The Cell Culture Dish

Ask the Expert
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Ask the Expert Session - Got mRNA? Solve DNA transfection issues with mRNA transfection

Typically, DNA enters the nucleus when cells divide because cell division creates small nuclear pores. In non-dividing primary cells DNA doesn't enter the nucleus making these cells very hard-to-transfect. If DNA entry is a bottleneck, why not deliver mRNA directly?

This Ask the Expert session was sponsored by Life Technologies and hosted by Nektaria Andronikou. Nektaria joined Life Technologies in 2010 and is currently working with the transfection team on the development of new delivery methods targeting relevant cellular models that will enable the use of exciting new technologies. She received a Bachelor of Science in Biochemistry with a minor in Cellular and Molecular Biology from UCSD. She began her professional career at ISIS Pharmaceuticals, as a research associate for the Cardiovascular Drug Discovery program, screening numerous pre-clinical targets that led to the discovery of the now FDA approved antisense drug, Kynamro.

Question:

We currently use DNA for transfection, can you tell me what the advantages are of mRNA transfection and it is cell line specific?

The Answer:

There are many advantages to using mRNA vs DNA for transfection and they are cell line specific.

A much higher level of transfection efficiency

If you are working with a difficult to transfect cell type, where DNA transfection yields less than 30% efficiency, transfecting an mRNA alternative can provide up to 80% transfection efficiency. Part of the hurdles that are encountered with DNA delivery has to do with the multiple steps required during transfection. In order to have a protein expressed in a cell, typically DNA is transfected, enters the nucleus, transcribed to mRNA, exported from the nucleus and translated into a protein in the cytoplasm. When delivering mRNA directly, the mRNA is present in the cytoplasm and ready for immediate translation to protein. However, if you are currently satisfied with the level of transfection achieved in the cell line that you are working with, there is no need to switch to mRNA.

It is a foot-print free method with no risk of genomic integration

mRNA transfection is transient and does not enter the nucleus or pose a risk of integrating with the cellular host DNA and is currently being researched for possible vaccine replacement and disease model development.

Additionally, transfection of mRNA with the newly developed Lipofectamine® MessengerMAX™ reagent, provides higher efficiency in a wider range of cell types

(e.g. primary neurons, primary hepatocytes, primary keratinocytes, primary fibroblasts, iPS cells, hNSC, mESC, Raw 264.7, SH-SY-5Y, HT-29). This is a result of its ability to delivery the highest amount of mRNA independent of the cell model being used.

Question:

I have been getting some toxicity in my mRNA transfections. What would you recommend for minimizing toxicity?

The Answer:

We also observed some toxicity in our initial experiments with mRNA. However, incorporating the proper 5' UTR and 3' UTR sequences into the template used for in vitro transcription quickly resolved it. Another key factor was the purity of the mRNA. Ensure that the 260/280 OD ratio is between 1.8 and 2.1. The quality of the mRNA can also be determined by running a small sample on a gel to check for the proper size. In some scenarios, it might be best to also incorporate chemically modified nucleotides to the transcription reaction. Another reason for toxicity may be a result of the cells themselves; if the cells are at too low a density then there will be significant toxicity (ideal viable cell density on the day of transfection is between 70-90% confluence).

Question:

We have seen quite a bit of variation in our transfection efficiency based on the number of passages prior to transfection. Do you have advice on the best passage number?

The Answer:
We have seen this as well throughout our labs. Since we identified this issue, we have implemented a standard best practice of only utilizing cells for transfection between 5-20 passages because at a low passage there is very low transfection efficiency and at a higher passage the optimal dose of transfection shifts. We have also implemented the standard use of a positive control within each transfection experiment to quantitatively determine and track efficiency from week-to-week.

Question:

Are there specific media requirements when using mRNA for transfection? Also, would I have to change the media after transfection?

The Answer:
No, there are no specific media requirements or restrictions when using mRNA for transfection. The only recommendation is to ensure that there is no serum or antibiotics present during the complexation process of the lipid and mRNA within the transfection protocol. We use OptiMEM® I Reduced Serum Medium in our transfection protocol.

The delivery of mRNA does not require a media change after transfection. Our common practice is to not change media within the first 24-hours following transfection so as to minimize handling of cells.

Question:

Is transfection with mRNA faster? What are standard incubation times?

The Answer:
Yes, transfection with mRNA results in faster and more immediate translation of protein and therefore, faster expression. We visually see expression of GFP in some cell lines as fast as 90-minutes after transfection. Additionally, transfection of mRNA with Lipofectamine® MessengerMAX™ also provides prolonged duration of expression (GFP expression lasting for 5-days post-transfection), due to its ability to protect mRNA from degradation during transfection.

Question:

I am trying to troubleshoot our process. Could you tell me some reasons why we may not be seeing expression after transfection? We are using primary cells, so I am not sure if it is due to the difficulty in transfecting these cells or another cause.

The Answer:
There are reasons that can influence expression after transfection, but before troubleshooting all the possibilities, a transfection experiment with a positive control reporter mRNA and the new Lipofectamine® MessegnerMAX™ mRNA transfection reagent could be the solution. If this does not yield good results it might be best to try an alternative delivery solution or different cells. However, if this gives acceptable results, the next step would be to try the mRNA of interest with the MessengerMAX™ reagent. If there are expression level concerns at this point, it might be the mRNA that is being used and troubleshooting from this perspective might be needed. For example, some questions to ask would be: Is there a 5' cap? Is there a poly(a) tail? Is the mRNA pure? Do I get a single band on a gel? Was the DNA template clean? There is information available to help answer many of these questions and more – click here.

Question:

What is the best transfection reagent to use to transfect mRNA to cells/tissue in a low pH environment? I have tried most of the mRNA transfection reagents on the market & they do not work.

The Answer:
The best reagent to transfect mRNA to cells is Lipofectamine® MessengerMAX™ because it has shown to further protect the mRNA molecules from degradation and has been shown to deliver the highest amount of mRNA into a wide variety of cell types. For tissues or small animal models it would be Invivofectamine® 2.0, which is a nanoparticle technology that can encapsulate and protect the payload for delivery. It can be used on cells in vitro as well as injected systemically via intravenous route or via direct injection (i.e. muscle, tumors, heart, brain). More information can be found at www.lifetechnologies.com/messengermax and www.lifetechnologies.com/invivofectamine

In addition, mRNA can also be synthesized with chemically modified nucleotides to improve stability and further minimize degradation. A positive control mRNA (e.g. GFP mRNA) is also a very helpful method to optimize transfection techniques.

Question:

When transfecting with mRNA, does the cell package the foreign RNA into vesicles reducing the efficiency of the mRNA, or are they left in the cytoplasm? If packaged away, what percent is packaged vs. what percent is left to be available for transcription? Is this observed with DNA plasmids?

The Answer:
We have not observed differences between how a cell packages an mRNA payload versus a DNA payload for the purpose of delivery. Transfection involves complexation formation between a liposome and mRNA, which create lipoplexes that are taken up by the cell via endocytosis. The liposome protects the mRNA during this process and also assists in endosomal escape, which releases the mRNA into the cytoplasm of the cell. The mRNA is immediately available for translation with the ribosome. The mRNA itself should be prepared using an in vitro transcription kit, such as mMessage mMachine® T7 Ultra Kit, which incorporate a 5' ARCA cap and a 3' poly(a) tail so that the mRNA mimics endogenous mRNA.