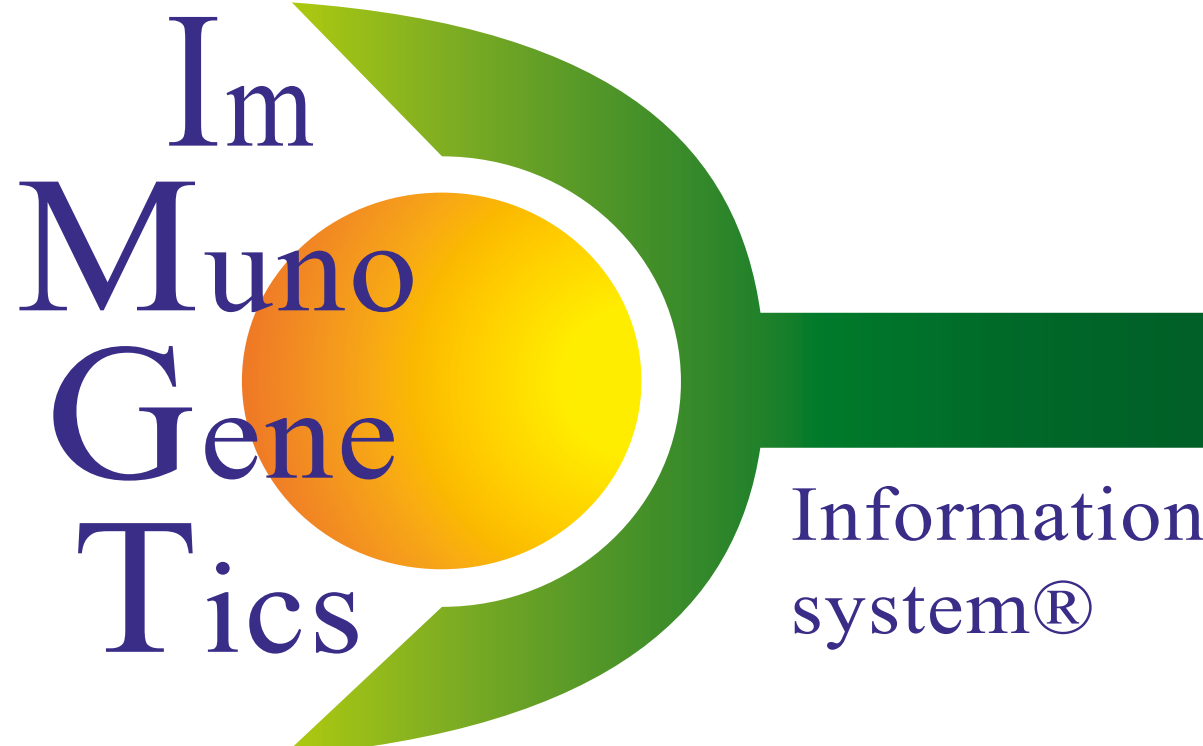


IMGT/HighV-QUEST for NGS antibody repertoire analysis



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<http://www.imgt.org>

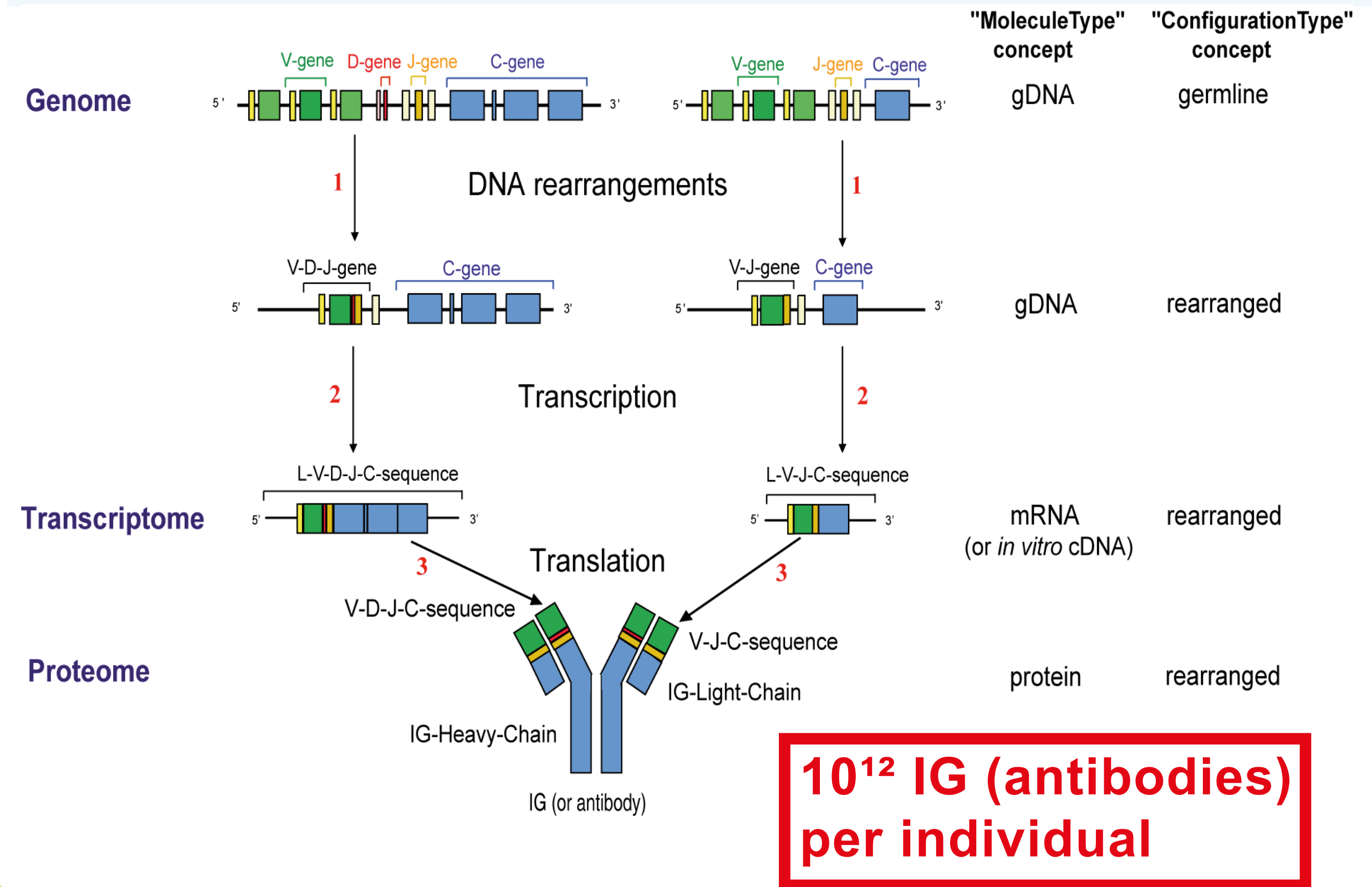
IMGT®, the international ImMunoGeneTics information system®, created in 1989 at Montpellier, France, by Marie-Paule Lefranc (CNRS and Université Montpellier 2), is at the birth of immunoinformatics. IMGT® manages the immunogenetics data, and more particularly the sequences, genes and structures of immunoglobulins (IG) or antibodies and T cell receptors (TR). Standardization and data integration are obtained through the IMGT-ONTOLOGY [1] concepts of identification (IMGT standardized keywords), classification (IMGT standardized nomenclature: IMGT gene and allele names approved by HGNC and used by NCBI Gene), description (IMGT standardized labels) and numerotation (IMGT unique numbering and IMGT Colliers de Perles: widely used for antibody engineering and humanization). IMGT® comprises seven databases (including IMGT/mAb-DB), seventeen tools and more than 15,000 pages of Web resources. To answer the needs of high throughput and Next Generation Sequencing (NGS) data, the IMGT/HighV-QUEST tool [2-4] was developed which analyses up to 500,000 long 454 sequences by run. The results, based on IMGT-ONTOLOGY, include identification of the closest germline genes and alleles for genotype and haplotype analysis, and standardized characterization of the 'IMGT clonotypes (AA)' for antibody clonal diversity and expression and achieve, for the first time, a degree of resolution for NGS verifiable by the user at the sequence level. Amino acid frequency can be determined at each CDR-IMGT and FR-IMGT positions. This tool provides a paradigm for IG clonal diversity and expression repertoire analysis from NGS and high resolution results for antibody engineering and combinatorial library construction.

TAKE HOME MESSAGE: * IMGT/HighV-QUEST analyses antibody NGS data at a sequence level verifiable by the user. * Standardized characterization of IMGT clonotypes, based on the IMGT-ONTOLOGY concepts, identifies clonal diversity and expression, * Amino acid frequency can be determined at each CDR-IMGT and FR-IMGT position.

[1] Giudicelli V and Lefranc M-P, *Front Genet*, 3:79, 2012. [2] Alamyar E et al. *Mol Biol* 882:569-604, 2012. [3] Alamyar E et al. *Immunome Res* 8(1):26, 2012. [4] Li S et al. *Nat. Commun.* 4:2333 doi: 10.1038/ncomms3333 (2013).

Biological Context

The adaptive immune response is characterized by an extreme diversity of the specific antigen receptors that comprise the immunoglobulins (IG) or antibodies and the T cell receptors (TR) (10^{12} different IG and 10^{12} different TR per individual, in humans). The complex molecular mechanisms (DNA rearrangements, N-diversity, and for IG, somatic hypermutations) that occur in B cells and T cells are at the origin of that huge diversity.



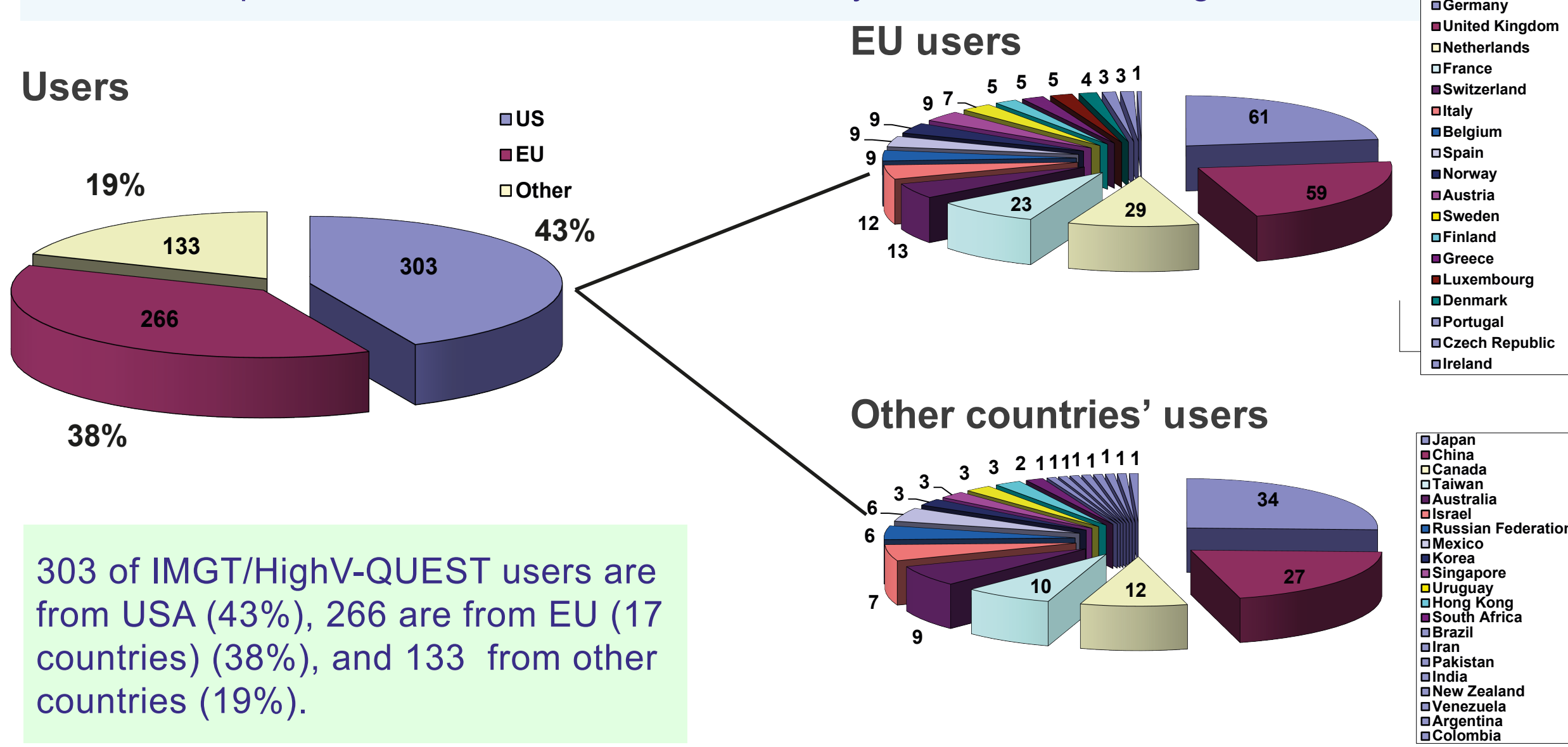
IMGT Clonotypes (AA)

In IMGT®, the clonotype designated as 'IMGT clonotype (AA)' is defined among the '1 copy' 'single allele' (for V and J) by a unique V-(D)-J rearrangement (IMGT genes and alleles determined at the nt level), conserved anchors (C104, W or F 118), and a unique CDR3-IMGT AA in frame junction [4]. Each 'IMGT clonotype (AA)' is characterized by a selected unique representative sequence.

ID	IMGT clonotype (AA) definition						IMGT clonotype (AA) representative sequence			Nb		IMGT clonotypes (nt)		
#	Exp. ID	V gene and allele	D gene and allele	J gene and allele	CDR3-IMGT length (AA)	CDR3-IMGT sequence (AA)	Anchor 104,118	V %	Sequence length	Sequence ID	Total nb of '1 copy'	Total nb of 'More than 1'	Total	Sequences file (1 copy)
Hommap IGHV1.2*02 F														
1	137-mi5	Hommap IGHV1.2*02 F	Hommap IGHV2-2*01 F	Hommap IGHV6*03	22 AA	ARDLYCSSTSCVGGWYYNDV	C,F	95.14	425	GINZTB402H8XK length=425	1	0	1	Sequences file
2	157-mi5	Hommap IGHV1.2*02 F	Hommap IGHV6-6*01 F	Hommap IGHV6*03	22 AA	ARERVGRSIAARRAPDYNDV	C,F	97.92	426	GINZTB402H4DK W length=426	1	0	1	Sequences file
3	305-mi5	Hommap IGHV1.2*02 F	Hommap IGHV3-22*01 F	Hommap IGHV4*02 F	21 AA	ARGPYHRPYYDSSGVYGDY	C,F	96.15	374	GINZTB402F5HFL length=374	1	0	1	Sequences file
4	331-mi5	Hommap IGHV1.2*02 F	Hommap IGHV3-10*02 F	Hommap IGHV3*02 F	21 AA	ARNVGHRRGSSDAWEDAFDI	C,F	99.31	422	GINZTB402F1J2Q length=422	1	0	1	Sequences file
5	374-mi5	Hommap IGHV1.2*02 F	Hommap IGHV3-3*01 F	Hommap IGHV3*02 F	21 AA	ATHEPAITFIVGVINDAFDI	C,F	96.18	422	GINZTB402H3Q02 length=422	1	0	1	Sequences file
6	647-mi5	Hommap IGHV1.2*02 F	Hommap IGHV6-19*01 F	Hommap IGHV6*03	19 AA	AKGAAVAGTNYYYYNDV	C,F	97.74	330	GINZTB402HYTV length=330	1	0	1	Sequences file
7	693-mi5	Hommap IGHV1.2*02 F	Hommap IGHV3-22*01 F	Hommap IGHV5*02 F	19 AA	ARDGTYDSSGVYWFDP	C,F	99.65	417	GINZTB402F7QZ length=417	1	0	1	Sequences file
8	709-mi5	Hommap IGHV1.2*02 ORF	Hommap IGHV4-23*01 ORF	Hommap IGHV2*01 F	19 AA	ARDMGRYGGNRRYYWFDL	C,F	97.57	416	GINZTB402G7EN4 length=416	1	0	1	Sequences file

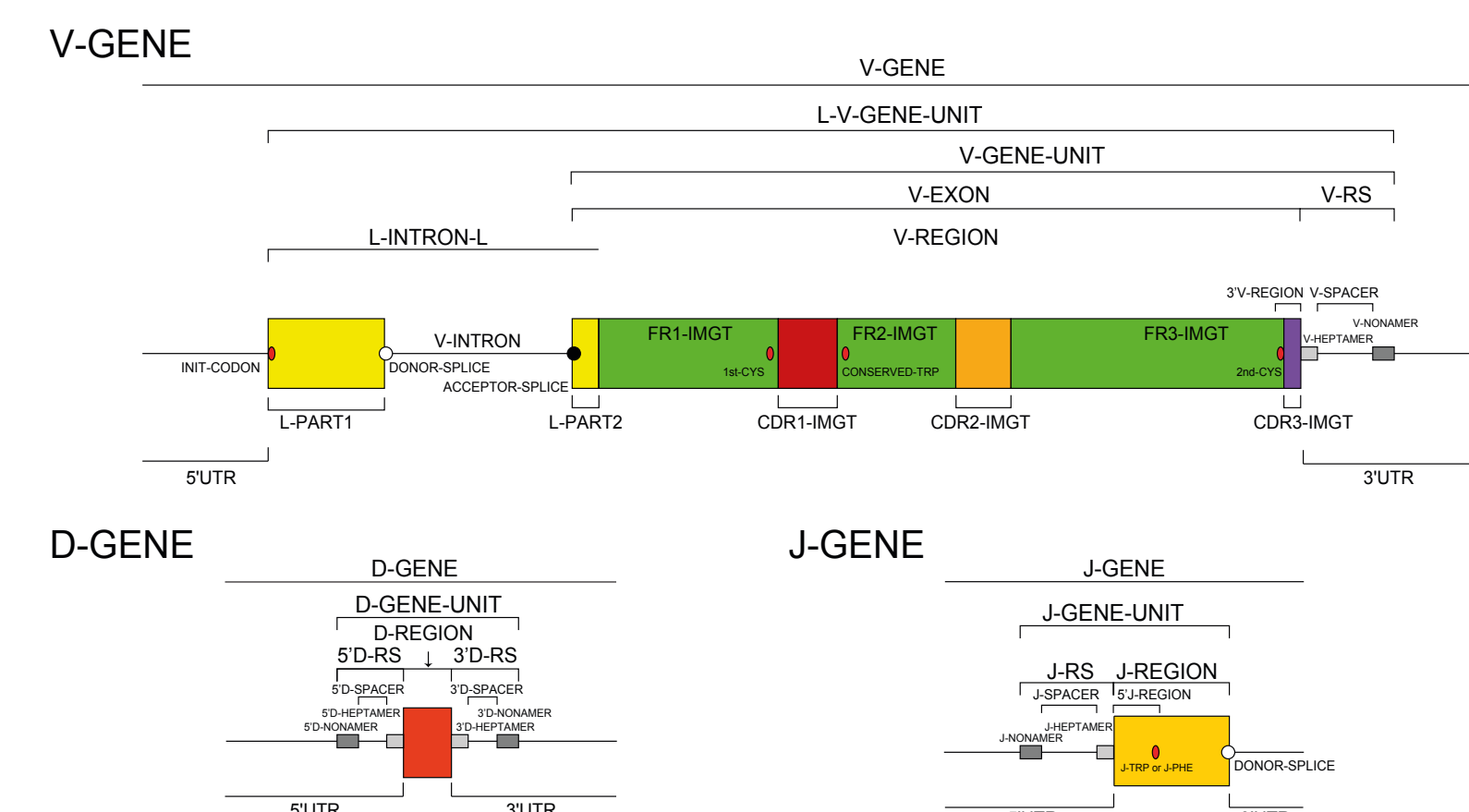
Users and Analyses

Since the availability of IMGT/HighV-QUEST in October 2010, more than 1.4 billions of sequences (from external users) have been submitted. They required more than 720,000 hours of computational resources. About 32 terabytes of results were generated.



IMGT-ONTOLOGY concepts

Prototypes of IG and TR V, D, J genes



Prototypes are graphical representation based on the concepts of description

IMGT-ONTOLOGY Concepts

DESCRIPTION

The concepts of description correspond to IMGT® standardized labels. They are more than 560 standardized labels (available in the IMGT Scientific chart), 277 for the nucleotide sequences and 285 for the 3D structures.

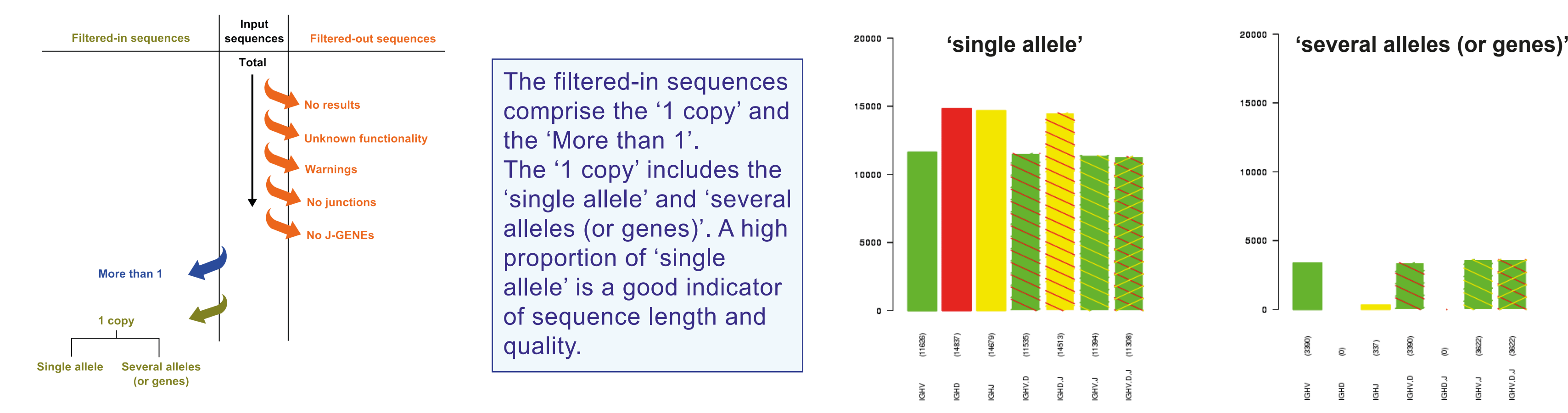
CLASSIFICATION

The concepts of classification allowed to classify and name the human IG and TR genes and alleles which were approved by HGNC and endorsed by WHO-IUIS. They provide the frame for the standardized IG and TR nomenclature of jawed vertebrates.

NUMEROTATION

The concepts of numerotation comprise the 'IMGT unique numbering' and 'IMGT Collier de Perles'.

IMGT/HighV-QUEST based on IMGT® standard



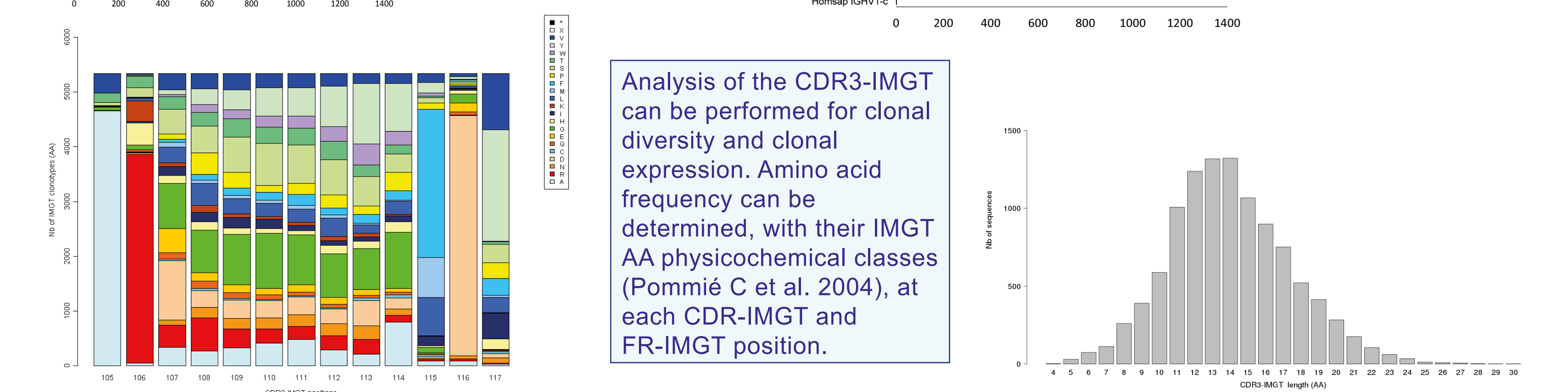
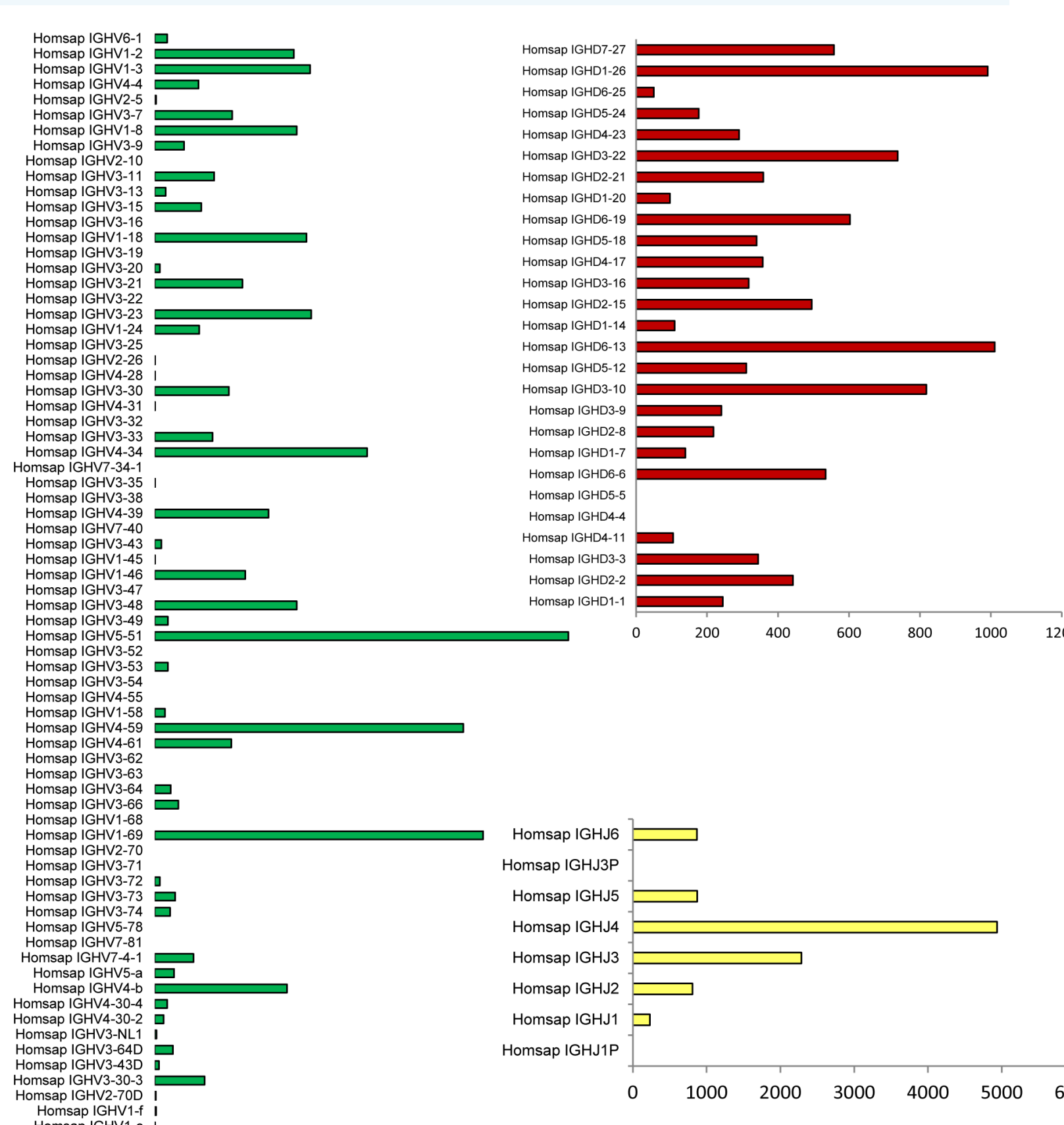
VH clonal diversity

Clonal diversity is the number of 'IMGT clonotypes (AA)' per V, D and J gene.



VH clonal expression

Clonal expression is the number of sequences assigned to 'IMGT clonotypes (AA)' per V, D and J gene.



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