Characterization of siRNA Asymmetry

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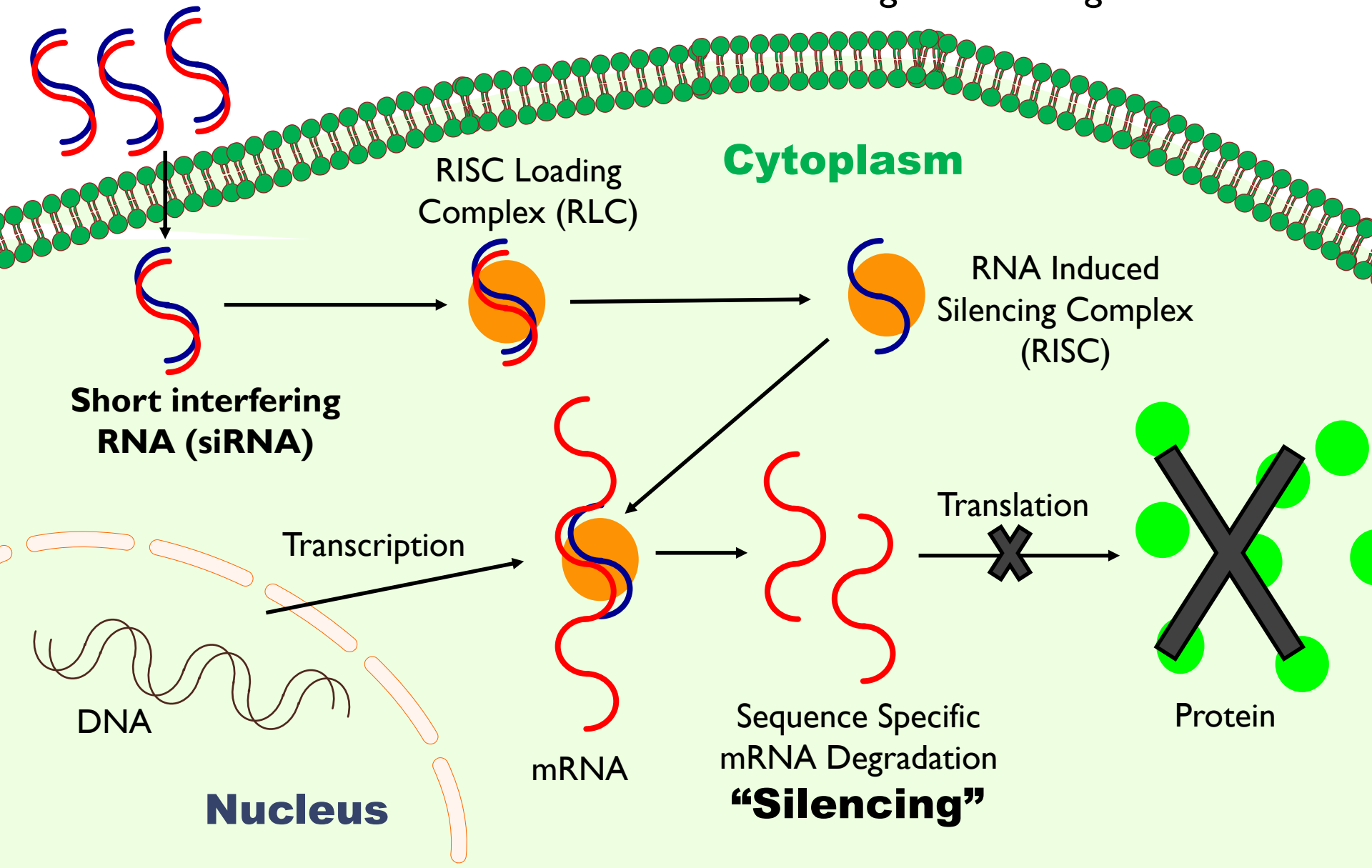
Introduction: RNA Interference Overview

RNA interference is a mechanism for gene silencing



Significance

- Used for a wide range of therapeutics
  - TKM-Ebola to combat Ebola virus*
    - Three siRNAs that combat L protein, VP24 and VP35 genes
    - 100% protection in macaques
    - 67% protection in rhesus monkeys
- Protein knockout experiments in functional characterization studies
- siRNAs can be designed *a priori* with knowledge of only the mRNA target sequence
- siRNA-programmed RISC is a multiple-turnover enzyme

*http://www.quora.com/How-does-TKM-Ebola-work

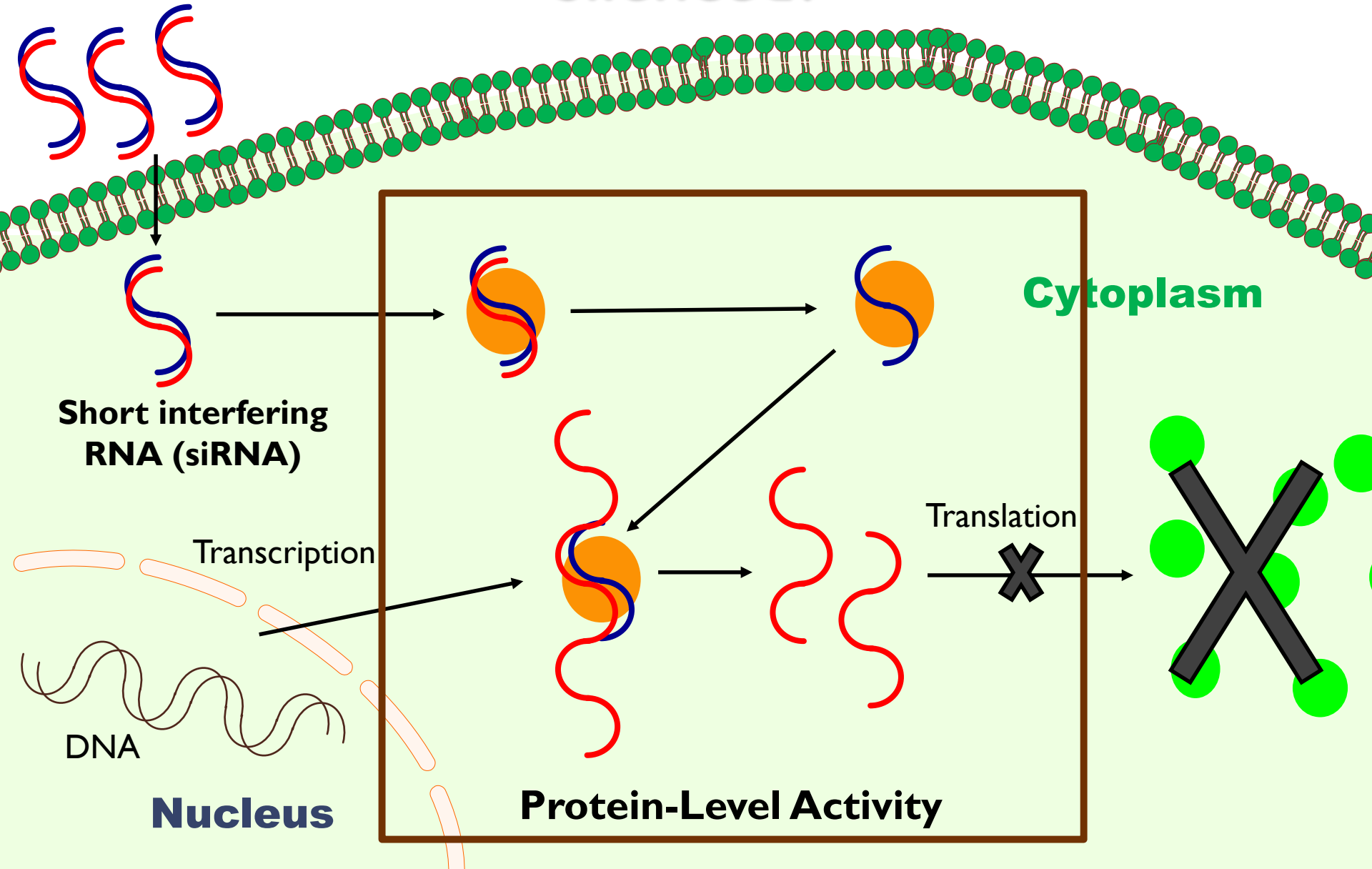
Question

How do we design highly active siRNAs?

Approach

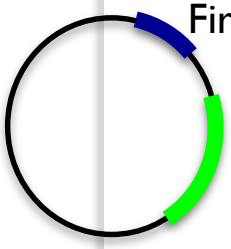
Determining the characteristics of siRNAs that influence relative strand activity and asymmetric strand loading.

Relative Strand Activity: How Much Is Silenced?

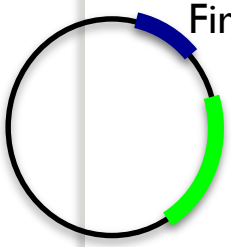


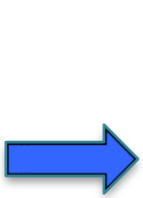
Investigating Strand Activity: PKR Silencing

1. Transfect Hela cells with plasmid



OR

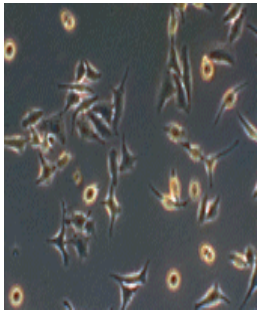


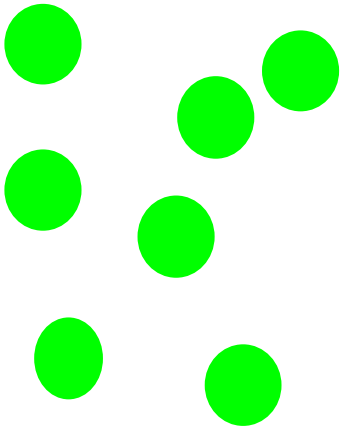


2. Transfect with PKR siRNA

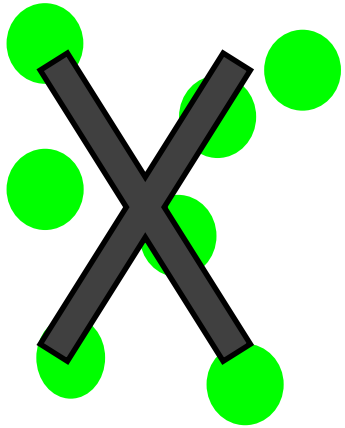
PKR siRNA

3. Measure protein expression by chemiluminescence





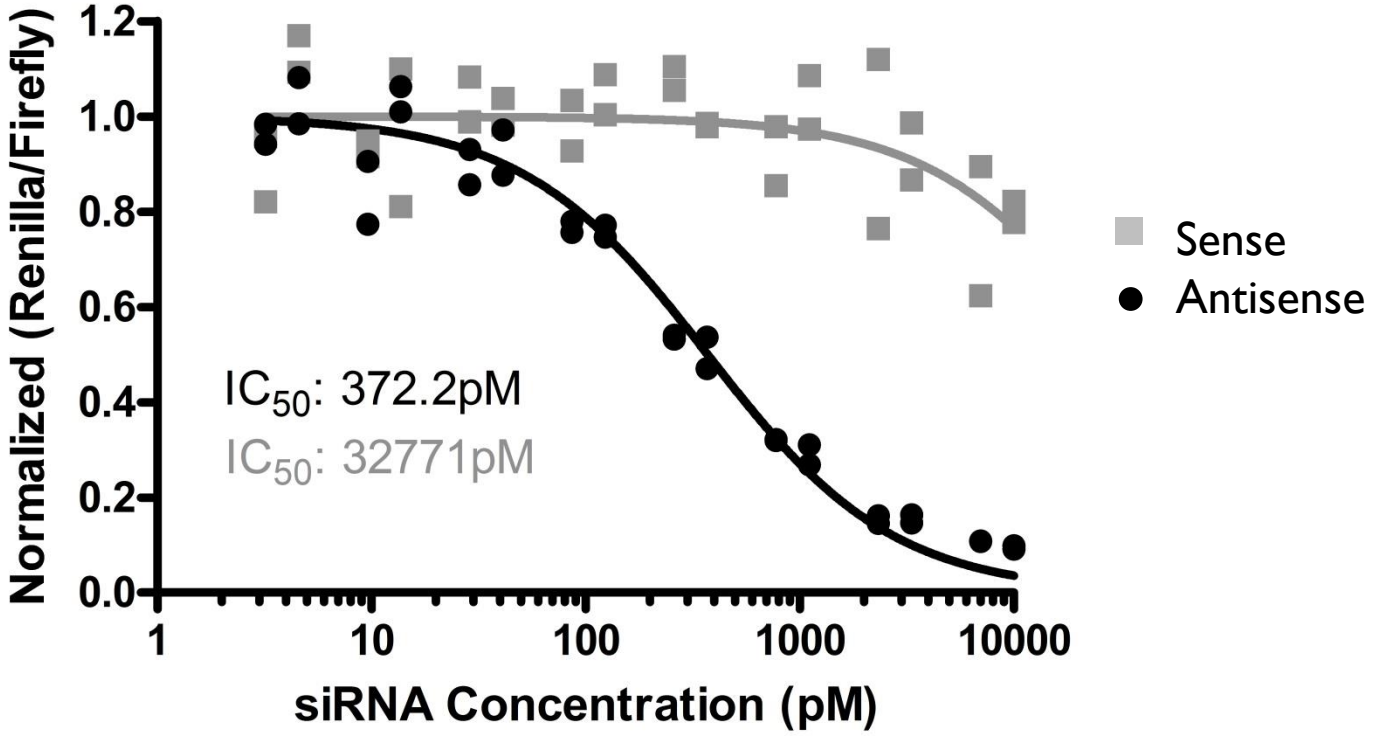
Renilla expression



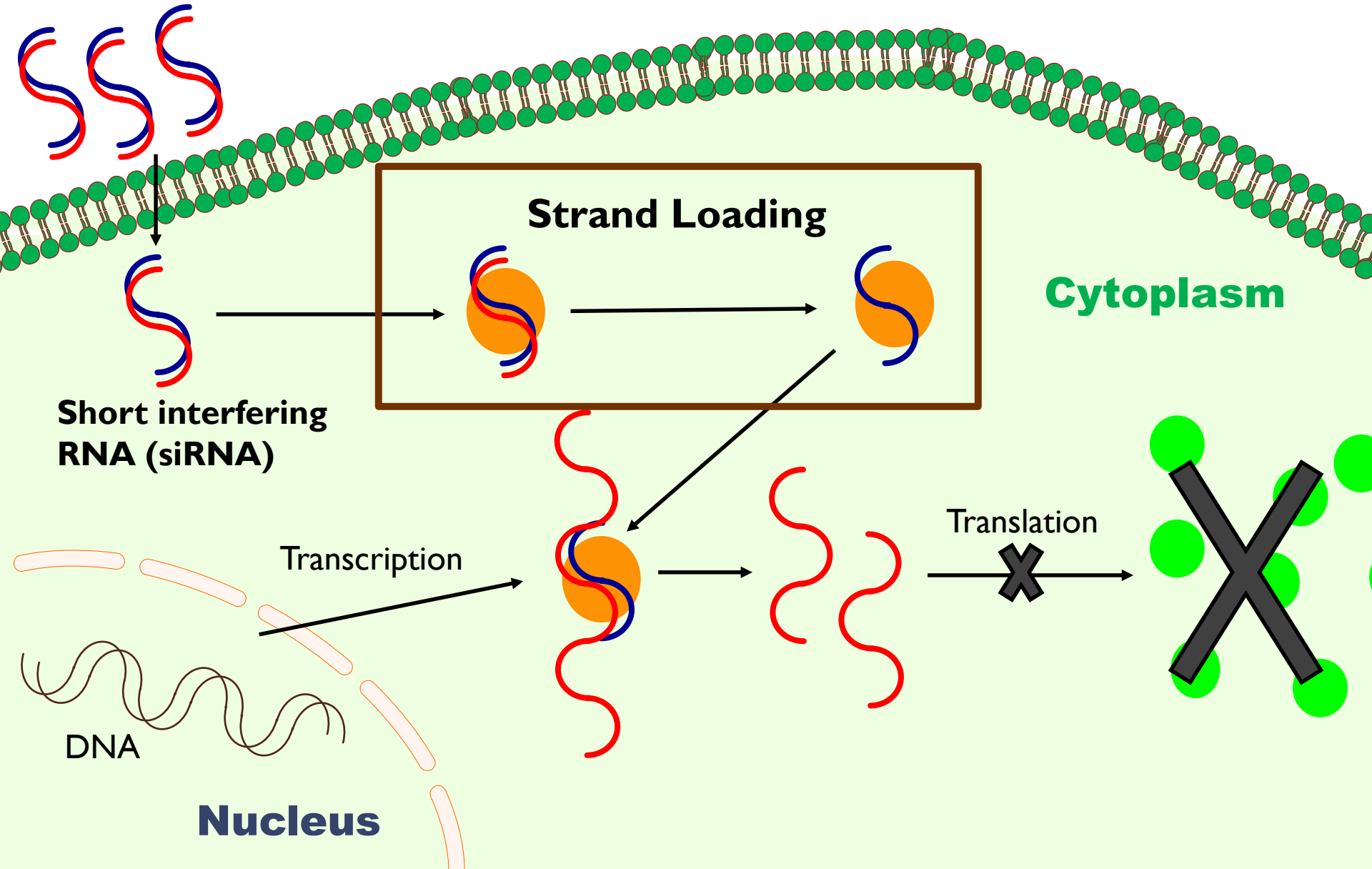
Renilla knockdown

IC_{50} Curve

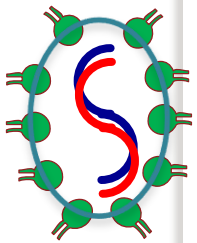
IC_{50} – Concentration at which 50% of maximum inhibition is achieved



siRNA Asymmetry: Ago2 Strand Loading

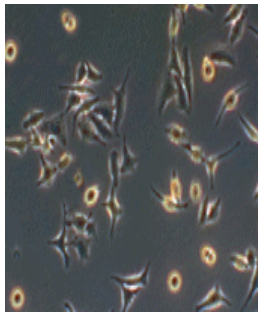


Investigating Strand Loading: Ago2 Immunoprecipitation and Stem-Loop RT-qPCR



siRNA

1. Transfect siRNA

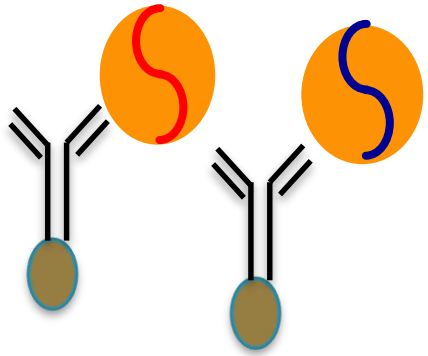


HeLa Cells

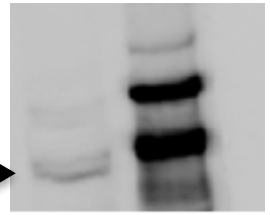
2. Collect cytoplasmic lysate

RLC/RISC in lysate

3. Immunoprecipitate Ago2



4. Confirm Ago 2 IP by Western

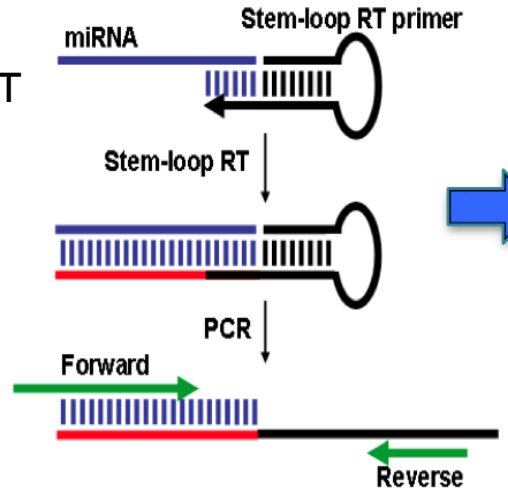


97kDa

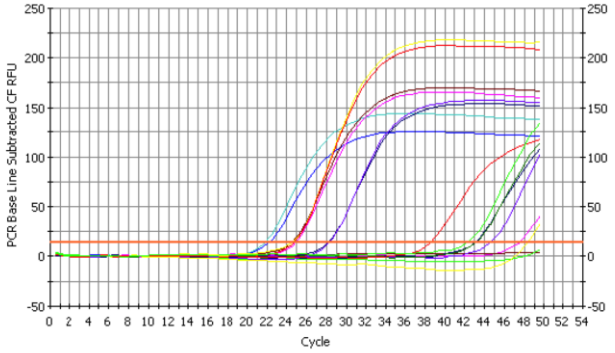
Investigating Strand Loading: Ago2 Immunoprecipitation and Stem-Loop RT-qPCR

5. Purify RNA

6. Stem-Loop RT

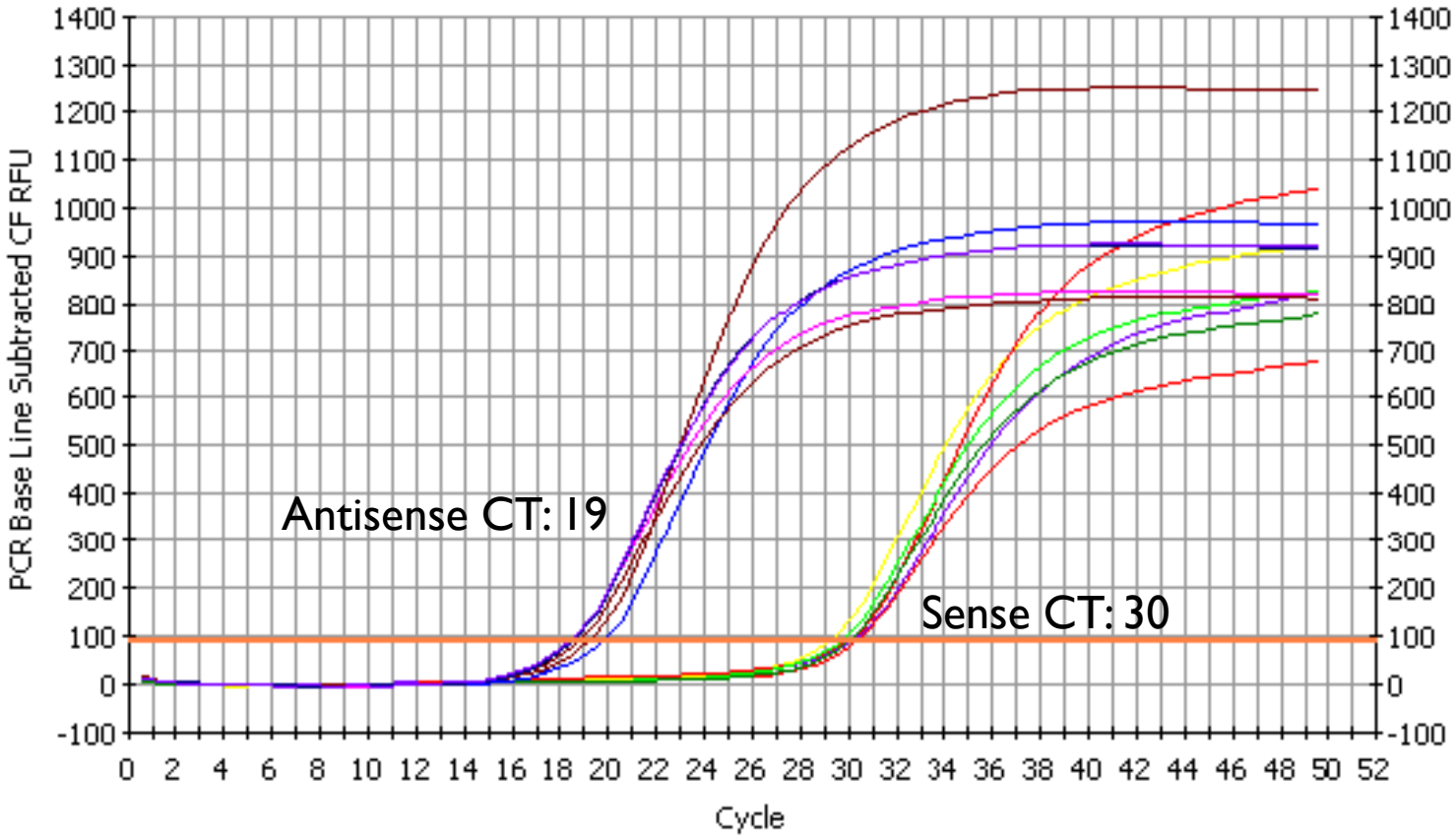


7. Measure strands by stem-loop RT-qPCR



Varkonyi-Gasic, E.. et al. *Plant Methods*, 2007.

qPCR Data


PCR Base Line Subtracted CF RFU
Cycle
Antisense CT: 19
Sense CT: 30
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52

Ongoing Work

- Develop parameters for designing highly active siRNAs based on experimental results
- Investigate the effects of other Ago proteins on different siRNAs
- Complete RNA-level activity studies by measuring strand cleavage

Acknowledgments

- PI, Dr. Walton for being an amazing mentor and adviser
- Our mentor, Phillip Angart for teaching us most of the skills we have in the lab today
- Sarah Thorwall for allowing us to bounce ideas when we needed to

Questions