STAPHYLOCIDE:

Delivering Antibiotic Resistance Gene Silencing Mechanisms to a MRSA Population using Bacterial Conjugation
"The problem is so serious that it threatens the achievements of modern medicine. “

Adapted from: Data collected from hospital intensive care units that participate in the National Nosocomial Infections Surveillance System of the Centers for Disease Control.

Infectious Diseases Society of America Clin Infect Dis. 2011; 52:S397-S428
MRSA resistance in a nutshell

Staphylococcus aureus

Chromosome

Cell Wall

Penicillin

PBP
MRSA resistance in a nutshell

Methicillin Resistant *Staphylococcus aureus*

*mecA* gene

Chromosome

Cell Wall

PBP2A

Penicillin
mecA mRNA

Transcription

Translation

PBP2 A

MRSA

mecA mRNA

Transcription

Translation

PBP2 A

STAPHYLOCIDE

mecA mRNA

Transcription

Translation

PBP2 A

mecA mRNA

Transcription

Translation

PBP2 A
IMPROVING THE REGISTRY
**Staphylococcal Parts**

**Promoters**
1. *sarA* P1 – Strong constitutive
2. Xylose inducible promoter construct

**Ribosome Binding Sites**
1. *sodA* RBS
2. Optimized TIR RBS

**Terminators**
1. *sarA* rho-independent

**Selection Markers**
1. *ermM* – Erythromycin resistance
2. *aadD* – Kanamycin resistance
3. *spC* – Spectinomycin resistance

**Origin of Replication**
1. pSK41
   - *S. aureus*
   - Theta Replication
   - Low copy

**Reporters**
- DsRed
- YFP
**Staphylococcal Strain**

*S. epidermidis* (ATCC 12228)

- Level 1 organism
- Native to human microbiota
- Able to conjugate with *S. aureus*
- No endogenous CRISPR system unlike other *S. epidermidis* strains
Reporter Gene: DsRed

E. coli  S. epidermidis  -ve control
Improved pSB1C3 by making it more versatile:

**E. coli-Staphylococcus Shuttle Vector**

BBa_K1323017

- VF2
- VR
- P
- S
- RFP Expression Cassette (BBa_J04450)
- Cm<sup>R</sup>
- ori<sub>E. coli</sub>V<sup>E. coli</sup>
- Erm<sup>R</sup>
- oriV<sub>S. aureus</sub>
Shuttle Vector: Antibiotic Resistance

- Stably maintained in *S. epidermidis*
- Confers erythromycin resistance
SILENCE

DELIVER

TRANSLATE
SILENCE

CRISPRi
Transcription

RNAi
Translation

Design
Silence

Transcription

YFP mRNA

Translation

YFP
Silence: CRISPRi

dCas9-sgRNA complex blocks RNA polymerase
Silence: CRISPRi Network

\[ \frac{\alpha_r}{k_+} \text{sgRNA} \rightarrow \text{dCas9-sgRNA} \]

\[ \frac{\beta_c}{k_-} \text{dCas9} \rightarrow \text{dCas9} \]

\[ \frac{\alpha_c}{\text{dCas9 mRNA}} \rightarrow \text{dCas9} \]

Transcription Repression

\[ \frac{\alpha_y}{\text{YFP mRNA}} \rightarrow \text{YFP} \]
Silence: CRISPRi Results

n-fold CRISPRi Repression of YFP mRNA

- YFP mRNA (no repression)
- 6-fold repressed YFP mRNA
- 35-fold repressed YFP mRNA

Concentration (nM) vs. Time (h)
• Sensitive to mRNA degradation rate

Therefore...

• Targeting of translation will improve silencing!

### Silence: CRISPRi Sensitivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>dCas9 mRNA Production</td>
<td>-0.0376</td>
</tr>
<tr>
<td>dCas9 mRNA Degradation</td>
<td>0.0411</td>
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<tr>
<td>dCas9 Protein Production</td>
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<tr>
<td>dCas9 Protein Degradation</td>
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<tr>
<td>YFP mRNA Production</td>
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<td>YFP mRNA Degradation</td>
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<td>sgRNA Production</td>
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<tr>
<td>sgRNA Degradation</td>
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<tr>
<td>dCas9-sgRNA Complex Degradation</td>
<td>0.0470</td>
</tr>
</tbody>
</table>
Silence: RNAi

sRNA-Hfq complex blocks ribosome
Silence: RNAi Network
Silence: RNAi Results

sRNA Silencing of YFP Expression

- YFP (Repressed)
- YFP (Unrepressed)
Silence: Design

CRISPRi

dCas9-sgRNA Complex

YFP mRNA

Translation

YFP
Silence: CRISPRi

XylR \rightarrow \text{xylose} \rightarrow \text{dCas9} \rightarrow \text{sgRNA} \rightarrow P_{\text{const}} \rightarrow \text{pSB1A3}

- oriV_{E. coli}
- Amp^R
- Erm^R
- oriV_{S. aureus}
Silence: RNAi

pSB1A3

xylR

Hfq

sRNA

xylose

P_{const}

oriV_{E.coli}

Amp^R

Erm^R

oriV_{S. aureus}
Silence: RNAi Design

YFP mRNA

5' TGATTAACCTTTTATAAGGAGGAAAAACATTACTAGATGTTTTCAAAAAAGGTGAAGAATTATTTATACAGGTC ... 3'

RBS

Scar

YFP CDS

sRNA 1

3'CTTTTTGTAATGATCTACCAAGTTT 5'

sRNA 2

3'TTGGTAATGATCTACCAAGTTTCCACT 5'

sRNA 3

3'TACCACAGTTTCCACTCTTTAATAAAT 5'
Silence: RNAi Preliminary Tests

**pSB1A3**
- sRNA
- oriV$_{E. coli}$
- Amp$^R$

**pSB3K3**
- YFP
- oriV$_{E. coli}$
- Km$^R$

Co-Transform

**E. coli DH5α**

Measure fluorescence
Silence: RNAi Preliminary Test

Silencing YFP Expression in *E. coli* using the RNAi system

**RFU/OD**

- **YFP Alone**: 1.00
- **Control**: 0.86
- **sRNA1**: 0.24
- **sRNA2**: 0.18
- **sRNA3**: 0.30
Silence: Future Directions

- Characterize silencing systems in *S. epidermidis*
- Integrate *yfp* into *S. epidermidis* genome
- Incorporate the *mecA* gene regulation
Conjugation in *Staphylococcus*

Donor → Solid Surface → Recipient
Deliver: Conjugation

Advantages:
• Large carrying capacity
• Independently propagates
• Opportunity to contribute to an underdeveloped area of research

Disadvantage:
• Not efficient
Conjugation Parts: pGO1

**pGO1:** *S. aureus* conjugational plasmid

**oriT-nes:** BBa_K1323003

**trs Region:** Still in progress
Conjugation Test Construct

pSBS1A3

Donor

Filter Mating Assays

Recipients

Transconjugants
Challenge: Modeling conjugation between cells spread across a lab plate or a patient’s skin
Deliver: Modeling

Two novel models:

Partial Differential Equation (PDE) is deterministic and computationally efficient

Agent-Based Approach is stochastic and considers the spatial relationships between individual cells

Output: time needed for silencing to spread
Deliver: Agent Based Model

Sufficient conjugation rate

\[ t = 0 \text{ h} \]

\[ \text{Susceptible } \text{Staphylococcus} \]

\[ \text{MRSA} \]

\[ t = 0 \text{ h} \]

\[ \text{Staphylococcus conjugation rate} \]
Deliver: Agent Based Model

Sufficient conjugation rate

\[ t = 6 \text{ h} \]

\[ \text{Susceptible } \text{Staphylococcus} \]

\[ \text{MRSA} \]

\[ Staphylococcus \text{ conjugation rate} \]

\[ t = 6 \text{ h} \]
Deliver: Agent Based Model

Sufficient conjugation rate

$t = 12 \text{ h}$

$Staphylococcus$ conjugation rate

$t = 12 \text{ h}$

Susceptible $Staphylococcus$

MRSA
Deliver: Agent Based Model

Sufficient conjugation rate

Staphylococcus conjugation rate

$\text{Susceptible } \text{Staphylococcus}$

$\text{MRSA}$

$t = 24 \text{ h}$

$t = 24 \text{ h}$
Deliver: Agent Based Results

Agent-Based Simulation

- Empty grid locations
- MRSA (Recipient) cells
- Methicillin Susceptible (Donor) cells

Number of cells

Time (h)
Deliver: PDE Model Results

PDE Conjugation Populations

Number of cells

Time (h)

25x10^6

Methicillin Susceptible (Donor) cells
MRSA (Recipient) cells
Deliver: Future Uses of Model

Find igem-waterloo on GitHub!
Deliver: Future Directions

• Improve conjugation efficiency with error prone PCR mutagenesis and selective mating assays

• Test conjugational efficiency in *S. epidermidis*
Translate: Commercialization

STAPHYLOCIDE
Plasmid

Conjugation Parts
Translate: Commercialization
Translate: Commercialization
Translate: Commercialization

β-Lactam Antibiotic
Translate: Commercialization
Translate: Adaptability
SILENCE
DELIVER
TRANSLATE
Accomplishments

- Submitted 19 BioBricks, 8 characterized
- Improved BioBrick backbone to develop shuttle vector
- Produced and validated several models of the silencing and delivery systems
- Explored scalability of project
- Collaborated on uOttawa iGEM & Virginia Tech project and assisted with oGEM
Accomplishments: Outreach

Sir John A. Macdonald Secondary School
Science Club

SHAD
UNCOMMON PURPOSE
High School Enrichment Program

YouTube
Lab Skills Video Series
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Questions?
References


