Validation of Protein Expression

Up- or down-regulated proteins discovered by proteomics analysis are generally validated by a second bioanalytical approach, typically Western-blot, via carefully choosing antibodies specific to the proteins of interest.

Our lab has a LI-COR Odyssey® Fc Imaging System that has two-color multiplex detection channels with a 700 channel laser source implemented by a solid-state laser diode at 685 nm and 800 channel laser source implemented by a solid-state laser diode at 785 nm. Membranes of Western-blot (WB) are first incubated with a primary antibody against a specific protein, that has been previously determined by proteomics analysis of interest and important in a protein pathway or being a potential biomarker, followed by a second incubation with a fluorescence-labeled secondary antibody (green or red) also supplied by LI-COR. By selecting secondary anti-bodies for targeting protein and control protein (such as β-actin) being able to be distinguished by two colors (green and red), one can run a WB analysis of both targeting and control proteins on the same gel and same transferring membrane which are respectively imaged at the two color channels.