Variable Region cDNA Sequence Analysis

Customer: GMA

Project: SAMPLE, Sample ID: SAMPLE

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.

Heavy Chain

DNA Sequence

caggtgcagctgaagcagtcaggacctggcctagtgcagccctcacagagcctgtccataacctgcacagtctctggtttctcattaactagatatggtgtaaattgg
gatcgccggtctccaggaaagggtctggagtggctgggagtgatgtggagaggtggttgcacagactacaatgcagctttcatgtccagactgaacatcaccaag
gacgactccaagagccaaatttttctttaaaatgaacagtctggaagttaatgacactgccatatattactgtgccaatgtgattacgacggttcctgatttacttactggg
gccaagggactctggtcaccgtctccgcag

Predicted Protein Sequence

Complementarity determining regions (CDRs) are underlined.

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTRYGVNWDRRSPGKGLEWLGVMWRGGCTDYNAAFMSRLNITKDDS
KSQIFFKMNSLEVNDTAIYYCANVITTVPDLLTGAKGLWSPSPQPAGLV

Light Chain

DNA Sequence

gacattgtgatgacccagtctcaaaaattcatgtccacatcagtaggagacagggtcagcgtcacctgcaaggccagtcagaatgtggatatttatgtagcctggta
tcaacagaaaccagggcaatctcctaaagcactgatttactcggcatcctaccggttcagtggagtccctgatcgcttcacaggcagtggatctgggacagatttca
ctctcagcatcagcaatgtgcagtctgaagacttggcagagtatttctgtcagcaatataacaactatccgtatacgttcggaggggggaccaagctggaaataaa
ac

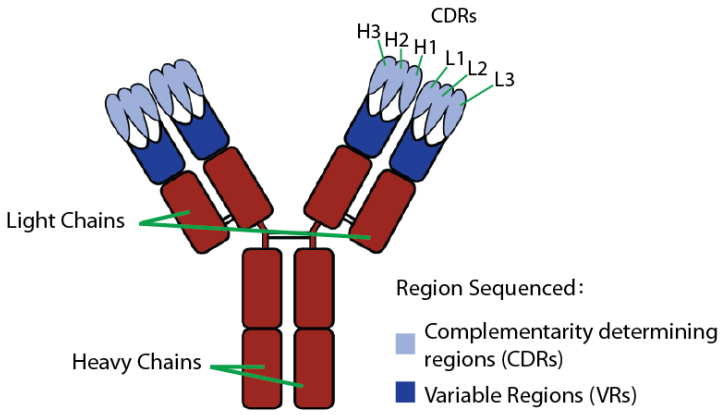
Predicted Protein Sequence

Complementarity determining regions (CDRs) are underlined.

DIVMTQSQKFMSTSVGDRVSVTCKASQNVDIYVAWYQQKPGQSPKALIYSASYRFSGVPDRFTGSGSGTDFTLSIS
NVQSEDLAEYFCQQYNNYPYTFGGGTKLEIK

Project Specifications

	Heavy Chain	Light Chain
Clones sequenced	6	6
Clones with >98% DNA sequence-identity	6	6
Consensus DNA sequence is consistent with murine immunoglobulin sequence	yes	yes



Methods

Sample Preparation

Total RNA was isolated from the hybridoma cell line culture (2 x 10^6 cells). RNA was treated to remove aberrant transcripts and reverse transcribed using oligo(dT) primers. Samples of the resulting cDNA were amplified in separate PCRs using framework 1 and constant region primer pairs specific for either the heavy or light chain. Reaction products were separated on an agarose gel, size-evaluated and recovered. In some cases, a second, nested PCR is performed to increase yield of the desired fragment(s). Amplicons were cloned into pCR®4-TOPO vector using the TA cloning strategy. 12 colonies were selected and plasmid DNA was amplified using primers specific for vector DNA sequences. PCR product size for each cloned insert was evaluated by gel electrophoresis, and 6 reactions were prepared for sequencing using a PCR clean up kit and sequenced at the Dartmouth College Molecular Biology & Proteomics Core Facility using cycle sequencing with fluorescent dye terminators and capillary-based electrophoresis.

Sequence Analysis

DNA sequence data from all constructs are analyzed and consensus sequences for heavy and light chain are determined. The consensus sequences are compared to all known Green Mountain Antibody variable region sequences to rule out artifacts and/or process contamination. Consensus sequences are then analyzed using an online tool to verify that the sequences could encode a productive immunoglobulin.

Important Notes

To our knowledge, the sequence reported here is accurate.
These results and the information contained in this report are for research use only. They are not intended for diagnostic or therapeutic use.
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