

Comparing Cost & Efficacy of Antibody Sequencing Techniques

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Abstract

The Defense Biological Product Assurance Office (DBPAO) antibody repository is a vital resource to the Warfighter, as it provides a stockpile of antibodies to a range of biological threat agents. Should any of these antibodies become compromised, either through damage to the repository itself or to the antibody-producing cell lines, it could render them useless. It is therefore imperative that the antibody sequences are known, in order to engineer new antibody should the need arise. This can be accomplished by leveraging de novo antibody sequencing, which is typically achieved by using a cocktail of enzymes to digest an antibody. The resulting overlapping peptide fragments are then pieced together to establish the full antibody sequence. However, the cost of these enzymes is steep, and only increases with the greater variety of enzymes used. In this work, we explored the efficacy and cost-effectiveness of alternative digestion methods: microwave-assisted acid hydrolysis (MAAH) and new, commercially available CinderBio enzymes, Krakatoa and Vesuvius. We used a commercially available NIST antibody standard to perform these comparisons. Sequence coverage and cost per sample were assessed.

Methods

Digestion: 50µg of antibody was prepared in each digestion. A 6-enzyme cocktail (Thermolysin, Elastase, Chymotrypsin, Lys C, Glu C, Asp N) was used with Protifi S-Trap cartridges. Microwave-assisted acid hydrolysis was performed as previously described¹. Briefly, HCl was added to a final concentration of 3M, then sample was microwaved on ice for 4 min. Finally, CinderBio enzymes Krakatoa and Vesuvius were used according to the manufacturer's instructions, at both 1:2 and 1:1 enzyme-to-protein ratios.

LC/MS/MS: 3µg injections were resolved on a Thermo Ultimate3000 HPLC, with mass spectral analysis performed on a Thermo Eclipse mass spectrometer with FAIMS interface. Data was collected in positive polarity and data dependent mode.

Data Processing: PEAKS de novo sequencing software was used for the initial de novo search. A canonical search against 2200 antibodies then acted as a scaffold that the de novo sequence was assembled upon. In-house script was leveraged to match the format of both search outputs. Assembly was performed in ALPS as described previously².

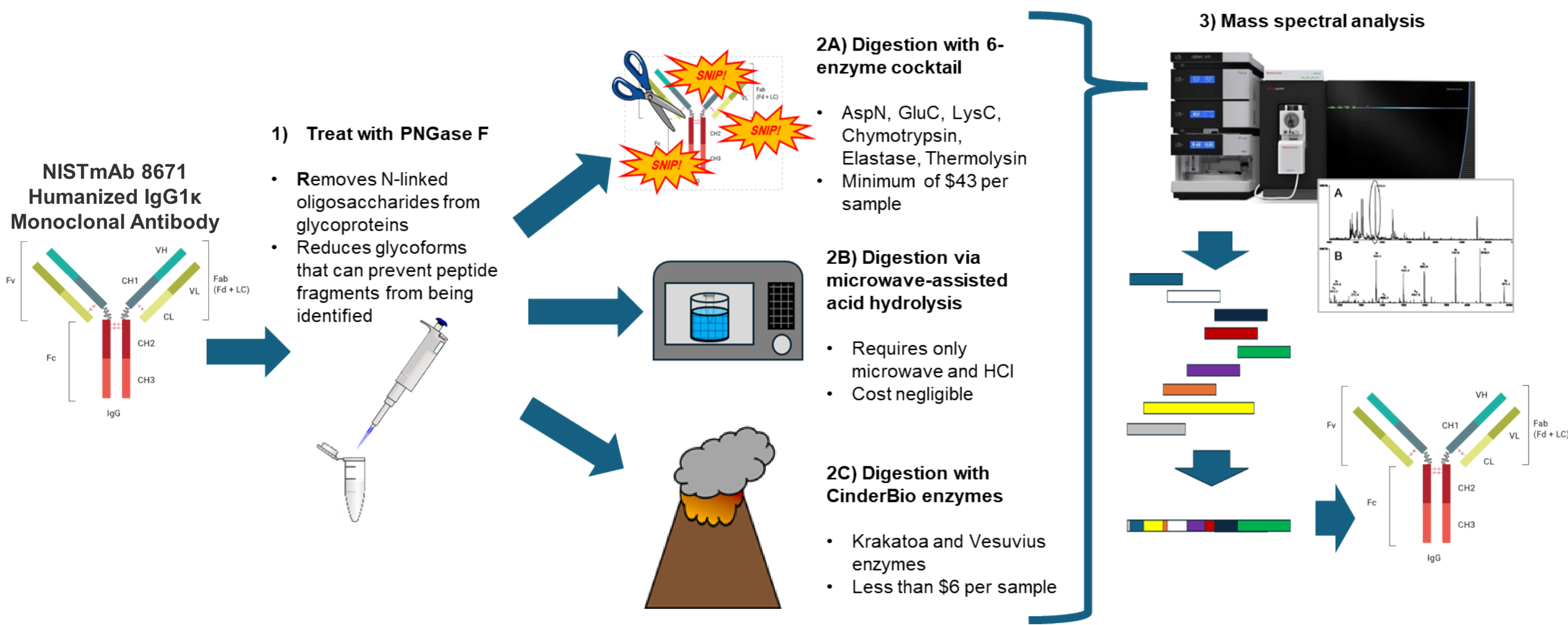


Figure 1. Sample prep workflow and cost per sample for each method.

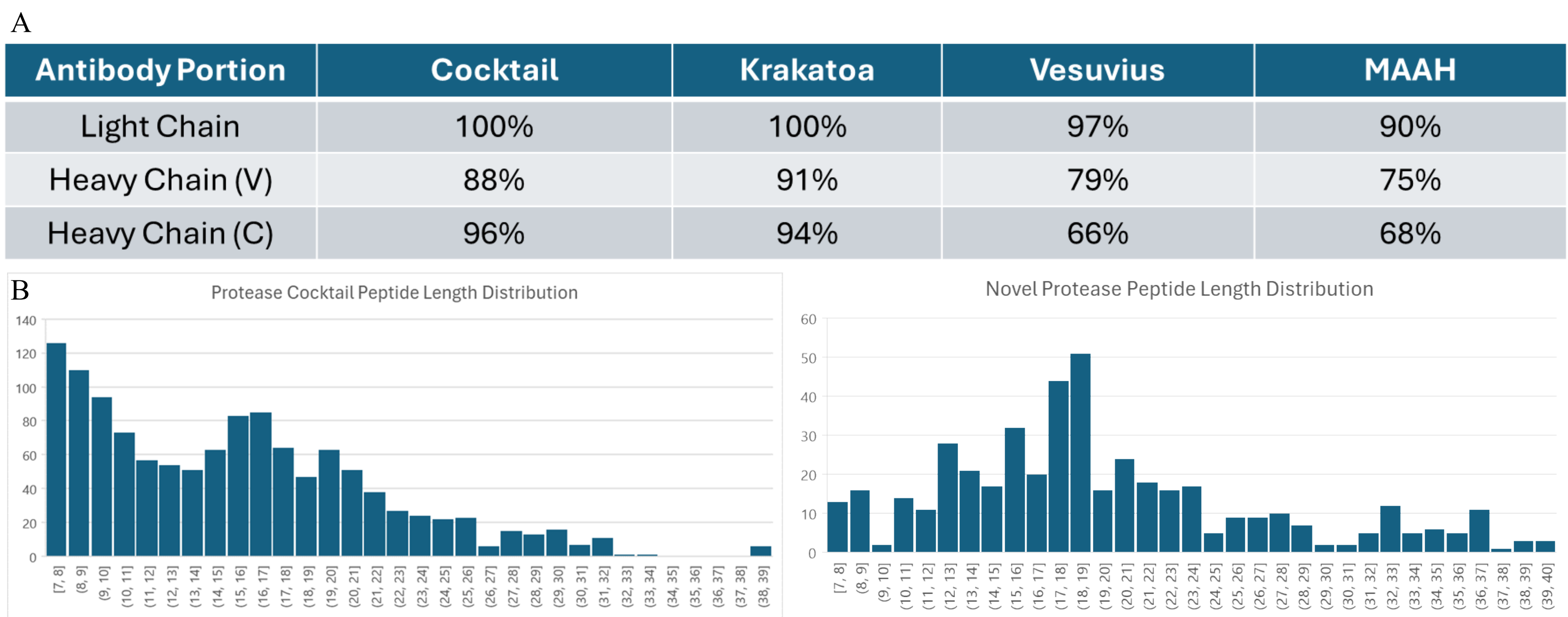


Figure 2. (A) Sequence coverage of heavy and light chains achieved with each method. (B) Peptide length distributions of the two methods that resulted in the highest sequence coverage.



Figure 3. NIST antibody sequencing achieved with near-100% accuracy and coverage using the data combined from all 4 digestion methods. (A) Peptide coverage map. (B) Light chain sequence alignment. (C) Heavy chain sequence alignment and mismatches in sequencing.

Conclusion

Safeguarding the DBPAO antibody repository is synonymous with protecting the Warfighter. Creating a backup of this repository is the best way to preserve it, and this can only be achieved by fully sequencing each antibody. While the effectiveness of the 6-enzyme cocktail is evident, it comes at a steep price point of \$43 per sample in digestion materials alone. This can create financial barriers when the goal is to perform sequencing for such a vast repository. On the other hand, the digestion materials for MAAH is virtually cost-negligible, making it an attractive alternative. But the sub-par sequence coverage of this method can not be ignored. CinderBio's Krakatoa and Vesuvius enzymes present a middle-ground in both cost and sequence coverage, which places it firmly in the lead between the three methods assessed in this work. The largest takeaway, however, lies in the usefulness of combining the data of all methods, as they each provided unique identifications that filled in sequence gaps, resulting in almost 100% coverage of the antibody standard. Where time and cost are not limiting factors, combined digestion methods should be employed for the best results.

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References: ¹Savador, A. et al. (2017). Database-independent Protein Sequencing (DiPS) Enables Full-length de Novo Protein and Antibody Sequence Determination. Molecular & cellular proteomics : MCP, 16(6), 1151–1161. <https://doi.org/10.1074/mcp.O116.065417>; ²Tran, N., Rahman, M., He, L. et al. Complete De Novo Assembly of Monoclonal Antibody Sequences. Sci Rep 6, 31730 (2016). <https://doi.org/10.1038/srep31730>



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