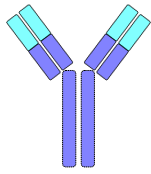
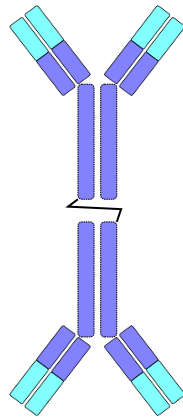


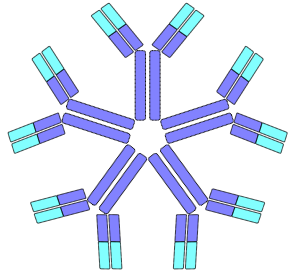
Isotypes



IgG, IgD, IgE
as monomer



IgA as monomer
or dimer

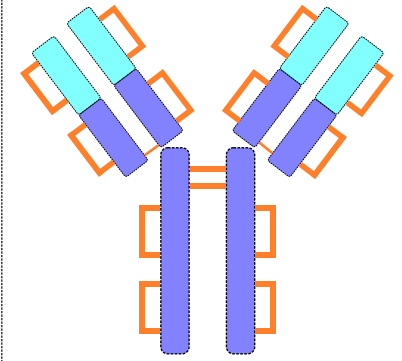


IgM as monomer
or pentamer

Protein A/G affinity

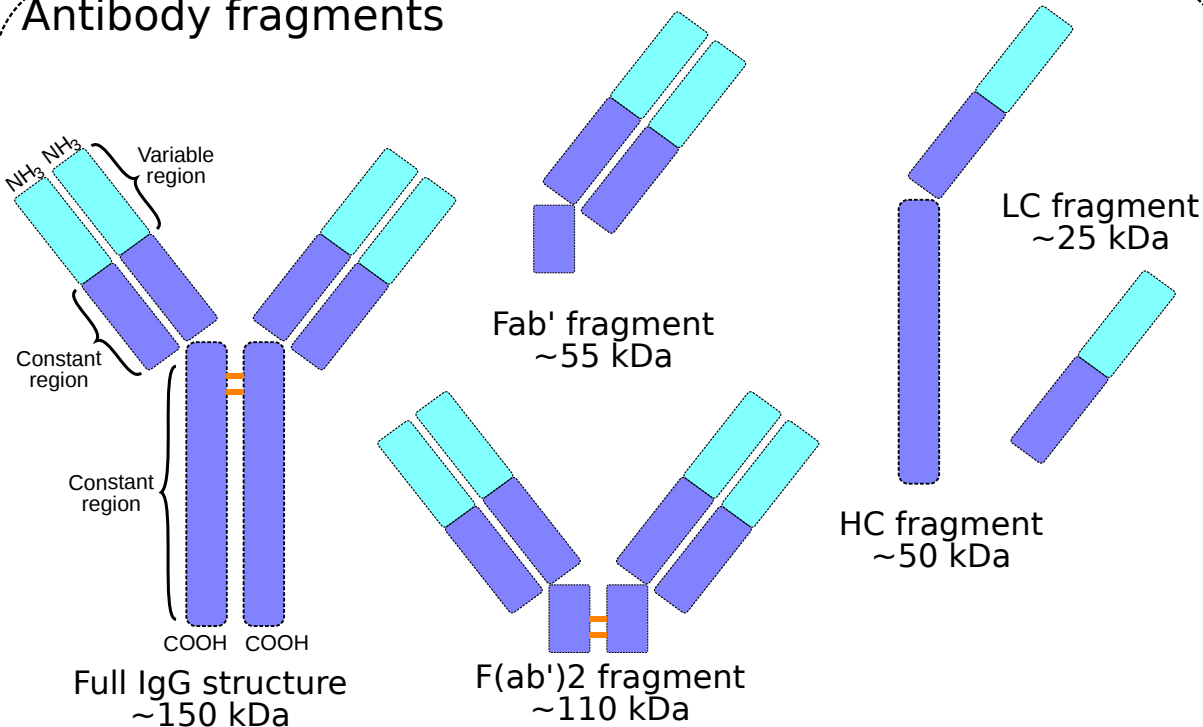
Species	Immunoglobulin	Protein A	Protein G
Human	IgG	++++	++++
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	-	++++
	IgG4	++++	++++
	IgM	-	-
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
Rat	IgG1	-	+
	IgG2a	-	++++
	IgG2b	-	++
	IgG2c	+	++
Rabbit	IgG	++++	+++
Goat	IgG	+/-	++
Sheep	IgG	+/-	++

Disulfide bonds



IgG1 shown, other isotypes differ, particularly in the hinge region.

Antibody fragments



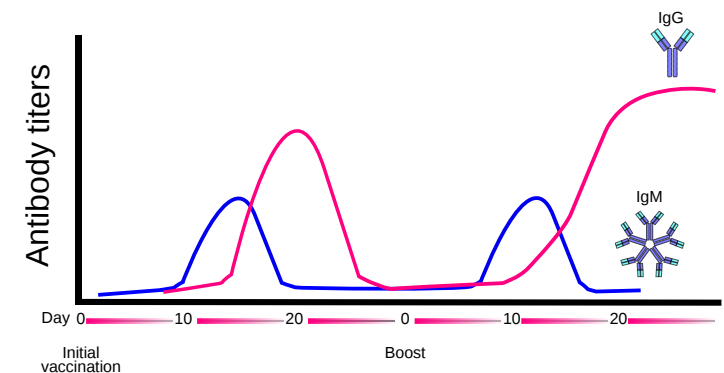
Commonly observed antibody fragments.

Fragment	Mw (Da)	How to obtain	Notes
IgG	150 kDa	protein A/G	Native molecule.
F(ab') ₂	110 kDa	Pepsin	Contains hinge, most of C-region.
Fab'	55 kDa	Reduction of F(ab') ₂	
HC	50 kDa	Reduce HC/LC	Single, reduced, heavy chain.
LC	25 kDa	Reduce HC/LC	Single, reduced, light chain.

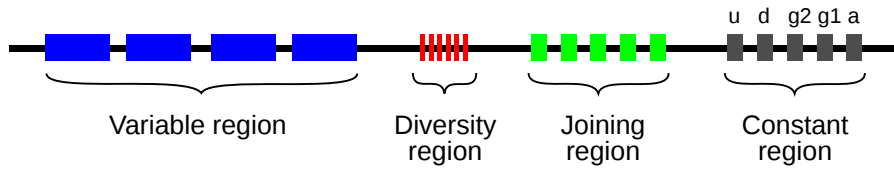
Isotype	# subclasses ¹	Description
IgA	2	Found in mucosal areas (e.g., gut, respiratory tract), saliva, tears, breast milk.
IgD	1	Ag-receptor to non-exposed B cells.
IgE	1	Binds to allergens, triggers histamine response.
IgG	4	Many locations, including placental crossing. Majority of Ab-based immunity.
IgM	1	Expressed on surface of B cells (monomer) and in a secreted form (pentamer).

¹: number of subclasses in human.

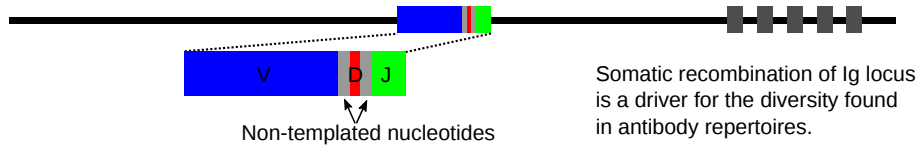
Antibody titer dynamics after immunization and boost. Expected serum titers for IgM and IgG are depicted.



Ig locus structure



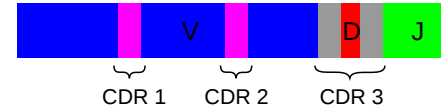
	Variable region	Diversity region	Joining region	Constant region
Human:	55 genes	24 genes	6 genes	
Mouse:	110 genes	11 genes	4 genes	
Rabbit:	45 genes	10 genes	6 genes	



Non-templated nucleotides

Ig somatic hypermutation

Complementarity determining regions (CDRs) are where diversification is localized, driven by recombination in CDR3 and somatic hypermutation (SHM) in CDRs 1 & 2.

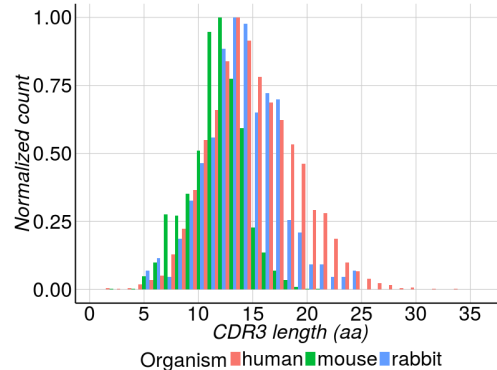
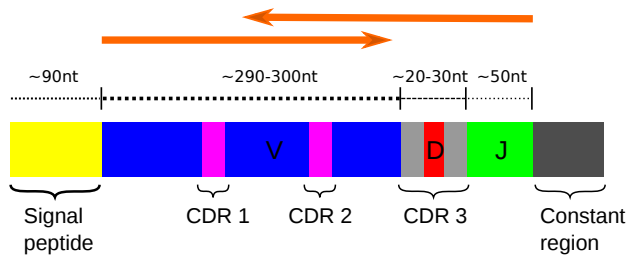


SHM motifs (in nt)	
5'	3'
WRC	GYW
[AT][AG]C	G[CT][AT]
WA	TW
[AT]A	[AT]

Common SHM motifs

IUPAC code	Base
A	Adenine
C	Cytosine
G	Guanine
T (or U)	Thymine (or Uracil)
R	A or G
Y	C or T
S	G or C
W	A or T
K	G or T
M	A or C
B	C or G or T
D	A or G or T
H	A or C or T
V	A or C or G
N	any base

Antibody sequence features

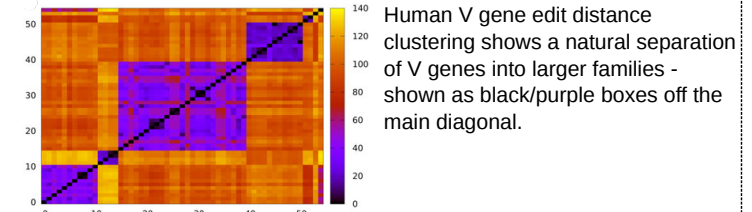


CDR3 length varies across different organisms.

Rearranged antibody transcript can vary considerably in length.

Antibody discovery directly from serum. Discover us at: <https://www.digitalproteomics.com/alicante>

VDJ genes



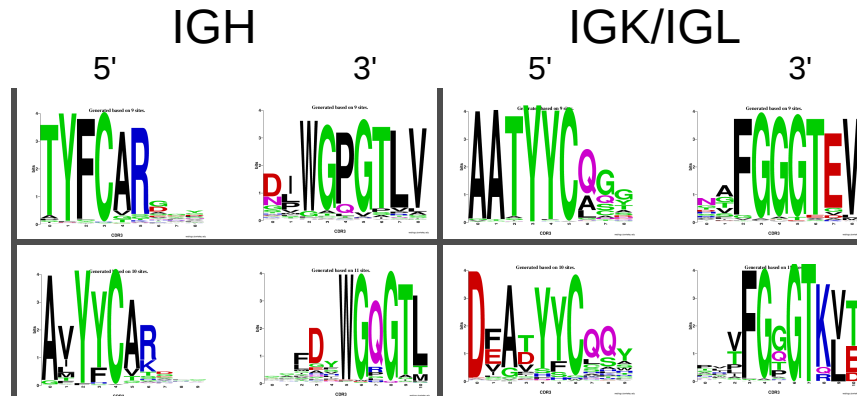
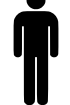
Locus	Gene segment lengths		
	V	D	J
Human IGH	296.6364 ± 3.0413	25.2000 ± 7.0351	52.8333 ± 4.8103
Human IGK	291.4634 ± 6.9531		38.3333 ± 0.4714
Human IGL	297.0606 ± 9.0752		38.0000 ± 0.0000
Mouse IGH	295.3268 ± 4.3134	17.4444 ± 3.1662	50.1111 ± 2.8846
Mouse IGK			
Mouse IGL			
Rabbit IGH	289.3333 ± 3.0758	30.1818 ± 5.4409	51.1818 ± 2.0369
Rabbit IGK	297.2353 ± 4.6751		39.0000 ± 1.0000

* all lengths in nt

CDR3 sequences can be identified by their common sequence motifs at the 5' and 3' ends.



These position weight matrices show motifs for rabbit and human.



Discovery from serum
Elucidation by mass spectrometry