



# MicroRNAs in dietary exosomes and their potential impact on the human body



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## Background

Exosomes or extracellular vesicles

- Secreted by cell types, including muscle, neural, epithelial, and stem cells into the extracellular space.
- Facilitate communication between cells by transferring various proteins, lipids, and RNAs.
- Found in nearly all biological fluids, including blood, tears, milk, saliva, urine, and amniotic fluid.
- Exosomes in bovine milk are consumed by humans and are considered one of the most important dietary source of nutrients during neonatal growth of mammalian species.

Mammalian miRNAs are very conserved.

- 239 of 243 miRNAs from bovine milk exosomes are identical to human miRNA sequences.
- Only 11 out 243 bovine milk miRNAs were originally thought to have sequence differences.
- miRNAs from milk are transported to peripheral tissues and affect gene regulation.

Implication: genes are regulated, in part, through dietary means across the species body.

## Hypothesis & Significance

### Hypothesis:

Bovine miRNAs from milk exosomes regulate human gene expression by becoming incorporated into the human miRISC complexes.

### Goals:

1. Identify homologous bovine and human miRNAs with sequence differences.
2. Optimize RNase H-dependent PCR (rhPCR) assay that can distinguish these miRNA homologs. This assay will determine if it possible to directly test whether bovine miRNAs from milk exosomes become incorporated into human miRISC complexes.

### Significance:

Understanding the mechanisms of how bovine miRNAs affect human gene expression is an important step towards improving health through the alteration of nutrient content in diets as well as utilizing its therapeutic potential.

## Materials & Methods

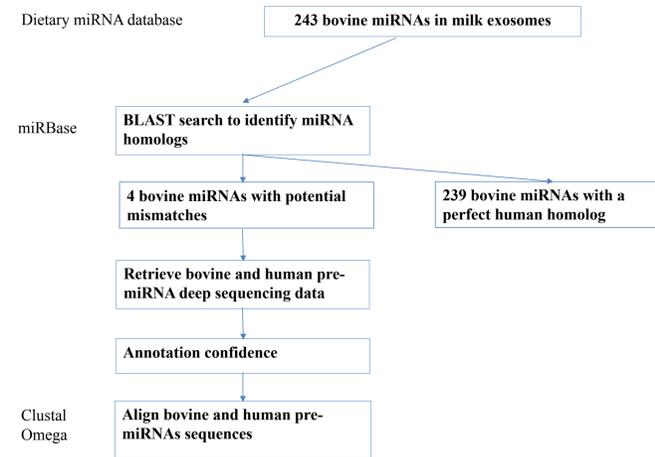


Figure 1. Demonstrates how sequence differences were identified between bovine and human miRNAs.

## Results

```

Pre-miR-146a
bta-mir-146a
CCCAUGUGUAUCCUCAGCUUUGAGAACUGAAUCCAUGGUUGUGUCAGUGUCAGACCUC 60
hsa-mir-146a
CCGAUGUGUAUCCUCAGCUUUGAGAACUGAAUCCAUGGUUGUGUCAGUGUCAGACCUC 54
*****
bta-mir-146a UGAAGUUUAGUUCUUUAGCUGGGUAUCUCUAUCAUCCU 99
hsa-mir-146a UGAAAUUCAGUUCUUCAGCUGGGUAUCUCUGUCAUCGU 99
*****

```

Figure 2. Bovine and human pre-miR-146a sequence is more conserved than expected.

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## Results

HEK-293 cells grown in standard medium supplemented with:	Human serum	Exosome-depleted fetal bovine serum	
		Bovine milk exosomes	Disrupted bovine milk exosomes
hsa-miRNA	positive	positive	positive
bta-miRNA	negative	positive	negative

Table 1. Expected results for distinguishing bovine miRNAs from human homologs.

### CLUSTAL Omega: multiple sequence alignment

```

bta-mir-146a UGAGAACUGAAUCCAUGGUUGU
hsa-mir-146a UGAGAACUGAAUCCAUGGUU

```

```

bta-mir-146b UGAGAACUGAAUCCAUGGCUGU
hsa-mir-146b UGAGAACUGAAUCCAUGGCUG
***** **

```

\*Bta-miR-146a can be distinguished from its bovine and human homologs

Figure 3. Alignment of bovine and human miR-146 sequences. An example of one of the microRNA with sequence differences from their human homologs.

## Conclusions

- Four bovine miRNAs from milk exosomes have sequence differences from their human homologs, which are good candidates for rhPCR.
- The remaining 239 bovine mRNAs found in milk exosomes have identical human homologs.
- The pre-miRNA sequences for the bovine miRNAs of milk exosomes are unusually highly conserved.

## Future Work

- Optimize rhPCR to specifically distinguish homologous human and bovine miRNAs.
- Determine whether bovine miRNAs enter human cells.
- Test whether bovine miRNAs become incorporated into human miRISC complexes.
- Identify human miRNAs targeted by bovine miRNAs.