SUPPLEMENTAL DATA - Price et al.

Engineered cell surface expression of membrane immunoglobulin as a means to identify monoclonal antibody-secreting hybridomas

Supplemental Table 1

Primers for V-region sequencing

Primer Name	**Location**
	Antisense outside primers in constant regions (first round PCR)
IgMV1A	ATGGCCACCAGATTCTTATCAGACA
IgDV1A	TGGCTCCCAGCTGATTTTCA
IgG3V1A	TTTACAGTTACCGGCTCAGGGAAGTA
IgG1V1A	TTCCAGGTCACTGTCACTGGCTCAGGGAAA
IgG2bV1A	ACTGACTCAGGGAAGTAGCCCTTGA
IgG2aV1A	AGAGTTCCAGGTCAAGGTCACTGGCTCA
IgEV1A	ACAGGACCAGGGAAGTAGTCCTTTACCA
IgAV1A	AGCCGATTATCACTGGGTCACTTGACA
	Antisense inside primers in constant regions (second round PCR)
IgMV2A	TCTCGCAGGAGACGAGGGGGAAGACATT
IgDV2A	AATTACTAAACAGCCCAGGTTT
IgG3V2A	TTGACAAGGCATCCCAGTGTCACCGAGGAT
IgG1V2A	AGGCATCCCAGGGTCACCATGGAGTT
IgG2bV2A	ATCCCAGAGTCACGGAGGAACCAGT
IgG2aV2A	AACCCTTGACCAGGCATCCTAGAGTCA
IgEV2A	TCATGGAAGCAGTGCCTTTACAGGGCTTCA
IgAV2A	AGCTGGTGGGAGTGTCAGTGGGTAGAT
	Sense primers in signal sequence[a]
MH1	AATSARGTNMAGCTGSAGSAGTC
MH2	AATSARGTNMAGCTGSAGSAGTCWGG

	Sense primers in leader region[b]
HLV3S	ACAGNCHCTGAASACASTGANYCYHAMSAT
HLV4S	AWYSARNMNTGAACASRGWMMHNTCAMCAT

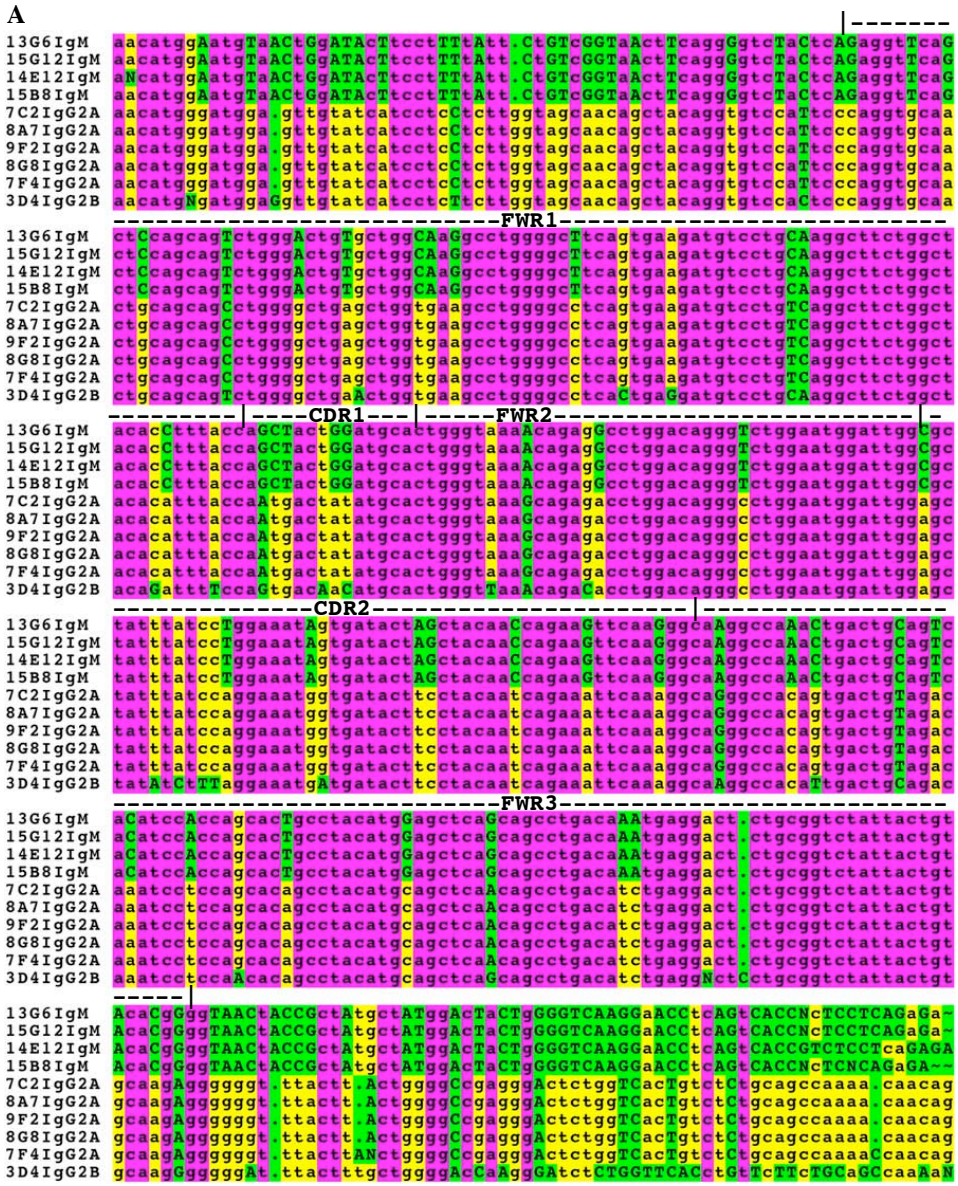
[a] Primers from Wang et al (1999)

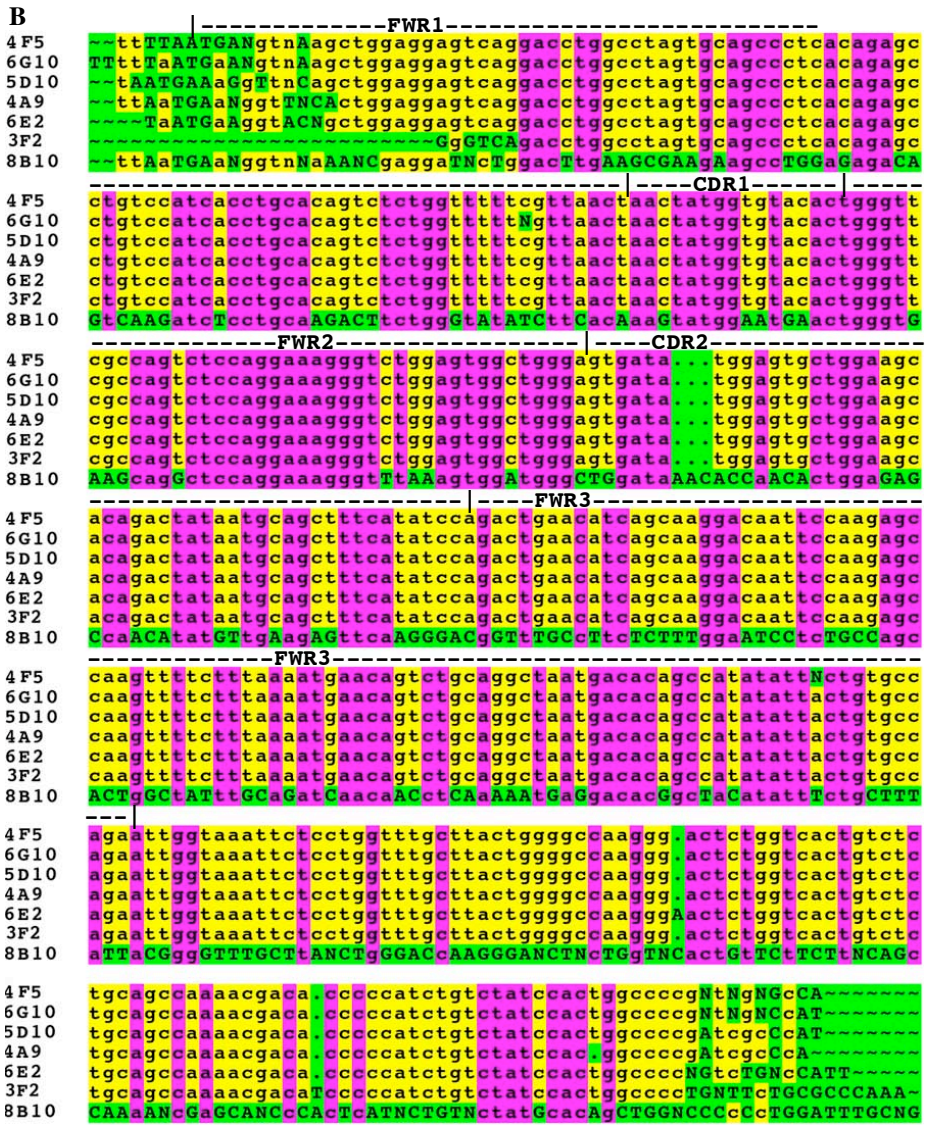
[b] Degenerate primers HLV3S and HLV4S were derived from the leader regions of IgG1 two mouse sequences (Accession #BC033451 and BC024405, respectively) and related sequences in the mouse database.

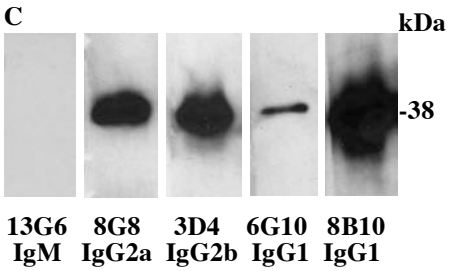
Supplemental Figure Legend 1.

Characterization of monoclonal antibodies from two ARP5 fusions. The ARP5-specific monoclonal antibodies were characterized. **A**. and **B**. HC V-region sequences obtained for hybridomas obtained from two SP2ab fusions with spleens from recombinant ARP5 immunized mice. Framework (FWR) and Complementarity-Determining Regions (CDR) are indicated. **C**. Western blot analysis of these monoclonal antibodies using partially purified recombinant ARP5 resolved on a SDS-PAGE on a 12% gel. Western blot analysis was performed as described in Kandasamy et al. (1999).

The rapid PCR sequencing protocol for the HC V-region (A & B) was performed as follows. Half-nested PCR was used to amplify the heavy chain V-region cDNA prior to sequencing. RNA was prepared from $5x10^6$ hybridoma cells using Shreder Kit #79654 and RNeasy Kit #74104 in succession (Qiagen, Inc., Valencia, CA). Invitrogen's Superscript III First Strand Synthesis Kit #18080-051 (Carlsbad, CA) and an oligo-dT primer were used to prepare cDNA. PCR and sequencing primers are described in **Supplemental Table 1**. PCR amplifications were performed using EX-Taq #RR001A (Takara BioInc., Madison, WI). An equal mixture MH1 and MH2 degenerate sense primers located in the signal sequence were used in amplifying the majority of V-region sequences (Wang et al., 1999). As an alternative, we designed degenerate sense primers HLV3S or HLV4S located in the mRNA leader region**,** which worked more effectively on most IgM clones. Nested pairs of antisense primers, specific for each isotype, located approximately 100 and 50 bp inside the constant region, were used in succession during the two rounds of PCR. The same degenerate sense primer was used in both PCR reactions. The inside antisense constant region primer was used during sequencing. This technique offered the advantage that PCR products were sequenced directly without prior molecular cloning. This saved time and effort, but also prevented the analysis of cloned mutant cDNAs that may be generated during PCR.


A
FWR1
CDR1
FWR2
CDR2
FWR3


B
FWR1
4F5
6G10
5D10
4A9
6E2
3F2
8B10
CDR1
FWR2
CDR2
FWR3
FWR3


C
kDa
-38
13G6 IgM
8G8 IgG2a
3D4 IgG2b
6G10 IgG1
8B10 IgG1

Supplemental References

Kandasamy, M.K., McKinney, E.C., Meagher, R.B. 1999. The late pollen-specific actins in angiosperms. Plant J 18, 681.

Wang, B., Chen, Y.B., Ayalon, O., Bender, J., Garen, A. 1999. Human single-chain Fv immunoconjugates targeted to a melanoma-associated chondroitin sulfate proteoglycan mediate specific lysis of human melanoma cells by natural killer cells and complement. Proc Natl Acad Sci U S A 96, 1627.