Homing Coli: Engineering *E. coli* to become tracking dogs
Homing Coli: Engineering \textit{E. coli} to become tracking dogs

Development of swimming agar

Improvement of flagella

Mutagenesis of chemoreceptors
Swimming Parameters

Parameter
Agar?
## Swimming Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar Composition?</td>
<td>0,3%</td>
</tr>
</tbody>
</table>

?
### Swimming Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.3% Tryptone / M9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>✓</td>
</tr>
<tr>
<td>Composition</td>
<td>✓</td>
</tr>
<tr>
<td>Growth conditions?</td>
<td></td>
</tr>
</tbody>
</table>
### Swimming Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.3% Tryptone / M9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>✔</td>
</tr>
<tr>
<td>Composition</td>
<td>✔</td>
</tr>
<tr>
<td>Growth conditions</td>
<td>✔</td>
</tr>
<tr>
<td>Volume?</td>
<td></td>
</tr>
</tbody>
</table>
### Swimming Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>✓</td>
</tr>
<tr>
<td>Composition</td>
<td>✓</td>
</tr>
<tr>
<td>Growth conditions</td>
<td>✓</td>
</tr>
<tr>
<td>Volume</td>
<td>✓</td>
</tr>
<tr>
<td>Chemoattractant?</td>
<td></td>
</tr>
</tbody>
</table>

0.3% Tryptone / M9

Diagram showing 5 μL injections at different locations.
## Swimming Parameters

### Parameter
- **Agar**: ✔
- **Composition**: ✔
- **Growth conditions**: ✔
- **Volume**: ✔
- **Chemoattractant**: ✔
- **Temperature?**: ?

### Composition
- **0.3% Tryptone / M9**

### Diagram
- Aspartate
- 5 µL
- 5 µL
- 5 µL
### Swimming Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>✔</td>
</tr>
<tr>
<td>Composition</td>
<td>✔</td>
</tr>
<tr>
<td>Growth conditions</td>
<td>✔</td>
</tr>
<tr>
<td>Volume</td>
<td>✔</td>
</tr>
<tr>
<td>Chemoattractant</td>
<td>✔</td>
</tr>
<tr>
<td>Temperature</td>
<td>✔</td>
</tr>
<tr>
<td>Bacterial strains</td>
<td>?</td>
</tr>
</tbody>
</table>

#### 0.3% Tryptone / M9

Aspartate

- 5 μL
- 33°C
Comparison of *E. coli* strains

Tryptone- vs. M9 agar

33°C over night vs. 33°C for 2 days
Homing Coli: Engineering *E. coli* to become tracking dogs

- Development of swimming agar
- Improvement of flagella
- Mutagenesis of chemoreceptors
Speed improvement of *E. coli*
Different targets for possible speed improvement were chosen.
fliC and motB can enhance the motility of E. coli

MG1655

Transformed BL21

RFP ctrl

fliC

motA

motB

M9 agar
Cyclic di-GMP is a second messenger in *E. coli*.
The c di-GMP binding protein YcgR acts as a flagellar brake

*yjhH* suppresses the function of flagellar brake protein *YcgR*.
yjhH suppresses the function of flagellar brake protein YcgR

$yjhH$ can enhance motility of $E. coli$ on certain media
Homing Coli: Engineering *E. coli* to become tracking dogs

- Development of swimming agar
- Improvement of flagella
  - WT
  - mutant
- Mutagenesis of chemoreceptors
The substrate binding pocket of chemoreceptor Tar
The substrate binding pocket of chemoreceptor Tar
Generating the Chemoreceptor Library

First round of saturated mutagenesis PCR

PCR

Parent plasmid

Linear mutant plasmid

Digest

digested with DpnI

Digestion with Bsal provides sticky ends

Second round of saturated mutagenesis PCR

PCR

Transformation, Plasmid Isolation

Ligation

Circular mutant plasmid

Plasmid library

After 2nd round

2 x 10^5
Generating the Chemoreceptor Library

Tar_QC original sequence

```
CNAGTCTCAGCGTGATGCAGANGAGA
```

69 73

Tar_QC mutated sequence

```
CTGAGTNNKTCAGCGTGATGCANKNATGAGA
```

69 73

```
GAGCTNNKNKKNGCTCAGCCANNKCAAGG
```

149 150 154
Controlling the Library Diversity

Tar_QC mutated DNA sequence

69  73
AGGTCAGCGGTATCTG
AGATTTCTG

149 150 154
TAGGGCCCTGGCTCAGCCAGCTTG
GCTTGGGP

Tar_QC mutated amino acid sequence

69  73
GSALLGR

149 150 154
GPAHLR
AQP
Library Selection

Library $2 \times 10^5$

I

II

III

Ref.

SelecFon
Library Selection
Reading the Library

- D-Aspartate
  - LTAQPD
  - SSAVP
- L-Aspartic acid 4-benzyl ester
  - PPAQPP
  - PSAVT
- Vanillin
  - LFAQPL
  - GSAVG
- Geraniol
  - D*AQPD
  - SSAVP
- 2-Ethyl-hexanol
  - LTAQPD
  - SSAVP
- Caffeine
  - PPAQPP
  - SSAVT
- Sodium cyclamate
  - LTAQPD
  - SSAVP
Outlook: Health & Medicine

2-Ethyl-hexanol

Filipiak W., et al., 2009

E. Coli Quick Test
Homing Coli: Engineering *E. coli* to become tracking dogs

- Perfect swimming agar
- Speed improvement
- Reading the receptor library
Our Human Practice Projects
Thank you for your Attention!