A Test Paper for Cancer
How Synthetic Biology Actually Change the World

PRESENTED BY Tsinghua-A
BACKGROUND

Why do we want to invent a test paper for cancer? Let me tell you a story first......
ESOPHAGEAL CANCER
ESOPHAGEAL CANCER

PART ONE
Background

PART TWO
Design

PART THREE
Experiment

PART FOUR
Model

PART FIVE
Practice

PART SIX
Future
The 8th most common cancer

The 6th leading cause of cancer mortality

Over 50% of esophageal cancer happens in China
Expensive

Inconvenient

Painful
Why Saliva? miRNA-144
We try to use synthetic biology to build a circuit on paper, which can indicate whether you get the cancer or not...
Function
As riboregulator through interaction between RNAs

Components
Two strand of RNA strands: the switch and the trigger

How it works
When trigger RNA appears, it will bind the hairpin in the switch and open the loop, exposing the RBS. Thus the downstream gene can be expressed.

**Part 1**

Toehold Switch for miRNA-144

- RBS
- Linker
- Repressed GFP

**Part 2**

Toehold Switch for GFP mRNA

- RBS
- Linker
- Repressed T3 RNA Polymerase

**Part 3**

T3 promoter

GFP Generator
Part 1

Toehold Switch for miRNA-144

- RBS
- AUG
- Linker
- Repressed
- GFP

miRNA-144
GFP mRNA

miRNA-144

GFP mRNA
PART ZER0
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Toehold Switch for GFP mRNA

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T3 promoter
GFP Generator

GFP mRNA
We try to build the circuit we designed and make it functions normally......
**PART THREE**

**Experiment**

**STEP 1**
Synthesis 3 parts using different ways

**STEP 2**
Link 3 parts in order into one plasmid

**STEP 3**
Test whether it can function normally
We try to use mathematical tools to guide the design of circuit...
4 miRNA
<table>
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<th>No.</th>
<th>WS-miR10*</th>
<th>WS-miR144</th>
<th>WS-miR451</th>
<th>SS-miR10*</th>
<th>SS-miR144</th>
<th>SS-miR21</th>
<th>SS-miR451</th>
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ROC Curve

Not good, so consider combination.
Fisher Linear Discriminant
Logistic Regression and SVM with Linear Kernel
Conclusion

<table>
<thead>
<tr>
<th></th>
<th>Fisher Discriminant</th>
<th>Logistic Regression</th>
<th>SVM</th>
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<tbody>
<tr>
<td>Whole Saliva</td>
<td>Accuracy = 0.7413</td>
<td>Accuracy ≥ 0.8</td>
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<tr>
<td></td>
<td>F11 = 0.57, F12 = 0.81</td>
<td>F1 ≥ 0.65</td>
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<tr>
<td>Supernatant</td>
<td>Accuracy = 0.6897</td>
<td>Accuracy ≥ 0.7</td>
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<tr>
<td></td>
<td>F11 = 0.47, F12 = 0.78</td>
<td>F1 ≥ 0.55</td>
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<tr>
<td></td>
<td></td>
<td>Accuracy ≥ 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>F1 ≥ 0.5</td>
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</tbody>
</table>
The Basic Component
Chemical Equilibrium Analysis

\[
[\text{trRNA}] + [\text{seRNA}] \iff [\text{tr.seRNA}] \iff [\text{reRNA}]
\]

\[K_1 = \frac{[\text{tr.seRNA}]}{[\text{trRNA}] \cdot [\text{seRNA}]}
\]

\[K_2 = \frac{[\text{reRNA}]}{[\text{tr.seRNA}]}
\]

We put \(X,Y\) as the total quantity of trRNA and seRNA we add to the system respectively.

\[X = [\text{trRNA}] + [\text{tr.seRNA}] + [\text{reRNA}] + \alpha_1
\]

\[Y = [\text{seRNA}] + [\text{tr.seRNA}] + [\text{reRNA}] + \alpha_2
\]

\[
\left( \frac{1}{K_2} + 1 \right)^2 [R]^2 - \left( \frac{1}{K_2} + 1 \right) ([X] + [Y] - 2\alpha) - \frac{1}{K_1 K_2} [R] + ([X] - \alpha)([Y] - \alpha) = 0
\]

\[\Delta G = -RT \ln K
\]
Thermodynamic Analysis

Appendix A. Pseudocode for the Multistranded Partition Function Algorithm.

Initialize $(Q, Q^0, Q^m)$ // $O(N^2)$ space
Set all values to 0 except $Q_{i-1, i} = 1$ for $i = 1, \ldots, N$

for $i = 1, N$

for $s = 1, N - i + 1$

$\delta = \delta + 1$

// $Q^m$ recursion equations
if $\delta = \delta - \frac{1}{2}$ then $0$ and $\delta = \delta - \frac{1}{2}$ then $0$

$Q_{i, s} = \exp\left(-\Delta G^\text{unw} / kT\right) Q_{i, s}$

for $d = s, s - 2$ // loop over all possible 3′ most pairs $d - s$

for $e = d + 1, d - 1$

if $\delta = \delta - \frac{1}{2}$ then $0$ and $\delta = \delta - \frac{1}{2}$ then $0$

$Q_{i, s} = \exp\left(-\Delta G^\text{nuc} / kT\right) Q_{i, s}$

if $\delta = \delta - \frac{1}{2}$ then $0$ and $\delta = \delta - \frac{1}{2}$ then $0$

// multi-loop: no top-level nicks
$Q_{i, s} = Q_{i, s} + \Delta G_{\text{unw}} + \Delta G_{\text{nuc}} + (\delta - e - 1) \Delta G_{\text{base}} / kT$

for $e \in \{1, \ldots, j - 1\}$ s.t. $\delta = \delta - \frac{1}{2}$ then $1$ // loop over all top-level nicks $\in [k + \frac{1}{2}, j - \frac{1}{2}]$

if $\delta = \delta - \frac{1}{2}$ then $0$ and $\delta = \delta - \frac{1}{2}$ then $0$

for $i = 1, N$

$Q_{i, s} = \exp\left(-\Delta G^\text{nuc} / kT\right) Q_{i, s}$

// exterior loops

if $\delta = \delta - \frac{1}{2}$ then $0$ then $Q_{i, s} = 1$ // empty substructure
else $Q_{i, s} = 0$ // unconnected substructure

for $d = e, f - 1$ // loop over all possible 3′-most pairs $d - e$

for $s = d + 1, e$

if $\delta = \delta - \frac{1}{2}$ then $0$

if $\delta = \delta - \frac{1}{2}$ then $0$

$Q_{i, s} = Q_{i, s} + Q_{i, s}$

if $\delta = \delta - \frac{1}{2}$ then $0$

// single pair in $Q^m$

$Q_{i, s} = \exp\left(-\Delta G_{\text{base}} / kT\right) Q_{i, s}$

if $\delta = \delta - \frac{1}{2}$ then $0$

// more than one pair in $Q^m$

return $[Q_{i, s} \exp\left(-\Delta G_{\text{base}} / kT\right)]$ // partition function $Q(x)$ for ordering $x$
Realization of the mathematical classifier —— in vivo

miRNA-144
Toehold Switch1

miRNA-10
Toehold Switch2

miRNA-451
Toehold Switch3

GFP miRNA

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Linker
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T3 RNA Polymerase

RBS
AUG
Realization of the mathematical classifier —— In silico
Practice

We try to "go beyond the lab" to imagine our projects in a social context, to better understand issues that might influence the design and use of our technologies...
Q1: Our country (China) is one of the countries with the highest incidence of esophageal cancer in the world, there are ten million people died of esophageal cancer each year. Do you know the causes of esophageal cancer? (Ultrasound, barium, endoscopy, etc.)

Q2: Have you ever heard of addiction, swallowing food, etc. 

Q3: Do you know how to go to the hospital to check? 

Q4: Do you know how to prevent indigestion, etc.)
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Future

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THANK YOU
FOR WATCHING

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