IGEM JOURNAL

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Source of Inspiration:

TSINGHUA IGEM 2023

- Find + kill breast cancer cell
- Product: bacteria with dual-input AND gate system (anaerobic temperature-controlled).

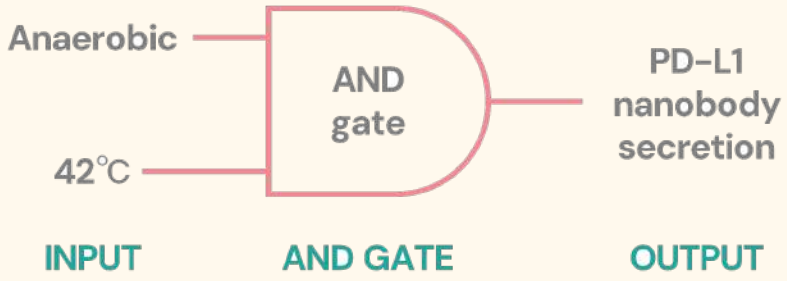
AND-GATE SYSTEM

Reason to make (so that)

- Engineered bacteria is only colonised within the tumour
- *Therapeutic* (healing of disease) function is control

Overall structure

- INPUT: *Anaerobic* (without oxygen) promoter, temperature-sensitive promoter
- PROCESS: And gate (work module)
- OUTPUT: *Secretion* (production) of PD-L1 *nanobody* (≈antibody, bind to specific antigen)
    - PD-L1: 抑制免疫系统，使它无法对抗癌症
    - PD-L1 nanobody: bind to PD-L1 antigen，让免疫细胞杀死癌细胞



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Input: Anaerobic promoter

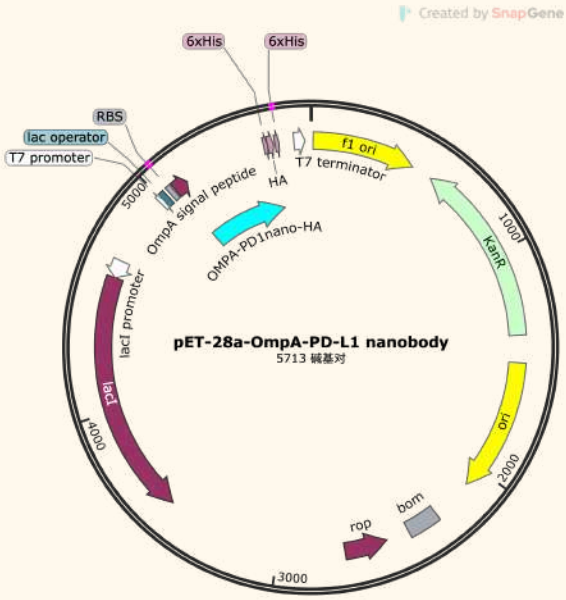
- using Hypoxia-inducible promoter P_{PepT}
- Activated in low-oxygen environments（cancer cell抢走了oxygen，在tumour region氧气含量特别低）→ Tumour localisation

Input: Temperature-sensitive Promoter

- using PL and PR promoter
- responding under a human tolerable heat shock (42 °C) generated by ultra-sound
- In *pBV220* (vector), they are located *tandemly* (串联地) → repressed by *Tcl857* (temperature sensitive mutant of phage λ)
- (under normal conditions) Tcl857 acts as dimers → bind to the operator site → hinder transcription
- (temperature rises to ≈42℃) Tcl857 repressor is denatured → transcription happens

Process: AND Gate

- Only input 2 detected
    - the promoter will be activated → T7ptag will be transcribed
    - T7ptag (derived from T7 RNA polymerase): contain two amber stop codons → translation will be terminated earlier at the amber stop codon (UAG) site
    - → T7ptag mRNA cannot be translated to functional T7 RNA polymerase
- Input 1 & 2 detected
    - the promoter will activate the transcription of supD (carries complementary anticodon of amber stop codon and Serine)
    - → T7ptag will be expressed to T7 RNA polymerase
    - → express output gene



Output: Secretion of PD-L1 nanobody

- using OmpA (N-terminal secretion signal peptide)

IMPLEMENTATION ON PCa

- Regions of prostate-specific antigen (PSA) promoter confer androgen-independent expression of PSA in prostate cancer cells
- The promoter of the prostate-specific antigen gene contains a functional androgen responsive element (abstract)
- The Promoter of the Prostate-Specific Antigen Gene Contains a Functional Androgen Responsive Element (full)
- INPUT: PSA promoter + x
    - x is unconfirmed, potential strategies are described below
- PROCESS: AND gate
- OUTPUT: Secretion of PD-L1 nanobody

Potential strategies to be used alongside PSA promoter

- **Enhancers or Response Elements:**
    - Eg: androgen receptor (AR) response element
    - AR signaling pathway is often dysregulated in prostate cancer
- **Tissue-Specific Promoters:**
    - Eg: prostate-specific membrane antigen (PSMA) promoter
    - PSMA promoter is known to be active primarily in prostate cancer cells
- **Cancer-Specific Regulatory Elements:**
    - Eg: DNA methylation pattern associated with prostate cancer
    - Prostate cancer cells often exhibit specific DNA methylation patterns, including hypermethylation of certain regions
- **Promoter Methylation:**
    - Example: methylation-sensitive regulatory elements or CpG islands
    - CpG islands: DNA regions with a high frequency of CpG dinucleotides, often associated with promoter regions

- → specifically activate gene expression in prostate cancer cells where the CpG islands are demethylated, while remaining inactive in normal prostate cells where the CpG islands are methylated.

Combinations of Input

- PSA promoter + PSMA promoter
- PSA promoter + AR promoter
- AR promoter + PSMA promoter

PSA Promoter

- PSA gene: KLK3 kallikrein related peptidase 3
- Gene summary: KLK3 upstream enhancer/promoter region
- Sequence: KLK3 promoter sequence

PSMA Promoter

- PSMA gene: FOLH1 folate hydrolase 1
- Gene summary: PSMA gene, promoter region
- Sequence: PSMA promoter sequence

AR Promoter

- AR gene: AR androgen receptor
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Findings on prostate cancer related- contents

1. Gene Mutations and Prostate Cancer Risk:
    - Researchers have identified several genes associated with prostate cancer risk.
    - These genes can have specific changes (mutations) that affect their function.
    - Some well-known gene related to prostate cancer risk include:

- **BRCA1** and **BRCA2**: Normally tumor suppressor genes, mutations in these genes can raise the risk of prostate cancer, especially **BRCA2**.
- **MSH2**, **MSH6**, **MLH1**, and **PMS2**: These genes help repair DNA damage. Inherited changes in these genes cause **Lynch syndrome**, which raises the risk of various cancers, including prostate cancer.
- **CHEK2**, **ATM**, **PALB2**, and **RAD51D**: These genes are involved in DNA repair and can impact prostate cancer risk.
- **RNASEL**: This gene helps cells self-destruct when something goes wrong. Inherited mutations can lead to abnormal cell survival and prostate cancer.
- **HOXB13**: Rare mutations in this gene raise the risk of early-onset prostate cancer (usually before age 55).

The scientists found that men who had higher testosterone levels also had higher PSA levels.

Testosterone is a type of androgen. Androgen helps prostate cancer cells to grow

PSMA promoters:

1. PSMA Enhancer-Promoter Element (PEPE):
    - **Function**: PEPE is derived from the **PSMA gene** and responds to **high PSMA levels**.
    - **Activity**: It drives **tissue-specific expression** in prostate cells where PSMA is naturally abundant.
    - **Applications**:
        - **Gene Therapy**: PEPE can selectively express therapeutic genes in prostate cancer cells.
        - **Transgenic Models**: Incorporating PEPE into transgenic mice allows studying PSMA-related biology.

Patients with prostate cancer may not have high PSMA levels

Enzalutamide, an androgen receptor inhibitor, has been shown to increase PSMA expression in prostate cancer cells. However, its impact on normal prostate cells is different:

1. Prostate Cancer Cells:
    - In prostate cancer cells, enzalutamide treatment leads to a **significant upregulation of PSMA levels**.
    - Studies have demonstrated that enzalutamide increases PSMA expression in PSMA-low prostate cancer cell lines 1.
    - This effect is beneficial for targeted therapies and imaging.
2. Normal Prostate Cells:
    - In **normal prostate cells**, enzalutamide does not typically cause significant PSMA upregulation.
    - PSMA expression in normal prostate tissue is minimal.
    - Enzalutamide's primary impact is on cancer cells with higher baseline PSMA expression.

PSMA promoter ($PSMA_{Pro}$) sequence (nucleotides 1453–2697, accession number AF007455) and PSMA enhancer ($PSMA_{Enh}$) sequence (nucleotides 14703–16351, accession number AF007455)

https://onlinelibrary.wiley.com/doi/10.1002/j.1939-4640.2005.tb01088.x

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