Development of an alternative method for the identification and production of antigen-specific monoclonal antibodies

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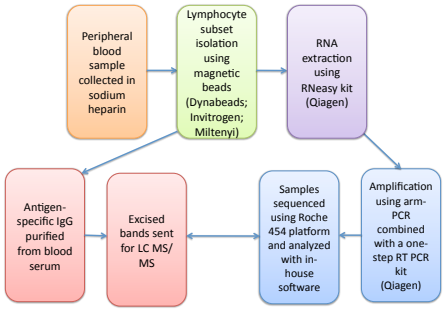
Abstract

A critical step in the production of monoclonal antibodies (Abs) is the initial identification of the antigen-specific Abs, which is usually performed by multiple rounds of panning in both hybridoma and phage display. We have developed an alternative method that allows for the rapid and direct identification of antigen-specific Abs from peripheral blood via high-throughput immune repertoire sequencing and LC MS/MS peptide matching. Our B-cell repertoire technology combines novel amplicon rescued multiplex PCR (arm-PCR, patent pending) with high-throughput gene sequencing to access the sequence of a broad spectrum of heavy and light chain V-regions. As a proof of concept, we have sequenced the immune repertoire of 2 healthy individuals at various time points after administration of the 2009-2010 seasonal influenza vaccine. Antigen-specific Abs were purified directly from immune peripheral blood serum and identified using LC MS/MS peptide sequencing, exploiting the B-cell repertoire gene sequencing results as a database for identification. During our study, several unique peptides were successfully matched for each individual's response to both Flu A strains in the vaccine. Future work includes the development of a method to rapidly clone and express these identified Abs in a human in vitro glycoexpression system. The recombinant Abs will then be tested for their ability to bind flu hemagglutinin, demonstrating the utility of our technology towards the production of mAbs.

Introduction

- The 2009-2010 Fluzone vaccine was administered to two healthy individuals as a booster against influenza A antigens: A/Brisbane/10/2007 and A/Brisbane/59/2007.
- Data analyzed at four time points: day 0, day3, day7, and day 21.



Method

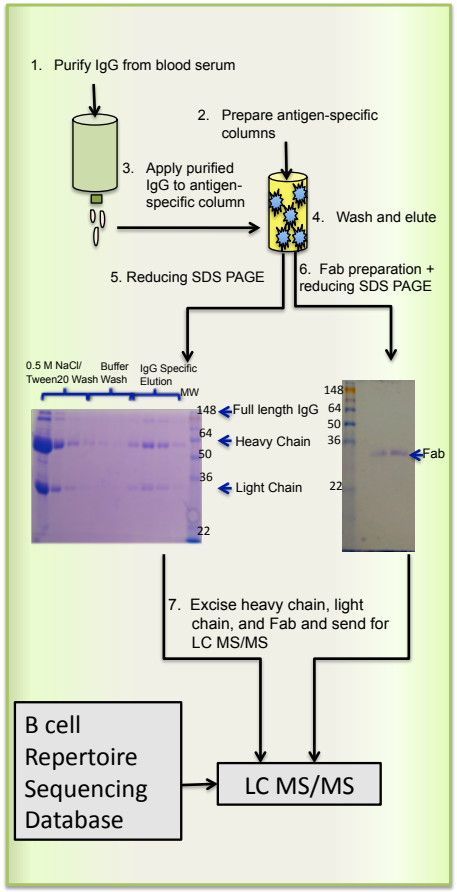


Figure 1. The outline of the method to identify antigen-specific IgG directly from blood serum is demonstrated. Unique CDR3 sequences identified through mass spec. will be cloned and expressed to assess their binding affinity for flu A antigens. In addition to utilizing the mass spec. data, we plan examine up-regulated CDR3s identified directly from the sequencing data.

Results

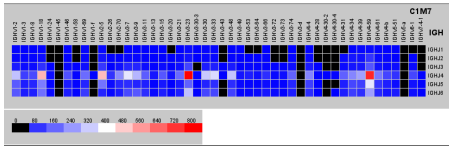


Figure 2. Two-dimensional heat-map indicating the frequency usage of both V and J germline gene segments for one individual's day 7 B-cell repertoire.

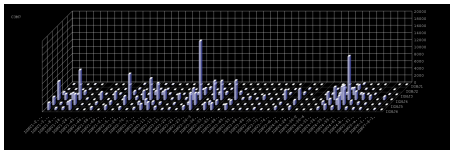


Figure 3. Three-dimensional bar graph indicating the frequency usage of both V and J germline gene segments for one individual's day 7 B-cell repertoire.

Show 10 entries Search:

Peptide	V	J	Frequency
VRGLIVGATYSER	hIGHV4-34	hIGHJ1	219
ARRGSNFYYYAMDV	hIGHV4-59	hIGHJ6	79
ARNDPRLETGMFMGY	hIGHV3-74	hIGHJ4	60
ARDHWGSLDC	hIGHV4-59	hIGHJ5	50
ARDEGLTYSSSFDY	hIGHV3-30	hIGHJ4	43
ARNDPRLATGMFMGY	hIGHV3-74	hIGHJ4	43
AREYSSLDY	hIGHV4-59	hIGHJ4	35
AHRRDRNSAWSLGDFDY	hIGHV2-5	hIGHJ4	33
ARVGAYCSSSSCFDY	hIGHV1-18	hIGHJ4	33
ARDPMGGYYGTFDY	hIGHV1-18	hIGHJ4	33

Figure 4. The top 10 CDR3 frequency of one individual's day 21 memory response is demonstrated.

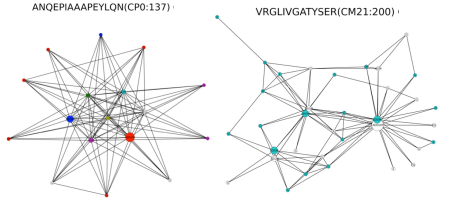


Figure 5. A figure demonstrating two hypermutation/class-switch "clusters" centered around two dominant CDR3s. Dots are linked with one AA difference in CDR3 sequences. Color indicates dates and either pan B (P) or memory (M): red-P, day 0; blue-P,3; green-P,7; cyan-P,21; magenta-M,3; yellow-M,7; white-M,21. The size of the circle represents the expression level (number of reads), and the letter inside the circle (ADMEG) indicates classes observed for that particular CDR3.

Results

SAMPLE A—DAY 7: A/BRISBANE/59/2007 HEAVY CHAIN

Scan No.	Peptide Mass	Peptide Sequence	Sequence Header
3344	2424.16	SYNYVNYWGQGTLVTVSSASTK	>gi|xxxxxxxx|CP0_IGH_GJQGNIM01EKNU5
2912	1367.72	NTLVLQFNSLR	>gi|xxxxxxxx|J3M7_IGH_GMPTOXV01A70D
3344	2424.27	VVRGYKSNWGQGTLVTVSSASTK	>gi|xxxxxxxx|J3M7_IGH_GMPTOXV02HNMK
3008	1635.81	DWGQGTLVTVSSASTK	>gi|xxxxxxxx|C2M21_IGH_GMNM01S01BJK0M
2716	1331.61	AEDTAVYYCIK	>gi|xxxxxxxx|C2M21_IGH_GMNM01S01BR76P
2716	1331.61	AEDTAVYYCLK	>gi|xxxxxxxx|C2M21_IGH_GMNM01S01CWUOL

SAMPLE A—DAY 7: A/BRISBANE/59/2007 LIGHT CHAIN

Scan No.	Peptide Mass	Peptide Sequence	Sequence Header
2980	1816.93	NYIAWYQQKPGQPPK	>gi|xxxxxxxx|C2M21_IGK_GMNM01S01DQ2LJ
2850	1302.61	FGSSGSGTDFTLK	>gi|xxxxxxxx|C2M21_IGK_GMNM01S02GGF4J
3034	1631.78	FGSSGSGTDFTLTISR	>gi|xxxxxxxx|C2M21_IGK_GMNM01S01C4RO5
2914	1218.66	SGTSASLAISGLR	>gi|xxxxxxxx|CP21_IGL_GJQGNIM02F2P7F
3084	2042.03	ANPTVTLFPPSSEELQANK	>gi|xxxxxxxx|F2_Bn_IGL_FWHEHJI01BUJBW

Figure 6. A partial sample output of the LC MS/MS data of one individual's antigen-specific heavy chain and light chain response to A/Brisbane/59/2007 hemagglutinin is shown. Unique CDR3 regions are highlighted in red (Fab data not shown).

Conclusions and Future Work

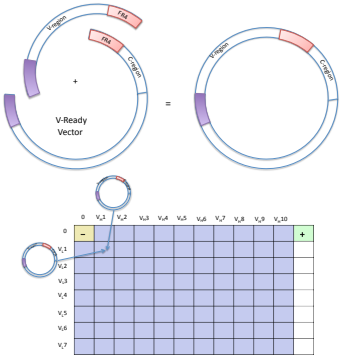


Figure 7. Currently, we have created V-ready vectors with the C-region as part of the vector. Homologous recombination will be used to clone identified V-regions into these prepared vectors. Heavy and light chain pairs will be expressed in a human *in vitro* transcription/translation system as Fab fragments. These Fab fragments will be tested for the ability bind influenza A antigens with ELISA.

References

Han, Jian *et al.*, 2006. Simultaneous Amplification and Identification of 25 Human Papillomavirus Types with Templex Technology. *J. Clin. Micro.* 44, 4157-4162.

Wang, Chunlin *et al.*, 2010. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. *PNAS.* 107, 1518-1523.