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Selection of Antibodies in Older People

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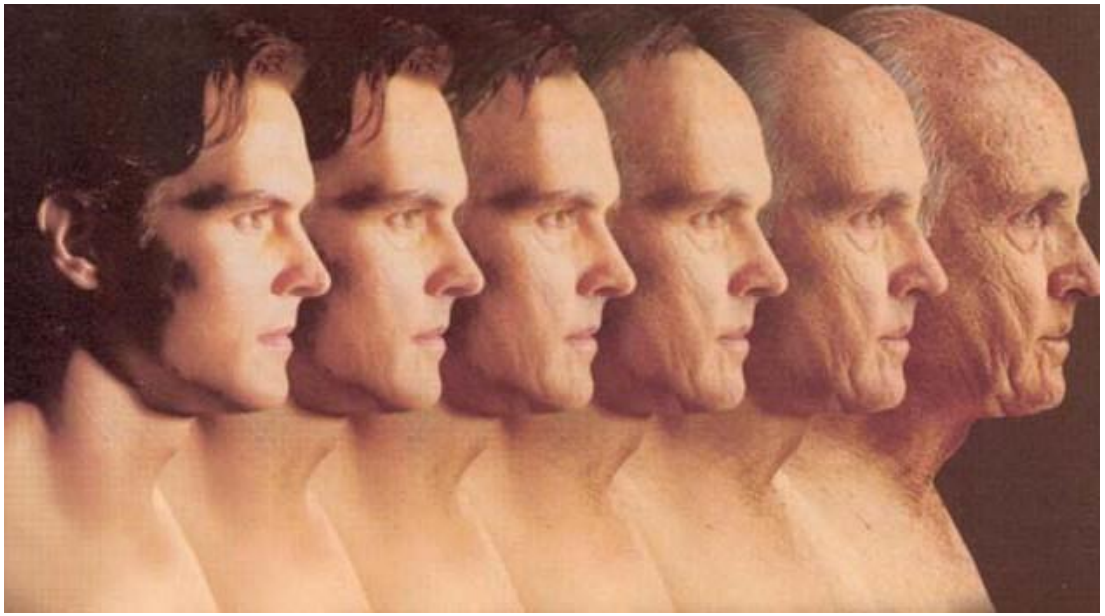
RUC 14th October 2015

Deborah Dunn-Walters Lab

The Older Population

- In the UK over-65s expected to rise from 16% in 2008 to nearly 30% by 2050
- Older people are less able to respond effectively to vaccination
- Pneumococcal vaccines: reduce the incidence of pneumonia by 10% in old, compared with 40–50% in younger patients

The Efficacy of the Immune System Decreases with Age



- Immunosenescence: Natural deterioration of the immune system occurring with age, resulting in dysregulation and failure to function efficiently:
  - An increased susceptibly to infections
  - An increased prevalence of inflammatory diseases
  - An increased morbidity and mortality

The Efficacy of the Immune System Decreases with Age

Aging Cell (2009) **8**, pp18–25

B-cell diversity decreases in old age and is correlated with poor health status

Kate L. Gibson,[1] Yu-Chang Wu,[1] Yvonne Barnett,[2] Orla Duggan,[2] Robert Vaughan,[3] Elli Kondeatis,[3] Bengt-Olof Nilsson,[4,5] Anders Wikby,[6] David Kipling[7] and Deborah K. Dunn-Walters[1]

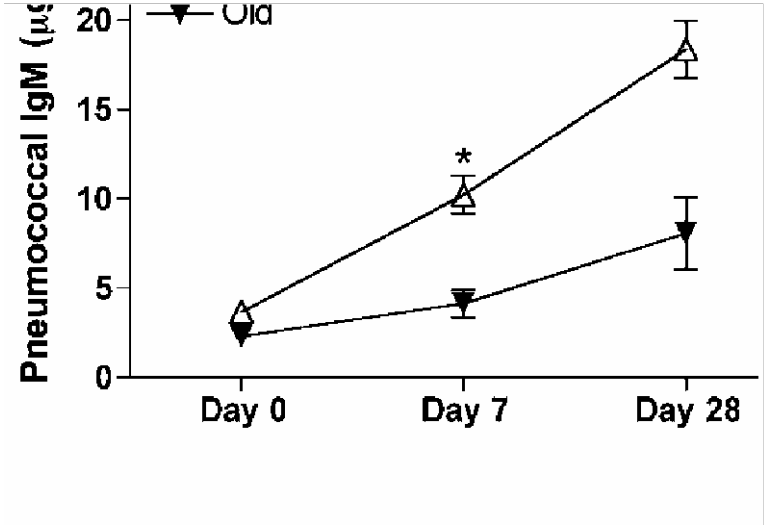
→ Loss of B cell diversity in the very old (86 to 94)

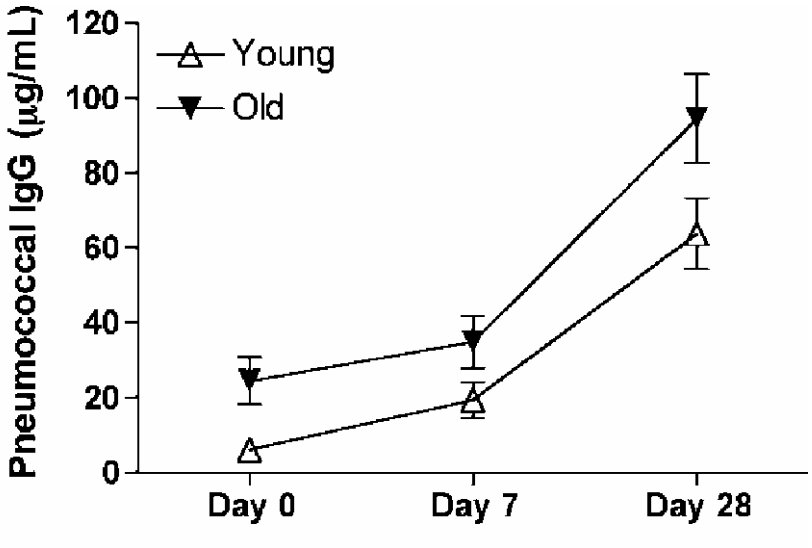
Aging Cell (2011) 10, pp922–930

Vaccination-induced changes in human B-cell repertoire and pneumococcal IgM and IgA antibody at different ages

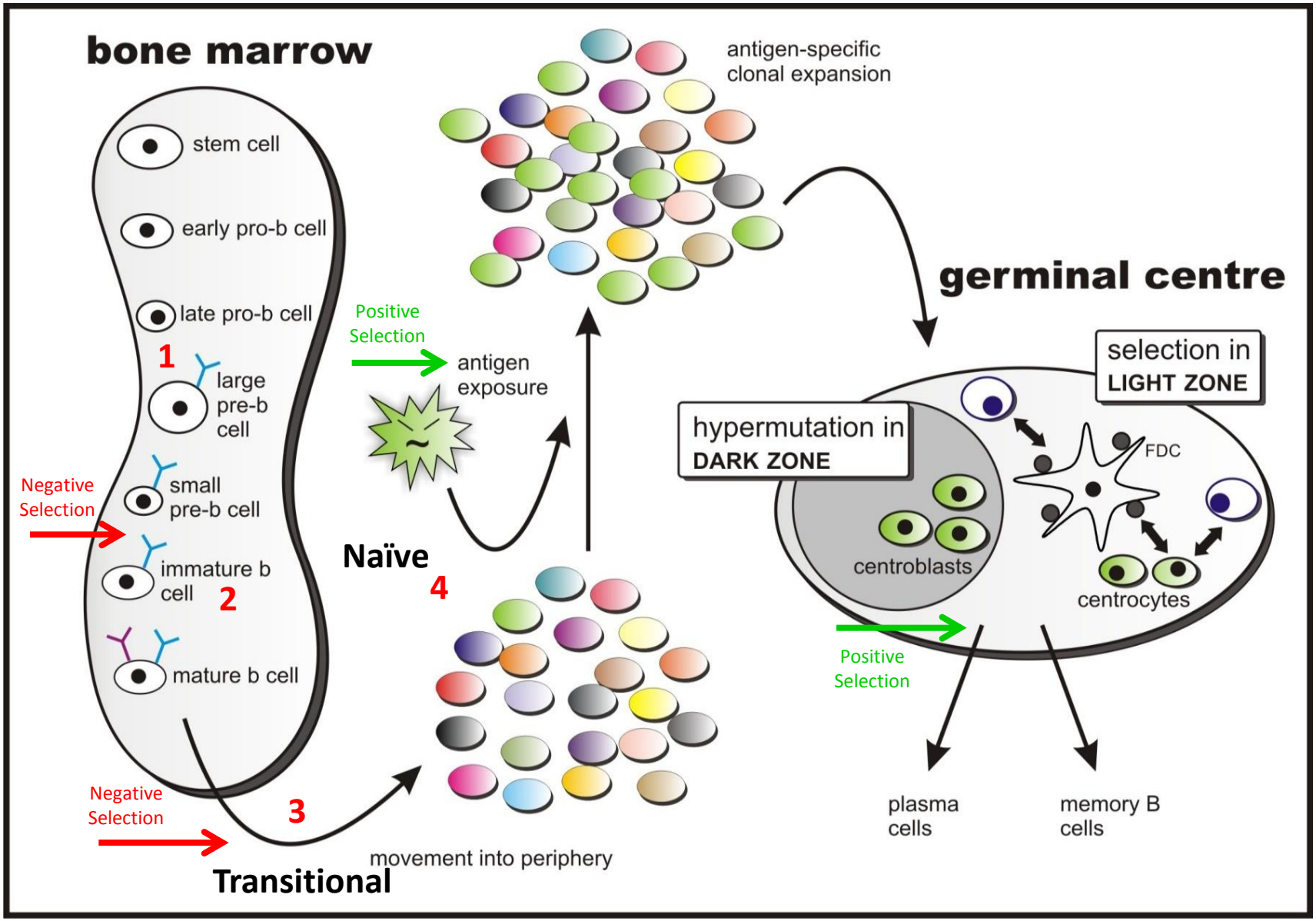
Alexander Ademokun,[1] Yu-Chang Wu,[1] Victoria Martin,[1] Rajive Mitra,[2] Ulrich Sack,[3] Helen Baxendale,[4] David Kipling[5] and Deborah K. Dunn-Walters[1]

→ Confirmed loss of diversity in age group (65 to 86)





B Cell Repertoire Shaping



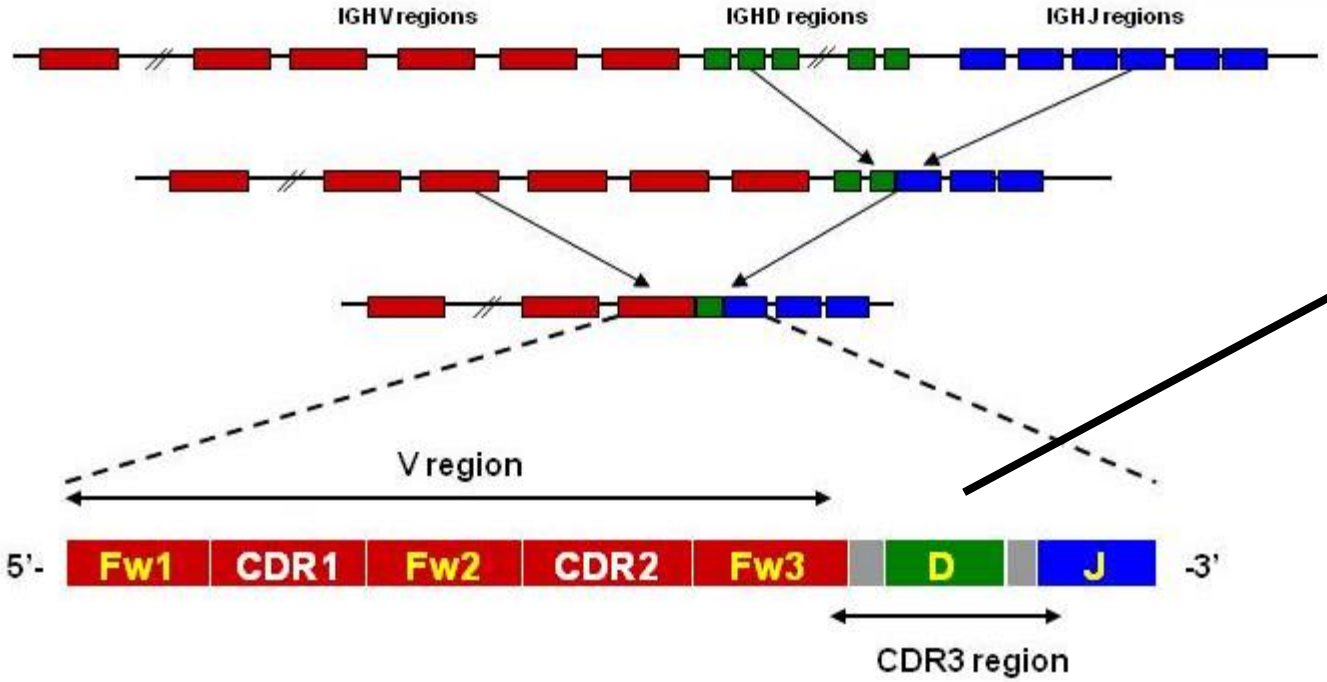
Our Aims

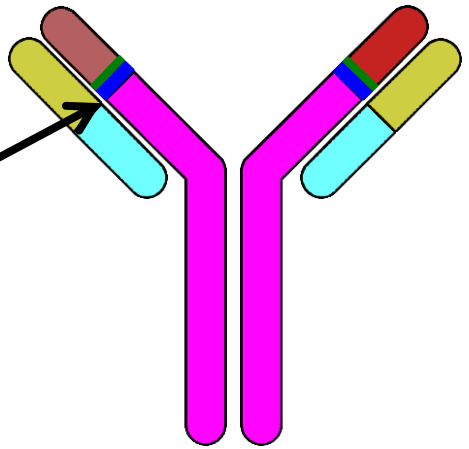
- Identify immunoglobulin genes that are differentially represented in the B cell repertoire of older people
- Define the characteristics of the antibody genes that are overexpressed in B cell development of older people
- Determine whether the age-related B cell immunoglobulin genes are more likely to bind self antigen or be polyspecific

B Cell diversity: Repertoire Generation

Human B Cell Antibody Repertoire ≈ 10^{11}

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	34–38	29–33	38–46
Diversity (D)	0	0	23
Joining (J)	5	4–5	6
Constant (C)	1	4–5	9

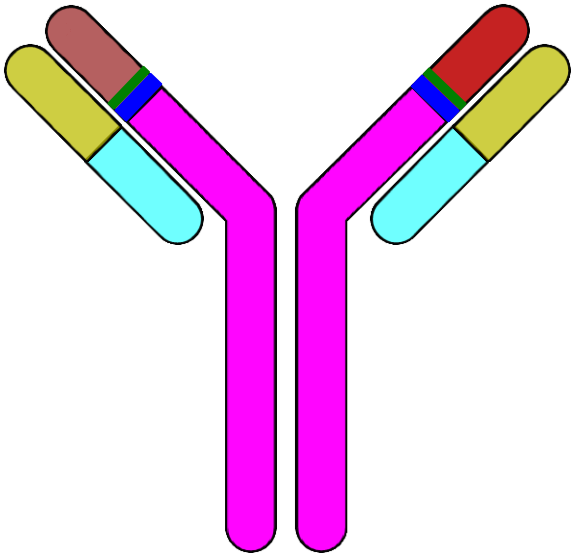




Whole Antibody

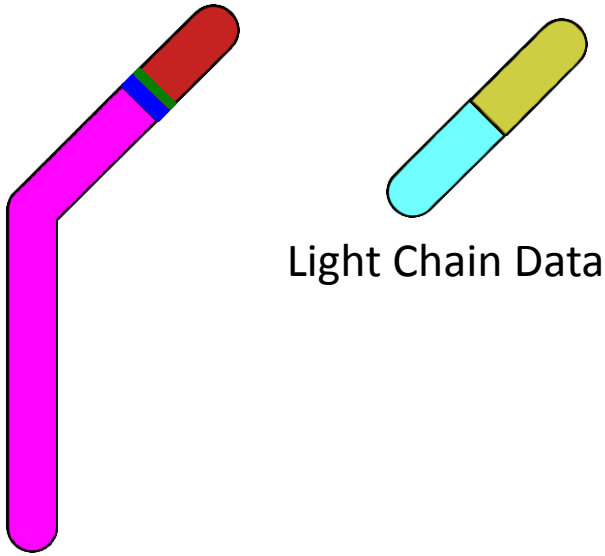
Single Cell Sequencing

Physiological state of Antibody



Matched Heavy and Light Chains Data

High-Throughput Sequencing

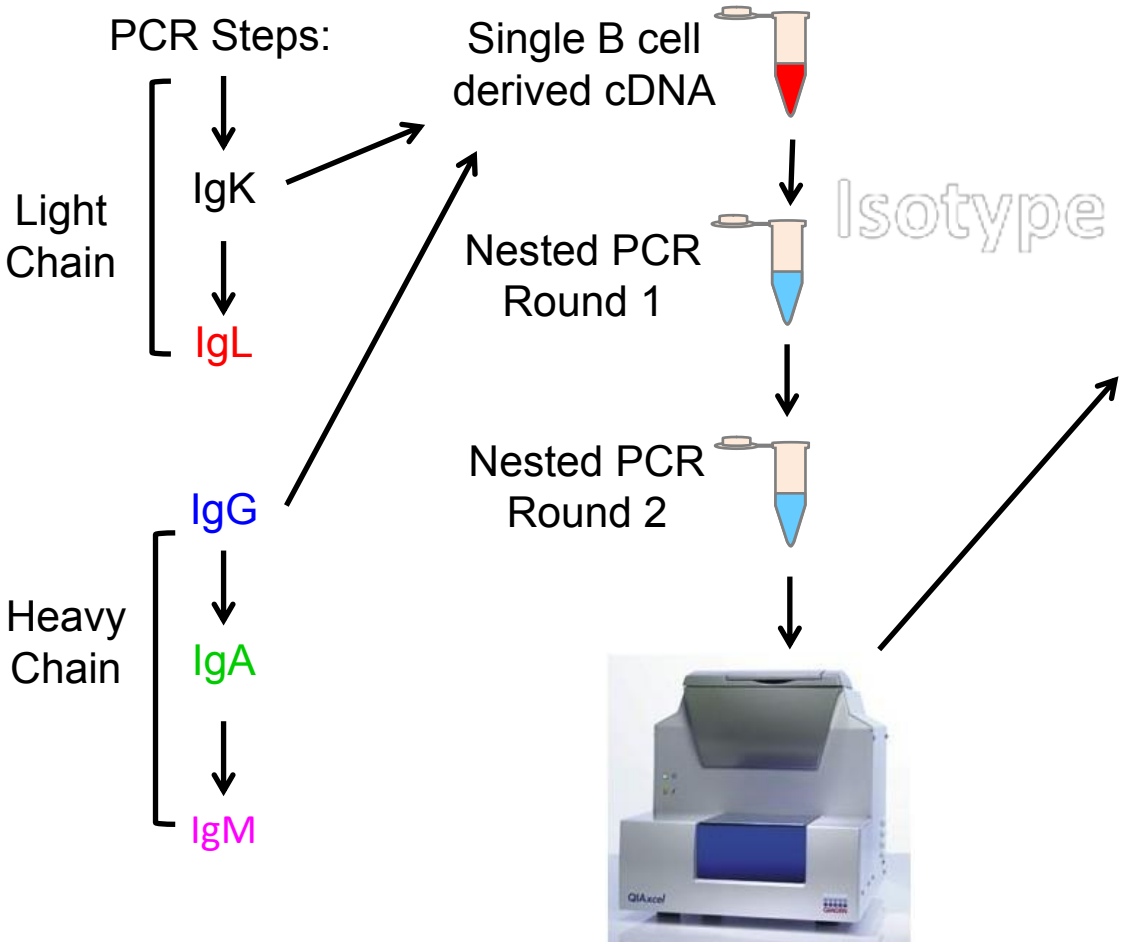


Light Chain Data

Heavy Chain Data

Single Cell Sequencing

- 76 years old man
- Sorted single plasmablasts (CD19+ CD38++)



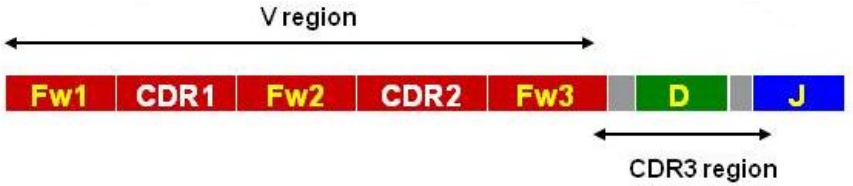
Isotype	Percentage
IgG	23%
IgA	63%
IgM	14%

150 Matched Heavy and Light Chain Sequences Identified

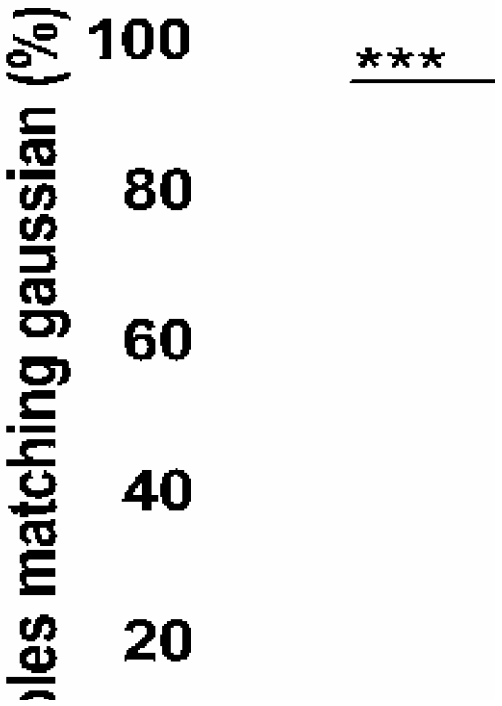
Single Cell Sequencing Data

Sequence ID	Class	V gene	J gene	D gene	CDR-H3 Size
006HA08H	IgA2	IGHV1-18	IGHJ4	IGHD2-21	11
006HH02H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006IA08H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006IE02H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006JE10H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006JG04H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006KB03H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006KC11H	IgA2	IGHV1-18	IGHJ4	IGHD6-6	11
006HD10H	IgA2	IGHV1-46	IGHJ3	IGHD2-21	9
006IB01H	IgA2	IGHV1-46	IGHJ3	IGHD2-21	9
006HD04H	IgA2	IGHV1-3	IGHJ4	IGHD6-13	13
006JD03H	IgA2	IGHV1-3	IGHJ4	IGHD6-25	13
006LA10H	IgA2	IGHV1-3	IGHJ4	IGHD6-13	13
006LD04H	IgA2	IGHV1-3	IGHJ4	IGHD6-13	13
006HB02H	IgA1	IGHV1-3	IGHJ5	IGHD3-10	19
006KD06H	IgA2	IGHV1-46	IGHJ4	IGHD5-12	13
006NF07H	IgA2	IGHV1-46	IGHJ4	IGHD5-12	13
006HD01H	IgA2	IGHV1-3	IGHJ5	IGHD4-17	9
006HE04H	IgA2	IGHV1-3	IGHJ5	IGHD4-17	9
006HE06H	IgA1	IGHV1-3	IGHJ6	IGHD3-22	22
006HB01H	IgG2	IGHV1-3	IGHJ4	IGHD2-2	13
006JC05H	IgG2	IGHV1-3	IGHJ4	IGHD2-2	13
006IC01H	IgG1	IGHV1-3	IGHJ3	IGHD2-21	21
006LA08H	IgG1	IGHV1-18	IGHJ2	IGHD1-7	19
006JC06H	IgM	IGHV1-18	IGHJ6	IGHD4-11	16
006LA06H	IgM	IGHV1-2	IGHJ4	IGHD6-19	17
006ID09H	IgA2	IGHV3-23	IGHJ5	IGHD3-10	20
006JF01H	IgA2	IGHV3-23	IGHJ5	IGHD2-2	20
006JH04H	IgA2	IGHV3-23	IGHJ5	IGHD3-10	20
006LH08H	IgA2	IGHV3-23	IGHJ5	IGHD2-2	20
006JG01H	IgA2	IGHV3-15	IGHJ4	IGHD3-22	14
006KD02H1	IgA2	IGHV3-15	IGHJ4	IGHD4-23	13
006HD11H	IgA1	IGHV3-48	IGHJ6	IGHD2-2	17
006JF05H	IgA1	IGHV3-48	IGHJ6	IGHD2-2	17
006ID11H	IgA2	IGHV3-23	IGHJ4	IGHD2-2	16
006JD09H	IgA1	IGHV3-15	IGHJ4	IGHD4-11	14
006NE03H	IgA2	IGHV3-15	IGHJ4	IGHD4-11	14
006IF03H	IgM	IGHV3-7	IGHJ4	IGHD2-15	12

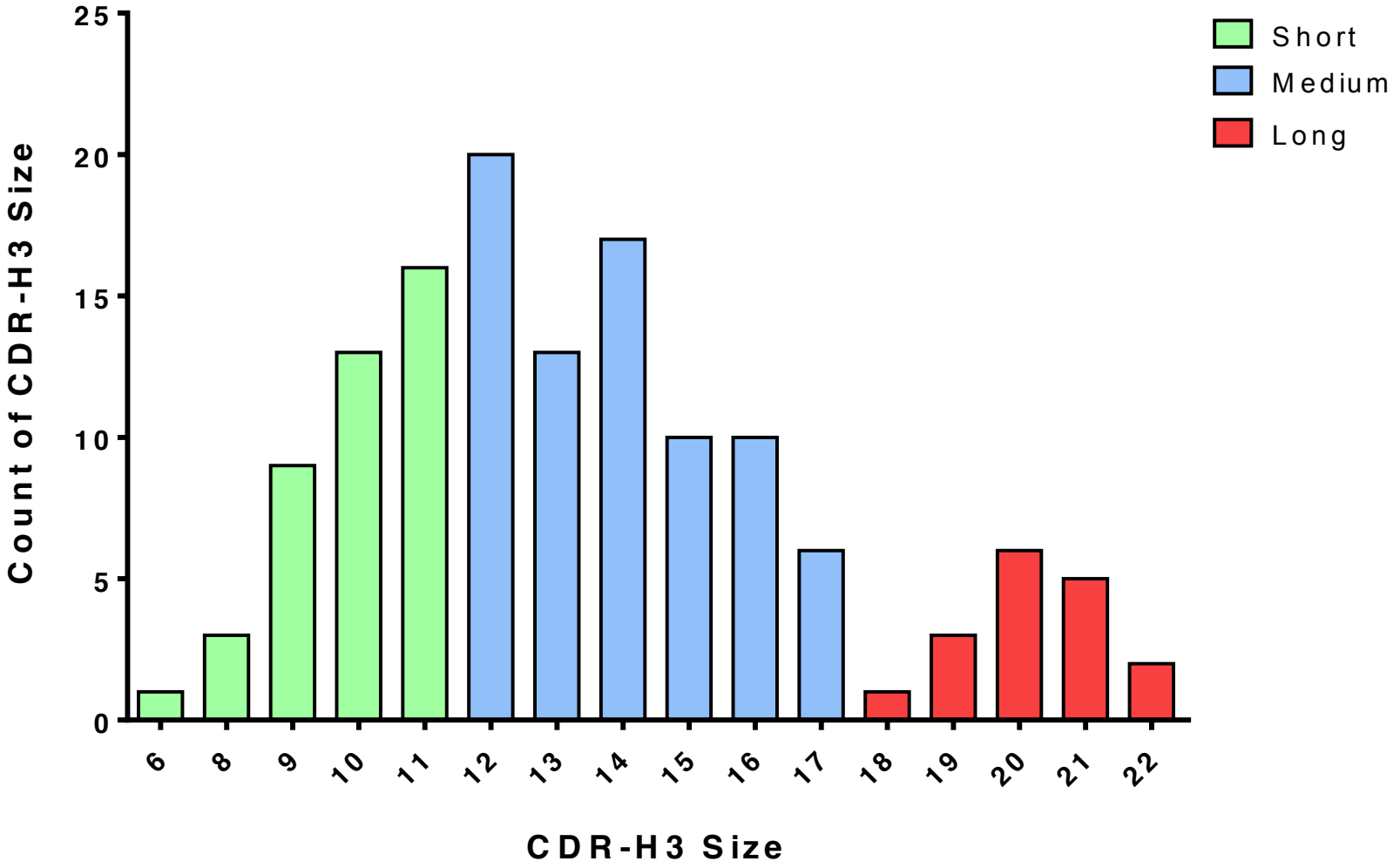
Heavy Chain: CDR-H3



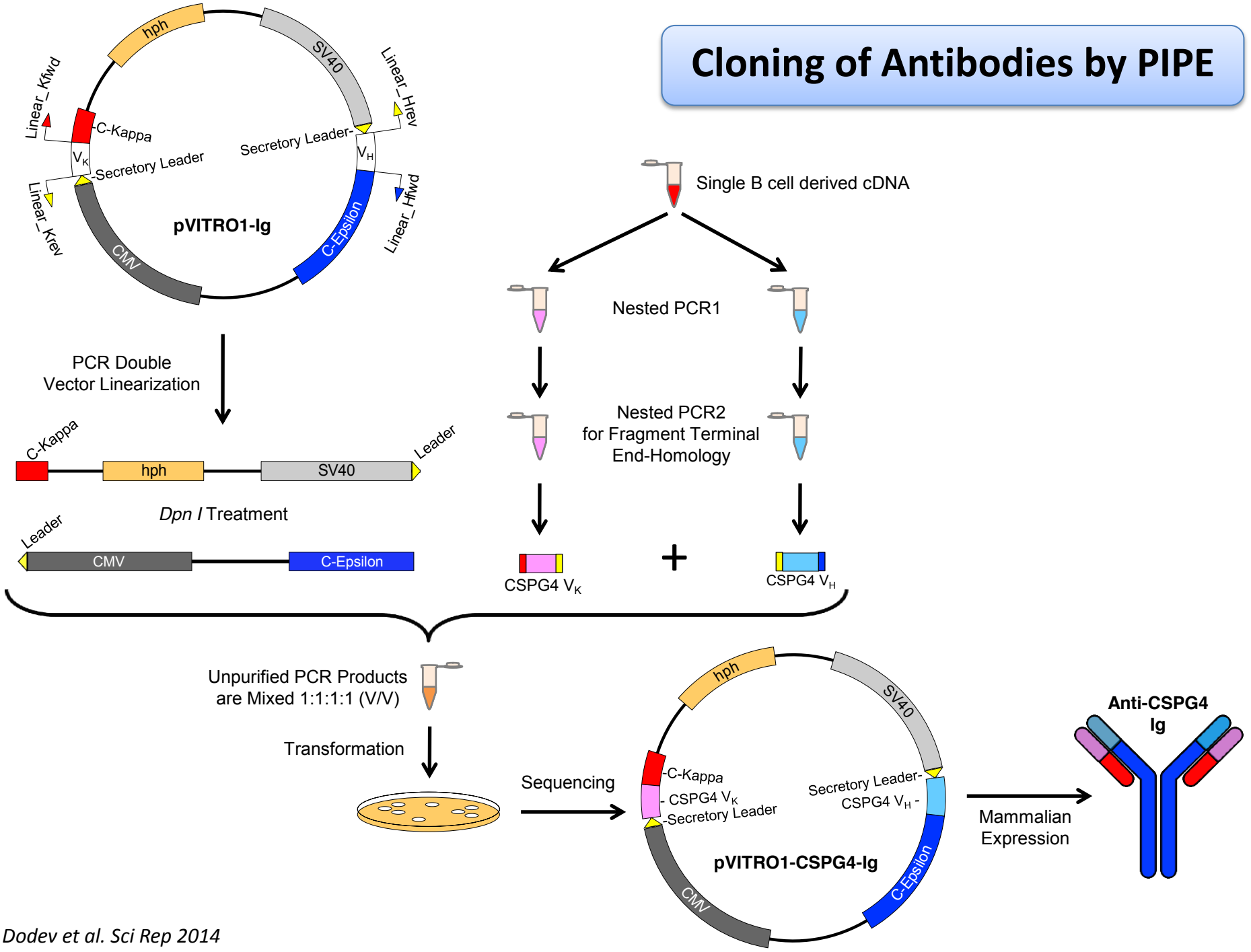
Pneumococcal and Flu Vaccines



CDR-H3 Length

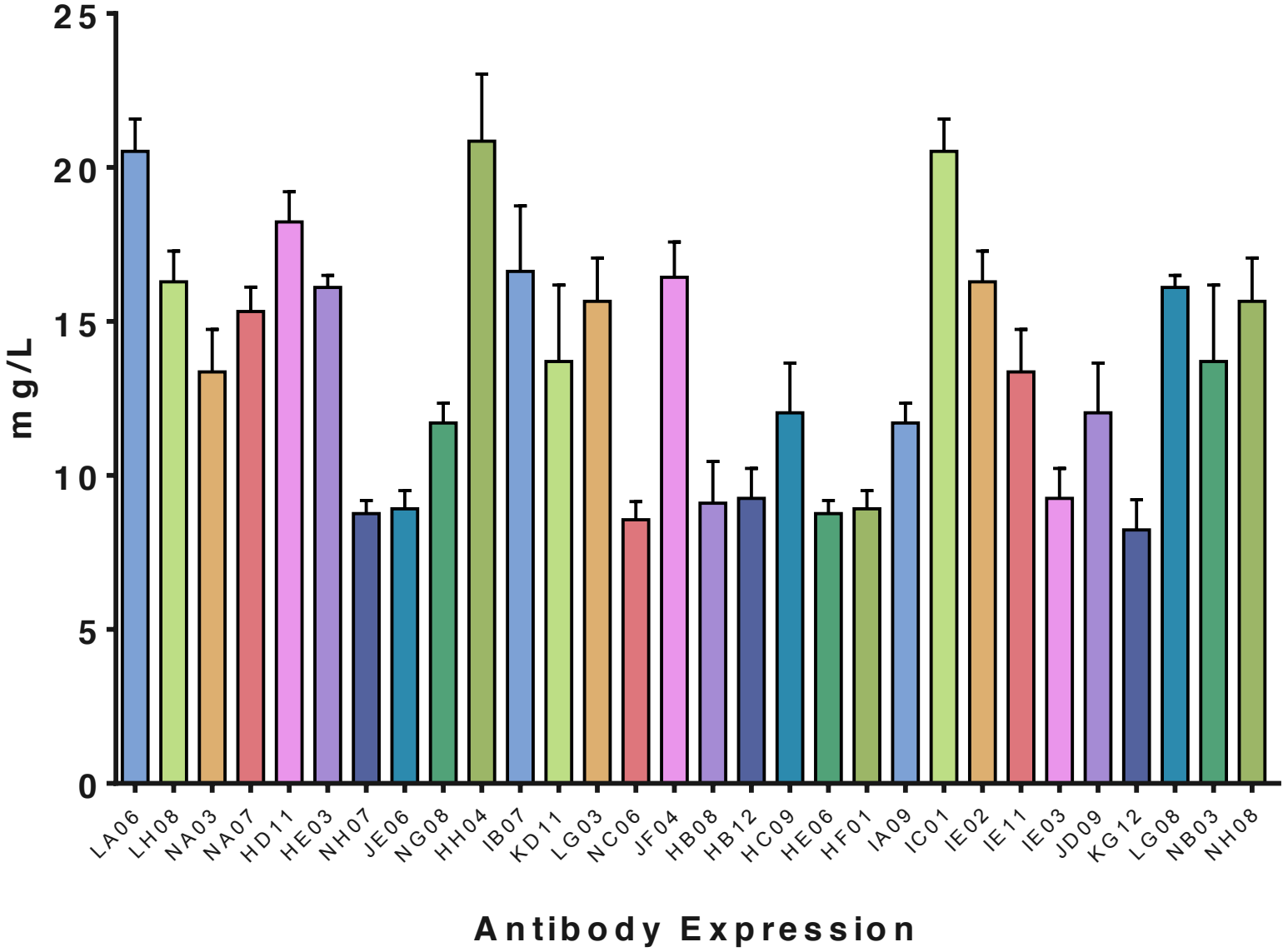


Cloning of Antibodies by PIPE

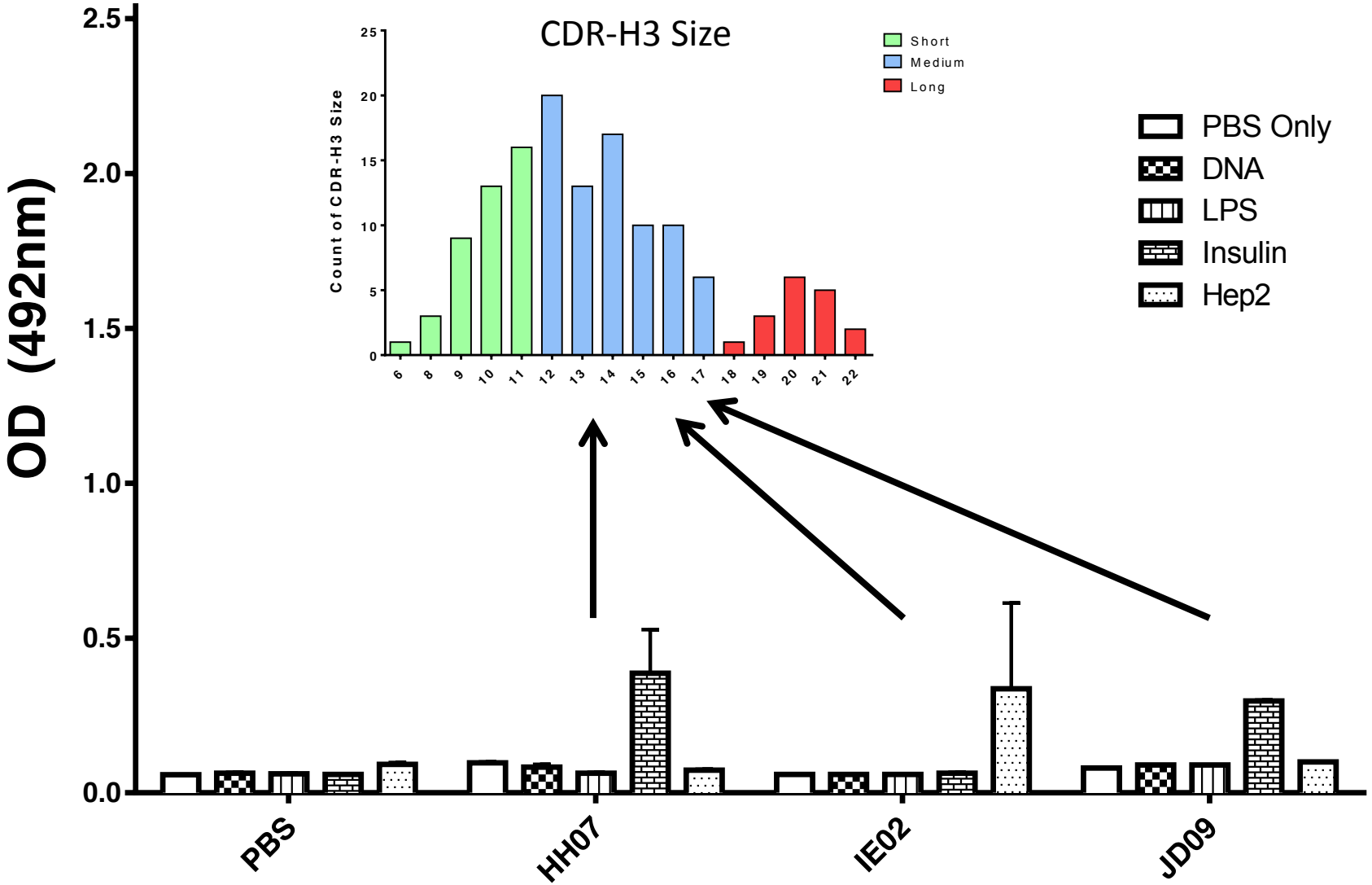


Dodev et al. Sci Rep 2014

Quantitative ELISA of 55 Cloned and Expressed Antibodies



Autoreactivity/Polyreactivity ELISA



High-Throughput Sequencing

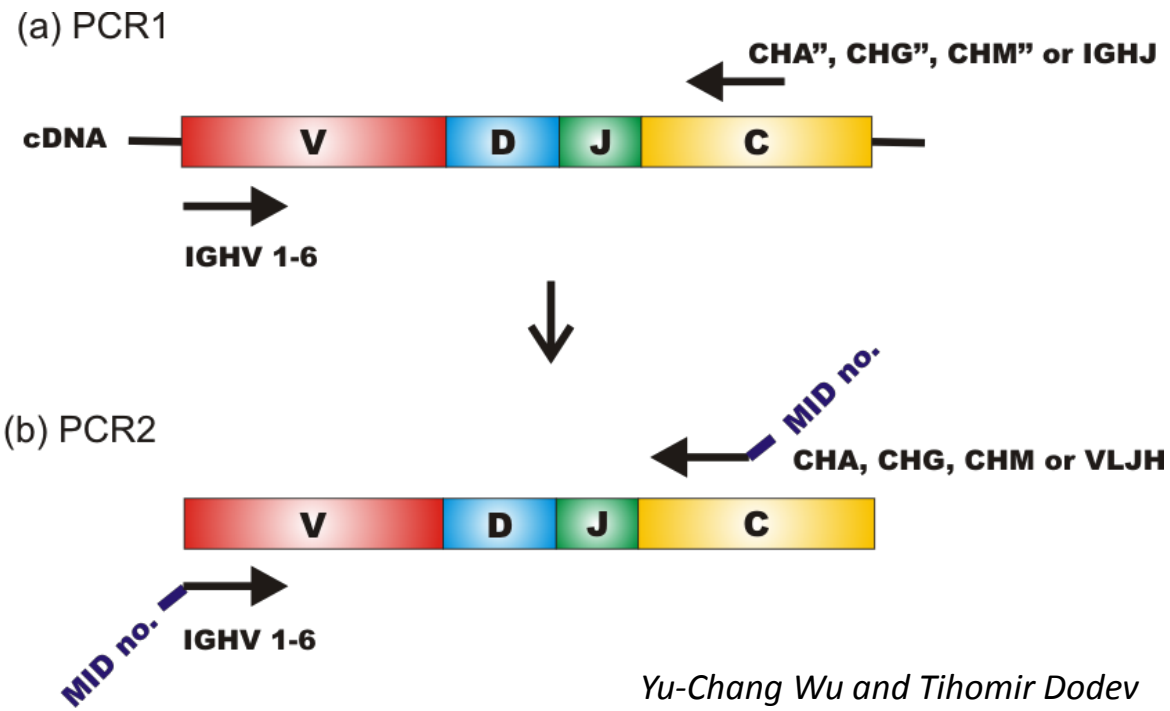
Bone marrow and matching peripheral blood lymphocytes from 12 patients aged 24 to 86

Lymphocyte sorting strategy

Type of B cell	Distinguishing surface markers
preB cell	CD19+ Igk- Igl- IgM+ CD38+
Immature B cell	CD19+ (Igk+ or Igl+) IgM+ CD38+ IgD- CD10+
Mature Transitional B cell	CD19+ (Igk+ or Igl+) IgM+ CD38+ CD27- IgD+ CD10+
Mature Naïve B cell	CD19+ (Igk+ or Igl+) IgM+ CD38+ CD27-IgD+ CD10-

RNA

cDNA



Yu-Chang Wu and Tihomir Dodev

Ig High-Throughput Sequencing Data Generation

Electrophoresis/Purification of PCR Products

↓

Assemble PCR Products from 12 MIDs

↓

Titanium Sequencer

Raw sequences

↓

Automatic Analysis QC, V-Quest, ProtPram

Excel Output

↓

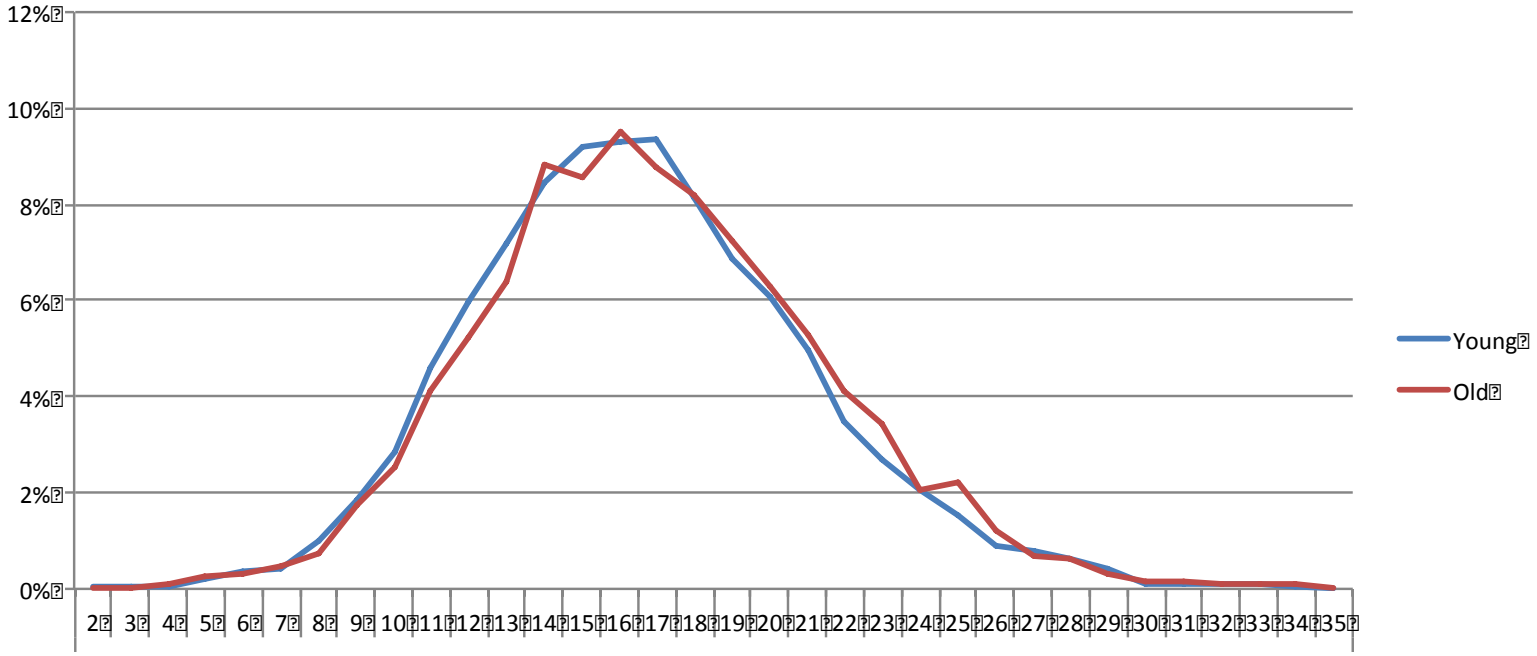
Statistical Analysis

↓

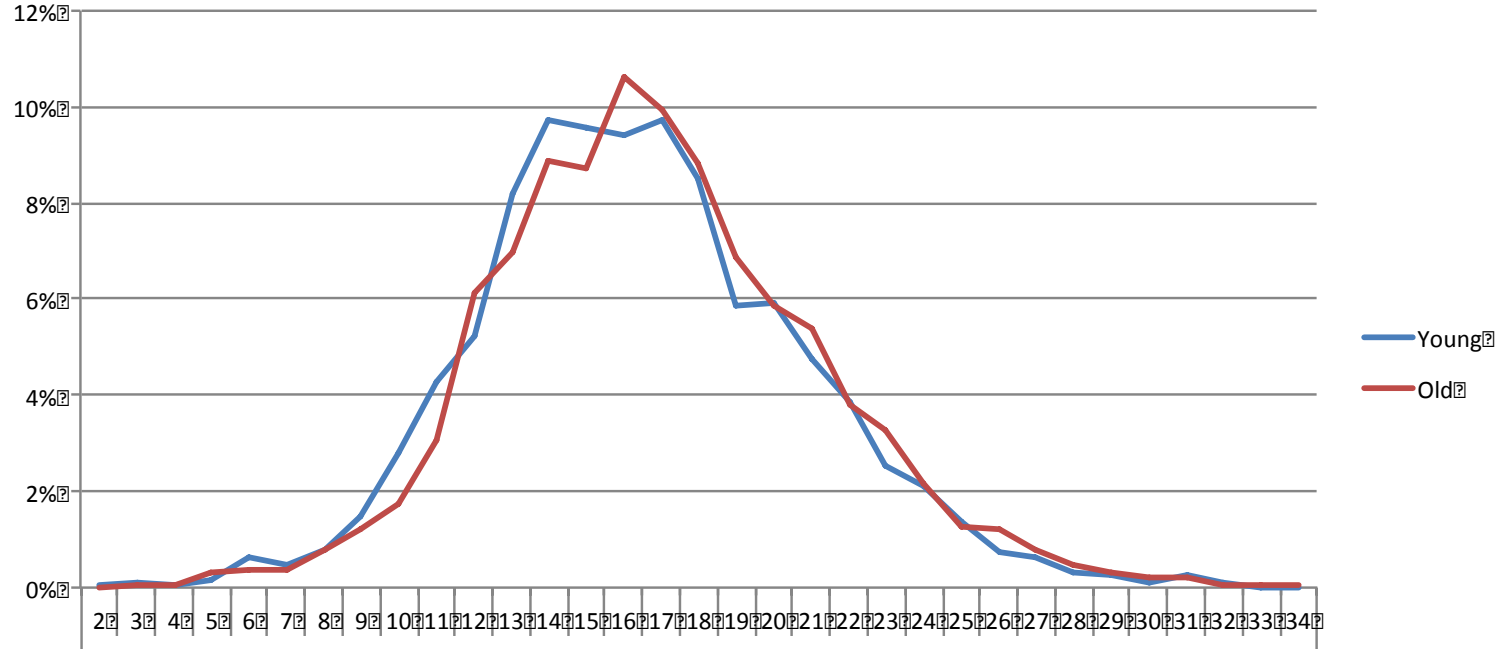
Age Group	Variable Sequences	preB		Immature		Transitional		Naïve		Grand Total
		Clones	Total	Clones	Total	Clones	Total	Clones	Total	
Young	Heavy	10817	35536	3758	7621	1864	3983	3603	6680	53820
	Light	-	-	6802	32877	7235	52655	7006	26925	157162
Old	Heavy	6248	15764	5025	12129	2522	5689	5801	9191	42773
	Light	-	-	10681	67969	4956	29429	6508	23154	149806

CDR-H3 Size

preB

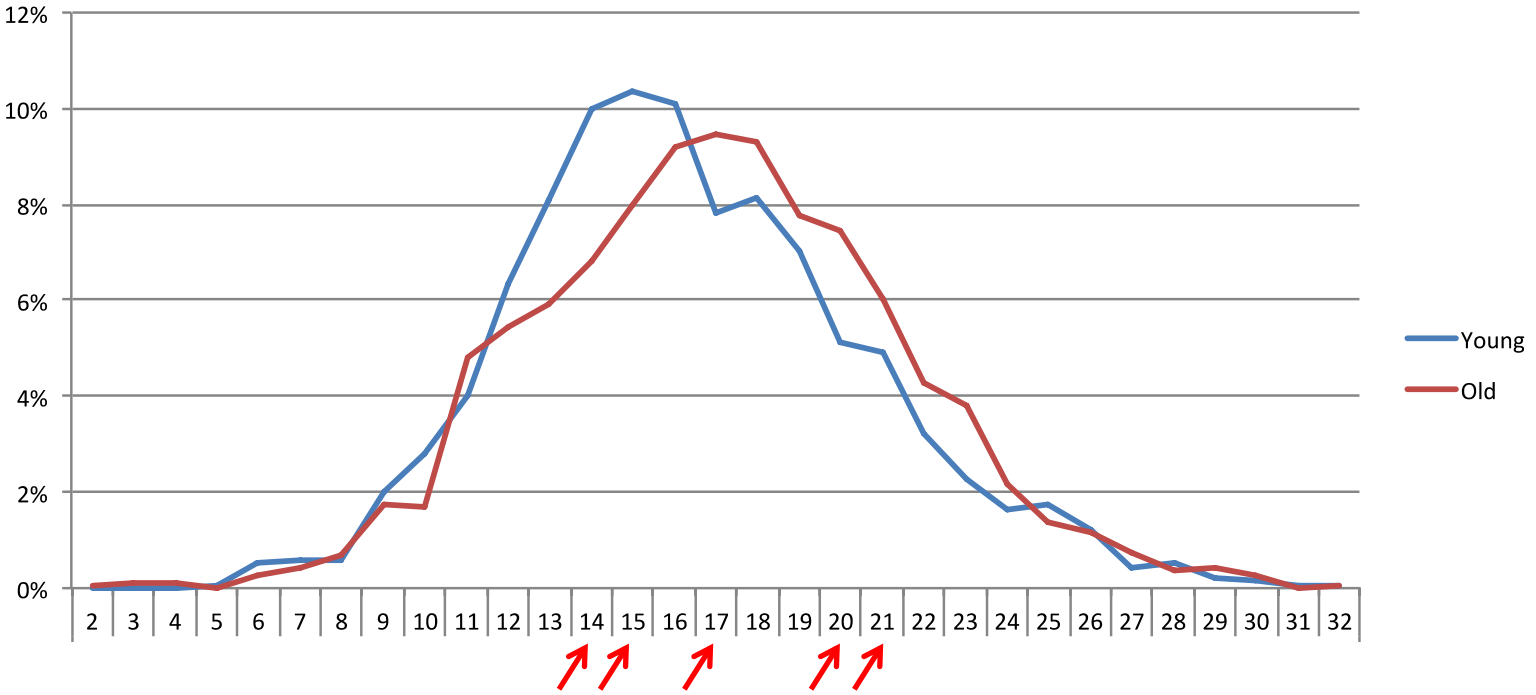


Immature

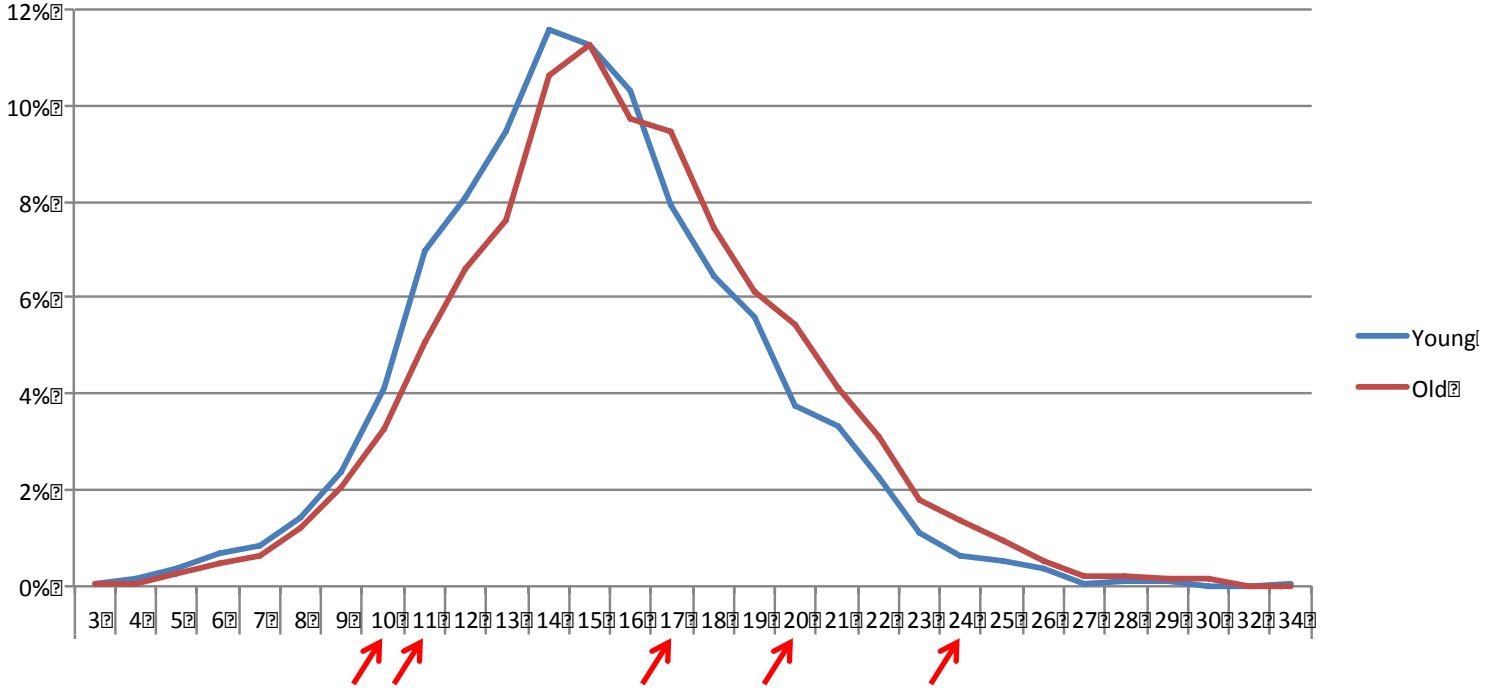


CDR-H3 Size

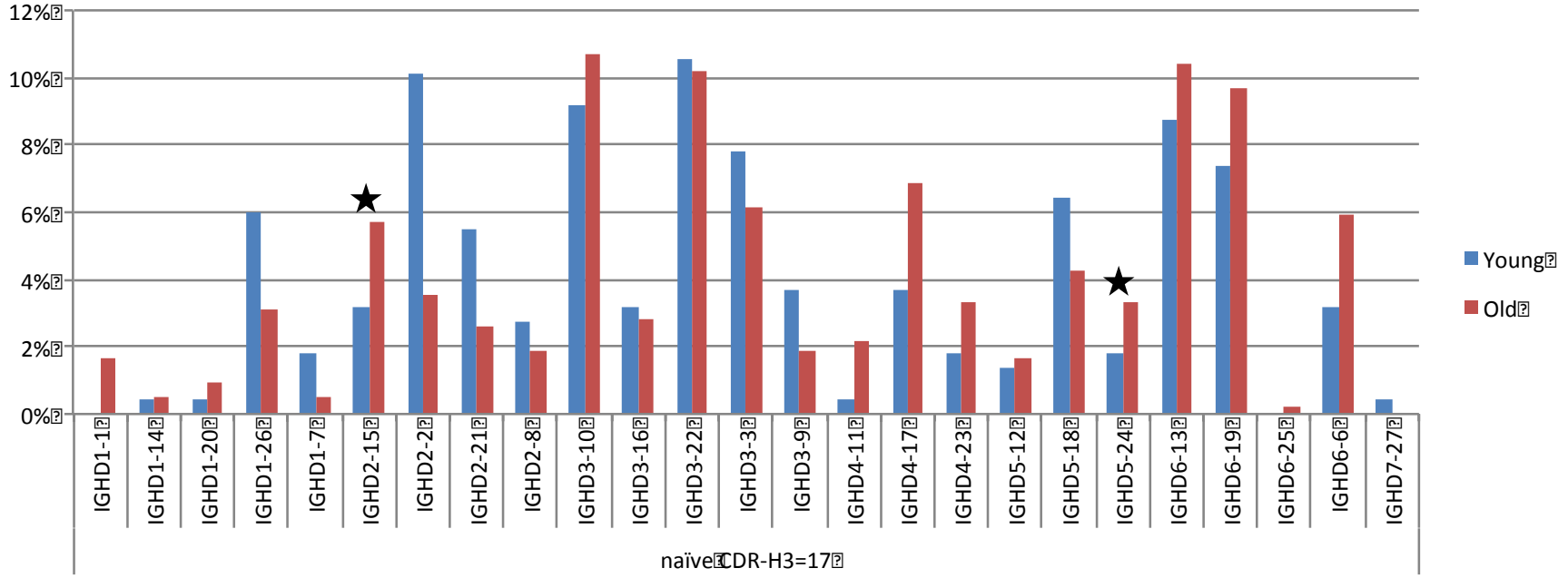
Transitional



Naïve

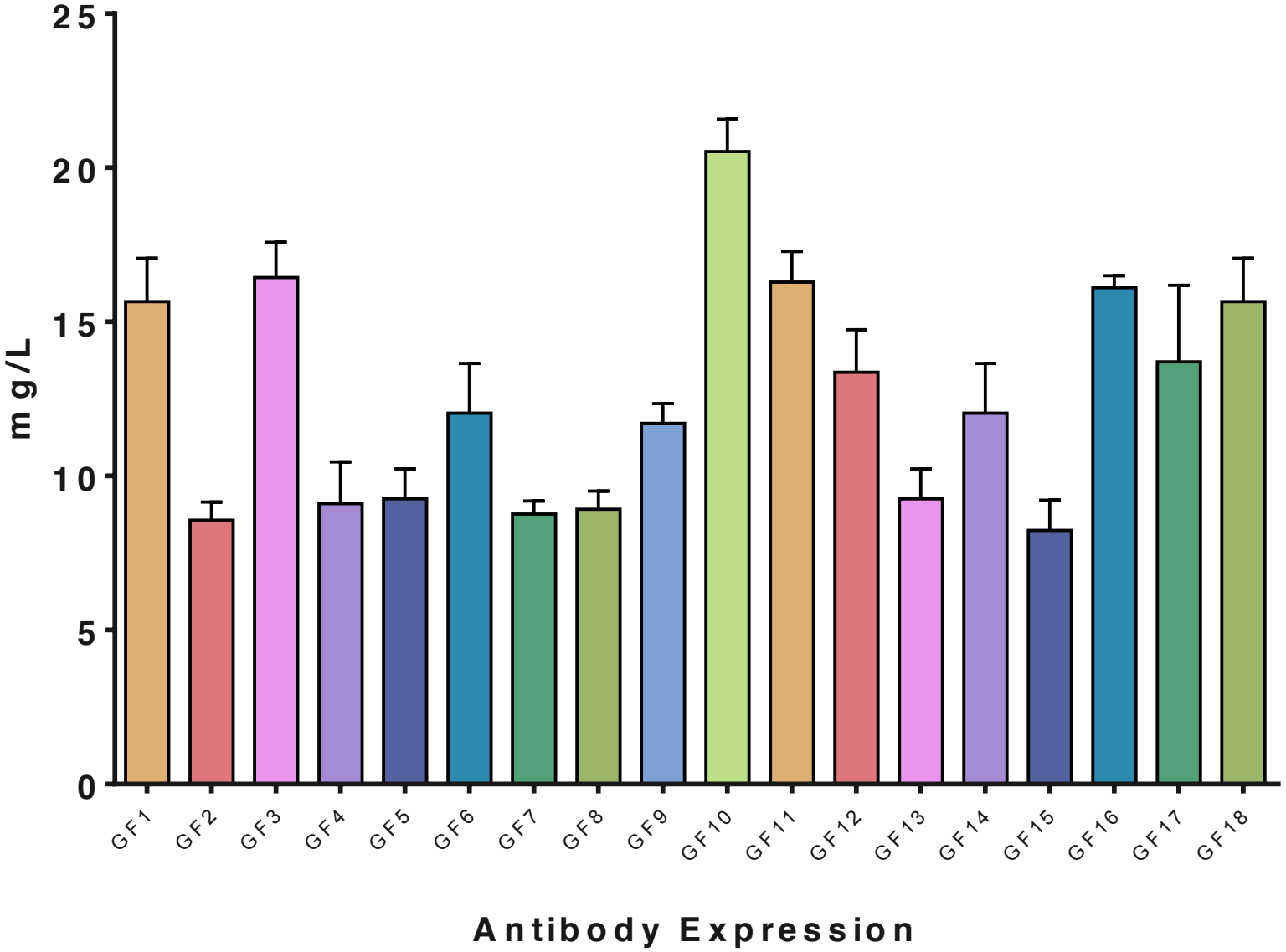


Increased Gene Usage in Old



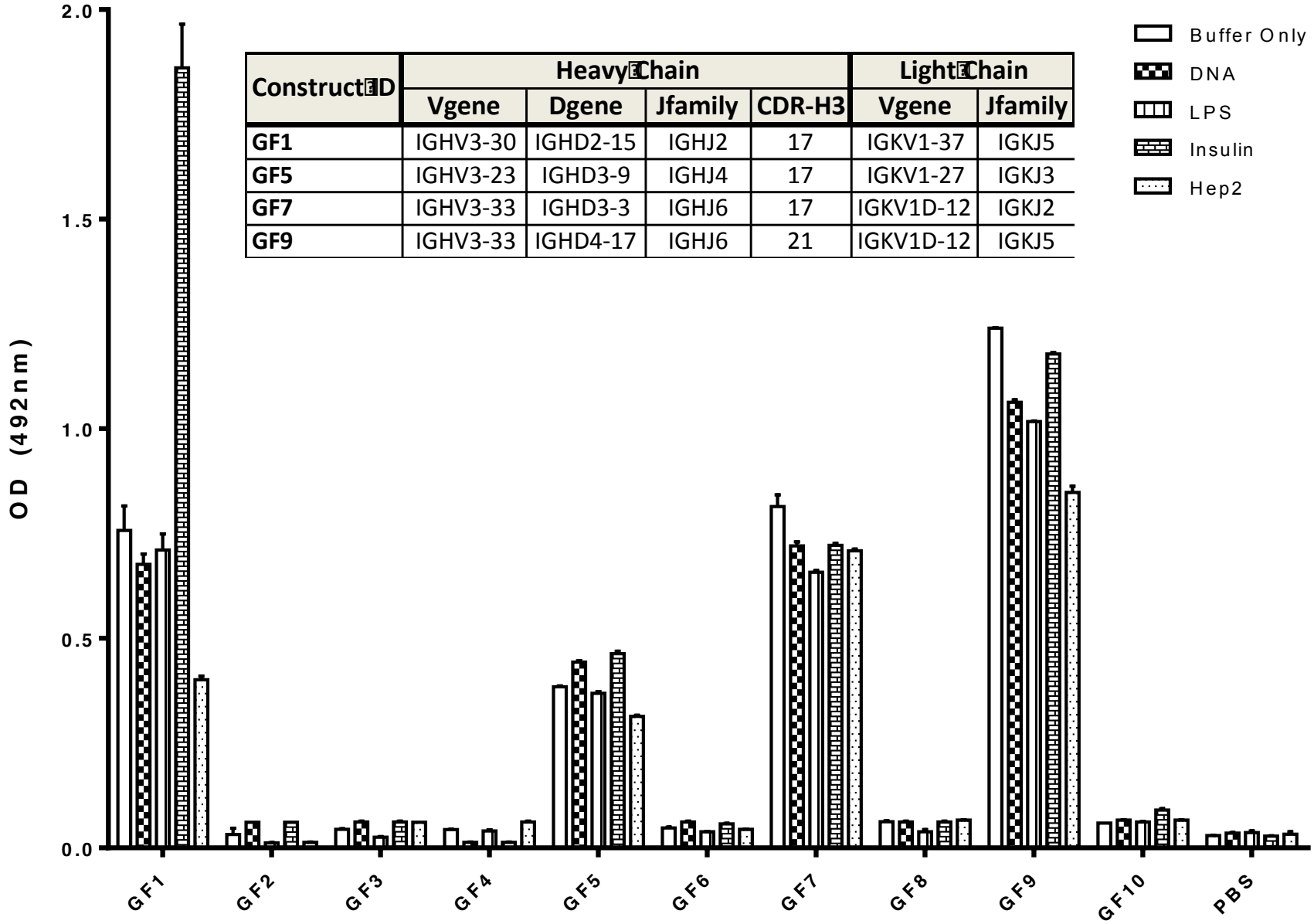
Cell Type	CDR-H3			Vgene			Dfamily			Dgene		
	Length	p≤0.05	Use in Old	Gene	p≤0.05	Use in Old	Family	p≤0.05	Use in Old	Gene	p≤0.05	Use in Old
Naïve	17	0.022	Increased	IGHV4-39	0.039	Increased						
Naïve	17	0.022	Increased				IGHD2	0.040	Decreased			
Naïve	17	0.022	Increased				IGHD4	0.049	Increased			
Naïve	17	0.022	Increased							IGHD2-15	0.038	Increased
Naïve	17	0.022	Increased							IGHD5-24	0.041	Increased
Naïve	20	0.040	Increased							IGHD2-21	0.043	Increased
Naïve	24	0.019	Increased							IGHD2-15	0.043	Increased
Transitional	17	0.043	Increased				IGHD1	0.042	Decreased			
Transitional	17	0.043	Increased				IGHD3	0.042	Increased			
Transitional	17	0.043	Increased							IGHD3-3	0.032	Increased
Transitional	20	0.013	Increased									
Transitional	21	0.044	Increased							IGHD4-17	0.041	Increased

Quantitative ELISA



Autoreactivity/Polyreactivity ELISA

Construct ID	Heavy Chain				Light Chain	
	Vgene	Dgene	Jfamily	CDR-H3	Vgene	Jfamily
GF1	IGHV3-30	IGHD2-15	IGHJ2	17	IGKV1-37	IGKJ5
GF5	IGHV3-23	IGHD3-9	IGHJ4	17	IGKV1-27	IGKJ3
GF7	IGHV3-33	IGHD3-3	IGHJ6	17	IGKV1D-12	IGKJ2
GF9	IGHV3-33	IGHD4-17	IGHJ6	21	IGKV1D-12	IGKJ5



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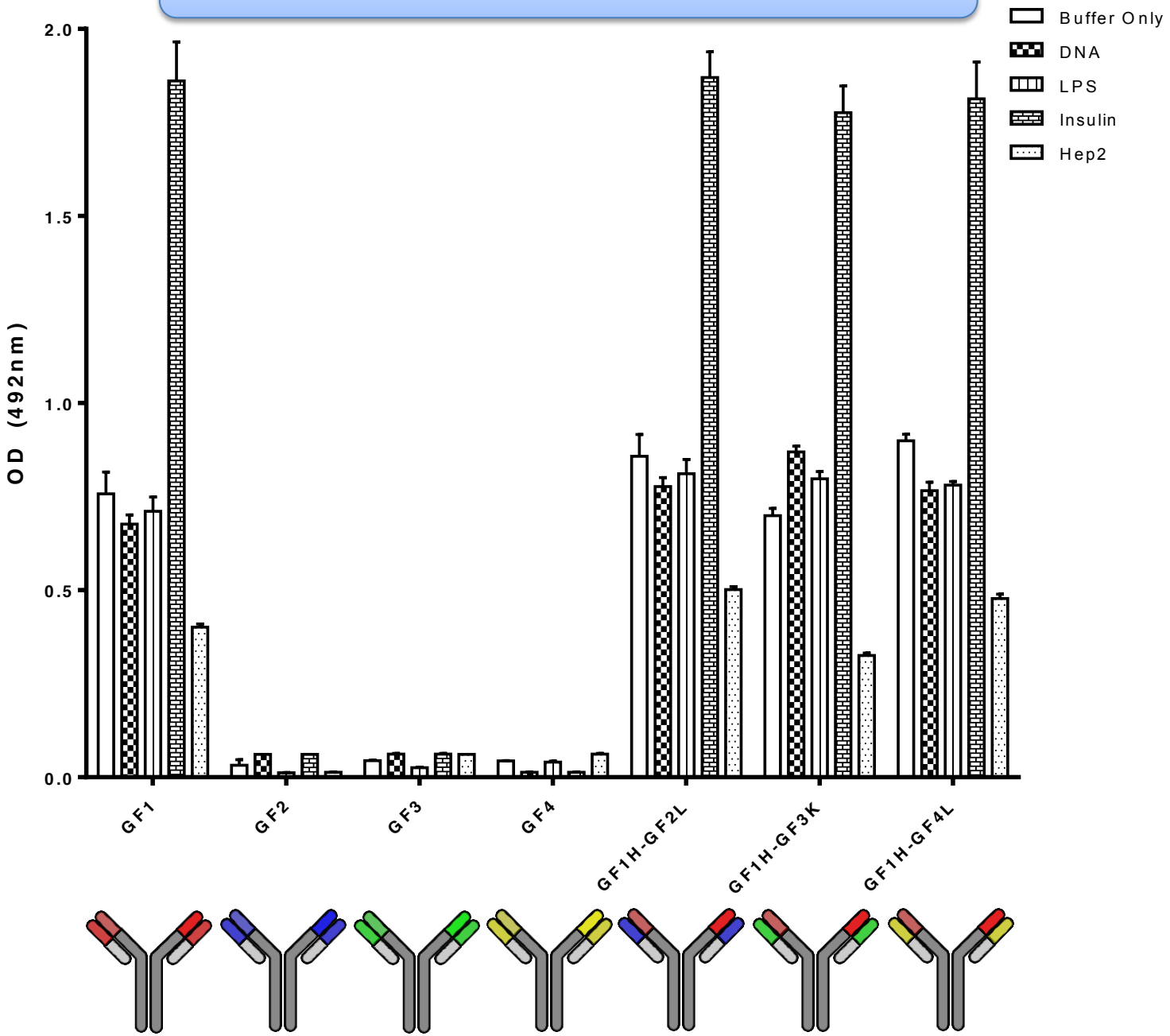
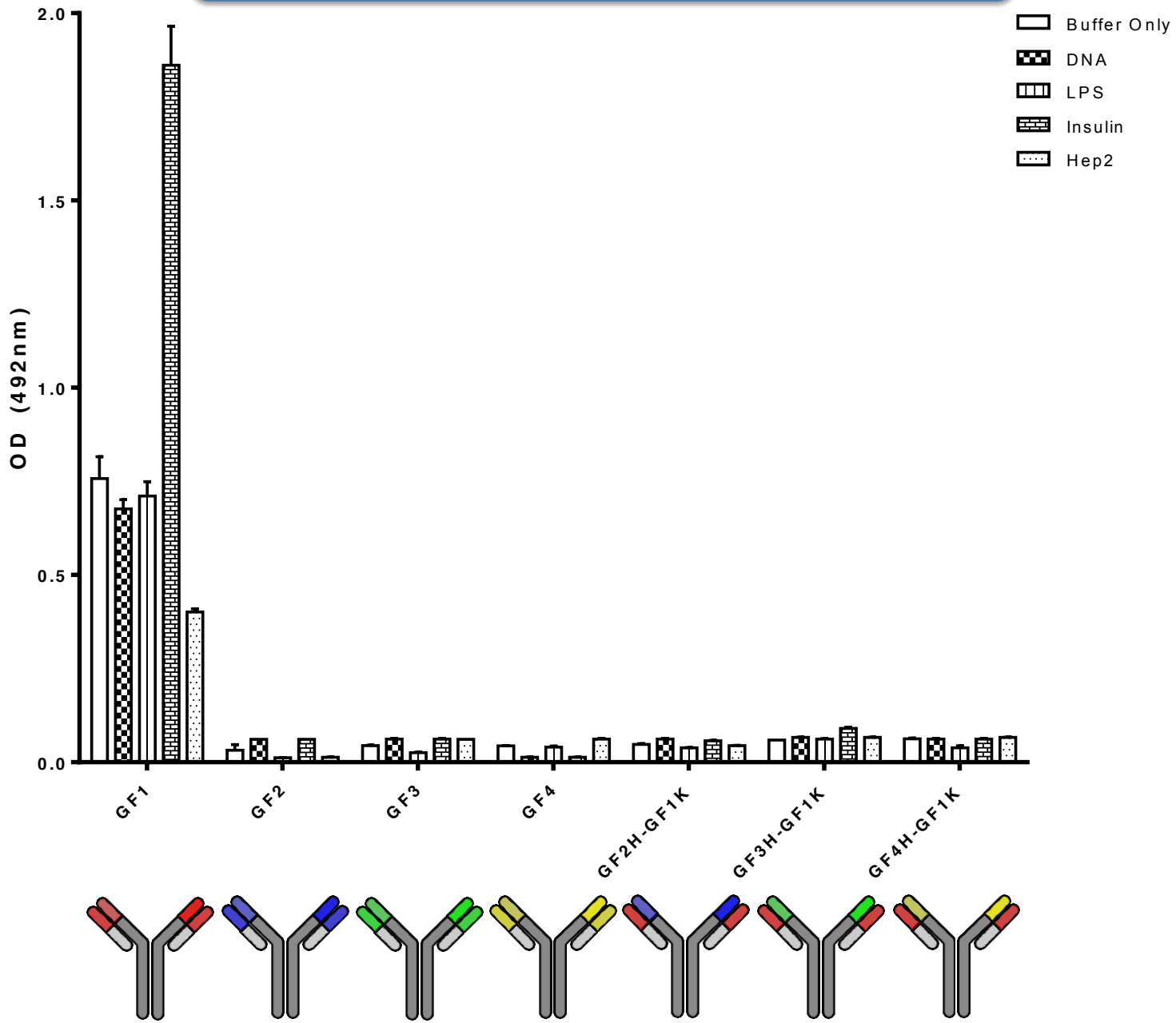
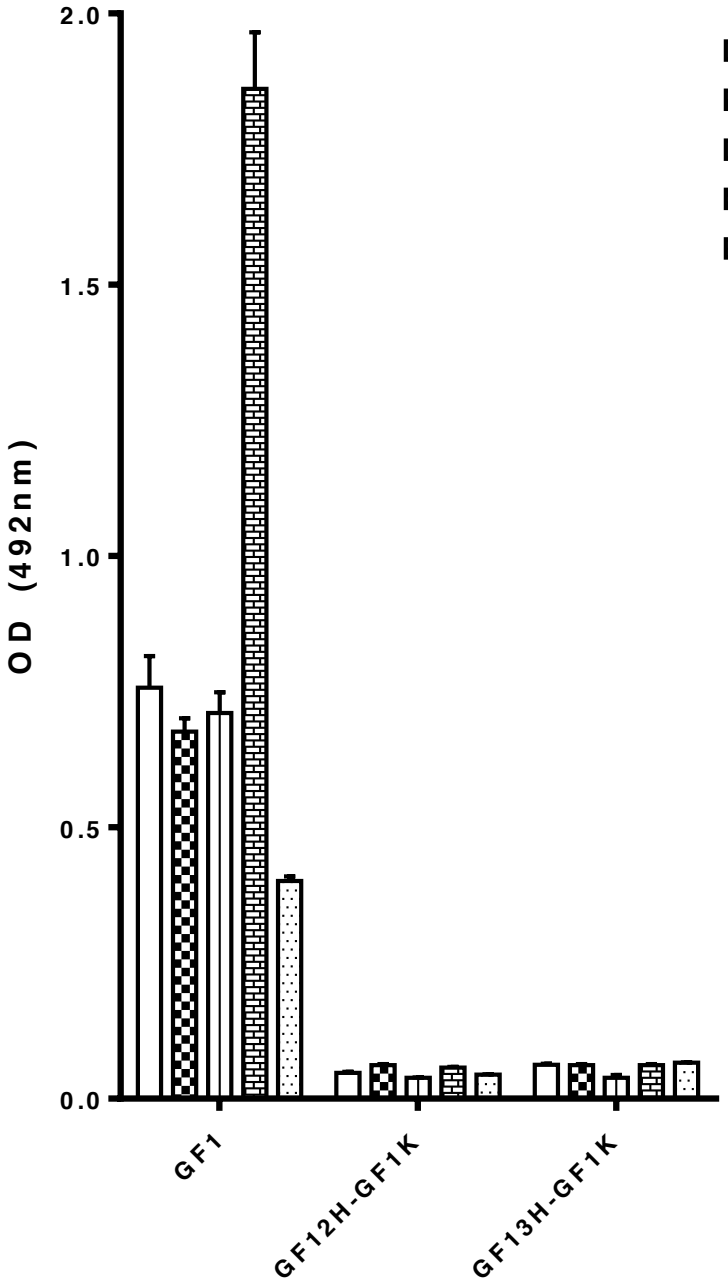


Autoreactivity/Polyreactivity ELISA

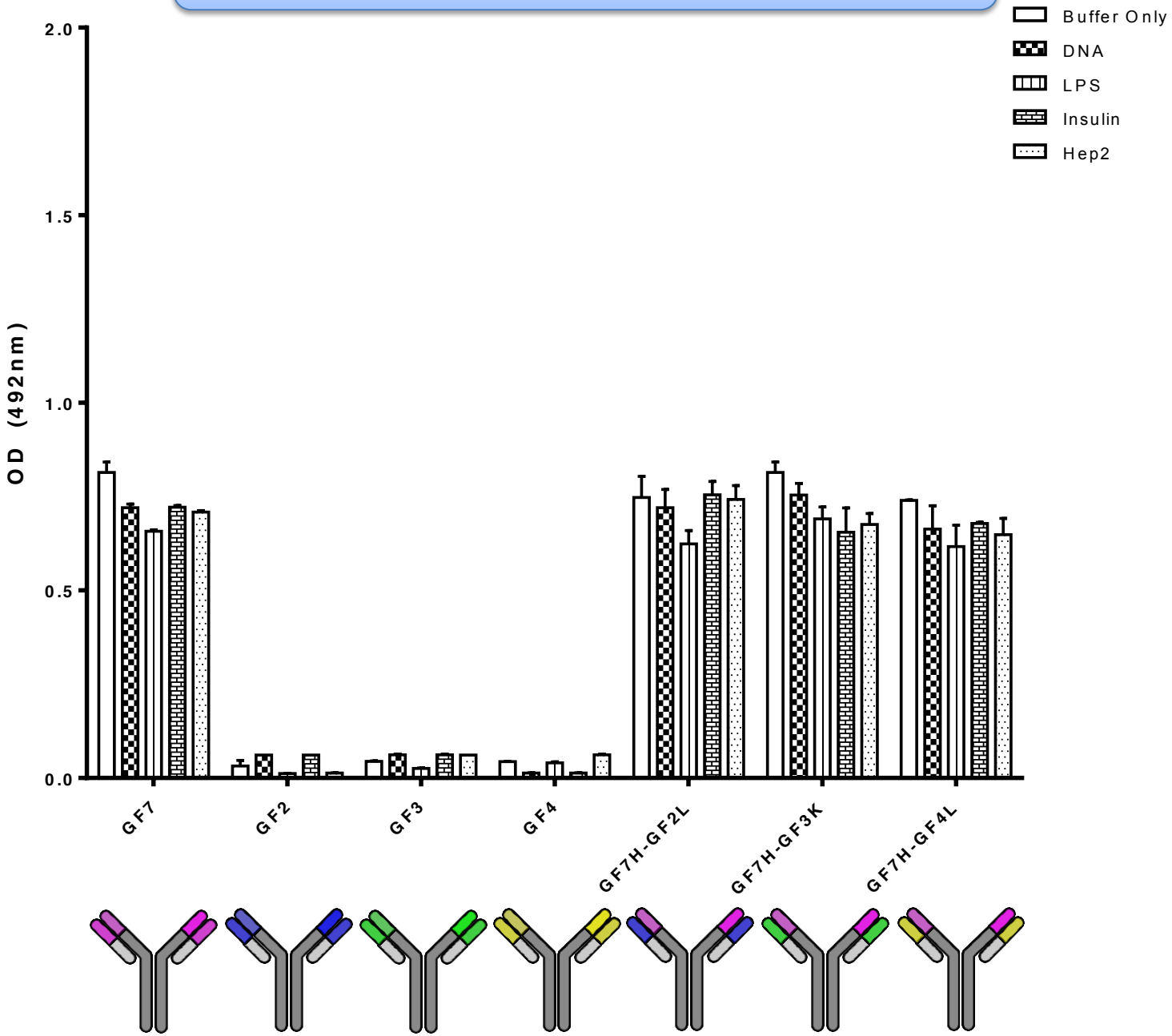


Polyreactivity ELISA

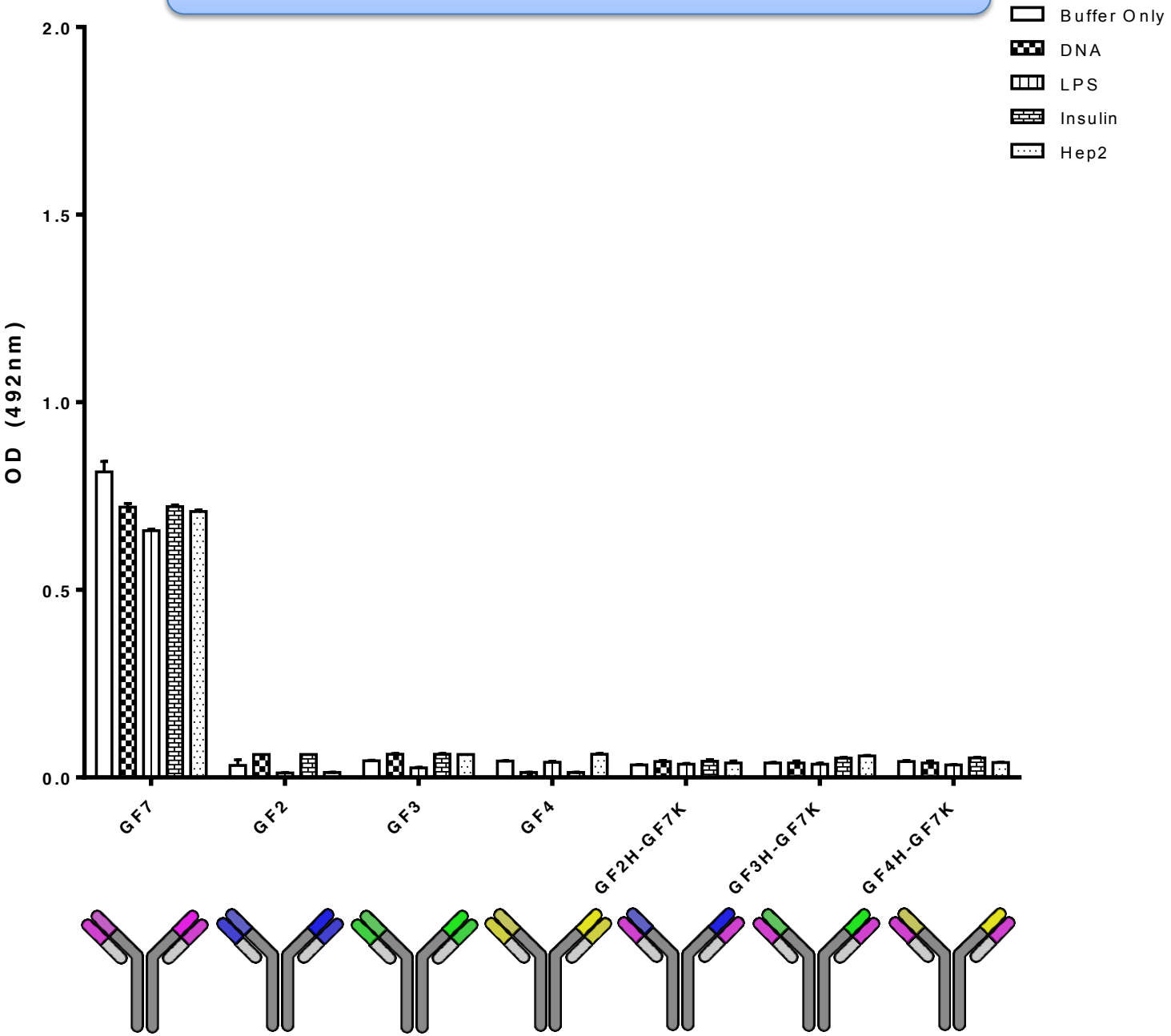


Construct ID	Heavy Chain				Light Chain	
	Vgene	Dgene	Jfamily	CDR-H3	Vgene	Jfamily
GF1	IGHV3-30	IGHD2-15	IGHJ2	17	IGKV1-37	IGKJ5
GF12_GF1K	IGHV3-30	IGHD2-15	IGHJ2	21	IGKV1-37	IGKJ5
GF13_GF1K	IGHV3-30	IGHD2-15	IGHJ2	16	IGKV1-37	IGKJ5

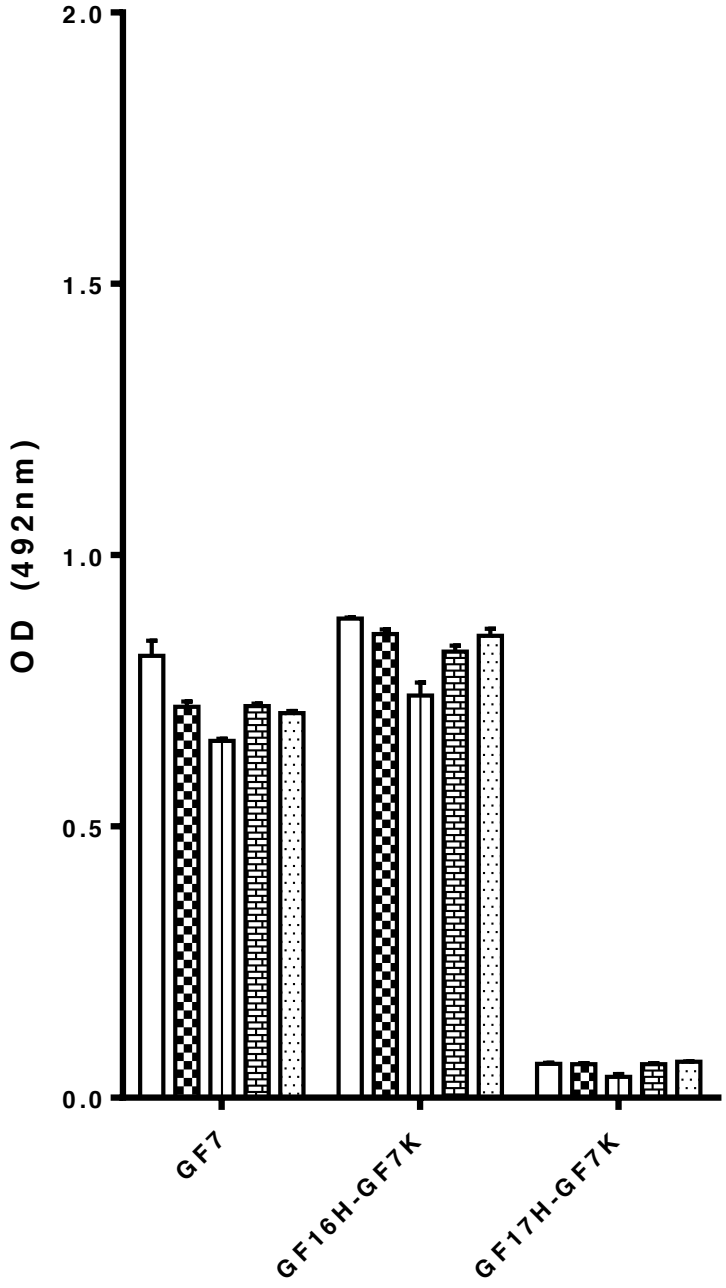
Autoreactivity/Polyreactivity ELISA



Autoreactivity/Polyreactivity ELISA



Polyreactivity ELISA



Construct ID	Heavy Chain				Light Chain	
	Vgene	Dgene	Jfamily	CDR-H3	Vgene	Jfamily
GF7	IGHV3-33	IGHD3-3	IGHJ6	17	IGKV1D-12	IGKJ2
GF16_GF7K	IGHV3-33	IGHD3-3	IGHJ6	28	IGKV1D-12	IGKJ2
GF17_GF7K	IGHV3-33	IGHD3-3	IGHJ6	22	IGKV1D-12	IGKJ2

Conclusions

- Isolated low autoreactive antibodies using matched heavy and light chains from the single sorted plasmablasts
- Identified polyspecific antibodies using statistical analysis of the HTS data from old vs young cell populations
- Our data suggest the variable heavy chain region contributes most to the antibody binding characteristics

Future Work

- Identify more age-related B cell Ig genes
- Expand library of cloned antibodies identified from the HTS data (using what we know of pairing from our library to match the most likely heavy/light chains)
- Testing the expressed antibodies for binding specificity in ELISAs: Including DNA, LPS, Insulin, Hep2 etc
- Liaise with collaborators in modelling structure of cloned polyreactive antibodies

Acknowledgements

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