

Article title: Hematopoiesis In The Equine Fetal Liver Suggests Immune Preparedness

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Supplemental Table 1. RT-PCR primer sequences for the hematopoietic genes studied.

Gene	GenBank ID	Forward (5'-3')	Reverse (5'-3')	Product (bp)
CD34	XM_001491596	CTAGGGTGTGCTCCTTGCTC	GACCAGTGCAATCAGGGTCT	209
CEBPA	NA <sup>a</sup>	TGGACAAGAACAGCAACGAG	GCGGCTCAGTTGTTCCAC	167
CXCL12	XM_001489644	CAGCCTGAGCTACAGATGTCC	CCTTTTCTGAGCAGCCTTTC	280
GATA1	XM_005614118	TGTTTCCAGCAGTGCCTATG	CCTGTTCTGCCATTTCATCT	247
IL7	CX592622	AAAGACGGCAAAGAATATCGA	ACAGAGGTTCTTATTATCATCAC	142
IL7R	CD469725	ACATGCCTGCTCTGGTCTCT	TCACGTGCATCCAATCATTT	580
IGHM	NW_001876796	CTTCACTACGGAAGAGGTGC	ACTCAGGCTGTCATAGGTGC	295
IRF8	XM_001502568	ACGTGGTGGTCAAGGTCTTC	AGCTCTTCCCAGTCTTCTCC	207
KIT	XM_001492243	AGACCTGGAAGACCTGCTCA	CATTCGGAAACCTTCCTTGA	376
NOTCH1	XM_001498582	TCCTTCTGACCTGGATGAC	CCATGTTGTCCTGGATGTTG	393
PAX5	XM_001504306	CATCAAGCCTGGGGTAATTG	CACGGTGTTCATTGTCACACA	157
PU.1	JN979560	GAGACCATCCAGCTCCAGAC	CAGCTCGGTGAAGTGGTTCT	315
β-Actin	NM_001081838.1	TCCCTGGAGAAGAGCTACGA	GTGGACAATGAGGCCACAAT	350

<sup>a</sup>The forward primer was designed based on the consensus sequence for the alignment of human (NM\_004364), cow (NM\_176784), mouse (NM\_007678), and pig (XM\_003127015) sequences, while the reverse primer was designed from the horse sequence NW\_001867363.

Supplemental Table 2. Immunoglobulin heavy chain germline segment use, nucleotide identity with reference genome sequence, and junctional nucleotides

Sequence	IGHV		VD	IGHD		DJ	IGHJ		
	Gene	Identity <sup>a</sup>	Junction <sup>b</sup>	Gene	Identity <sup>a</sup>	Junction <sup>b</sup>	Gene	Identity <sup>a</sup>	
Fetal Liver (n = 30)									
Fetus 1	IGVDJ1	IGHV2S2	100.0	13	IGHD7S1	100	2	IGHJ1S3	100
	IGVDJ2	IGHV2S3	99.1	7	IGHD15S2	100	3 <sup>c</sup>	IGHJ1S2	100
	IGVDJ3	IGHV2S3	98.8	14	IGHD18S1	84.2	0	IGHJ1S2	100
	IGVDJ4	IGHV2S3	97.4	35	IGHD15S2	100	0	IGHJ1S3	100
	IGVDJ5	IGHV2S3	98.8	19	IGHD10S1	84.6	3 <sup>c</sup>	IGHJ1S3	100
	IGVDJ6	IGHV2S3	97.4	10	IGHD18S1	92.3	9	IGHJ1S3	100
	IGVDJ7	IGHV2S3	97.7	4 <sup>c</sup>	IGHD18S1	84.2	0	IGHJ1S5	100
	IGVDJ8	IGHV2S3	98.0	7	IGHD20S2	84.6	0	IGHJ1S5	100
	IGVDJ9	IGHV2S4	98.8	2	IGHD18S1	100	0	IGHJ1S2	100
	IGVDJ10	IGHV2S4	99.4	9	IGHD20S2	80	0	IGHJ1S5	100
Fetus 2	IGVDJ11	IGHV2S2	99.7	1	IGHD17S1/S2	100	0	IGHJ1S5	94.3
	IGVDJ12	IGHV2S2	100.0	3	IGHD17S1/S2	100	0	IGHJ1S5	100
	IGVDJ13	IGHV2S3	99.4	0	IGHD13S1	100	0	IGHJ1S2	100
	IGVDJ14	IGHV2S3	98.8	5	IGHD9S1	100	2	IGHJ1S3	96.6
	IGVDJ15	IGHV2S3	98.5	7	IGHD15S2	100	0	IGHJ1S5	96.8
	IGVDJ16	IGHV2S3	98.8	3	IGHD17S1/S2	91.7	0	IGHJ1S5	96.8
	IGVDJ17	IGHV2S3	99.4	0	IGHD10S1	100	0	IGHJ1S5	94.1
	IGVDJ18	IGHV2S3	99.4	ND <sup>c</sup>	ND	ND	ND	IGHJ1S5	96.7
	IGVDJ19	IGHV2S3	99.4	0	IGHD17S1/S2	100	0	IGHJ1S5	96.8
	IGVDJ20	IGHV2S3	97.7	16	IGHD15S2	92.3	0	IGHJ1S5	96.2
Fetus 3	IGVDJ21	IGHV2S3	97.4	6	IGHD18S1	84.2	2	IGHJ1S3	100
	IGVDJ22	IGHV2S3	96.8	6	IGHD17S1/S2	100	0	IGHJ1S3	93.1
	IGVDJ23	IGHV2S3	97.7	7	IGHD9S1	72	3	IGHJ1S3	100
	IGVDJ24	IGHV2S3	98.6	33 <sup>c</sup>	IGHD10S1	100	0	IGHJ1S5	97.1
	IGVDJ25	IGHV2S3	98.5	17	IGHD10S1	100	0	IGHJ1S5	97.1
	IGVDJ26	IGHV2S3	97.7	13	IGHD18S1	100	8	IGHJ1S5	100
	IGVDJ27	IGHV2S3	98.8	4	IGHD18S1	90	1	IGHJ1S5	100
	IGVDJ28	IGHV2S3	97.7	8 <sup>c</sup>	IGHD24S1	100	5	IGHJ1S5	100

	IGVDJ29	IGHV2S3	98.6	1	IGHD17S1/S2	100	1	IGHJ1S5	100	
	IGVDJ30	IGHV2S4	98.8	3	IGHD20S1	100	5	IGHJ1S5	100	
Fetal bone Marrow (n = 33)										
Fetus 1	IGVDJ31	IGHV2S2	100.0	0	IGHD18S1	66.7	0	IGHJ1S6	97.4	
	IGVDJ32	IGHV2S3	97.7	2	IGHD15S2	91.3	0	IGHJ1S2	100	
	IGVDJ33	IGHV2S3	97.1	4	IGHD17S1/S2	100	0	IGHJ1S3	95.7	
	IGVDJ34	IGHV2S3	98.8	15	IGHD7S1	100	0	IGHJ1S3	100	
	IGVDJ35	IGHV2S3	97.4	9	IGHD24S1	100	3	IGHJ1S3	100	
	IGVDJ36	IGHV2S3	96.8	8	IGHD15S1	100	1 <sup>c</sup>	IGHJ1S5	100	
	IGVDJ37	IGHV2S3	97.4	ND <sup>c</sup>	ND	ND	ND	IGHJ1S5	100	
	IGVDJ38	IGHV2S3	96.8	16	IGHD17S1/S2	100	1	IGHJ1S5	100	
	IGVDJ39	IGHV2S3	98.8	9	IGHD18S1	100	0	IGHJ1S5	100	
	IGVDJ40	IGHV2S4	98.8	1	IGHD17S1/S2	93.8	0	IGHJ1S3	100	
Fetus 2	IGVDJ41	IGHV2S4	99.4	15	IGHD18S1	92.3	0	IGHJ1S5	100	
	IGVDJ42	IGHV2S2	99.4	5	IGHD18S1	84.2	0	IGHJ1S3	100	
	IGVDJ43	IGHV2S3	98.8	5	IGHD9S1	100	0	IGHJ1S3	96.6	
	IGVDJ44	IGHV2S3	98.8	5	IGHD7S2	95.7	0	IGHJ1S5	97	
	IGVDJ45	IGHV2S3	98.8	3	IGHD20S2	83.3	0	IGHJ1S7	100	
	IGVDJ46	IGHV1S3	99.7	1	IGHD18S1	100	0	IGHJ1S3	96.3	
	IGVDJ47	IGHV4S2	99.7	1	IGHD17S1/S2	100	0	IGHJ1S3	100	
	IGVDJ48	IGHV4S2	99.7	0	IGHD17S1/S2	100	0	IGHJ1S5	96.8	
	IGVDJ49	IGHV4S5	99.7	1	IGHD17S1/S2	100	0	IGHJ1S3	96	
	IGVDJ50	IGHV4S5	98.6	0	IGHD15S2	100	0	IGHJ1S5	100	
Fetus 3	IGVDJ51	IGHV4S5	98.6	3	IGHD7S2	100	0	IGHJ1S5	100	
	IGVDJ52	IGHV2S2	100.0	3 <sup>c</sup>	IGHD11S1	100	2	IGHJ1S5	100	
	IGVDJ53	IGHV2S3	97.7	10	IGHD18S1	100	4	IGHJ1S2	100	
	IGVDJ54	IGHV2S3	99.1	1 <sup>c</sup>	IGHD18S1	85	5	IGHJ1S3	96.4	
	IGVDJ55	IGHV2S3	98.8	10	IGHD17S1/S2	100	2 <sup>c</sup>	IGHJ1S3	100	
	IGVDJ56	IGHV2S3	97.4	13	IGHD22S1	100	0	IGHJ1S5	100	
	IGVDJ57	IGHV2S3	97.7	12	IGHD22S1	100	3	IGHJ1S5	100	
	IGVDJ58	IGHV2S3	96.8	6	IGHD17S1/S2	95	0	IGHJ1S5	100	
	IGVDJ59	IGHV2S3	98.5	17	IGHD18S1	85	2	IGHJ1S5	100	
	IGVDJ60	IGHV2S3	97.4	9	IGHD4S1	100	8	IGHJ1S5	97.1	
Adult Horse Bone Marrow (n = 32)	IGVDJ61	IGHV2S3	97.4	9	IGHD22S1	100	4	IGHJ1S5	100	
	IGVDJ62	IGHV4S2	98.6	5	IGHD18S1	100	0	IGHJ1S3	93.1	
	IGVDJ63	IGHV2S4	98.8	4	IGHD4S1	100	14	IGHJ1S4	91.7	
	Adult A	IGVDJ64	IGHV2S2	94.8	19	IGHD4S1	100	6	IGHJ1S5	96
		IGVDJ65	IGHV2S2	89.4	ND	ND	ND	ND	IGHJ1S5	82.8
		IGVDJ66	IGHV2S2	82.9	0	IGHD5S6 <sup>d</sup>	100	0	IGHJ1S7	77.8
		IGVDJ67	IGHV2S3	88.5	0	IGHD15S2	100	7	IGHJ1S4	95.2
		IGVDJ68	IGHV2S3	90.7	33	IGHD26S1	87.5	2	IGHJ1S5	94.4
		IGVDJ69	IGHV2S3	92.4	14	IGHD1S1	81.8	7	IGHJ1S5	86.1
		IGVDJ70	IGHV2S3	88.7	5	IGHD5S5	100	50	IGHJ1S5	90
IGVDJ71		IGHV2S3	89.0	5	IGHD18S1	95	19	IGHJ1S5	94.4	
IGVDJ72		IGHV2S3	95.4	16 <sup>c</sup>	IGHD15S2	90.9	9	IGHJ1S7	90.9	
IGVDJ73		IGHV2S3	93.3	ND	ND	ND	ND	IGHJ1S7	88.9	
Adult B	IGVDJ74	IGHV2S4	86.3	ND	ND	ND	ND	IGHJ1S5	81.5	
	IGVDJ75	IGHV2S2	79.7	0	IGHD26S1	88.9	34	IGHJ1S5	100	
	IGVDJ76	IGHV2S2	82.4	24	IGHD15S1	70	2	IGHJ1S5	100	
	IGVDJ77	IGHV2S2	89.4	7	IGHD18S1	73.9	6	IGHJ1S5	78.1	
	IGVDJ78	IGHV2S3	83.6	ND	ND	ND	ND	IGHJ1S2	88.2	
	IGVDJ79	IGHV2S3	92.2	23	IGHD16S1	92.9	4	IGHJ1S3	95.8	
	IGVDJ80	IGHV2S3	90.5	11	IGHD5S2	70	12	IGHJ1S5	86.7	
	IGVDJ81	IGHV2S3	89.0	23	IGHD8S3	87.5	4	IGHJ1S5	88.9	
	IGVDJ82	IGHV2S3	89.7	12	IGHD10S1	88.5	5	IGHJ1S5	100	
	IGVDJ83	IGHV2S3	89.5	ND	ND	ND	ND	IGHJ1S5	92.9	
Adult C	IGVDJ84	IGHV2S3	91.1	ND	ND	ND	ND	IGHJ1S5	100	
	IGVDJ85	IGHV2S3	89.1	23 <sup>c</sup>	IGHD13S1	82.4	6	IGHJ1S6	76.3	
	IGVDJ86	IGHV2S2	87.1	10	IGHD18S1	83.3	18	IGHJ1S5	93.1	
	IGVDJ87	IGHV2S3	88.9	0	IGHD14S1	100	8	IGHJ1S3	90.5	
	IGVDJ88	IGHV2S3	91.0	19	IGHD18S1	100	8	IGHJ1S3	100	

IGVDJ89	IGHV2S3	87.8	ND	ND	ND	ND	IGHJ1S5	89.7
IGVDJ90	IGHV2S3	80.5	18	IGHD26S1	85.7	4	IGHJ1S5	95
IGVDJ91	IGHV2S3	87.4	ND	ND	ND	ND	IGHJ1S5	85.7
IGVDJ92	IGHV2S3	92.4	10	IGHD18S1	76.2	6	IGHJ1S5	78.1
IGVDJ93	IGHV2S3	89.8	ND	ND	ND	ND	IGHJ1S5	73.5
IGVDJ94	IGHV2S3	91.4	14	IGHD9S1	100	2	IGHJ1S7	82.8
IGVDJ95	IGHV2S3	91.3	ND	ND	ND	ND	IGHJ1S7	87.1

a. Identity represents the percent identical nucleotides shared with the germline sequence

b. Junction indicates the number of N + P-nucleotides present at segment junction

c. Junctional nucleotides may include P-nucleotides

d. The annotated IGHD segment of IGVDJ66 was only 4 nucleotides long, however there were only 4 nucleotides between the annotated IGHV and IGHJ segments that were identical to the genomic IGHD5S6.

'ND' indicates that the germline gene segment could not be determined

Supplemental Table 3. Immunoglobulin lambda light chain germline segment use, nucleotide identity with reference genome sequence, and junctional nucleotides

	Sequence	IGLV		VJ		IGLJ	
		Gene	Identity <sup>a</sup>	Junction <sup>b</sup>	Gene	Identity <sup>a</sup>	
Fetal Liver (n = 22)							
Fetus 1	IGLVJ1	IGLV4-66	95.7	2 <sup>c</sup>	IGLJ1	97.4	
	IGLVJ2	IGLV4-66	99.4	0	IGLJ1	100	
	IGLVJ3	IGLV4-66	99.4	1	IGLJ5	100	
	IGLVJ4	IGLV4-69	99.7	0	IGLJ5	100	
	IGLVJ5	IGLV4-75	100.0	0	IGLJ1	97.4	
	IGLVJ6	IGLV8-128	99.2	0	IGLJ7	100	
	IGLVJ7	IGLV8-20	98.6	0	IGLJ1	100	
	IGLVJ8	IGLV8-20	98.6	0	IGLJ4	92.3	
	IGLVJ9	IGLV8-20	98.6	1	IGLJ5	100	
	IGLVJ10	IGLV8-12	100.0	0	IGLJ5	100	
Fetus 2	IGLVJ11	IGLV4-66	100.0	0	IGLJ1	97.1	
	IGLVJ12	IGLV4-66	100.0	0	IGLJ1	97.1	
Fetus 3	IGLVJ13	IGLV2-41	100.0	0	IGLJ1	100	
	IGLVJ14	IGLV4-75	100.0	1 <sup>c</sup>	IGLJ5	100	
	IGLVJ15	IGLV4-75	100.0	0	IGLJ7	100	
	IGLVJ16	IGLV6-98	99.4	0	IGLJ4	94.4	
	IGLVJ17	IGLV8-133	94.9	0	IGLJ5	100	
	IGLVJ18	IGLV8-24	99.7	0	IGLJ1	97.1	
	IGLVJ19	IGLV8-24	98.8	2	IGLJ5	97.1	
	IGLVJ20	IGLV8-24	97.1	1	IGLJ5	97.1	
	IGLVJ21	IGLV8-12	100.0	2	IGLJ5	97.1	
	IGLVJ22	IGLV8-12	100.0	0	IGLJ5	100	
Fetal Bone Marrow (n = 30)							
Fetus 1	IGLVJ23	IGLV2-41	99.7	0	IGLJ1	100	
	IGLVJ24	IGLV2-41	100.0	0	IGLJ1	100	
	IGLVJ25	IGLV4-66	99.4	1	IGLJ4	94.4	
	IGLVJ26	IGLV4-66	99.4	2	IGLJ4	91.7	
	IGLVJ27	IGLV4-66	99.4	1	IGLJ5	97.1	
	IGLVJ28	IGLV6-98	99.4	0	IGLJ5	100	
	IGLVJ29	IGLV8-137	100.0	0	IGLJ5	100	
	IGLVJ30	IGLV8-24	100.0	1	IGLJ5	97.1	
	IGLVJ31	IGLV8-24	100.0	0	IGLJ5	100	
	IGLVJ32	IGLV8-12	99.7	0	IGLJ5	100	
	Fetus 2	IGLVJ33	IGLV4-66	100.0	0	IGLJ1	97.1
		IGLVJ34	IGLV4-66	99.7	0	IGLJ1	97.1
		IGLVJ35	IGLV8-122	96.6	0	IGLJ4	97.3
IGLVJ36		IGLV8-122	91.0	0	IGLJ4	97.2	
IGLVJ37		IGLV8-128	98.9	1	IGLJ5	97.1	
IGLVJ38		IGLV8-28	99.4	0	IGLJ4	97.4	
IGLVJ39		IGLV8-28	99.1	1	IGLJ4	97	
IGLVJ40		IGLV8-28	99.4	0	IGLJ4	97.2	
IGLVJ41		IGLV8-24	99.7	0	IGLJ4	97.3	

Fetus 3	IGLVJ42	IGLV8-24	99.4	1	IGLJ7	97.1	
	IGLVJ43	IGLV4-66	99.4	2 <sup>c</sup>	IGLJ1	100	
	IGLVJ45	IGLV4-66	99.4	1 <sup>c</sup>	IGLJ5	100	
	IGLVJ46	IGLV4-66	99.4	0	IGLJ5	100	
	IGLVJ47	IGLV4-75	100.0	1	IGLJ5	97.1	
	IGLVJ48	IGLV4-75	100.0	4	IGLJ5	97.1	
	IGLVJ49	IGLV8-26	98.9	1	IGLJ5	97.1	
	IGLVJ50	IGLV8-24	99.7	3 <sup>c</sup>	IGLJ7	100	
	IGLVJ51	IGLV8-20	98.3	1	IGLJ4	97	
	IGLVJ52	IGLV8-20	98.6	0	IGLJ7	100	
	IGLVJ53	IGLV8-12	100.0	0	IGLJ7	100	
	Adult Horse Bone Marrow (n = 33)						
	Adult A	IGLVJ54	IGLV8-122	93.2	0	IGLJ4	94.7
IGLVJ55		IGLV8-128	86.5	14	IGLJ4	91.7	
IGLVJ56		IGLV8-128	90.9	20	IGLJ5	96.9	
IGLVJ57		IGLV8-128	90.8	0	IGLJ5	100	
IGLVJ58		IGLV8-128	92.5	2	IGLJ5	100	
IGLVJ59		IGLV8-128	88.0	2	IGLJ7	91.4	
IGLVJ60		IGLV8-128	89.7	5	IGLJ7	96.6	
IGLVJ61		IGLV8-133	88.6	3 <sup>c</sup>	IGLJ5	89.5	
IGLVJ62		IGLV8-24	88.6	17	IGLJ1	93.8	
IGLVJ63		IGLV8-24	86.6	19	IGLJ5	85.3	
Adult B	IGLVJ64	IGLV8-20	90.1	8	IGLJ5	90.3	
	IGLVJ65	IGLV6-109	85.6	30	IGLJ7	90.3	
	IGLVJ66	IGLV8-122	84.5	1	IGLJ7	94.3	
	IGLVJ67	IGLV8-128	84.8	20	IGLJ7	93.5	
	IGLVJ68	IGLV8-128	88.8	19	IGLJ7	100	
	IGLVJ69	IGLV8-128	85.7	1	IGLJ7	96.8	
	IGLVJ70	IGLV8-128	88.5	17	IGLJ7	86.7	
	IGLVJ71	IGLV8-128	90.9	10	IGLJ7	93.3	
	IGLVJ72	IGLV8-128	85.1	5	IGLJ7	93.5	
	IGLVJ73	IGLV8-128	79.2	25	IGLJ7	81.6	
Adult C	IGLVJ74	IGLV8-24	89.4	15	IGLJ7	83.8	
	IGLVJ75	IGLV6-101	93.2	18	IGLJ7	78.1	
	IGLVJ76	IGLV8-122	86.1	4	IGLJ4	78.8	
	IGLVJ77	IGLV8-122	87.8	16	IGLJ5	93.5	
	IGLVJ78	IGLV8-122	83.8	7	IGLJ7	89.5	
	IGLVJ79	IGLV8-128	93.3	0	IGLJ5	91.4	
	IGLVJ80	IGLV8-128	71.7	20	IGLJ5	77.4	
	IGLVJ81	IGLV8-128	82.3	15	IGLJ7	87.1	
	IGLVJ82	IGLV8-128	92.0	4	IGLJ7	94.3	
	IGLVJ83	IGLV8-128	83.3	11	IGLJ7	87.5	
	IGLVJ84	IGLV8-128	88.1	5	IGLJ7	100	
	IGLVJ85	IGLV8-28	88.1	15	IGLJ7	93.9	
	IGLVJ86	IGLV8-24	89.3	14	IGLJ7	85.7	

a. Identity represents the percent identical nucleotides shared with the germline sequence

b. Junction indicates the number of N + P-nucleotides present at segment junction

c. Junctional nucleotides may include P-nucleotides

Supplemental Table 4: Nucleotides removed from the 3' ends of IGHV and IGLV segments and 5' ends of IGHJ and IGLJ segments

	Heavy Chain		Lambda Light Chain	
	IGLV	IGLJ	IGLV	IGLJ
	Median (range)	Median (range)	Median (range)	Median (range)
Fetal liver	4 (0 – 5)	2 (0 – 17)	1 (0 – 11)	3 (0 – 8)
Fetal bone marrow	4 (0 – 6)	2 (0 – 11)	1 (0 – 9)	3 (0 – 6)
Adult horse bone marrow	4 (0 – 7)	7 (0 – 20)	10 (0 – 28)	6 (0 – 8)