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REPORT

Proteomic sequencing and resurrection of a monoclonal antibody

Natalie Castellana[1], Kexin Huang[2], Jingxing Li[2], and Hua Tu[2]

[1] Digital Proteomics LLC, San Diego, CA, USA
[2] Curia, Inc., Belmont, CA, USA

While hybridoma sequencing has become a routine part of drug development workflows, there are few options for antibody primary sequence determination when the source hybridoma is unavailable. Mass spectrometry offers a high-throughput method for interrogating the proteome. Digital Proteomics developed Valens, which combines an antibody-specific mass spectrometry protocol with sophisticated informatics to address the challenge of proteomic sequencing of antibodies.

FLAG® tag is a polypeptide tag that can be conjugated to a protein, enabling quantitative investigation of a protein(s) of interest. The ANTI-FLAG® M2 antibody targets FLAG tag and is a crucial component of the proteomic assays. A preliminary protein sequence of an ANTI-FLAG tag antibody was recently published for the purposes of elucidating the crystal structure of the antibody in complex with trans-membrane proteins (Roosild *et al*, 2006).

Protein sequencing was performed using Edman degradation; however, this method only revealed the first 40 amino acids and several gaps persisted, including in the CDR3. Recently, Digital Proteomics developed a monoclonal antibody sequencing technology, Valens, which employs mass spectrometry to determine the full antibody sequence. In collaboration with Curia, Digital Proteomics determined the antibody sequence for the ANTI-FLAG M2 antibody.

Protein digestion

A single MS run (~4500 spectra) of a single enzyme only covers about 50% of the antibody sequence. Using our recommended five enzymes (trypsin, chymotrypsin, pepsin, elastase, and AspN) routinely achieves over 99% coverage.

QIQLQQSGAELASPGASVKLSCKASGYTFTDHIMNWVKKRPGQGLEWIGRIYPVKKETNY		
QIQLQQSGAELASPGASVK		
	ASGYTFTDHIMNWVK	
		RPGQGLEWIGR
LQQSGAEL		
	SCKASGY	
		VKKRPGQGLEW
QIQLQQSGAELASPGASVKLSCKASGYTFT		
		DHIMNWVKKRPGQGLEWIGRIYPVKKETNY
Trypsin	Chymotrypsin	AspN

Final sequences

Heavy Chain	
Valens	EVQLQQSAAELARPGASVKMSCKASGYXFTXYXIHWVKQRPGQGLEWIGYIXPXXGXXXYNQNFKDETTLTADPSSS
Published	EVQLQQSGGEL?KPGASVKMSCK?SGYTFT?Y?IHW?KQ?-G?GLEWIGYI?P??G??-YN??FKGK?TL??DKSSS
Valens	TAYMELNSLTSEDSAVYYCARXXXXXXDYWGQGATLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPV
Published	TAYM????LTSEDSAVY-C?R????G?DYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPV
Valens	TVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIV . . .
Published	TLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSPRPSETVTCNVAHPASSTKVDKKIV
Light Chain	
Valens	DVLMTQIPLSLPVSLGDQASISCRSSQXIVHXNGNTYLEWYLLKPGQSPKLLIYKVXNRFSGVPDRFSGSGSGTDFT
Published	DVLMTQ?PLSLPVSLGDQASISCRSSQ?IVH?NGNTYLEWYLQKPGQSP?LLIYKV?NRFSGVPDRFSGSGSGTDFT
Valens	LKISRVEAEDLGVYYCFQGXHXPYTFGGGTKLEIRRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWK
Published	LKISRVEAEDLGVYYCFQG?H?PYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWK
Valens	IDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNR . . .
Published	IDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNR

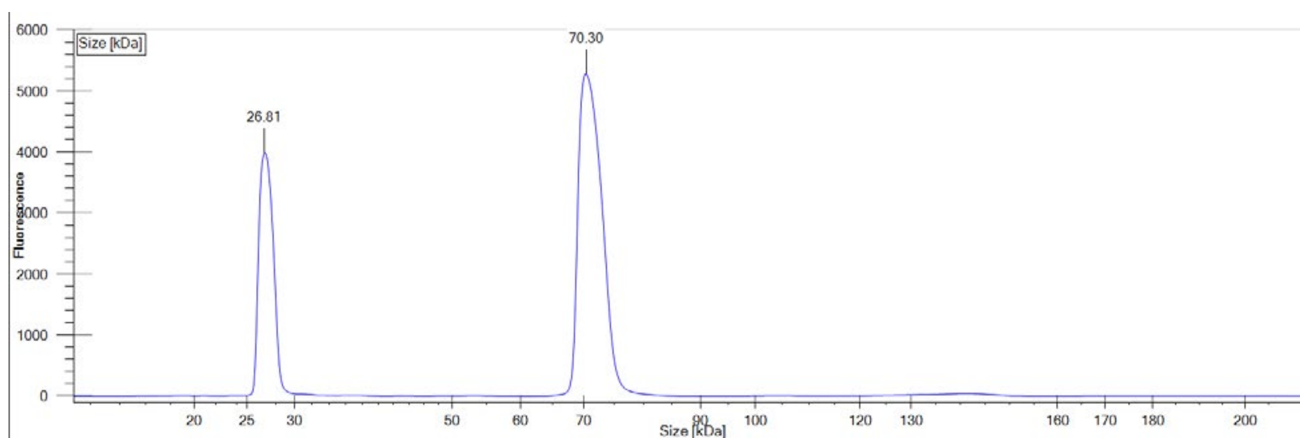
34 unknown residues in the the published sequence (periwinkle)

14 amino acid differences (plum)

Some amino acids are replaced by 'X' for proprietary reasons.

Making recombinant HM2 antibody

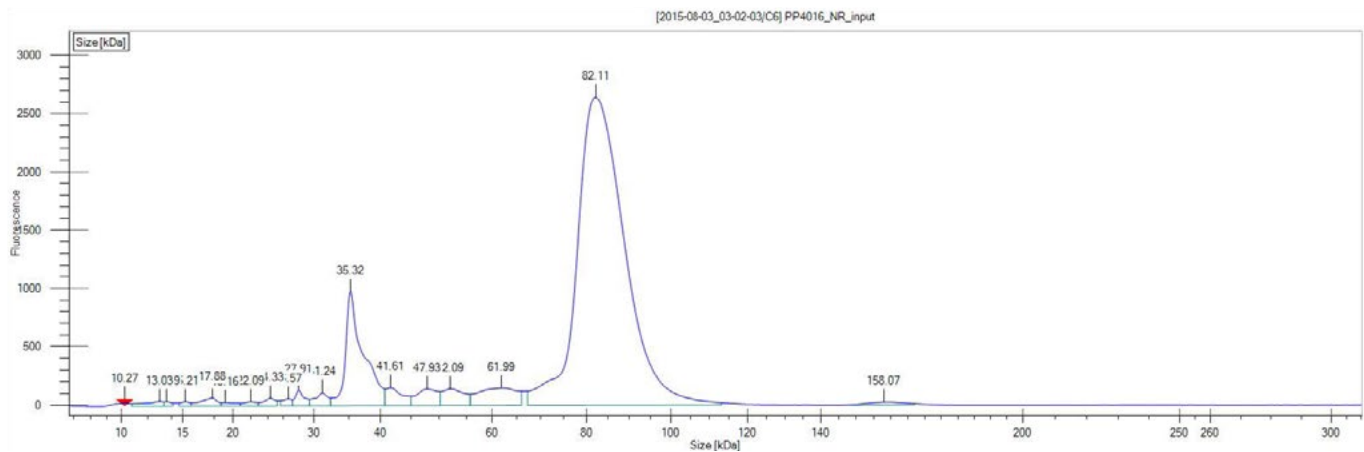
A recombinant antibody with human constant regions and the amino acid sequences identified by Valens as the variable regions were made. Plasmid DNA encoding the full-length antibody was transfected to CHO cells to produce the HM2 antibody. HM2 antibody can be purified very efficiently from the conditioned media with Protein A beads. Purified HM2 antibody was tested for binding to multiple proteins in comparison to Sigma ANTI-FLAG® M2 antibody, and the results showed that they have similar binding profile and affinity (Kd).



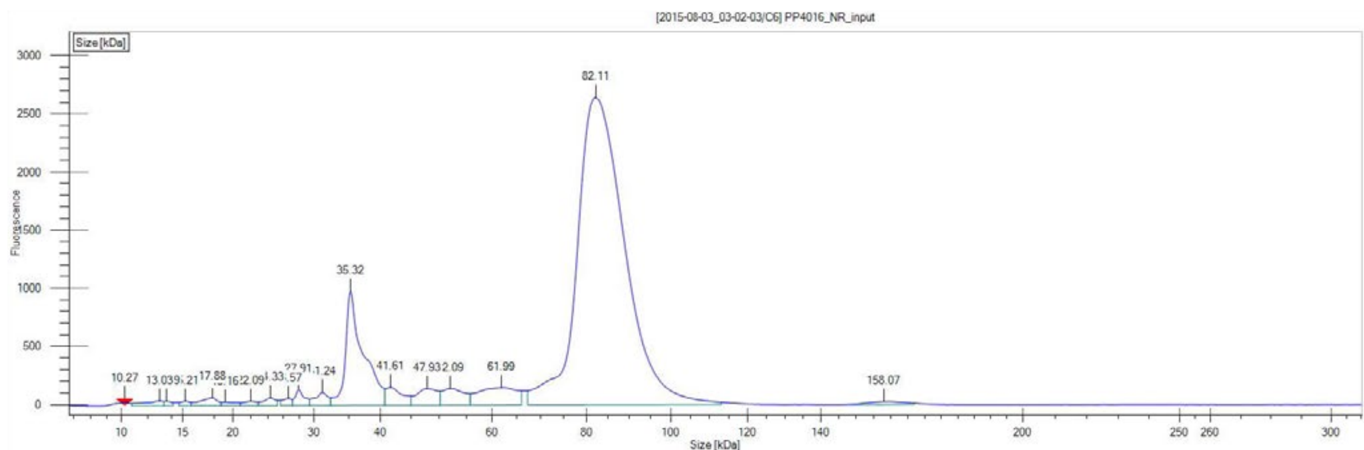
	Kd against FLAG tagged protein (Octet® system)	Kd against His-tagged protein (Octet® system)	Kd against 3x FLAG tagged protein (Biacore® system)
HM2	26 ± 3 nM	No binding	< 1 nM
M2 (reference)	25 ± 4 nM	No binding	< 1 nM

Using HM2 for purification

Input

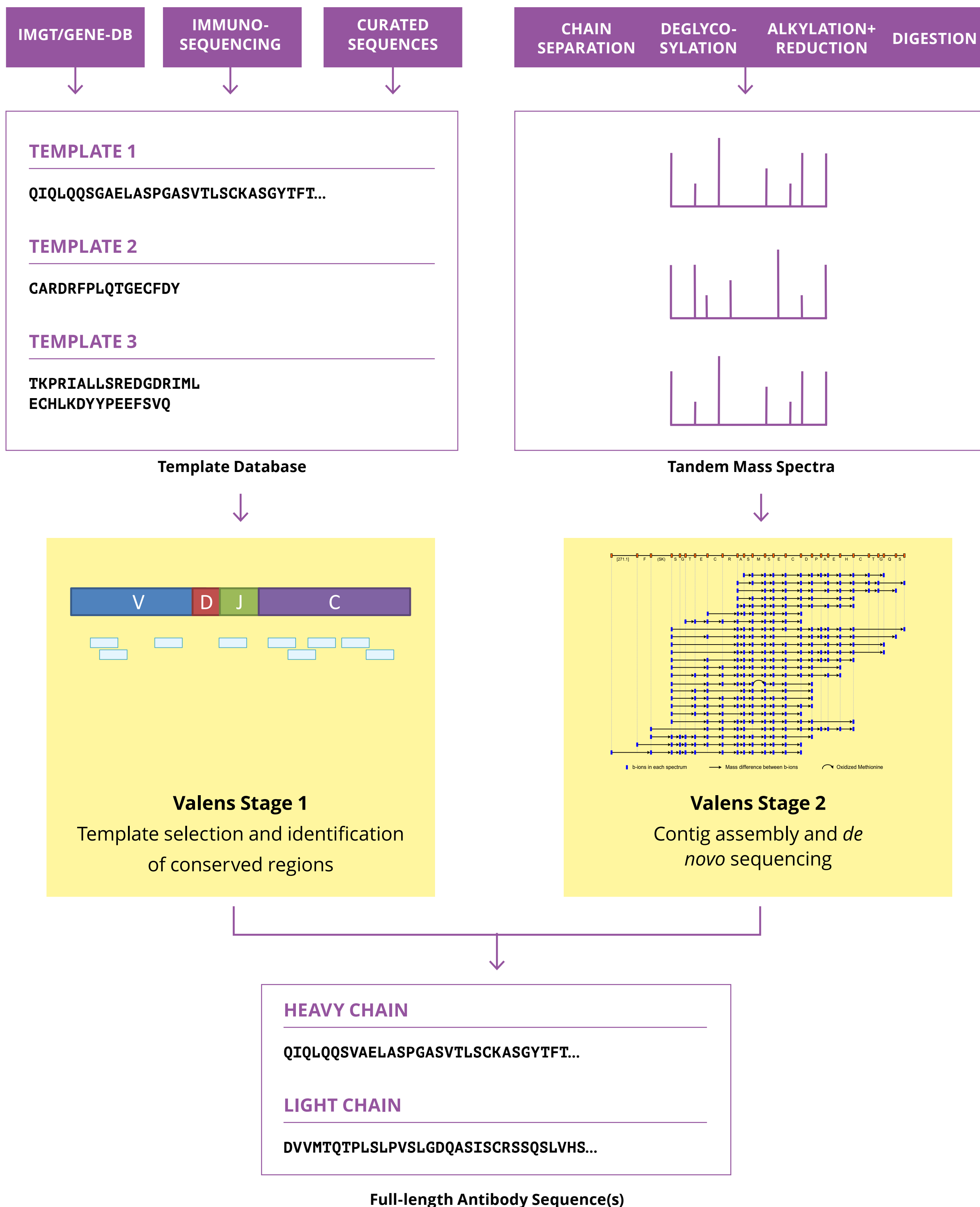


Elute



HM2 antibody was chemically conjugated to sepharose beads. The resulting beads were used to purify from a mixture containing a FLAG tagged protein. As shown in the graphs above, the HM2 beads can specifically and efficiently purify FLAG tagged proteins, with a recovery rate greater than 90%. The target protein binding capacity is 13.5 mg per mL of HM2 resin, which is at least 10x better than Sigma M2 resin.

Proteomic sequencing using Valens



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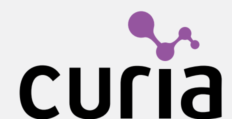

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