PLASMID TRANSFORMATION OF *Escherichia coli* WITH BIOLUMINESCENCE GENES

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Plasmid transformation of *Escherichia coli* can be easily performed within 20 minutes using a modification of the rapid colony transformation procedure (Hanahan, 1986; Smith and Danner, 1981). The *lux* genes, contained on the plasmid pLUX-Ap, provide a vivid demonstration of plasmid transformation by conferring on *E. coli* the phenotype of bioluminescence.

**Introduction**

There are numerous examples of organisms which demonstrate bioluminescence. These include bacteria, mollusks, worms, insects, fungi, jellyfish, and diatoms. Many marine organisms such as fish and cephalopods symbiose with bioluminescent bacteria and use the light for intraspecific communication. The enzymes involved in bioluminescence are known as luciferases.

The genes for bioluminescence (*lux* genes) have been cloned from a few marine bacteria (Shaw and Kado, 1986) and coleopterans (De Wet *et al.*, 1985). The plasmid pLUX-Ap contains the *lux* gene system from a marine bacterium (*Vibrio*) and an ampicillin antibiotic resistance marker. The plasmid pLUX-Ap was constructed in this lab using the *lux* genes previously cloned by Shaw and Kado (1986).

The two major biochemical reactions for bacterial bioluminescence are:

1) \[ \text{RCHO} + \text{FMNH}_2 + \text{O}_2 \rightarrow \text{RCOOH} + \text{FMN} + \text{H}_2\text{O} + \text{hv (490nm)} \]  
   \[ \text{Luciferase} \]  
   \[ \text{light rxn} \]

2) \[ \text{RCOOH} + \text{NADPH}_2 + \text{ATP} \rightarrow \text{RCHO} + \text{NADP} + \text{AMP} + \text{PPi} \]  
   \[ \text{Recycling rxn} \]

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**Materials**

Colony transformation buffer (TFB), final pH 6.20 per liter:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl (ultra pure)</td>
<td>7.4g</td>
<td>(100mM)</td>
</tr>
<tr>
<td>MnCl$_2$ · 4H$_2$O</td>
<td>8.9g</td>
<td>(45mM)</td>
</tr>
<tr>
<td>CaCl$_2$ · 2H$_2$O</td>
<td>1.5g</td>
<td>(10mM)</td>
</tr>
<tr>
<td>HACoCl$_3$</td>
<td>0.8g</td>
<td>(3mM)</td>
</tr>
<tr>
<td>K-MES</td>
<td>20ml</td>
<td>of a 0.5M stock (pH 6.3)</td>
</tr>
</tbody>
</table>
Luria Broth (LB), final pH 7.5 per liter (Maniatis, Fritsch, and Sanbrook, 1992):

Yeast Extract 5g  
Tryptone 10g  
NaCl 10g

LB petri plates: Add 15g agar/liter 
LBA petri plates: 50μg/ml ampicillin

E. coli strain TG-1 (JM109 and DH5α have also been used).


Crushed Ice and a Water bath at 37-42°C.

Methods

1. Take one or two colonies directly from an LB plate (which has been previously prepared within the last 72 hours) and suspend in 100μl of TFB. Add one μl of plasmid pLUX-Ap DNA (approximately 50ng) and incubate on ice for ten minutes. (A control is also set up at this point. The control consists of E. coli undergoing the same treatment except for the addition of plasmid DNA).

2. Place the bacteria in a 37 - 42°C water bath for 60 - 90 seconds. The timing here is critical and must not exceed 90 seconds. This procedure is referred to as "heat shocking".

3. After heat shocking, place the bacteria on ice for two minutes.

4. Remove the bacteria from the ice and add 400μl LB. Plate directly onto the LB and LBA plates.

5. Allow the plates to dry and incubate overnight at 37°C.

6. Observe the colonies in a completely darkened room. Bioluminescence should become visible within two to five minutes.

Results

The untransformed E. coli (control) when compared to the transformed E. coli (both on the ampicillin containing plates) demonstrate that ampicillin prevents growth of all untransformed E. coli. This absence of growth in the presence of antibiotic demonstrates that E. coli does not contain endogenous ampicillin resistance. The growth of E. coli on plates without antibiotic demonstrates that the transformation treatment was not harmful to E. coli. Additionally, this plate demonstrates that E. coli is not bioluminescent. Colonies growing on the plates with ampicillin have the phenotypes of ampicillin resistance and bioluminescence.

A flask of LBA also provides a vivid example of the transformed E. coli’s bioluminescence. Inoculate the flask and grow for approximately ten hours. Observe in a dark room. Swirling the flask will enhance the bioluminescence (see the light reaction). If the bacteria have grown too long the media will have been depleted of nutrients and the bioluminescence will not be very visible. If this occurs, add more LB and observe after a period of ten to twenty minutes.
Literature Cited


Acknowledgement: We thank Dr. C. I. Kado for providing the lux genes.

First Call for Papers
AMCBT 1993 Convention

Millikin University, host of the 1993 AMCBT convention, invites all members of AMCBT to make plans to attend our annual fall meeting scheduled for October 28th through the 30th, 1993. Suggestions for papers, speakers, special topics or other items to be considered for the meeting agenda can be sent to any member of the steering committee or to Sister Jeanene Yackey, vice president in charge of the program. Harold Wilkinson is the local arrangements vice president. Please send your abstract and/or any suggestions concerning the meeting before February 1, 1993.

Abstract:

Send Abstracts/Suggestions to: Sister Jeanene Yackey, Fontbonne Science Academy, Fontbonne College, St. Louis, MO 63105-3098.