Highly Efficient Delivery of Viral RNA Genomes Using a Lipid-Based Transfection Reagent Compared to Electroporation

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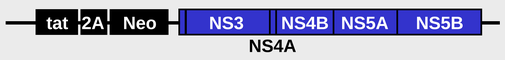
Abstract

The production of infectious virus by the transfection of viral RNA genomes is an integral tool in dissecting the life cycles and pathogenesis of RNA viruses. Historically, electroporation has been the method of choice for the transfection of viral RNA genomes and mini-replicons, but it suffers from many limitations including high cell death, serum-free transfection conditions, and the requirements for large numbers of cells and large amounts of transfected RNA. To optimize and facilitate the introduction of viral RNAs into mammalian cells, we compared lipid-based transfection reagents and electroporation for their ability to deliver hepatitis C virus (HCV) and yellow fever virus replicons to HuH7 cells. We have identified a lipid-based transfection reagent that enables highly efficient delivery of the viral replicons to HuH7 cells in the presence of serum. Compared to electroporation, the lipid-based reagent requires significantly less RNA to obtain the same level of expression from an HCV replicon. In addition, because there is low cellular toxicity associated with the lipid-based reagent, many fewer HuH7 cells are required for each transfection compared to electroporation.

General Methods

Viral RNA replicons were produced by *in vitro* transcription of linearized plasmid templates using the AmpliScribe™ T7 High Yield Transcription Kit (Epicentre). The following replicons were used for these studies:

HCV Ntat2ANeo Replicon [1]



Yellow Fever YFRP-IRES-Luc Replicon [2]

Electroporations and transfections performed using the lipid-based *Trans*IT®-mRNA Transfection Kit (Mirus Bio), were performed as outline in Figure 1. Transfections using other reagents were performed as recommended by the manufacturer, and the YFRP-IRES-Luc replicon was assayed post-transfection using standard firefly luciferase assays.

[1] Yi, M., Bodola, F., and S. Lemon. (2002) *Virology* **304**:197
[2] Jones, C.T., Patkar, C.G., and R.J. Kuhn. (2005) *Virology* **331**:247

Results and Discussion

Figure 1. Electroporation and Lipid-based *Trans*IT-mRNA Transfection Kit Protocol Comparison

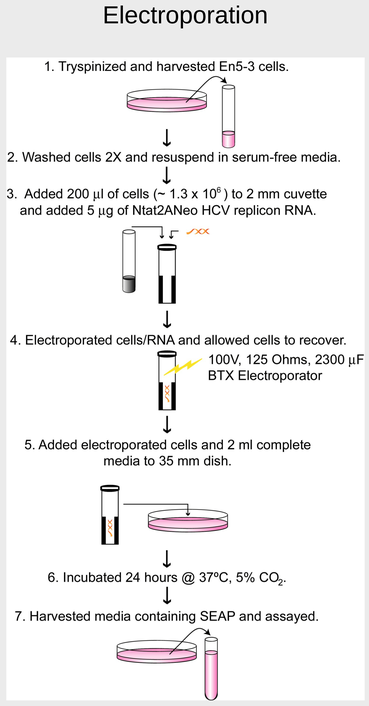
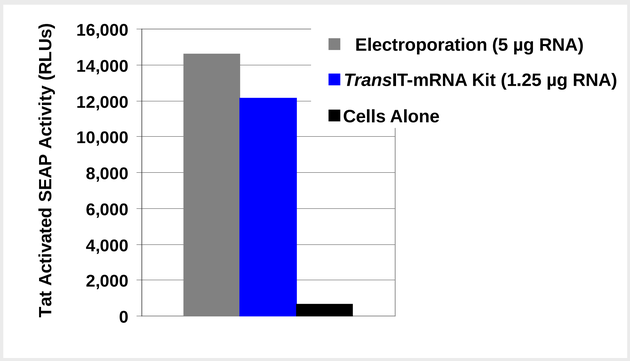
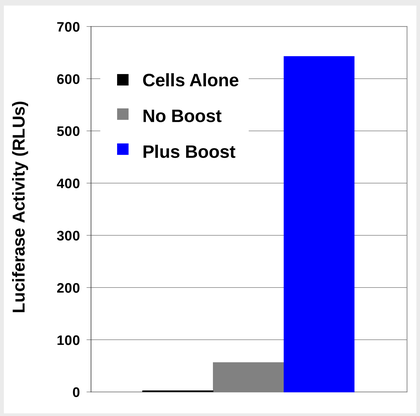


Figure 2. Comparable Transfection Efficiency with the *Trans*IT-mRNA Kit Using 75% Less RNA



En5-3 cells containing a Tat-activated reporter gene (1), were transfected with the Ntat2ANeo HCV replicon RNA as outlined in Figure 1. Expression of the Tat protein from the replicon after transfection, activates expression of the SEAP reporter in the En5-3 cells. In this experiment, SEAP activity serves as an indicator of successful transfection and the relative efficiency of the two transfection methods. As shown, the level of SEAP expression between the two methods differed by approximately 18%. Significantly, 4X less Ntat2ANeo RNA transcripts were transfected using the *Trans*IT-mRNA Kit compared to electroporation.

Figure 3. The Boost Reagent in the *Trans*IT-mRNA Kit is Required for Optimal Transfection Efficiency

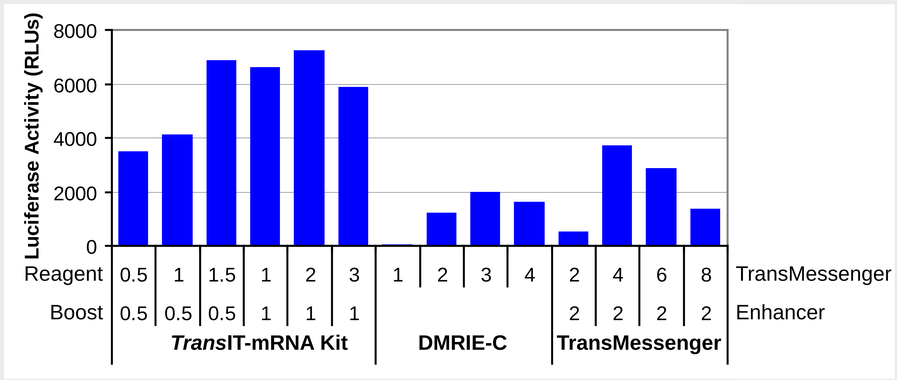


The YFRP-IRES-Luc replicon RNA was transfected into HuH7 cells using the *Trans*IT-mRNA Transfection Kit and the following conditions per well of a 24-well plate:

- 0.5 µg YF-luc replicon RNA
- 0.5 µl TransIT-mRNA Reagent
- +/- 0.25 µl RNA Boost Reagent

Cells were harvested 24 hours post-transfection and assayed for luciferase activity.

Figure 4. Performance Comparison of Different Lipid-based RNA Transfection Reagents



Because the *Trans*IT-mRNA Transfection Kit performed comparably to electroporation (Figure 2), we wanted to determine if other lipid-based transfection reagents were as effective at delivering viral RNA replicons. We transfected HuH7 cells in 12 well plates with 1 µg per well of the YFRP-IRES-Luc replicon RNA, and 24 hours later, we harvested the transfected cells and assayed for luciferase activity. We tested the *Trans*IT-mRNA Kit side-by-side with DMRIE-C (Invitrogen) and TransMessenger™ (Qiagen) following the recommended protocols. We tested a range of reagent levels, and when the reagents had two components, we tested different levels of each component as suggested by the manufacturers. As shown, the *Trans*IT-mRNA Transfection Kit outperformed both DMRIE-C and TransMessenger Reagents. In addition, the DMRIE-C and TransMessenger reagents require that the transfections be performed in the absence of serum with a media change 4-6 hours post-transfection.

Conclusions

Electroporation and the *Trans*IT-mRNA Transfection Kit deliver viral RNA replicons with similar efficiency, but the *Trans*IT-mRNA Kit offers several advantages over electroporation including:

1. **4X less viral RNA is required when using the *Trans*IT-mRNA Kit compared to electroporation.**
2. **Because the *Trans*IT-mRNA Kit transfects adherent cells and is not toxic, large numbers of cells are not required for transfection.**
3. **The *Trans*IT-mRNA Kit protocol has fewer steps than electroporation including no trypsinization, no washing, and no transfer steps.**

Compared to other commercially available RNA transfection reagents, the *Trans*IT-mRNA kit outperforms those reagents by approximately 2-3X. It has the added advantage that transfections can be performed in the presence of serum with no media change necessary.