

Modelling toehold switches *in silico* for the detection of miRNA signatures for the diagnosis of colorectal cancer

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Under the direction of Dr Elizabeth Wood, JURA Bio, Inc.

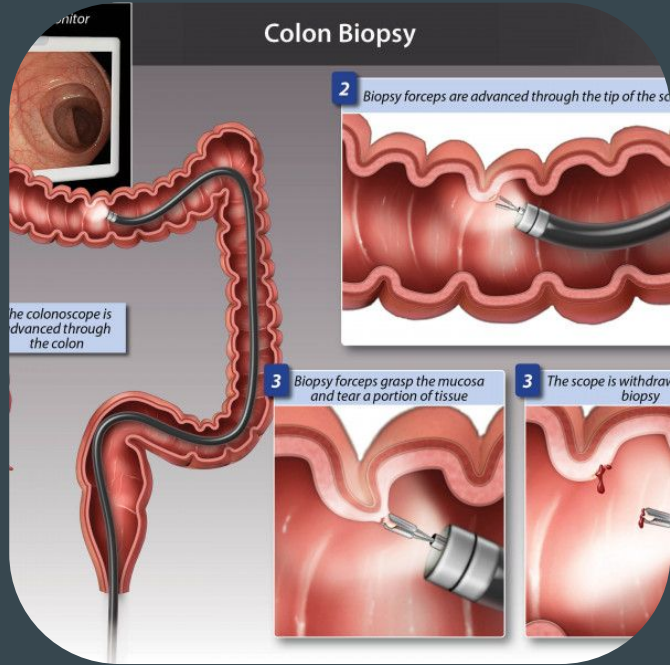
1,900,000

cases of colorectal cancer globally in 2021

60%

of colorectal cancer deaths can be prevented by early screening

The issue with Colorectal Cancer (CRC) diagnosis



<https://www.trialexhibitsinc.com/library-item/colon-biopsy>

- Diagnostic method is invasive procedure
 - Severe side effects eg. perforation of the colon
 - Purging may have detrimental effects for patient health
 - Only fully effective 88% of the time

miRNA signatures can be used to diagnose CRC

- Short noncoding RNA molecules circulating in the bloodstream.
- Aberrant microRNA expression causes carcinogenesis
- Certain 'panels' of miRNAs can be indicative of disease

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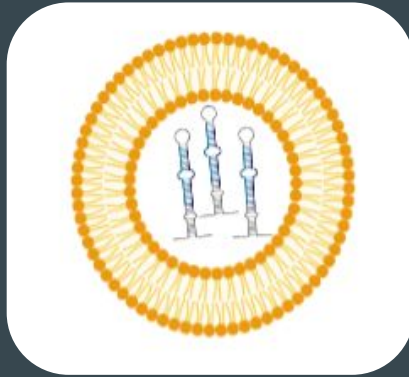
Non-invasive
early-stage detection

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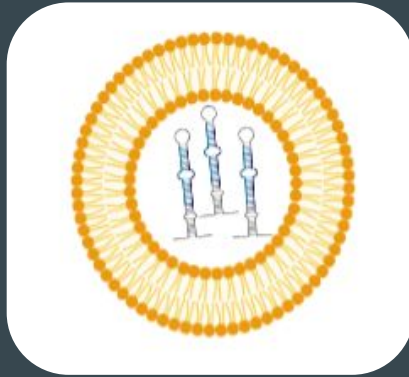
Stable in
lipoprotein complexes

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Non-invasive
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Stable in
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Specific binding for
accurate diagnosis

miRNA signatures for CRC


- miR-145-5p
- miR-200c-3p
- miR-375-3p
- miR-203a-3p

miRNA signatures for CRC


- miR-145-5p



- miR-200c-3p





- miR-375-3p





- miR-203a-3p

miRNA signatures for CRC

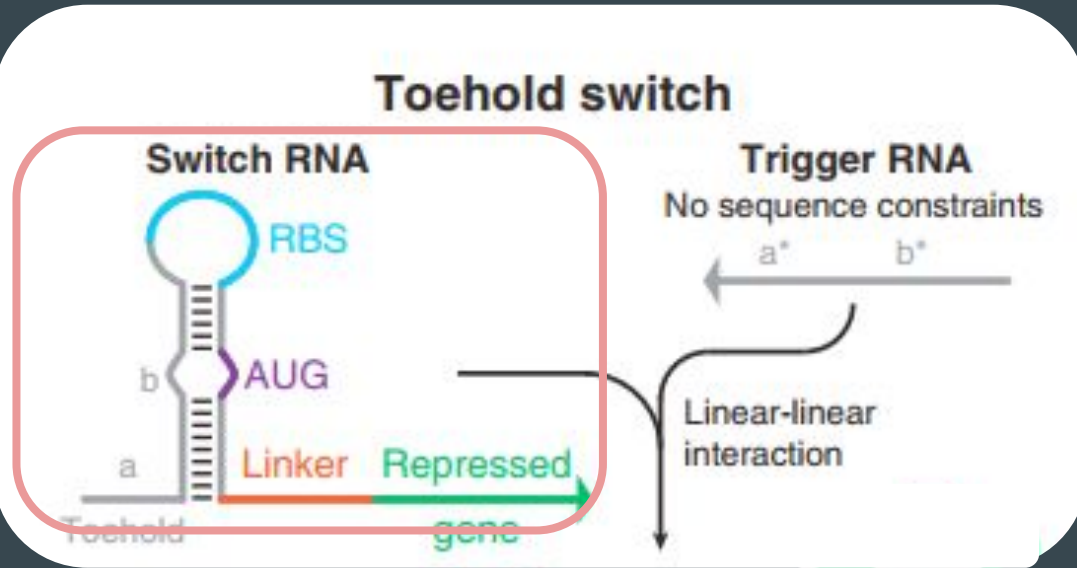
- miR-145-5p 

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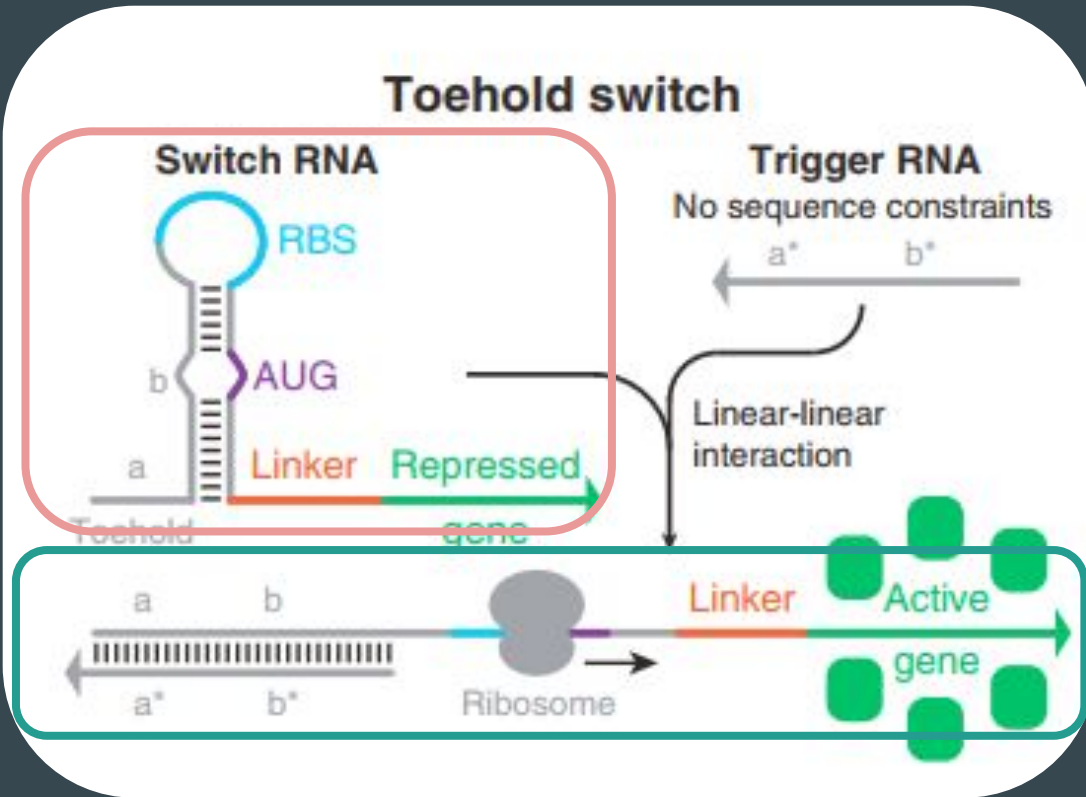
- miR-203a-3p 

Toehold switches to detect miRNA targets



OFF state

Toehold switches to detect miRNA targets



OFF state

Conformational
change occurs
upon binding

ON state

Goals for this project



Designing toehold switches *in-silico* to detect the target miRNAs of CRC



Evaluating the viability of the switches generated by analysing biophysical data



1

Generating toehold switches with Toehold

1

Generating toehold switches

Toehold generates RNA sequences for multiple potential switches based on the target gene region

1

Generating toehold switches with Toeholder

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Toeholder generates RNA sequences for multiple potential switches based on the target gene region.

2

Elimination of invalid structures

Invalid switches are eliminated by Toeholder.

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Generating toehold switches with Toehold

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Generating toehold switches

Toehold generates RNA sequences for multiple potential switches based on the target gene region.

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Elimination of invalid structures

Invalid switches are eliminated by Toehold.

3

Evaluating properties of each switch

Secondary structures and biophysical data for each switch are obtained.



2

Evaluation of the efficiency of toehold switches generated

2

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GC Content

- Implications for:
 - Stability
 - Ease of synthesis

Acceptable GC content:

20-60%

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Minimum Free Energy (MFE)

- Used for direct comparison of stability

Acceptable MFE:
Lower than unbound switch
and target

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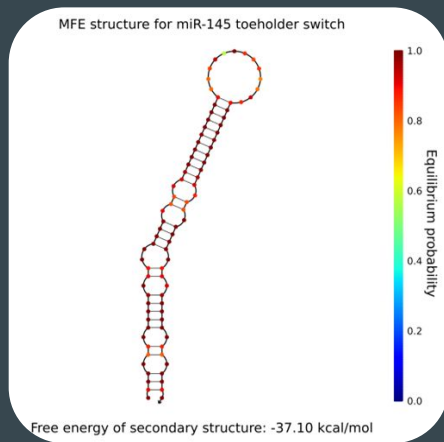
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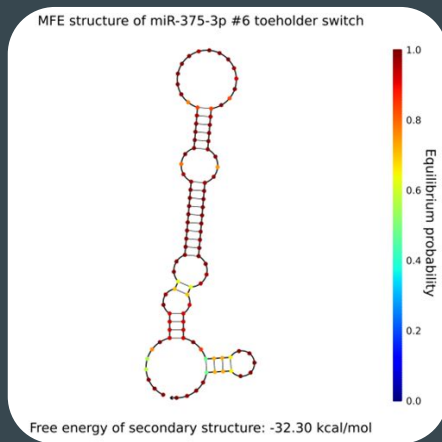
Pair probability graphs

- Illustrates equilibrium base pairing probabilities for each base in ordered complexes
- Can calculate overall probability of complex forming

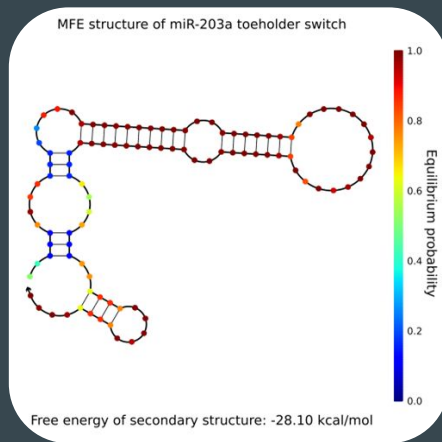
Results: Secondary structures generated for switches



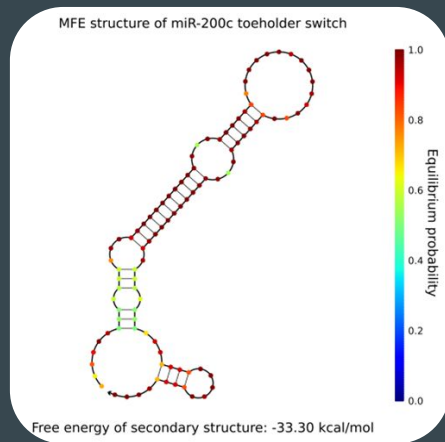
miR-145



miR-375-3p



miR-203a



miR-200c

GC content in % 53.33

60.00

40.00

56.67

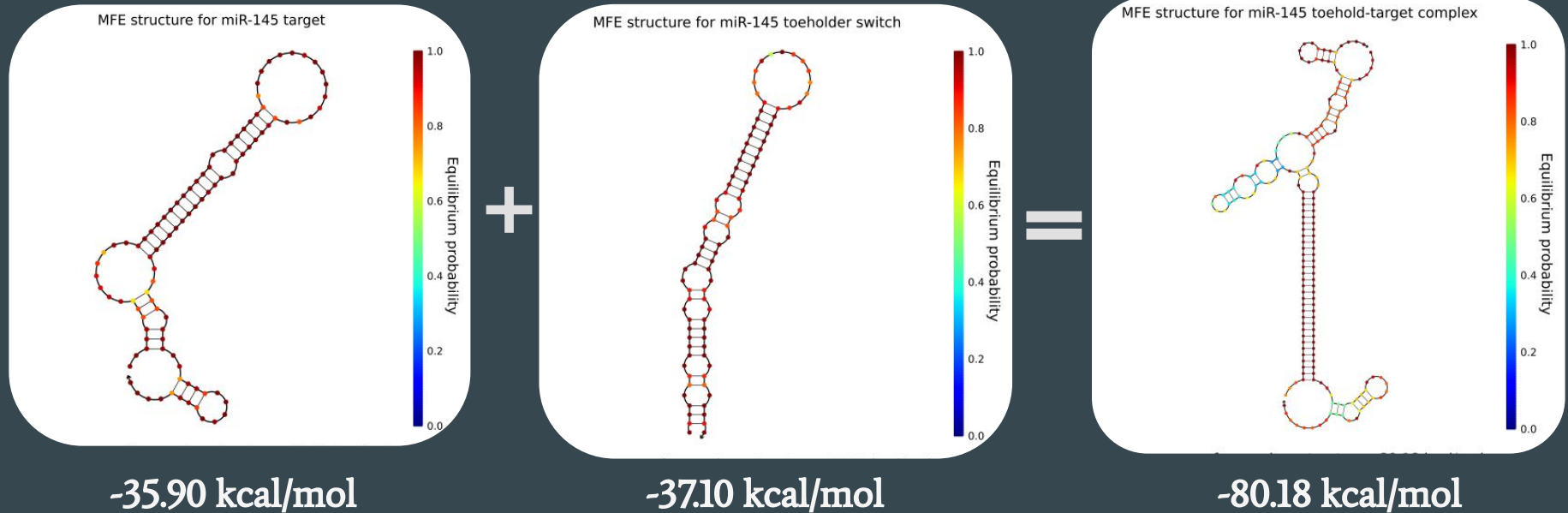
MFE of
complex in
kcal/mol -80.18

-77.48

-79.98

-82.38

Results: Secondary structures for miR-145

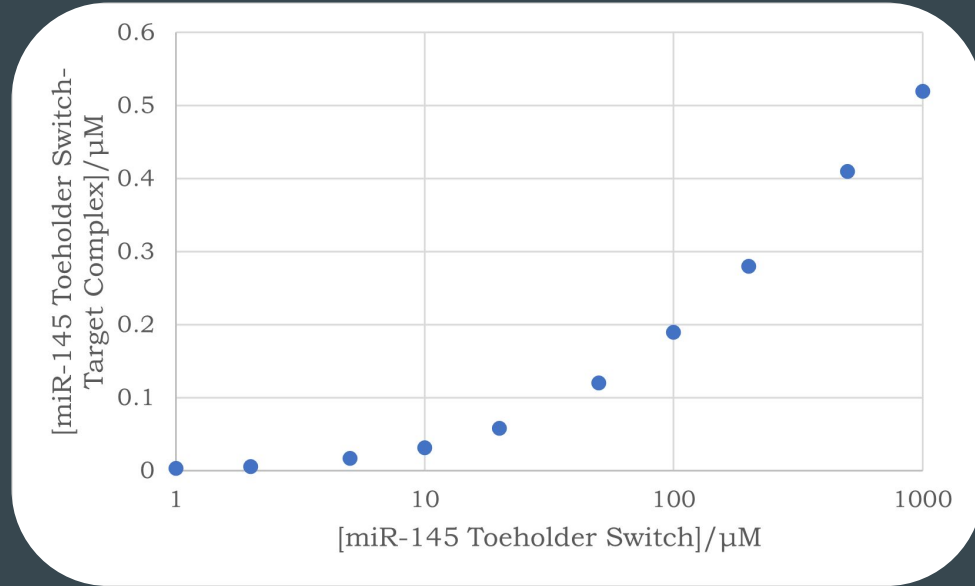


Conformational change occurs upon binding

Investigating Aberrant Result: miR-145 switch-target complex

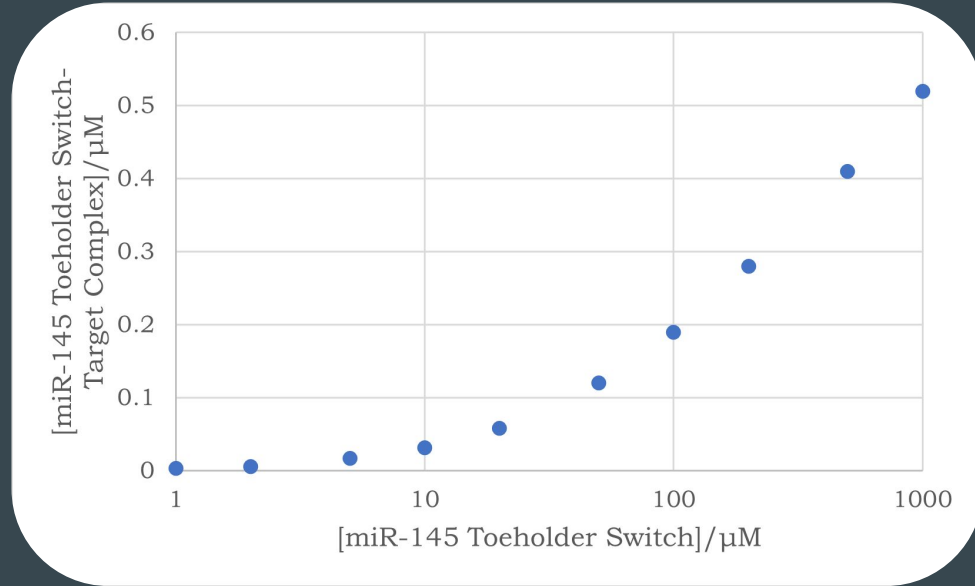
Investigating Aberrant Result: miR-145 switch-target complex

- Observed that binding ratio of miR-145 switch and target was significantly less than 1:1
- 1000 μ M of switch
 - Signal: 0.52 μ M of switch-target complexes
 - Noise: 350 μ M of switch-switch complexes



Investigating Aberrant Result: miR-145 switch-target complex

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Low signal to noise ratio \longrightarrow Unviable sensor for diagnosis

Conclusions

We have identified that to detect miRNA signatures for diagnosing CRC:

- 1 miR-145 switch is **invalid**
- 2 miR-203a switch should detect **upregulation**
- 3 miR-375-3p and miR-200c switches should detect **downregulation**

In the future, this logic can be applied to point-of-care diagnostic devices for CRC.

Acknowledgements

Special thanks to:

- Dr Elizabeth Wood
- Dr Matt Cain
- Dr Andre Nguyen
- Tutor Joy Xu
- Tutor Catherine Xue
- Ali Christine Yang
- RSI 2022 peers
- Singapore Ministry of Education
- Research Science Institute (RSI)
- Massachusetts Institute of Technology (MIT)
- The Center for Excellence in Education (CEE)

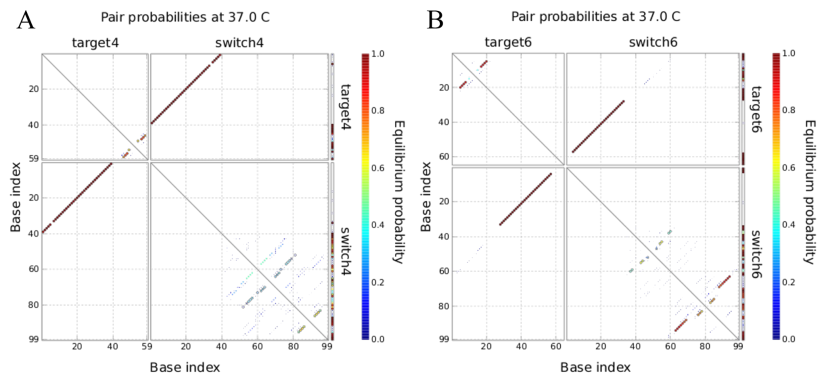
References

- [1] American Society of Clinical Oncology. What is colorectal cancer?: How does colorectal cancer start? <https://www.cancer.org/cancer/colon-rectal-cancer/about/what-is-colorectal-cancer.html>, 2020.
- [2] Centers for Disease Control and Prevention. What are the symptoms of colorectal cancer? https://www.cdc.gov/cancer/colorectal/basic_info/symptoms.htm, Feb 2022.
- [3] American Society of Clinical Oncology. Colorectal cancer statistics. <https://www.cancer.net/cancer-types/colorectal-cancer/statistics>, May 2022.
- [4] S. Wang, L. Wang, N. Bayaxi, J. Li, W. Verhaegh, A. Janevski, V. Varadan, Y. Ren, D. Merkle, X. Meng, et al. A microRNA panel to discriminate carcinomas from high-grade intraepithelial neoplasms in colonoscopy biopsy tissue. *Gut*, 62(2):280{289, 2013.
- [5] C. C. AF, F. Rouleau, C. Bautista, P. Lemieux, and N. Dumont-Leblond. Toehold: a software for automated design and in silico validation of toehold riboswitches. 2021.

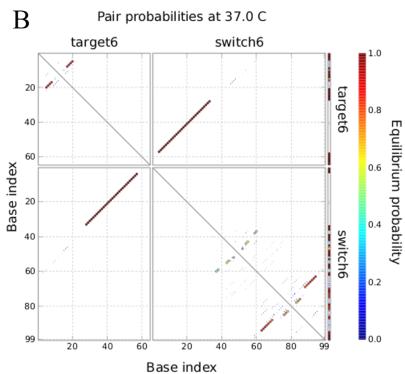
References

- [6] J. N. Zadeh, C. D. Steenberg, J. S. Bois, B. R. Wolfe, M. B. Pierce, A. R. Khan, R. M. Dirks, and N. A. Pierce. NUPACK: Analysis and design of nucleic acid systems. *Journal of Computational Chemistry*, 32(1):170{173, 2011.
- [7] C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, and K. Bealer. BLAST+: Architecture and applications. *BMC Bioinformatics*, 10:421, 2009.
- [8] G. R. Brown, V. Hem, K. S. Katz, M. Ovetsky, C. Wallin, O. Ermolaeva, I. Tolstoy, T. Tatusova, K. D. Pruitt, D. R. Maglott, et al. Gene: A gene-centered information resource at NCBI. *Nucleic Acids Research*, 43(D1):D36{D42, 2015.
- [9] S. Griths-Jones, R. J. Grocock, S. Van Dongen, A. Bateman, and A. J. Enright. BLAST+: Architecture and applications. *Nucleic Acids Research*, 34(suppl 1):D140{D144, 2006.

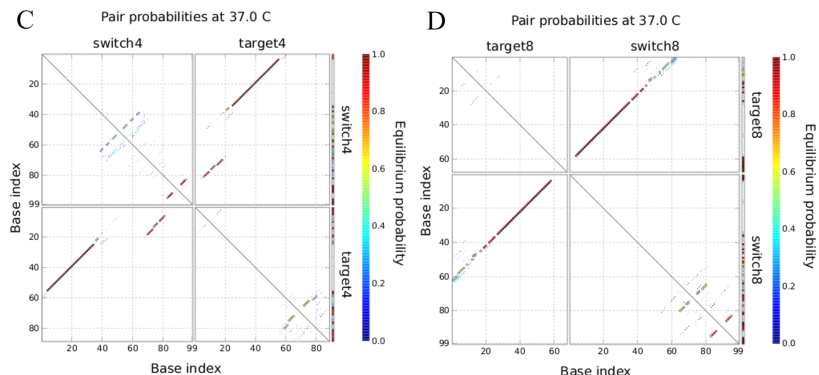
Results: Pair probability graphs for switches



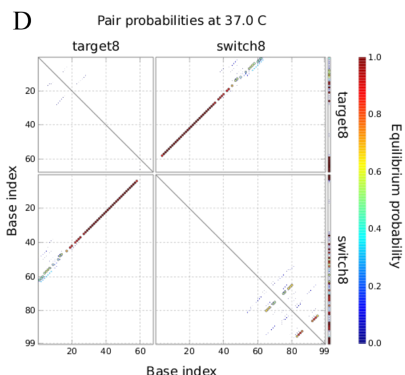
Free energy of ordered complex (-kT log Q): -82.34 kcal/mol



Free energy of ordered complex (-kT log Q): -79.63 kcal/mol



Free energy of ordered complex (-kT log Q): -82.64 kcal/mol



Free energy of ordered complex (-kT log Q): -84.82 kcal/mol

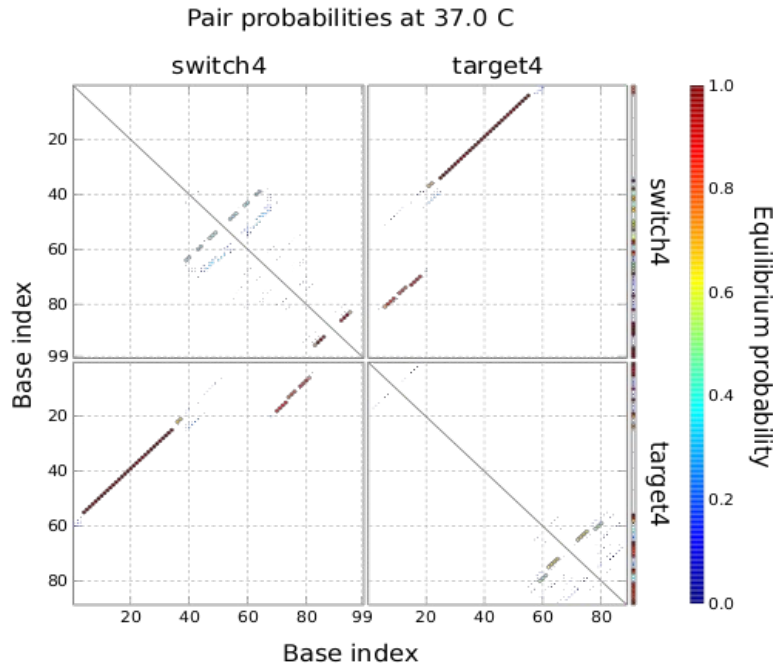
(A) miR-145

(B) miR-375-3p

(C) miR-203a

(D) miR-200c

Results: Pair probability graph for miR-145



Each point on the graph represents one of the following binding base pairs:

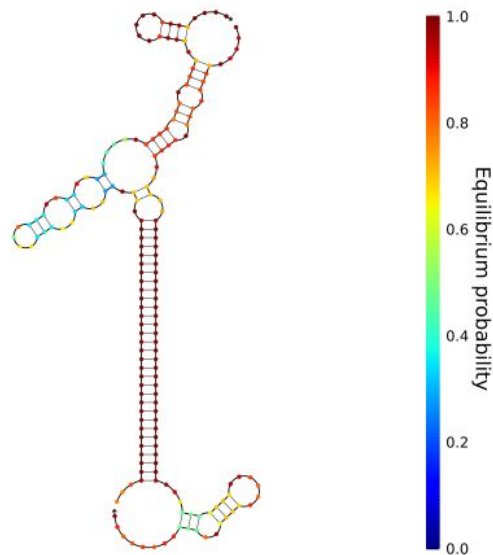
1. Switch-target
2. Switch-switch
3. Target-target

Strongest binding:

- (Target) Bases 25 – 55
- (Switch) Bases 3 – 33

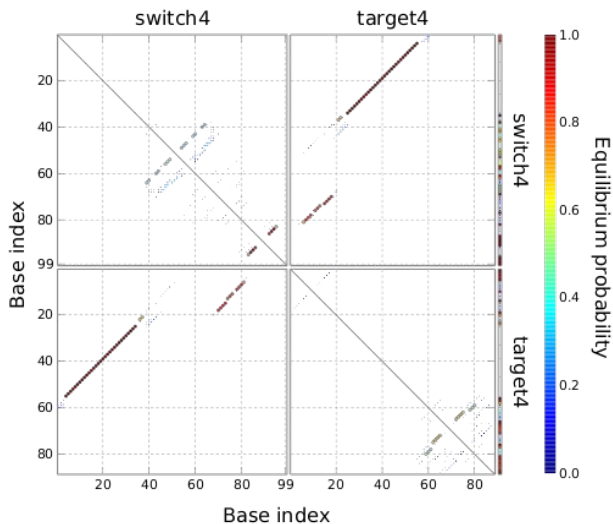
Results: miR-145

MFE structure for miR-145 toehold-target complex



Free energy of secondary structure: -80.18 kcal/mol

Pair probabilities at 37.0 C



Free energy of ordered complex ($-kT \log Q$): -82.64 kcal/mol

Complex MFE:
-80.18kcal/mol

GC Content:
53.33

Strongest binding:

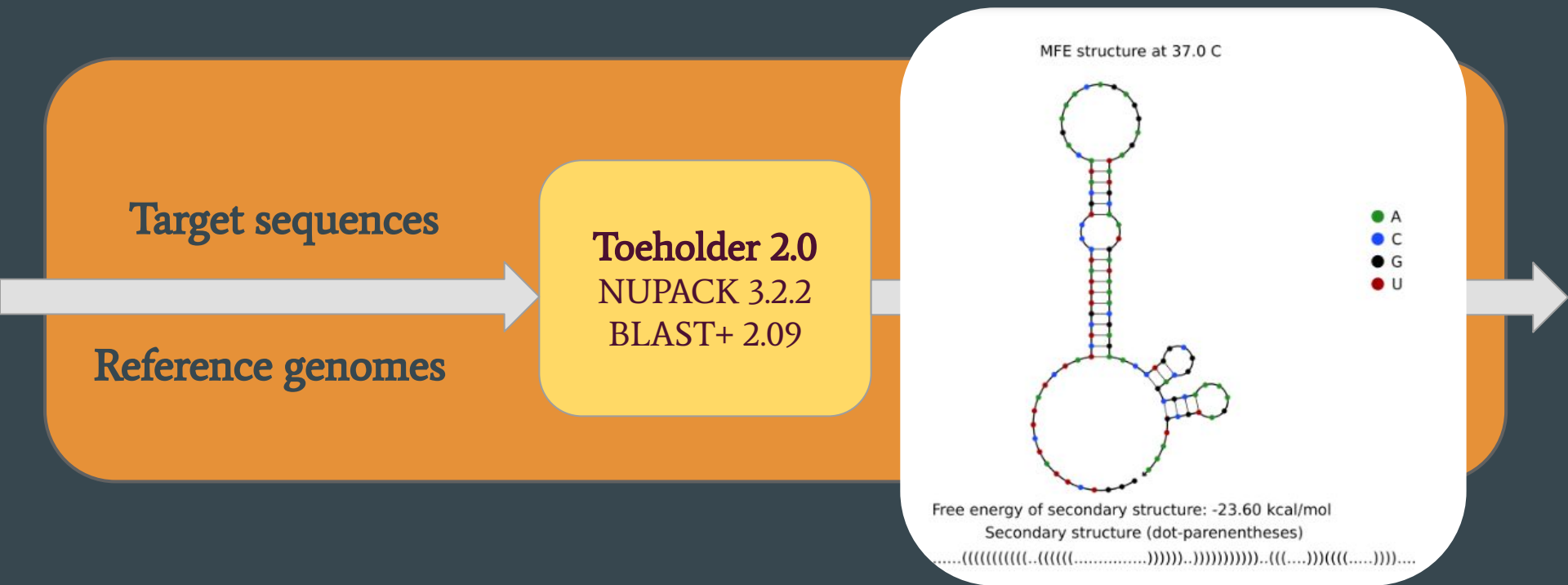
- Bases 25–55 of target
- Bases 3-33 of switch

Choosing reference genomes

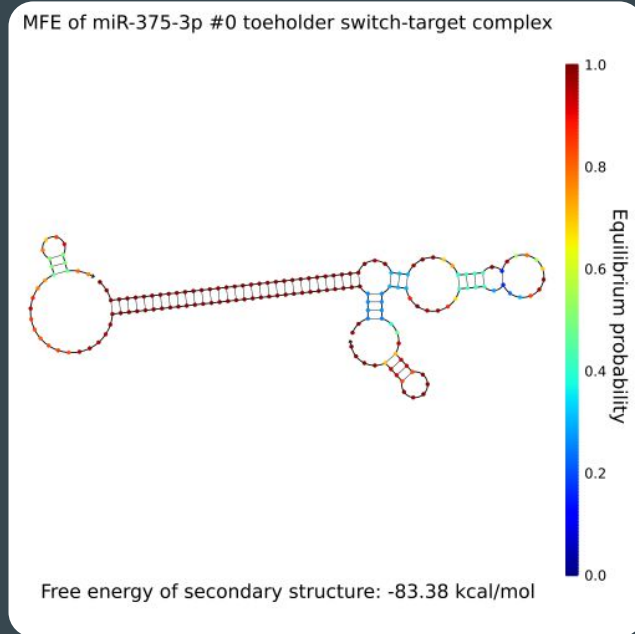
- Testing predicted specificity and versatility of generated switches using **BLAST+** tool
- *Escherichia coli*, *Homo sapiens*, MS2 phase, PM2 Phase ^[10]
- JC Polyomavirus, HPV, *Helicobacter pylori*, *Streptococcus bovis* ^[11]
- Obtained from NCBI genome database

[10] C. C. AF, F. Rouleau, C. Bautista, P. Lemieux, and N. Dumont-Leblond. Toehold: a software for automated design and in silico validation of toehold riboswitches. 2021.
[11] A. N. Burnett-Hartman, P. A. Newcomb, and J. D. Potter. Infectious agents and colorectal cancer: a review of helicobacter pylori, streptococcus bovis, jc virus, and human papillomavirus. Cancer Epidemiology Biomarkers & Prevention 17(11):2970–2979, 2008

Methods: Designing toeholder switch with Toeholder 2.0

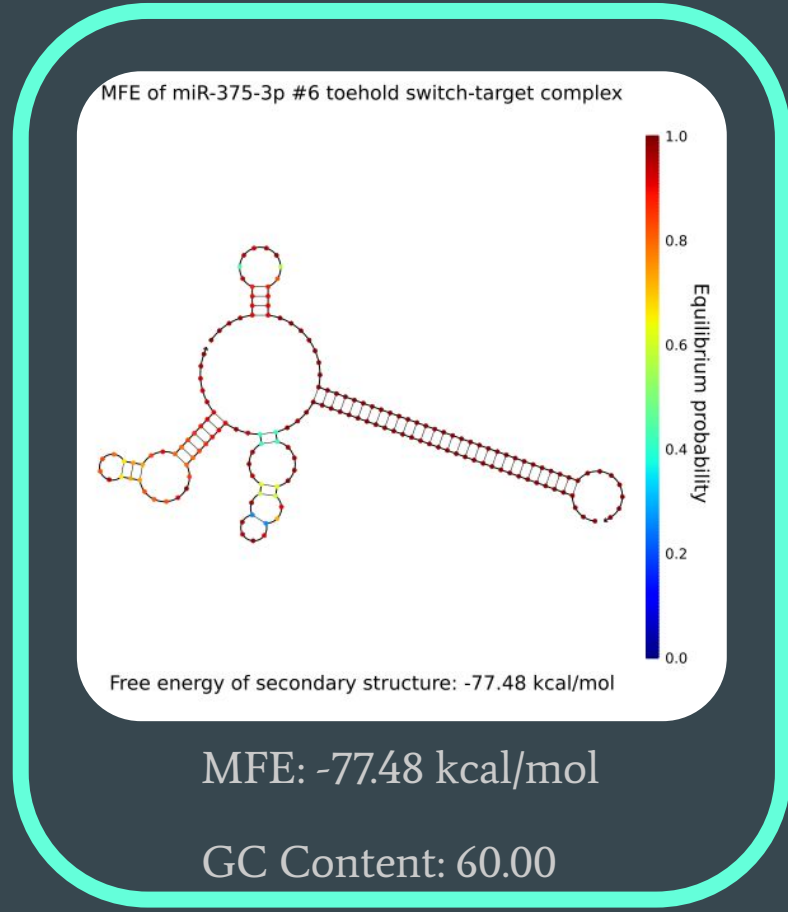


Key Results: Selection of miR-375-3p switch



MFE: -83.38 kcal/mol

GC Content: 66.67



MFE: -77.48 kcal/mol

GC Content: 60.00

Issue at hand

The gold standard for CRC diagnosis is a **colonoscopy biopsy**.

However, this technique makes it difficult for pathologists to distinguish high-grade intraepithelial neoplasms (adenomas) from invasive carcinomas.^[3]

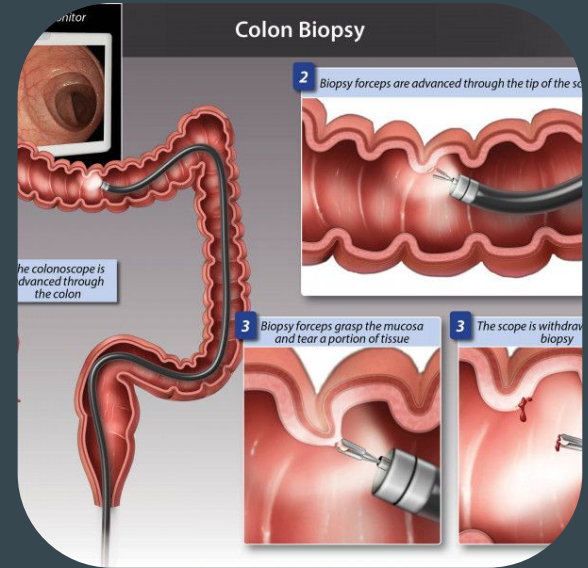


Figure 2: Colonoscopy biopsy procedure
<https://www.trialexhibitsinc.com/library-item/colon-biopsy>

^[3] Wang S, Wang L, Bayaxi N, Li J, Verhaegh W, Janevski A, et al. A microRNA panel to discriminate carcinomas from high-grade intraepithelial neoplasms in colonoscopy biopsy tissue. Gut 2012; 62: 280–9.