

Regulation of miR-34a

Miranda Houtman

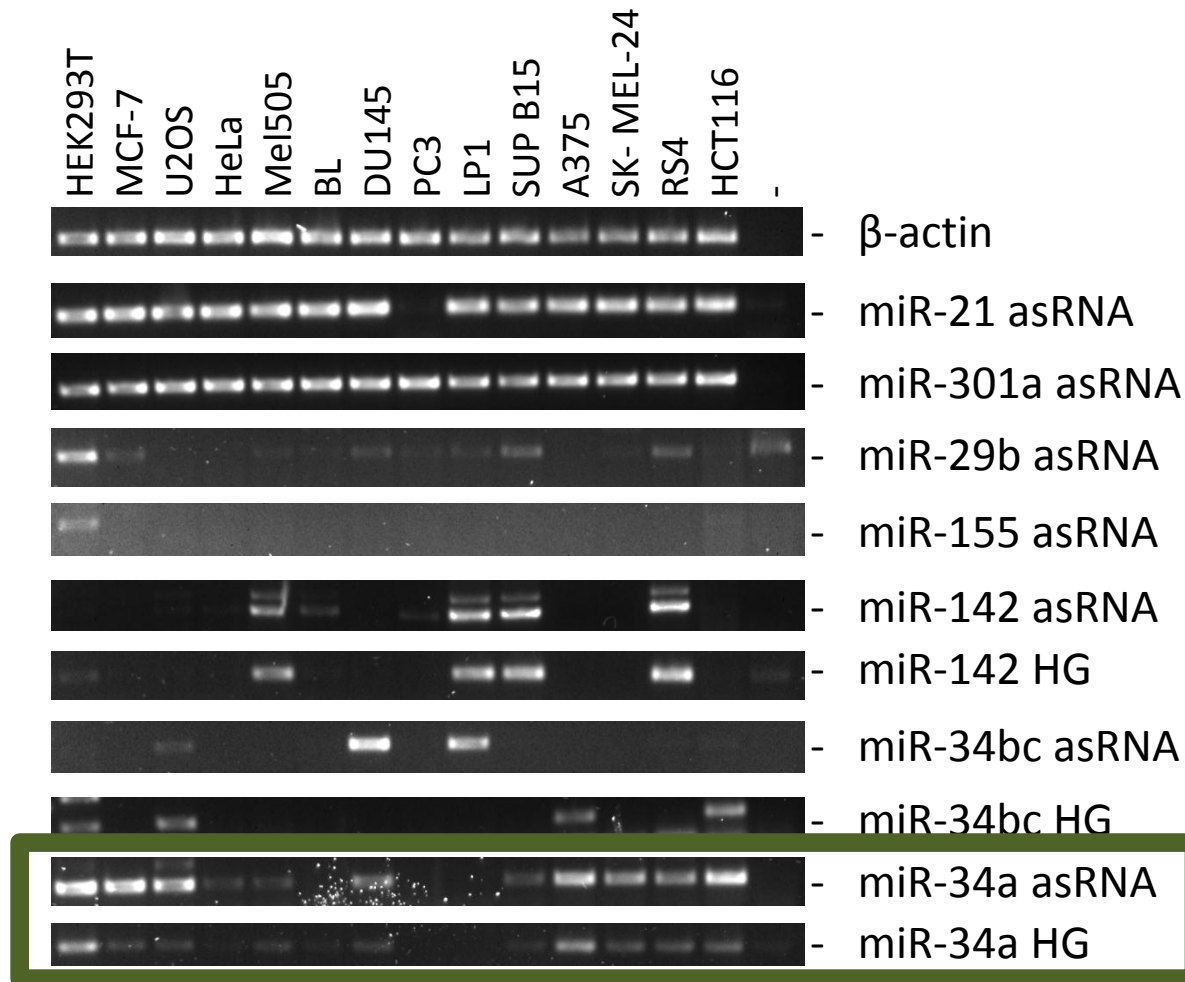
2012-06-04

Introduction

- ~70% of the protein encoding genes have an antisense encoded transcript
- asRNAs regulate protein encoding genes
 - p21, p53, Ecad

Are miRNA host genes (HG) regulated by asRNAs?

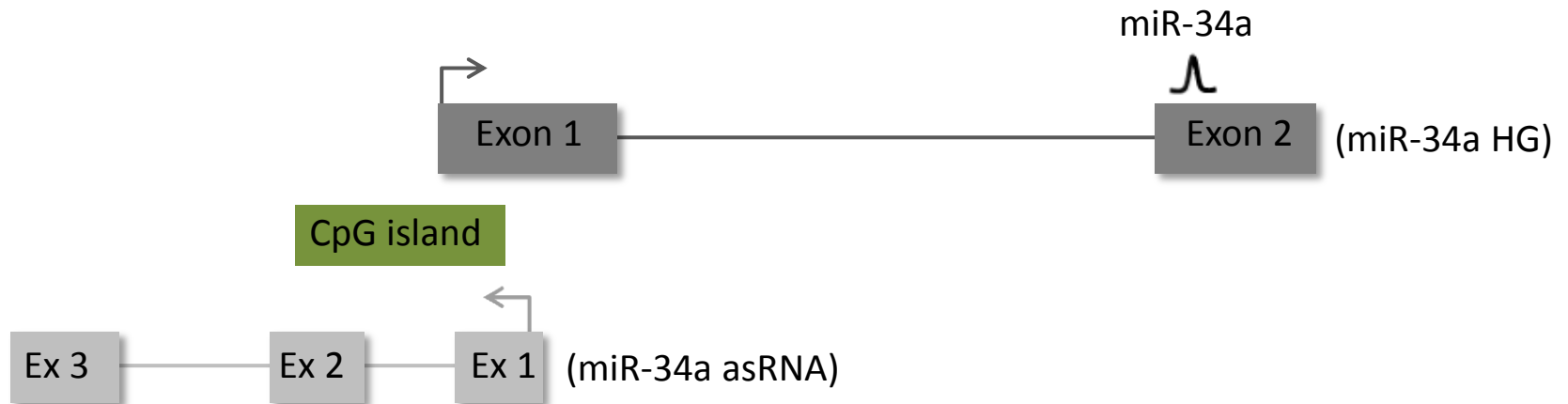
Identification of miRNA genes with asRNA transcripts



Is miR-34a HG regulated by miR-34a asRNA?

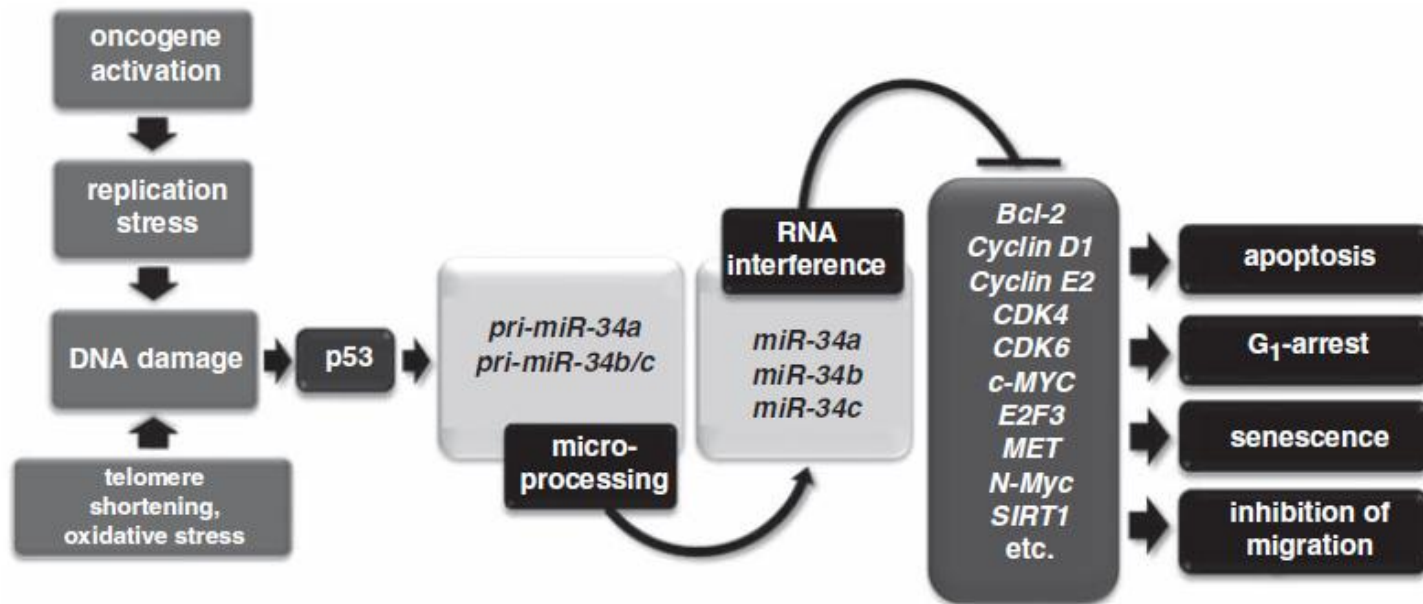
Background: miR-34a

- Localizes to chromosome 1p36
- Non-coding HG, non-coding asRNA



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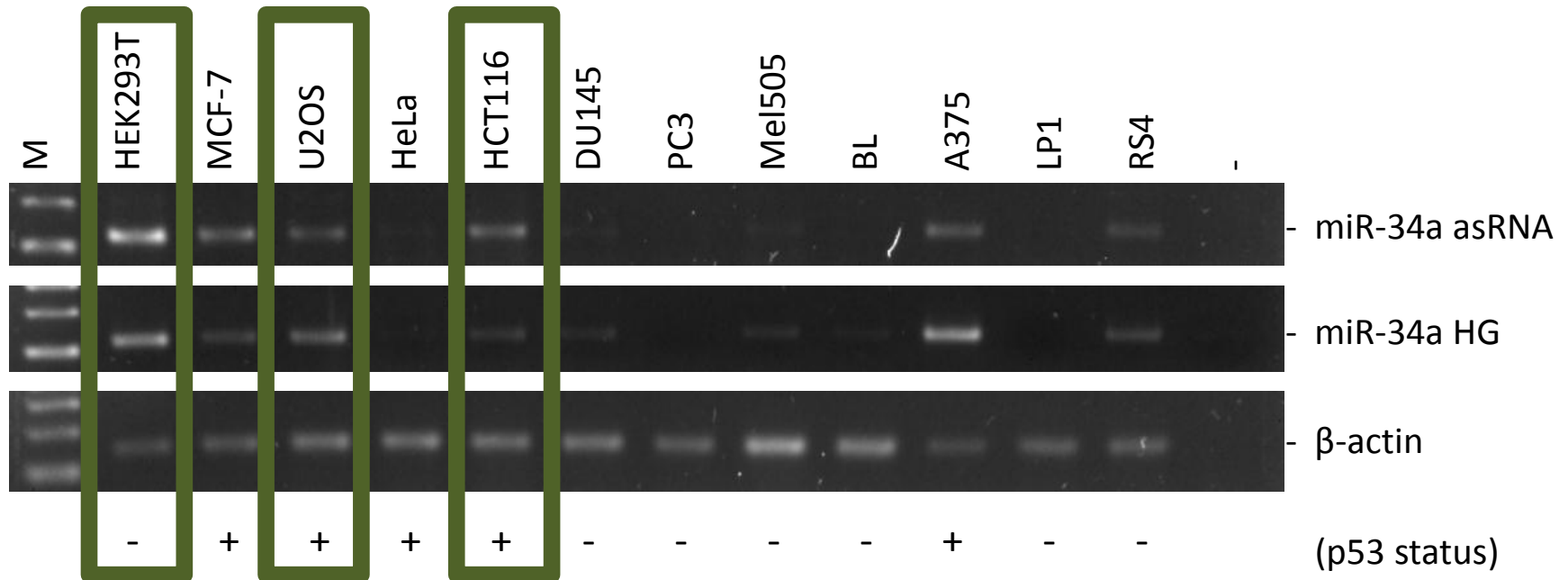
- Localizes to chromosome 1p36
- Non-coding HG, non-coding asRNA
- Direct target of p53



(Hermeking, 2010)

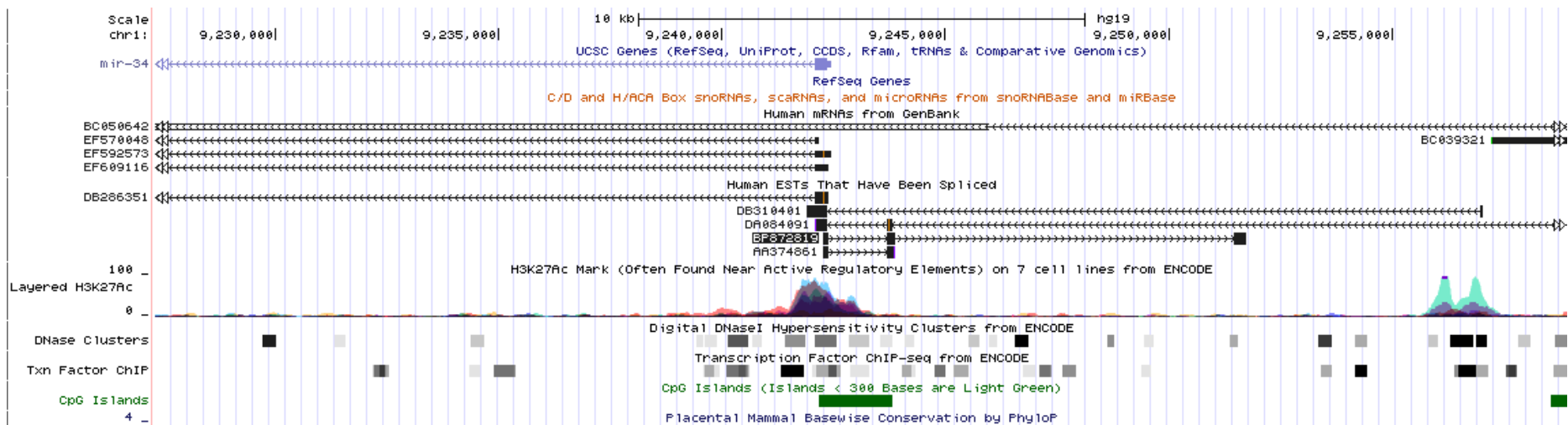
Results

miR-34a expression in different cell lines



→ miR-34a asRNA and HG are co-expressed

Is miR-34a asRNA a real asRNA?



Is miR-34a asRNA a real asRNA?

- Strand specific PCR

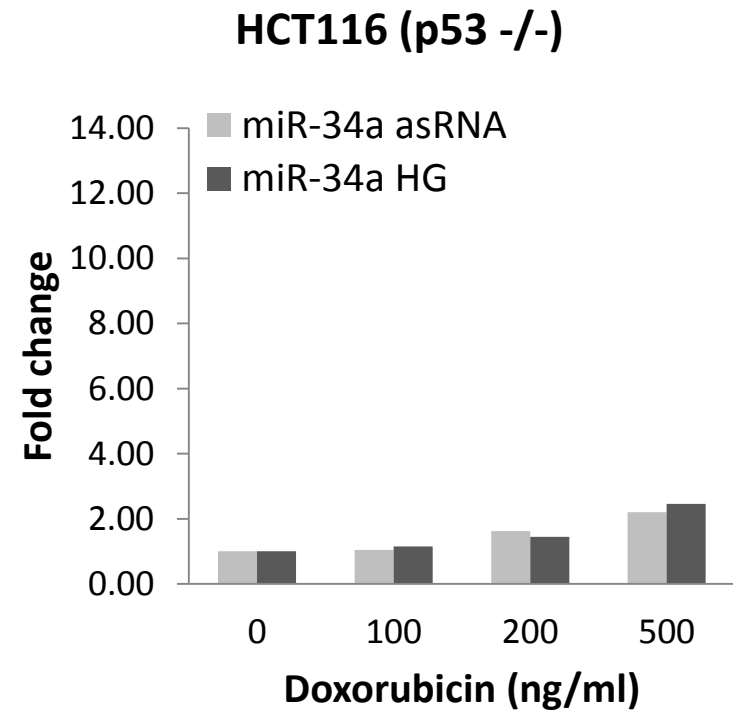
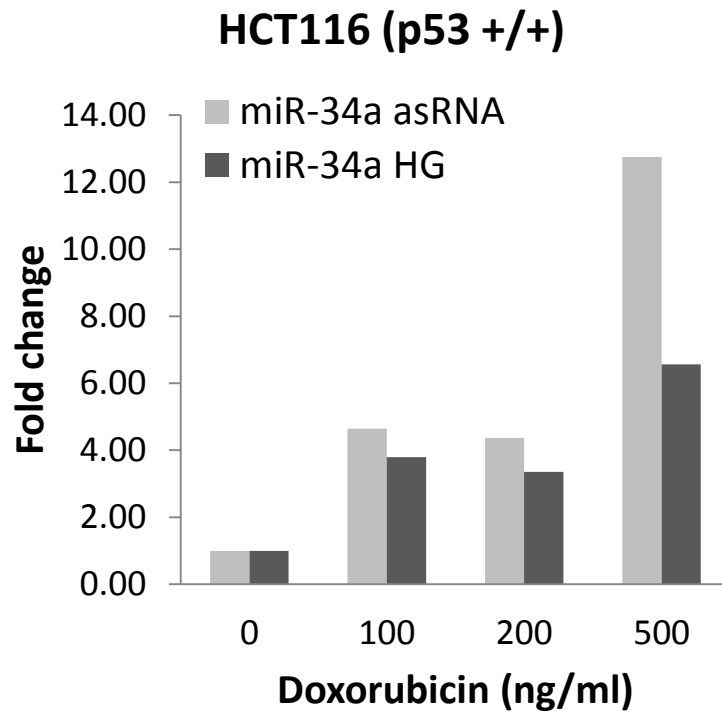


Many asRNAs have been described, but their transcriptional regulation is largely unknown

- miR-34a is a transcriptional target of p53
- What about miR-34a asRNA?

Is miR-34a asRNA a target of p53?

- Different concentrations of doxorubicin (24 h)

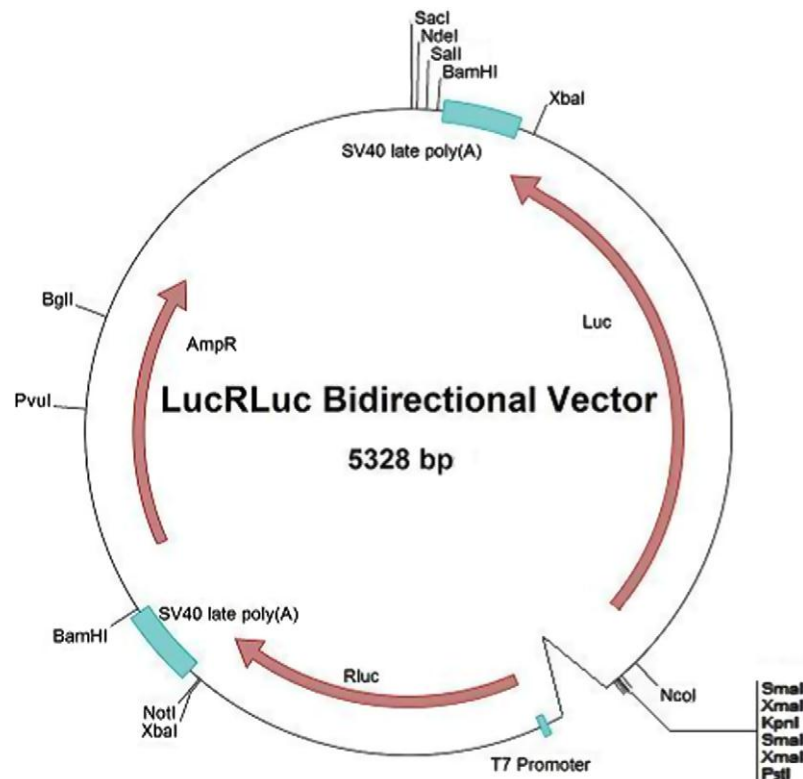


→ miR-34a asRNA is also a target of p53

Is miR-34a asRNA regulated by the same promoter?

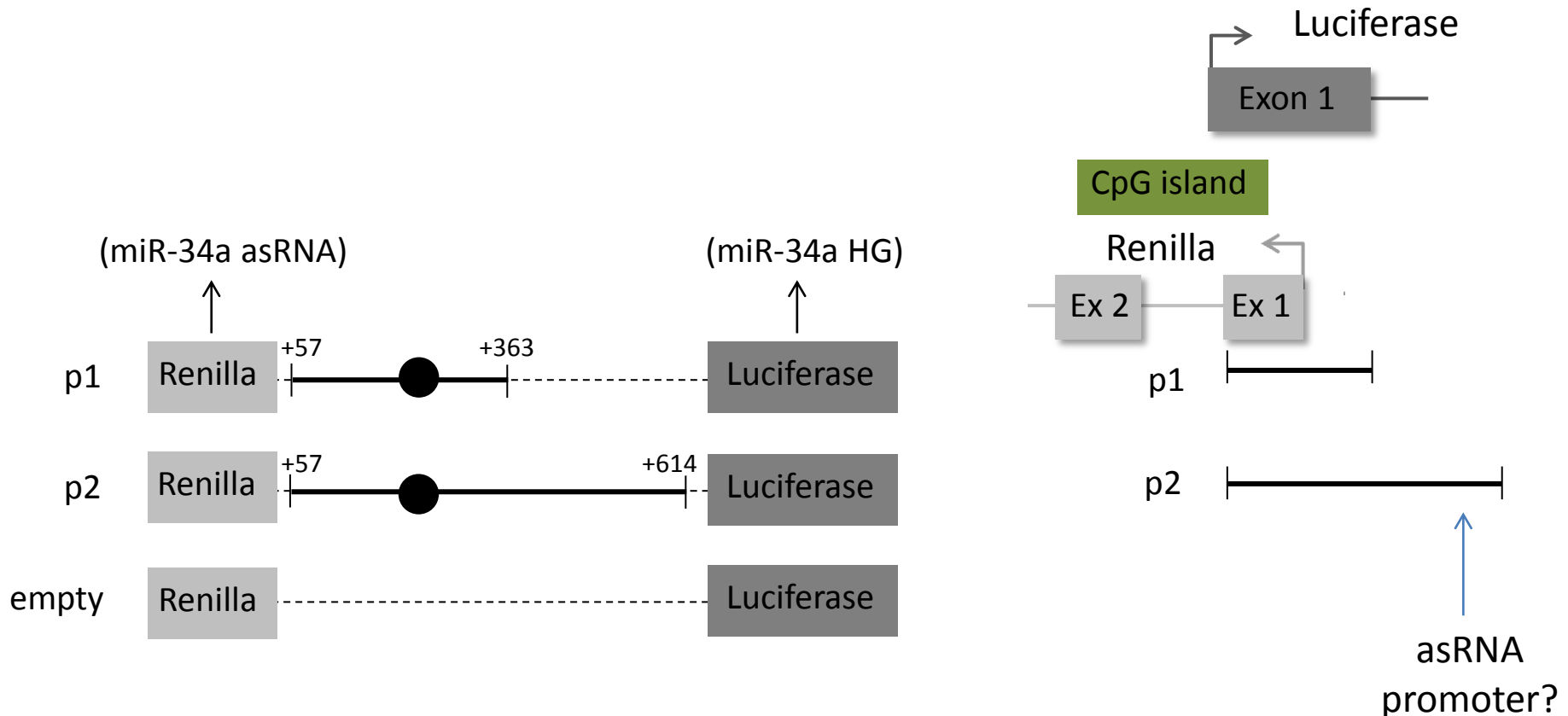
Is miR-34a asRNA regulated by the same promoter?

- Clone constructs into a bidirectional expression vector



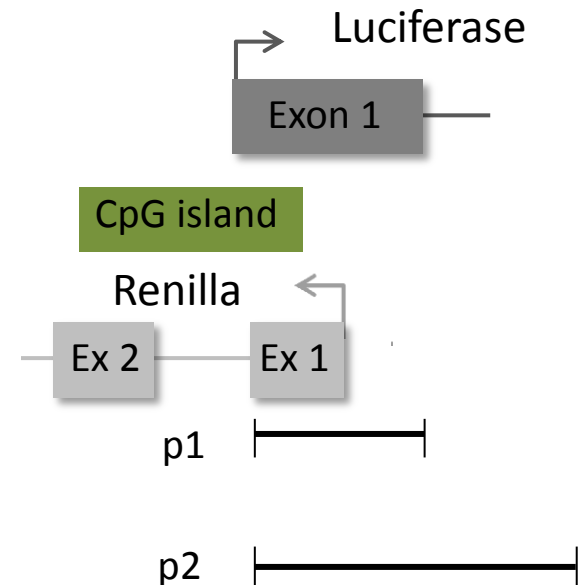
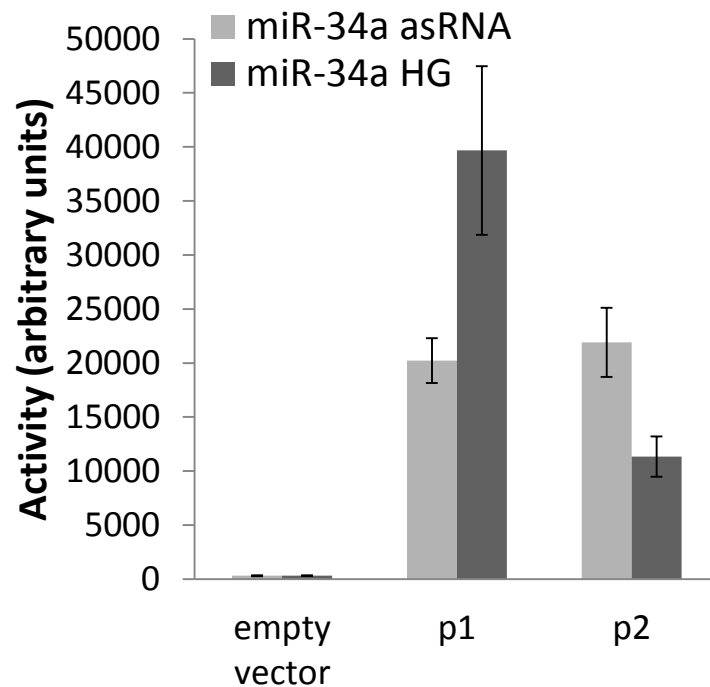
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Is miR-34a asRNA regulated by the same promoter?

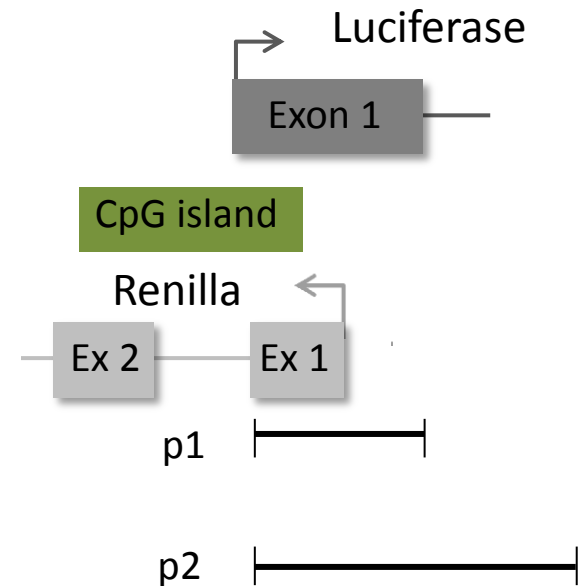
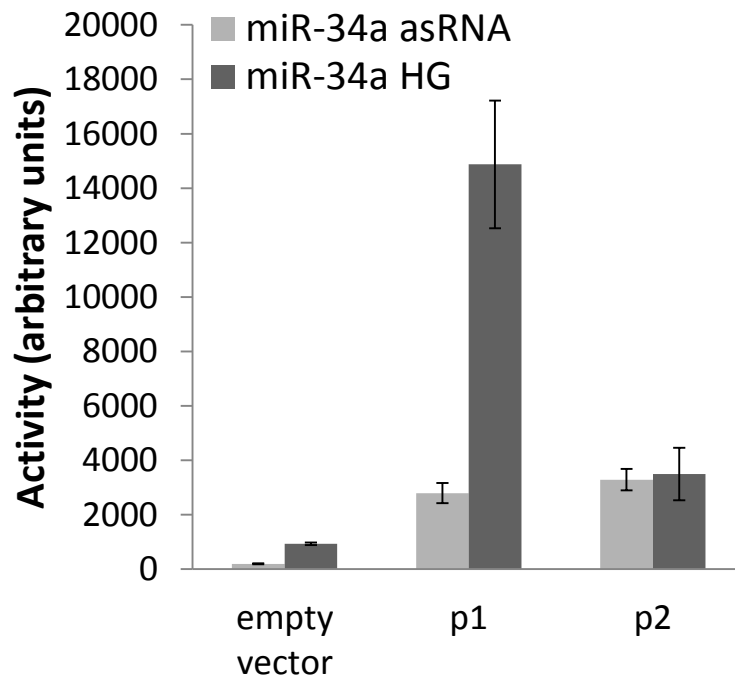
- Transfect vector into HCT116 cells (p53 +/+)



→ miR-34a asRNA is regulated by the same promoter₁₆

Is miR-34a asRNA regulated by the same promoter?

- Transfect vector into HEK293T cells (p53 inactivated)

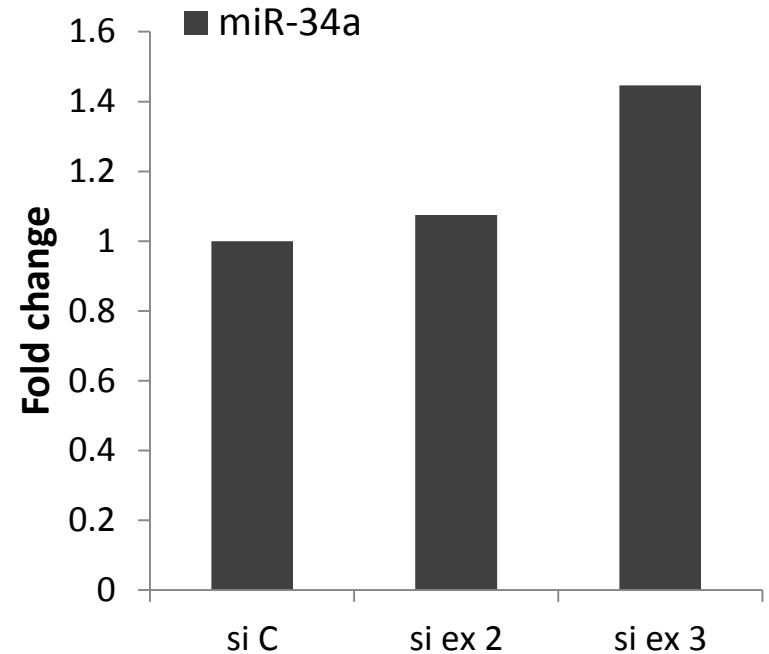
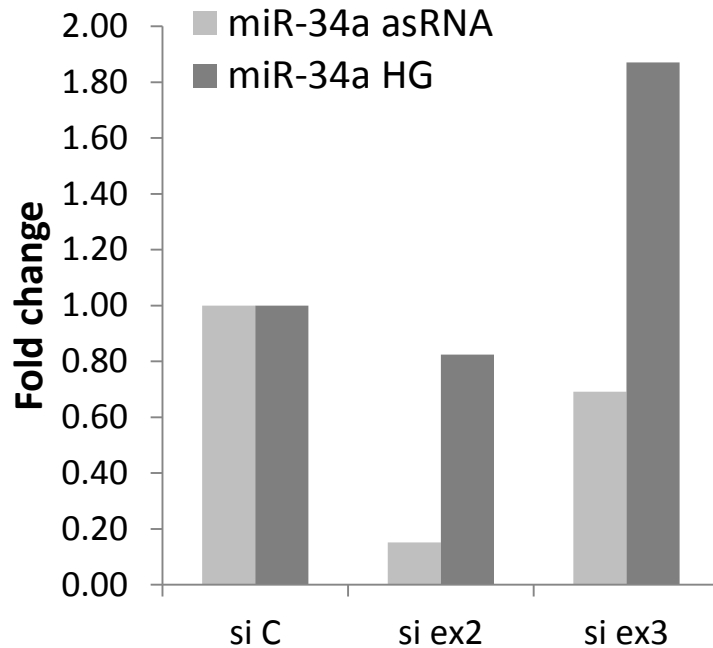


→ miR-34a asRNA is regulated by the same promoter, not restricted to p53 expression

What is the effect of miR-34a asRNA knockdown?

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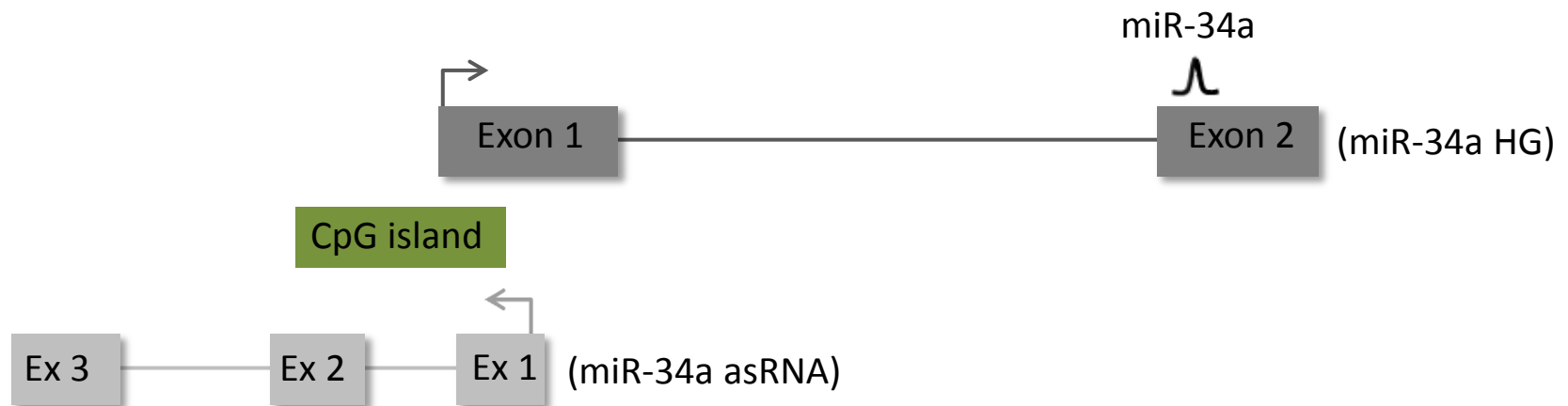
- miR-34a asRNA knockdown in U2OS cells (10 nM; 72 h)

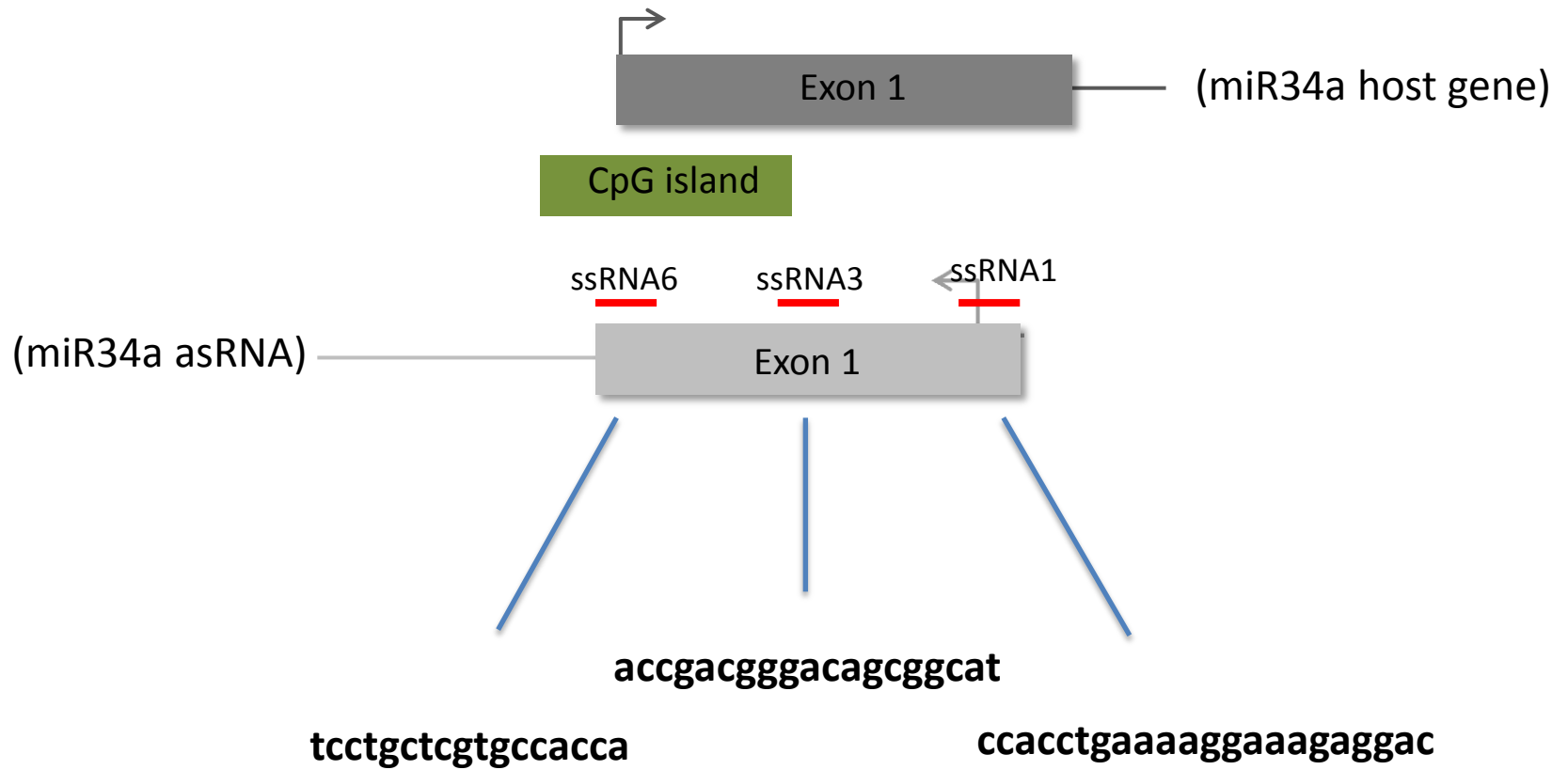


→ Induction of miR-34a after KD of asRNA

We were interested in targeting of the miR-34a HG and asRNA overlapping region (exon1)

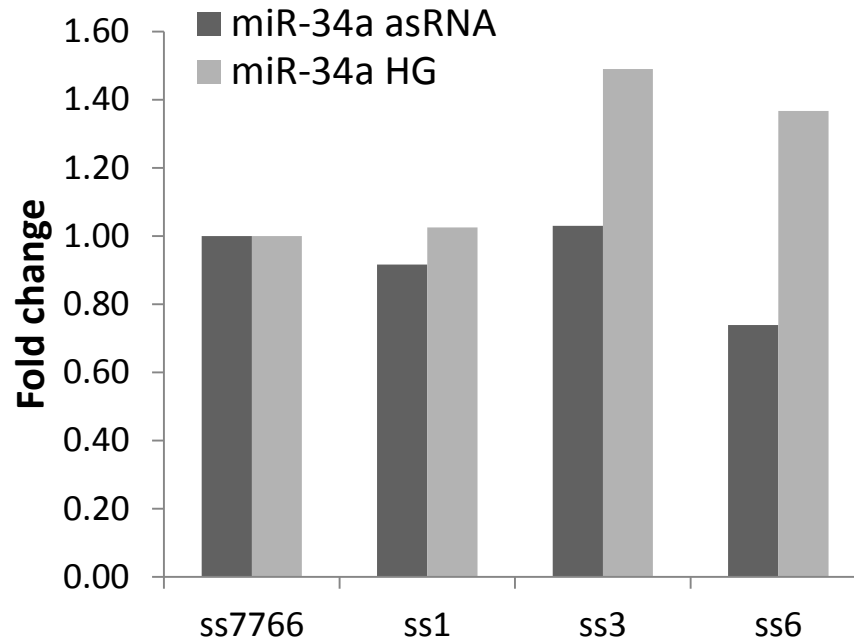
- siRNAs are not strand specific
- U6 encoded single stranded RNAs
→ nuclear expressed transcripts





What is the effect of ssRNAs targeting exon 1 (asRNA)?

- Transfect ssRNAs into HEK293T cells

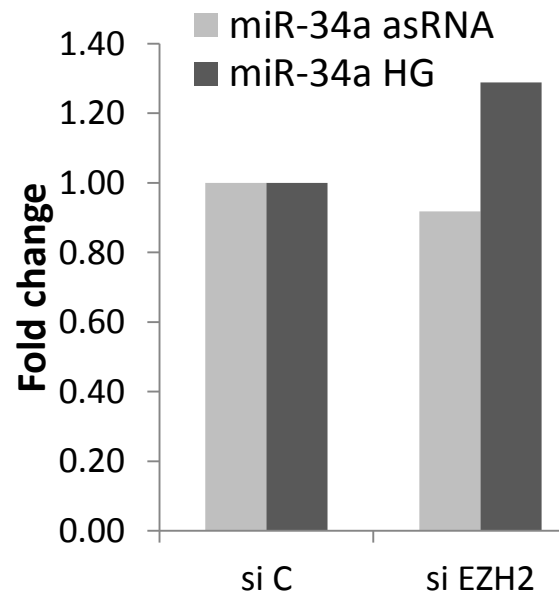


→ Induction of miR-34a HG

A hint into the molecular mechanism...

What is the effect of EZH2 knockdown?

- EZH2 (a known asRNA mediator) knockdown in U2OS



→ Possibly activation of miR-34a after KD of EZH2

Conclusions

- Many microRNA host genes encodes asRNAs
- miR-34a asRNA and HG are co-expressed
- miR-34a promoter is bidirectional
- miR-34a asRNA is a target of p53
- Knockdown of miR-34a asRNA induces the HG

miR-34a HG is negatively regulated by miR-34a asRNA

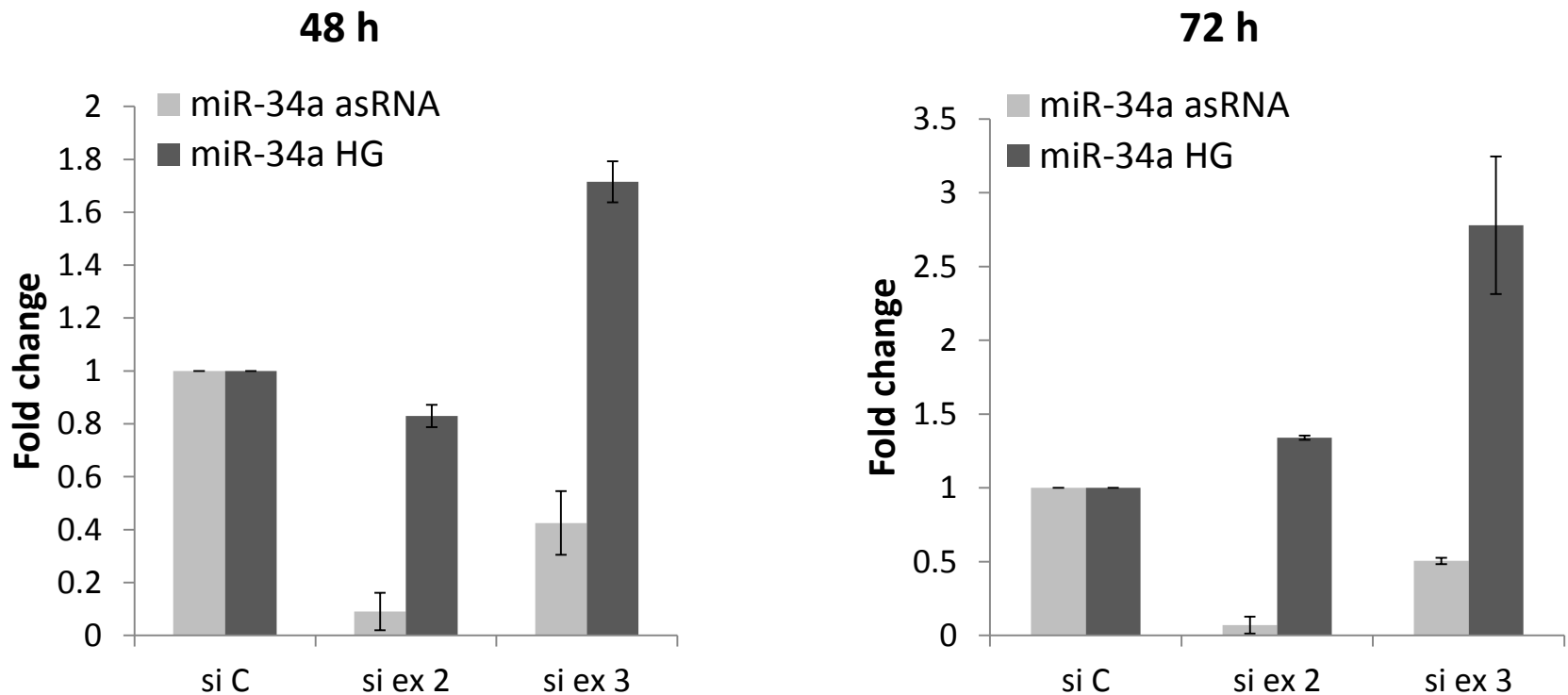
Upcoming experiments

- Doxorubicin treatment after asRNA knockdown
- asRNA knockdown followed by WB for validated miR-34a targets (BCL2)
- Overexpression of miR-34a asRNA
- Generate stable cell lines with knockdown of miR-34a asRNA
- Investigate the asRNA mechanism in more detail

Questions?

What is the effect of miR-34a asRNA knockdown?

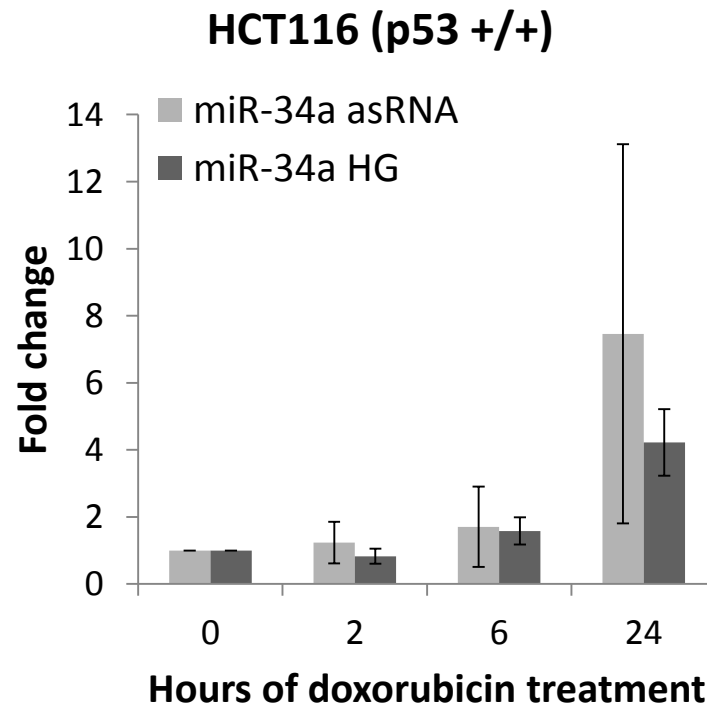
- miR-34a asRNA knockdown in U2OS cells (15 nM)



→ Induction of miR-34a HG

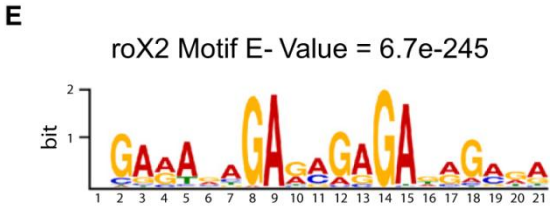
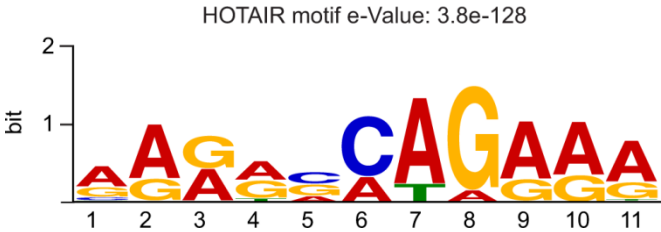
Is miR-34a asRNA a target of p53?

- Time course experiment with doxorubicin (200 ng/ml)

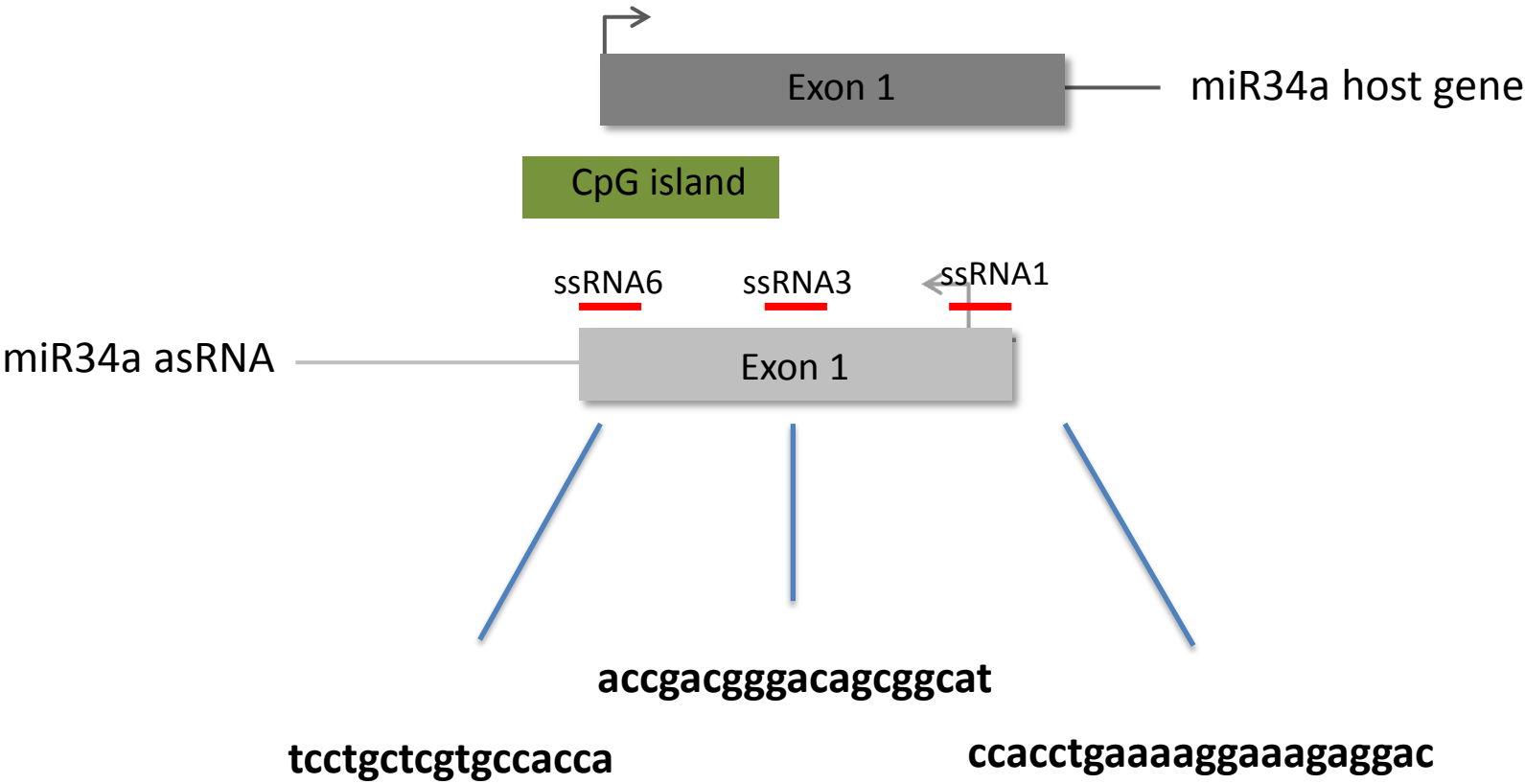


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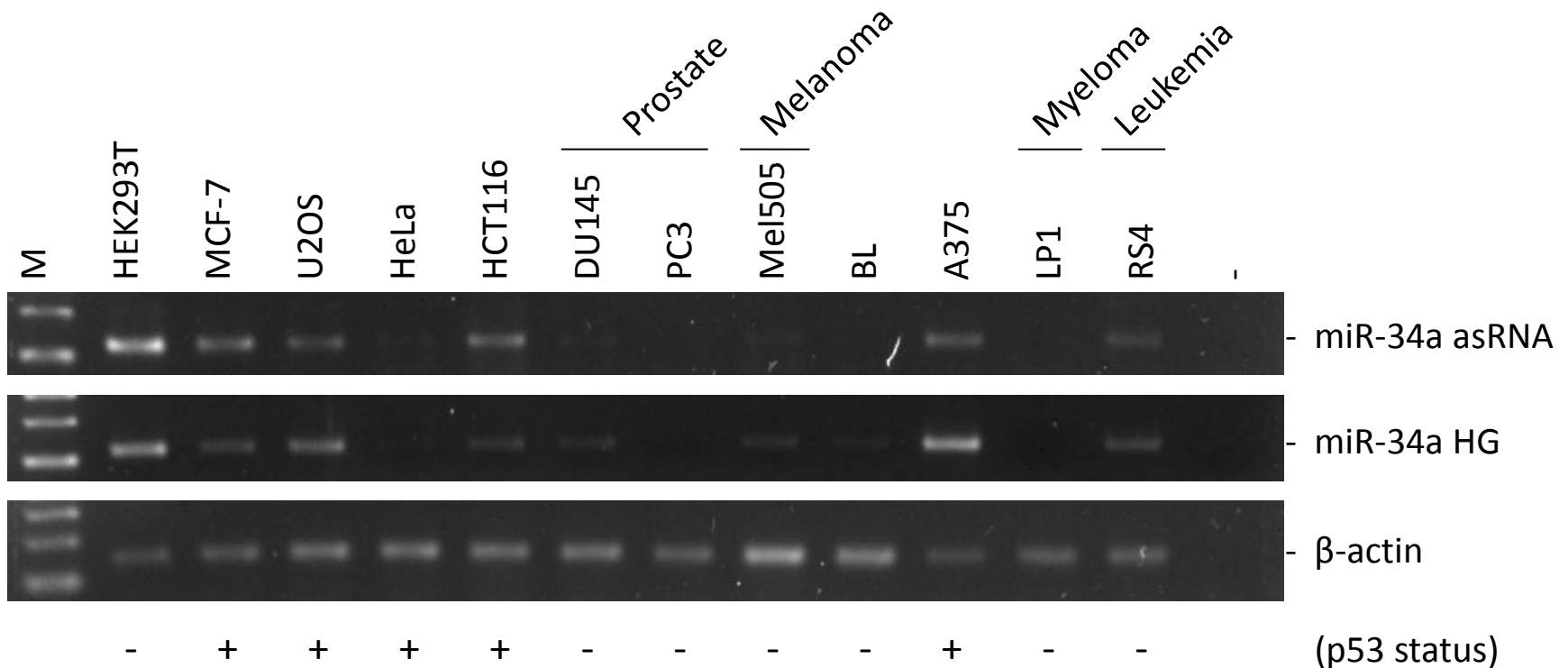
Genome wide studies have identified active elements for ncRNA / asRNAs



Kevin Morris have designed an algorithm for the identification of active elements;



miR-34a expression in different cell lines



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