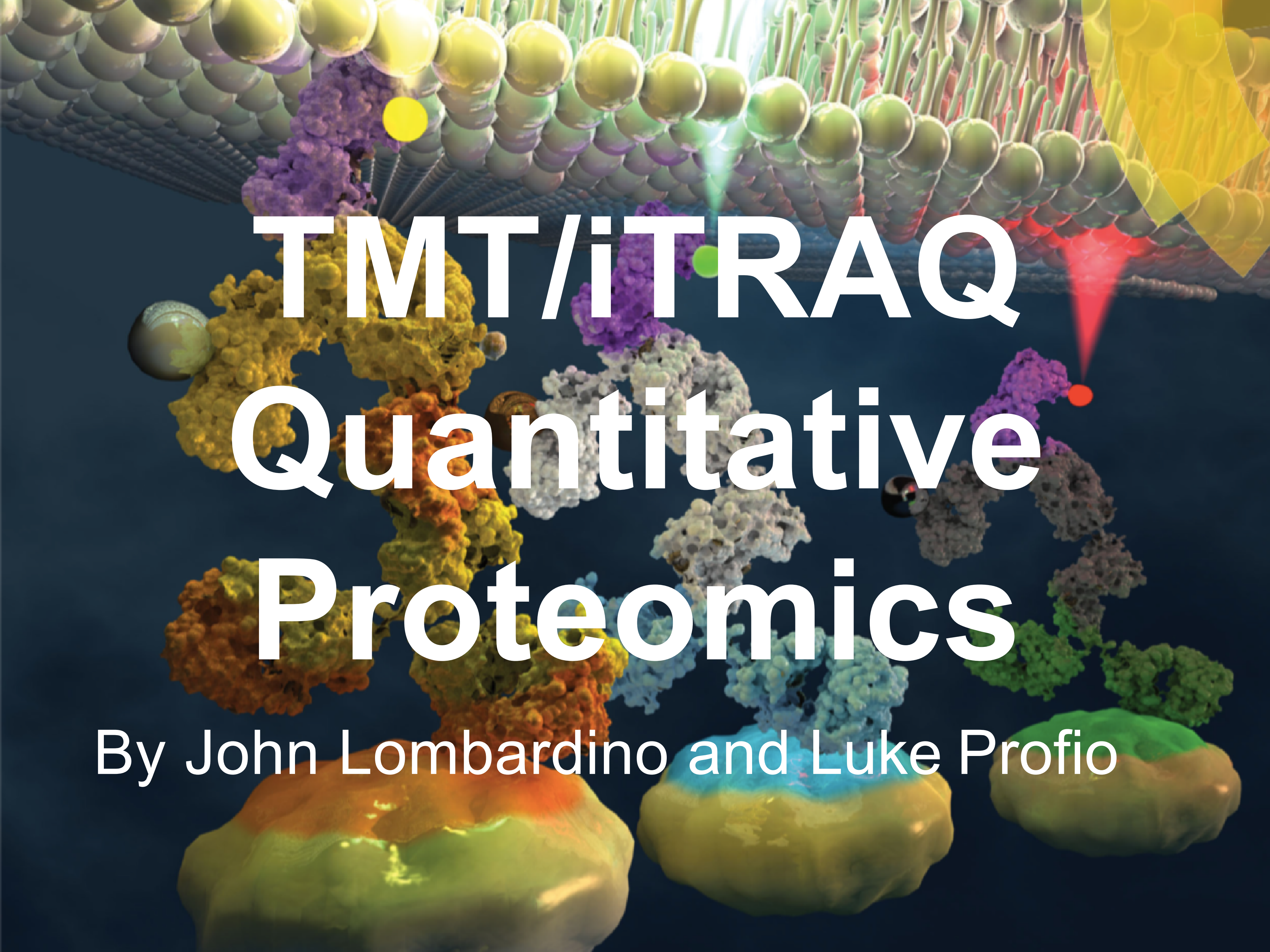
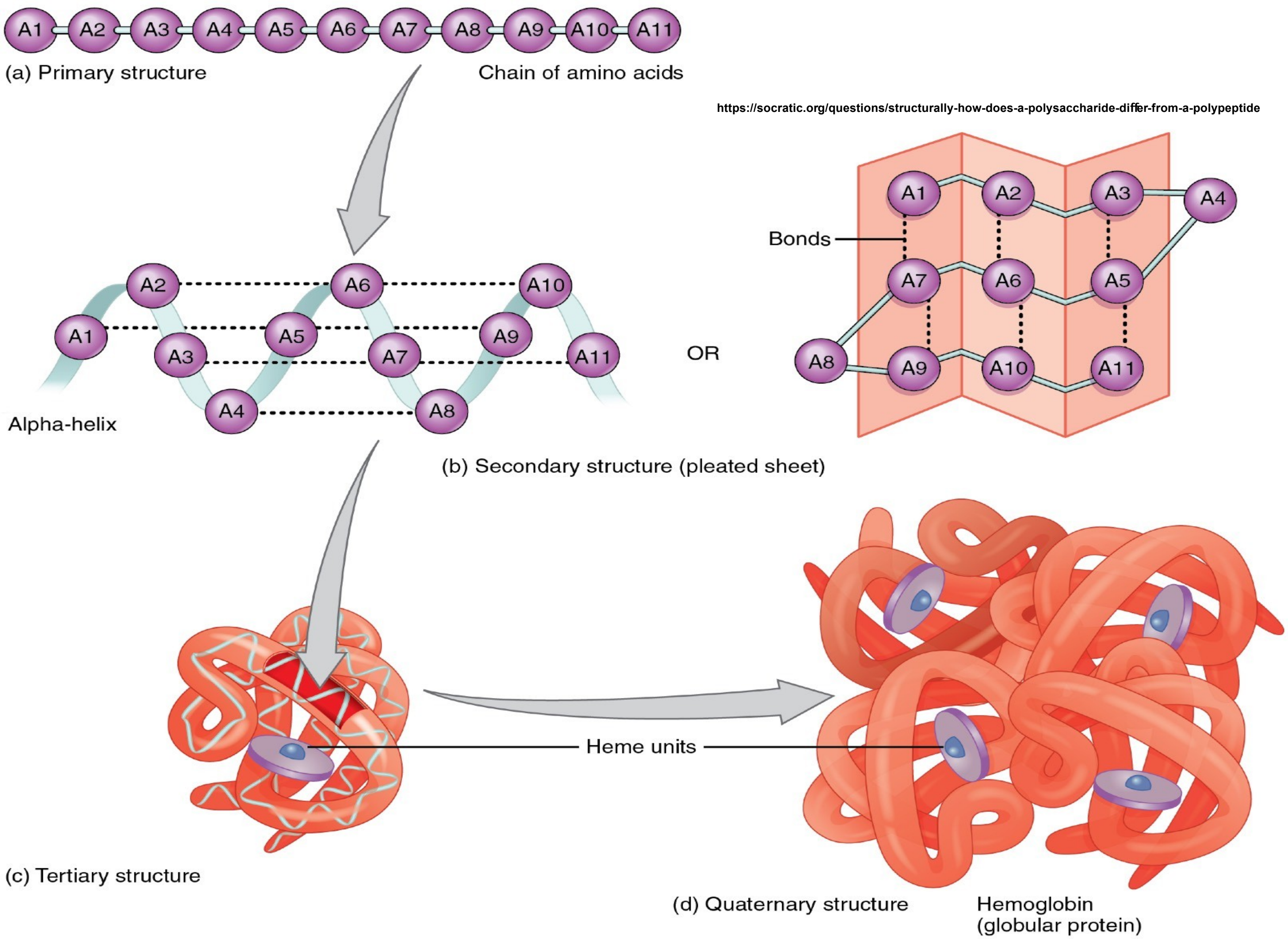


TMT/iTRAQ Quantitative Proteomics

By John Lombardino and Luke Profio

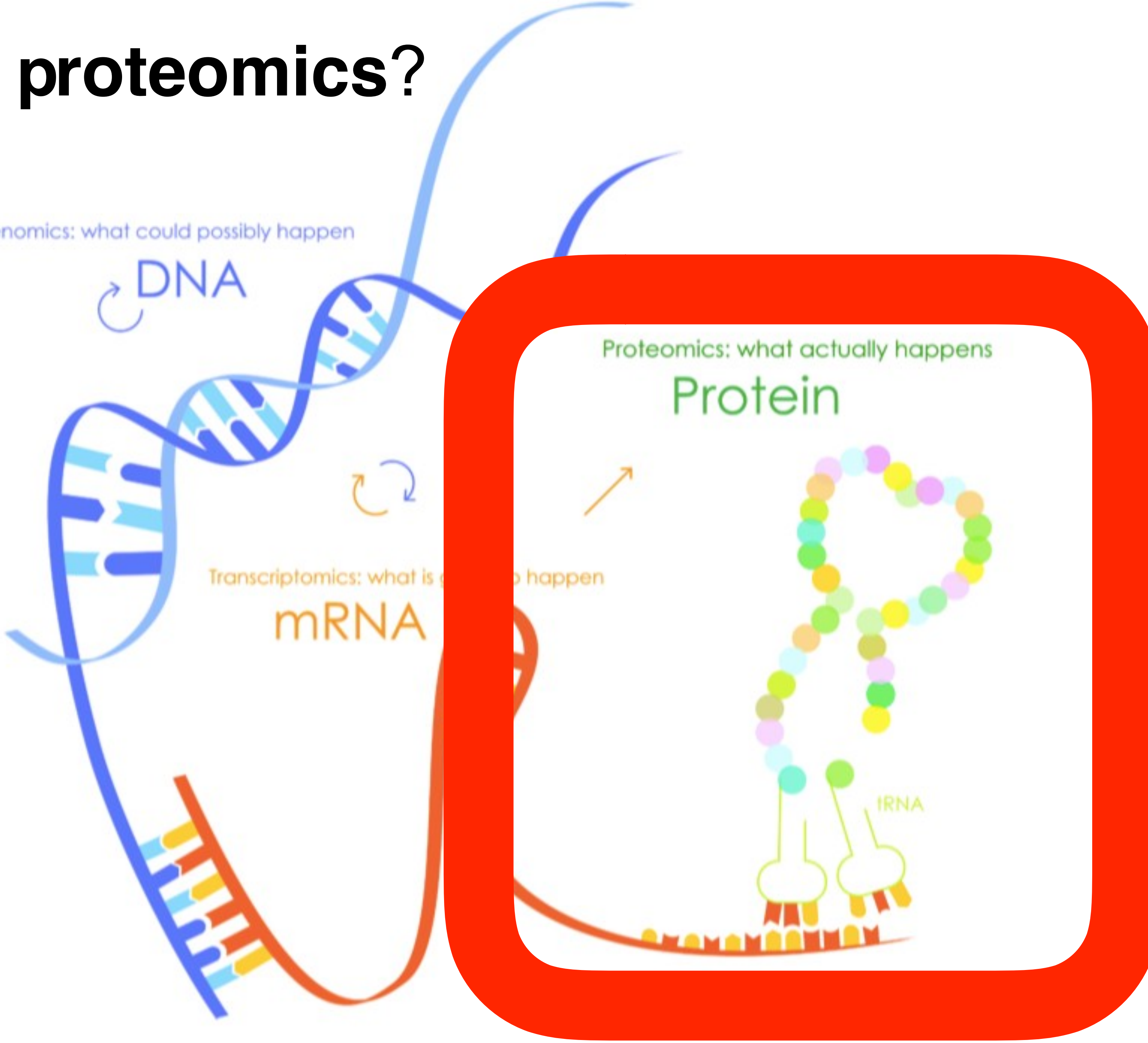
Overview: Why is **protein structure** important?



https://socratic.org/questions/structurally-how-does-a-polysaccharide-differ-from-a-polypeptide

Protein structures are the basic units of cellular and molecular work.

What is **proteomics**?



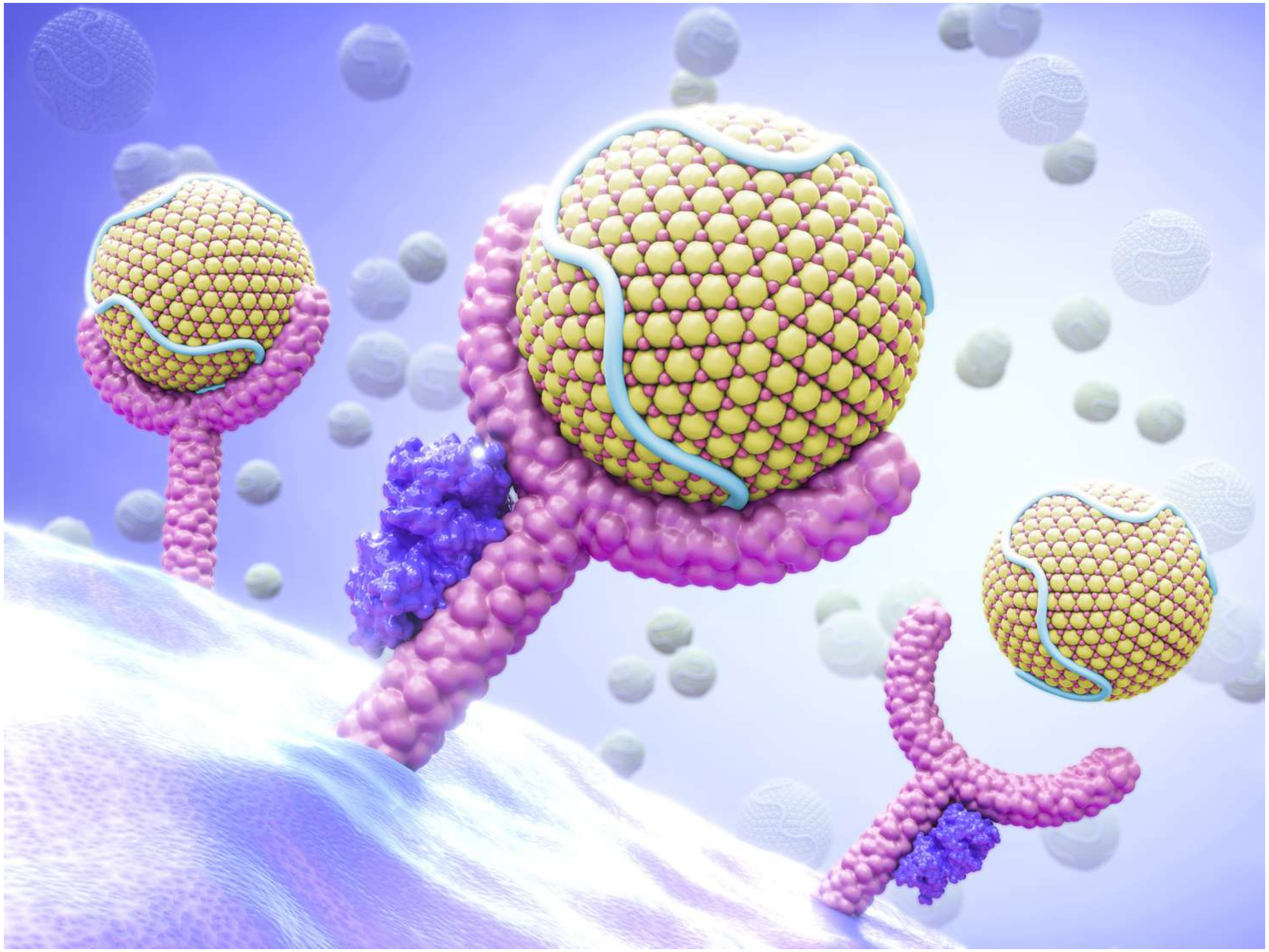
https://openarchive.ki.se/xmlui/bitstream/handle/10616/45601/Thesis_Bo_Zhang.final.pdf?sequence=1&isAllowed=y

What is **quantitative proteomics**?

http://sneezetoronto.com/corptest/clinical-operations/high-throughput-screening/

Quantitative proteomics is an analytical chemistry technique that determines the amount of the proteome's proteins in a variety of contexts.

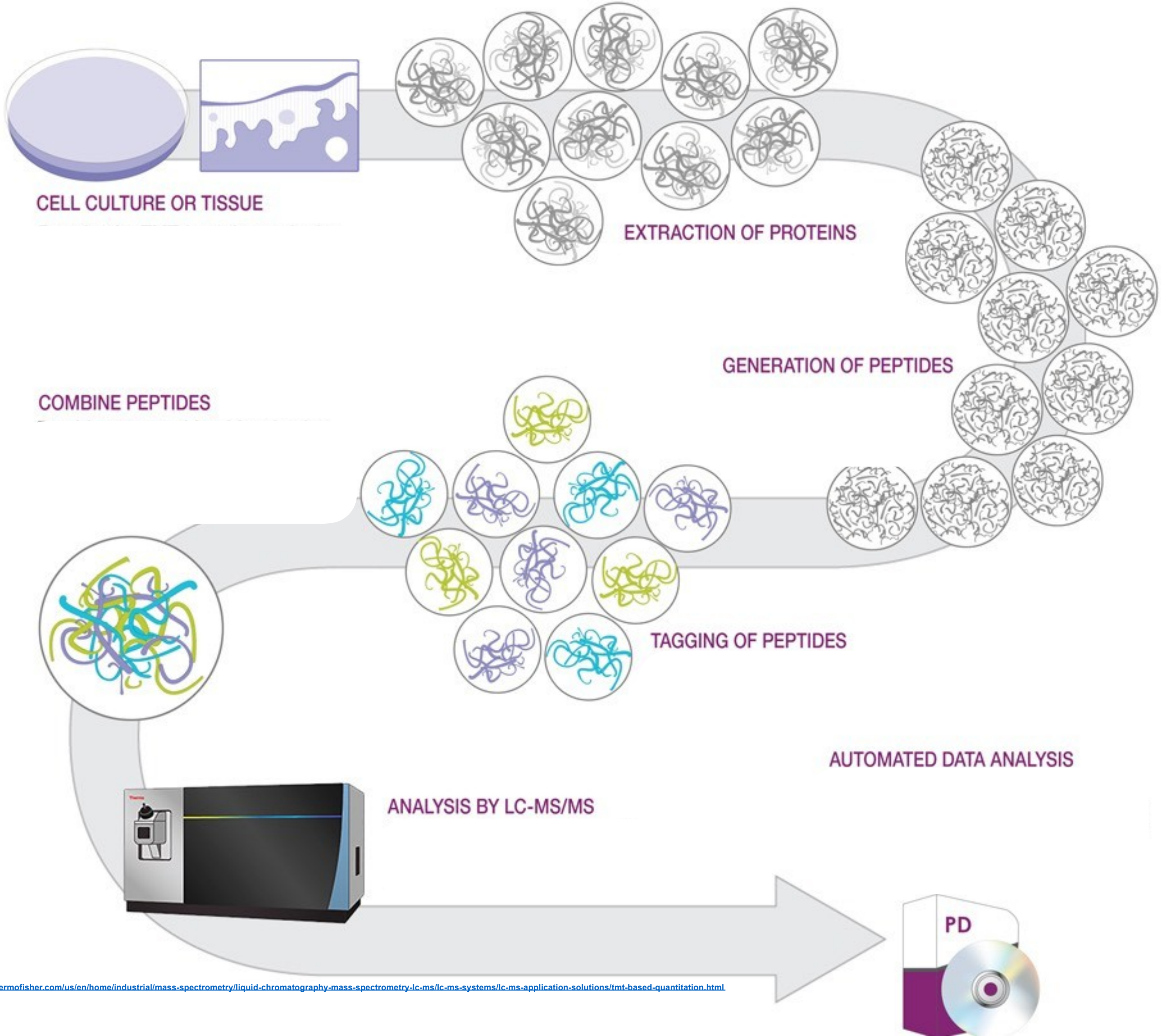
Why is quantitative proteomics important?



Quantitative proteomics enables both the *identity* and *quantification* of proteins essential to cellular and molecular mechanisms.

https://www.thoughtco.com/cell-membrane-373364

What’s the quantitative proteomic workflow?



CELL CULTURE OR TISSUE

EXTRACTION OF PROTEINS

GENERATION OF PEPTIDES

TAGGING OF PEPTIDES

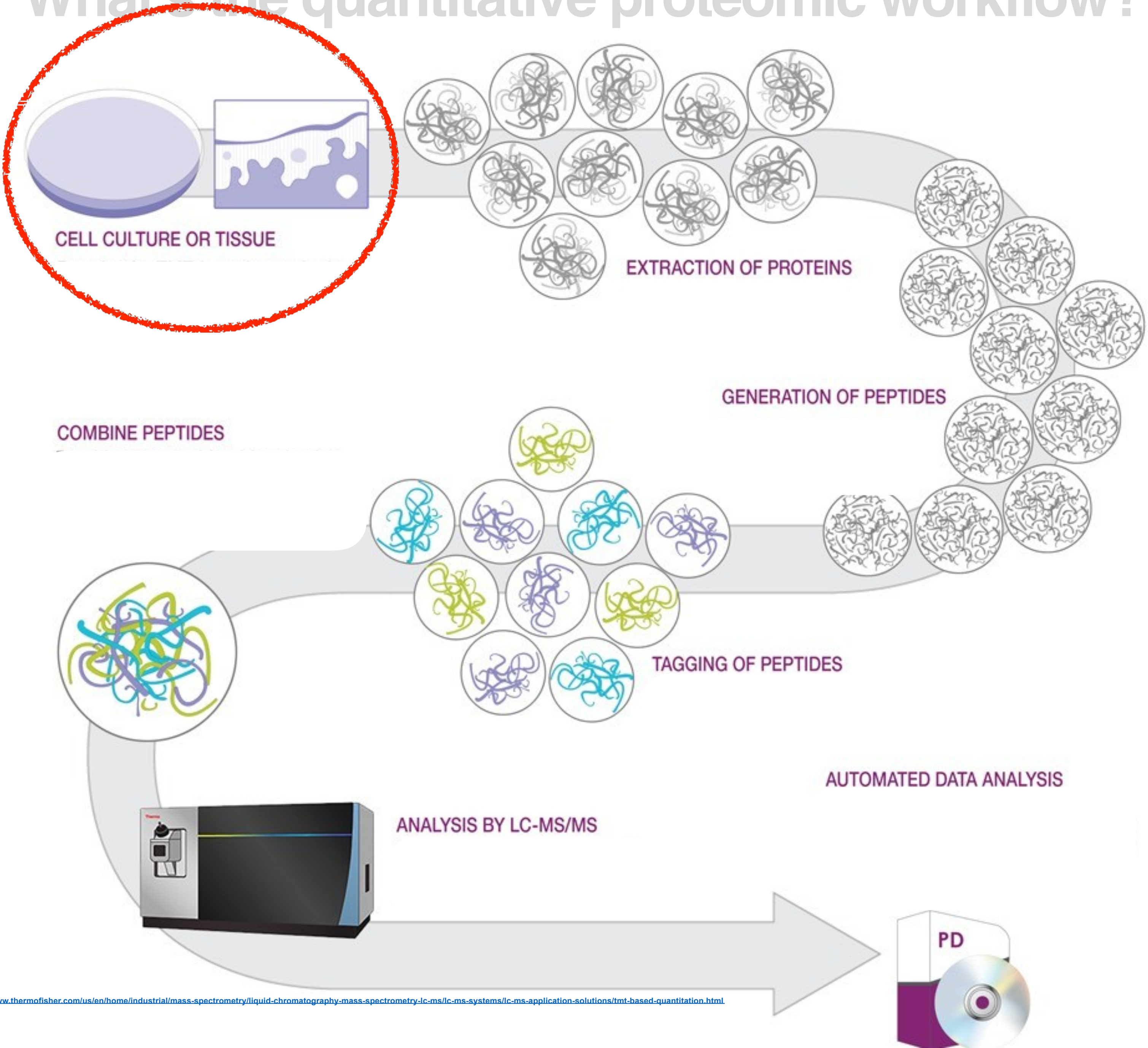
COMBINE PEPTIDES

ANALYSIS BY LC-MS/MS

AUTOMATED DATA ANALYSIS

https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html

What’s the quantitative proteomic workflow?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https://www.rdmag.com/article/2016/07/avoiding-contamination-cell-cultures

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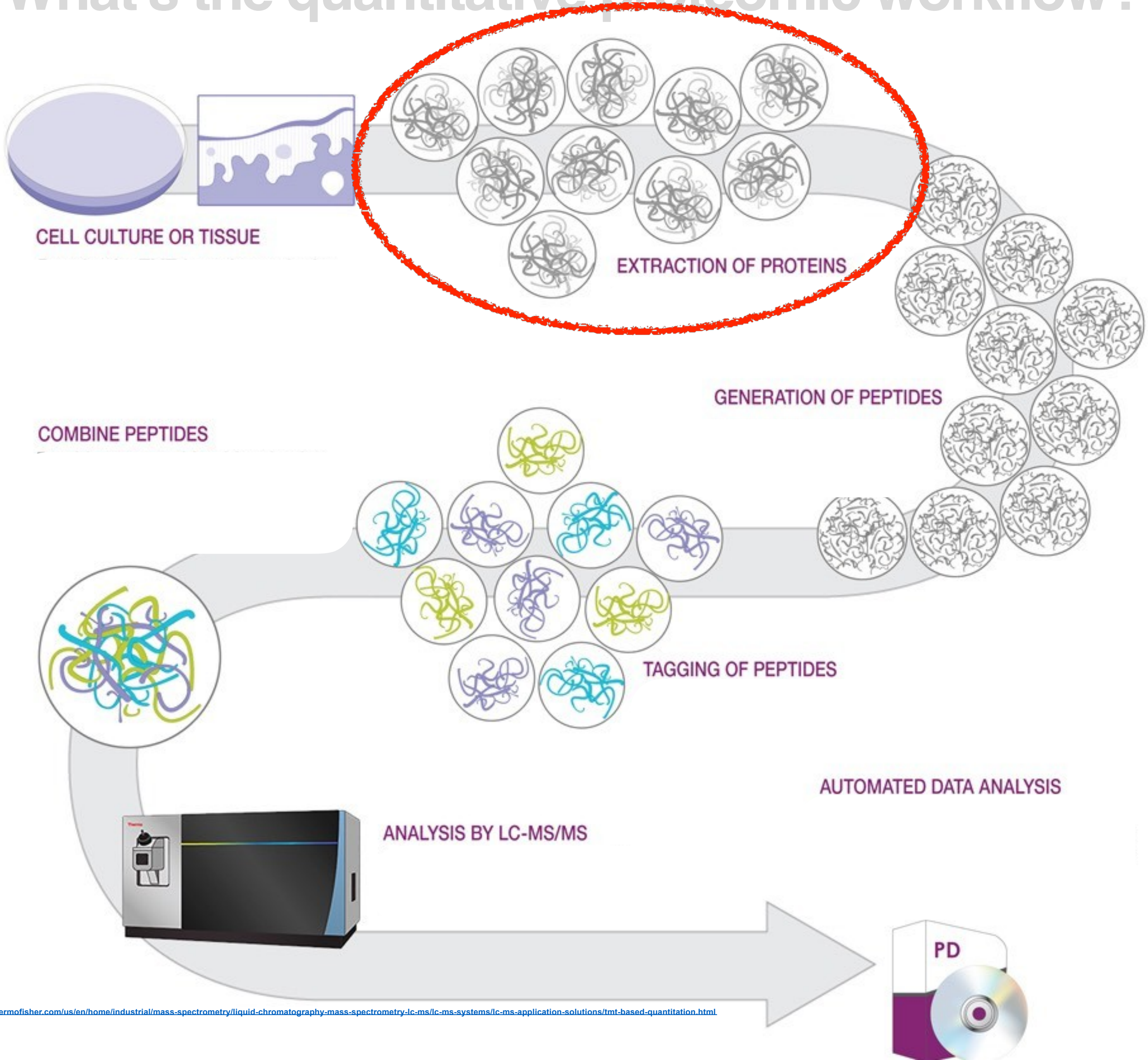

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http://www.geneaid.com/products/dna-rna-protein-extraction/dna-rna-protein-extraction-kit

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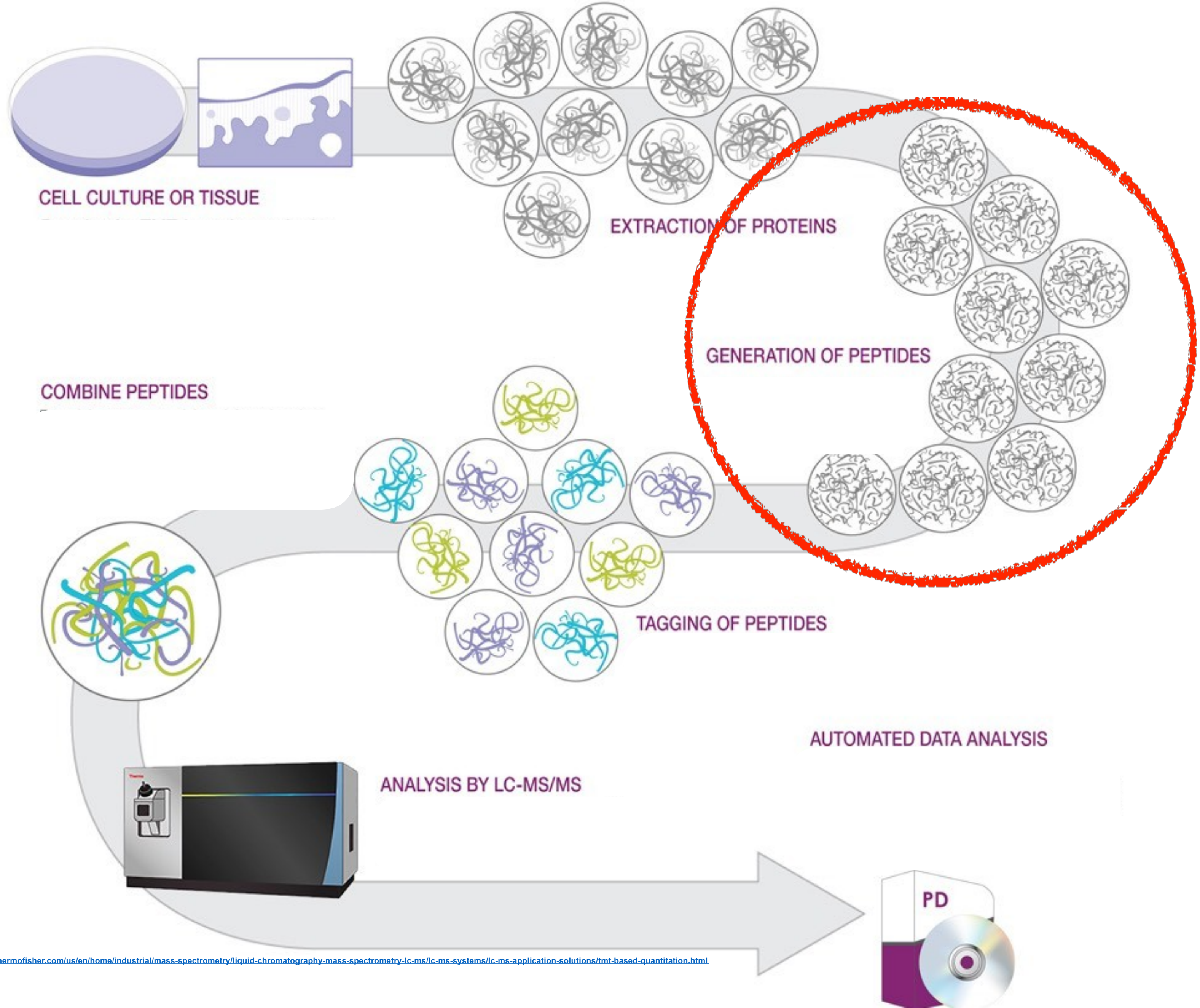
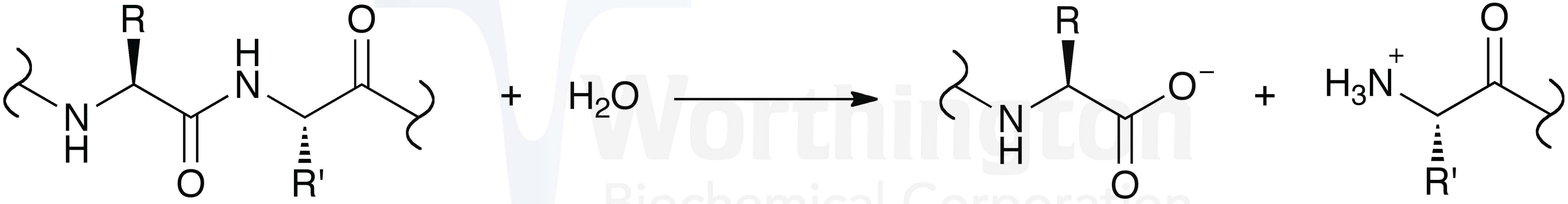


https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html

Where does **trypsin** cut proteins?

Trypsin



Polypeptide

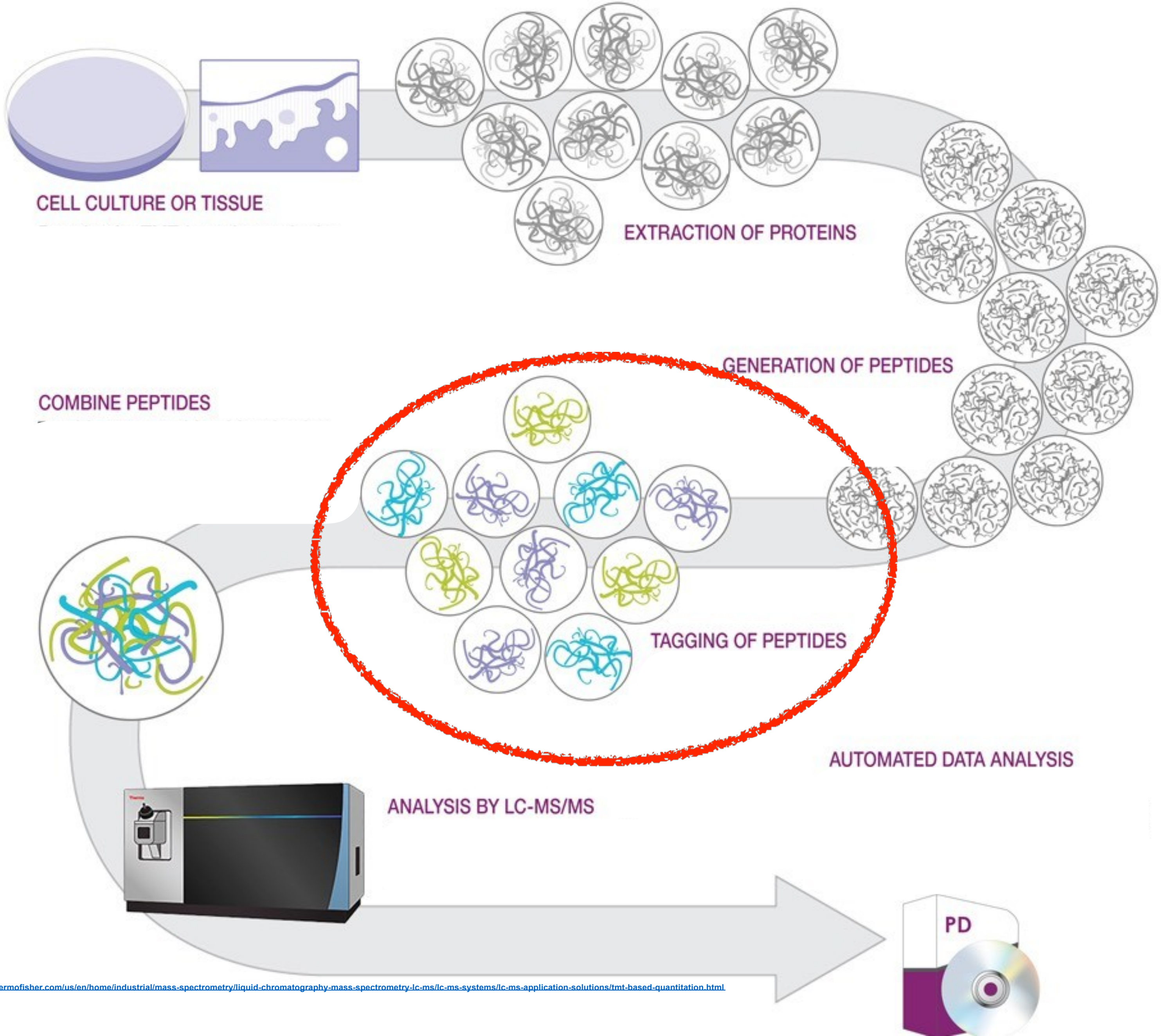
R = Arg and Lys

Polypeptide fragments

Trypsin is a serine protease

http://www.worthington-biochem.com/TRY/

What’s the quantitative proteomic workflow?

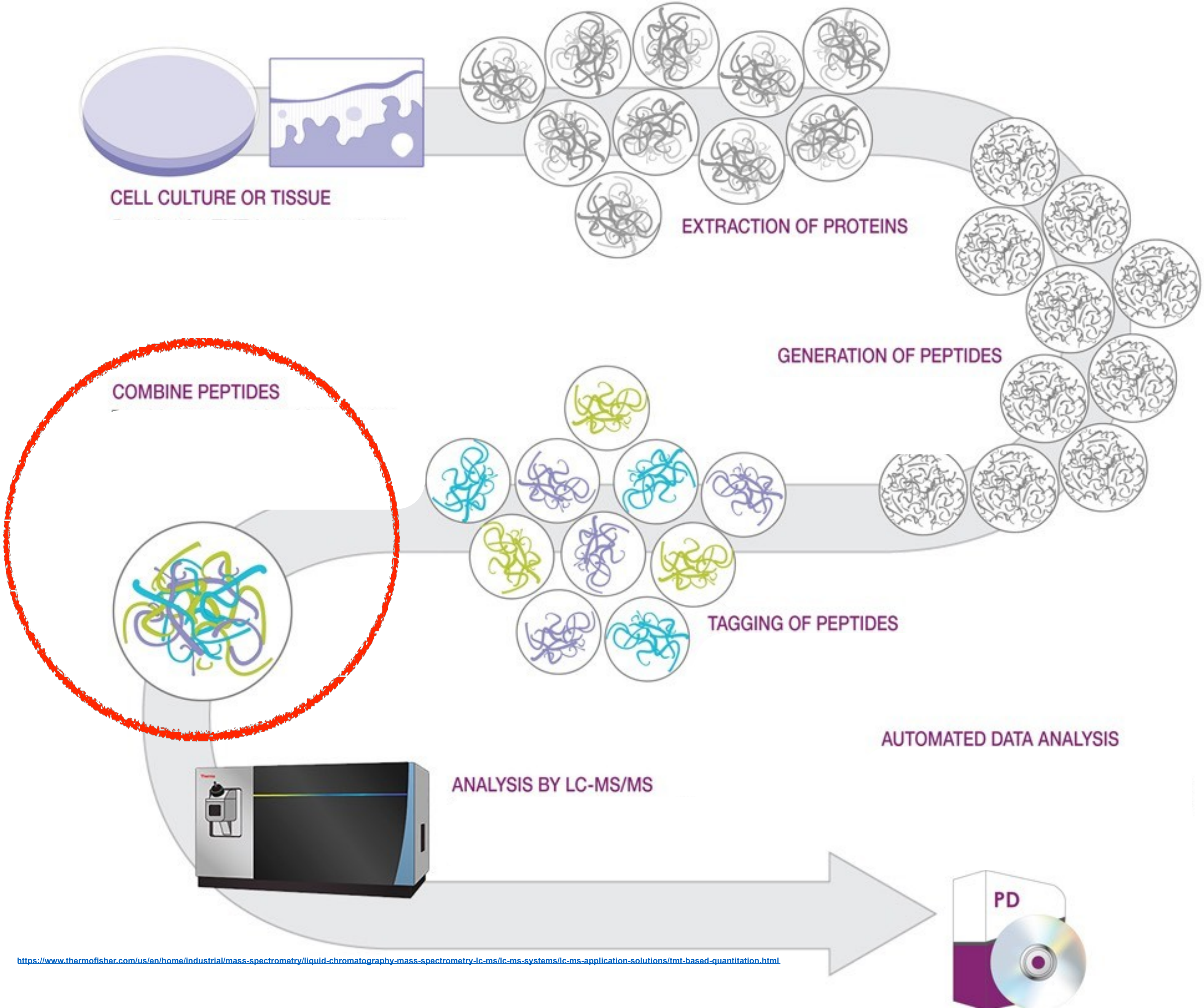


https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html

What might a tag look like?

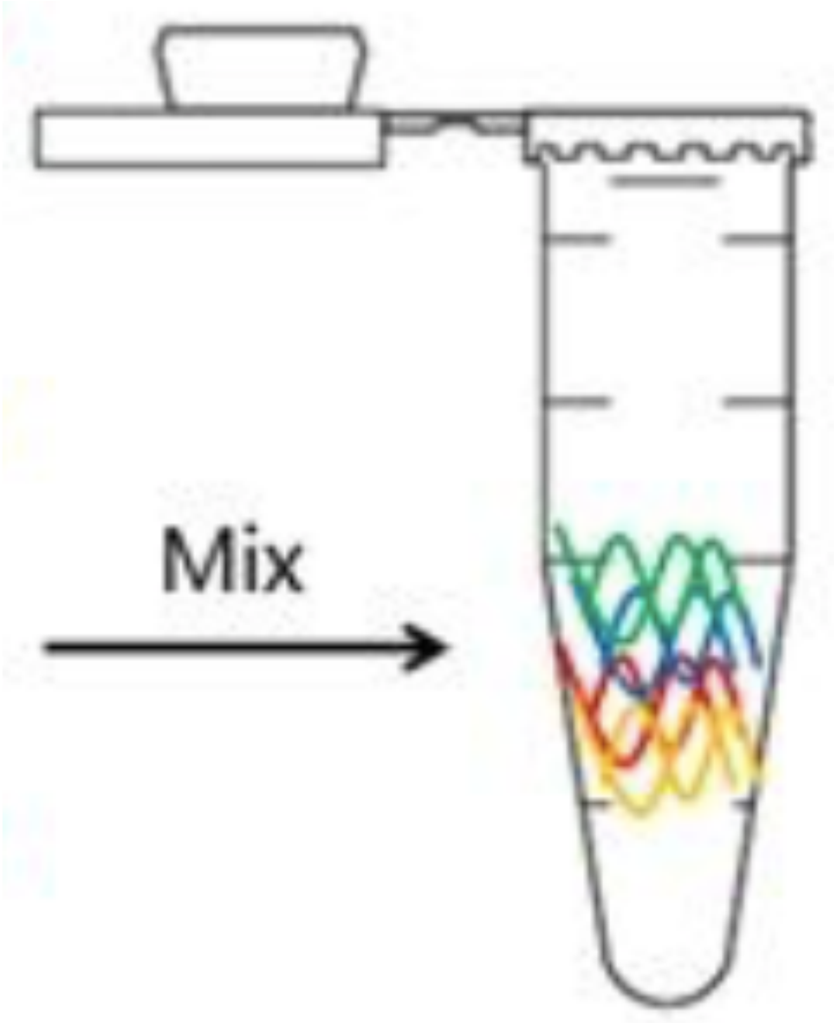
https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

What’s the quantitative proteomic workflow?



https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html

How are tags mixed within a treatment sample?



https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

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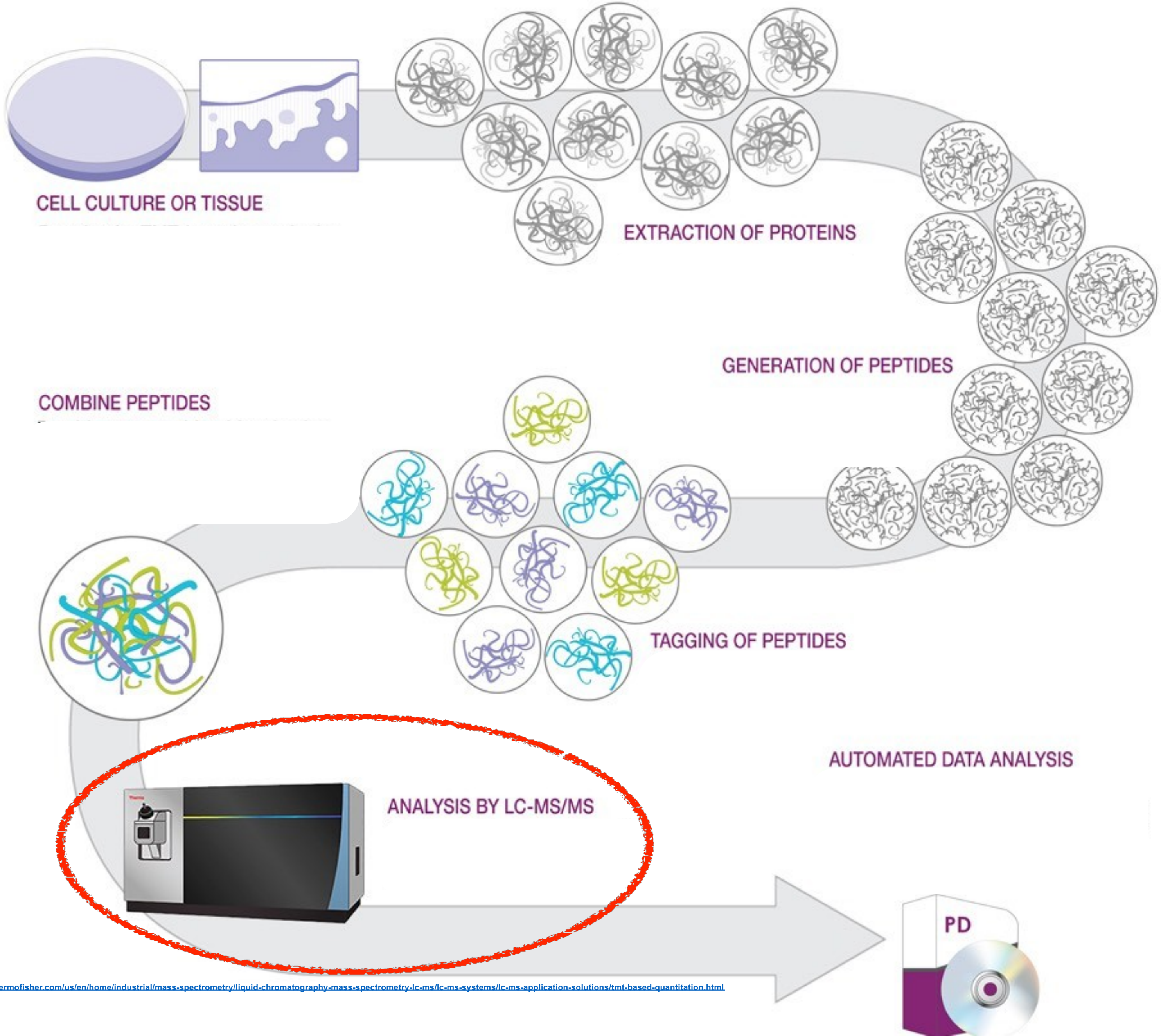
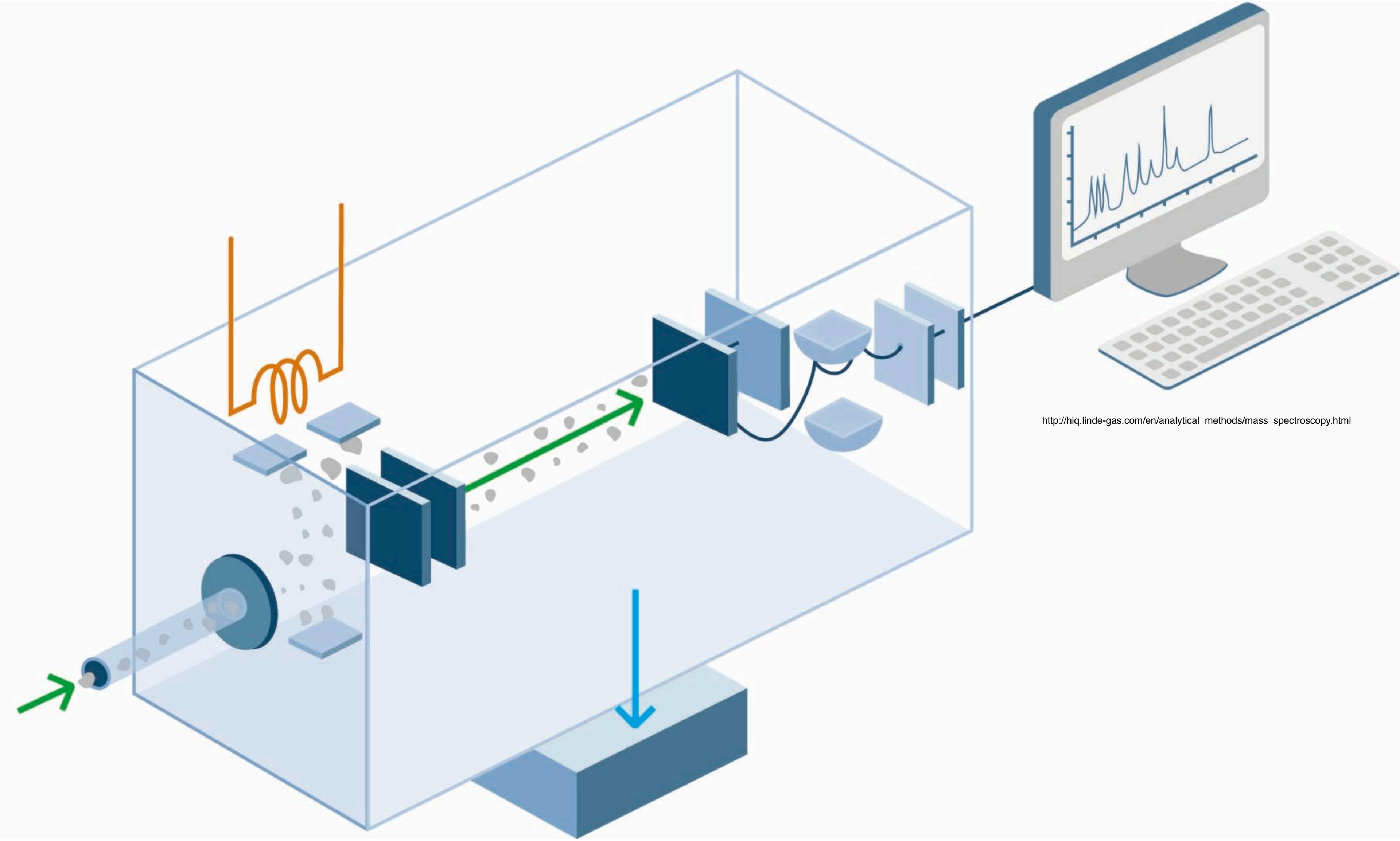


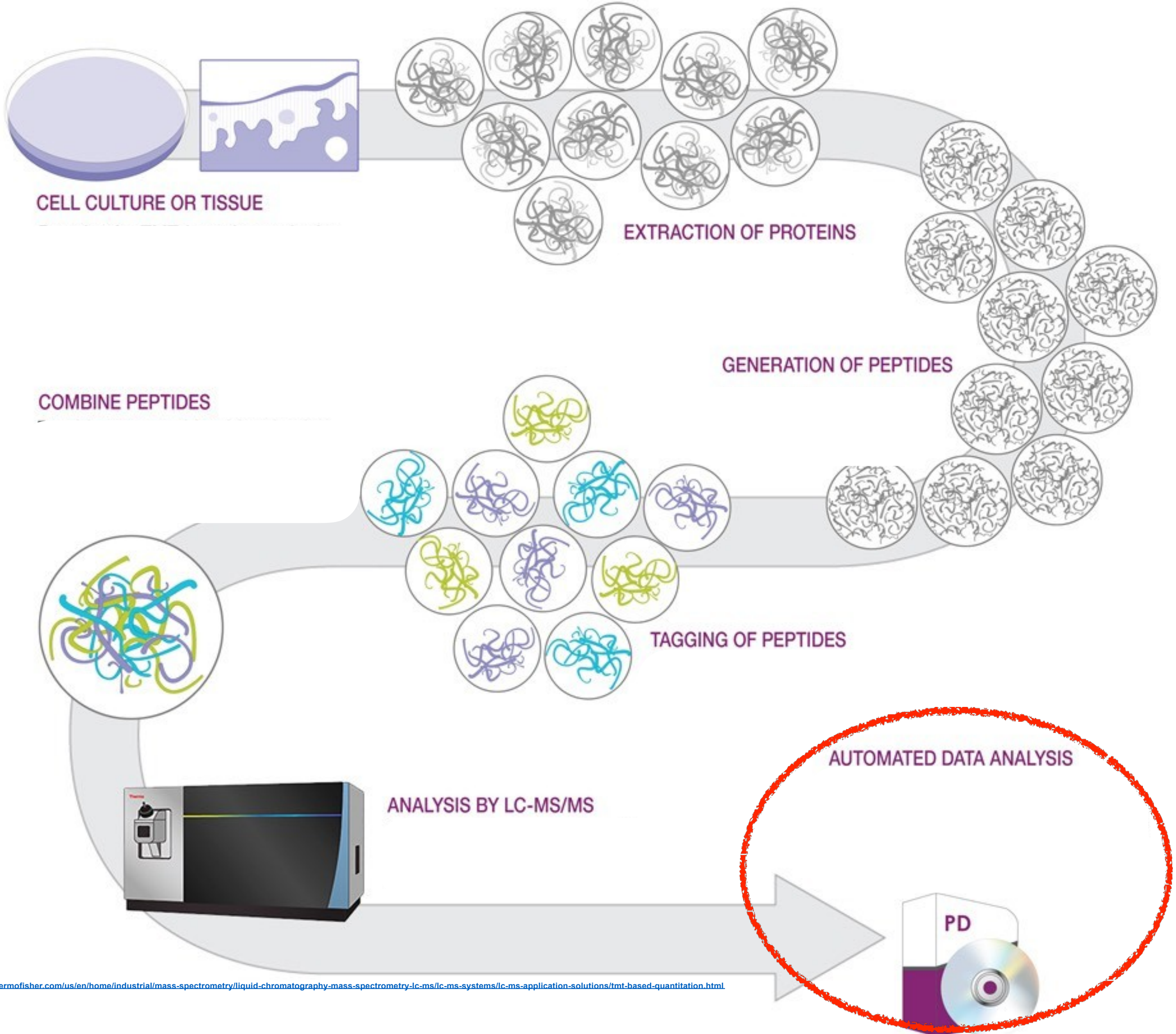
https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html

Overview: Mass Spectrometry



http://hiq.linde-gas.com/en/analytical_methods/mass_spectroscopy.html

What's the quantitative proteomic workflow?



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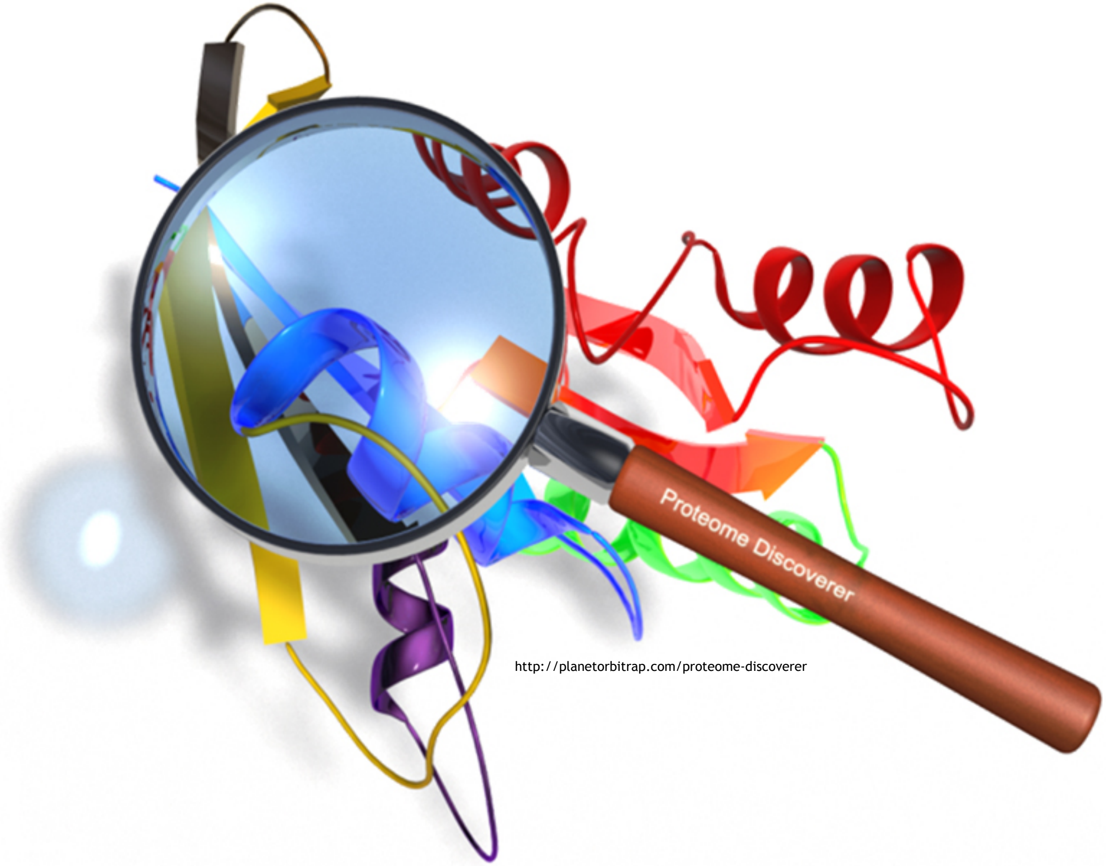
What are the automated data analysis programs?

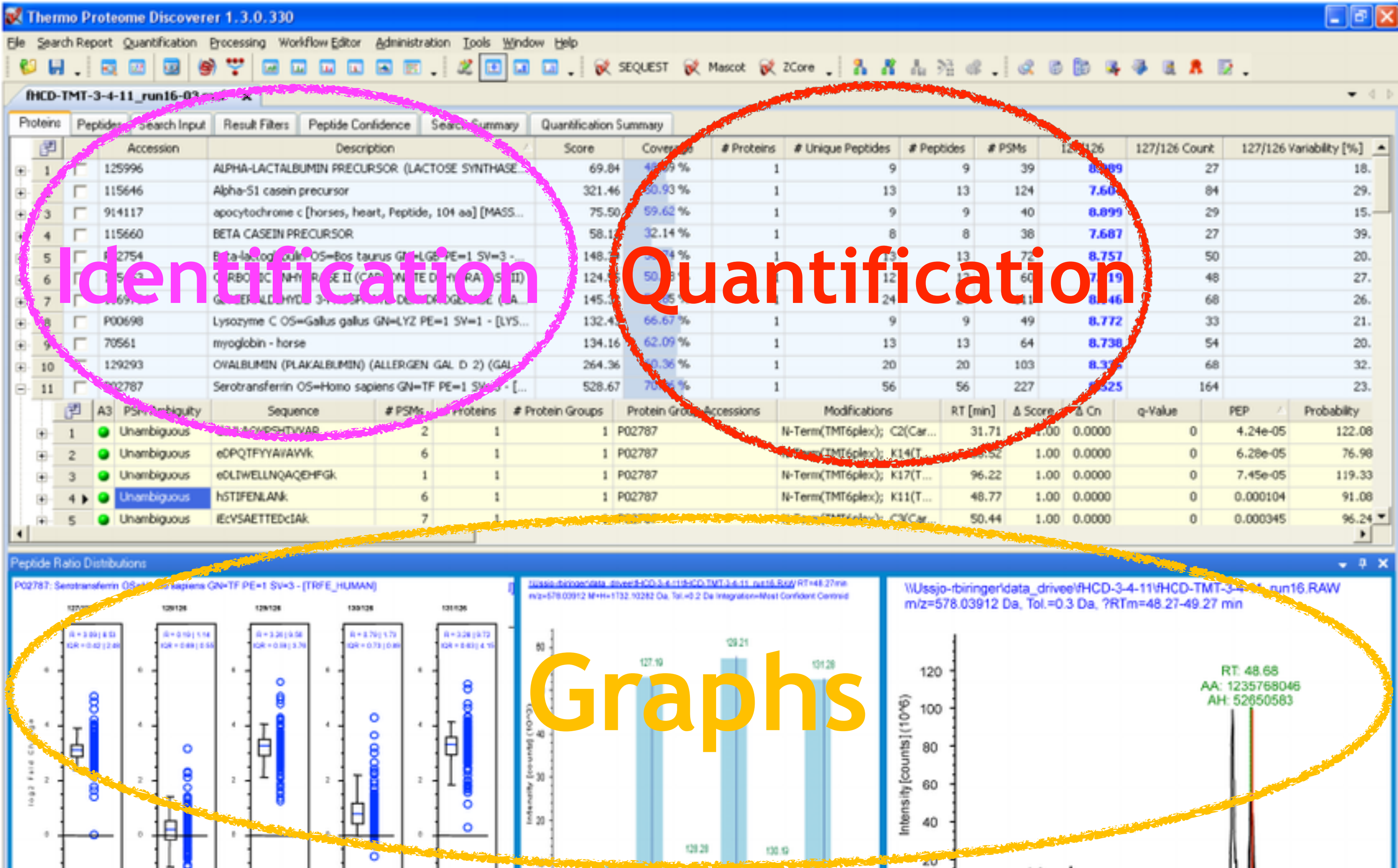


http://planetorbitrap.com/proteome-discoverer

Tools like **Proteome Discoverer** run mass-to-charge ratios through algorithms to quantify identified proteins.

https://www.researchgate.net/figure/Proteome-Discoverer-software-output-for-three-replicate-samples-with-alternating-TMT-tag_fig1_267229694

How does a Proteome Discoverer output look like?



Identification

Quantification

Graphs

	Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	127/126	127/126 Count	127/126 Variability [%]
1	125996	ALPHA-LACTALBUMIN PRECURSOR (LACTOSE SYNTHASE...	69.84	[illegible]	1	9	9	39	[illegible]	27	18.
2	115646	Alpha-S1 casein precursor	321.46	[illegible]	1	13	13	124	[illegible]	84	29.
3	914117	apocytochrome c [horses, heart, Peptide, 104 aa] [MASS...	75.50	59.62 %	1	9	9	40	8.899	29	15.
4	115660	BETA CASEIN PRECURSOR	[illegible]	32.14 %	1	8	8	38	7.687	27	39.
5	[illegible]	Beta-lactoglobulin OS=Bos taurus GN=LGB PE=1 SV=3 -...	[illegible]	[illegible]	1	13	13	72	8.757	50	20.
6	[illegible]	CARBONIC ANHYDRASE II (CARBONATE DEHYDRATASE II)	[illegible]	[illegible]	[illegible]	12	[illegible]	60	[illegible]	48	27.
7	[illegible]	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GA...	[illegible]	[illegible]	[illegible]	24	[illegible]	[illegible]	[illegible]	68	26.
8	P00698	Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1 - [LYS...	[illegible]	66.67 %	1	9	9	49	8.772	33	21.
9	70561	myoglobin - horse	134.16	62.09 %	1	13	13	64	8.738	54	20.
10	129293	OVALBUMIN (PLAKALBUMIN) (ALLERGEN GAL D 2) (GAL...	264.36	[illegible]	1	20	20	103	[illegible]	68	32.
11	[illegible]	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3 - [...	528.67	[illegible]	1	56	56	227	[illegible]	164	23.

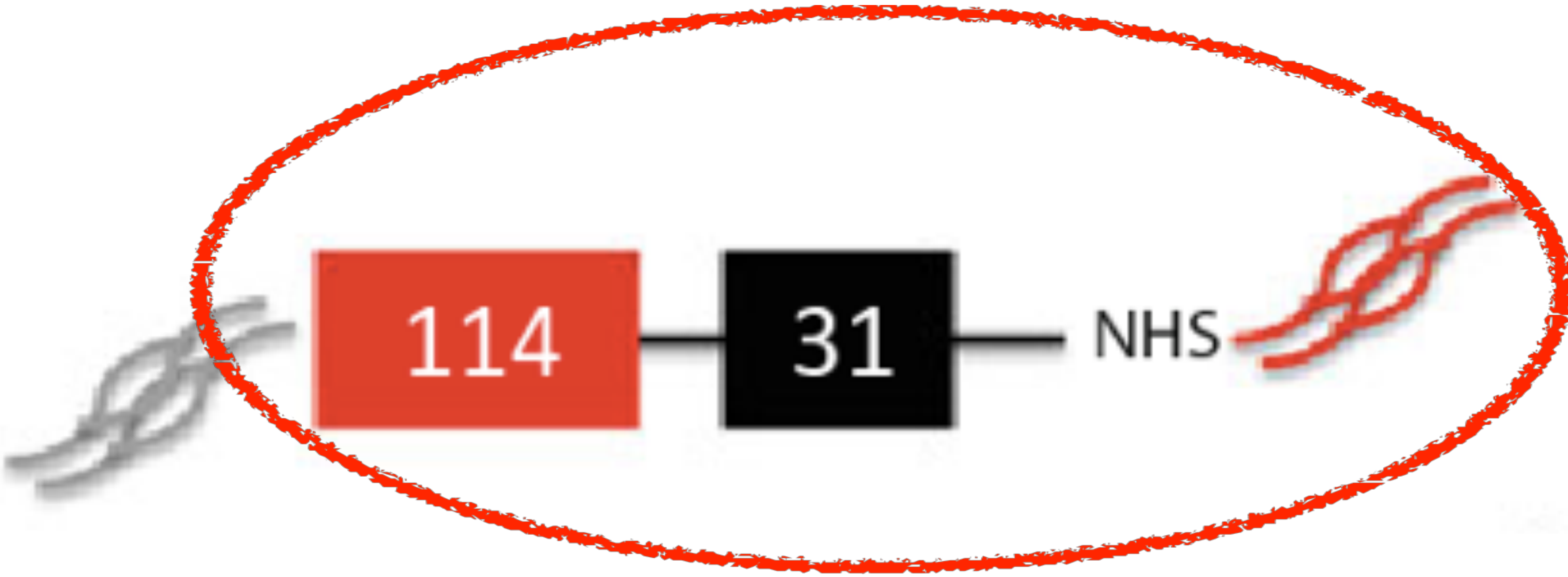
	A3	PSM Ambiguity	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	RT [min]	Δ Score	Δ Cn	q-Value	PEP	Probability
1	●	Unambiguous	[illegible]	2	1	1	P02787	N-Term(TMT6plex); C2(Car...	31.71	[illegible]	0.0000	0	4.24e-05	122.08
2	●	Unambiguous	eDPQTFYYAVAVVk	6	1	1	P02787	N-Term(TMT6plex); K14(T...	[illegible]	1.00	0.0000	0	6.28e-05	76.98
3	●	Unambiguous	eDLIWELLNQAQEHFGk	1	1	1	P02787	N-Term(TMT6plex); K17(T...	96.22	1.00	0.0000	0	7.45e-05	119.33
4	●	Unambiguous	hSTIFENLANk	6	1	1	P02787	N-Term(TMT6plex); K11(T...	48.77	1.00	0.0000	0	0.000104	91.08
5	●	Unambiguous	lEcVSAETTEDcIAk	7	1	[illegible]	[illegible]	[illegible]	50.44	1.00	0.0000	0	0.000345	96.24

What are two types of quantitative proteomics?

iTRAQ

TMT

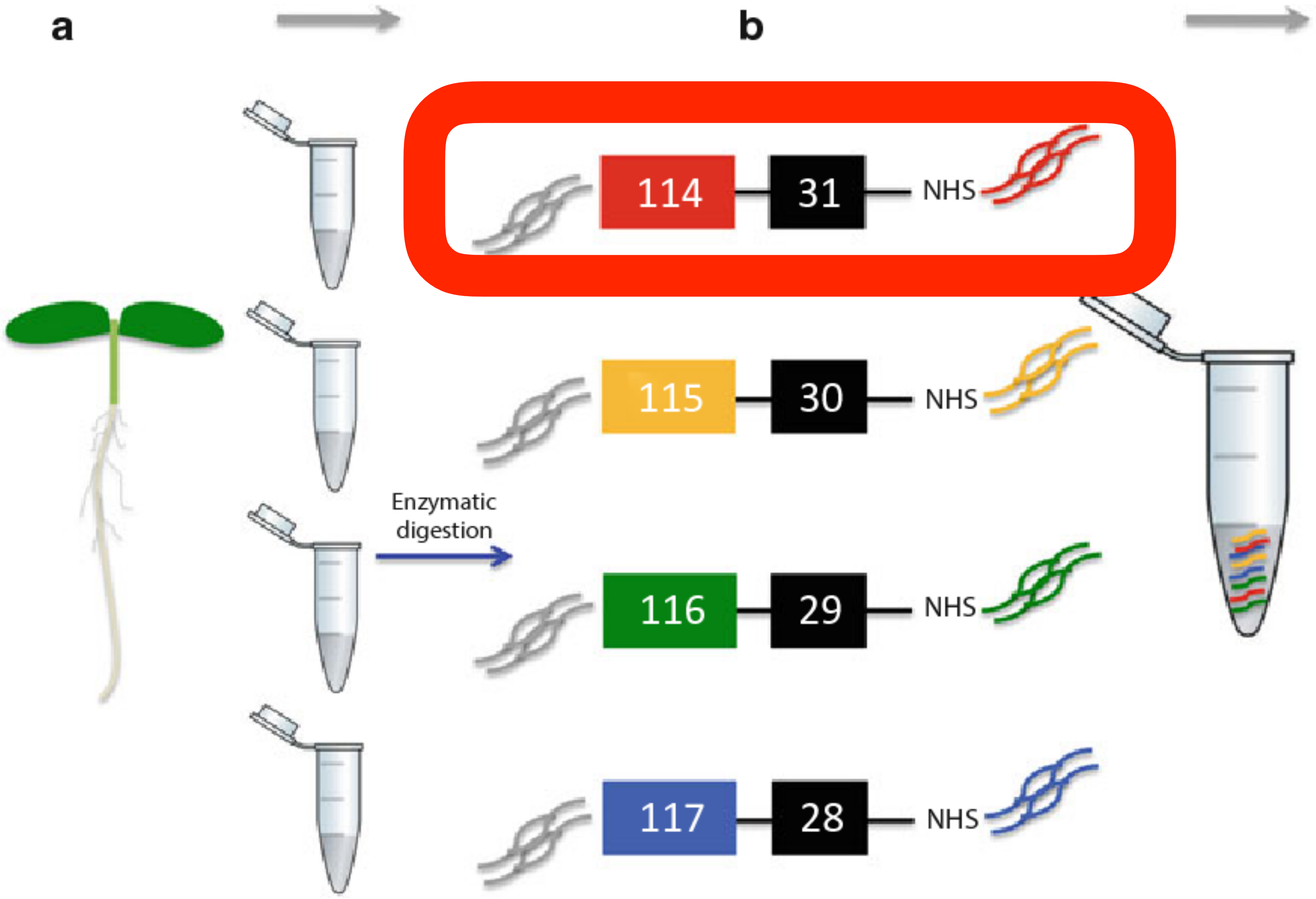
What makes a tagging reagent **isobaric**?



Chemical tag groups with identical masses

https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

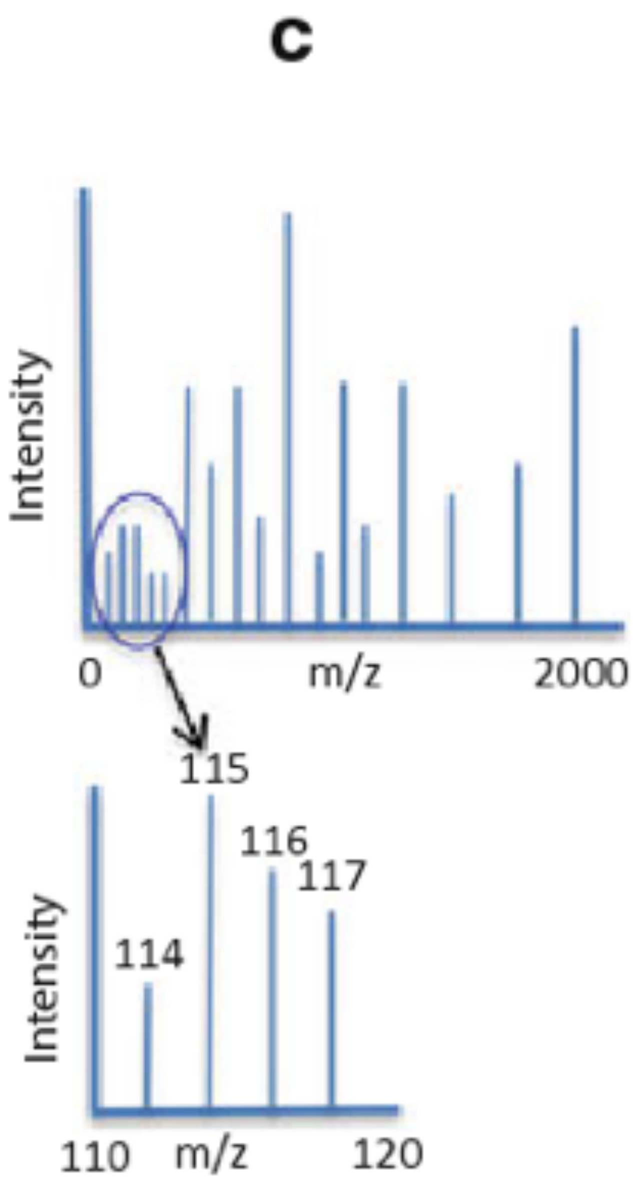
What is iTRAQ?

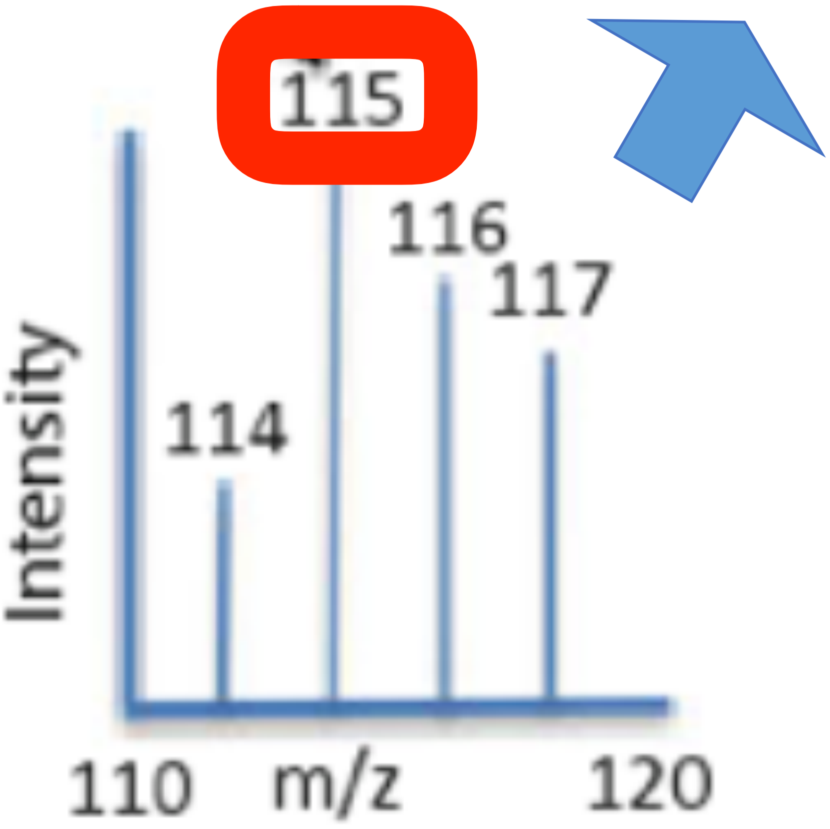


Isobaric tags for relative and absolute quantification

https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

What is iTRAQ?

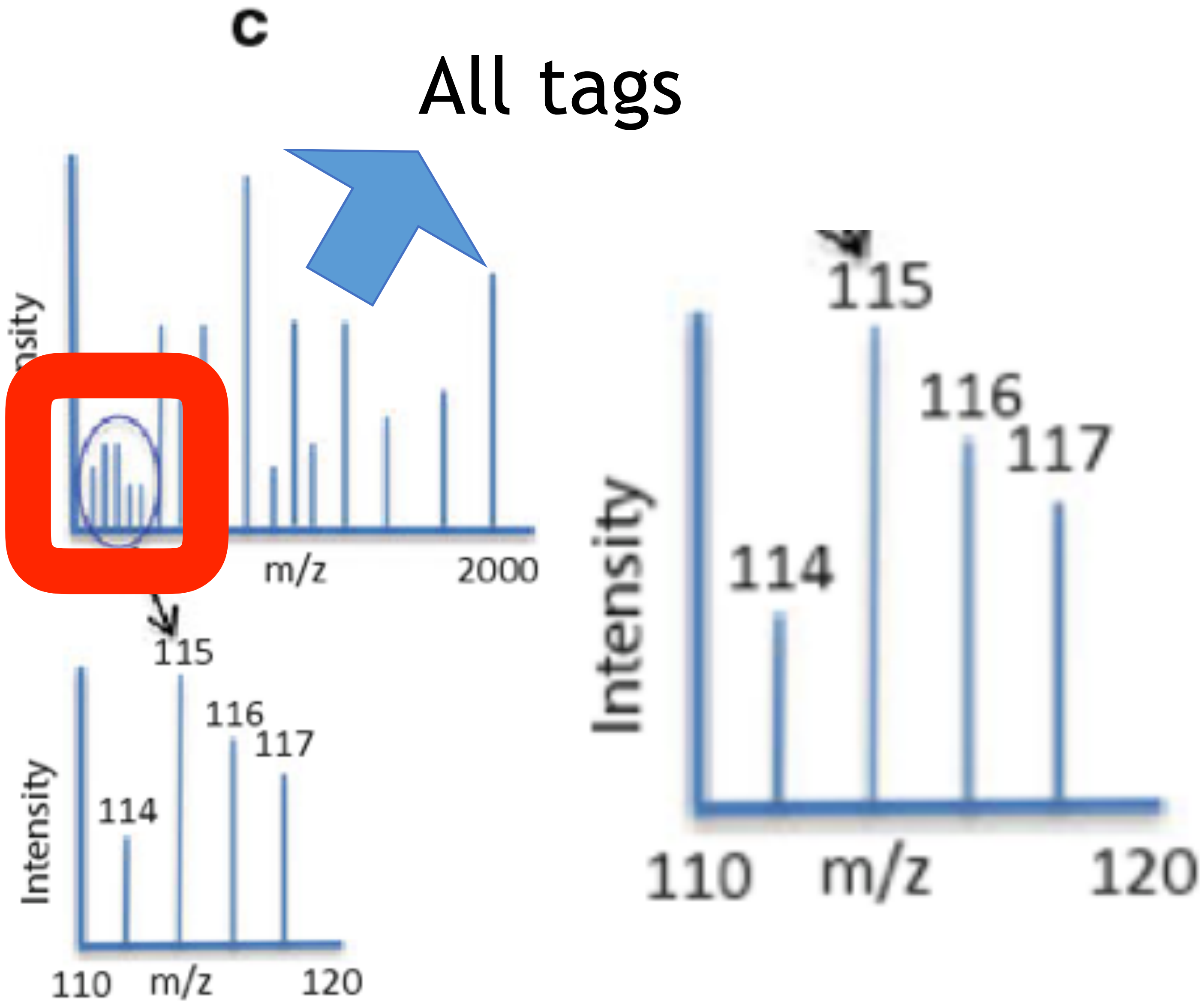




Quantification of identified protein

https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

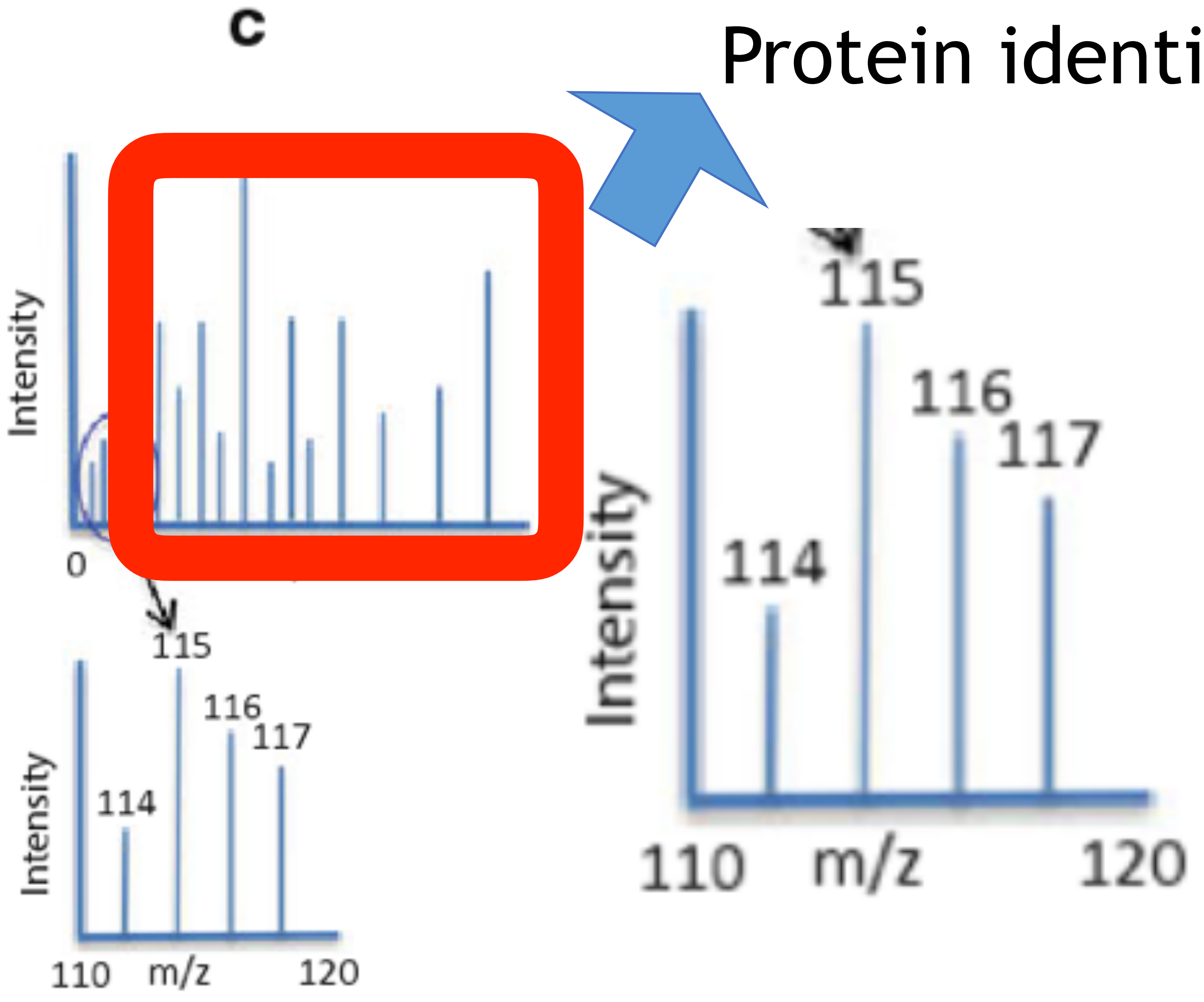
What is iTRAQ?



All tags

https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

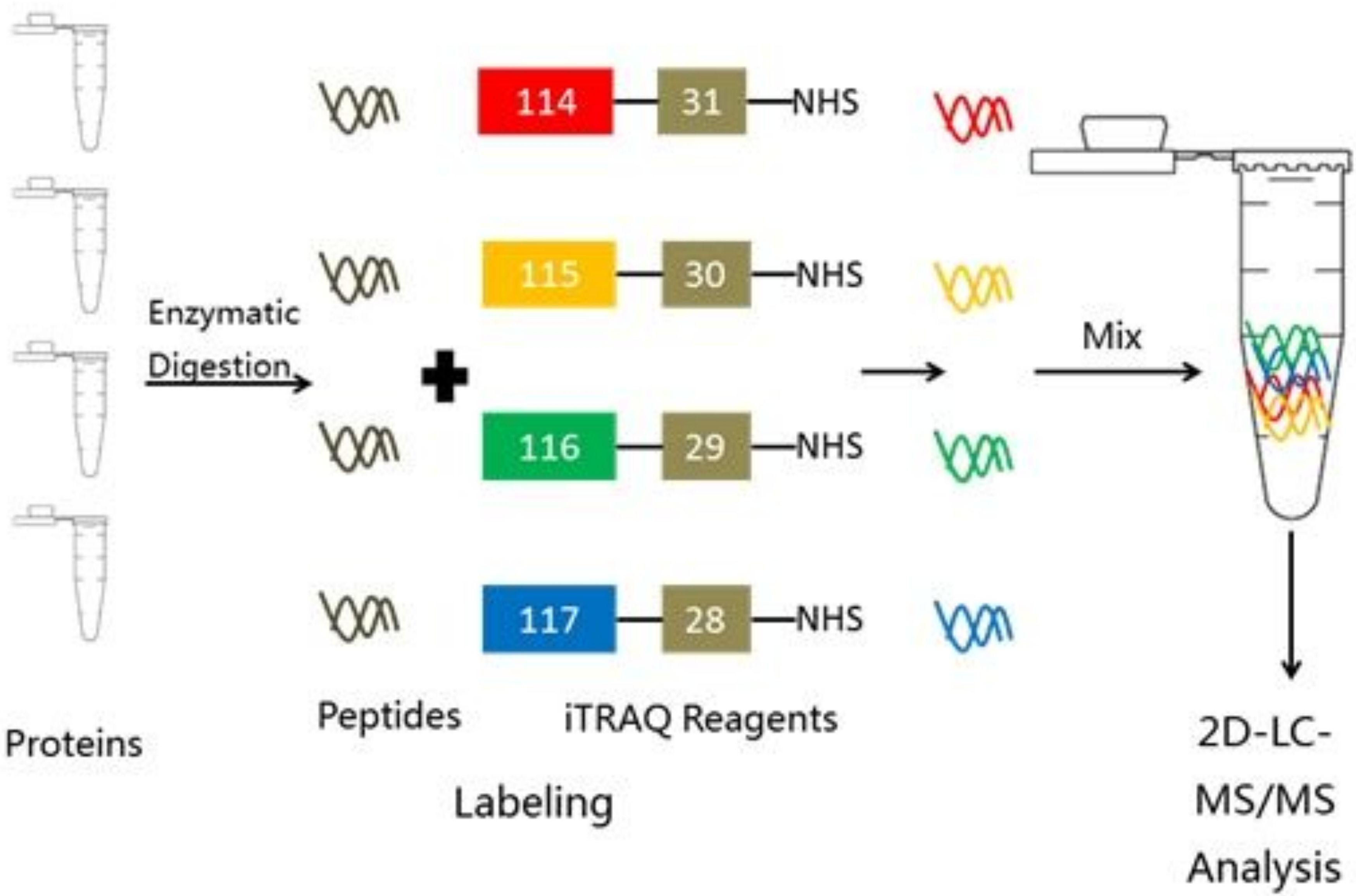
What is **iTRAQ**?



Protein identification

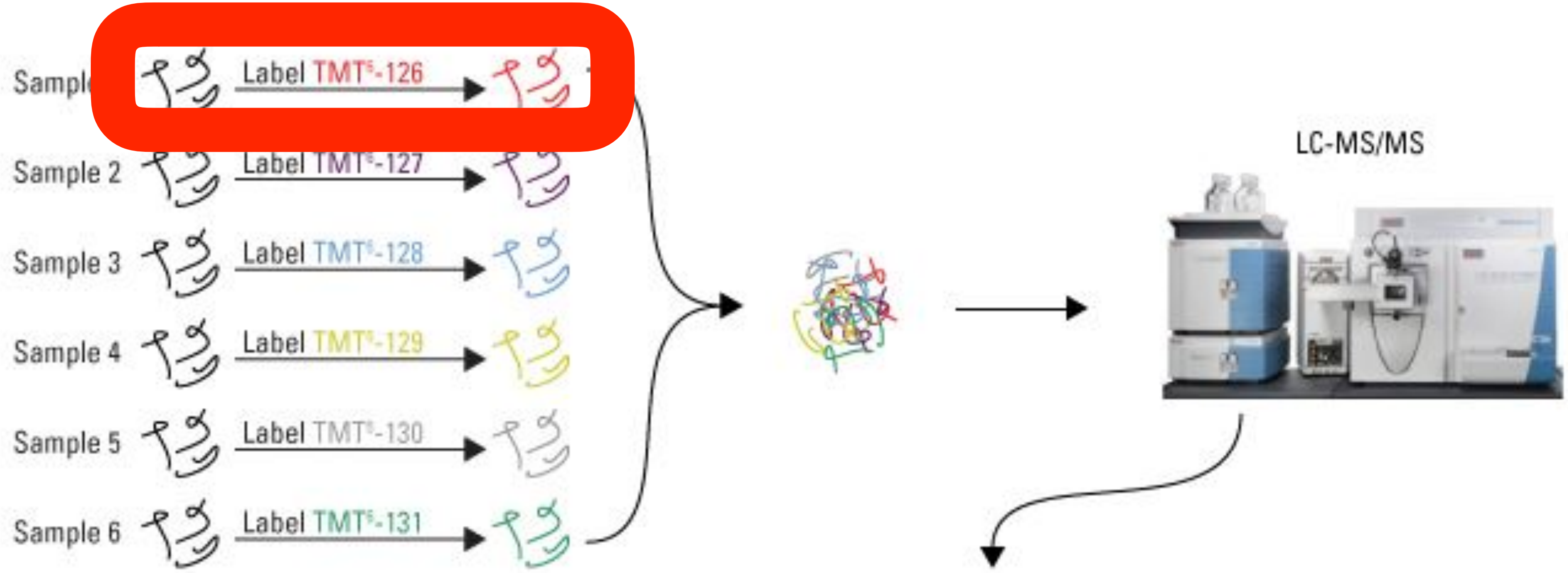
https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

What’s the iTRAQ workflow?



https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

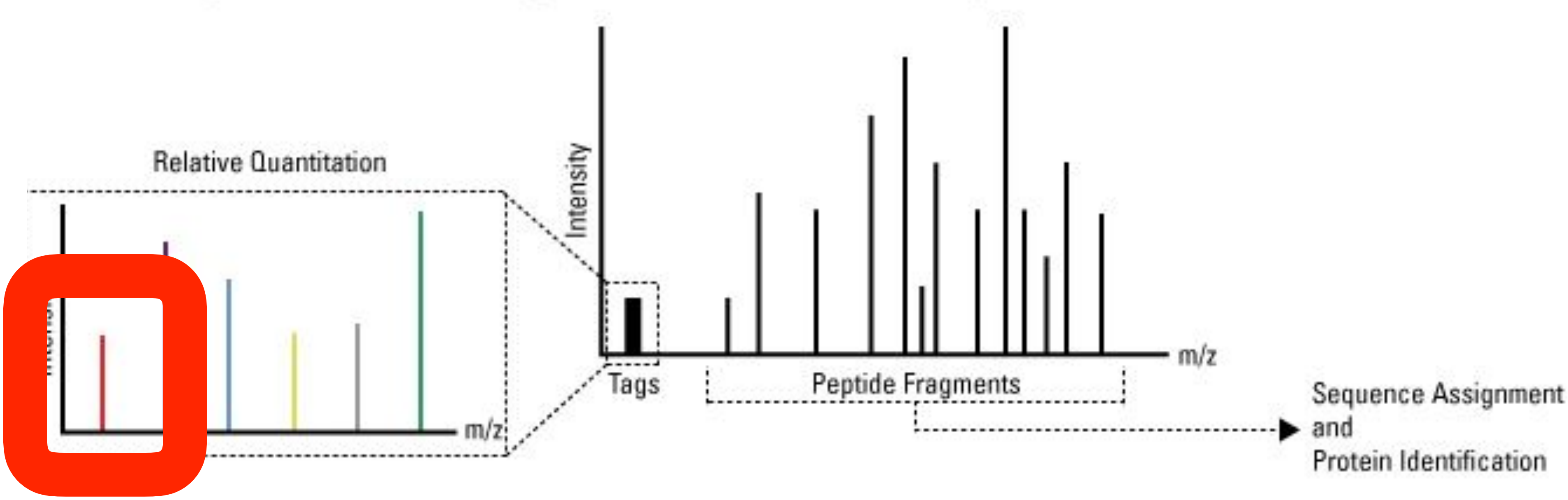
What is the TMT workflow?



Tandem mass tagging

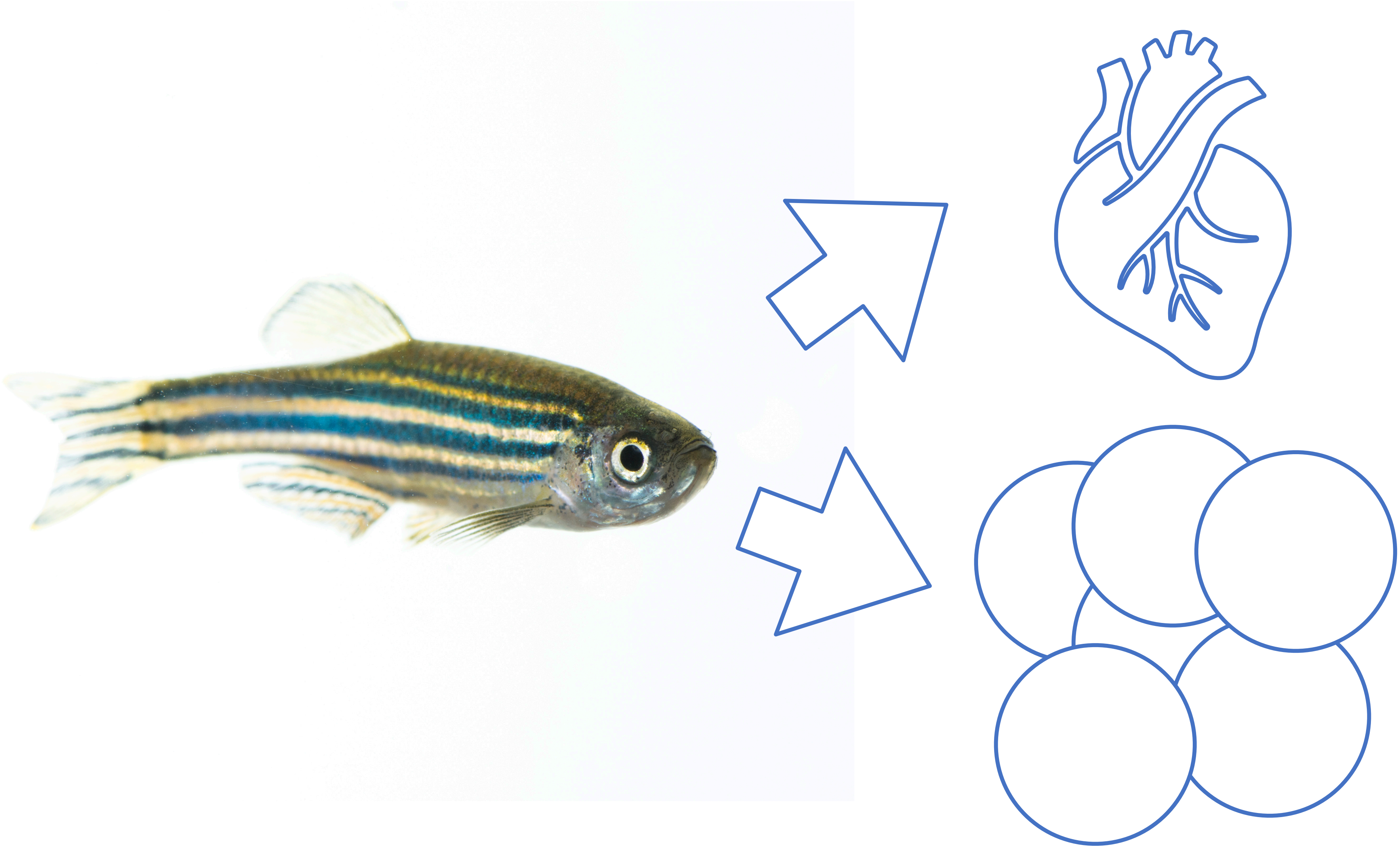
https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

What is the **TMT** workflow?



https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

How can you use quantitative proteomics in your project?



https://www.umass.edu/magazine/fall-2016/small-fish-big-impact

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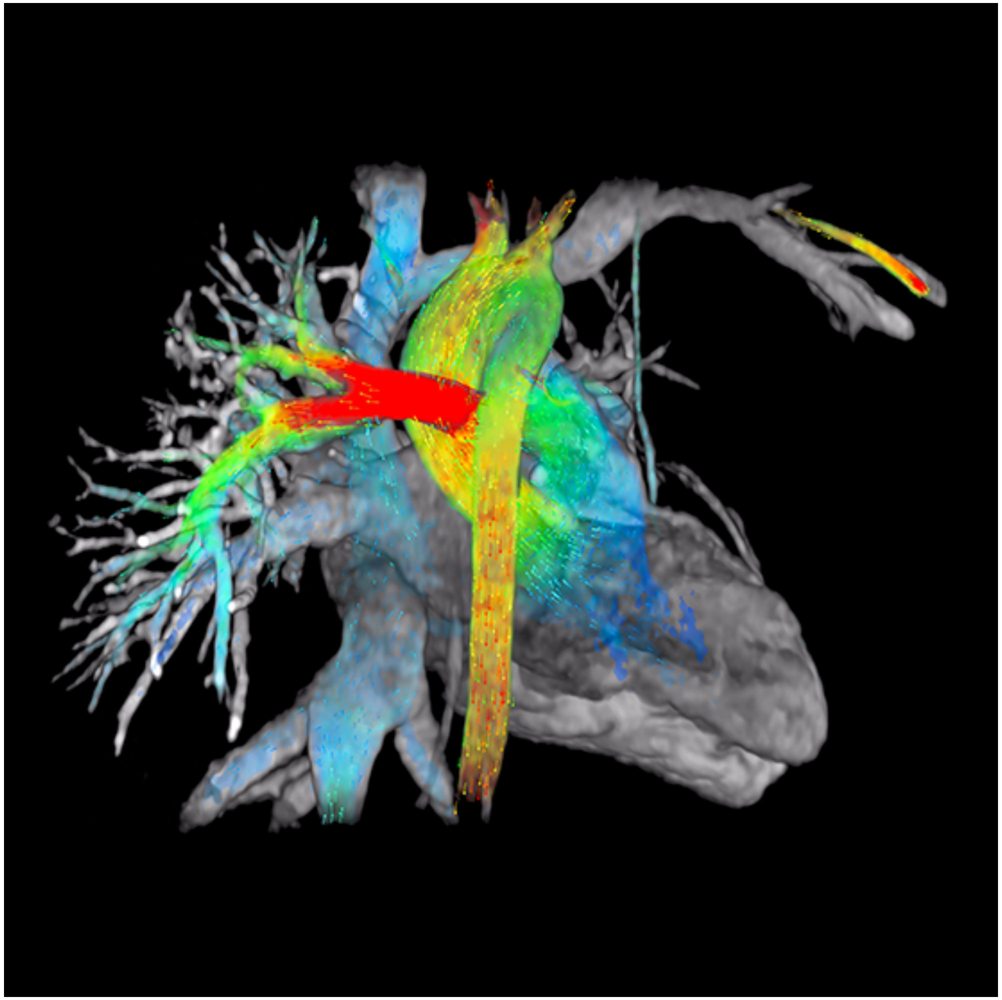


http://newsroom.gehealthcare.com/thepulse/20-ways-to-see-the-heart/

Protein Localization

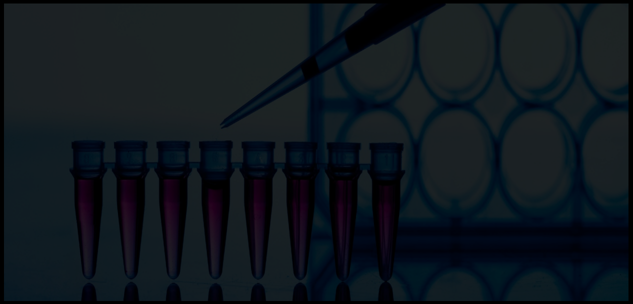
Protein Quantification

Protein Identification

What are the take-home points?

https://openarchive.ki.se/xmlui/bitstream/handle/10616/45601/Thesis_Bo_Zhang.final.pdf?sequence=1&isAllowed=y

Proteomics is the study of all proteins, the drivers of cellular and molecular work.



Quantitative Proteomics is a subset of proteomics which enables both the identification and quantification of proteins.

iTRAQ/TMT Quantitative Proteomics utilize **isobaric tagging reagents** in order to identify and quantify protein in a cost-effective manner.

What are the take-home points?


http://sneezetoronto.com/corptest/clinical-operations/high-throughput-screening/

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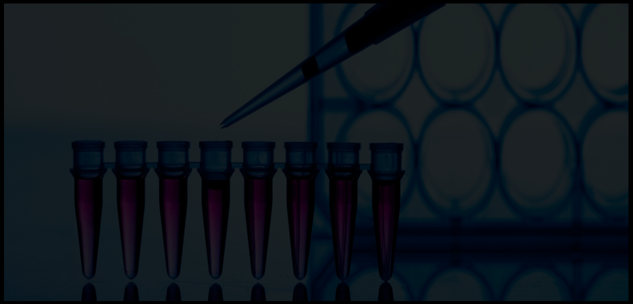

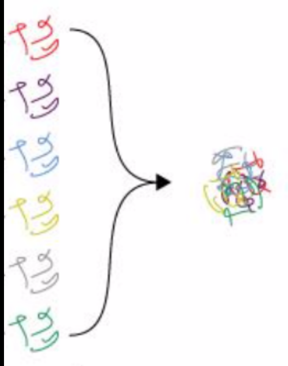

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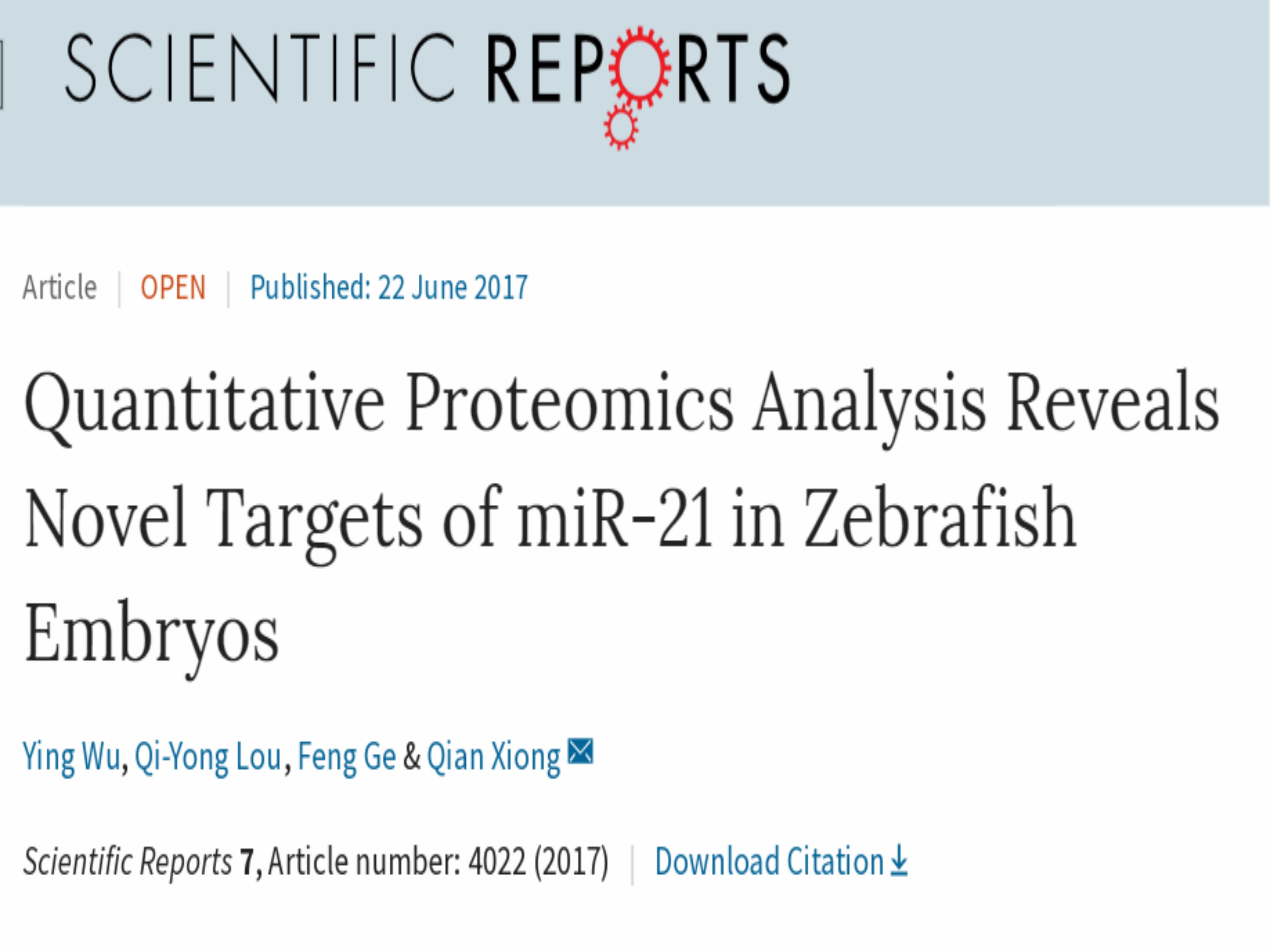


SCIENTIFIC REPORTS

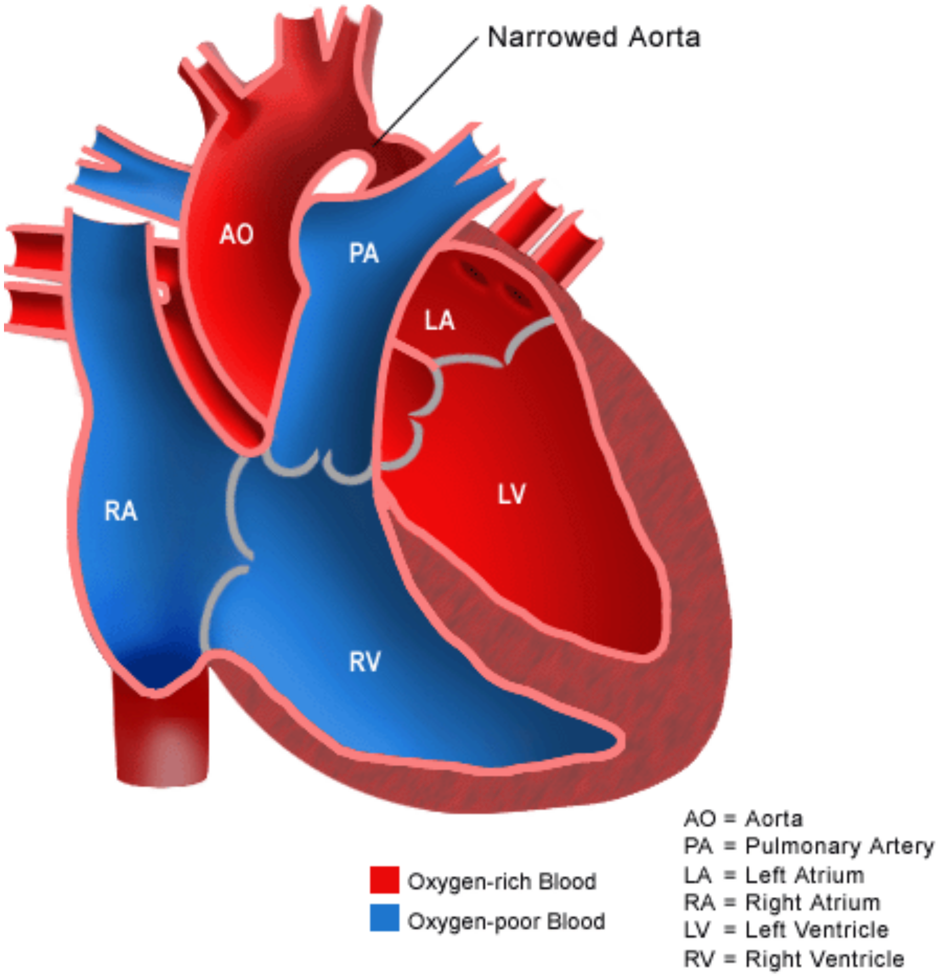
Article | OPEN | Published: 22 June 2017

Quantitative Proteomics Analysis Reveals Novel Targets of miR-21 in Zebrafish Embryos

Ying Wu, Qi-Yong Lou, Feng Ge & Qian Xiong ✉

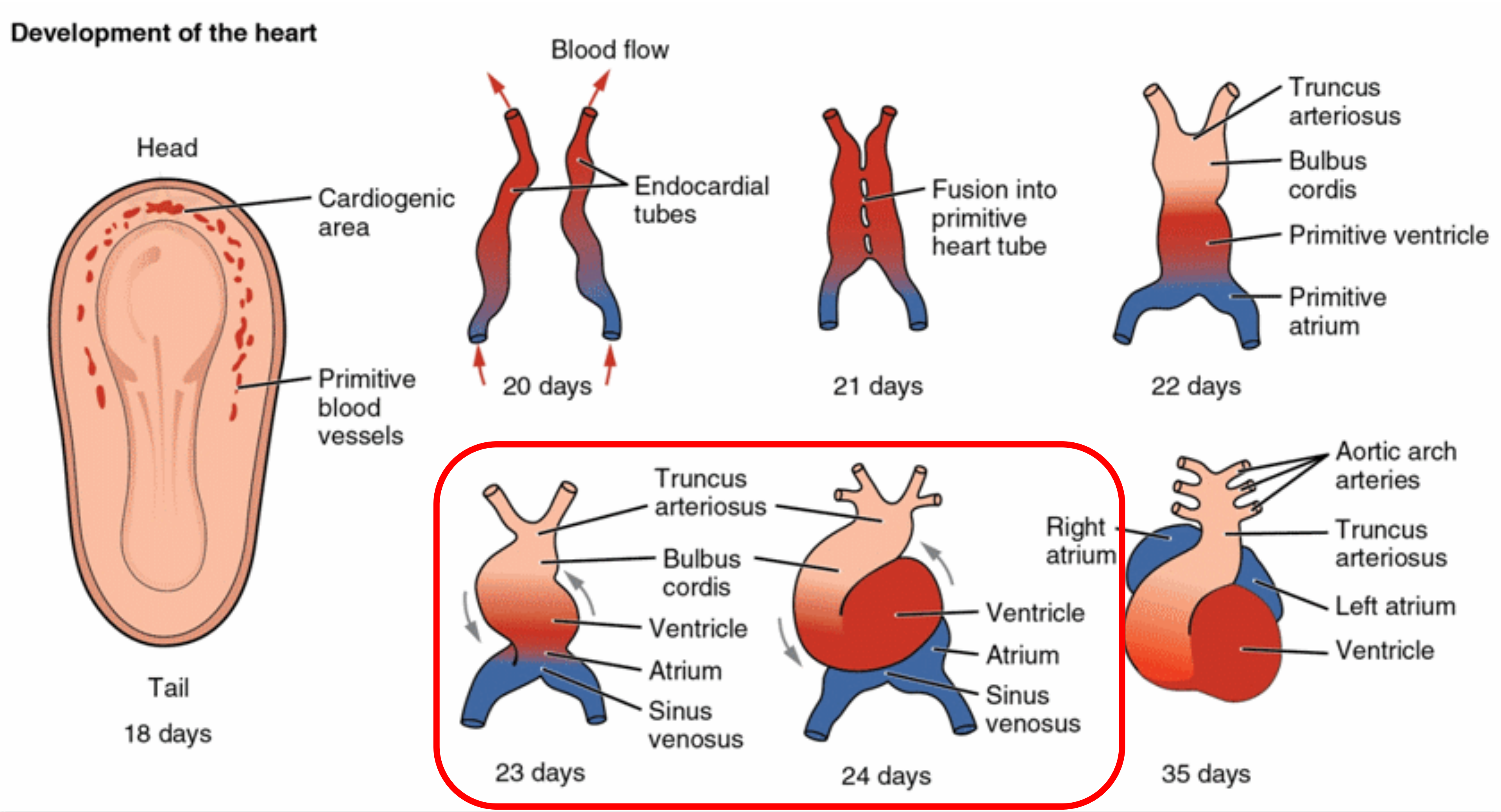
Scientific Reports **7**, Article number: 4022 (2017) | Download Citation ⤓

Why study congenital heart disease?



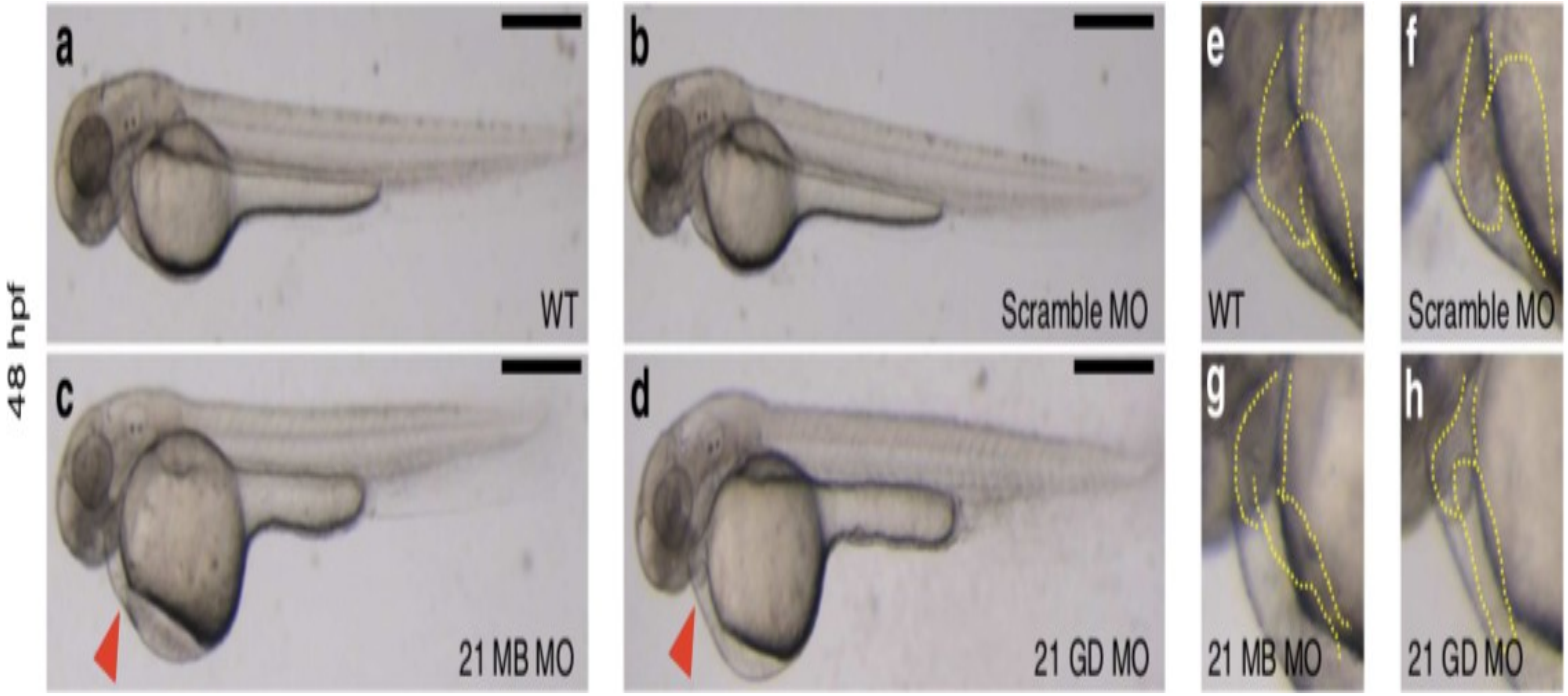
Congenital Heart Disease is the most common form of birth defect; specifically, heart valve defects affect 1% of Americans

What is valvulogenesis?



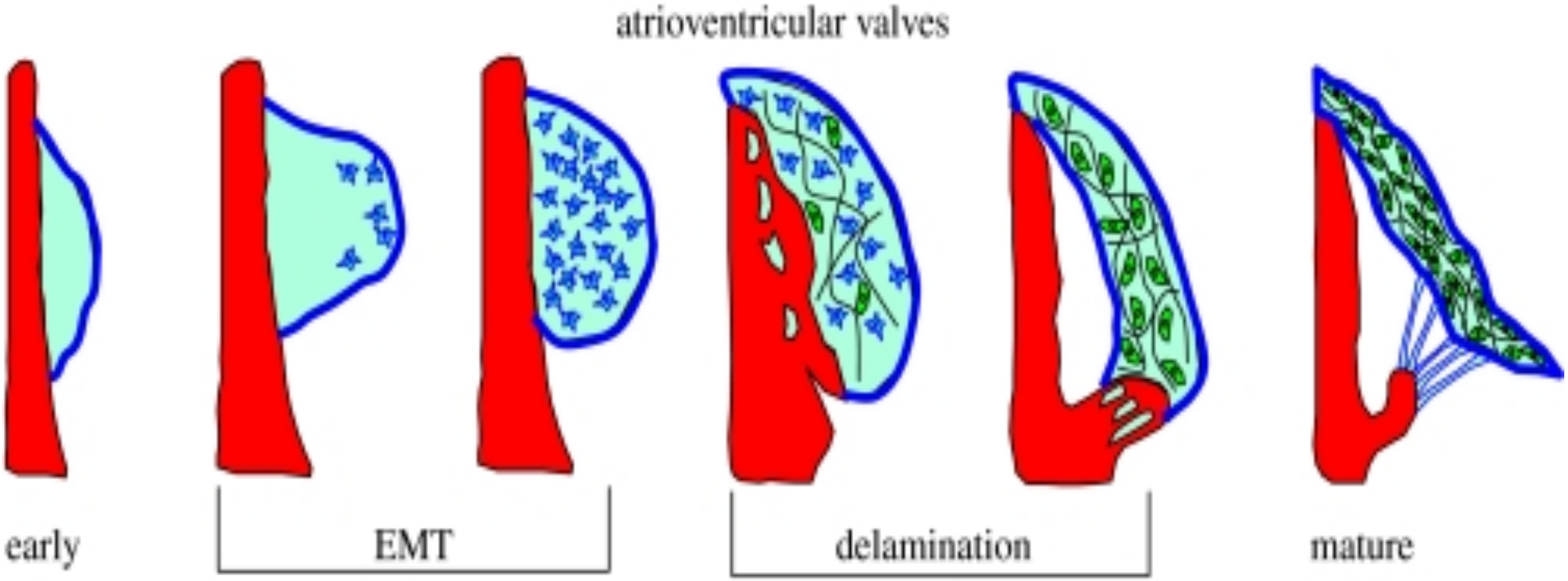
Valvulogenesis is the process in which the valves of the heart are developed and coincides with the looping of the heart

What is a good model organism to study valvulogenesis?



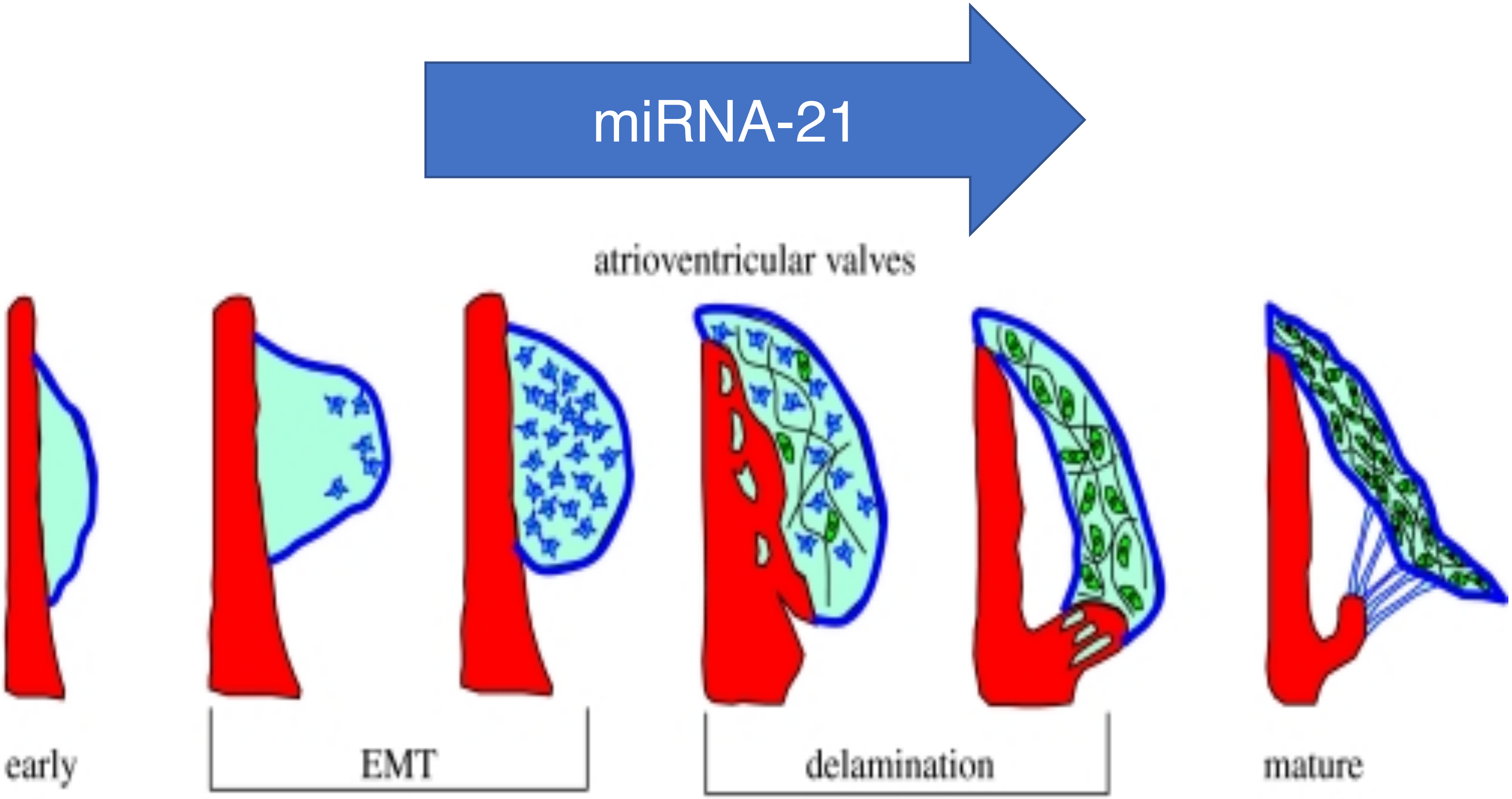
What is an important regulator of valvulogenesis?

Cell Migration and Proliferation



During heart looping cells must proliferate and migrate into the cardiac jelly to remodel and constrict the atrioventricular valve ring

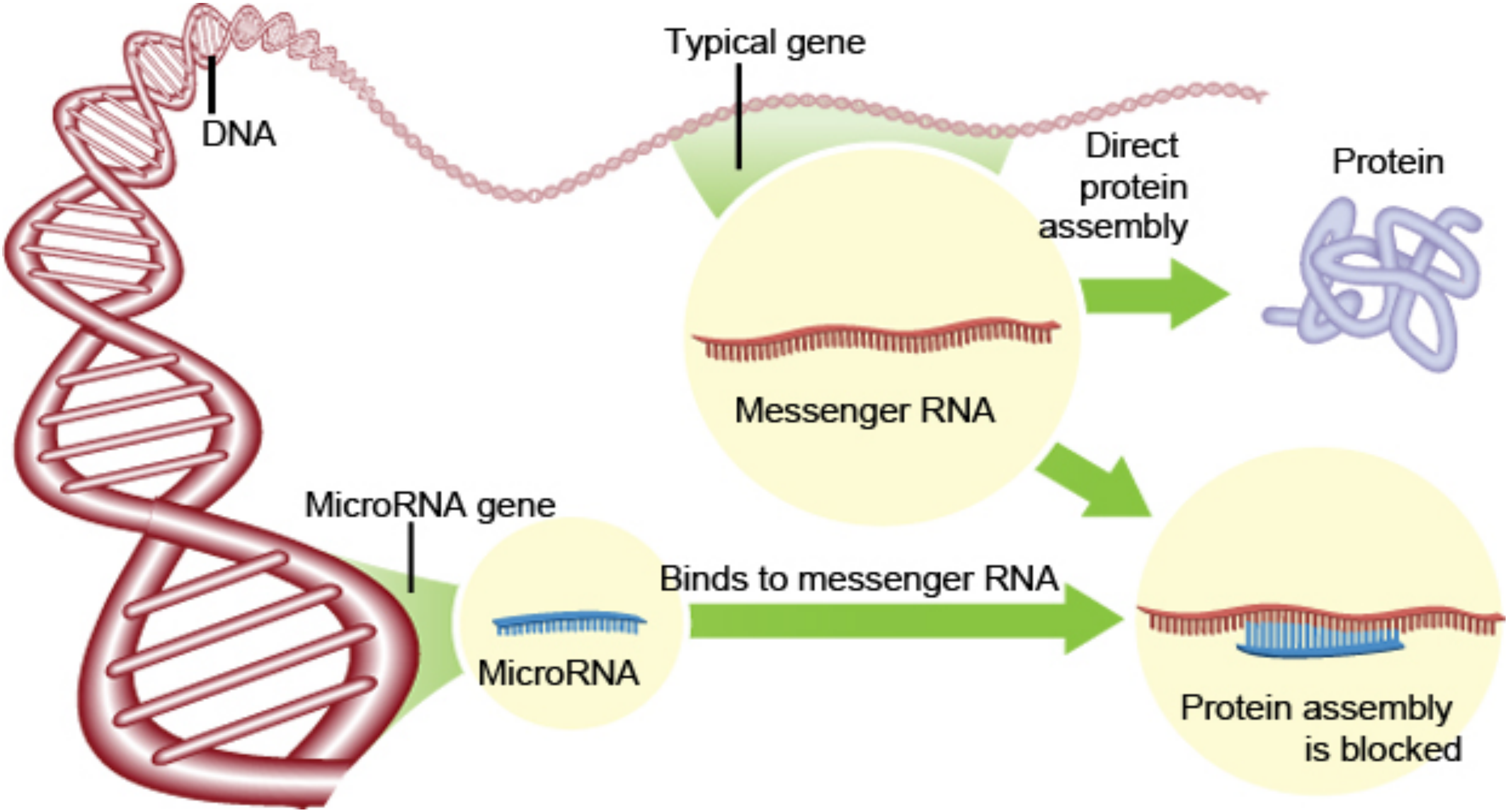
What is an important regulator of valvulogenesis?



miRNA-21

MicroRNA plays important roles in heart development. In valvulogenesis, miRNA-21 is essential for proper valve development

What are micro RNAs?



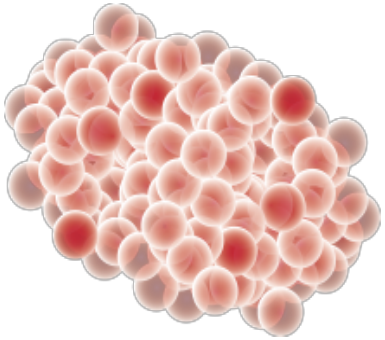
miRNA are non-coding RNA that bind to multiple transcripts to modulate protein translation

How does miR-21 function?

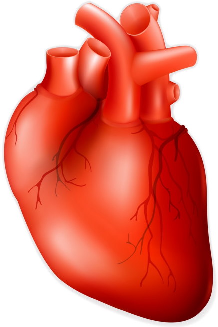
Expressed In:

Brain

+

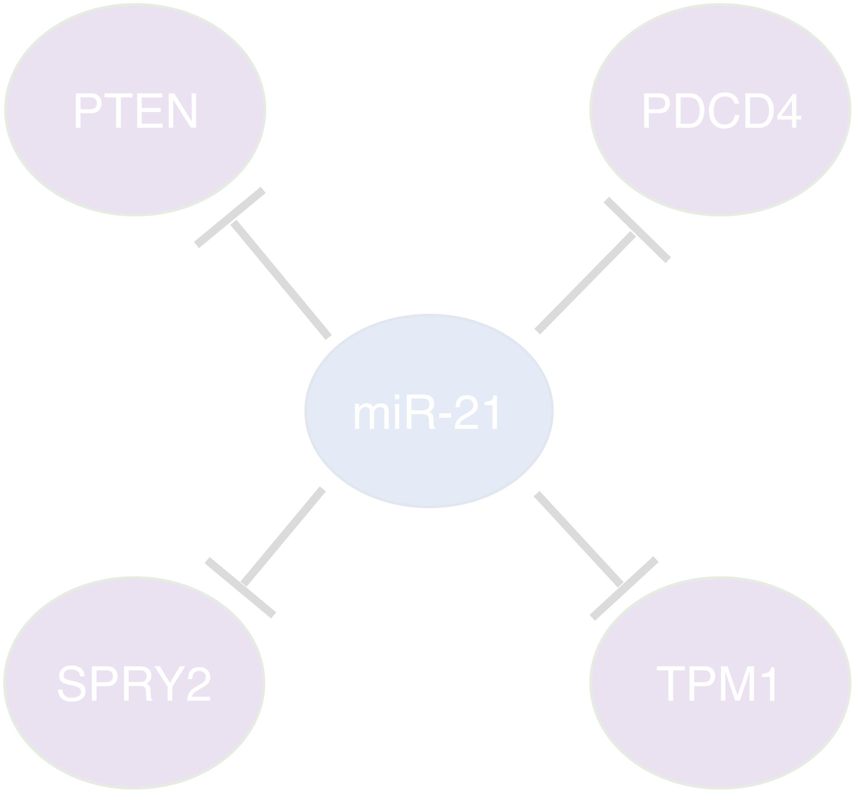


Cancer



Heart

Human Targets:

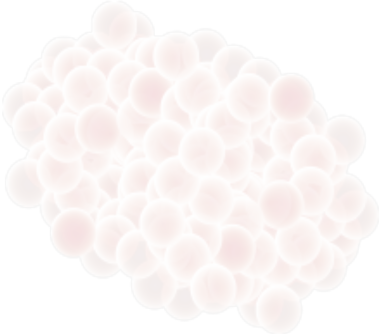


What does miR-21 regulate?

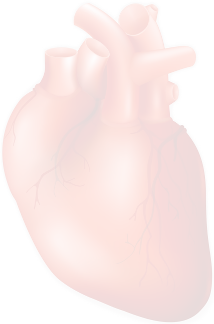
Expressed In:

Brain

+

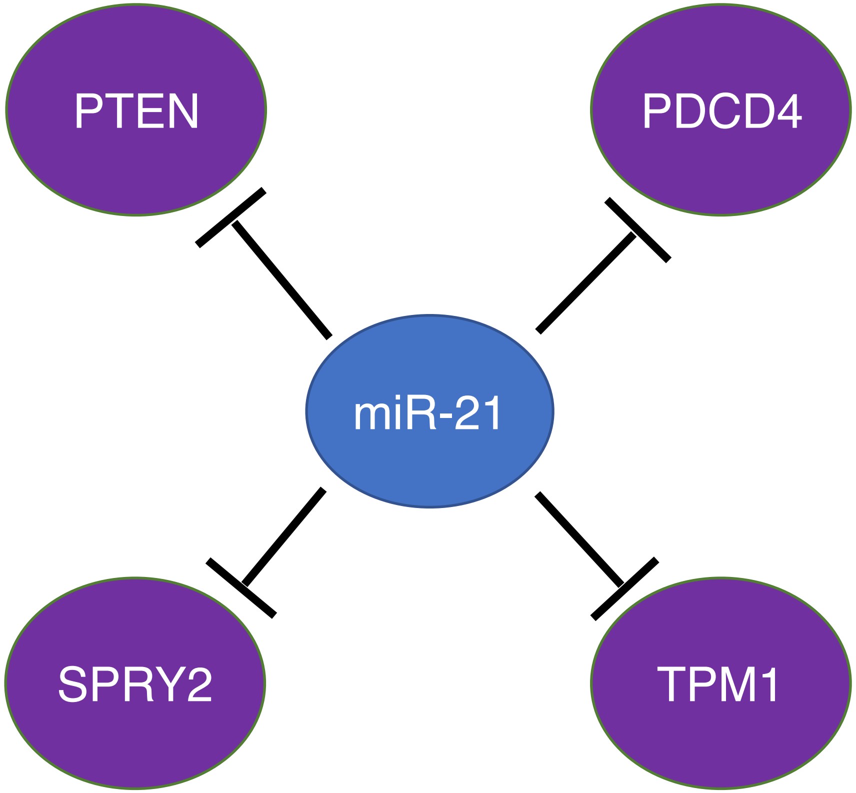


Cancer



Heart

Human cancer targets:



PTEN

PDCD4

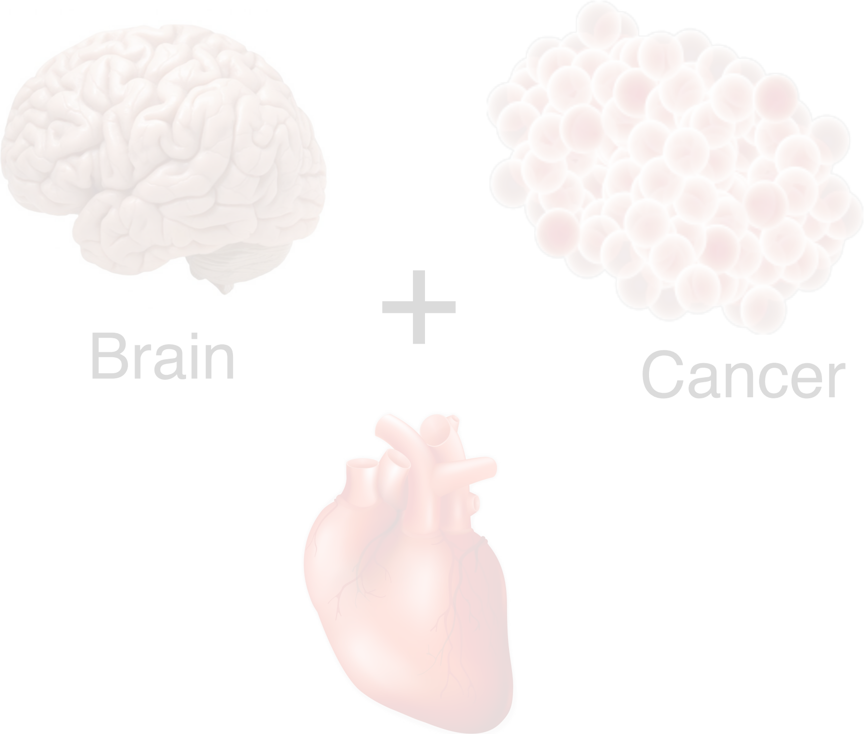
miR-21

SPRY2

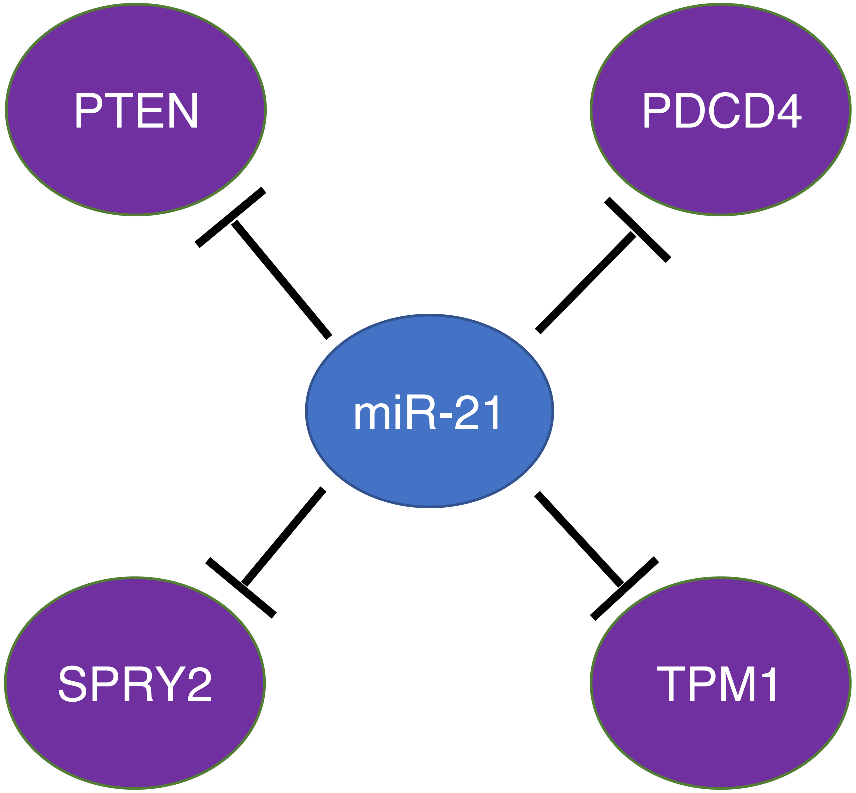
TPM1

What does miR-21 regulate?

Expressed In:



Human cancer targets:



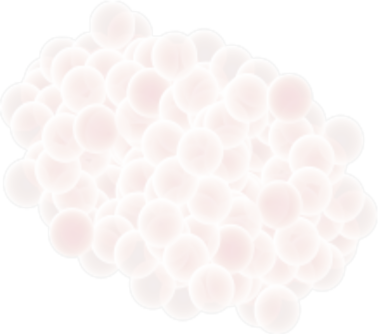
miR-21 inhibits tumor suppressors to promote cell proliferation

What does miR-21 regulate?

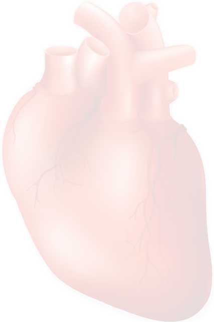
Expressed In:

Brain

+

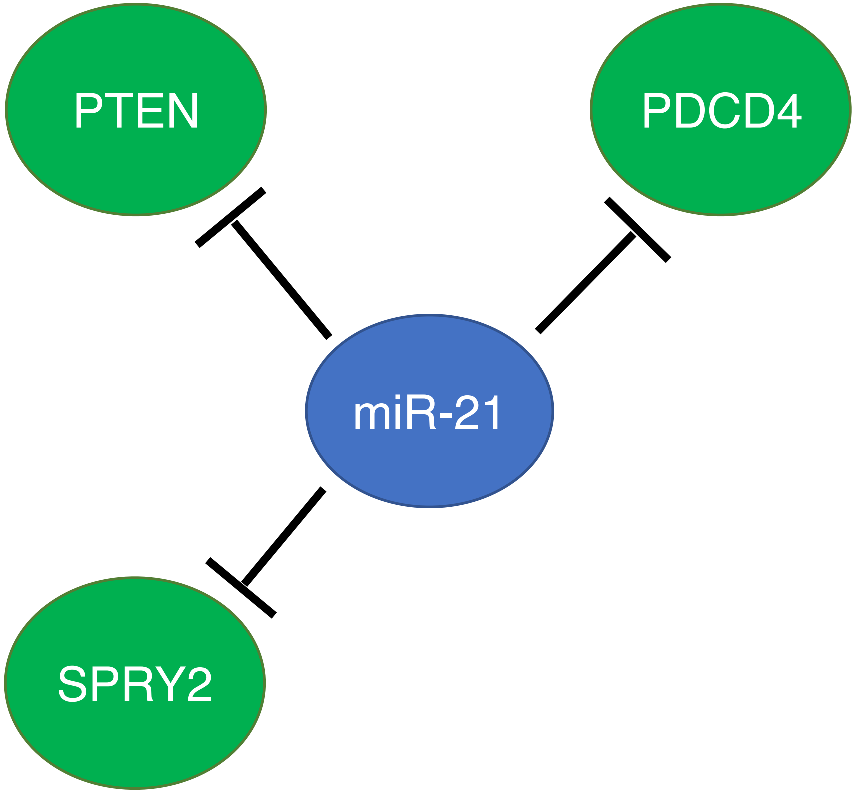


Cancer



Heart

Zebrafish heart targets:



PTEN

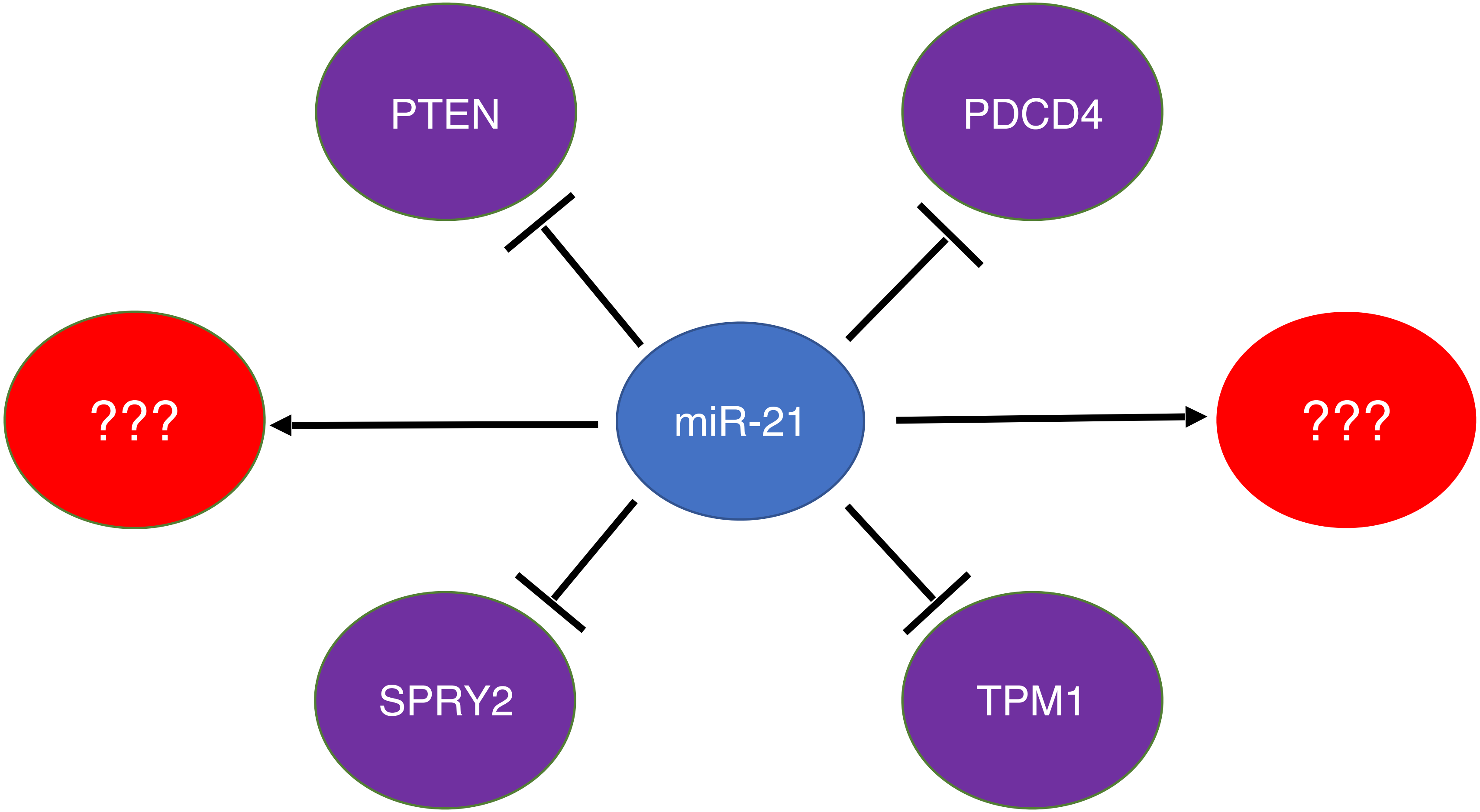
PDCD4

miR-21

SPRY2

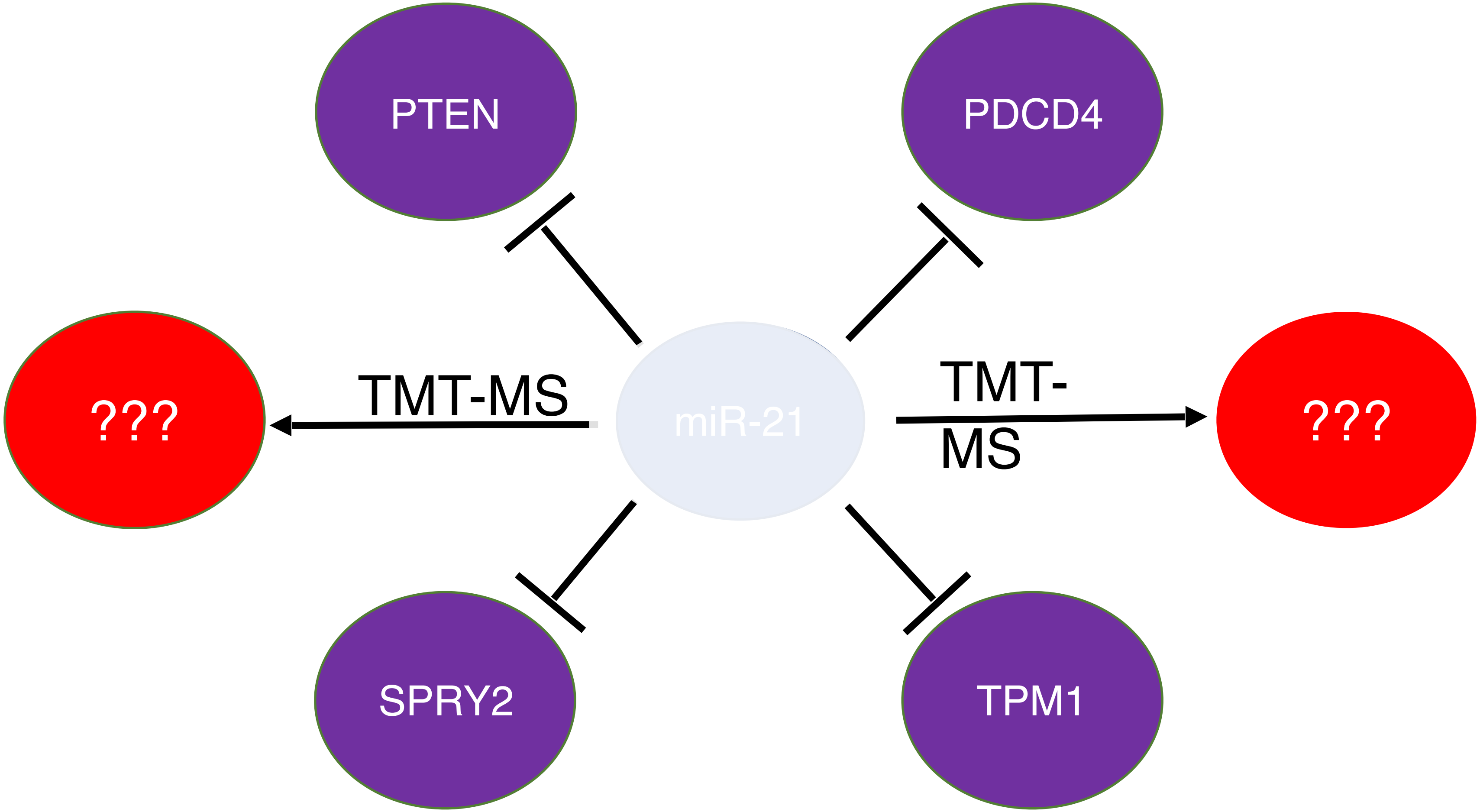
miR-21 has similar targets in both tumor invasion and zebrafish valvulogenesis

What is the goal of the study?



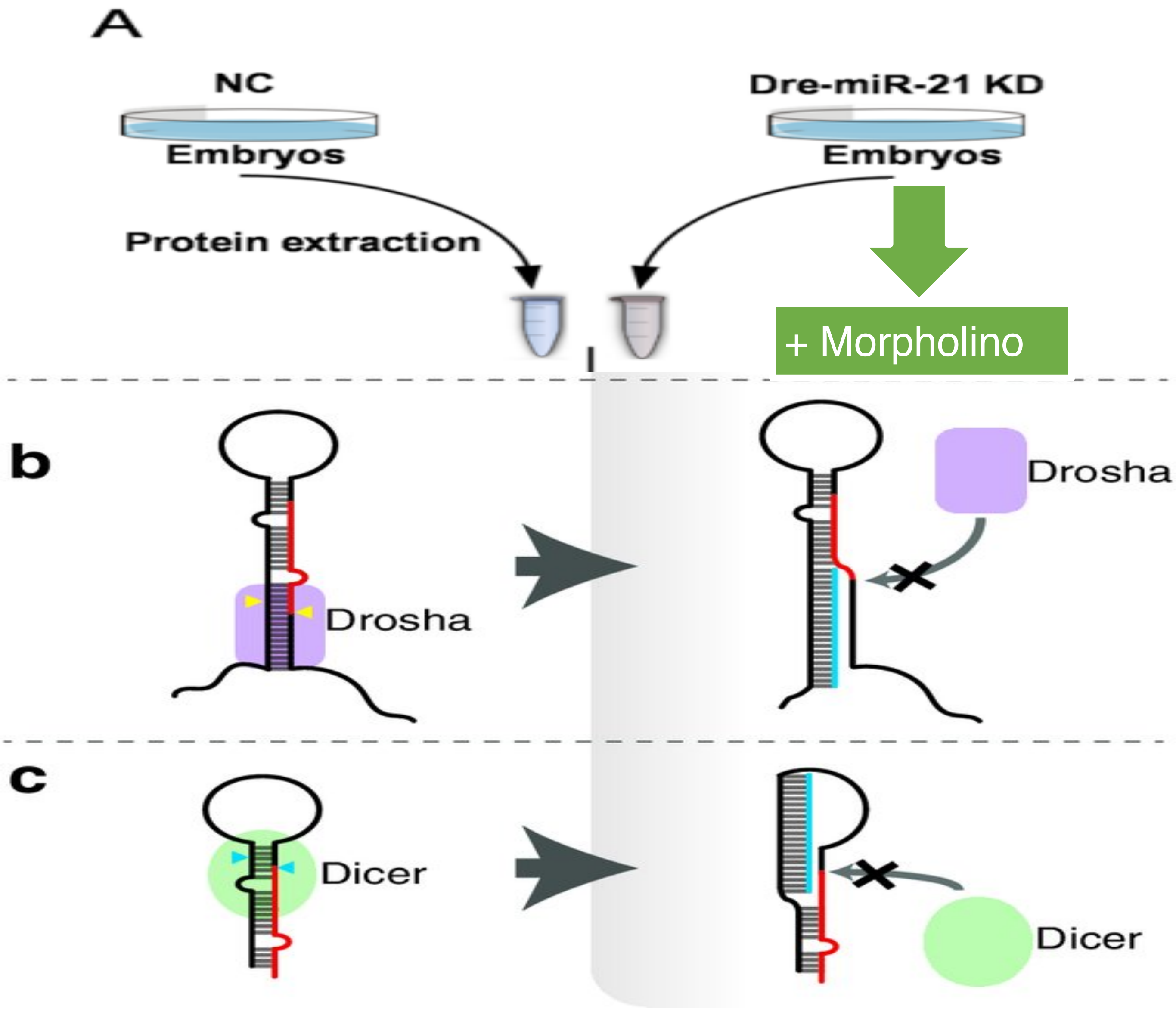
Identify new global targets of miR-21 and their relationship to Heart Development/Cancer

How to identify miRNA targets?



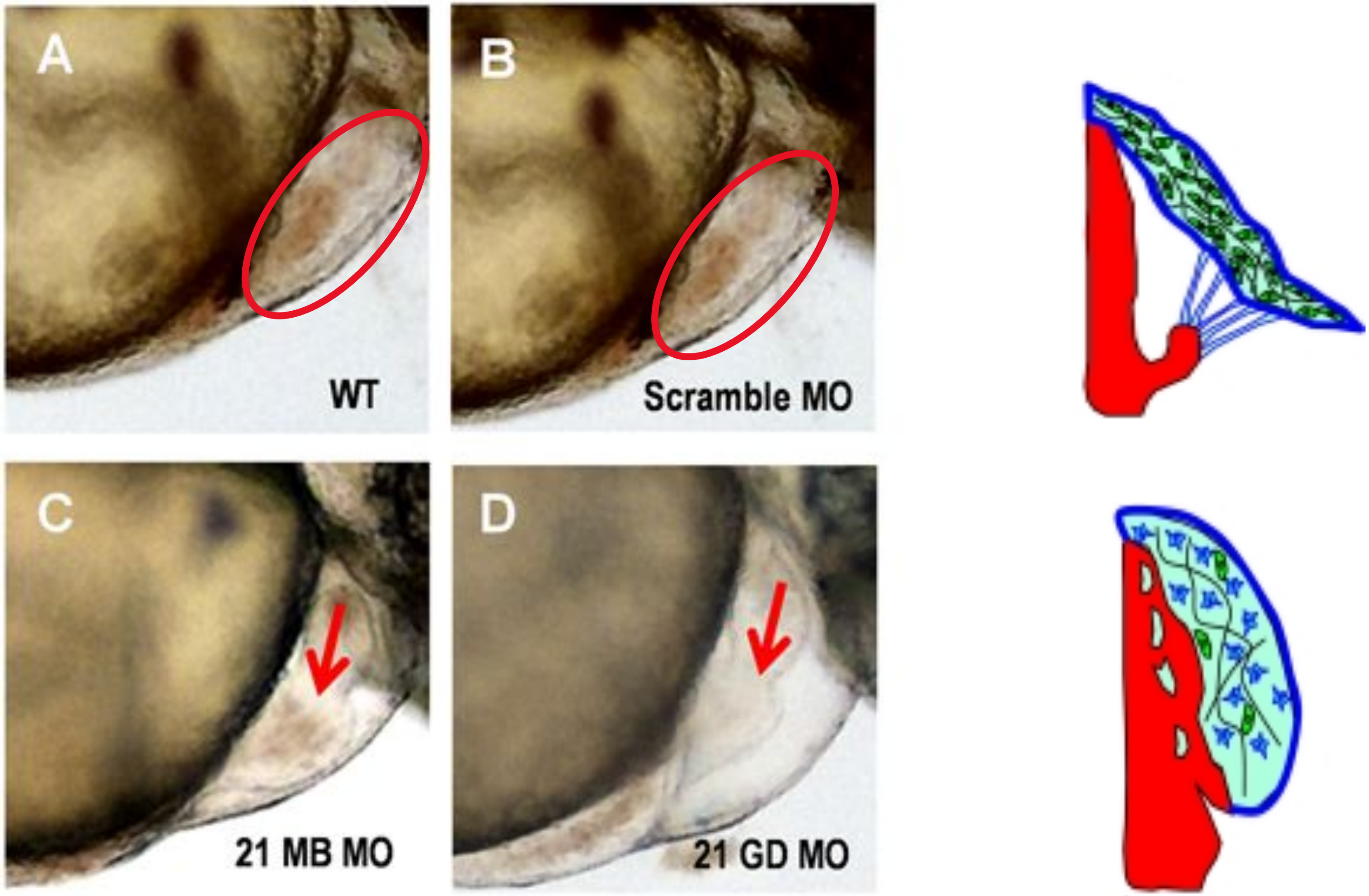
miRNA targets can be identified by high-throughput TMT Mass Spectrometry

Fig. 2A: Experimental workflow



Morpholino injections inhibit miR-21 maturation

Fig. 1: Does a miR-21 KD affect valvulogenesis?



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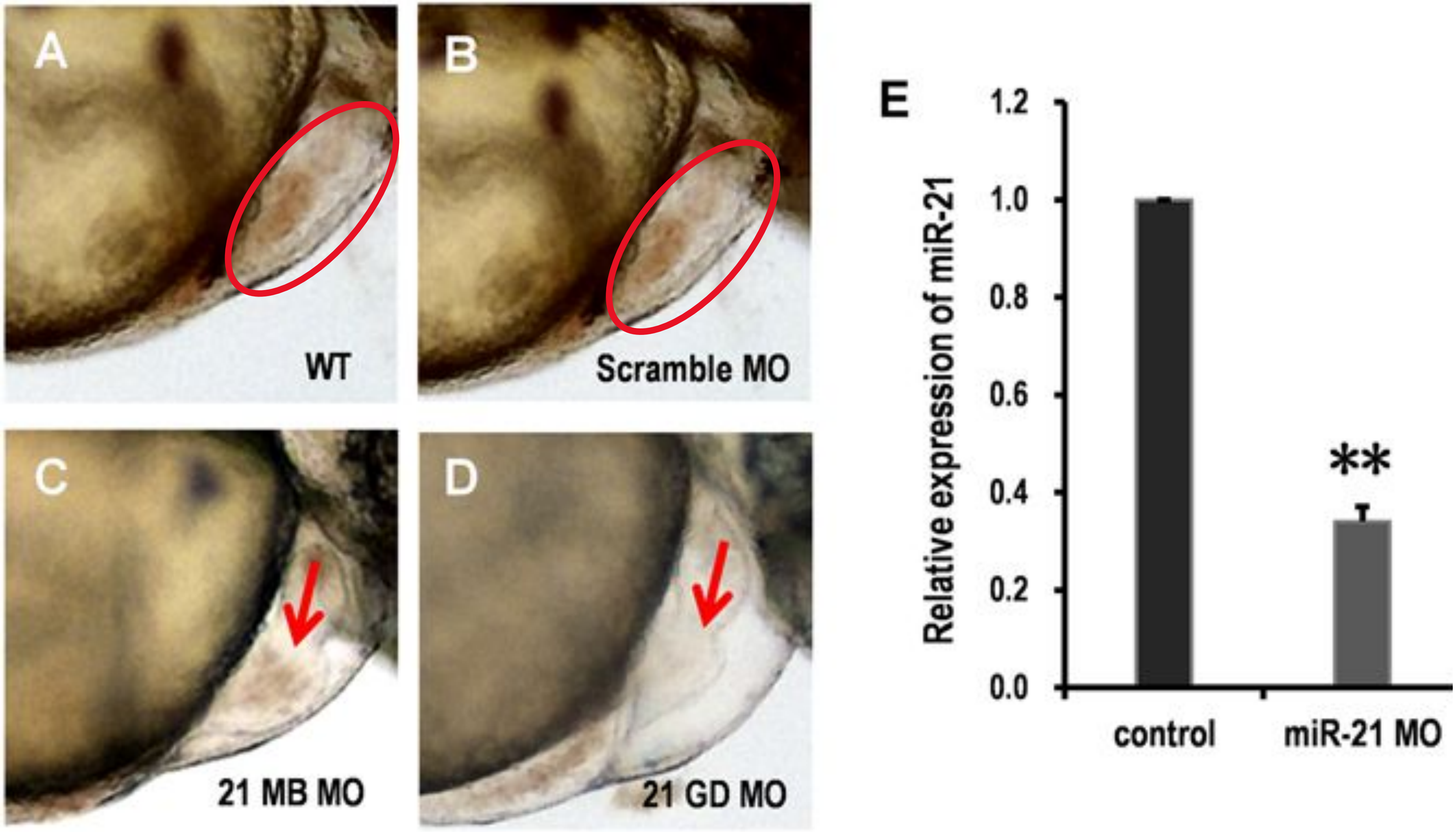


Fig. 2A: Experimental workflow

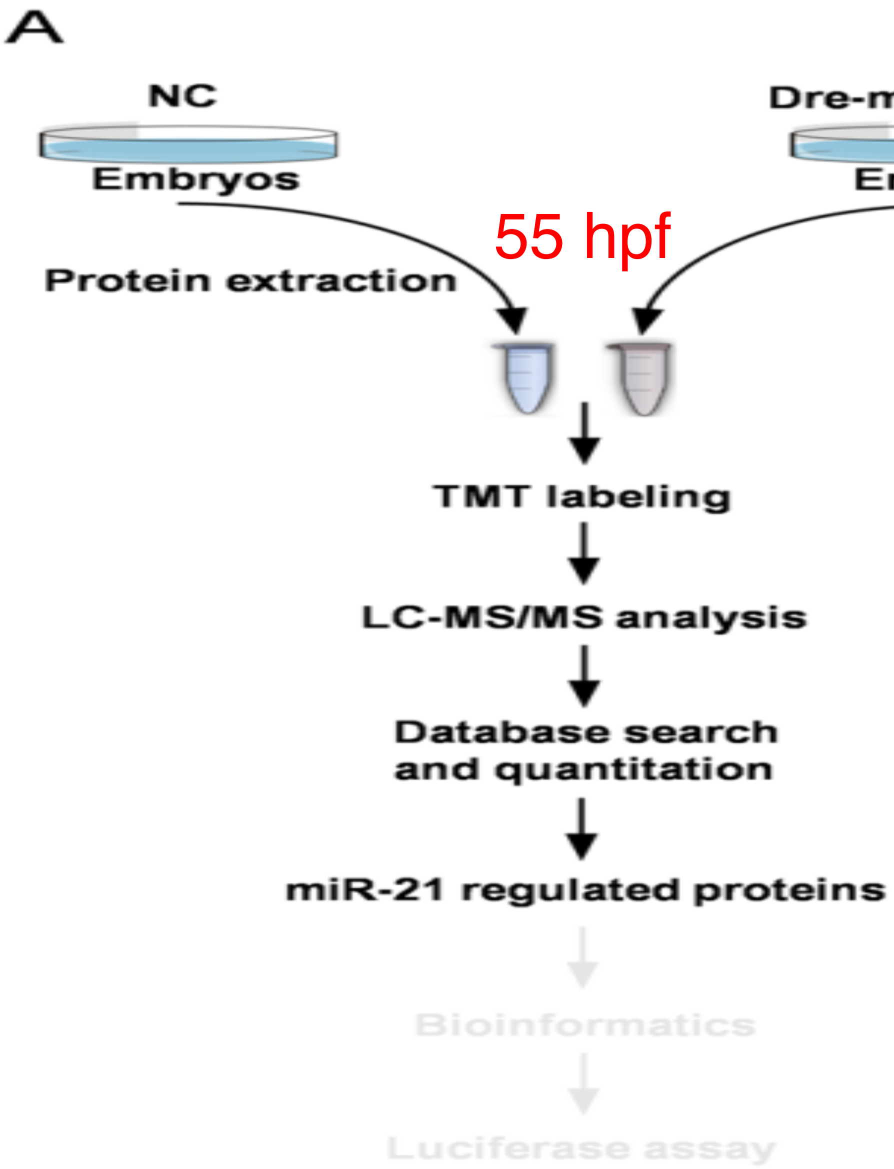


Fig. S2: How were differentially expressed proteins identified?

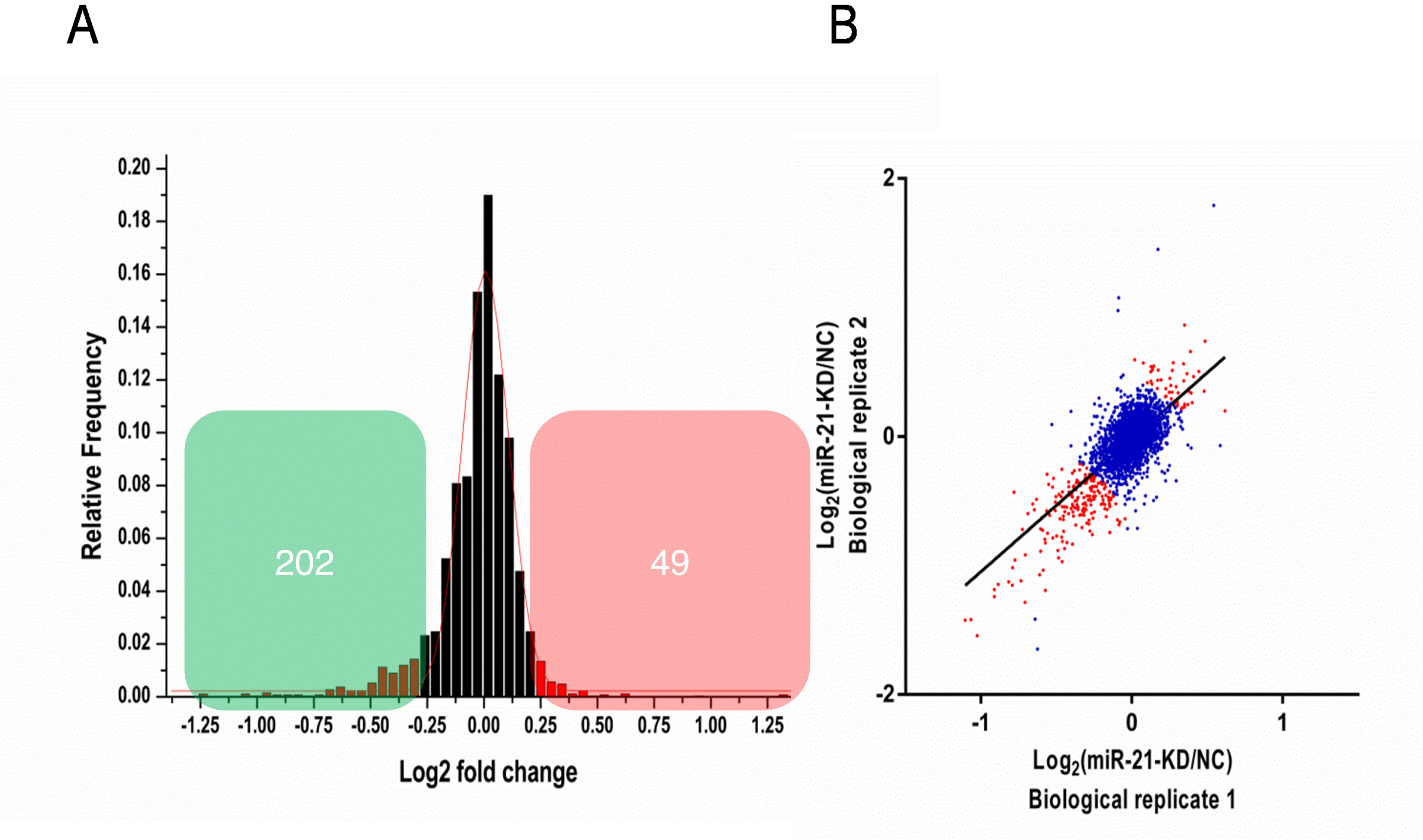
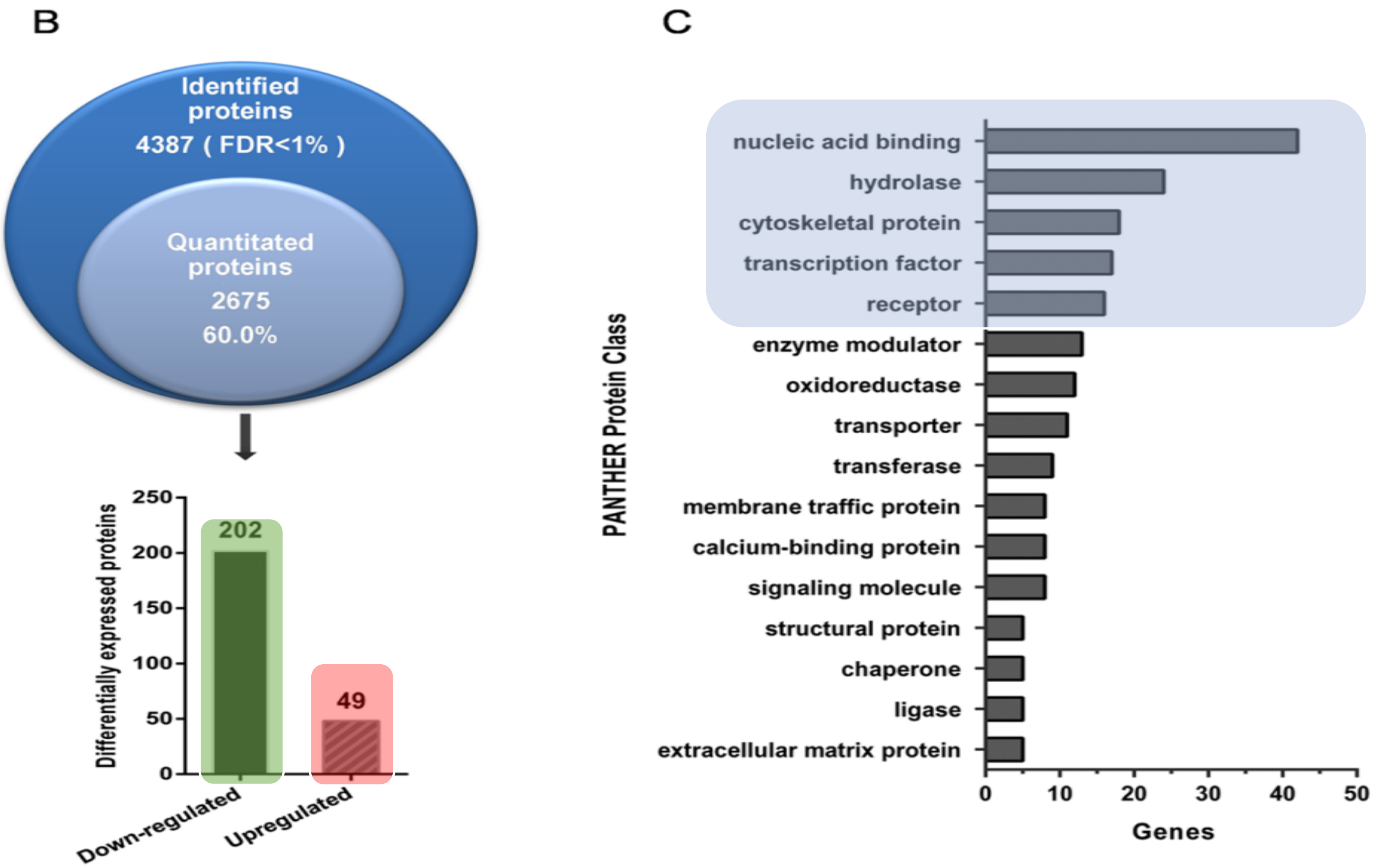
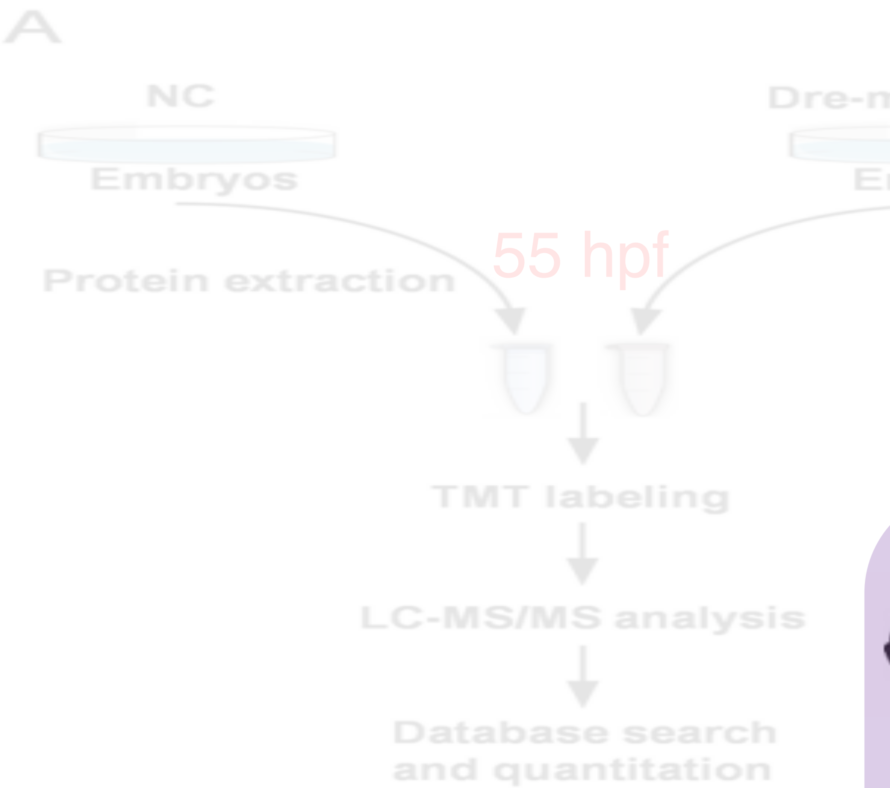


Fig. 2B,C: How many differentially expressed proteins were identified?



251 differentially expressed proteins were identified by TMT-MS and have diverse protein functions.

Fig. 2A: Experimental workflow


A

NC

Dre-miR-21 KD

Embryos

Embryos

55 hpf

Protein extraction

TMT labeling

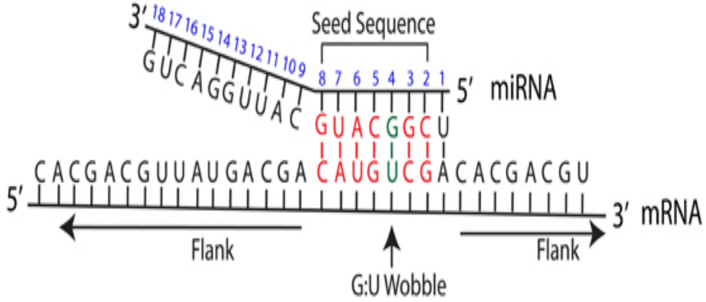
LC-MS/MS analysis

Database search
and quantitation

miR-21 regulated proteins

Bioinformatics

Luciferase assay

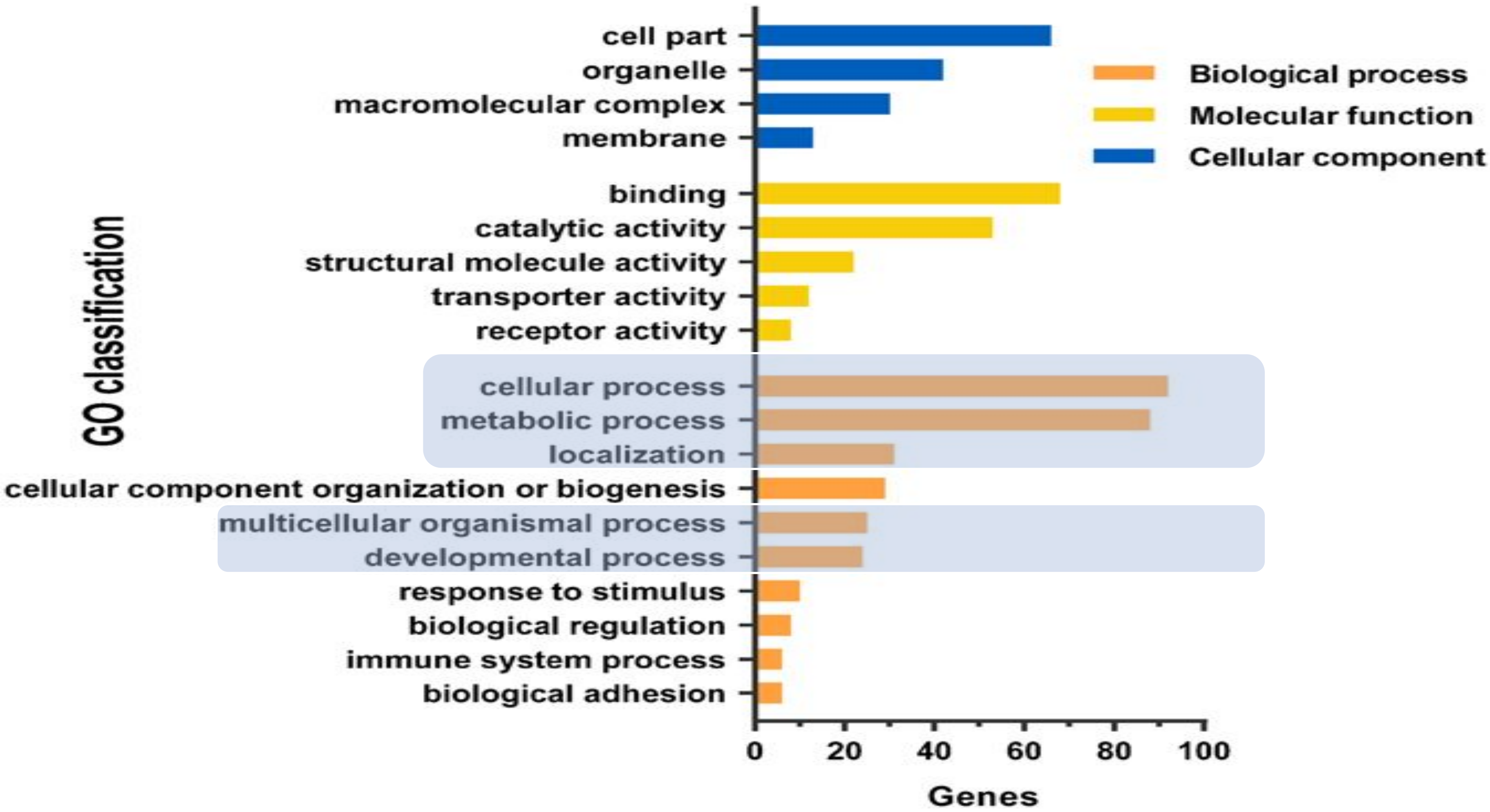


Six miRNA Prediction Software

PANTHER
Classification System

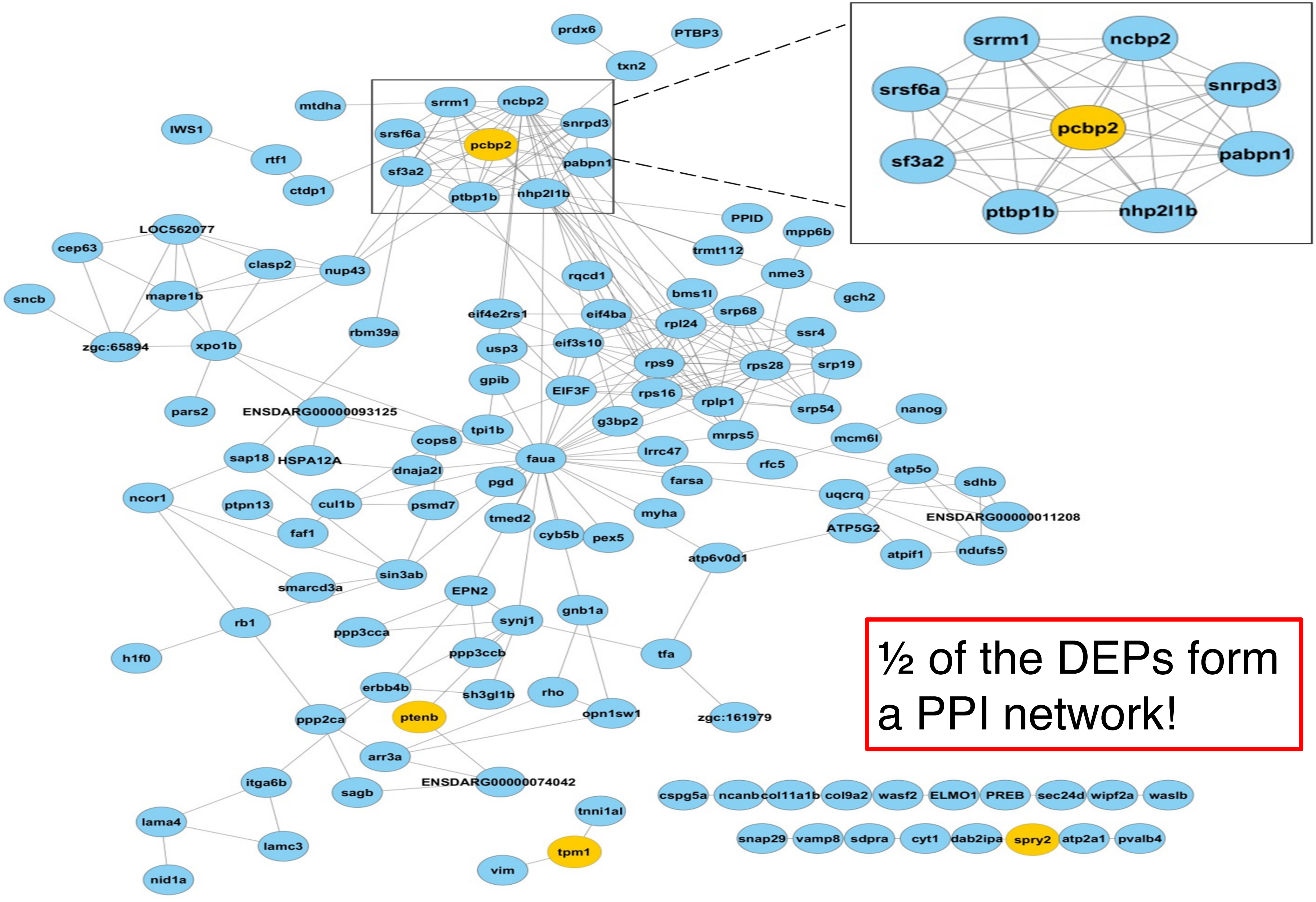
String DB

Fig. 3: What is the GO for the 251 differentially expressed proteins?



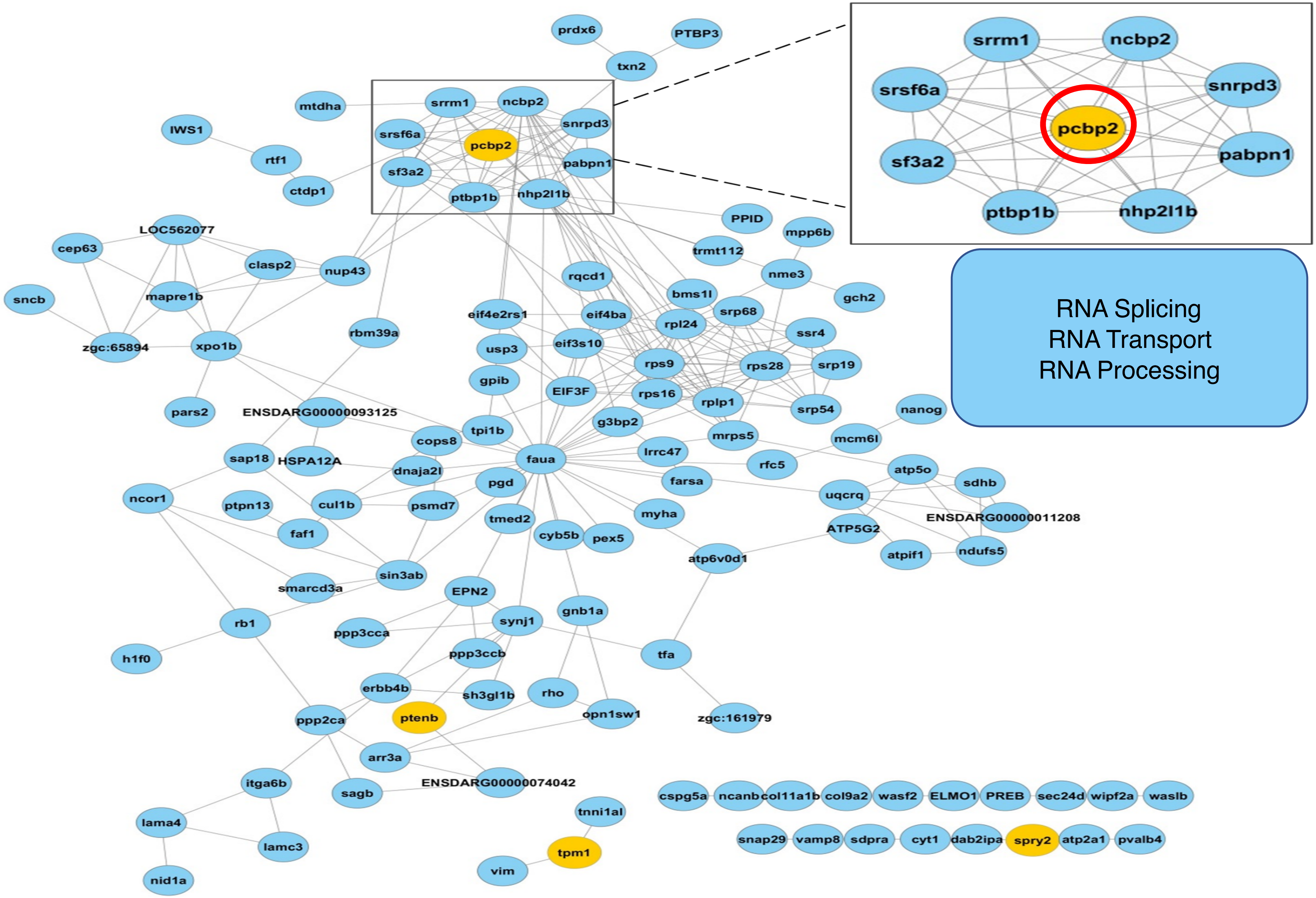
miR-21 likely regulates multiple biological processes

Fig. S3: Do any of candidates interact?



½ of the DEPs form a PPI network!

Fig. S3: Do any of the candidates interact?



RNA Splicing
RNA Transport
RNA Processing

Fig. 2A: Experimental workflow

A

NC

Dre-miR-21 KD

Embryos

Embryos

55 hpf

Protein extraction

TMT labeling

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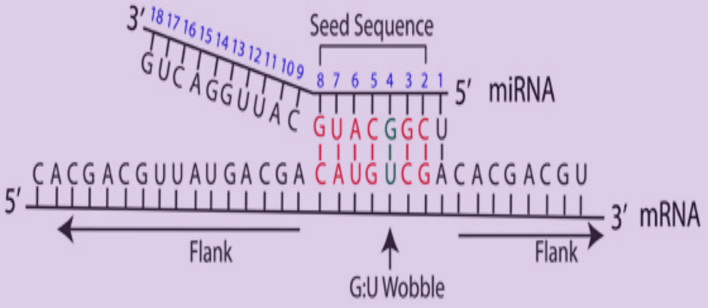
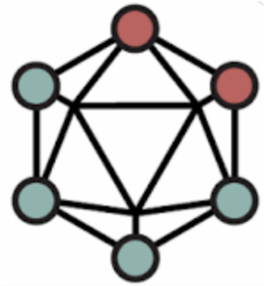


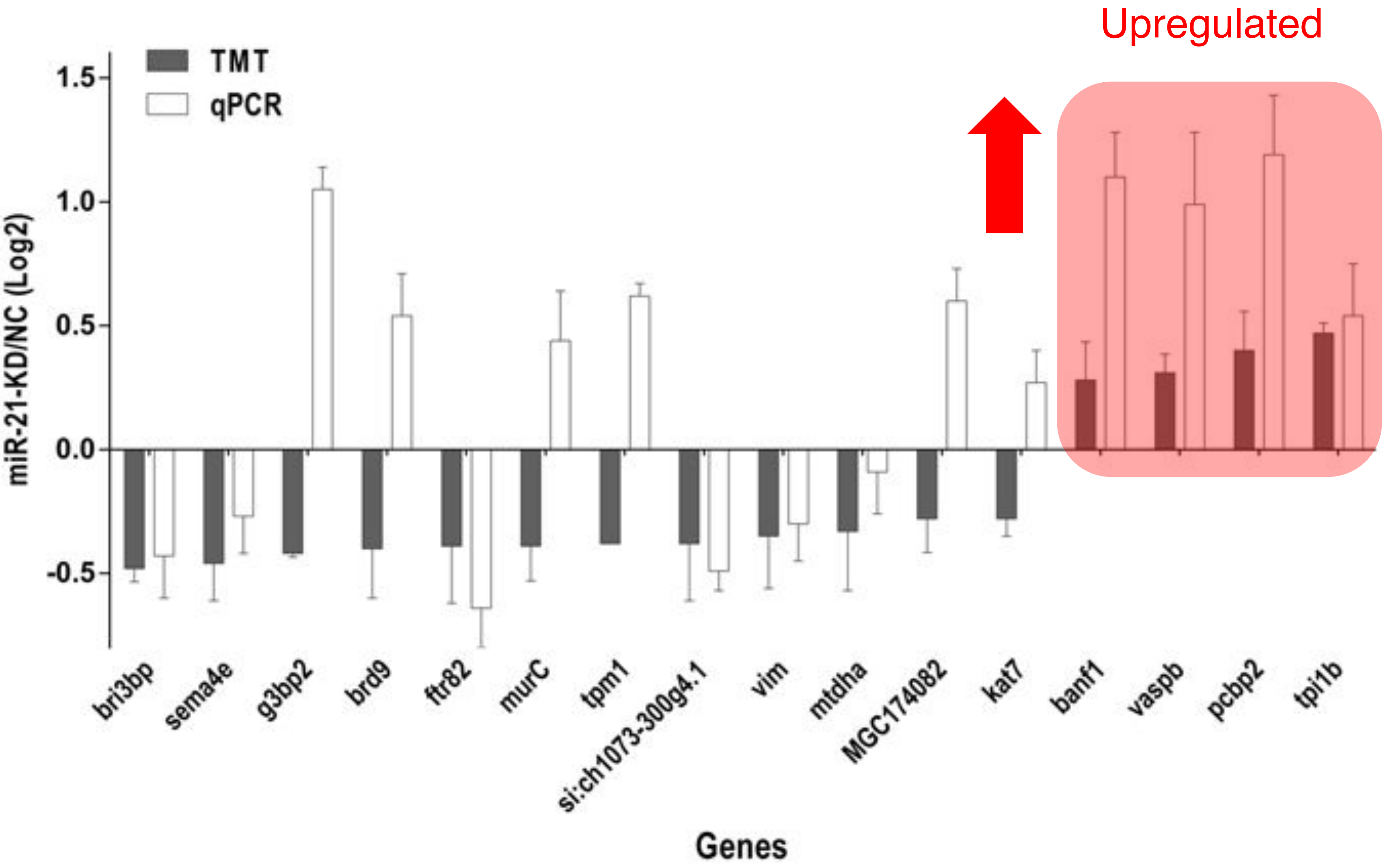
Six miRNA Prediction Software

PANTHER
Classification System



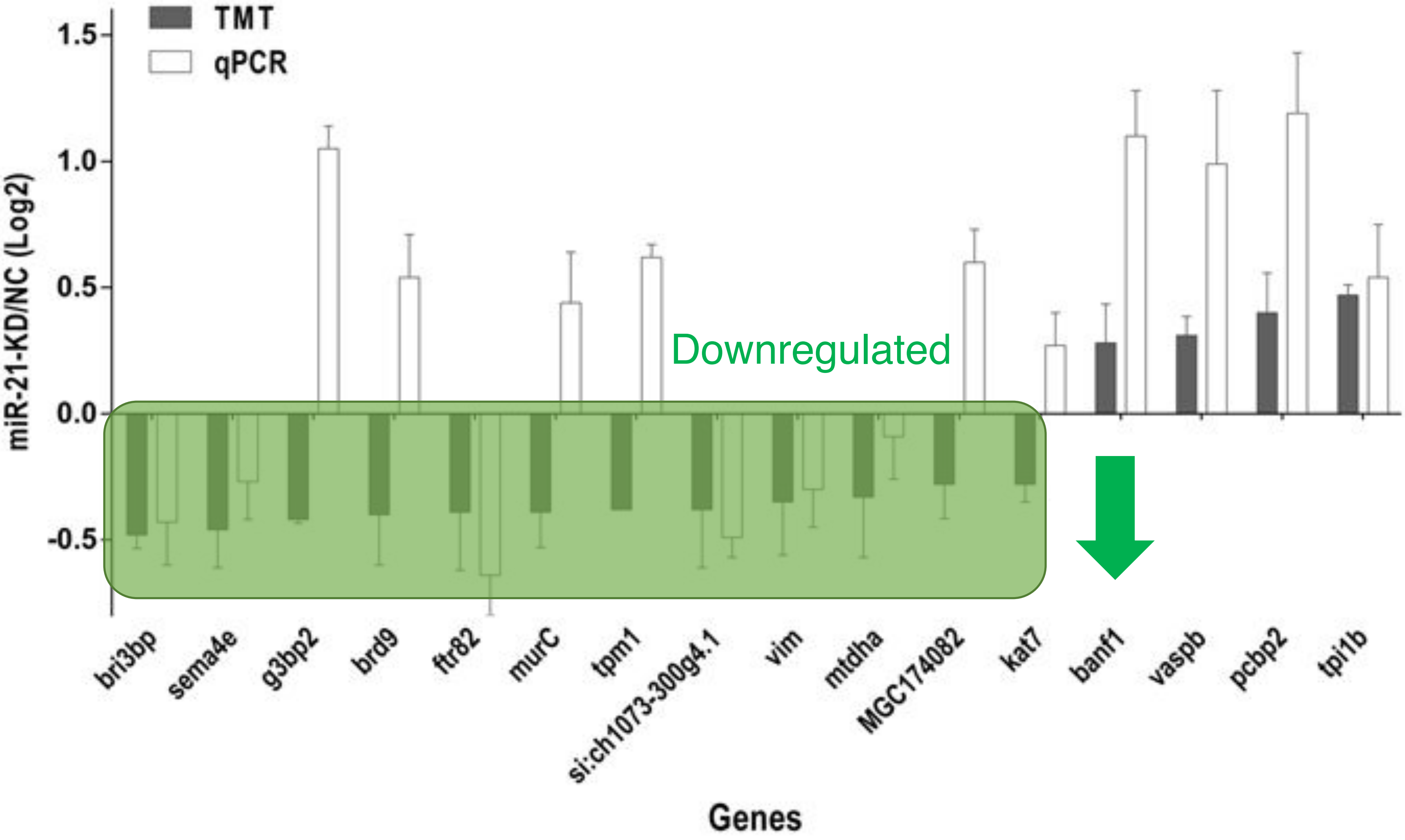
String DB

Fig. 4 Do transcript levels correlate with protein abundance?



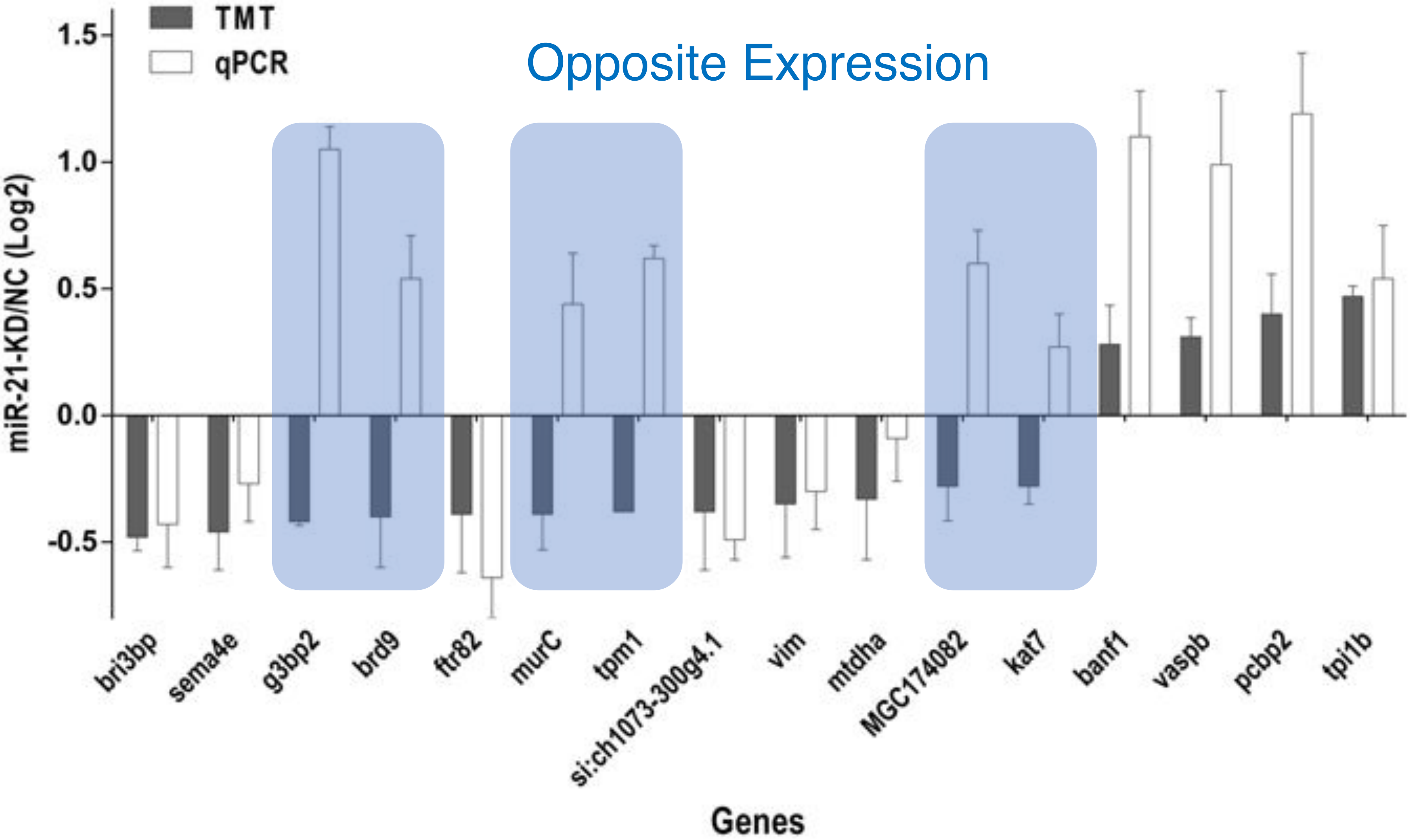
Sixteen targets predicted to be targeted by miR-21

Fig. 4 Do transcript levels correlate with protein abundance?



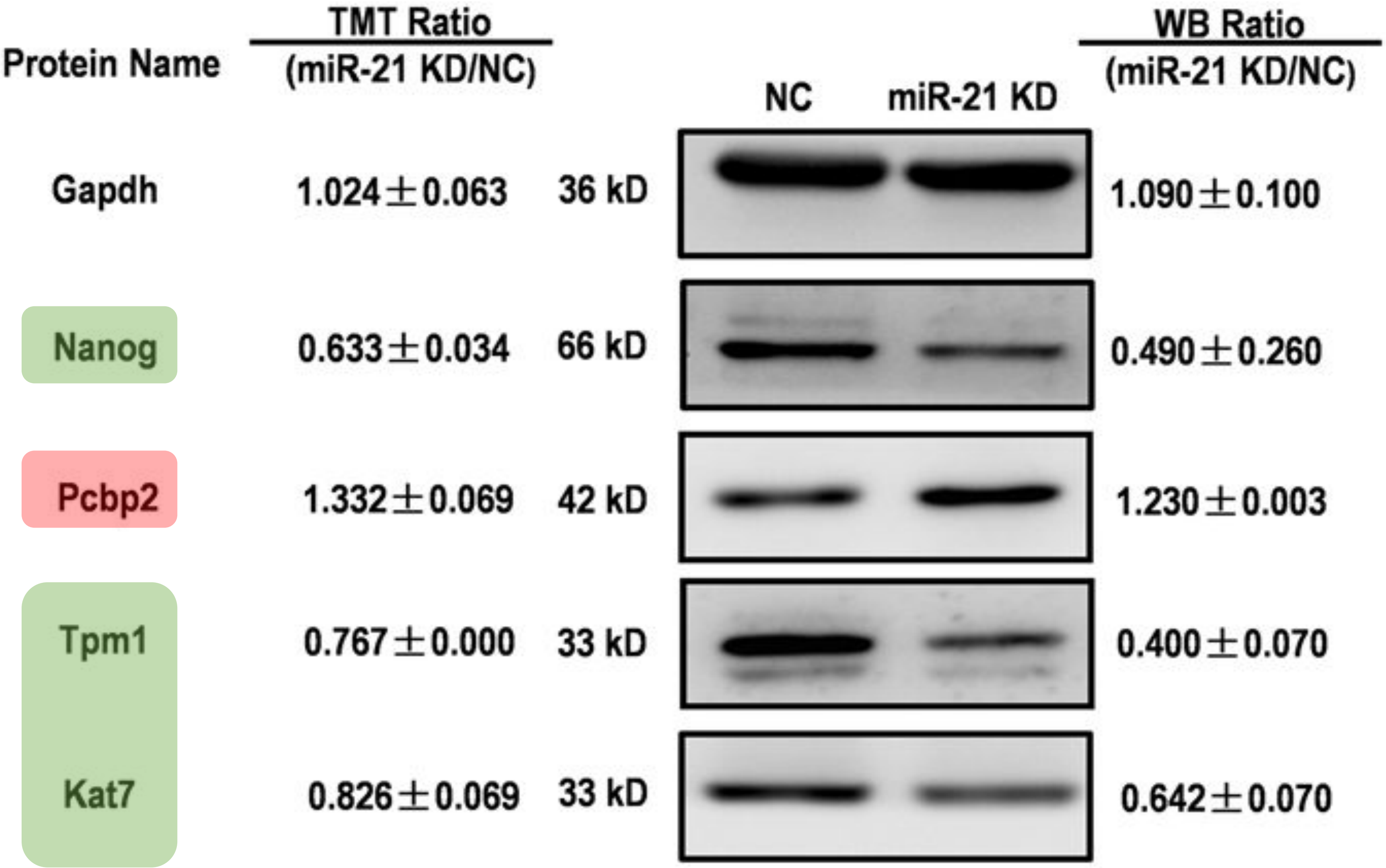
Ten targets show similar trends in gene expression

Fig. 4 Do transcript levels correlate with protein abundance?



Six targets show opposite expression patterns

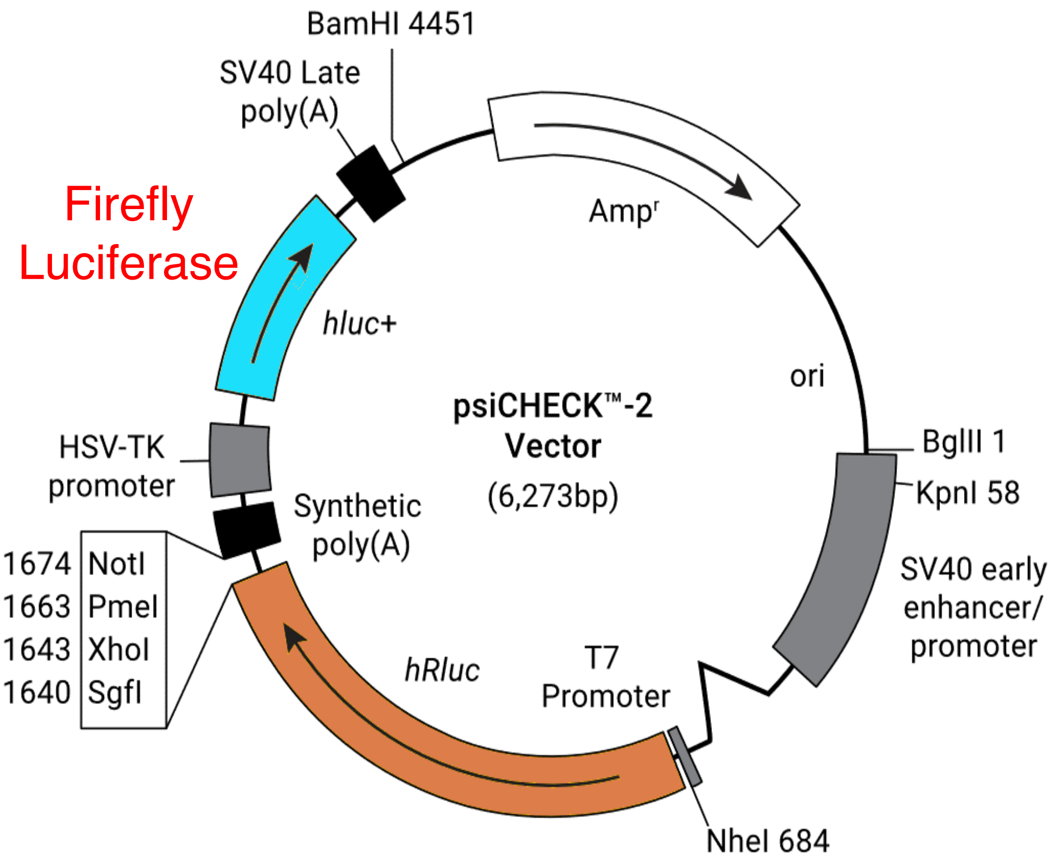
Fig. 5: How valid is the proteomic data?



Protein Name	TMT Ratio (miR-21 KD/NC)		NC	miR-21 KD	WB Ratio (miR-21 KD/NC)
Gapdh	1.024±0.063	36 kD			1.090±0.100
Nanog	0.633±0.034	66 kD			0.490±0.260
Pcbp2	1.332±0.069	42 kD			1.230±0.003
Tpm1	0.767±0.000	33 kD			0.400±0.070
Kat7	0.826±0.069	33 kD			0.642±0.070

Western Blot assays show similar results to TMT-MS

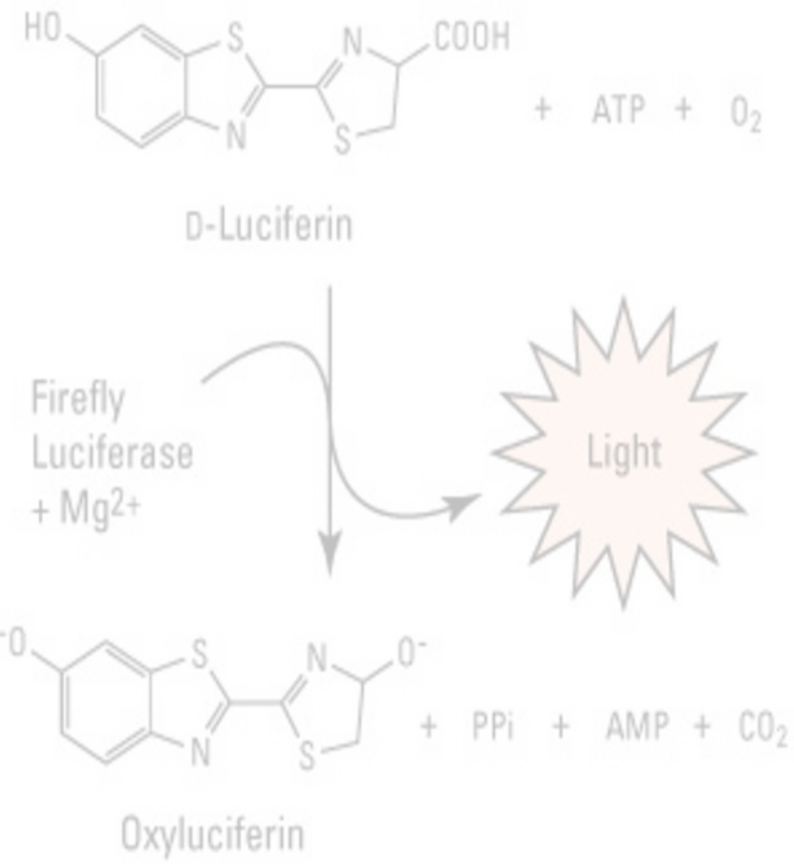
What is a luciferase assay?



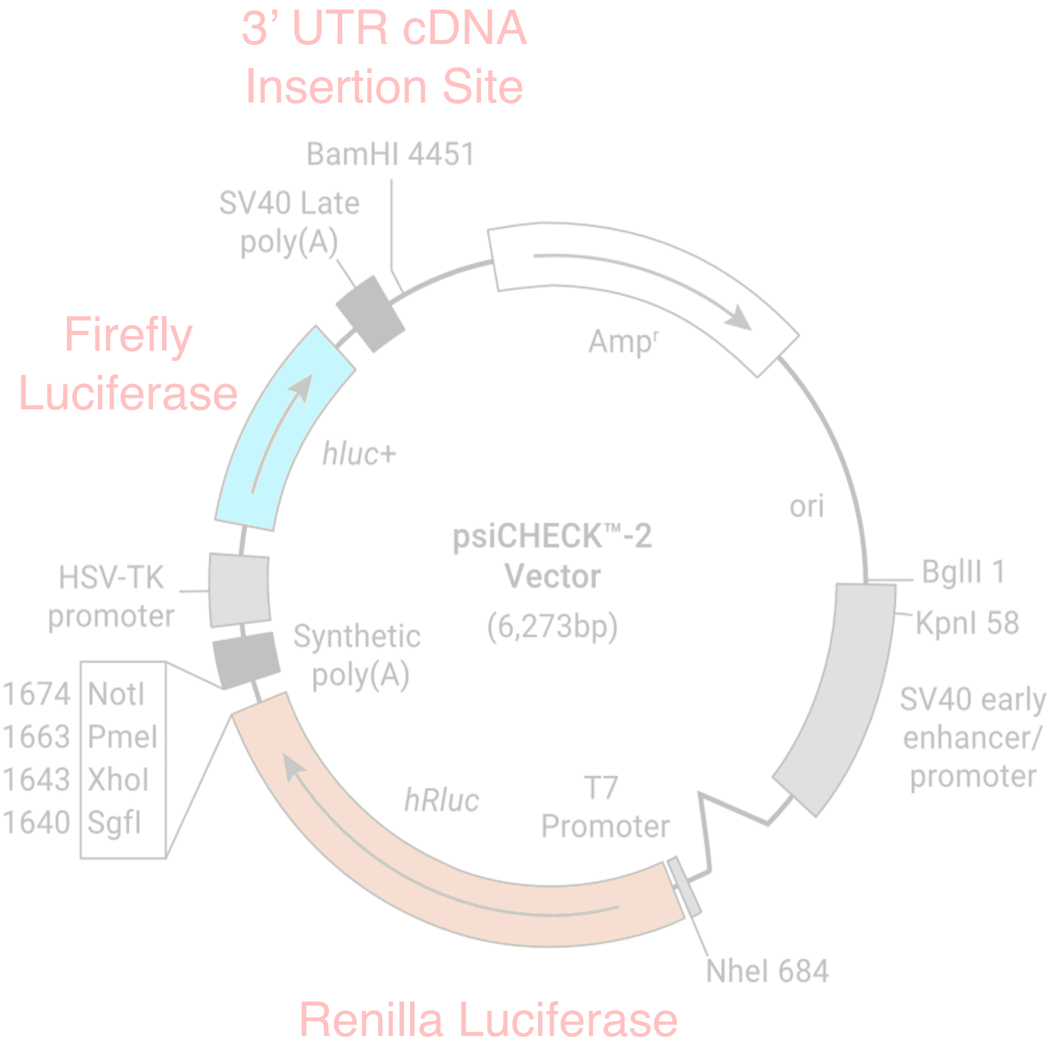
3’ UTR cDNA Insertion Site

Firefly Luciferase

Renilla Luciferase



What is a luciferase assay?



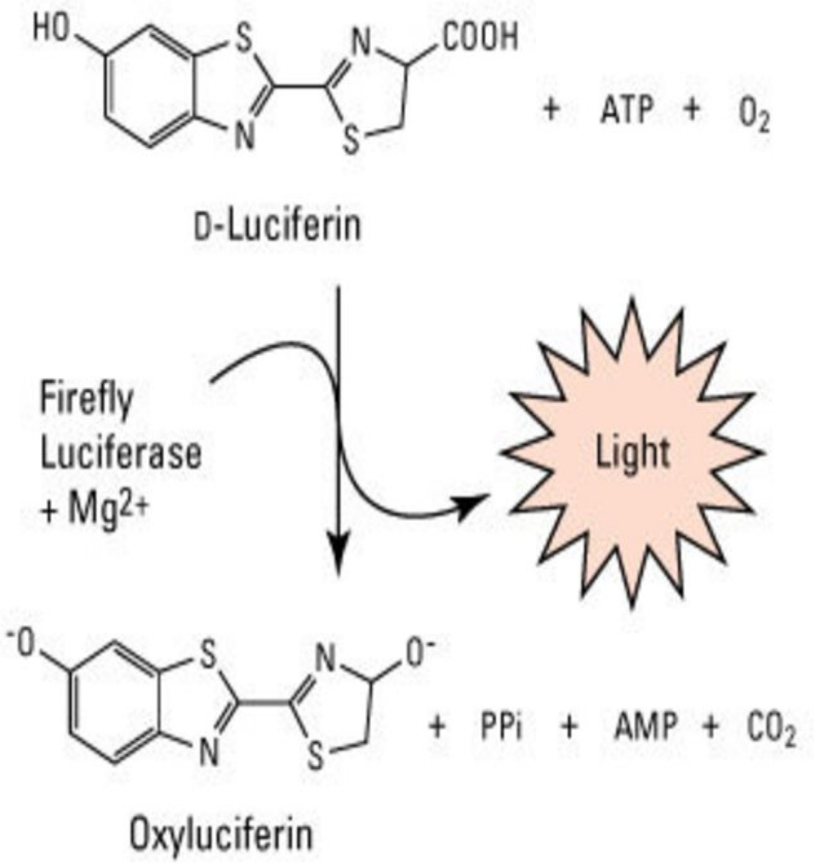
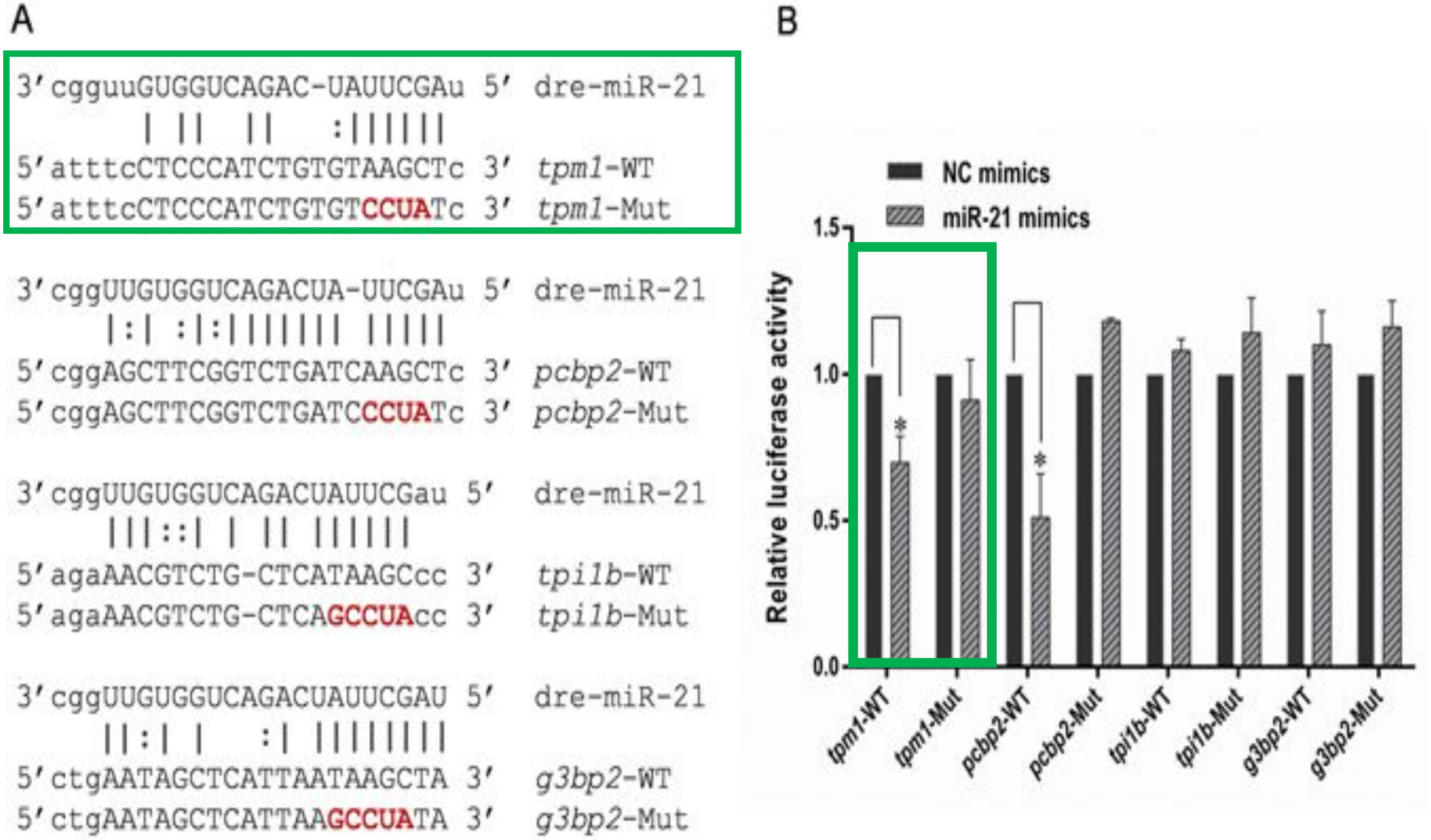
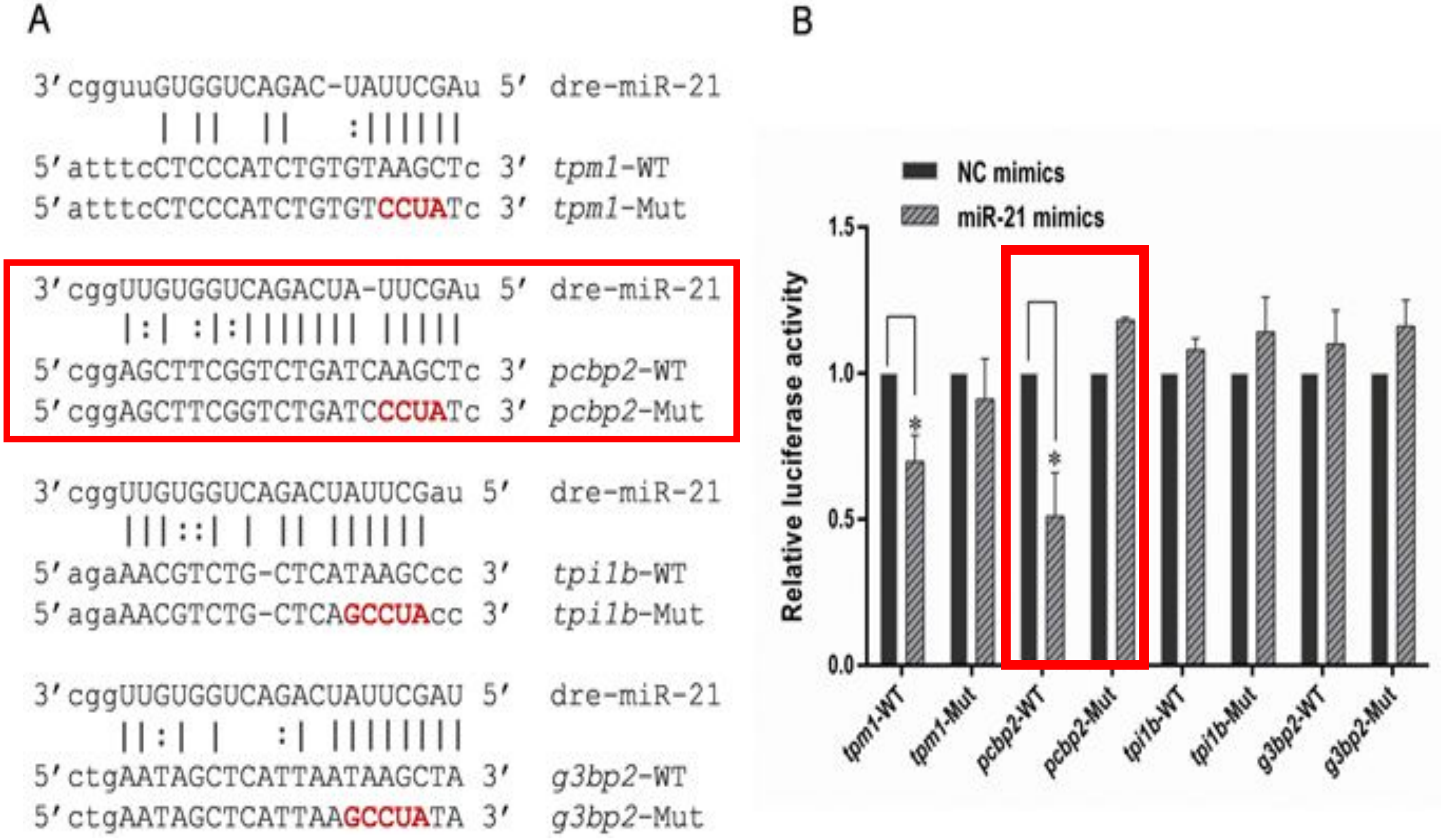


Fig. 6: How valid is the miR-21 bioinformatic data?



tpm1 is targeted and repressed by miR-21 but decreases in the K

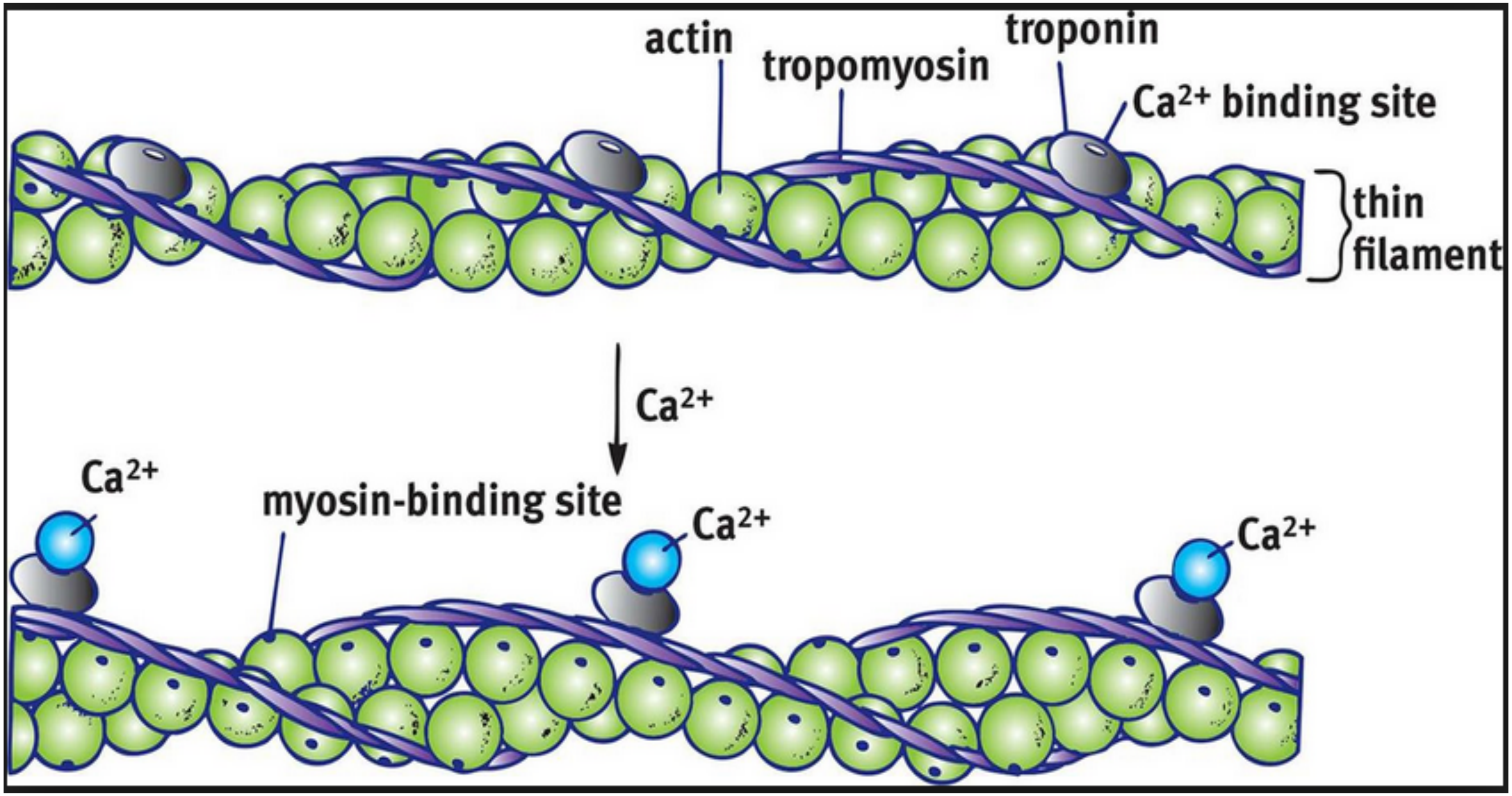
Fig. 6: How valid is the miR-21 bioinformatic data?



```
3'cgguuGUGGUCAGAC-UAUUCGAu 5'  dre-miR-21
       | ||  ||   :||||||
5'atttcCTCCCATCTGTGTAAGCTc 3'  tpm1-WT
5'atttcCTCCCATCTGTGTCCUATc 3'  tpm1-Mut

3'cggUUGUGGUCAGACUA-UUCGAu 5'  dre-miR-21
     |:| :|:||||||| |||||
5'cggAGCTTCGGTCTGATCAAGCTc 3'  pcbp2-WT
5'cggAGCTTCGGTCTGATCCCUATc 3'  pcbp2-Mut

3'cggUUGUGGUCAGACUAUUCGau 5'   dre-miR-21
     |||::| | || ||||||
5'agaAACGTCTG-CTCATAAGCcc 3'   tpi1b-WT
5'agaAACGTCTG-CTCAGCCUAcc 3'   tpi1b-Mut

3'cggUUGUGGUCAGACUAUUCGAU 5'   dre-miR-21
     ||:| |   :| ||||||||
5'ctgAATAGCTCATTAATAAGCTA 3'   g3bp2-WT
5'ctgAATAGCTCATTAAGCCUATA 3'   g3bp2-Mut
```

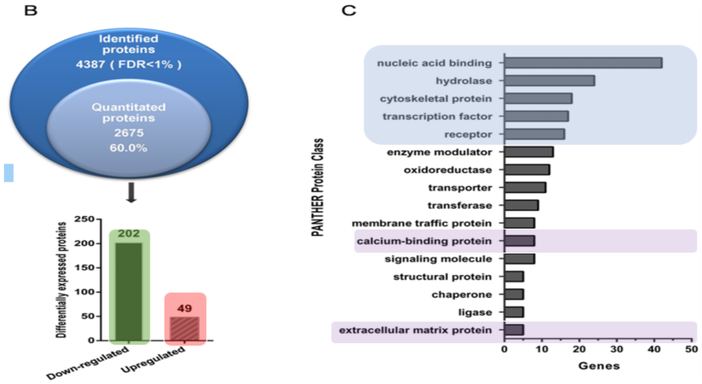
pcbp2 is directly targeted and repressed by miR-21

What is *tpm1*?

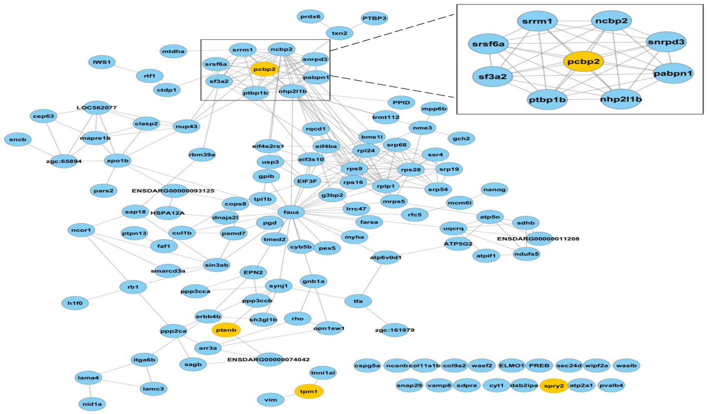


Tropomysin regulates muscle contractions and is a known tumor suppressor gene

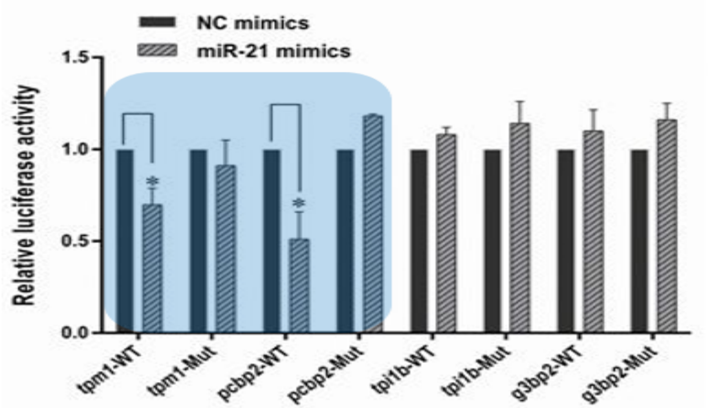
Summary



Quantitative Proteomics is a tool for measuring protein abundance and identified 251 differentially expressed proteins.



miR-21 regulates valvulogenesis through a network of different proteins with diverse biological roles.



Luciferase reporters demonstrate that both tpm1 and pcbp2 are directly regulated by miR-21 during valvulogenesis.

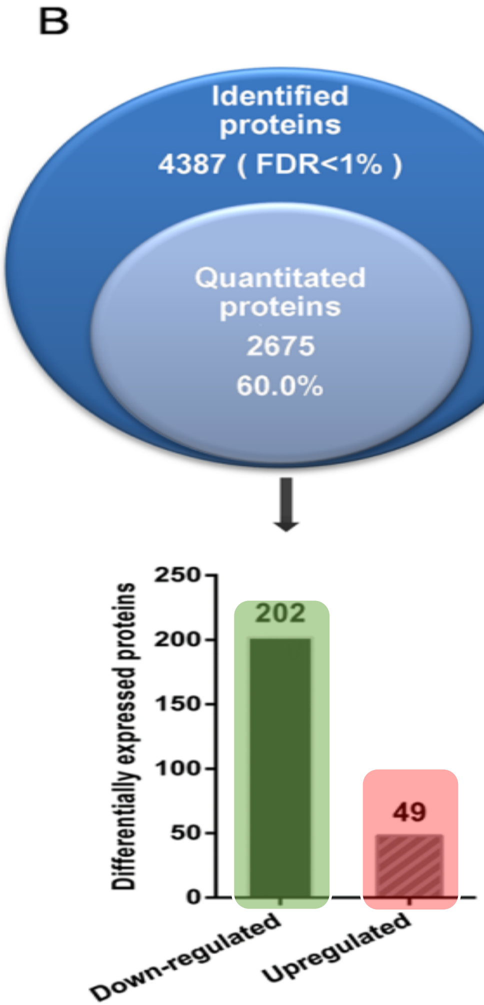
Questions or Comments?

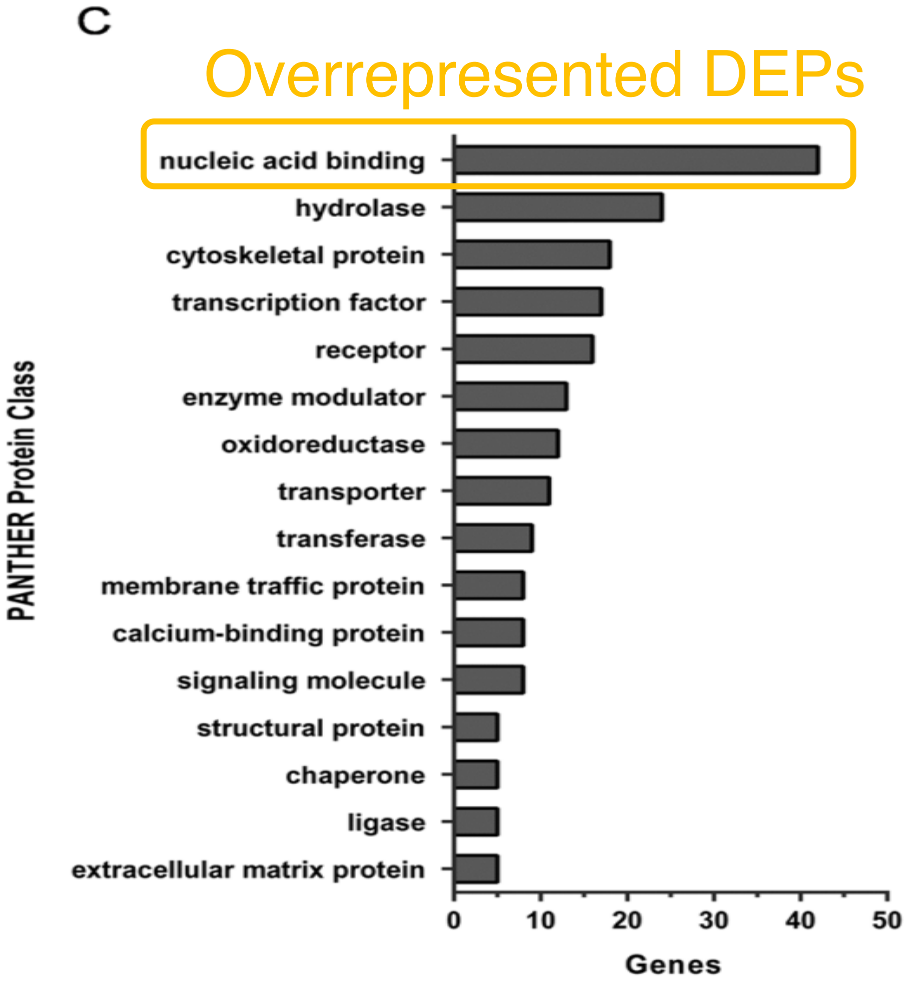
References – Images (JL)

1. https://www.nature.com/articles/s41598-017-04166-x#ref-CR18
2. https://stanfordhealthcare.org/medical-conditions/blood-heart-circulation/congenital-heart-disease/types/obstructive.html
3. https://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-24/CB24.html
4. https://en.wikipedia.org/wiki/Heart_development
5. https://www.nature.com/articles/ncomms2978.pdf?origin=ppub
6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2440410/
7. https://www.geneticistinc.com/blog/microrna
8. https://pouringmyartout.com/2014/02/14/some-thoughts-on-valentines-day-part-6-and-i-swear-this-is-the-last-one/
9. http://spmphysics.onlinetuition.com.my/2013/08/uses-of-radioisotope-in-medical.html
10. http://2012.igem.org/wiki/images/8/8a/Tumor.png
11. https://www.researchgate.net/publication/315803167_Morpholino_Oligomers/figures?lo=1&utm_source=google&utm_medium=organic
12. http://www.pantherdb.org/
13. https://en.wikipedia.org/wiki/STRING
14. https://www.frontiersin.org/articles/10.3389/fgene.2014.00023/full
15. https://www.nature.com/articles/s41598-017-04166-x#ref-CR18
16. https://www.promega.com/products/rna-purification-and-analysis/rna-interference/psicheck-1-and-psicheck-2-vectors/?catNum=C8011
17. http://bpsbioscience.com/dual-luciferase-firefly-renilla-luciferase-assay-system-60683
18. http://physiologyplus.com/intro-mcq-skeletal-muscle-structure/

Supplemental Slides

Fig. 2B,C: How many differentially expressed proteins were identified?





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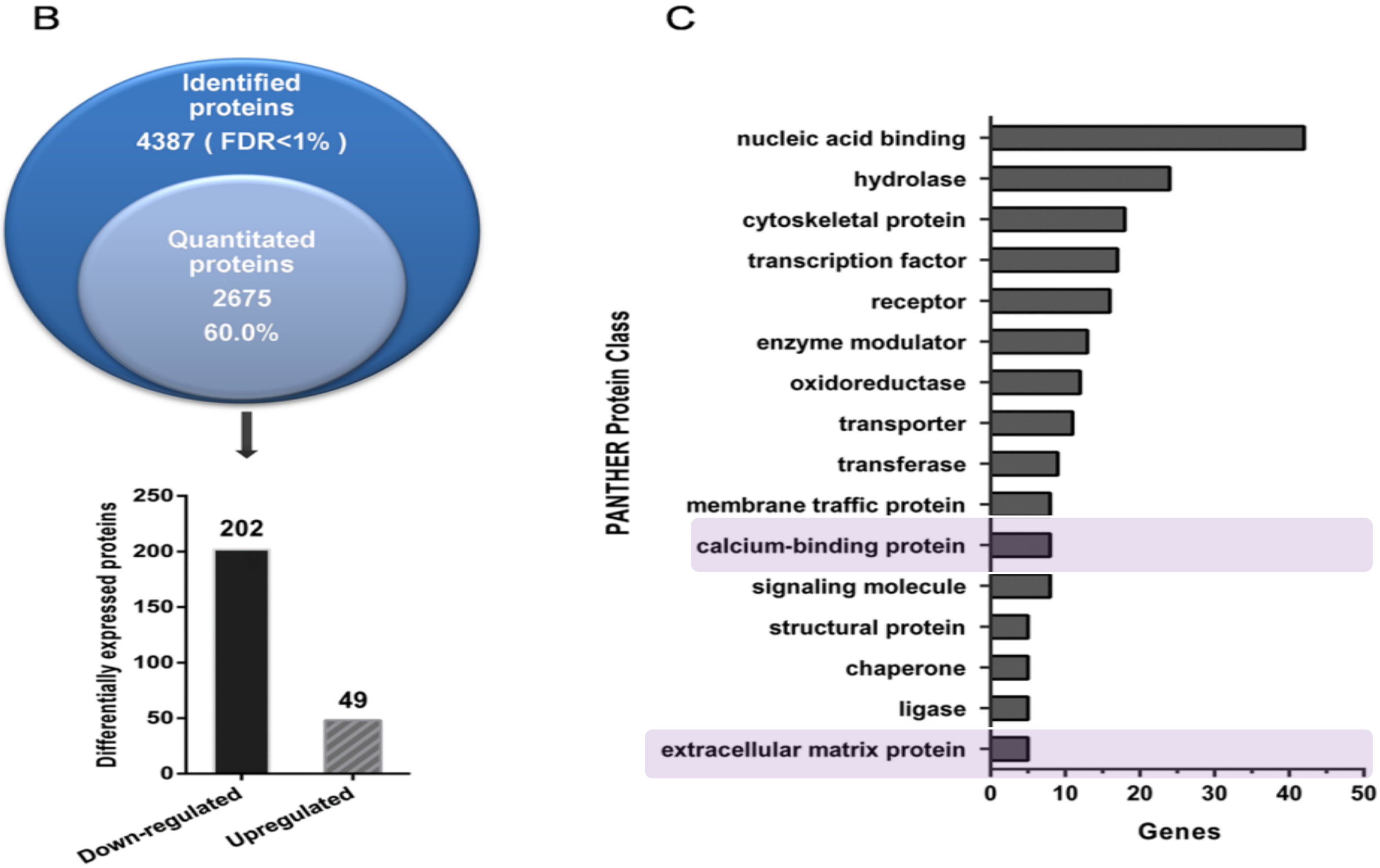

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