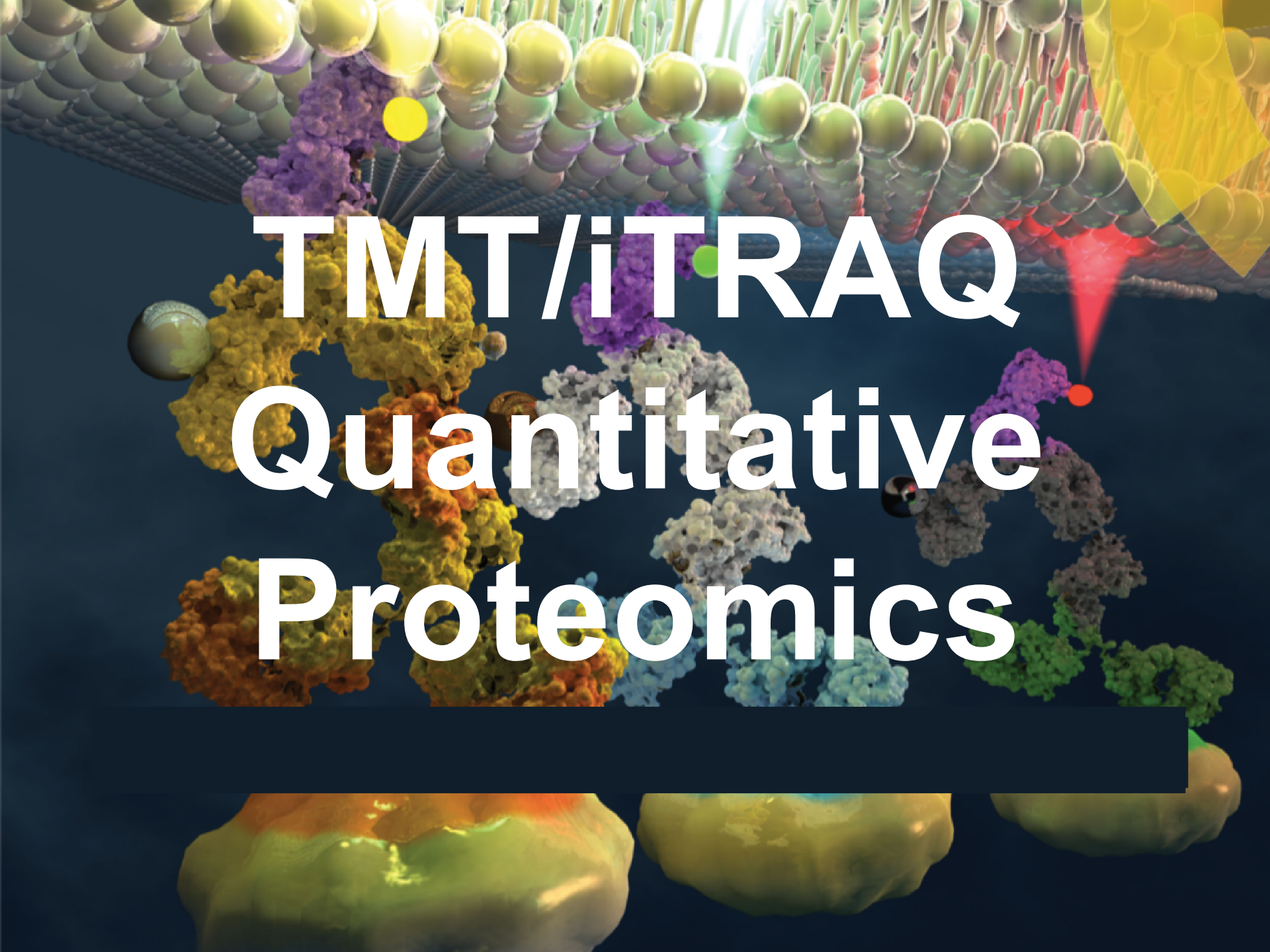
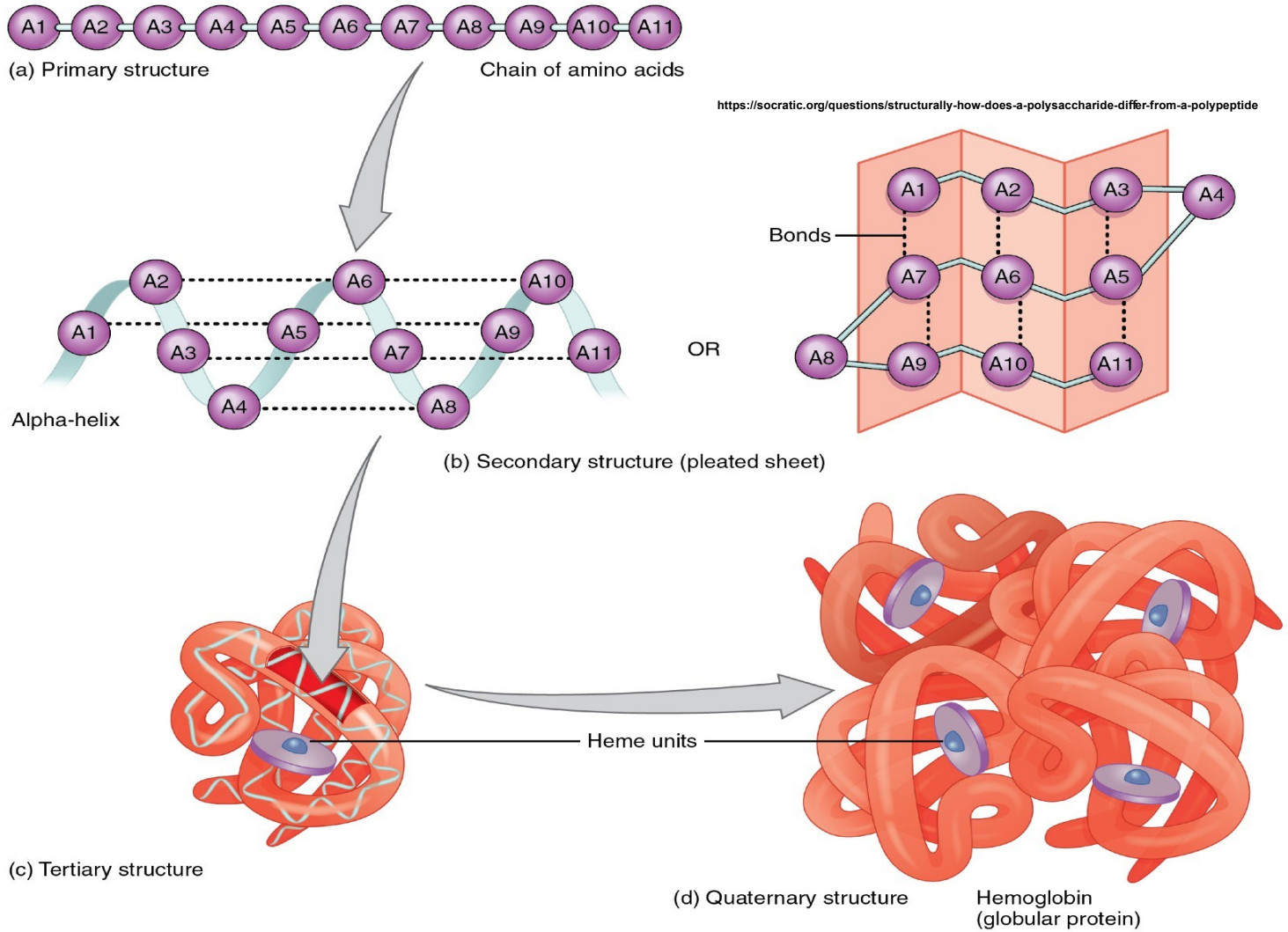
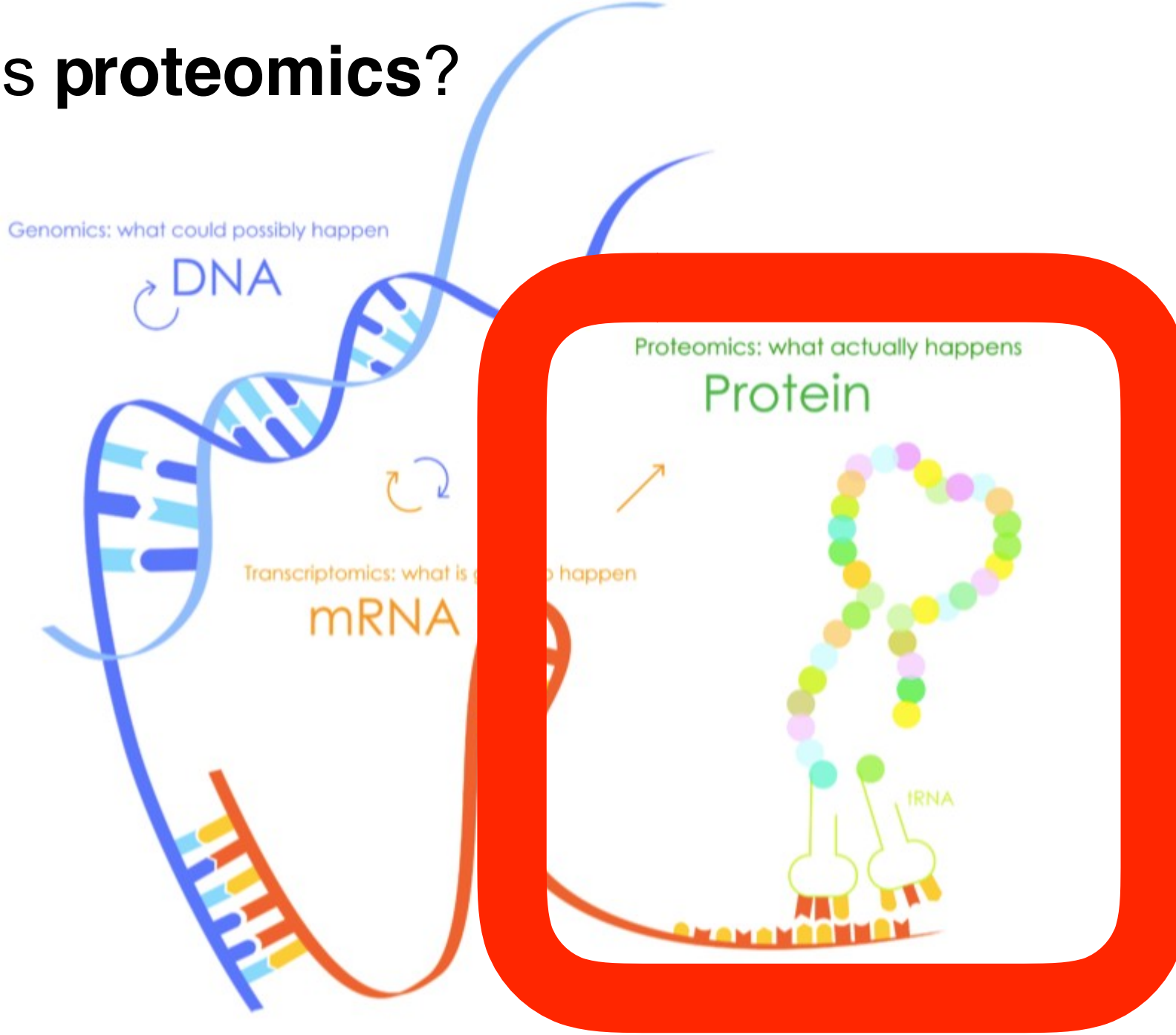

TMT/iTRAQ
Quantitative
Proteomics

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



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

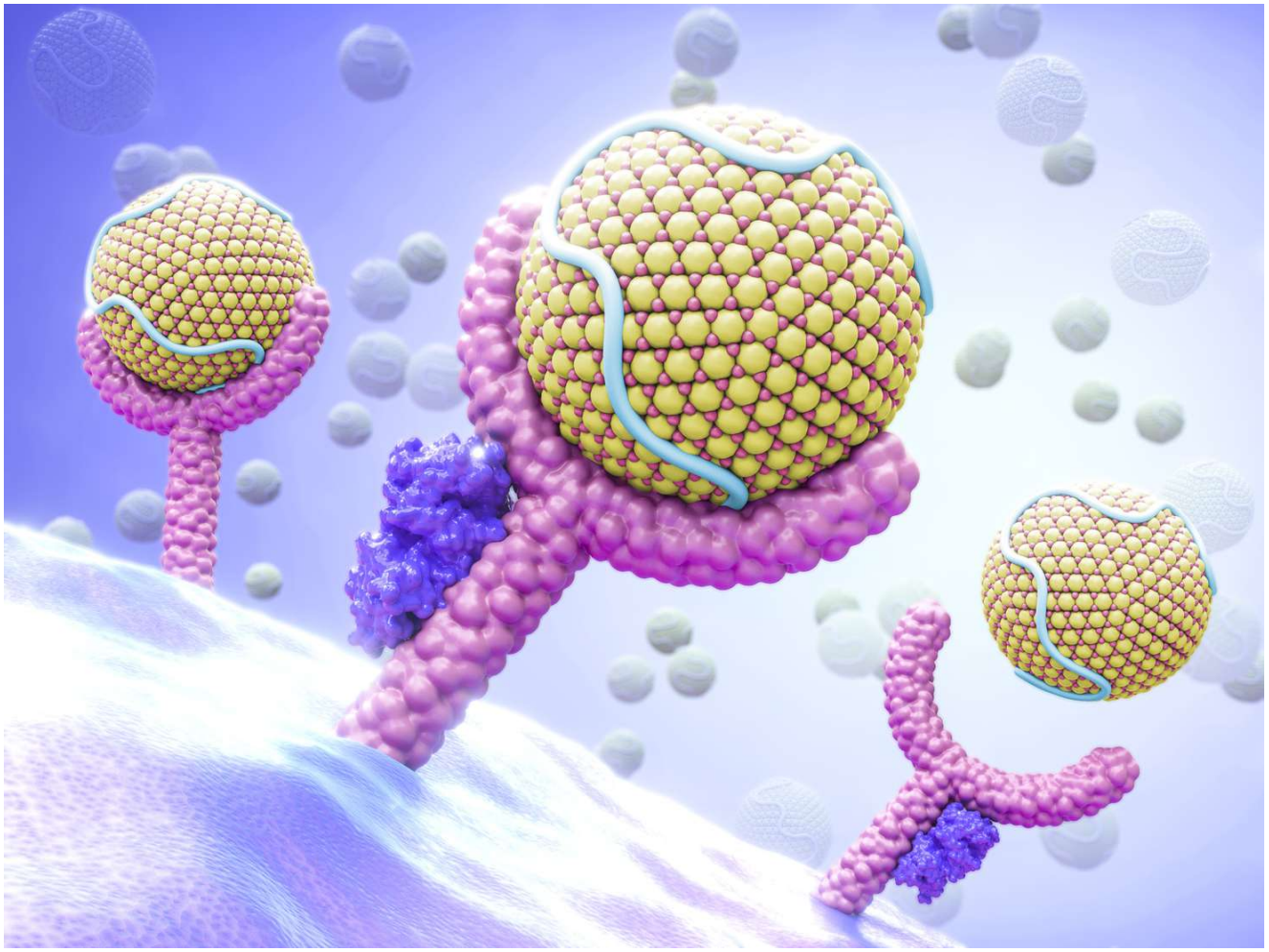
What is **quantitative proteomics**?



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.

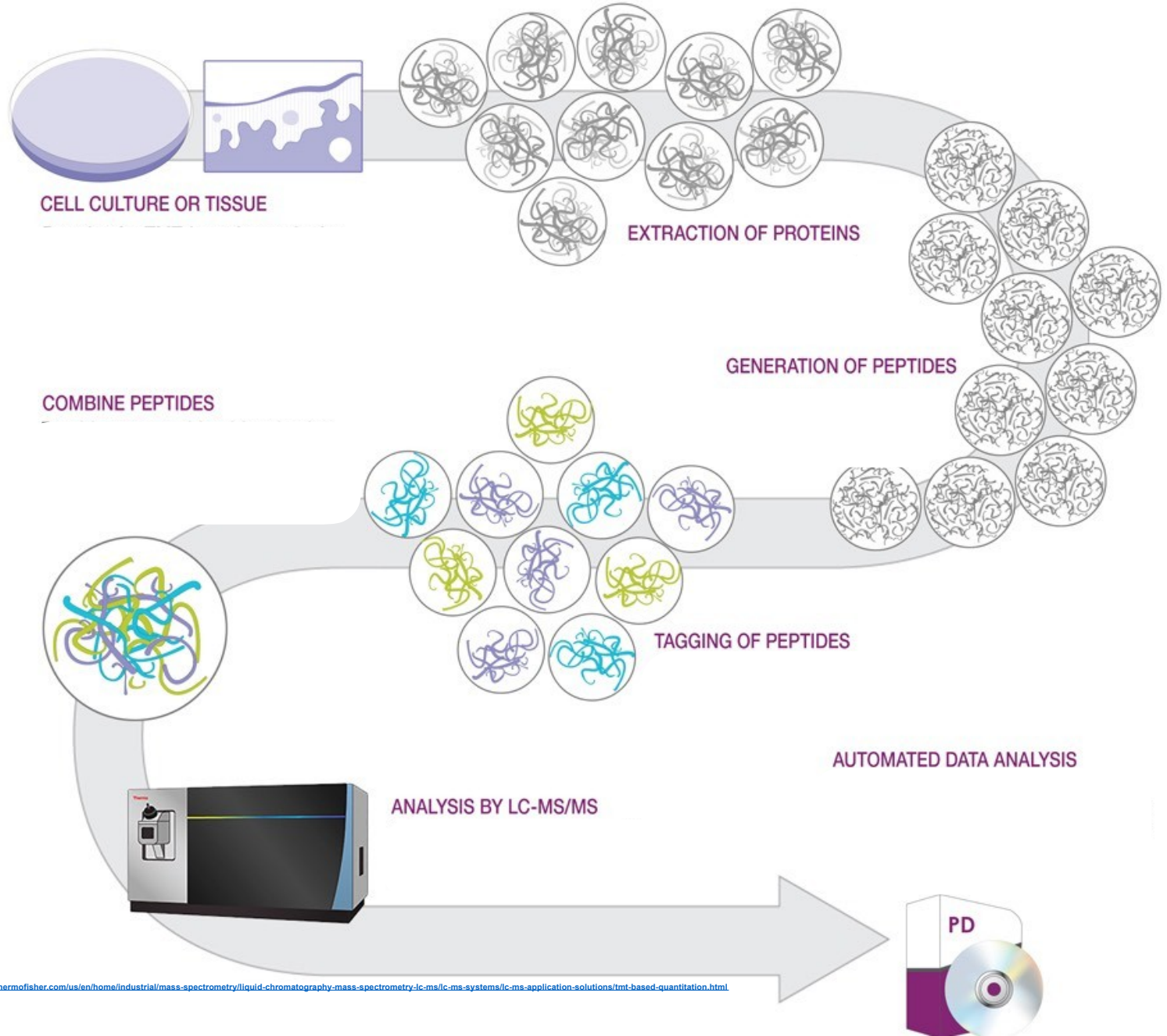
Why is quantitative proteomics important?



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

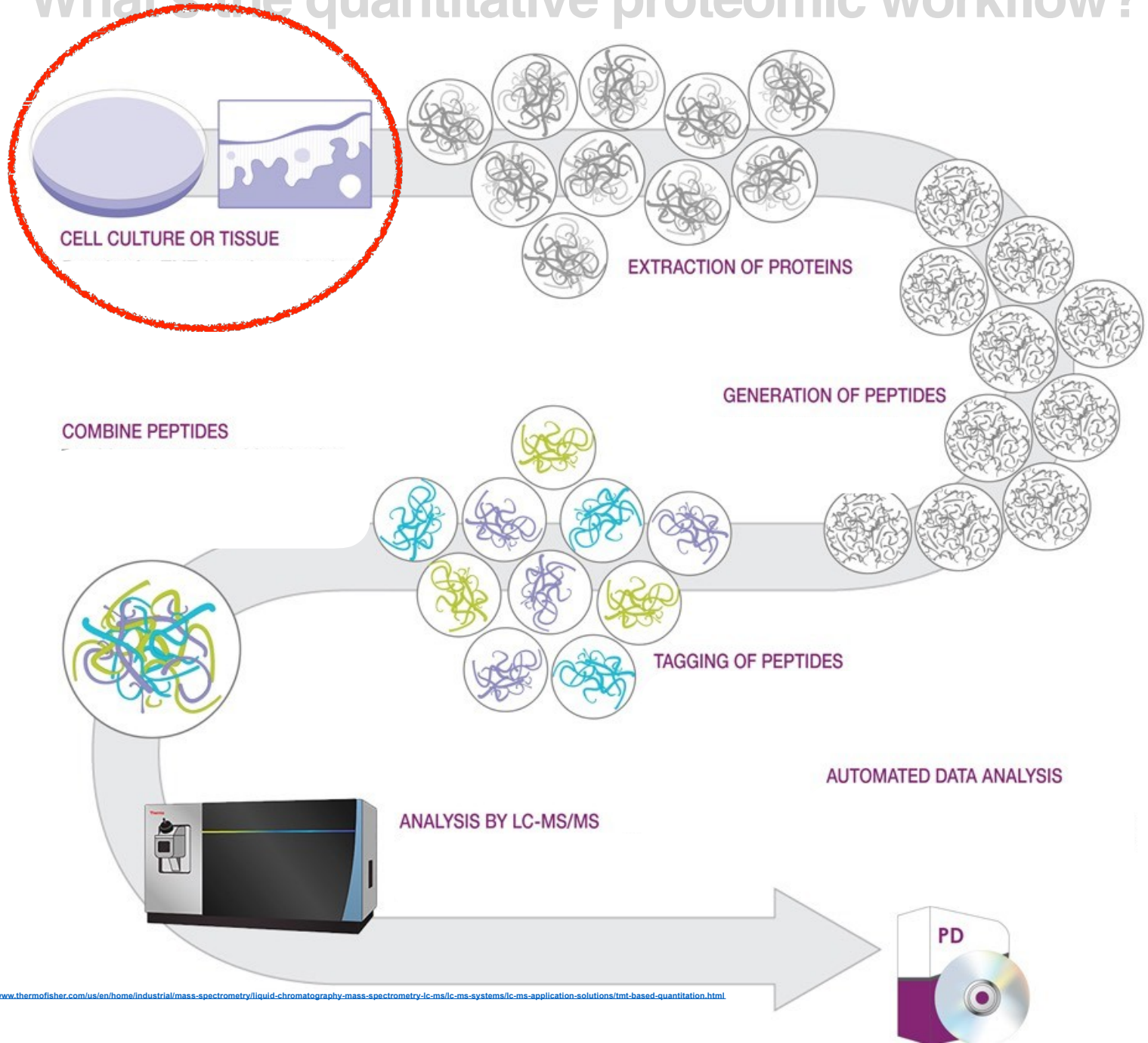
https://www.thoughtco.com/cell-membrane-373364

What's the quantitative proteomic workflow?



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?

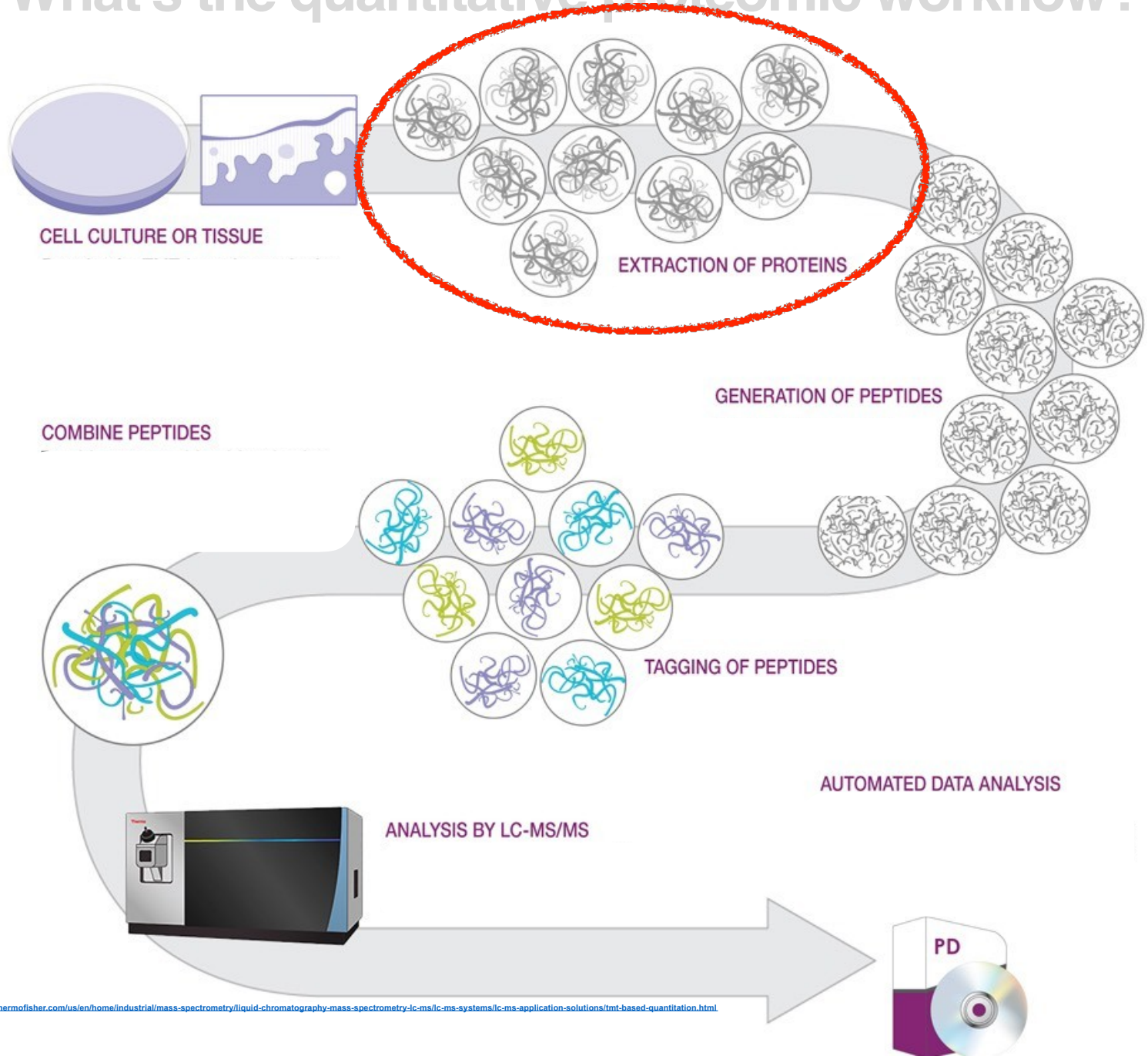


https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html



https://www.rdmag.com/article/2016/07/avoiding-contamination-cell-cultures

What's the quantitative proteomic workflow?

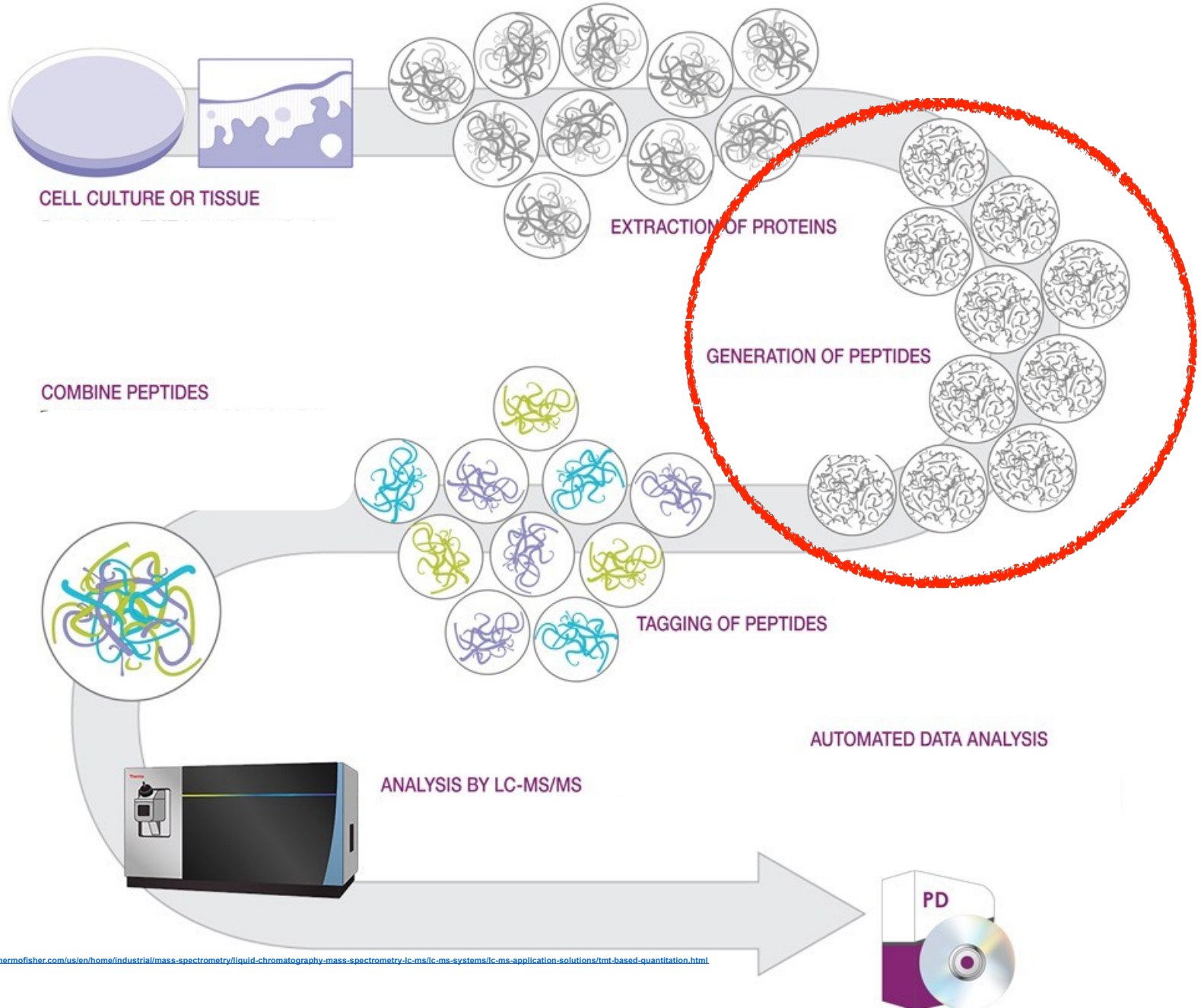


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http://www.geneaid.com/products/dna-rna-protein-extraction/dna-rna-protein-extraction-kit

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

Where does **trypsin** cut proteins?

Trypsin



Polypeptide

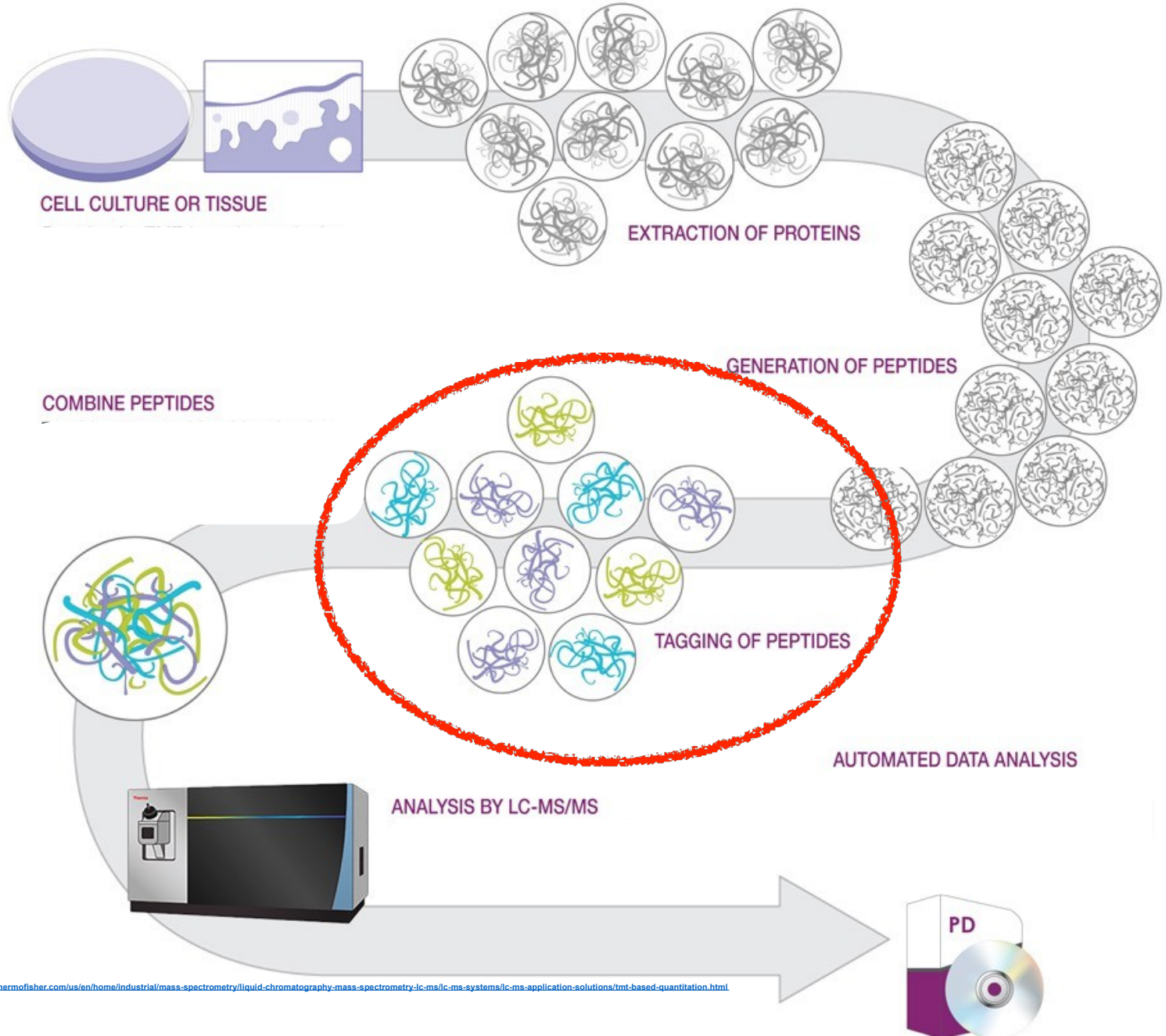
R = Arg and Lys

Polypeptide fragments

Trypsin is a serine protease

Worthington Biochemical Corporation

What's the quantitative proteomic workflow?



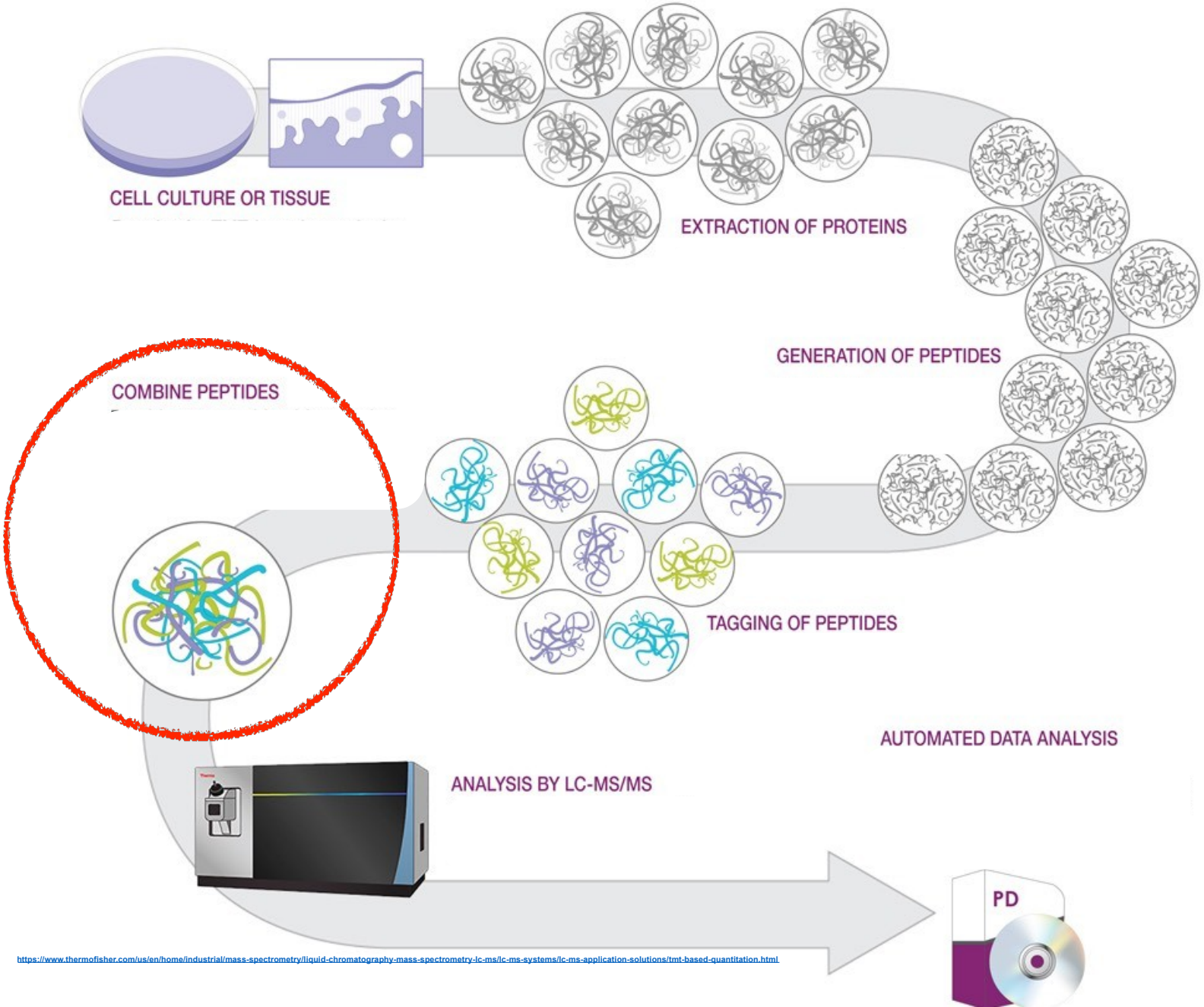
https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-application-solutions/tmt-based-quantitation.html

What might a tag look like?



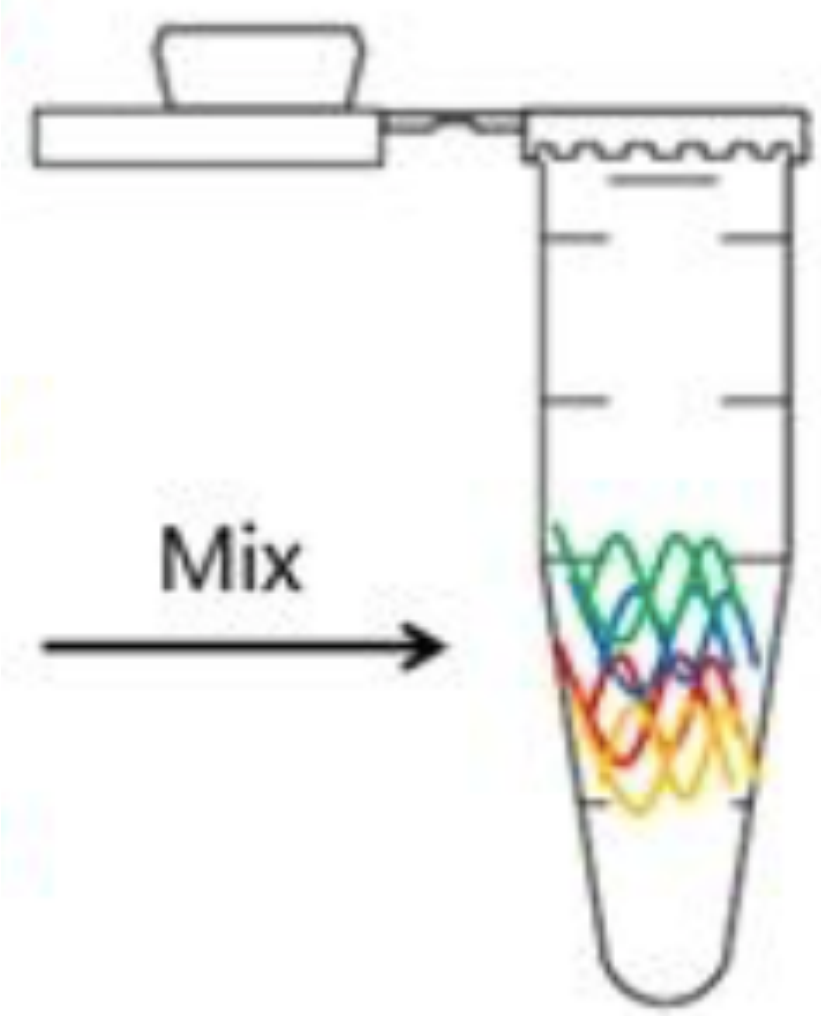
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What's the quantitative proteomic workflow?



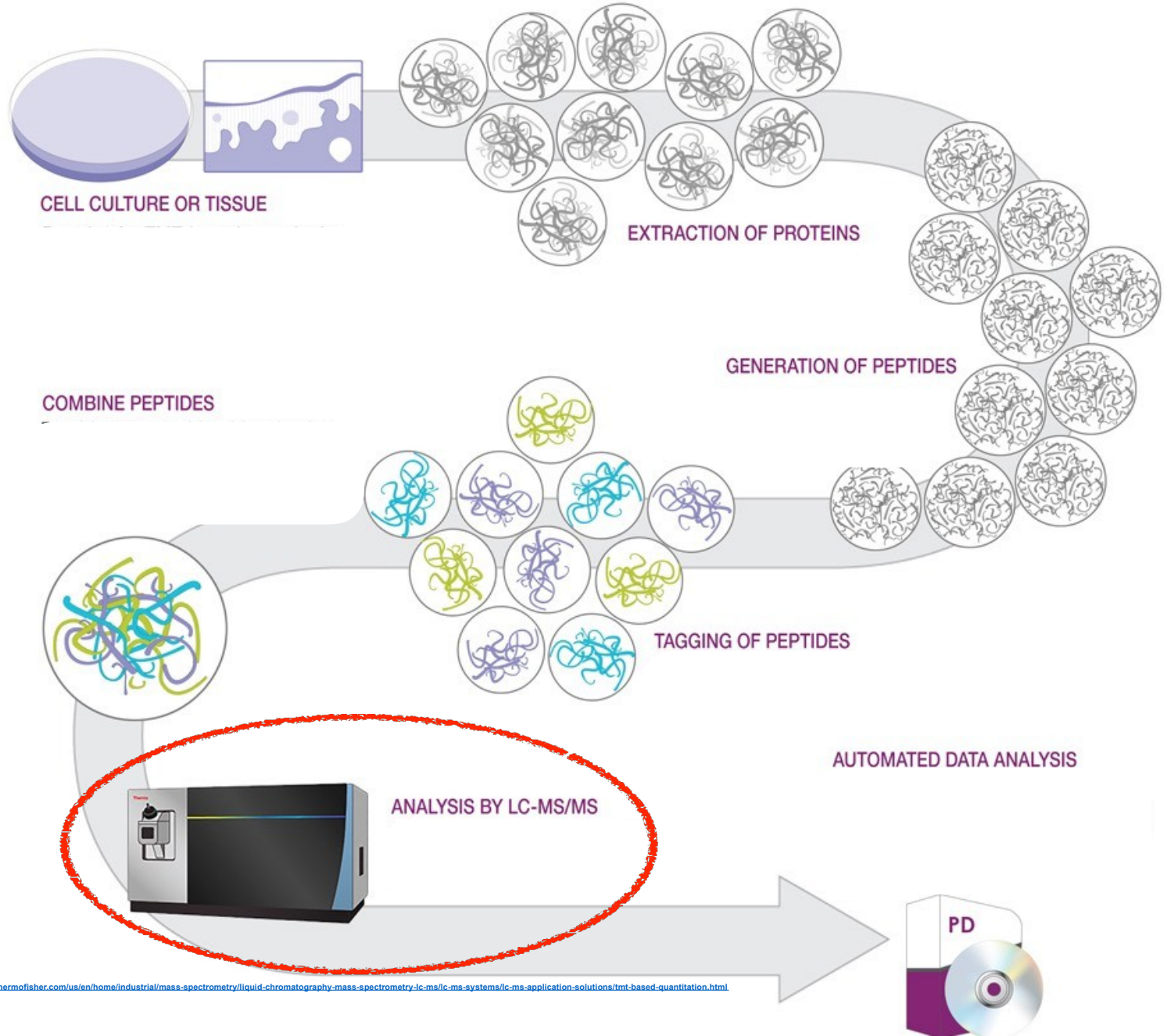
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How are tags mixed within a treatment sample?



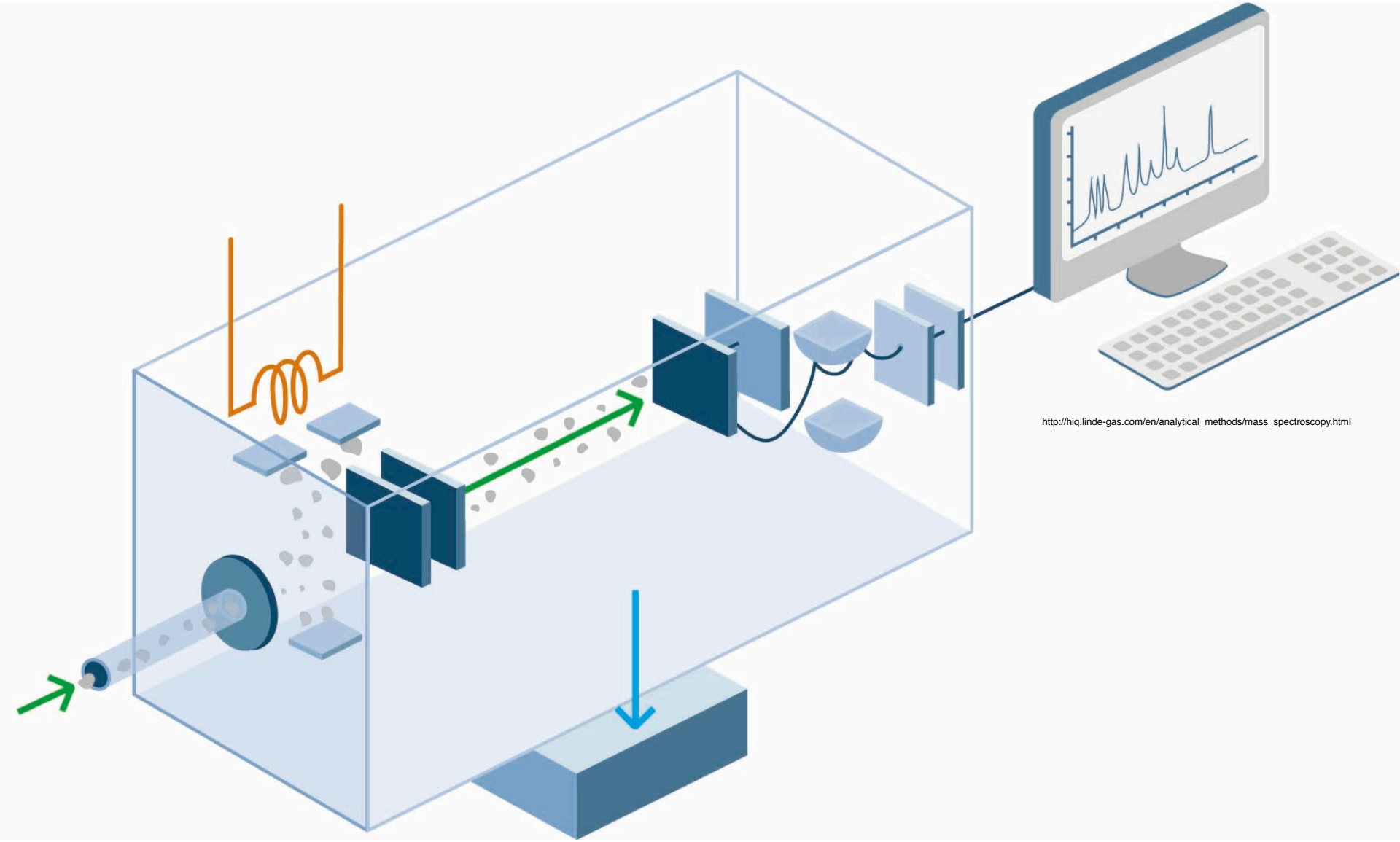
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What's the quantitative proteomic workflow?



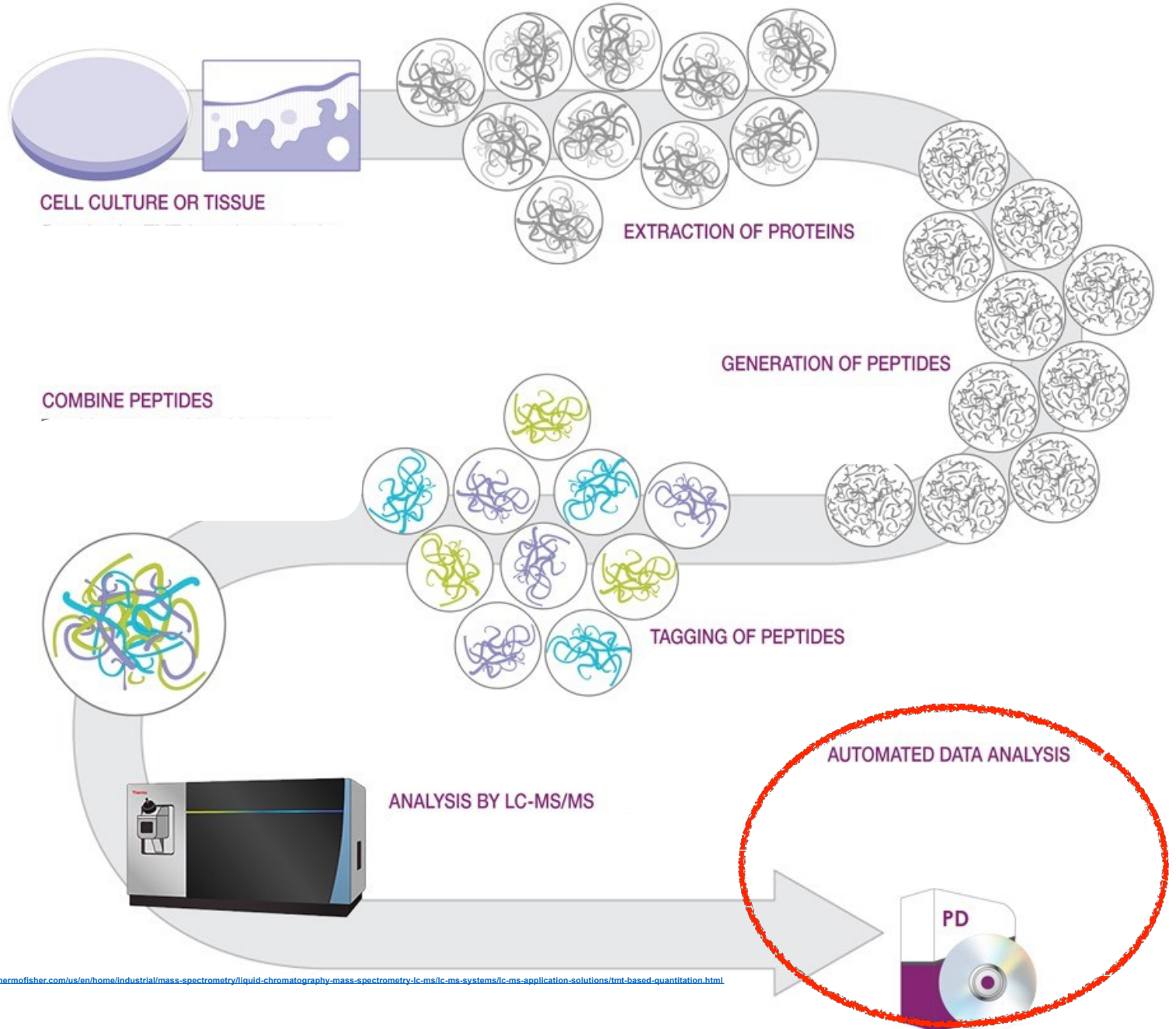
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Overview: Mass Spectrometry



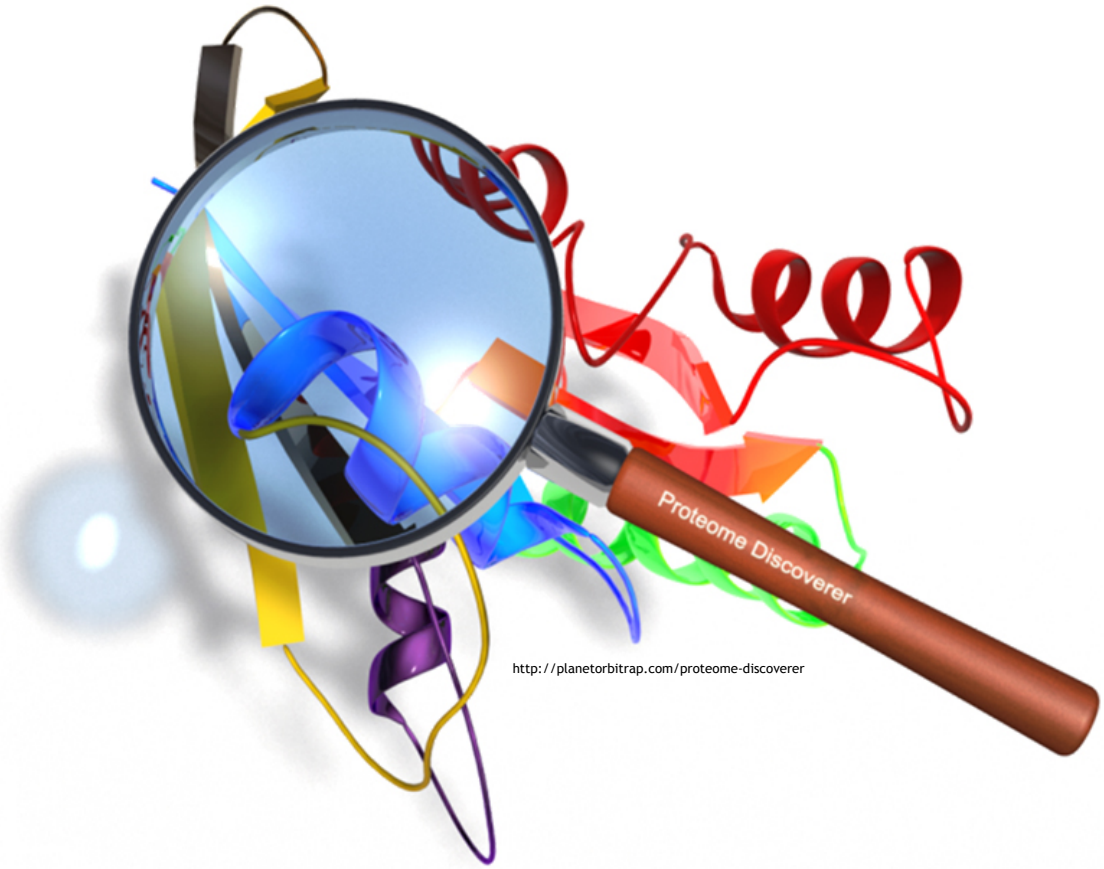
http://hiq.linde-gas.com/en/analytical_methods/mass_spectroscopy.html

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

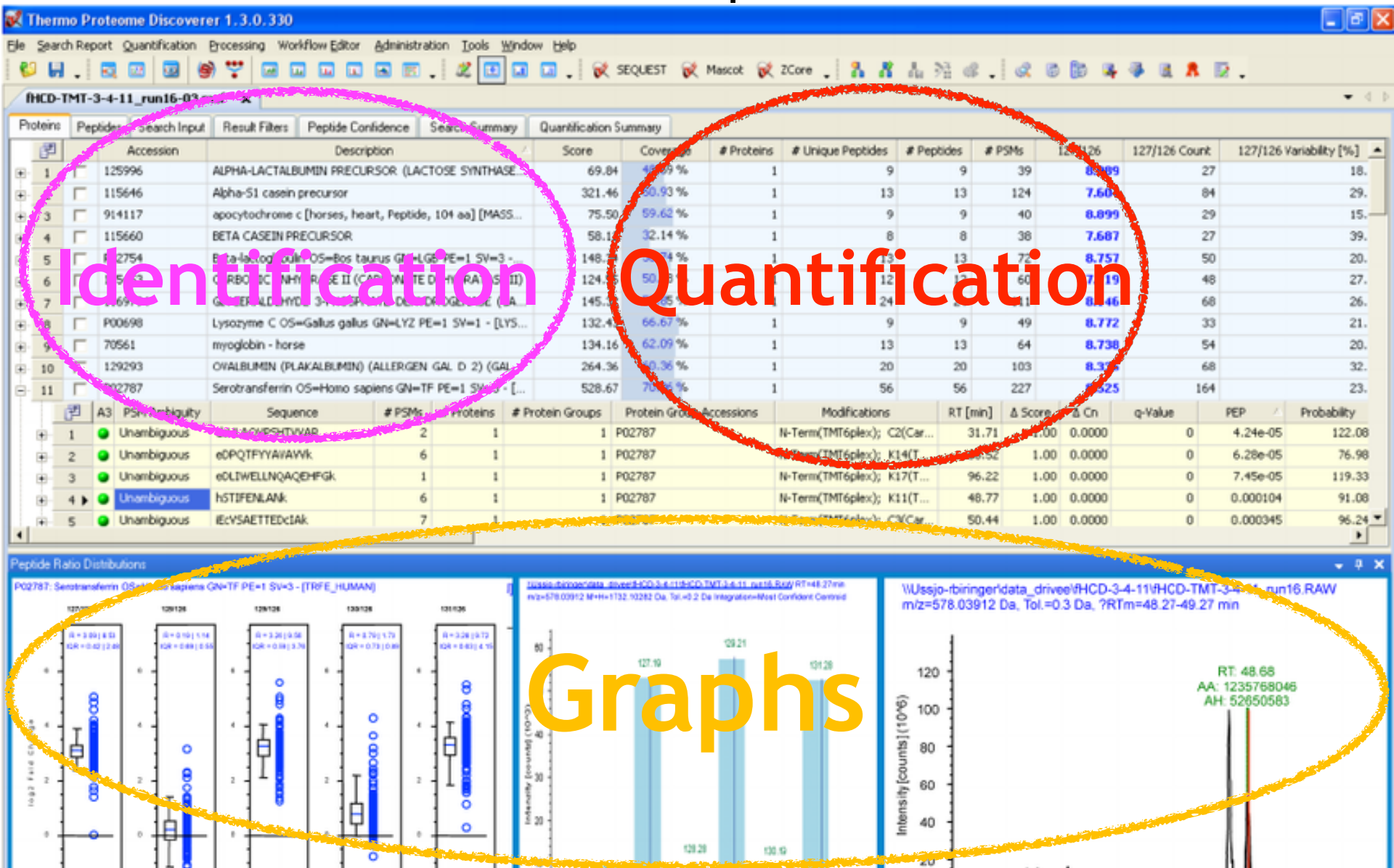
What are the automated data analysis programs?



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

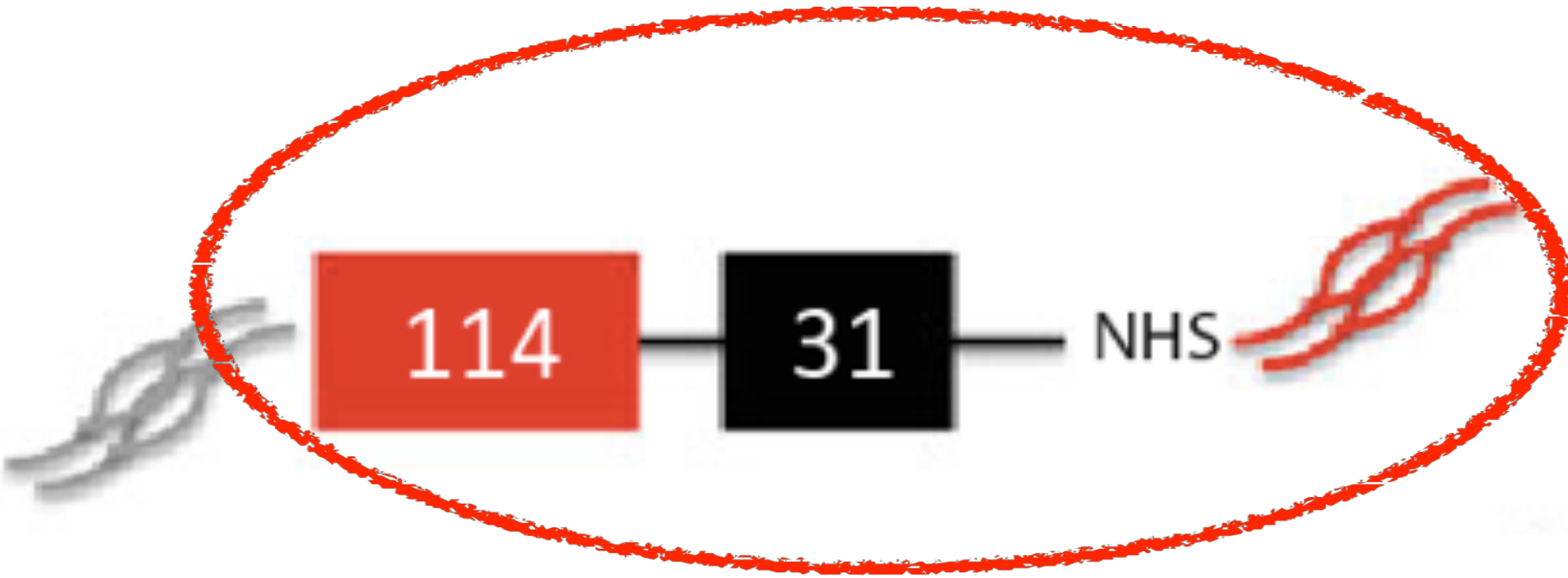
How does a Proteome Discoverer output look like?



What are two types of quantitative proteomics?



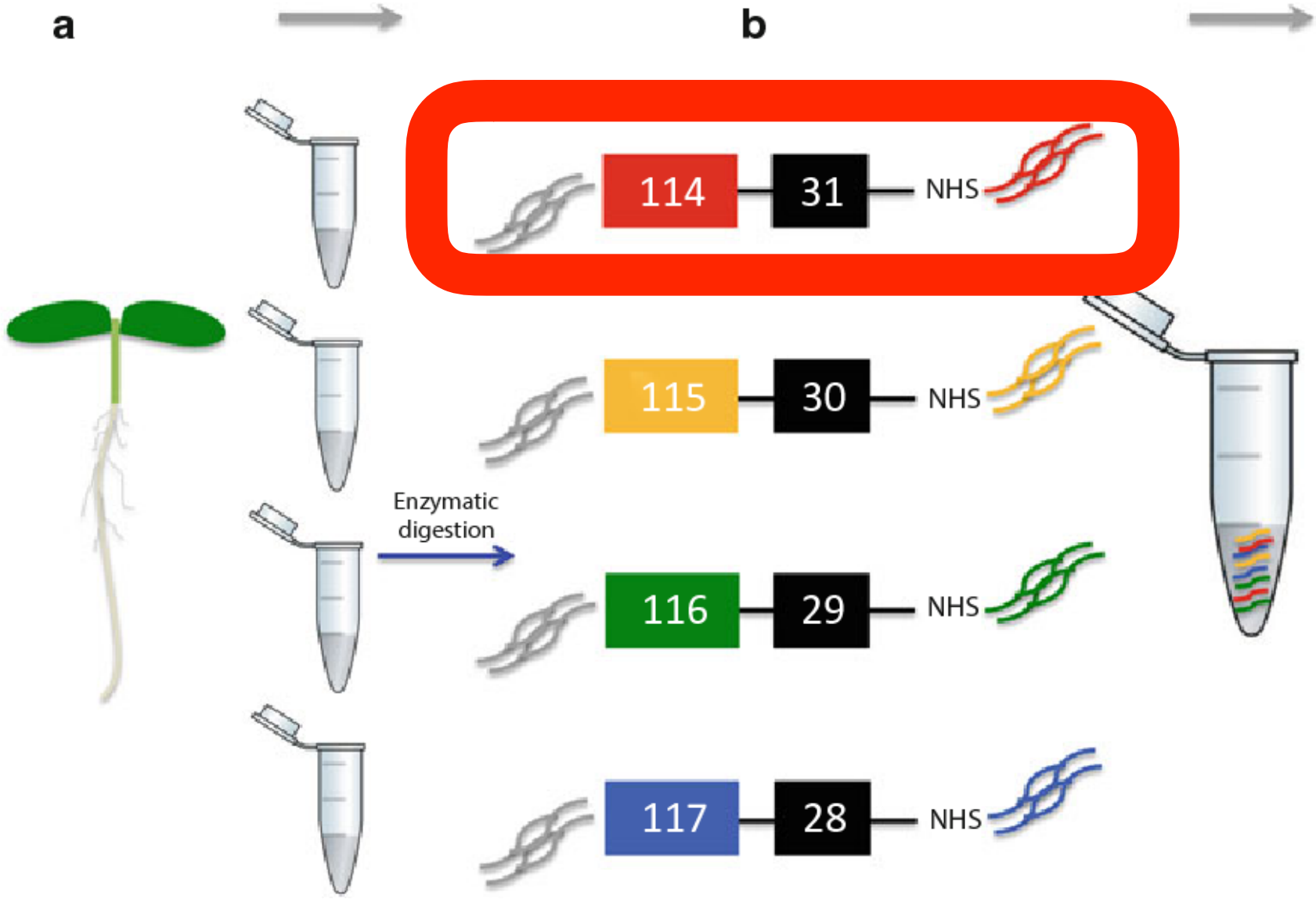
What makes a tagging reagent **isobaric**?



Chemical tag groups with identical masses

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

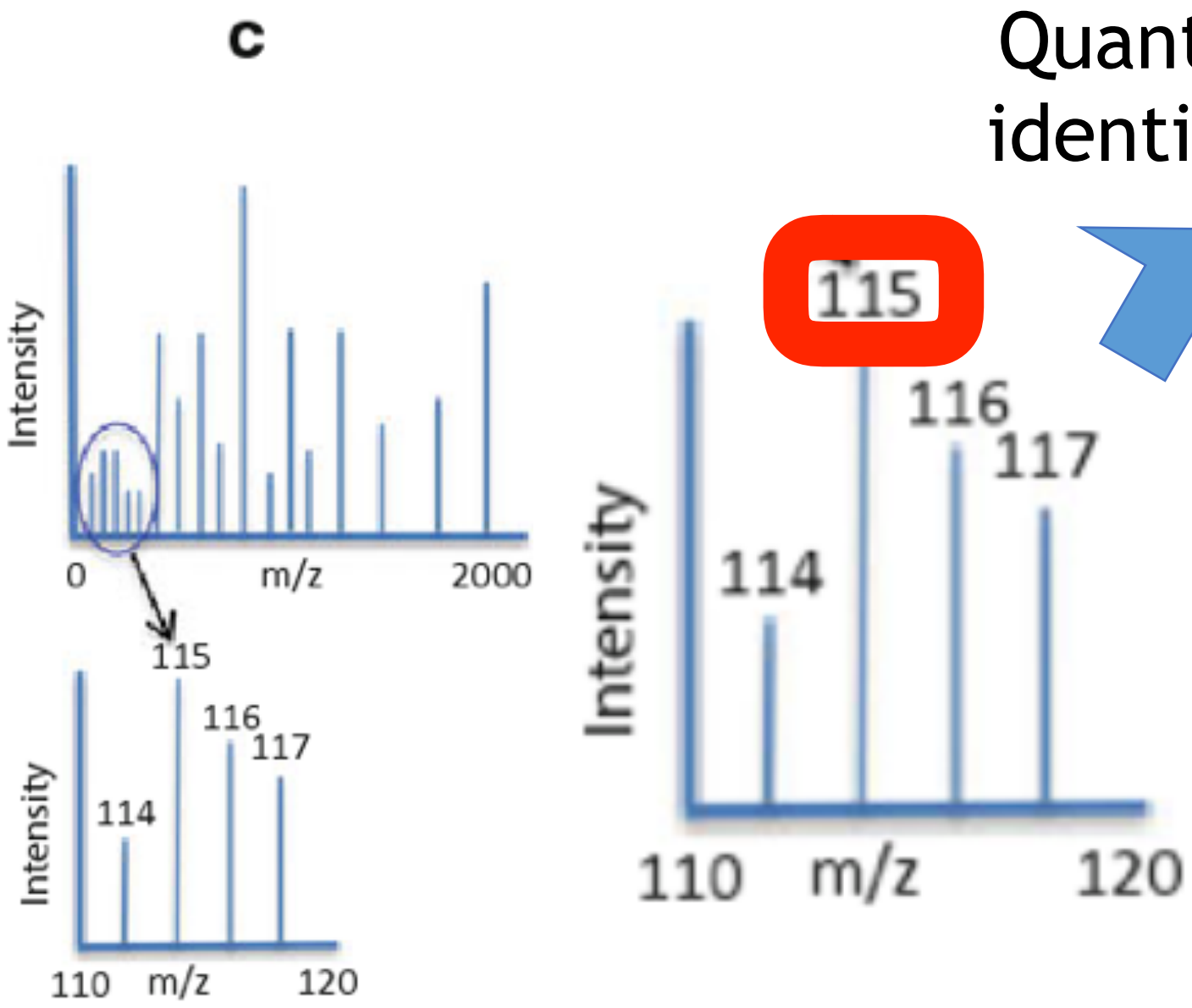
What is iTRAQ?



Isobaric tags for relative and absolute quantification

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

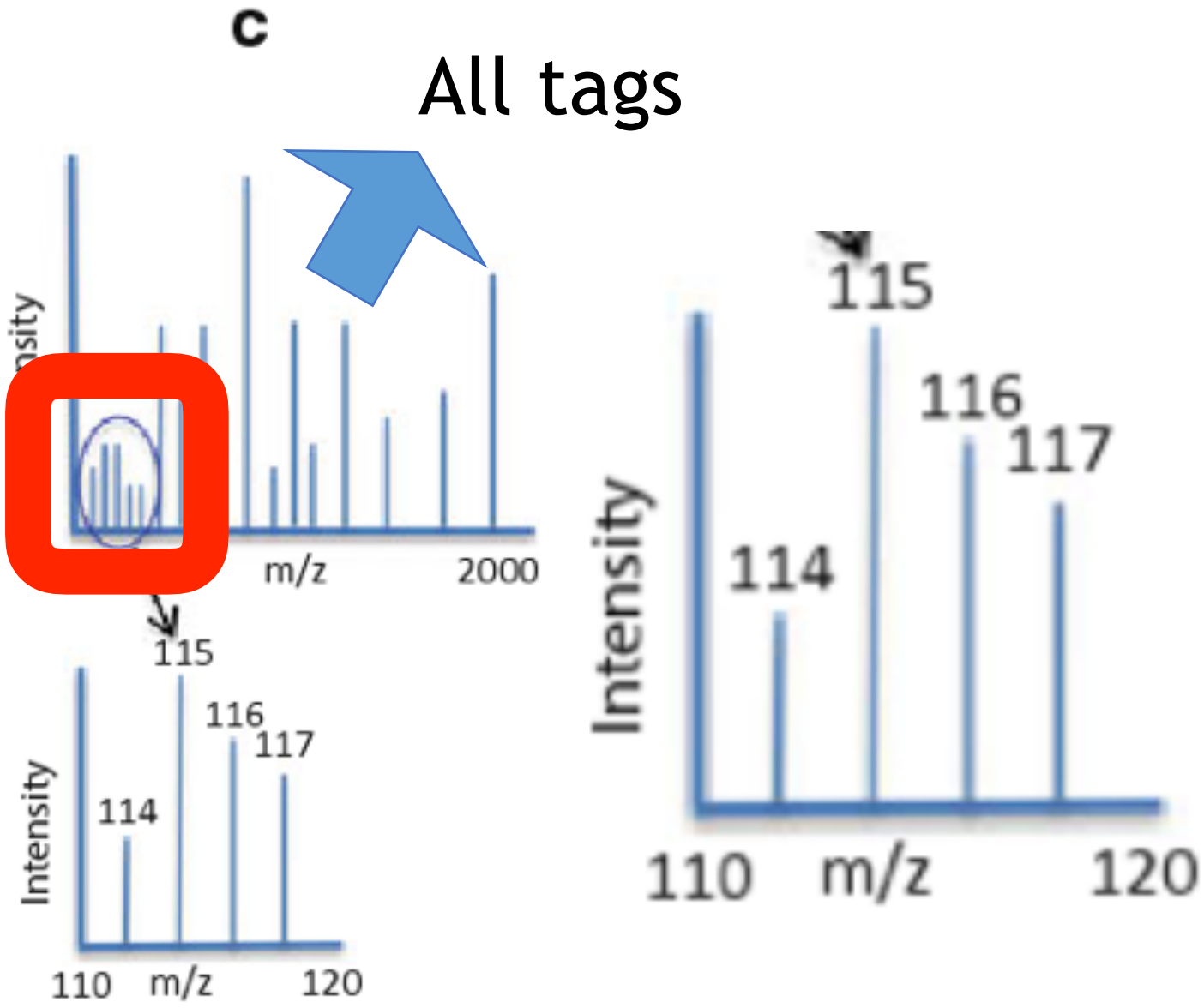
What is iTRAQ?



Quantification of identified protein

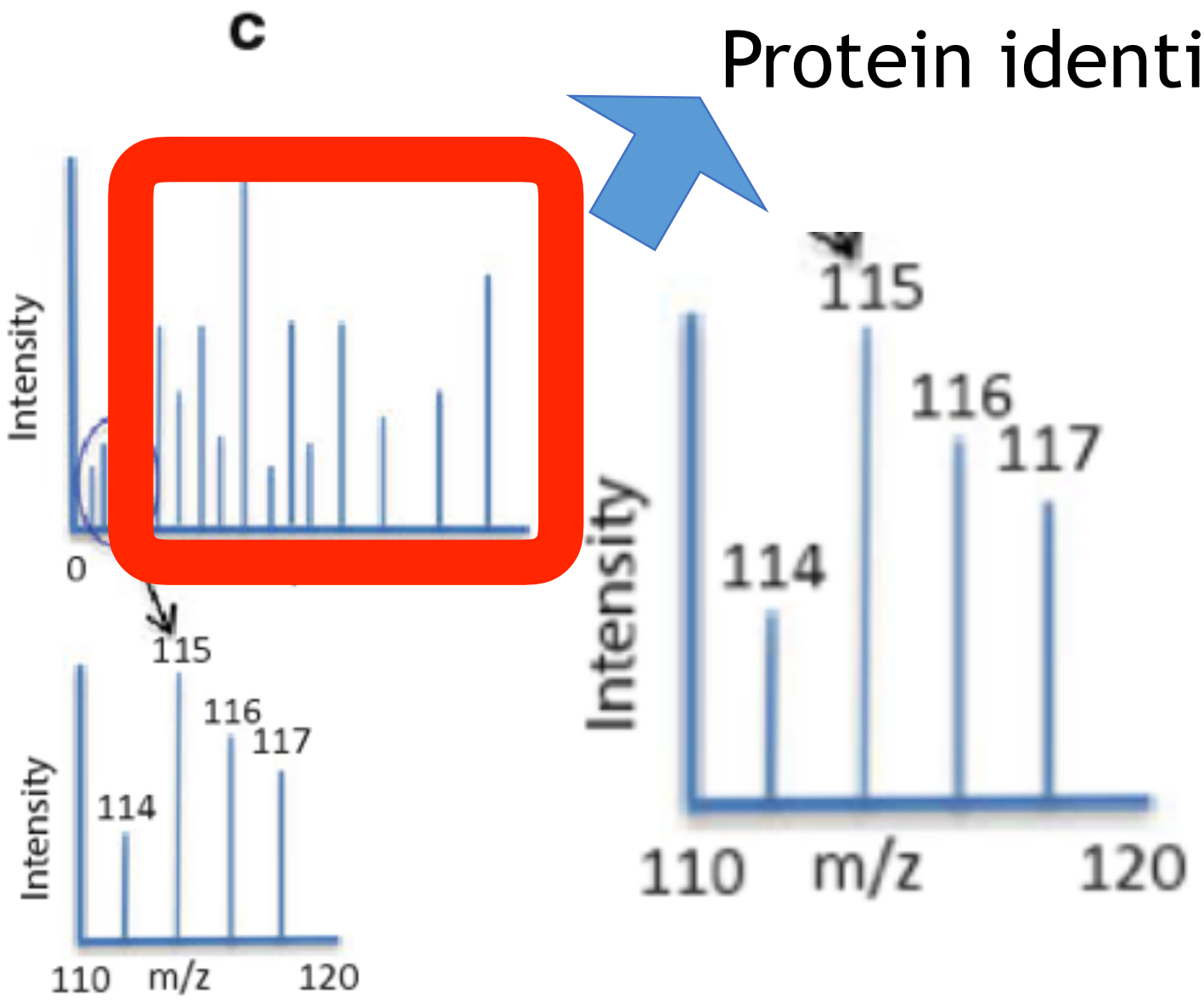
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What is iTRAQ?



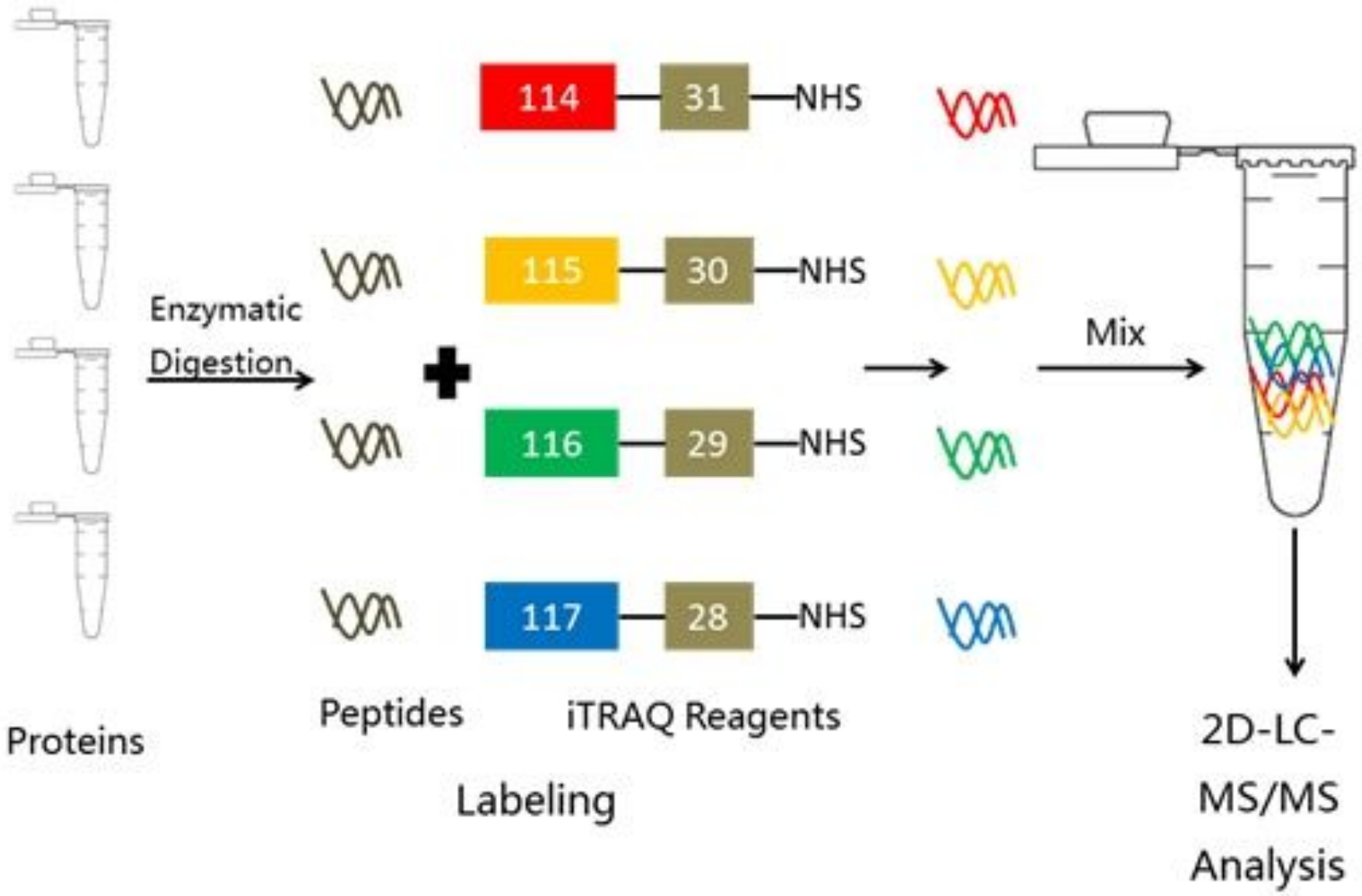
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What is iTRAQ?



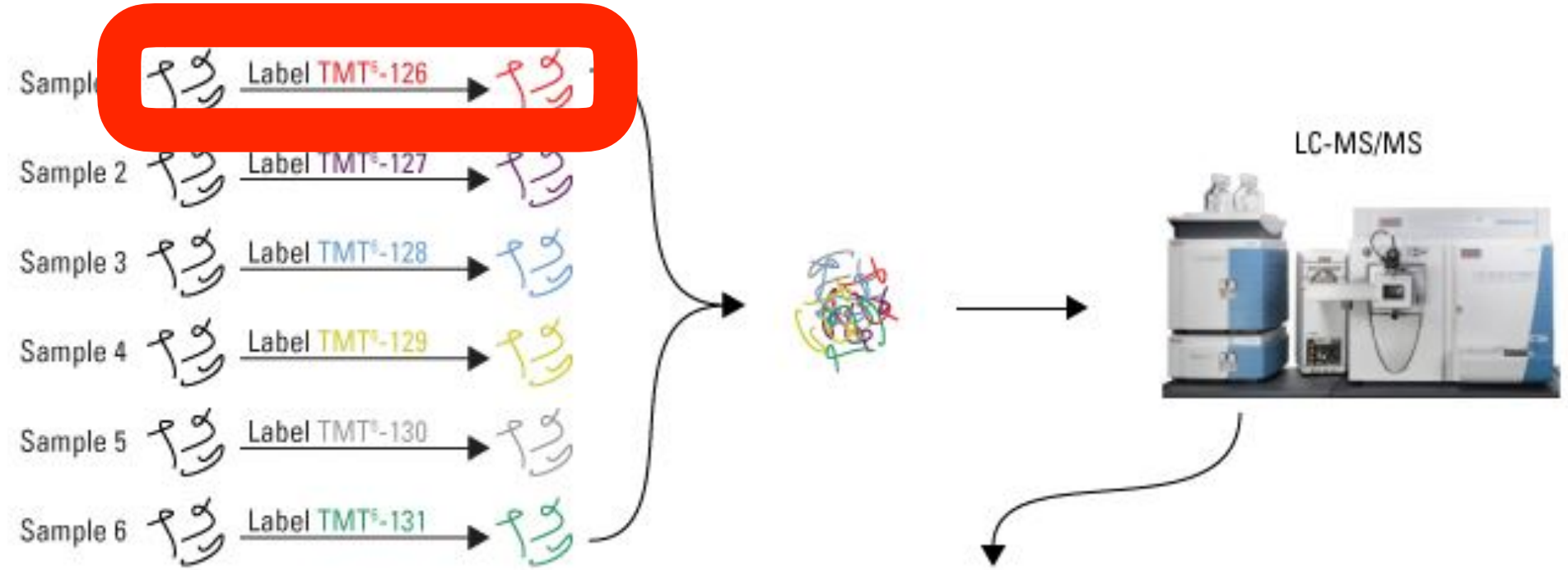
https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

What's the iTRAQ workflow?



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

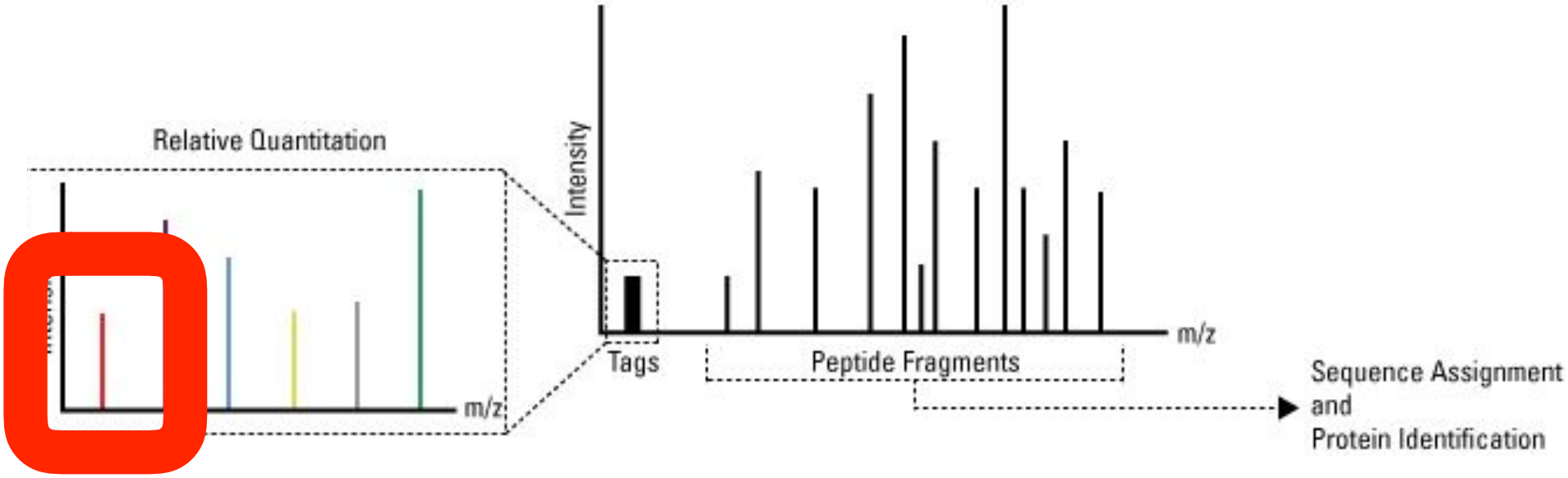
What is the TMT workflow?



Tandem mass tagging

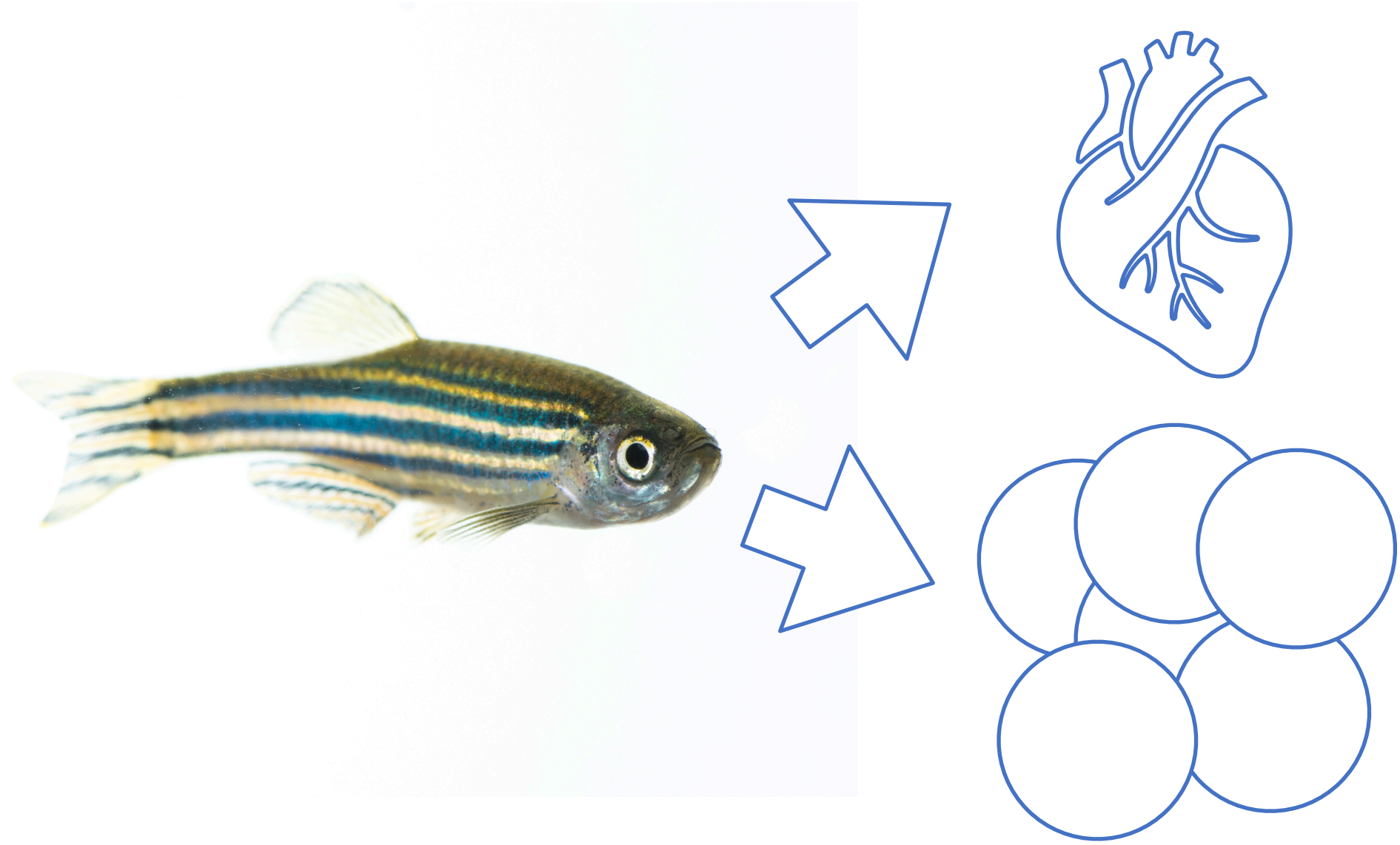
https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

What is the TMT workflow?



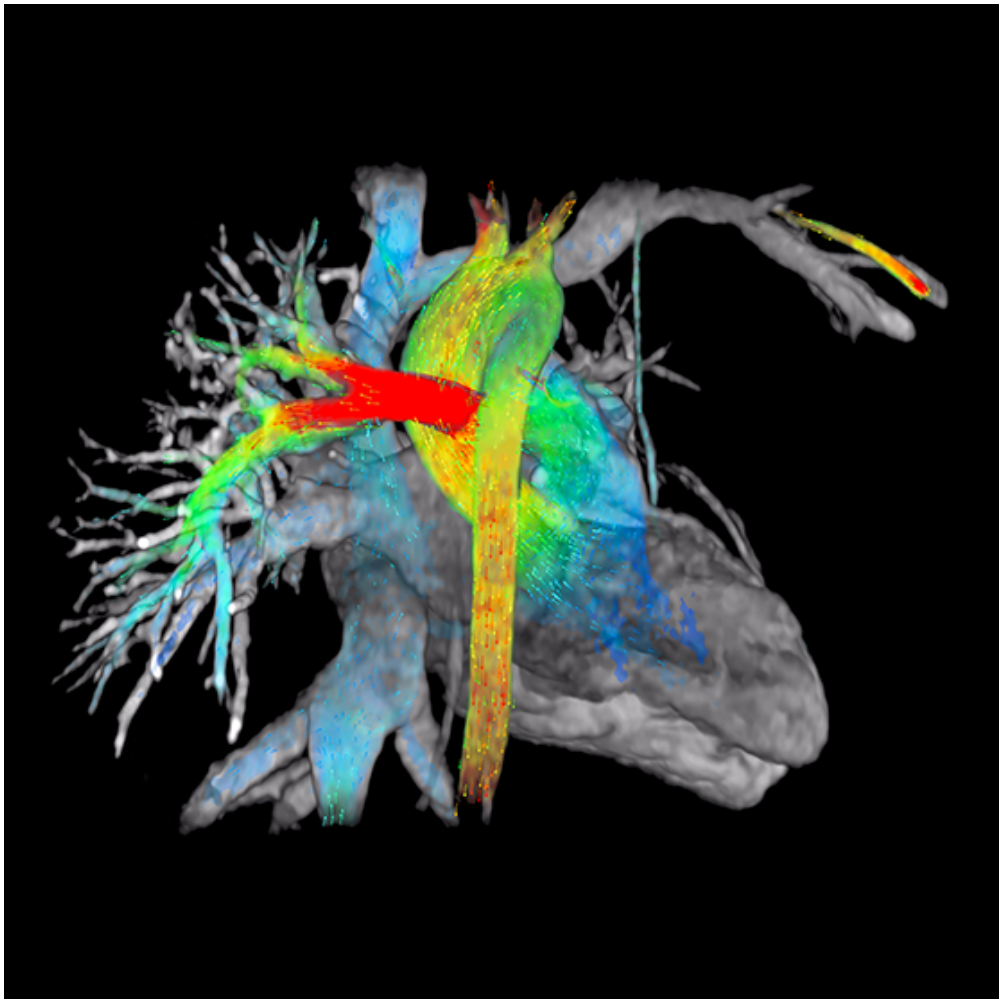
https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0112482.g001

How can you use quantitative proteomics in your project?



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

How can you use quantitative proteomics in your project?



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

Protein Localization

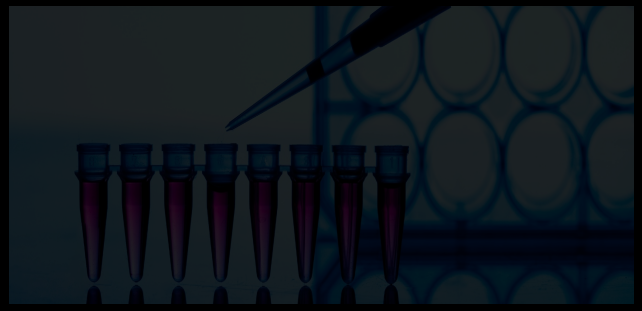
Protein Quantification

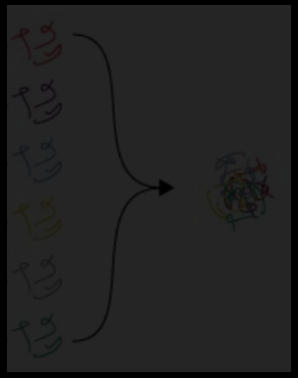
Protein Identification

What are the take-home points?

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







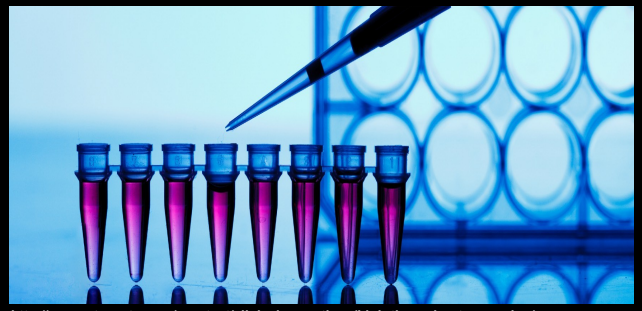
Proteomics is the study of all proteins, the drivers of cellular and molecular work.

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

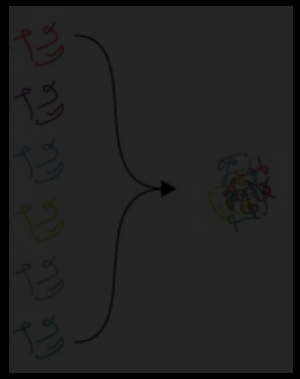
iTRAQ/TMT Quantitative Proteomics utilize **isobaric tagging reagents** in order to identify and quantify protein in a cost-effective manner.

What are the take-home points?





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



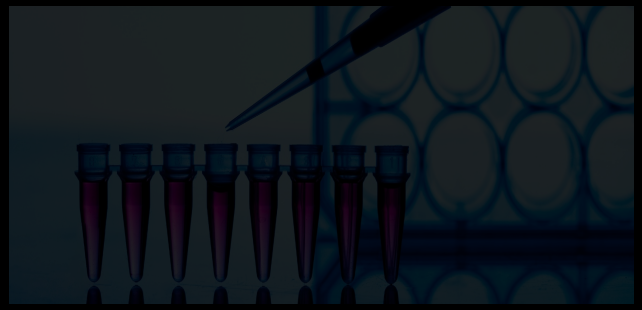
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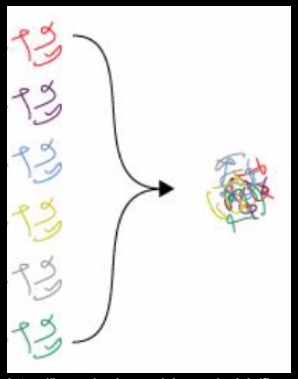
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Proteomics is the study of all proteins, the drivers of cellular and molecular work.

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

SCIENTIFIC REPORTS

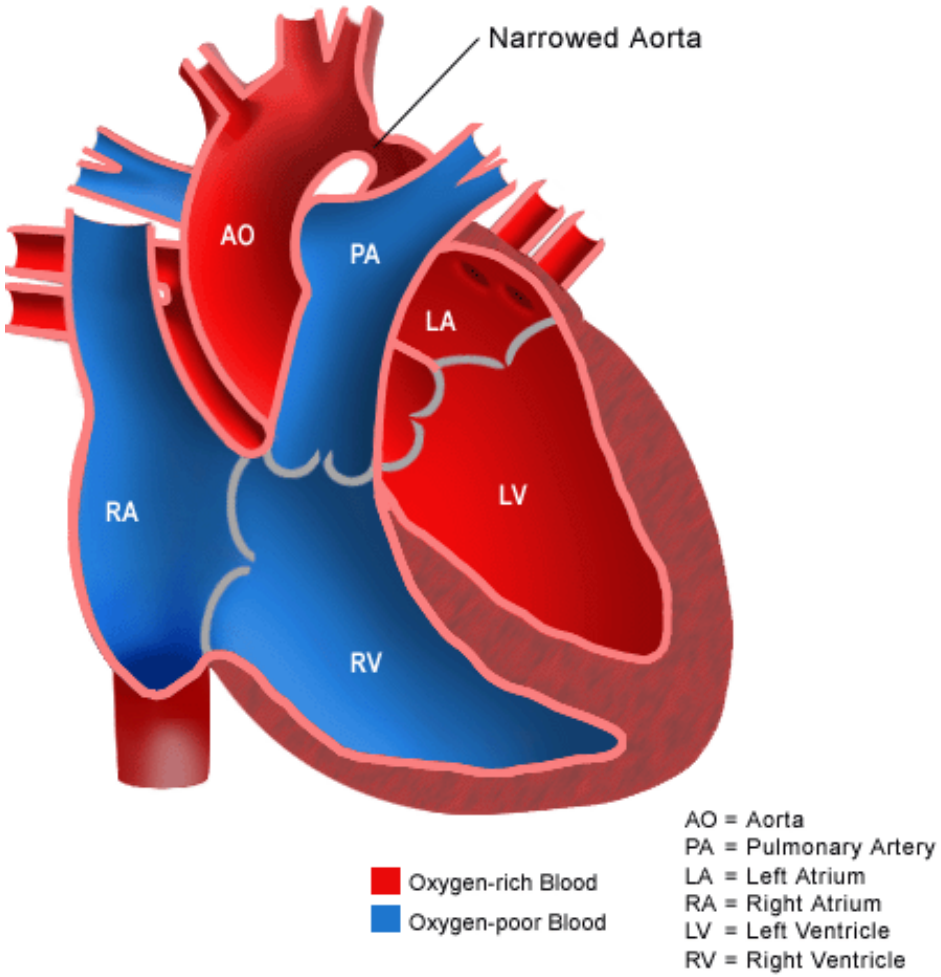
Article | OPEN | Published: 22 June 2017

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

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

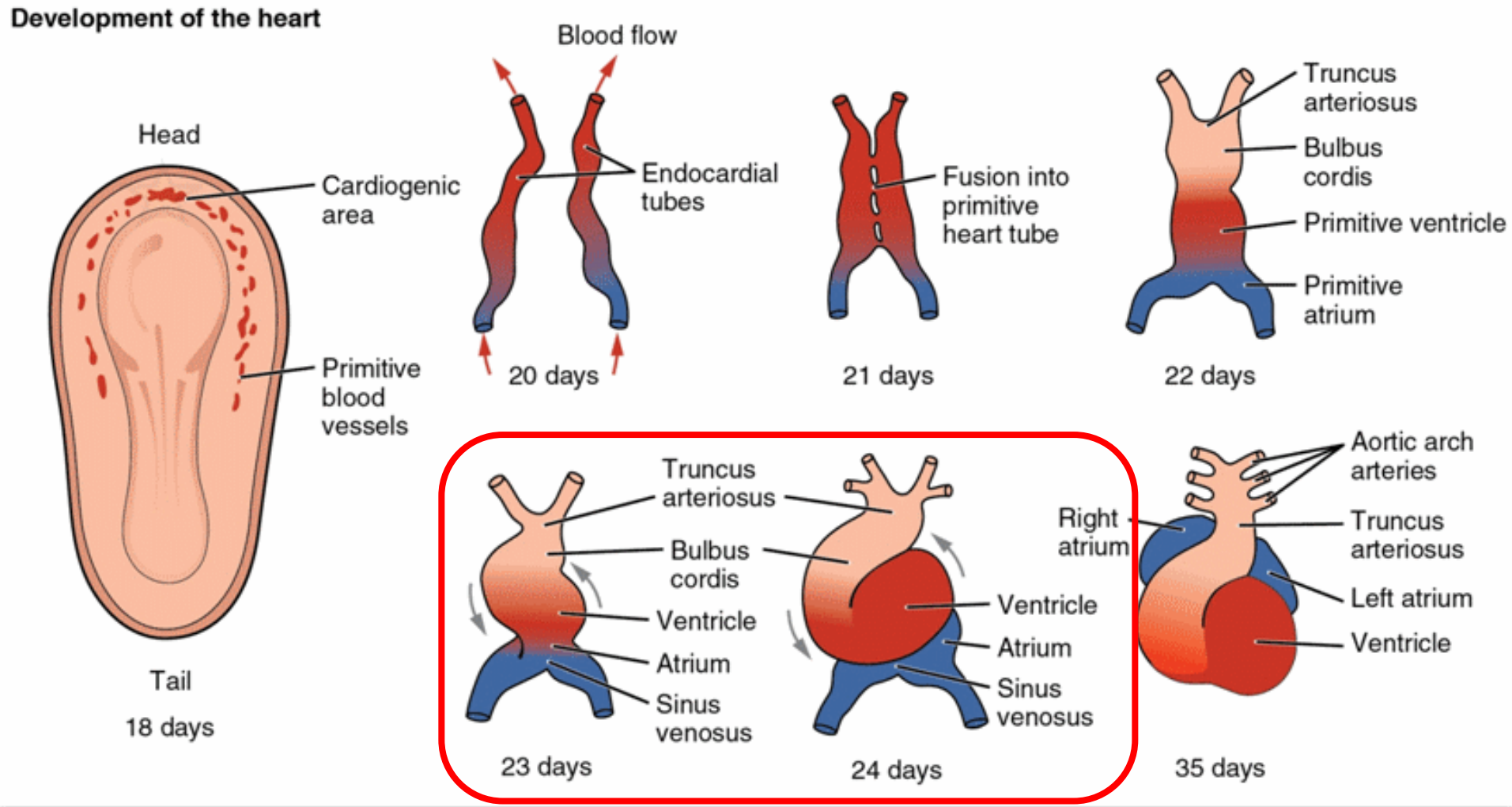
Scientific Reports **7**, Article number: 4022 (2017) | Download Citation

Why study congenital heart disease?



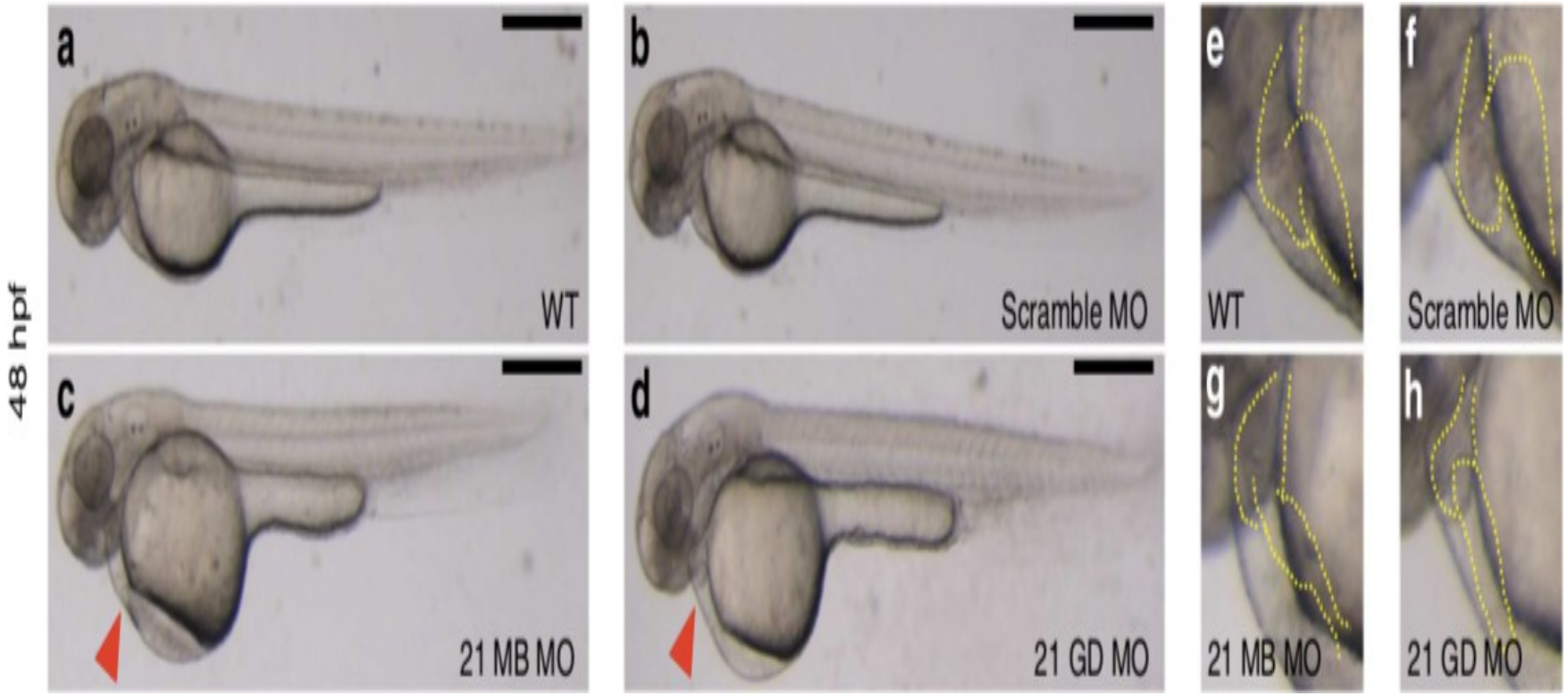
Congenital Heart Disease is the most common form of birth defect; specifically, heart valve defects affect 1% of Americans

What is valvulogenesis?



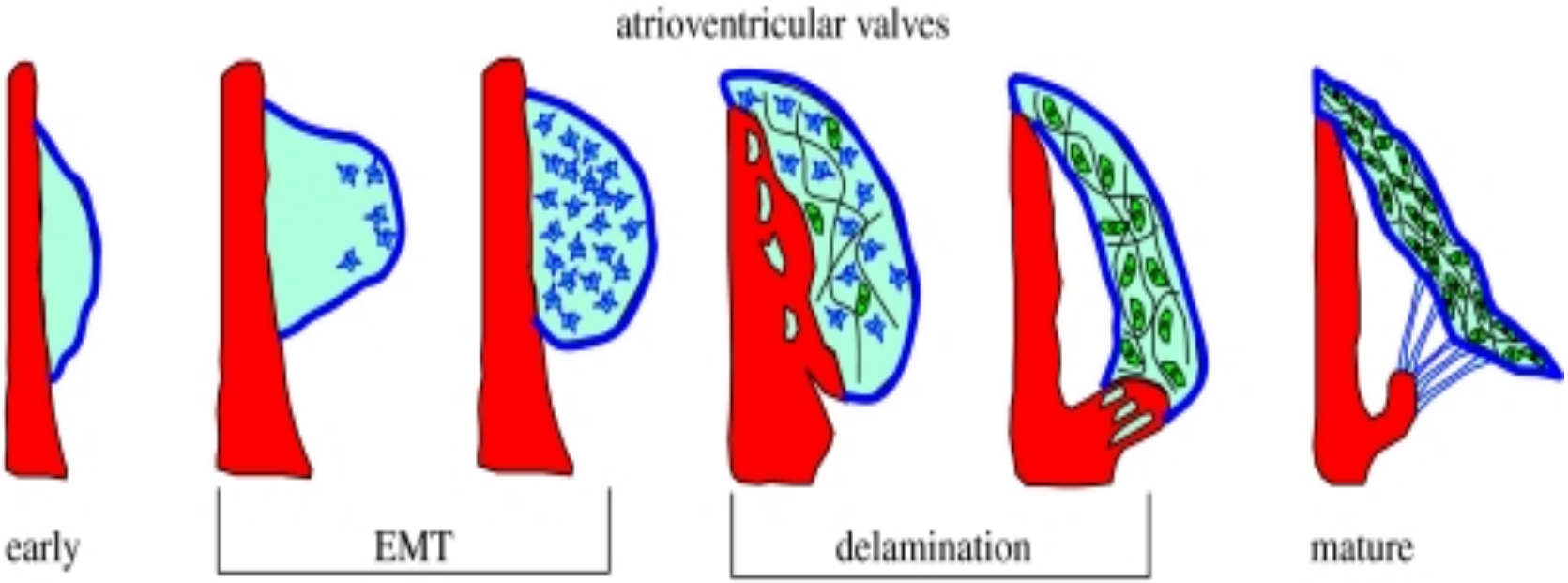
Valvulogenesis is the process in which the valves of the heart are developed and coincides with the looping of the heart

What is a good model organism to study valvulogenesis?



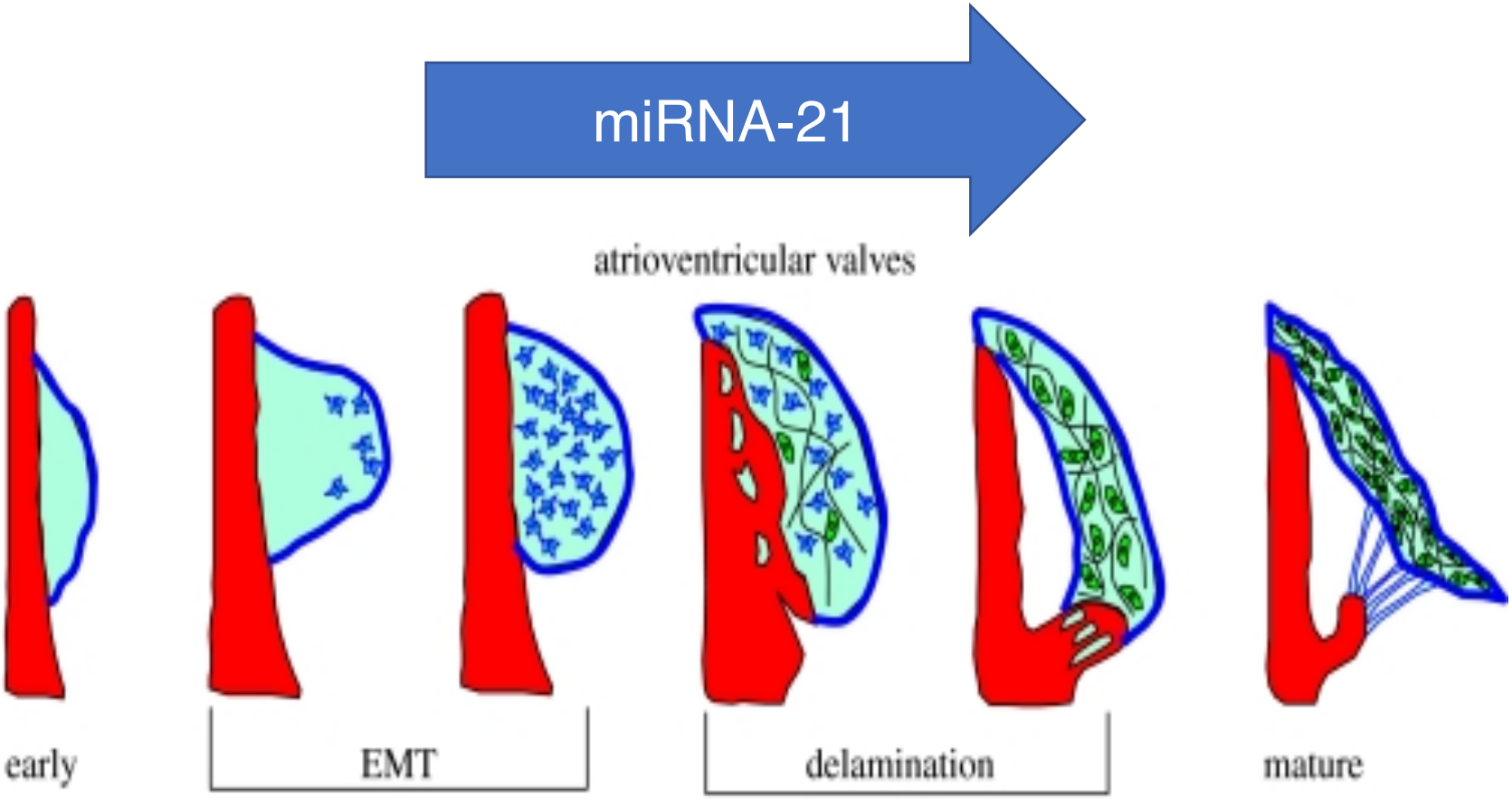
What is an important regulator of valvulogenesis?

Cell Migration and Proliferation



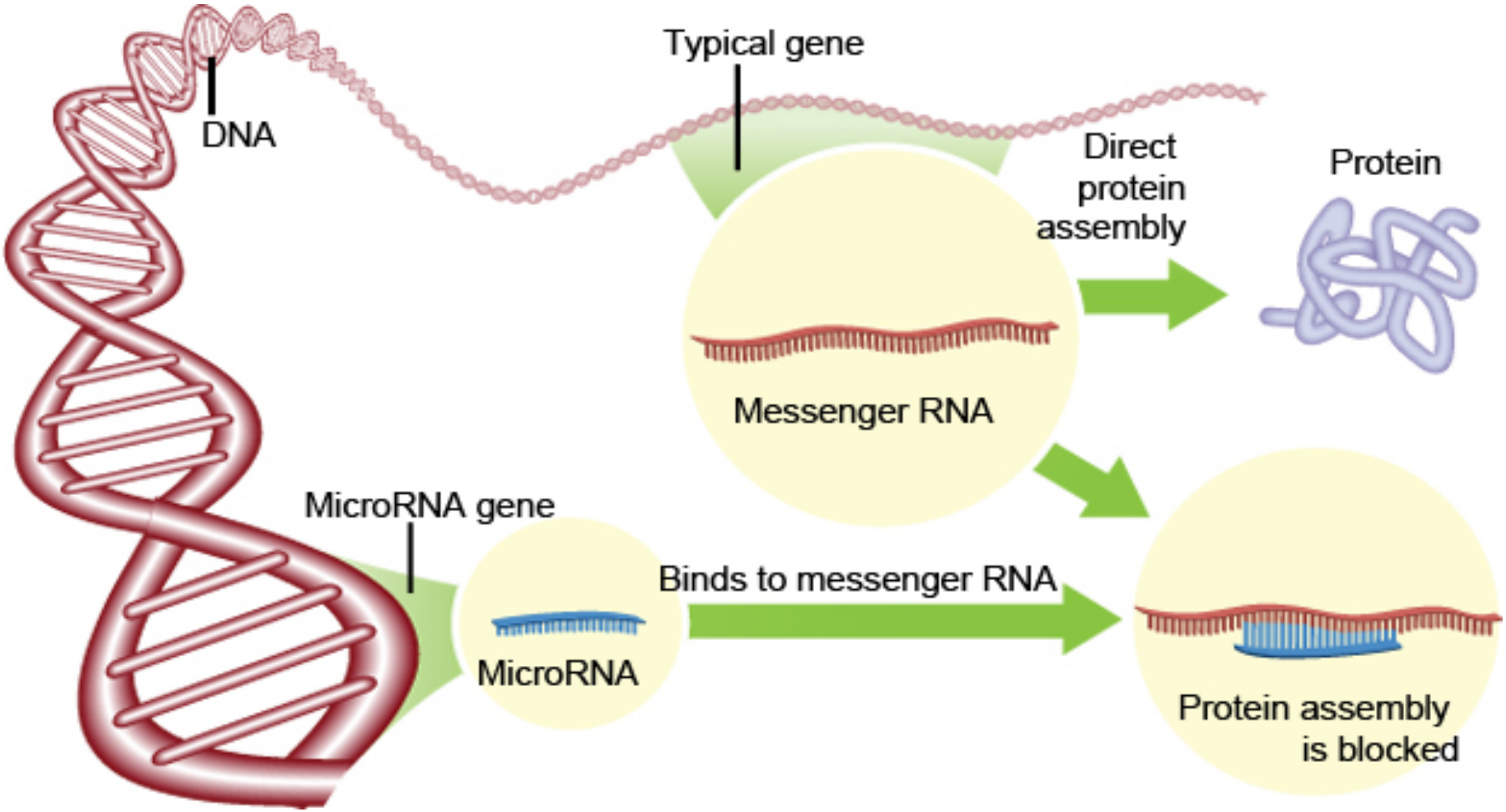
During heart looping cells must proliferate and migrate into the cardiac jelly to remodel and constrict the atrioventricular valve ring

What is an important regulator of valvulogenesis?



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

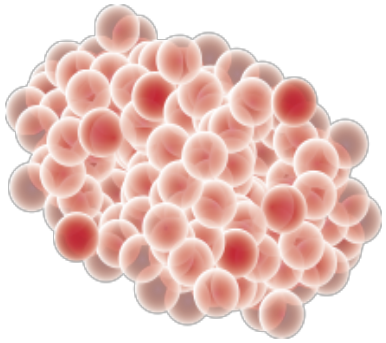
How does miR-21 function?

Expressed In:

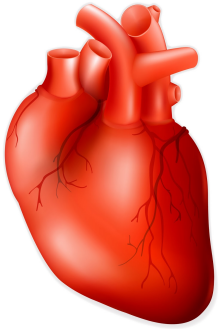


Brain

+



Cancer



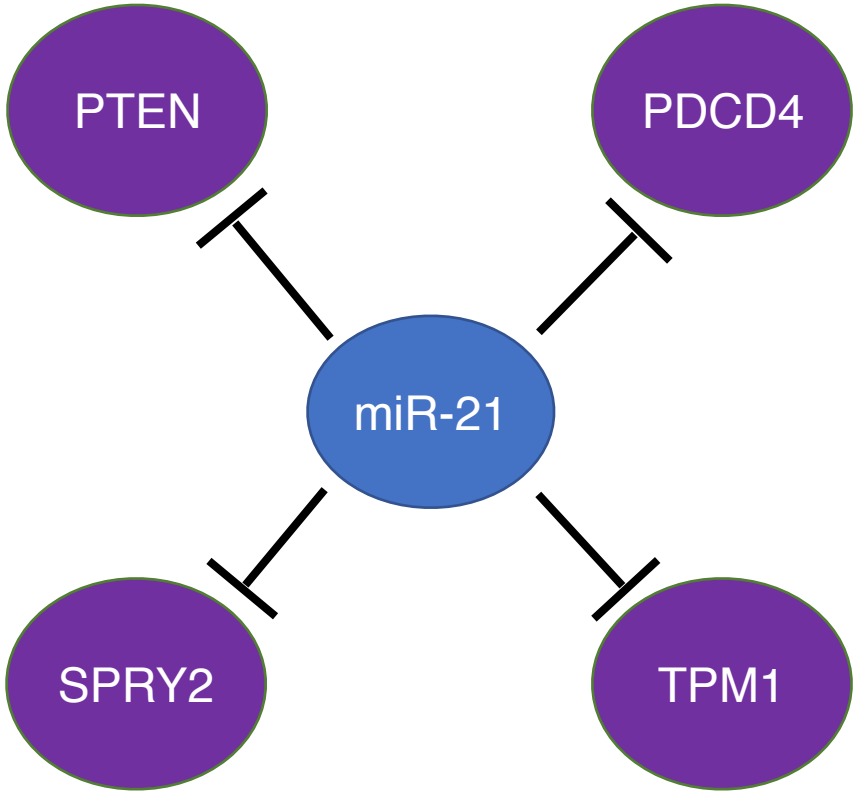
Heart

Human Targets:

What does miR-21 regulate?

Expressed In:

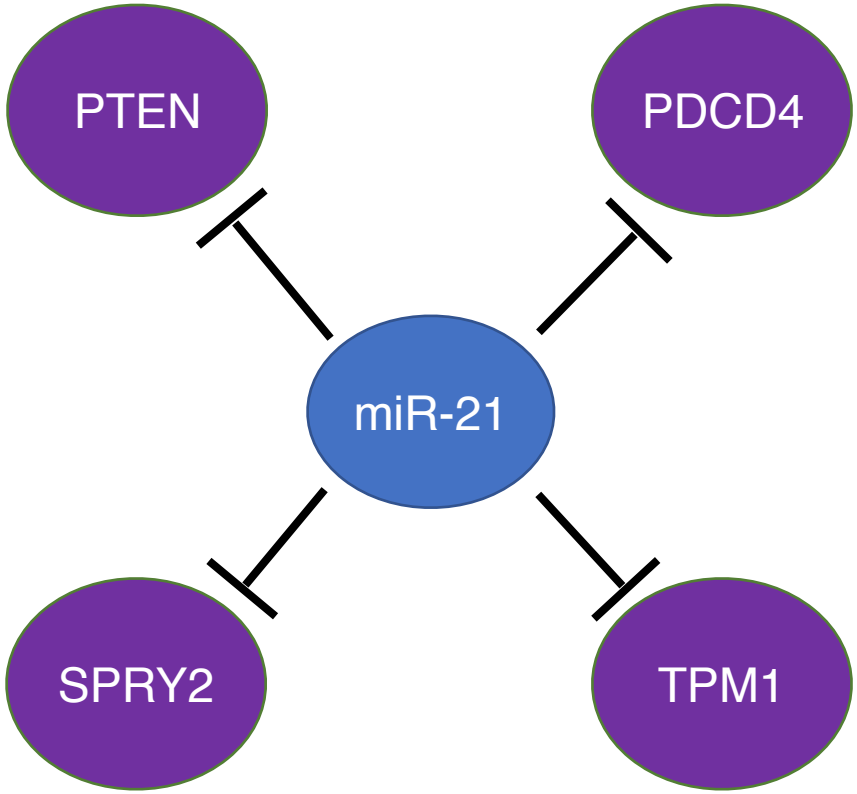
Human cancer targets:



What does miR-21 regulate?

Expressed In:

Human cancer targets:

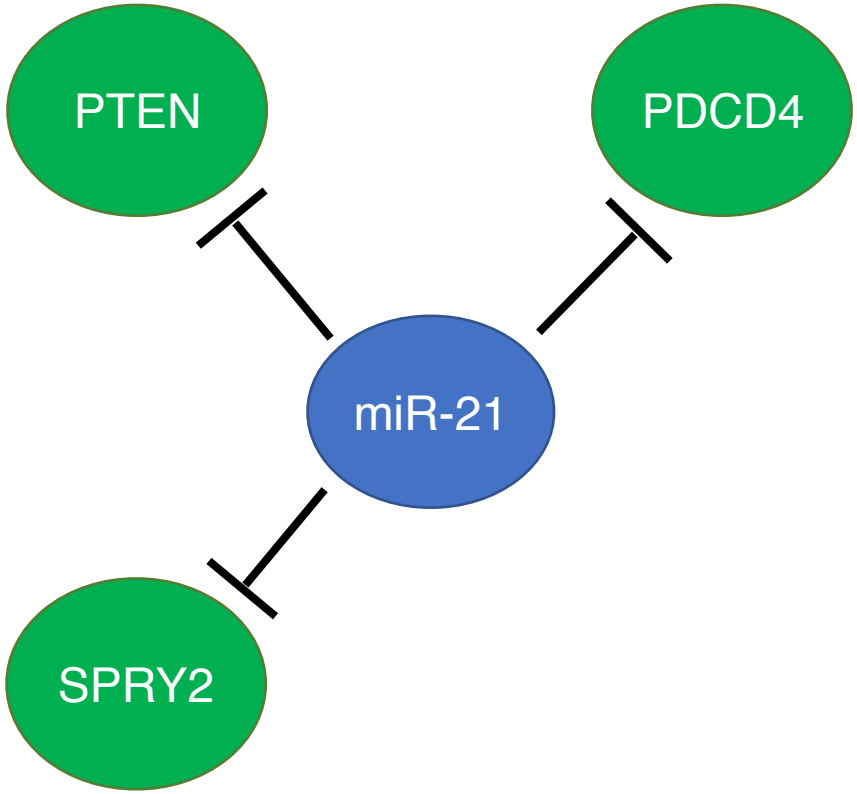


miR-21 inhibits tumor suppressors to promote cell proliferation

What does miR-21 regulate?

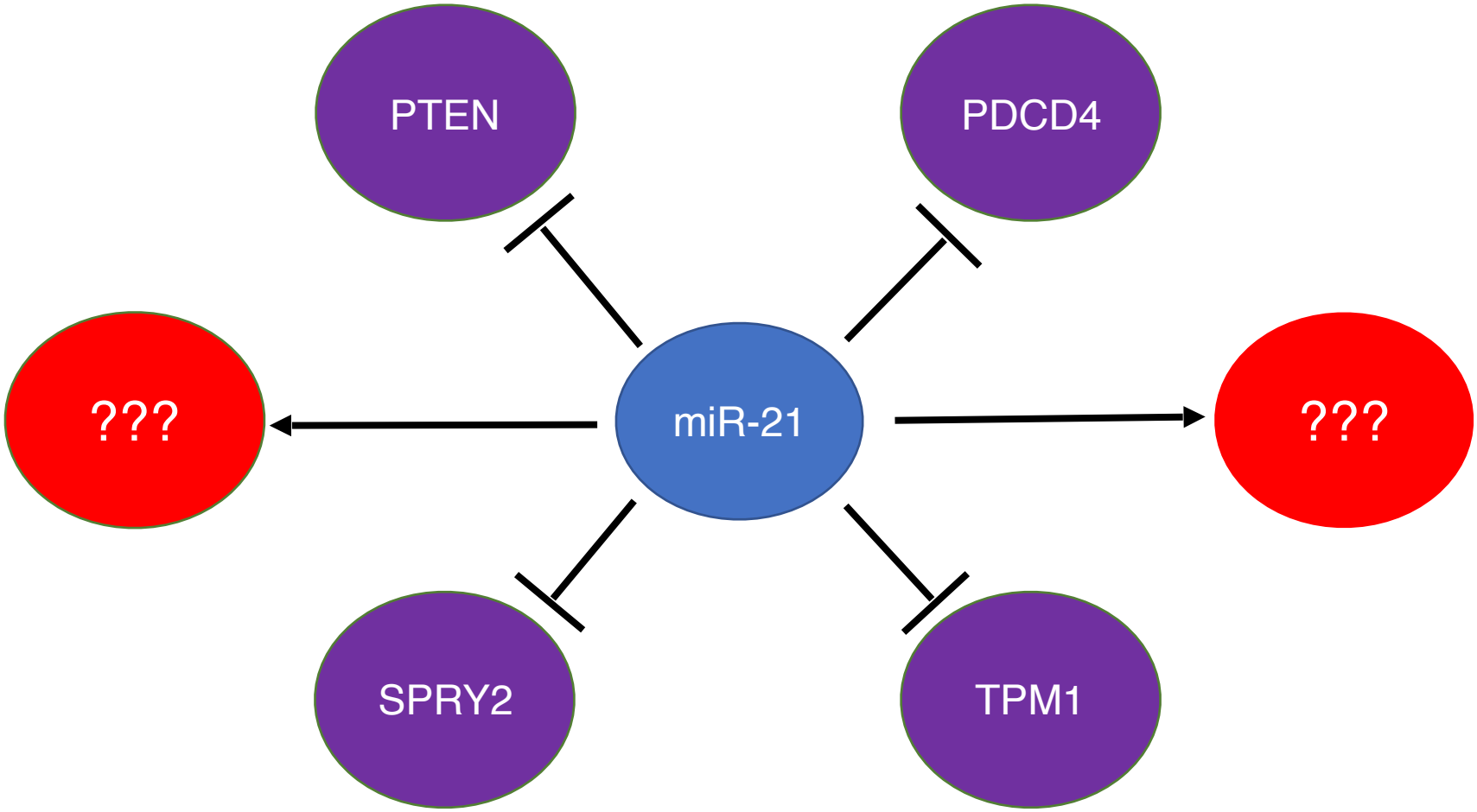
Expressed In:

Zebrafish heart targets:



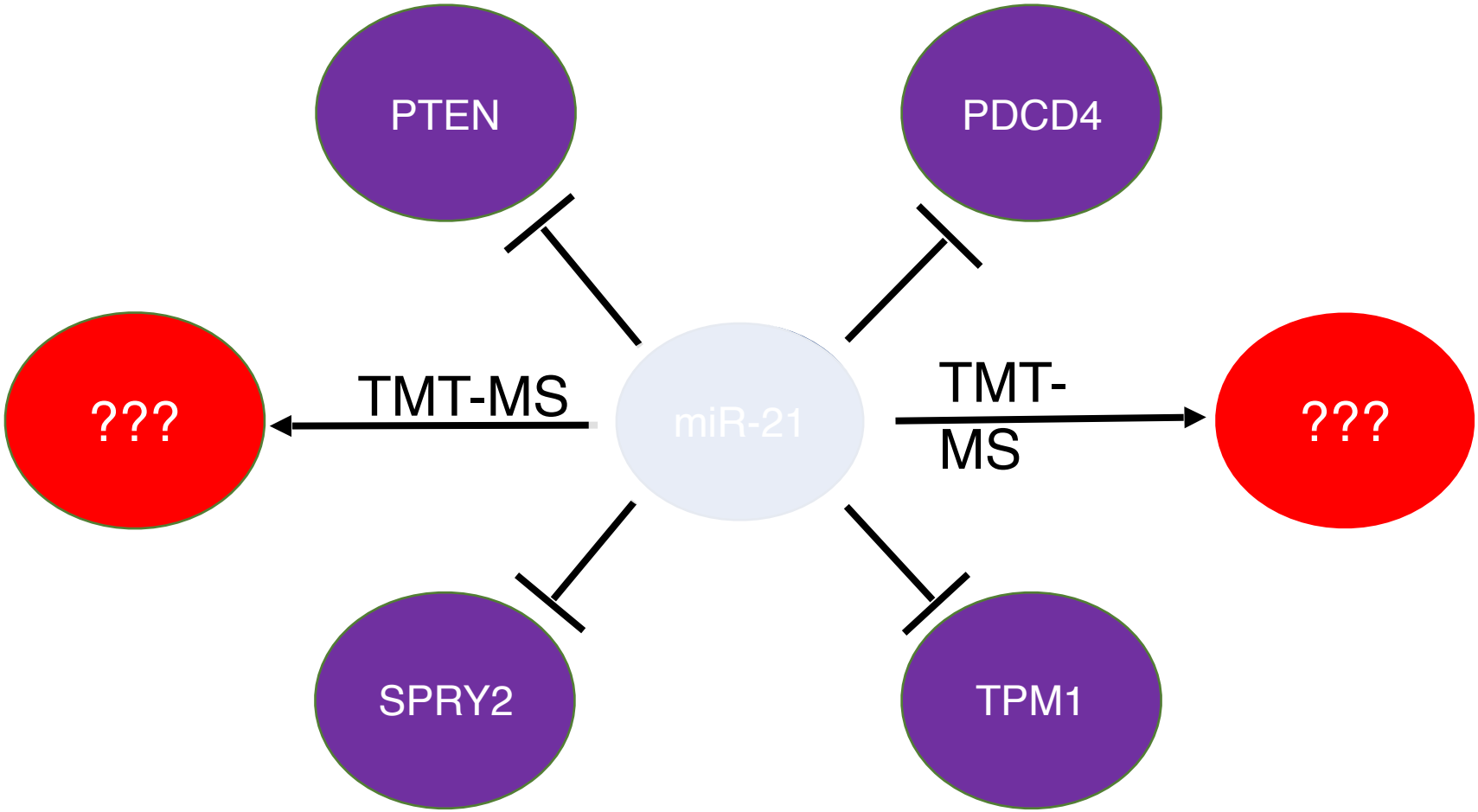
miR-21 has similar targets in both tumor invasion and zebrafish valvulogenesis

What is the goal of the study?



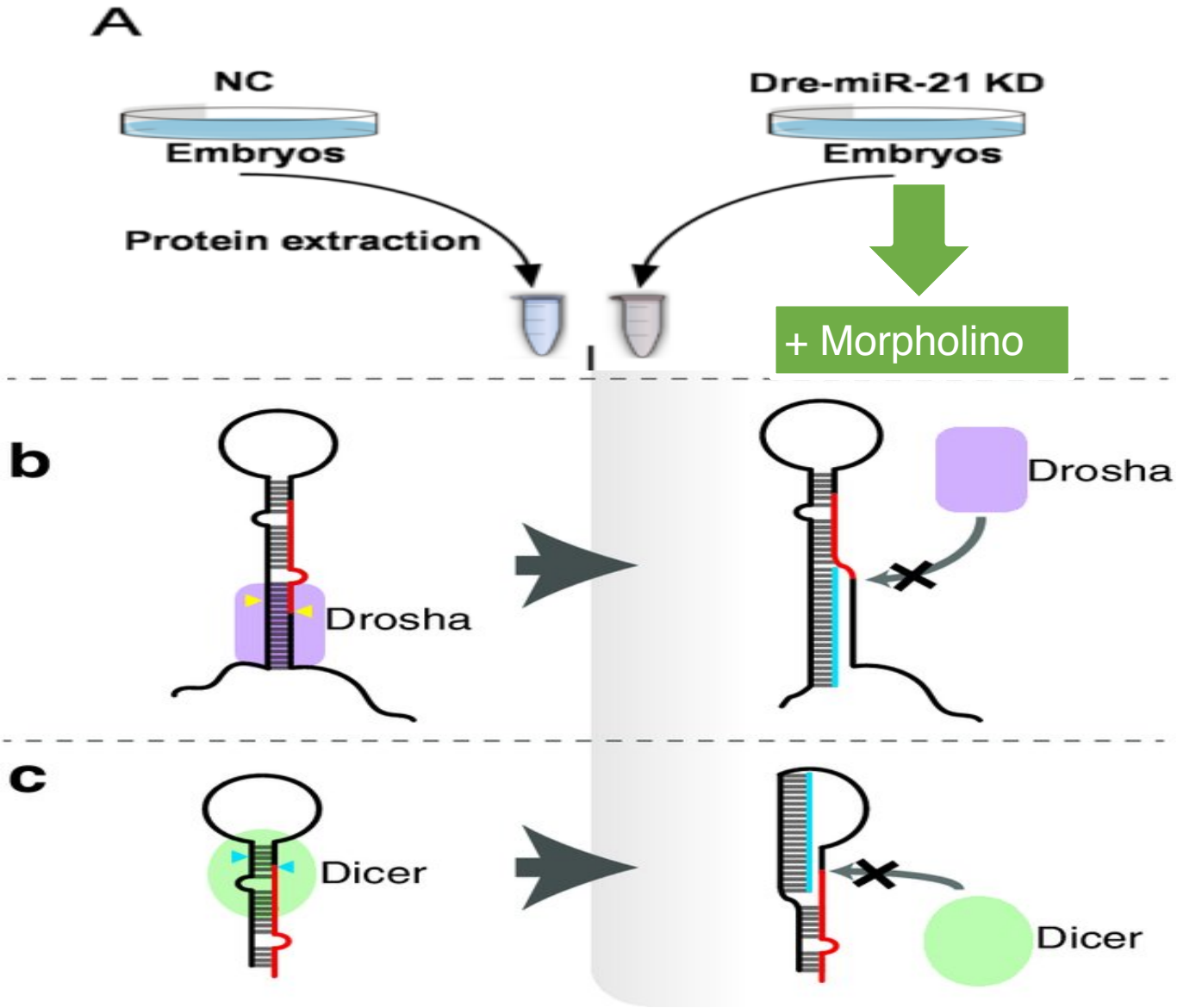
Identify new global targets of miR-21 and their relationship to Heart Development/Cancer

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?

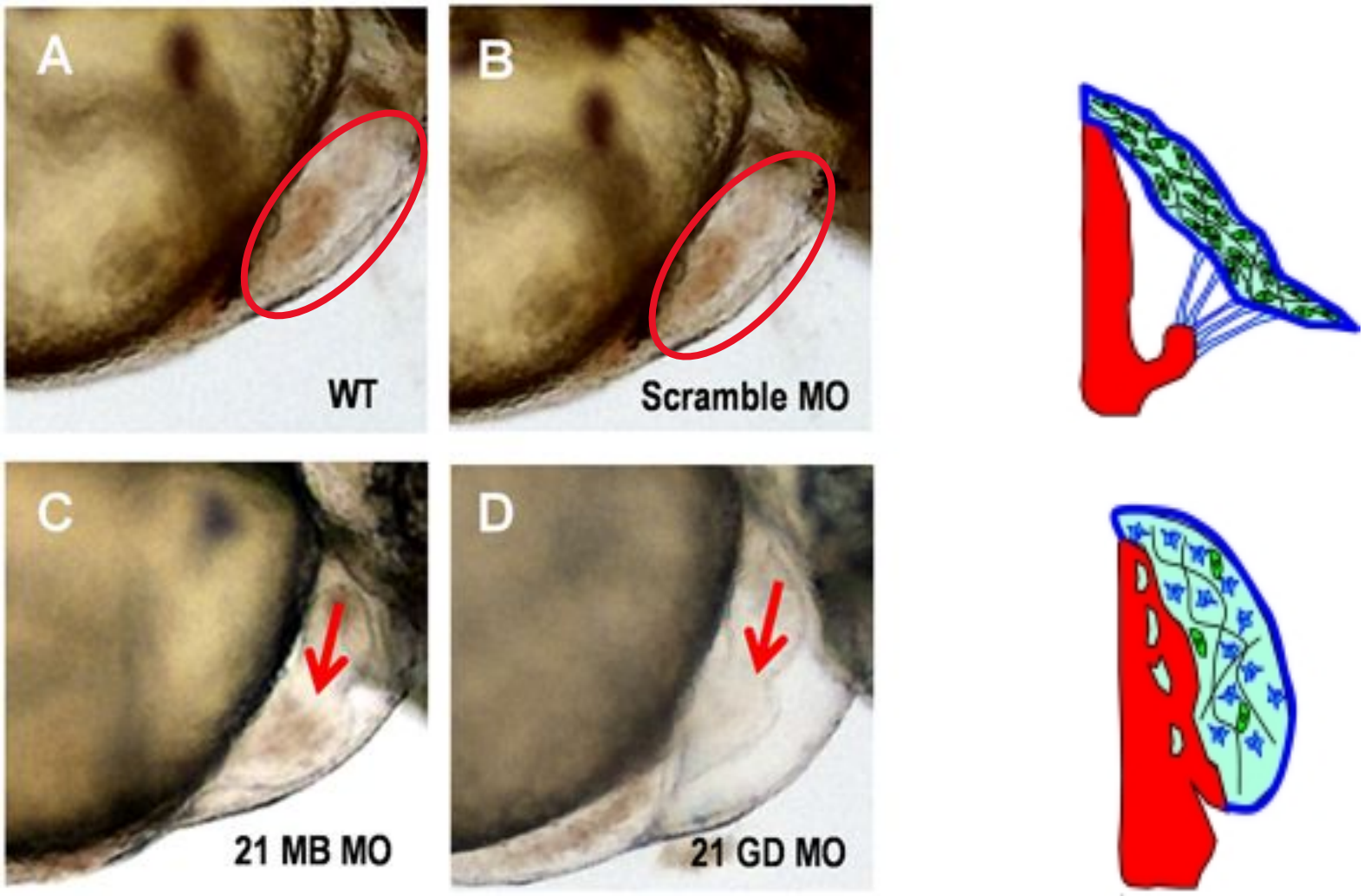
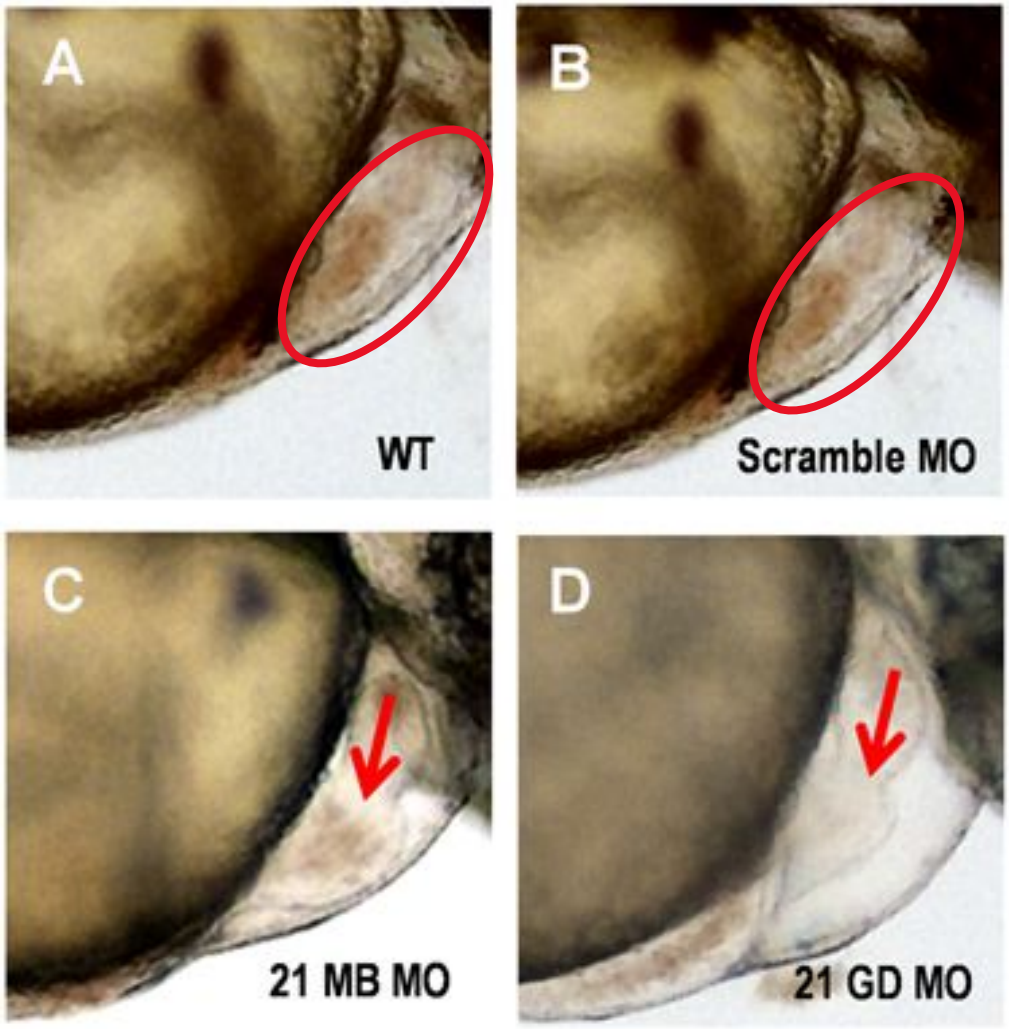


Fig. 1: Does a miR-21 KD affect valvulogenesis?



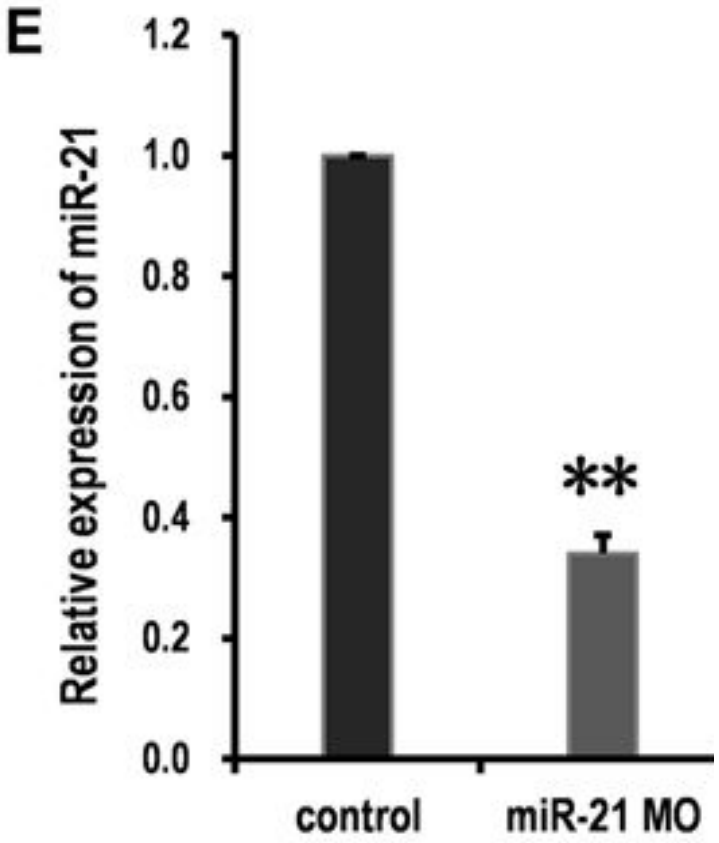


Fig. 2A: Experimental workflow

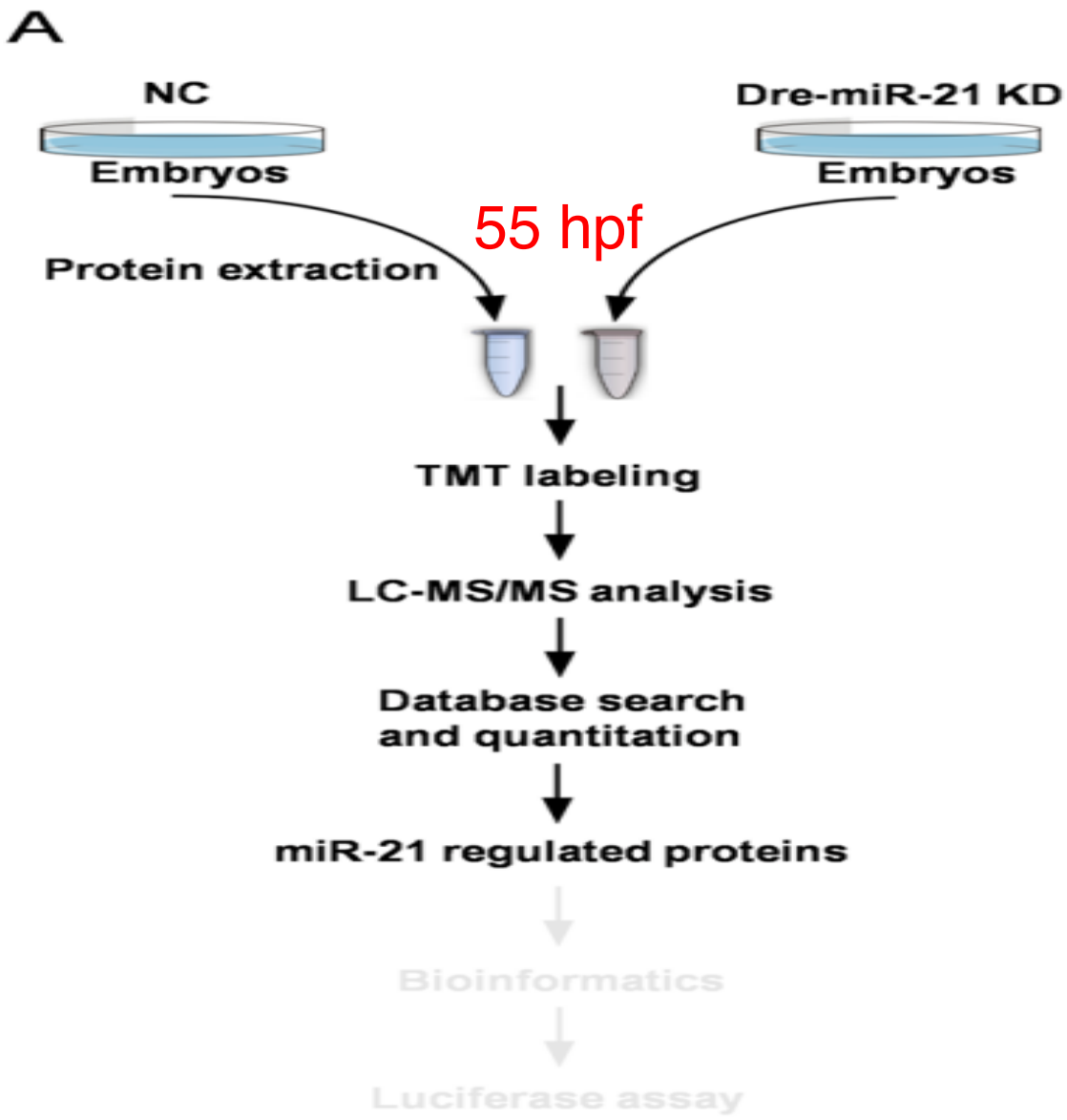


Fig. S2: How were differentially expressed proteins identified?

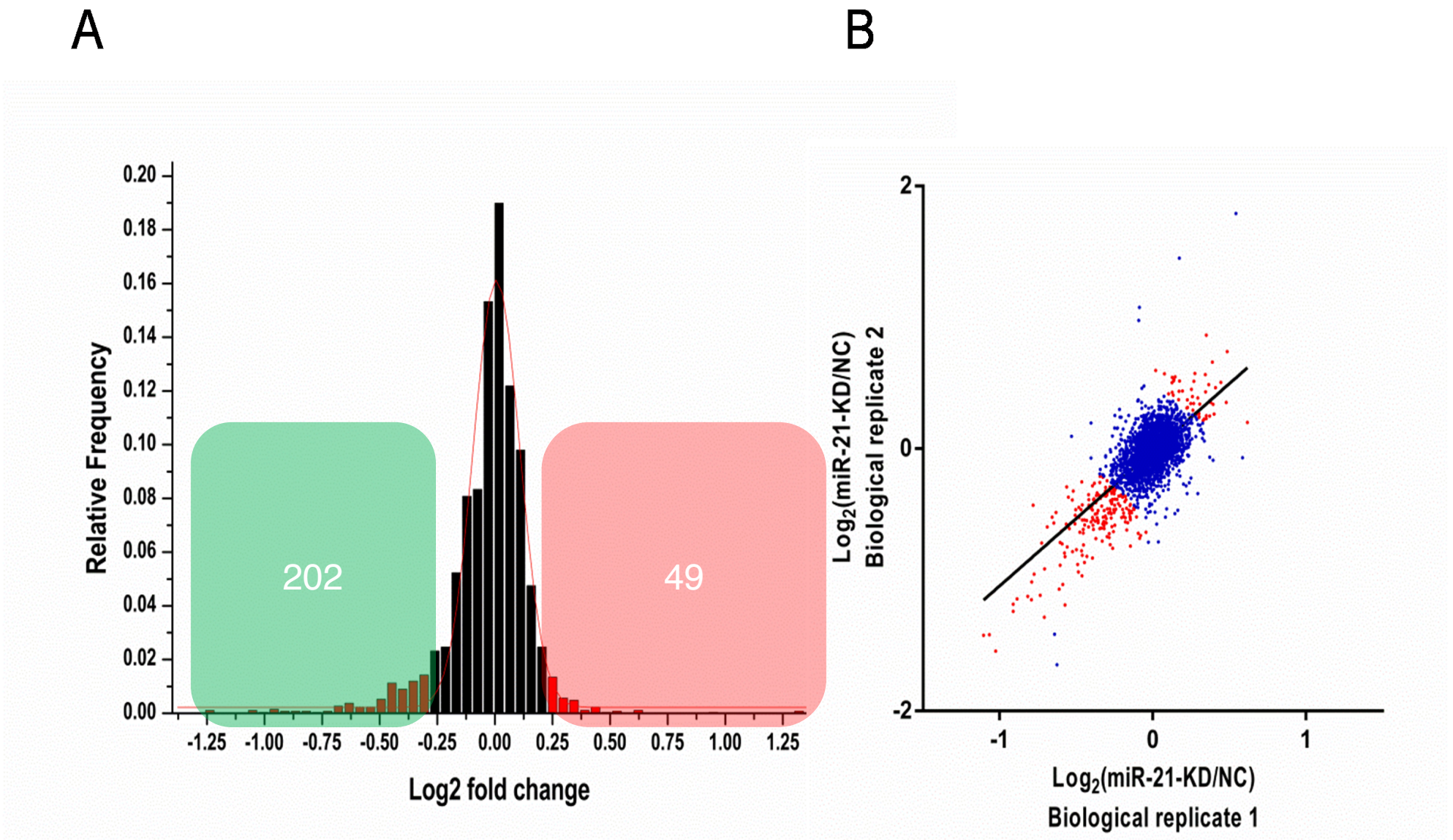
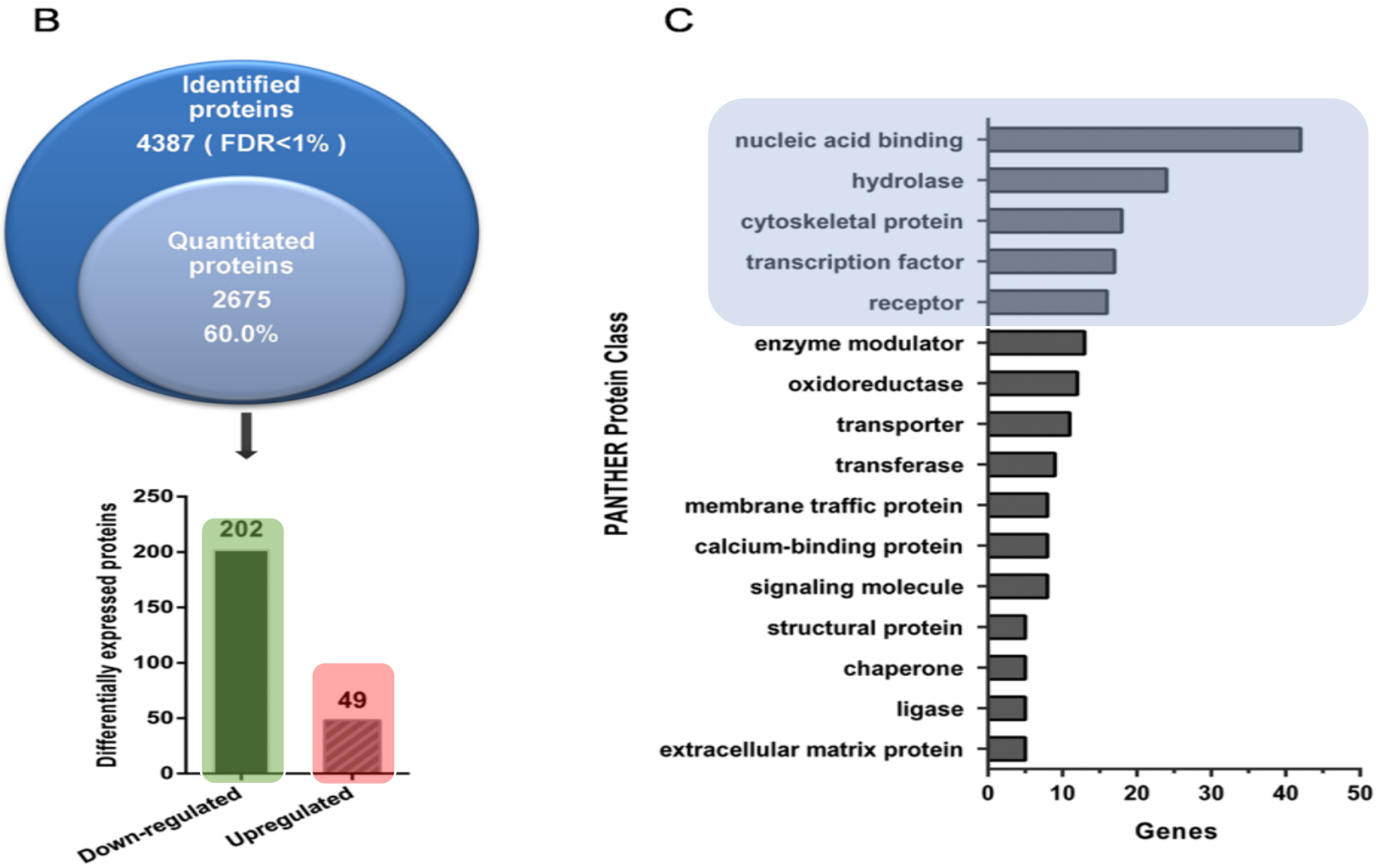
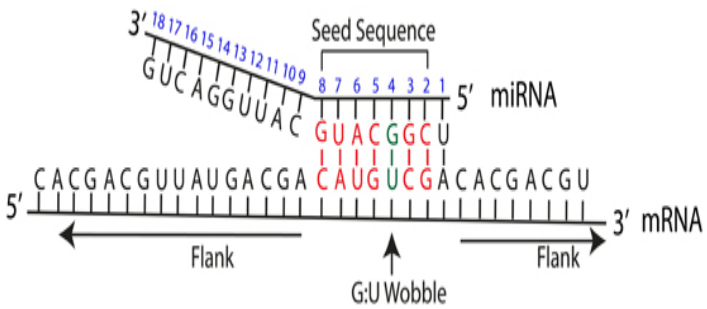


Fig. 2B,C: How many differentially expressed proteins were identified?



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

Fig. 2A: Experimental workflow



Six miRNA Prediction Software

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

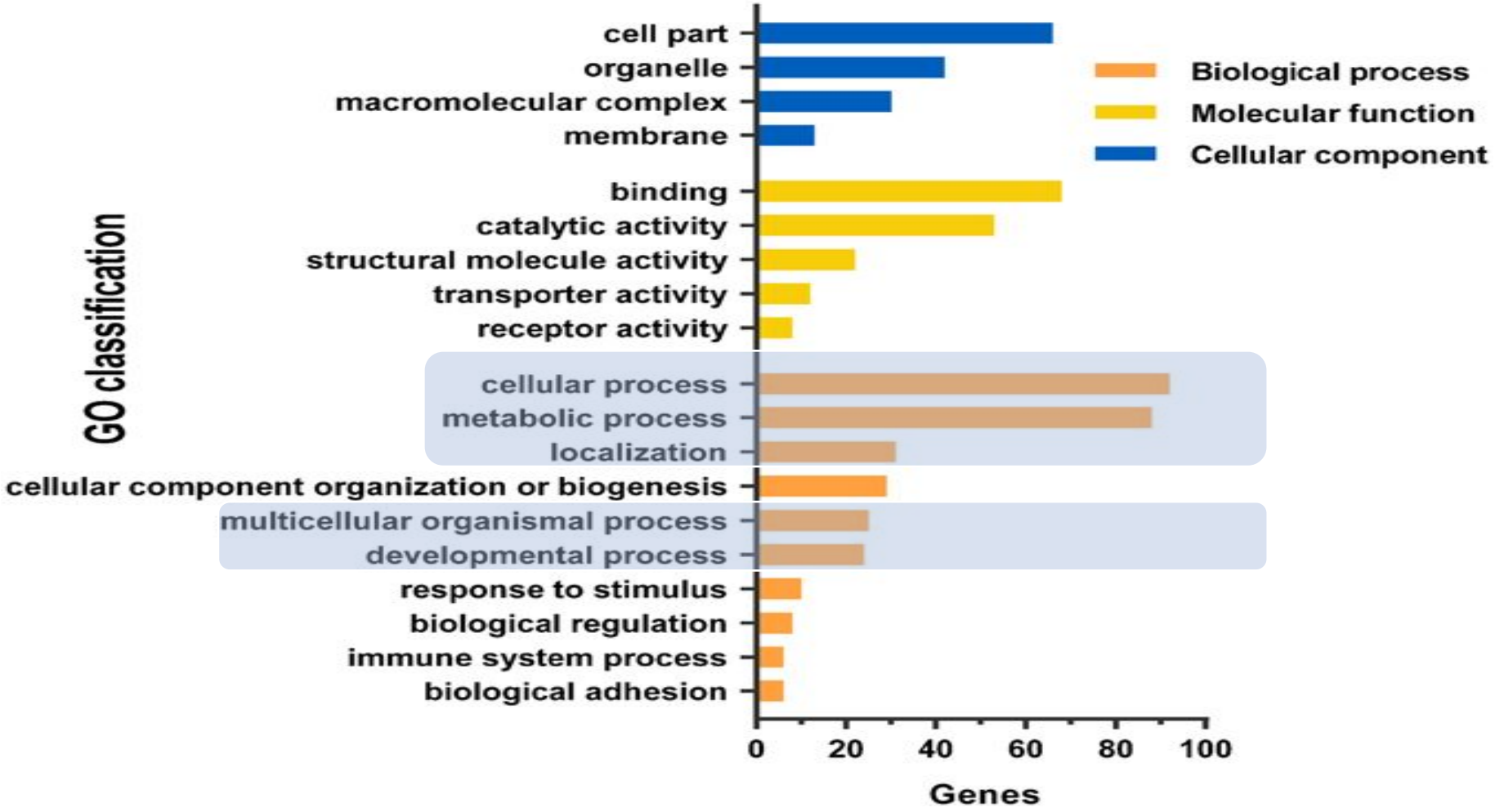
Bioinformatics

Luciferase assay

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?

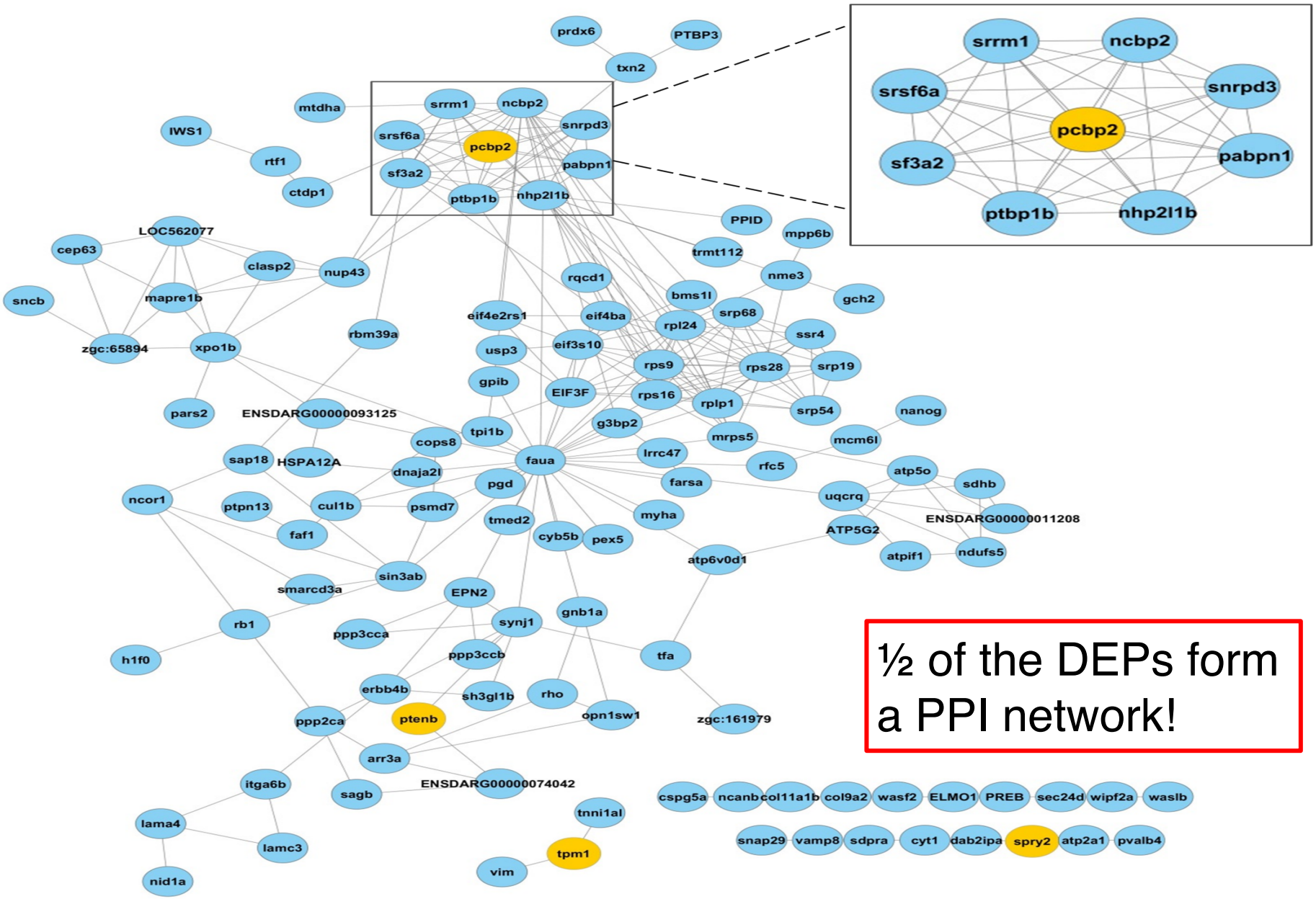


Fig. S3: Do any of the candidates interact?

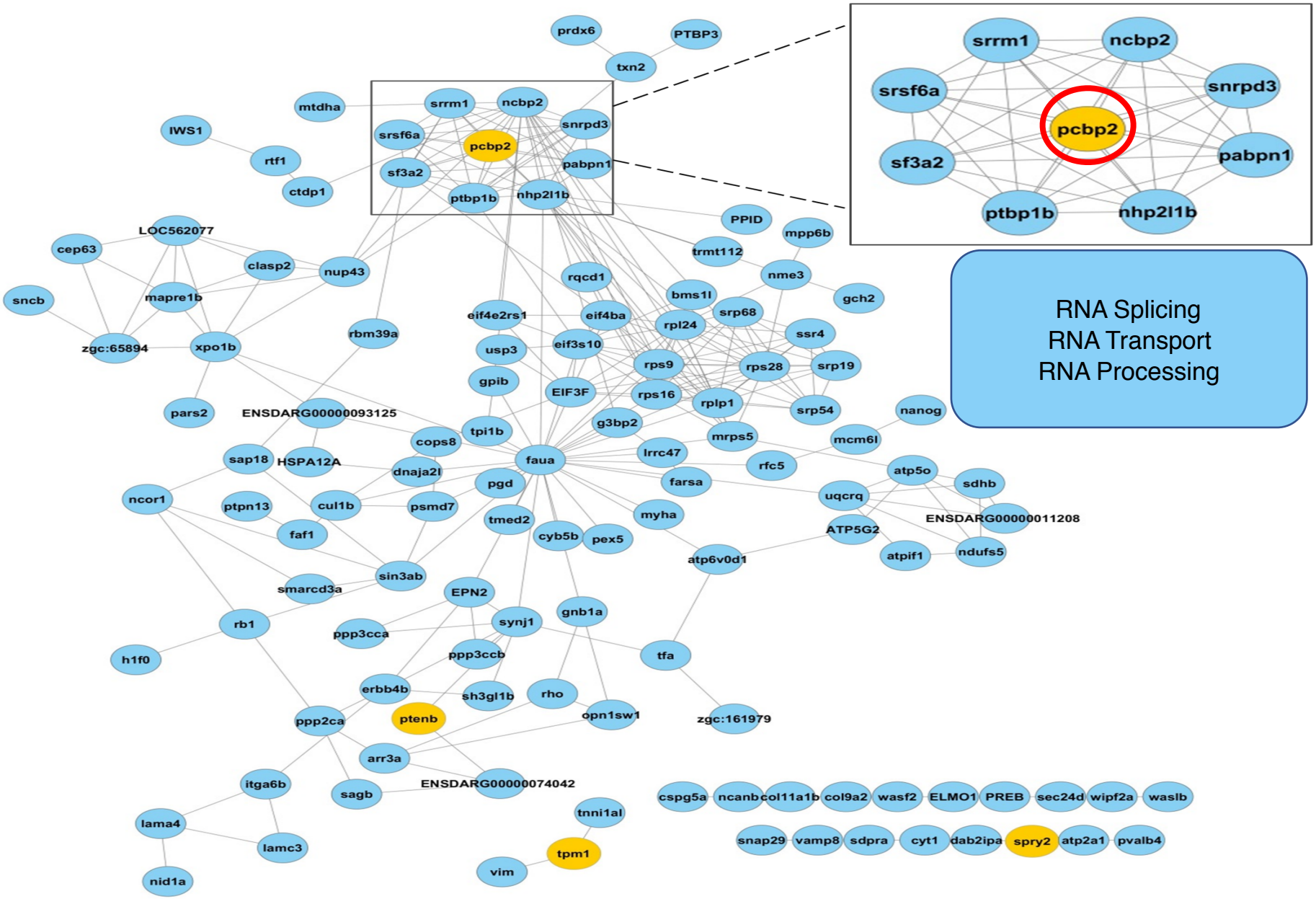
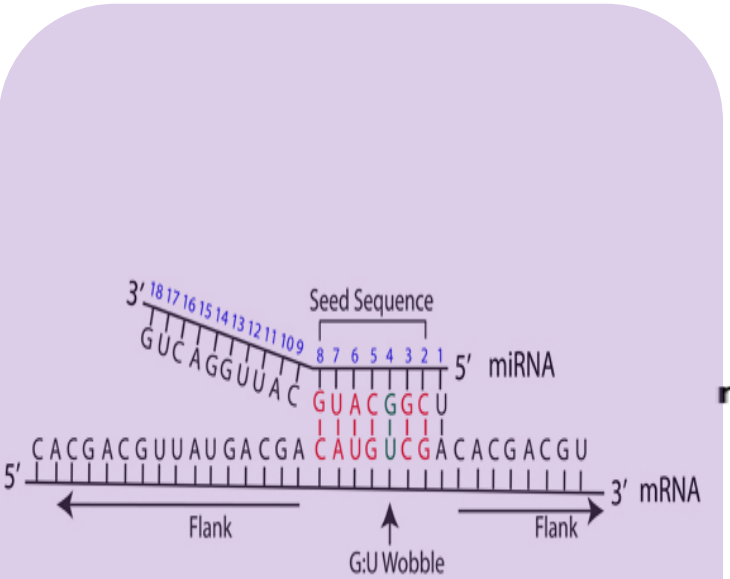


Fig. 2A: Experimental workflow



Six miRNA Prediction Software

miR-21 regulated proteins

↓

Bioinformatics

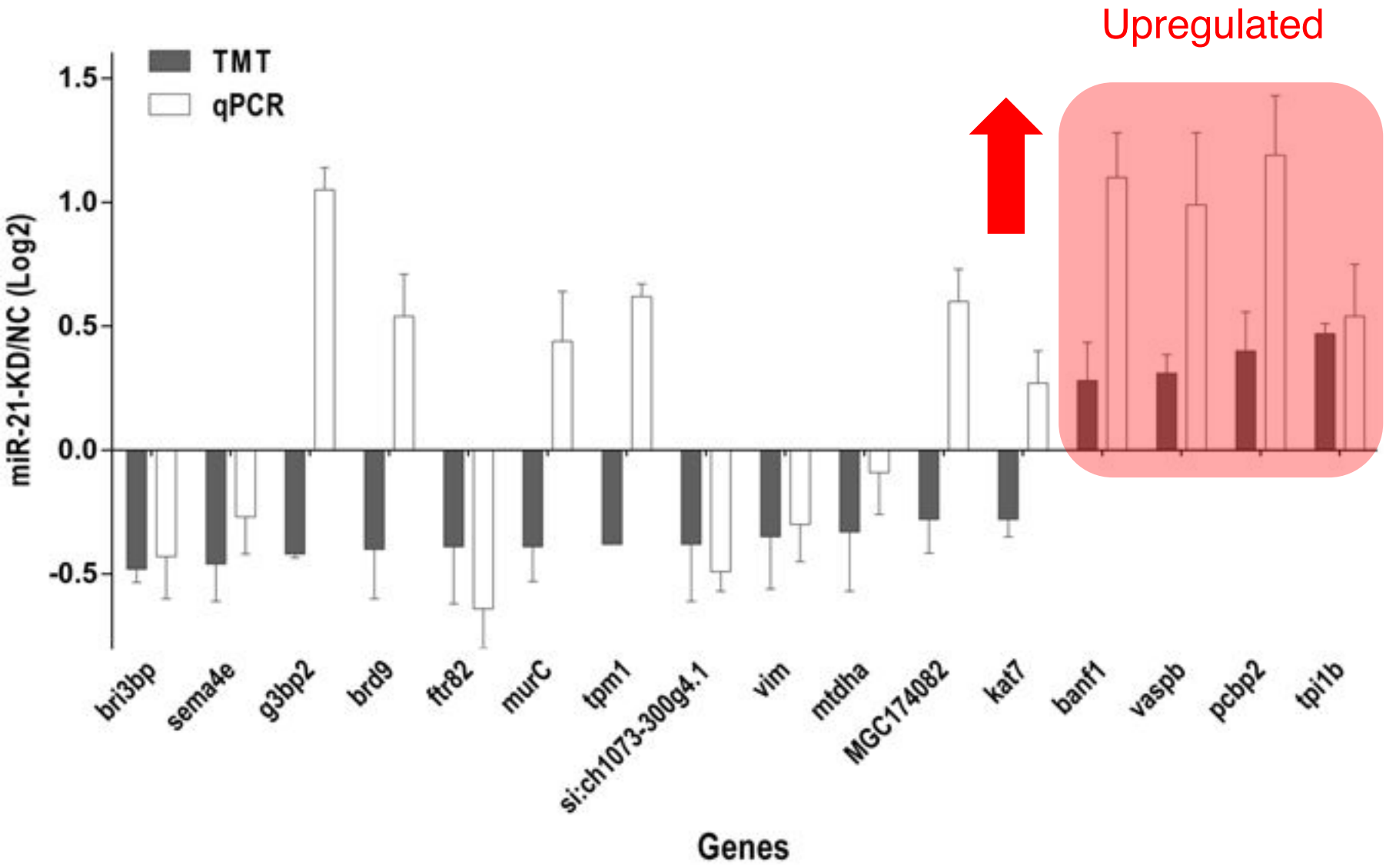
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Luciferase assay

PANTHER Classification System

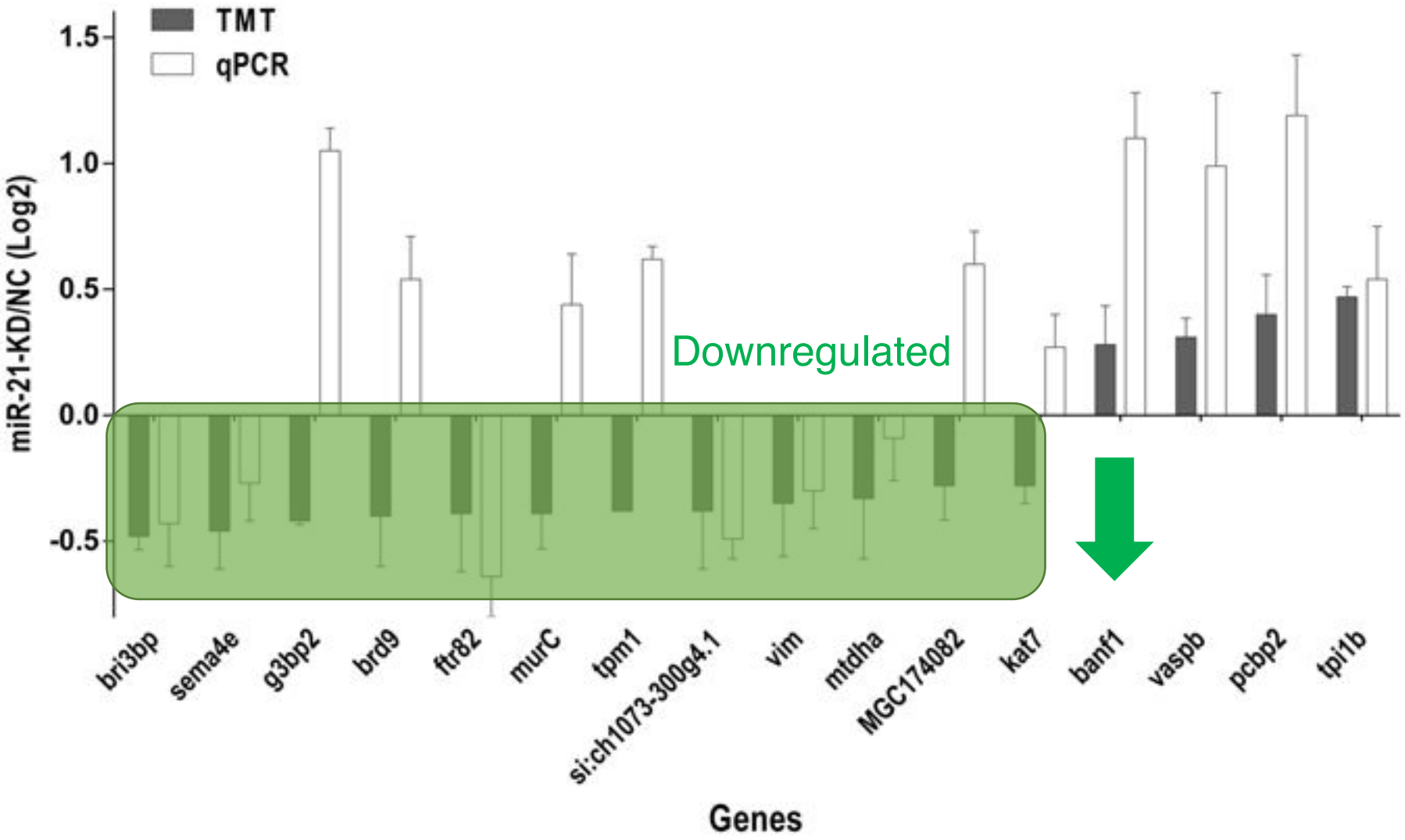
String DB

Fig. 4 Do transcript levels correlate with protein abundance?



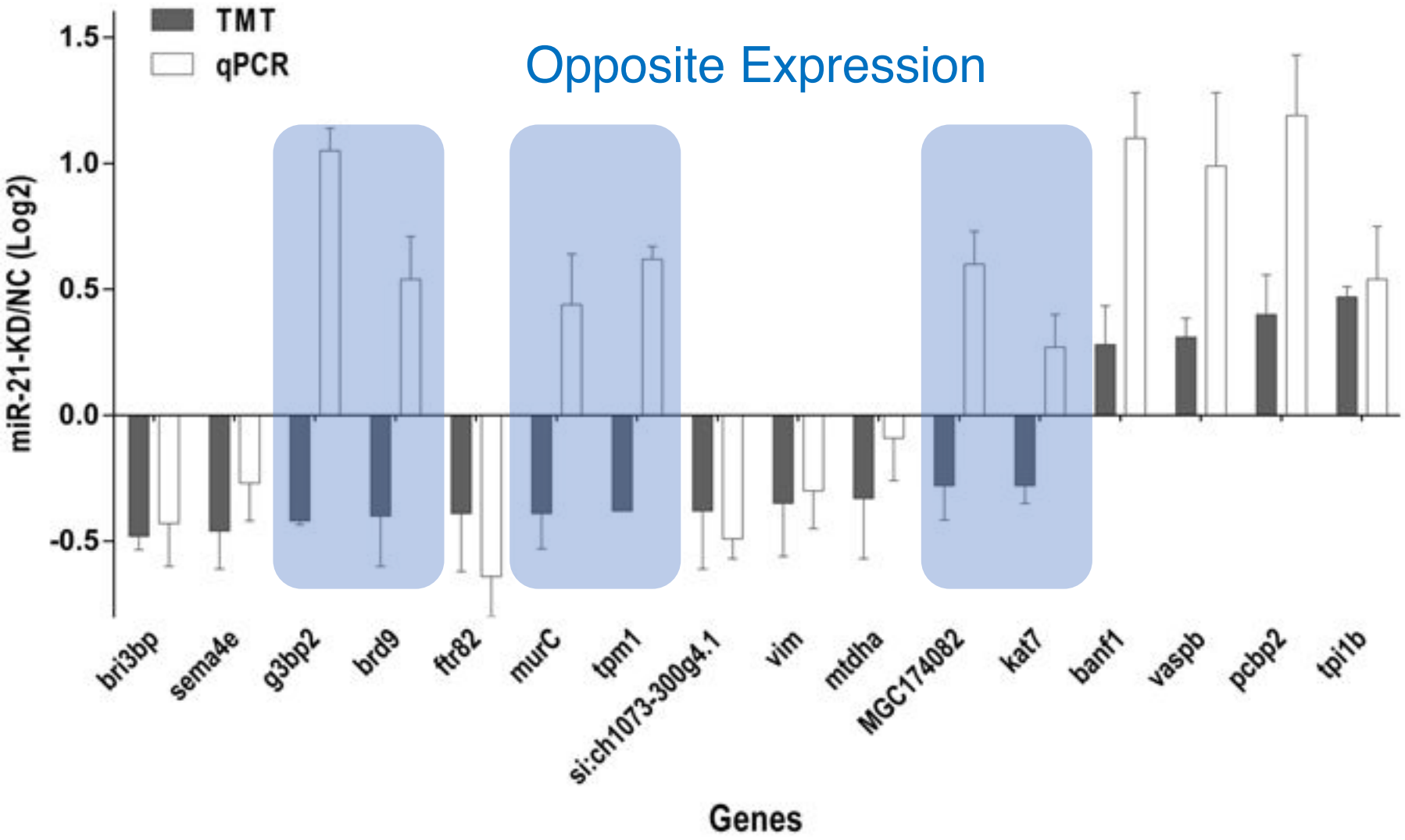
Sixteen targets predicted to be targeted by miR-21

Fig. 4 Do transcript levels correlate with protein abundance?



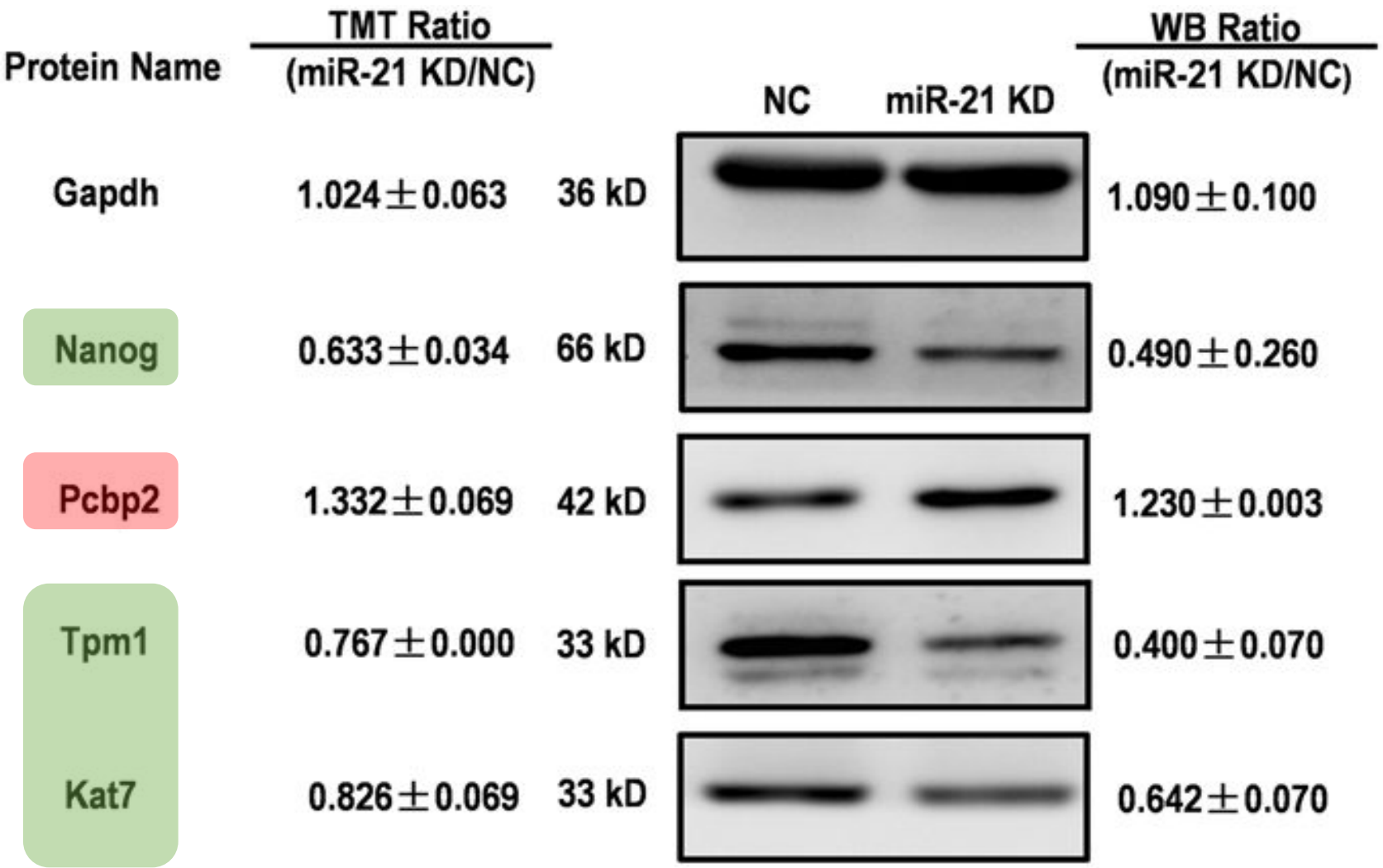
Ten targets show similar trends in gene expression

Fig. 4 Do transcript levels correlate with protein abundance?



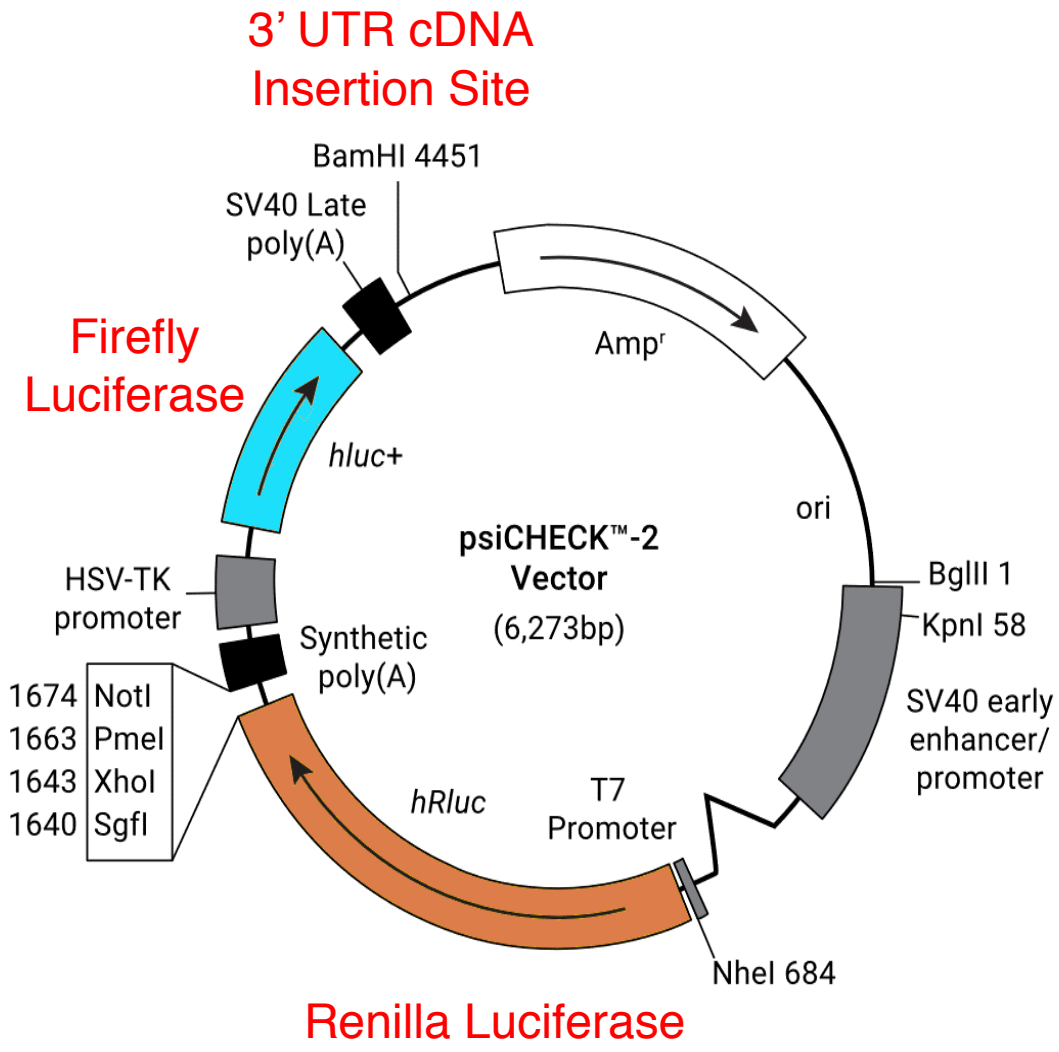
Six targets show opposite expression patterns

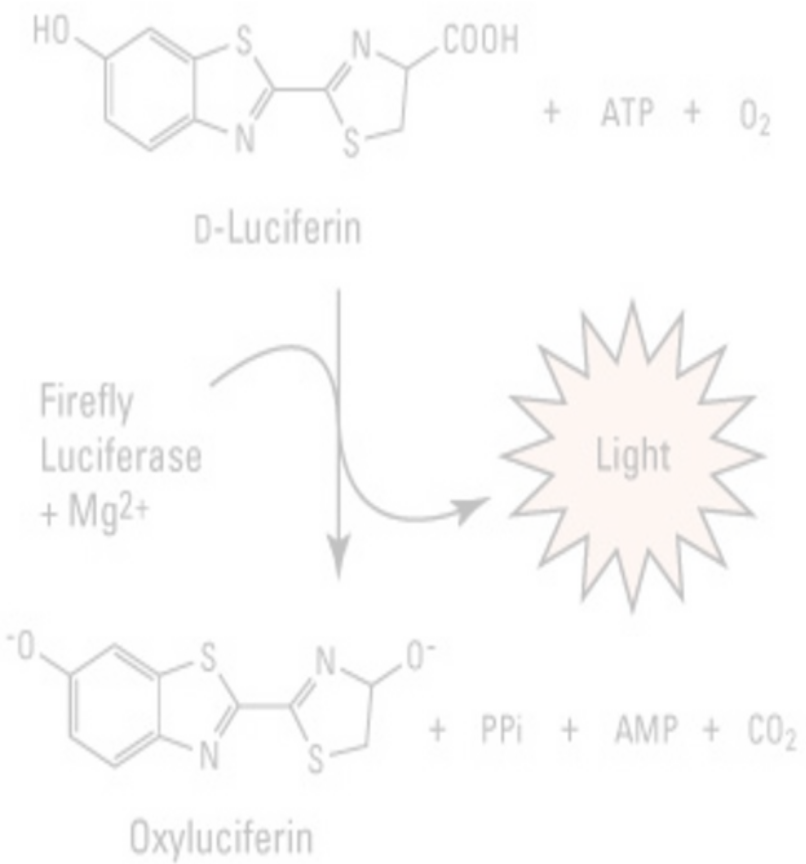
Fig. 5: How valid is the proteomic data?



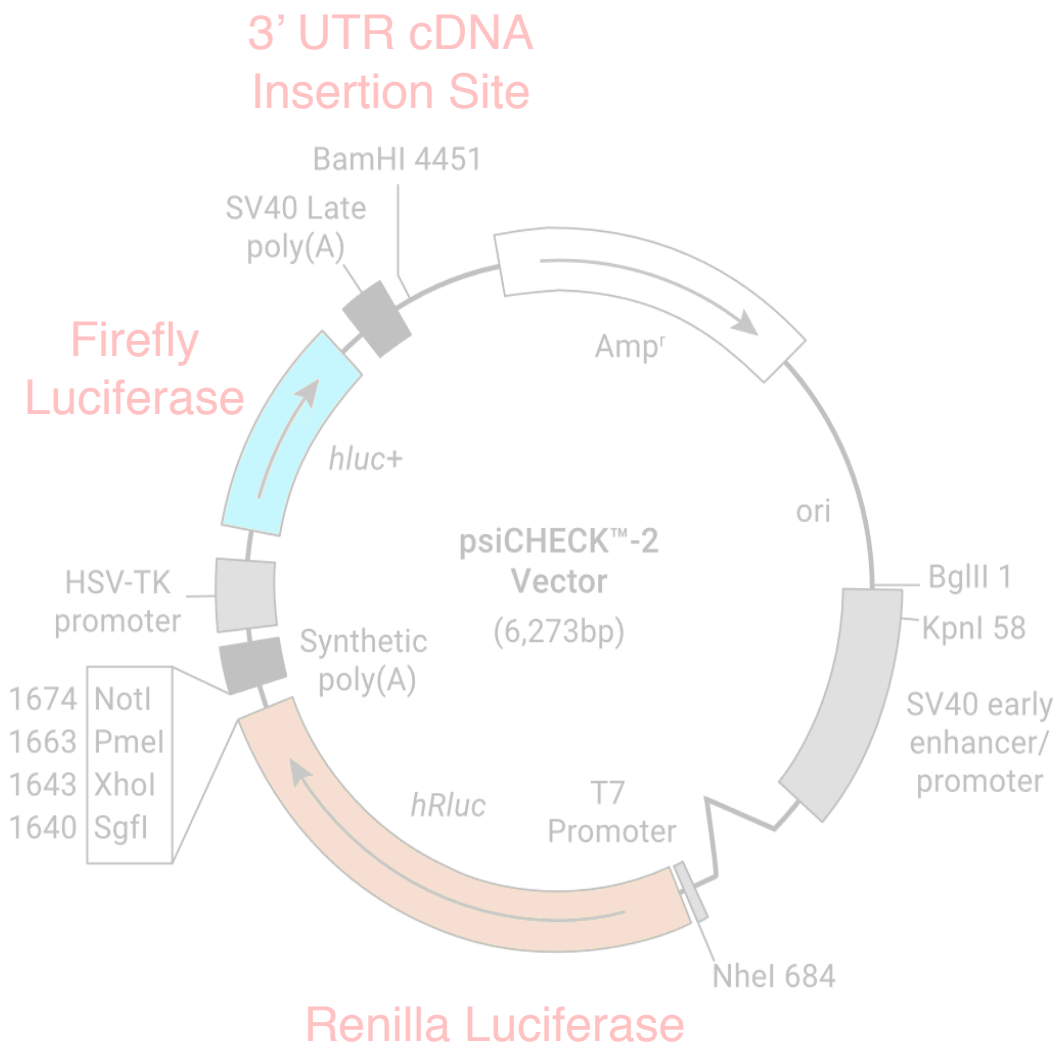
Western Blot assays show similar results to TMT-MS

What is a luciferase assay?





What is a luciferase assay?



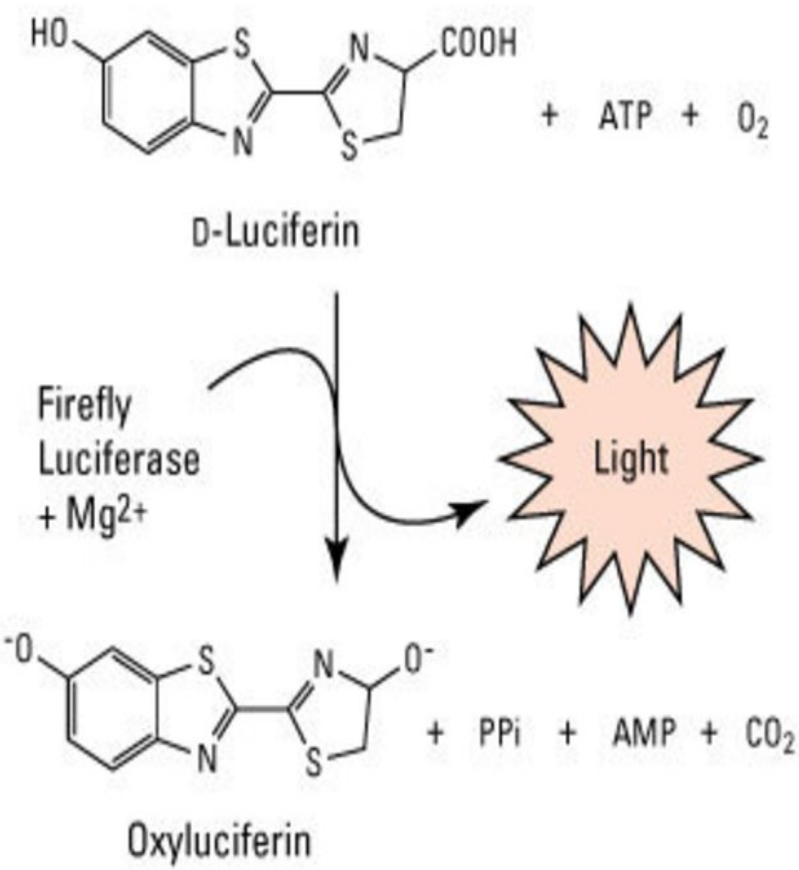


Fig. 6: How valid is the miR-21 bioinformatic data?

A

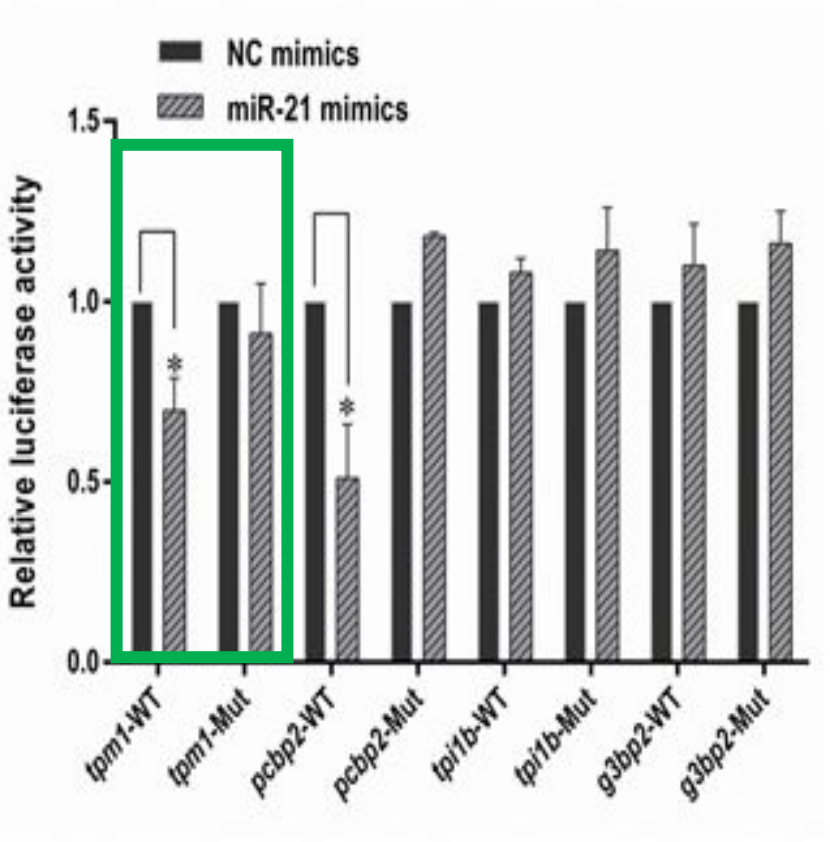
```
3′ cgguuGUGGUCAGAC-UAUUCGAu 5′  dre-miR-21
       | ||  ||   :||||||
5′ atttcCTCCCATCTGTGTAAGCTc 3′  tpm1-WT
5′ atttcCTCCCATCTGTGTCCUATc 3′  tpm1-Mut

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
```

B



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

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

A

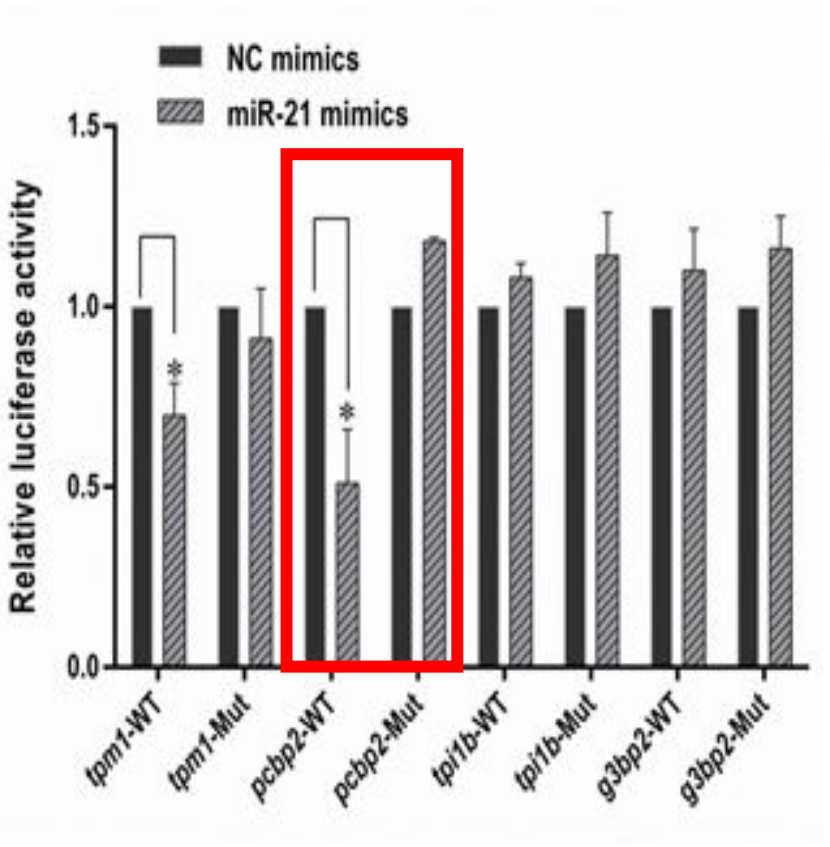
```
3′ cgguuGUGGUCAGAC-UAUUCGAu 5′  dre-miR-21
       | ||  ||   :||||||
5′ atttcCTCCCATCTGTGTAAGCTc 3′  tpm1-WT
5′ atttcCTCCCATCTGTGTCCUATc 3′  tpm1-Mut

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

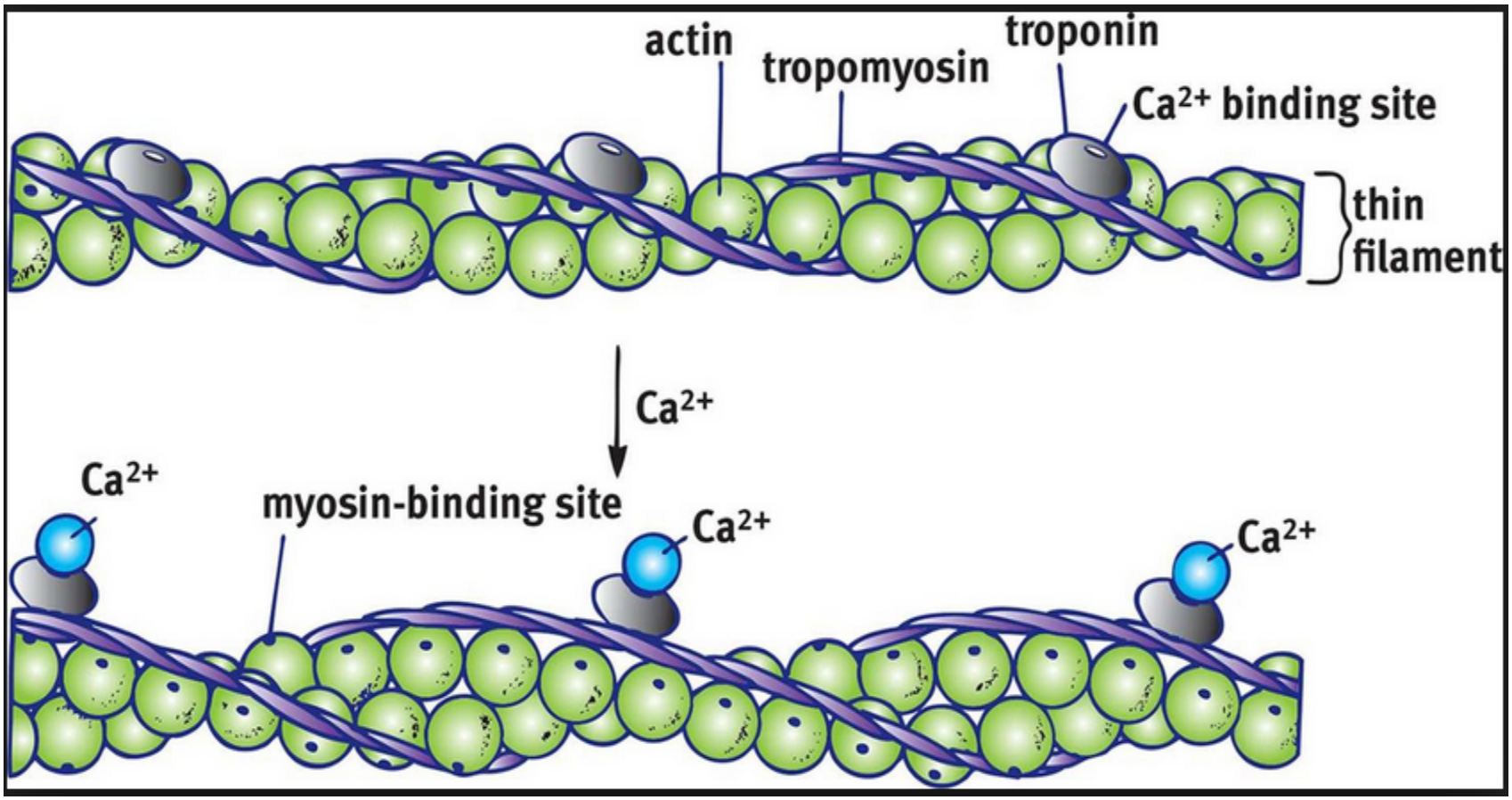
3′ cggUUGUGGUCAGACUAUUCGAU 5′  dre-miR-21
     ||:| |   :| ||||||||
5′ ctgAATAGCTCATTAATAAGCTA 3′  g3bp2-WT
5′ ctgAATAGCTCATTAAGCCUATA 3′  g3bp2-Mut
```

B



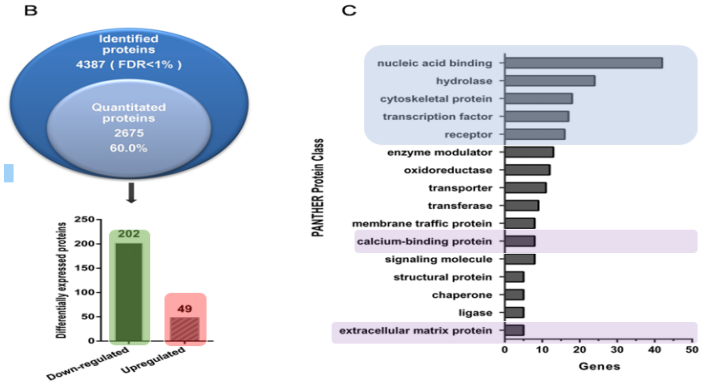
pcbp2 is directly targeted and repressed by miR-21

What is *tpm1*?

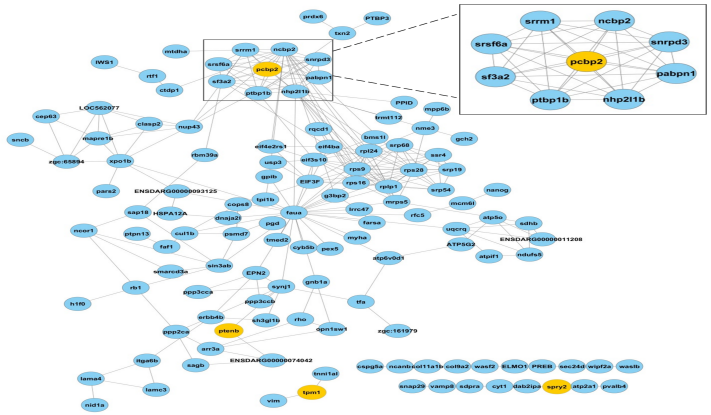


Tropomysin regulates muscle contractions and is a known tumor suppressor gene

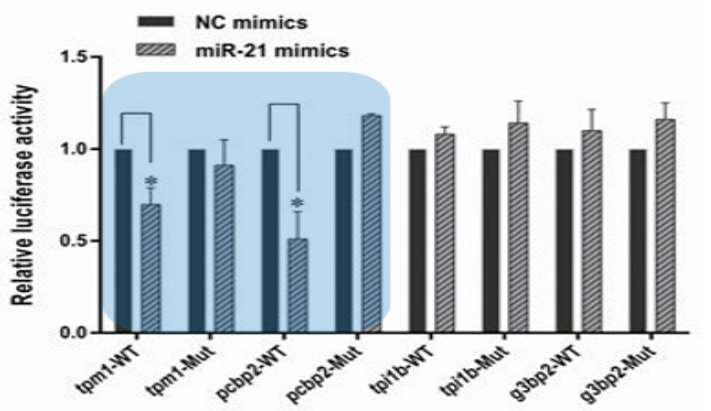
Summary



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.

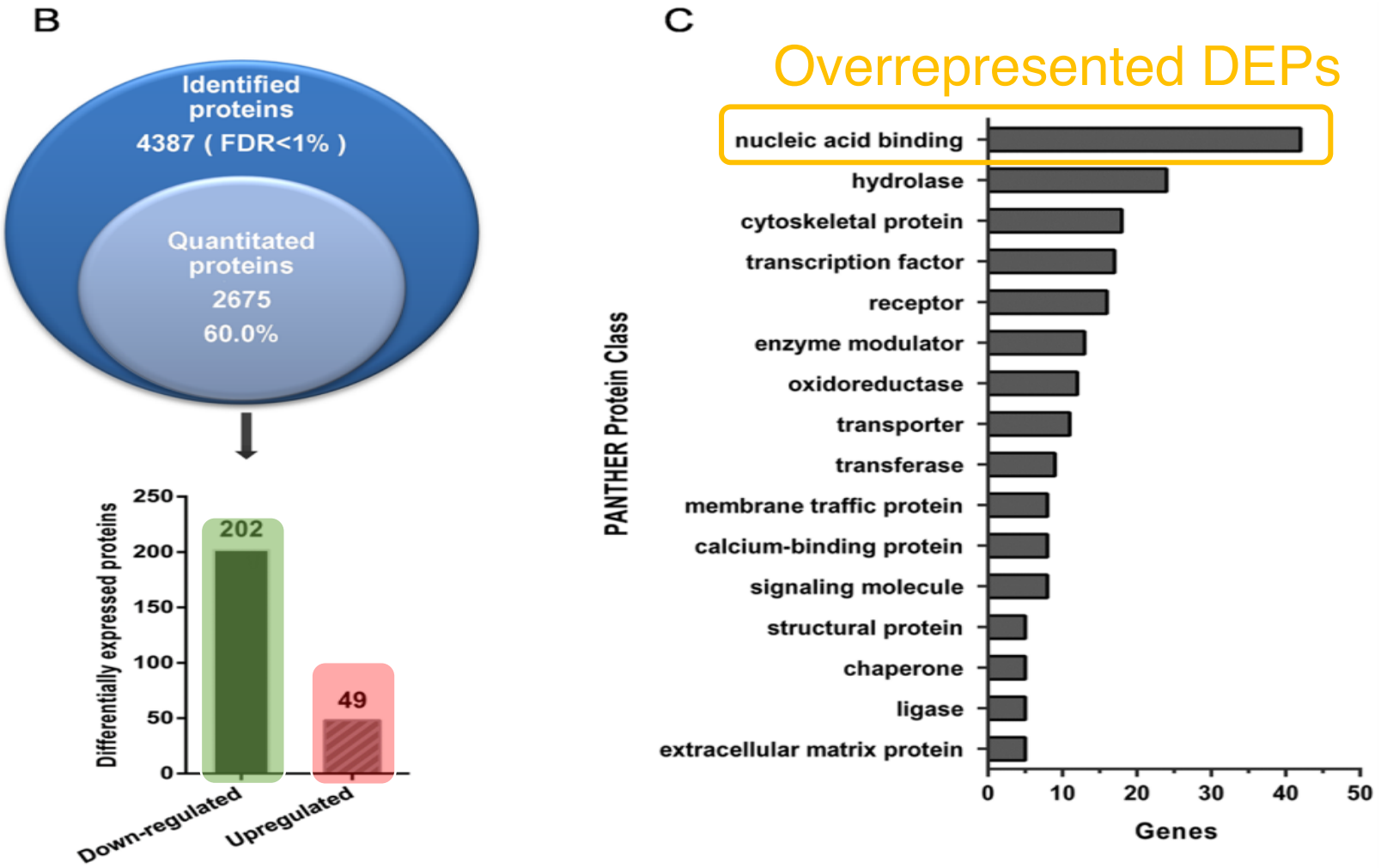
Questions or Comments?

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/

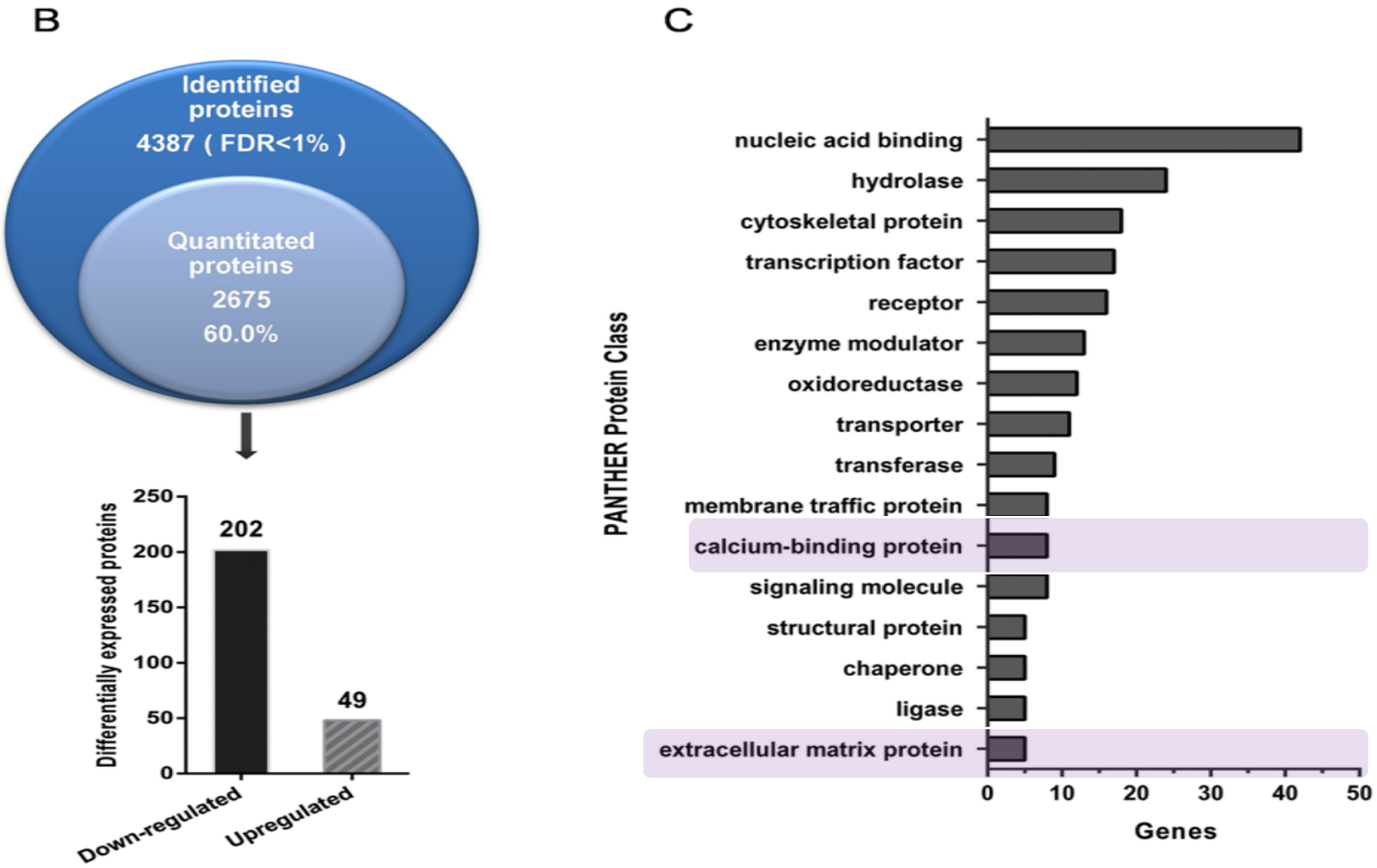
Supplemental Slides

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



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

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