

2.3.8 DNA Agarose Gel Electrophoresis

Agarose (350 mg) was dissolved in Tris/Borate EDTA buffer (50 ml; TBE) by heating in a microwave until boiling, this solution was allowed to cool and ethidium bromide was added (0.05 µg/ml). Gels (0.7 %) were then cast in a 10 x 20 cm gel tank, any bubbles were removed with a pipette tip and then gel comb was added and the gel allowed to set. Gel electrophoresis was carried out in a horizontal tank containing TBE buffer and was run at 100 V for 1.5 h. Gels were imaged using the Typhoon 9410 Variable Mode Imager. This technique was used for the analysis of polyplexes (Chapters 4 and 6) and in the confirmation of plasmid preparation and purification (Fig. 2.6).

2.3.9 Western blotting

2.3.9.1 Preparation of a Cell Lysate

First a cell lysis buffer was prepared: 1 % (v/v) triton-X-100, 0.5 mM EDTA, 15 mM NaCl, 2 mM Tris base. This solution was aliquoted and stored at -20 °C. Before use the following protease inhibitors were added: leupeptin (2 µg/ml), pepstatin A (1 µg/ml), aprotinin (2 µg/ml) and phenylmethylsulphonyl fluoride (100 µg/ml).

In the case of the adherent cell lines: Caco-2; COS-1; COS-7; DU145; MCF-7 and PC3 cells, the cells were grown to 70-90 % confluence in a 75 cm² flask, washed with ice cold PBS on ice and 1 ml of the lysis buffer was added to cells. They were incubated on ice with rocking for 5 min before transfer to a -80 °C freezer. The flasks were removed from -80 °C and defrosted on ice, cells were scraped from the flask and the contents transferred to 1.5 ml eppendorfs. These were vortexed for 30 s then centrifuged at 13000 RCF at 4 °C for 10 min. The supernatant was removed and analysed for protein content using the BCA assay as described in Section 2.3.5. This solution was aliquoted and the samples stored at -80 °C until use in western blot experiments. In the case of the suspension cell line: U937, 20 ml of culture (at 70-90 % maximum cell density) was centrifuged at 200 RCF and resuspended in 1 ml of lysis buffer. This was incubated on ice for 5 min then frozen at -80 °C. This was removed from the freezer and defrosted on ice before a 30 s vortex. The lysate was then cleared by centrifugation at 13000 RCF for 10 min at 4 °C then the supernatant was removed. This solution was analysed for protein content using the BCA assay as described in Section 2.3.5. The remaining solution was aliquoted and stored at -80 °C until use in western blot experiments.