

Analysis of the miR-34 family functions in breast cancer reveals annotation error of miR-34b

Authors:

Engkvist ME¹, Stratford EW¹, Lorenz S^{1,2}, Meza-Zepeda LA^{1,2}, Myklebost O^{1,3*}, Munthe E^{1*}

*shared last authorship

¹Department of Tumour Biology, Institute for Cancer Research, Oslo University Hospital

²Department of Core Facilities, Institute for Cancer Research, Oslo University Hospital

³Department of Clinical Science, University of Bergen

gugcucgguuuguaggcagugucauuagcugauuuacugugggguuacaaucacuaacuccacugccaucaaaacaaggcac

.....uagCcagugucauuagcug.....	6	1	seq
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.....uaggcCgugucauuagcug.....	4	1	seq
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.....uaggcagugucauGagcug.....	4	1	seq
.....uaggcagugucauGagcug.....	3	1	seq
.....uaggcagugucauuagcU.....	4	1	seq
.....uaggcagugucauuAacug.....	1	1	seq
.....uaggcagugucaCuagcug.....	2	1	seq
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.....Gaggcagugucauuagcug.....	8	1	seq
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.....uaggcagugucauuGgcugauu.....	2	1	seq
.....uaggcagugucauuUcugauu.....	11	1	seq
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.....uUggcagugucauuagcugauu.....	3	1	seq
.....uagUcagugucauuagcugauu.....	58	1	seq
.....uaggcagugucauuagcugaCu.....	77	1	seq
.....uaggcagugucauGagcugauu.....	1	1	seq
.....uagAcagugucauuagcugauu.....	2	1	seq
.....uaggcagugucauuagcugauG.....	23	1	seq
.....uaggcagugucauuagGugauu.....	1	1	seq
.....uaggcagUucauuagcugauu.....	2	1	seq
.....uaggUagugucauuagcugauu.....	1	1	seq

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.....uaggGagugucuuagcugauu.....	3	1	seq
.....uaggcagugucuuagcugauA.....	24	1	seq
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.....uaggcagugucuuagcugauuC.....	24	1	seq
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.....uaggcagugucuuagcugauuA.....	95	1	seq
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.....uaggcagugucuuagUgauug.....	34	1	seq
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.....uaggcagugucuuagcuUauug.....	8	1	seq
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.....uaggcaguuUucuuagcugauug.....	14	1	seq
.....uaAgcagugucuuagcugauug.....	1	1	seq
.....uaggcaguuAucuuagcugauug.....	4	1	seq
.....uaggcagugucuuagcGgauug.....	2	1	seq
.....Gaggcagugucuuagcugauug.....	9	1	seq
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.....uaggcCgugucuuagcugauug.....	1	1	seq
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.....uaggcagugucuuagcugauuUu.....	68	1	seq
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.....uUggcagugucauuagcugauugu.....	11	1	seq
.....uaggcagugucauuagcugauuGgu.....	1	1	seq
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.....uaggcagugAcuuagcugauugu.....	2	1	seq
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.....uaggcagAgucauuagcugauugu.....	4	1	seq
.....uaggcagugucauuagcugaCugu.....	4	1	seq
.....uaggcagugucauuagcugUuugu.....	10	1	seq
.....uaggcagugucauuagcugauuG.....	57	1	seq
.....Caggcagugucauuagcugauugu.....	1	1	seq
.....uaggcagCgucuuagcugauugu.....	1	1	seq
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.....uaggcagugucauuagcugauCgu.....	1	1	seq
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.....uagAcagugucauuagcugauugua.....	1	1	seq
.....uagUcagugucauuagcugauugua.....	24	1	seq
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.....uaggcagugucauuagcugauuGga.....	1	1	seq
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.....uaggcagugucauuagcUauugua.....	1	1	seq
.....uaggcagugucauuagcugauuGuC.....	27	1	seq
.....uaggcaUugucauuagcugauugua.....	1	1	seq
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.....uaggcagugucauuagcugauuGaa.....	3	1	seq
.....uaggcagugucauuagcugauuGAc.....	3	1	seq
.....uaggcagugucauuagcugauuGuaU.....	4	1	seq
.....uaggcagugucauuagcugauuGuaG.....	1	1	seq
.....uaggcagugucauuagcugauuGuaUu.....	2	1	seq
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.....uaggcagugucauuagcugauuGuUcu.....	1	1	seq
.....aggcagugucaGuagcug.....	1	1	seq
.....aggcagugucauuagcuC.....	1	1	seq
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.....Uggcagugucauuagcug.....	2	1	seq
.....aggcagugucauuUgcug.....	1	1	seq
.....aggcagugucUuuagcuga.....	1	1	seq
.....aggcagugucauuGgcuga.....	1	1	seq
.....aggcagugucauuagcuga.....	26	0	seq

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..... aggcagugucuuagcugUu.....	10	1	seq
..... aggcUgugucuuagcugauu.....	1	1	seq
..... aggGagugucuuagcugauu.....	1	1	seq
..... aggcagugucuuagcugaCu.....	3	1	seq
..... aggcagugCcauuagcugauu.....	1	1	seq
..... aggcagugucuuagcugauu.....	12	0	seq
..... aggUagugucuuagcugauug.....	2	1	seq
..... agUcagugucuuagcugauug.....	2	1	seq
..... aggcagugGcauuagcugauug.....	1	1	seq
..... aUgcagugucuuagcugauug.....	4	1	seq
..... aggcagugucuuagcugaAu.....	1	1	seq
..... Gggcagugucuuagcugauug.....	1	1	seq
..... aggGagugucuuagcugauug.....	1	1	seq
..... aggcagugucuuagcugauuA.....	1	1	seq
..... aggcagUucauuagcugauug.....	1	1	seq
..... aggcagugucuuagcugauuU.....	3	1	seq
..... aggcUgugucuuagcugauug.....	2	1	seq
..... aggcagAgucuuagcugauug.....	1	1	seq
..... aggcagugucuuUgcugauug.....	2	1	seq
..... aggcagCgucuuagcugauug.....	1	1	seq
..... aggcagugucuuagcugauug.....	174	0	seq
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..... aggcagugucuuagcGgauug.....	1	1	seq
..... aggcagugucuuagcCgauug.....	1	1	seq
..... aggcagugucuuagcugauugA.....	3	1	seq
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..... aggcagugucuuagcugauuCu.....	1	1	seq
..... aggcagugucuuagcugUuug.....	1	1	seq
..... agUcagugucuuagcugauug.....	1	1	seq
..... aggcagugGauuagcugauug.....	1	1	seq
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..... aggcagugucuuagcugauug.....	48	0	seq
..... aggcagugucuuagcugauugC.....	2	1	seq
..... aggcagugucuuagUgauug.....	1	1	seq
..... aggcagugucuuagcugauugU.....	20	1	seq
..... aggcagugucuuagcugauugua.....	8	0	seq
..... aggcagugucuuagcugauuguaU.....	1	1	seq
..... ggcagugucuuagcuga.....	4	0	seq
..... ggcagUucauuagcuga.....	1	1	seq
..... ggcagugucuuagcugUu.....	1	1	seq
..... ggcagugucuuagcugaCu.....	1	1	seq
..... ggcagugucuuagcugauu.....	2	0	seq
..... ggcagugucuuagcugaGug.....	1	1	seq
..... ggcagugucuuUcugauug.....	1	1	seq
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..... ggcagugucuuagcugauuU.....	1	1	seq
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..... ggcagugucuuagcugauug.....	10	0	seq
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..... ggcagugucuuagcugauugua.....	1	0	seq
..... ggcagugucuuagcugauugC.....	1	1	seq
..... gcagugucuuagcuUauug.....	1	1	seq
..... gcagugucuuagUgauug.....	3	1	seq
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..... cagugucuuagcugauu.....	3	0	seq
..... cagugucuuagcugauA.....	1	1	seq
..... cagugucuuagcugauuU.....	1	1	seq
..... cagugucuuagcAgaug.....	1	1	seq

hsa-miR-34b-5p

hsa-miR-34b-3p

gag ucgguuuguaggcagugucauuagcugauuguacuguggugguuacaaucaacuauaccacugccaucaaaa caaggcac

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.....cagugucauuUcugauug.....	1	1	seq
.....cagugucauuagcugauugu.....	2	0	seq
.....agugucauuagcugauuU.....	1	1	seq
.....agugGcauuagcugauug.....	1	1	seq
.....agugucauuagcugauug.....	18	0	seq
.....aguUucauuagcugauug.....	2	1	seq
.....agugucauuagcugauugA.....	1	1	seq
.....gugucauuagcugauugu.....	1	0	seq
.....gugucauuacCugauugu.....	1	1	seq
.....caucacuaacuccacugccau.....	1	0	seq
.....aaucacuaacuccacugccauc.....	3	0	seq
.....aaucacuaacuccacugccauU.....	1	1	seq
.....aucacuaacuccacugccauc.....	1	0	seq

Supplementary data S4: miRDeep analysis of miR-34b sequencing data for MDA-MB-231 cells transfected with annotated miR-34b mimic.

The plot shows the percentage of reads for each individual base in the mature miRNA. The annotated sequences are given in red, and the identified sequences and their number of reads are listed below.

Cell line : MDA-MB-231 cells transfected with Negative control

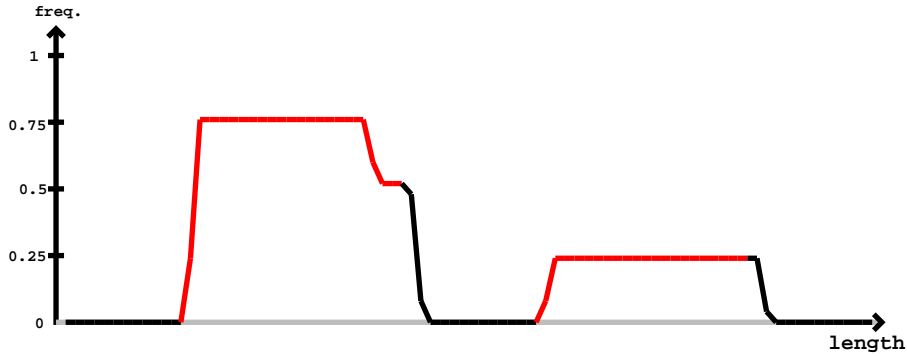
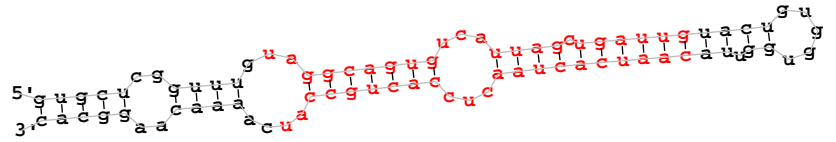
miRBase precursor : hsa-mir-34b

Total read count : 25

hsa-miR-34b-3p read count : 6

hsa-miR-34b-5p read count : 19

remaining reads : 0

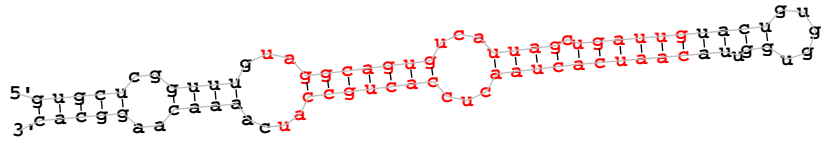


hsa-miR-34b-5p

hsa-miR-34b-3p

5' -	gugcucgguuuagggcagugucuuagcugauuguaucuguggguucaaucacuaacuccacugccaucaaaaaaggcac	-3'	exp			
	(((((((((....(((.....))			reads	mm	
uaggcagugucuuagcug.....			4	0	seq
uaggcagugucuuagcuga.....			2	0	seq
aggcagugucuuagcugauug.....			1	0	seq
aggcagugucuuagcugauuga.....			10	0	seq
aggcagugucuuagcugauugua.....			2	0	seq
caucacuaacuccacugccauc.....			2	0	seq
aucacuaacuccacugccauc.....			3	0	seq
aucacuaacuccacugccaucU.....			1	1	seq

Cell line : IOR-MOS
 miRBase precursor : hsa-mir-34b
 Total read count : 703
 hsa-miR-34b-3p read count : 100
 hsa-miR-34b-5p read count : 603
 remaining reads : 0



hsa-miR-34b-3p

hsa-miR-34b-5p		reads	mm	sample
5' -	gugcucgguuuugaggcagugucuauuagcugauuguacuguggguuacaaucaacuacuccacugccaucaaaacaaggcac	-3'	exp	
	((
uaggcagugucuauuagcug.....	2	0	seq
uaggcagugucuauuagcugauu.....	1	0	seq
uaggcagugucuauuagcugauug.....	3	0	seq
Caggcagugucuauuagcugauug.....	1	1	seq
uaggcagugucuauuagcugauugu.....	7	0	seq
uaggcagugucuauuagcugauugua.....	3	0	seq
uaggcagugucuauuagcugauuguaU.....	1	1	seq
aggcagugucuauuagcug.....	1	0	seq
aggcagugucuauuagcugauu.....	1	0	seq
aggcagugucuauuagcugauug.....	21	0	seq
aggcagGgucuauuagcugauug.....	1	1	seq
aggcagugucuauuagcugauugu.....	415	0	seq
aggcagugucuauuagcugauuAu.....	1	1	seq
aggcagugucuauuagcugauugu.....	1	1	seq
aggcagugucuauuagcugauugu.....	1	1	seq
aggcagugucuauuagcugauugu.....	2	1	seq
aggcagugucuauuagcugauugu.....	2	1	seq
aggcagugucuauuagcugauuguC.....	1	1	seq
aggcagugucuauuagcugauuguU.....	8	1	seq
aggcagCgucuauuagcugauugu.....	1	1	seq
aggcagugucuauuagcugauugu.....	121	0	seq
aggcagugucuauuagcugauuguA.....	2	1	seq
aggcagugucuauuagcugauuguU.....	5	1	seq
cagugucuauuagcugauugu.....	1	0	seq
cauacuaaaccucacugcc.....	2	0	seq
cauacuaaaccucacugccU.....	1	1	seq
cauacuaaaccucacugccau.....	1	0	seq
cauacuaaaccucacugccauc.....	18	0	seq
cauacuaaaccucacugccauU.....	3	1	seq
cauacuaaaccucacugccauca.....	1	0	seq
cauacuaaaccucacugccaucU.....	1	1	seq
aaucauaaaccucacugcc.....	1	0	seq
aaucauaaaccucacugccauU.....	2	1	seq
aaucauaaaccucacugccauc.....	46	0	seq

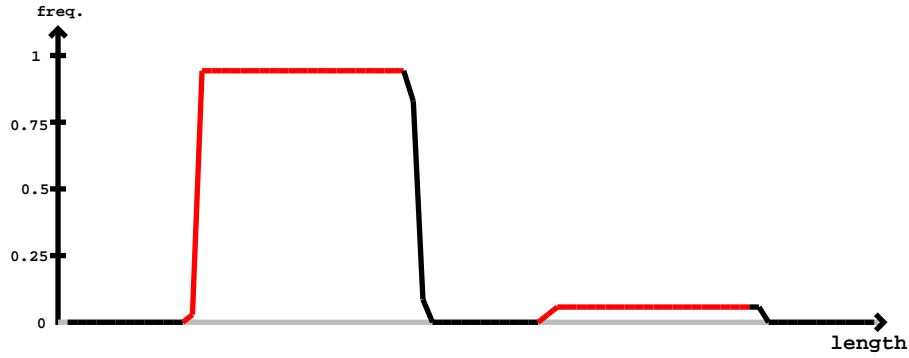
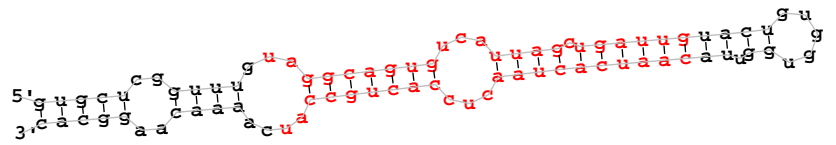
hsa-miR-34b-3p

hsa-miR-34b-5p

gugcucgguuuguaggcagugucauuagcugauuguacuguggguua**caauca**cuaacuccacugccaucaaaacaaggcac

.....aauca <u>cu</u> a <u>acuccacugccau</u> U.....	13	1	seq
.....aauca <u>cu</u> a <u>acuccacugccau</u> ca.....	1	0	seq
.....auca <u>cu</u> a <u>acuccacugccau</u> c.....	5	0	seq
.....auca <u>cu</u> a <u>acuccacugccau</u> ca.....	4	0	seq
.....auca <u>cu</u> a <u>acuccacugccau</u> ca.....	1	0	seq

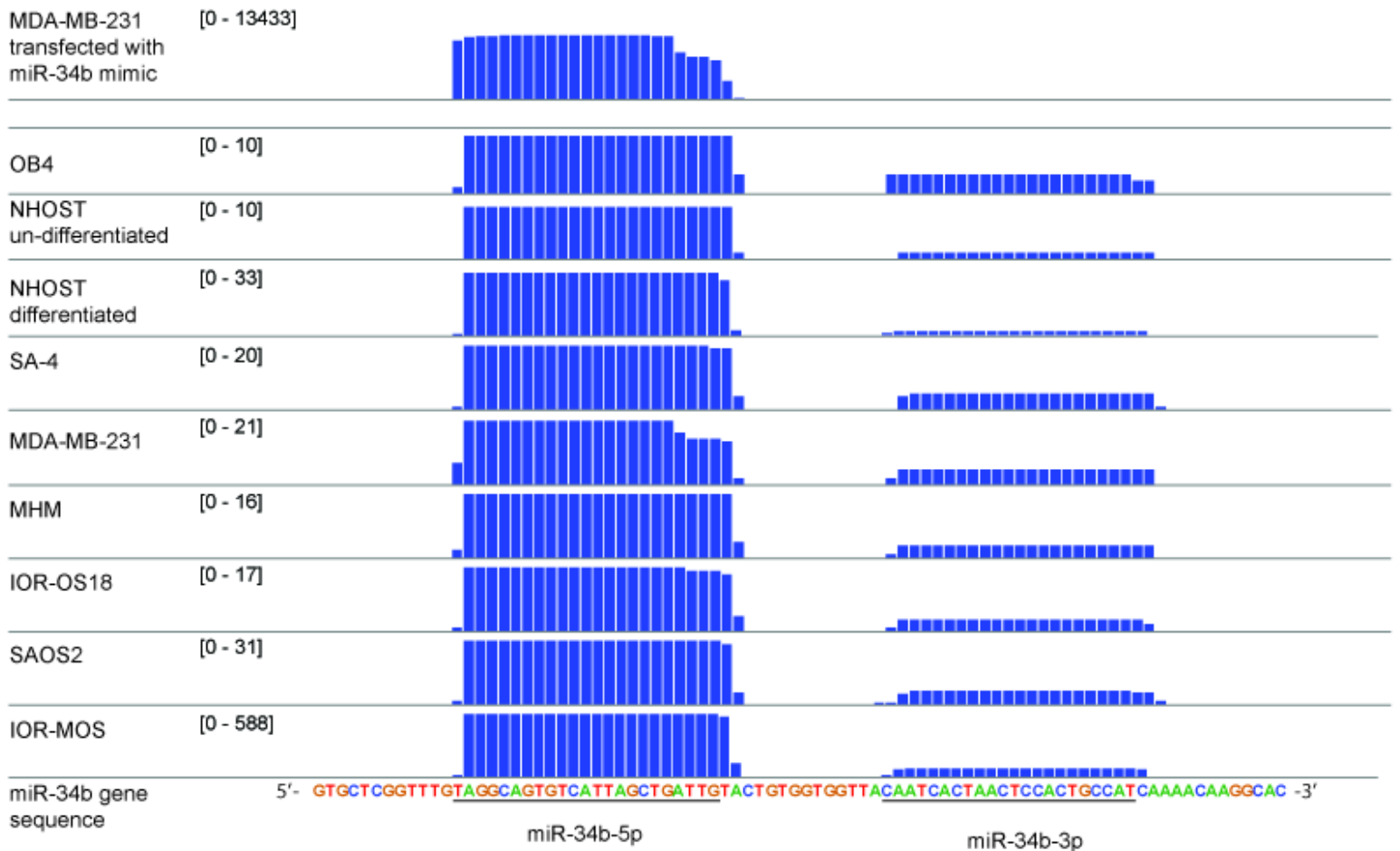
Cell line : NHOST differentiated
 miRBase precursor : hsa-mir-34b
 Total read count : 35
 hsa-miR-34b-3p read count : 2
 hsa-miR-34b-5p read count : 33
 remaining reads : 0



hsa-miR-34b-3p

hsa-miR-34b-5p

5'	seq	3'	exp	reads	mm	sample
5' -	gugcucgguuuuuaggcagugucauuagcugauuguacuguggguua <u>caaucacuaacuccacugcca</u> caaaaagggcac	-3'	exp			
	(((((.....(((.....(((.....(((.....))))))))).....)))))).....))))		reads	mm	sample	
uaggcagugucauuagcugauug.....		1	0	seq	
aggcagugucauuagcugauug.....		3	0	seq	
aggcagugucauuagcugauugu.....		26	0	seq	
aggcagugucauuagcugauugua.....		3	0	seq	
caaucacuaacuccacugccauc.....		1	0	seq	
aaucaacuaacuccacugccauc.....		1	0	seq	



Supplementary data S7: Raw sequencing reads of mature miR-34b from both non-malignant and malignant cells.

Bars indicating the percentage of reads for the individual bases in the miR-34b gene sequence, obtained from raw sequencing data of MDA-MB-231 and noncancerous mesenchymal stromal cells (NHOST) before and after osteogenic differentiation, osteoblast primary culture (OB4), and liposarcoma (SA-4) and osteosarcoma cell lines (MHM, IOR-OS18, SAOS2, IOR-MOS). The annotated sequence is underscored. The numbers in brackets represent the number of total reads for each cell line.