**Competent cells**

**Project:** InterLab Measurements  
**Authors:** Laura Laiho  
**Dates:** 2016-07-05 to 2016-07-13

**TUESDAY, 7/5**  
Prepared agar plates

**WEDNESDAY, 7/6**

Testing the competent cells according to the iGEM protocol:  
iGEM protocol for competent cells was used: http://parts.igem.org/Help:Competent_Cell_Test_Kit.

**TOP10* Escherichia coli** competent cells and SOC medium from Gibson assembly were used.

**THURSDAY, 7/7**

Testing the competent cells according to the iGEM protocol:  
iGEM protocol for competent cells was used: http://parts.igem.org/Help:Competent_Cell_Test_Kit.

**TOP10* Escherichia coli** competent cells and SOC medium from Gibson assembly were used.

*One difference in methods from Wednesday was that we added plasmids onto cells not other way around.

**MONDAY, 7/11**

Prepared agar plates for transformation  
20 plates, 15 for iGEM plasmids

**TUESDAY, 7/12**

Testing the competent cells according to the iGEM protocol for the 3rd time.  
iGEM protocol for competent cells was used: http://parts.igem.org/Help:Competent_Cell_Test_Kit.

**TOP10* Escherichia coli** competent cells and SOC medium from Gibson assembly were used.

Testing the competent cells also with another plasmid, pJR18, for control. Three different concentrations of DNA were used: for control 500 pg/ul, 50 pg/ul and 5 pg/ul. and 2 plates of each. Each concentration was plated on two ampicillin plates.

**WEDNESDAY, 7/13**

Counting colonies according to the protocol with excel sheet, transformation efficiency was quite low (average 1.8E7 cfu/uL and weighted 1.6E7 cfu/uL), but good enough for transformations.

Table 1.
<table>
<thead>
<tr>
<th>DNA concentration [pg/ul]</th>
<th>0.5</th>
<th>1.0</th>
<th>3.0</th>
<th>6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency</td>
<td>3.6</td>
<td>4.0</td>
<td>3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Efficiency</td>
<td>2.51E-07</td>
<td>1.06E-07</td>
<td>1.78E-07</td>
<td>2.08E-07</td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>average</td>
<td>1.30E-05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weighted average</td>
<td>1.48E-05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The first day of the InterLab study. The DNA tubes were empty so they were resuspended in 200uL of dH2O and vortexed.

Transformation of TOP10 competent cells was performed according to the iGEM protocol: http://parts.igem.org/Help:Protocols/Transformation.

Instead of pipetting 1uL of resuspended DNA, 5uL was pipetted. Following plasmids were transformed:

Device 1: J23101+I13504  
Device 2: J23106+I13504  
Device 3: J23117+I13504  
Positive control  
Negative control

Transformation had to be done again because of the LUDOX problem. Transformation of TOP10 competent cells was performed according to the iGEM protocol: http://parts.igem.org/Help:Protocols/Transformation.

Following plasmids were transformed:

Device 1: J23101+I13504  
Device 2: J23106+I13504  
Device 3: J23117+I13504  
Positive control  
Negative control

1 ul of plasmids were used for transformation instead of 5 (which was indicated in the exceptional protocol which stated that one should use 5 ul after resuspending them if the plasmids are dried up)

The second day of the Interlab study. Preparing the liquid culture:

5ml of LB  
Chloramphenicol working concentration 25ug/ml.

Two colonies from each plate were picked and inoculated. Liquid cultures were grown overnight at 37°C 230 rpm.

Also restreaking the previous transformants if they happen to be too old.

Practicing the 3rd day protocol and preparing the liquid culture of the restreaked cells.
The 3rd day of the Interlab study. Measuring the OD600 of overnight cultures, diluting them to the OD of 0.02 and performing the assay:

Incubating cultures at 37°C 220 rpm and taking 100ul samples from each tube at 0, 1, 2, 3, 4, 5 and 6 hours. Measuring the ODs and FIs of each sample.

Table 1.

Table 2.

Figure 1.

Table 3.
Figure 2.

![interlab11.png](image)

![interlab12.png](image)
Preparing of the FITC solution according to the iGEM plate reader protocol:  

20x PBS was diluted into H2O to get 1x PBS - 1 part PBS and 19 parts H2O -> 3.8ml of H2O and 200ul of PBS (4mL).
We made 4ml of 1xPBS, not 1 mL because we needed more of it later for the plate reader, but obviously for the FITC solution we used just 1 mL of 1xPBS.

So the protocol says "Prepare 2x FITC stock solution (500 µM) by resuspending FITC in 1 mL of 1xPBS"

\[
\begin{align*}
  n &= \frac{m}{M} = C \cdot V = \frac{500 \text{µM} \cdot 1 \text{mL}}{0.5 \text{µmol}} = 1000 \text{µmol} \\
  m &= n \cdot M = 1000 \text{µmol} \cdot 389,382 \text{g/mol} = 389,382 \text{g}
\end{align*}
\]

In order to have 500µM concentration of FITC we take 194.7µg of FITC because it's a dry reagent. We add 194.7µg of FITC into 1ml of 1xPBS and we get 2xFICT solution.

Then 2xFITC was incubated in 42°C for 4 hours.

Later to prepare 1xFITC solution we added 1 ml of 2xFITC into 1ml of 1xPBS. Because 2xFITC got diluted by half its concentration became 250uM in 1xFITC solution.

Measured fluorescence of all samples in standard measurement modes in plate reader. Result can be found in table 1.

Table 1.
Obtaining a ratiometric conversion factor to transform absorbance data into a standard OD600 measurement by using LUDOX-S30 as a single point reference. Results can be seen in table 2. The same iGEM protocol as above was used.

Table 2.

<table>
<thead>
<tr>
<th></th>
<th>LUDOX</th>
<th>H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>replicate 1</td>
<td>0.045</td>
<td>0.037</td>
</tr>
<tr>
<td>replicate 2</td>
<td>0.045</td>
<td>0.037</td>
</tr>
<tr>
<td>replicate 3</td>
<td>0.045</td>
<td>0.037</td>
</tr>
<tr>
<td>replicate 4</td>
<td>0.045</td>
<td>0.037</td>
</tr>
<tr>
<td>average</td>
<td>0.045</td>
<td>0.037</td>
</tr>
<tr>
<td>corrected Abs500</td>
<td>0.008</td>
<td></td>
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<tr>
<td>reference OD600</td>
<td>0.01475</td>
<td></td>
</tr>
<tr>
<td>correction factor</td>
<td>1.84375</td>
<td></td>
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