Run Sperm Run !

School of Life Sciences, Tsinghua, China

Introduction

1.1 Reproductive Health

With increasing infertility rate among the population, **reproductive health** has become a hotspot of concern. According to available data from WHO, **15%** of reproductive-aged couples worldwide are affected by infertility, among which **low sperm quality** is considered to be the major cause.

1.2 Sperm Quality Test

Currently, sperm quality can be examined mainly through **hospital appointments** which are **time-consuming** and **inconvenient**, adding additional challenge to tackling the issue of reproductive health. **Fig.1** shows the current methods. Therefore, our team has proposed **a novel diagnostic method** to test sperm quality for **household use**.

Fig. 1 Current methods to test sperm quality

1.3 Our Solution

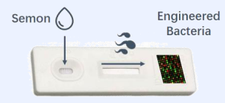

Fig. 2 Our chip-based product

Fig. 3 The relationship between colour and sperm properties

The **chip-based** product **(Fig.2)** is designed to measure **sperm motility and fertility** simultaneously. Just **a drop of semen** loaded onto the chip could reveal results of sperm quality within hours. Engineered bacteria are organized through intricate design of a **two-component system** to recognize **Sp10 and EGFR** protein on the surface of sperm, which are critical index of sperm concentration and fertility respectively. When the bacteria recognize different protein, it will express different **color protein**, which further indicate the quality of the sperm.

Wet Lab

The recognition through the **two-component system** would then trigger downstream signling transduction to induce the expression of reporter genes for the two indexes respectively. **A logic gated system** based on **cro/cI** in lambda repressor is introduced to separate the **two signals** indicating **concentration** and **fertility** via conditional computation.

2.1 Two-component System Ⅰ

2.1.1 Nisin induced two component system

Nisin, an antimicrobial peptide produced originally by L. lactis, can activate downstream gene expression by interacting with two component system **nisK/R**.

Fig. 4 Function of nisK/R system

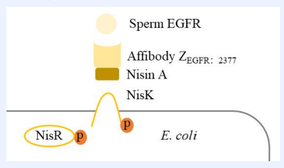

Fig. 5 Design for sperm EGFR detection

2.1.2 Our Design

We planned to construct nisK/R two component system in **E. coli** to response to nisin induction. We also design a **fusion protein** containing **nisin** and **EGFR affibody** to detect sperm EGFR using nisK/R system.

2.1.3 Current Progress

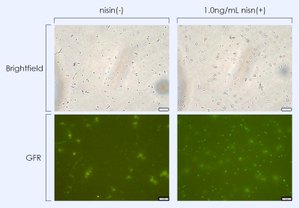

Fig. 6 Nisin induction assay by fluorescence microscope

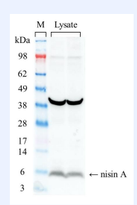

Fig. 7 Western blots of bacteria lysate using anti-6×His antibody

We have constructed the **nisK/R system** in BL21(DE3) strain and proved its function using EGFP as reporter gene **(Fig.6)**, and we have also constructed **nisin A expression** BL21(DE3) strain together with **nisA-affi fusion protein expression** strain and confirm the expression of nisin A **(Fig.7)**.

2.2 Two-component System Ⅱ

2.2.1 Background

Pmr A/B is a two-component system in E.coli sensing the concentration of **ferric ion.** Meanwhile affibody is a kind of small engineered protein with the function of recognizing other proteins, which has a structure of three a-helix while two of them participate in recognition.

2.2.2 Design

Due to the structural similarity of affibody's recognition part and the extracellular section of PmrB, we designed **a new receptor recognizing Fc segment of antibody**. Then we predicted the structure of our new receptor by AlphaFold2, the structure of our new receptor is almost the same as that of PmrB itself.

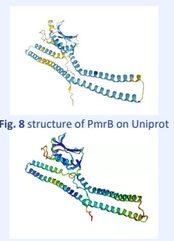

Fig. 8 structure of PmrB on Uniprot

Fig. 9 structure of Affi-PmrB predicted by AlphaFold2

2.2.3 Progress

We expressed the **recombined Affi-PmrA/B system** in BI21(DE) and use **GFP** as a reporter gene to test its function, the result is recorded by confocal microscope.

Fig. 10 No IgG

Fig. 11 100μM IgG

2.3 Serine Integrase

2.3.1 Serine integrase

Serine integrase, adapted from bacteriophage, is capable of **catalyzing site-specific recombination** between **two attachment sites** (attP and attB) and **inverting the DNA sequence flanked** by these two opposing sites.

2.3.2 Our Design

We designed **a logic gated system** based on **cro/cI** in lambda repressor via conditional computation to separate the **two signals** from different two-component systems upstream. When **signal 1** is recognized, cro will **disinhibit** cI repressor to allow downstream GFP to express. In contrast, when **signal 2** is recognized, serine integrase will **invert the DNA sequence flanked by attP and attB sites** to allow transcription of **mCherry**.

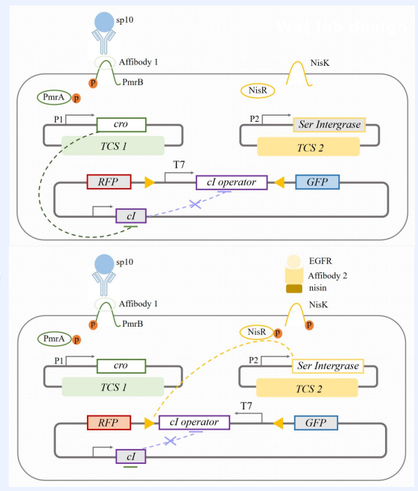

Fig. 12 Two component system

2.3.3 Current Progress

We have constructed the plasmids **(Fig.13)** and co-transformed into BL21(DE3) to induce expression. The qPCR result demonstrated **8-fold inversion efficiency after induction (Table 1)**.

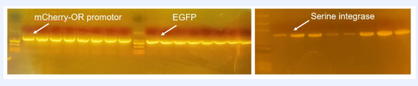

Fig. 14 colony PCR of plasmid construction

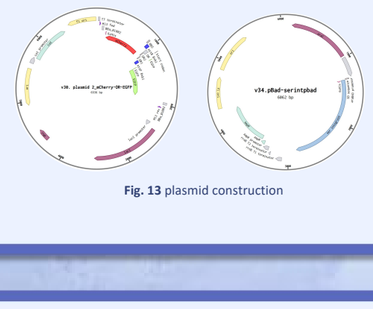

Fig. 13 plasmid construction

Colony	Threshold (dRn)	Ct (dRn)
Before induction	0.0140	24.48
After induction	0.0140	20.98

Table1 qPCR result of inversion efficiency

Dry Lab

3.1 Modeling

Our basic model demonstrates the **change in fluorescent protein expression** over time for sp10 and EGFR at different relative concentrations.

The basic equations are as follows.

3.1.1. Binding equation.

The theoretical model should be: $[ab] = K_a[a][b]$.

However, because there are too many binding reactions involved in this system, it is difficult to determine specific parameters, so it is temporarily simplified to:

$$[ab] = \frac{[a] + [b]}{[a][b]}$$

3.1.2 Expression/degradation equation

$$\frac{d[c]}{dt} = K_b[a]$$

3.1.3 The Michaelis Menten equation

$$\frac{d[c]}{dt} = v$$

$$v = \frac{V_{max}[a]}{K_m + [a]},\quad V_{max} = k_{cat}[e]$$

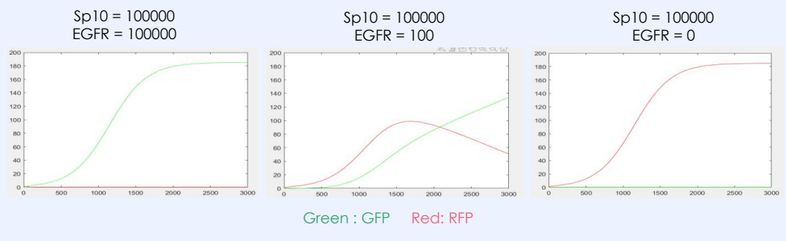

Fig. 15 Modeling

3.2 software

3.2.1 kmer2vec:

We want to design an **alignment-free sequence comparison method based on word2vec** for exploring the scope of application of our system rapidly.

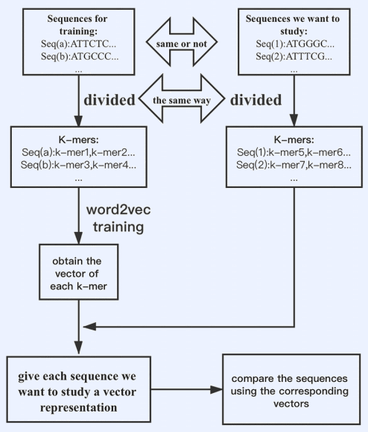

Fig. 17 The flowchart of kmer2vec

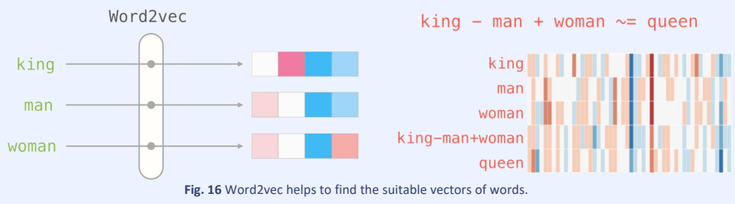

Fig. 16 Word2vec helps to find the suitable vectors of words.

3.2.2 AI promoter evolution:

We hope to predict the **promoter strength** by sequences based on **a deep learning model** (Transformer) to find better promoters for our two-component system. The structure of the traditional Transformer is as follows.

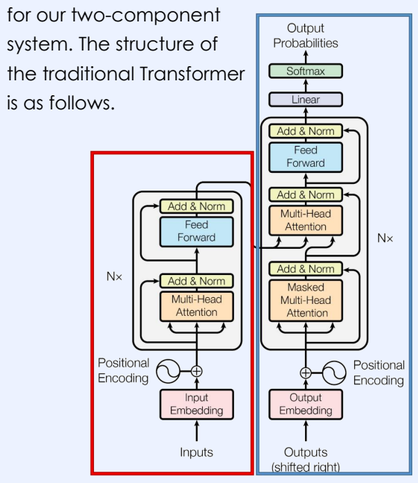

Fig. 18 The structure of traditional Transformer model

3.3 hardware

Through a designed **microfluidic system**, we can create a suitable **concentration gradient** of chemotactic substances.

The concentration gradient can give sperm cells the driving force to 'run on the racetrack'.

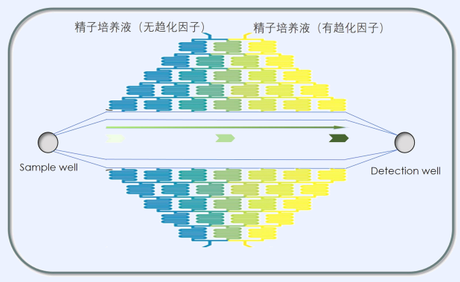

Fig. 19 The design of microfluidic system

Then, sample addition and detection can be performed through the hardware system.

Reference

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Contact Us

THU iGEM
(wechat public account)

Zhangzhi Huizi
(student leader)

Our idea may still have a lot of shortcomings, looking forward to your suggestions!