IMGT/Automat and IMGT® biocuration pipeline for IG and TR rearranged cDNA sequences

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Im Muno Gene Tics Information system®

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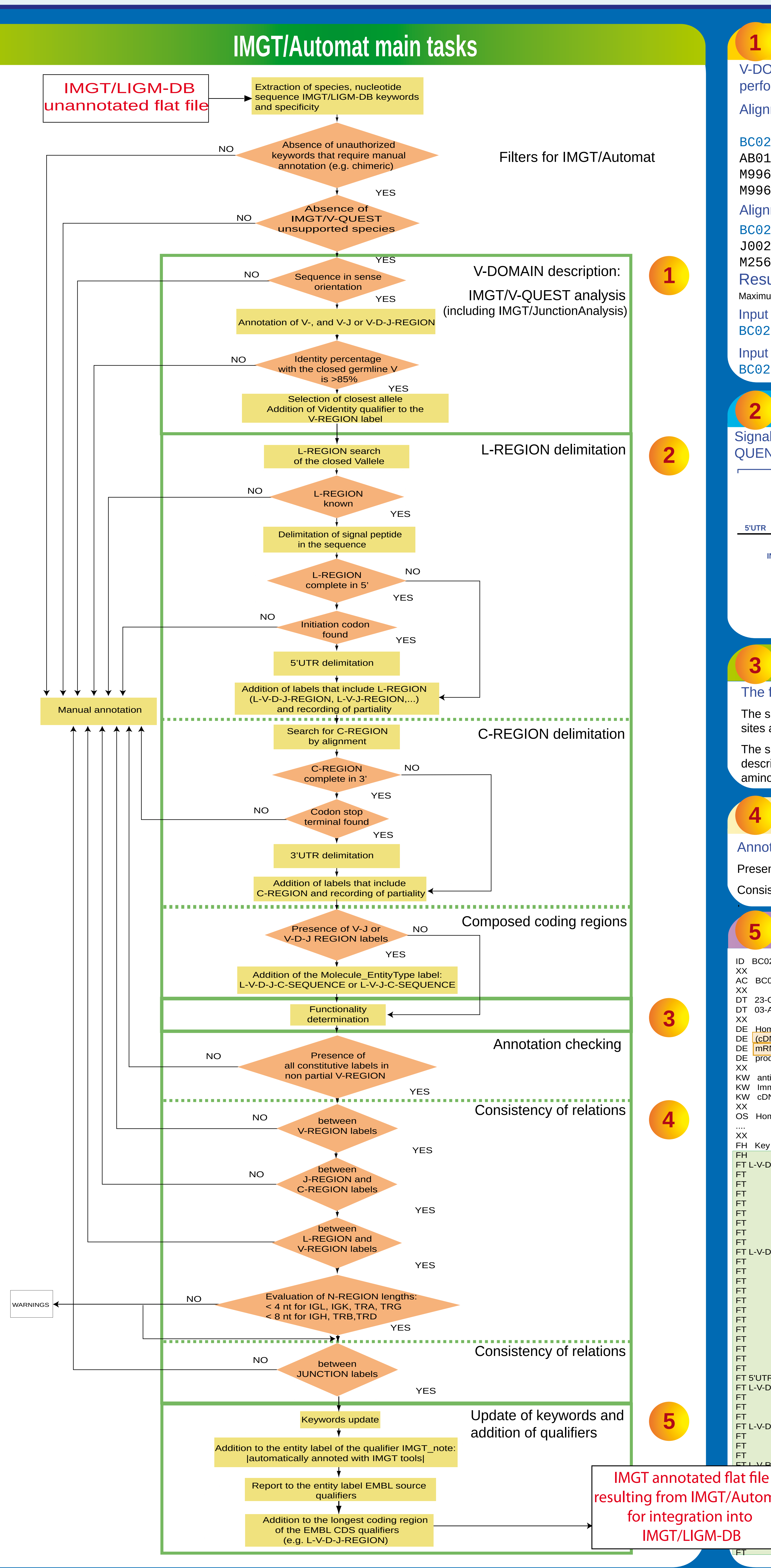
IMGT®, the international ImMunoGeneTics information system®, http://www.imgt.org, has developed the IMGT/Automat tool and an expert biocuration pipeline for immunoglobulin (IG) and T cell receptor (TR) rearranged cDNA sequences. Synthesis of the IG and TR proteins requires rearrangements of a variable (V) and junction (J) genes for the IGK, IGL, TRA and TRG chains, and of a V, diversity (D) and J genes for the IGH, TRB and TRD chains. The rearrangements occur at the DNA level, and are followed by the splicing at the RNA level of the rearranged V-J and V-D-J gene to the C gene. IG or TR rearranged cDNA sequences correspond to two major Molecule_EntityType L-V-J-C-SEQUENCE and L-V-D-J-C-SEQUENCE (L for L-REGION and C for C-REGION). IMGT/Automat and the IMGT® biocuration pipeline take into account the particularities of IG and TR cDNA structures and the annotation is based on the IMGT standardized rules generated from the IMGT-ONTOLOGY axioms and concepts [1]. In a first step, the analysis of the V-DOMAIN, that corresponds to the V-J-REGION or V-D-J-REGION, is performed with the IMGT/V-QUEST tool (standalone or incorporated in IMGT/Automat). IMGT/V-QUEST compares and aligns the cDNA sequences with the IMGT reference directory sequences and identifies the closest germline V, D and J genes and alleles (CLASSIFICATION). It delimits the framework regions (FR-IMGT) and complementarity determining regions (CDR-IMGT) (DESCRIPTION) and numbers the codons according to the IMGT unique numbering (NUMEROTATION). The detailed description of the V-D-J and V-J junction is performed by the IMGT/JunctionAnalysis tool. In a second step, IMGT/Automat delimits the L-REGION, the C-REGION and the composed coding regions (e.g., L-V-D-J-C-REGION). In a third step, the functionality of the sequence (productive or unproductive) 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. To finalize cDNA annotation, data consistency controls are checked by biocurators (position errors, missing IMGT labels, organization...). Curated data are integrated in the IMGT/LIGM-DB database. IMGT annotations are visible via a friendly interface which gives the possibility to query with labels.

Thus the IMGT/Automat tool provides a totally automatic and complete annotation of rearranged cDNA IG and TR sequences. The results provided by IMGT/Automat are of a quality identical to expert biocuration. For that reason, IMGT/Automat has been integrated in IMGT/HighV–QUEST, the high throughput version of IMGT/V-QUEST that gives users the possibility to analyse rearranged IG and TR sequences from NGS and Deep Sequencing by batches of 150,000 for human and mouse. IMGT/Automat can potentially been used for any other species once the IMGT reference directories become available, following genomic biocuration and entry in IMGT/GENE-DB.

[1] IMGT booklet (11 papers), Cold Spring Harb Protoc, 124 pages (2011) (pdf, IMGT References, http://www.imgt.org). *With generous provision from Cold Spring Harbor (CSH) Protocols.*

IMGT/Automat main tasks


IMGT/LIGM-DB unannotated flat file
Extraction of species, nucleotide sequence IMGT/LIGM-DB keywords and specificity
Filters for IMGT/Automat
Absence of unauthorized keywords that require manual annotation (e.g. chimeric)
NO
YES
Absence of IMGT/V-QUEST unsupported species
NO
YES
V-DOMAIN description:
IMGT/V-QUEST analysis (including IMGT/JunctionAnalysis)
1
Sequence in sense orientation
NO
YES
Annotation of V-, and V-J or V-D-J-REGION
Identity percentage with the closed germline V is >85%
NO
YES
Selection of closest allele Addition of Videntity qualifier to the V-REGION label
L-REGION delimitation
2
L-REGION search of the closed Vallele
L-REGION known
NO
YES
Delimitation of signal peptide in the sequence
L-REGION complete in 5'
NO
YES
Initiation codon found
NO
YES
5'UTR delimitation
Addition of labels that include L-REGION (L-V-D-J-REGION, L-V-J-REGION,...) and recording of partiality
Manual annotation
C-REGION delimitation
Search for C-REGION by alignment
C-REGION complete in 3'
NO
YES
Codon stop terminal found
NO
YES
3'UTR delimitation
Addition of labels that include C-REGION and recording of partiality
Composed coding regions
Presence of V-J or V-D-J REGION labels
NO
YES
Addition of the Molecule_EntityType label: L-V-D-J-C-SEQUENCE or L-V-J-C-SEQUENCE
3
Functionality determination
Annotation checking
Presence of all constitutive labels in non partial V-REGION
NO
YES
Consistency of relations
4
between V-REGION labels
NO
YES
between J-REGION and C-REGION labels
NO
YES
between L-REGION and V-REGION labels
YES
WARNINGS
NO
Evaluation of N-REGION lengths: < 4 nt for IGL, IGK, TRA, TRG < 8 nt for IGH, TRB,TRD
YES
Consistency of relations
between JUNCTION labels
NO
YES
Update of keywords and addition of qualifiers
5
Keywords update
Addition to the entity label of the qualifier IMGT_note: |automatically annoted with IMGT tools|
Report to the entity label EMBL source qualifiers
Addition to the longest coding region of the EMBL CDS qualifiers (e.g. L-V-D-J-REGION)
IMGT annotated flat file resulting from IMGT/Automat for integration into IMGT/LIGM-DB

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-IMGT ---
BC024289                        gaggtgcagctggtggagtctgggga...ggcctggtcaagcctggg
AB019439 IGHV3-21*01             ---------------------------...------------------
M99658  IGHV3-21*02             --------a------------------...------------------
M99675  IGHV3-48*01             ---------------------------...---t----ac--------
```

Alignment for J-GENE and allele identification

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

Results of IMGT/JunctionAnalysis

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

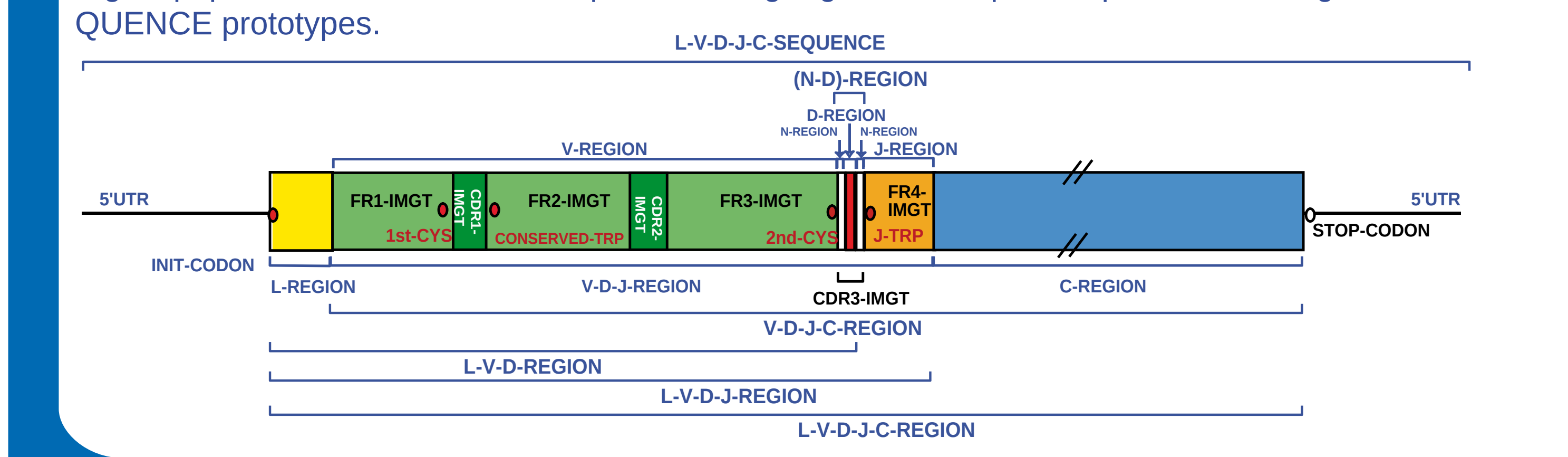
```
Input      V name       3'V-REGION   N1          D-REGION              N2
BC024289 IGHV3-21*01 tgtgcgagaga    t   .............tccgccagcta.......  acttc

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)

2 L-REGION, 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 amino acids demonstrated as leading to uncorrect folding.

4 Annotation checking

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

Presence of all constitutive labels by comparison with the prototype (e.g., L-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/LIGM-DB 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-REGION       64..429
FT                    /translation="MELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSC
FT                     AASGFTFSSYSMNWVRQAPGKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKN
FT                     SLYLQMNSLRAEDTAVYYCARDLRQL"
FT L-V-REGION          64..416
                      /translation="MELGLRWVFLVAILEGVQCEVQLVESGGGLVKPGGSLRLSC
                      AASGFTFSSYSMNWVRQAPGKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKN
                      SLYLQMNSLRAEDTAVYYCAR"
                       64..120
                      /translation="MELGLRWVFLVAILEGVQC"
              GION    121..1476
                      /translation="EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAP
                       GKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
FT                     ARDLRQLTSYWYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
FT   V-D-J-REGION       121..486
FT                    /translation="EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAP
FT                     GKGLEWVSSMSSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
FT                     ARDLRQLTSYWYFDLWGRGTLVTVSS"
FT   V-REGION         121..416
FT                    /IMGT_allele="IGHV3-21*01"
FT                    /IMGT_gene="IGHV3-21"
FT                    /Videntity="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                    /IMGT_allele="IGHD3-10*01"
FT                    /IMGT_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                    /IMGT_allele="IGHJ2*01"
FT                    /IMGTgene="IGHJ2"
FT                    /putative_limit="5' side"
FT                    /Jidentity="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                    /IMGT_allele="IGHG1*02"
FT                    /IMGT_gene="IGHG1"
FT                    /translation="ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
```

IDENTIFICATION (IMGT® standardized keywords)

CLASSIFICATION (IMGT® standardized nomenclature)

OBTENTION

DESCRIPTION (IMGT® standardized labels)

NUMEROTATION (IMGT® unique numbering)

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