Transformation Experiment Using Bioluminescence Genes of *Vibrio fischeri*

James Slock

Transformation experiments are an important tool in recombinant DNA technology. The addition of new genes to a recipient cell introduces a heritable modification in the recipient cell's phenotype. Most laboratory teaching exercises involve the transformation of plasmid DNA into *E. coli* followed by selection of transformants containing an antibiotic resistance marker (Moss 1991). While these experiments are successful and instructive, they lack the wonderment that is the essence of genetic engineering.

Research on the regulation of bioluminescence in *Vibrio fischeri* has provided us with exciting teaching materials that can be used in a series of molecular biology experiments, including transformation. There are seven genes required to produce bioluminescence in *E. coli*. These seven *lux* genes from *Vibrio fischeri* have been cloned into two plasmids. The plasmid pHK724 (Figure 1) contains the *lux R* gene whose gene product is a transcriptional regulatory protein. The plasmid pHK555 (Figure 2) (*lux ICDABE*) contains the structural genes required to make light. The *lux R* gene of pHK555 is inactive because of the insertion of phage DNA. When pHK724 is transformed into *E. coli* containing pHK555, the resultant colonies grow on selective media bioluminesce (Kaplan & Greenberg 1987; Slock et al. 1990). Bioluminescence transformation experiments show students the excitement and power of recombinant DNA technology.

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Procedure

As was stated by Moss (1991) in his article on transformation, sterile technique and good laboratory practice must be carried out at all times.

Micro Tube Procedure for Preparation of Competent Cells of *E. coli* (Hanahan 1985), Modified by Slock

1. Culture desired bacterial strain overnight in Luria broth (LB). If using *E. coli*/pHK555, chloramphenicol (Cm) must be added to a final concentration of 50 μg/ml. Note: The plasmid pHK555 contains a chloramphenicol resistance gene (Figure 2). Therefore, Cm is added to the growth medium to apply selective pressure on *E. coli* to maintain the plasmid.
2. Inoculate 200 μl of overnight culture into 50 ml of LB (plus antibiotic Cm to final concentration of 50 μg/ml LB) in an Erlenmeyer flask plugged with cotton or capped with an inverted beaker.
3. Incubate at 37° C with agitation. Monitor culture density with Spectronic 21 or Klett-Summerson photoelectric colorimeter (Baxter Diagnostics, Inc.).
4. Allow the culture to reach an optical density of 0.35 to 0.50 at a wavelength of 550 nm. This is midlog phase of growth and generally takes 2½ hours. This is equivalent to 40–80 Klett units when using a Klett colorimeter.
5. Pipet 1 ml culture to 1.5-ml Eppendorf tube. Microfuge 2 minutes and pour off supernatant. Add a second 1 ml and repeat microfuge. Pour off supernatant. If a microfuge is not available, any centrifuge can be used to pellet the *E. coli* cells.
6. Resuspend pellet in 200 μl of frozen storage buffer (FSB).
7. Incubate on ice for 15 minutes.
8. Microfuge 2 minutes and pour off the FSB.
9. Resuspend pellet in 200 μl FSB.
10. Bacteria are now "competent" and ready for transformation. Competent cells can also be stored in the refrigerator for up to 24 hours or for up to 6 months if stored in a −70° C freezer. The FSB ($CaCl_2$ solution) should be stored at 4° C and made fresh every 6 months.

Plasmid Preparation

Numerous techniques are available for extracting plasmids from *E. coli*. A

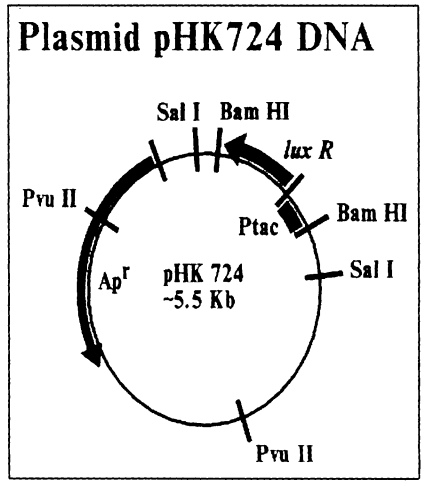

Figure 1. Plasmid pHK724 is a 5.5 Kb plasmid containing an ampicillin resistance gene (Ap^r). Ptac is the promotor for the *lux R* gene. Sal I, Bam H I and Pvu II are restriction endonuclease sites.

good source of information regarding molecular biology experiments, including plasmid mini preparations, is *DNA Science, A First Course in Recombinant DNA Technology*, by David Micklos and Greg Freyer. (It is distributed by Carolina Biological Supply Company 800-334-5551).

For this experiment, I use a commercial kit supplied by QIAGEN (800-426-8157). The kit (#27104) is a fast and simple plasmid miniprep method that can be completed in about 45 minutes (50 preps for $50). The procedure does require a microfuge. At the end of the extraction, the plasmid is resuspended in 100 μl of 10 mM Tris/EDTA buffer, pH 8.3–8.5.

Purified plasmid pHK724 may be obtained by writing the author.

Transformation of E. coli

1. Add plasmid pHK724 DNA (5 μl or less) to 200 μl of competent cells.
2. Incubate on ice 15 minutes.
3. Place tubes in 42° C water bath for 90 seconds.
4. Incubate at room temperature for 5 minutes.
5. Add 1 ml of LB and incubate at 37° C for 1 hour.
6. Add 100 μl to selective solid medium. Spread with glass spreader.
7. If transforming for luminescence, incubate agar plates at 30° C or room temperature for 30–48 hours.
8. The bioluminescent colonies must be viewed in a dark room. It takes about 5 minutes for one's eyes to "dark adapt." The bioluminescent colonies are readily visible under these conditions.

Note: An experiment to get students to think about the influence of temperature on microbial growth would be to incubate selective agar plates at both 30° C and 37° C. *E. coli* will grow at both temperatures but will grow faster at 37° C since its optimal growth temperature is 37° C (the temperature of the human gut).

However, the genes for bioluminescence are from the marine bacterium, *Vibrio fischeri*. *Vibrio fischeri* has adapted to a cooler environment (the ocean) and, therefore, the proteins for bioluminescence are heat denatured at 37° C.

Reagents

Inorganic chemicals were purchased from Sigma Chemical Company (800-325-3010).

Name	Catalog Number	Price
Ampicillin	A-9518	$28.35/ 5 g
Calcium Chloride	C-3881	$17.80/ 500 g
Chloramphenicol	C-0378	$11.25/ 5 g
Glycerol	G-7893	$18.10/ 500 ml
Potassium Acetate	P-3542	$10.65/ 100 g
Potassium Chloride	P-4504	$7.15/ 250 g
Sodium Chloride	S-9625	$10.65/ 500 g
Sodium Hydroxide	S-0899	$17.70/ 500 g
Tris-EDTA Buffer (10x concentrate)	T-9285	$8.95/ 100 ml

Bacteriological media was purchased from Fisher Scientific (800-766-7000).

Name	Catalog Number	Price
LB Broth	DFO446-17-3	$26.20/500 g
Agar	DFO140-15-4	$44.40/100 g
LB Agar	DFO445-17-4	$57.20/500 g

Reagent Preparation

1M Potassium Acetate	g per 100 ml	final [ ]
$KC_2H_3O_2$	9.8 g	1M

- Dissolve in 80 ml water.
- Adjust pH to 7.5 with KOH or HCl.
- Make up to 100 ml in a volumetric flask.
- Autoclave.
- Store at 4° C.

Frozen Storage Buffer (FSB)

	g or ml per 1L	final [ ]
KCl	7.4 g	100 mM
$CaCl_2$-$2H_2O$	7.5 g	50 mM
Glycerol	100 ml	10%
1 M $KC_2H_3O_2$	10 ml	10 mM

- Dissolve in 900 ml water.
- Adjust pH to 6.2 with HCl (1M).
- Make up to 1000 ml in a volumetric flask.
- Dispense 200-ml aliquots into 250-ml bottles.
- Autoclave.
- Store at 4° C.

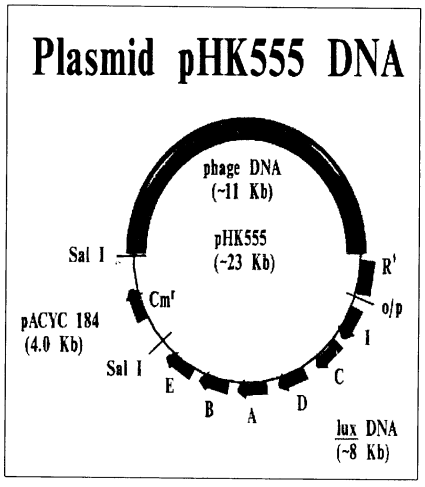

Figure 2. Plasmid pHK555 is a large plasmid containing a chloramphenicol resistance gene (Cm^r). R is the *lux R* gene inactivated with phage DNA; o/p is the operator/promotor region for transcription of *lux ICDBE*; Sal I is a restriction endonuclease site; and pACYC 184 is a portion of a plasmid containing the Cm^r gene (2).

L-Broth 10 g Tryptone; 5 g Yeast Extract; 5 g NaCl; make up to 1L and adjust pH to 7.5 with 20% NaOH. Autoclave. Store at room temperature.

L-Agar Add 15 g Bacto-agar per 1L L-Broth. Autoclave. Store at 4° C.

Antibiotics

Chloramphenical

Stock Cm = 25 mg/ml of EtOH
Final [Cm] in LB = 50 μg/ml LB

Sample Calculation:

$$C_1V_1 = C_2V_2$$

(25,000 μg/ml)(Xml) =

(50 μg/ml)(50 ml LB)

X ml = 0.1 ml

X = 100 μl stock Cm per 50 ml LB

Selective Medium for Transformation of E. coli/ pHK555 with pHK724

Stock Ampicillin (Ap) = 40 mg/ml dH_2O; filter sterilize (or add Ap to sterile water)

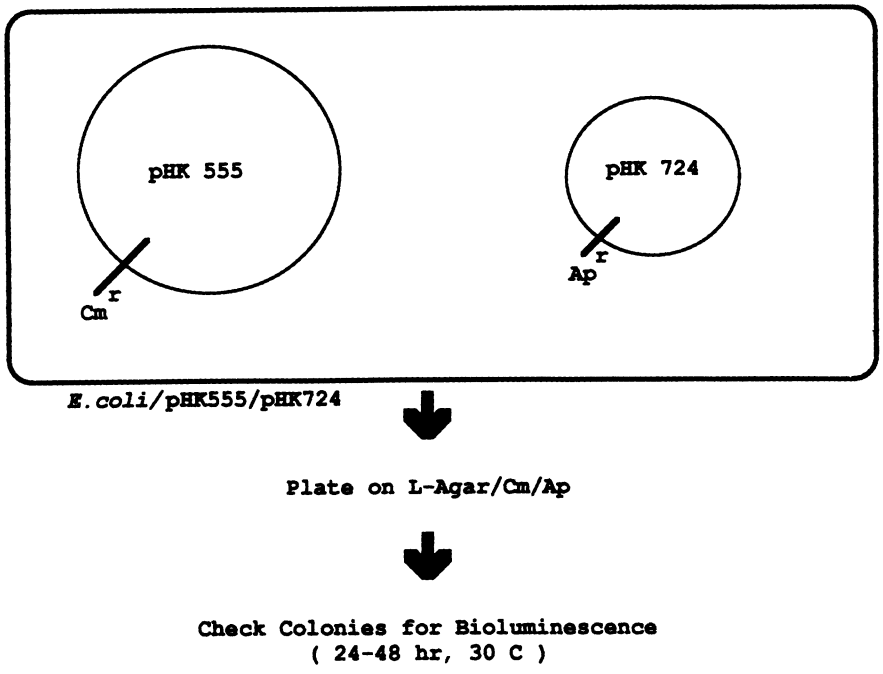

Figure 3. Drawing of *E. coli*/pHK555 transformed with pHK724.

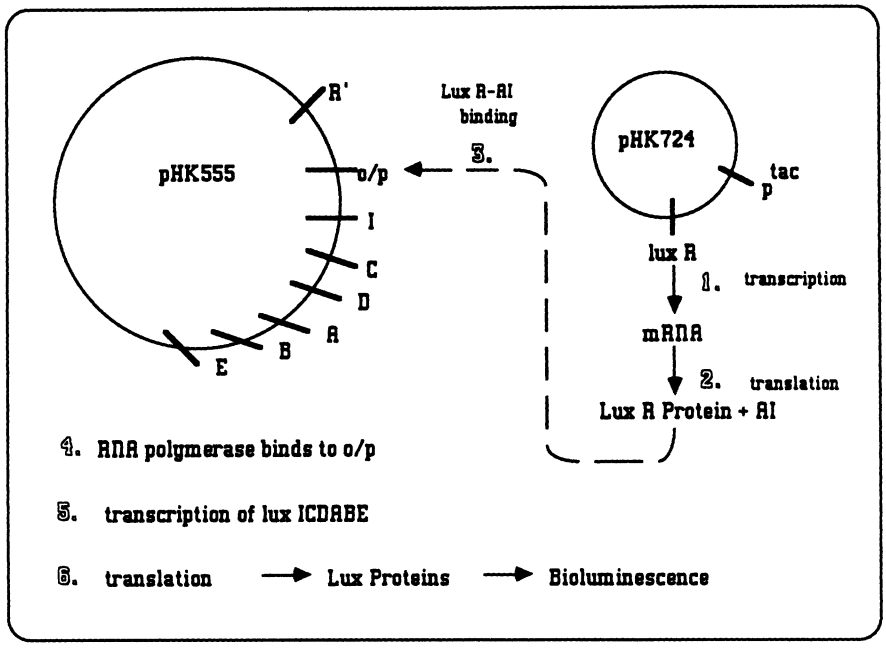

Figure 4. Cellular events leading to bioluminescence.

Final [Ap] = 80 μg/ml L-Agar
Final [Cm] = 50 μg/ml L-Agar

After autoclaving the L-Agar, cool to 60° C in a water bath, add Cm and Ap, then pour plates. Store at 4° C. Plates are good for up to 4 weeks.

Controls for Transformation Experiment

E. ccoli/pHK555 Viability Control.
Plate competent cells on L-Agar plates (without antibiotics). Cells on these plates should show growth.

Selectivity Control.
Plate competent cells on LA/Cm/Ap plate. One would expect no growth of competent cells on these plates.

Final Note

To get students involved in the learning process, I give them the diagram shown in Figure 3 and ask them to complete the diagram by showing the cellular events that are necessary to produce bioluminescence. A completed diagram would look similar to Figure 4. Information regarding transcription, translation, *lac* operon and *trp* operon would have been provided in the lecture portion of the course.

Cultures & Plasmid pHK724

The bacterial strains and plasmid pHK724 necessary to conduct this experiment may be obtained free of charge by writing the author.

Acknowledgment

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