

JETPREP Extended Trouble Shooting Guide

Low Yield of Plasmid DNA

RNA Contamination

Contamination with Bacterial Chromosomal DNA

Additional Plasmid Forms Running in Front of the Supercoiled DNA on an Agarose Gel

Subsequent Enzymatic Reactions with the JETPREP-purified DNA are Inhibited

DNA Degradation/Background Smear

Inquiries from customers

Problem: Low yield of plasmid DNA	
<u>Question / Problem</u>	<u>Hints and suggestions</u>
What was the total plasmid DNA yield from your prep?	<p>Depending on the individually used host-plasmid system one can expect 12-15 µg of plasmid DNA from 3 ml culture of an <i>E. coli</i> strain bearing a high-copy plasmid. The yield can be increased to about 20-25 µg by using 6 ml of bacterial culture without major losses in DNA quality (e.g. less susceptibility against digestion with restriction enzymes). A further increase of the volume of the bacterial culture (e.g. up to 10 ml) will give only a marginally higher DNA yield but the quality of the DNA decreases significantly.</p> <p>Therefore, only high-copy plasmids should be prepared with the JETPREP kit in order to have acceptable yields. If the customer has to prepare a low-copy plasmid (present with $\leq 1\mu\text{g}$ plasmid DNA per ml of bacterial culture), we recommend to work up 15-20 ml of bacterial culture, which are processed with doubled volumes of the respective buffers of the JETPREP system.</p>
Did you shake buffer D4 (containing the JETPREP resin) very well before taking an aliquot?	<p>The JETPREP resin has a 'glassy' appearance in the guanidine hydrochloride-containing buffer D4, making it not very good visible.</p> <p>The D4 solution (even if well-shaken) appears more or less clear.</p> <p>But as the resin is easy to resuspend even after a longer period of standing, a good shake for 4-5 seconds is usually sufficient to obtain a homogenous suspension.</p>
Were the mixing steps during the bacterial lysis procedure carried out as described?	<p>Obviously one has to be careful with the mixing steps during the preparation of the cleared lysate (steps 3 and 4) in order to not release bound chromosomal bacterial DNA from the precipitated debris. Therefore do not vortex the samples during steps 3 and 4. Nevertheless the mixing has to be carried out thoroughly. So invert the tubes multiple times until you obtain a homogeneous mixture.</p>

<p>Has buffer D5 been properly reconstituted and has the bottle been kept tightly closed when not in use?</p>	<p>After reconstitution of buffer D5 with ethanol it is important to keep this bottle tightly closed when not in use. If the bottle is not kept tightly closed, the ethanol tends to evaporate from the buffer. Continuously evaporating ethanol will lead to a too low alcohol concentration in the washing buffer D5, resulting in the loss of the DNA sample during the washing steps. An ethanol concentration of 70% (v/v) is necessary for optimal performance.</p>
<p>Was the elution step carried out properly?</p>	<p>The drying of the JETPREP resin after the final washing step is quite critical. On one hand the pelleted resin must be free of ethanol, on the other hand the resin must not be overdried. Be careful with an oil pump and a speed vac. For a standard purification assay 1-2 minutes in an oil pump-generated vacuum should be sufficient. We prefer to use a water jet filter pump or a vacuum chamber to dry the JETPREP pellet. With this handling we got good recoveries even after drying the sample for 8-10 min. If you have overdried the pellet, try to elute the DNA for an extended period of time at elevated temperatures (10-15 min at 65-70°C).</p> <p>A good alternative for the use of vacuum is a 10 min-incubation of the JETPREP pellet at 50-60°C in a thermo bloc or water bath with the cap open after removing the buffer D5 supernatant of the final washing step.</p>

<p>Problem: RNA contamination</p>	
<p><u>Question / Problem</u></p>	<p><u>Hints and suggestions</u></p>
<p>How old is your JETPREP kit?</p>	<p>Bacterial RNA is removed mostly by the action of RNase. We have found enough RNase activity to get rid of all bacterial RNA even after storage of buffer D1 for 6 months at room temperature. After this period of time (or maybe earlier if the ambient temperature exceeds 20°C most of the time) add fresh RNase to a final concentration of 100 µg/ml.</p>
<p>Were the supernatants after the binding and washing steps removed quantitatively, e.g. with a pipet?</p>	<p>Washing is carried out by a series of diluting-out steps. Simply pouring out the supernatants may lead to an inefficient removal of residual RNA and other impurities.</p>

Problem: Contamination with bacterial chromosomal DNAQuestion / ProblemHints and suggestions

Has the bacterial lysate been vortexed during steps 3 or 4?

Chromosomal bacterial DNA is bound to the inner membrane of the *E. coli* cells. It forms a network during neutralization and is subsequently precipitated together with the bacterial debris. This is only successful if shearing of the chromosomal DNA after cell lysis is kept to a minimum. Shearing of the chromosomal DNA occurs if the sample is vortexed after the addition of buffers D2 and/or D3. For this reason high amounts of bacterial cells (LB cultures \geq 15 ml or growth in very rich media) may cause trouble. Upon lysis of the cells a highly viscous lysate which requires vigorous mixing will be obtained. This may lead to shearing of the chromosomal DNA. Therefore **double** the volumes of all JETPREP buffers in this case (see above).

Problem: Additional plasmid forms running in front of the supercoiled DNA on an agarose gelQuestion / ProblemHints and suggestions

Did the incubation time in step 3 (cell lysis) exceed 5 min?

An additional plasmid form, running on agarose gels in front of the supercoiled form is due to irreversibly denaturated plasmid DNA. This irreversible denaturation occurs if the cell lysis (step 3) was carried out for longer than the recommended 5 min under the strongly alkaline conditions. It seems that this effect is intensified by the subsequent interaction of the DNA with the chaotropic salt. This so-called form IV-DNA can not be renaturated. Try a shorter incubation next time. We have found 1-2 minutes incubation with buffer D2 to be sufficient for the lysis of the bacterial cells from up to 3 ml culture.

Problem: Subsequent enzymatic reactions with the JETPREP-purified DNA are inhibitedQuestion / ProblemHints and suggestions

Did you carry over particles of the precipitate after centrifugation of the cleared lysate (step 4)?

Any carryover of particles should be avoided. Normally this should not be a problem, because due to the formulation of the neutralization buffer D3 the debris pellet is very compact and the supernatant is particle-free and clear. The centrifugation step is performed best in an **unchilled** micro-centrifuge. If it is carried out at 4°C, some SDS, which precipitates at low temperatures, is probably floating in the supernatant. This SDS does not affect binding of the plasmid DNA to the resin and is efficiently removed during the following washing steps with reconstituted buffer D5. Therefore these washing steps should be carried out very carefully (see comments on the next points).

<p>Did you resuspend the pelleted JETPREP resin between each washing step?</p>	<p>This is obviously vital! We had users, who didn' t do this and therefore failed with their downstream applications.</p> <p>The efficiency of the washing steps has an high impact on the quality of the subsequently eluted DNA particularly for the following reason: After addition of solution D4 to the bacterial lysate (step 5) the mix becomes turbid. This is partially caused by the precipitation of residual SDS in the high-salt environment. This SDS precipitates together with the JETPREP resin, resulting in an irregular shaped pellet after the first spin after the binding step. The SDS portion of this pellet is easily soluble in the ethanol-containing buffer D5. Therefore, after pelleting the JETPREP resin from the binding assay one should resuspend the pellet in both following washing steps very thoroughly.</p>
<p>Did you remove the supernatants after the binding and each washing step quantitatively, e.g. with a pipet?</p>	<p>Washing is carried out by a series of diluting-out steps. Simply pouring out the supernatants will lead to an inefficient removal of residual RNA, soluble cell components and guanidine hydrochloride, because there will be a significant remainder of the supernatant in the reaction tube. In such a case the washing efficiency is poor and downstream applications can be inhibited.</p>
<p>Did you remove the ethanol completely from the JETPREP resin before the elution step?</p>	<p>If you have too much ethanol in your sample (the JETPREP resin still appears ' greyish' , or the eluate smells of ethanol, or the gel samples come out of the slots when being applied onto the agarose gel), try to evaporate residual ethanol by incubating the tube in a thermo bloc or water bath at 50-60°C for 10 to 15 min with the cap of the reaction tube open.</p>
<p>Do you have too many JETPREP beads in your eluate (e.g. is your eluate visibly turbid after recovering the supernatant from the elution step)?</p>	<p>The JETPREP resin forms a very compact pellet, so that this problem should be a minor one. However, if processing many samples in parallel, the pellet of a later sample may already have become instable when pipetting off the supernatant. If the eluate is turbid, simply recentrifuge it for 30 seconds and take off the supernatant. This should rectify almost all problems associated with this point.</p>

<p>Problem: DNA degradation / Background smear</p>	
<p><u>Question / Problem</u></p>	<p><u>Hints and suggestions</u></p>
<p>What is the size of the plasmid you have purified?</p>	<p>We have tested plasmids with sizes up to 20 kb in the JETPREP procedure and found excellent recoveries with no degradation. Even cosmids can be purified with JETPREP – the binding/releasing properties of the resin are excellent. However, due to their size cosmids are susceptible against degradation, mostly because of shearing forces generated during the procedure. So, for the preparation of cosmids all steps, especially the resuspending steps, must be carried out very carefully.</p> <p>Don't vortex, but resuspend the JETPREP resin by gently pipetting up and down with a wide-bored 1 ml-pipet tip.</p>

Which medium was used for the growth of the bacteria and how long was the growth time of the culture?

LB is the recommended medium for the growth of *E. coli*. In very rich media (i.e. 2xYT, TB, Super Broth) the *E. coli* cells grow to extreme high cell densities in shorter times than in LB. This means that the saturation phase is already reached after 8-10 h. After this time, more and more cells die. If such dead cells get lysed, their chromosomal DNA goes into the surrounding medium and may contaminate the subsequent preparation, becoming visible as a background smear in the respective lane on an agarose gel.

Therefore use LB medium for bacterial growth and use incubation times being not longer than 16 h.

Inquiries from customers

Question

Answer

After adding solution D4 to my cleared lysate, I obtained a turbid suspension. When spinning down the resin, I get an irregular shaped pellet spread over the wall of my tube. Some particles resuspend very easy and are floating in the supernatant. What is wrong?

Nothing! After adding solution D4, your mix is adjusted to high-salt conditions. These conditions precipitate residual SDS from the cleared lysate which causes the irregular shaped pellet and the small particles that are floating in your supernatant. Residual SDS and these particles are completely removed by the first wash with the ethanol-containing buffer D5. So, after this wash your pellet will look regular shaped and you will find no more particles in your supernatant.

When vortexing or flicking my tube with the plasmid-containing eluate, I am observing foam. Is there residual SDS in the eluate?

No. We are aware of this phenomenon and have made several checks for SDS.

First, we tried to precipitate residual SDS by cooling down the eluate, but it remained clear. Additionally, we tried to precipitate SDS by adding potassium-ions. Due to the very low solubility coefficient of potassium dodecyl sulfate (10^{-30} M) this test is very sensitive. We didn't detect SDS in any of these tests.

Second, we used a couple of enzymes to do various experiments with the JETPREP-purified plasmid DNA (21 different restriction enzymes, T4 DNA ligase, different Taq DNA polymerases, T7 DNA polymerase, T7 RNA polymerase and more). None of these enzymes was inhibited in its activity, even those which are known to be susceptible against contaminations in plasmid preps.