

**PROGRESS FOR REAGENT DEVELOPMENT:
Gene cloning and expression, bioactivity of expressed molecules and mAb production**
(Updated 9-25-08)

CHEMOKINES & CYTOKINES1

CATFISH.....1

CATTLE.....2

CHICKEN.....3

EQUINE.....4

SWINE.....5

TROUT.....6

CELL SURFACE MOLECULES FOR MONOCLONAL ANTIBODIES.....7

CATFISH.....7

CATTLE.....7

CHICKEN.....8

EQUINE.....8

SWINE.....9

TROUT.....9

CHEMOKINES & CYTOKINES

CATFISH

Gene	WHOLE CODING SEQUENCE INCLUDING SIGNAL or PROPEPTIDE				PROGRESS FOR EXPRESSION OF MATURE PROTEIN			
	Clone #	FASTA File on website	US-VIRN GenBank #	Clone available	Stage	Transformed in expression vector	Expressed in yeast	Bioactivity affirmed
IFN-γ with signal sequence	√	√	DQ124250	UMiss labs	KF primers made			
IFN-γ without signal sequence	√	no	Not applicable	UMiss labs	KF primers made			
Type I IFN	√	√	AY847295	UMiss labs				

CATTLE

Gene	WHOLE CODING SEQUENCE INCLUDING SIGNAL or PROPEPTIDE				PROGRESS FOR EXPRESSION OF MATURE PROTEIN				
	Clone #	FASTA file on website	US-VIRN GenBank #	Clone available	Clone # without s.s.	Stage	Transformed in expression vector	Expressed in yeast	Bioactivity affirmed
CCL2	TH-33	√	EU276069	√	TH-80	Sent to KF	√		
CCL5	TH-21	√	EU276060	√	TH-76	Sent to KF	√		
CCL11	TH-333	√	EU744565	√	TH-172	Sent to KF	√		
CXCL9	TH-6	√	EU276061	√	TH-152	Sent to KF	√	√	
CXCL10	TH-13	√	EU276062	√	TH-88	Sent to KF	√		
CXCL11	TH-11	√	EU276063	√	TH-101	Sent to KF	√	√	YES
IFN- γ	TH-30	√	EU276066	√	TH-72	Sent to KF	√	√	in progress
IFN- α	TH-1	√	EU276064	√	TH-148	Sent to KF	√	√	in progress
IFN- β	TH-113	√	EU276065	√	TH-141	Sent to KF	√		
IL-1 β	TH-94	√	EU276067	√	TH-94	Sent to KF	√	√	
IL-2	TH-36	√	EU276068	√	TH-129	Sent to KF	√		
IL-4	TH-132	√	EU276069	√	TH-132	Sent to KF	√	√	YES
IL-5	TH-185	√	EU276070	√	TH-177	Sent to KF	√		
IL-6	TH-135	√	EU276071	√	TH-135	Sent to KF	√	√	
IL-7	TH-114	√	EU276072	√	TH-114	Sent to KF	√		
IL-8	TH-18	√	EU276073	√	TH-84	Sent to KF	√		
IL-10	TH-104	√	EU276074	√	TH-160	Sent to KF	√		
IL-12p35	TH-180	√	EU276075	√	TH-165	Sent to KF	√		
IL-12p40	TH-43	√	EU276076	√	TH-139	Sent to KF	√		
IL-13	TH-90	√	EU276077	√	TH-90	Sent to KF	√	√	YES
IL-15	VML94	√	EU682380	√	TH-348	Sent to KF	√	√	
IL-17	VLM96	√	EU682381	√	TH-121	Sent to KF	√	√	
IL-18	TH-118	√	EU276078	√	TH-118	Sent to KF	√	√	
IL-23	CC13	√	EU616677		TH-126	Sent to KF	√		
TNF- α	TH-96	√	EU276079	√	TH-96	Sent to KF	√		

CHICKEN

Gene	WHOLE CODING SEQUENCE INCLUDING SIGNAL or PROPEPTIDE				PROGRESS FOR EXPRESSION OF MATURE PROTEIN				
	Lillehoj Clone #	US-VIRN GenBank #	Clone available	FASTA file on website	Clone # without s.s.	Stage	Transformed in expression vector	Expressed in yeast	Bioactivity affirmed
CCL4	H112	NM001030360	Lillehoj lab	√	DT-362	Sent to KF	√		
CCL20	H113	NM204438	Lillehoj lab	√	DT-358	Sent to KF	√		
IFN-γ	H029	AH009942	Lillehoj lab	√	TH-264	Sent to KF	√		
IL-1β	H242	Y15006	Lillehoj lab	√	TH-259	Sent to KF	√		
IL-2	H030	AF017645	Lillehoj lab	√	TH-265	Sent to KF			
IL-4	H107	NM 001007079	Lillehoj lab	√	DT-352	Sent to KF	√		
IL-10	H108	NM 001004414	Lillehoj lab	√	RN-034	Sent to KF	√	√	YES
IL-12p35	H109	NM213588	Lillehoj lab	√	DT-650	Sent to KF			
IL-12p40	H110	Ay262752	Lillehoj lab	√	DT-654	Sent to KF			
IL-15	H238	NM 204571	Lillehoj lab	√	DT-363	Sent to KF	√		
IL-16	H033	AJ508678	Lillehoj lab	√	DT-606	Sent to KF	√	√	YES
IL-17	H032	AJ493595	Lillehoj lab	√	TH-262	Sent to KF			
IL-17D	H008	Ef570583	Lillehoj lab	√	DT-660	Sent to KF			
IL-18	H020	AJ277865	Lillehoj lab	√	DT-325	Sent to KF	√		
LITAF	H103	AY765397	Lillehoj lab	√	TH-265	Sent to KF	√		
Lymphotactin	H026	AF006742	Lillehoj lab	√	DT-341	Sent to KF	√		
MIF	H013	M95776	Lillehoj lab	√	DT-665	Sent to KF	√		
TNFSF15 (TL1A)	H105	NM001024578	Lillehoj lab	√	TH-269	Sent to KF	√		

EQUINE

Gene	WHOLE CODING SEQUENCE INCLUDING SIGNAL or PROPEPTIDE				PROGRESS FOR EXPRESSION OF MATURE PROTEIN				
	clone #	FASTA file on website	US-VIRN GenBank #	Clone available	Clone # without s.s.	Stage	Transformed in expression vector	Expressed in yeast	Bioactivity affirmed
CCL2	TH-295	√	EU438774	√	TH-281	Sent to KF	√		
CCL3	TH-337	√	EU438775	√	TH-343	Sent to KF	√		
CCL5	TH-361	√	EU744564	√	n.d.	Sent to KF to finish			
CCL11	TH-419	√	In progress	√		Sent of KF			
CXCL9	TH-351	√	EU438776	√	TH-341	Sent to KF			
CXCL10	TH-345	√	EU438777	√	TH-357	Sent to KF	√		
GM-CSF	TH308	√	EU438778	√	RN-027	Sent to KF	√		
IFN-γ	Wagner BW143	√	U04050	√	TH-201	Sent to KF	√	√	
IFN-α1	TH-382	√	EU682378	√	TH-278	Sent to KF	√	√	
IL-1β	DT-111	√	EU438767	√	RN-003	Sent to KF	√		
IL-2	DT-113	√	EU438768	√	RN-014	Sent to KF	√	√	YES
IL-4	DT-117	√	EU438769	√	RN-005	Sent to KF	√	√	YES
IL-5	Horohov lab	√	U91947						
IL-6	DT-122	√	EU438770	√	RN-016	Sent to KF	√	√	
IL-10	DT-126	√	EU438771	√					
IL-12 p35/p40	Dr. Mealey, WSU	no	Not needed	no		Being sent directly			
IL-13	Wagner BW133	√	EF645663	√					
IL-15	TH-379	√	EU682379	√	RN-029	Sent to KF	√	√	
IL-17	TH-416	√	EU744563-no s.s.	√	RN-020	Sent to KF	√	√	
IL-18	DT-001	√	EU438772	√	RN-011	Sent to KF	√	√	
IL-23	TH-287	√	EU438773	√	TH-279	Sent to KF	√	√	
TGF-β	Wagner BW106	√	X99438	√	TH-273	Sent to KF	√		
TNF-α	TH322	√	EU438779	√	TH-285	Sent to KF	√	√	

SWINE

Gene	WHOLE CODING SEQUENCE INCLUDING SIGNAL or PROPEPTIDE				PROGRESS FOR EXPRESSION OF MATURE PROTEIN				
	Clone #	FASTA file on website	US-VIRN GenBank #	Clone available	Clone # without s.s.	Stage	Transformed in expression vector	Expressed in yeast	Bioactivity affirmed
CCL2	DT-304	√	EU682382	√	DT-616	Sent to KF	√	√	
CCL3L1	DT-401	√	EU364893	√	DT-639	Sent to KF	√		
CCL4	TH-409	√	EU364894	√		Sent to KF to finish			
CCL5	DT-515	√	EU44561 – no s.s.	√	DT-515	Sent to KF	√	√	
CXCL9	TH-400	√	EU36897	√	DT421	Sent to KF	√		
CXCL10	DT-308	√	EU364898	√	TH-249	Sent to KF	√	√	In progress
CXCL11	TH-404	√	EU682377	√	DT-432	Sent to KF	√	√	YES
IL-7	DT-300	√	EU364895	√	DT-413	Sent to KF	√	√	
IL-13	DT-321	√	EU682383	√	TH-253	Sent to KF	√	√	In progress
IL-15	Zarlenga	√	NM 214390	n.a.	DT-228	Sent to KF	√	√	
IFN- α	DT-316	√	EU364896	√	DT-440	Sent to KF	√	√	
IFN- β 1	DT-627		EU744562 – no s.s.		DT-627	Sent to KF	√	√	
TNF- α	DT-211	√	EU682384	√	DT-624	Sent to KF	√	√	

TROUT

Gene	WHOLE CODING SEQUENCE INCLUDING SIGNAL or PROPEPTIDE				PROGRESS OF EXPRESSING MATURE PROTEIN			
	Clone #	FASTA file on website	US-VIRN GenBank #	Clone available	Stage	Transformed in expression vector	Expressed in yeast	Bioactivity affirmed
IFN1	John Hansen cloned	√	AM489418	Contact USGS	KF primer design	√		
IFN2	John Hansen cloned	√	AJ582754	Contact USGS	KF primer design	√		
IFN3	John Hansen cloned	√	AM235738	Contact USGS	KF primer design	√		
IFN γ	John Hansen cloned	√	AJ616215	Contact USGS	KF primer design	√		

CELL SURFACE MOLECULES FOR MONOCLONAL ANTIBODIES

CATFISH

Molecule	Full length cDNA with signal sequence	GenBank	Gene cloned into expression system	Stable transfectant	Protein being produced/purified	Monoclonal antibody status
TCR α	√ (C-region only)	U58505	Yes	Yes	Yes	Positive titers in mice
TCR β	√ (C-region only)	U39193	Yes	Yes	Yes	
TCR δ	√ (C-region only)		Yes	Yes		
TCR γ	√ (C-region only)	DQ435303	Yes	Yes	Yes	Positive titers in mice- reacts with catfish cells
IgD	√	U67437	Yes*	Yes*	Yes*	Made mAbs but only IgMs were obtained*
CD4-1	√	DQ435301				
CD4-2	√	DQ435302				
FcRI	√	DQ286290				
LITR2	√	AY885644				
NITR10	√	AF397463				

*Indicates made in Catfish species lab at UMiss Medical Center

CATTLE

Molecule	Full length cDNA with signal sequence	GenBank	Gene cloned into expression system	Stable transfectant	Protein being produced/purified	Monoclonal antibody status
TCR δ	√	BC142411	Yes	Yes	Yes	
TCR γ C3	√	BC149622	Yes	Yes	Yes	Positive titers in mice
TCR γ C5	√	AY735449				
TCR α	√	D10394				
TCR β	√	D90139				
IL-23R	√	EU616678	Yes	Yes		
IL-10R	√	BC123561	Yes	Yes		
CCR7	√	AY834253	Yes	Yes		Made by BBSRC Toolkit

CHICKEN

Molecule	Full length cDNA with signal sequence	GenBank	Gene cloned into expression system	Stable transfectant	Protein being produced/purified	Monoclonal antibody status
IL-2R α	√	NM_204596	Yes	Yes		
IL-21R	Primers made	NM_001030640				
CXCR4	√	NM_989948	Yes	Yes		
CD80	Lillehoj – in progress	NM_001079739	Yes			
CD83 or CD86	Lillehoj – in progress	XM_418929	Yes			
CD86	Lillehoj	NM_001037839	Yes			
CTLA4	√		Yes*	Yes*		

EQUINE

Molecule	Full length cDNA with signal sequence	GenBank	Gene cloned into expression system	Stable transfectant	Protein being produced/purified	Monoclonal antibody status
CD40	√		Yes	Yes	Yes	Fusion ongoing*
CD23	√		Yes	Yes	Yes	
CD25 (IL-2R α)	√		Yes	Yes	Yes	Mabs being characterized
CD28	√		Yes	Yes	Yes	Mabs being characterized
Fc ϵ R1 α	√		Yes	Yes	Yes	Fusion ongoing
TCR α	√ (C-region)		Yes			
TCR β						
TCR γ	√ (C-region)					
TCR δ	√ (C-region)					
IgD	√		Yes			
IgG2	√					
IgG3	√					
IgG6	√		Yes	Yes	Yes	Made mAb*
CD16	√					
<i>Tbd</i>						
<i>Tbd</i>						
<i>Tbd</i>						

SWINE

Molecule	Full length cDNA with signal sequence	GenBank	Gene cloned into expression system	Stable transfectant	Protein being produced/purified	Monoclonal antibody status
TCR γ	√	EU364901				
TCR α	√	EU364899	Yes	Yes	Yes	mAb made
TCR β	√	EU364900	Yes	Yes	Yes	Fusion to be repeated
IL-4R α	√	AY266143	Yes	Yes	Yes	mAb being characterized
IL-13R α 1	√	AY266142	Yes	Yes	Yes	
CD45RO	Not needed		Not needed	peptide	Not needed	Mice immunized
IGSF2						
IL-7R						
CCR7						
CXCR3			Pending at USDA-ARS			

TROUT

Molecule	Full length cDNA with signal sequence	GenBank	Gene cloned into expression system	Stable transfectant	Protein being produced/purified	Monoclonal antibody status
Blimp	√		Yes*	Yes*	Yes*	Made mAbs*
CD3g/d	√	CA357253	Yes*	Yes*	Yes*	
CD4	√	AY973028	Yes*	Yes*	In progress	
CD4-REL	√	AY973029	Yes*	Yes*	In progress	
CD8	√	AF178054	Yes*	Yes*	Yes*	
CD28	√	AY789435	Yes*	Yes*		
CD79A	Partial, missing stop	CA369371	Pending*			
CD79B	Partial, missing stop	CA378285	Pending*			
CD83	√	AY263796	Pending at Cornell			
CTLA4	√	AY789436	Yes*	Yes*		
IgD	√	AY870261				
IgT	√	AY870265				
MHC IA (UBA)	√	AF11519				
MHC IIB	√	AF115533				
p56LCK	√	AY973032	Yes*	Yes*	Yes*	Made mAbs*
Pax5	√	EU147491	Yes*	Yes*	Yes*	Made mAbs*
TCR α	√	EU072698	Yes			
TCR β	√	EU072699	Yes			
TCR γ	√	EU072700	Yes			

*Indicates made in “Trout species lab” at USGS

MOLECULES FOR MONOCLONAL ANTIBODY PRODUCTION

- The cell surface molecules being expressed at Cornell in the Wagner Lab (see table above) will all be used for producing monoclonal antibodies
- In addition, there are plans to make monoclonal antibody against about 30-40 cytokines or chemokines for different species as noted in the Tables above once they have shown bioactivity
- Some species labs are producing monoclonal antibody against molecules in their own labs as well.

Grant goal is 12 monoclonal antibodies per species	SPECIES					
	HORSE	BOVINE	CHICKEN	SWINE	CATFISH	TROUT
#1	CD16	TCR α	IL-2R α	TCR α	TCR α	TCR α
#2	CD23	TCR β	IL-21R	TCR β	TCR β	TCR β
#3	CD28	TCR γ	CXCR4	CD45RO	TCR δ	TCR γ
#4	CD40	TCR δ	CD80	IL-4R α	TCR γ	IFN γ
#5	TCR α	IL-23R	CD83 or CD86	IL-13R α 1	IgD	IFN α
#6	TCR β	IL-10R	IL-12 p40	CXCL10	IFN γ	<i>Tbd</i>
#7	TCR γ	CCR7	IL-12 p35	CCL5	<i>Tbd**</i>	<i>Tbd</i>
#8	TCR δ	IL-6	IL-17	CCL2	<i>Tbd</i>	<i>Tbd</i>
#9	IL-5	IL-13	IL-17D	CXCL11	<i>Tbd</i>	<i>Tbd</i>
#10	IL-8	IL-17	IL-16	IL15	<i>Tbd</i>	<i>Tbd</i>
#11	IL-12	IL-23	IL-10	IL13	<i>Tbd</i>	<i>Tbd</i>
#12	IL-13	IFN α	IL-4	IL7	<i>tbd</i>	<i>tbd</i>
Additional monoclonal antibodies beyond minimum proposed						
#13	IgG6			IFN α	IgD	P56 lck
#14	CD25			TNF α		Pax5
#15	Fc ϵ R1 α			IFN β 1		Blimp
#16	IgD			CCL4		CD8
#17	IgG2			CCL3L1		CD28
#18	IgG3			IGSF2		CTLA4
#19				IL-7R		CD3
#20				CCR7		CD4
#21				CXCR3		CD4-REL

*Cyto/chemokine: cytokines or chemokines; order of priority for antibody production is dependent on Pichia expression and bioactivity.

***Tbd*: To be determined