

JETQUICK Extended Trouble Shooting

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A) 'JETQUICK Plasmid' Extended Troubleshooting

Problem: Low yield of plasmid DNA	
<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 up to 30 µg 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 20 µg for a JETQUICK column?	<p>From 3 ml <i>E. coli</i> culture containing a typical high-copy plasmid (≥ 7 µg plasmid DNA per ml of culture) one can expect approximately 20 µg of DNA. The DNA yield can't be increased to more than 30 µg by raising the culture volume above the recommended value of 3-5 ml. If these volumes are exceeded, a severe <u>decrease</u> in DNA yield may be observed. In this 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.</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 2 ml of bacterial culture and that even 3 ml may lead to a decreased DNA yield.</p> <p>In such a case, if more than 2 ml of bacterial culture have to be worked up, use the 'Modified Protocol' (see below).</p>
Has wash buffer G4 been reconstituted properly with ethanol and was the bottle stored tightly closed?	<p>After reconstitution of buffer G4 with ethanol it is important to keep this bottle tightly closed 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 G4 resulting in the loss of the DNA sample during the washing step. An ethanol concentration of 70% (v/v) is necessary for optimal performance.</p>

How much bacterial culture has been used?

If the recommended culture volumes are exceeded (more than 5 ml per column), a **severe decrease in DNA yield may be observed**. In this 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 **2 ml** of bacterial culture and that even 3 ml 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 JETQUICK protocol.

Modified Miniprep Protocol for JETQUICK: Pellet up to **20 ml** of bacterial culture in a 1.5 ml or 2 ml reaction tube and resuspend the bacterial cells in **1 ml** of *buffer G1* (the suspension must be homogeneous; no cell clumps must be visible). Lyse the cells with **1 ml** of *buffer G2* for 5 min at room temperature. Neutralize the lysate with **1 ml** 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 5 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 **500 µl** of sterile water or TE buffer and adjust binding conditions by adding **350 µl of buffer G3**. Continue with step 5 of the JETQUICK 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 G1 and G2 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.

<p>Has been checked whether the <i>E. coli</i> cells contain the expected amount of plasmid?</p>	<p>Is the plasmid DNA-content of the <i>E. coli</i> culture known (determined by other methods)?</p> <p>If not, take samples from different steps of the procedure:</p> <ul style="list-style-type: none"> - <i>supernatant after centrifugation following lysis (step 4)</i>; 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). - <i>flow-through after binding (step 5)</i>; 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). - <i>column wash (step 7)</i>; 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), - <i>eluate (step 8)</i>; take 5% of the eluate and load directly onto an agarose gel (SAMPLE 4). <p>Compare all samples on an 1% agarose gel. Only the lanes with the samples 1 and 4 should contain significant amounts of DNA.</p> <p>The total amount of plasmid DNA in <i>E. coli</i> 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.</p>
<p>Was the DNA precipitated with alcohol after the elution from the JETQUICK micro-spin column?</p>	<p>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.</p>
<p>Were buffers G2 and G3 mixed completely with the resuspended cells (steps 3 and 4)?</p>	<p>The resuspended <i>E. coli</i> cells must be mixed thoroughly with the buffers G2 and G3 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.</p> <p>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.</p>

Problem: RNA contamination	
<u>Question / Problem</u>	<u>Help and comments</u>
What kind of bacterial growth-medium has been used?	<p>If the bacterial culture grows to much higher cell densities than 1×10^9 cells per ml the micro-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.</p> <p>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.</p> <p>In some cases, when the bacterial culture is growing to <u>very high densities</u> (i.e. <i>E. coli</i> strains XL1-Blue, TG1, HB101) we observe that the JETQUICK system is already saturated with 2 ml of bacterial culture and that even 3 ml may lead to a decreased DNA yield.</p> <p>In such a case reduce the maximum amount of bacterial culture that is stated in the protocol by 2/3.</p>
Was buffer G1 stored at 4 °C? How old is your JETQUICK kit?	<p>Bacterial RNA is removed mostly by the action of RNase. The RNase-containing buffer G1 should be stored at 4°C. We have found enough RNase activity to get rid of all RNA even after storage of buffer G1 for 6 months <u>at room temperature</u>. 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>
Has a very "dirty" <i>E. coli</i> strain been used?	<p>Some <i>E. coli</i> host strains are producing extreme amounts of RNA. In such a case add fresh RNase to buffer G1 to a final concentration of 400 µg/ml.</p> <p>As an alternative supplement buffer G1 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.</p>
Was the amount of wash buffer G4 used too low?	<p>Make sure that the recommended amount of the wash buffer is used.</p>

Problem: Low $A_{260/280}$ ratio	
<u>Question / Problem</u>	<u>Help and comments</u>
Were buffers G2 and G3 mixed completely with the resuspended cells (steps 3 and 4)?	<p>Mixing must be done carefully (see above), but nevertheless thoroughly (e.g. by multiple inverting), until a homogeneous phase is obtained.</p> <p>If buffers G1, G2 and G3 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 Micro-Spin 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 Micro-Spin 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 microfuge.</p>

Problem: Long column run times	
<u>Question / Problem</u>	<u>Help and comments</u>
Same column used more than one time?	<p>Upon repeated usage the filter at the bottom of the Micro-spin column will clog.</p>
Buffers G2 and G3 mixed completely with the resuspended cells (steps 3 and 4)?	<p>Mixing must be done carefully (see above), but nevertheless thoroughly (e.g. by multiple inverting), until a homogeneous phase is obtained.</p> <p>If buffers G1, G2 and G3 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 5) 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 at least 13,000 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. If possible ncrease centrifugal force to 15,000 x g.</p>
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Problem: Poor performance of the plasmid DNA in subsequent reactions (e.g. sequencing ...)

<u>Question / Problem</u>	<u>Help and comments</u>
Were buffers G2 and G3 mixed <i>completely</i> with the resuspended cells (steps 3 and 4)?	<p>Mixing must be done carefully (see above), but nevertheless thoroughly (e.g. by multiple inverting), until a homogeneous phase is obtained.</p> <p>If buffers G1, G2 and G3 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.</p>
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 microfuge.
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?	<p>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 5 min. Make sure that your microfuge runs at top speed which should be at least 13,000 x g.</p> <p>Alternatively, let the open micro-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.</p>

Problem: Contamination with bacterial chromosomal DNAQuestion / ProblemHelp 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 G2 or G3. In this context, high amounts of bacterial cells (LB cultures ≥ 15 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 / ProblemHelp 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 G3.

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

Problem: Degraded DNAQuestion / ProblemHelp 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.
Use the optional wash with buffer GX (step 6) to enhance the removal of endo- and/or exonucleases.

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.
Use the optional wash with buffer GX (step 6) to enhance the removal of endo- and/or exonucleases.

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 75 µl to 50 µl will result in a reduction from 100 to 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.

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.

B) ' JETQUICK PCR' Extended Trouble Shooting

Problem: Low yield of DNA	
<u>Question / Problem</u>	<u>Hints and suggestions</u>
Did your PCR perform well?	Run an aliquot (e.g. 10 µl of a 100 µl PCR assay) on an agarose gel and check the amount of the expected PCR product.
Did you set up the correct binding conditions?	Apply high-salt conditions by adding buffer H1 and mixing very thoroughly . This is essential for an efficient binding of the DNA to the silica membrane in the Micro-spin cup. If PCR assays with volumes >100 µl have to be processed, the purification assay has to be scaled up, e.g. if one starts with a sample volume of 200 µl , add 800 µl of buffer H1 . Because of the higher volume of this sample the Micro-spin cup must be loaded twice. It is normally not necessary to increase the volume of the washing buffer H2. <i>The DNA capacity of the Micro-spin cups in the JETQUICK PCR kit is >20 µg, so that this kit is suitable for the purification of preparative PCR assays.</i>
Did you use the detergent Tween-20 in your PCR assay in concentrations >0.5%?	While other detergents (Triton X-100, Non-Idet P40) as well as gelatin don' t affect the DNA binding to the silica membrane, the JETQUICK system is susceptible against Tween 20 concentrations >0.5%. This component will cause a low DNA yield, but normally that high Tween concentrations are not present in a PCR assay).
Was the bottle with wash buffer H2 always tightly closed when not in use?	After reconstitution of buffer H2 with ethanol it is important to keep this bottle tightly closed 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 H2 resulting in the loss of the DNA sample during the washing step. An ethanol concentration of 70% (v/v) is necessary for optimal performance.
How long is the fragment you want to purify?	We didn' t observe any differences in binding with regards to the fragment size. The JETQUICK system purifies all sizes from 80 bp to 12 kb equally well.

Problem: Subsequent enzymatic reactions are inhibitedQuestion / ProblemHints and suggestions

Was all ethanol completely removed from the Micro-Spin before the elution step?

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 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 5 min. Make sure that your microfuge runs at top speed which should be at least 13,000 x g.
Alternatively, let the open micro-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.

Did your purification perform as described in the protocol, or was it necessary to extend centrifugation times?

Normally the JETQUICK PCR kit performs very well, even if multiple rounds of sample loading (e.g. when doing a preparative PCR assay) are necessary. If still some reduction in performance occurs, i.e. if the sample or the wash buffer doesn't pass the silica membrane in the time stated in the protocol, extend the centrifugation time to up to 5 min. Make sure that your microfuge runs at top speed which should be at least 13,000 x g.

Special problems of special usersQuestion / ProblemAnswer

Can I purify nucleic acid species which are partially single stranded and partially double-stranded with JETQUICK?

Yes, this is possible.

Can I purify heteroduplexes, e.g. RNA/DNA hybrids with JETQUICK?

YES! It seems only to be important to have a double-stranded structure of the nucleic acid. We have customers using JETQUICK for the purification of their products after first-strand cDNA synthesis. This is a DNA strand, synthesized by reverse transcription of a mRNA template. The purified product was used successfully for second-strand cDNA synthesis.

C) ' JETQUICK Gel Extraction' Extended Trouble Shooting

Problem: Low DNA recovery	
<u>Question / Problem</u>	<u>Help and comments</u>
Was ethanol added to solution L2?	<p>Ethanol is necessary to keep the DNA bound to the silica membrane.</p> <p>After reconstitution of buffer L2 with ethanol the bottle must be kept <u>tightly closed</u>, when it is not in use. Otherwise the ethanol tends to evaporate, leading to a loss of the bound DNA during the washing step with buffer L2.</p>
Was the bottle with buffer L2 kept tightly closed when not in use?	If evaporation reduces the ethanol content in buffer L2, recovery of DNA will be increasingly poor. The ethanol concentration for an optimal performance of the JETQUICK Gel Extraction kit must be <u>70% (v/v)</u> .
Has the exact weight of the agarose gel slice been determined?	For an optimal solubilization the ratio between the weight of the gel slice and the volume of binding buffer L1 must be at least 1 : 3 . Ratios higher than 1,5 : 3 will lead to an incomplete dissolving of the agarose, the DNA will remain bound to residual unsolubilized agarose and therefore DNA recovery and quality will be poor.
Was the weight of the agarose gel higher than 100 mg? (see also ' Handling of larger gel slices')	<p>For gel slices with a higher weight than 100 mg the volume of buffer L1 must be scaled up proportionally.</p> <p>It is usually not necessary to scale up the volume of wash buffer L2.</p> <p>We recommend an additional wash with buffer L1 if the size of the gel slice exceeds 250 mg. A second wash with buffer L2 can be included if the performance of the system is poor for some reason, i.e. if the centrifugation steps must be extended significantly (> 5 min).</p>

DNA fragment smaller than 500 bp?	<p>JETQUICK works well for dsDNA fragments being as small as 80 bp. But there are several aspects which may raise problems with small fragments.</p> <p><i>First</i>, the typical amounts of small DNA fragments are quite low giving problems in handling.</p> <p><i>Second</i>, and most important, the diffusion-rate in agarose gels is higher for small fragments than for big ones, so that losses may occur by diffusion into the overlaying buffer or by band-broadening.</p> <p><i>Third</i>, for small fragments the yield may decrease due to denaturation caused by elevated temperatures and/or the interaction with the chaotropic salt. ssDNA is not bound very well by JETQUICK. So if the DNA fragment is small, do the solubilization of the agarose (step 1 of the protocol) at ambient temperature or at 37°C for an extended period of time with repeatedly vortexing the tube.</p> <p>It may be helpful not to overlay the gel with buffer and to minimize the migration distance during electrophoresis.</p>
DNA fragment larger than 10 kb?	<p>Using the standard protocol the recovery rate for DNA species will decrease above sizes of 10 kb. There are several modifications mentioned in the protocol to partially compensate a poor recovery of larger fragments:</p> <ol style="list-style-type: none"> 1. Raise the temperature of the elution buffer to 60-65°C. 2. Before centrifugation allow an incubation of the elution buffer after pipetting it onto the silica membrane for 1-2 min.
Was the DNA to be eluted supercoiled?	<p>Supercoiled DNA (e.g. undigested plasmids) can principally be bound to and eluted from the JETQUICK membrane, but cannot be extracted from agarose gels by the JETQUICK kit.</p>
Does the JETQUICK spin cup still smell of ethanol after the final wash with buffer L2?	<p>Ethanol, which is part of buffer L2, reduces the elution efficiency. In particular, elution of large fragments is affected.</p> <p>After the final centrifugation before the elution step the JETQUICK membrane shall appear dry and must not smell of ethanol. If this is the case, extend the centrifugation time to up to 5 min. Make sure that your microfuge runs at top speed and reaches at least 13,000 x g.</p> <p>Alternatively let the open micro-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 the influence on the elution efficiency remains to be checked.</p>

Do you work with AT-rich DNA?	AT-rich DNA (average AT-content >70%, i.e. intron-DNA) denaturates easier (which means separation into the two DNA strands) than "normal" DNA. Denaturation is enhanced by elevated temperatures and the chaotropic salt. So do the solubilization step of the agarose (step 1 of the protocol) at <u>ambient</u> temperature or at 37°C for an extended period of time (approx. 20-30 min) with repeatedly vortexing the tube.
What is the storage temperature of the JETQUICK kit?	Other gel extraction kits are recommended to be stored cold at 4°C if they are not in use. JETQUICK should be kept at ambient temperature . All buffers and ingredients of JETQUICK are completely different from competitor' s products. There is absolutely no need for storing the kit in the cold.

Problem: Handling of larger gel slices	
<u>Question / Problem</u>	<u>Help and comments</u>
Was the weight of the agarose gel slice higher than 100 mg?	For gel slices larger than the standard size of 100 mg the volume of buffer L1 must be scaled up proportionally . It is usually not necessary to scale up the volume of the wash buffer L2. We recommend an additional wash with buffer L1 if the size of the gel slice exceeds 250 mg. A second wash with buffer L2 can be included if the performance of the system is poor for some reason, i.e. if the centrifugation steps must be extended significantly (> 5 min).
Did you melt your agarose gel slice in a glass tube?	<u>This must not be done!!</u> Due to the presence of the chaotropic salt in the solubilization process the DNA binds to the wall of the glass tube and will be lost.
Was the larger agarose gel slice completely solubilized?	Larger gel slices take longer to dissolve in the buffer L1. Therefore extend the solubilization step in any case for 10-15 min. The solubilization can be enhanced by cutting the large gel slice into smaller pieces. If the size of the DNA fragment that is to be purified is not too small (<500 bp), the temperature for solubilization can be increased to 55°C

Problem: Agarose gel slice doesn' t solubilize	
<u>Question / Problem</u>	<u>Help and comments</u>
What was the buffer L1-to-gel-ratio?	The standard protocol can be used for gel concentrations of up to 2% . For higher gel concentrations use a higher buffer-to-gel-ratio (600 µl of buffer L1 for 100 mg of agarose gel slice). If the buffer L1-to-gel-ratio is too low, the gel won' t solubilize completely, leading to poor DNA recovery and quality . Determine the weight of the gel slice exactly with a balance and add the correct volume of buffer L1.
What was the temperature used for gel solubilization?	If the solubilization step is carried out at temperatures below 50°C, the time required for completely dissolving the gel slice will increase . The gel should only be solubilized at room temperature if low agarose concentrations ($\leq 1\%$) are used or if one is handling small and/or AT-rich DNA fragments. DNA binding is not affected at room temperature.
Was the weight of the agarose gel slice higher than 100 mg?	If the agarose gel slice weighs more than 100 mg it should be cut down to small pieces in order to enhance solubilization.

Problem: Formation of a precipitate after addition of buffer L1	
<u>Question / Problem</u>	<u>Help and comments</u>
Did your assay contain SDS?	SDS, which may be present from proceeding steps at concentrations of more than 0.1% will precipitate in solutions containing high concentrations of sodium perchlorate. Any precipitate should be removed by a short centrifugation (5 min at 13,000 x g) before loading the binding mix onto the Micro-Spin column.
Did your assay contain potassium ions?	Potassium perchlorate is highly insoluble. If your assay contains potassium ions, add first buffer L1 and spin down the resulting pellet of potassium perchlorate. Transfer the supernatant directly into the JETQUICK micro-spin tube.

Problem: Subsequent enzymatic reactions with the JETQUICK-purified DNA are inhibited

Question / Problem

Hints and suggestions

Applications, which are sensitive to NaClO₄

If the performance of the system is poor for some reason (i.e. long centrifugation times necessary), the washing step with buffer L2 can be not as efficient as it should be. Under these circumstances the final concentration of NaClO₄ in the eluate may exceed 50 mM. To avoid this, extend the centrifugation time to up to 5 min and/or wash 2 times with buffer L2. Make sure that your microfuge runs at top speed and reaches at least 13,000 x g.

Applications, which are sensitive to ethanol

If the centrifugation efficiency during the washing step is poor, too much ethanol will remain in the membrane layer and go into the eluate. In such cases the eluate will smell rather strongly of ethanol and/or come out of the slot if a part is loaded onto a gel. In such a case extend the centrifugation time to up to 5 min. Make sure that your microfuge runs at top speed and reaches at least 13,000 x g.

Alternatively let the open micro-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 the influence on the elution efficiency remains to be checked.

Did your purification perform as described in the protocol, or was it necessary to extend centrifugation times?

Normally the JETQUICK Gel Extraction kit performs very well, even if multiple rounds of sample loading (e.g. when doing a preparative assay) are necessary. If still some reduction in performance occurs, i.e. if the sample or the wash buffer doesn't pass the silica membrane in the time stated in the protocol, extend the centrifugation times to up to 5 min. Make sure that your microfuge runs at top speed and reaches at least 13,000 x g.

Additionally consider the optional wash with buffer L1 (see comment to step 2 of the protocol)!

Applications, which are sensitive to EDTA

Use 10 mM Tris/HCl, pH 8.0 without EDTA or sterile water for elution.

Some special inquiries that came from customers

Smaller elution volumes?

The elution of DNA can be carried out with only 30 µl of elution buffer per assay. In such a case, reduced recovery rates of approximately 30 % must be tolerated.

This can partially be compensated by using warm (60-65°C) elution buffer and/or leaving the tube for 1-2 min 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 total DNA yield.

Elution in water?

Elution with sterile water is possible, but in some cases the recovery rates of larger fragments may be lower. DNA dissolved in water is less stable than in a Tris-buffered elution buffer.

What are the minimal, what the maximal sizes of DNA fragments I can elute from my gel?

Lower limit: 80 bp, upper limit: 10 kbp. Below 50 bp and above 10 kbp reduced recoveries (about 50%) must be expected.

For single stranded DNA and RNA the lower limit is about 200 nt.

How much DNA can I isolate ?

Each Micro-spin tube has a nominal capacity of 20 µg of dsDNA.

Ligation problems

The problem of poor efficiencies in fragment ligation is caused in most cases by an incomplete removal of the solubilized agarose and/or perchlorate. If these components are not totally removed, they may interfere with ligation or other enzymatic reactions. This should only be a problem if the performance of the kit is poor. Normally the JETQUICK Gel Extraction kit performs very well, even if multiple rounds of sample loading (e.g. when doing a preparative assay) are necessary. If still some reduction in performance occurs, i.e. if the sample or the wash buffer doesn't pass the silica membrane in the time stated in the protocol, extend the centrifugation times to up to 5 min. Make sure that your microfuge runs at top speed and reaches at least 13,000 x g.

Additionally consider the optional wash with buffer L1 (see comments on step 2 of the protocol)!

My DNA is very AT-rich; does the sequence influence the recovery with JETQUICK?

If a double-stranded sequence is AT-rich (average AT-content $\geq 70\%$, i.e. in introns), it is possible that the DNA melts into its single strands. This may happen during the solubilization of the gel, because under the high-salt conditions in perchlorate-containing solutions the melting temperature

of the double-stranded DNA is reduced. As a consequence, the DNA is poorly visible on agarose gels and cloning and enzymatic reactions will become impossible.

To avoid this, the temperature during the solubilization process can be reduced to 37°C or even to ambient temperature. To compensate for the lower temperatures, the time for the solubilization must be extended.

Lower grade agaroses

We have tested JETQUICK with the GIBCO-BRL and the FMC product-line. It is not necessary to use special DNA-grade agaroses (e.g. SeaKem GTG Agarose) or low gelling-temperature agaroses (e.g. NuSieve). The lower quality FMC SeaKem LE, HE and LE or BRL ultrapure agaroses perform equally well. For other brands and qualities no statement can be made.

Polyacrylamide gels

JETQUICK can not directly be used in combination with polyacrylamide gels. This is because the chaotropic salt in the buffer L1 is able to dissolve the non-covalent linkages between the agarose molecules. As polyacrylamide is a covalent-linked network, this method for dissolving the gel matrix can't be used.

What a user can TRY is the following:

- a. Grind the PAA-gel to fine pieces (i.e. squash the gel between a blue pipet tip and the wall of a reaction tube).
- b. Add a twofold volume of sterile TE buffer and incubate for 1 hr at 50 °C. Shake several times during incubation. Fragments of sizes smaller than approximately 200 bp will diffuse out of the gel into the surrounding buffer.
- c. Centrifuge briefly and transfer the eluate to a new tube.
- d. For each 100 µl of eluate add 300 µl of buffer L1.
- e. Carry out the JETQUICK procedure as described in the protocol.
- f. The volume of buffer L1 must be scaled up according to step d. It is normally not necessary to change the volume of buffer L2 for the low-salt wash. A twofold wash with buffer L2 and/or the optional wash with buffer L1 (see comment in step 2) should only be considered if the performance of the JETQUICK system is poor for some reason.

As the elution of DNA fragments from polyacrylamide gels was not a major goal during the development of the JETQUICK kit, this procedure has not been extensively tested with subsequent biochemical applications. Therefore we can not guarantee any success.