Nascent protein profiling

Method	Label	Cell Specific?	Genetic Modification Necessary	Already Applied in	Enrichment of Labeled Proteins Possible	Time Scale and Applications	References
Stable isotope labeling of amino acids in cell culture (SILAC)	heavy isotope containing amino acids	no	no	wide range of cell lines and model organisms	no	5 doubling times to achieve complete labeling of a proteome. Pulsed labeling possible but number of identifications is compromised	[1,4–13]
Bioorthogonal labeling of amino acids in cell culture (BONCAT)	methione analogues AHA or HPG	no	no	wide range of cell lines and model organisms	yes (covalent capture with click chemistry)	short pulses (down to minutes) and subsequent enrichment of newly synthesized proteins. Prolonged labeling possible	[14–17,23–32]
Cell specific BONCAT	biorthogonal amino acids that require a modified tRNA sythetase (azidonorleucine or p-azido-L-phenylalanine)	yes	yes (mutated tRNA synthetase)	cell lines, worm, fly	yes (covalent capture with click chemistry)	short pulses (down to minutes) and subsequent enrichment of newly synthesized proteins. Prolonged labeling is possible but dependent on the system side effects are possible	[18–20,33–37]
Stochastic orthogonal recoding of translation (SORT)	bioorthogonal amino acid in combination with an orthogonal tRNA and tRNA synthetase	yes	yes (mutated tRNA synthetase and tRNA)	cell lines, fly, mouse brain	yes (covalent capture with click chemistry)	short pulses (down to minutes) and subsequent enrichment of newly synthesized proteins. Prolonged labeling is possible. Many codons can be tagged	[21,39–41]
O-propargyl-puromycin labeling (OP-Puro)	Puromycin analogue (OP-Puro) binding to nascent polypeptides	yes	yes (penicillin G acylase)	cell lines	yes (covalent capture with click chemistry)	very short labeling (minutes). Provides a snapshot of actively translated proteins in a cell	[48,49]
isotopic labeling of amino acid precursors (CTAP)	heavy isotope containing lysine	yes	yes (lysine synthesizing enzymes)	cell lines	no	labeling comparable to SILAC. Cell specific labeling of cells in co-culture	[44–46]
GFP -labeling and sorting	GFP	yes	yes (GFP)	cell lines, unicellular organisms, mouse	sorting of labeled cells with FACS	steady state proteome of a subpopulation of cells	[59,60]
proximity-dependent biotin identification with a promiscous biotin ligase (BioID)	biotin	yes	yes (promiscous biotin ligase fused to protein of interest)	cell lines, unicellular organisms	yes (affinity purification with streptavidin)	proximity labeling of interacting proteins	[51–53]
biotinylation with sequence specific biotin ligase BirA	biotin	yes	yes (BirA and Avi tagged protein of interest)	cell lines, wide range of model organisms	yes (affinity purification with streptavidin)	biotinylation of tagged proteins only in cells expressing BirA. Purification of interacting proteins	[50]
	biotin-ubiquitin	yes	yes (Avi tagged Ubiquitin in fusion with BirA ligase)	cell lines, fly, mouse	yes (affinity purification with streptavidin)	biotinylation of ubiquitin and enrichment of ubiquitinated proteins	[62–65]
labeling with an engineered ascorbate peroxidase (APEX)	biotin phenol	yes	yes (APEX)	cell lines, fly, worm	yes (affinity purification with streptavidin)	proximity labeling of interacting proteins or cellular compartment specific proteins	[54–58]

Ramberger et al. Proteomes 2017