

# JETSPIN Extended Trouble Shooting

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<b>Problem: Low yield of plasmid DNA</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
What was the total yield of plasmid DNA from your prep?	More than the nominal yield of <b>up to 100 µg (Midi) / 500 µg (Maxi)</b> per preparation should not be expected. Depending on the conditions somewhat more DNA can be obtained, but that's it...
Was the DNA yield higher than 100 µg (Midi) / 500 µg (Maxi) for a JETSPIN column?	<p>From 25 ml (Midi) / 100 ml (Maxi) bacterial culture containing a typical high-copy plasmid (<math>\geq 4\text{-}5</math> µg plasmid DNA per ml of culture) one can expect approximately 100 µg (Midi) / 500 µg (Maxi) of DNA.</p> <p>The DNA yield can't be increased by raising the culture volume above the recommended values of 25 ml (Midi) / 100 ml (Maxi). <b>If these volumes are exceeded, a severe <u>decrease</u> in DNA yield may be observed.</b> In such a case the lysis procedure will remain incomplete because the volumes are insufficient to process such large volumes of bacteria. Furthermore, the spin column will be <b>overloaded</b> - it seems that a large excess of unwanted components in the cleared lysate (i.e. bacterial proteins, remnants of RNA etc.) are hindering the plasmid DNA to bind to the matrix.</p> <p>In cases, when the bacteria are growing to <u>very high cell densities</u> (i.e. <i>E. coli</i> strains XL1-Blue, TG1, HB101), we observe that the JETQUICK system was already saturated with <b>15 ml (Midi) / 66 ml (Maxi)</b> of bacterial culture and that even the nominal amounts may lead to a decreased DNA yield.</p> <p>In such a case please use the 'Modified Protocol' (see below).</p>
Has wash buffer F4 been reconstituted properly with ethanol and was the bottle stored tightly closed?	After reconstitution of buffer F4 with ethanol it is important to keep this bottle <b>tightly closed</b> when it is not in use. In several cases there was ethanol evaporating from the bottles, if they had not been closed very carefully. Continuously evaporating ethanol will lead to too low alcohol concentrations in buffer F4 resulting in the loss of the DNA sample during the washing step. <b>An ethanol concentration of 70% (v/v) is necessary for optimal performance.</b>

How much bacterial culture has been used?

If the recommended culture volumes are exceeded (more than 25 ml (Midi) / 100 ml (Maxi) per column), **a severe decrease in DNA yield may be observed**. In such a case the lysis procedure will remain incomplete because the volumes are insufficient to process such large volumes of bacteria. Furthermore, the spin column will be **overloaded** - it seems that a large excess of unwanted components in the cleared lysate (i.e. bacterial proteins, remnants of RNA etc.) are hindering the plasmid DNA to bind to the matrix.

In some cases, when the bacterial culture is growing to very high densities (i.e. *E. coli* strains XL1-Blue, TG1, HB101) we observe that the JETQUICK system is already saturated with **15 ml (Midi) / 66 ml (Maxi)** of bacterial culture and that even the nominal amounts may lead to a decreased DNA yield.

Bacteria with low copy plasmids or expression vectors may contain plasmid DNA at concentrations of less than 4 µg per ml culture. These vector/host combinations usually lead to lower DNA yields when the recommended culture volumes are used. If larger culture volumes of these strains are to be processed, use a **modified procedure** of the JETSPIN protocol.

**Modified Miniprep Protocol for JETSPIN:** Pellet up to **100 ml (Midi) / 500 ml (Maxi)** of bacterial culture and resuspend the bacterial cells in **8 ml (Midi) / 20 ml (Maxi)** of *buffer F1* (the suspension must be homogeneous; no cell clumps must be visible). Lyse the cells with **8 ml (Midi) / 20 ml (Maxi)** of *buffer F2* for 5 min at room temperature. Neutralize the lysate with **8 ml (Midi) / 20 ml (Maxi)** of *3 M potassium acetate (pH 5.5 adjusted with acetic acid)*. Mix thoroughly until a homogeneous phase is obtained and spin down bacterial debris for 10 min at maximum speed ( $\geq 13.000 \times g$ ) in a centrifuge. Transfer the supernatant into a new reaction tube and precipitate the nucleic acids with 0.7 vol of isopropanol. Spin down the nucleic acids for 15-30 min in a chilled centrifuge, discard the supernatant and let the pellet drain for a few minutes on an absorbent sheet of paper towel. Dissolve the nucleic acid pellet **completely** in **2.4 ml (Midi) / 12 ml (Maxi)** of sterile water or TE buffer and adjust binding conditions by adding **1.6 ml (Midi) / 8.0 ml (Maxi) of buffer G3**. Continue with step 5 of the JETSPIN protocol (Column loading step).

What kind of growth medium was used?

**Very rich media** (e.g. 2 x YT, terrific broth) should be avoided. These media lead to ferocious growth thus yielding very high amounts of bacterial cells; **what may follow is a severe decrease of the DNA yield even with lower volumes of bacterial culture**. In this case the lysis procedure will remain incomplete because the volumes of the buffers F1 and F2 are insufficient to process such large amounts of bacteria. Furthermore, the micro-spin column can be **overloaded** - it seems that a large excess of unwanted components in the cleared lysate (i.e. bacterial proteins, remnants of RNA etc) are hindering the plasmid DNA to bind to the matrix.

Has been checked whether the *E. coli* cells contain the expected amount of plasmid?

Is the plasmid DNA-content of the *E. coli* culture known (determined by other methods)?

If not, take **samples** from different steps of the procedure:

- *supernatant after centrifugation following lysis (step 4)*; sample should correspond to 1 ml of initial culture. Precipitate with 0.7 vol of isopropanol, dissolve in a suitable volume of TE buffer and load the whole sample onto an agarose gel (**SAMPLE 1**).

- *flow-through after binding (step 5)*; sample should correspond to 1 ml of initial culture. Precipitate with 0.7 vol of isopropanol, dissolve in a suitable volume of TE buffer and load the whole sample onto an agarose gel (**SAMPLE 2**).

- *column wash (step 7)*; dry the flowthrough of the wash-fraction in a speed vac. Dissolve the resulting pellet in a small amount of water and load the whole sample onto an agarose gel (**SAMPLE 3**),

- *eluate (step 8)*; take 5% of the eluate and load directly onto an agarose gel (**SAMPLE 4**).

Compare all samples on an 1% agarose gel. Only the lanes with the samples 1 and 4 should contain significant amounts of DNA.

The total amount of plasmid DNA in *E. coli* is very much dependent on the individual host-plasmid system. Depending on the category, plasmids can exist in low-/medium-/high-copy number per cell. Also the size and sequence of specific DNA inserts influence the copy number of a particular plasmid.

Was the DNA precipitated with alcohol after the elution from the JETSPIN column?

The DNA is precipitated best with 0.1 vol of 3 M sodium acetate (pH 5.2) and 2 vols of ethanol. Isopropanol requires longer centrifugation times. For a quantitative DNA precipitation the centrifugation should be performed for **at least 30 min at 4 °C** and with **at least 13,000 x g**. **We found that after treatment with chaotropic salt the DNA most likely doesn't precipitate as a compact pellet but gives a 'glassy', nearly invisible pellet which is spread over the wall of the centrifuge tube.** Carefully redissolve also the DNA that sticks to the tube' s wall.

Were buffers F2 and F3 mixed **completely** with the resuspended cells (steps 3 and 4)?

The resuspended *E. coli* cells must be mixed **thoroughly** with the buffers F2 and F3 by inverting the tubes several times until a **homogeneous phase** is obtained. Otherwise the cells will not be lysed efficiently and the plasmid DNA will remain partially bound to the bacterial debris.

In the case of bigger bacterial cell pellets more vigorous **shaking** to reach homogeneity is allowed. **But DON'T vortex:** Vortexing will release the bacterial chromosomal DNA and must therefore be avoided.

**Problem: RNA contamination**Question / ProblemHelp and comments

What kind of bacterial growth-medium has been used?

If the bacterial culture grows to much higher cell densities than  $1 \times 10^9$  cells per ml the spin columns may be overloaded. This can be the case if 2 x YT, TB (Terrific Broth) or similar very rich media are used. These media lead to ferocious growth thus yielding a very high amount of bacterial cells; **what may follow is a decrease of the DNA yield with a simultaneously increased amount of cellular RNA**. In such a case the lysis procedure of the cells and the digestion of the cellular RNA with RNase remain incomplete because the buffer volumes are insufficient to process such large amounts of cells.

Furthermore, the micro-spin column will be **overloaded** - it seems that a large excess of unwanted components in the cleared lysate (i.e. bacterial proteins, residual RNA etc) are hindering the plasmid DNA to bind to the matrix.

In cases, when the bacteria are growing to very high cell densities (i.e. *E. coli* strains XL1-Blue, TG1, HB101), we observe that the JETQUICK system was already saturated with **15 ml (Midi) / 66 ml (Maxi)** of bacterial culture and that even the nominal amounts may lead to a decreased DNA yield.

In such a case please use the ' Modified Protocol' (see above) or reduce the maximum amount of bacterial culture that is stated in the protocol by 2/3.

Was buffer F1 stored at 4 °C?  
How old is your JETSPIN kit?

Bacterial RNA is removed mostly by the action of RNase. The RNase-containing buffer F1 should be stored at 4°C. We have found enough RNase activity to get rid of all RNA even after storage of buffer F1 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.

Has a very "dirty" *E. coli* strain been used?

Some *E. coli* host strains are producing extreme amounts of RNA. In such a case add fresh RNase to buffer F1 to a final concentration of 400 µg/ml.

As an alternative supplement buffer F1 with 200 U/ml of RNase T1. The combined activities of RNase A and T1 will result in a better digestion efficiency of the bacterial RNA, thus leading to a better removal of the RNA during the procedure.

Was the amount of wash buffer F4 used too low?

Make sure that the recommended amount of the wash buffer is used.

**Problem: Low  $A_{260/280}$  ratio**

<u>Question / Problem</u>	<u>Help and comments</u>
Were buffers F2 and F3 mixed <b><i>completely</i></b> with the resuspended cells (steps 3 and 4)?	<p>Mixing must be done carefully (see above), but nevertheless <b>thoroughly</b> (e.g. by multiple inverting), until a <b>homogeneous phase</b> is obtained.</p> <p>If buffers F1, F2 and F3 are not mixed completely during the preparation of the cleared lysate, bacterial debris and SDS are not totally removed by the subsequent centrifugation. The excess of bacterial cell contents, e.g. proteins, may not be totally washed away during the spin column procedure, thus increasing the OD value at 280 nm.</p>
Centrifugation step after neutralization (step 4) performed with at least 13,000 x g?	<p>Upon centrifugation with lower centrifugal forces the bacterial debris and salt-SDS-particles may not be removed completely. The excess of bacterial cell contents, e.g. proteins, may not be totally washed away during the spin column procedure, thus increasing the OD value at 280 nm.</p>
Floating particles on the surface of the cleared lysate?	<p>Sometimes not all of the salt-SDS-particles are removed completely by centrifugation. Avoid the carryover of any particle onto the column because they contain also bacterial cell contents, e.g. proteins. Proteins increase the OD value at 280 nm.</p> <p>If necessary re-centrifuge the lysate for 5 min at top speed in a centrifuge or clear it through a folded filter.</p>

### **Problem: Long column run times**

<u>Question / Problem</u>	<u>Help and comments</u>
Same spin column used more than one time?	<p>Upon repeated usage the filter at the bottom of the spin column will clog.</p>
Buffers F2 and F3 mixed completely with the resuspended cells (steps 3 and 4)?	<p>Mixing must be done carefully (see above), but nevertheless <b>thoroughly</b> (e.g. by multiple inverting), until a <b>homogeneous phase</b> is obtained.</p> <p>If buffers F1, F2 and F3 are not mixed completely, bacterial debris and SDS are not totally removed by centrifugation. The column may then be blocked by remainders of cell debris and/or precipitated SDS-particles.</p>
Centrifugation step after neutralization (step 4) performed with at least 13,000 x g?	<p>Upon centrifugation with lower centrifugal forces the bacterial debris and potassium-SDS-particles may not be removed completely and block the Micro-spin unit.</p> <p>Increase centrifugal force to 15,000 x g. If too many floating particles will remain in the cleared lysate, re-centrifuge the lysate for 5 min at top speed in a microfuge.</p>

**Problem: Poor performance of the plasmid DNA in subsequent reactions (e.g. sequencing ...)**

Question / Problem

Help and comments

Were buffers F2 and F3 mixed **completely** with the resuspended cells (steps 3 and 4)?

Mixing must be done carefully (see above), but nevertheless **thoroughly** (e.g. by multiple inverting), until a **homogeneous phase** is obtained.

If buffers F1, F2 and F3 are not mixed completely, bacterial debris and SDS are not totally removed by the following centrifugation. The excess of bacterial debris contents and SDS may not be totally washed away, thus contaminating the eluate and inhibiting subsequent applications.

Has the centrifugation step after neutralization (step 4) been carried out with at least 13,000 x g?

Upon centrifugation with lower centrifugal forces the bacterial debris and potassium-SDS-particles are not removed completely. The excess of bacterial debris contents and SDS may not be totally washed away, thus contaminating the eluate and inhibiting subsequent applications.

Floating particles on surface of the cleared lysate?

Sometimes not all of the salt-SDS-particles are removed completely by centrifugation. Avoid to carry over any particles onto the column. If necessary, re-centrifuge the lysate for 5 min at top speed in a centrifuge or clear it through a folded filter.

What can I do, if my eluate still smells of ethanol and a subsequent application doesn't work because i.e. an enzyme is inhibited?

If centrifugation efficiency during the washing step is poor (i.e. due to too low g-force), too much ethanol will remain in the membrane and will reach the eluate. In such cases the eluate will smell quite strongly of ethanol and/or come out of the slots if a part is loaded onto an agarose gel. In such a case extend the centrifugation time to up to 10 min.

Alternatively, let the open spin cup stand for up to 15 min at room temperature, so that residual ethanol can evaporate. Elution efficiency is not affected by leaving the spin cup standing open for this period of time. One can also try to incubate for 5 min at elevated temperatures (i.e. 50°C in a thermo block), but here the influence on the elution efficiency remains to be checked.

**Problem: Contamination with bacterial chromosomal DNA**

Question / Problem

Help and comments

Has the lysate been vortexed during step 3 or 4?

Intact bacterial DNA forms a network during the neutralization step and is subsequently precipitated together with the SDS. This is only successful if shearing of the chromosomal DNA after cell lysis does not occur.

Shearing of the chromosomal DNA occurs if the sample is vortexed after the addition of either buffer F2 or F3. In this context, high amounts of bacterial cells (LB cultures  $\geq 25$  ml or growth in very rich media) may cause problems. Lysis will result in a highly viscous bacterial lysate which requires vigorous mixing and so may lead to a shearing of the genomic DNA.

**Problem: Additional forms of plasmid DNA running faster on agarose gels (form IV-DNA)**

Question / Problem

Help and comments

Has the incubation time of step 3 (cell lysis) exceeded 5 min?

An additional plasmid form which runs on agarose gels in front of the supercoiled plasmid DNA is due to irreversibly denaturated plasmid DNA. The irreversible denaturation is likely if the cell lysis (step 3) was carried out for longer than the recommended 5 min under the strongly alkaline conditions. This effect is probably enhanced by the chaotropic salts in the neutralization buffer F3.

This form IV-DNA can't be re-naturated. So, don't exceed the 5 min time interval for lysis.

**Problem: Degraded DNA**

Question / Problem

Help and comments

Is the *E. coli* strain known as problematic?

Certain bacterial strains contain high amounts of endo- and exonucleases. If usage of this strains can't be avoided, don't let the bacterial culture grow for more than 16 hrs. Harvest bacteria immediately and continue directly with the plasmid isolation.

Bacterial growth time longer than overnight?

Don't let bacteria grow for more than 16 hrs. Harvest bacteria immediately and continue directly with plasmid isolation.

**Problem: The elution volume is too high / the DNA concentration is too low**

- Can I reduce the elution volume?

If the DNA concentration in the eluate is too low, the elution volume can be reduced. Smaller elution volumes lead to higher DNA concentrations, but the total yield of DNA does decrease. For instance, reducing the elution volume from 800 µl to 400 µl (Midi) or 4.0 ml to 2.0 ml (Maxi) will result in a reduction from 100 to approximately 77 % in the overall DNA yield. This can partially be compensated by using warm (60-65°C) elution buffer and/or leaving the tube for 1-2 min before the final centrifugation after pipetting the elution buffer onto the silica membrane. A further reduction of the elution volume will not further increase the DNA concentration but will only decrease the absolute DNA yield.

### **- Can I concentrate the DNA by an alcohol precipitation?**

If DNA concentrations higher than 0,2 - 0,3 µg/ml are required, the DNA must be precipitated with alcohol and redissolved in a smaller volume of TE buffer or water. The DNA is precipitated best with 0.1 vol of 3 M sodium acetate (pH 5.2) and 2 volumes of ethanol. Isopropanol requires longer centrifugation times. For a quantitative DNA precipitation the centrifugation should be performed for **at least 30 min, at 4°C and with at least 13,000 x g** **We observed that after a treatment with chaotropic salt the DNA very often doesn't precipitate as a compact pellet, but comes down as a 'glassy', almost invisible pellet that is spread over the wall of the centrifuge tube.** Carefully redissolve also the DNA sticking to the tube's wall.

### **-Can I use the JETSPIN-purified DNA for transfection?**

We **don't recommend** JETSPIN for transfection-quality DNA. JETSPIN-purified DNA still contains a significant amount of bacterial endotoxins (lipopolysaccharides) (~85,000 EU/mg DNA) which can affect a subsequent transfection. For transfection-quality DNA please use the JETSTAR system - the JETSTAR-purified DNA contains only very low amounts of endotoxin (<100 EU/mg DNA), thus being the optimal solution for transfection.