

MICRORNA-TRIGGERED DCAS9 BEACON FOR CANCER DETECTION

by

Lauren Dorsey

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TABLE OF CONTENTS

LIST OF FIGURES	v
ABSTRACT	vii
Chapter	
1 INTRODUCTION	1
1.1 The CRISPR-Cas System to Engineer Biological Systems	1
1.2 MicroRNA Detection for Cancer Diagnosis	2
2 DEVELOPMENT OF THE MICRORNA-TRIGGERED DCAS9 BEACON ASSAY	6
2.1 Introduction	6
2.2 Materials and Methods	7
2.2.1 Expression and Protein Purification by Gravity-Flow Chromatography	7
2.2.2 In Vitro Transcription of Single-Guide RNA	7
2.2.3 Ethanol Precipitation of Single-Guide RNA	8
2.2.4 Conditions for In Vitro Cleavage Assay	8
2.2.5 Blocked Beacon and Blocked PAM Complexes	9
2.2.6 Conditions for Beacon Assay	10
2.2.7 Conditions for Electromobility Shift Assay	10
2.3 Results and Discussion	11
2.3.1 Design of the dCas9 Beacon Assay	11
2.3.2 Initial Functionality of the dCas9 Beacon Assay	12
2.3.3 Complex Formation by Electromobility Shift Assay	12
2.3.4 Optimized Functionality of the dCas9 Beacon Assay	13
2.4 Recommendations for Future Work	14
3 SUMMARY AND CONCLUSION	15
FIGURES	16
REFERENCES	22

LIST OF FIGURES

- Figure 1. FRET-based Cas9 Beacon Assay. (A) Design of Cas9 beacon DNA. Molecular beacon consists of three annealed DNA oligonucleotides, a fluorophore-labeled strand, a quencher-labeled strand, and a strand containing the Sp PAM sequence. (B) Upon mixing of beacon DNA with complexed Cas9 (or dCas9, not shown) and sgRNA, increase in fluorescence is detected corresponding to binding to DNA target. When the PAM sequence of oligo 2 is mutated, Cas9 does not displace quencher and no increase in fluorescence is detected. 16
- Figure 2. dCas9 Beacon Assay for miRNA Detection. (A) Addition of miR-122 triggers two sequential toehold displacement reactions resulting in a fully assembled beacon containing a PAM sequence. (B) Addition of precomplexed dCas9 – sgRNA displaces the quencher-labeled strand from the fluorophore-labeled strand resulting in an increase in fluorescence. 17
- Figure 3. PAM Sequence on Beacon Results in Increase in Fluorescence. Toehold displacement reactions were run for an hour at 100 nM before mixing with precomplexed dCas9 – sgRNA and fluorescence measured. (1) Fluorescent-labeled strand hybridized with PAM strand, (2) Unblocked beacon with PAM strand, (3) Unblocked beacon without PAM strand, (4) Blocked beacon complex, (5) Blocked beacon and blocked PAM complexes. 18
- Figure 4. Evaluation of Complex Formation by Electromobility Shift Assay. Toehold displacement reactions were run for an hour at 100 nM before loaded onto 20% native acrylamide gels. (1) Fluorophore-labeled strand, (2) Fluorophore-labeled strand hybridized with PAM strand, (3) Blocked beacon complex, (4) Blocked beacon and blocked PAM complexes. 19
- Figure 5. dCas9 Beacon Assay Responds to MiR-122 Input. Toehold displacement reactions were run for an hour at 100 nM before mixing with precomplexed dCas9 – sgRNA and fluorescence measured. (1) Unblocked beacon with PAM strand, (2) Blocked beacon complex, (3) Blocked beacon and blocked PAM complexes, (4) Blocked beacon complex, blocked PAM complex, and miR-122. Molar excess of 1.2:1

and 4:1 of blocking strand to PAM and beacon complexes used, respectively. For miR-122 input, 2-fold molar excess used. 20

Figure 6. Dosage Response Curve for MiR-122 – Triggered dCas9 Beacon Assay. The linear range of detection was from 50 nM to 200 nM miR-122 input concentration. 21

ABSTRACT

Cancer is the second leading cause of death in the United States. Early detection has a major influence on receiving effective treatment and increasing the chance of survival. One such target for detection of cancer is microRNA. Deregulation of microRNA have been linked to a variety of cancers; which combined with their small size of 22 nucleotides makes them a popular target for cancer detection.

In this work, an in vitro method for sensitive, rapid detection of cancer-relevant microRNA has been developed. The key of the assay relies on the ability of the type II catalytically inactive Cas9 protein, when complexed with its single-guide RNA, to recognize and bind to any designed double-stranded DNA target. The presence of a cancer-relevant microRNA triggers a series of toehold displacement reactions resulting in a fully assembled molecular beacon labeled by a fluorophore-quencher pair. Upon assembly of the beacon, a dCas9-sgRNA complex binds and displaces a quencher strand for fluorescence signal. This dCas9 beacon provides a microRNA detection limit at nanomolar concentrations.

Chapter 1

INTRODUCTION

1.1 The CRISPR-Cas System to Engineer Biological Systems

The clustered regularly interspaced palindromic repeats (CRISPR) – associated system (Cas) is an RNA-guided nuclease complex discovered in bacteria and archaea as an adaptive immune defense response (Mali, Esvelt, and Church 2013). The type II system involves a CRISPR RNA (crRNA) that binds to a trans-activating crRNA (tracrRNA) and the single effector Cas9 protein to facilitate recognition and destruction of foreign invaders. Fragments of the foreign DNA are incorporated into the CRISPR loci in regions called spacers, separated by short repeat sequences. The hybridized crRNA-tracrRNA, or a chimaeric single-guide RNA (sgRNA), bound to the Cas9 protein guides the nuclease complex to bind and cleavage double-stranded DNA sequences complementary to the spacer region of the crRNA (Jinek et al. 2012). Recognition and cleavage will only occur if the foreign target contains a short sequence at the 3' of its protospacer sequence called the protospacer-adjacent motif (PAM). The discovery of CRISPR-Cas9 technology has made genome engineering simple and scalable, allowing genomic perturbation for functional organization determination as well as changes to the genomic sequence for therapeutic purposes (Hsu, Lander, and Zhang 2014, Doudna and Charpentier 2014). Engineering of a catalytically inactive Cas9 (dCas9) has broadened the application of the CRISPR-Cas9 technology to include transcriptional activation and repression, epigenetic modulation,

genome architecture modulation, and multiplexed localization activity (Mali, Esvelt, and Church 2013).

In addition to these applications, catalytically inactive Cas9 recently demonstrated potential as an *in vitro* nucleic acid sensor for the detection of foreign pathogens. Two dCas9 proteins were genetically fused to split firefly luciferase for reconstitution in the presence of two DNA target sequences. In the presence of both DNA target sequences, an increase of 2 – 3-fold in luminescence was detected compared with either single DNA target present (Zhang et al. 2016). However, detection of nucleic acid targets for this paired dCas9 reporter system was limited to double-stranded nucleic acid targets. It would be advantageous to develop a dCas9 *in vitro* sensor for the detection of single-stranded nucleic acids because of their involvement in human diseases, particularly in cancer pathogenesis (Volinia et al. 2006, Esteller 2011).

1.2 MicroRNA Detection for Cancer Diagnosis

Cancer is the second leading cause of death in the United States. Approximately 1600 Americans will die of cancer every day this year (Siegel, Miller, and Jemal). Providing effective treatment to cancer patients is contingent upon providing accurate, sensitive methods of detection. Since cancer detected at advanced stage is more likely to cause death than if it is detected before it spreads from its organ of origin, a strong effort has been placed on improving methods for early cancer detection (Etzioni et al. 2003).

Advanced in genomics, transcriptomics, and proteomics provide insight into the regulatory changes that occur during the progression of cancer. One class of molecules, small non-coding RNA molecules called microRNAs (miRNAs), are

extensively involved in cancer pathogenesis (Volinia et al. 2006). miRNAs are non-coding, single-stranded RNA molecules about twenty-two nucleotides in length that function as gene regulators by binding perfectly or imperfectly to their protein-coding messenger RNA targets. miRNAs negatively regulate expression of genes involved in cell cycle regulation and differentiation, genes which are damaged in cancer. They are frequently located in areas of the genome associated with cancer (Esquela-Kerscher and Slack 2006). Since their discovery, deregulation of miRNAs has been correlated with a variety of cancer types, as well as tumor stage and response to treatments (Fabbri et al. 2008, Esquela-Kerscher and Slack 2006, Catuogno et al. 2011).

Although a variety of methods exist to quantify miRNA expression, fluorescence is the preferred method for signal detection due to the ease of use. A variety of methods are employed to detect miRNA expression. The major approaches used are microarrays and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Microarrays rely on hybridization of fluorescently-labeled miRNAs to complementary target oligonucleotide probes that are immobilized to a support surface. After washing, the hybridized miRNAs are excited and fluorescence is detected. An advantage of microarrays is high-throughput analysis of many samples for a profile of miRNA expression. However, due to the short length of miRNAs considerable effort must be devoted to design of the probes to obtain a suitable and uniform melting temperature (Catuogno et al. 2011, Pritchard, Cheng, and Tewari 2012).

In addition to microarrays, qRT-PCR is a very common method used to quantify expression. The method quantifies expression by binding of fluorescent dyes such as SYBR Green or TaqMan probes to target DNA for real-time analysis. It relies

on enzymes to reverse transcribe miRNAs into complementary DNA then to amplify the cDNA products. To specifically amplify mature miRNAs, either a poly(A) tail or a stem-loop primer is added during reverse transcription. Compared to the microarray, qRT-PCR is more sensitive, but also more costly (Catuogno et al. 2011, Pritchard, Cheng, and Tewari 2012).

Similarly to microarrays, molecular beacons are another method of miRNA detection that relies on hybridization of fluorescently-labeled oligonucleotides to complementary targets. Molecular beacons traditionally consist of a hairpin loop region and a stem region with a fluorescent dye and corresponding quencher pair at the end. Initially, the stem-loop probe is stable and the fluorophore is quenched. In the presence of a miRNA target, the molecular beacon opens by hybridization of its hairpin loop to the complementary miRNA sequence. Opening of the hairpin loop physically displaces the quencher from its fluorophore, resulting in an increase in fluorescence upon excitation. The advantages of using molecular beacons include high specificity to the target miRNA and practical ease of performing miRNA assessment in one step. Molecular beacons are capable of *in vitro* and *in vivo* quantification of expression of various miRNAs (Baker, Bao, and Searles 2011).

Furthermore, toehold-mediated strand displacement (TMSD) technology exploits the specificity and predictability of Watson-Crick base pairing to construct artificial nanoscale structures and dynamic devices (Šulc et al. 2015). Strand displacement is a process by which two single-stranded oligonucleotides with partial or full complementarity hybridize by displacement of one or more previously hybridized strands. Strand displacement is initiated at single-stranded overhanging regions called toeholds; varying the sequence and length of the toehold domains

provides control over the kinetics of the reaction (Zhang and Seelig 2011). TMSD technology has been utilized for miRNA detection, such as during the creation of an AND logic gate computational device to identify the presence of miR-21 and miR-122 in mammalian cells (Hemphill and Deiters 2013).

The purpose of this work is to engineer catalytically inactive Cas9 for *in vitro* sensor detection of cancer-relevant single-stranded miRNA by utilizing TMSD technology for conversion from a single-stranded RNA input to a double-stranded output.

Chapter 2

DEVELOPMENT OF THE MICRORNA-TRIGGERED DCAS9 BEACON ASSAY

2.1 Introduction

The dCas9 beacon design was inspired by previous work using a fluorescence-based assay to detect target binding of CRISPR-Cas9 with a set of structurally distinct sgRNAs. Cas9 from *Streptococcus pyogenes* (Sp) and its nuclease-inactive variant dCas9 bound to sgRNA were demonstrated to displace a quencher-labeled oligonucleotide from its complementary fluorophore-labeled strand as long as the double-stranded molecular beacon contained a protospacer sequence and a functional PAM (Figure 1) (Mekler et al. 2016).

This design relies on the recognition by the type II dCas9 – sgRNA complex to a double-stranded DNA target containing a functional PAM sequence (5' – NGG – 3') at the end of its protospacer sequence (O'Connell et al. 2014). The presence of a target miR-122, which is overexpressed during breast cancer metastasis, triggers two sequential toehold displacement reactions that upon completion result in a fully assembled molecular beacon labeled by a fluorophore-quencher pair (Figure 2) (Fong et al. 2015). Upon assembly of the beacon, a dCas9-sgRNA complex binds and displaces a quencher strand resulting in an increase in fluorescence.

2.2 Materials and Methods

2.2.1 Expression and Protein Purification by Gravity-Flow Chromatography

The plasmids for expression of *Streptococcus pyogenes* (Sp) Cas9-his₆ and Sp dCas9-mCherry-his₆ were cloned by other students in the lab, obtained from frozen stocks CR088 and CR089, and expressed in *Escherichia coli* BL21 [F⁻ ompT hsdSB (r^B m^B) gal dcm (DE3); Novagen, Madison, WI]. Overnight cultures were inoculated into 30 mL Lysogeny broth media (10.0 g/L tryptone, 5.0 g/L yeast extract, 10.0 g/L sodium chloride) at an initial OD₆₀₀ of 0.05 supplemented with 100 µg/mL ampicillin and incubated in a culture shaker at 37 °C until OD₆₀₀ reached 0.6. Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 500 µM and cells were grown at 20 °C overnight. Cells were harvested by centrifugation at 5000xg for 10 min at 4 °C and resuspended in tris-buffered saline supplemented with 5 µM imidazole (TBS; 0.24 g/L Tris-HCl, 3.0 g/L mM NaCl, 0.034 g/L imidazole, pH 7.9). Cells were lysed by ultrasonic disruption using a sonicator. Soluble cell lysate was separated by centrifugation at 10000xg for 20 min at 4 °C and passed through a nickel-nitrilotriacetic acid resin on a polypropylene column using standard manufacturer protocol (Novagen, Madison, WI). Proteins were eluted with 250 µM imidazole and dialyzed overnight into phosphate-buffered saline (8.0 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na₂HPO₄, 0.24 g/L KH₂PO₄, pH 7.4) by magnetic stirring in 4 °C. The cell lysates and purified proteins were loaded onto a 4% stacking/7.5% resolving SDS-PAGE gel and stained with coomassie blue for analysis.

2.2.2 *In Vitro* Transcription of Single-Guide RNA

SpT2-sgRNA was *in vitro* transcribed using HiScribe T7 High Yield RNA Synthesis Kit following manufacturer protocol. The template DNA for *in vitro*

transcription was prepared by extraction of the plasmid (cloned by another student in the lab) from an overnight culture of freezer stock CR097, amplified by polymerase-chain (PCR) reaction with the forward primer 5' – aagc TAATACGACTCACTATAGGTATCACATGACTAAACGA gtttagagctagaatag – 3' and the reverse primer 5' – aaaaaaagcaccgactcggtgccacttttcaagtgataacg – 3' using the high fidelity Q5 polymerase, then purified from a 1% agarose gel (New England Biolabs, Ipswich, MA). Components for *in vitro* transcription mixed were reacted overnight at 37 °C on a S1000™ Thermal Cycler to produce the following sequence of RNA: 5' – GGU AUC ACA UGA CUA AAC GAG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC UUU U – 3' (Bio-Rad, Hercules, CA).

2.2.3 Ethanol Precipitation of Single-Guide RNA

RNase-free sodium acetate (3 M, pH 5.2) and 100% ethanol (v/v) were added to the reaction mixture and frozen in -20 °C for 20 minutes. Mixture was centrifuged at max speed at 4 °C for 10 minutes. Liquid was decanted off, pellet was washed with 70% ethanol (v/v), and centrifuged again at max speed at 4 °C for 10 minutes. Wash was repeated once more. Pellet was air dried, resuspended in RNase-free Tris-EDTA buffer (TE, 10 mM tris, 1 mM EDTA, pH 7.5), and stored in -20 °C until use. SpT2-sgRNA was loaded onto a 6% denaturing urea gel and stained with ethidium bromide for analysis of full-length product.

2.2.4 Conditions for *In Vitro* Cleavage Assay

To check the quality of *in vitro* transcription, sgRNA was incubated with Cas9 and the complex was checked for cleavage activity. Target DNA for cleavage was

cloned by another student in the lab, expressed from freezer stock CR083, extracted, and linearized by digest with NotI enzyme. Sp Cas9 was pre-incubated in a 37 °C water bath for 30 minutes then reacted with target DNA at room temperature for 10 minutes. Reaction was loaded on 0.5% agarose gel for analysis of target DNA cleavage.

2.2.5 Blocked Beacon and Blocked PAM Complexes

All DNA oligonucleotides were synthesized by Integrated DNA Technologies. The 3' fluorescein-labeled beacon target strand contained 50 nucleotides (5' – CCAGAG CCGTAAGTTAGTTGGAGA CCT TCGTTTAGTCATGTGATACctat – 3') with a toehold sequence colored pink, a branch migration region colored purple, and a protospacer sequence for crRNA recognition colored red. The 5' Iowa Black® FQ-labeled beacon non-target strand contained 23 nucleotides (5' – ataGGTATCACATGACTAAACGA – 3'). The blocking strand on the beacon complex contained 19 nucleotides with one nucleotide of overlap into the Sp PAM sequence (5' – G TCTCCA ACTA ACTTACGG – 3'). The PAM strand with the full Sp PAM sequence contained 40 nucleotides (5' – AGG TCTCCA ACTA ACTTACGG CTGTGG AGTGTGACAATGG – 3') with the Sp PAM sequence colored dark blue. The blocking strand on the PAM complex contained 25 nucleotides (5' – CAAACA CCATTGTCACACT CCAGAG – 3') with a toehold sequence colored light blue and a branch migration region colored orange. A DNA mimic of miR-122 contained 22 nucleotides (5' – TGG AGTGTGACAATGG TGTTTG – 3'). Oligonucleotide complexes were hybridized on a S1000™ Thermal Cycler at 1 μM in a hybridization buffer (40 mM Tris, 100 mM NaCl, 5 mM MgCl₂, pH 7.9) by heating to 95 °C and slow cooling to 4 °C overnight (Bio-Rad, Hercules, CA).

2.2.6 Conditions for Beacon Assay

Sp dCas9-mCherry-his₆ and SpT2-sgRNA were incubated for 30 minutes in a 37 °C water bath to initiate complex formation. Toehold displacement reactions were run in hybridization buffer in the dark for one hour at 100 nM. Before fluorescence measurements the reactions were diluted to 1 nM by mixing with 10 nM pre-complexed dCas9 – sgRNA in a beacon binding buffer (20 mM Tris, 120 mM NaCl, 1 mM MgCl₂, 5% glycerol, 0.1 mM DTT, 0.02% Tween 20, pH 7.9). Fluorescence measurements were performed on a microplate reader by excitation at 485 nm and emission at 520 nm (Synergy4; BioTek, VT). The sensitivity was adjusted to 150. Measurements were carried out in a 96-well Corning microtiter plate.

2.2.7 Conditions for Electromobility Shift Assay

Oligonucleotide complexes were hybridized on a S1000TM Thermal Cycler at 1 μM in the hybridization buffer (40 mM tris, 100 mM NaCl, 5 mM MgCl₂, pH 7.9) by heating to 95 °C and slow cooling to 4 °C overnight (Bio-Rad, Hercules, CA). To achieve high intensity visualization, the beacon non-target strand labeled Iowa Black[®] FQ was not included in oligonucleotide complex hybridization for electromobility shift assays. Toehold displacement reactions were run in hybridization buffer for one hour at 100 nM then loaded onto a 20% native acrylamide gel. The gels were run in the dark for 2.5 hours at 100 V before fluorescence imaging on a Typhoon 9400 Variable Mode Imager.

2.3 Results and Discussion

2.3.1 Design of the dCas9 Beacon Assay

The objective of this work is to design a sensor based on binding of catalytically inactive Cas9 to a double-stranded beacon by triggering the addition of a cancer-relevant miRNA to initiate reconstitution of a functional PAM on the beacon via TMSD (Figure 2). Previous work demonstrated that a mutant non-functional PAM (Sp Cas9 and dCas9 recognize 5' – NGG – 3' where N is any nucleotide) was unable to initiate displacement of a quencher-labeled oligonucleotide on a double-stranded molecular beacon (Mekler et al. 2016, O'Connell et al. 2014). We anticipate that a partially double-stranded blocked beacon complex with fluorophore and quencher-labeled oligonucleotides hybridized to a complementary blocking strand would not result in displacement of the quencher strand and an increase in fluorescence after addition of pre-complexed dCas9 – sgRNA because of lack of a full functional PAM sequence on the non-complementary strand. However, addition of miR-122 initiates toehold exchange with another partially double-stranded complex called the blocked PAM by binding of miR-122 with the 5' complementary toehold on the its blocking strand. The previously sequestered PAM strand containing the Sp PAM sequence triggers a second toehold displacement and subsequent branch migration at the 5' complementary toehold on the fluorophore-labeled strand of the blocked beacon complex (Figure 2). Upon addition of pre-complexed dCas9 – sgRNA we expect to observe an increase in fluorescence corresponding to binding and displacement of the quencher-labeled strand due to reconstitution of a full PAM sequence on the non-complementary strand of the beacon.

2.3.2 Initial Functionality of the dCas9 Beacon Assay

We first aimed to verify that binding of the RNA-guided dCas9 complex and displacement of the quencher-labeled oligonucleotide strand was dependent on the presence of a functional PAM sequence on the beacon. When an unblocked beacon was reacted with an unblocked PAM strand for an hour, a full response was observed compared with the background signal when no PAM strand was present (Figure 3). The maximal ON signal was determined by hybridizing the fluorophore-labeled strand with the PAM strand without quencher. However, when the blocked beacon complex was mixed with the blocked PAM complex, a moderate background signal was observed. Background signal in the absence of any miR-122 trigger may hinder sensitivity of the assay, so we sought to determine the source and reduce the background signal.

2.3.3 Complex Formation by Electromobility Shift Assay

Electromobility shift assay was utilized to determine the extent of toehold displacement reaction. Complex formation was confirmed for the fluorophore-labeled strand hybridized to the PAM strand as well as for the blocked beacon complex by detecting a slower mobility compared with the fluorophore-labeled strand by itself (Figure 4A, lanes 1-3). The presence of background signal was confirmed when the blocked beacon was mixed with the blocked PAM in the absence of a miR-122 input, as two additional larger complexes were observed in addition to the complex corresponding to blocked beacon by itself (Figure 4, lanes 3-4). The smaller of the two additional complexes corresponded to the same size as the fluorophore-labeled strand hybridized with the PAM strand, suggesting that this leak resulted from the blocked PAM complex. Indeed, with 1.2 molar excess of the blocking strand on the PAM

complex the smaller of the two additional complexes was eliminated (Figure 4A, lane 4*). Furthermore, with 4 molar excess of the blocking strand on the beacon complex, the larger of the two additional complexes was greatly reduced, indicating that this leak resulted from the blocked beacon complex (Figure 4B, lane 4*).

2.3.4 Optimized Functionality of the dCas9 Beacon Assay

After reducing the sources of background signal by electromobility shift assay, the miRNA trigger was introduced into dCas9 beacon assay. Upon addition of precomplexed dCas9 – sgRNA to the toehold displacement reactions, a full response was detected in the presence of miR-122 (Figure 5). The maximal ON signal was determined from mixing of the unblocked beacon with the PAM strand. Furthermore, the background signal from the blocked beacon and blocked PAM complexes in the absence of miR-122 was considerably lower, indicating that 1.2:1 molar excess and 4:1 molar excess of the blocking strands on the PAM and beacon complexes, respectively, was successful in reducing leak. In addition, the sensitivity of the dCas9 beacon assay was evaluated using a range of concentrations of miR-122 input (Figure 6). The detection of miR-122 was linear from approximately 50 nM to 200 nM concentration of input. Since the volume of a typical mammalian cell is 1000 – 25,000 μm^3 , the intracellular miRNA concentrations can range from as low as about 80 pM (1 copy in a 25,000 μm^3 cell) to as high as about 22 μM (10,000 copies in a 1,000 μm^3 cell) (Ragan, Zuker, and Ragan 2011). Additionally, in tumor cells certain miRNAs can be expressed at an unusually high concentrations (Lu et al. 2005). From this information, this method based on dCas9 binding to a beacon should be able to detect at least 100 copies/cell. To be able to detect concentrations at 10 copies/cell or lower, it would necessitate incorporating an amplification scheme such as catalytic hairpin

assembly which relies on hybridization of metastable hairpins and toehold displacement for regeneration of a linear catalyst template strand to enable 50 to 100-fold signal amplification (Li, Ellington, and Chen 2011).

2.4 Recommendations for Future Work

The next step of this work would be to shift from detection of a DNA analog of a cancer-relevant miRNA to a miRNA itself. Furthermore, it would be of interest to demonstrate that the dCas9 beacon assay is adaptable as a sensor of diverse miRNA sequences by using another cancer-relevant miRNA as a trigger for beacon assembly. The ultimate goal would be to demonstrate successful detection of overexpressed miRNA extracted from cancer cells.

Chapter 3

SUMMARY AND CONCLUSION

In this thesis, a catalytically inactive Cas9 was designed and utilized to aid in cancer diagnosis by detection of relevant miRNA. Although the type II Cas9 and dCas9 proteins have been applied for a variety of applications from genome editing to transcriptional control, to our knowledge this is the first utilization of the catalytically inactive variant for *in vitro* sensor detection of single-stranded RNA molecules.

The RNA-guided dCas9 complex was triggered for binding to a molecular beacon by miR-122, a miRNA involved in breast cancer metastasis (Fong et al. 2015). Optimization of the series of toehold-mediated strand displacement reactions enabled successful conversion of the single-stranded input into the double-stranded output recognizable by the dCas9 complex. Future work resides demonstrating adaptability of the assay for diverse miRNA sequences. Ultimately, improvements to methods of miRNA detection will lead to more accurate cancer diagnoses and prolonging higher quality of life for cancer survivors (Etzioni et al. 2003).

FIGURES

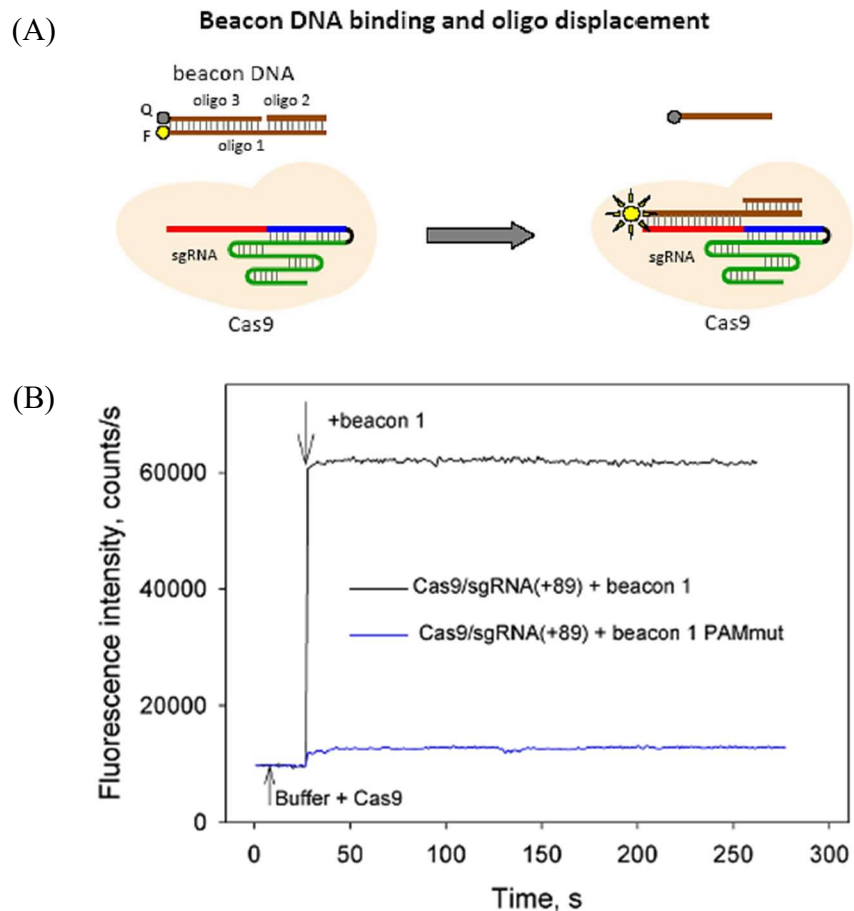


Figure 1. **FRET-based Cas9 Beacon Assay.** (A) Design of Cas9 beacon DNA. Molecular beacon consists of three annealed DNA oligonucleotides, a fluorophore-labeled strand, a quencher-labeled strand, and a strand containing the Sp PAM sequence. (B) Upon mixing of beacon DNA with complexed Cas9 (or dCas9, not shown) and sgRNA, increase in fluorescence is detected corresponding to binding to DNA target. When the PAM sequence of oligo 2 is mutated, Cas9 does not displace quencher and no increase in fluorescence is detected.

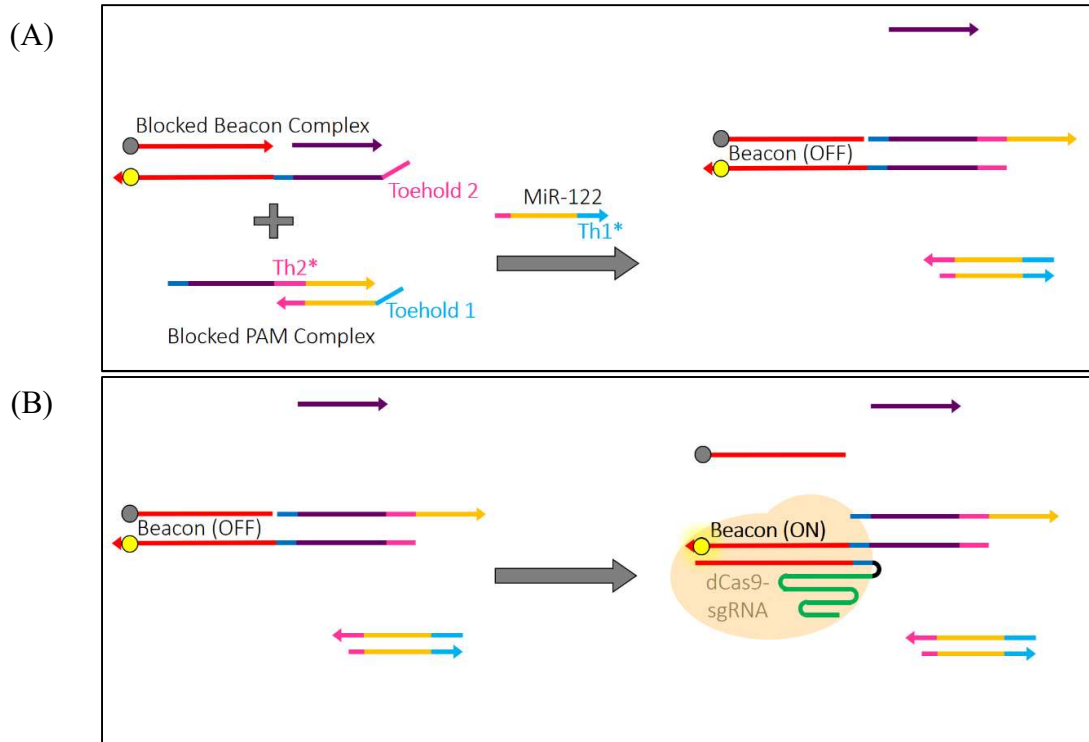


Figure 2. dCas9 Beacon Assay for miRNA Detection. (A) Addition of miR-122 triggers two sequential toehold displacement reactions resulting in a fully assembled beacon containing a PAM sequence. (B) Addition of precomplexed dCas9 – sgRNA displaces the quencher-labeled strand from the fluorophore-labeled strand resulting in an increase in fluorescence.

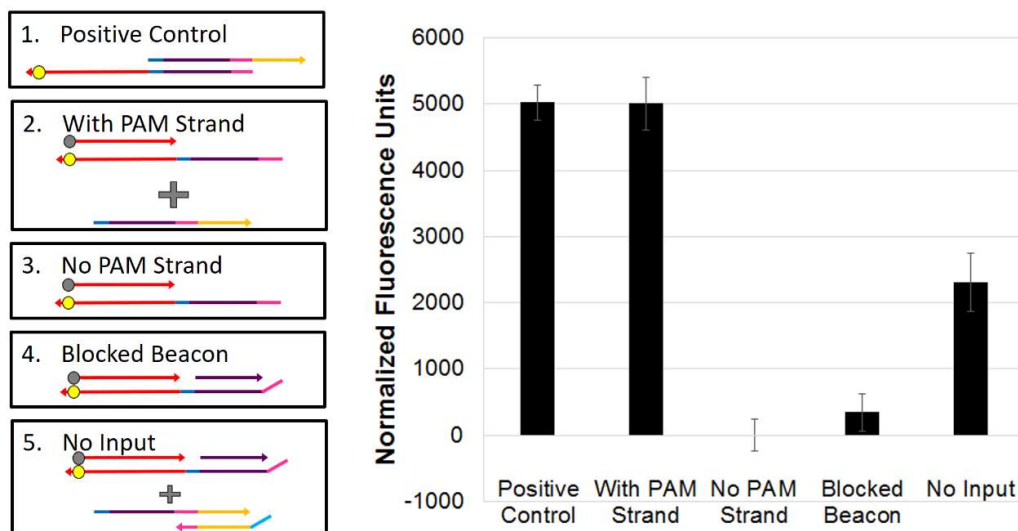


Figure 3. PAM Sequence on Beacon Results in Increase in Fluorescence. Toehold displacement reactions were run for an hour at 100 nM before mixing with precomplexed dCas9 – sgRNA and fluorescence measured. (1) Fluorescent-labeled strand hybridized with PAM strand, (2) Unblocked beacon with PAM strand, (3) Unblocked beacon without PAM strand, (4) Blocked beacon complex, (5) Blocked beacon and blocked PAM complexes.

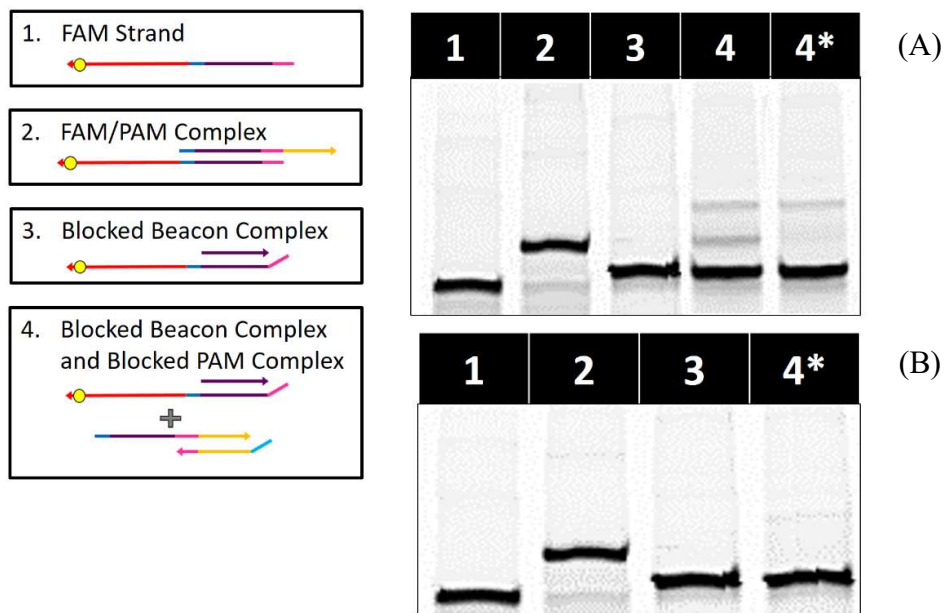


Figure 4. **Evaluation of Complex Formation by Electromobility Shift Assay.** Toehold displacement reactions were run for an hour at 100 nM before loaded onto 20% native acrylamide gels. (1) Fluorophore-labeled strand, (2) Fluorophore-labeled strand hybridized with PAM strand, (3) Blocked beacon complex, (4) Blocked beacon and blocked PAM complexes.

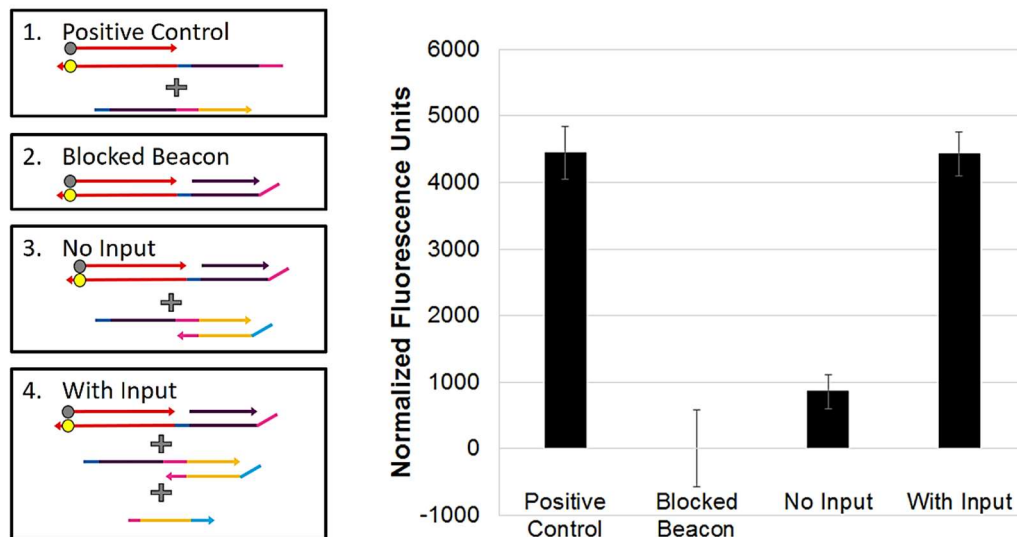


Figure 5. **dCas9 Beacon Assay Responds to MiR-122 Input.** Toehold displacement reactions were run for an hour at 100 nM before mixing with precomplexed dCas9 – sgRNA and fluorescence measured. (1) Unblocked beacon with PAM strand, (2) Blocked beacon complex, (3) Blocked beacon and blocked PAM complexes, (4) Blocked beacon complex, blocked PAM complex, and miR-122. Molar excess of 1.2:1 and 4:1 of blocking strand to PAM and beacon complexes used, respectively. For miR-122 input, 2-fold molar excess used.

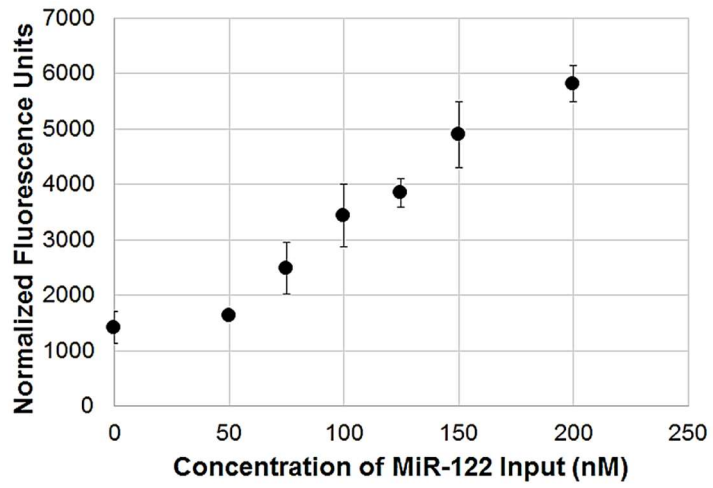


Figure 6. Dosage Response Curve for MiR-122 – Triggered dCas9 Beacon Assay. The linear range of detection was from 50 nM to 200 nM miR-122 input concentration.

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