SPEED UP DISCOVERY

Mass-spec based protein sequencing

Antibodies are an invaluable tool in the life sciences. They are ubiquitously used as research tools, diagnostics, and therapeutics. However, despite their importance, many antibodies are in a reproducibility crisis [1].

For over a decade, we have recognized that more than half of antibodies do not exclusively bind their target [2], these inconsistencies cost an estimated $350 million USD annually in the United States alone.

Researchers are turning towards mass spectrometry-based protein sequencing as a solution to improve reproducibility, rescue lost cell lines and identify post-translational modifications; information not capturable via DNA sequencing.

Rapid Novor's REmAb™ service is the world-leading protein sequencing platform offering 100% sequencing accuracy for any monoclonal antibody.

- Are your mAbs immortalized?
- How confident are you with your vendors' QC?
- What has DNA sequencing missed?

KEY TAKEAWAYS:

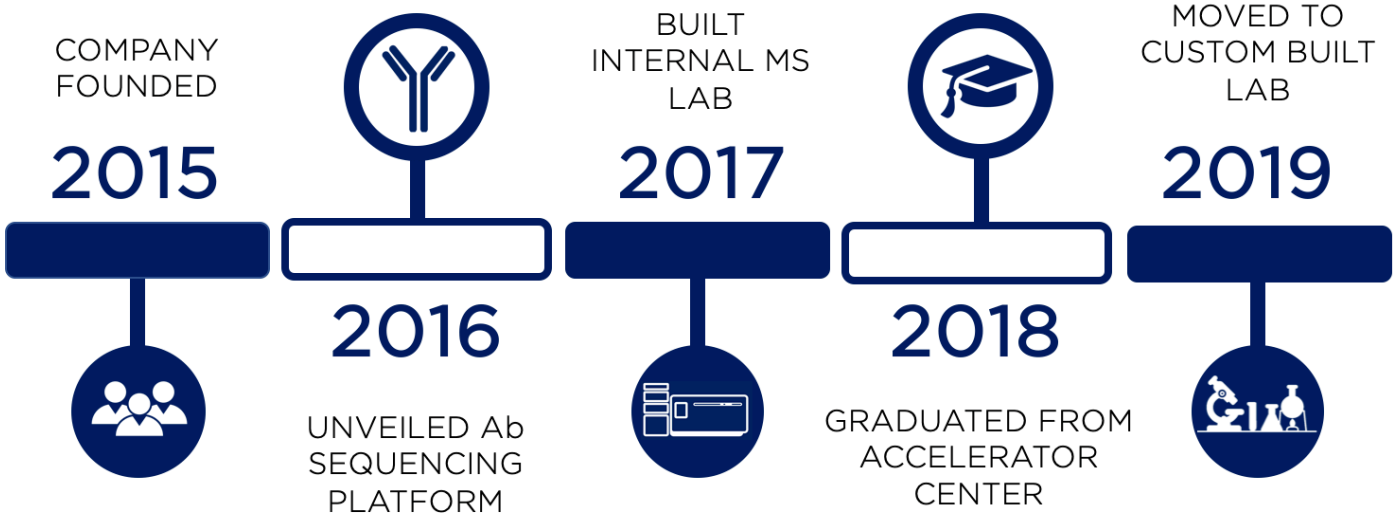
- Rapid Novor's protein sequencing is highly accurate, sensitive and high-throughput
- ~10-20% of mAbs have additional glycosylation sites that go unnoticed with DNA sequencing [3]
- Protein sequencing relies on bioinformatic evidence, not only homology

COMPANY HISTORY

Rapid Novor is a protein sequencing contract research organization founded in 2015 as a spin-off from the University of Waterloo. Starting as three friends working out of local coffee shops, we have grown its team to over 20 members including bioinformatic and mass. spec. experts, while simultaneously establishing the largest privately-owned proteomics mass-spec facility in Ontario.

Our President & CSO, Dr. Bin Ma, has been an active researcher in proteomics for over two decades. Building on this knowledge, Dr. Ma developed new algorithms incorporating machine learning and artificial intelligence to focus explicitly on protein sequencing, resulting in our primary service offering: REmAb™.

Within only four years of operation, we have successfully derived full coverage for more than 1,000 protein sequencing projects and continue to iterate their processes. By focusing our efforts on their internal R&D pipeline, we are finding new ways to address the emerging challenges facing the life science community.



WHAT SETS RAPID NOVOR APART

We specialize in *de novo* sequencing of antibodies directly from the protein, bypassing the need for DNA sequences, cell lines or laboratory animals. We are the world leaders in antibody protein sequencing and are home to the largest private mass. spec. proteomics lab in Ontario, combining high-throughput MS instruments and machine learning algorithms. Through the union of these technologies, our team yields a continuous data feedback loop that benefits both the software and proteomic experimentation. As a result, we are able to consistently deliver unparalleled throughput and accuracy and have derived an unprecedented number of successful protein sequences.

WILD™

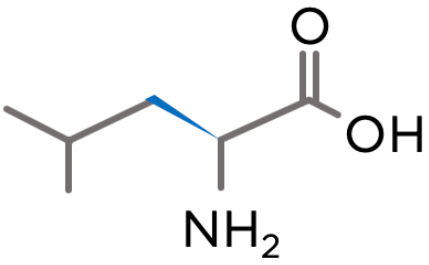
Remove the guesswork and get to your great work.

Mass spec-based de novo sequencing has the ability to measure the mass difference of detected ions from MS/MS fragmentation spectra, allowing researchers to figure out the complete AA sequences of their antibodies. However, until recently it was not possible to distinguish between leucine (Leu) and isoleucine (Ile) at a commercial scale due to their identical masses. That was until early 2017 when Rapid Novor became the first to commercially offer a high-throughput solution: WILD™.

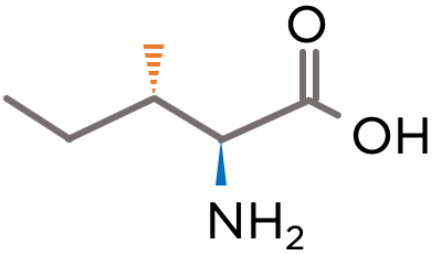
Standard MS experiments measure b and y peptide ions that arise from fragmentation events along the peptide backbone. Our high-resolution MS instrument has a built-in electron-transfer high collision dissociation (EThcD) cell where peptides undergo fragmentation at side chains, resulting in rare w-ions. In using this approach, we are able to confirm sequences with evidence rather than homology.

The exact sequence of antibodies is important for structure and function. It only takes one incorrect amino acid to severely affect the antibody avidity, so guessing Ile and Leu positions can be both costly and time-consuming to your research. To confirm whether a given position is an Ile or Leu, typically researchers express multiple variations of their antibodies trying to guess the correct sequence. A typical antibody contains at least 3 Ile/Leu in the CDRs, however in complicated samples there can be as many as 9. This translates into a minimum of 8 to upwards of 512 expression variants.

By sequencing your antibodies with REmAb + WILD™, your team can remove the guesswork and generate recombinant antibodies with ease and 100% certainty.



Leucine (Leu)
131.175 Da



Isoleucine (Ile)
131.175 Da

HOW VALUABLE IS THE TIME & MONEY ASSOCIATED WITH EXPRESSING 8 SEQUENCES? 16? 64? 512?

OUR METHODOLOGY

SAMPLE RECEIVED

Ship your sample(s) to us using your courier of choice.

1

PROTEASE DIGEST

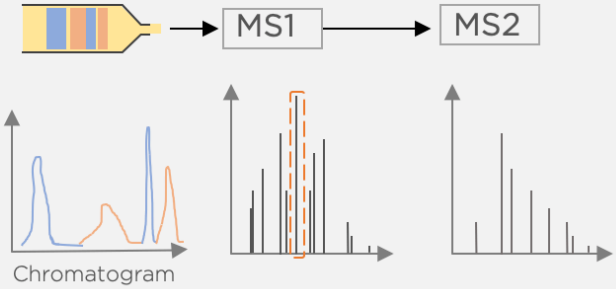
Sample is digested with multiple proteases (5-10)

2

LC-MS/MS

Peptides will be run on our Thermo Orbitrap Fusion

3



PEPTIDE SEQUENCING

Mass spectra are analyzed and peptide sequences are derived

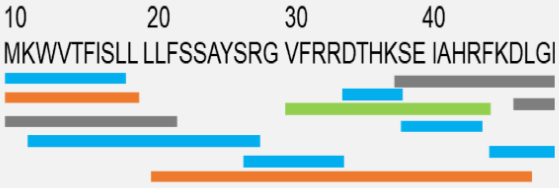
4

MKWVTFISL
LLFSSAYSR
LFSSAYSR
GVFRRDTHK
SEIAHRFKDLGE
DTHK
SEIAHR
VFRRDTHKSEIAHRF
DFAEDK

ASSEMBLY

Resulting peptide sequences are assembled with min. 30x coverage at every position

5

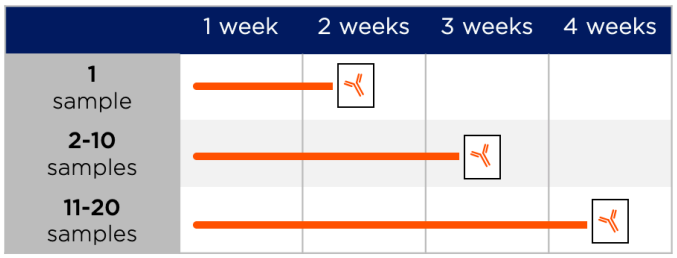


PROJECT DELIVERY

Results are delivered electronically encrypted with password access

6

TURNAROUND TIME



**Expedited options are available*

SAMPLE REQUIREMENTS

- **100 µg** of pure mAb
- **>80%** purity
- **Free of protein contaminants** (e.g. BSA, Keratins)
- **Samples in a standard buffer**

APPLICATIONS

*Why **9 of the top 10** pharmas choose to REmAb their mAbs:*

- There is no access to the cell line
- There is no complete database for antibody sequences
- Genomic sequences aren't explaining biological activity
- Important antibodies are running out
- Antibody supply is unreliable or non-existent
- Batch-to-batch variability is negatively influencing project outcomes

SCHEDULE A FREE CONSULTATION TODAY
1-855-899-9990
info@rapidnovor.com

Client Testimonial:

UNIVERSITY OF DUNDEE

Do you have the capabilities to do protein sequencing in your lab?
"Yes, we have an MS facility however we did not want to perform the bioinformatics analysis ourselves. Also, due to the costs of enzymes used for cutting the Ab and no guaranteed success we chose to outsource to Rapid Novor."

Why was it important for you to know the protein sequence?
"We're focused on species switching for our research, so switching a rabbit mAb into a mouse back bone, donkey backbone etc. As there is high species diversity, there is no hybridoma or cell line."

How would you quantify the benefits of using our service?
"It saved us money, time in evaluating targets and time working with them."

Select References

[1] Bauer, A. Blame it on the anibodies. *Lakartidningen* **71,** 2618-2619 (1974).
[2] van de Bovenkamp, F. S., Hafkenscheid, L., Rispens, T. & Rombouts, Y. The Emerging Importance of IgG Fab Glycosylation in Immunity. *J. Immunol.* **196,** 1435-1441 (2016).
[3] Bradbury, A. & Plückthun, A. Reproducibility: Standardize antibodies used in research. *Nature* **518,** 27-29 (2015).