CHRONIC KIDNEY DISEASE
ESCAPE ROOM
Nearly 12% of the population in Taiwan is the Kingdom of Dialysis with the highest national prevalence.
Chronic Kidney Disease (CKD)
850,000,000 people suffer from Chronic Kidney Disease.
YOU?
Death Caused by CKD

Mortality Rate

4X

Number of death (millions)

Year

1990: 0.6
2000: 1.2
2010: 1.8
2018: 2.4
Death Caused by CKD

Over 6500 deaths everyday

Year

1990 2000 2010 2018

Number of death (millions)

0.6 0.8 1.2 1.6 2.0 2.4
Death Caused by CKD

Number of death (millions)

Year

1990
2000
2010
2018

0.6
1.2
1.6
2.4

6th
Fastest growing cause of death
$64 Billion

US
Oral Adsorbent

Hemodialysis

?
Major Hidden Problem

*p*-Cresol accumulation
para-Cresol ($p$-Cresol)

Chronic Kidney Disease Deterioration

20X more likely to get Cardiovascular Disease
Clues Give Away!
START!
Stage 1

SCIENCE
Synthetic Biology

\( p \)-Cresol time bomb

Live therapeutic CreSolve
Tyrosine Ammonia-lyase (TAL)
Tyrosine

\[
\text{Tyrosine} \quad \text{HO} - \text{C} - \text{NH}_2 - \text{COOH}
\]

TAL

\[
\text{TAL} \quad \text{HO} - \text{C} - \text{C} - \text{HO} - \text{C} - \text{C} - \text{HO}
\]

\[
\text{\(\beta\)-Coumaric acid} \quad \text{HO} - \text{C} - \text{C} - \text{HO} - \text{C} - \text{C} - \text{HO}
\]

18
\( p\)-Coumaric acid
**P_{fnr} Characterization**

- FNR Promoter (K1123000)
- RBS (B0034)
- TAL (I1742142)
- Double terminator (B0015)

**Fluorescence Intensity**

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<td>P_{fnr}-GFP</td>
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Improve Biobrick with RBS

Promoter (J23100) → Native RBS (I742146) → TAL (I1742142) → Double terminator (B0015)

Promoter (J23100) → RBS (B0034) → TAL (I1742142) → Double terminator (B0015)

pSB1C3

(I742106) → (K2997011) → pSB1C3
$p$-Coumaric Acid Measurement

$n$-octanol

Nanodrop 310 nm

Growth medium

$p$-Coumaric Acid Standard Curve

\[
Y = 0.004725 \times X - 0.01851
\]

$R^2 = 0.9729$
Improve Biobrick with RBS

Promoter (J23100) → Native RBS (I1742146) → TAL (I1742142) → Double terminator (B0015) → 2-fold

Promoter (J23100) → RBS (B0034) → TAL (I1742142) → Double terminator (B0015) → [p-Coumaric acid (µM)/OD600]

Native: 5
B0034: 15

****
Tyrosine Conversion Pathway

Native promoter → Native RBS → tyrP → Double terminator (B0015) → Promoter (J23100) → RBS (B0034) → TAL (11742142) → Double terminator (B0015)

E. coli Nissle 1917 → p-Coumaric acid
Tyrosine Conversion Pathway

Tyrosine to \( p \)-Coumaric Acid Conversion Comparison

**1.3-fold**
Key fragment I
$p$-Cresol Production Pathway

Tyrosine $\rightarrow$ $p$-Cresol

Clostridium
Bacteriocin
Minimum Inhibitory Concentration Test

- Clostridium difficile
- Clostridium perfringens
- Clostridium tetani
- Klebsiella pneumoniae
- Proteus mirabilis
- Staphylococcus epidermidis

MIC (µg/ml)
Spot-on-lawn assay

Purified bacteriocin
Spot-on-lawn assay

Purified bacteriocin

Key fragment 2
Probiotic
E. coli Nissle 1917
Probiotic Nissle

Won’t colonize for more than a week!
Carbonic Anhydrase

\[ \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \]
In 5% CO₂ (In guts)

E. coli Δ can::FRT
Alive

In 0.04% CO₂ (In the atmosphere)

E. coli Δ can::FRT
Dead
In 5% CO₂ (In guts)

Cm Control  |  Wild Type
Δ can::CmR  |  Δ can::FRT

In 0.04% CO₂ (In the atmosphere)

Cm Control  |  Wild Type
Δ can::CmR  |  Δ can::FRT
Key fragment 3
Stage 2
ENGINEERING
CreSolve → Target → p-Cresol
TAL Effectiveness Simulation

*p-Coumaric Acid and p-Cresol Production*

- p-Cresol (C. difficile monoculture)
- p-Cresol (co-culture)
- p-Coumaric acid (co-culture)

Produced compounds (µM)

Time (Hours)
Current Method

HPLC

High-Performance Liquid Chromatography
Inspiration

Current method

Expensive
Inaccessible
Complicated
Inspiration

Current method
- Expensive
- Inaccessible
- Complicated

Our method
- Low cost
- Accessible
- Easy
CreSense

- Inaccessible
- Expensive
- Low cost
- Accessible
- Complicated
- Easy
CreSense

*p*-Cresol sensing bacteria

Light sensor

Blood separation fluidic disk
CreSense
Blood Separation

3000 rpm

900 rpm

Reaction chamber
CreSense

*p*-Cresol Sensing Bacteria

![Diagram of CreSense system with molecular structures and gene expressions](image-url)
CreSense

Sample injection

Plasma separation

GFP excitation & results

Biosensor injection
CreSense’s Advantages

User-friendly
- Load the samples
- Press a button
- Record the result

Affordable
- $1.6 only

Customizable
- Switch biosensor
Customizable

$p$-Cresol sensing bacteria

Switch Biosensor

$X$ bacteria

$p$-Cresol

$X$ compound
OM?
Stage 3
SOCIETY
Map of society stage

Meeting with Experts

Enterprise visit

Meeting with iGEMers

Education & Engagement
Meeting with Experts

Dialysis Center

Medical Device Innovation Center

Professor

Nurse
Meeting with Experts

Enhancing device’s function
Map of society stage

- Completed
- Meeting with Experts
- Enterprise visit
- Meeting with iGEMers
- Education & Engagement
Enterprise Visit

Potential Manufacturer:

Winston Medical Supply Co.

Understanding Drug Development:

- Define main compound
- Biosafety

CreSolve
Meeting with iGEMers

2019, August 5-6
Taiwan iGEM Conference
National Chung Hsing University, Taichung
Collaborations

- iGEM Washington
- iGEM Tübingen
- Adieu, Residues
- KORHS
- iGEM UNSW Sydney
- iGEM Costa Rica
Collaborations (Mentoring)

iGEM Korea_HS

iGEM UNSW
Collaborations (Mentoring)

Global Business Scaffold

iGEM UNSW

Australia and Asia Policy and Regulations
Education & Engagement

DIY Kidney Model
NanKe International School, Taiwan

Teaching Synthetic Biology
Kemurnian II Senior High School, Indonesia

Snake Game
ESCAPE ROOM

- Applications of Synthetic Biology
- Basic CKD Knowledges
- Oh My Gut Project
Science

Reduce $p$-Cresol accumulation rate

Engineering

$p$-Cresol bio-sensing system

Society

CreSense

CreSolution
Criteria Checklist

S
- Project inspiration
- Part characterization
- Validated new part

E
- Improve previous parts
- Modeling
- Demonstrate project

S
- Collaborations
- Human practices
- Integrated human practices
CONGRATULATIONS!
850,000,000
Suffer from Chronic Kidney Disease
CreSolve

Reduce p-Cresol accumulation rate

CreSense

p-Cresol biosensing system
CreSolve

Reduce $p$-Cresol accumulation rate

CreSense

$p$-Cresol biosensing system

OH MY GUT

Better quality of life for CKD patients
OMG! CreSolution!
Thank You!
Attributions