

Recovery plasmids from dead bacterial/plasmid -80 stocks

We store our all of our plasmids as bacterial host stocks at -80 stored in 7% DMSO. Such stocks are very long-lived giving robust bacterial growth even after 10-15 years. However, occasionally we have had a stock that performs poorly. Perhaps DMSO was forgotten, or the stock was thawed by accident, which resulted in greatly reduced viability.

Plasmids from such stocks can easily be recovered by minipreping the cells, or even the supernatant from the frozen culture.

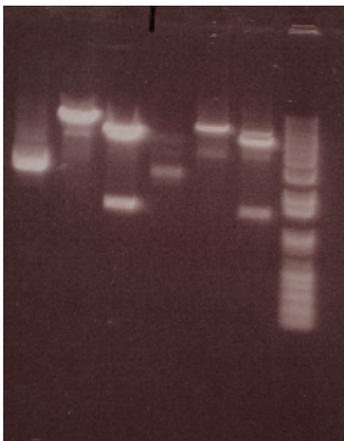
Protocol. (For a 1 ml frozen stock)

Thaw culture quickly at 37°C. Mix gently, remove 0.9 ml, and refreeze the last 0.1 ml.

Spin the culture. Usually we observe that a fraction of the bacteria have lysed resulting a flocculent pellet. Remove as much supernatant as possible using a P1000 blue tip that has been cut to have a wide bore.

Save the supernatant. Prep up the pellet using a standard Qiagen miniprep protocol. In our experience the quality of the DNA from such a prep is pretty comparable to a fresh miniprep.

Isolating DNA from the supernatant. To 750 µl of supernatant, add 150 µl of Qiagen P1 buffer with RNase. Mix 500 µl of 10% SDS and 100 µl of 5 M NaOH. The solution will not be clear (This is essentially a 10X rather than 2X Lysis buffer). Add 120 µl of this mix to your supernatant/Qiagen P1 mixture, mix by inverting 5 to 6 times. The solution should clarify. Split the sample into 2 tubes. Add 350 N3 neutralization buffer to each tube, mix gently by inverting and centrifuge at 16,000g for 10 minutes. We load the supernatant of both tube in series on a single Qiagen column, and finish the Qiagen plasmid isolation procedure.



Example:

HindIII digested minipreps from preps of the pellet and supernatant of 3 8 year old dead stocks. 2 µl out of 60 µl of elution from the Qiagen column was digested and 50% of the digest was loaded on the gel. The marker lane contains 300 ng total DNA. The left 3 are the preps of the pellet. The right 3 are the preps of the supernatants.

Updated 2/15/08 with an example.