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A wide range of significant products, from small molecules to large proteins, can be made using cells as production hosts or cell factories. Using genome editing it is possible to greatly improve the organism ability as a cell factories. An important aspect of this is to knock out genes, thereby altering the phenotype of the cell. Following KO it is therefore important on a protein level to ensure that the corresponding protein is no longer present in the cell.

For this, we uses MS^e (DIA) in which the instrument alternates between scans of low (MS spectra are collected) and high energy (all precursor ions are fragmented).

Data were acquired on Synapt G2 (Waters) Q-TOF instrument operated in positive mode using ESI with a NanoLock-spray source. During MS^E acquisition, the mass spectrometer alternated between low and high energy mode using a scan time of 0.8 s for each mode over a 50-2000 Da interval. Nanoscale LC separation of the tryptic digested samples was performed using a nanoACQUITY ^{TM} System (Waters) equipped a nanoaAcquity BEH130 C18 1.7 µm, 75 µm x 250 mm analytical reversed-phase column (Waters, USA). A reversed-phase gradient was employed to separate peptides using 5–40 % acetonitrile in water over 90 min with a flow rate of 250 nL.min-1 and a column temperature of 35°C.The data were analyzed using the progenesis QI software (NonLinear dynamics), which aligns the different runs ensuring that the precursor ions and identifications can be shared in between runs.

First based on data from Wild type cell line, precursor ions originating from peptides that are unique for the KO proteins are identified. This is in principle used as inclusion list, however the KO samples are also run in DIA mode. When analyzing data from the KO cell type one can specifically investigate the MS trace for these precursor ions, which if the KO was successful are no longer present. This can clearly also be accomplished by running a targeted method, however DIA also allows to observe if/how the KO affects the overall proteome. Of five investigated KO genes four where completely absent in the KO cell line while one KO resulted in a small part of the protein being present. KO the genes did not significantly alter the overall proteome.