Every Bacteria Counts!
Pop-counter       Memo-cell

iGEM Paris Liliane Bettencourt
Principle of pop-counter

Counter

Recombined Cells, %

Time

NO pulse 1 pulse N pulses

0000 0001 00NN
Principle of pop-counter

Counter

[Diagram showing different pulse scenarios with corresponding cellular representations and counter values]

Recombined Cells, %

Time

NO pulse  1 pulse  N pulses

0000  0001  00NN

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Principle of pop-counter

Counter

<table>
<thead>
<tr>
<th>NO pulse</th>
<th>1 pulse</th>
<th>N pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Recombined Cells, %

Time

iGEM Paris Liliane Bettencourt Sunday, November 7, 2010
Principle of pop-counter
Principle of pop-counter

Counter

<table>
<thead>
<tr>
<th>NO pulse</th>
<th>1 pulse</th>
<th>N pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Recombined Cells, %

Time

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Principle of the timer

Timer

N pulses

Quorum sensing

AHL

Recombined Cells, %

GFP (100% cells)

Time

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Principle of the timer

Timer

Quorum sensing

Recombined Cells, %

GFP (100% cells)

Time

[AHL]
Principle of the timer

Timer

N pulses

N pulses

Quorum sensing

AHL

Recombined Cells, %

GFP (100% cells)

Quorum sensing threshold

Time

Principle of the timer

Timer

N pulses

Quorum sensing

N pulses

AHL

Recombined Cells, %

GFP (100% cells)

Quorum sensing threshold

Time
Controlled integrase biobrick for rare recombination event
Design: timer

N events $\Rightarrow$ quorum sensing
$\Rightarrow$ timer triggering
$\Rightarrow$ GFP expression
Microfluidic chemostat

- chemostat
- cells can be observed under microscope
- laminar flux (only diffusion)
Microfluidic chemostat

- chemostat
- cells can be observed under microscope
- laminar flux (only diffusion)

Microfluidics is an ideal tool for our system
Microfluidic chip design

Based on the model of Yamada et al. 2010
Microfluidic chip design

Based on the model of Yamada et al. 2010
Modeling

Simulation tool

Equations

Diffusion of arabinose

$$\frac{\partial [A]}{\partial t}(x, t) = k_A \frac{\partial^2 [A]}{\partial x^2}(x, t)$$

Cells’ recombinations
(Gillespie algorithm)

Cells’ movement

$$X \sim Bin(dt \ k_{diff \ cells} \ f(t, x), \ f_2(t, x))$$

AHL profile

$$\frac{\partial [AHL]}{\partial t}(x, t) = k_I[LuxI](x, t) + k_{diff} \frac{\partial^2 [AHL]}{\partial x^2}(x, t)$$

Parameters: diffusion coefficients, recombination probability, time constants
Modeling

Arabinose

Switched cell

Unswitched cell

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
The number of recombined cells rises stepwise as we expected.
Recombination rate and RFP expression increase with the length of Ara pulse

Quantification of recombination rate: \[
\frac{\text{signal}}{\text{background}} > 100
\]
Population counter achievements

- ✓ Proof of feasibility by modelling
- ✓ Design and construction of the system from biobricks
- ✓ Characterization of the recombinase
- ✓ Proof of principle of the system
Memo-cell
Memo-cell
Memo-cell

Memo-cell module

0 0 0 0 0

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Memo-cell

Several signals

Memo-cell module

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Principle
Principle
Principle
Major challenges

1. Regeneration of a functional integration site

2. Single integration per counting signal

3. Specificity of the excision mechanism
Recombinases biobricks

1. Phage integration

Recombination site  →  Integration site

iGEM Paris Liliane Bettencourt
Recombinases biobricks

1. Phage integration

First signal

Phage \( \lambda \) recombinases
Recombinases biobricks

1. Phage integration

First signal

Phage λ recombinases
Recombinases biobricks

1. Phage integration
# Recombinases biobricks

1. **Phage integration**

## Lambda phage

- Phage λ recombinases

## HK022 phage

- Phage HK recombinases

---

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Recombinases biobricks

1. Phage integration
2. Transposon Tn916 excision

Tn 916

Left arm Right arm

Chromosome
Recombinases biobricks

1. Phage integration
2. Transposon Tn916 excision

Tn 916

Left arm  Right arm

Chromosome

Mobile transposon
Memo-cell device

2 plasmids

Chromosomal module

Inducible recombinases

Phage λ recombinases

Phage HK recombinases

Transposon Tn916 recombinases

iGEM Paris Liliane Bettencourt
Memo-cell device

2 plasmids

Chromosomal module

Inducible recombinases

Phage λ recombinases

Phage HK recombinases

Transposon Tn916 recombinases

iGEM Paris Liliane Bettencourt
Memo-cell device

Phage λ recombinases
Phage HK recombinases
Transposon Tn916 recombinases

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Memo-cell device

[i'm countin' it]

Sunday, November 7, 2010
Memo-cell device

1st signal
Memo-cell device

1st signal

Memo-cell module

00000

Phage λ recombinases

Phage HK recombinases

Transposon Tn916 recombinases

Sunday, November 7, 2010
Memo-cell device

1st signal

Memo-cell module

0 0 0 0

Functional right arm regenerated!

Phage λ recombinases

Phage HK recombinases

Transposon Tn916 recombinases
Memo-cell device

1st signal
Memo-cell device

1st signal

Memo-cell module

Functional integration site! regenerated!
Memo-cell device

1st signal

Memo-cell module

0 0 0 1

One counting scar

Functional integration site! regenerated!
Memo-cell device

1st signal
2nd signal

Memo-cell module

0 0 0 1

Phage λ recombinases
Phage HK recombinases
Transposon Tn916 recombinases

Sunday, November 7, 2010
Memo-cell device

1st signal

2nd signal
Memo-cell device

1st signal
2nd signal

Memo-cell module

0 0 0 1

Functional right arm regenerated!
Memo-cell device

1st signal

2nd signal

Memo-cell module

00001

Functional integration site regenerated!
Memo-cell device

1st signal
2nd signal

Memo-cell module

Second counting scar

Functional integration site regenerated!

Phage λ recombinases
Phage HK recombinases
Transposon Tn916 recombinases

Sunday, November 7, 2010
Memo-cell device

1st signal
2nd signal
N signals

Memo-cell module

0 0 0 2

1 2 3 ...

Phage λ recombinases
Phage HK recombinases
Transposon Tn916 recombinases

Sunday, November 7, 2010
Memo-cell device

1st signal
2nd signal
N signals

Memo-cell module

0 0 0 N

1 2 3 ...

Phage λ recombinases
Phage HK recombinases
Transposon Tn916 recombinases

Sunday, November 7, 2010
Rational design

- Tinkering with the transposon
  - Size reduction (from 18kb to 2.4kb)
  - Chimera between the Tn916 arm and the phage recombination site
  - Microcin C as the selection marker

Wild-type Tn916

Engineered Tn916

iGEM Paris Liliane Bettencourt
• Tinkering with the transposon
• Tinkering with the phage

Wild-type phage recombination site

Engineered phage recombination site

Mutated Tn916

Coupling sequence
Results: Efficient plasmid integration

Lambda site

HK022 site

Integration frequency

WT
Mutant

100%

25%

50%

75%

100%

WT
Mutant

100%

76%

25%

50%

75%

100%

74%
Results: Efficient transposon excision

Chimeric Tn916-Lambda arm

Chimeric Tn916-HK022 arm

Excision frequency

0 2 4 6 8 10 30

Induction time (h)

75%
50%
25%

0 2 4 6 8 10 30

Induction time (h)

75%
50%
25%

WT
λ

WT
HK

Kan
LacZ

Kan
LacZ

iGEM Paris Liliane Bettencourt
Memo-cell proof of principle

After an induced plasmid integration and transposon excision.....

• PCR verification

• Regenerated integration site checked by sequencing

iGEM Paris Liliane Bettencourt
Memo-cell achievements

- ✔ Rational design of chimeric sites
- ✔ Synthesis and cloning of the parts
- ✔ Characterisation of recombinases
- ✔ Full counting cycle achieved, with all components efficiently working

iGEM Paris Liliane Bettencourt
Statistically Improbable Phrases

Snapshot of our project
Statistically Improbable Phrases

Our project snapshot

iGEM Paris Liliane Bettencourt
Statistically Improbable Phrases

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
SynBioWorld: openProtocols

The Perfect Overnight Culture

What You’ll Need

- Lysogeny broth (LB)
- 10 mL pipette
- Plate with Colonies
- Growth tubes
- Tube rack
- Toothpicks
- Pipette-Aid
- Bunsen burner

Step One
Assemble all of your equipment inside a sterile bench. Put all the growth tubes in the rack with their lids loosened but resting atop the tube. Ensure your burner is not leaking, and light it carefully to establish a sterile environment.

• Easily create standardized protocols
• Share, find and edit them online
• Comment and vote on protocols
Acknowledgements


Advisors: D. Bikard, A.S. Coquel, T. Lombès, E. Meltzer, Y. Yang

Instructor: A. Lindner
Issue raised

**Integration**
Plasmids population that did not integrate

**Excision**
Excision of Transposons within non-integrated plasmids
After Transposon Excision

3 Different Possibilities

1. 1 entity on mobile Transposon
   2 entities on chromosome

2. 2 entities on mobile Transposon

3. 3 entities on mobile Transposon
   0 chromosomal entities left
We made minipreps of the induced cultures and transformed other cells with the mix of obtained plasmids.
We made **minipreps** of the induced cultures and **transformed** other cells with the mix of obtained plasmids.

Sunday, November 7, 2010
We made **minipreps** of the induced cultures and **transformed** other cells with the mix of obtained plasmids.
We made **minipreps** of the induced cultures and **transformed** other cells with the mix of obtained plasmids

**Transformation :** 1 plasmid/cell

Then copying plasmids : **amplification**
Integrase and Excisase biobricks

Phage integrases

Plasmid

Recombination site

Integration site

Chromosome
Principle
Principle

Int+Xis Phage

pBAD
Counting toolkit

• Pop-counter device
  • Inducible integron recombinases

• Memo-cell device
  • Inducible phage recombinases
  • Wild-type and mutant HK and Lambda recombination sites
  • Inducible transposon Tn916 recombinases
  • Synthetic functional transposon
  • Wild-type and mutant transposon arms
Principle
Principle

Disrupted integration site
Principle

Disrupted integration site
No more integration possible
Principle

Disrupted integration site
No more integration possible

Need to regenerate a new integration site to add another DNA entity!
Principle

Half of integration site on the integrated plasmid
Principle

Half of integration site on the integrated plasmid

Other half on the chromosome
Principle
Principle

Hijacking of transposon Tn916
Principle

Hijacking of transposon Tn916

Left end on plasmid

Right end on chromosome
Principle

Hijacking of transposon Tn916
Principle
Hijacking of transposon Tn916
Principle

Hijacking of transposon Tn916
Principle

Integration site
REGENERATED!
Principle

To count more, we could just add other sites on the chromosome
Principle

To count more, we could just add other sites on the chromosome
Principle

To count more, we could just add others
• right ends on the chromosome
Principle

To count more, we could just add others
• right ends on the chromosome
Principle

To count more, we could just add others

- right ends on the chromosome
- left ends on the plasmids
Principle

To count more, we could just add others

• right ends on the chromosome
• left ends on the plasmids
Principle

To count more, we could just add others

- right ends on the chromosome
- left ends on the plasmids
Principle

No problem for the plasmid integration
BUT...

iGEM Paris Liliane Bettencourt

Sunday, November 7, 2010
Principle

No problem for the plasmid integration
BUT...

iGEM Paris Liliane Bettencourt
Principle

No problem for the plasmid integration

BUT...

NO specificity for transposon excision!

Left end
(from the plasmid)

Right ends
(from the chromosome)
Principle

No problem for the plasmid integration

BUT...

NO specificity for transposon excision!

Left end
(from the plasmid)

Right ends
(from the chromosome)
Principle

No problem for the plasmid integration

BUT...

NO specificity for transposon excision!

[Diagram of molecular structures and interactions]
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome

Left part (on the plasmids)

Right part (on the chromosome)
Principle

Solution: Disruption of the right arms on the chromosome

Left part (on the plasmids)

Right part (on the chromosome)
Principle

Solution: Disruption of the right arms on the chromosome

Left part (on the plasmids)

Right part (on the chromosome)

Int+Xis Phage

Int+Xis Tn916

ARA

pBAD
Principle

Solution: Disruption of the right arms on the chromosome

Left part (on the plasmids)

Right part (on the chromosome)
Principle

Solution: Disruption of the right arms on the chromosome

Left part (on the plasmids)

Right part (on the chromosome)

iGEM Paris Liliane Bettencourt
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome
**Principle**

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome

Integration site REGENERATED!
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome
Principle

Solution: Disruption of the right arms on the chromosome
SynBioWorld : openProtocols
Engineering the recombination sites

- Transposon engineering

![Diagram of recombination sites]

Left arm

Left coupling sequence

Right arm

Right coupling sequence
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)

Left arm

Left coupling sequence

Right arm

Right coupling sequence
Engineering the recombination sites

• Transposon engineering
  • Size reduction  (from 18kb to 2.4kb)
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)
  - Coupling sequences mutations
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)
  - Coupling sequences mutations
Engineering the recombination sites

• Transposon engineering
  • Size reduction (from 18kb to 2.4kb)
  • Coupling sequences mutations
Engineering the recombination sites

• Transposon engineering
  • Size reduction (from 18kb to 2.4kb)
  • Coupling sequences mutations
  • Insertion of a phage recombination site inside the right arm
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)
  - Coupling sequences mutations
  - Insertion of a phage recombination site inside the right arm
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)
  - Coupling sequences mutations
  - Insertion of a phage recombination site inside the right arm
Engineering the recombination sites

• Transposon engineering
  • Size reduction  (from 18kb to 2.4kb)
  • Coupling sequences mutations
  • Insertion of a phage recombination site inside the right arm
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)
  - Coupling sequences mutations
  - Insertion of a phage recombination site inside the right arm
Engineering the recombination sites

- Transposon engineering
  - Size reduction (from 18kb to 2.4kb)
  - Coupling sequences mutations
  - Insertion of a phage recombination site inside the right arm
Acknowledgements
First, we tested our construct on a high-copy-number plasmid even though the event (excision) is rare, we expect to have at least 1 recombined plasmid per cell after Ara induction.
To be able to observe events on a single-cell level, we used microfluidics

- constant media flow ➔ acts as a chemostat  avoiding AHL accumulation
- can be observed under a microscope ➔ detection of single cell events
- laminar flux ➔ easy to control Ara pulses and model the system
To be able to observe events on a single-cell level, we used microfluidics

- constant media flow
  - acts as a chemostat
  - avoiding AHL accumulation
- can be observed under a microscope
  - detection of single cell events
- laminar flux
  - easy to control Ara pulses and model the system

Microfluidics is an ideal tool to model and test our system