**Data Description of**

***HyperSCP: Combining Isotopic and Isobaric Labeling for Higher Throughput Single-Cell Proteomics***

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In this work, we have developed a hyperSCP method combining isotopic and isobaric labeling to double the throughput of MS-based single-cell proteomic analysis. The two-plex stable isotope labeling of amino acids in cell culture (SILAC) and isobaric tandem mass tag labeling (TMTpro) enabled up to 28 single cells to be analyzed in a single LC-MS analysis, in addition to carrier, reference and negative control channels. Using a 145-min LC separation with a 60-min active elution profile, we analyzed ~280 cells per day.

Particularly, we have demonstrated the feasibility of the developed hyperSCP method and analyzed 598 single cells in total with a carrier channel and 312 cells without a carrier channel across 4 different cell lines (HeLa, A549, K562 and HFL1). In each TMTpro-labled sample set, a 0.5 ng reference sample in channel 134N was included for normalization, and a prepared blank sample served as negative control. The carrier consisted of a 10-ng protein digest was labeled with TMTpro-126. Channel 127C was not used due to isotopic contamination.

**Metadata table**

The experimental design is included in the supporting materials as *Table S1. 2\_cells in channels.xlsx*.

Columns:

*Location* indicates the nested wells on a chip. F1-F27 are the ID numbers of the nested wells. This “field” number meets the default definition of wells in CellenONE system.

*126, 127N, 127C, 128N, 128C, 129N, 129C, 130N, 130C, 131N, 131C, 132N, 132C, 133N, 133C, 134N, 134C, 135N* are channels arranged in nanowells. 2 different cell lines (“Cell\_1” & “Cell\_2”) and control samples are randomized in each nanowell (each TMTpro channel). *NotUsed* means no reagent was added to the nanowell, including the TMTpro reagent.

Cells assignment:

For hyperSCP experiments, the raw file names are formed as “chip number\_cell lines\_nested well loation”. For chip 1 and chip 2, “Cell\_1” is HeLa and “Cell\_2” is K562; In chip 3, “Cell\_1” is HeLa and “Cell\_2” is A549; In chip 4, “Cell\_1” is HFL1 and “Cell\_2” is A549.

**Data accessible**

All the raw files, fasta file and database searching files are available via ProteomeXchange with identifier PXD040455.

**Data processing workflow**

The R script used to process the data are available on <https://github.com/RTKlab-BYU/HyperSCP>.