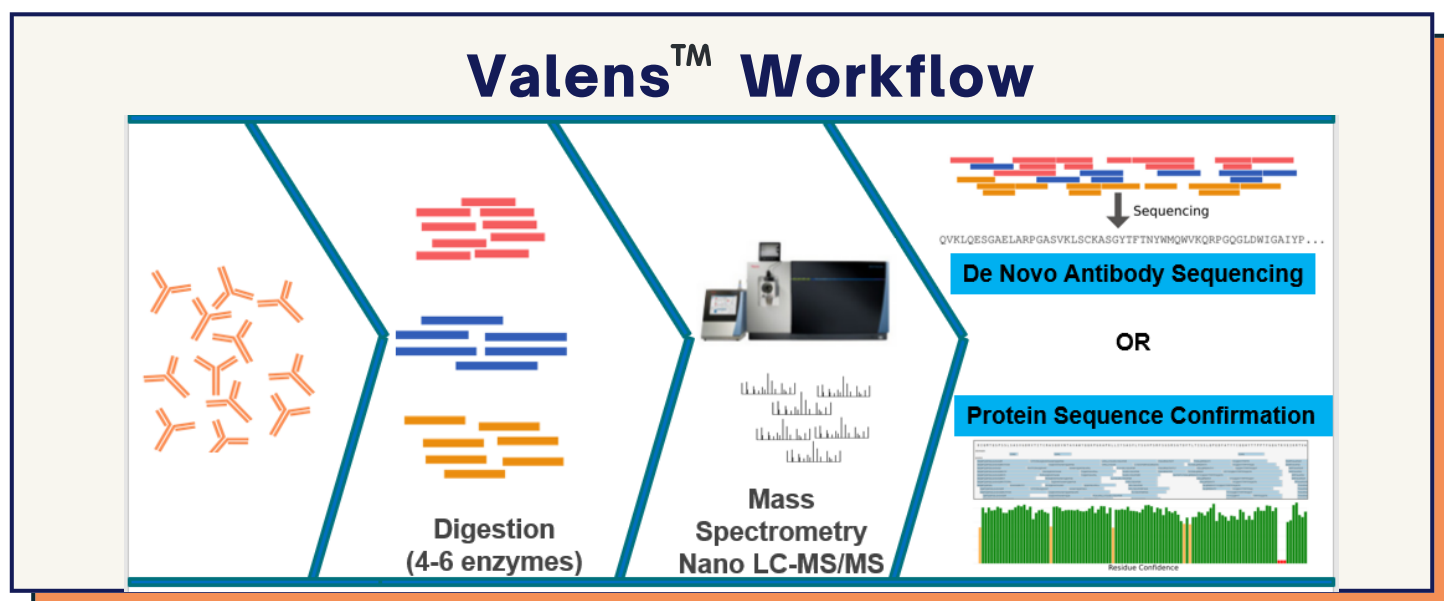


Case Study: Valens recovers missing amino acids from a published anti-FLAG M2 antibody sequence.

Using our Valens de novo antibody sequencing technology, we were able to determine the full-length sequence of an anti-FLAG M2 antibody and fill in missing regions of an Edman sequenced publication (Roosild et al, 2006).



Results

	Heavy Chain
Valens	EVQLQQS AAELAR PGASVKMSCK ASGYXFTYX IIHWVKQRPGQGLEWIGYI XPXXGXXX Y
Published	EVQLQQSGGEL?KPGASVKMSCK?SGYTF?Y?IHW?KQ?-G?GLEWIGYI?P??G??-Y
Valens	NQNFKDETTLTADPSSSTAYM ELNS LTSEDSAVY YCARXXXXX DYWGQ GA TLTVSS...
Published	N??FKGK?TL??DKSSSTAYM????LTSEDSAVY-C?R????G?DYWGQGTTLTVSS...
	Light Chain
Valens	DVLM TQ IPLSLPVSLG DQASISCRSSQ XIVH X NGNTYLEWY L KPGQSP KLLIYK V X NRF
Published	DVLM TQ ?PLSLPVSLG DQASISCRSSQ ?IVH?NGNTYLEWY L QKPGQSP?LLIYK V ?NRF
Valens	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVY YCFQG X H XPYTFGGGT KLEIR ...
Published	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVY YCFQG ?H?PYTFGGGT KLEIK ...

- Valens recovered 31 unknown residues (in blue) and identified 13 different amino acids (in orange) in the published sequence.

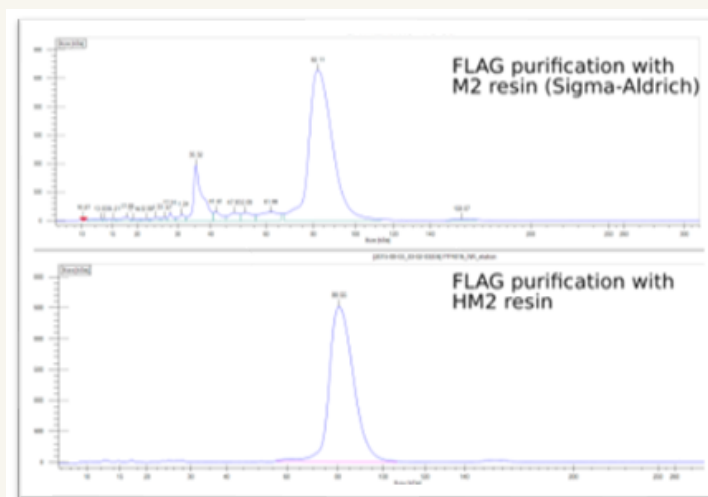
Validation

- In collaboration with LakePharma Inc., we expressed the predicted antibody.
- HM2 antibody sequence (by Valens) and M2 antibody (Sigma Aldrich) have similar binding profiles and affinity (Kd).

	Kd against FLAG-tagged protein (Octet)	Kd against His-tagged protein (Octet)	Kd against 3x FLAG-tagged protein (Biacore)
HM2 (Valens™)	26 ± 3nM	no binding	< 1 nM
M2 (Sigma)	25 ± 4nM	no binding	< 1 nM

Purification using HM2 (Valens™)

- Target protein binding capacity of HM2 resin is at least 10x better than Sigma M2 resin.



Conclusion

- Valens aligned our predicted sequences to the published sequences for the anti-FLAG M2
- We recovered the full-length sequences for both the heavy and light chains.

Valens™ Services:

- De Novo Antibody Sequencing
- Protein Sequence Confirmation

Key features:

- Full-length heavy and light chain sequences
- Isoleucine and Leucine determination using w-ions
- Guaranteed full spectral coverage of variable regions