

## A NOVEL APPROACH TO STUDY PACKAGING OF RETROVIRAL RNA BY RNA TRANSFECTION

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We are interested in designing novel strategies to generate helper-free high titre recombinant retroviruses. We reasoned that direct introduction of viral RNA into packaging cell lines ( $\psi$ 2 or PA317) should yield infectious virions. Since the conventional techniques of calcium phosphate or DEAE dextran transfection of RNA onto cells was inefficient (at least 100-fold lower than DNA transfection), we developed an efficient RNA transfection system based on the DOTMA containing liposome - (lipofectin) transfection technique (Felgner et al. PNAS 84:7413-7417, 1987). As model systems we chose bacterial luciferase and chloroamphenicol transferase (CAT) genes which were inserted in a T7 RNA polymerase transcription plasmid. Polyadenylated luciferase and/or CAT-mRNA were generated, mixed with lipofectin and transfected onto NIH/3T3 cells. The luciferase enzymatic activity from transfection of 1  $\mu$ g of luciferase RNA in  $10^6$  NIH/3T3 cells was equivalent to 4  $\mu$ g of transfected DNA.

We have constructed a T7 RNA polymerase transcription plasmid capable of generating polyadenylated mRNA similar to the genomic RNA of N2-retroviral vector. We would present the results of transfecting the in vitro synthesized viral RNA into packaging cell lines  $\psi$ 2 and PA317. An efficient RNA-transfection system would be beneficial for the direct cellular analysis of many facets of RNA metabolism including stability, translational efficiency, splicing, the effects of covalent RNA modification and the identification and characterization of RNA binding proteins. Furthermore, direct RNA transfection into packaging cell lines would offer an opportunity to study mechanisms of viral assembly.

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