Analisis myoglobin dengan digesti proteolitik

Strategy

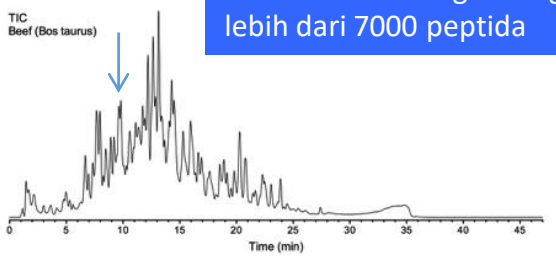


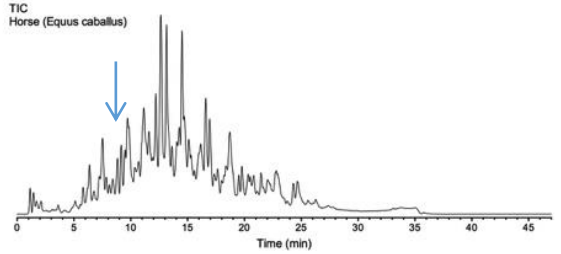
Proteotypic peptides		Mass (m/z)
Beef	HPSDFGADAQAAMSK	x_1
Horse	HPGDFGADAQGAMTK	x_2
Pork	HPGDFGADAQGAMSK	x_3
Lamb	HPSDFGADAQGAMSK	x_4

Beef	Horse	Pork	Lamb
766.8435 (z=2)	751.8383 (z=2)	744.8304 (z=2)	759.8357 (z=2)

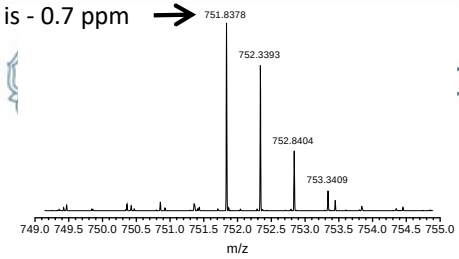
Extracted ion chromatograms with $m_n \pm 5$ ppm

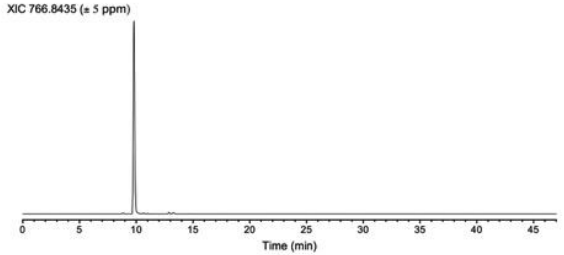
TIC masih mengandung lebih dari 7000 peptida

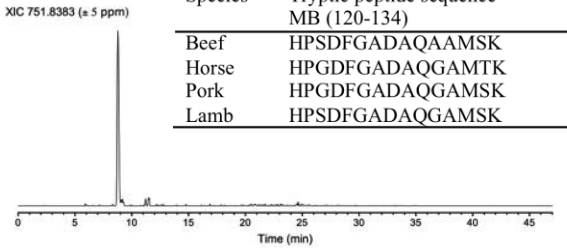




Δm is - 0.7 ppm

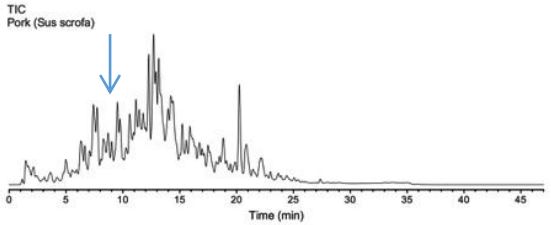


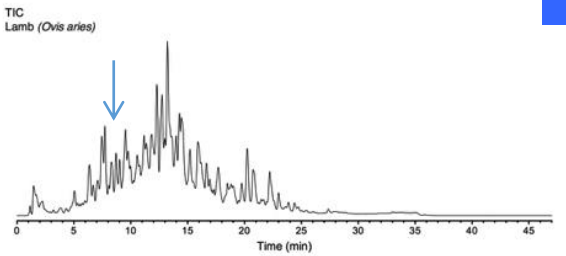


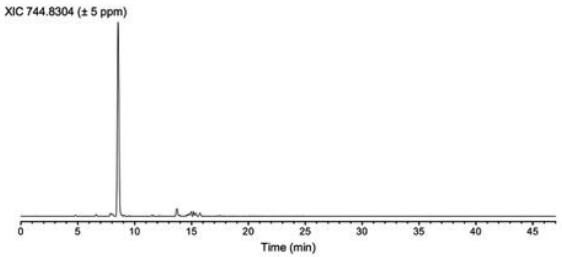


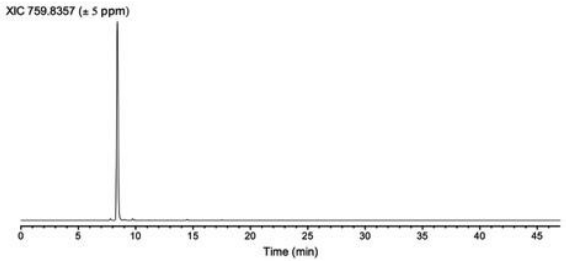
Species	Tryptic peptide sequence MB (120-134)	Theoretical mass (z=2)	Observed mass (z=2)	Mass accuracy (ppm)
Beef	HPSDFGADAQAAMSK	766.8435	766.8436	0.13
Horse	HPGDFGADAQGAMTK	751.8383	751.8378	-0.67
Pork	HPGDFGADAQGAMSK	744.8304	744.8314	1.34
Lamb	HPSDFGADAQGAMSK	759.8357	759.8363	0.79

Setiap target peptide diekstrak dari TIC

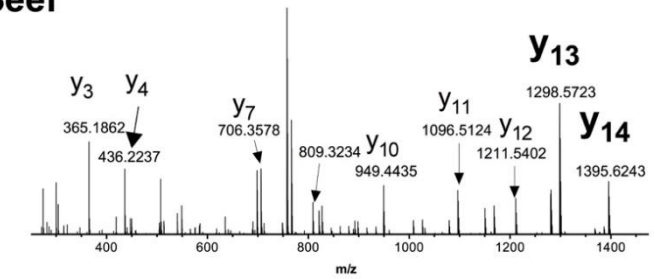






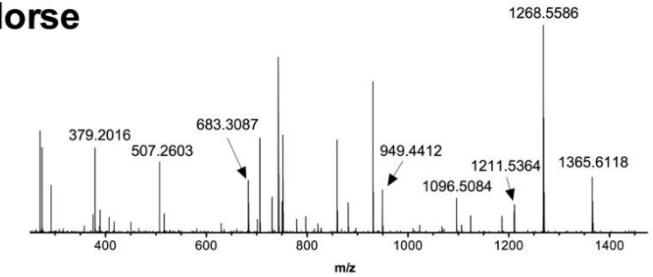


Beef



HPSDFGADAQAAMSK
y_{14} y_{13}

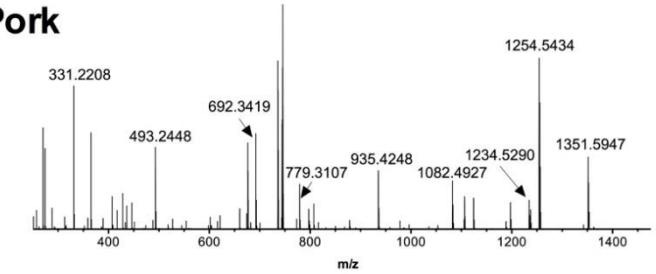
Horse



HPGDFGADAQGAMTK
y_{14} y_{13}

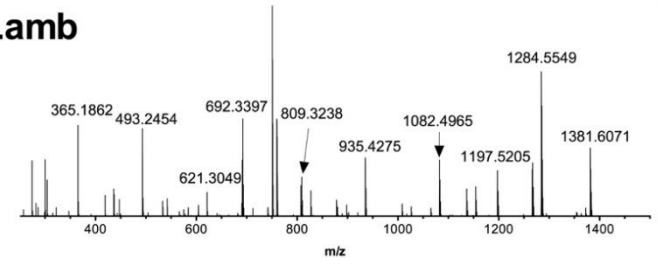
HPGDFGADAQGAMSK
y_{14} y_{13}

Pork



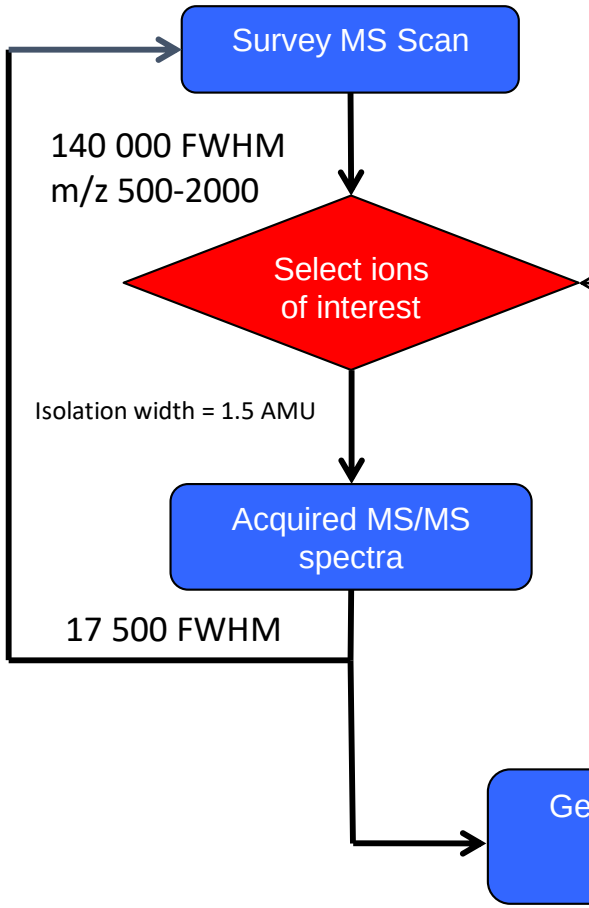
HPSDFGADAQGAMSK
y_{14} y_{13}

Lamb



Identifikasi spesifik produk ion

Beef	Horse	Pork	Lamb
766.8435 (z=2)	751.8383 (z=2)	744.8304 (z=2)	759.8357 (z=2)

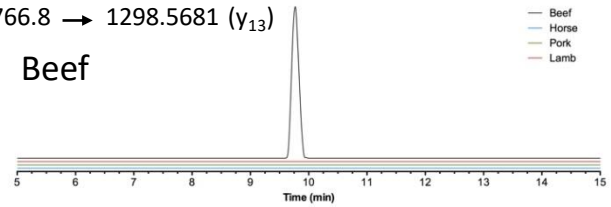


Beef	Horse	Pork	Lamb
1298.5681 (y_{13})	1268.5576 (y_{13})	1254.5419 (y_{13})	1284.5525 (y_{13})
1395.6209 (y_{14})	1365.6103 (y_{14})	1351.5947 (y_{14})	1381.6053 (y_{14})

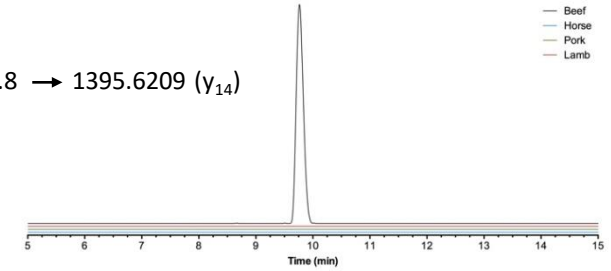
XIC untuk y_{14} atau y_{13} ± 5 ppm

766.8 → 1298.5681 (y_{13})

Beef

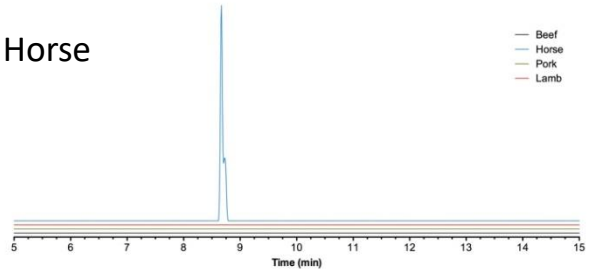


766.8 → 1395.6209 (y_{14})

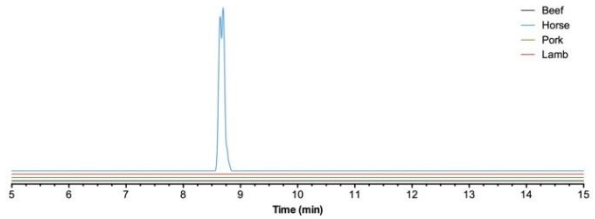


751.8 → 1268.5576 (y_{13})

Horse

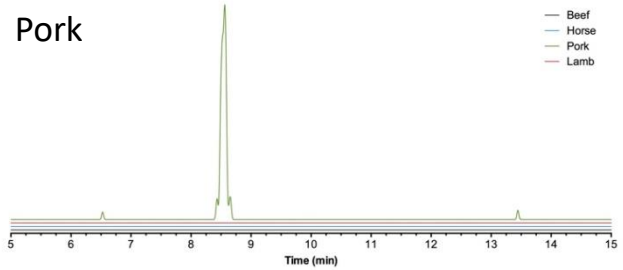


751.8 → 1365.6103 (y_{14})

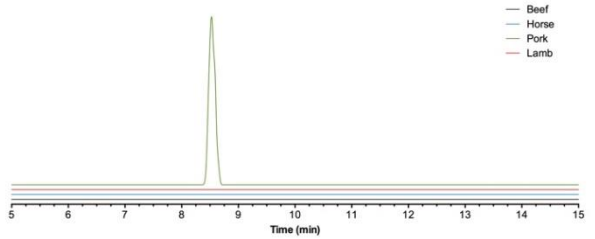


744.8 → 1254.5419 (y_{13})

Pork

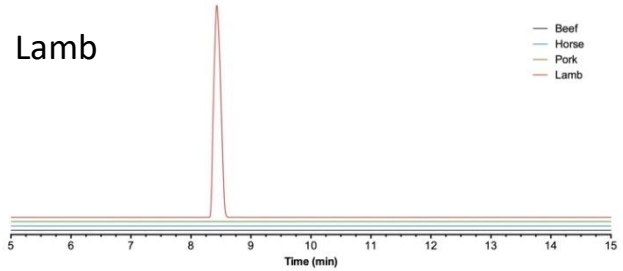


744.8 → 1351.5947 (y_{14})

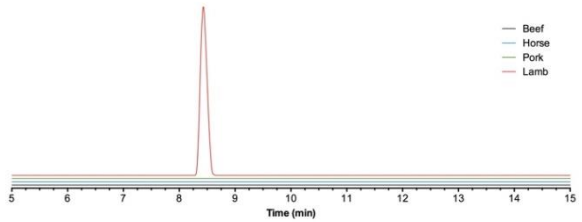


759.8 → 1284.5525 (y_{13})

Lamb

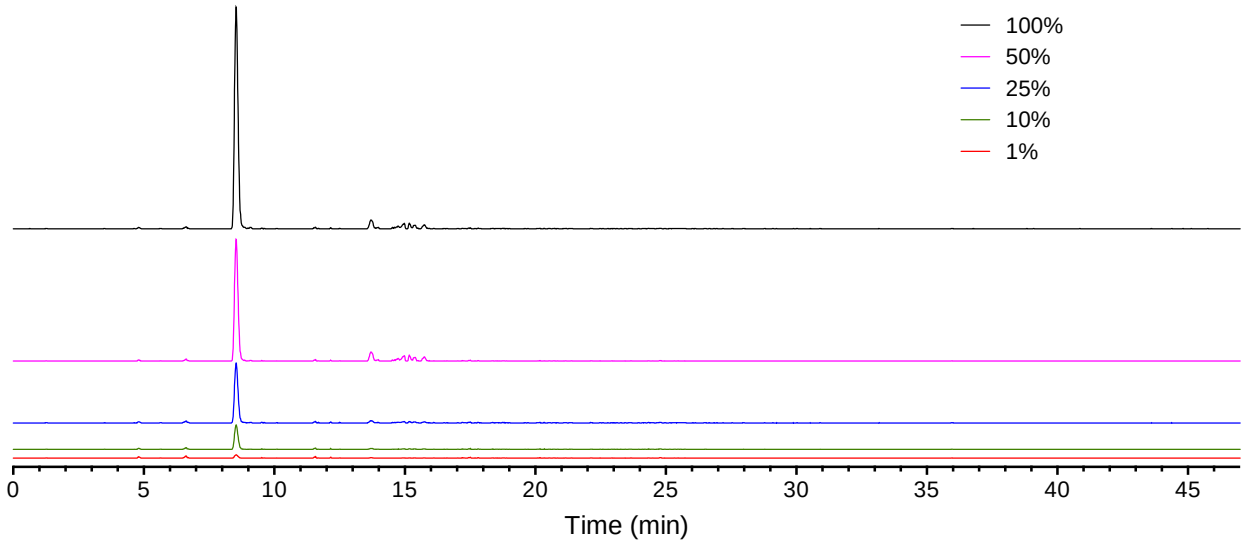


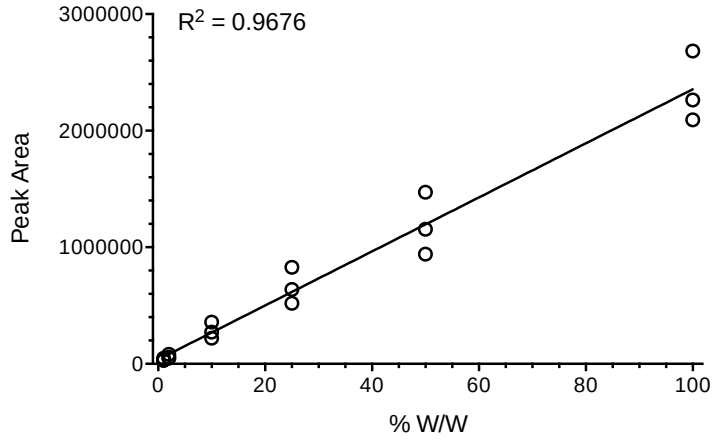
759.8 → 1381.6053 (y_{14})



XIC spesifik dapat digunakan untuk membedaan antar spesies , tetapi….

Overlay XIC (extracted ion chromatogram) m/z 744.8304 ± 5 ppm (z=2)
Peptida myoglobin babi

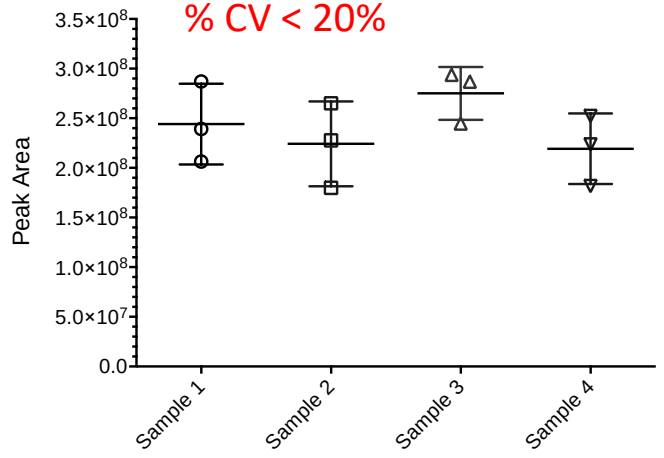




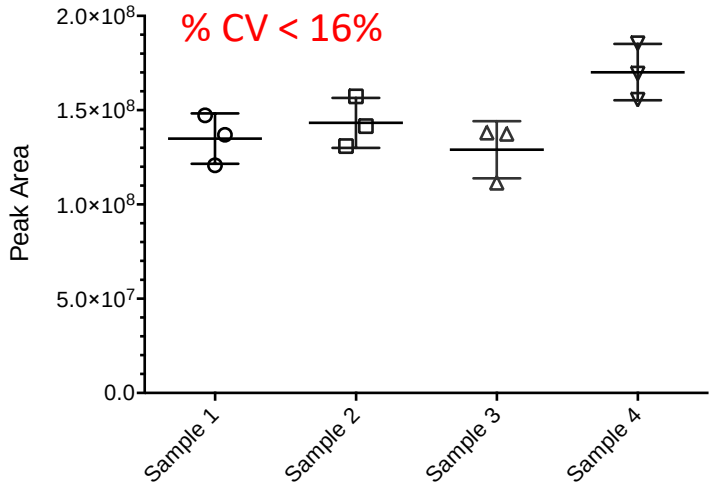
Peak area XIC vs konsentrasi

Presisi

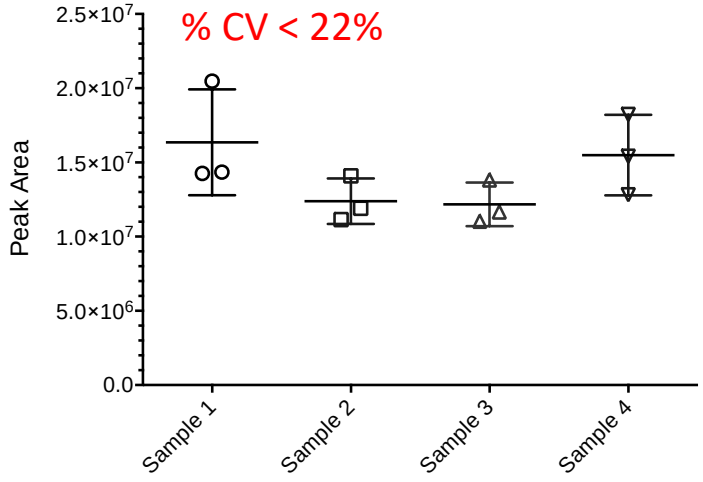
Beef (Myoglobin peptide 120-134)



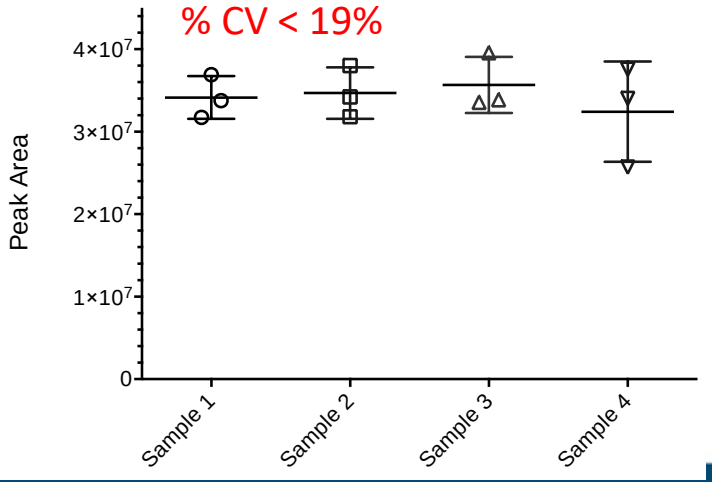
Horse (Myoglobin peptide 120-134)



Pork (Myoglobin peptide 120-134)



Lamb (Myoglobin peptide 120-134)



Sampel daging dispike dengan 1% (w/w) babi

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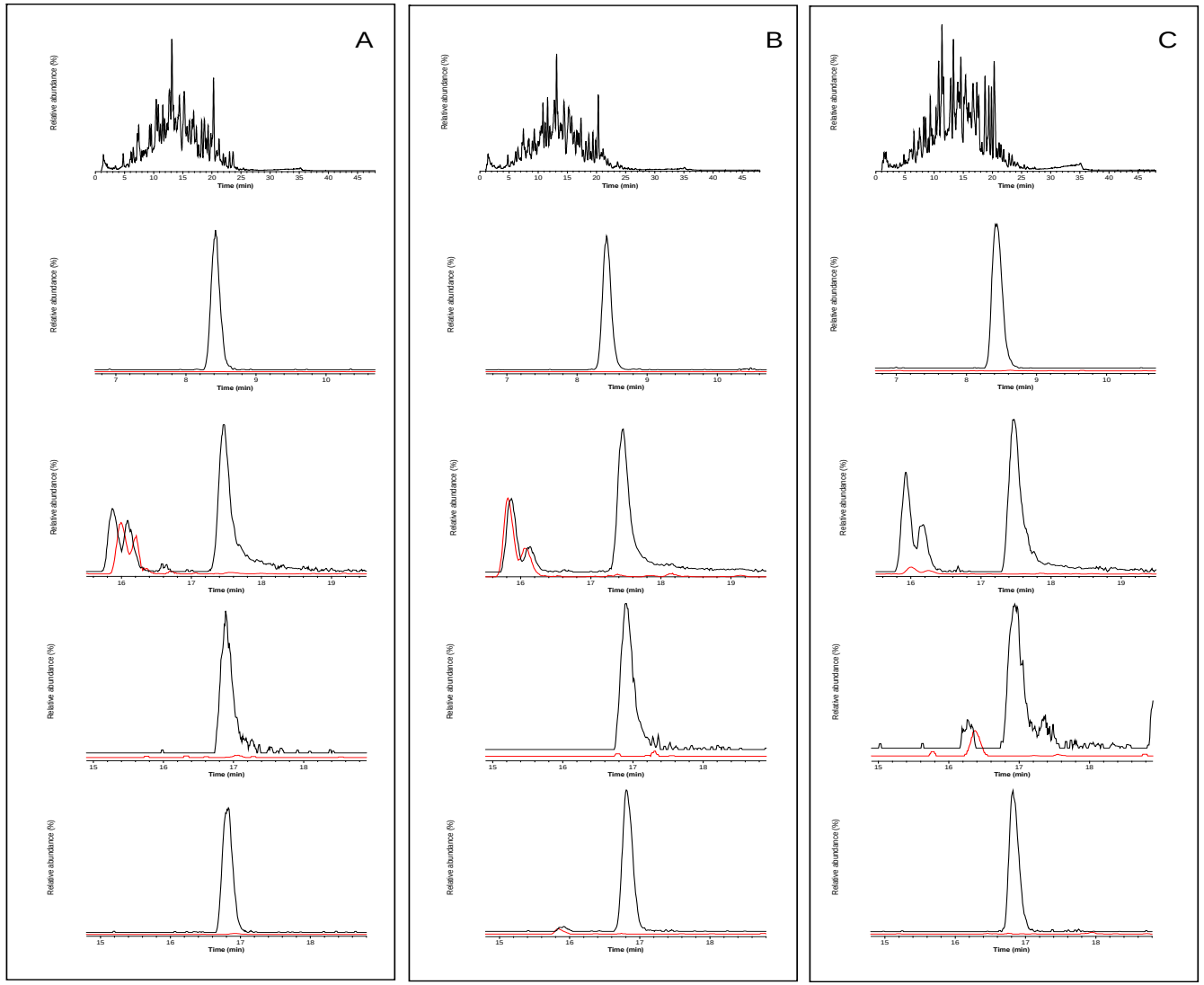
sapi | kambing | ayam



Myoglobin (120-134)
HPGDFGADAQGAMSK
XIC 744.8304 (± 5 ppm)

Myosin-1 (619-638)
TLAFLFTGAAGADAEAGGGK
XIC 912.9600 (± 5 ppm)

Myosin-2 (619-638)
TLAFLFSGAQTGEAEAGGTK
XIC 978.4891 (± 5 ppm)

β-Haemoglobin
FFESFGDLSNADAVMGNPK
XIC 1023.4673 (± 5 ppm)

Warna merah untuk daging tanpa spiking

Data-independent acquisition (DIA)

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Full scan m/z 600 – 1200 70, 000 FWHM

MS/MS isolation range

17 500 FWHM
AGT: $1e^6$
Isolation with : 50 DA
MSX count: 1
Loop count: 6
Max IT: Auto

- m/z 600-650
- m/z 650-700
- m/z 700-750
- m/z 750-800
- m/z 800-850
- m/z 850-900
- m/z 900-950
- m/z 950-1000
- m/z 1000-1050
- m/z 1050-1100
- m/z 1100-1150
- m/z 1150-1200

Example: Pork myoglobin proteotypic peptide (120-134)

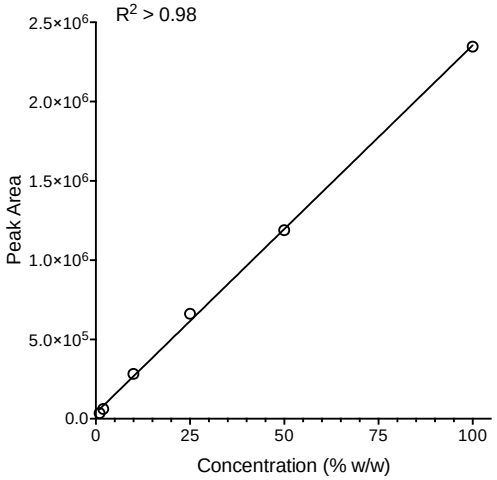
HPGDFGADAQGAMSK

Precursor mass m/z
744.8304 (+2)

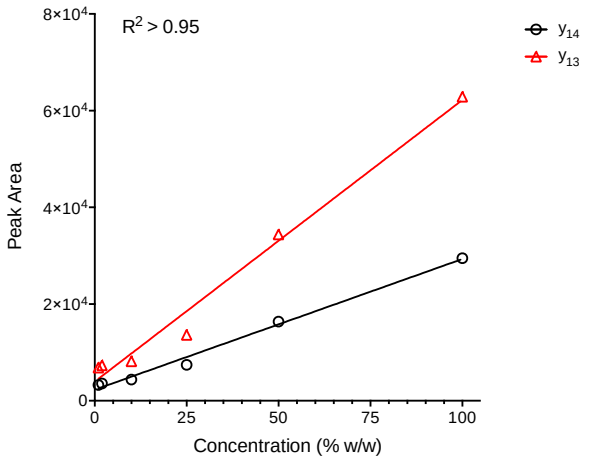
Specific fragment ions

- 1254.5419 (y_{13})
- 1351.5947 (y_{14})

MS1 data



DIA data



STRATEGI UMUM

Extraction of gene information

In silico sequence alignment

In silico protein translation and alignment

Sequence analysis and *In silico* tryptic digestion

Generate proteotypic mass lists

Generate MS/MS product ion mass lists

Survey MS Scan

Targeted MS/MS or DIA (25 or 50 Da)

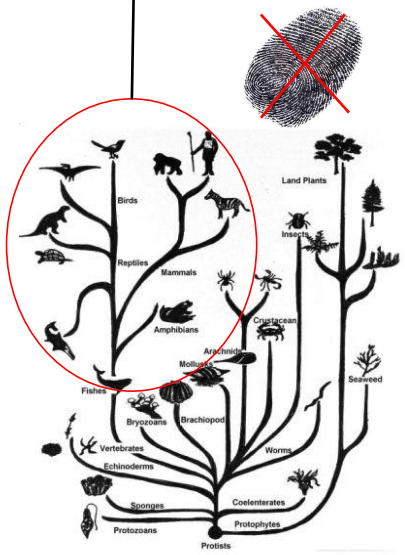
Acquired MS/MS spectra

Select ions of interest

Generation of post-analysis XICs for selected proteotyping peptides

Precursor and specific production ions

Speciation is therefore based on genetic differences



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TERIMA KASIH

LOCALLY ROOTED, GLOBALLY RESPECTED

UGM.AC.ID