

Transfection of Raji cells, a commonly used human B-cell line, using Metafectene Pro

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Introduction

One of the most amazing features of higher organisms is the ability to recognise and even remember a seemingly infinite number of pathogenic microorganisms and respond specifically to them. In the heart of this response is the interaction between an antigen-presenting cell's MHCII molecule and a regulatory T-cell's TCR molecule, which ensures that only those cells recognising the particular enemy at hand will be activated. Transcriptional control of the MHCII molecules is thus of central importance to the immune system's regulation, and while their expression can be induced in most cell types, there are very few and specific cell types that constitutively express them, thus having a pivotal role in the immune system's regulation. One such cell type is the B-cells, which are of haemopoietic lineage, therefore giving rise to suspension cell lines in the laboratory. While usually electroporation is used to transfect cells in suspension, there are particular B-cell lines that are particularly hard to transfect. In order to establish alternative protocols for routine B-cell transfection we tested Metafectene Pro on the more amenable Raji cells, one of the most frequently used B-cell lines.

Materials and methods

Materials: DRA-luc was previously constructed in our laboratory and contains the MHCII DRA promoter (bases -433 – +84 in pGL3-basic). HLA-DRA is constitutively expressed in B-cells.

RPMI was purchased from Biological Industries, gentamycin from Fluka, FBS from Biochrom AG and luciferin from Promega.

Luc assay: Harvested cells were resuspended in 200 µl of Tris pH7.5, 0.25M and lysed by sonication. The lysate was cleared by centrifugation at 14000 rpm, 1 min, then 40 µl were mixed with 20 µl luciferin just prior measuring light in a luminometer.

Experimental procedures / transfection protocol

Preparation of cells: Raji cells cultured at an approximate density of $0.4-0.8 \times 10^6$ cells/ml in RPMI1640 supplemented with 10% serum and 50 $\mu\text{g/ml}$ gentamycin were diluted in an equal amount of full media on the day prior transfection. For the transfection, a 24-well plate was used, containing 0.4×10^6 cells per well (collected by centrifugation at 1200rpm and resuspended in 500 μl RPMI1640 supplemented with serum and gentamycin).

Complex formation: The appropriate amounts of DNA or Metafectene Pro were diluted in serum- and antibiotic-free RPMI to a total amount of 30 μl each. The DNA solution was subsequently added to the Metafectene Pro solution and incubated at room temperature for 15-20 minutes before being added to the cells. The cells were left for 24 hours and then fed an additional 1 ml of medium. Finally they were harvested at approximately 48 hours and processed as described.

Results and discussion

Optimal Metafectene:DNA ratio

Varying amounts of Metafectene Pro were complexed with 1 μg of DRA-luc. Luc assay results are shown in Figure 1. A strong preference to a ratio of 5:1 (μl Metafectene Pro : μg DNA) is evident.

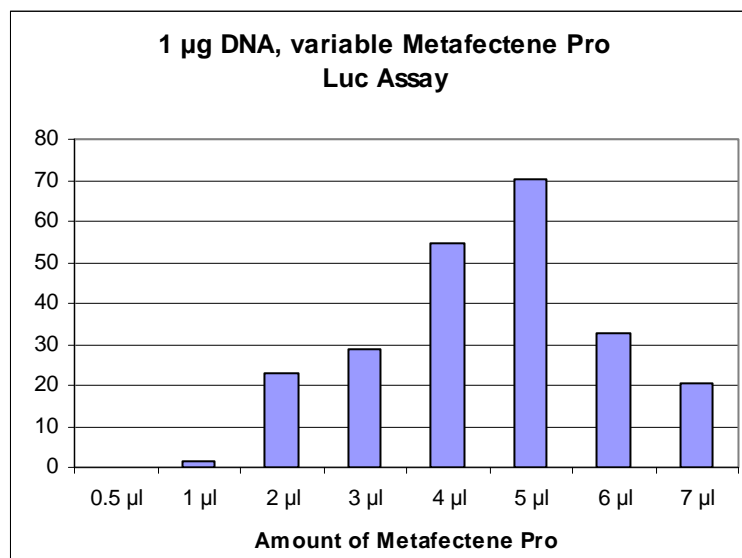


Fig.1

Quantity of transfection complex

Since no apparent mortality was observed in the initial experiment, increased amounts of complex were tested. The Metafectene Pro : DNA ratio was kept constant at 5 μl : 1 μg . As seen in Figure 2, the precipitate is well tolerated by the

cells at even twice the maximum recommended quantity – however the increase did not correspond to increased transfection (Figure 3).

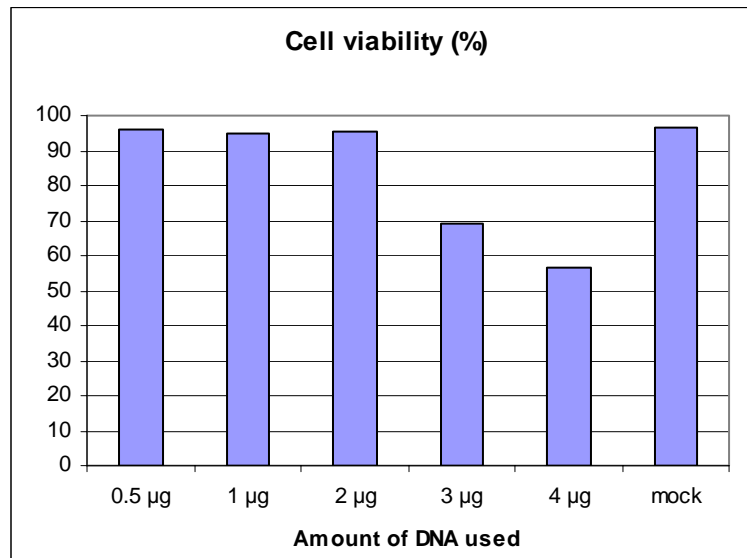


Fig. 2

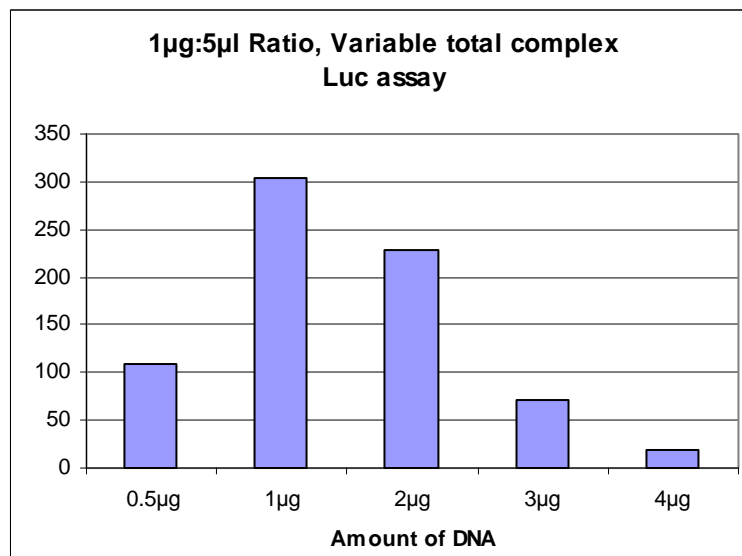


Fig.3

Conclusion / summary

Metafectene Pro provides an easy and effective alternative to electroporation in a well characterised suspension cell line. Minimal optimisation was required and no toxicity was observed in the particular cell line used, even at twice the maximum recommended amount of reagent.