Variable Domain cDNA Sequence Analysis



Customer: Your Company

Project: Your Favorite Antigen

Cell Line: Your Hybridoma

Sequencing ID: SEQ####

CONFIDENTIAL

Sequencing Overview

Productive Immunoglobulin Domain Sequence	Reads Aligned to Consensus	V Domain Sequence Q Score >40	lsotype from Sequence
V _H	≥15	100.0%	IgG1
VL	≥15	100.0%	Kappa

Recommended Analysis Tools

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

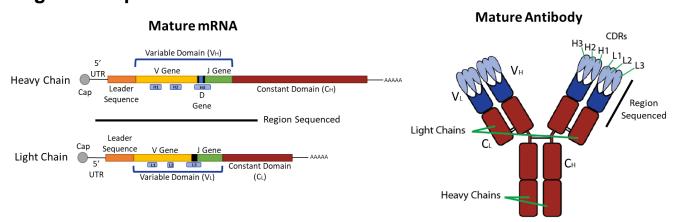
IMGT/V-Quest program

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_H)

Complementarity determining regions (CDRs) are underlined.

EVQLQQSGTVLARPGASVKMSCKTS<u>GYTFTSYW</u>MHWVKQRPGQGLEWIGA<u>IYPGNSDT</u>SYNQKFKGKAKLTAVT SASTAYMELSSLTNEDSAVYYCTRSGGNQYYYSMDSWGQGTSVTVSS

Light Chain Sequence

DNA Sequence

Leader sequence is underlined.

ATGAAGTCACAGACCCAGGTCTTCGTATTTCTACTGCTCTGTGTGTCTCGTGTGCTCATGGGagtattgtgatgacccagac tcccaaattcctgcttgtttcagcaggagacagggttaccataacctgcaaggccagtcagattgtgagtaatgatgtagcttggtaccaacagaagtcagggcagt ctcctaaactgctgatatactatgcatccaatcgctacactggagtccctgatcgcttcactggcagtggatatgggacggatttcactttcaccatcagcactgtgcag gctgaagacctggcagtttatttctgtcagcaggattataggtctcccacgttcggtgctgggaccaagctggagctgaaac

Predicted Protein Sequence (V_L)

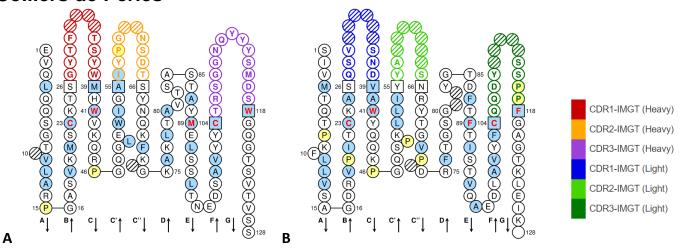
Complementarity determining regions (CDRs) are underlined.

SIVMTQTPKFLLVSAGDRVPITCKASQSVSNDVAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTIST 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 10⁶ 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 sequenced using Oxford nanopore sequencing. A minimum of 15x coverage is required (>99.99% sequence accuracy) and all sequences were analyzed 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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