

Toehold Switch Sensor for miRNA Cancer Biomarker

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Background

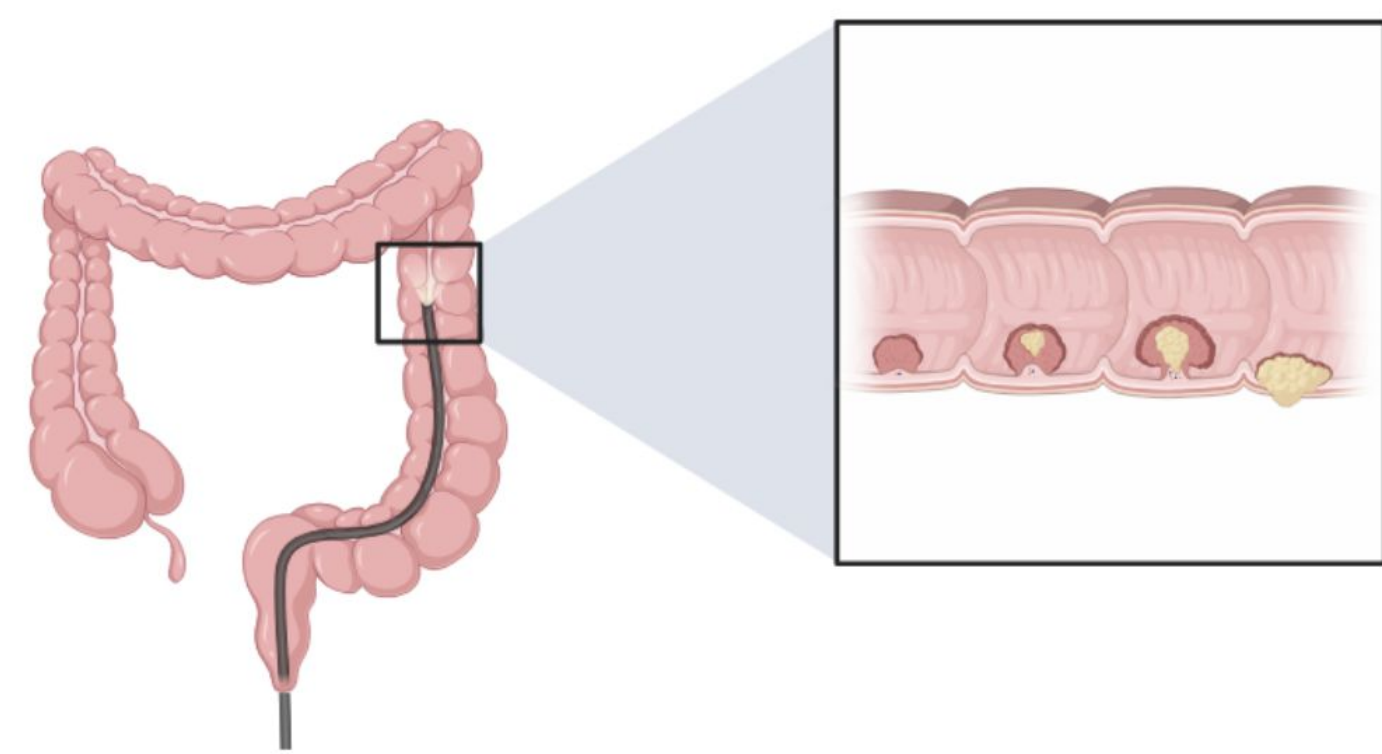


Figure 1. Colon with polyps. Colonoscopy shows the abnormal tissue growth. The structure of the right colon delays symptom onset and reduces test detection accuracy.

Colorectal Cancer (CRC) Diagnostics

- Characterized by abnormal growths in the inner lining of the colon or rectum (polyps)¹
- 3rd most common cancer in the U.S²
- Current diagnostics are invasive deterring people from seeking treatment²

miRNA Biomarker miR-29a

- MicroRNAs (miRNAs) are short, non-coding RNA sequences that contribute to the regulation of gene expression³
- In CRC patient blood plasma, miR-29a levels are upregulated³

Cell Free Reaction with Toehold

- Uses cellular components to carry out transcription/translation, which is useful for portable screening applications⁴, such as miRNA detection

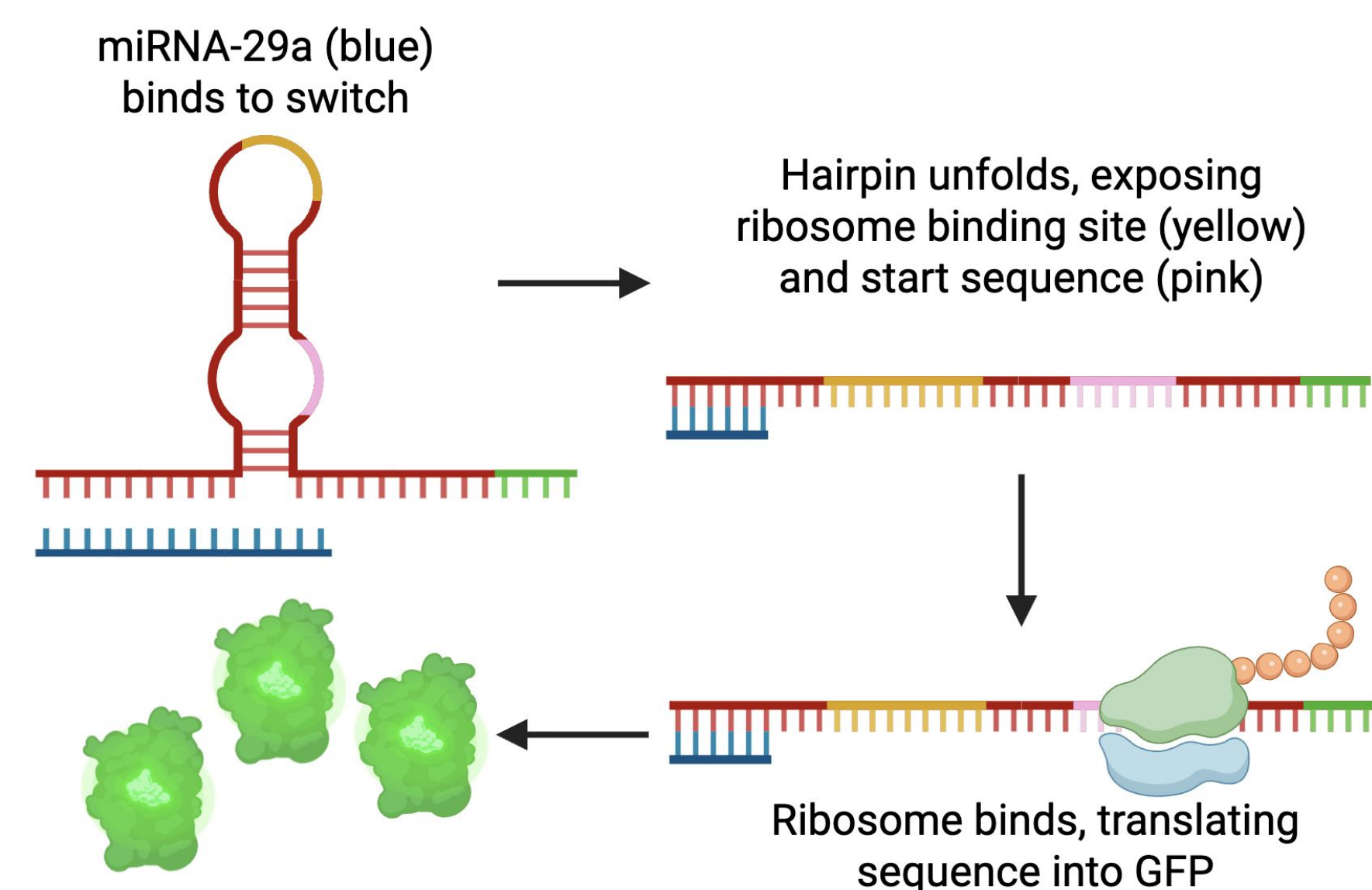
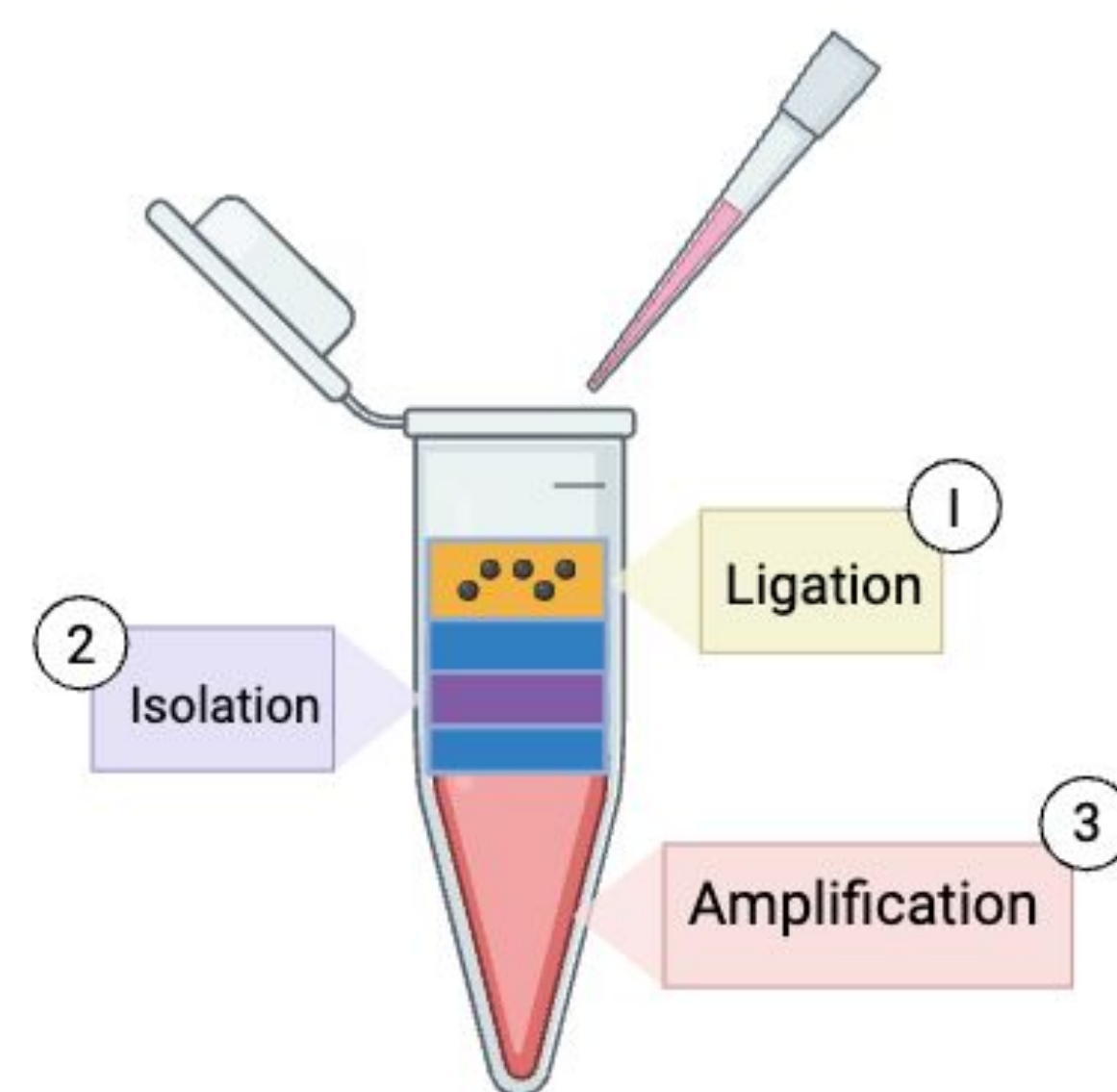


Figure 1. Target miRNA binds to the complementary site, unraveling the hairpin loop containing a ribosome binding site allowing for the translation of the reporter region, producing a fluorescent signal.

Figure 2. TRAP⁷ System

- Separates reactions in one tube using wax layers (blue) of increasing melting points⁸
- Plasma in first layer containing magnetic beads coated with DNA probes bind to the miRNA
- As layers are heated to their melting points, a magnet pulls the beads through layers for washing before the final layer, where the toehold switch initiates the One-Pot Ligation Assay Reaction to express GFP



Intent & Motivation

- **Objective:** To develop an early screening tool for CRC using a toehold switch sensor that expresses fluorescence (GFP) in the presence of miRNA-29a.
- **Significance:** To offer a less invasive, accessible, and cost-effective CRC screening method that will enable patients to seek diagnosis and treatment sooner.

Methods

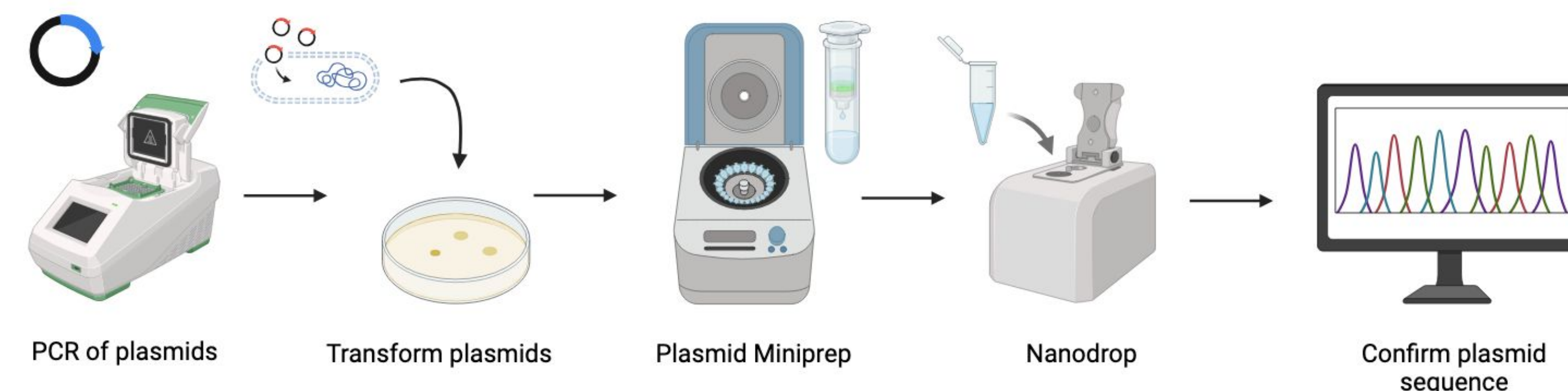


Figure 3. Gibson Assembly⁹ combines vector backbone and toehold switch sequence⁶, into a plasmid. This plasmid is transformed into NEB 5- α Competent *E. coli* cells for replication. The plasmids are then isolated from the bacterial cell using plasmid miniprep. Nanodrop checks plasmid purity and plasmid is sequenced to check for correct structure.

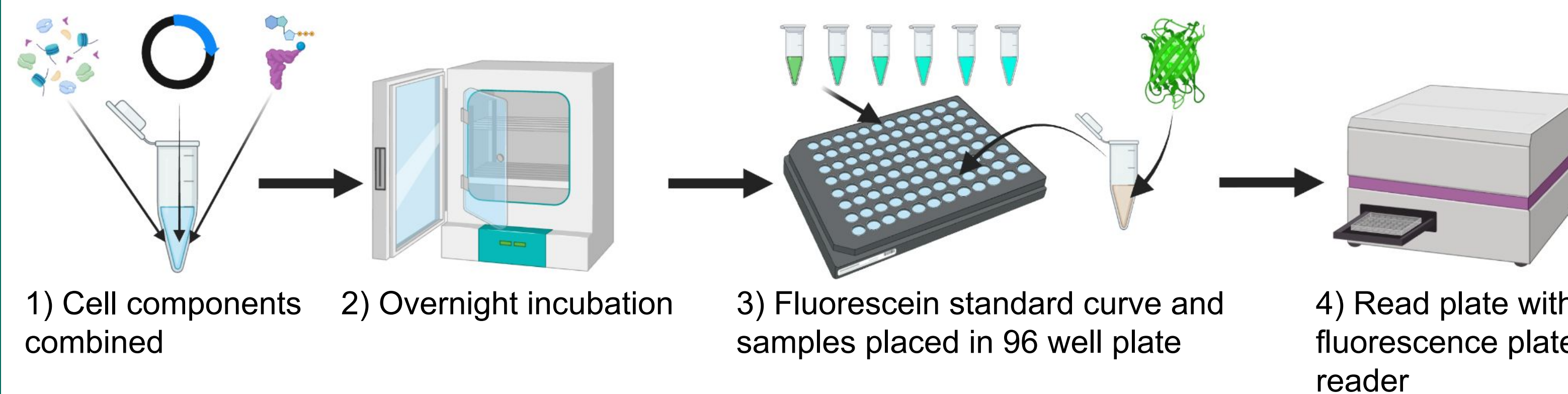
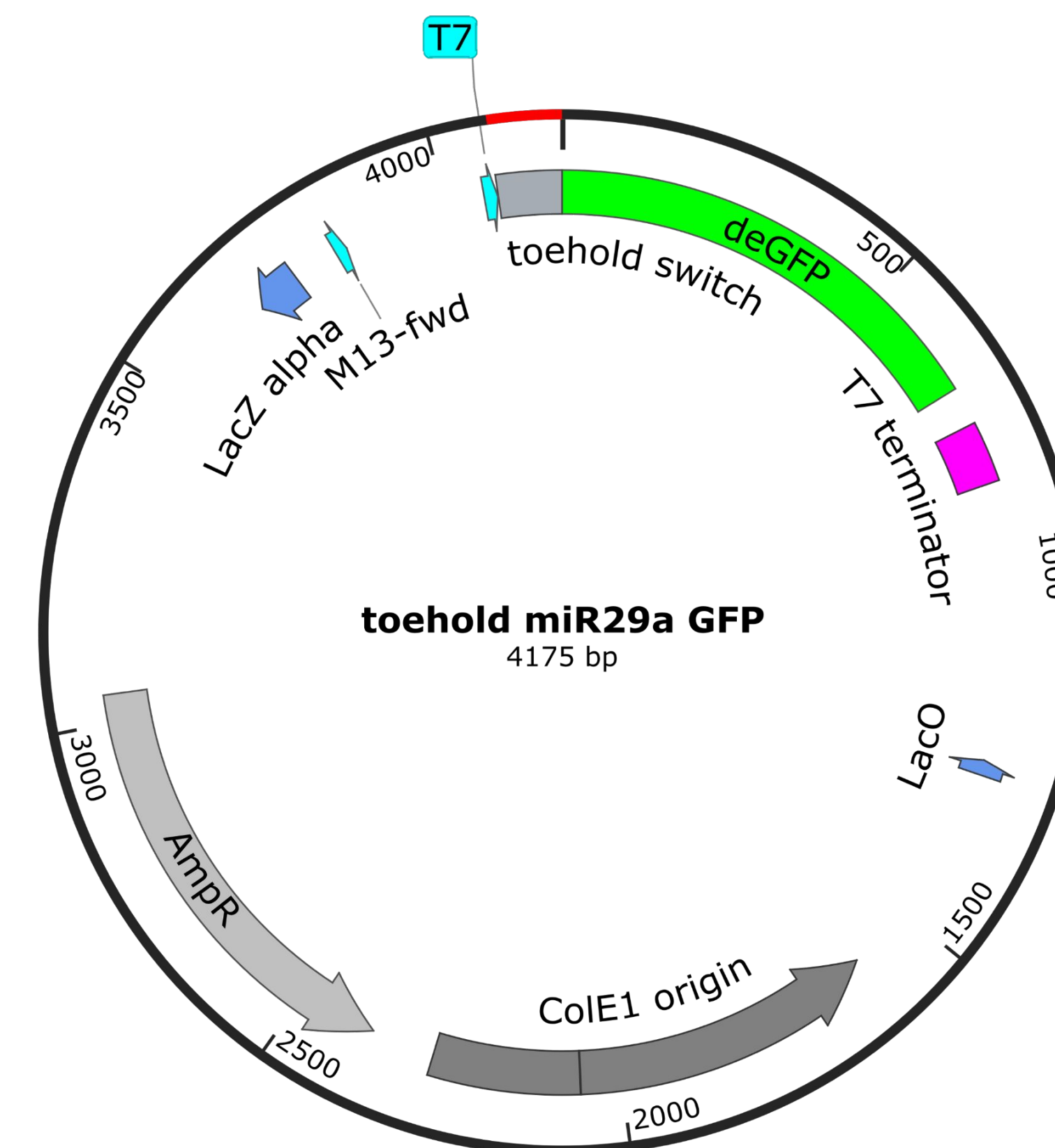


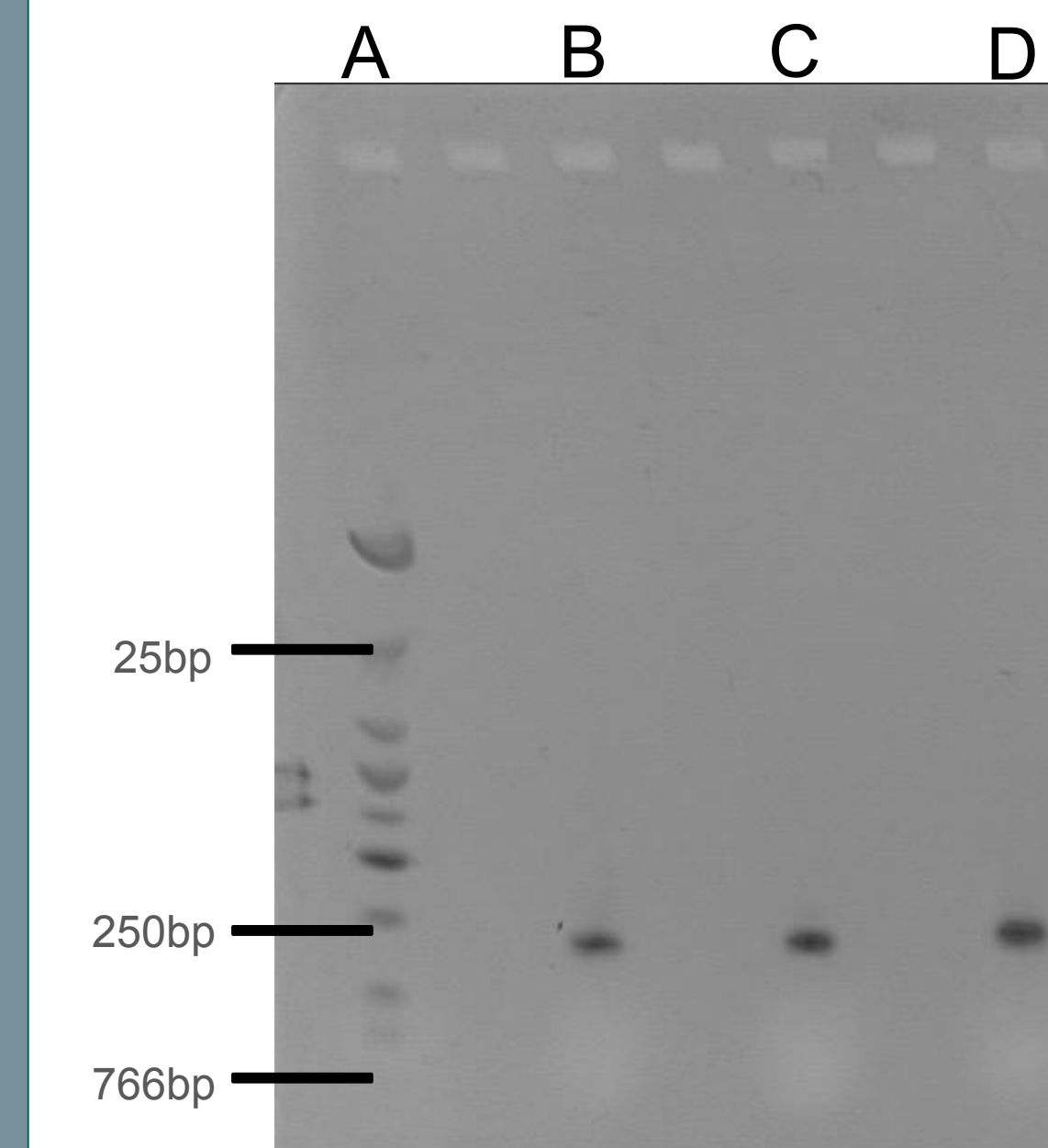
Figure 4. Cell-Free Reaction Testing. Toehold switch plasmid, target miRNA-29a (encoded in a linear PCR product controlled by T7 promoter), ribosomes, and Panox Energy Mix are combined in a microcentrifuge tube and incubated overnight at 29°C. Preparation of the 96-well plate involves creating a fluorescence standard curve and diluting overnight reactions in PBS buffer. The plate is then read by a fluorescence plate reader.

Figure 5. Toehold miR-29a GFP plasmid map.

- T7 promoter toehold structure unfolds when target miRNA-29a present (refer to Figure 1 for mechanism) allowing deGFP synthesis
- AmpR is for ampicillin resistance selection
- ColE1 origin is for replication
- LacO/LacZa is for inducible expression, cloning, and standard sequencing primer site (M13-fwd)



Results & Discussion

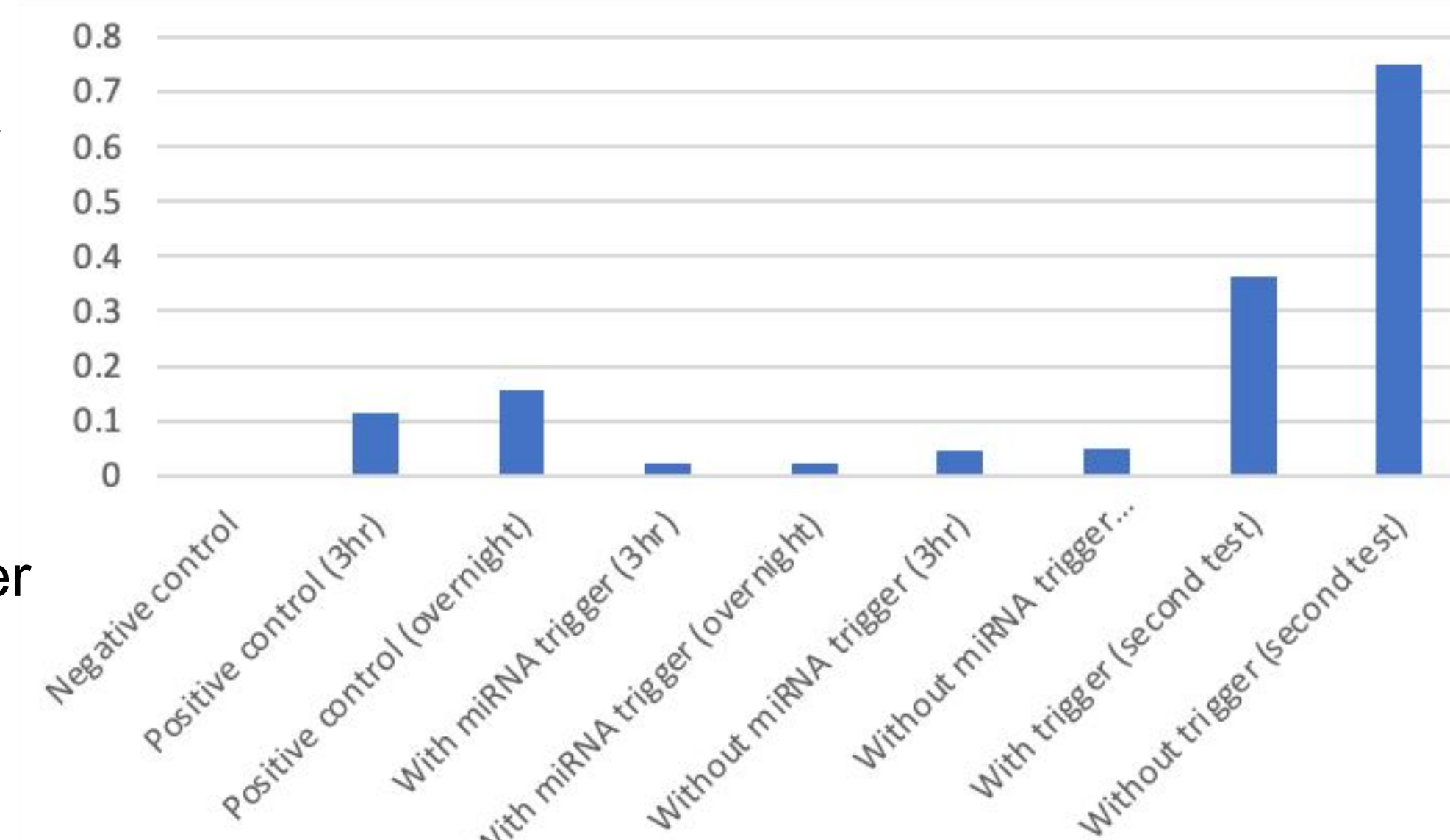


A: Purple Low MW DNA Ladder
B: PCR Toehold Switch Insert "F" (66 °C)
C: PCR Toehold Switch Insert "E" (66.9 °C)
D: PCR Toehold Switch Insert "D" (68 °C)

Figure 6. Gel of toehold switch insert PCR products at varying annealing temperatures (66 °C, 66.9 °C, 68 °C). 25 cycles of PCR were performed of toehold switch insert (using DreamTaq Mastermix) to prepare for Gibson Assembly. A 2% gel stained with SYBR safe dye was then ran for 30 minutes at 135V of the PCR products to confirm purity and size.

Figure 7.

Fluorescence reading of toehold biosensor in a cell-free system (containing in-house CellEx Lysate, PANOX and GamS) with and without miRNA biomarker after incubating for 3hrs and overnight.



Discussion:

- Toehold insert was successfully amplified and of the correct size (~280bp) and pure (concentrated bands)
- Sequencing using Oxford Nanopore long-read confirmed sequence
- Successful assembly of plasmid
- Toehold shows trend of being an off switch rather than an on switch

Future Work

- Re-test cell-free reactions with more controls (non-trigger miRNA and trigger in positive control)
- Assemble the TRAP system.
- Determine miRNA detection threshold, and ranges for negative and positive tests
- Test with human plasma samples and check the performance of the biosensor.

References & Acknowledgements



References

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