ATLAS ANTIBODIES

QPrEST standards enable absolute and multiplex protein quantification in cell lines

Introduction

The possibility to determine absolute protein concentrations or copy numbers in different biological samples offers great possibilities in the field of proteomics. Mass spectrometry (MS) enables accurate quantification of proteins by the use of isotope-labeled standards as internal references[1,2]. QPrEST standards are recombinantly produced heavy isotope-labeled multipeptide standards, each containing a stretch of 50-150 amino acids identical to a human protein sequence including at least two unique tryptic peptides. All QPrEST standards contain an N-terminal Quantification Tag (QTag) sequence and are accurately quantified with high precision in an MS-based setup using an unlabeled, amino acid analysed QTag protein as internal reference[3]. The QPrEST product catalog offers broad proteome coverage with over 20,000 QPrEST standards targeting 13,000 human proteins.

Workflow for multiplex protein quantification using QPrEST standards

Individually quantified QPrEST standards targeting 23 different human proteins were used to create a QPrEST master mix for multiplex copy number analysis in HeLa cells[4]. In order to generate heavy to light (H/L) ratios close to one for all 23 included proteins, a primary experiment was first performed with a master mix of equimolar amounts of all QPrEST standards. For the following experiments, adjusted molar amounts of the QPrEST standards were added to the master mix. All experiments were performed in triplicate.

The QPrEST master mix was added to the cell sample directly after cell lysis (Fig 1). Adding the QPrEST standards at this early stage decreases the variation introduced during subsequent sample handling steps, as compared to for example peptide standards that are spiked in at a later stage of the workflow. The sample was directly digested with trypsin after addition of the QPrEST master mix using the filter aided sample preparation (FASP) methodology[5]. Peptides were further fractionated using strong anion exchange (SAX) chromatography in a pipette tip format[6]. The resulting peptide fractions were desalted and analyzed on a QExactive mass spectrometer and the generated data was analyzed using the MaxQuant software[7]. Copy numbers of individual peptides were calculated based on the generated H/L peptide ratios and the median value was used to determine the corresponding copy number of the protein in HeLa cells. The coefficient of variation (CV) was determined for all proteins as the relative standard deviation observed between the three replicate experiments.

Multiple tryptic peptides used for quantification

Fig 2 illustrates generated H/L peptide ratios for a subset of the proteins included in the experiment. Only peptides quantified in all three replicates are presented and the median peptide ratio is shown for each peptide. Each horizontal bar represents a tryptic peptide and its relative position on the QPrEST sequence with the size of the bar showing the peptide sequence length. The blue and orange coloring of the bars represents fully cleaved peptides and

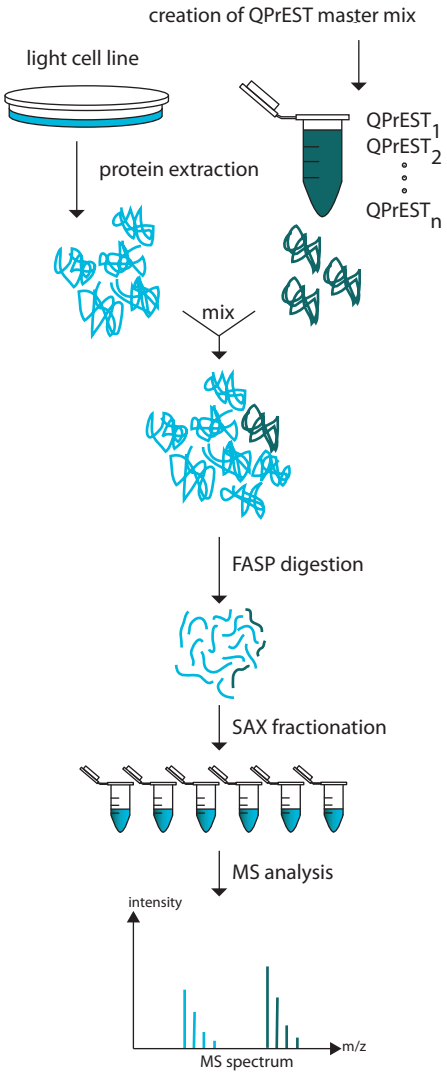

Figure 1.
Applied workflow for protein quantification using QPrEST standards. FASP = filter aided sample preparation, SAX = strong anion exchange chromatography.

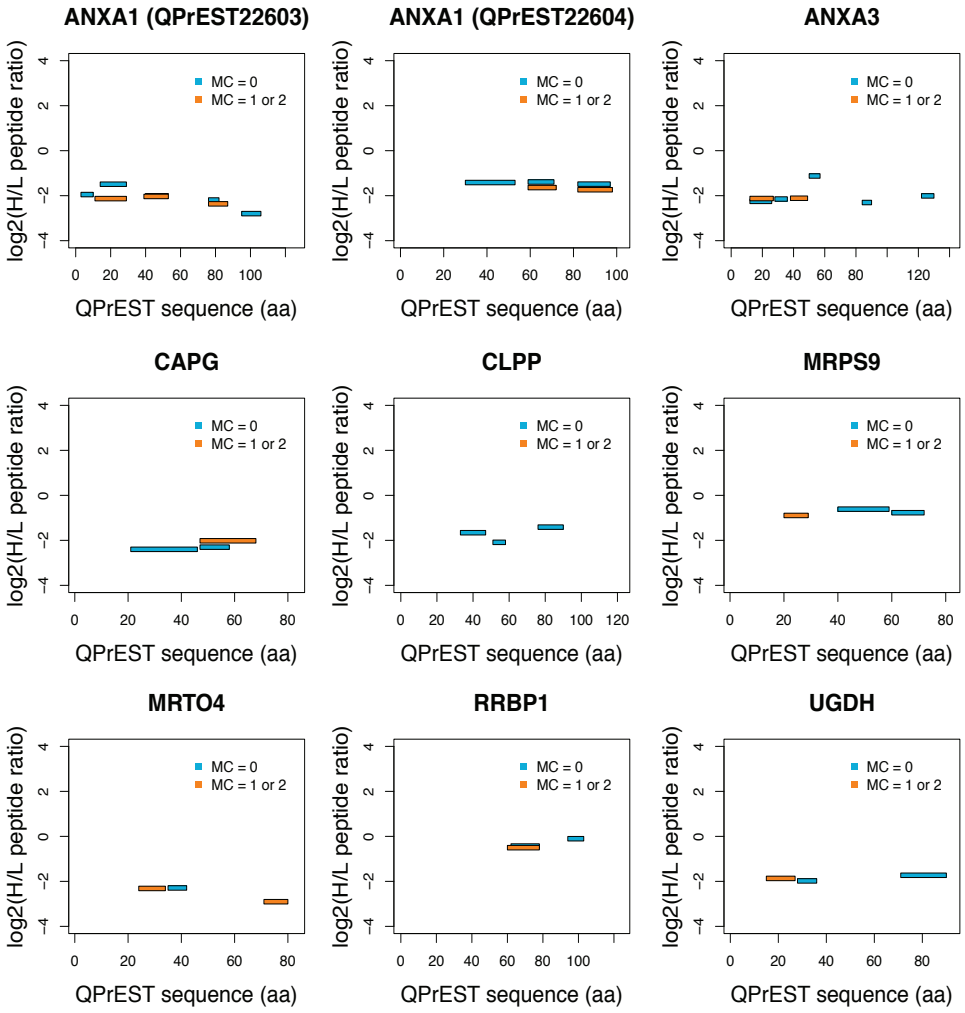

Figure 2.
Quantification of proteins using QPrEST standards. Horizontal bars represent quantified peptides, where fully cleaved peptides and peptides with one or two missed cleavages (MC) are shown in blue and orange, respectively. Only peptides quantified in all three replicates are included. Peptide location within the QPrEST sequence is displayed on the x axis and the y axis shows heavy to light (H/L) peptide ratios. ANXA1 QPrEST1 = QPrEST22603, QPrEST2 = QPrEST22604.

peptides with one or two intact cleavage sites, respectively. It can be seen that the peptides align nicely on the y axis, meaning that the H/L peptide ratios are consistent for peptides across the QPrEST sequence. These data clearly show the benefits of including multiple peptides in protein quantification, as the ratio of one peptide can be verified by data from additional tryptic peptides. Peptides with one or two missed cleavages in general show very similar ratios compared to the fully

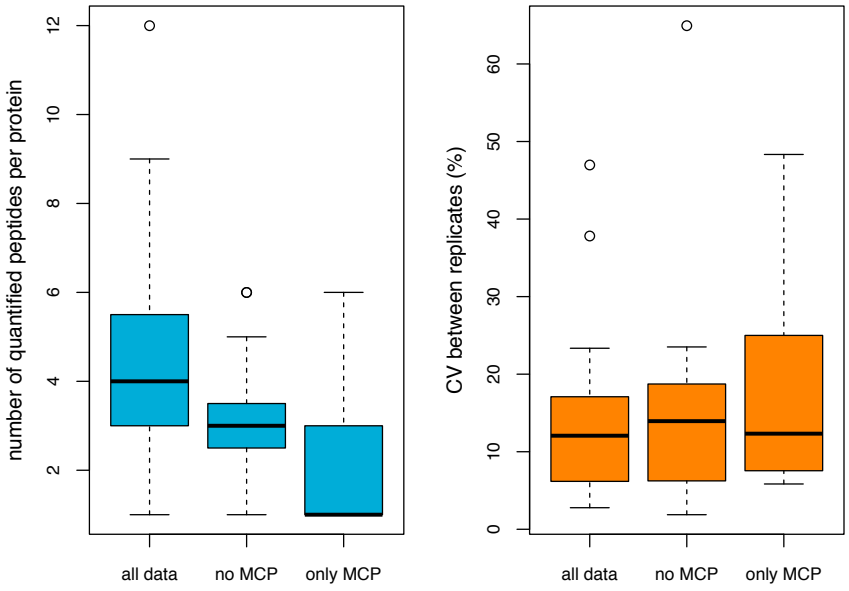

Figure 3.
Multiplex quantitative analysis of 23 proteins in HeLa cells using QPrEST standards. The number of peptides per protein ranged from 1 to 12 (left) and the median coefficient of variation was 12% when including all data (right). Black lines within each box show the median values. Outlying values are shown as circles. MCP = miscleaved peptides.

cleaved peptides. By including them in the analysis these peptides can therefore contribute with additional quantitative information, further increasing the reliability of the analysis. The median peptide ratios used for quantification of these proteins are shown in table 1.

The average number of peptides used for quantification for each protein in this data set was 3.1 (median 3) when only including fully cleaved peptides. When also adding peptides with one or two intact tryptic cleavage sites, the average number of quantified peptides per QPrEST standard was increased to 4.6 (median 4) (Fig 3). The number of peptides used for quantification ranged from one to six when only using fully cleaved peptides. This number increased to twelve when also including peptides with one or two missed cleavage sites.

Quantitative precision using QPrEST standards

All analyses were performed in triplicate and the median value from the three replicates was used to determine the protein copy number in HeLa cells (Fig 4). The protein abundances ranged from 44,000 copies per cell for GOLGA4 to 20 million copies per cell for S100A4. For the 23 proteins included in this multiplex quantification, the median CV was 12.1% when including both fully cleaved peptides and peptides with one or two intact cleavage sites (Fig 3). After removing peptides with one or two missed cleavages from the dataset, the CV was slightly increased (median CV 13.9%) and hence including peptides with missed cleavages does not negatively affect quantitative precision. If only including peptides with one or two intact cleavage sites in the analysis, the obtained median CV was 12.3%. Product numbers for QPrEST standards included in the experiments are shown in table 2.

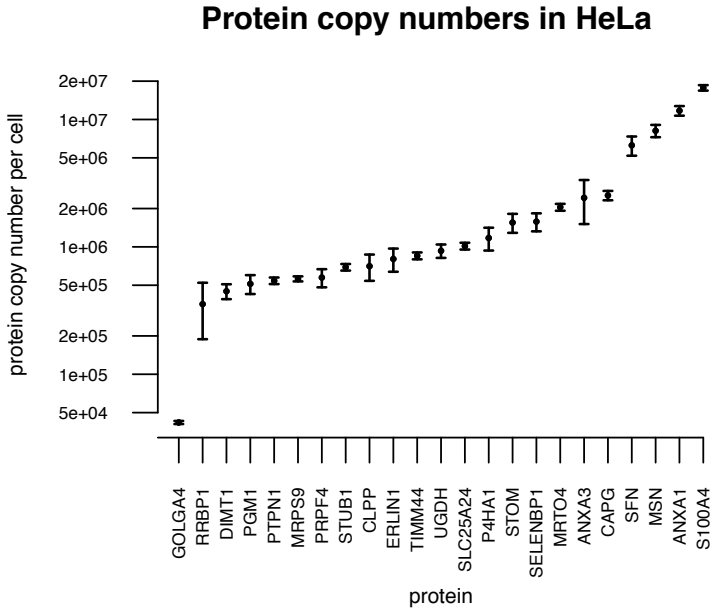

Figure 4.
Copy numbers for 23 proteins in HeLa cells determined using QPrEST standards. Error bars show standard deviation between three replicate experiments.

Table 1.
Quantified peptides for eight example proteins. Only peptides quantified in all three replicates are shown. Gene name, peptide sequence, median heavy to light (H/L) ratio and number of missed cleavages (MC) is shown for each quantified peptide.

Gene	Sequence	H/L ratio	MC	Gene	Sequence	H/L ratio	MC
ANXA1	CATSKPAFFAEK	0.14	0	CAPG	AQVEIVTDGEEPAEMIQVLGPKPALK	0.19	0
	GDRSEDFGVNEDLADSDAR	0.23	1		EGNPEEDLTADK	0.20	0
	GTDVNVFNTILTTR	0.25	0		EGNPEEDLTADKANAQAAALYK	0.25	1
	KGTDVNVFNTILTTR	0.24	1	CLPP	GQATDIAIQAEEIMK	0.32	0
	NALLSLAK	0.26	0		QLYNIYAK	0.24	0
	SEDFGVNEDLADSDAR	0.36	0		YMSPMEAQEFGILDK	0.38	0
	VLDLELK	0.22	0	MRPS9	AIAYLFPSGLFEK	0.59	0
	VLDLELKGDIEK	0.19	1		ETYTEDFIKK	0.54	1
	AAYLQETGKPLDETLK	0.36	0		HLANMMGEDPETFTQEDIDR	0.65	0
	AAYLQETGKPLDETLKK	0.30	1	MRTO4	QLGLPTALKR	0.13	1
	GGPGSAVSPYPTFNPSSDVAALHK	0.37	0		TKEEVNEWFTK	0.20	1
	GVDEATIIDILTK	0.38	0		YTEMDYAR	0.20	0
	GVDEATIIDILTKR	0.32	1	RRBP1	EAEETQSTLQAECDQYR	0.74	0
ANXA3	ALLTLADGR	0.23	0		LREAEETQSTLQAECDQYR	0.71	1
	DESLKVDEHLAK	0.23	1		SVEEEEQVWR	0.93	0
	LTFDEYR	0.20	0	UGDH	EQIVVDLSHPGVSEDDQVSR	0.30	0
	NTPAFLAER	0.25	0		IAILGFAFK	0.25	0
	QDAQILYK	0.46	0		IIDSLFNTVTDKK	0.27	1
	RDESLKVDEHLAK	0.21	2				
	SLGDDISSETSGDFR	0.21	0				

Table 2.
QPrEST standards included in the experiments.

QPrEST	Gene	QPrEST	Gene
QPrEST22603	ANXA1	QPrEST24287	PGM1
QPrEST22604	ANXA1	QPrEST21062	PRPF4
QPrEST23118	ANXA3	QPrEST23435	PTPN1
QPrEST24357	CAPG	QPrEST38025	RRBP1
QPrEST27335	CLPP	QPrEST23857	S100A4
QPrEST38889	DIMT1	QPrEST26348	SELENBP1
QPrEST21717	ERLIN1	QPrEST24094	SFN
QPrEST26431	GOLGA4	QPrEST29056	SLC25A24
QPrEST25609	MRPS9	QPrEST23874	STOM
QPrEST38590	MRTO4	QPrEST38867	STUB1
QPrEST23840	MSN	QPrEST21208	TIMM44
QPrEST23170	P4HA1	QPrEST21478	UGDH

Summary

- QPrEST standards have been used in a multiplex fashion for copy number determination of 23 different protein in HeLa cells
- Multiple peptides, on average 4.6 in this dataset, were used for quantification of each protein
- Peptides with missed cleavage sites can be included in the analysis and increase the overall reliability without significantly decreasing precision

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