sliceSILAC : a short explanation

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References :

SILAC : (Ong et al., 2002)

sliceSILAC : (Morikawa et al., 2014) http://www.ncbi.nlm.nih.gov/pubmed/?term=23929719

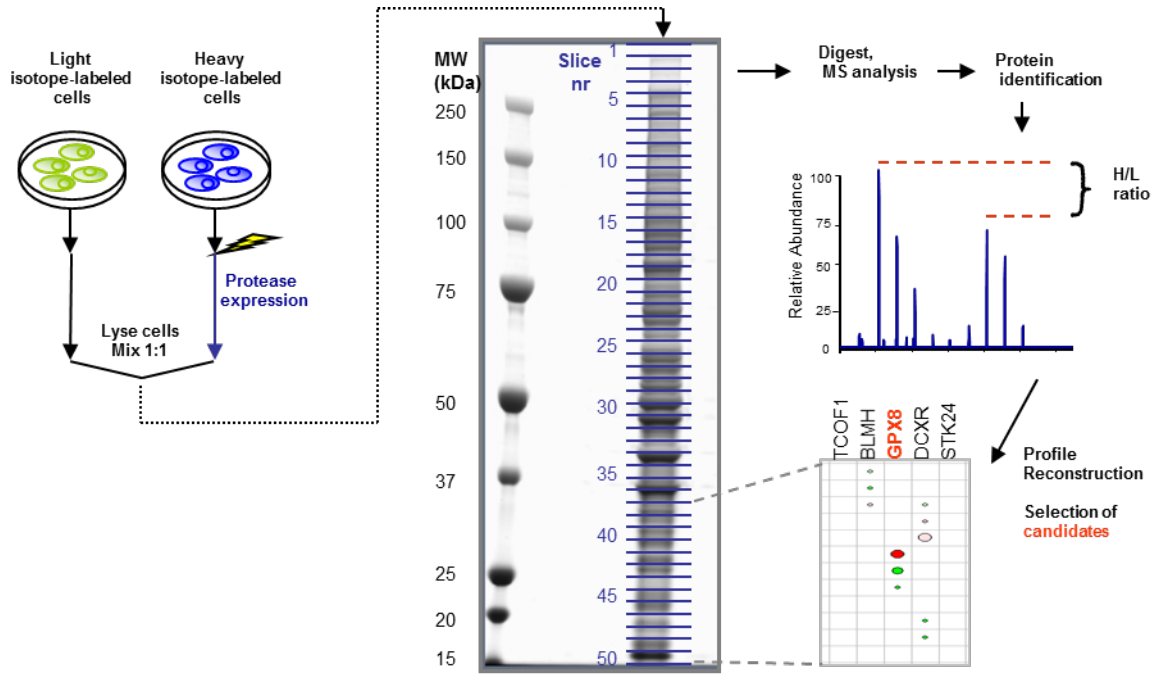
sliceSilac is a variant of a normal SILAC comparative analysis. Most of the times, biologists want to know how total levels of a maximum of proteins change following a treatment, a knockdown etc. For this, SILAC labelling is used, in conjunction with trypsin digestion and mass spectrometry.

However powerful, these techniques, it is usually necessary to fractionate very complex proteomes to decrease the complexity of individual samples submitted for MS analysis. This can be done at the protein level before trypsin digestion, or it can also be done at the peptide level after digestion.

Separation of proteins by electrophoresis prior to trypsinolysis is often used to reduce complexity. After individual MW ranges are digested and MS analysed, data are pooled to obtain total protein quantification data.

However, if the focus of the study is rather on protein modifications, it can be interesting to exploit the information on electrophoretic migration of each protein, in addition to the SILAC quantification ratio.

Here is the general principle of sliceSILAC (Figure 1) :



The lane is cut into a certain number of identical size slices, which are then all digested and MS analysed. The idea is that for every protein identified in every slice, a SILAC ratio H/L is measured, which reflects the amounts of this particular protein isoform in the two samples. Therefore it is possible to find out if this particular isoform with a precise migration property has changed between the two conditions. In addition, it is possible to monitor the overall presence of a given protein at all molecular weights, producing a sort of virtual, MS-based western blot. Very often indeed, proteins are in fact detected in several slices and the combination of the migration patterns with the SILAC ratios generates a large, complex dataset. We developed some tools to select and visualize proteins corresponding to patterns of interest, based on specific hypotheses. First the table is parsed to look for proteins that are,for example, present in at least two different slices with ratios of opposite sign. Then the SILAC ratio is represented in the colour of the "bubbles" shown for each protein in each slice in which the protein was found.

Example : proteolysis (Fig.1)

Electrophoretic migration is thought to reflect primarily molecular weight. Any event that induces a shift in gel migration is potentially detectable by sliceSILAC, although the number of slices cut somewhat limits the resolution. Inducible expression of a protease is the classical case that is expected to cause cleavage and thus decrease in mass of several proteins, and such shifts can be detected by sliceSILAC. Other PTM's that induce MW changes are Ubiquitination, glycosylation and in some cases phosphorylation. In most of these cases sliceSILAC does not provide conclusive proof of which modification is causing the shift (although some information on cleavage sites and sequence coverage can be obtained) but can lead to identification of plausible candidates.

Quantity matters

When analysing for example cleavage events it is important to have an idea if the bulk of a protein is cleaved or only a minor fraction, since this will have an impact on the ratios that can be expected for the full length and the cleaved fragments. Luckily, some information is available in the output files in the form of a parameter called "Ratio count". This gives a measure on how quantifications have been successfully determined for peptides associated to a given protein. This parameter is important to estimated the reliability of the quantitation (the higher "Ratio count" is, the better) but it also gives a measure of the abundance of the protein form detected in a given gel slice. In our visualization mode, the "Ratio count" parameter is reflected in the size of the bubbles (it is however maxed out above a certain level).

It is best to have a clear hypothesis

SliceSILAC datasets are very rich and contain a wide variety of patterns. Systematic interpretation of all phenomena observed is virtually impossible. It is essential to have a clear starting hypothesis at the beginning of the analysis.

An example of a partial sliceSILAC output image (for a group of 20 proteins, *Fig. 2*); the legend specifies the parameters used for visualization. It is a good idea to perform filtering of the table with

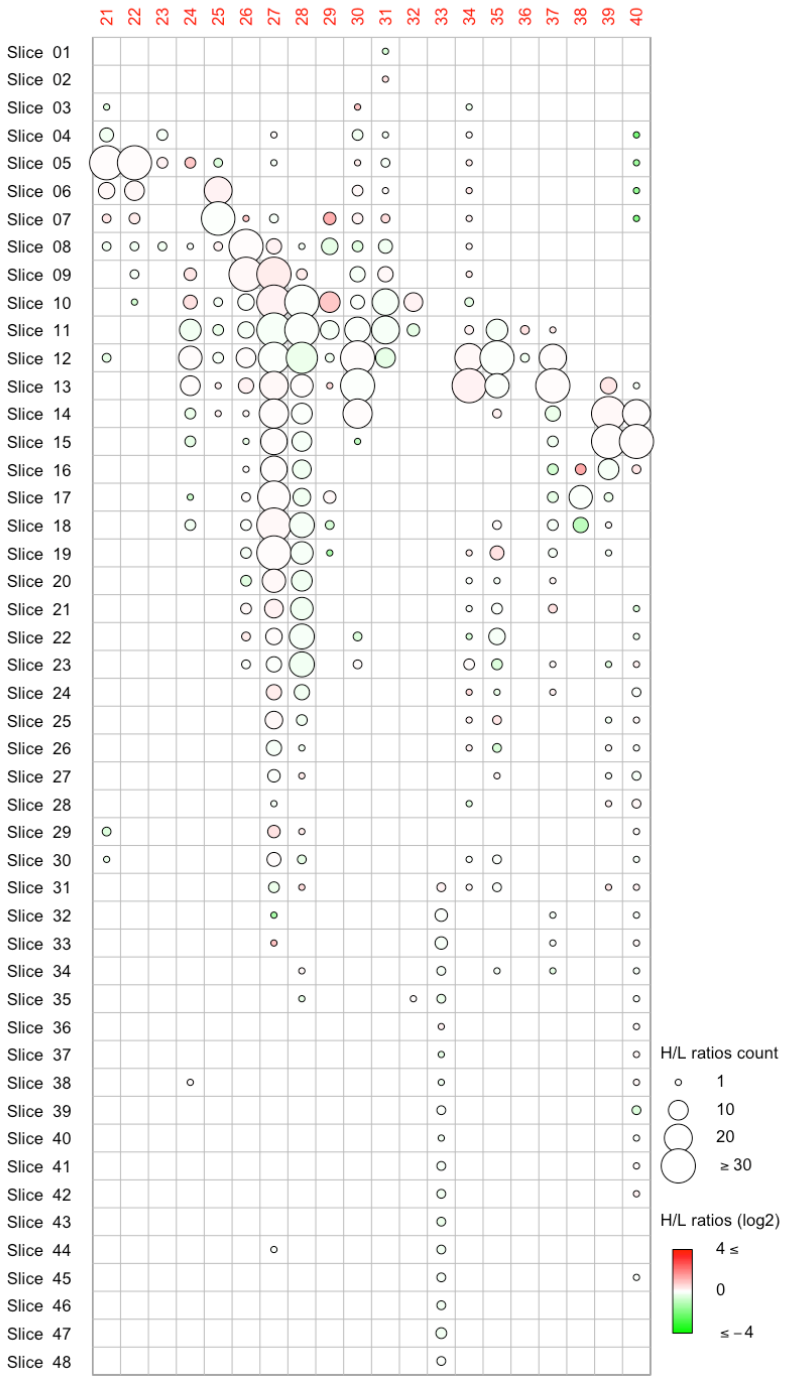
at least two sets of threshold for minimal changes of different stringency, to make sure to cover a maximum of observable phenomena. For example, one can filter as follows :

a) Select all proteins satisfying the following filter :
    a. present in at least two gel slices,
    b. with log2(H/L) value >0.5 for one slice AND
    c. with log2(H/L) value <-0.5 for another slice AND
    d. a minimum ratio count =2 in both slices (minimum quality of quantitation and amount of material)

A slightly different filter could be the following :

b) Select all proteins with:
    a. present in at least two gel slices,
    b. with log2(H/L) value >0.2 for one slice AND
    c. with log2(H/L) value <-0.2 for another slice AND
    d. a minimum ratio count =4 in both slices (minimum quality of quantitation and amount of material)

Figure 2 : output from filtering and visualization of proteins satisfying the filter



21 Chromodomain-helicase-DNA-binding protein 4
22 Nuclear pore complex protein Nup214
23 YLP motif-containing protein 1
24 Host cell factor 1; ...
25 Nuclear pore membrane glycoprotein 210
26 Eukaryotic translation initiation factor 3 subunit A
27 Carbamoyl-phosphate synthase [ammonia], mitochondrial
28 ATP-dependent RNA helicase A
29 Neural cell adhesion molecule L1
30 Splicing factor 3B subunit 3
31 Plasma membrane calcium-transporting ATPase 1
32 Integrin alpha-2
33 POTE ankyrin domain family member E; ...
34 ATP-citrate synthase
35 Vinculin
36 Abelson tyrosine-protein kinase 2
37 Poly [ADP-ribose] polymerase 1
38 Dynamin-like 120 kDa protein, mitochondrial; ...
39
40 Exportin-2

Slice SILAC vs. global protein ratio output :

SliceSILAC results are produced by MaxQUant, the same software that is used for general SILAC quantitation. The MaxQuant output table proteinGroups.txt contains both the slice-by-slice H/L ratios used to generate the images as well as a global ratio for every protein. The latter is calculated by pooling all the peptide ratios measured in all gel slices, to try to give a global measure of whether a protein is increased or decreased. There can be discrepancies between the global ratio and the values for individual protein isoforms corresponding to various gel bands. Here for example 2 cases which reflect the considerable complexity of protein processing behaviors.

1) Global level of a protein stays the same, but levels of individual isoforms change. In this case, the global H/L ratio will be close to 1.0, but two or more forms of the protein can be detected as green and red bands, indicating an interconversion among isoforms

2) A protein is cleaved and the fragment is unstable and is rapidly degraded. Since cleaved fragments are not detected in the gel, such a phenomenon appears as a global downregulation. Although biologically an interesting situation, the exact mechanism must be confirmed by other means

Given the multiple possibilities that exist it is important to consider global SILAC ratios along with sliceSILAC data to make sure all possible interesting cases have been considered.

References

Morikawa, K., Gouttenoire, J., Hernandez, C., Dao Thi, V. L., Tran, H. T. L., Lange, C. M., … Morikawa, Kenichi; Gouttenoire, Jerome; Hernandez, Celine; Dao Thi, Viet Loan; Tran, Huong; Lange, Christian; Dill, Michael; Heim, Markus; Donze, Olivier; Penin, Francois; Quadroni, Manfredo; Moradpour, D. (2014). Quantitative proteomics identifies the membrane-associated peroxidase GPx8 as a cellular substrate of the hepatitis C virus NS3-4A protease. *Hepatology (Baltimore, Md.)*, *59*(2), 423–33. doi:10.1002/hep.26671

Ong, S.-E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., & Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Molecular & Cellular Proteomics : MCP*, *1*(5), 376–86. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12118079