Variable Domain cDNA Sequence Analysis

Green Mountain Antibodies

Customer: Your Company

Project: Your Favorite Antigen

Cell Line: Your Hybridoma

Sequencing ID: SEQ###

C O N F I D E N T I A L

Sequencing Overview

Productive Immunoglobulin Domain Sequence	Reads Aligned to Consensus	V Domain Sequence Q Score >40	Constant Region from Sequence
V_H	≤15	100.0%	IgG1
V_L	≤15	100.0%	Kappa

Recommended Analysis Tools

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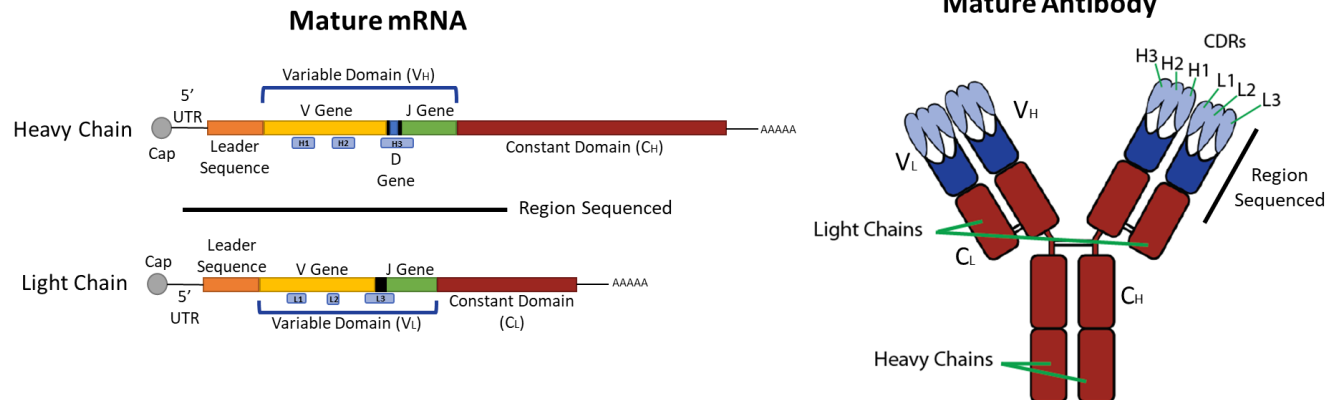
NCBI Nucleotide BLAST

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.

Green Mountain Antibodies

Regions Sequenced

Mature mRNA

Mature Antibody

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.

ATGGAAAGGCACTGGATCTTTCTCTTCCTGTTTTCAGTAACTGCAGGTGTCCACTCCgaggttcagctccagcagtctggg actgtgctggcaaggcctgggcttcagtgaagatgtcctgcaagacttctggctacacatttaccagctactggatgcactgggtaaaacagaggcctggacag ggtctggaatggataggggctatttatcctggaaatagtgatactagctacaaccagaagttcaagggcaaggccaaactgactgcagtcacatccgccagcact gcctacatggagctcagcagcctgacaaatgaggactctgcggtctattactgtacaagatccggggggtaaccaatattactattctatggactcctggggtcaagg aacgtcagtcaccgtctcctcag

Predicted Protein Sequence (V$_H$)

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.

<u>ATGAAGTCACAGACCCAGGTCTTCGTATTTCTACTGCTCTGTGTGTCTGGTGCTCATGG</u>agtattgtgatgacccagac tcccaaattcctgcttgtttcagcaggagacagggttaccataacctgcaaggccagtcagattgtgagtaatgatgtagcttggtaccaacagaagtcagggcagt ctcctaaactgctgatatactatgcatccaatcgctacactggagtccctgatcgcttcactggcagtggatatgggacggatttcacttttcaccatcagcactgtgcag gctgaagacctggcagtttatttctgtcagcaggattataggtctcccacgttcggtgctgggaccaagctggagctgaaac

Predicted Protein Sequence (V$_L$)

Complementarity determining regions (CDRs) are underlined.

DIVMTQTPKFLLVSAGDRVPITCKAS<u>QSVSND</u>VAWYQQKPGQSPKLLIY<u>YAS</u>NRYTGVPDRFTGSGYGTDFTFTIST VQAEDLAVYFC<u>QQDYSSPP</u>FGAGTKLEIK

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

Methods

Total cytoplasmic RNA was isolated from the hybridoma cell line culture (1×10^6 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 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.

Thank you for choosing Green Mountain Antibodies, custom monoclonal antibody production specialists. Our goal is to help you succeed—contact us to discuss your next project