

was added with gentle stirring of the white precipitate. Then the vacuum reapplied and the solution was combined with the previous filtrate. A sample (120 μ l) of the cleared lysate was removed and stored for analysis.

The buffer ER (12.5 ml) was then added to the filtered lysate and mixed by inversion 10 times before a 30 min incubation on ice. A QIAGEN-tip 2500 was equilibrated with 35 ml of buffer QBT (750 mM NaCl, 50 mM MOPS pH 7.0, 15 % isopropanol (v/v), 0.15 % triton X-100) with flow being gravity driven and flow stopping when the meniscus reached the top of the column. The filtered lysate was then added to the column. The QIAGEN-tip was washed with 200 ml of buffer QC (1.0 M NaCl, 50 mM MOPS pH 7.0, 15 % isopropanol (v/v)) and a 160 μ l sample of the eluent was taken for analysis.

DNA was then eluted from the column with 35 ml of buffer QN (1.6 M NaCl, 50 mM MOPS pH 7.0, 15 % isopropanol). A sample of the eluent (22 μ l) was taken for analysis. The DNA was precipitated from the eluent solution by addition of isopropanol (26 ml). The centrifuge tube was marked on the back to indicate the expected position of the pellet and the solution centrifuged at 15000 RCF for 30 min at 4 °C. The supernatant was carefully decanted to avoid loss of the DNA pellet. Ethanol (70 % v/v) was used to wash the pellet and then re-centrifuged at 15000 RCF for 10 min at 4 °C. The ethanol was decanted and the pellet air dried for 10 min. The dried pellet was re-dissolved in 2 ml of buffer TE (10 mM Tris.Cl pH 8.0, 1 mM EDTA).

2.3.7.5 Quantification of pGL3 luc

Quantification of plasmid concentration was made using both UV absorbance and quantification of band intensity in agarose gel. The UV absorbance (280 nm) of a 50 μ g/ml solution of pDNA has an absorbance of 1. Therefore, by measuring the UV absorbance (280 nm) of the isolated pGL3 luc (as described above), it was possible to calculate the concentration in a sample of plasmid.

For the agarose gel characterisation a known concentration of pDNA was loaded onto a lane and the band intensity of the sample compared, intensity was measured with ImageQuant (Amersham, UK). Agarose gel electrophoresis also confirms the identity of the plasmid through the comparison of restriction digests of the stock and plasmid preparation. As can be seen in Fig. 2.6 the retention factor (rf) of the complete plasmid and EcoR1/BamH1 restriction digest fragments are the same in the stock (lanes 3 and 4) and plasmid preparation (lanes 5 and 6) confirming the plasmid production.