



Proteomics

Silantes SILAC Media

Stable Isotope Labelling of Amino Acids in Cell Culture

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SILAC (Stable Isotopic Labelling of Amino Acids in Cell Culture) (Ong et Mann, 2006) has been proven as a powerful technique for quantitative proteomics in cell culture. The method is robust and provides accurate results (Ong et al., 2002).

The procedure in short:

Cells are grown in culture media that are identical with exception of amino acids. The “light” culture (L) contains all the amino acids in unlabelled form and the “heavy” culture (H) contains one or two amino acids in labelled form. The labelled amino acids are usually lysine labelled with ^2H (D4), ^{13}C or $^{13}\text{C}^{15}\text{N}$ and/or arginine labelled with ^{13}C or $^{13}\text{C}^{15}\text{N}$. The labelled amino acids increase the molecular weight allowing identification and quantification of the proteins/peptides stemming from culture (L) and (H), respectively.

Workflow:

Fig. 1 shows the work flow of the procedure. In order to determine quantitatively differences in the protein pattern of culture (L) and (H),

- the cells from both cultures are mixed;
- the proteins are isolated, then cleaved into peptides (by either LysC or trypsin), and finally
- subjected to LS-MS analysis.

Silantes SILAC-Media-kits:

Each kit consists of

- 2x500mL DMEM media with all amino acids except lysine and arginine;
- 2x50mL FBS, dialyzed;
- Lys(0) and Arg(0) for preparing culture (L);
- Lysine and arginine in all isotopic combinations for preparing culture (H). (See product list)

Biological competence of the labeled amino acids:

A growth kinetics of MDCK cells on Silantes SILAC media is shown in Fig. 2 using different stable isotopically labelled lysine, namely:

- Lys (2) ^{15}N -labelled lysine;
- Lys (4) ^2H (D4)-labelled lysine;
- Lys (6) ^{13}C -labelled lysine;
- Lys (8) $^{13}\text{C},^{15}\text{N}$ -labelled lysine.

Similar results were obtained with labelled arginine (data not shown).

Time course of Lys(6) enrichment in a HeLa cell line.

Fig. 3 shows that the cell culture is fully labelled after 4 passages.

Literature:

Ong, S.E., and Mann, M. (2006). A practical recipe for stable isotope labelling by amino acids in cell culture (SILAC). Nat. Protoc. 1, 2650–2660.

Ong, S.E., Blagoev, B., Kratchmarova, I., Kristensen, D.B., Steen, H., Pandey A., and Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics, Mol. Cell Proteomics 1, 376–386.

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Fig. 1 Work flow of the SILAC- approach

Stable isotope labeling of amino acids in cell culture (SILAC)

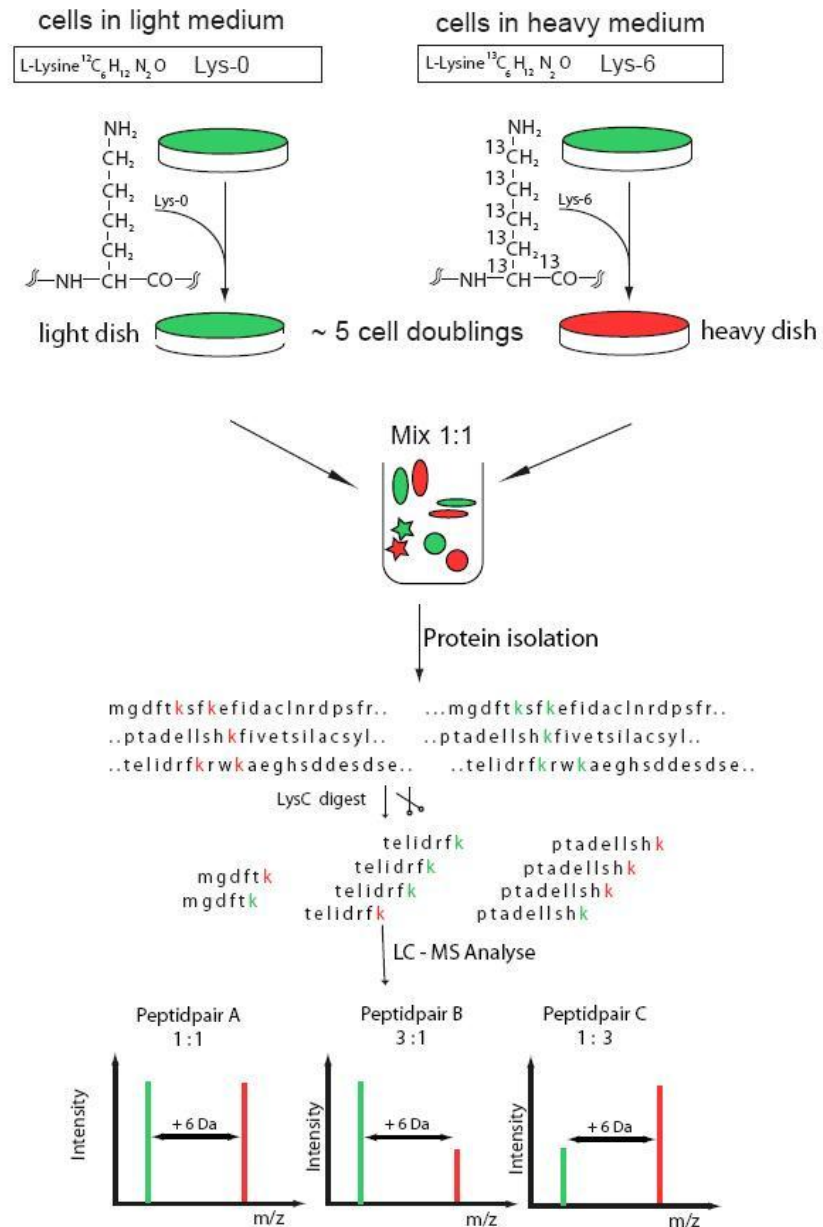


Fig. 1 shows that cells are grown in cell cultures A (light) and B (heavy), whereby culture B is labeled with e.g. ^{13}C -lysine by metabolic enrichment. This is achieved by preparing a cell culture medium without lysine. The medium of cell culture A is supplemented with $^{12}\text{C}_6$ -lysine (L) and that of B with $^{13}\text{C}_6$ -lysine (H). Cells from both cultures are mixed in a 1:1 ratio. The proteins of interest are extracted and digested with LysC, a protease which specifically cleaves at lysines. The proteolytic cleavage creates pairs of peptides stemming from culture A and B, differing by a molecular weight of 6Da due to the molecular weight difference on end standing $^{13}\text{C}_6$ -lysine. The ratio of the amount of light and heavy peptides is determined by mass spectrometry

Fig. 2 Growth curves for MDCK cells using lysines with different labels

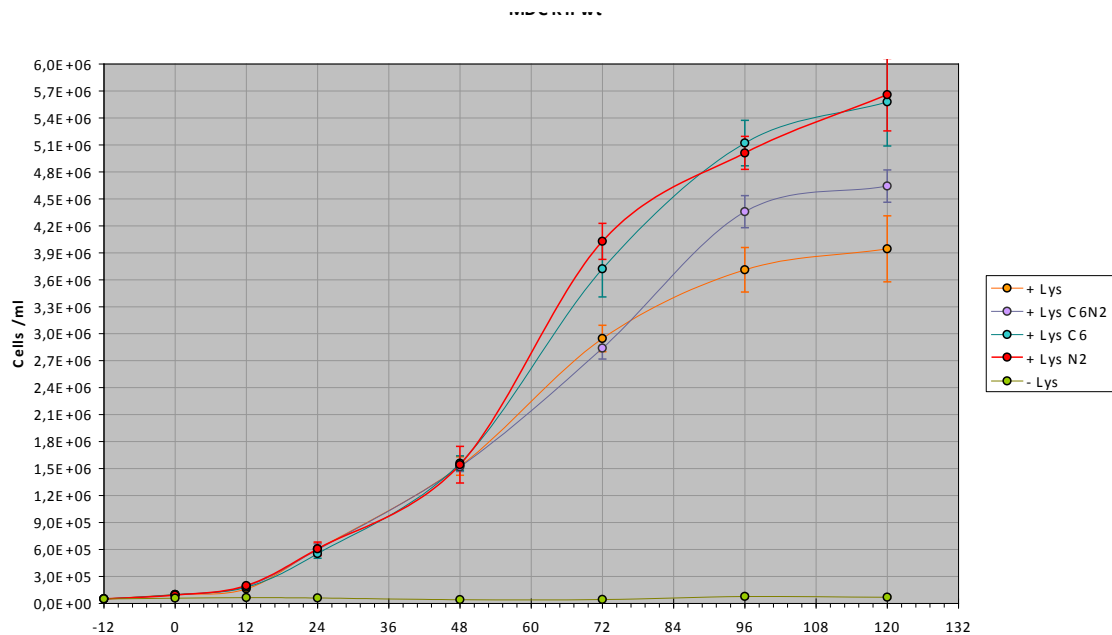


Fig. 2 shows that the Silantes SILAC media are competent for growing cells.

Fig. 3 Time dependent incorporation of $^{13}\text{C}_6$ -lysine

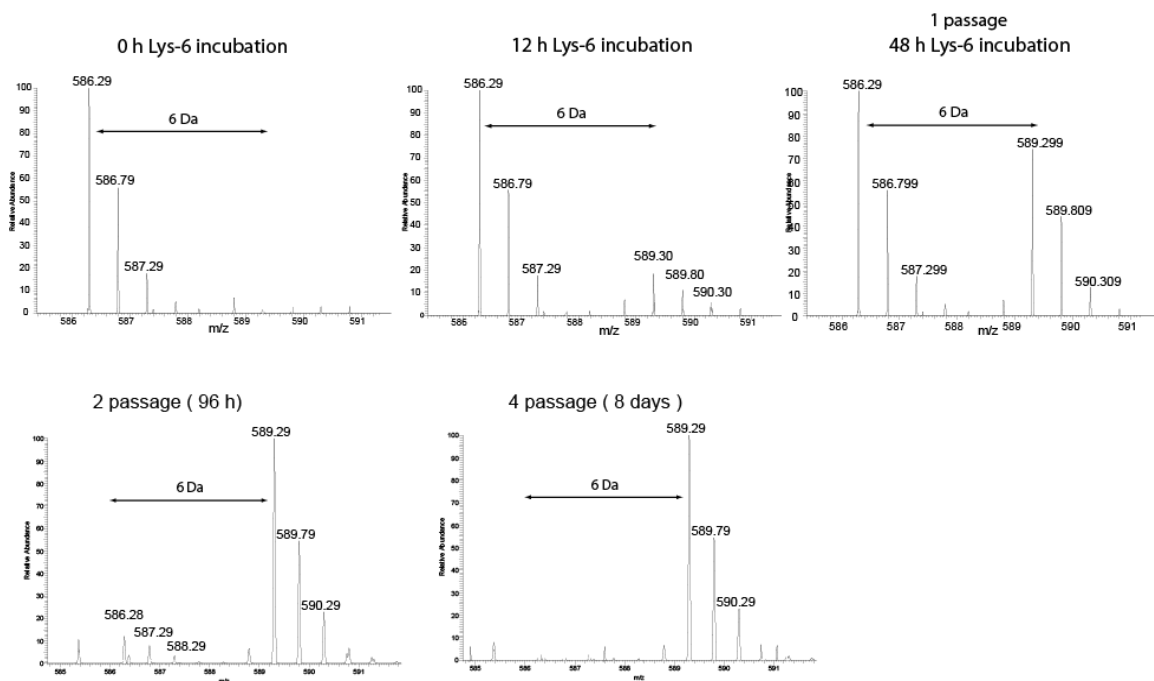


Fig. 3 Time-dependent incorporation of $^{13}\text{C}_6$ -lysine in an actin peptide with a molecular mass of 586Da. Comparing the time course of the 586Da peak stemming from unlabelled actin peptide and the 589Da peak stemming from the corresponding labelled actin peptide indicates that the cell culture is fully labeled after 8 days (4 passages). That the nominal difference of the peaks is 3Da (and not 6Da) is due to the fact that the ratio m/z (x-coordinate) is 2 (instead of 1). The correctness of this statement is demonstrated by the fact that the topoisomers e.g. the 586.29Da peak and 586.79Da peak are 0.5.