ATLAS ANTIBODIES QPrEST

QPrEST™—isotope-labeled multipeptide standards for quantitative mass spectrometry–based proteomics

Mass spectrometry (MS) enables absolute quantification of endogenous proteins by the use of isotope-labeled standards as internal references. QPrEST standards, currently available for >13,000 human proteins, represent a novel class of recombinantly produced heavy isotope–labeled standards that are added early in the quantification workflow. These multipeptide standards contain 50–150 amino acids identical to a human target sequence, resulting in absolute quantification data based on multiple tryptic peptides.

QPrEST isotope-labeled standards

The QPrEST standards are derived from the Human Protein Atlas project[1,2], with unlabeled (light) recombinant protein fragments used as antigens for antibody generation. Antigen sequences are selected so as to minimize sequence identity with respect to other human proteins. The protein fragments, named protein epitope signature tag antigens (PrEST Antigens), are produced with incorporated heavy isotope–labeled amino acids to generate proteins suitable for use as internal standards for MS-based quantification, termed QPrESTs[3–5]. All QPrESTs contain a stretch of 50–150 amino acids identical to a human protein target including at least two unique tryptic peptides (**Fig. 1**). The QPrEST standard is added to the sample at an early stage in the sample-preparation workflow, decreasing the potential for quantitative errors introduced by, for example, variations in the proteolytic cleavage reaction.

The QPrEST product catalog contains over 20,000 products, and more than 70% of them have at least one experimentally verified proteotypic peptide in PeptideAtlas[6] (**Fig. 2**). In total, the currently available QPrEST standards target more than 13,000 human proteins, of which more than 40% are covered by multiple (up to five) QPrESTs.

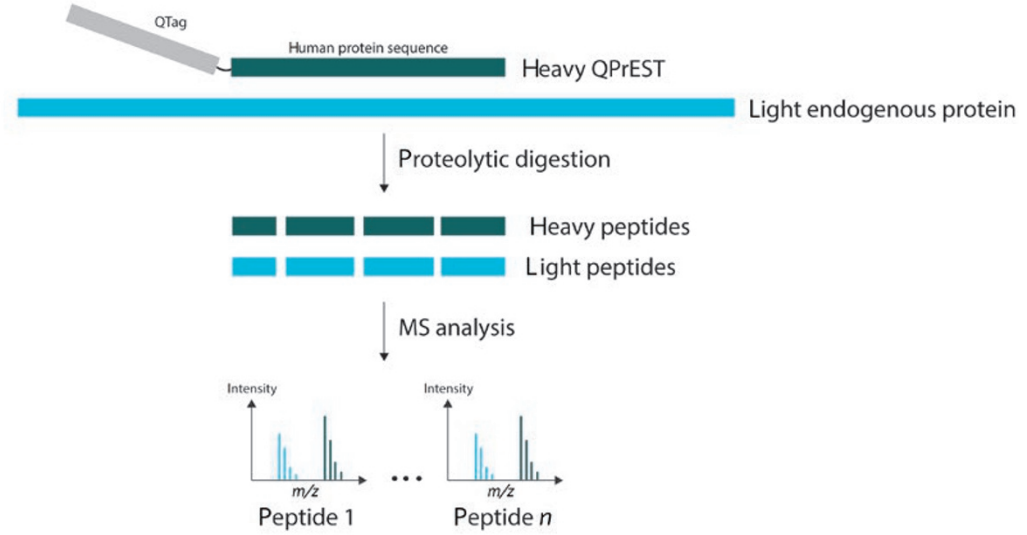

Figure 1 | Schematic of a QPrEST standard and its use. The standard contains an N-terminal sequence for affinity purification and quantification of the QPrEST standard. The C-terminal part consists of a stretch of 50–150 amino acids identical to a corresponding human protein. Each standard contains multiple tryptic peptides that can be used to determine the absolute quantity of the target protein in an unknown sample.

Tove Boström

Atlas Antibodies AB, AlbaNova University Center, Stockholm, Sweden. Correspondence should be addressed to Tove Boström (tove.bostrom@atlasantibodies.com).

APPLICATION NOTES

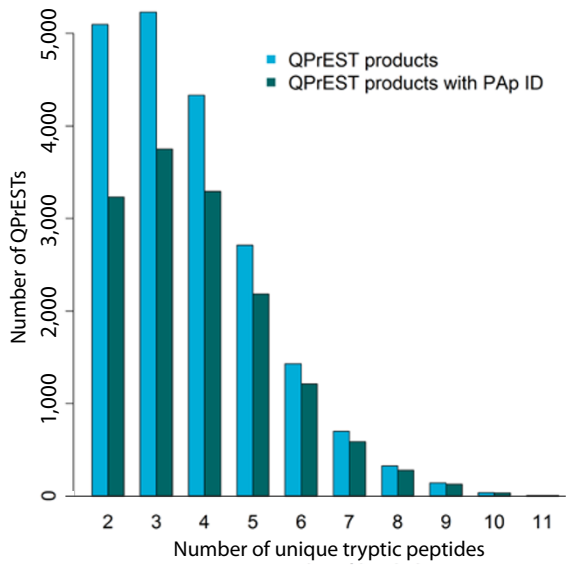

Figure 2 | Distribution of QPrEST products based on the number of unique tryptic peptides. The number of QPrESTs in each group with at least one tryptic peptide present in PeptideAtlas (PAp) is also presented.

Production and quality control

QPrESTs are expressed in an *Escherichia coli* BL21(DE3) derivative, auxotrophic for lysine and arginine, using a minimal autoinduction medium supplemented with heavy isotope–labeled lysine and arginine (^{13}C, ^{15}N) residues, resulting in near complete (>99%) isotopic incorporation[7]. An N-terminal quantification tag (QTag) is present in all QPrEST standards and is used for both affinity purification and accurate quantification of the QPrEST product. The quantification is performed in an MS-based setup through quantitative analysis of QTag-derived tryptic peptides, using unlabeled QTag protein as an internal reference[4]. High purity and an accurately determined concentration of the unlabeled QTag protein are ensured through multiple affinity-purification steps and subsequent amino acid analysis. This targeted QPrEST quantification results in a highly accurate product concentration for which data from multiple QTag peptides have been taken into account.

Absolute protein quantification using QPrEST standards

QPrEST standards can be used to determine absolute quantities of target proteins in human cell lines with both single-plex and multiplex approaches. Addition of the QPrEST standards directly after cell lysis decreases the variation introduced during subsequent sample handling steps as compared to spiking-in of peptide standards at a later stage of the workflow. Quantities of individual peptides are calculated on the basis of the generated heavy-to-light (H/L) peptide ratios, and because each QPrEST contains multiple peptides, several data points are usually obtained for each protein. **Figure 3** shows an example of quantification of two proteins in a HeLa cell lysate. The protein CAPG was quantified using four peptides derived from one QPrEST standard, and the copy number was determined to be 2.5 million molecules per cell. UGDH was quantified using a total of five peptides generated from two separate QPrEST standards, resulting in a determined cellular abundance of 890,000 copies per cell.

Figure 3 shows that data for the different peptides aligned nicely on the *y*-axis, meaning that the H/L peptide ratios were consistent for peptides in the QPrEST sequence. The data clearly show the benefits of including multiple peptides in protein quantification, as the H/L ratio of one peptide can be verified by data from additional tryptic peptides. Peptides containing missed cleavage sites have H/L ratios very similar to those of the fully cleaved peptides; including these peptides in the analysis can generate additional quantitative information, further increasing the reliability of the analysis. The high correlation between data from different peptides—both fully cleaved peptides and peptides containing missed cleavage sites—indicates that the digestion efficiency is similar for the QPrEST standard and the endogenous protein. This is of great importance and is one of several advantages of using larger protein-fragment standards as opposed to peptide standards, with which incomplete cleavage results in quantitative errors.

QPrEST standards used in research applications

The QPrEST-based protein-quantification method was originally developed by Matthias Mann and Mathias Uhlén, who in recent years have used the approach in their respective laboratories, resulting in a number of publications. In early publications, the QPrEST standards were referred to as SILAC-PrESTs or heavy isotope–labeled PrESTs. In 2012, Zeiler *et al.*[4] showed how QPrEST standards can be used for multiplex protein quantification in HeLa cells. In that proof-of-principle study, absolute quantification of 40 human proteins was performed in parallel. The included proteins spanned a large concentration range, from FOS, with a cellular abundance of 6,000 copies, to vimentin, with 20 million copies per cell.

The concept was then developed further, and in a 2014 publication, Edfors *et al.*[3] included a peptide-enrichment step in the sample-preparation procedure. After digestion of a HeLa cell lysate spiked with QPrEST standards, antibodies were used to capture both heavy and light versions of their corresponding target peptides, resulting in a significant decrease in sample complexity and target-peptide enrichment. The authors showed that this system generated results with equally high accuracy while significantly simplifying MS analysis compared to the previous method.

Recently, QPrEST standards have been used by Mann's group in platelet and muscle fiber proteomics. In one study, Zeiler *et al.*[5] used the QPrEST method to target 13 mouse platelet proteins. Working with the absolute quantitative data generated using the standards, they were able to estimate copy numbers per platelet for all other identified proteins on the basis of their corresponding normalized intensities.

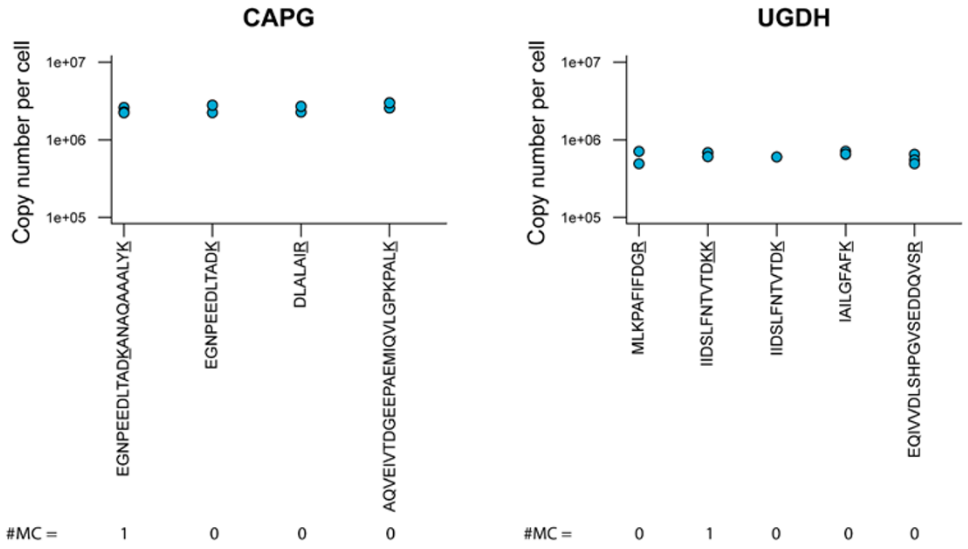

Figure 3 | Absolute quantification of two human proteins using QPrEST standards. The copy numbers of CAPG and UGDH were determined in HeLa cells. Quantified peptides are shown on the *x*-axis, and determined copy numbers are on the *y*-axis. Each circle represents data from one of three replicate analyses. The number of missed cleavage sites (#MC) is shown along the bottom for each peptide. KP and RP motifs were not considered to be missed cleavage sites.

Further, Murgia *et al*.[8] used QPrEST standards when investigating the mouse muscle fiber proteome, in which various muscle fiber types differ in their myosin heavy chain isoforms. For determination of the relative isoform contributions, QPrEST standards were produced targeting the different isoforms.

Conclusion

QPrESTs represent a new generation of isotope-labeled standards for MS-based absolute protein quantification. The standards are recombinant proteins expressed in a bacterial host with >99% isotopic incorporation and precisely determined concentrations. Each standard contains a stretch of a human protein including at least two unique tryptic peptides, ensuring multiple data points from a single standard. Furthermore, the QPrEST standard is added to the unknown sample prior to digestion, resulting in decreased variation from the proteolytic cleavage step compared to approaches using peptide standards. The presence of endogenous cleavage sites in the QPrEST enables the use of peptides containing missed cleavage sites, as they usually generate H/L ratios similar to those of the corresponding fully cleaved peptides. The QPrEST product catalog covers a large portion of the human proteome, and protein quantification using QPrEST standards can easily be multiplexed.

1. Uhlen, M. *et al. Nat. Biotechnol.* **28**, 1248–1250 (2010).
2. Uhlén, M. *et al. Science* **347**, 1260419 (2015).
3. Edfors, F. *et al. Mol. Cell. Proteomics* **13**, 1611–1624 (2014).
4. Zeiler, M., Straube, W.L., Lundberg, E., Uhlén, M. & Mann, M. *Mol. Cell. Proteomics* **11**, O111.009613 (2012).
5. Zeiler, M., Moser, M. & Mann, M. *Mol. Cell. Proteomics* **13**, 3435–3445 (2014).
6. Farrah, T. *et al. J. Proteome Res.* **13**, 60–75 (2014).
7. Matic, I. *et al. J. Proteome Res.* **10**, 4869–4875 (2011).
8. Murgia, M. *et al. EMBO Rep.* **16**, 387–395 (2015).