Comparison of five different RNA sources to examine the lactating bovine mammary gland transcriptome using RNA-Sequencing

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Supplementary File 1 Markers of myoepithelial cells, milk epithelial cells and immune cells used to validate the selectivity and specificity of laser capture microdissection by qPCR.

Genes	Primers	Sequence 5'>3'	Amplicon size (nt)
κ-casein (*CSN3*)	forward	AGG TGC AAT GAT GAA GAG TTT TTT C	66
GenBank:NM_174294.2	reverse	CCC AAA AAT GGC AGG GTT AA	
Cluster of differentiation 3 epsilon (*CD3e*)	forward	ACG CTG TAC CTG AAA GCA AGA	118
GenBank: NM_174011.3	reverse	AAT ACA CCA GCA GCA GCA AG	
Cluster of differentiation 68 (*CD68*)	forward	GAT CTG CTC TCC CTG AAG CTAC A	79
GenBank: NM_001045902.1	reverse	CAT TGG GAC AAG AGA AAC TTG GT	
Cluster of differentiation 18 (*CD18*)	forward	AGC GAC CTC AGG GAG TAC CA	65
GenBank: M81233.1	reverse	TTA TCG TTG TTC CAC TGG GAC TT	
Keratin 14 (*KRT14*)	forward	CCC AGC TCA GCA TGA AAG C	57
GenBank: NM_001166575.1	reverse	AGC GGC CTT TGG TCT CTT C	

Supplementary File 2 Quantification of specific markers for mammary myoepithelial cells (Krt14), lymphocytes (CD3e), neutrophils (CD18), macrophages (CD68 and CD18) and MEC (CSN3) by qPCR.
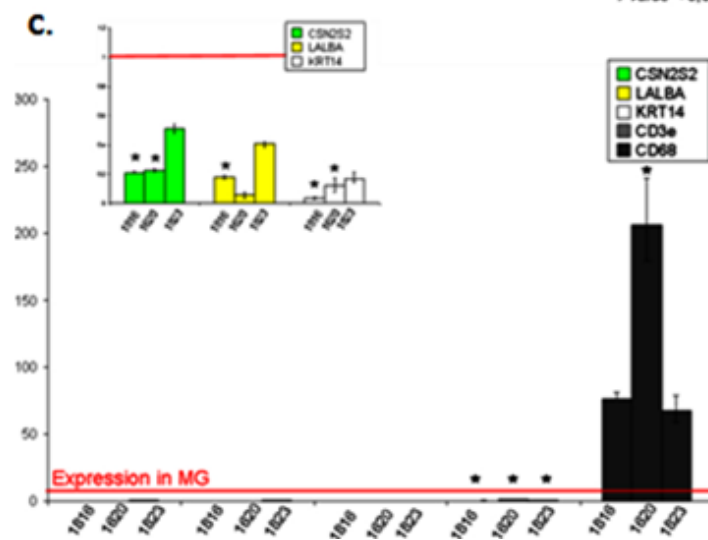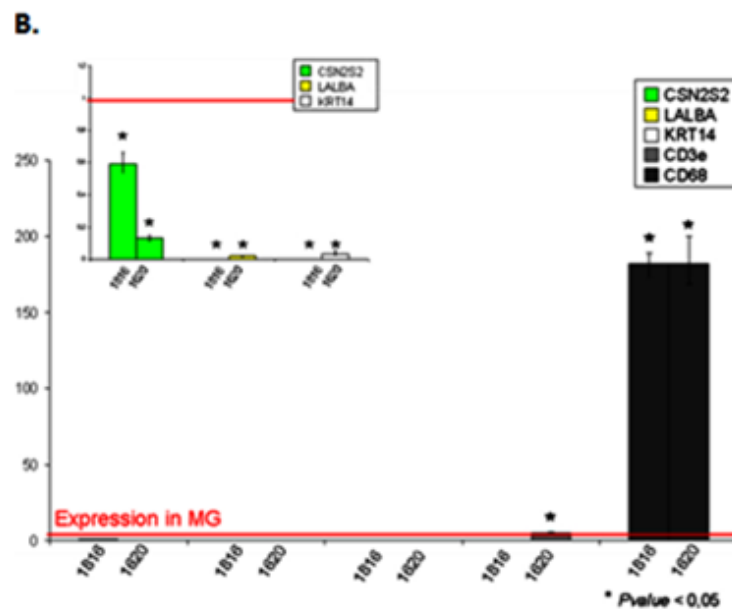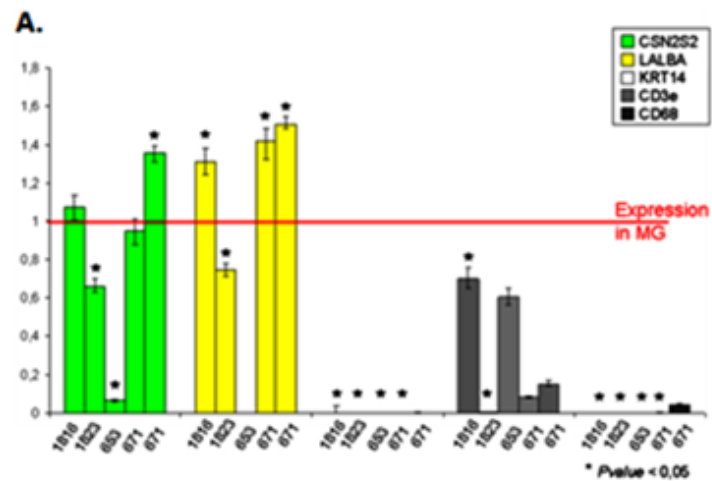
Supplementary File 3 Correlations between highly expressed genes (≥500 RPKM) of MGT, SC, LCMEC, MFG and mMEC

transcriptome in Jersey and Normande cows.

		MGT		SC	LCMEC		MFG		mMEC	
		Jersey	Normande	Jersey	Jersey	Normande	Jersey	Normande	Jersey	Normande
MGT	Jersey	1.00	-	-	-	-	-	-	-	-
	Normande	0.97	1.00	-	-	-	-	-	-	-
SC	Jersey	0.98	0.91	1.00	-	-	-	-	-	-
LCMEC	Jersey	0.99	0.99	0.95	1.00	-	-	-	-	-
	Normande	0.99	0.99	0.95	1.00	1.00	-	-	-	-
MFG	Jersey	0.94	0.85	0.97	0.90	0.89	1.00	--	-	-
	Normande	0.96	0.93	0.96	0.95	0.94	0.96	1.00	-	-
mMEC	Jersey	0.08	0.01	0.16	0.04	0.01	0.36	0.24	1.00	-
	Normande	0.05	0.02	0.09	0.04	0.02	0.19	0.16	0.58	1.00

MGT: mammary gland tissue; SC: milk somatic cells; LCMEC: laser microdissected mammary epithelial cells; MFG: milk fat globules; mMEC: antibody- captured milk mammary epithelial cells.

Supplementary File 4 Correlations between normally/medium expressed genes (ranged from 500 to 10 RPKM) of MGT, SC, LCMEC, MFG and mMEC transcriptome in Jersey and Normande cows.

		MGT		SC	LCMEC		MFG		mMEC	
		Jersey	Normande	Jersey	Jersey	Normande	Jersey	Normande	Jersey	Normande
MGT	Jersey	1.00	-	-	-	-	-	-	-	-
	Normande	0.91	1.00	-	-	-	-	-	-	-
SC	Jersey	0.95	0.97	1.00	-	-	-	-	-	-
LCMEC	Jersey	0.91	0.91	0.95	1.00	-	-	-	-	-
	Normande	0.84	0.93	0.99	0.95	1.00	-	-	-	-
MFG	Jersey	0.87	0.87	0.81	0.95	0.96	1.00	--	-	-
	Normande	0.96	0.86	0.87	0.90	0.96	0.95	1.00	-	-
mMEC	Jersey	0.40	0.14	0.75	0.36	0.10	0.42	0.12	1.00	-
	Normande	0.35	0.32	0.39	0.34	0.22	0.35	0.34	0.27	1.00

MGT: mammary gland tissue; SC: milk somatic cells; LCMEC: laser microdissected mammary epithelial cells; MFG: milk fat globules; mMEC: antibody- captured milk mammary epithelial cells.

Supplementary File 5 Correlations between lowly expressed genes (<10 RPKM) of MGT, SC, LCMEC, MFG and mMEC

transcriptome in Jersey and Normande cows.

		MGT		SC	LCMEC		MFG		mMEC	
		Jersey	Normande	Jersey	Jersey	Normande	Jersey	Normande	Jersey	Normande
MGT	Jersey	1.00	-	-	-	-	-	-	-	-
	Normande	0.83	1.00	-	-	-	-	-	-	-
SC	Jersey	0.77	0.78	1.00	-	-	-	-	-	-
LCMEC	Jersey	0.75	0.77	0.73	1.00	-	-	-	-	-
	Normande	0.84	0.78	0.87	0.75	1.00	-	-	-	-
MFG	Jersey	0.81	0.82	0.82	0.73	0.73	1.00	--	-	-
	Normande	0.87	0.81	0.83	0.63	0.67	0.76	1.00	-	-
mMEC	Jersey	0.13	0.06	0.81	0.06	0.06	0.46	0.34	1.00	-
	Normande	0.12	0.13	0.21	0.08	0.10	0.12	0.61	0.18	1.00

MGT: mammary gland tissue; SC: milk somatic cells; LCMEC: laser microdissected mammary epithelial cells; MFG: milk fat globules; mMEC: antibody- captured milk mammary epithelial cells.

Supplementary File 6 Correlation values using the ranking/position of the RPKM gene expression values ranged from the most expressed gene to the lowest expressed gene in mammary gland tissue, SC, LCMEC, MFG and mMEC transcriptome in Jersey and Normande cows.

		MGT		SC	LCMEC		MFG		mMEC	
		Jersey	**Normande**	**Jersey**	**Jersey**	**Normande**	**Jersey**	**Normande**	**Jersey**	**Normande**
MGT	**Jersey**	1.00	-	-	-	-	-	-	-	-
	Normande	0.96	1.00	-	-	-	-	-	-	-
SC	**Jersey**	0.94	0.94	1.00	-	-	-	-	-	-
LCMEC	**Jersey**	0.93	0.93	0.92	1.00	-	-	-	-	-
	Normande	0.92	0.94	0.90	0.90	1.00	-	-	-	-
MFG	**Jersey**	0.94	0.91	0.95	0.93	0.89	1.00	-	-	-
	Normande	0.91	0.91	0.92	0.89	0.94	0.90	1.00	-	-
mMEC	**Jersey**	0.91	0.82	0.94	0.90	0.80	0.93	0.80	1.00	-
	Normande	0.82	0.76	0.73	0.80	0.79	0.80	0.84	0.69	1.00

MGT: mammary gland tissue; SC: milk somatic cells; LCMEC: laser microdissected mammary epithelial cells; MFG: milk fat globules; mMEC: antibody- captured milk mammary epithelial cells.