SILAC experiments – guidelines and protocols

Overview

SILAC (Stable Isotope Labelling with Amino acids in Culture) is used for comparative, quantitative proteome analysis in mammalian cultured cells. It bases on the metabolic incorporation into proteins of different stable isotope labeled essential amino acids (AA).

Such AAs have different molecular masses due to the presence of isotopes of carbon (^{13}C) and nitrogen (^{15}N) heavier than the "normal" ones (^{12}C, ^{14}N). This results in the production of "heavy" proteins in a labeled culture, which are then mixed 1:1 with "light" proteins from an unlabelled culture before analysis. Processing and measuring samples together ensures a maximum of reproducibility and accuracy of quantitation.

For reasons linked to the requirements of mass spectrometric analysis, the amino acids used are mainly Lysine (K) and Arginine (R). After trypsin digestion this results in a majority of peptide fragments carrying one C-terminal labeled AA.

Delta mass	0 ("Light")	+4	+6	+8	+10
Lys (K) isotope label	^{12}C,^{14}N	^{2}H4	^{13}C6	^{13}C6,^{15}N2	--
Arg (R) isotope label	^{12}C,^{14}N	--	^{13}C6	--	^{13}C6,^{15}N4

Variants of labels available for SILAC

Duplex vs Triplex : the availability of various isotope labeled variants of AAs makes it possible to mix and compare two (Heavy : Light) or three samples (Heavy : Medium : Light), each coming from a corresponding culture. Although triplex analysis is important for some experiments, it also results in an increasing in sample complexity and somewhat lower performance of protein ID and quantification.
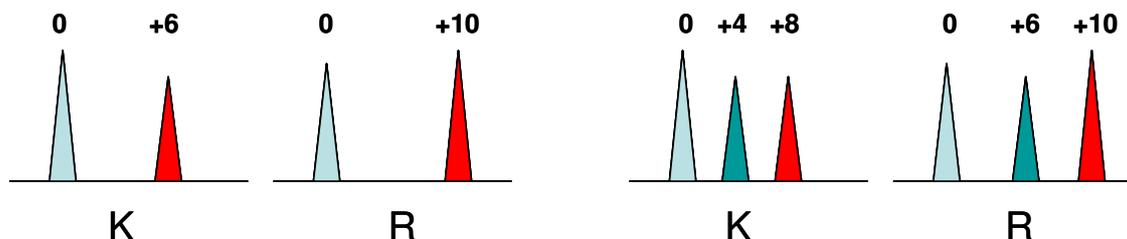
*Schematic mass difference patterns obtained for K- and R-containing peptides in **duplex** (**left**) vs. **triplex** (**right**) SILAC labeling*

Cell culture and labeling : principles and requirements

Labelling is performed with essential amino acids, i.e. AA's that the cells should not be able to synthesize themselves. Ideally, labeling should be near to 100% to obtain clear discrimination of the signal originating from each sample. This poses some requirements on the cell culture medium employed:

    i)   a special formulation medium is necessary which lacks the labeling AAs. These are added later according to desired labeling,

    ii)   dialysed FBS (dFBS) has to be used since standard FBS also contains unlabelled (light) AAs in unknown concentrations.

The second point especially can be problematic for some cell types. Indeed, dFBS lacks (poorly defined) low-mw factors that are important for the growth or phenotypes of some cells. Preliminary tests are necessary.

To obtain near-100% isotope labeling at least 5 or more cell divisions in the SILAC labeling medium are usually required. Labeling levels should be checked by MS before proceeding with experiments (see below).

Partial labeling or use of standard FBS : recently, we have successfully tried to perform SILAC experiments in systems in which labeling is not complete (e.g. primary cell cultures that divide only 1-2x) or using standard FBS instead of dFBS, by using a triplex SILAC approach in which the "L" channel is disregarded. This is has also been reported by other groups (Imami et al). However greater care is required in setting up such experiments, and some restrictions apply (for example, cells to compare should have the same growth rate).

Refs and web links

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

Mann, M. (2006). Functional and quantitative proteomics using SILAC. *Nature reviews. Molecular cell biology*, *7*(12), 952-8. doi:10.1038/nrm2067

Imami, K., Sugiyama, N., Tomita, M., & Ishihama, Y. (2010). Quantitative proteome and phosphoproteome analyses of cultured cells based on SILAC labeling without requirement of serum dialysis. *Molecular bioSystems*, *6*(3), 594-602. doi:10.1039/b921379a

http://en.wikipedia.org/wiki/Silac

http://www.silac.org/

Reagents

1) Media
We can provide you with SILAC-grade RPMI 1640; for all other SILAC media please refer to commercial suppliers (http://www.piercenet.com/browse.cfm?fldID=03020501)

2) dFBS
We keep stocks of frozen, filtered dFBS that we can provide you at low cost.

3) Isotope labeled AAs
We bulk purchase and stock frozen isotope labeled AAs that we can provide you at low cost. We only give you the amount you need, so you do not have to purchase an excess of these.

Practical Steps

1) define growth medium to use and experimental design ; pay attention to what you want to compare with what.

2) Prepare media (L/H or L/M/H) and test growth of cells in SILAC medium. For efficient labeling, wash cells before passage and dilute cells at least once 10x or more

3) If possible, test biological stimulation or desired phenotype of cells grown in SILAC medium. Does is correspond to what is observed in normal medium ?

4) Grow cells for at least 4-5 cell divisions in heavy medium ; prepare a cell extract (at least 50 ug protein) in a buffer compatible with SDS gel electrophoresis and bring it to us. We will run a gel, cut a standard protein (usually actin) and run MS to assess the labeling attained. It should be >90% at this point. We also test Arg to Pro conversion, an interfering phenomenon observed in some cell lines.

5) If everything is OK, proceed with your experiment

Sample preparation

For total proteome analysis please refer to our other protocol "FASP_desalting_offgel.doc" for cell lysis and protein extraction. For a full proteome profiling at least 300 ug of protein / sample are necessary, at a concentration of about 5 ug/ul.
Rule of thumb : 10xe6 human cultured cells lysed in 100 ul FASP buffer should give at least 700 ug protein at 7 ug/ul.

Otherwise sample preparation is largely experiment-dependent.