An automated in-gel digestion/iTRAQ-labeling workflow for robust quantification of gel-separated proteins.

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Simple protein separation by 1DE is a widely used method to reduce sample complexity and to prepare proteins for mass spectrometric identification via in-gel digestion. While several automated solutions are available for in-gel digestion particularly of small cylindric gel plugs derived from 2D gels, the processing of larger 1D gel-derived gel bands with liquid handling work stations is less well established in the field. Here, we introduce a digestion device tailored to this purpose and validate its performance in comparison to manual in-gel digestion. For relative quantification purposes, we extend the in-gel digestion procedure by iTRAQ labeling of the tryptic peptides and show that automation of the entire workflow results in robust quantification of proteins from samples of different complexity and dynamic range. We conclude that automation improves accuracy and reproducibility of our iTRAQ workflow as it minimizes the variability in both, digestion and labeling efficiency, the two major causes of irreproducible results in chemical labeling approaches.