Barcoding Kit

A multiplexed protein screening solution that quantifies the relative expression of peptide barcodes to accelerate protein research, engineering, and drug development.

Key Innovations

Direct protein detection: Quantify expression and differentiate functional performance of proteins

Multiplexing power: Test multiple candidates in a single model organism with up to 24× the power of single-plex analysis

Analytical validity: Detect low-abundance proteins and differentiate expression changes across up to 120-fold on a half chip or 240-fold dynamic range on a full chip

Workflow optimization: Total barcode library workflow time <6 hours with 1 hour hands-on time

Introduction

Protein barcodes are short sequences of amino acids encoded by DNA sequences that can be added to the protein-coding DNA. They are designed to be easily distinguishable and sequenceable, allowing for the sequencing of only the barcode of interest. There is a critical need for methods to directly read protein barcode sequences and to identify protein barcodes with single-molecule resolution.

Next-Gen Protein Sequencing™ (NGPS™) offers researchers the ability to directly sequence protein barcodes with single-molecule resolution for the first time. The Quantum-Si Barcoding Kit when combined with NGPS on the Platinum® Pro sequencer, enables robust, multiplexed functional protein screening and characterization for several applications, including screening mRNA vaccine candidates, optimizing drug delivery systems, tracking protein subcellular localization, engineering proteins, and studying protein-protein interactions.

Barcode Design and Workflow

Quantum-Si has optimized barcode constructs to enable efficient enrichment and recovery of custom barcodes for specific genes. These constructs are designed to effectively enrich barcoded proteins expressed in cells or tissues. They use available affinity tags at the C- or N-terminus of the target protein, allowing a single-step reaction and cleavage that can be completed in under four hours. The Platinum Barcoding Kit supplies the necessary reagents to prepare peptide barcode libraries for sequencing with the Platinum Pro benchtop protein sequencer.

Protein barcodes are cloned into a plasmid containing an N-terminal *FLAG* tag, a polyG linker (GS Linker), the protein barcode sequence, a sortase recognition motif (LPETG), and an optional C-terminal *His* tag. A representation of the barcode construct is as follows:

C-Terminal Design

Protein of Interest-FLAG Tag-GS Linker-LysC Cleavage-Barcode-Sortase Tag-His Tag

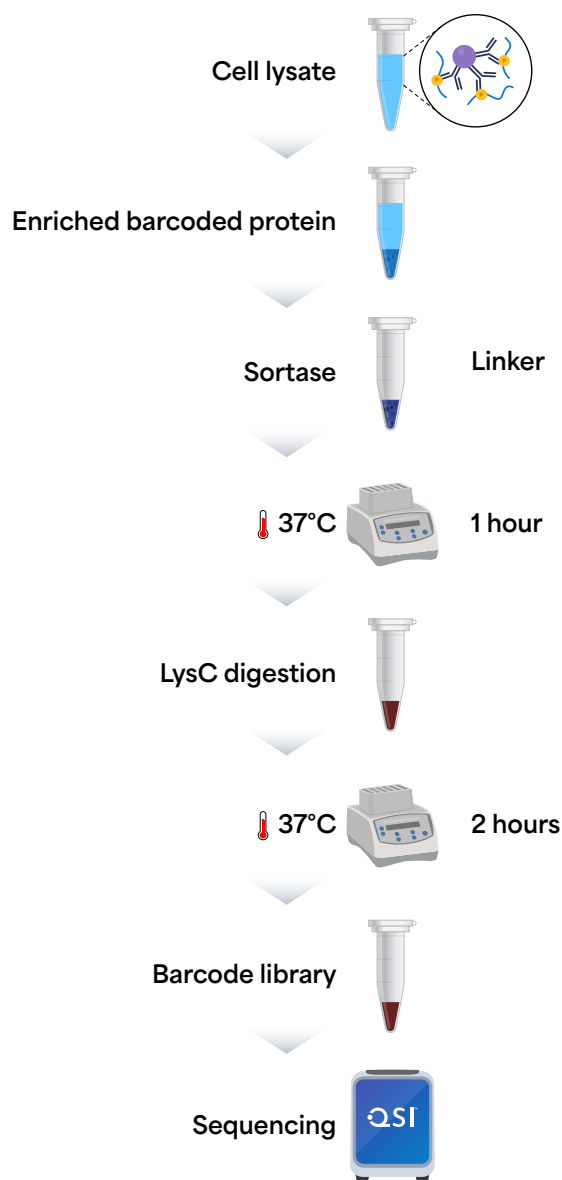**Protein of Interest-DYKDDDDK-GGGGSGGGGS-K-BARCODE-LPETG-HHHHHH**

N-Terminal Design

FLAG Tag-GS Linker-LysC Cleavage-Barcode-Sortase Tag-Protein of Interest-His Tag

DYKDDDDK-GGGGSGGGGS-K-BARCODE-LPETG-Protein of Interest-HHHHHH

24 barcode designs were computationally generated and empirically validated for optimal sequencing on Platinum Pro.

Protein purification from cell lysates depend on the protein and expression system. FLAG enrichment purification is leveraged to isolate the barcoded proteins of interest. Quantum-Si has validated the construct using the C-terminal design with a *Flag* tag for enrichment and an optional secondary purification *His* tag. Other tags and an N-terminal design may also be considered, depending on the application.

The Barcoding Kit includes reagents necessary to prepare peptide barcode libraries for sequencing with the Platinum Sequencing Kit. Peptide barcodes are functionalized for immobilization on the sequencing chip with a sortase reaction and cleaved from FLAG beads and the protein of interest with LysC digestion. Recovered protein libraries can be loaded for sequencing or stored at –20°C.

Cell lysate

Enriched barcoded protein

Sortase   Linker

🌡 37°C   1 hour

LysC digestion

🌡 37°C   2 hours

Barcode library

Sequencing

Figure 1. Barcoding workflow from cell lysate to sequenceable barcode library.

Barcode Quantitation on Platinum Pro

Multiplexed analysis of barcodes on the Platinum Pro saves researchers time and resources. The Barcode Kit workflow was optimized to support a 24-plex peptide barcode run. Peptide alignments provided by the software graph the reads aligned to all the peptides identified in a run that are successfully aligned to the reference with a false discovery rate (FDR) of less than 10%.

The Barcoding Kit calls for standard sample input of 5 pmol per peptide barcode and 25 pmol or higher per 24-plex mixture. Peptide barcodes, together with the Platinum Sequencing Kit, offer a highly sensitive solution for low expressing barcodes, where the limit of detection for each barcode in the complexity of 24 barcodes are in the sub-pmol range (~104 fmol) sample input (just 1% of mixture) and on-chip about 1 fmol. This sensitivity aligns with the requirements for accurate and quantitative protein analysis in a range of applications.

Quantifying barcodes is crucial for evaluating protein expression, refining drug delivery systems, and examining protein trafficking and other protein characteristics. The barcodes in the Barcoding Kit were empirically tested to determine normalization factors for 24-plex, aiming to improve linearity and minimize bias in multiplex mixtures.

As shown in Figure 2, the alignments were converted to normalized alignments by dividing the normalization factor for each barcode. Then, each of the normalized alignments was divided by the sum of the normalized alignments to extract the relative fraction of each observed barcode. The normalization factors yield the expected relative fraction of around 4.16% for a 24-plex mixture. These results establish the reproducible recovery of 24 barcodes in expected ratios across multiple runs.
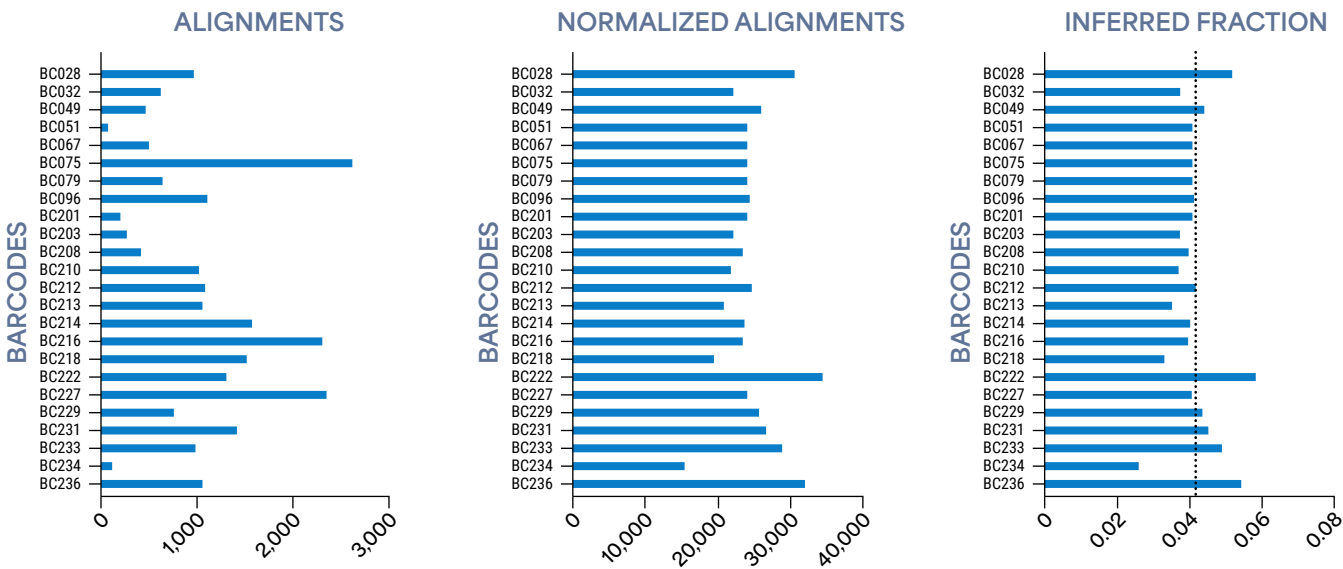

Figure 2. Normalized relative fraction of 24 equimolar barcodes.

Relative quantitation of the multiplexed barcodes was confirmed across a dynamic range of 240-fold on a full sequencing chip and 120-fold on a half chip. As shown in Figure 3, all barcodes were identified with an FDR <10%, and the recovered relative abundance shows a good linear correlation after normalization. In applications where researchers seek the strongest expressors, the ability to differentiate between the highest- and lowest-abundance proteins reproducibly is supported within a defined rate of error.
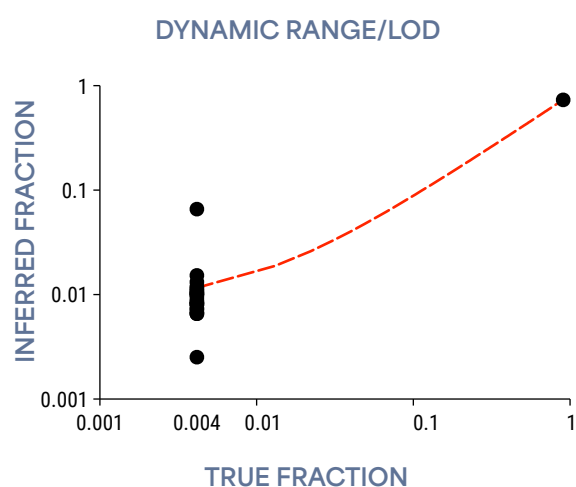
DYNAMIC RANGE/LOD

Figure 3. 24 multiplexed barcodes across 240-fold dynamic range.

Flexible Solution Enables a Broad Range of Applications

The greatest advantage of the Barcoding Kit is its application across a broad range of protein research areas. The kit supports evaluation of drug delivery systems, relative expression of mRNA therapeutics, and assessment of multiplexed protein characteristics. The multiplexing capability of the Barcoding Kit has utility across protein engineering, cell and gene therapy, mRNA therapeutics, and primary research.

Summary

The Barcoding Kit – together with the Platinum Sequencing Kit and Platinum Pro – enables robust, multiplexed functional protein screening and characterization for a number of applications, including screening mRNA vaccine candidates, optimizing drug delivery systems, tracking protein subcellular localization, engineering proteins, and studying protein-protein interactions.

mRNA Therapeutics

Optimize drug delivery systems like LNPs and AAVs by integrating protein barcodes

Cell and Gene Therapy

Screen relative expression of nucleic acid gene therapy targets with protein barcodes *in vitro* and *in vivo*

Protein Engineering and Research

Assess protein characteristics and cell trafficking by integrating protein barcodes

Product Specifications

Attribute	Detail
Barcoding Kit catalog number	910-00047-01
Description	Reagents for protein barcoding; generates eight libraries of up to 24 barcodes each library for sequencing
Samples per kit	Eight reactions per kit
Workflow	Two days expressed barcode to results with <1 hour hands-on time
Multiplexed barcodes per sample	Up to 24-plex barcodes with Sequencing Kit V4
Relative quantitation dynamic range	Up to 120-fold on a half chip or 240-fold dynamic range on a full chip with 25 pmol protein input

Ordering Information

Product	Part Number
Platinum Pro instrument	910-10904-00
Sequencing Kit V4	910-00038-04
Barcoding Kit	910-00047-00