

Are all miRNA family members functionally equivalent?

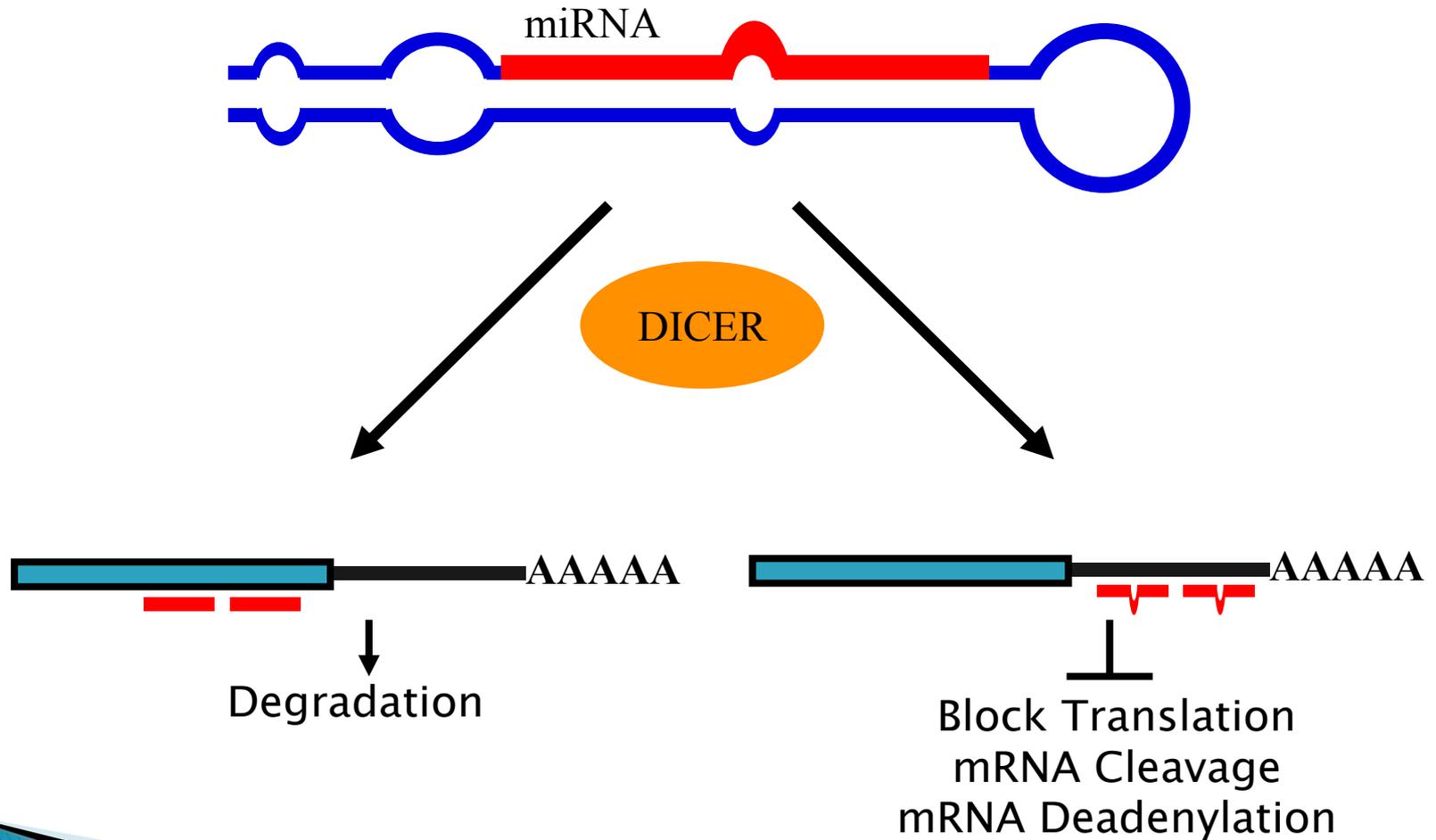
Dissection of the *lin-4* family using
the *C. elegans* animal model.

Nicholas Feuer

What are microRNAs?

- ▶ Small non-coding RNAs that are ~22 nucleotides long
- ▶ Function to block expression of their targets
- ▶ Thousands of miRNA genes identified in plant, virus and animal genomes (>1500 in humans alone!)
- ▶ Play essential roles in cell proliferation, differentiation, apoptosis, metabolism, immune response, aging

MicroRNAs negatively regulate their gene targets



MiRNA targets are dictated by “seed” sequence

- ▶ MicroRNA targets (messenger RNAs) are thought to be dictated by the “microRNA seed” [Doench and Sharp, 2004] – this region binds with 100% complementarity to its target
- ▶ The “microRNA seed” sequence consists of the 2nd through 8th nucleotide of the miRNA
- ▶ miRNAs are grouped into families by “seed” sequence homology [Bartel, 2009]

Why study microRNAs in *Caenorhabditis elegans*?

- ▶ Translucent soil-dwelling nematodes
- ▶ Short life cycle, mature in about 1 week
- ▶ Predominantly exist as hermaphrodites and lay 250–300 eggs
- ▶ Genome fully sequenced
- ▶ Many genes are evolutionarily conserved in humans [Pasquinelli, et al 2000]

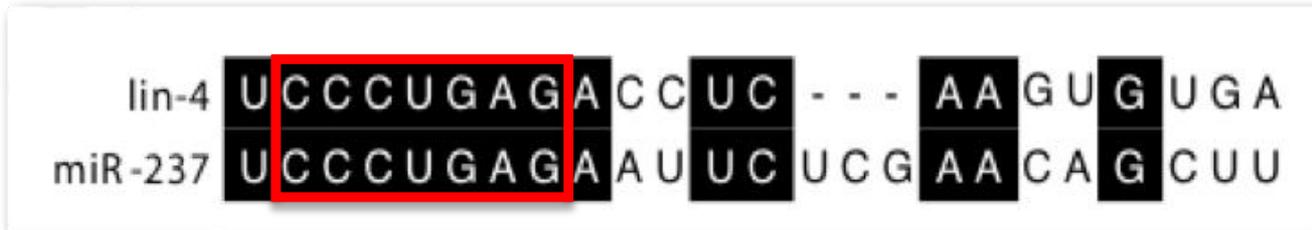


Study will focus on *lin-4*

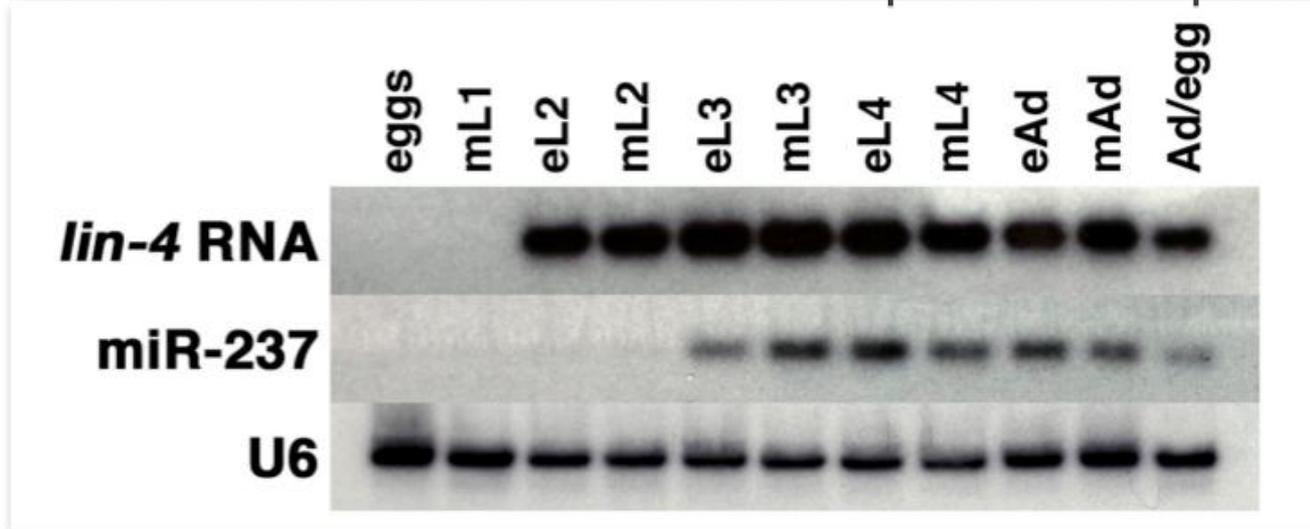
- ▶ First miRNA ever identified. Found in *C. elegans* to regulate the timing of the larval transitions during the worm lifecycle.
- ▶ Deletion of *lin-4* results in defects in cell cycle exit and differentiation of certain cell types, characteristics also shared by cancerous cells.
- ▶ *lin-4* is conserved in mammals and implicated to function as tumor suppressor genes in humans. Little is known of its role during mammalian development or in the adult.
- ▶ By studying the biological roles of these families in the simple *C. elegans* model, we hope to understand how these miRNAs relate to human development and disease.

The *lin-4* microRNA family in *C. elegans*

Sequence alignment of family members *lin-4* and *mir-237*

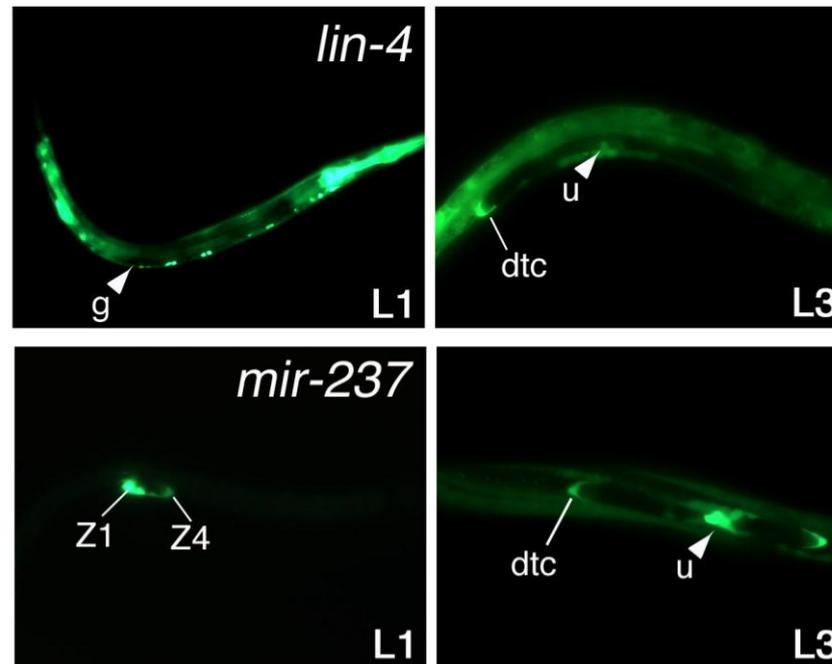


Northern blot shows difference of expression temporally



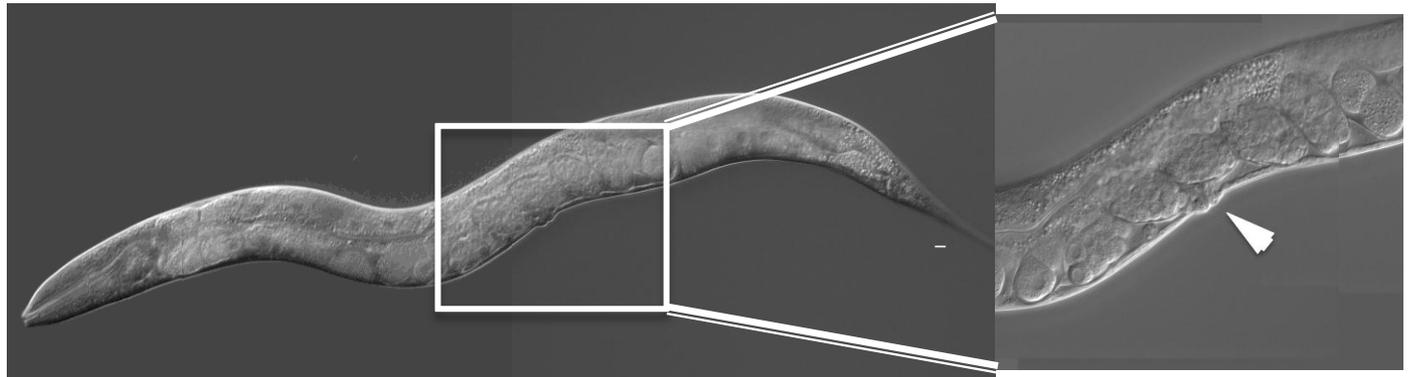
lin-4 and miR-237 expression is distinct temporally and spatially during development

Does this indicate separable biological roles?

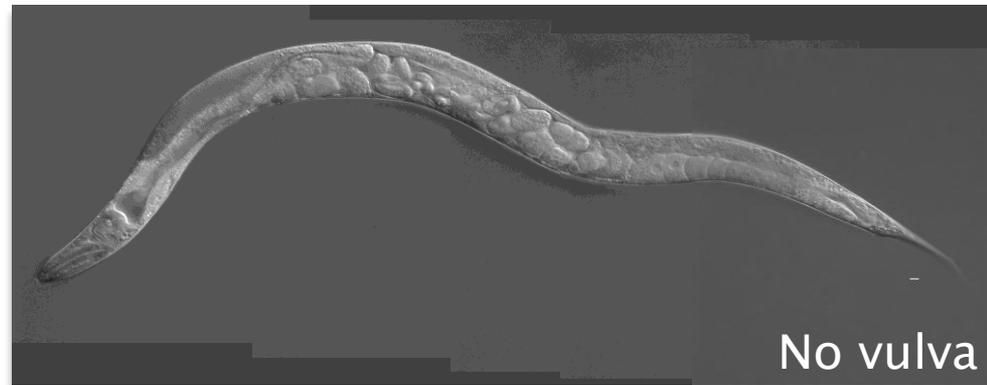


The *lin-4* and miR-237 mutants are distinct

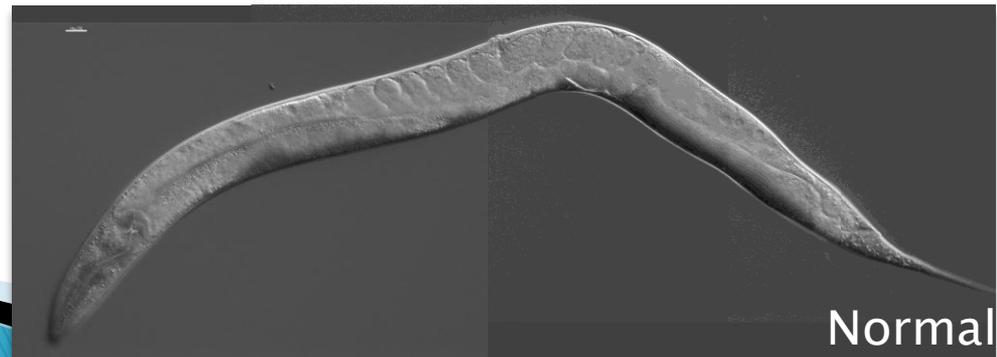
Wild type



lin-4
deletion

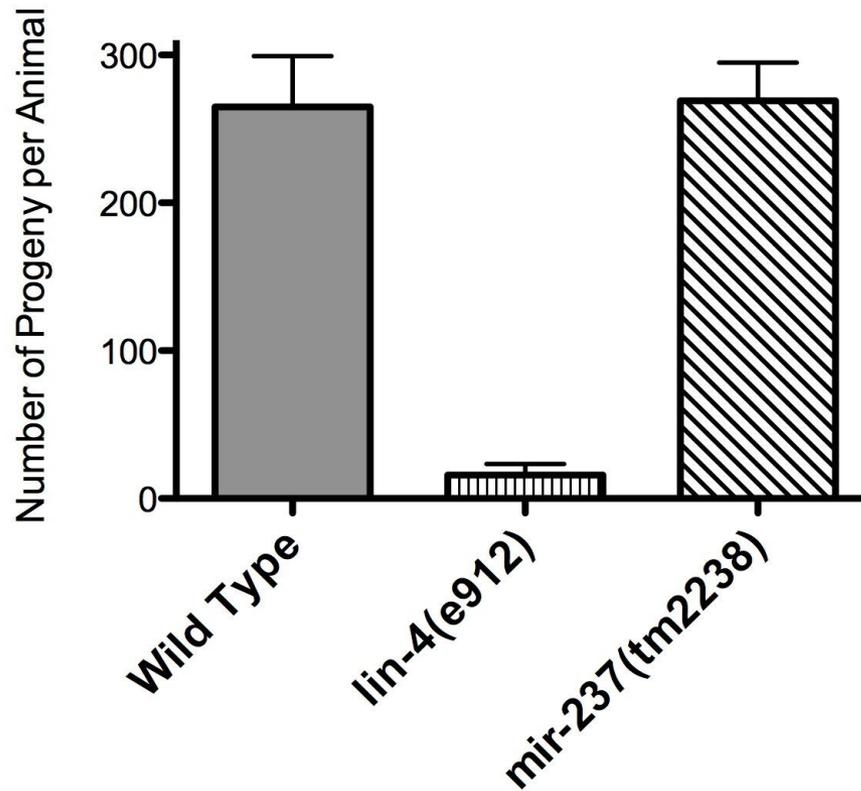


mir-237
deletion



The *lin-4* and miR-237 mutants are distinct

Brood Size Analysis



The *lin-4* miRNA family is evolutionarily conserved in *C. elegans* and Humans

	Human
TCCCTGAGAA--CCCTTTAACTGTGA	hsa-mir-125a
TCCCTGAGAA--CCCT--AACTGTGA	hsa-mir-125b-1
TCCCTGAGAA--CCCT--AACTGTGA	hsa-mir-125b-2
TCCCTGAGAA--CCTC--AAGTGTGA	cel- <i>lin-4</i>
TCCCTGAGAAATTCTC--GAAC-AGCT	cel-mir-237 <i>C. elegans</i>

Little is known about the developmental role of miR-237 in nematodes or the mammalian homologues

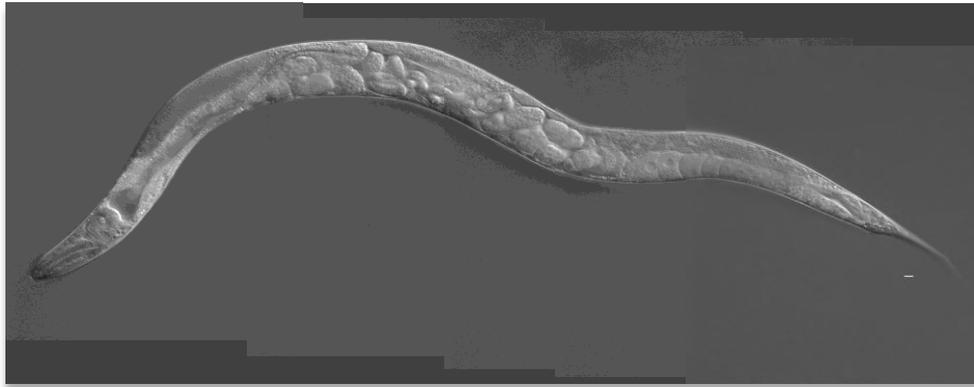
Hypothesis

The hypothesis to be tested is if members within miRNA families are functionally equivalent but are expressed in distinct temporal & spatial ways.

- ▶ It is important when thinking therapeutically about delivering miRNAs to humans to treat diseases such as cancer.

The Experiment

- ▶ Rescue *lin-4* deletion mutant (lack egg-laying structures)



by using microRNA family member
mir-237

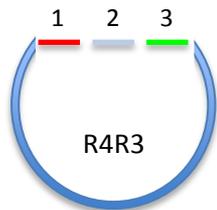
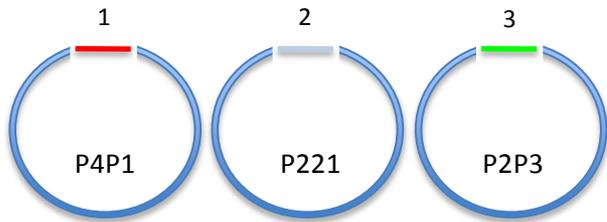
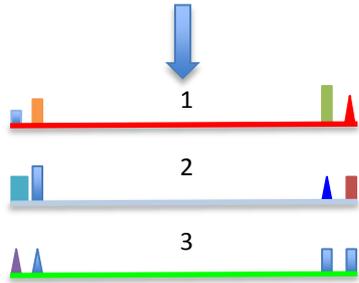
Experimental Approach: build a synthetic miRNA expression construct

lin-4 promoter pre-mir-237 *unc-54* 3'UTR

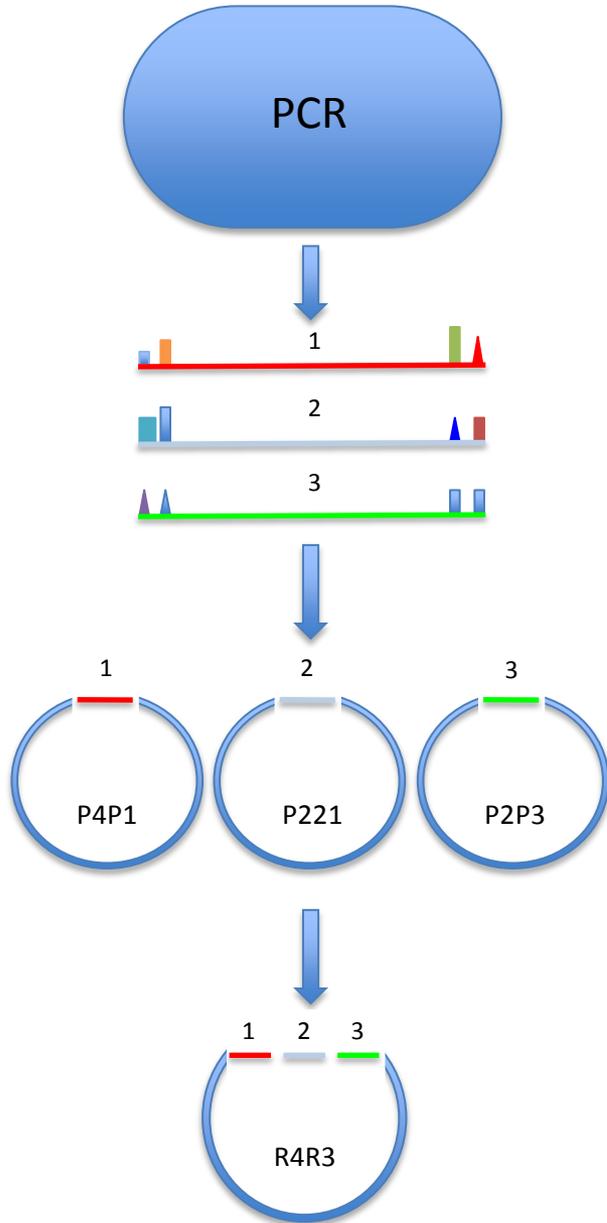
- ▶ Use *lin-4* promoter to drive *miR-237* expression in specific cells and at certain times that *lin-4* is normally expressed
- ▶ *mir-237* hairpin is flanked by ~150 bp on either side to ensure proper Drosha/Dicer processing
- ▶ *unc-54* 3'UTR region lacks regulatory elements but allows for proper splicing and polyadenylation of the pri-miRNA transcript. (prevents RNA transcript from being degraded)

Construction

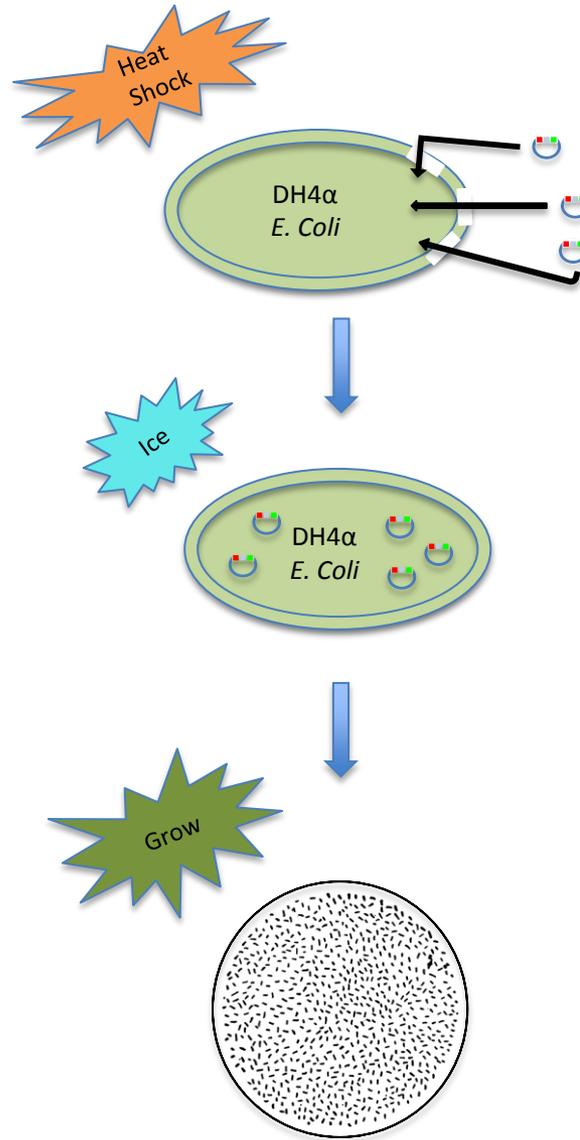
PCR



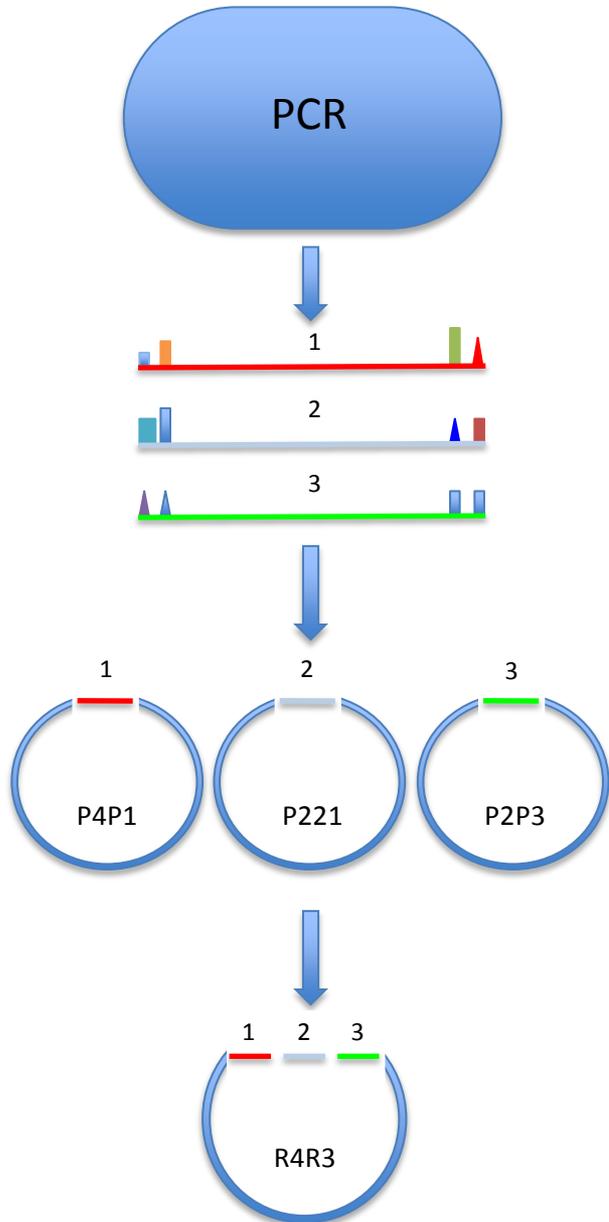
Construction



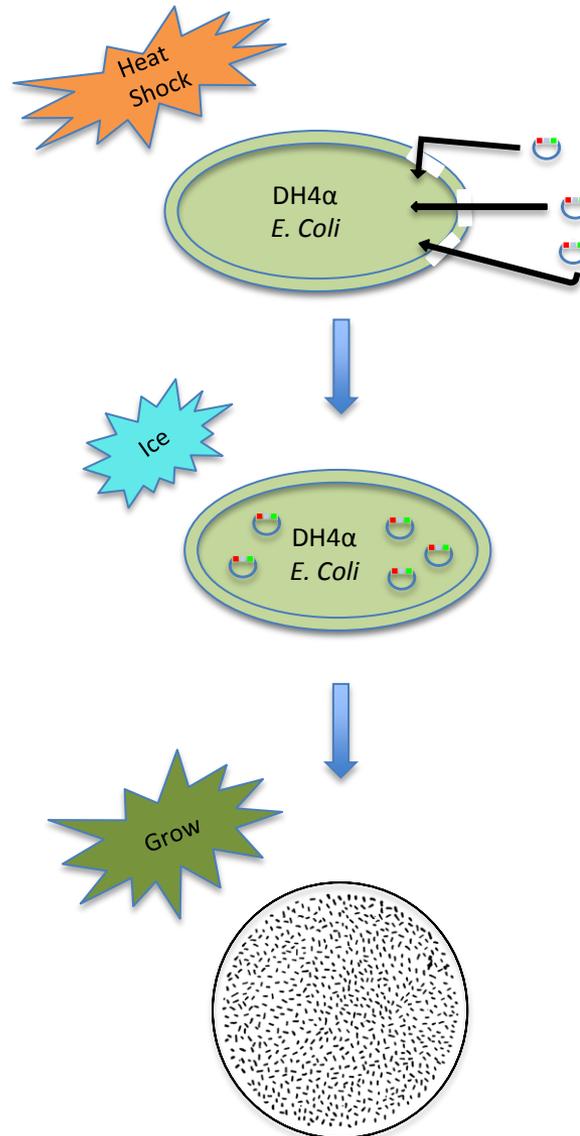
Transformation



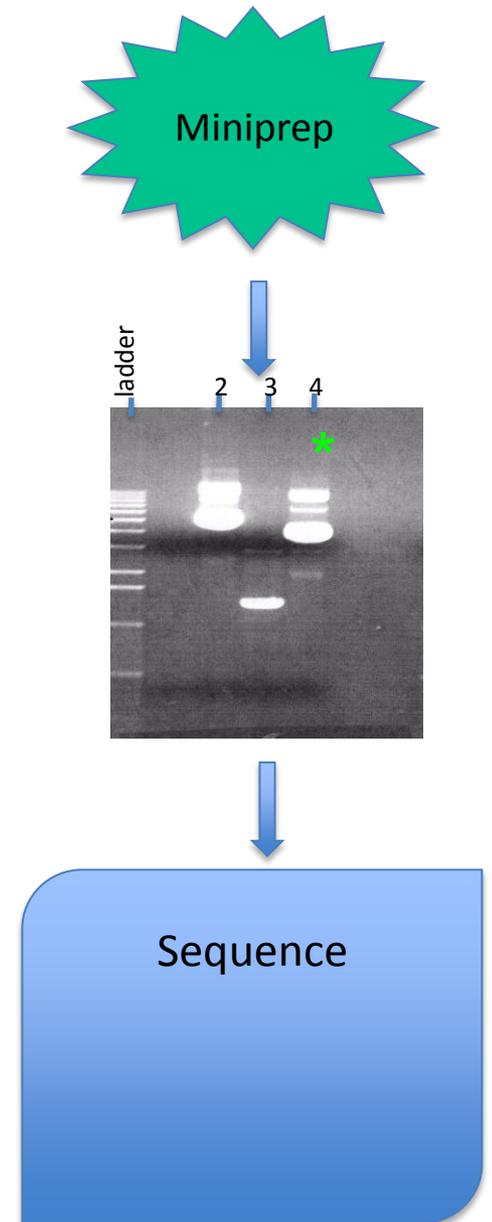
Construction



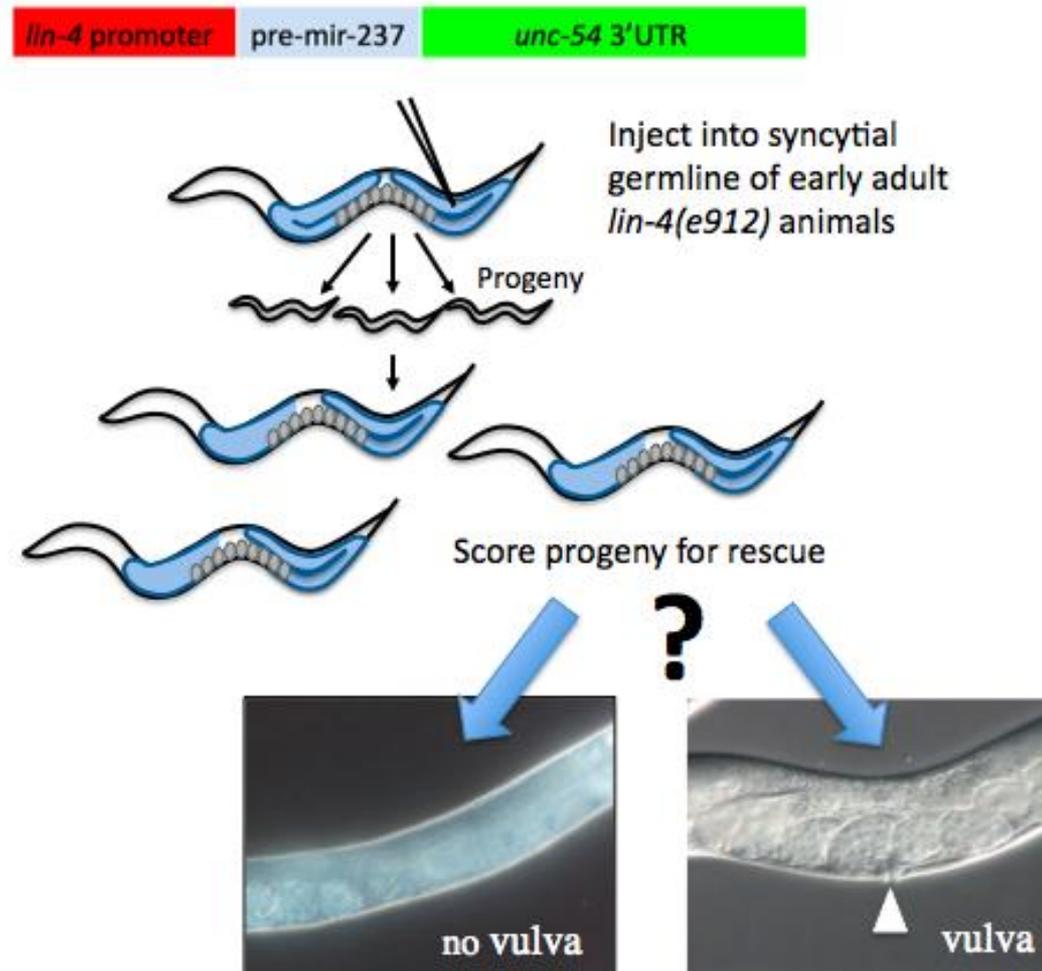
Transformation



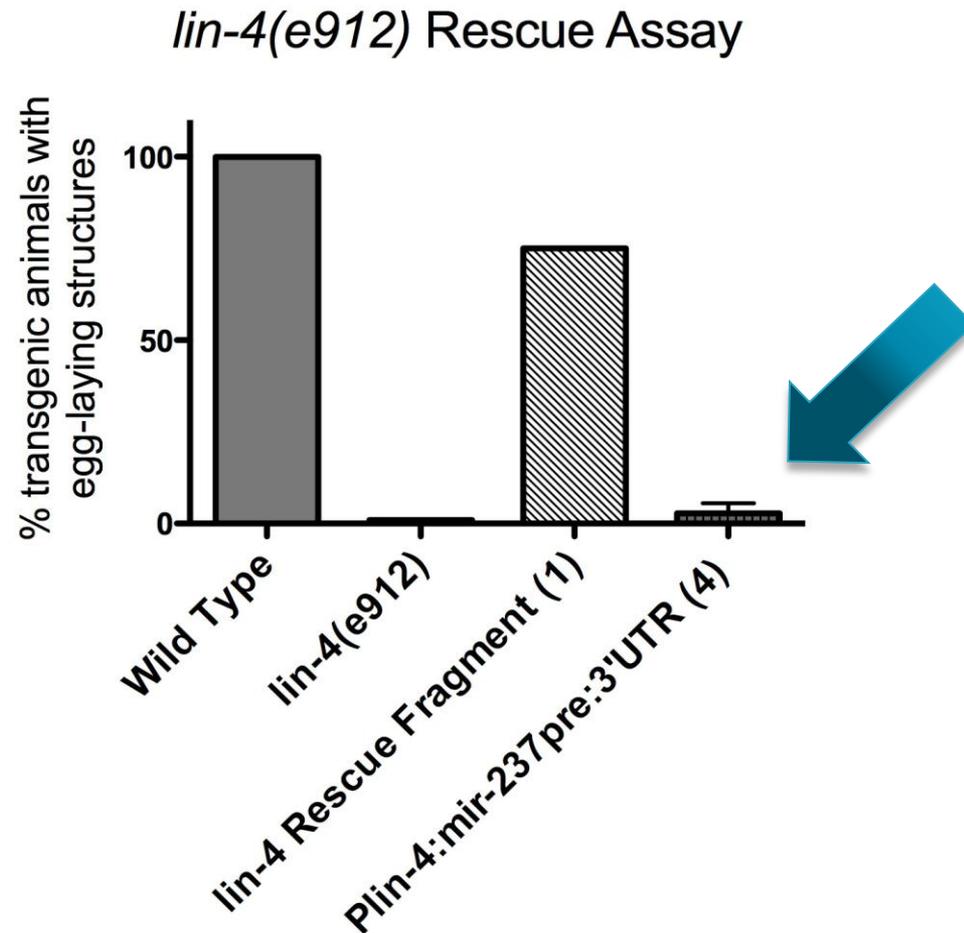
Extraction



Rescue Assay used to test the “microRNA seed” theory



Result: The *lin-4* homologue, miR-237, was unable to rescue the vulvaless phenotype!



What does this mean for microRNAs?

- ▶ MicroRNA family members cannot be treated as being interchangeable
- ▶ Novel finding which challenges the established “microRNA seed” theory [Doench and Sharp, 2004; Kloosterman et al., 2004; Brennecke et al., 2005; Lai et al., 2005]
- ▶ Important when thinking therapeutically about delivering miRNAs to humans to treat diseases such as cancer.

Future Research

- ▶ Generate and score more lines. Test if the synthetic mir-237 is being expressed in these transgenic lines.
 - ▶ Is this also true for other microRNA families?
 - ▶ What other factors are involved in dictating microRNA targets other than the “seed”?
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A Special Thanks

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 - ▶ My parents for all their support
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Questions?

