Troubleshooting Forum

Molecular Biology Techniques Q&A

Retroviral Transfection

This month's question from the Molecular Biology Forums (online at molecularbiology.forums.biotechniques.com) comes from the "General Methods" section. Entries have been edited for concision and clarity. Mentions of specific products and manufacturers have been retained from the original posts, but do not represent endorsements by, or the opinions of, *BioTechniques*.

How can I troubleshoot a failed retroviral vector infection? (Thread 3862)

Q I subcloned GFP into the retroviral vector pLXSN so that I could monitor the efficiency of transfection of EcoPack 2-293 packaging cells. When I infected the NIH-3T3 and CHO target cells, I didn't see any GFP fluorescence, nor did the cells become resistant to neomycin. What can I do to resolve the failed infection?

A I recommend that you begin troubleshooting by checking virus titers; your viral titer might be too low for efficient infection. If you find that the titer is high enough, then you must ensure that it is accurate.

How did you titer the viral stock produced by the packaging cell lines? How are you handling and storing the viral stocks? Repeated thawing or poor thawing practices may kill the virus. It is also possible that a step during subcloning affected the neomycin resistance gene somehow. Did you sequence the vector before you started the viral production?

A A quick and easy way to check that the GFP is expressed properly from your plasmid is to compare fluorescence of bacteria with the vector vs. bacteria with vector-GFP. Then you will be able to determine what step of the procedure to begin troubleshooting.

Q The transfection was good as monitored through the expression of GFP, but when I tried to titer, I didn't see a signal. To titer the viral stock, I prepared six 10-fold serial dilutions and infected NIH 3T3 cells, which I had plated the day before in 6-well plates at a density of 0.5×10^5 cells per well. I sequenced the vector in the subcloning region and found it to be as expected.

Q2 I am trying to transfect hamster fibroblasts with a MoMuLV-derived vector from Clontech. I used an amphotropic packaging cell line since the vector is mouse specific. When it didn't work, I called technical support and Clontech said the vector may not work with hamster cell lines.

Can anyone working with similar approaches suggest alternative vectors to use in these cell lines? Would pLXSN work for this situation?

A pLXSN is a MoMuLV-derived vector from Clontech. I usually use it to transfect ecotropic cells to obtain viral particles to infect both mouse and rat cells. What vector are you using? Can you change the packaging cells?

Q2 My vector is pRetro-Lib, which is also a MoMuLV-derived vector from Clontech. I am using amphotropic packaging cells since my target cells are from hamster. I previously tried ecotropic cells, which also failed. Clontech was skeptical about the efficiency of this vector with hamster cells, but the previous post mentioned using a similar vector with CHO cells.

A I think that the vector is not causing your problem. The type of viral particles you obtain depends upon the packaging cells. You can infect CHO with virus from either ecotropic cells or amphotropic packaging cells using pRetro-Lib or pLXSN. Did the cells properly package the virus?

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A To address this problem, you should first check your plasmid by sequencing for potential open reading frame mutations. You should also verify whether the packaging cells show green fluorescence or not.

The transfection rate of your packaging line should be high enough. Your packaging cells should be confluent when you collect the supernatant. The packaging cells I have had the most success with are Phoenix (amphotropic or ecotropic env proteins). You might try using those cells instead.

My advice is to cotransfect your cells with VSV-G (glycoprotein of the vesicular stomatitis virus). This increases the viral particle half life so that you can even subject your supernatant to ultracentrifugation at 15,000×g for 45 min. With this modification, you will be able to infect more cell types because the protein is ubiquitous. I have even successfully infected frog cells.

A There is a lot of good information on the Nolan lab home page hosted by Stanford University. You may find your answers there.

A From your comments, my best guess is that something is wrong with your transfection method. I suggest transfecting COS7 cells as a positive control; these cells are easy to transfect and culture. If the COS7 cells express GFP, you will know that the transfection method and GFP-carrying construct are okay. If not, it will be easier to troubleshoot in this cell line until you get it working.

Q3 I have a similar problem. I transfected the Phoenix cell line amphotrophic cells with my plasmid. The transfection efficiency was 80-90%, monitored by GFP expression. However, when I collected the supernatant and infected NIH3T3 cells with it, I didn't see any fluorescent cells at all.

I obtained both the NIH3T3 and Phoenix cells directly from ATCC and only passaged the NIH3T3 cells once and the Phoenix cells twice, so I don't believe I could have lost env expression in the Phoenix cells.

I did viral production at 32°C and collected the supernatant 48 hours post-transfection, which should have allowed sufficient time for a high titer. The cells were confluent when I collected the supernatant.

I did not freeze the viral supernatant; instead I collected it, filtered it with a PES 0.45 µm membrane, and put it directly onto the target cells with 4µg/ml polybrene.

A I tried the EcoPack cell line for making infectious viruses once, but it returned really low titers. After that I tried the pVPack system where I cotransfected the gag-pol, and env cDNA under the CMV promoter together with my vector into 293 cells. Thereafter I collected the supernatant as usual. With this system I obtained much higher titers and also was able to choose tropism just by changing the env plasmid. You might try the pVPack system.

A We use the Phoenix ecotropic line regularly and I have not had many problems. On one occasion, my transfection worked beautifully, but no virus was produced. But I just reselected for the gag-pol genes using hygromycin and env gene using diphtheria toxin. After a couple of weeks, the cells worked fine again. Since you said your cells were fresh from the ATCC, I assume they must already be well selected.

We culture our Phoenix producer lines at 30°C for virus production, which is the only difference I can see between our protocol and yours. You might try spin-concentration of your virus to see if it is produced in low titer.

Selected by Kristie Nybo, Ph. D.

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