

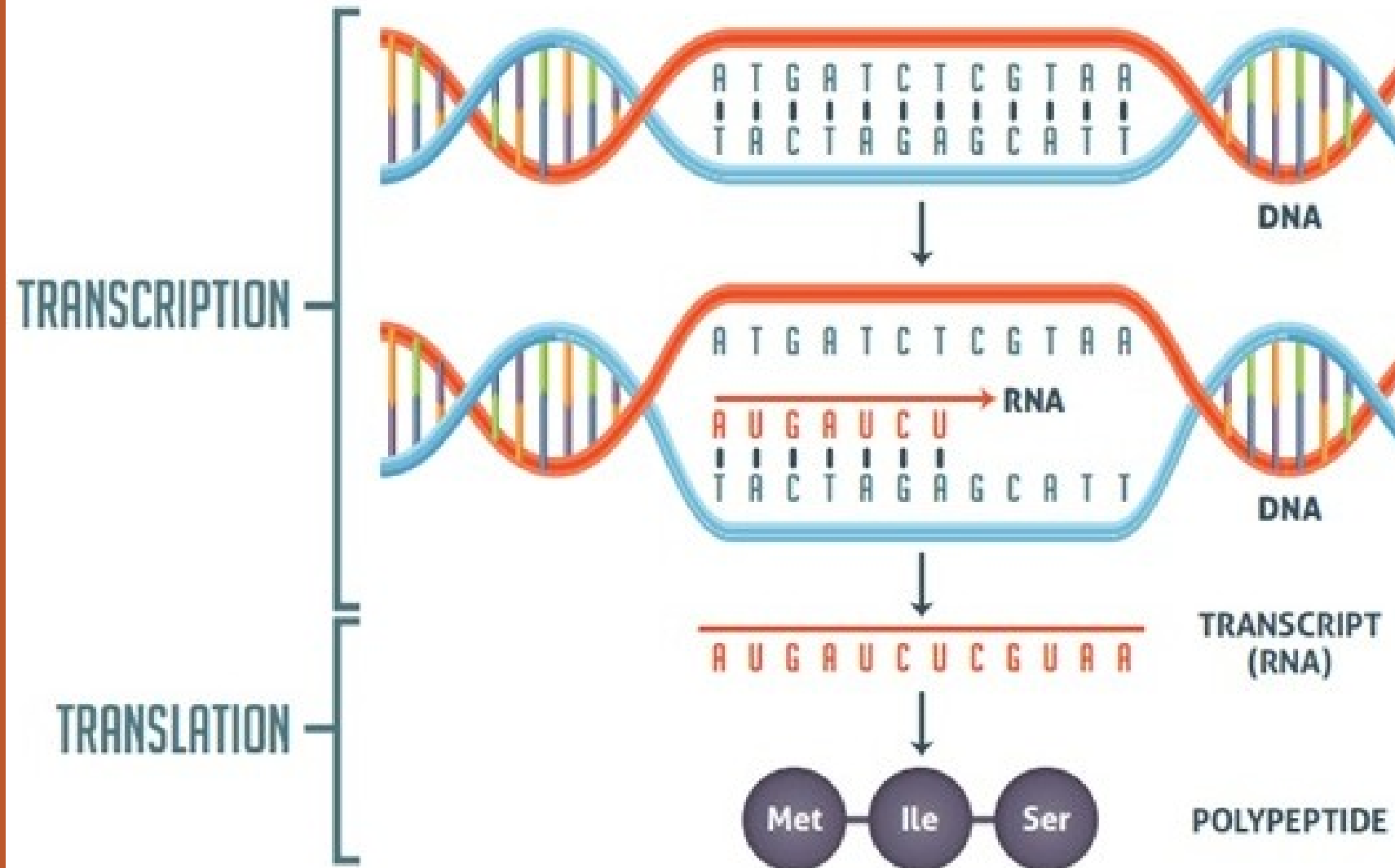
SEKVENOVÁNÍ DNA A PROTEINŮ

Norbert Gašparik

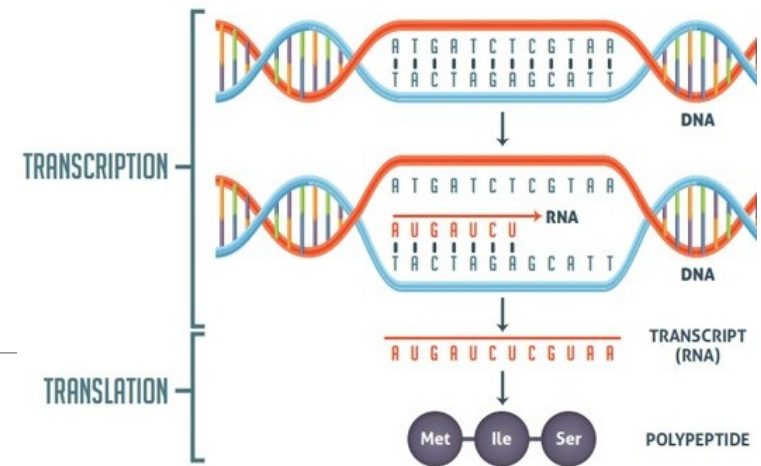
22/10/2021

C9531 Strukturní Biochemie – seminář

Transcription Translation



Synthesis of mRNA



coding strand

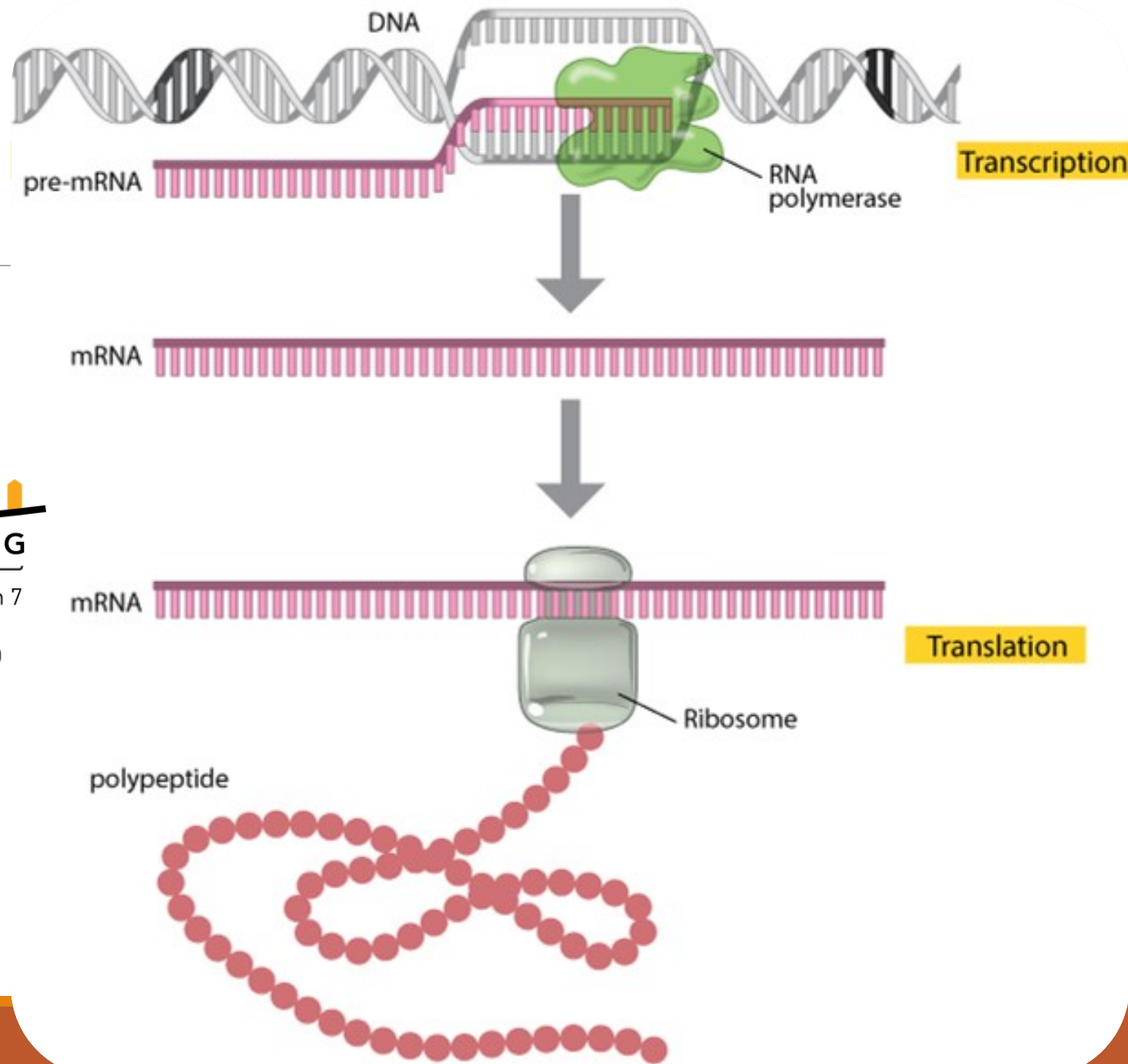
5' - TTAGCTTTCATACG - 3'

5' - UUAGCUUUCAUACG

3' - AATCGAAAGTATGC - 5'

non-coding strand

Protein biosynthesis




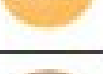

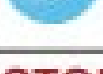







































RNA																					
Base	G	C	U	A	C	G	G	A	G	C	U	U	C	G	G	A	G	C	U	A	G
Codon	Codon 1			Codon 2			Codon 3			Codon 4			Codon 5			Codon 6			Codon 7		
Aminoacid	Alanine			Threonine			Glutamate			Leucine			Arginine			Serine			Stop		

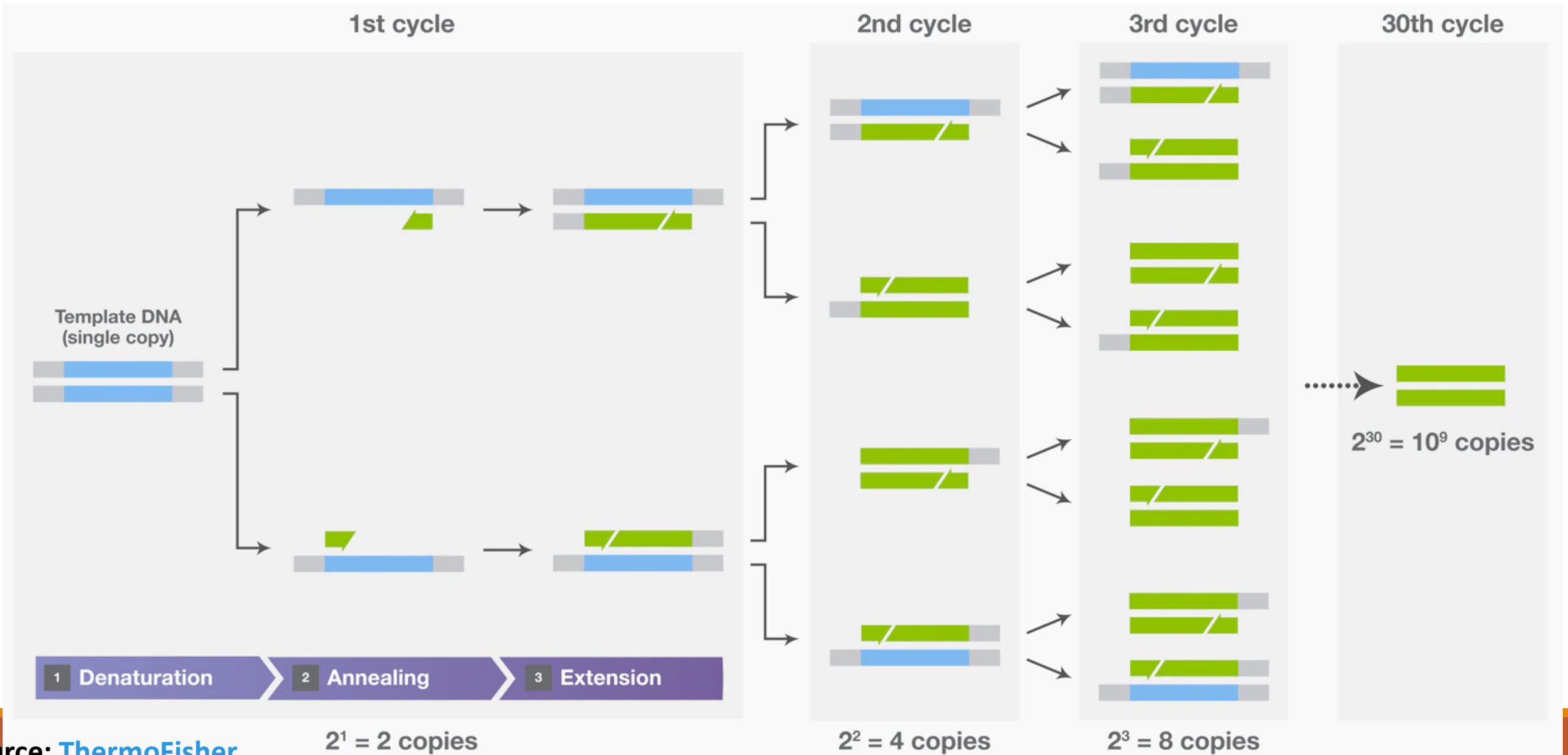
Codon Table

Genetic code:

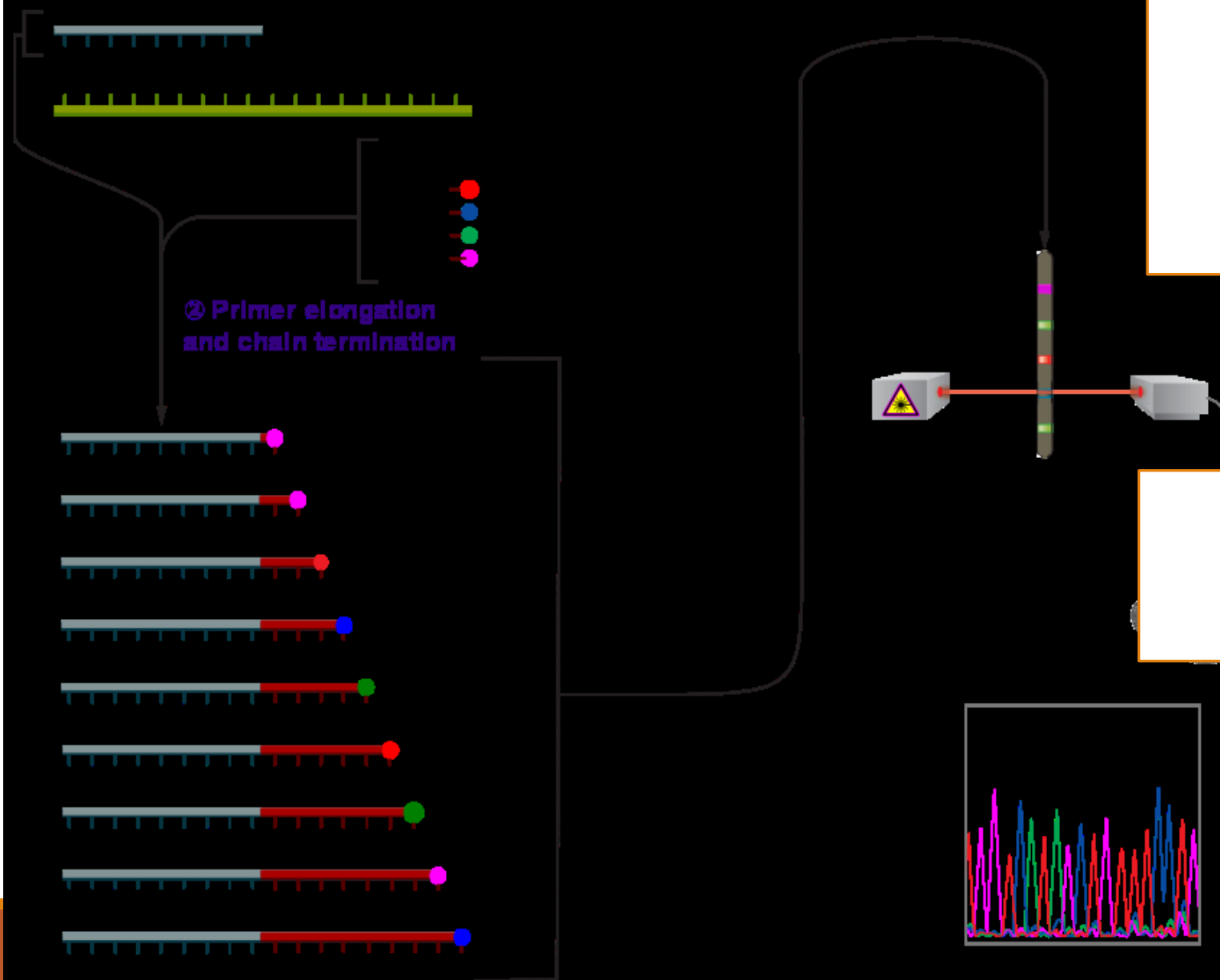
- Universal
- Degenerated
- Specific
- Non-overlapping

		Second nucleotide				
		U	C	A	G	
U	U	UUU 	UCU	UAU 	UGU 	U C A G
		UUC 	UCC 	UAC 	UGC 	
		UUA 	UCA 	UAA STOP	UGA STOP	
		UUG 	UCG	UAG STOP	UGG 	
C	C	CUU 	CCU	CAU 	CGU	U C A G
		CUC 	CCC 	CAC 	CGC 	
		CUA 	CCA 	CAA 	CGA 	
		CUG 	CCG	CAG 	CGG	
A	A	AUU 	ACU	AAU 	AGU 	U C A G
		AUC 	ACC 	AAC 	AGC 	
		AUA 	ACA 	AAA 	AGA 	
		AUG 	ACG	AAG 	AGG 	
G	G	GUU 	GCU	GAU 	GGU	U C A G
		GUC 	GCC 	GAC 	GGC 	
		GUA	GCA	GAA	GGA	
		GUG	GCG	GAG	GGG	

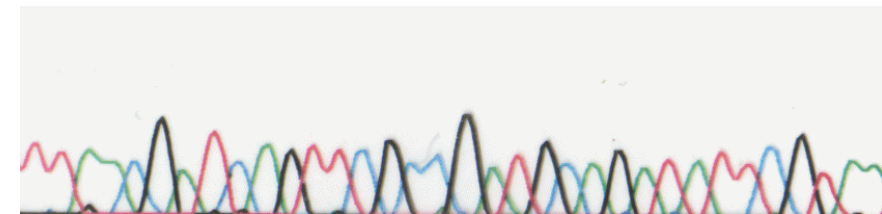
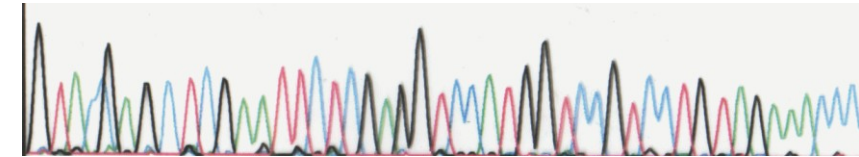
Polymerase Chain Reaction



- ① Reaction mixture
 - ▶ Primer and DNA template
 - ▶ DNA polymerase
 - ▶ ddNTPs with flouochromes
 - ▶ dNTPs (dATP, dGTP, dGTP, and dTTP)



Sanger sequencing



Sequencing primers

coding strand

5' - **T T A G C T T T C A T A C G** - 3'
A A T C G A A A G **T A T G C** - 5' Rev

For

5' - **T T A G C T T T C A T A C G**
3' - **A A T C G A A A G T A T G C** - 5'

non-coding strand

Open Reading Frames

5 ' - TTAGCTTTACACG - 3 '

3 ' - AATCGAAAGTATGC - 5 '

Open Reading Frames

5 ' - TTAGCTTT CATACG - 3 '

3 ' - AATCGAAA GTATGC - 5 '

ORF1

5' - TTAGCTTTTCATACG - 3'

Leu

Ala

Phe

Ile

L

A

F

I

ORF2

5' - TTAGCTTTCATACG - 3'

stop

Leu

Ser

Tyr

-

L

S

Y

ORF3

5' - TTAGCTTTTTCATACG - 3'

Ser

Phe

His

Thr

S

F

H

T

Open Reading Frames

5 ' - TTAGCTTTTCATACG - 3 '

3 ' - AATCGAAAGTATGC - 5 '

Open Reading Frames

5 ' - TTAGCTTTTCATACG - 3 '

3 ' - AATCGAAAGTATGC - 5 '

5 ' - CGTATGAAAGCTAA - 3 '

ORF4

5' - CGTATGAAAAGCTAA - 3'

Arg

Met

Lys

Ala

R

M

K

A

ORF5

5 ' - C|G|T|A|T|G|A|A|A|G|C|T|A|A - 3 ' |

Val

stop

Lys

Leu

V

-

K

L

ORF6

5' - CGTATGAAAGCTAA - 3'

Tyr

Glu

Ser

stop

Y

E

S

-

Exercise 1

CODING STRAND

5' - AGATGAAACCGCGATTCTG -3'

R - N R D S

D E T A I L

M K P R F



NON-CODING STRAND

5' - GCGTTCCACCAGAATGCTCA -3'

↓
rev comp

5' - TGAGCATTCTGGTGGAACGC -3'

- A F W W N

E H S G G T

S I L V E R



Exercise 2

Coding strand

5' -
CATGATTCTGAAAGCG -3'

**ORF2:
MILKA**

Coding strand

3' -
AAACGTTTACGCCCAT -5'

↓
reverse

5' -
TACCCGCATTTGCAAA -3'

**ORF2:
TRICK**

Rev primer

5' -
TCACTTCTTTGCGCATGT
-3'

↓
rev comp

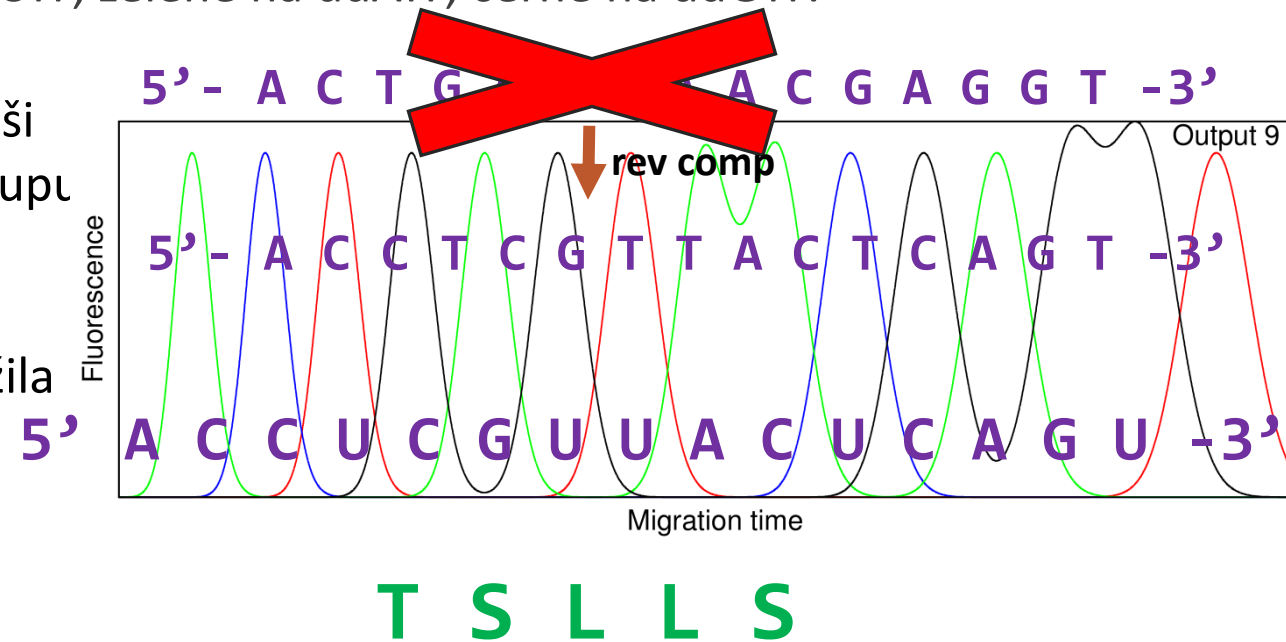
5' -
ACATGCGCAAAGAAGTG -
3'

**ORF3(6):
MRKEV**

Exercise 3

Získali jste cDNA vašeho proteinu, a naklonovali jste ji do expresního plasmidu tak, aby krátce za 3' koncem cDNA následovala plasmidová sekvence ACG GCC GTC GTT TTA C. Pro kontrolu sekvence vašeho genu pomocí Sangerovy metody jste použili primer 5'- GTA AAA CGA CGG CCG T -3' . Výstup sekvenátoru obsahoval přiložený fragment, odpovídající přibližně stému nukleotidu v sekvenci. Červeně je značen signál fluoroforu navázaného na ddTTP, modře na ddCTP, zeleně na ddATP, černě na ddGTP.

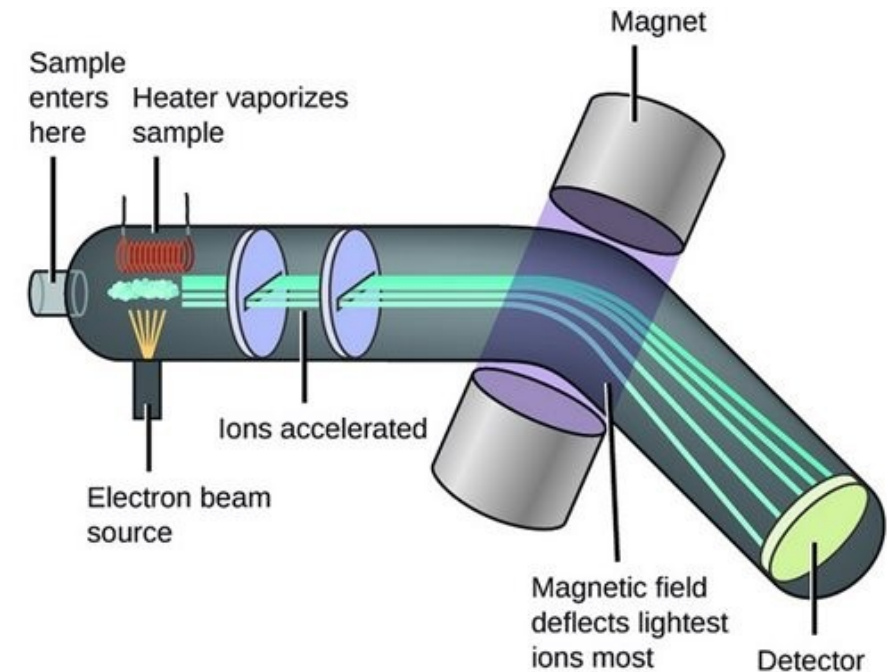
1. zapište úsek sekvence vlákna DNA obsahujícího vaši cDNA, který odpovídá uvedenému fragmentu výstupu sekvenátoru:
2. zapište příslušný úsek sekvence mRNA, která sloužila jako templát pro vaši cDNA
3. zapište úsek sekvence proteinu, který je kódován úsekem vašeho genu



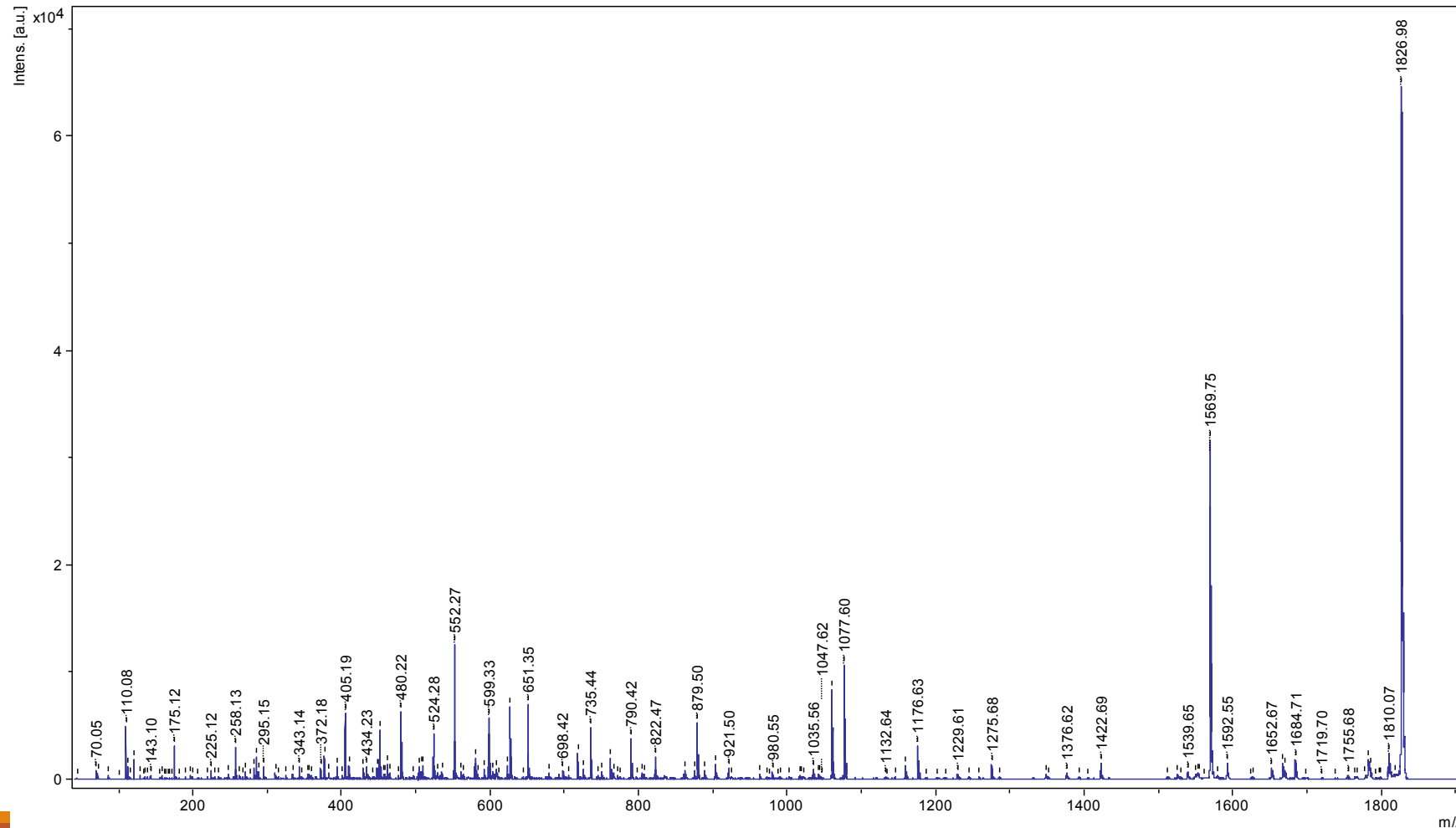


Mass Spectrometry

- Detection of the ratio between relative mass and charge of ions
 - Whole protein (intact) (MS)
 - Proteolytic digest (MS)
 - Physical fragmentation (MS/MS)
- Ionisation
 - Matrix Assisted Laser Desorption Ionisation (MALDI) (+ $1H^+$)
 - ElectroSpray Ionisation (ESI) (+ nH^+)
- Detection
 - Time-Of-Flight (TOF)
 - Ion Trap (IT)
 - Fourier Transform Ion Cyclotron Resonance (FT-ICR)
 - OrbiTrap

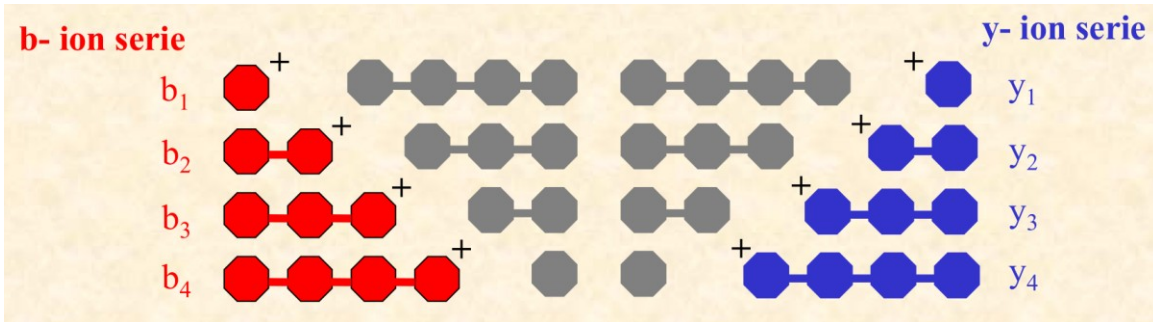
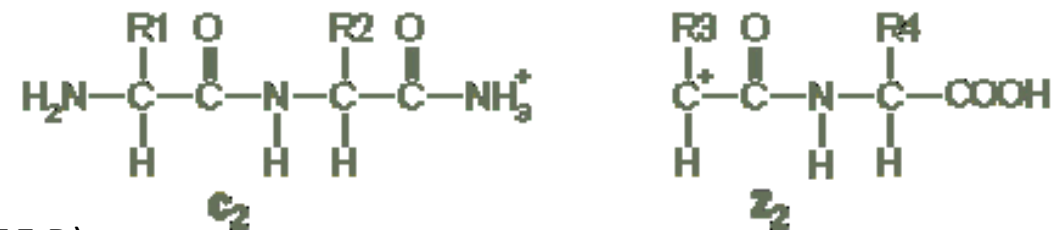
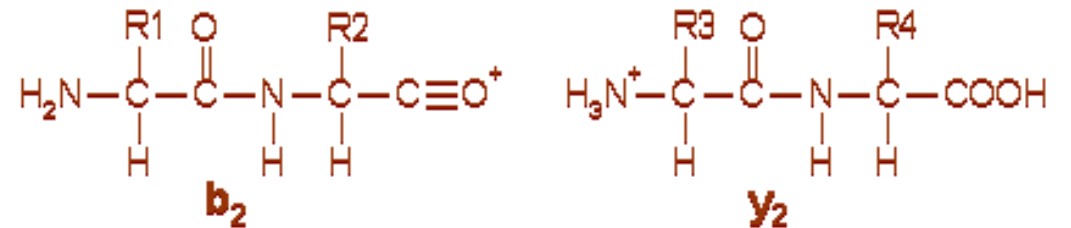
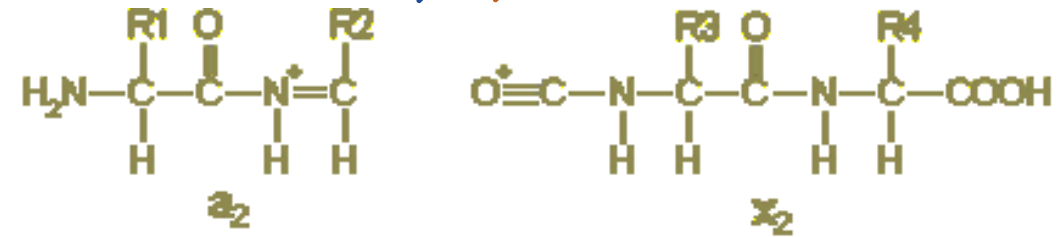
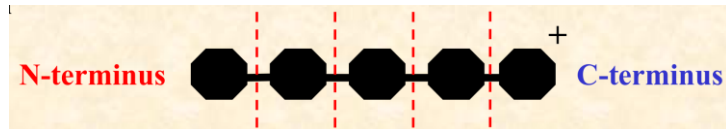
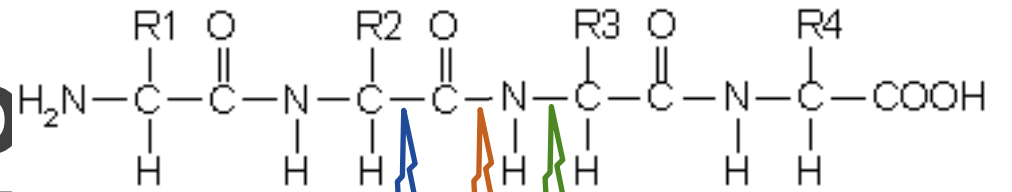


MS/MS spectra example



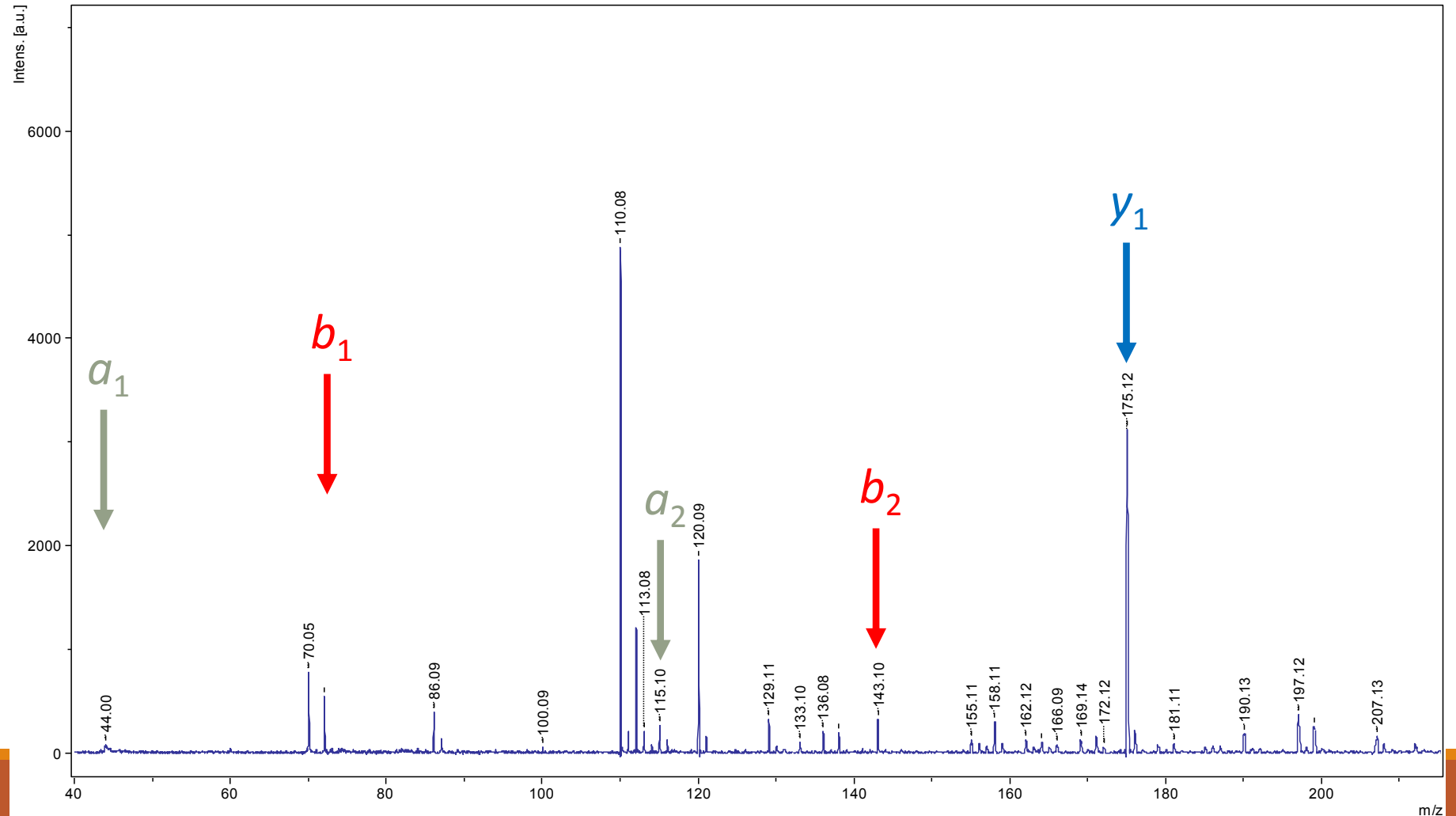
Peptide MH⁺ = 1826.98:
AADFFVPTGSHFYLR

Peptide fragmentation

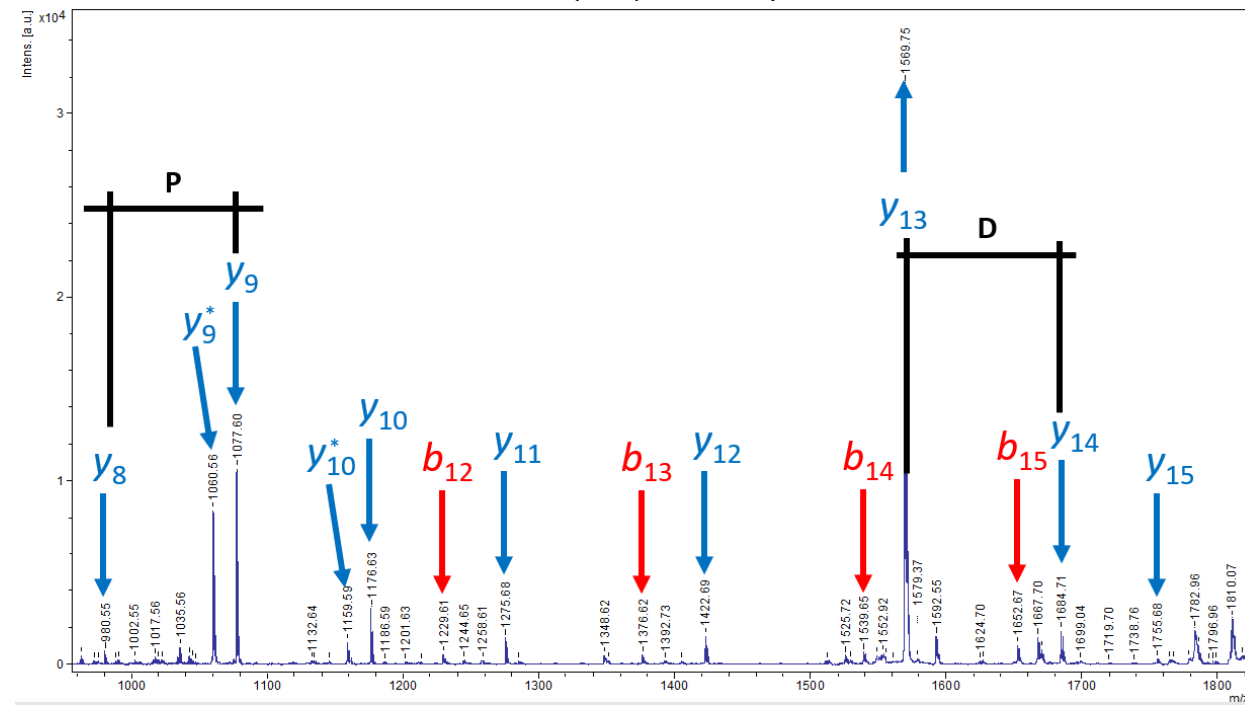
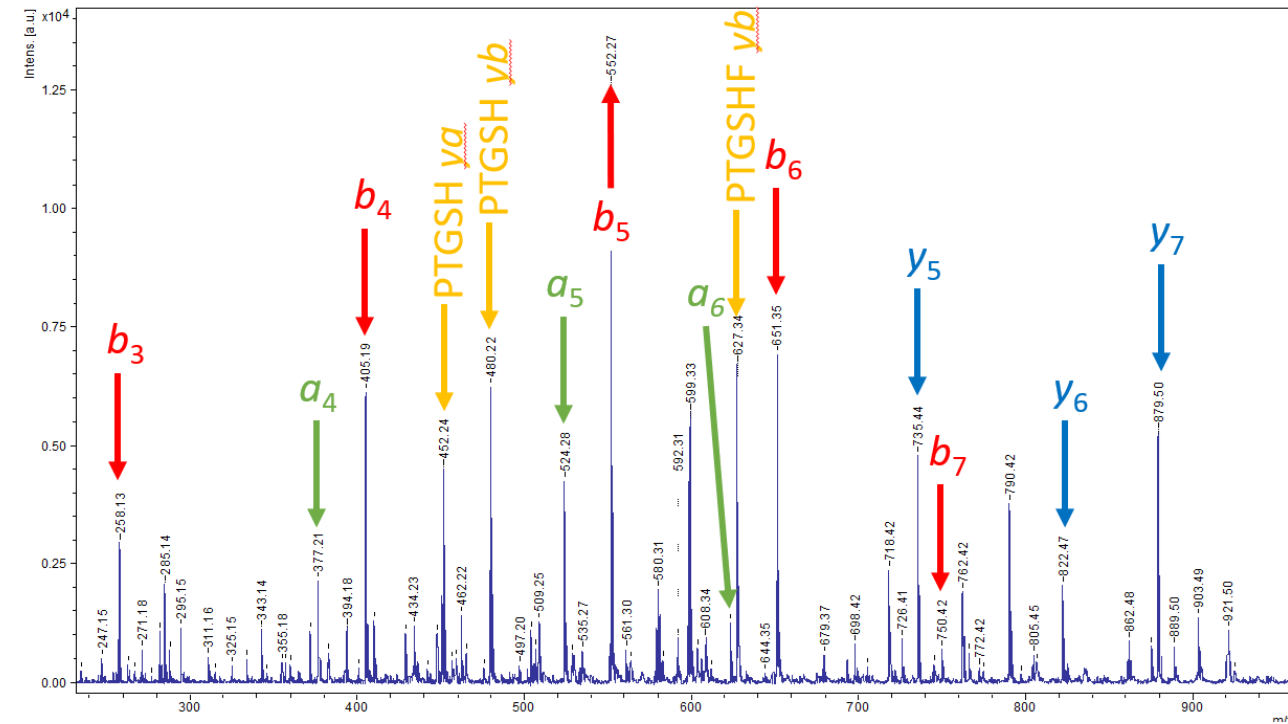


a⁰, b⁰, y⁰ = ztráta H₂O (S,T,E,D)
a^{*}, b^{*}, y^{*} = ztráta NH₃ (R,K,N,Q)

MS/MS spectra example



MS/MS spectra example



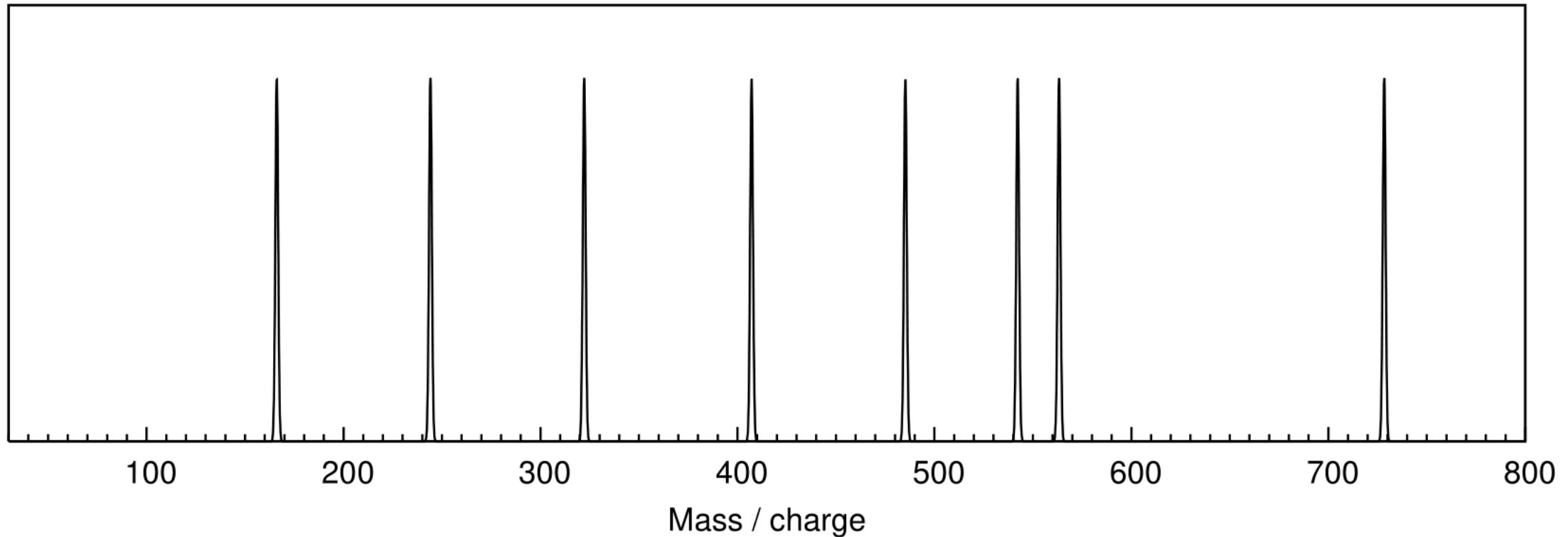
MS/MS sequencing example

MS/MS sequencing exercise 1

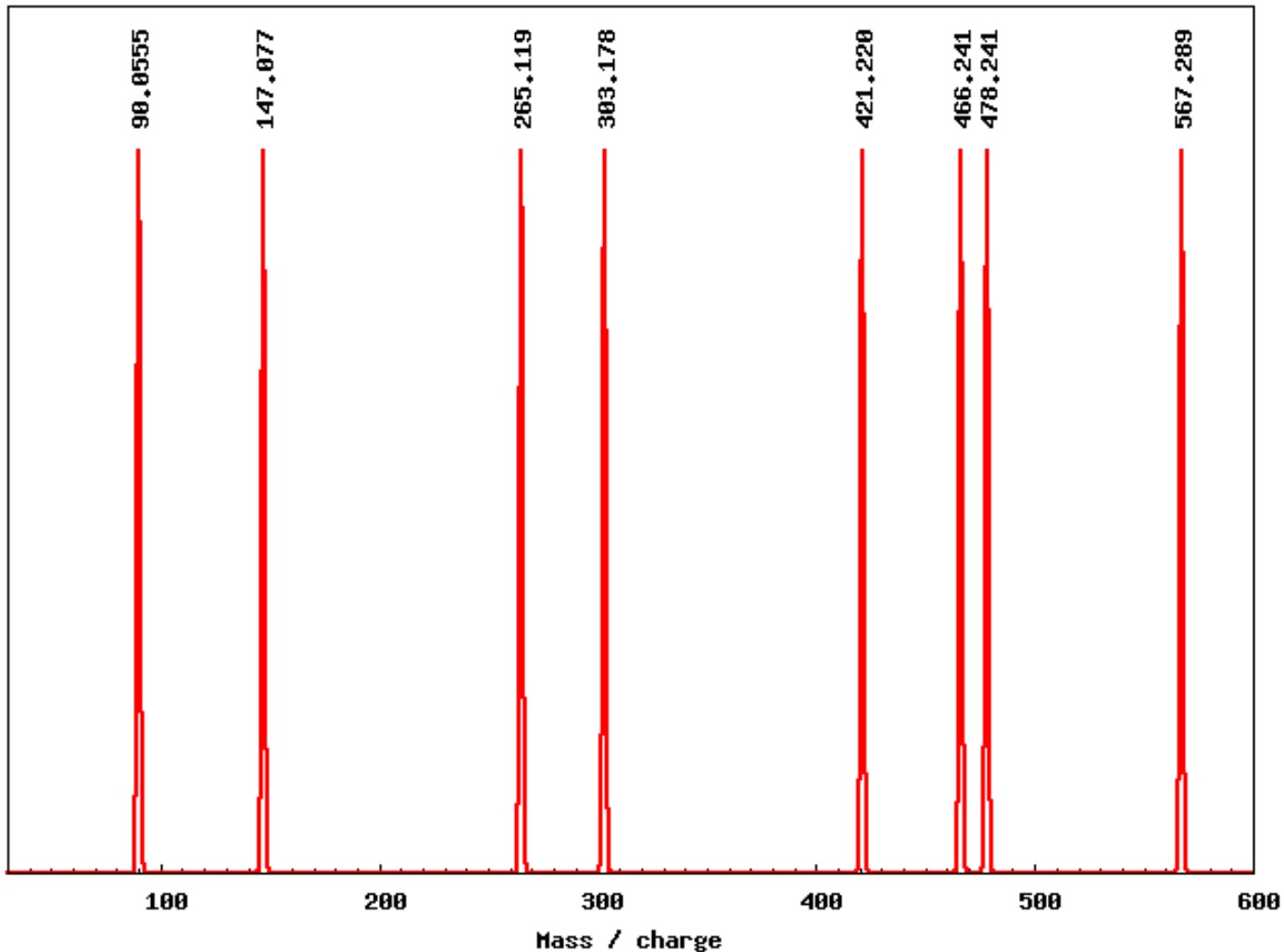
Peptide 2

WGYRF

166.087
244.109
322.188
407.172
485.251
542.273
563.273
728.352



MS/MS sequencing exercise 2

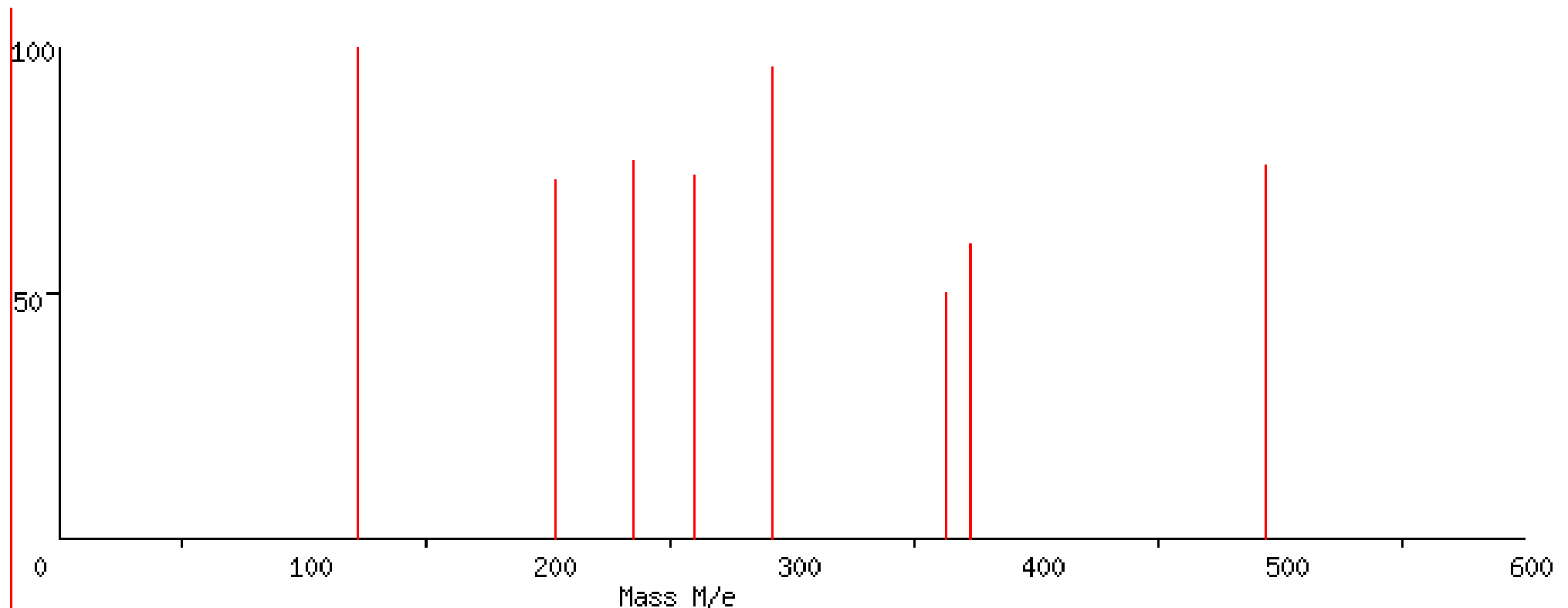


TYRGA

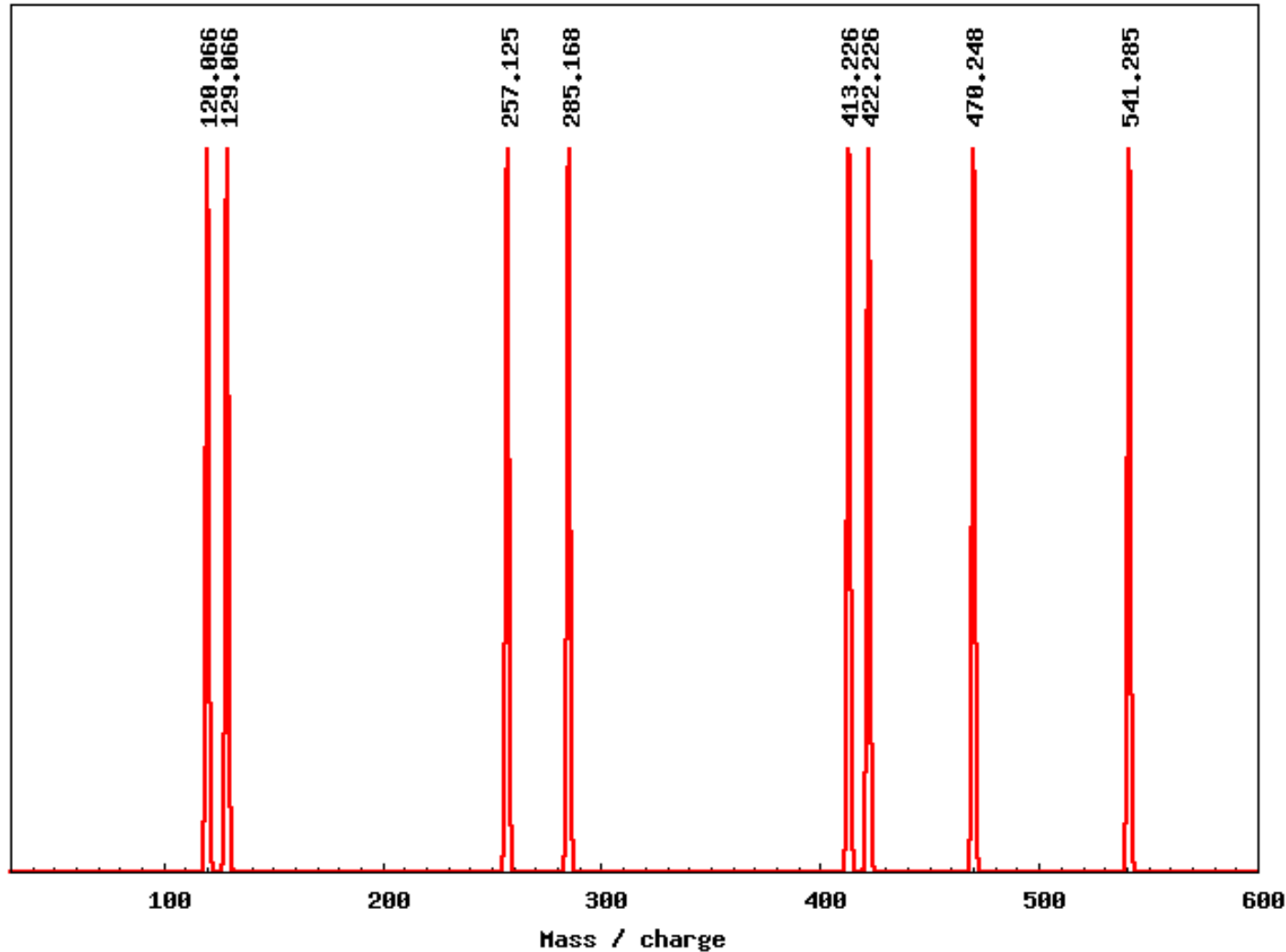
MS/MS sequencing exercise 3

MAGIC

122.02707
203.08492
235.11114
260.10638
292.1326
363.16971
373.19045
494.2102



MS/MS sequencing exercise 4



AGRHT