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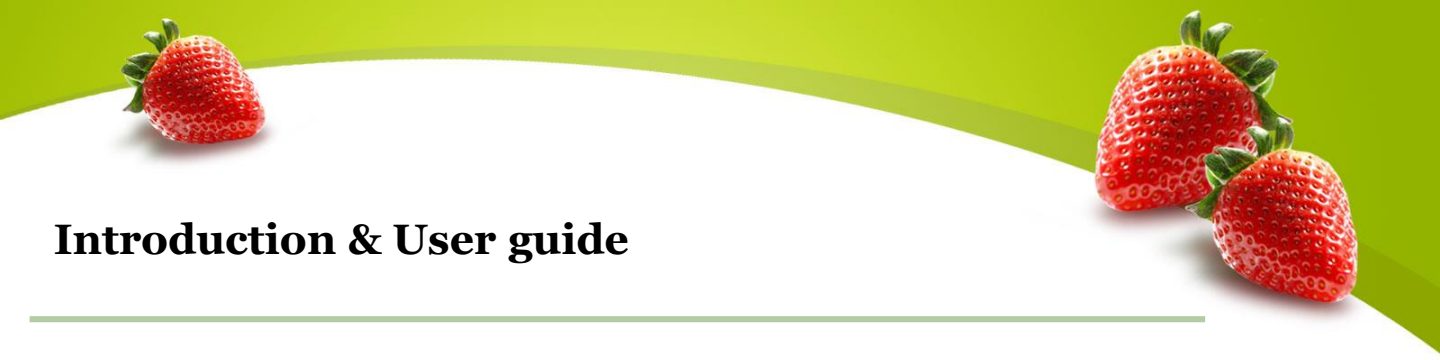
The Proteomics Cookbook

A user-friendly guide on what the Proteomics Core can do for you

by
Simone Sidoli &
the Proteomics Core

Beta version 0.6 (August 2020)


Index



Introduction & User guide

Welcome!

The Proteomics Cookbook is a collection of protocols and ideas of experiments that can be performed with the Proteomics Core. Each "recipe" describes established and robust protocols that provide quantitative data about the proteome of your system. This includes identification and quantification of proteins, but also other interesting aspect of your proteome such as turnover, localization and more.

You will notice that each protocol is flanked by a page displaying data graphs. The goal is to assist brainstorming regarding the type of information that are achievable with your experiment. We hope this will help you select the most appropriate protocol and stimulate your creativity, including planning novel experiments that you did not expect were feasible.

You are more than welcome to design other experiments not described here; we love new challenges! The members of the Core are here at Einstein to help you and discuss with you potential limitations.

User guide

Each "recipe" lists the required reagents and the procedure. The color coding of the page header represents the difficulty of the procedure

Green: simple and highly robust

Yellow: medium complexity, some optimization might be required

Red: feasible experiments, but consider a learning curve

The Proteomics Core can perform for you sample preparations for a selected number of protocols. However, we strongly encourage all the users to consider preparing the samples within their lab. We assure that none of these protocols are challenging nor require expensive reagents. We are certain that, in the long run, you will appreciate the commodity of being familiar with these simple steps. They will always work best in your own hands, because nobody knows your samples more than you do. It is also very likely that samples ready for analysis might have shorter waiting times for obvious reasons.

For the best appreciation of this document, please print it front and back, so you can have side-by-side the protocol (on the left) with the type of results obtained from this type of experiment (on the right).

Message from the author

Dear everyone, thank you very much for the very warm welcome I received at Einstein! We will work very hard to repay your trust by making all of you very proud of the proteomics we do at the School. The Core is here to assist you and work with you. Please, feel free to contact us to discuss new projects, collaborations and more. I trust this Edition of the Cookbook will not be the last one, as it will grow with all of your new ideas and challenges. Looking forward to work with all of you!

Simone Sidoli

Standard identification and quantification of a protein mixture (in-solution digestion)

Ingredients (reagents)

- Cell lysis buffer: 6 M urea + 2 M thiourea in 50 mM ammonium bicarbonate
- Protease inhibitor cocktail (e.g. Thermo Halt Protease Inhibitor Cocktail, 100X, catalog # 78445)
- 50 mM ammonium bicarbonate, pH 8.0
- Dithiothreitol concentrated stock solution (e.g. 1 M)
- Iodoacetamide, powder
- Trypsin sequencing grade (e.g. Promega, catalog # V5111)

Recipe (protocol)

Estimated preparation time: 3 hours + overnight trypsin digestion

- Collect cells in an Eppendorf tube, or a Falcon tube in case the pellet is more than 50-100 µl. Make sure they have been washed with PBS (otherwise proteins from the media will interfere with the analysis). Recommended amount: 100,000 to 1,000,000 cells

- Mix the lysis buffer with the protease inhibitor cocktail. Add the minimum volume possible of buffer with the cell pellet. You can start from ~5 times the volume of the pellet, e.g. 20 µL of buffer. Pipette up and down to lyse the cells. The solution should become viscous due to the DNA. You can freeze and thaw to assist cell lysis. Do not heat or sonicate, because urea reacts with proteins at high temperatures

- *Optional: If you have a solution excessively viscous, i.e. does not pipette well, you can add 1U of benzonase to digest DNA into individual nucleic acids. Check after 1 hour if the solution is less viscous*

- Dilute sample with 5 times the initial lysis buffer volume using the following buffer: 50 mM ammonium bicarbonate and 5 mM dithiothreitol (DTT). The solution should be at pH 8

- Leave the solution for 1 hour at room temperature. DTT is a reducing agent. This step opens all protein disulfide bonds, which will assist digestion

- Add iodoacetamide to a final concentration of 20 mM, and incubate 30 min in the dark. Try to prepare concentrated iodoacetamide, so you can put a small volume in the final sample. Prepare iodoacetamide fresh right before the incubation, as it is a photosensitive reagent

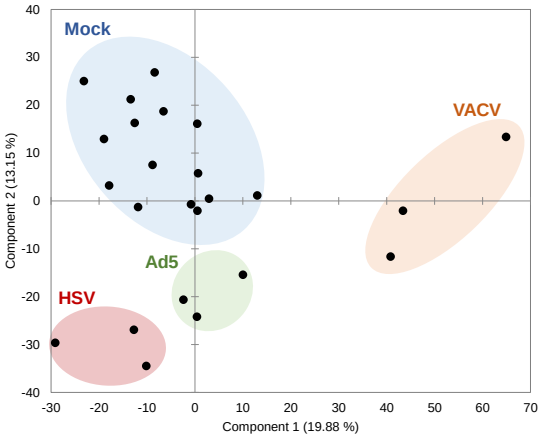
- Add trypsin at an approximate concentration of 1:50 enzyme:sample (w:w) overnight at room temperature. For rapid sample usage, just add 0.1% trifluoroacetic acid (TFA) the following morning; for long term, dry the samples using a SpeedVac centrifuge and store in -80 freezer

Estimated costs

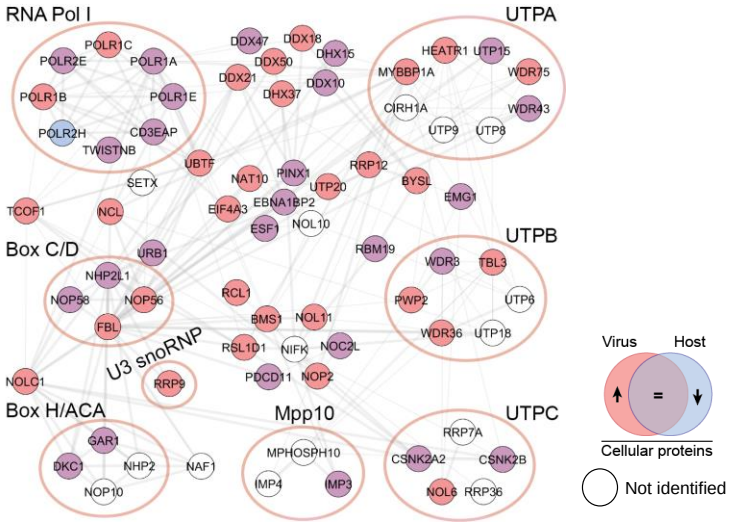
All reagents adopted in this protocol are cost effective and likely present in other labs. Trypsin is about $100 per 100 µg, so the costs should be contained unless you start from enormous starting materials

The Core charges per time. An entire proteome will be run with a 2-3 hours gradient, implying that in a day you can fit around 8 runs. A simpler protein mixture would be run with 1 hour gradient

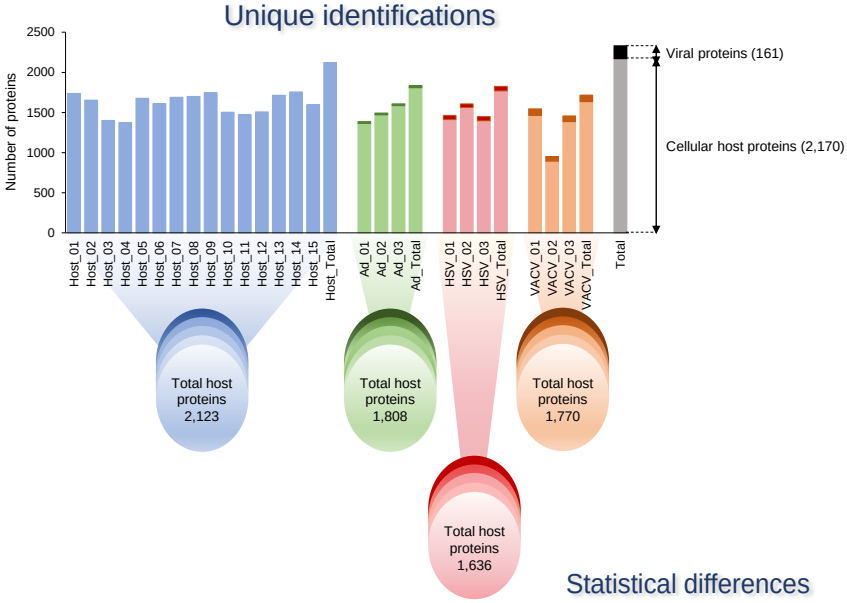
PCA plot – Sample similarity



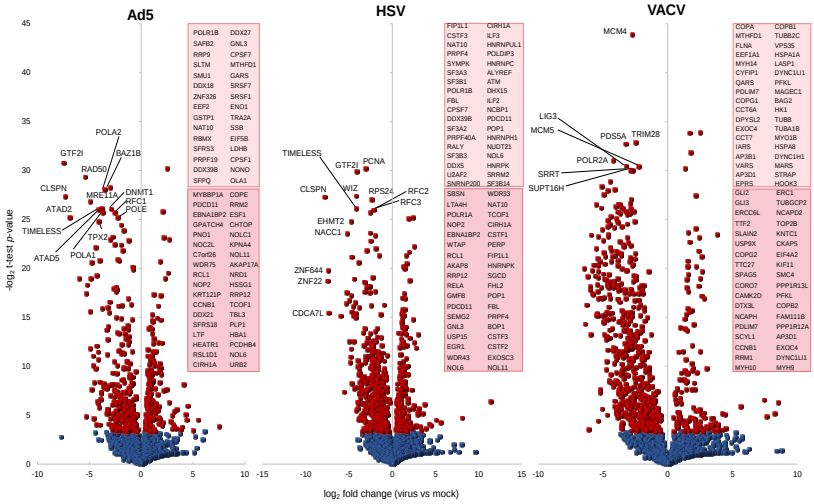
Network of regulated proteins with their abundance (node color)



Unique identifications



Statistical differences



Artwork of Katarzyna Kulej

Identification and quantification of selected gel bands (in-gel digestion)

Ingredients (reagents)

- HPLC grade acetonitrile
- 100 mM ammonium bicarbonate, pH 8.0
- Dithiothreitol concentrated stock solution (e.g. 1 M)
- Iodoacetamide, powder
- Trypsin sequencing grade (e.g. Promega, catalog # V5111)
- 5% formic acid in HPLC grade water

Recipe (protocol)

Estimated preparation time: 4 hours + overnight trypsin digestion

- Cut around the protein band(s). Cut the excised piece into roughly 1 mm^3 cubes and transfer them to a clean 1.5 mL or 0.5 mL tube. *Note: If you cannot see even a very pale band after Coomassie staining, it is unlikely that you will have good results in the analysis*

- Wash the gel pieces with 5 times gel volume using water (~ 100 µL) 10 min on a shaker. Remove and add 50% acetonitrile 10 min. Repeat the operation twice, then shrink with 100% acetonitrile (~ 50 µL)

- Swell the gel particles in 10 mM dithiotreitol/0.1 M NH_4HCO_3 (~ 50 µL) and incubate for 45 min at 56°C. Chill tubes to room temperature. Remove excess liquid and replace it quickly with roughly the same volume of iodoacetamide solution (10 mg/ml (55 mM) iodoacetamide in 0.1 M NH_4HCO_3). Incubate for 30 minutes in the dark at room temperature

- Wash the gel pieces with 5 times gel volume using water (~ 100 µL) 5 min on a shaker. Remove and add 50% acetonitrile 5 min. Repeat the operation twice, then shrink with 100% acetonitrile (~ 50 µL)

- On ice (4°C) rehydrate gel particles in 50 mM NH4HCO3 and 12.5 ng/µL of trypsin. Add enough digestion buffer to cover the gel pieces. (~ 10 µL). Add more buffer if all the initially added volume has been absorbed by the gel pieces. After 45 min remove remaining supernatant and replace it with 5-20 µL of 50 mM NH4HCO3 without trypsin (37°C, overnight)

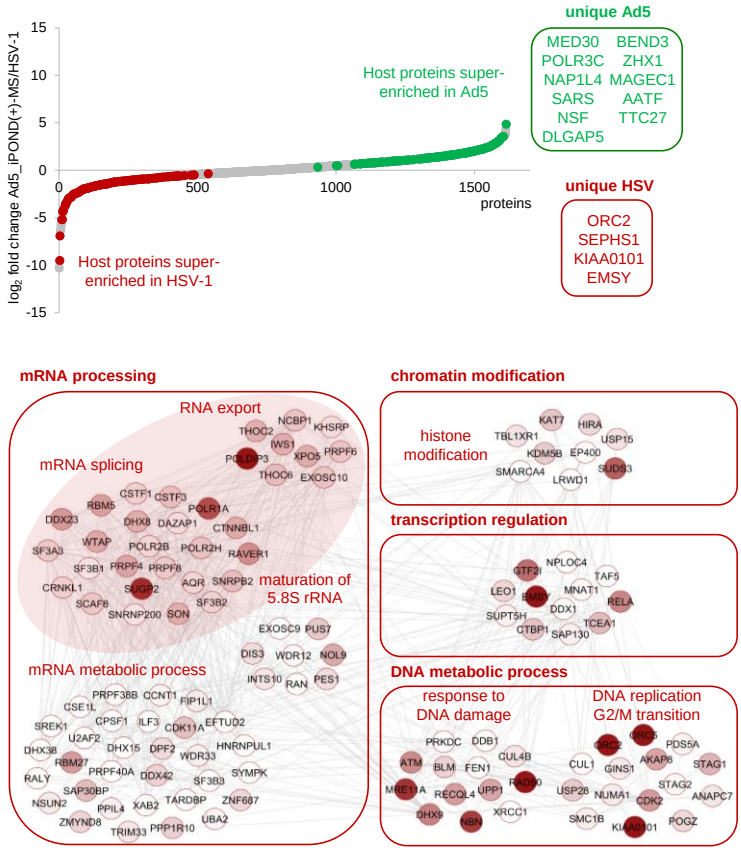
- Transfer supernatant with peptides to a clean 1.5 mL tube. Extract more peptides from the gel by addition enough to cover the gel pieces: 5% formic acid - 15 min (~ 20 µL). Add the same volume of acetonitrile - 15 min. (~ 20 µL). Recover the supernatant. Repeat twice and dry the recovered supernatant in a vacuum centrifuge

Estimated costs

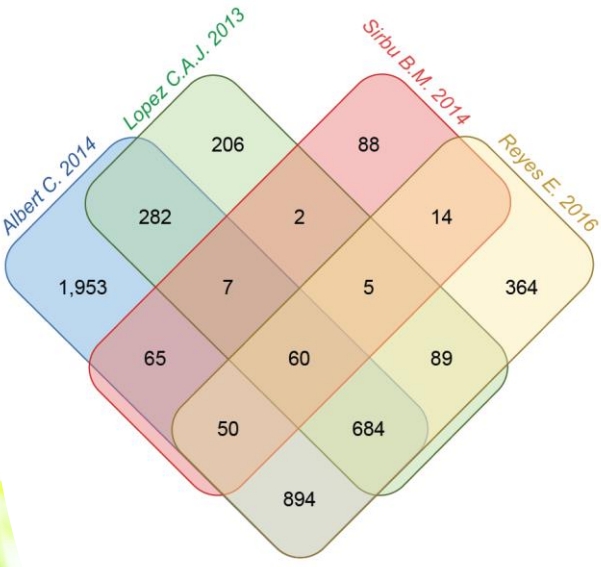
All reagents adopted in this protocol are cost effective and likely present in other labs. Trypsin is about $100 per 100 µg, so the costs should be contained unless you start from enormous starting materials

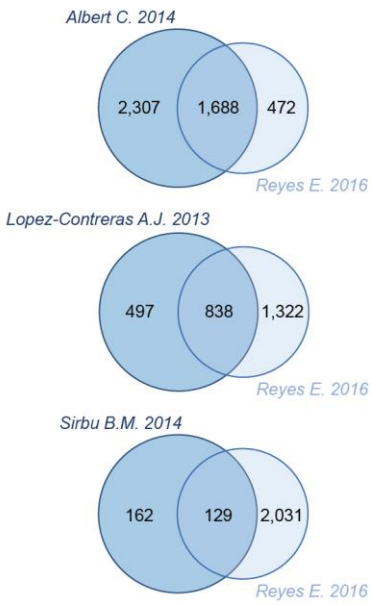
The Core charges per time. A single gel band is a relatively simple mixture, which can run with a gradient of about 1 hour. This implies that in one day you can fit about 15-20 samples

* All figures with in-gel and in-solution digestion are interchangeable



Comparison with existing datasets





Artwork of Katarzyna Kulej

Identification and quantification of proteins in detergents, e.g. immunoprecipitations

Ingredients (reagents)

- S-traps (ProtiFi, https://www.protifi.com/s-trap). Use the "micro" version for <100 µg of proteins
- 12% phosphoric acid
- Loading buffer: 90% methanol, 10 mM ammonium bicarbonate, pH 8.0
- Dithiothreitol concentrated stock solution (e.g. 1 M)
- Iodoacetamide, powder
- Trypsin sequencing grade (e.g. Promega, catalog # V5111) + 50 mM ammonium bicarbonate
- 0.2% formic acid in HPLC grade water

Recipe (protocol)

The protocol is intended for S-trap micro. For larger S-traps, adjust volumes as described in the ProtiFi website (https://www.protifi.com/s-trap)

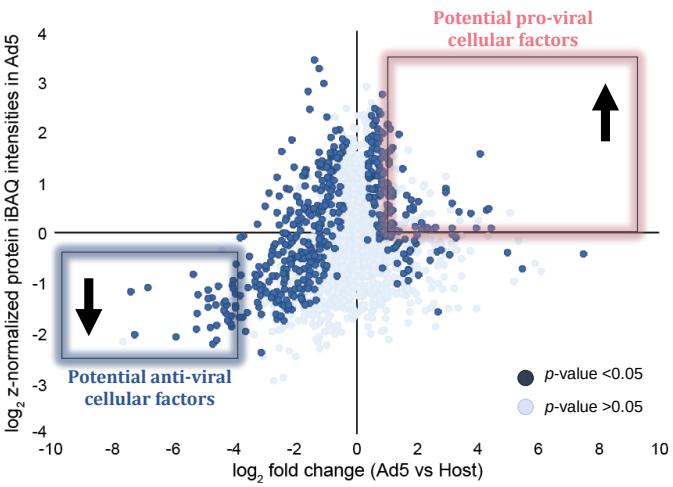
- Reduce and alkylate proteins as in Page 4 (5 mM DTT for 1 hour, followed by 20 mM iodoacetamide or 30 min in the dark). The ideal final volume is 25 µL
- Add to the sample ~12% aqueous phosphoric acid at 1:10 for a final concentration of ~1.2%
- Add 165 µL of Loading buffer to the sample
- Load the sample into the S-Trap micro. Use an Eppendorf tube to hold the S-trap
- Centrifuge gently, until all the volume passed through, e.g. 500 g for 30 sec. Repeat the load if you had a bigger sample volume
- Wash twice with 150 µL of Loading buffer. Centrifuge gently at each wash
- Move the S-Trap into a clean Eppendorf tube for the digestion
- Add 20 µL of trypsin at the concentration of 0.1 µg/µL in 50 mM ammonium bicarbonate. Leave the digestion at least 1 hr at 47°C. Do not shake
- Centrifuge gently
- Elute peptides with 40 µL of 0.2% aqueous formic acid
- Perform a second elution with 35 µL of 50% acetonitrile containing 0.2% formic acid. Pool elutions
- Dry eluted peptides

Estimated costs

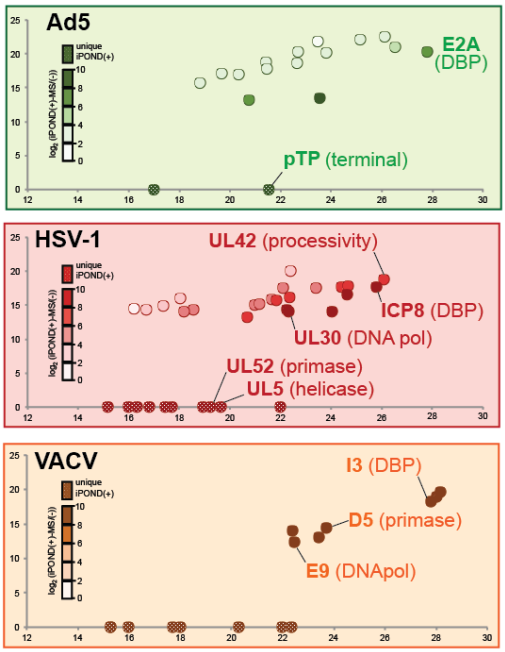
Reagents are all cost effective, but the S-Trap cartridges need to be purchased for SDS-containing samples. The price is about $250 for 40 preparations.

The Core charges per time. An immunoprecipitation is a relatively simple mixture, which can run with a gradient of about 1 hour. This implies that in one day you can fit about 15-20 samples

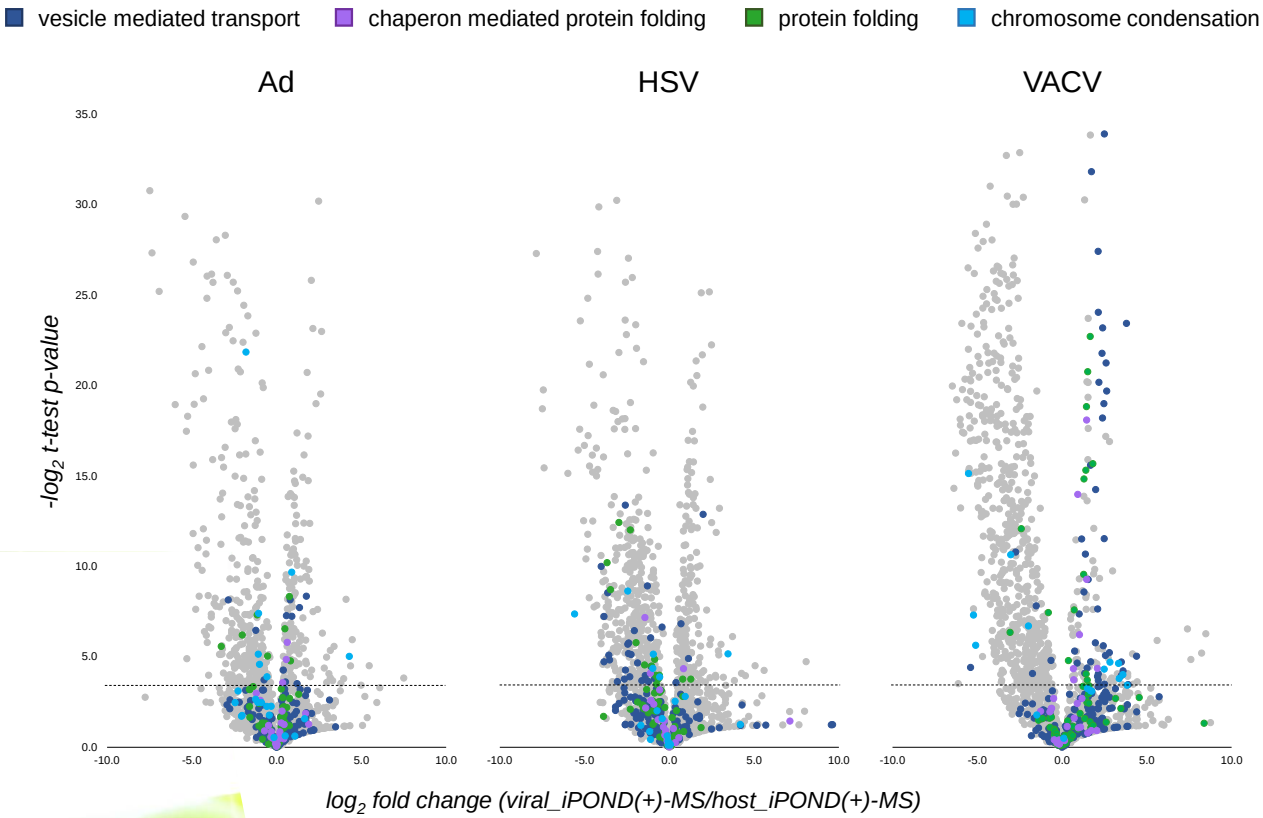
Fold change vs control (x-axis) and protein abundance (y-axis)



Enrichment



Reyes et al. Mol Cell Proteomics (2017)



Artwork of Katarzyna Kulej

Time-resolved proteomics (multiple time points to be analyzed as regulation profiles)

Ingredients (reagents)

- HPLC grade acetonitrile
- 100 mM triethylammonium bicarbonate, pH 8.0
- Dithiothreitol concentrated stock solution (e.g. 1 M)
- Iodoacetamide, powder
- Trypsin sequencing grade (e.g. Promega, catalog # V5111)
- Tandem Mass Tag (TMT) kit (Thermo, catalog # 90061 for 6-plex or # 90406 for 10-plex)

Recipe (protocol)

Please, follow the link below for the detailed protocol

- The protocol is very similar to the canonical in solution digestion described in Page 4. After protein digestion, the different samples are labeled using the TMT kit, and then mixed together to run them as a single sample. This reduces cost and missing values between time points, because peptides need to be identified just once to obtain quantification for all the mixed conditions

http://www.biotech.cornell.edu/sites/default/files/uploads/Documents/Proteomics_protocols/Protocol%2017_TMT%20labeling.pdf

Very important! At the stage of peptide labeling with the TMT kit, you cannot utilize any buffer containing free amine groups. This includes:

- Ammonium bicarbonate (use triethylammonium bicarbonate instead)
- Tris
- Urea
- RIPA

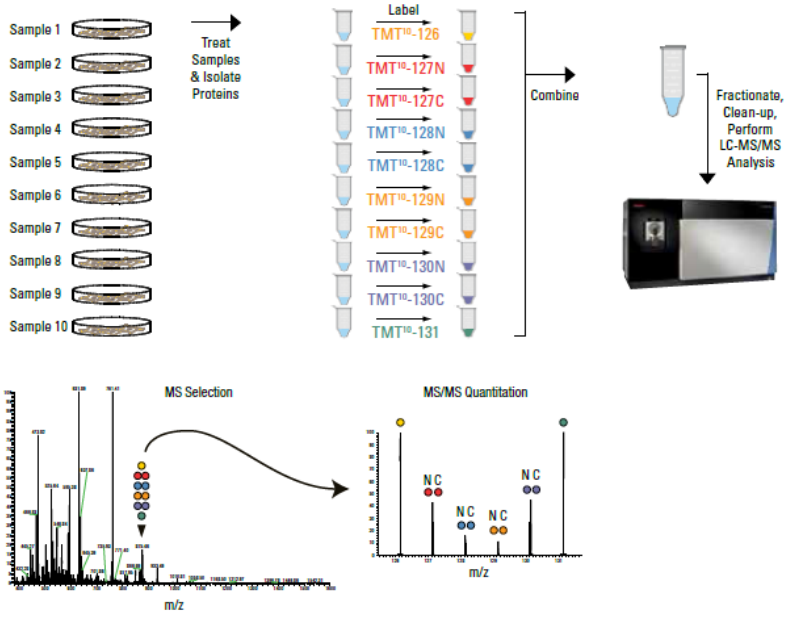
If urea and/or RIPA buffers are needed for e.g. to lyse cells, it is fundamental to desalt the peptides prior labeling. Use for example the C18 cartridges WAT023501 (Waters)

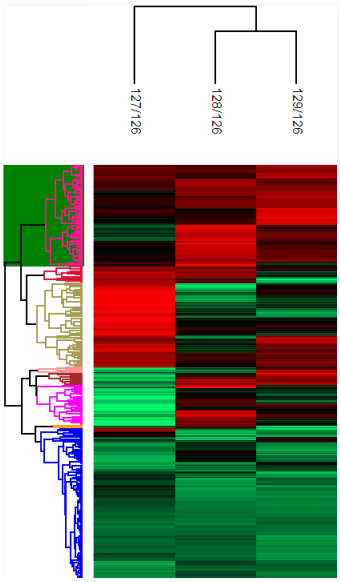
Estimated costs

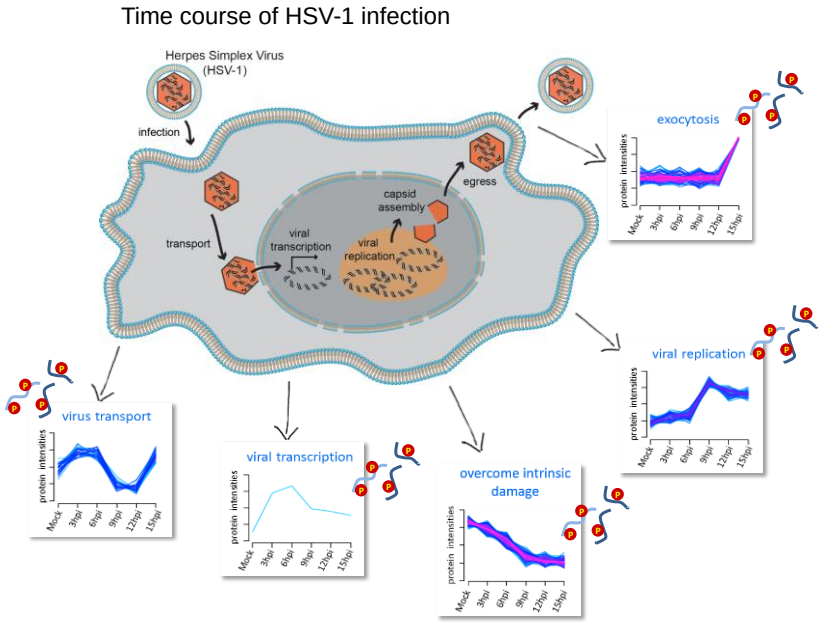
All reagents adopted in this protocol are cost effective and likely present in other labs. The TMT kit is the most expensive; it can vary between $600 and $1,100 depending if you want 6 or 10 time points

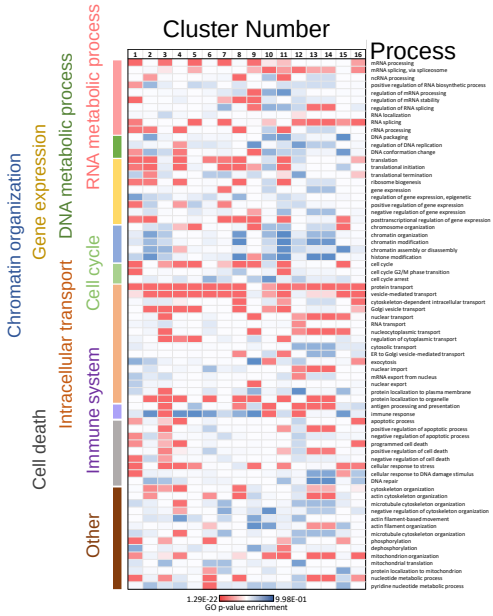
Please, consider that the TMT kit can be aliquoted, but it is not stable for long time once resuspended. Also, consider that all time points are analyzed as a single sample, reducing the overall Core service cost

Commercial description of the method (Thermo Scientific)

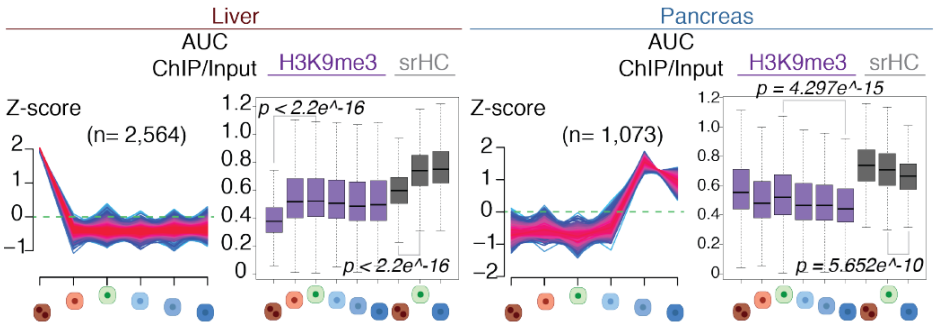








Kulej, Augusti, Sidoli et al. – Molecular and Cellular Proteomics (2017)



Nicetto et al. Science (2019) – Example with RNA-seq data

Comprehensive quantification of histone post-translational modifications

Ingredients (reagents)

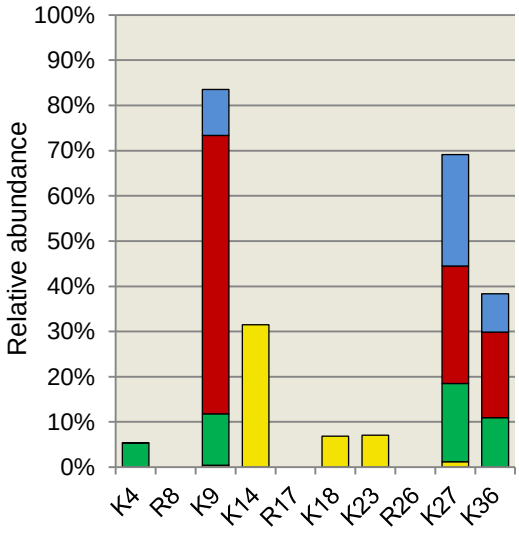
- HPLC grade acetonitrile
- 100 mM ammonium bicarbonate, pH 8.0
- Propionic anhydride
- Ammonium hydroxide
- Glacial acetic acid
- Trypsin sequencing grade (e.g. Promega, catalog # V5111)

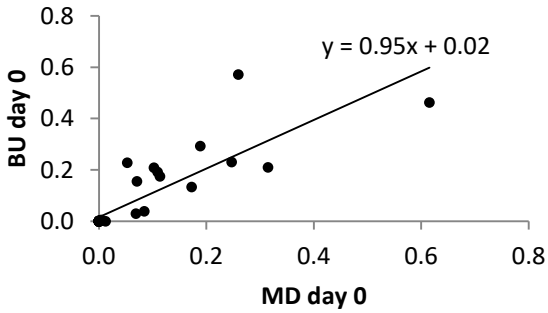
Recipe (protocol)

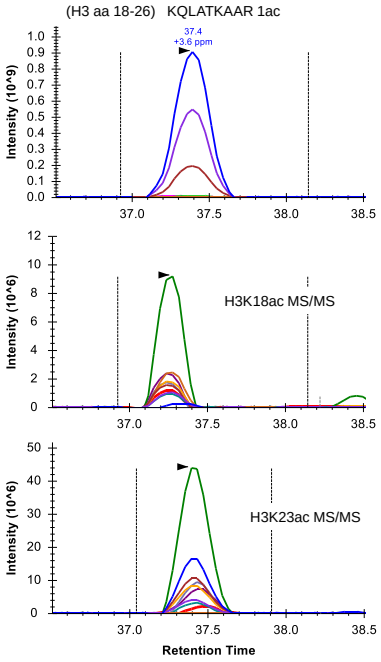
Estimated preparation time: 1 hour + overnight digestion + 1 hour.

The protocol is designed for multi-channel pipettes to process many samples. Obviously, it can also be performed with single Eppendorf tubes

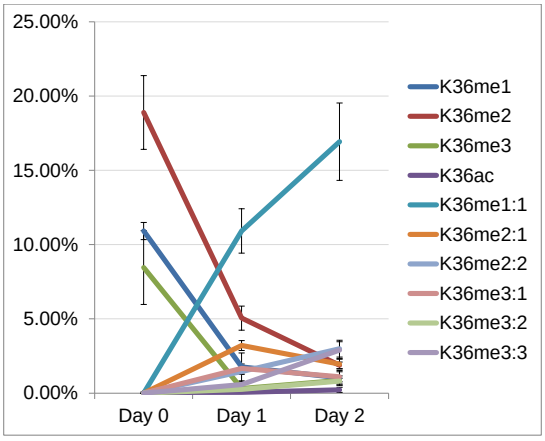
1. Resuspend at least 20 µg of histones in 20 µL of 50 mM ammonium bicarbonate (NH_4HCO_3), pH 8.0. The amount of material does not need to be precise, but do not exceed 100 µg.
2. Place each sample in a well of a 96-well plate (e.g. link). Mark in an excel table which sample is in which well.
3. Add 5 µL of acetonitrile in each well using a multi-channel pipette (8 or 12 channels). Solutions can be poured into a polypropylene reservoir (e.g. link).
4. (Under the hood) Propionylate histones by adding 2 µL of propionic anhydride (e.g. link) rapidly followed by 9 or 10 µL of ammonium hydroxide (NH_4OH). Pipette up and down a few times just with the NH4OH pipette to mix the reaction.
5. By using a multi-channel pipette, dip into one row or one column with P10 pipette tips (no need to aspirate). Touch with the tips a pH indicator strip. NH_4OH and glacial acetic acid can be used to adjust the pH to around 8.0. If the result seems ambiguous (different pH across samples), test more rows/columns and adjust the pH.
6. Wait 10-15 min.
7. Repeat steps 4-6 (step 4 under the hood).
8. Dry plate(s) in a SpeedVac centrifuge. They will likely not dry completely, because of the viscosity of the propionic anhydride. There is no need to leave them more than 2 hours.
9. Resuspend trypsin (e.g. link) to a concentration of 25 ng/µL in 50 mM NH_4HCO_3 and pour it into the reservoir. Add 30 µL of solution in each sample with the multi-channel pipette. Pipette up and down a few times to eliminate the viscosity of the residual liquid in the bottom of the wells.
10. Check the pH as step 5. This is important! In acidic pH trypsin is not active. For extra safety, it is suggested to check the pH again after e.g. 1 hour of trypsin incubation.
11. Wait overnight or at least 6 hours.
12. Repeat steps 3-8.



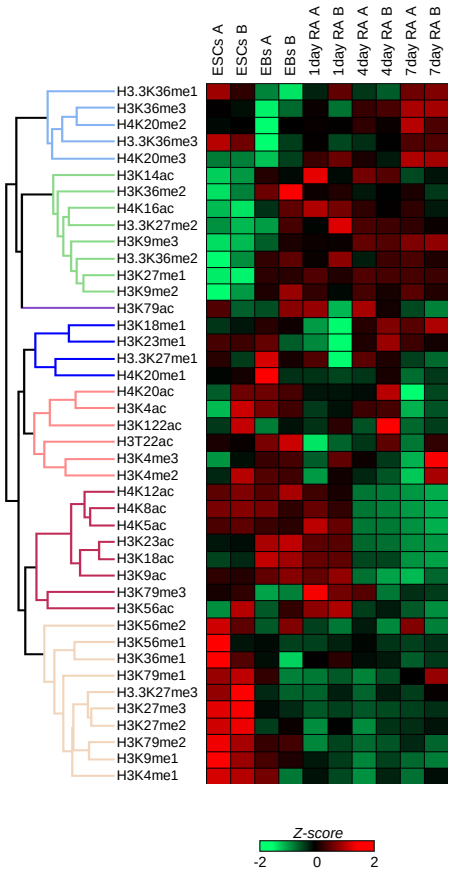




Sidoli et al. – Analytical Chemistry (2017)



Sidoli et al. – Epigenetics & Chromatin (2017)



Gonzales-Cope, Sidoli et al. – BMC Genomics (2016)

Quantification of synthesis/degradation rate of proteomes using pulsed metabolic labeling

Ingredients (reagents)

- Media for cell growth requires additional lysine and arginine with stable isotopes (13C and 15N)
- All the other reagents are the same as Page 4

Recipe (protocol)

Follow the same protocol as Page 4

This procedure provides a complementary quantification dimension to protein abundance, which is the turnover rate of the protein synthesis/degradation. A protein might not change much its abundance between two conditions, but it might be expressed and degraded faster/slower.

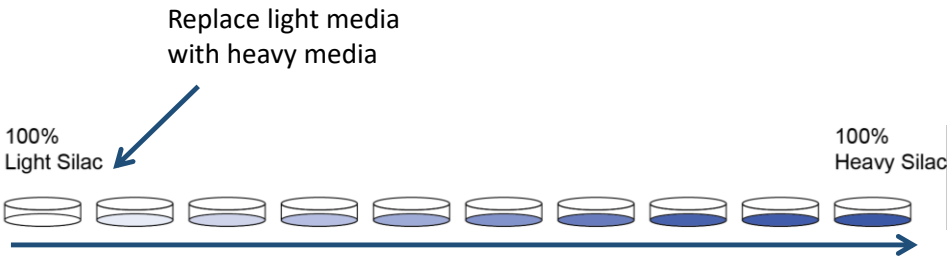
To obtain this information, proteins should be only “partially labeled”, meaning that a complete incorporation of labeled amino acids should not be achieved. To obtain this effect, we recommend labeling proteins for an interval of time corresponding to 1-2 cell cycles

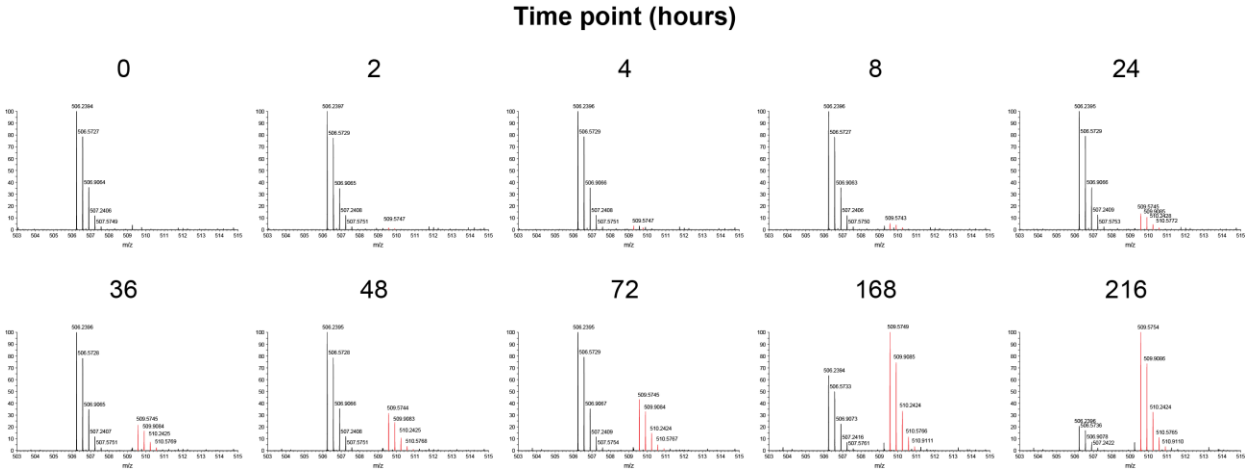
Estimated costs

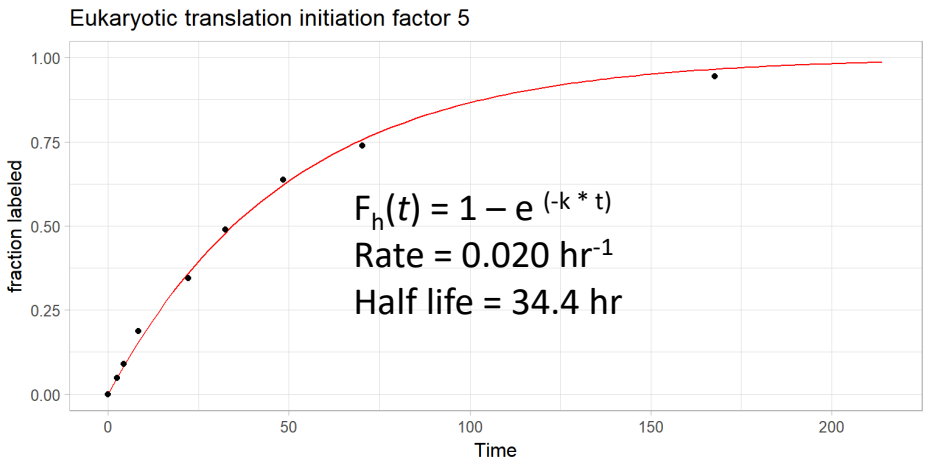
All reagents adopted in this protocol are cost effective and likely present in other labs. Trypsin is about $100 per 100 µg, so the costs should be contained unless you start from enormous starting materials

The Core charges per time. A single gel band is a relatively simple mixture, which can run with a gradient of about 1 hour. This implies that in one day you can fit about 20 samples

Heavy arginine and lysine are more expensive than the canonical ones. The cost is highly dependable on the volume where the cells are grown into. Consider the amount of material required to minimize the costs


Replace light media
with heavy media
100%
Light Silac
100%
Heavy Silac


Time point (hours)
0
2
4
8
24
36
48
72
168
216


Eukaryotic translation initiation factor 5
fraction labeled
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
Time
$F_h(t) = 1 - e^{(-k * t)}$
Rate = 0.020 hr^{-1}
Half life = 34.4 hr

Protein – RNA interaction analysis to identify nucleic acid binding domains of proteins

Ingredients (reagents)

- Canonical proteomics reagents (Page 4)
- 4-Thiouridine (4sU), e.g. Sigma-Aldrich catalog # T4509
- UV light
- Benzonase or RNAse A to digest RNA on peptides
- Cell culture to incorporate 4sU in the nascent RNA

Recipe (protocol)

Please, follow the protocol described in this manuscript:

https://www.jove.com/video/56004/sample-preparation-for-mass-spectrometry-based-identification-rna

An application of this workflow can be found at:

https://www.ncbi.nlm.nih.gov/pubmed/27768875

The Proteomics Core will then assist you with data processing, statistics and interpretation

Estimated costs

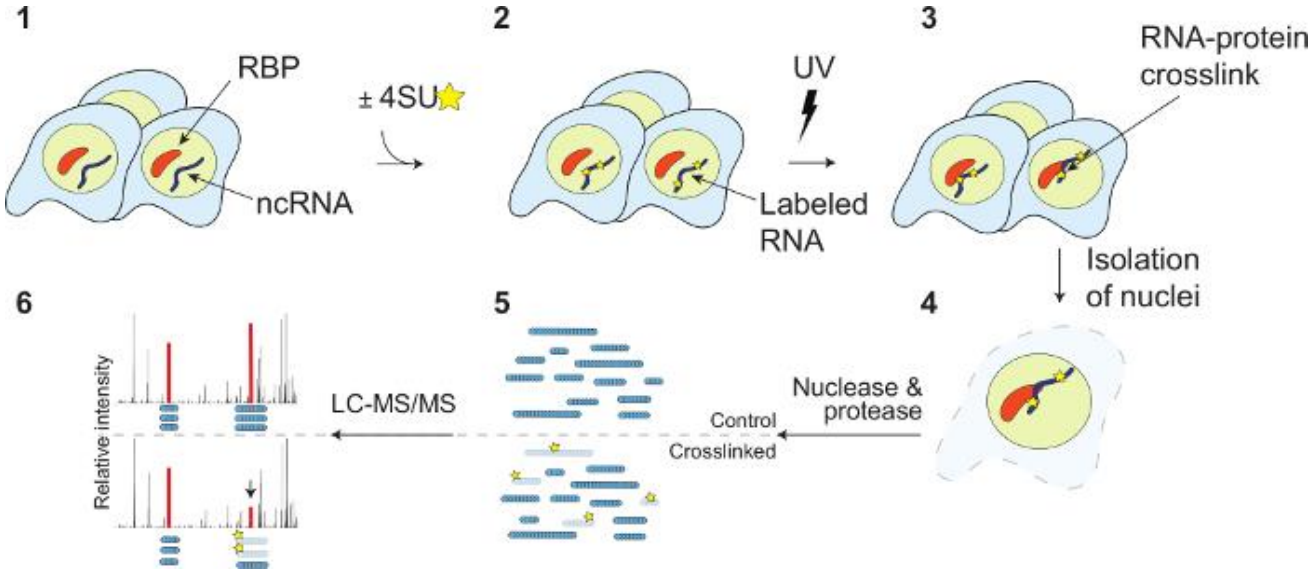
All reagents adopted in this protocol are cost effective and likely present in other labs. Trypsin is about $100 per 100 µg, so the costs should be contained unless you start from enormous starting materials

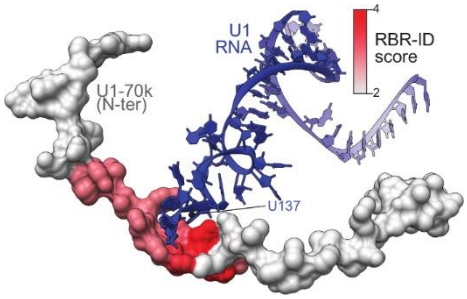
The Core charges per time. An entire proteome will be run with a 2-3 hours gradient, implying that in a day you can fit around 8 runs. A simpler protein mixture would be run with 1 hour gradient

4-thiouridine is about $170 for 25 mg

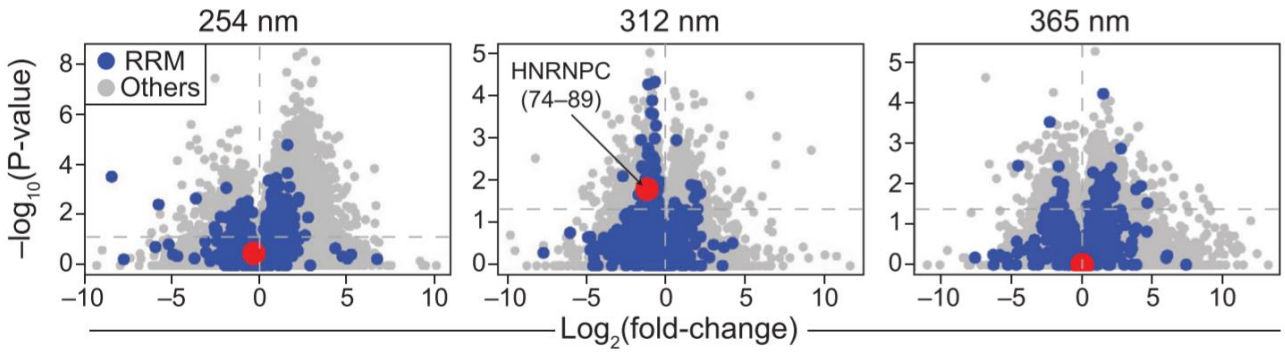
The cost of a UV-B lamb can be below $1,000

Protocol (RBP = RNA binding protein)

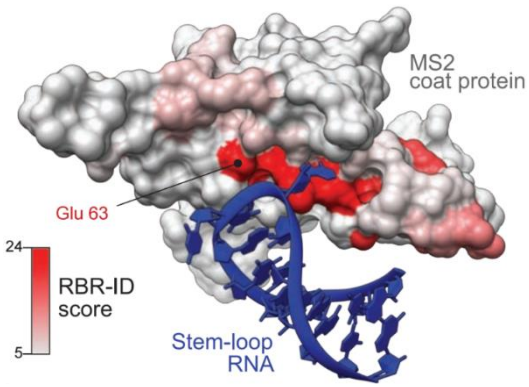




Warneford-Thomson, He, Sidoli et al. JoVE (2017)



He, Sidoli et al. Molecular Cell (2016)



Identification of protein complexes associated on the chromatin (ChIP-SICAP)

Ingredients (reagents)

- Canonical proteomics reagents (Page 4)
- Other reagents described in the link

Recipe (protocol)

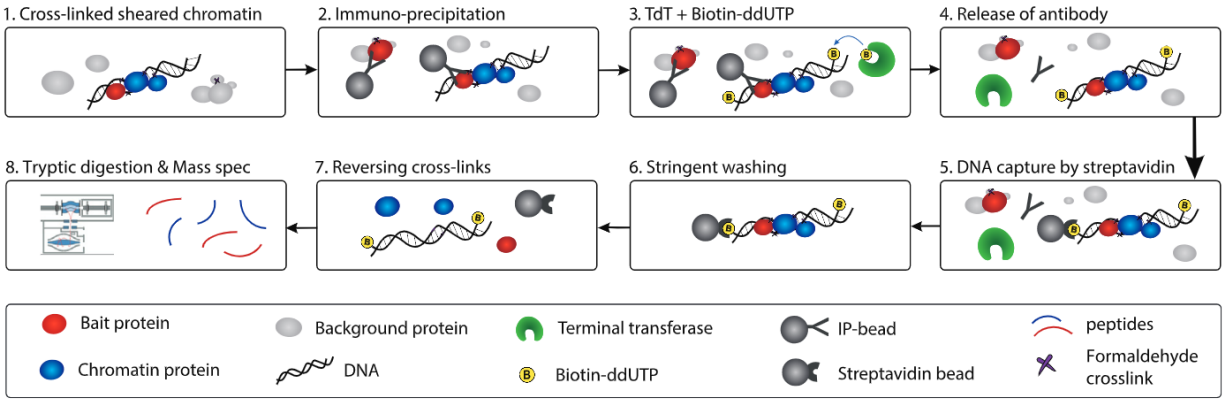
This protocol allows for the identification of protein-protein interactions specifically on chromatin. The protocol can be found at the following publication:

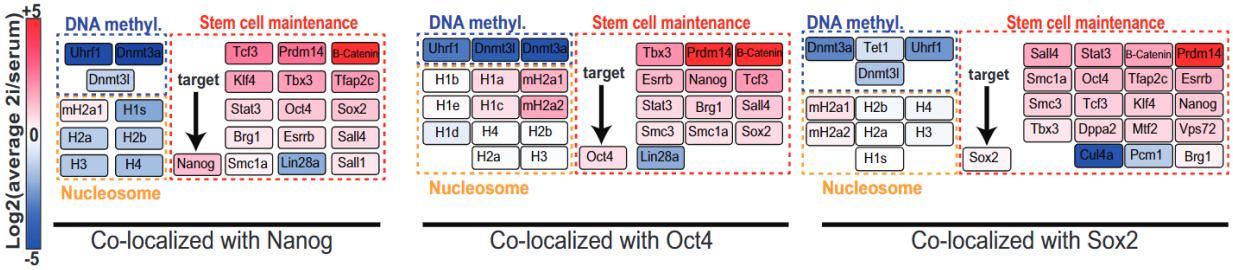
https://www.cell.com/molecular-cell/pdfExtended/S1097-2765(16)30568-8

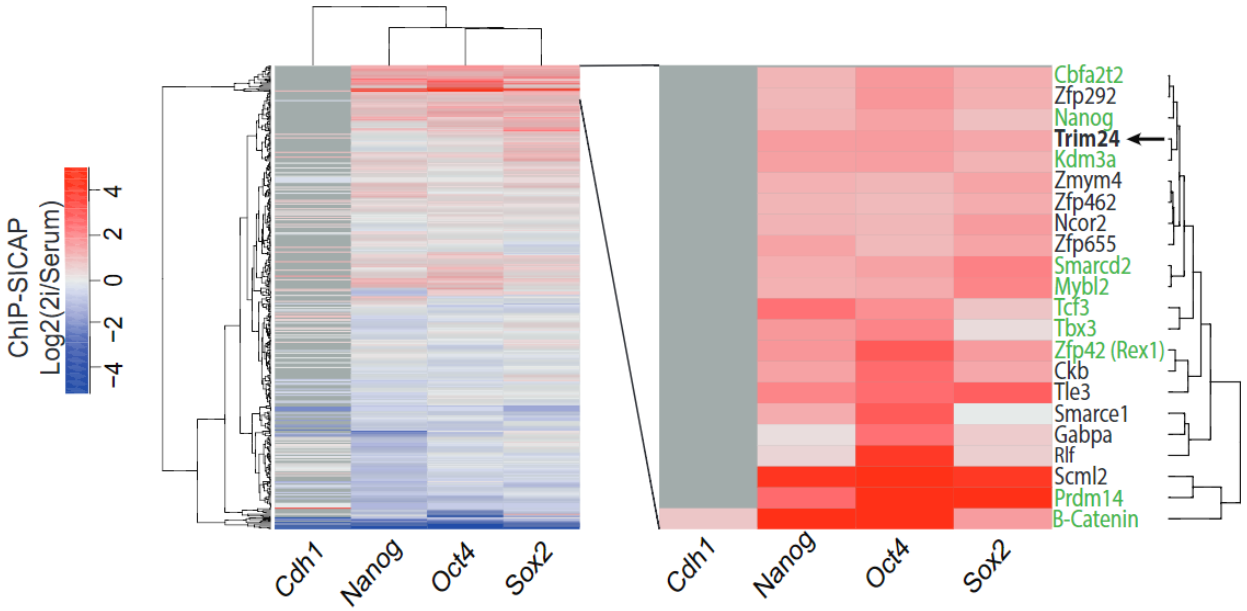
Please, consider a learning curve and optimization for this procedure. The Proteomics Core is glad to assist with guidance and edits to the protocol.

Estimated costs

N/A







Rafiee et al. – Molecular Cell (2016)

Estimation of the turnover rate of post-translational modifications

Ingredients (reagents)

- Please, see next page for the list of metabolites that can be used to label newly synthesized post-translational modifications
- The rest of the reagents are the same as described at Page 4

Recipe (protocol)

Follow the same protocol as Page 4

This procedure provides a complementary quantification dimension to the abundance of post-translational modifications, which is their turnover rate. A modification might not change much its abundance between two conditions, but it might be catalyzed and removed faster/slower.

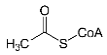
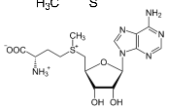
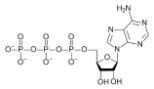
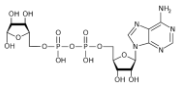
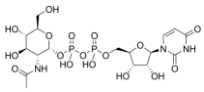
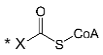
To obtain this information, modifications should be only "partially labeled". Please, see next page to have an approximate idea about the catalysis speed of different modifications.

To obtain a real complete estimation of the turnover rate of a modification, it is necessary to perform a normalization by the turnover rate of the protein itself. For estimating the turnover rate of a protein, please see the protocol at Page 14

Estimated costs

All reagents adopted in this protocol are cost effective and likely present in other labs. Trypsin is about $100 per 100 µg, so the costs should be contained unless you start from enormous starting materials

The Core charges per time. An entire proteome will be run with a 2-3 hours gradient, implying that in a day you can fit around 8 runs. A simpler protein mixture would be run with 1 hour gradient

ac	acetylation		acetyl-CoA	min – hours
me	methylation		S-adenosylmethionine (SAM)	hours – days
ph	phosphorylation		ATP	sec – min
Ar	ADP-ribosylation		ADP ribose	???
Og	O-GlcNAc		UDP-N-Acetylglucosamine	hours – days
suc/pr/bu/cr hib/bhb/ma/glu	other acylations (succinylation, *propionylation, butyrylation, crotonylation, hydroxyisobutyrylation, β-hydroxybutyrylation, malonylation, glutarylation*)		* acyl-CoA	min – hours?

Simithy, Sidoli and Garcia – Proteomics (2018)

Sidoli et al. – Epigenetics & Chromatin (2017)

Phosphoproteomics

Ingredients (reagents)

- Loading buffer: 80% Acetonitrile, 5% TFA and 1 M Glycolic acid (76 mg/mL)
- Washing buffer 1: 80% Acetonitrile, 1% TFA
- Washing buffer 2: 20% Acetonitrile, 0.2% TFA
- Elution buffer: 40 µL Ammonia solution (28%) in 980 µL H_2O, pH 11,3
- Titanium Dioxide (TiO_2) beads, e.g. GL Sciences catalog # GL-5010-21315

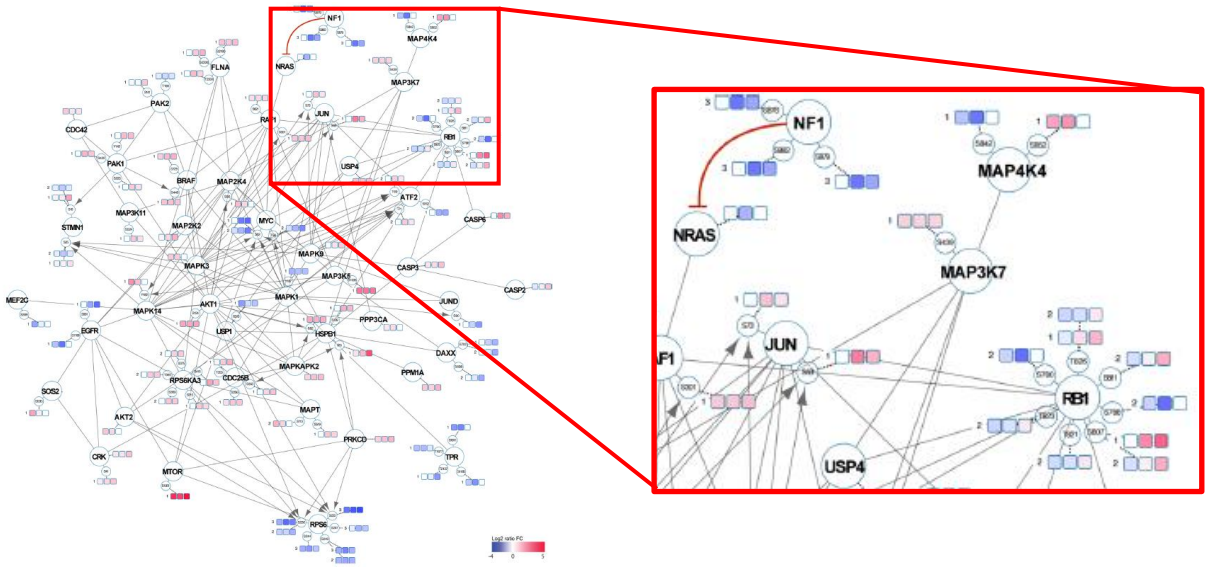
Important reminder

Please, ensure that cell lysis and follow-up steps up to trypsin digestion are performed on ice (when possible) and with the presence of phosphatases inhibitors (e.g. Thermo catalog # 78426)

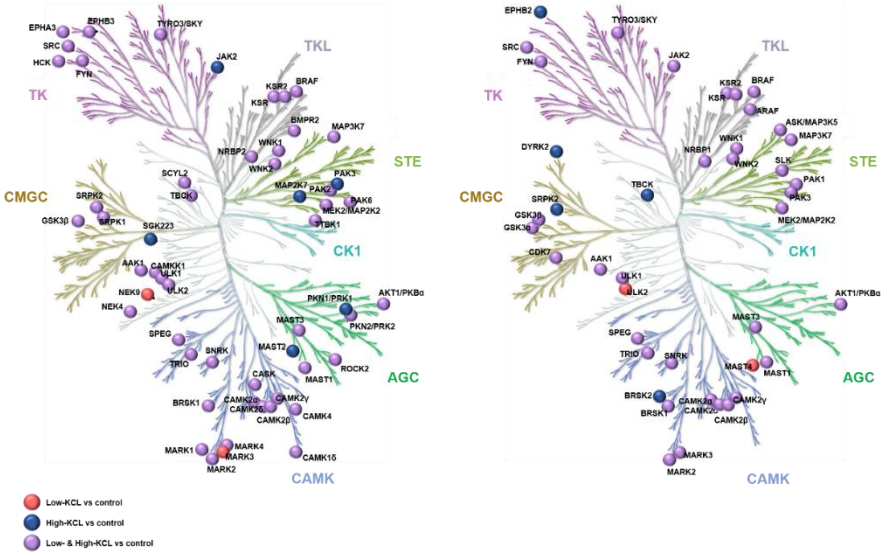
Recipe (protocol)

Follow the same protocol as Page 4 to obtain peptides from your cells. Then:

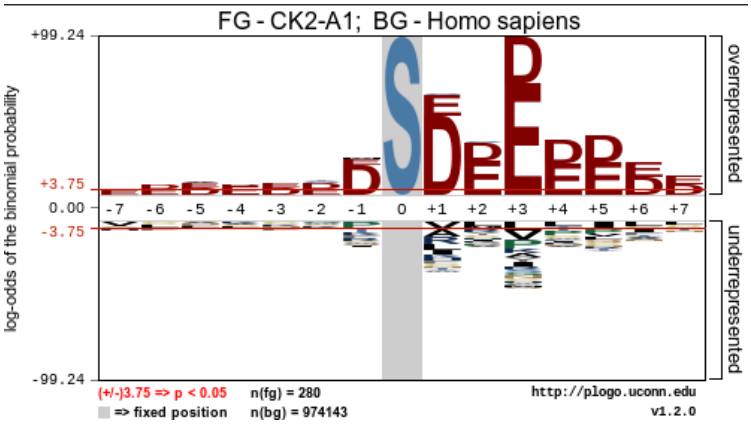
- Dilute your peptide solution at least 10 times with loading buffer (alternatively if you have 100 µL sample you can add 50 µl water, 50 µL 100% TFA, 800 µL Acetonitrile and 76 mg Glycolic acid to make the sample up to the proper loading buffer)
- Add 0.6 mg TiO_2 beads per 100 µg peptide solution
- Place the tubes on the shaker (highest shaking) at room temperature for 5-10 min
- Centrifuge to pellet the beads (table centrifuge <15 sec)
- Remove the supernatant
- Wash the beads with 70-100 µL loading buffer - mix for 15 sec, transfer to another eppendorff tube and then centrifuge to pellet the beads (the transfer is performed due to the fact that peptides and phosphopeptides stick to surfaces and can be eluted in the last elution step)
- Wash with 70-100 µL washing buffer 1 - mix for 15 sec and then centrifuge to pellet the beads. Remove supernatant.
- Wash with 50-100 µL washing buffer 2 - mix for 15 sec and then centrifuge to pellet the beads. This step is important to remove peptides that bind in a HILIC mode to TiO_2. Remove supernatant.
- Dry the beads for 5-10 min in the vacuum centrifuge or on the table.
- Elute the phosphopeptides with 50 µL Elution buffer – mix well and leave the solution and the beads for 5-10 min to allow an efficient elution
- Centrifuge the solution for 1 min, recover the supernatant (as it contains the phosphopeptides) and dry it in a SpeedVac centrifuge



PhosphoPath: Raaijmakers et al. J. Proteome Research (2015)



Kinome Render: Chartier et al. PeerJ (2013)



Motif-X: O'Shea et al. Nature Methods (2013)