2.3.7.2 Transformation of Competent DH5α

An aliquot of competent DH5α in glycerol was removed from storage at -80 ºC and thawed on ice. pGL3 luc plasmid (~10 ng) was added to the eppendorf (in less than 10 µl) and mixed gently with the pipette tip to avoid shearing the pDNA. This aliquot was incubated on ice for 30 min, then at 42 ºC for 1 min before returning to ice for 2 min (heat shock). LB medium (800 µl) was added and the eppendorf incubated for 45 min at 37 ºC with shaking at 150 rpm. This culture was then pour plated onto an LB agar plate containing ampicillin (100 µg/ml).

2.3.7.3 Amplification of Transformed DH5α

Amplification of DH5α was performed according to the QIAGEN® plasmid purification method (Qiagen, 2000) and all buffers used are included in the Megaprep plasmid purification kit. A single colony from the ampicillin selection plate was picked with an inoculation loop and inoculated into 10 ml of LB medium containing ampicillin (100 µg/ml) and incubated for 8 h at 37 ºC under shaking at 220 rpm. All of this starter culture was inoculated into 500 ml of LB medium in a 1L aerated flask and grown overnight (16 h) at 37 ºC under shaking at 220 rpm.

2.3.7.4 Isolation and Purification of the pGL3 luc Plasmid

Transformed DH5α (prepared as described above) were pelleted by centrifugation at 6000 RCF for 15 min at 4 ºC. A QIAfilter Mega-Giga cartridge was attached to a 1 L Pyrex bottle. The bacterial pellet was re-suspended thoroughly using a pipette in a total volume of 50 ml of the buffer P1 (50 mM Tris.Cl pH 8.0, 10 mM EDTA, 100 µg/ml RNase A) and transferred to a 500 ml bottle. To this, 50 ml of the buffer P2 (200 mM NaOH, 1 % SDS (w/v)) was added and mixed thoroughly by inversion of the bottle 4-6 times. This solution was incubated at room temp for 5 min. Neutralisation of pH was achieved by addition of 50 ml of chilled buffer P3 (3.0 M potassium acetate pH 5.5) and the solution was mixed by inversion 4-6 times. A white precipitate formed.

The lysate was transferred to the QIAfilter Mega-Giga cartridge prepared at the start and incubated at room temperature for 10 min to allow the protein precipitate to float on top of the rest of the solution. The precipitate was removed by vacuum filtration and the solution collected. The vacuum was stopped and 50 ml of buffer FWB (750 mM NaCl, 50 mM 4-Morpholinepropanesulfonic acid (MOPS) pH 7.0, 15 % isopropanol (v/v))