IMGT/Automat: the strategy for the annotation of human and mouse cDNA nucleotide sequences of IG and TR

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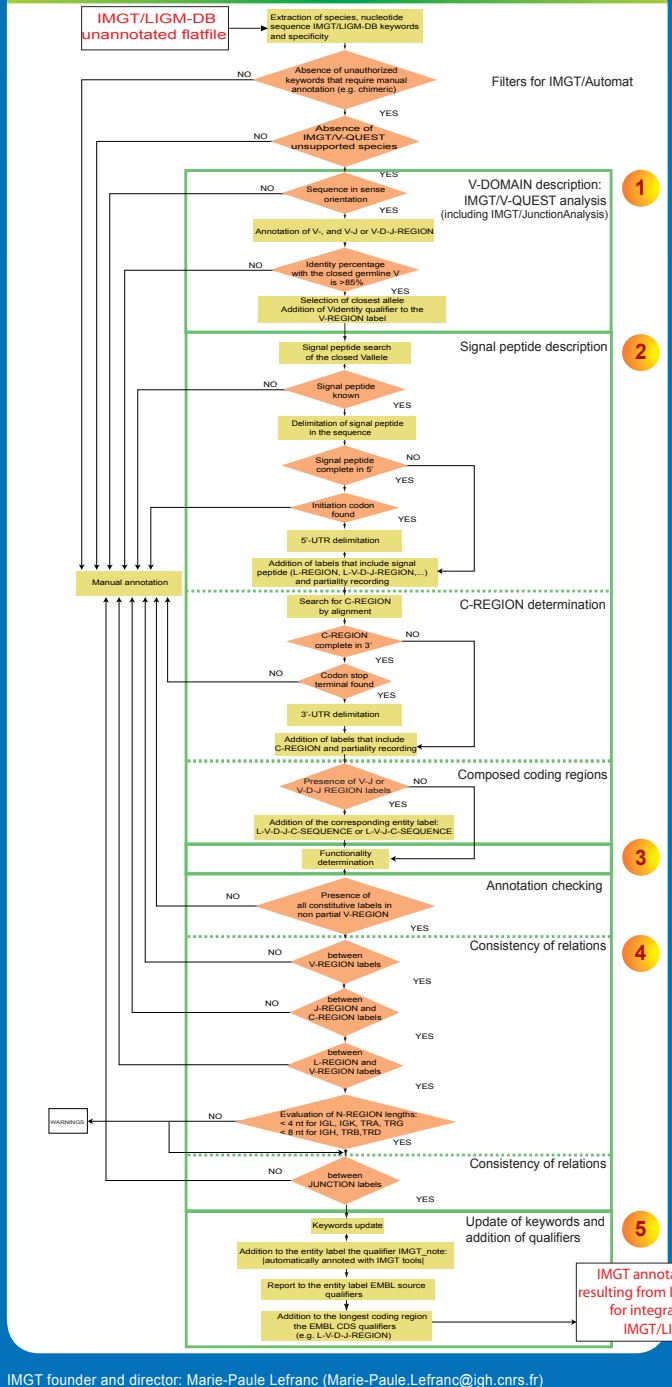
Im Muno Gene Tics Information system®

http://www.imgt.org

The cDNA sequences of immunoglobulins (IG) and T cell receptors (TR) represent more than one half of the sequences in the IMGT® nucleotide database IMGT/LIGM-DB [1] and 75% of them are from human and mouse. A few cDNA are germline but the great majority results from a V-D-J or V-J gene rearrangement, spliced to a C gene. The IG and TR genes have been studied extensively in IMGT® (http://www.imgt.org) [2], which allowed to set up their nomenclature and the corresponding germline reference sequences. These standardized reference directory sets (one for each group of each locus) and the IMGT-ONTOLOGY axioms and derived concepts [3] are the key elements indispensable to perform the annotation of IG and TR cDNA sequences. A Java program, IMGT/Automat [4], was developed by IMGT®, to automatically annotate the IG and TR cDNA sequences and to produce a totally automatic and complete annotation. More than 9,000 human and mouse cDNA have already been successfully automatically annotated. The quality of the cDNA automatic annotation is equivalent to the quality of the annotation achieved by a human expert. The IMGT® strategy is currently the only way, in the field of immunogenetics, to guarantee the annotation quality and the management of an always increasing number of IG and TR cDNA nucleotide sequences.

[1] Giudicelli V. et al. Nucleic Acids Res., 34, D781-784 (2006).
[2] Lefranc M.-P. et al. Nucleic Acids Res., 37, D1006-1012 (2009).
[3] Duroux P. et al. Biochimie, 90, 570-583 (2008).
[4] Giudicelli V. et al. Stud. Health Technol. Inform., 116, 3-8 (2005).
[5] Brochet X. et al. Nucleic Acids Res., 36, W503-508 (2008).
[6] Yousfi Monod M. et al. Bioinformatics, 20, i379-385 (2004).

IMGT/Automat main tasks



IMGT/Automat includes five main tasks:
In a first step IMGT/Automat implements IMGT/V-QUEST [5] . The description of the V-D-J and V-J junction is performed by the IMGT/JunctionAnalysis [6] tool. In a second step, IMGT/Automat delimits the signal peptide, the constant region and the composed coding regions (for example: L-V-D-J-C-REGION). In a third step, the functionality of the sequence (a concept of identification) is defined. The fourth step corresponds to a thorough annotation checking. In a fifth and final step, keywords are updated and qualifiers on biological origin and methodology used (concepts of obtention) are integrated, and the annotated flat file is generated.

1 V-DOMAIN description: IMGT/V-QUEST Analysis (including IMGT/JunctionAnalysis)

V-DOMAIN description (V-J-REGION and V-D-J-REGION is performed by IMGT/V-QUEST analysis. Detailed analysis of JUNCTION is performed by the integrated IMGT/JunctionAnalysis tool

Alignment for V-GENE and allele identification

```
                                                  <---------------------------------- FR1-IMG
BC024289                                          gaggtgcagctggtggagtctgggggga...ggcctggtcaagg
AB019439 IGHV3-21*01                              ------------------------------...-----------
M99658  IGHV3-21*02                               --------a---------------------...-----------
M99675  IGHV3-48*01                               ------------------------------...----t----ac--
AB019438 IGHV3-48*02                              ------------------------------...----t----ac--
AJ879484 IGHV3-h*01(P)                            ------------------------------...----t----a---
```

Alignment for J-GENE and allele identification

```
BC024289                                          tctccgccagctaacttcctactggtacttcgatctctgggg
J00256 IGHJ2*01                                   ...........------------------------------
M25625  IGHJ4*03                                  ....................-c-----t--cta-------
```

Results of IMGT/JunctionAnalysis

Maximum number of accepted mutations in 3'V-REGION = 2, D-REGION = 4, 5'J-REGION = 2

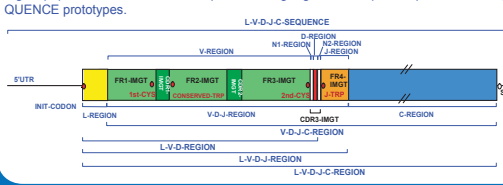
Input	V name	3'V-REGION	N1	D-REGION	N2
BC024289	IGHV3-21*01	tgtgcgagaga	t	...........tccgccagcta.....	actt

Input	5'J-REGION	J name	D name	Vmut	Dmut	Jmut	Ngc
BC024289	ctactggtacttcgatctctgg	IGHJ2*01	IGHD3-10*01	0	4	0	3/7

IMGT/V-QUEST analysis provides:
- **Identification** of the sequence (chain type for ex: IG-Heavy)
- **Classification** of the V, D, J genes and alleles
- **Description** of the IG and TR specific constitutive motifs
- **Delimitation** of the framework regions (FR-IMGT) and complementarity determining regions (CDR-IMGT)
- **Numbering** of the codons

2 Signal peptide, C-REGION and composed coding regions

Signal peptide, C-REGION and composed coding regions description is performed using the L-V-J-C-SEQUENCE and L-V-D-J-C-SEQUENCE prototypes.



Composed coding regions for:
- Entity : L-V-D-J-C-SEQUENCE
L-REGION, L-V-D-REGION, L-V-D-J-REGION, L-V-D-J-C-REGION, V-D-J-C-REGION, C-REGION

Composed coding regions for:
- Entity : L-V-J-C-SEQUENCE
L-REGION, L-V-J-REGION, L-V-J-C-REGION, V-J-C-REGION, C-REGION

3 Functionality determination

The functionality of the sequence is defined according to the biological rules of the IMGT Scientific chart.

The sequence is PRODUCTIVE if the coding region has an open reading frame, with no stop codon and no defect described in the initiation codon, splicing sites and/or regulatory elements, and an in-frame JUNCTION.

The sequence is UNPRODUCTIVE if the JUNCTION is out-of-frame and/or the presence of stop codon(s) and/or frameshift mutation(s), and/or a defect described in the splicing sites and/or the regulatory element(s), and/or unusual features (TRANSLOCATED, GENE FUSION...) and/or changes of conserved

4 Annotation checking

Annotation checking comprises several steps (see figure), for examples:

Presence of all constitutive labels by comparison with the prototype (e.g. I-REGION, V-REGION, D-REGION, ...)

Consistency of relations between labels (e.g. L-REGION adjacent_in_its_3_prime_with V-REGION, FR1-IMGT is_included_with_same_5_prime_in V-REGION)

5 Annotated IMGT FLAT-FILE resulting from IMGT/Automat

```
ID  BC024289 IMGT/LIGM annotation : automatic; mRNA; HUM; 1630 BP.
XX
AC  BC024289;
XX
DT  23-OCT-2003 (Rel. 200343-4, arrived in LIGM-DB )
DT  03-APR-2009 (Rel. 200914-5, Last updated, Version 4)
XX
DE  Homo sapiens immunoglobulin heavy constant gamma 3 (G3m marker), mRNA
DE  (cDNA clone MGC:39273 IMAGE:5440834), complete cds. ; ;
DE  mRNA; rearranged configuration; IG-Heavy; regular; functionality
DE  productive; group IGHV; subgroup IGHV3.
XX
KW  antigen receptor; Immunoglobulin superfamily (IgSF);
KW  Immunoglobulin (IG); constant; variable; diversity; joining; regular;
KW  cDNA; undefined; rearranged; productive; L-V-D-J-C-sequence.
XX
OS  Homo sapiens (human)
...
XX
FH  Key             Location/Qualifiers
FH
FT  L-V-D-J-C-SEQUENCE  1..1630
FT                  /db_xref="RZPD:IRALp962O2042"
FT                  /clone_lib="NIH_MGC_113"
FT                  /IMGT_note="automatically annotated with IMGT tools"
FT                  /clone="MGC:39273 IMAGE:5440834"
FT                  /lab_host="DH10B-R"
FT                  /tissue_type="Spleen"
FT                  /organism="Homo sapiens"
FT                  /productive
FT  L-V-D-J-C-REGION    64..1476
FT                  /db_xref="REMTREMBL:AAH24289"
FT                  /product="IGHG3 protein"
FT                  /protein_id="AAH24289.1"
FT                  /translation="MELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSC
FT                  AASGFTFSSYSMNWVRQAPGKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKN
FT                  SLYLQMNSLRAEDTAVYYCARDLRQLTSYWYFDLWGRGTLVTVSSASTKGPSVFP
FT                  LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
FT                  SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
FT                  PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
FT                  EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
FT                  VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
FT                  SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK"
FT  5'UTR           1..63
FT  L-V-D-J-REGION  64..486
FT                  /translation="MELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSC
FT                  AASGFTFSSYSMNWVRQAPGKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKN
FT                  SLYLQMNSLRAEDTAVYYCARDLRQLTSYWYFDLWGRGTLVTVSS"
FT  L-V-D-J-REGION  64..429
FT                  /translation="MELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSC
FT                  AASGFTFSSYSMNWVRQAPGKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKN
FT                  SLYLQMNSLRAEDTAVYYCARDLRQL"
FT  L-V-REGION      64..416
FT                  /translation="MELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSC
FT                  AASGFTFSSYSMNWVRQAPGKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKN
FT                  SLYLQMNSLRAEDTAVYYCAR"
FT  L-REGION        64..120
FT                  /translation="MELGLRWVFLVAILEGVQC"
FT  V-D-J-C-REGION  121..1476
FT                  /translation="EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAP
FT                  GKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
FT                  ARDLRQLTSYWYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
FT                  KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
FT                  VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
FT                  PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
FT                  DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
FT                  CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
FT                  VFSCSVMHEALHNHYTQKSLSLSPGK"
FT  V-D-J-REGION    121..486
FT                  /translation="EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAP
FT                  GKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
FT                  ARDLRQLTSYWYFDLWGRGTLVTVSS"
FT  V-REGION        121..416
FT                  /allele="IGHV3-21*01"
FT                  /gene="IGHV3-21"
FT                  /identity="99.31%  (286/288 nt)"
FT                  /CDR_length="[8.8.15]"
FT                  /putative_limit="3' side"
FT                  /translation="EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAP
FT                  GKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
FT                  AR"
FT  FR1-IMGT        121..195
FT                  /AA_IMGT="AA 1 to 26, AA 10 is missing"
FT                  /translation="EVQLVESGGGLVKPGGSLRLSCAAS"
FT  1st-CYS         184..186
FT  CDR1-IMGT       196..219
FT                  /AA_IMGT="AA 27 to 38, AA 31, 32, 33, 34 are  missing"
FT                  /translation="GFTFSSYS"
FT  FR2-IMGT        220..270
FT                  /AA_IMGT="AA 39 to 55"
FT                  /translation="MNWVRQAPGKGLEWVSS"
FT  CONSERVED-TRP   226..228
FT  CDR2-IMGT       271..294
FT                  /AA_IMGT="AA 56 to 65, AA 60, 61 are  missing"
FT                  /translation="MSSSSSYI"
FT  FR3-IMGT        295..408
FT                  /AA_IMGT="AA 66 to 104, AA 73 is missing"
FT                  /translation="YYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC"
FT  2nd-CYS         406..408
FT  CDR3-IMGT       409..453
FT                  /AA_IMGT="AA 105 to 117 including 112.1, 111.1"
FT                  /translation="ARDLRQLTSYWYFDL"
FT  JUNCTION        406..456
FT                  /in_frame
FT                  /translation="CARDLRQLTSYWYFDLW"
FT  3'V-REGION      406..416
FT  N1-REGION       417..418
FT                  /codon_start=2
FT  D-REGION        419..429
FT                  /allele="IGHD3-10*01"
FT                  /gene="IGHD3-10"
FT                  /codon_start=3
FT                  /translation="RQL"
FT  N2-REGION       430..434
FT                  /translation="T"
FT  5'J-REGION      435..456
FT  J-REGION        435..486
FT                  /allele="IGHJ2*01"
FT                  /gene="IGHJ2"
FT                  /putative_limit="5' side"
FT                  /identity="100.00% (53/53 nt)"
FT                  /codon_start=2
FT                  /translation="YWYFDLWGRGTLVTVSS"
FT  J-TRP           454..456
FT  FR4-IMGT        454..486
FT                  /AA_IMGT="AA 118 to 128"
FT                  /translation="WGRGTLVTVSS"
FT  C-REGION        487..1476
FT                  /allele="IGHG1*02"
FT                  /gene="IGHG1"
FT                  /translation="ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
FT                  NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
FT                  KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
FT                  EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
FT                  SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
FT                  VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
FT                  NHYTQKSLSLSPGK"
FT  STOP-CODON      1477..1479
```

IDENTIFICATION Keywords

CLASSIFICATION nomenclature

OBTENTION

DESCRIPTION Labels

NUMEROTATION

IMGT founder and director: Marie-Paule Lefranc (Marie-Paule.Lefranc@igh.cnrs.fr)
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