Bovine RNA-Seq Data Analysis of Liver and Pituitary Gland

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ABSTRACT

Two key applications of RNA-seq were investigated to analysis of bovine liver and pituitary gland transcriptome. Here, we are presenting ONLY the results of bovine pituitary gland. Results summary of read alignment mapping of bovine pituitary transcriptome revealed a total of 318,400877, 215,392157 and 408, 045666 unique mRNA pituitary gland transcripts of Polish HF, Polish Red and Hereford. Results showed that majority of the transcripts were mapped to known transcriptional units (98%-100%). Out of obtained breed specific sequence reads: 295,416188, 203,310212 and 335,507332 sequenced reads were properly paired in Polish HF, Polish Red and Hereford. Furthermore, a total of 9,396729, 2,622076 and 3,667587 singleton reads were obtained for the Polish HF, Polish Red and Hereford breeds. Results summary of variant calling / SNPs detection in bovine pituitary transcriptome revealed a total 13 775 885 SNPs. The obtained results identified SNPs unique to a particular breed and age, and also randomly repetitive or completely unrelated to each other. After the initial assessment, the statistical model for categorization and filtering was designed, aiming at the highest quality of end results. The criteria include two rigorous filtering conducted in parallel: at least ten reads in the locus with the probability of A ≥ 90% (598 815 SNPs), and B = 100% (495 626 SNPs). The final results comprise only a common part (A∩B), so results are valid for the whole range of 90% -100%. Using an appropriate categorization, the final results were obtained with great accuracy. Highly possible candidates for further study include: 53 nucleotides to map *de novo* *Bos taurus* specie, 567 annotation candidates, 98 single nucleotide polymorphisms specific to Hereford breed, 14 SNPs for Polish Holstein-Frisian and one polymorphism specific and unique to young bulls of all breeds.

1. METHODS

1.a. Workflow of RNA-seq Laboratory Methods

Total RNA of bovine Liver samples (n=18) from young bulls (3 Breeds) (Trizol, Life Technologies, USA)

Total RNA of Pituitary samples (n=18) from young bulls (3 Breeds) (Trizol, Life Technologies, USA)

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DNA-nized and clean up of Total RNA (n=36) from both liver and Pituitary samples (Rapid Out DNA Removal Kit, Thermo Scientific, USA)

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mRNA isolation from Total RNA (n=36) both liver and Pituitary samples (Dynabeads® mRNA DIRECT™ Kit, Life Technologies, USA)

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For dUTP directional mRNA libraries, 25-100ng mRNA was fragmented by chemical hydrolysis, converted to first strand cDNA with random hexamers, and second strand synthesized with dUTP according to the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Bio Labs) protocol.

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Nextseq illumina library indices

SI no	Index name	index seq
1	A001	ATCACG
2	A002	CGATGT
3	A003	TTAGGC
4	A004	TGACCA
5	A005	ACAGTG
6	A006	GCCAAT
7	A007	CAGATC
8	A008	ACTTGA

SI no	Index name	index seq
9	A009	GATCAG
10	A010	TAGCTT
11	A011	GGCTAC
12	A012	CTTGTA
13	A013	AGTCAA
14	A014	AGTTCC
15	A015	ATGTCA
16	A016	CCGTCC

SI no	Index name	index seq
17	A018	GTCCGC
18	A019	GTGAAA
19	A020	GTGGCC
20	A021	GTTTCG
21	A022	CGTACG
22	A023	GAGTGG
23	A025	ACTGAT
24	A027	ATTCCT

↓

NextSeq Illumina sequencing of bovine liver and pituitary transcriptome. Six full Runs of NextSeq® 500 High Output /300 cycles (Illumina, USA)

1.b. Workflow of RNA-seq data

Obtained RNA-seq data of bovine liver and pituitary transcriptome generated from NextSeq Illumina instrument were loaded at BaseSpace illumina, followed by bioinformatics analysis performed at Genomics Core Facility, Waksman Institute of Microbiology, The, State university of New Jersey, **RUTGERS**, NJ, USA. (RNA-seq data analysis workflow is shown as below).

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Pre-alignment analysis

I. Removing the adaptors: using the cutadapt software [Martin, 2011]. For all 18 samples, the minimum overlap length was set to 10 and error rate was set to 0.05 at cutadapt software.
II. Filtering / trimming reads by quality: After cutting adaptor, the low quality bases were trimmed from 3'- end.

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Post-alignment analysis

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I. Read alignment mapping

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Alignment score distribution The processed short paired end reads were aligned, or mapped to the reference genome Ensembl75_UMD3-1.1 plus the Chromosome Y from Btau_4.6.1 assembly, by using BWA version 0.7.5-r404 for both alignment and SNP calling [Li and Durbin *et al.*, 2009]. The HT-Seq framework, version 0.5.3p9, was used to count the aligned reads in genes using the STAR BWA tools [Dobin, 2015; Dobin *et al.*, 2013]. The read alignment of bovine pituitary transcriptome representing three investigated breeds to the bovine reference genome (UMD3.1 assembly) are summarized and presented in Table 9 (Polish HF), Table 10 (Polish Red) and Table 11 (Hereford), respectively.

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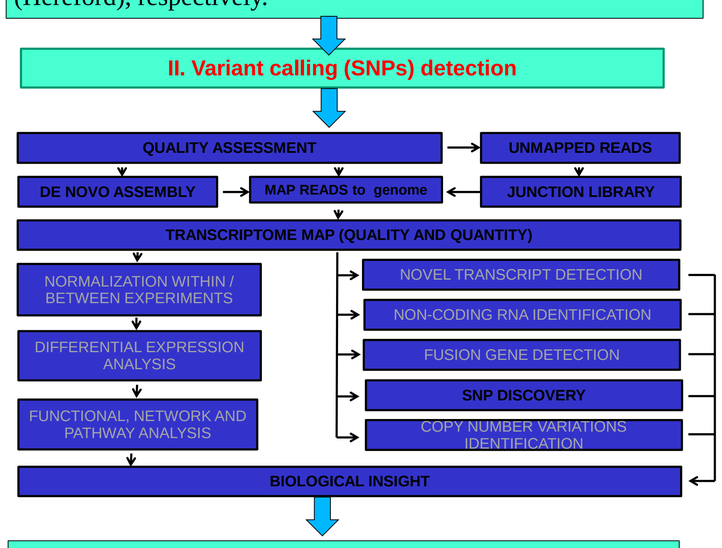
II. Variant calling (SNPs) detection



For the analysis of SNPs detection (variant calling), the samtools mPileUp package to call SNPs and indels [Li *et al.*, 2009] was utilized to detect the putative SNPs in bovine pituitary gland transcriptome.

2. RESULTS

2.a. Results on alignment (mapping) presented in Tables 1 to 3:

Table 1. Alignment (Mapping) of Polish HF pituitary gland transcriptome to bovine reference genome (UMD3.1 assembly).

Age	Total	Mapped	Paired in sequencing	Read1	Read2	Properly paired	With itself and mate mapped	Singletons	With mate mapped to a different chromosome
	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶
6m	218,45	218,45	109,26	109,19	173,99	202,27	4,34	8,13	1,89
9m	159,83	159,83	79,84	79,98	132,45	148,29	2,38	3,17	0,59
12m	137,10	137,10	68,58	68,52	118.33	128,94	1,77	2,07	0,48
SUM	515,53	515,38	257,38	257,70	424,78	479,51	8,49	13,38	2,97

Table 2. Alignment (Mapping) of Polish Red pituitary gland transcriptome to bovine reference genome (UMD3.1 assembly).

Age	Total	Mapped	Paired in sequencing	Read1	Read2	Properly paired	With itself and mate mapped	Singletons	With mate mapped to a different chromosome
	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶
6m	108,49	108,49	54,27	54,22	94,08	102,68	1,36	1,14	628,78
9m	56,89	56,89	28,36	28,53	48,48	54,14	0,70	0,69	329,58
12m	50,00	50,00	25,03	24,96	42,02	46,48	0,71	0,78	287,20
SUM	215,39	215,39	107,66	107,72	184,60	203,31	2,78	2,62	1 245,58

Table 3. Alignment (Mapping) of Hereford pituitary gland transcriptome to bovine reference genome (UMD3.1 assembly).

Age	Total	Mapped	Paired in sequencing	Read1	Read2	Properly paired	With itself and mate mapped	Singletons	With mate mapped to a different chromosome
	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶	1x10⁶
6m	207,07	202,72	207,07	102,64	104,43	174,57	200,69	2,03	6,30
9m	105,39	103,66	105,39	52,01	53,37	82,16	102,80	0,86	6,31
12m	95,58	94,07	95,59	47,41	48,17	78,77	93,30	0,78	2,94
SUM	408,04	401,97	408,04	202,06	205,98	335,51	396,79	3,67	15,54

2.a. Results on SNPs detection presented in Table 4 to 8:

Table 4. Statistical analysis of post-processed reads and construction of bovine SNP database of pituitary gland transcriptome.

	raw data	≥10reads≥90%	%raw	≥10reads≥95%	%raw	≥10reads=100%	%raw
CP01	738 394	35 332	4,8%	33 429	4,5%	30 215	4,1%
CP02	828 367	36 036	4,4%	34 136	4,1%	31 152	3,8%
CP03	790 832	33 381	4,2%	31 534	4,0%	29 123	3,7%
CP04	785 933	38 424	4,9%	36 370	4,6%	33 656	4,3%
CP05	833 475	39 441	4,7%	37 517	4,5%	34 362	4,1%
CP06	551 315	25 336	4,6%	24 054	4,4%	21 808	4,0%
CP07	676 583	26 167	3,9%	24 433	3,6%	20 804	3,1%
CP08	737 090	27 529	3,7%	25 814	3,5%	22 233	3,0%
CP09	925 447	43 001	4,6%	40 538	4,4%	35 393	3,8%
CP10	550 977	19 901	3,6%	18 691	3,4%	16 275	3,0%
CP11	429 917	14 595	3,4%	13 424	3,1%	11 428	2,7%
CP12	644 903	21 292	3,3%	19 829	3,1%	17 307	2,7%
CP13	1 046 054	53 695	5,1%	49 835	4,8%	43 121	4,1%
CP14	861 383	47 371	5,5%	44 119	5,1%	37 453	4,3%
CP15	834 981	37 419	4,5%	34 934	4,2%	29 594	3,5%
CP16	756 302	24 637	3,3%	23 042	3,0%	19 935	2,6%
CP17	948 955	39 679	4,2%	37 072	3,9%	32 476	3,4%
CP18	834 977	35 579	4,3%	33 393	4,0%	29 291	3,5%
	13 775 885	598 815	4,3%	562 164	4,1%	495 626	3,6%

Table 5. Identification of unmapped nucleotides in the *Bos taurus* reference genome. (Variant A∩B). In this particular case A∩B = B.

				HEREFORD			POLISH RED			POLISH HF		
GENE	POSITION	REF	NEW	6M	9M	12M	6M	9M	12M	6M	9M	12M
gnl|UMD3.1|GK000002.2	13 666 806	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000011.2	16 159 476	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000019.2	20 698 850	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000019.2	20 699 376	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000030.2	40 311 600	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000029.2	44 977 738	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000013.2	58 017 388	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000008.2	63 634 684	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000002.2	66 268 098	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000013.2	66 450 509	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000005.2	75 097 696	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000030.2	86 606 893	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000004.2	95 100 034	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000004.2	95 100 035	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000004.2	95 100 038	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000004.2	95 100 041	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000005.2	112 711 342	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000002.2	130 709 059	N	T	T T	T T	T T	T T	T T	T T	T T	T T	T T
gnl|UMD3.1|GK000004.2	1 293	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000002.2	22 473 839	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000025.2	32 373 861	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000030.2	40 074 361	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000016.2	50 690 794	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000004.2	77 886 141	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000030.2	86 606 892	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000004.2	95 100 039	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000004.2	95 109 287	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000002.2	105 397 839	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000002.2	105 397 840	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000005.2	110 675 552	N	G	G G	G G	G G	G G	G G	G G	G G	G G	G G
gnl|UMD3.1|GK000027.2	25 434 307	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000030.2	37 660 032	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000029.2	44 977 737	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000030.2	59 772 837	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000018.2	62 670 837	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000018.2	63 725 859	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000013.2	72 922 644	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000005.2	75 097 695	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000011.2	96 117 503	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000011.2	105 877 948	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000011.2	106 196 227	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000005.2	112 176 316	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000004.2	113 600 675	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000001.2	146 208 312	N	C	C C	C C	C C	C C	C C	C C	C C	C C	C C
gnl|UMD3.1|GK000004.2	1 294	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000016.2	49 314 108	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000016.2	50 690 793	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000022.2	51 489 659	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000015.2	53 024 230	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000013.2	58 026 564	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000002.2	66 271 736	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000004.2	95 100 037	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A
gnl|UMD3.1|GK000005.2	110 675 723	N	A	A A	A A	A A	A A	A A	A A	A A	A A	A A

RESULTS….continue….

Table 6. Hereford – Identification of fully-unique SNPs.

			HEREFORD					
GENE	LOCATION	REF	6M		9M		12M	
gnl|UMD3.1|GJ059828.1	2 900	A	G	G	G	G	G	G
gnl|UMD3.1|GJ059828.1	2 911	A	C	C	C	C	C	C
gnl|UMD3.1|GK000001.2	31 242 812	A	G	G	G	G	G	G
gnl|UMD3.1|GK000001.2	70 697 404	A	G	G	G	G	G	G
gnl|UMD3.1|GK000001.2	31 242 815	A	C	C	C	C	C	C
gnl|UMD3.1|GK000002.2	23 299 129	A	G	G	G	G	G	G
gnl|UMD3.1|GK000002.2	128 857 605	A	G	G	G	G	G	G
gnl|UMD3.1|GK000002.2	133 632 476	A	G	G	G	G	G	G
gnl|UMD3.1|GK000006.2	98 929 560	A	T	T	T	T	T	T
gnl|UMD3.1|GK000006.2	98 929 562	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 219 841	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 219 896	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 219 898	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 219 915	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 221 176	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 221 196	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 221 205	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 221 342	A	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 219 905	A	C	C	C	C	C	C
gnl|UMD3.1|GK000008.2	17 425 531	A	G	G	G	G	G	G
gnl|UMD3.1|GK000008.2	58 590 932	A	G	G	G	G	G	G
gnl|UMD3.1|GK000011.2	43 949 110	A	G	G	G	G	G	G
gnl|UMD3.1|GK000012.2	12 496 583	A	G	G	G	G	G	G
gnl|UMD3.1|GK000013.2	74 496 915	A	G	G	G	G	G	G
gnl|UMD3.1|GK000015.2	20 235 437	A	C	C	C	C	C	C
gnl|UMD3.1|GK000016.2	39 622 819	A	G	G	G	G	G	G
gnl|UMD3.1|GK000018.2	11 171 889	A	G	G	G	G	G	G
gnl|UMD3.1|GK000018.2	14 983 973	A	G	G	G	G	G	G
gnl|UMD3.1|GK000019.2	23 864 192	A	G	G	G	G	G	G
gnl|UMD3.1|GK000019.2	42 682 253	A	G	G	G	G	G	G
gnl|UMD3.1|GK000022.2	57 859 356	A	T	T	T	T	T	T
gnl|UMD3.1|GK000025.2	32 367 501	A	G	G	G	G	G	G
gnl|UMD3.1|GK000025.2	35 240 086	A	G	G	G	G	G	G
gnl|UMD3.1|GK000026.2	5 717 177	A	G	G	G	G	G	G
gnl|UMD3.1|GK000028.2	3 160 036	A	G	G	G	G	G	G
gnl|UMD3.1|GK000029.2	48 144 401	A	C	C	C	C	C	C
gnl|UMD3.1|GK000001.2	83 653 181	C	T	T	T	T	T	T
gnl|UMD3.1|GK000001.2	31 242 827	C	A	A	A	A	A	A
gnl|UMD3.1|GK000004.2	84 628 048	C	A	A	A	A	A	A
gnl|UMD3.1|GK000007.2	89 221 204	C	G	G	G	G	G	G
gnl|UMD3.1|GK000011.2	65 246 617	C	A	A	A	A	A	A
gnl|UMD3.1|GK000012.2	12 418 931	C	G	G	G	G	G	G
gnl|UMD3.1|GK000014.2	83 264 839	C	T	T	T	T	T	T
gnl|UMD3.1|GK000016.2	56 183 550	C	T	T	T	T	T	T
gnl|UMD3.1|GK000019.2	42 680 833	C	A	A	A	A	A	A
gnl|UMD3.1|GK000020.2	49 533 411	C	T	T	T	T	T	T
gnl|UMD3.1|GK000022.2	57 859 501	C	T	T	T	T	T	T
gnl|UMD3.1|GK000025.2	32 367 601	C	G	G	G	G	G	G
gnl|UMD3.1|GK000030.2	9 377 864	C	G	G	G	G	G	G
gnl|UMD3.1|GK000004.2	17 769 142	G	A	A	A	A	A	A
gnl|UMD3.1|GK000007.2	89 221 216	G	C	C	C	C	C	C
gnl|UMD3.1|GK000008.2	85 248 262	G	A	A	A	A	A	A
gnl|UMD3.1|GK000011.2	10 923 748	G	T	T	T	T	T	T
gnl|UMD3.1|GK000013.2	51 930 773	G	C	C	C	C	C	C
gnl|UMD3.1|GK000013.2	47 904 563	G	A	A	A	A	A	A
gnl|UMD3.1|GK000014.2	83 828 338	G	C	C	C	C	C	C
gnl|UMD3.1|GK000014.2	83 842 002	G	C	C	C	C	C	C
gnl|UMD3.1|GK000014.2	338 832	G	A	A	A	A	A	A
gnl|UMD3.1|GK000015.2	38 876 724	G	A	A	A	A	A	A
gnl|UMD3.1|GK000015.2	42 332 661	G	A	A	A	A	A	A
gnl|UMD3.1|GK000017.2	28 834 221	G	A	A	A	A	A	A
gnl|UMD3.1|GK000018.2	64 278 854	G	T	T	T	T	T	T
gnl|UMD3.1|GK000018.2	64 286 567	G	T	T	T	T	T	T
gnl|UMD3.1|GK000019.2	7 901 367	G	C	C	C	C	C	C
gnl|UMD3.1|GK000019.2	5 065 717	G	A	A	A	A	A	A
gnl|UMD3.1|GK000019.2	42 679 832	G	A	A	A	A	A	A
gnl|UMD3.1|GK000019.2	42 680 738	G	A	A	A	A	A	A
gnl|UMD3.1|GK000019.2	42 902 904	G	A	A	A	A	A	A
gnl|UMD3.1|GK000019.2	42 959 610	G	A	A	A	A	A	A
gnl|UMD3.1|GK000021.2	69 621 011	G	C	C	C	C	C	C
gnl|UMD3.1|GK000023.2	29 529 749	G	T	T	T	T	T	T
gnl|UMD3.1|GK000030.2	9 377 707	G	C	C	C	C	C	C
gnl|UMD3.1|GJ059828.1	2 875	T	C	C	C	C	C	C
gnl|UMD3.1|GJ059828.1	2 893	T	C	C	C	C	C	C
gnl|UMD3.1|GK000001.2	31 242 761	T	G	G	G	G	G	G
gnl|UMD3.1|GK000001.2	31 242 831	T	C	C	C	C	C	C
gnl|UMD3.1|GK000001.2	31 242 847	T	C	C	C	C	C	C
gnl|UMD3.1|GK000001.2	31 242 851	T	C	C	C	C	C	C
gnl|UMD3.1|GK000002.2	107 981 556	T	C	C	C	C	C	C
gnl|UMD3.1|GK000002.2	107 981 559	T	C	C	C	C	C	C
gnl|UMD3.1|GK000004.2	11 079 570	T	C	C	C	C	C	C
gnl|UMD3.1|GK000007.2	89 219 893	T	G	G	G	G	G	G
gnl|UMD3.1|GK000007.2	89 221 220	T	C	C	C	C	C	C
gnl|UMD3.1|GK000011.2	43 949 134	T	G	G	G	G	G	G
gnl|UMD3.1|GK000011.2	43 949 130	T	C	C	C	C	C	C
gnl|UMD3.1|GK000012.2	26 267 050	T	C	C	C	C	C	C
gnl|UMD3.1|GK000015.2	20 235 461	T	C	C	C	C	C	C
gnl|UMD3.1|GK000018.2	15 186 414	T	C	C	C	C	C	C
gnl|UMD3.1|GK000018.2	53 955 290	T	C	C	C	C	C	C
gnl|UMD3.1|GK000019.2	7 939 290	T	C	C	C	C	C	C
gnl|UMD3.1|GK000020.2	6 355 801	T	C	C	C	C	C	C
gnl|UMD3.1|GK000021.2	2 696 277	T	G	G	G	G	G	G
gnl|UMD3.1|GK000023.2	28 579 736	T	C	C	C	C	C	C
gnl|UMD3.1|GK000025.2	32 367 478	T	C	C	C	C	C	C
gnl|UMD3.1|GK000025.2	32 367 491	T	C	C	C	C	C	C
gnl|UMD3.1|GK000025.2	32 367 513	T	C	C	C	C	C	C
gnl|UMD3.1|GK000025.2	32 367 517	T	C	C	C	C	C	C
gnl|UMD3.1|GK000025.2	32 367 519	T	C	C	C	C	C	C

Table 7. Polish Red – Identification of fully-unique SNPs.

			HEREFORD			POLISH RED			POLISH HF		
GENE	LOCATION	REF	6M	9M	12M	6M	9M	12M	6M	9M	12M
gnl|UMD3.1|GK000007.2	5 657 758	G	G G	G G	G G	A A	A A	A A	G G	G G	G G

Table 8. Polish HF – Identification of fully-unique SNPs Common part of Variant A (22 SNPs) and Variant B (15 SNPs).

			POLISH HF					
GENE	LOCATION	REF	6M		9M		12M	
gnl|UMD3.1|GK000010.2	102 942 684	A	G	G	G	G	G	G
gnl|UMD3.1|GK000025.2	37 378 666	A	G	G	G	G	G	G
gnl|UMD3.1|GK000004.2	77 555 734	C	T	T	T	T	T	T
gnl|UMD3.1|GK000015.2	25 118 578	C	T	T	T	T	T	T
gnl|UMD3.1|GK000025.2	37 379 038	C	G	G	G	G	G	G
gnl|UMD3.1|GK000006.2	73 396 770	G	A	A	A	A	A	A
gnl|UMD3.1|GK000019.2	27 086 284	G	A	A	A	A	A	A
gnl|UMD3.1|GK000019.2	56 201 240	G	C	C	C	C	C	C
gnl|UMD3.1|GK000025.2	33 248 237	G	C	C	C	C	C	C
gnl|UMD3.1|GK000011.2	74 120 662	T	C	C	C	C	C	C
gnl|UMD3.1|GK000015.2	25 118 428	T	C	C	C	C	C	C
gnl|UMD3.1|GK000016.2	81 545 856	T	C	C	C	C	C	C
gnl|UMD3.1|GK000021.2	68 782 355	T	C	C	C	C	C	C
gnl|UMD3.1|GK000023.2	9 540 557	T	C	C	C	C	C	C

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CONCLUSIONS

1. **The large set of aligned/mapped sequencing reads of bovine pituitary gland can be considered as short expression sequence tags (ESTs) of cattle genome. These ESTs might be suitable to construct the expression array chips, *i.e.*, custom designed microarray, specific to bovine pituitary gland expression. All the generated bovine pituitary transcriptome ESTs database will be submitted to the NCBI bovine transcriptome database resources.**
2. **The large set of SNPs db might be suitable to construct the SNPs chips, *i.e.*, custom designed SNP-chip, specific to bovine pituitary gland expression. All the generated bovine pituitary transcriptome SNPs database will be submitted to the NCBI bovine transcriptome database resources.**
3. **In context to bovine body growth and developmental traits, construction of bovine pituitary (hormonome) specific custom designed microarray and/or SNP-chip will have the great impact in identification of potential candidate genes for body growth. However, for validation, such microarray and/or SNP-chip should be tested on the resource cattle population.**

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