Influence of the genetic background to the bovine milk microRNA composition

Le Guillou S., Leduc A., Laubier J., Barbey S., Launay F., Rossignol M-N., Lefebvre R., Marthey S., Laloë D., Le Provost F.

UMR GABI Animal Genetics and Integrative Biology

INRA Le Pin Research Farm

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microRNAs

- Small non-coding endogenous RNA (~22 nt)
- High degree of conservation between species
- Physiological stage-specific, tissue-specific or ubiquitous expression

- Involved in all biological processes
- Post-transcriptional regulation of the genes

4,533 microRNAs are reported in Bovine

miRBase v.22.1, Oct. 2018 & RumimiR, June 2019

microRNAs in milk

- Large concentration, with a **wide variety**

Weber *et al.*, 2010

Sample	**Median total RNA concentration, µg/L (interquartile range)**	**Number of detectable miRNA**
Amniotic fluid	570 (354)	359
Breast milk	**47 240 (73 180)**	**429**
Bronchial lavage	1 128 (886)	260
Cerebrospinal fluid	111 (66)	212

- **Described in different species**, including Human and Bovine

Kosaka *et al.*, 2010; Hata *et al.*, 2010 ; Izumi *et al.*, 2012; Ji *et al.*, 2012

- Affect functions such as **immunity, growth, development or cell proliferation**

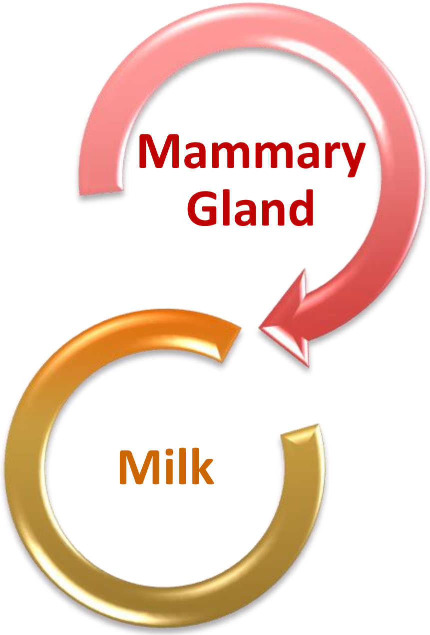
Kosaka *et al.*, 2010, 2013

- Present in different milk **fractions** Li *et al.*, 2016

Bovine milk

- Milk microRNAs **primarily originate from the mammary gland,** with a small contribution of the maternal circulation Alsaweed *et al.*, 2016
- Direct link between microRNAs expression in the mammary gland and their level in milk Laubier *et al.*, 2015

Factors for microRNA composition variation



- Lactation stage Ji *et al.*, 2017
- Nutrition Mobuchon *et al.*, 2015
- Breeds


Yorkshire


Jinhua

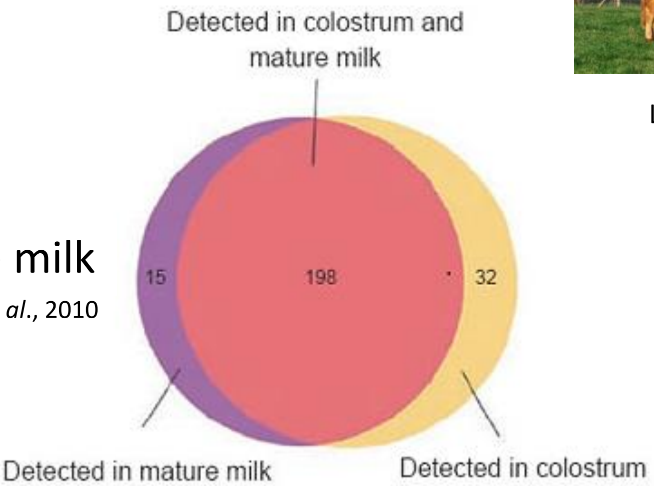
Peng *et al.*, 2015

- Lactation stage


Limousine


Holstein

Wicik *et al.*, 2016



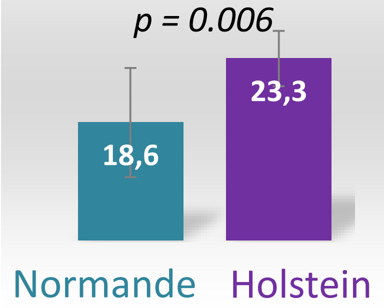
Bovine milk

Chen *et al.*, 2010

Does milk microRNA composition vary according to the genetic background?

Characterization & comparison of milk miRNomes from two dairy breeds with contrasted lactation performances

Normande
n = 8

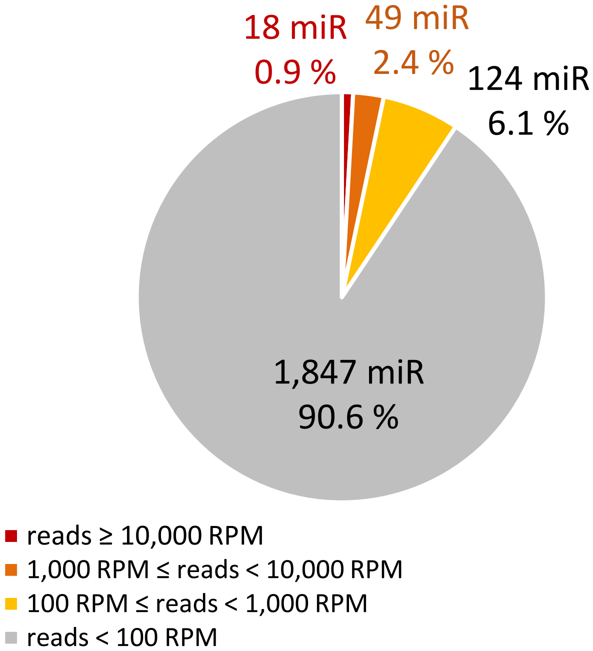


Dairy milk production over 5 weeks (kg/day)

Holstein
n = 9

Milk miRNomes

	 **Holstein**	 **Normande**
Number of microRNAs	**2,038**	**2,030**
Annotated	1,107	1,135
Predicted	931	895



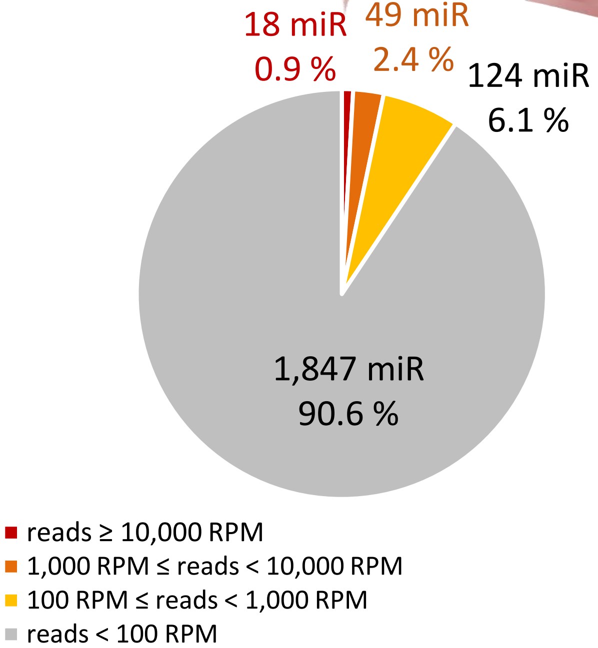
With various abundances

18 microRNAs represent

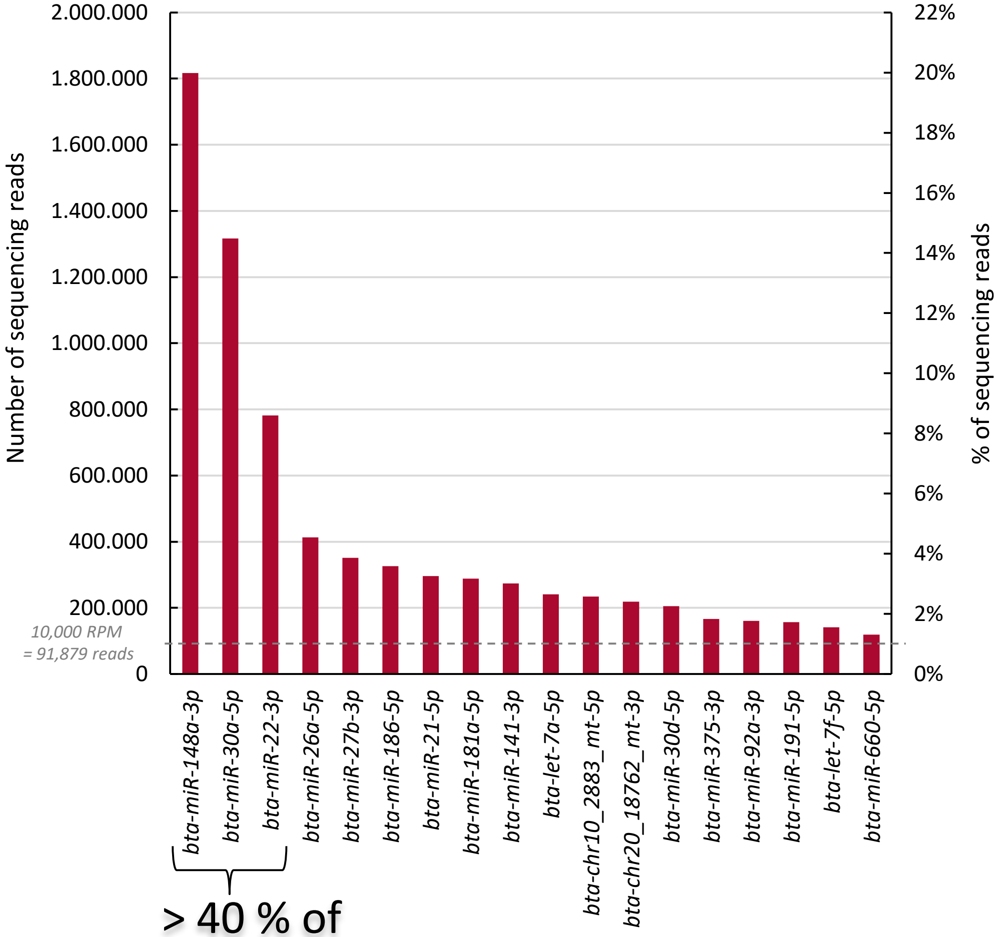
82% of the miRNome

Milk miRNomes

Holstein	**Normande**
2,038	**2,030**
1,107	1,135
931	895

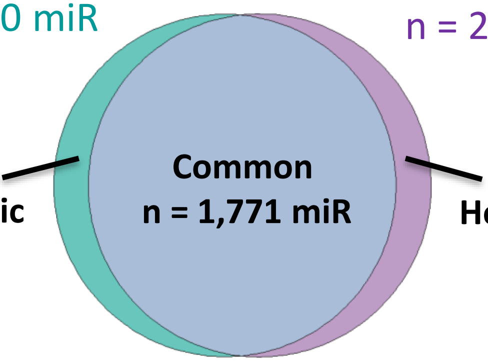


Major milk microRNAs



> 40 % of milk reads

Breed comparison of milk miRNomes



Normande
n = 2,030 miR

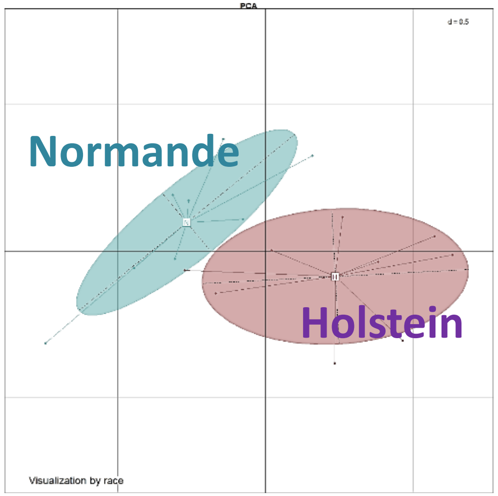
Holstein
n = 2,038 miR

Normande specific
n = 259 miR

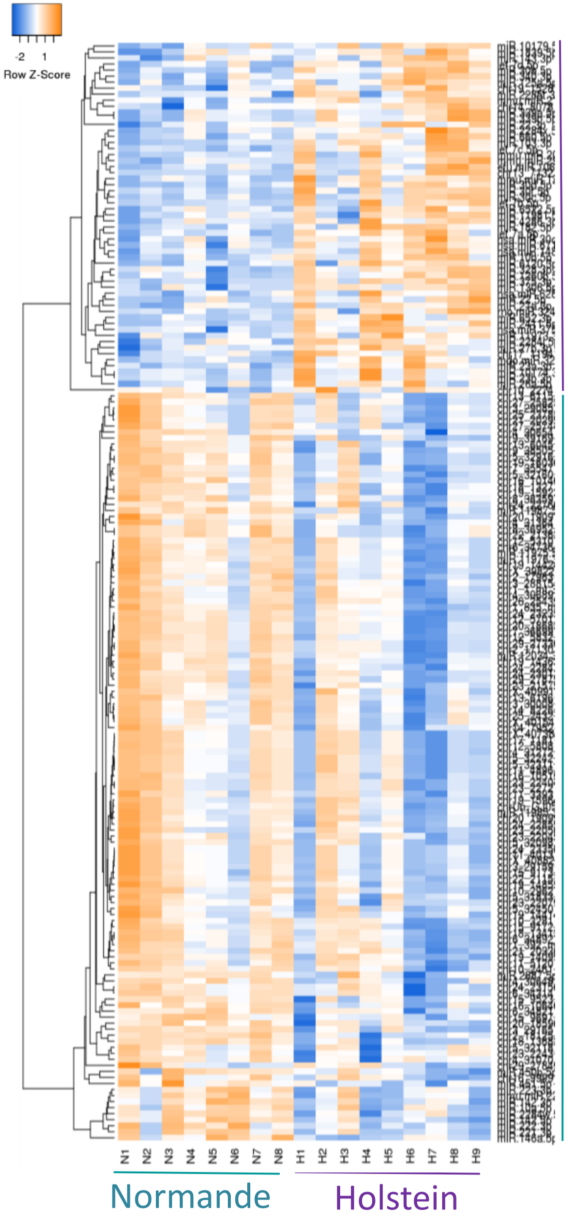
Common
n = 1,771 miR

Hosltein specific
n = 267 miR

- Majority of milk microRNAs common to both breeds with variable abundancies
- 267 Holstein-specific microRNAs
  259 Normande-specific microRNAs
- 182 milk microRNAs are significantly different between breeds ($p<0.05$)



Principal component analysis



Holstein > Normande: n = 58

Holstein < Normande: n = 124

Regulation of genes and pathways related to

- ➢ milk fat synthesis and metabolism
- ➢ protein processing in endoplasmic reticulum
- ➢ mammary epithelial cell differentiation

Essential for lactation

microRNAs variation could lead to differences in milk production

The milk microRNA composition depends on dairy cow breed

Comparison between milk and mammary miRNomes

- Holstein lactating mammary miRNome performed in the same way previously

Le Guillou *et al.*, 2014

Mammary miRNome
n = 484 microRNAs reported in Bovine

- **n = 433 annotated microRNAs in common with the milk miRNome**
  Including 16 microRNAs of the top 30 (and the 3 most present in milk)

= Majority of the microRNAs expressed in the mammary gland ⇔ ~ 11% of the mammary microRNAs are not present in milk

The milk miRNome is a partial mirror of the lactating mammary miRNome

Synthesized by cell types other than luminal epithelial cells, like basal cells or adipocytes?

And / or with selective secretion mechanisms?

Conclusion

- Dual characterization of Holstein and Normande milk miRNomes
- 182 microRNAs with significantly different levels between breeds

Milk microRNA composition vary according to the genetic background

- The milk miRNome is a partial mirror of the lactating mammary miRNome
- Milk microRNAs could be informative of what occurs in the mammary gland

Highlighting microRNAs candidates for further investigations

MicroRNAs' evaluation in milk opens a field of investigation

Variation of milk miRNome according to genetic variables

Why do they vary? What explains these differences between breeds?

Which impact on milk quality?

Characterization of milk fractions, particularly of extracellular vesicles

Identification of milk biomarkers for the mammary gland status ?

Thank you for your attention

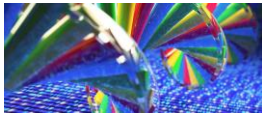
From biological sample to high throughput data

- Primiparous cows raised together with same farming conditions (environment, diet, farmers)

- Milk collection at 2 months of lactation

- Total RNA extraction ⇨ Small RNA-seq Illumina

- Sequences processing



  - Adaptors removing (Cutadapt)
  - Sizing (17-28 nt)
  - Genome blasting (BosTau8)

Means	**Holstein**	**Normande**
Raw reads	32,653,400	33,810,306
Cleaned and filtered reads	13,555,745	15,710,520
Mapped reads	9,299,953	9,836,034

Computational analysis of sequencing data: annotation & quantification

- All species referenced in miRBase relase 22
- RumimiR (detailed microRNA database focused on ruminant species, from the literature) Bourdon *et al.*, 2019
- miRDeep2 predictions

Milk miRNomes

Exhaustive description including new data

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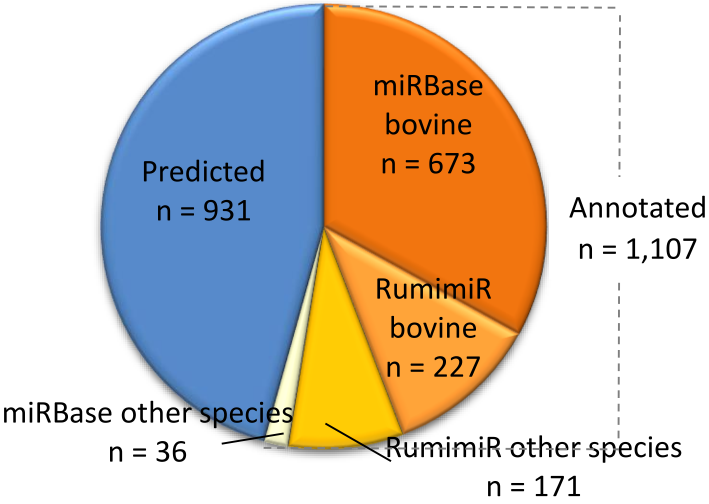

Holstein