

# Abstract

The US Veterinary Immune Reagent Network (US-VIRN, [www.vetimm.org](http://www.vetimm.org)) aims to develop new tools for ruminants, swine, horse, poultry and aquaculture species to improve immunological research in infectious diseases and animal health, and to contribute to new vaccine development strategies and food safety. For cattle, several new reagents were developed including recombinant cytokines and chemokines and monoclonal antibodies (mAbs) to cytokines and cell surface molecules. Several key reagents were needed, such as CCR7, a chemokine receptor, to distinguish central memory cells and naive cells. Such a reagent is important in studying responses to vaccines. In addition, there was no reagent to identify alpha beta T cells or the subpopulations of gamma delta T cells by their expression of TCR gamma constant genes. Thus these have been targeted. Also there are a limited number of reagents to identify cytokines and essentially none to identify chemokines. Thus these were prioritized by survey of the bovine immunology research community for expression of bioactive proteins and subsequent production of mAb pairs to them. The community was surveyed and the currently commercially available reagents determined and is posted at [www.vetimm.org](http://www.vetimm.org). Other international groups were contacted to find out their priorities to avoid redundancies. In total, for cattle we cloned, sequenced and aligned the complete coding sequence of 25 genes for cytokines and chemokines including the signal sequence. Recombinant cytokines and chemokines were produced by Kingfisher Biotech in a yeast system. These can be obtained from Kingfisher ([www.kingfisherbiotech.com](http://www.kingfisherbiotech.com)). We cloned and sequenced 10 genes coding for cell surface molecules and transferred these to the Cornell lab for cell surface molecule expression in a mammalian expression system and these sequences are deposited in GenBank. MAb to cytokines and cell surface markers were produced at University of Massachusetts. For cell surface molecules, recombinant proteins were expressed in mammalian IgG or IL-4 fusion protein system and used for immunization of mice. Fully characterized mAbs with proven specificity to the native protein may be obtained from University of Massachusetts.

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## Cytokine and Chemokine Progress

Molecule	VIRN GenBank#	Expression in Yeast	Bioactivity Testing	Monoclonals Produced
CCL2	EU276 069	✓	Not needed	Tested at Cornell with α-swine CCL2 mAb, some+
CCL5	EU276 060	✓	Protein at Umass	Will target: Protein at Umass
CCL11	EU744 565	✓		
CXCL9	EU276 061	✓	YES (Umass) but variable results	
CXCL10	EU276 062	✓		
CXCL11	EU276 063	✓	YES (Umass) but less than R&D's	
IFN-γ	EU276 066	✓	YES (Umass) low for 1 lot and negative for others stored incorrectly	Will target: Protein at Umass
IFN-α	EU276 064	✓	YES (Umass) low activity-new lot expressed in 2009?	5 hybridomas reactive with recombinant protein
IFN-β	EU276 065	✓		
IL-1β	EU276 067	✓	low expresser, being retransformed and expressed	
IL-2	EU276 068	✓	YES on TF1 cells; YES (Umass) bovine PBMC	
IL-4	EU276 069	✓	YES on TF1 cells; YES (Umass) ConA-actd cells	Will target: Protein at Umass
IL-5	EU276 070	✓	low expresser, being retransformed and expressed	
IL-6	EU276 071	✓	NO (Umass) on B9 cells as reported by others for bovine; working out Q-RT-PCR for IL-23 expression	Will target: protein at Umass
IL-7	EU276 072	✓	3/17/09 low expresser, being retransformed and expressed	
IL-8	EU276 073	✓		Sheep mAb made by UK Toolkit cross-react with our bovine IL-8
IL-10	EU276 074	✓		
IL-12p35	EU276 075	✓		
IL-12p40	EU276 076	✓		
IL-13	EU276 077	✓	YES on TF1 cells; YES (Umass) increased MHC classII expression on bovine PBMC	Fusion done 2x's at Umass; no mAbs produced
IL-15	EU682 380	✓	YES (Umass) on bovine PBMC had some activity at 300ng/ml but less than for human IL-15	
IL-17	EU682 381	✓	YES (Umass) increased mRNA for IL6 and IL8 at 48 and 72 hr	Will target: protein at Umass & UK
IL-18	EU276 078	✓		
IL-23A	EU616 677	✓	One chain expressed so won't be tested in bioassay	Fused 5 weak mAbs in ELISA turned negative
TNF-α	EU276 079	✓		Will target: protein at Umass

## Cell Surface Molecule Progress

Molecule	GenBank#	Protein produced	mAb fusion status	mAb on recombinant protein
TCR δ		✓	Fusion performed	3 hybridomas
TCRγ-C3	BC149622	✓	mAb made (Umass)	3-5 stable hybridomas
TCR α	D10394	✓		
TCR β	D90139	✓		
IL-23R	EU616678			
IL-10R	BC123561	✓	Mice immunized	
CCR7	AY834253	✓	Fusion performed	7 candidates

# US Veterinary Immune Reagent Network - Progress report for Bovine Dec 2009

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([www.vetimm.org](http://www.vetimm.org))



## Examples of expressed gene sequences for cattle that we cloned, sequenced and aligned (All gene alignments may be found at [www.vetimm.org](http://www.vetimm.org))

**CCL5**

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Hs CCL5 NP_002976   MKVSAALAVLILATALCAPASA--SPYSSDTPCCFYAIRLPLRAHIEVFYTSKCKSNPAVVFTRKRGVCANPEKKVREINSLMS*
Mm CCL5 NP_038681   .I..I..T.I..T.A..T..P.....G.....L..L..L..V.....S.....L.....R.....Q.....Y.....*
Ec CCL5 AF506970    .P.S.A.V..F..L.G..G..T..T..A..N..H..D.D.G..R.M.A.I.G..G..L..S.....L.....D..Q.....T..M..F..Q.K.....A..*
Ec CCL5 VIRN ACB0217 .P.S.A.V..F..L.G..G..T..T..A..N..H..D.D.G..R.M.A.I.G..G..L..S.....L.....D..Q.....T..M..F..Q.K.....A..*
Bt CCL5 AJ007043    .T..F..L..M..A.....A.....S.....T..V..Q.....S.....M..A..I..K.....A.....L..*
Bt CCL5 VIRN ABX72058 .T..F..L..M..A.....A.....S.....T..V..Q.....S.....M..A..I..K.....A.....L..*
Bt CCL5 mature      .T..F..L..M..A.....A.....S.....T..V..Q.....S.....M..A..I..K.....A.....L..*
Ss CCL5 DQ372066    .T..T..F..L..M..A.....A.....S.....L..S.....L..Q.....S.....M..A..I..K.....A.....L..*
Ss CCL5 mature      .T..T..F..L..M..A.....A.....S.....L..S.....L..Q.....S.....M..A..I..K.....A.....L..*
    
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**CXCL10**

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Hs CXCL10 NP_027778   MNPQTALILCCLIFLFLSLGIGQ--VPLSRVRCVCCISISNQGVMNPLRSLKLELIIIPASQPCFRVRELIATMKKKGKRCCLNPEKSKAIKLLKAVSKERSKRP*
Mm CXCL10 NP_067249   .P.S.A.V..F..L.G..G..T..T..A..N..H..D.D.G..R.M.A.I.G..G..L..S.....L.....D..Q.....T..M..F..Q.K.....A..*
Mm CXCL10 P17515     .P.S.A.V..F..L.G..G..T..T..A..N..H..D.D.G..R.M.A.I.G..G..L..S.....L.....D..Q.....T..M..F..Q.K.....A..*
Ec CXCL10 VIRN ABZ91986 .P.S.A.V..F..L.G..G..T..T..A..N..H..D.D.G..R.M.A.I.G..G..L..S.....L.....D..Q.....T..M..F..Q.K.....A..*
Ec CXCL10 mature     .I..I..A..N..D..R..I..P.....M.....S..Q.....N.....N.....T..V.....I..Q.....I..Q.....R..T..L..R..E..V..*
Bt CXCL10 VIRN ABX72060 .K.S.G.F..F..L..Q..G.V.....N..T..S..E..G..S.....V.....S.....N.....N.....T.....I..N..Q..T.....R..T..R..K..E..A..*
Bt CXCL10 mature     .K.S.G.F..F..L..Q..G.V.....N..T..S..E..G..S.....V.....S.....N.....N.....T.....I..N..Q..T.....R..T..R..K..E..A..*
Bt CXCL10 BC112547    .K.S.G.F..F..L..Q..G.V.....N..T..S..E..G..S.....V.....S.....N.....N.....T.....I..N..Q..T.....R..T..R..K..E..A..*
Bt CXCL10 QZK1Q8     .K.S.G.F..F..L..Q..G.V.....N..T..S..E..G..S.....V.....S.....N.....N.....T.....I..N..Q..T.....R..T..R..K..E..A..*
Ss CXCL10 VIRN ABY66144 .S..V..F..L..L..T..G..I.....K..D..R.....M.....S..H.....N.....T.....I.....I.....R..T..O..R..E..A..*
Ss CXCL10 mature     .S..V..F..L..L..T..G..I.....K..D..R.....M.....S..H.....N.....T.....I.....I.....R..T..O..R..E..A..*
Ss CXCL10 AY789646    .S..V..F..L..L..T..G..I.....K..D..R.....M.....S..H.....N.....T.....I.....I.....R..T..O..R..E..A..*
Ss CXCL10 Q5S1S3     .S..V..F..L..L..T..G..I.....K..D..R.....M.....S..H.....N.....T.....I.....I.....R..T..O..R..E..A..*
    
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**IFN-α1**

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Hs IFNα1 NP_076918   MAFTEHSLDNRRTIMLILQMRKISPSICMORIDVSPQEEEDQWQKPAKISVLIHELIGQFFPLETTIDSS
Mm IFNα1 NP_034632   .R.I.C..F..A..A..Y..M..P.....Q..N..R..K..A..T..V..R..L..L..K..K.....K..V..A..Q..I..K..Q..P..S..T..L..I..S.....*
Bt IFNαA M10952     .P.M.S.F..L..L..N..A.I.....H..H..A..V.....Q..L..R..V.....Q..N..E..L..A..G..S..L..L..Q.....V..T..H..T..Q..S..E..G..P..*
Bt IFNαA VIRN ABX72062 .P.M.S.F..L..L..N..A.I.....H..H..A..V.....Q..L..R..V.....Q..N..E..L..A..G..S..L..L..Q.....V..T..H..T..Q..S..E..G..P..*
Bt IFNαA mature     .H..H..A..V.....Q..L..R..V.....Q..N..E..L..A..G..S..L..L..Q.....V..T..H..T..Q..S..E..G..P..*
Ec IFNα1 A33683     .L..V..S.....A.....H..I.....H..G..T..V.....G..R.....F..K..N.....V.....R..P..Q.....A..V..T.....H..S..D..G.....*
Ec IFNα1 VIRN ACD81649 .L..V..S.....A.....H..I.....H..G..T..V.....G..R.....F..K..N.....V.....R..P..Q.....A..V..T.....H..S..D..G.....*
Ec IFNα1 KF TH-278   .H..G..T..V.....G..R.....F..K..N.....V.....R..P..Q.....A..V..T.....H..S..D..G.....*
Ss IFNα DQ872654    .P.T.S..F..T..A..L..N..A.I.....Q.....A..H..T..A..R.....R.....F..D..H..R.....S..H..A..G.....V..Q..M..A..V..M..L..T..Q..S..E..G..A..*
Ss IFNα VIRN ABY66142 .P.T.S..F..T..A..L..N..A.I.....Q.....A..H..T..A..R.....R.....F..D..H..R.....S..H..A..G.....V..Q..M..A..V..M..L..T..Q..S..E..G..A..*
Ss IFNα mature     .P.T.S..F..T..A..L..N..A.I.....Q.....A..H..T..A..R.....R.....F..D..H..R.....S..H..A..G.....V..Q..M..A..V..M..L..T..Q..S..E..G..A..*
    
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**IL-13**

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Hs IL-13 NP_002179   MHPFLNPLLLALGLMALLTFLVIALTCLGGFASP--GFVPPSTA--LRELIIELVHITQNKAPLFCNGSMVSNLIPAGM-CYCAELSLINVSQCSA
Hs IL-13 Q4VB52     .A..L..W..V..T..A..V.....L.....A..P.....R..V..S..L..P..I..T..K.....S.....D..T.....V..D..A..G..F..V..D..T..I..N..N..*
Mm IL-13 NP_032381   .A..L..W..V..T..A..V.....L.....A..P.....R..V..S..L..P..I..T..K.....S.....D..T.....V..D..A..G..F..V..D..T..I..N..N..*
Mm IL-13 P20109     .A..L..W..V..T..A..V.....L.....A..P.....R..V..S..L..P..I..T..K.....S.....D..T.....V..D..A..G..F..V..D..T..I..N..N..*
Bt IL-13 VIRN ABX72075 .A.....T..A..V..I..L.....F..L..T.....S.....S..A.....A..K.....V.....L..S..S.....D.....S..I..N..V..*
Bt IL-13 mature     .A.....T..A..V..I..L.....F..L..T.....S.....S..A.....A..K.....V.....L..S..S.....D.....S..I..N..V..*
Bt IL-13 AF072807    .A.....T..A..V..I..L.....F..L..T.....S.....S..A.....A..K.....V.....L..S..S.....D.....S..I..N..V..*
Bt IL-13 Q9XSV9      .A.....T..A..V..I..L.....F..L..T.....S.....S..A.....A..K.....V.....L..S..S.....D.....S..I..N..V..*
Ss IL-13 VIRN ACD81654 .A..L..W..T..L.....F..L.....H..S..T.....A..K.....V.....T.....V.....T..S.....I..D.....*
Ss IL-13 mature     .A..L..W..T..L.....F..L.....H..S..T.....A..K.....V.....T.....V.....T..S.....I..D.....*
Ss IL-13 AF385625    .A..L..W..T..L.....F..L.....H..S..T.....A..K.....V.....T.....V.....T..S.....I..D.....*
Ss IL-13 Q95568      .A..L..W..T..L.....F..L.....H..S..T.....A..K.....V.....T.....V.....T..S.....I..D.....*
Ec IL-13 DQ889711    .NS.....S.....F.....L.....A..L..S..M.....A..K.....K.....V.....D..T.....R.....S.....T.....*
Ec IL-13 Wagner clone .A..L..W..T..L.....F..L.....A..L..S..M.....A..K.....K.....V.....D..T.....R.....S.....T.....*
    
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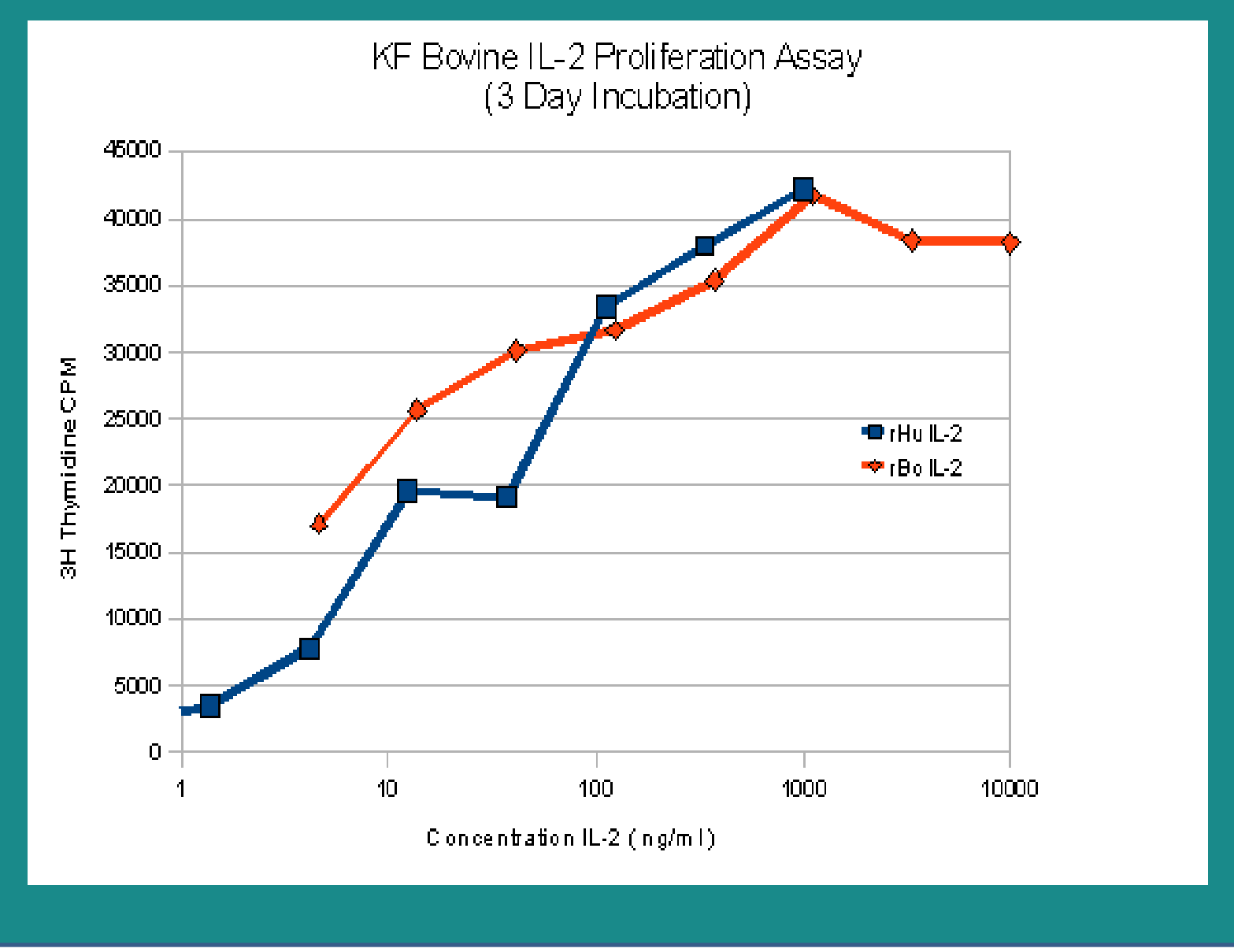
**IL-13**

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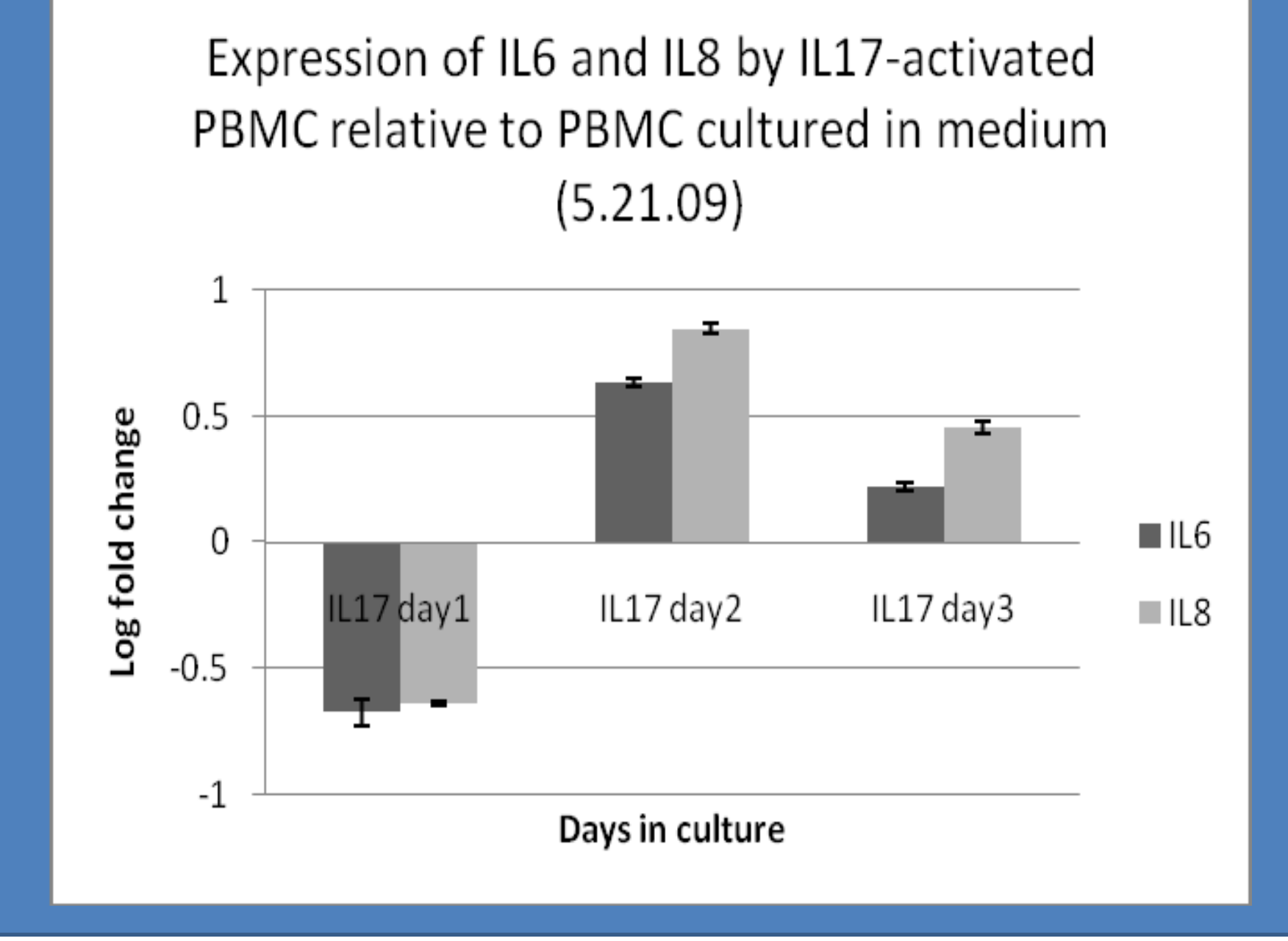
Hs IL-13 NP_002179   IEKTRMLSGFCPHKVSAGQFSSLHVDRDKIEVAGFVKDLLLKKLFRGQFN*
Hs IL-13 Q4VB52     .Y..R.....I..H..L..N..R..A..P..T..V..S..L..P.....H..I..T..K.....S..Y..T..Q.....H..P..*
Mm IL-13 NP_032381   .Y..R.....I..H..L..N..R..A..P..T..V..S..L..P.....H..I..T..K.....S..Y..T..Q.....H..P..*
Mm IL-13 P20109     .Y..R.....I..H..L..N..R..A..P..T..V..S..L..P.....H..I..T..K.....S..Y..T..Q.....H..P..*
Bt IL-13 VIRN ABX72075 .Q..R..K.....N..A..L.....P..K..V..E..Y.....L.....R..S..R..I..V.....N..E..R.....*
Bt IL-13 mature     .Q..R..K.....N..A..L.....P..K..V..E..Y.....L.....R..S..R..I..V.....N..E..R.....*
Bt IL-13 AF072807    .Q..R..K.....N..A..L.....P..K..V..E..Y.....L.....R..S..R..I..V.....N..E..R.....*
Bt IL-13 Q9XSV9      .Q.....A..L..S.....P..P..S..E..V..P..K..I.....K..R..M..I.....H..*
Ss IL-13 mature     .Q.....A..L..S.....P..P..S..E..V..P..K..I.....K..R..M..I.....H..*
Ss IL-13 AF385625    .Q.....A..L..S.....P..P..S..E..V..P..K..I.....K..R..M..I.....H..*
Ss IL-13 Q95568      .Q.....A..L..S.....P..P..S..E..V..P..K..I.....K..R..M..I.....H..*
Ec IL-13 DQ889711    .Q..N..R..K.....T..K..L.....Q..L.....V..E..R..A.....E.....*
Ec IL-13 Wagner clone .Q..N..R..K.....T..K..L.....Q..L.....V..E..R..A.....I..V..L.....K..N..R..I..H..G..K..H.....V..D..A..*
    
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## Examples of Bioassay Results

Bovine IL-2. Cultures of bovine total PBMC were incubated for three days with the different concentrations of recombinant bovine IL-2 (rBo IL-2). Negative control cultures received no treatment, and positive cultures received recombinant human IL-2 (rHu IL-2). Cells were pulsed with tritium for 18 hours and harvested, and radioactivity was measured by scintillation counting. Bovine IL-2 treatment resulted in proliferation in a manner similar to the human IL-2 control.



Bovine IL-17. Effect of KF boIL-17 was evaluated by setting up cultures of bovine total PBMC with 300 ng/ml boIL-17 with three time points (Day 1, Day 2 and Day3). Negative controls included equivalent cells set up with the same time points and cultured in medium. Cells were collected and RNA was isolated for evaluation of IL-6 and IL-8 (both induced by IL-17) via quantitative PCR. Increased expression of both was observed for Day 2 and Day3.

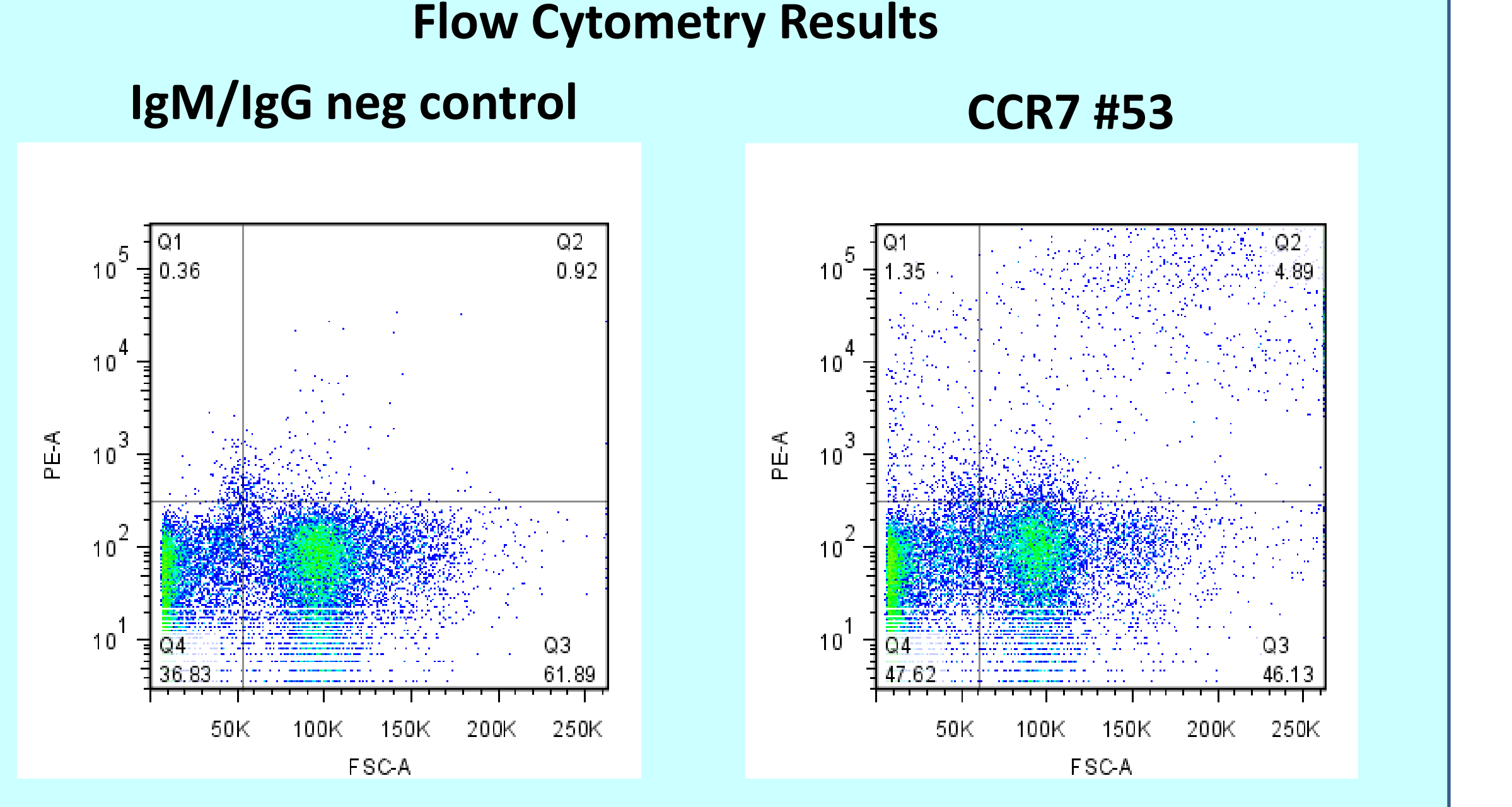


## Example Monoclonal antibody Results Bovine CCR7

The extracellular domain of bovine CCR7 was expressed in CHO cells as a fusion protein with equine IgG4 by Dr. Bettina Wagner at Cornell. The fusion protein was treated with enterokinase and purified. Mice were immunized with 20mg of the purified protein, boosted with 10mg 2 and 3 weeks after immunization, then boosted 3 successive days with 10mg. Splenocytes were collected and fused with X63-Ag.653 cells on the day after the final boost. After screening for ELISA-positive clones, bovine PBMC was subject to flow cytometric analysis of indirect immunofluorescence using a secondary Ab that reacts with all mouse IgG subclasses and IgM. According to the analysis, two of the 9 ELISA-positive clones yielded signal on flow (clone #53 is shown here).

### ELISA Results

Clone #	Antigen	
	CCR7	BSA
4	0.104	0.088
7	0.186	0.08
20	0.211	0.093
36	1.416	0.109
44	1.113	0.118
53	0.262	0.092
53	0.302	0.096
79	0.111	0.089



## Publications

Chen, C., C.T.A.H. Herzig and C.L. Baldwin. Expressed gene sequence of bovine IL-23p19 and IL-23 receptor. *Vet Immunol Immunopathol* 128:425-430.

Entrican, G., J.K. Lunney, V.P. Rutten, and C.L. Baldwin. (2009) A Current Perspective on Availability of Tools, Resources and Networks for Veterinary Immunology (A workshop report). *Vet Immunol Immunopathol* 128:24-29.

Hudgens, E., D. Tompkins, P. Boyd, M. Wysocki, J. Lunney, D. Horohov, and C.L. Baldwin. Expressed gene sequence of the IFNγ-response chemokine CXCL9 of cattle, horses, and swine, submitted.

Hudgens, E., D. Tompkins, P. Boyd, M. Wysocki, J. Lunney, and C.L. Baldwin. Expressed gene sequence of the IFNγ-response chemokine CXCL11 of cattle and swine, submitted.

Tompkins, D., E. Hudgens, D. Horohov, and C.L. Baldwin. Expressed gene sequences of the equine cytokines interleukin-17 and interleukin-23 p19. *Vet Immunol Immunopathol*, in press.

Tompkins, D., E. Hudgens, C. Steffens, L. Kakach, J. Labresh, and C.L. Baldwin. Expressed gene sequences of the bovine and swine cytokine Interleukin-13. *Vet Immunol Immunopathol*, revision submitted.

Tompkins, D., E. Hudgens, D. Horohov, P. Boyd, J. Lunney and C.L. Baldwin. Expressed gene sequences of the bovine and equine IFNγ-response chemokine CXCL10, *Veterinary Immunology and Immunopathology*, in revision.