# Identifying Monoclonal Antibodies with Quantum-Si's Next-Generation Protein Sequencing Technology


## SUMMARY

Identifying low-abundance monoclonal antibodies in a population of polyclonal antibodies can provide critical insights for diverse applications such as understanding disease mechanisms, disease diagnostics, vaccine development, therapeutic antibody discovery, and serological surveillance. Existing methodologies, despite their utility, come with significant disadvantages including high costs, complex procedures, and time-intensive workflows, which often limit their broad application. In this application note, we present a method utilizing Quantum-Si's next-generation protein sequencing technology on the Platinum® instrument to successfully identify two low-abundance monoclonal antibodies in a population of polyclonal antibodies.


### Q-SI TECHNOLOGY

Quantum-Si's benchtop Platinum® instrument enables protein sequencing from biological samples in a simple user-friendly workflow. Our technology utilizes dye-tagged N-terminal amino acid recognizers and semiconductor chip technology to detect the binding characteristics and binding order of N-terminal amino acids, resulting in unique kinetic signatures that can be used to differentiate and identify amino acid residues and PTMs. A more detailed overview of the workflow and technology can be found in our Science Paper.

## INTRODUCTION

The detection of low-abundance monoclonal antibodies from a diverse population of polyclonal antibodies is critical in various research and clinical settings. In disease diagnostics, particularly in cancers and autoimmune diseases, the presence of monoclonal antibodies can serve as valuable biomarkers for early detection or monitoring disease progression.[1-3] They also play a significant role in immunology research, where understanding the diversity and specificity of an immune response to an antigen can offer insights into disease mechanisms. Additionally, in the field of vaccine development and therapeutic antibody discovery, researchers need to isolate specific monoclonal antibodies that exhibit high affinity for target antigens, even if present in low amounts. Serological

surveillance also relies on detecting such antibodies to track infectious disease exposure in populations over time.[4] Similarly, the identification of low-abundance monoclonal antibodies can lead to the development of novel antibody-drug conjugates (ADCs), where an antibody selectively targets and delivers a drug to tumor cells, sparing normal cells.[5]

Several methods exist to identify low-abundance monoclonal antibodies in a population of polyclonal antibodies. ELISA and Western blots are commonly used as an easy way to screen for antibodies; however, these assays might not exhibit the required sensitivity to detect very low-abundance antibodies and often demand larger sample volumes. Similarly, flow cytometry and fluorescence-activated cell sorting (FACS) have the potential for high sensitivity but demand sophisticated and expensive equipment and entail a technically challenging process of deriving monoclonal antibodies from sorted B cells. Mass spectrometry (MS), while offering a detailed view of the antibody repertoire, is complex, time-consuming, and requires high levels of expertise to interpret data, which in turn requires more time and resources. Finally, phage display, while advantageous for high-throughput screening, is labor-intensive and requires specialized molecular biology techniques.

Quantum-Si's Platinum next-generation protein sequencing platform is a compact, affordable benchtop instrument that effectively addresses many challenges of traditional antibody identification techniques. Its user-friendly interface eliminates the need for specialized expertise to operate or interpret data, making it a practical choice for a wide range of labs. Moreover, it offers a fast and efficient way to identify low-abundance monoclonal antibodies, accelerating the pace of research and clinical diagnostics without the time-consuming processes associated with many traditional methods. To demonstrate the capabilities of Quantum-Si's technology to identify low-abundance monoclonal antibodies, we sequenced the Fab fragment of two monoclonal antibodies individually and successfully identified the Fab fragments in a mixed population of polyclonal antibodies.

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## METHODOLOGY & WORKFLOW

The Fab fragments of two monoclonal antibodies (mAb) were isolated, enriched, and individually sequenced on Platinum using our previously established library preparation and real-time sequencing procedure.[6] Subsequently, the mAbs were mixed into a population of polyclonal antibodies (pAb) at the mAb abundance levels of 12.5% and 2.5% before sequencing on the Platinum.

The total antibody concentrations at the start of the library preparation process were 5-10 µM. In brief, the process involves digesting proteins into peptide fragments and conjugating the peptides to macromolecular linkers at the C-terminus. The peptides are then immobilized on Quantum-Si's semiconductor chip at a loading concentration of 7 nM, exposing the N-termini for sequencing. Dye-tagged recognizers bind on and off to N-terminal amino acids (NAAs), creating pulsing patterns with distinct kinetic properties. Sequential removal of individual NAAs is achieved using aminopeptidases present in the solution, allowing for the detection of subsequent amino acids. Fluorescence lifetime, intensity, and binding kinetics of each NAA binding event are collected and processed using Cloud-based software to determine the peptide sequence of the Fab fragment and the corresponding antibody.

## RESULTS & DISCUSSION

### THE MONOCLONAL ANTIBODIES ARE SUCCESSFULLY IDENTIFIED INDIVIDUALLY

We first sought to demonstrate the capability of the Platinum in sequencing the monoclonal antibodies by analyzing their unique peptide sequences. We sequenced each antibody on the Platinum instrument using five NAA recognizers with specificity for 11 NAAs (F, Y, W, L, I, V, R, A, S, N, and Q)—and analyzed data to identify recognition segments (RSs), determine the mean pulse duration (PD) of each RS, and characterize the kinetic signature of the peptides of interest. For antibody A, we focused on a unique peptide sequence of TTLFLQMNSLRAEDTAVYYCAK, located in the heavy chain of its variable region. Similarly, antibody B has a unique peptide sequence of NSLYLQMNSLRAEDTAVYYCAREGNSGYEPLDYWGQGTLVTVSSASTK in its variable region's heavy chain. The successful identification of these unique peptides indicates the accurate sequencing of the corresponding antibodies.

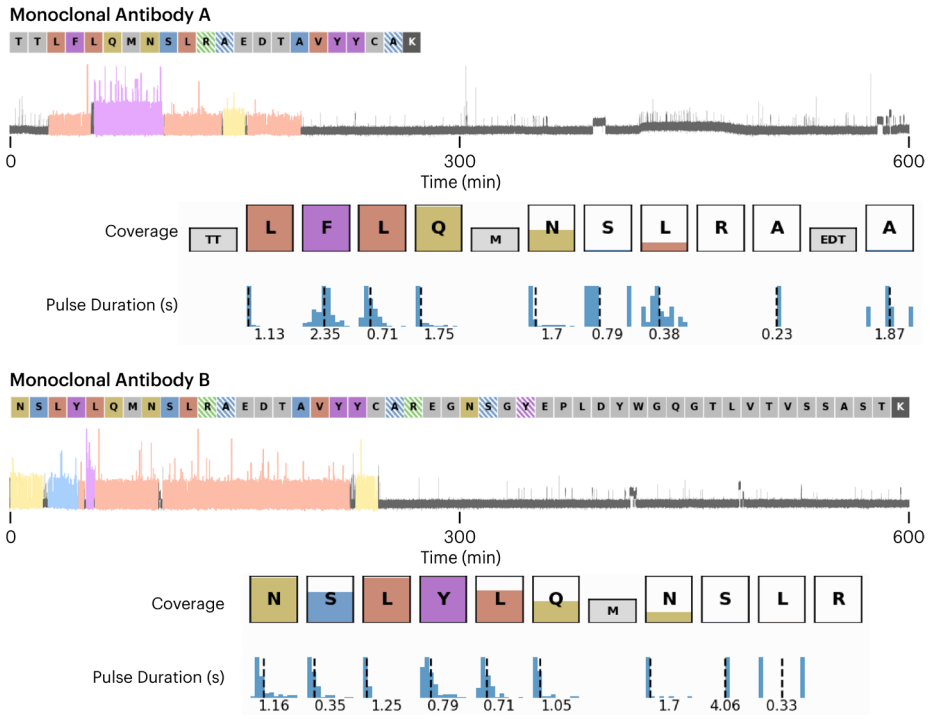


**FIGURE 1**

The Sequencing Data of Unique Peptides From Two Monoclonal Antibodies. Each residue in the sequence is colored by the corresponding recognizer. Pulse data is collected over a 10-hour period of sequencing, with each recognition segment (RS) colored by the corresponding recognizer. The coverage for each RS is indicated by the height of color in the box, and the corresponding pulse duration distribution.

The two peptides were detected in the sequencing data and displayed characteristic pulsing patterns (Figure 1). The unique peptide of antibody A was sequenced up to at least the tenth amino acid (L), while the unique peptide of antibody B was sequenced up to at least the eighth amino acid (N), as demonstrated by the representative traces. The kinetic properties of these peptides were demonstrated through the mean PD values for each recognition segment (RS). These results demonstrate the effectiveness of Quantum-Si Platinum in accurately sequencing monoclonal antibodies, enabling the identification of unique peptide sequences specific to each antibody.

### THE MONOCLONAL ANTIBODIES ARE SUCCESSFULLY IDENTIFIED AT LOW ABUNDANCE IN A POPULATION OF POLYCLONAL ANTIBODIES

Next, we sought to demonstrate the capability of the Platinum in sequencing the monoclonal antibodies at low abundance in a population of polyclonal antibodies by identifying their unique peptide sequences. Antibodies A and B were mixed with polyclonal antibodies at mAb:pAb ratios of 1:7 and 1:39, which correspond to mAb abundance levels of 12.5% and 2.5%, respectively.

Similar to individual sequencing results above, the two unique peptides of antibodies A and B were readily detected in the sequencing data. The unique peptide of antibody A was sequenced up to at least the tenth amino acid (L), while the unique peptide of antibody B was sequenced up to at least the eighth amino acid (N). These results demonstrate the effectiveness of the Platinum's ability to sequence and identify unique peptides of monoclonal antibodies.

## CONCLUSION

In this application note, we demonstrated the successful identification of individual and low-abundance monoclonal antibodies in a population of polyclonal antibodies using the Platinum protein sequencing platform. The results underscore the platform's potential to accelerate research involving monoclonal antibody detection and characterization. By overcoming traditional challenges of cost, complexity, and time consumption, Quantum-Si's technology can be an effective tool for advancing immunological research, disease diagnostics, and therapeutic development, potentially opening new opportunities for precision medicine.

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