# **Variable Domain cDNA Sequence Analysis**



**Customer: Your Company Project: Your Favorite Antigen** 

**Cell Line: Your Hybridoma** 

**Sequencing ID: SEQ0000** 

## CONFIDENTIAL

# **Sequencing Overview**

Productive Immunoglobulin Domain Sequence	Clones Sequenced	V Domain Sequence Identity	lsotype from Sequence
V <sub>H</sub>	≥5	100%	lgG1
$V_L$	≥5	100%	Kappa

## **Recommended Analysis Tools**

We recommend the following free online tools for DNA-sequence analysis of immunoglobulin variable regions: NCBI <u>Nucleotide BLAST</u>

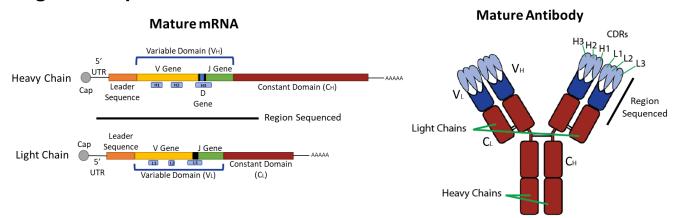
IMGT/V-Quest program

NCBI IgBLAST

**Note:** Be aware that if you copy sequence directly from this pdf, your text will contain paragraph returns that must be removed prior to BLAST analysis.



## **Regions Sequenced**



# **Heavy Chain Sequence**

#### **DNA Sequence**

Leader sequence (underlined) is translated and targets the nascent polypeptide to the endoplasmic reticulum (ER). It is cleaved during translocation into the ER and is not part of the mature antibody.

### Predicted Protein Sequence (V<sub>H</sub>)

Complementarity determining regions (CDRs) are underlined.

EVQLQQSGTVLARPGASVKMSCKTS<u>GYTFTSYW</u>MHWVKQRPGQGLEWIGA<u>IYPGNSDT</u>SYNQKFKGKAKLTAVT SASTAYMELSSLTNEDSAVYYC<u>TRSGGNQYYYSMDS</u>WGQGTSVTVSS

## **Light Chain Sequence**

### **DNA Sequence**

Leader sequence is underlined.

ATGAAGTCACAGACCCAGGTCTTCGTATTTCTACTGCTCTGTGTGTCTCATGGGagtattgtgatgacccagac tcccaaattcctgcttgtttcagcaggagacagggttaccataacctgcaaggccagtcagattgtgagtaatgatgtaggttagcaacaggagtcaggggcagt ctcctaaactgctgatatactatgcatccaatcgctacactggagtccctgatcgcttcactggcagtggatatgggacggatttcactttcaccatcagcactgtgcag gctgaagacctggcagtttatttctgtcagcaggattataggtctcccacgttcggtgggaccaagctggagctgaaac

### Predicted Protein Sequence (V<sub>L</sub>)

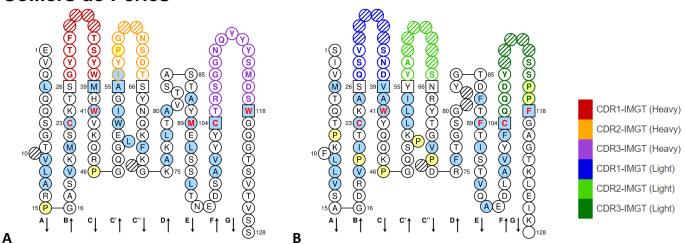
Complementarity determining regions (CDRs) are underlined.

 ${\tt SIVMTQTPKFLLVSAGDRVPITCKAS} \underline{{\tt QSVSND}} {\tt VAWYQQKPGQSPKLLIY} \underline{{\tt YAS}} {\tt NRYTGVPDRFTGSGYGTDFTFTIST} {\tt VQAEDLAVYFCQQDYSSPPFGAGTKLEIK}$ 

Note: Additional sequence annotation, including 5' UTR, partial constant region, and V/D/J gene identity, are available upon request.



## Colliers de Perles



Two-dimensional graphical representations of the predicted V-domains, generated by IMGT (Ruiz, M. and Lefranc, M.-P., Immunogenetics, 53, 857-883 (2002) PMID: 11862387) for the heavy (A) and light (B) chain. Amino acids are shown as one-letter abbreviation. Positions with hydrophobic amino acids or tryptophan (W) at a given position in more than 50% of analyzed sequences are shown in blue, with highly conserved residues emphasized with red font. All prolines (P) are shown in yellow. Squares show the limits of the CDR regions and belong to the neighboring framework region. Arrows indicate the predicted direction of the beta sheets and their different designations in 3D structures. Red font is highly conserved hydrophobic amino acids. Hatched circles or squares correspond to missing positions for these chains (but may be present in other antibodies) according to the IMGT unique numbering for V-DOMAIN.

### **Methods**

Total cytoplasmic RNA was isolated from the hybridoma cell line culture (1 x  $10^6$  cells). RNA was reverse transcribed into cDNA using isotype-specific antisense primers and SMARTScribe Reverse Transcriptase with a template switch oligonucleotide to capture the 5' end of the mRNA. The resulting  $V_H$  and  $V_L$  cDNA was amplified by PCR, size confirmed by agarose gel electrophoresis, and cloned separately into a standard cloning vector. The sequences of no less than five colonies were aligned and the consensus sequence of these clones was analyzed for verification that the sequences could encode a productive immunoglobulin and to ensure no process contamination. Additional sequence analysis is available upon request.

#### **Important Notes**

To our knowledge, the sequence reported here is accurate.

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