

# **Non-canonical human microRNAs:**

## **Characterization of the mirtron pathway in higher eukaryotes**

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# Mirtrons

➤ first described in *D. melanogaster* and *C. elegans*

(Ruby et al., Nature, 2007; Okamura et al., Cell, 2007)

➤ special miRNAs:

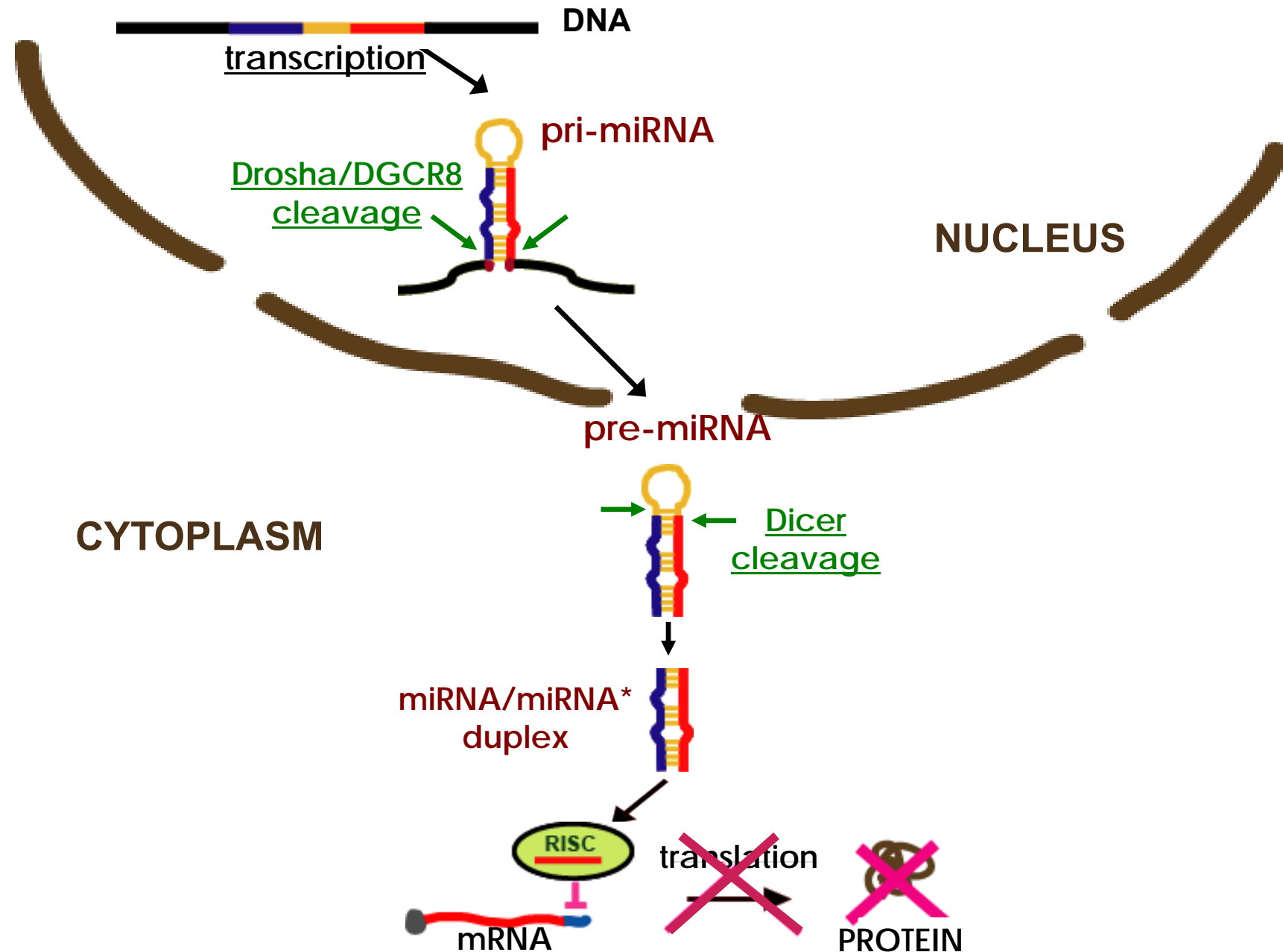
=> localization in small introns (50-100nt)

=> non-canonical biogenesis

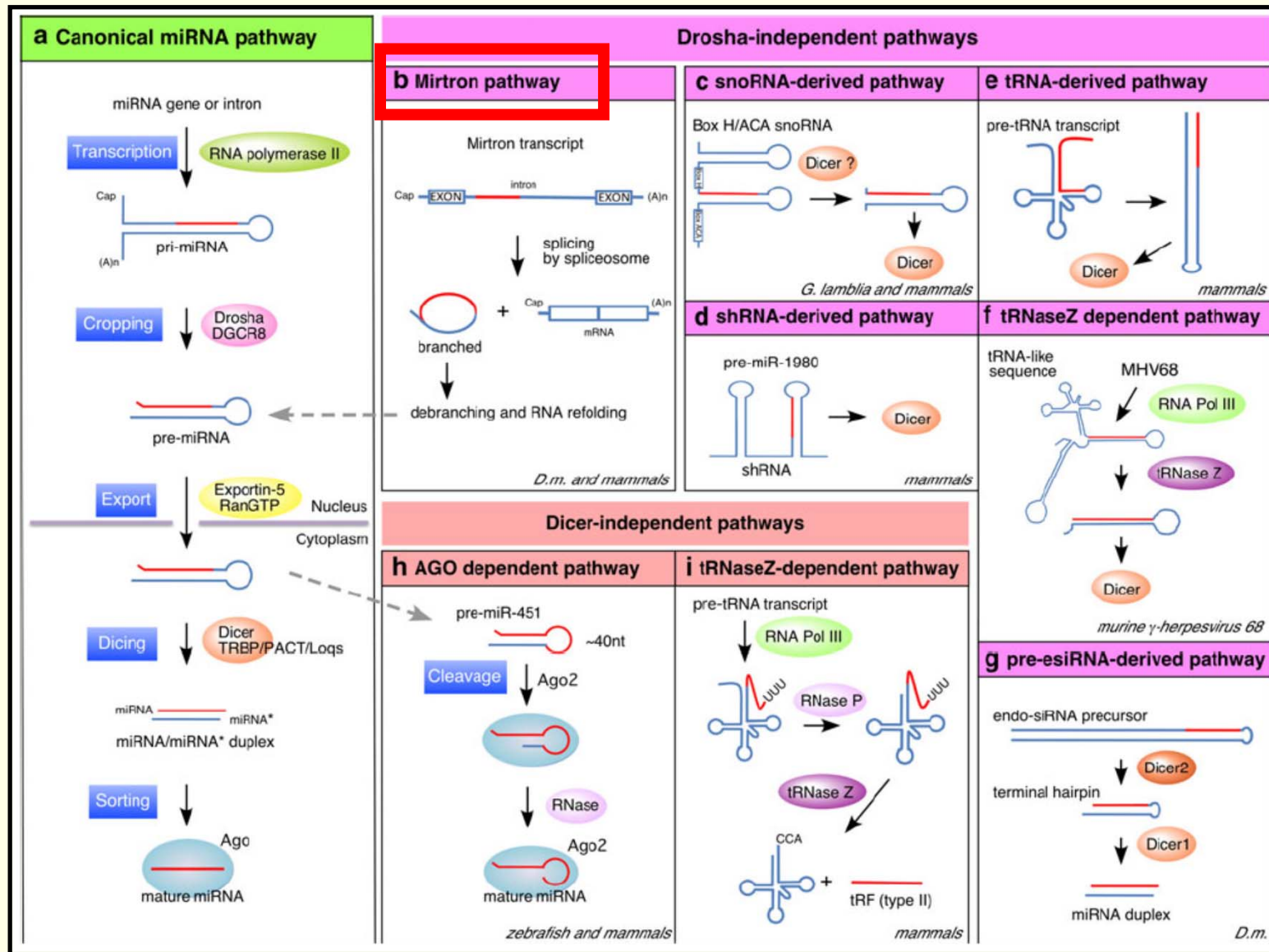
# miRNAs

- **~20-24nt endogenous, non-coding RNAs**
- **important roles in many cellular processes**  
(e.g. regulation of ontogenesis, cell proliferation, differentiation, apoptosis, cancer and metabolic diseases )
- **form a regulatory network similar to that of transcription factors**
- **regulatory function by post-transcriptional gene silencing**
  - **translational repression**
  - >> mRNA degradation

# Canonical miRNA biogenesis



# Various miRNA biogenesis pathways

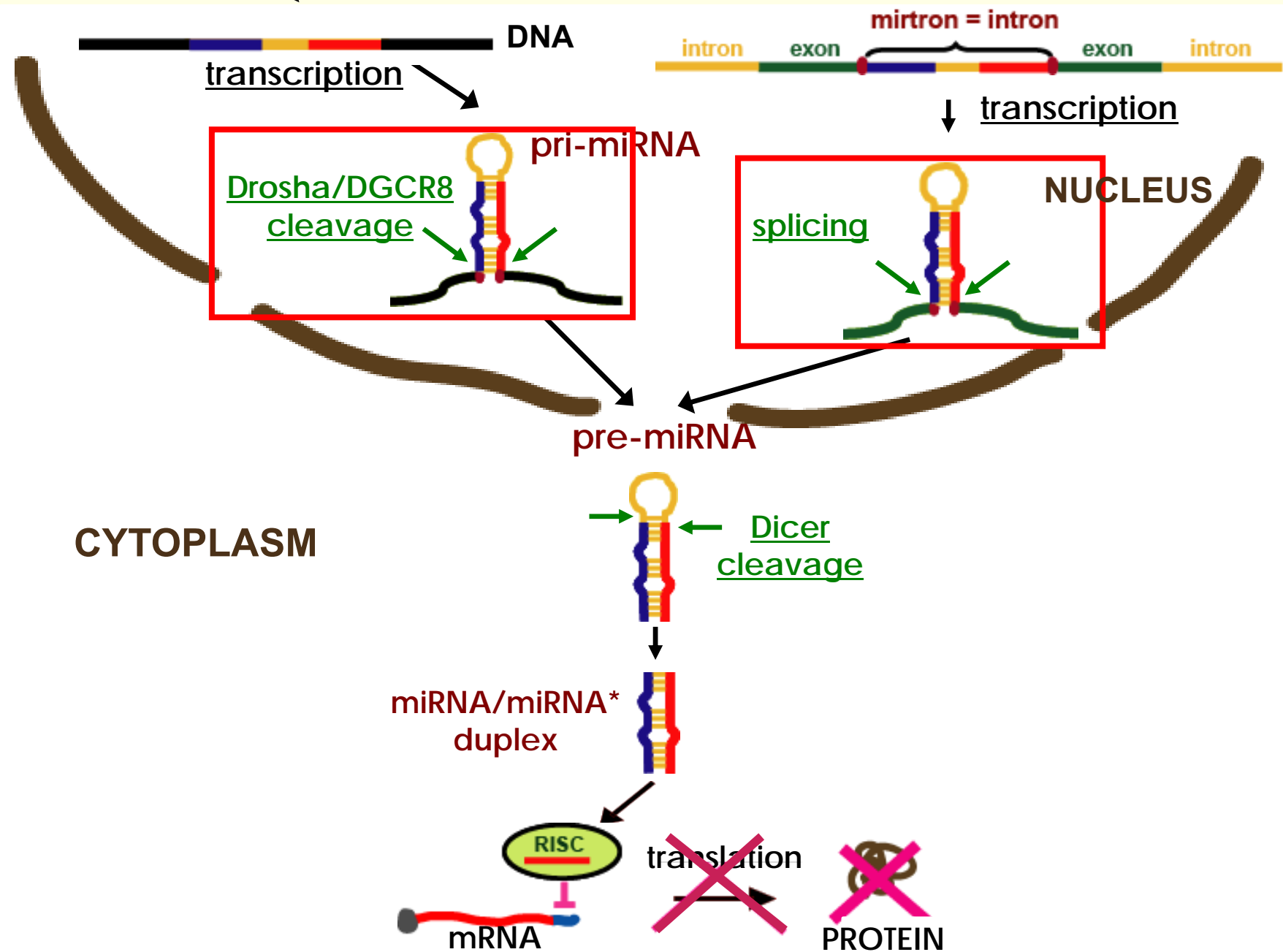


Miyoshi et al., Mol Genet Genomics (2010) 284:95.

# miRNA biogenesis

canonical

mirtron



# Mirtrons

➤ discovery and experimental validation in *D.m.* and *C.e.*

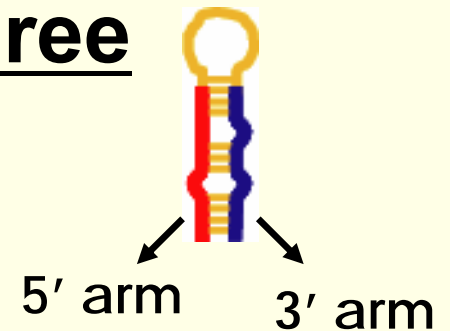
=> short average intron length ( = ~ pre-miRNA)

➤ in mammals (long average intron length) :

- bioinformatic predictions for mirtrons

=> is this alternative miRNA maturation pathway exist in human?

# Evolutional conservation of three predicted mirtrons

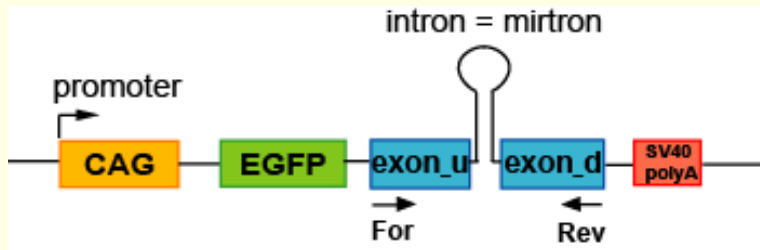


	mature miRNA	mir-877	miRNA*	
hs a- mi r- 877	GUAGAGGAGAUGGCCGACAGGGGACACGGGCAAGACUUGGGGUUCCUGGGACCCUCAGACGUGUGUCCUCUUCUCCUCCUCCAG			86
ppy- mi r- 877	GUAGAGGAGAUGGCCGACAGGGGACACGGGCAAGACUUGGGGUUCCUGGGACCCUCAGACGUGUGUCCUCUUCUCCUCCUCCAG			86
mml- mi r- 877	GCUAGAGAAGGUAGAGGAGAUGGCCGACAGGGGACACGGGCUAAGACUUGGGGUUCCUGGGACCCUCAGACGUGUGUCCUCUUCUCCUCCUCCAGGUGUAUG			103
bt a- mi r- 877	GCUCGAGAAGGUAGAGGAGAUGGCCGACAGGGGACACGGGCUAAGACUUGGGGUUCCUGGGACCCUCAGACGUGUGUCCUCUUCUCCUCCUCCAGGUGUACG			102
mmu- mi r- 877	GUAGAGGAGAUGGCCGACAGGGGACACAAAGGUAGGACUUGGGGUUCCUGGGACCCUCAGACGUGUGUCCUCUUCUCCUCCUCCUCCAG			85
r no- mi r- 877	GUAGAGGAGAUGGCCGACAGGGGACACAAAGGUAGGACUUGGGGUUCCUGGGACCCUCAGACGUGUGUCCUCUUCUCCUCCUCCUCCAG			85
conserved	*****			
	miRNA*	mir-1226	mature miRNA	
hs a- mi r- 1226	GUGAGGGCAUGCAGGCCUGGAUGGGGCAGCUGGGAUGGUCCAAAAGGGUGGCCUCACCAGCCUGUGUUCCCUAG			75
ppy- mi r- 1226	GUGAGGGCAUGCAGGCCUGGAUGGGGCAGCUGGGAUGGUCCAAAAGGGUGGCCUCACCAGCCUGUGUUCCCUAG			75
mml- mi r- 1226	GUGAGGGCAUGCAGGCCUGGAUGGGGCAGCUGGGAUGGUCCAAAAGGGUGGCCUCACCAGCCUGUGUUCCCUAG			75
pt r- mi r- 1226	GUGAGGGCAUGCAGGCCUGGAUGGGGCAGCUGGGAUGGUCCAAAAGGGUGGCCUCACCAGCCUGUGUUCCCUAG			75
conserved	*****			
		mir-1233	mature miRNA	
hs a- mi r- 1233- 1	GUGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCUAUGGGAGGGGCCCCAGCCUCUGAGCCCUUCCUCCCGCAG			82
hs a- mi r- 1233- 2	GUGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCUAUGGGAGGGGCCCCAGCCUCUGAGCCCUUCCUCCCGCAG			82
ppy- mi r- 1233	GUGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCUAUGGGAGGGGCCCCAGCCUCUGAGCCCUUCCUCCCGCAG			82
mml- mi r- 1233	GUGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCUUCCGGAGGGGCCCCAGCCUCUGAGCCCUUCCUCCCGCAG			82
pt r- mi r- 1233- 1	GUGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCGUCGGAGGGGCCCCAGCAUCUGAGCCCUUCCUCCCGCAGG			83
pt r- mi r- 1233- 2	GUGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCGUCGGAGGGGCCCCAGCCUCUGAGCCCUUCCUCCCGCAGG			83
pt r- mi r- 1233- 3	UGAGUGGGAGGCCAGGGCAAGGCAGGGGGAGCUGCAGGGGCGUCGGAGGGGCCCCAGCCUCUGAGCCCUUCCUCCCGCAG			80
conserved	*****			

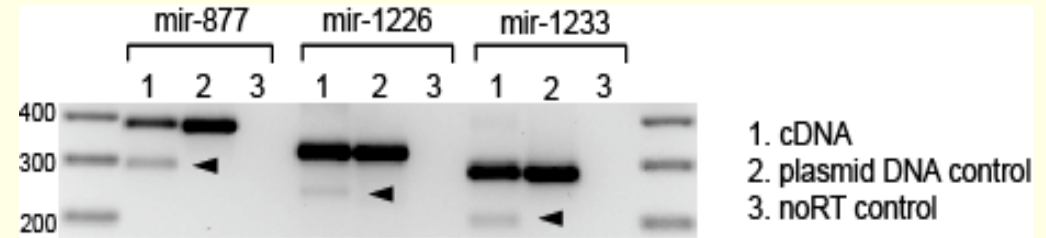


# Original exonic environment

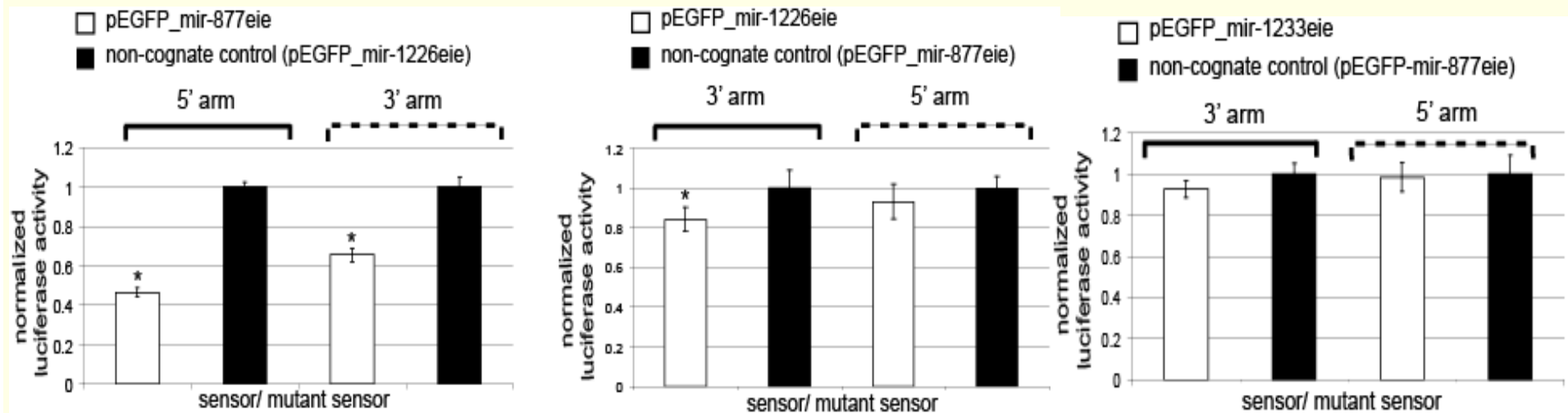
## ➤ Construct



## ➤ Splicing (RT-PCR)



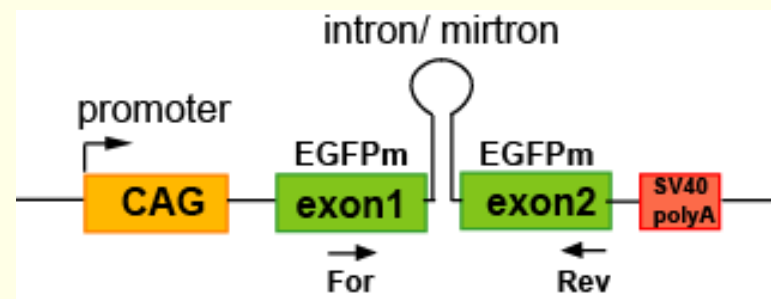
## ➤ Luciferase assay



# Role of flanking exons?

= > heterologous (GFP) exonic environment

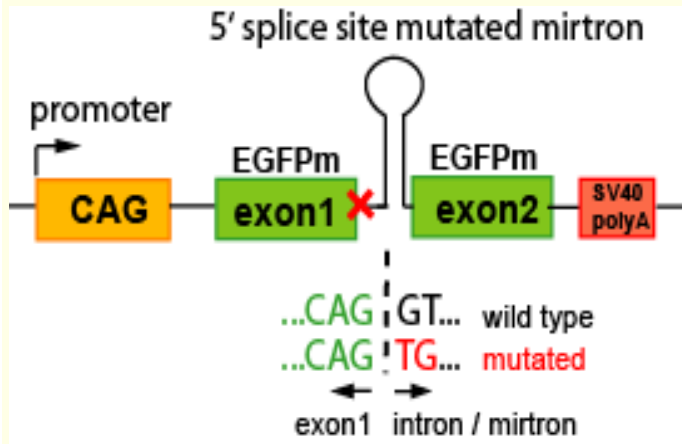
➤ Construct



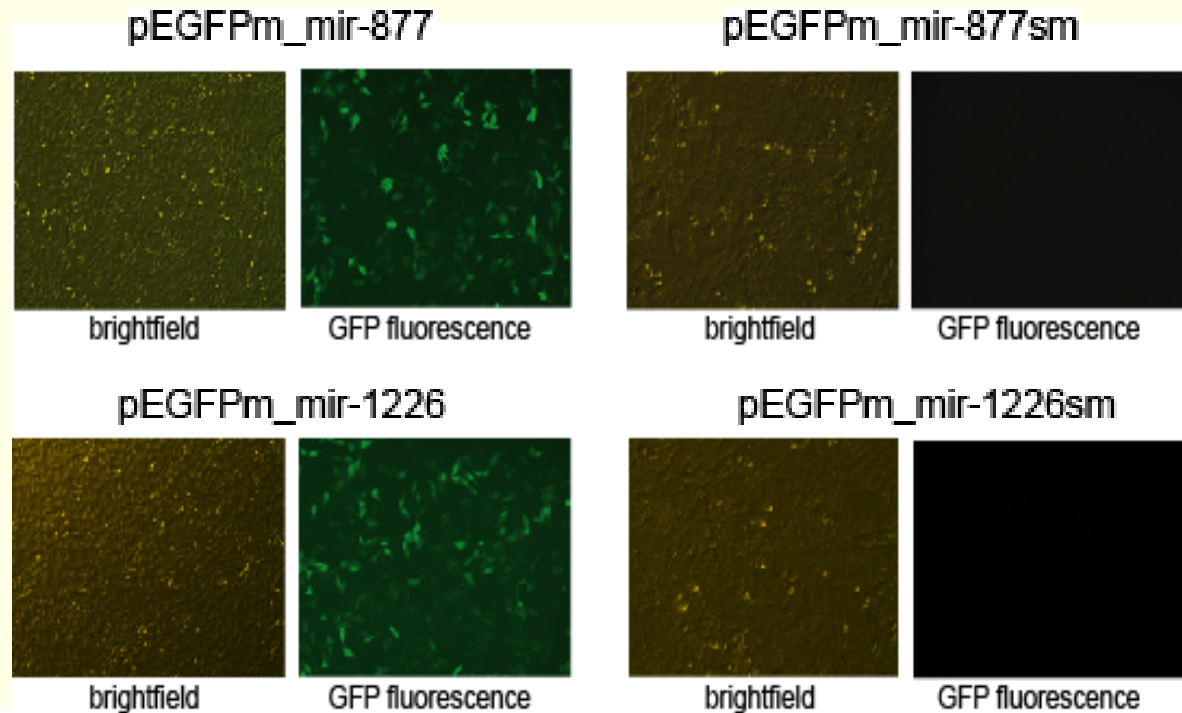
Essentially same results as from the original environment

# Splicing dependency I.

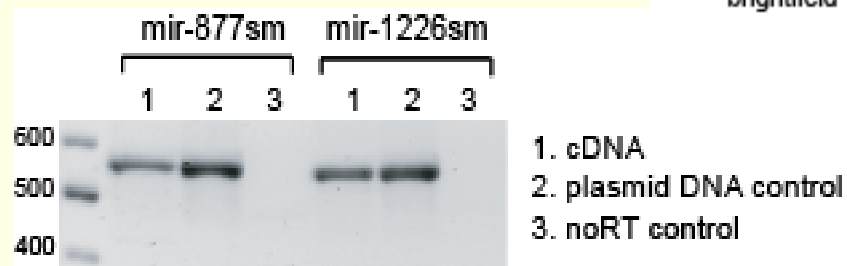
## ➤ Construct



## ➤ Fluorescent microscopy



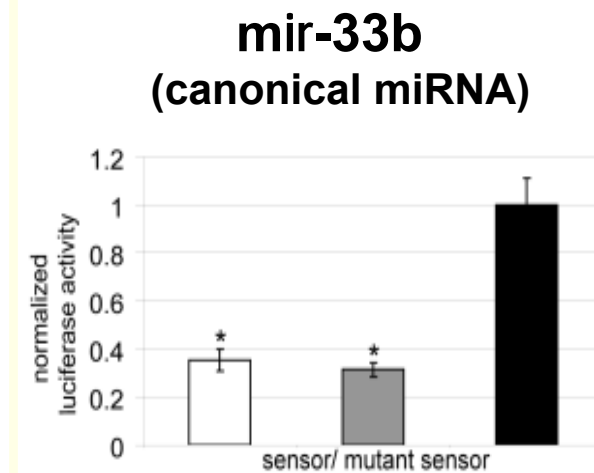
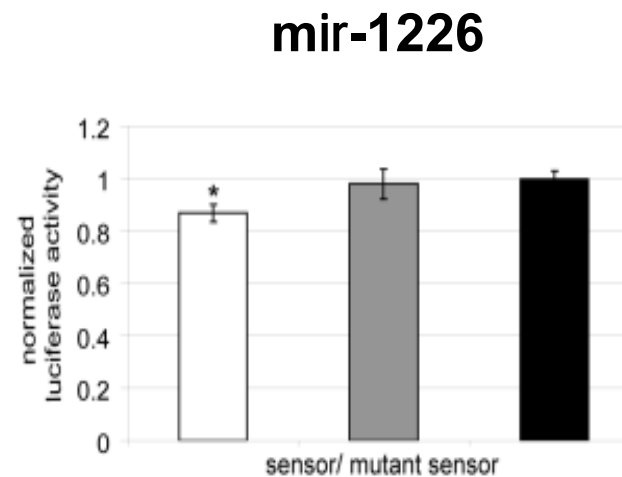
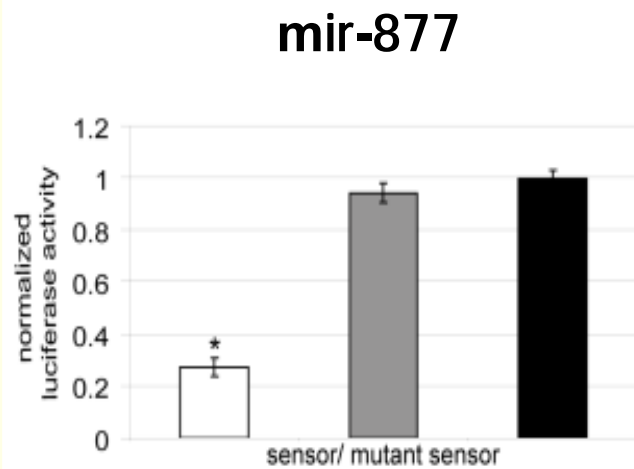
## ➤ Splicing (RT-PCR)



# Splicing dependency II.

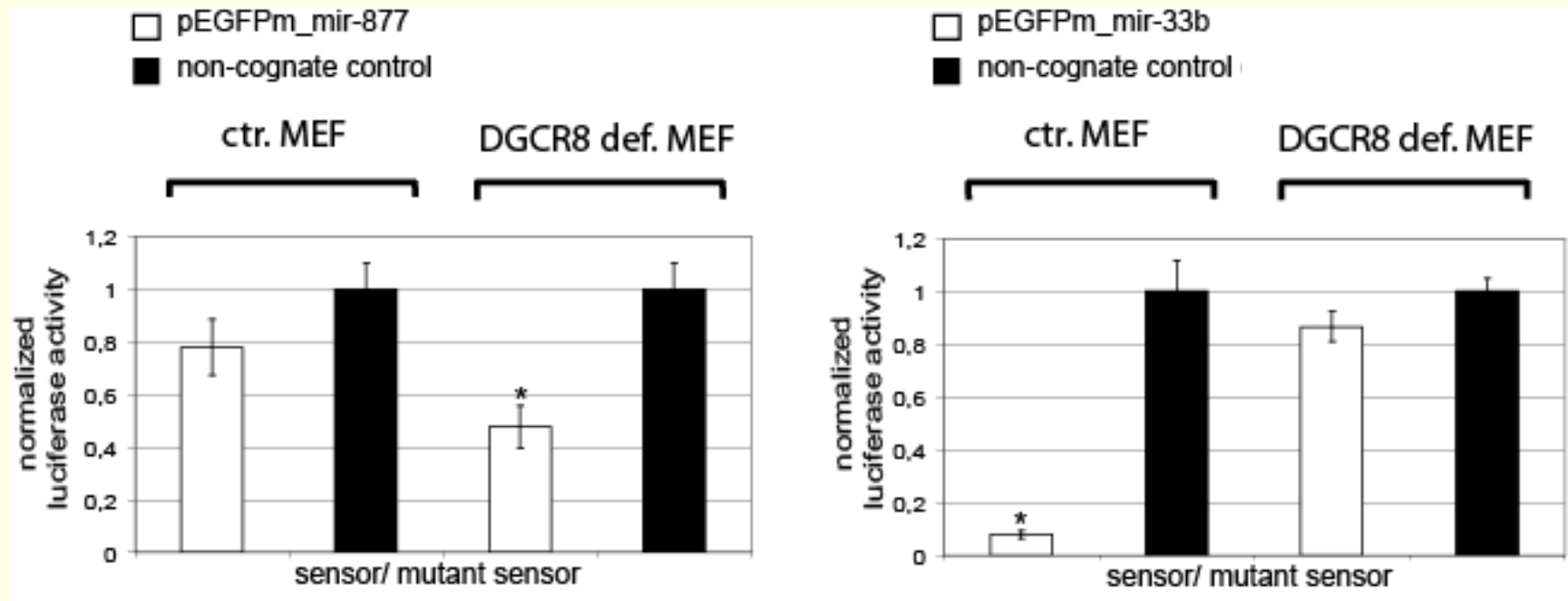
## ➤ Luciferase assay

- wild type
- mutant
- control



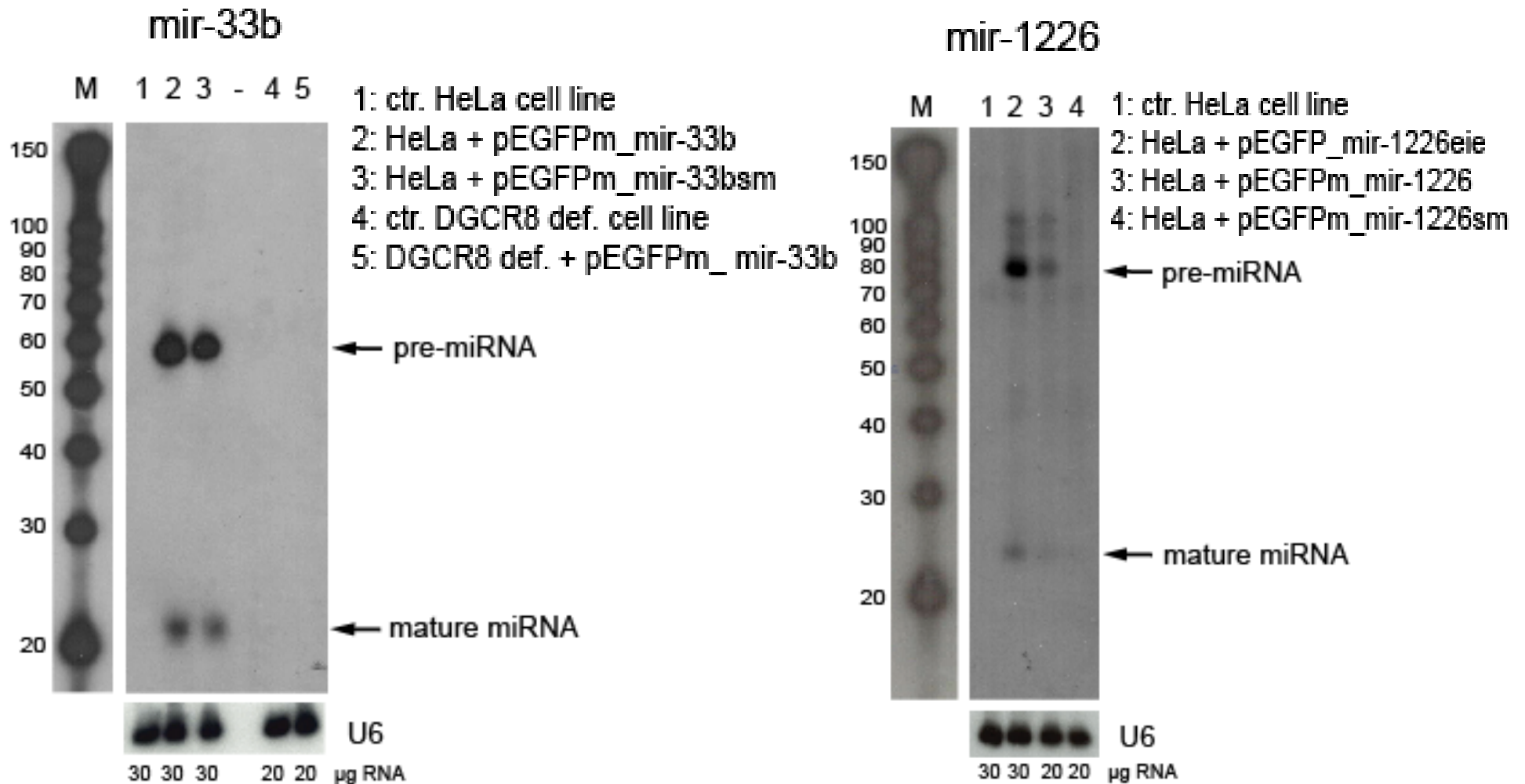
# Independence of Drosha/DGCR8 processing

- Luciferase assay in DGCR8 deficient cell line:



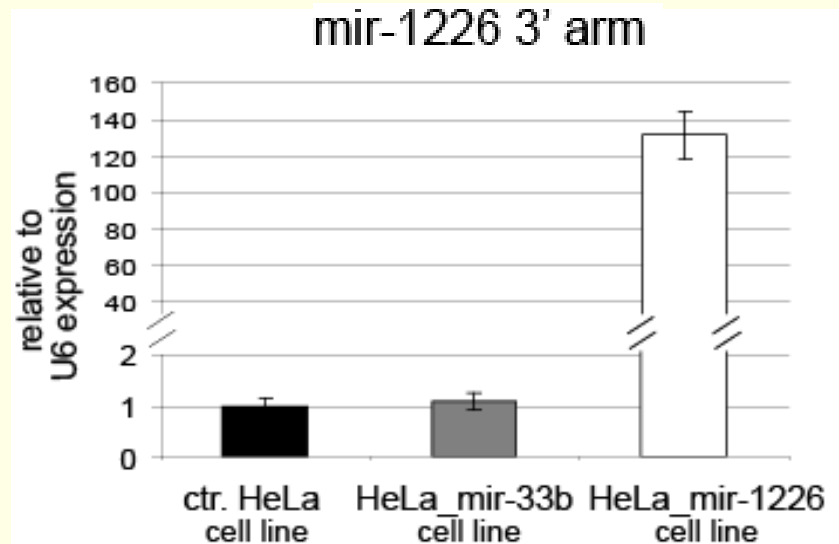
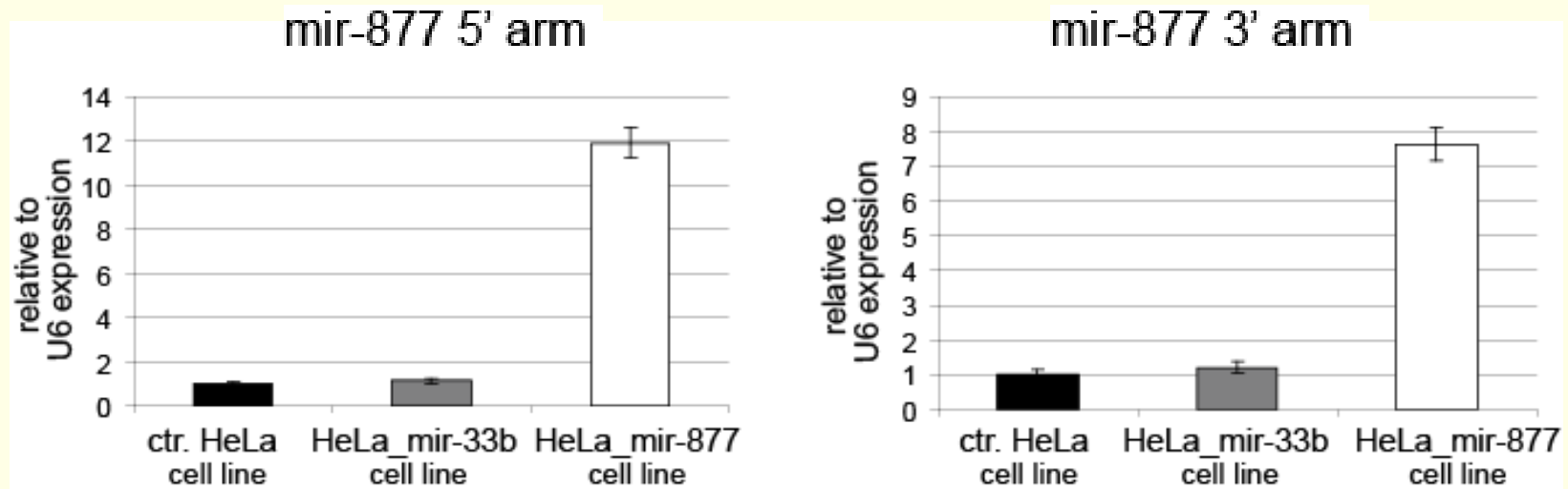
# IV. Mature miRNA detection /1

## ➤ Northern blot analysis



# IV. Mature miRNA detection /2

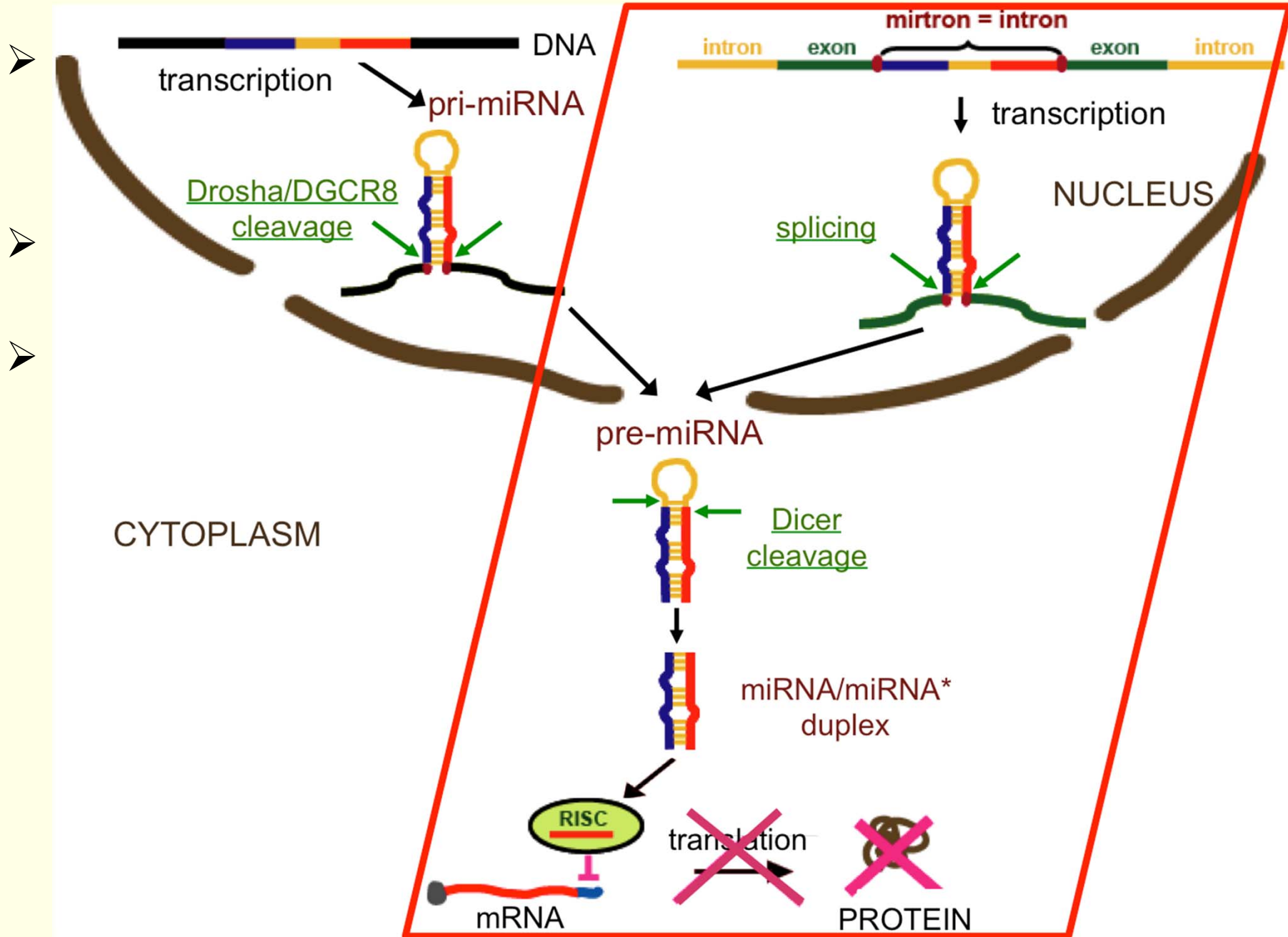
## ➤ qRT\_PCR (miRNA TaqMan)



# miRNA biogenesis Summary

canonical

mirtron





# Summary

- human mirtrons can be spliced from:
  - original environment
  - heterologous coding context
- their processing is splicing dependent and Drosha/DGCR8 independent
- mature miRNA detection by Northern blot analysis and qRT-PCR
- not every predicted sequence is mirtron regardless of being localised in a short intron
- mir-877 is capable of producing functional mature miRNAs simultaneously from both arms
- neither good splicing efficiency nor high overall miRNA expression level is a prerequisite for mirtron functionality

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*Schamberger et al.,  
RNA Biology, 2012;9(9):1177-1185.*

# Advantages of mirtrons as gene silencers

- **'easy to follow' miRNA expression**  
(strong correlation between miRNA and marker gene e.g. GFP expression; cell sorting etc.)
- **Pol II promoter regulated expression (inducible, tissue specific)**
- **'2 or more' in 1: more than one mirtron embedded in a gene**

Mirtrons are promising genetic tools for basic research and gene therapy applications.

# Thanks to...

... Everyone, who helped me in my project,  
especially ...

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