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Conservation of bta-miR-29b and its effect on human gene expression

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Conservation of bta-mir-29b and its effect on human gene expression

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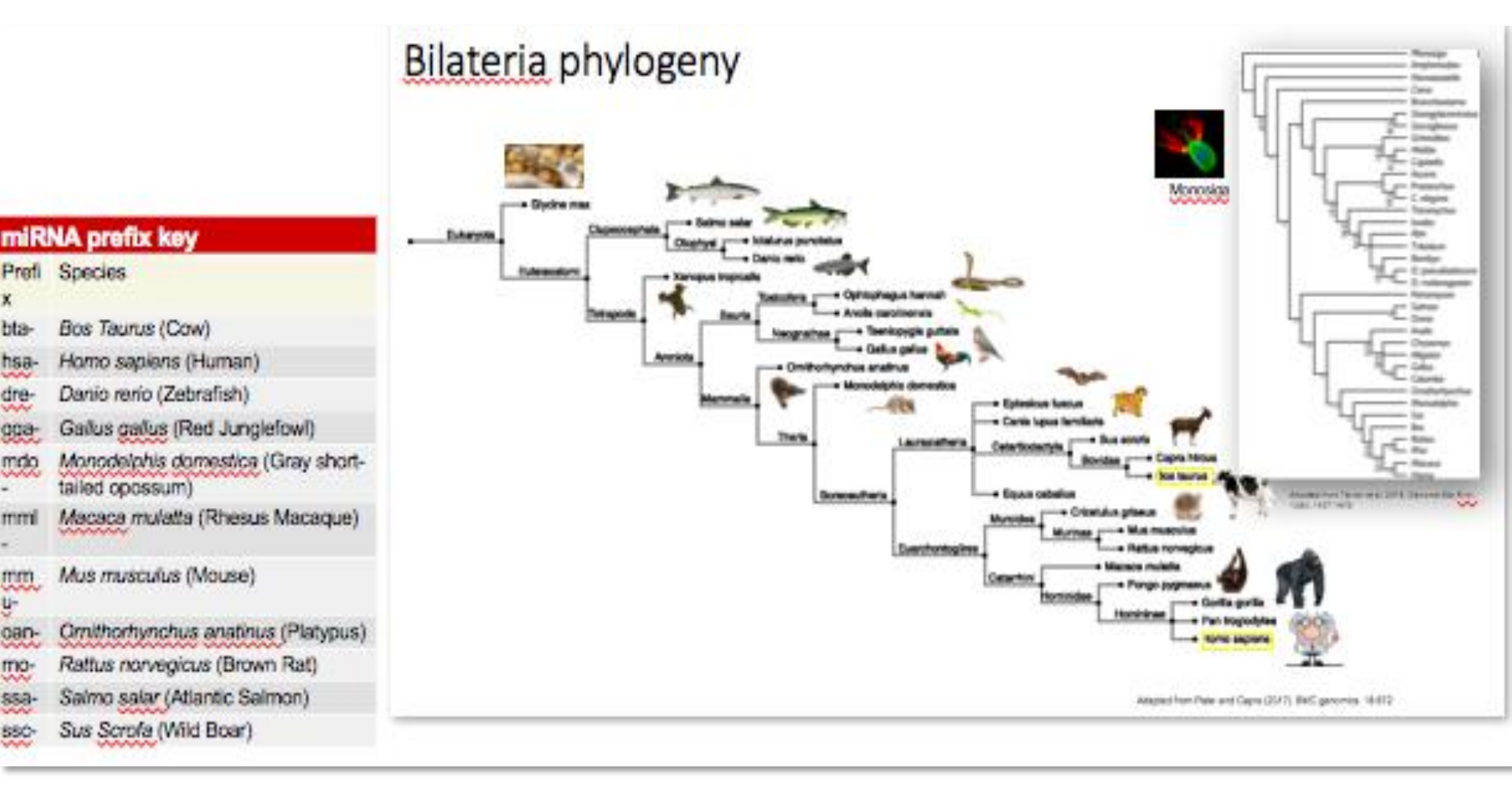
Introduction

- miRNAs are often highly conserved across species and bind to target mRNAs resulting in decreased gene expression.
- Bovine milk exosomes contain miRNAs and most of these miRNAs are identical to human miRNAs.
- Because these miRNAs are so conserved, it is hypothesized that bovine miRNAs can regulate gene expression and be linked to disease risk.

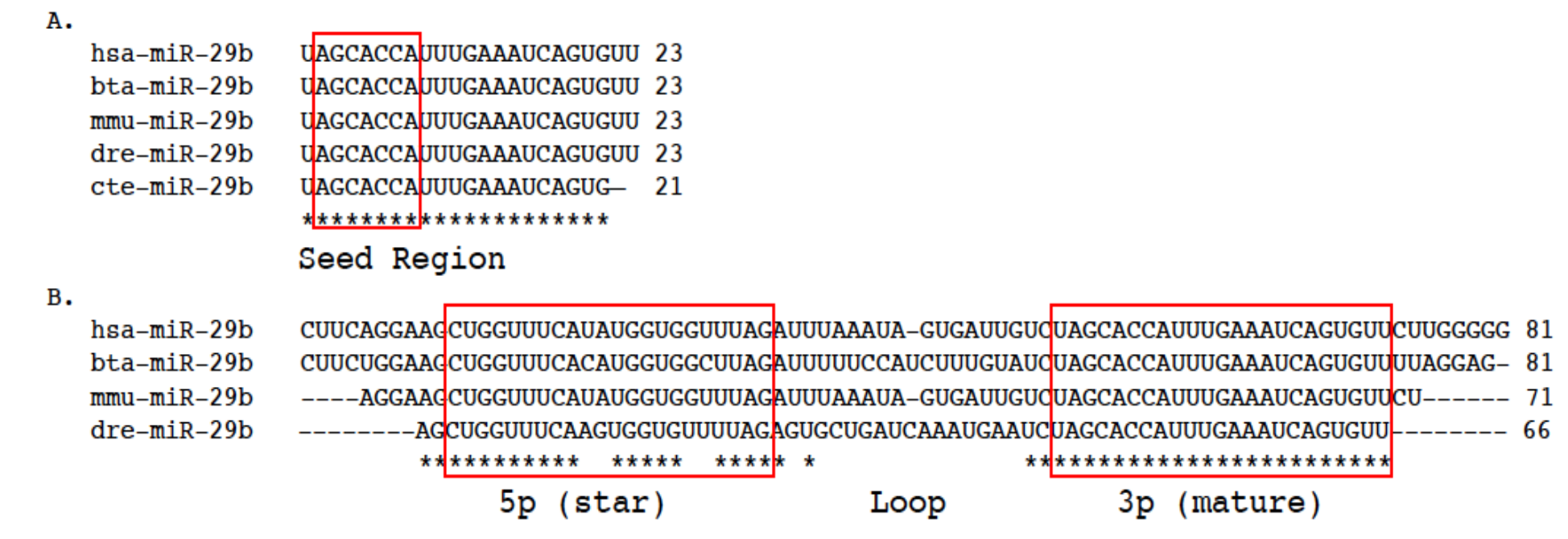
Purpose

The purpose of this work was to examine conservation of miR-29b and its binding sites in target mRNAs as well as co-expression of bta-miR-29b in bovine milk exosomes and its target mRNAs in HEK293 cells.

Results

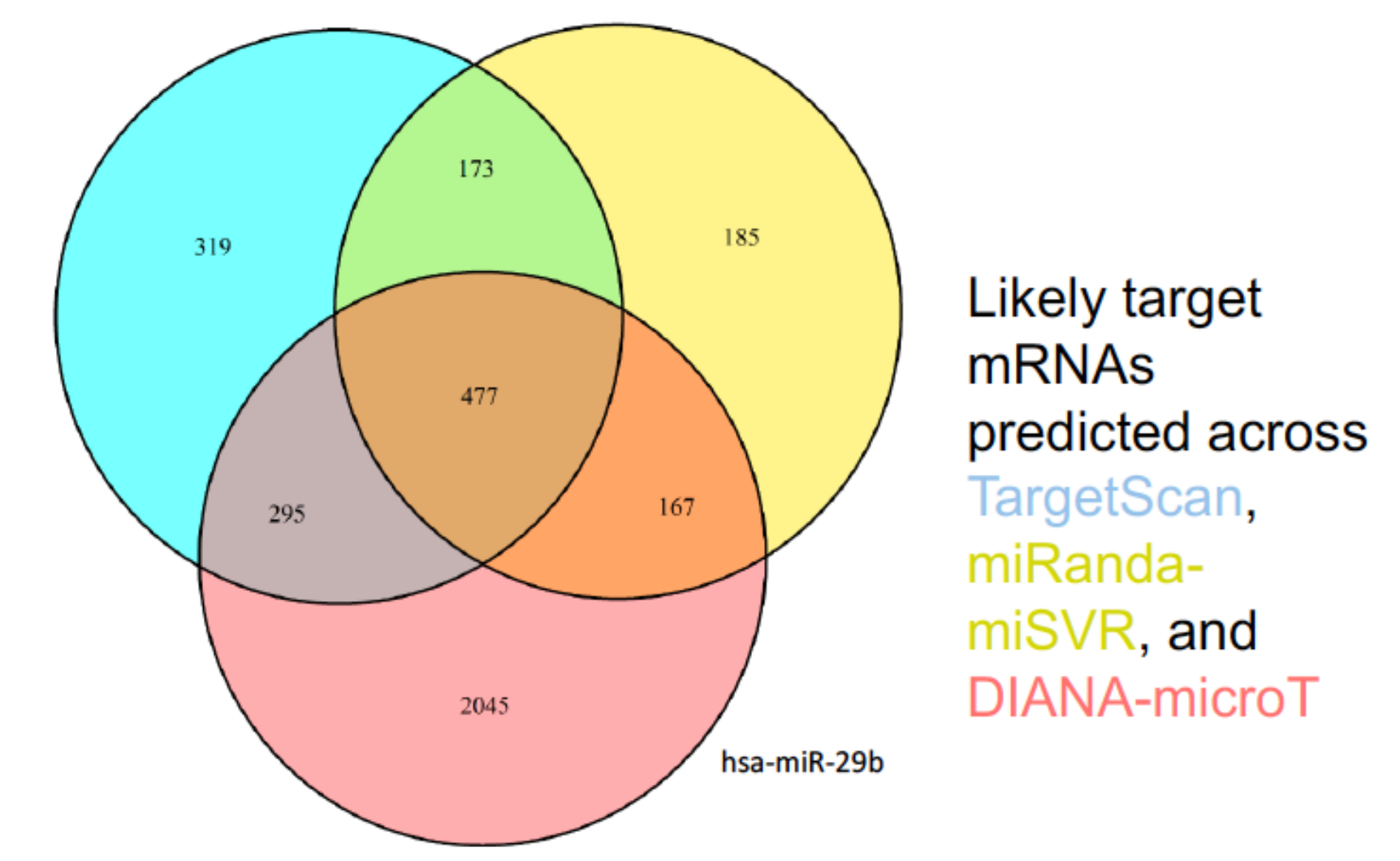


miR-29b is broadly conserved

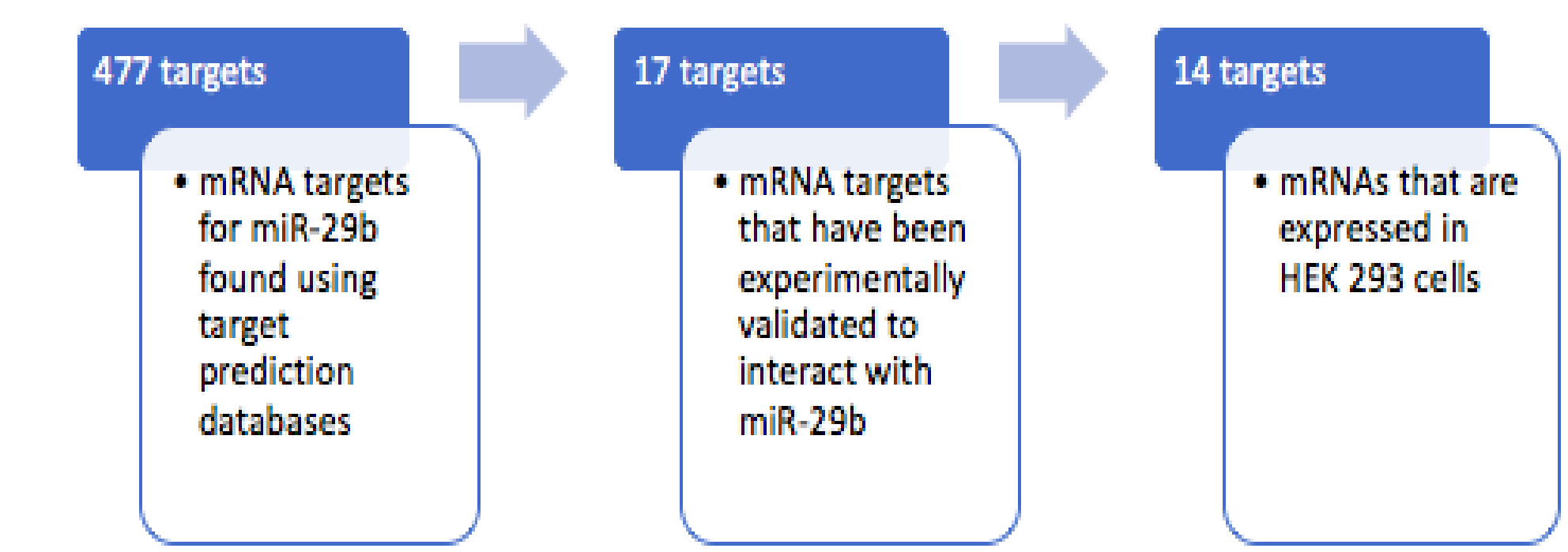


Alignment of representative miR-29b sequences. The miRNA (A) and pri-miRNA (B) sequences of miR-29b from *Bos taurus* (bta; cow), *Homo sapiens* (has; human), *Mus musculus* (mmu; mouse), *Capitella teleta* (cte; segmented worm), and *Danio rerio* (dre; zebrafish) were obtained from miRbase and aligned with Clustal Omega. The mature, loop and star regions of the sequence are marked. * indicate conserved sequences.

mRNAs targeted by miR29b

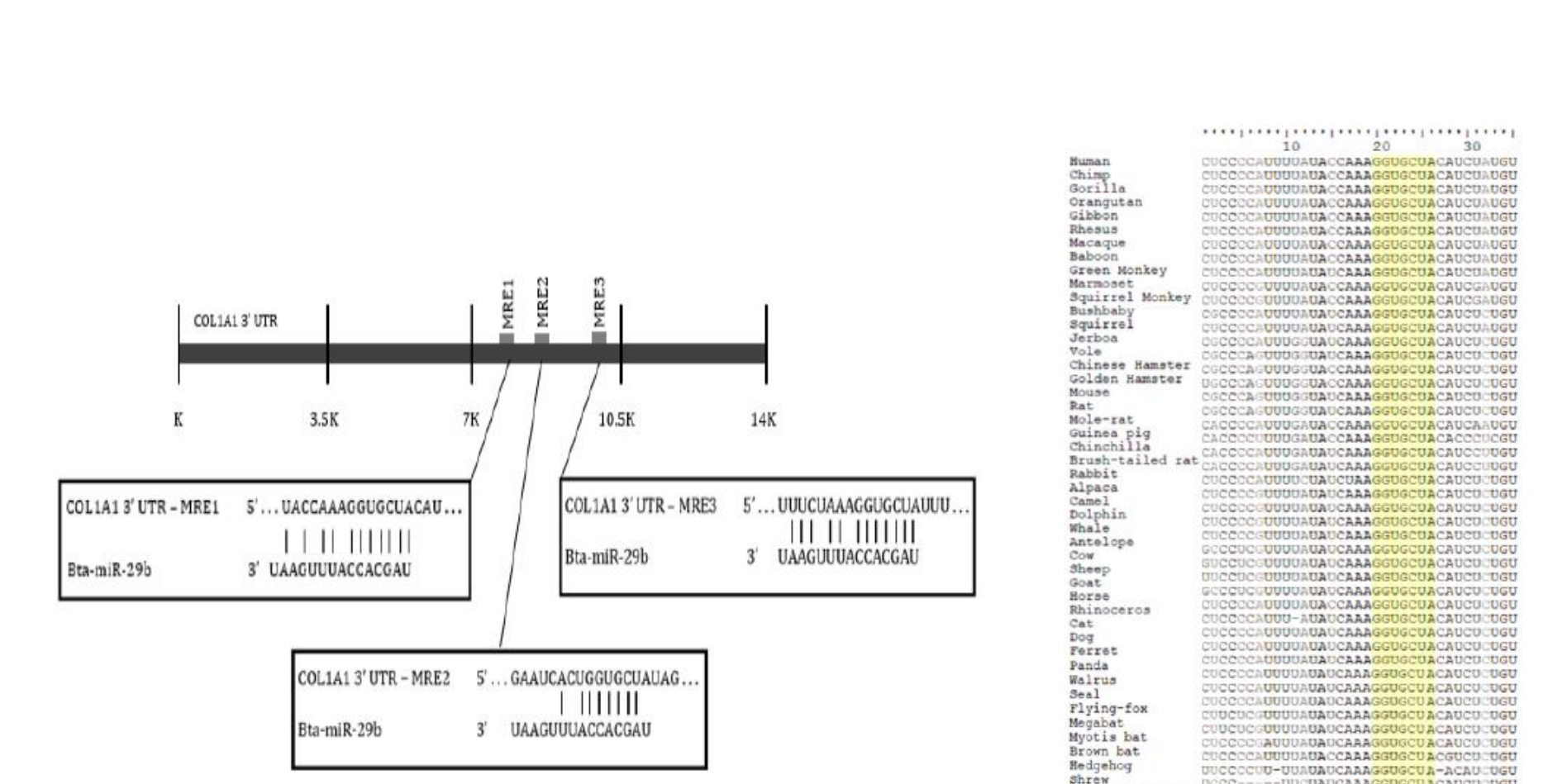


mRNAs with predicted binding sites for miR-29b were retrieved from the TargetScan (version 7.2), miRanda-miSVR, and DIANA-microT (version 5) databases.



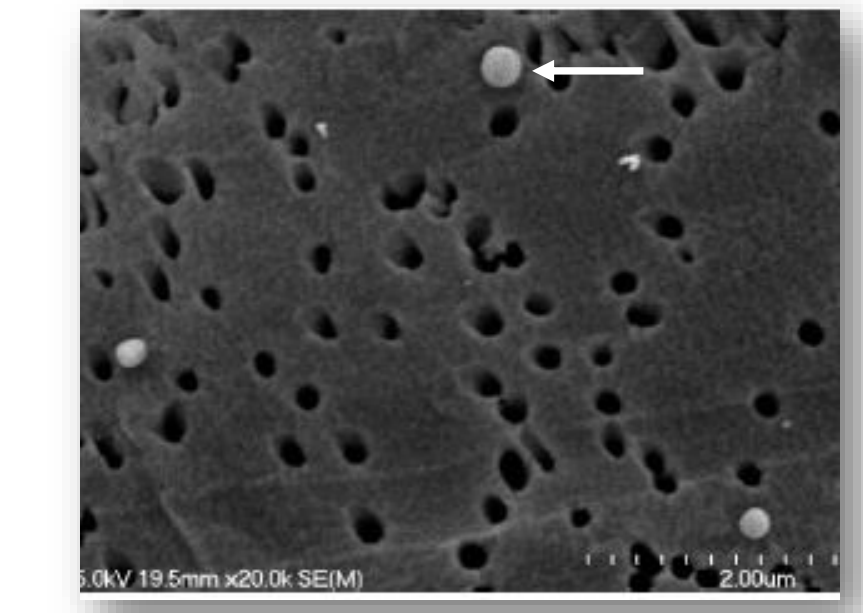
The 477 high confidence predicted mRNA targets for bta-miR-29b were found using target prediction databases: TargetScan, miRanda-miSVR, and DIANA-microT. 17 of these targets are experimentally validated. 14 of these mRNA targets are expressed in HEK293 cells, the *in vivo* model for this experiment.

Conservation of miR-29b binding sites



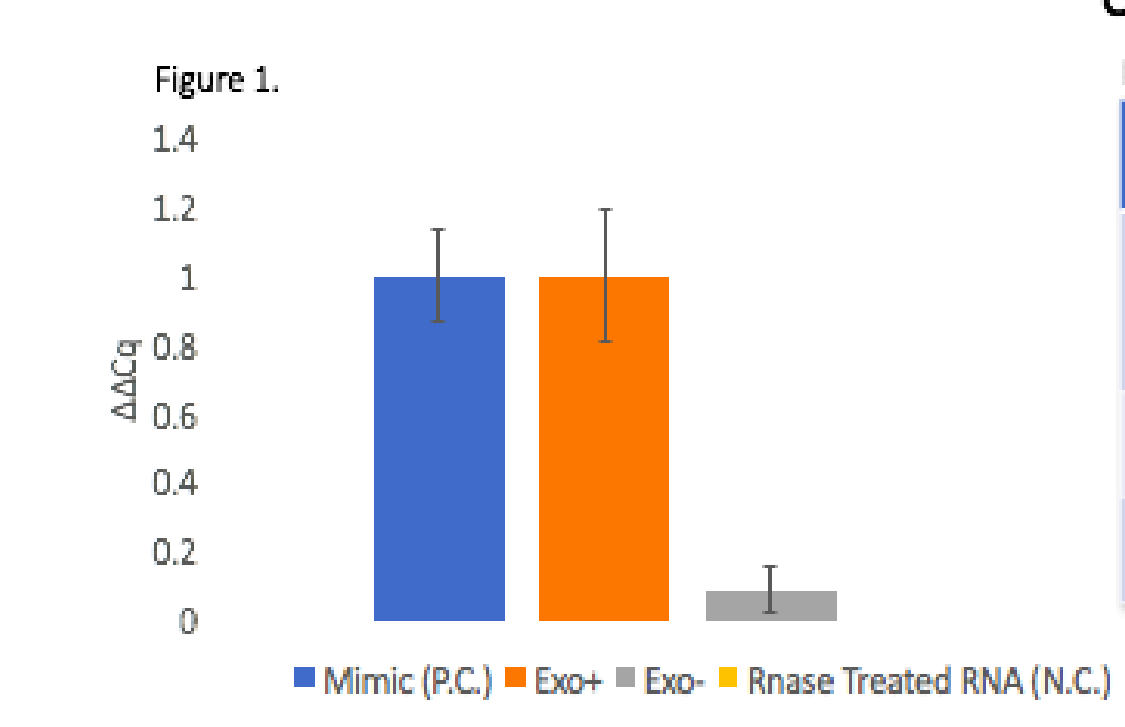
The binding sites for bta-miR-29b in Col1A1 mRNA are conserved across the bilateria.

Experimental validation of miRNA and mRNA expression



SEM (scanning electron microscopy) was used to visualize the exosomes (see arrow) separated from bovine milk. These exosomes were used for downstream qPCR to analyze miR-29b levels.

bta-miR-29b is present in bovine milk exosomes



Predicted bta-miR-29 target is present in HEK293 cells

Figure 2.

Replicate	Cq value
1	29.2
2	28.9
3	29

TaqMan microRNA assay used to analyze levels of bta-miR-29b from exosomes separated from bovine milk (Left). Intact exosomes had a higher level of bta- miR-29b as compared to disrupted exosomes and exosomes treated with RNase. qPCR was use to show that the predicted bta-miR-29b TET2 mRNA is present in HEK293 cells (Right). A Cq value of less than 30 shows the RNA is present in the sample.

miR-29b disease association

Disease Association	HMDD Experimentally Supported Causality
Obesity	Linked
Diabetes	Causal
Metabolic Syndrome	Not related
Immune Diseases	Not related
Inflammation	Causal

Analysis of bta-miR-29b bovine milk exosomal miRNA link to disease risk via the Human microRNA Disease Database (HMDD) version v3.2

Discussion

- miR-29b is highly conserved.
- Identified 17 experimentally validated miR-29b target mRNAs.
- The Col1A1 mRNA has a highly conserved miR-29b binding site.
- Conservation of these miRNAs and their target sites suggests that bovine miRNAs delivered into human cells by exosomes through the consumption of cow's milk, have the potential to regulate human gene expression.
- miR-29b is linked to obesity in humans.

Future Direction

- Treat HEK293 with bovine milk exosomes to determine if bta-miR-29b reduces expression of its target mRNAs.
- Model the effect of decreased expression of the bta-miR-29b on human gene expression to determine if there is a change in disease risk associated with consumption of milk.

Acknowledgements

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