Reducing Protein Input for Next-Generation Protein Sequencing™ on the Platinum® Instrument

Poster P18.02

Presenter: Madelyn Davis

Authors: Cassie Lv, Marla Charron, Dister Deoss, Juan Del Valle, Golriz Ghahreman, Tanuj Sapra, Emily Sexton, Catharine Shipps, Cassie Simonides, Mandeep Singh, Abigail Tyree, Dmitriy Gremyachinskiy, and John Vieceli

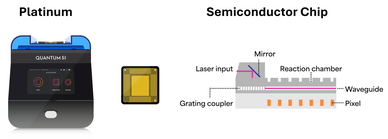
Quantum-Si Incorporated, 29 Business Park Drive Branford, CT 06405, USA

ABSTRACT

Next-Generation Protein Sequencing™ (NGPS™) on the Quantum-Si Platinum® and Platinum® Pro instruments enable the direct analysis of individual proteins at an unprecedented resolution.[1] However, one challenge in applying this technology to biological and clinical samples is the limited availability of input protein. Quantum-Si's Library Preparation Kit, V1 recommends a protein input of 500 pmol for library preparation, which often means enrichment is required when working with clinical and biological samples. Quantum-Si recently developed an updated version (V2) of the Library Prep Kit and protocol to address this limitation, introducing new chemistry to improve conjugation efficiency and reduce protein input requirements.

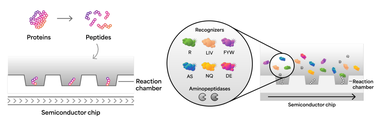
To evaluate the performance of the Library Preparation Kit, V2, we tested 20 proteins across a range of molecular weights, from 6 kDa to 86 kDa, corresponding to approximately 0.6 µg to 8.6 µg per reaction. This protein input requirement is comparable to or lower than that used in some mass spectrometry sample preparation protocols.[2,3] We prepared libraries with 500 pmol and 100 pmol of protein input using the Library Prep Kit, V2, followed by Platinum NGPS. We evaluated sequencing performance based on alignment, number of peptides, and inference precision metrics. The 100 pmol input libraries demonstrated equal inference results for all 21 proteins, equal or more peptides for 18 of 20 proteins, and equal or higher alignment for 10 of 20 proteins. These data demonstrate the potential for successful protein sequencing on Platinum even with a five-fold lower input, which paves the way for broader applications in biological research and clinical diagnostics.

METHODS



STEP 1

Prepare proteins for sequencing

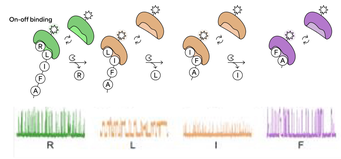


- Semiconductor chip uses a filter-less system that excludes excitation light based on photon arrival time.
- Evanescent illumination at reaction chamber bottoms from nearby waveguide.

- Proteins with a wide range of molecular weights (Table 1) were reduced, alkylated, and digested with LysC.
- Peptides are functionalized, conjugated, and immobilized on the surface of Quantum-Si's semiconductor chip.
- Fluorescently labeled N-terminal amino acid (NAA) recognizers and aminopeptidases are added to the semiconductor chip.

STEP 2

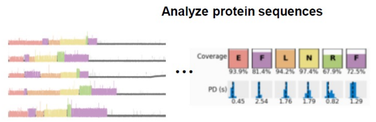
Sequence proteins on Platinum



- **Recognition:** 10s–100s of pulsing events per NAA.
- Fluorescence lifetime differentiates dye-labeled recognizers.
- **Aminopeptidases:** Cleavage events stochastic at the single-trace level.

STEP 3

Analyze protein sequences



- Fluorescent intensity, lifetime, and duration of each NAA binding event generate a **unique kinetic signatures**.
- Kinetic signatures are analyzed to align reads to reference peptides and compute **false discovery rate (FDR).**
- Kinetic signatures are converted into **amino acid calls** to identify peptides and proteins.

RESULTS

Performance of the Library Preparation Kit, V2

The performance of Library Preparation Kit, V2, Lys-C **(catalog #910-00012-02)** was evaluated on 20 proteins with molecular weights from 6 to 86 kDa at input concentrations of 100 pmol and 500 pmol.

The library preparation time was 2 days with under 2 hours of hands-on time.

Protein	Molecular weight (kDa)	Protein	Molecular weight (kDa)
ADML	6	K1C19	47
APOE4	34	K2C8	56
CAPN1	86	LMNB1	30
CDK5	33	PDL1	25
CDNF	21	SFN	30
FBXL2	48	SSNA1	18
FCER2	32	TBP	39
FGF2	17	TMLHE	34
IL4	15	TTPA	40
IL6	24	VIME	55

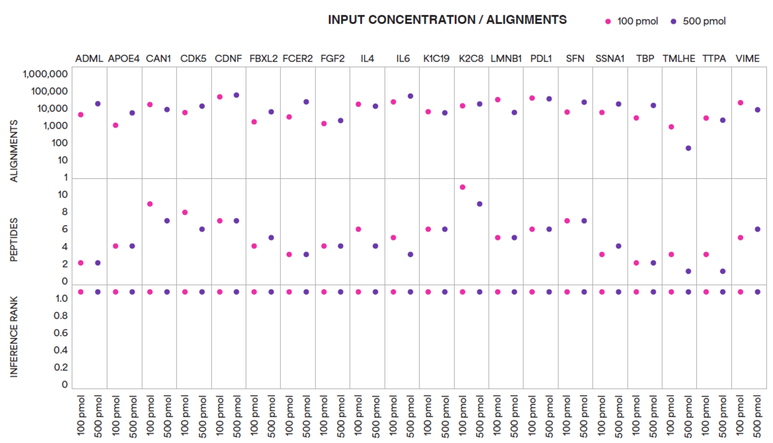
Subsequently, each resulting library underwent sequencing on the Platinum instrument with the Sequencing Kit V3 (**Catalog #910-00038-00**).

Protein identification was completed by aligning kinetic signatures to peptide sequences with predictable kinetic signatures using the Peptide Alignment analysis workflow. The composition of each protein determines the number of alignments per protein, specifically the concentration of peptides with at least four amino acids recognized by at least three distinct recognizers.

Sequencing performance was evaluated based on alignment, number of peptides, and inference precision metrics.

Accurate identification of proteins at 100 pmol and 500 pmol

The 100 pmol input libraries demonstrated equal or better inference results for 20 of 21 proteins, equal or more peptides for 17 of 21 proteins, and equal or higher alignment for 10 of 21 proteins.



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

SUMMARY

- The Library Preparation Kit, V2, Lys-C offers a simple and efficient workflow for preparing proteins and peptides of various molecular weights and compositions for Next-Generation Protein Sequencing (NGPS) on Platinum.
- Researchers conducting proteomics studies to identify protein variants, examine post-translational modifications, investigate protein-protein interactions, and characterize antibodies can perform single-molecule sequencing using Platinum technology.
- With the ability to successfully sequence proteins using an input concentration of 100 pmol — five times lower than the standard recommendation of 500 pmol — unlocks new possibilities for applications in biological research and clinical diagnostics.

For more information, visit quantum-si.com/products

REFERENCES

1. Brian D. Reed et al, *Science* 2022, 378 (6166) 186–192.
2. https://www.thermofisher.com/order/catalog/product/A40006 (Thermo Fisher Scientific EasyPep™ MS Sample Prep Kits)
3. https://cdn.prod.website-files.com/6322f21c2bfa08926805e0fe/6322f21c2bfa08736f05e666_PreOmics_TechPaper_iST_v3.pdf (PreOmics)

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