Library Preparation Kit, V2, Lys-C

Easily prepare proteins for Next-Generation Protein Sequencing™ in a simple, user-friendly workflow

INTRODUCTION

Platinum®, the world's first Next-Generation Protein Sequencer™, delivers single-molecule and single-amino acid resolution in a user-friendly benchtop platform. Platinum enables protein identification and variant detection without complex workflows and advanced expertise, making new discoveries possible for every lab.

The Platinum instrument, kits, and software contain everything you need to prepare, sequence, and analyze proteins. Library preparation is the initial step in Quantum-Si's Next-Generation Protein Sequencing workflow. The Library Preparation Kit, V2, Lys-C (catalog #910-00012-02) contains the necessary components to digest proteins into peptides and functionalize and conjugate peptides for immobilization on a semiconductor chip.

In the library preparation process (figure 1), intact proteins first undergo reduction and alkylation to break disulfide bonds and cap free thiol groups. The proteins are then digested at the carboxyl side of lysine residues using the endoprotease Lys-C. The resulting peptides are functionalized at the C-terminal lysine residues with azide groups for conjugation to a macromolecular K-linker via click chemistry. The peptide libraries are then ready for chip immobilization and sequencing with Sequencing Kits on the Platinum instrument.

Dye-labeled N-terminal amino acid (NAA) recognizers bind on-off NAA residues and aminopeptidases cleave each NAA exposing the next NAA for recognition until the entire peptide is sequenced. Fluorescent intensity, lifetime, and binding kinetics data from each NAA binding event and the order of recognition make up kinetic signatures for each peptide which can be automatically transferred to the Platinum Analysis Software for peptide alignment and protein identification.[1]

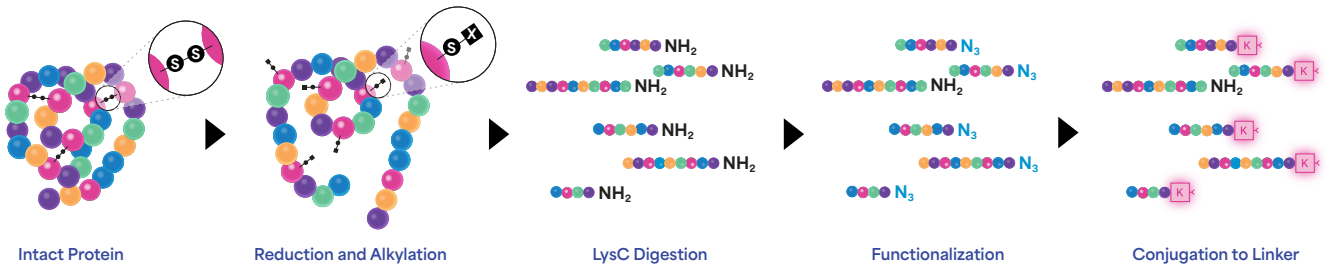

Figure 1. Overview of the library preparation process using the Library Preparation Kit, V2, Lys-C.

The performance of Library Preparation Kit, V2, Lys-C was evaluated on various sample types, on samples at various input concentration, and on proteins with a wide range of molecular weights (table 1). Peptide and protein sequencing data of various sample types demonstrates the utility of the Library Preparation Kit, V2, Lys-C for proteins with various molecular weights, and concentrations.

Libraries per Kit	8
Recommended Input Amount	100 µL of 1-5 µM solution (100-500 picomol input)
Molecular Weights Tested	6-86 kDa
Library Preparation Time	Hands-on time: <2 hours; Total time: 2 days

Table 1. Specifications of the Library Preparation Kit, V2, Lys-C specifications for protein samples.

PROTEIN IDENTIFICATION OF PROTEINS WITH A RANGE OF MOLECULAR WEIGHTS DEMONSTRATES ROBUSTNESS

The average molecular weight of a protein in the human proteome is around 50 kDa.[2] To demonstrate the performance of the Library Preparation Kit, V2, Lys-C on proteins with a range of molecular weights spanning the average size, 27 different proteins ranging in molecular weight from 6 to 86 kDa were prepared (table 2). Subsequently, each resulting library underwent sequencing on Platinum with Sequencing Kit V3 (Catalog #910-00038-00. Protein identification was completed by aligning kinetic signatures to peptide sequences with predictable kinetic signatures using the Peptide Alignment analysis workflow. The number of alignments per protein is determined by the composition of each protein, specifically the concentration of peptides with at least four amino acids recognized by at least three distinct recognizers. Confidence in peptide alignment and protein identification can be evaluated by the false discovery rate (FDR) reported for each peptide within a protein.

Protein	Molecular Weight (KDa)
ADML	6
APOE4	34
CAPN1	86
CDK5	33
CDNF	21
FBXL2	48
FCER2	32
FGF2	17
FOXO4	56
GSK3A	55
IL18R	62
IL4	15
IL6	24
K1C19	47
K2C8	56
KCNJ5	51
LMNB1	30
PDL1	25
SFN	30
SSNA1	18
TBP	39
TMLHE	34
TPM1	35
TPM2	37
TTPA	40
Ubiquitin	9
VIME	55

Table 2. 27 proteins with various sizes tested with Library Preparation Kit, V2, Lys-C.

Of the 27 proteins sequenced, 23 proteins (>85%) were successfully identified on Platinum (figure 2).

Differences in the observed number of alignments and high-quality peptides can be attributed to the protein's composition. For example, proteins with a low number of lysine residues will not generate as many peptides for sequencing, and proteins with less visible residues (residues that are not detectable by the current sequencing chemistry) will result in fewer alignments.

These data demonstrate that a variety of proteins differing in molecular weight and amino acid composition can be confidently identified.

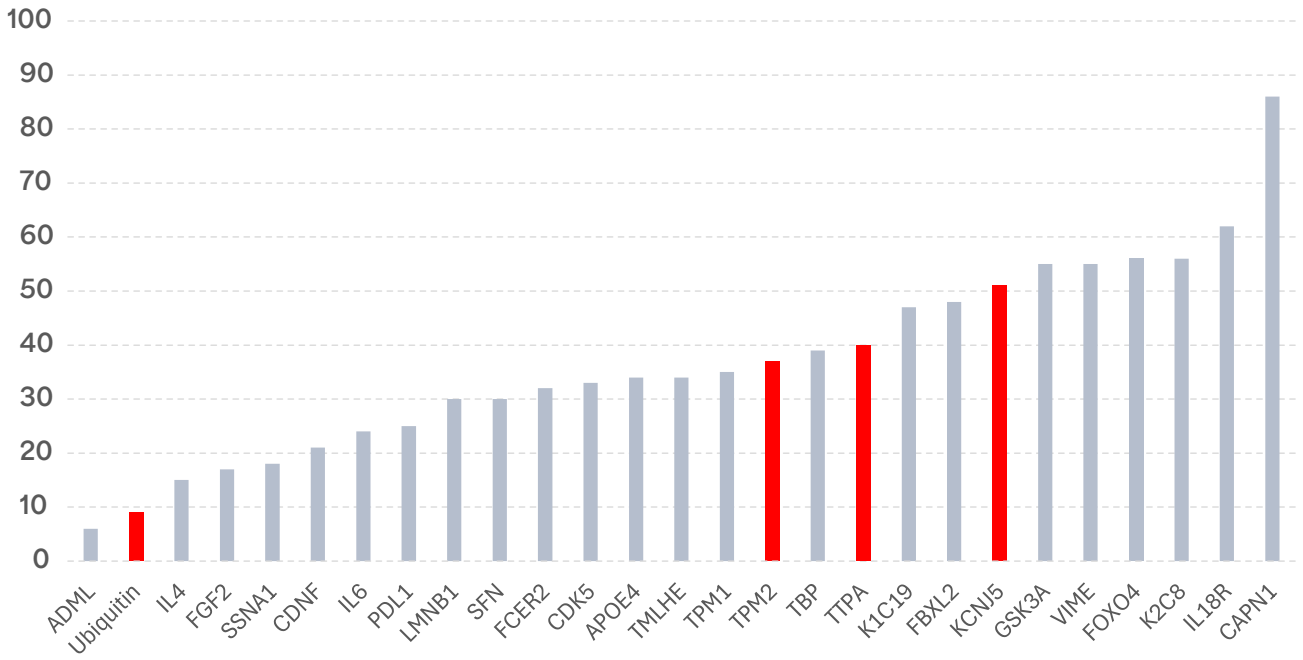

Figure 2. Increasing molecular weight of proteins attempted.

PROTEIN INPUT AND SEQUENCING ALIGNMENT

Proteins exist at various concentrations in nature. Understanding how protein sequencing performance is impacted by different protein input amounts will provide guidance on assay optimization and results interpretation. The current library preparation protocol recommends an input concentration of 1-5 µM. A concentration range of 5 µM and 1 µM were tested to evaluate the performance of the Library Preparation Kit, V2, Lys-C at lower input concentrations.

Sequencing data demonstrated a correlation between input concentrations and the number of alignments (figure 3). Libraries prepared with 1 µM and 5 µM protein input both generated sufficient alignments to uniquely identify each protein (figure 3). These results demonstrated that the proteins tested were properly aligned to their respective reference at an input concentration fivefold lower than the standard recommendation.

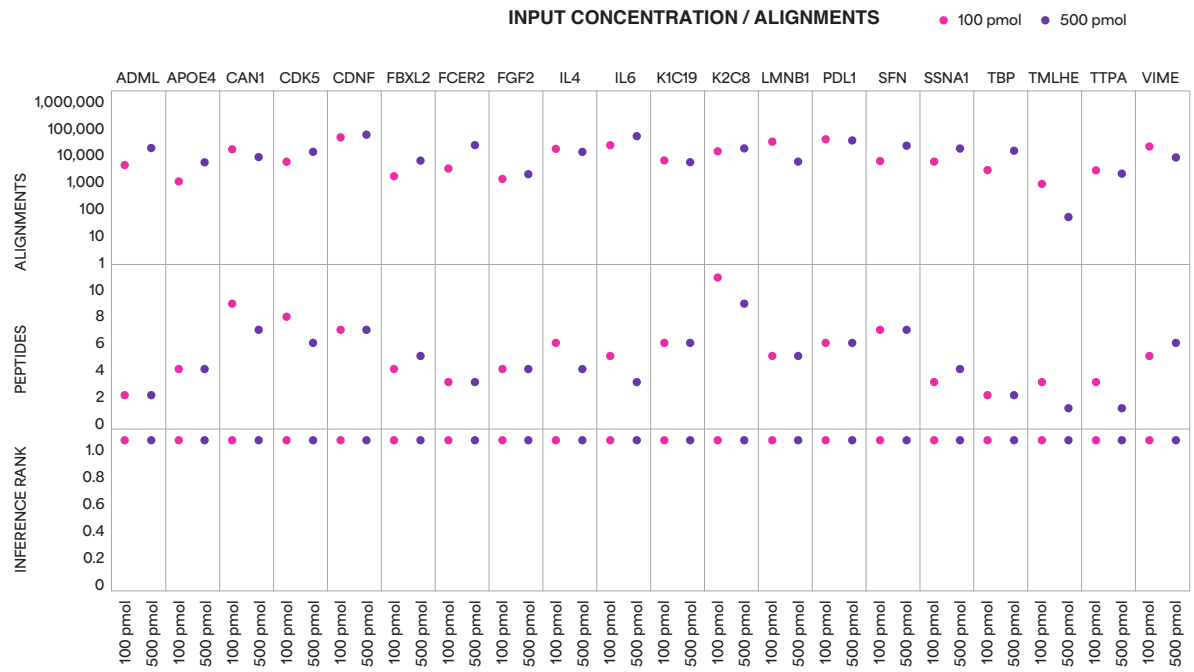

Figure 3. Performance of the Library Preparation Kit, V2, Lys-C at input concentrations of 1-5 µm, assessed by number of alignments from platinum sequencing.

SUMMARY

The Library Preparation Kit, V2, Lys-C enables researchers to easily prepare proteins and peptides with different molecular weights and peptide compositions for Next-Generation Protein Sequencing on Platinum. Researchers can integrate this simple workflow for preparing libraries for protein identification with single-molecule resolution on Platinum. For more information, visit www.quantum-si.com/products.

ORDERING INFORMATION

Product	Catalog Number
Library Preparation Kit, V2, Lys-C	910-00012-02

RELATED PRODUCTS

Product	Catalog Number
Sequencing Kit v3.0	910-00038-00

REFERENCES

1. Reed, B. D. et al. Real-time dynamic single-molecule protein sequencing on an integrated semi-conductor device. *Science* 378, 186-192 (2022).
2. Hendil, K. B., Hartmann-Petersen, R., Tanaka, K. 26 S proteasomes function as stable entities. *J Mol Biol* 315, 627-636 (2002).
3. Data Sheet: Platinum® Software Analysis Data Sheet.