# Final Report

# Antibody Full Length Sequencing of Hybridoma 5G1.1 T175

## Order Number: U856UEK140-1

## November 15, 2019

Prepared by

Jinquan Chen

Jinquan Chen, Production Supervisor

This study was conducted by strictly following the procedures described in this report. All data presented are authentic and accurate to the best of our knowledge. Information and materials provided by this investigation are for research use only.


860 Centennial Ave., Piscataway, NJ 08854, USA
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ALXN2075
IPR2023-01069
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IPR2019-00739

## Recommended Analysis Tools

We recommend the following free online tools for sequence analysis of immunoglobulin variable regions:

i. NCBI Nucleotide BLAST
ii. IMGT/V Quest program
iii. NCBI IgBLAST

## Antibody sequences of 5G1.1 T175 were listed as below:

### Heavy chain: DNA sequence (1416 bp)

Signal sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

ATGGAATGGACCTGGGTCTTTCTCTTCCTCCTGTCAGTAACTGCAGGTGTCCACTCCCAGGTTCAGC
TGCAGCAGTCTGGAGCTGAGCTGATGAAGCCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTACTGG
CTACATATTCAGTAACTACTGGATACAGTGGATAAAGCAGAGGCCTGGACATGGCCTTGAGTGGATT
GGTGAGATTTTACCTGGAAGTGGTTCTACTGAGTACACTGAGAACTTCAAGGACAAGGCCGCATTCA
CTGCAGATACATCCTCCAACACAGCCTACATGCAACTCAGCAGCCTGACATCAGAGGACTCTGCCGT
CTATTACTGTGCAAGATATTTCTTCGGTAGTAGCCCCAACTGGTACTTCGATGTCTGGGGCGCAGGG
ACCACGGTCACCGTCTCCTCAGCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTGTGTG
GAGATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAGGGTTATTTCCCTGAGCCAGTGAC
CTTGACCTGGAACTCTGGATCCCTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGAC
CTCTACACCCTCAGCAGCTCAGTGACTGTAACCTCGAGCACCTGGCCCAGCCAGTCCATCACCTGCA
ATGTGGCCCACCCGGCAAGCAGCACCAAGGTGGACAAGAAAATTGAGCCCAGAGGGCCCACAATCAA
GCCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCT
CCAAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGA
GCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGAC
ACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAG
GACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGA
GAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGA
AGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCTGAAGACATTTAC
GTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCCTGGACTCTG
ATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTA
CTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCG
GGTAAATGA

### Heavy chain: Amino acid sequence (471 aa)

Signal peptide-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

MEWTWVFLFLLSVTAGVHSQVQLQQSGAELMKPGASVKMSCKATGYIFSNYWIQWIKQRPGHGLEWI


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GEILPGSGSTEYTENFKDKAAFTADTSSNTAYMQLSSLTSEDSAVYYCARYFFGSSPNWYFDVWGAG
TTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSD
LYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFP
PKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQ
DWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIY
VEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTP
GK-

**Light chain: DNA sequence (702 bp)**

Signal sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

ATGGGTGTACCCACTCAGCTCCTGTTGCTGTGGCTTACAGTCGTAATTGTCAGATGTGACATCCAGA
TGACTCAGTCTCCAGCTTCACTGTCTGCATCTGTGGGAGAAACTGTCACCATCACATGTGGAGCAAG
TGAGAATATTTACGGTGCTTTAAATTGGTATCAGCGGAAACAGGGAAAATCTCCTCAGCTCCTGATC
TATGGTGCAACCAACTTGGCAGATGGCATGTCATCGAGGTTCAGTGGCAGTGGATCTGGTAGACAGT
ATTATCTCAAGATCAGTAGCCTGCATCCTGACGATGTTGCAACGTATTACTGTCAAAATGTGTTAAA
TACTCCTCTCACGTTCGGTGCTGGGACCAAGTTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTA
TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACA
ACTTCTACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCT
GAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACC
AAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCA
TTGTCAAGAGCTTCAACAGGAATGAGTGTTAG

**Light chain: Amino acid sequence (233 aa)**

Signal peptide-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-Constant region-Stop codon

MGVPTQLLLLWLTVVIVRCDIQMTQSPASLSASVGETVTITCGASENIYGALNWYQRKQGKSPQLLI
YGATNLADGMSSRFSGSGSGRQYYLKISSLHPDDVATYYCQNVLNTPLTFGAGTKLELKRADAAPTV
SIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC-


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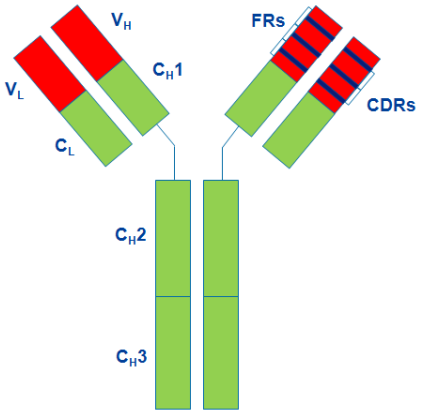
## Project Specifications

| Sequence | Clones sequenced | Clones with >99% sequence identity |
|---|---|---|
| $V_H$ | 5 | 5 |
| $C_H$ | 5 | 5 |
| $V_L$ | 5 | 5 |
| $C_L$ | 5 | 5 |




## IMGT Analysis of V(D)J Junctions

| Sequence | V-GENE and allele | Functionality | V-REGION identity % (nt) | J-GENE and allele | D-GENE and allele | AA Junction | Junction frame |
|---|---|---|---|---|---|---|---|
| $V_H$ | Musmus IGHV1-9*01 F | productive | 93.06% (268/288 nt) | Musmus IGHJ1*01 F | Musmus IGHD1-1*01 F | CARYFF GSSPNW YFDVW | in-frame |
| $V_L$ | Musmus IGKV12-89*01 F | productive | 99.28% (277/279 nt) | Musmus IGKJ5*01 F | | CQNVLN TPLTF | in-frame |

## Isotype of antibody

Isotype is mouse IgG2a/kappa, as analyzed by the sequences of constant region.

## Materials

Hybridoma cells provided by the client;

TRIzol® Reagent (Ambion, Cat. No. : 15596-026);

PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Cat. No. : 6110A).

## Methods

Total RNA was isolated from the hybridoma cells following the technical manual of TRIzol® Reagent. Total RNA was then reverse-transcribed into cDNA using either isotype-specific anti-sense primers or universal primers following the technical manual of PrimeScript™ 1st Strand cDNA Synthesis Kit. Antibody fragments of heavy chain and light chain were amplified according to the standard operating procedure (SOP) of rapid amplification of cDNA ends (RACE) of GenScript. Amplified antibody fragments were cloned into a standard cloning vector separately. Colony PCR was performed to screen for clones with inserts of correct sizes. The consensus sequence was provided.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