

### **2.3.6 Luciferase Assay – Preparation of Cell Lysate**

Luciferase enzyme catalyses the conversion of luciferin to an oxidated form in a chemiluminescent reaction (Fig 2.4). The manufacturer's method was followed as described (Promega, 2003). Passive cell lysate solution was prepared from passive cell lysate solution (5x) by a 5 fold dilution with ddH<sub>2</sub>O. Following transfection, cells were lysed through addition of 200 µl of passive cell lysate solution to each well. They were then incubated on ice for 5 min whilst rocking. Following this, they were frozen -80 °C to ensure complete cell rupture. Wells were then scraped with a rubber policeman, solutions transferred to eppendorfs and vortexed briefly (2 s), centrifuged at 12000 RCF at 4°C for 5 min. Supernatants were transferred using a pipette into fresh eppendorfs and stored at -80 °C until analysis.

#### **2.3.6.1 Luciferase Assay – Analysis of Cell Lysate**

This assay was performed with 50 µl of luciferase substrate, added to a luminometer tube and 10 µl of cell lysate mixed in using the pipette. Readings were taken at room temperature with a 3 s delay and 10 s acquisition time in duplicate and results are expressed as relative light units per mg protein.

### **2.3.7 Plasmid (pGL3 luc) Amplification, Isolation and Characterisation**

#### **2.3.7.1 Preparation of Competent *E.coli* (DH5α)**

pGL3 luc (Fig. 2.5) was brought to the group by Dr M. Thanou. A glycerol stock of DH5α was taken out of the -80 °C freezer and defrosted on ice. It was loop inoculated into a sterile bijou containing 2.5 ml of LB medium and grown overnight at 37 °C with shaking at 225 rpm. This culture was inoculated into 250 ml of LB medium containing 20 mM MgSO<sub>4</sub> in a 1 L aerated flask and grown for 6 h ( $A_{600} = 0.4-0.6$ ). The cells were then pelleted by centrifugation at 4500 RCF for 5 min at 4 °C. The cell pellets were then re-suspended in a total volume of 50 ml ice cold 0.1 M CaCl<sub>2</sub> and all subsequent procedures were made on ice. Cells were incubated for 5 min at 4 °C then centrifuged (4500 RCF) for 5 min at 4 °C. The pellet was re-suspended in 10 ml of ice cold CaCl<sub>2</sub> (0.1 M) and incubated on ice for 40 min then 40 % glycerol (10 ml) was added (v/v in 0.1 M CaCl<sub>2</sub>). Aliquots (0.2 ml) of this stock solution were then quick frozen in isopropanol/dry ice and stored at -80 °C until use.