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# Transient Expression Using the T7 RNA Polymerase Recombinant Vaccinia Virus System

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## I. INTRODUCTION

Gene transfer by transient transfection of cultured cells is a widely used method to express proteins for a variety of applications, including antibody testing, determining the intracellular localisation of proteins, or studying their function. The T7 RNA polymerase recombinant vaccinia virus (vT7) has become an important tool for the transient expression of proteins in cell culture (Fuerst *et al.*, 1986). Compared to traditional transient expression systems, the vT7 system is versatile, efficient, rapid, and simple. It is based on a two-step process (see Fig. 1): (1) the infection of cells with vT7 and (2) a regular lipofection with a plasmid containing the gene of interest behind a T7 promoter. Such plasmids are routinely used for the transcription of RNA *in vitro* and for the subsequent translation of the encoded protein in a cell-free system (e.g., rabbit reticulocyte lysate). The vT7 rapidly produces T7 RNA polymerase in the cytosol, and the DNA to be expressed will be rapidly and efficiently transcribed in the cytosol of the infected/transfected cell. Cells can be analyzed as early as 3–4 hr after infection/transfection.

## II. MATERIALS AND INSTRUMENTATION

Glasgow minimal essential medium (GMEM, Cat. No. 2170-025) is from GIBCO-BRL. HEPES (Cat. No. H-3375), hydroxyurea (Cat. No. H-8627), and soybean trypsin inhibitor (Cat. No. T-9003) are from Sigma. DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate, Cat. No. 1 202 375) is from Boehringer Mannheim.

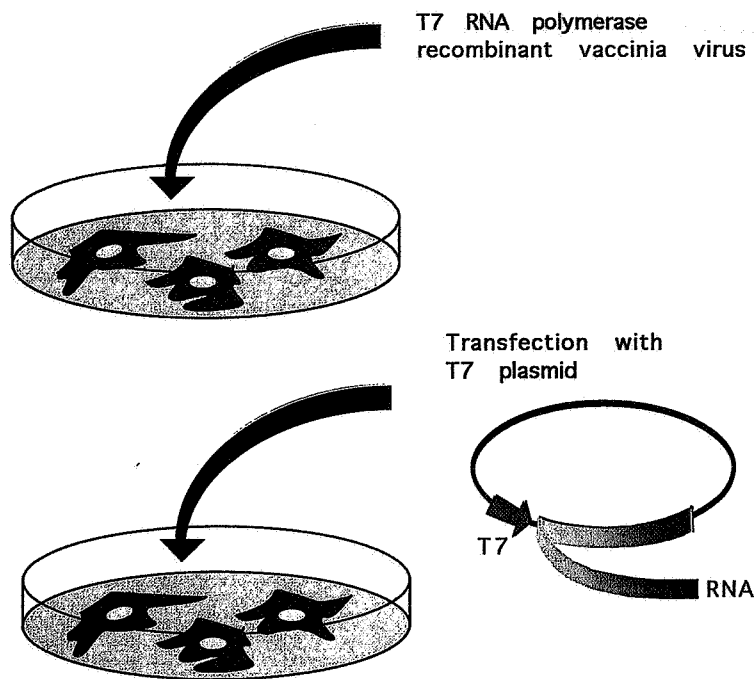
## III. PROCEDURES

### A. Plasmid Preparation

#### Steps

1. Clone the cDNA to be expressed in a plasmid vector, behind the T7 promoter. Vectors of the pGEM (Promega), pcDNA (Invitrogen) and pBluescript (Stratagene) series are useful for this purpose, as they contain T7 promoters adjacent to polylinker regions.

2. Purify the plasmid DNA. Although conventional minipreps of plasmids DNA can be used, we recommend using DNA prepared by anion-exchange chromatography, using disposable columns such as Qiagen.



**FIGURE 1** Vaccinia T7 RNA polymerase-based transient expression system.

## B. Infection/Transfection

The following protocol is adapted for the immunofluorescence analysis of the transfected cells.

### Solutions

1. *1 M hydroxyurea*: To make 10 ml, dissolve 760 mg hydroxyurea in 10 ml water. Sterilize by filtration and store at 4°C.
2. *Soybean trypsin inhibitor (20 mg/ml)*: To make 5 ml, dissolve 100 mg soybean trypsin inhibitor in 5 ml of distilled water. Sterile filter and store in aliquots at -80°C.
3. *Cationic liposomes (commercial or "home-made") at 1-2 mg/ml*.

### Steps

1. Plate cells on 11-mm glass coverslips in a 24-well plate (~50,000-100,000 cells, depending on the cell type). Incubate overnight at 37°C.
2. Wash the cells once with medium.
3. Add 200  $\mu$ l of medium containing the appropriate dilution (according to virus titration experiments) of vT7. Leave at room temperature for 30 min.
4. Mix ca. 1  $\mu$ g of the plasmid DNA into 110  $\mu$ l transfection medium in a polystyrene tube.
5. In a separate tube, mix 5  $\mu$ g liposomes with 110  $\mu$ l transfection medium.
6. Mix the diluted DNA with the diluted liposomes. Leave at room temperature for 10 min.
7. Remove the virus medium from the cells, add the DNA/liposome mixture, and incubate at 37°C for 3.5 to 8 hr.
8. Assay the expression, e.g., by immunofluorescence microscopy.

### NOTE

*Caution:* All manipulations, except for the centrifugations, should take place in a safety sterile bench (vertical flow) in a laboratory certified for work with vaccinia virus (safety level 2 or equivalent). Gloves and eye protection are required. Virus-contaminated items should be autoclaved or chemically disinfected.

## IV. COMMENTS

This protocol can easily be scaled up for preparative purposes. Hydroxyurea inhibits replication of viral DNA. This efficiently prevents the generation of new viral particles and inhibits the formation of viral factories, which often give rise to background staining in immunofluorescence microscopy.

## V. PITFALLS

1. Vaccinia virus may cause severe infections if it comes in contact with the eyes or with open wounds. Wear eye protection and gloves. Vaccination against smallpox is recommended. The virus is stable and resists drying. Any spills therefore have to be chemically disinfected.

2. Cells growing in colonies or in intact monolayers are inefficiently transfected. Plate the cells as a single-cell suspension, at a maximal density of 80% confluency.

3. For optimal results, always use cells of low passage number that have not been allowed to grow overconfluent, even in previous passages.

4. The presence of serum inhibits the infection step. It is therefore essential to wash the cells with serum-free medium prior to infection and to infect the cells in the absence of serum.

5. Linearized plasmids or plasmids that have been nicked (e.g., by repeated freezing/thawing) result in low transfection efficiencies. Use supercoiled plasmid DNA for lipofection.

6. Mixing liposomes and DNA before they have been diluted results in precipitation, yielding poor transfection efficiency.

7. Polypropylene tends to adsorb lipid/DNA complexes, and polystyrene tubes should be used to ensure optimal transfection efficiency.

8. In some cases, precipitated material can be observed when the transfected cells are viewed in a microscope. This usually lowers the transfection efficiency and may cause toxic effects to the cells. Precipitates may come from impurities from the DNA preparations, especially when "miniprep" DNA is used. They also occur when an inappropriate ratio between DNA and liposomes is used or when the pH of the transfection medium is too high. For the best results, different amounts of DNA (1–5  $\mu\text{g}$ ) and liposomes (2–10  $\mu\text{l}$ ) should be tested in combination by transient transfection and immunofluorescence microscopy. The pH of the incubation medium should always be kept below 7.2.

9. The optimal time of the transfection step will vary, depending on the purpose. The expression level of the protein and the cytotoxic effect due to the vaccinia infection will increase with time, so the transfection time should be kept as short as possible.

10. Vaccinia infection of cells has been reported to lead to the disassembly of stress fibers (Cudmore *et al.*, 1995). These effects are prevented by treatment with hydroxyurea. Nevertheless, special caution should be taken in the interpretation whenever the function of actin-interacting proteins is studied.

## Acknowledgment

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## References

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