JETNICK Extended Trouble Shooting Guide

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Problem: Low DNA recovery	
Question / Problem	Help and comments
What was the method which gave „higher recoveries“?	The JETNICK kit removes the unincorporated nucleotides from the labelled DNA nearly quantitatively. In contrast, Sephadex® column chromatography or a simple precipitation of the labeling assay are leaving high amounts of radioactive nucleotides in the assay, leading the user to believe that a higher recovery rate of the labelled probe was obtained.
Has buffer B3 been properly reconstituted and has the bottle been kept tightly closed when not in use?	After reconstitution of buffer B3 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 B3, resulting in the loss of the DNA sample during the washing steps. **An ethanol concentration of 70% (v/v) is necessary for optimal performance.**
Was the initial reaction volume brought to 100 µl?	For optimal binding of the DNA the ratio between the volume of the DNA-containing solution and the volume of binding buffer B2 must be **1 : 3**. Too low amounts of buffer B2 will lead to increasing contaminations and incomplete binding.

Has the initial reaction volume exceeded 100 µl?	For larger assay volumes than the standard volume of 100 µl the volumes of **all** assay components must be scaled up: a. the volume of binding buffer B2 for optimal binding and for optimal washing, b. the volume of the JETNICK suspension. Even if the capacity is high enough, too low concentrations of the JETNICK resin in the assay can cause longer binding times of the DNA. **However, the maximally used amount of JETNICK resin should not exceed 30 µl. This amount of resin is capable of binding enough DNA, which is sufficient for all applications. Higher amounts of resin may trap too much liquid which may lead to an insufficient removal of residual perchlorate and other salts.** c. the volume of wash buffer B3 for an optimal washing of the resin.
Were the supernatants after binding and washing simply **poured out** of the reaction tube or were they removed by a pipet tip?	Incomplete removal of the supernatants will not only lead to poor removal of unincorporated nucleotides and to salt contaminations in the eluate, but may also cause ethanol still being present in the assay during the elution step. In this case DNA recovery and quality will be compromised.
Has the JETNICK resin a homogeneously snow-white appearance after the drying step (step 5)?	If the whole pellet or even parts of it are still appearing 'greyish', the resin has been dried **incompletely** after the final washing step with buffer B3. This means that trace amounts of buffer B3 and ethanol are still present. In this case DNA recovery and quality will be compromised.
Was an oil pump used for drying the JETNICK resin (step 5)?	More than a 2 min oil-pump vacuum can cause overdrying of the JETNICK resin and therefore poor DNA elution. Better use a water jet filter pump for approx. 5-7 min. In each case, the drying should be stopped when the pellet is appearing completely snow-white. **A good alternative to vacuum is a 10 min-incubation of the JETNICK pellet at 50-60°C in a thermo bloc or water bath with the cap open after having removed the B3 supernatant of the final washing step.**

Fragment larger than 5 kb?	Using the standard protocol, the recovery rate of the DNA may decrease slightly for fragments being larger than 5 kb in size. There are several modifications mentioned in the protocol to avoid a poor recovery for larger fragments: 1. Extend the incubaton time during elution (to up to 15 min). 2. Raise the elution temperature to 60°C. 3. Don´t vortex. Shearing of large fragments can be avoided if the JETNICK matrix is resuspended by flicking the tube with the finger tip or by pipetting up and down with a wide-bored pipet tip. 4. Don' t overdry the JETNICK pellet after the final washing step with buffer B3. To ensure this, don' t use vacuum. **A good alternative for the use of vacuum is a 10 min-incubation of the JETNICK pellet at 50-60°C in a thermo bloc or water bath with the cap open after removing the buffer A2 supernatant of the final washing step.**
Was the labelled probe single-stranded or RNA?	The kit was not developed for the use with single-stranded DNA or RNA. But they can be isolated if a reduced recovery (about 30-40%) can be tolerated.

Problem: Dried-out JETNICK resin	
Question / Problem	Help and comments
Has the suspension already been used?	We observed that the suspension will not dry out during storage (even after one year). But if the tube has been opened several times to take out aliquots of the resin, beads may be trapped between the rim of the tube and the tube´s cap. Additionally, if the tube is not tightly closed, evaporation of the liquid can occur. As a consequence there must be to paid attention **that there is no resin at the tube´s rim that can be trapped between the rim and the cap** or to **remove such resin before closing the tube**. Nevertheless, a dried-out resin can still be used: The resin is supplied as an approx. 65 % (v/v) slurry. In order to reconstitute the dried-out resin, give the vial a quick spin, estimate the height of the resin in the vial, and add half the volume of deionized water. The JETNICK resin is not damaged at all by dehydration. *General guidelines are*: -Store the vial with the resin upright. Take out aliquots of the JETNICK resin without contaminating the rim of the vial, -seal the cap of the vial with parafilm, or -carefully remove remnants of the JETNICK resin from the rim of the vial with a clean Kimwipe.

Problem: JETNICK beads in the eluate	
Question / Problem	Help and comments
Are JETNICK particles carried over together with the eluate?	We have extensively tested JETNICK contaminations in enzymatic reactions. We have found that only amounts of JETNICK beads causing a visibly turbid eluate may partially inhibit enzymatic reactions. To perfectly avoid getting JETNICK beads into the eluate follow these simple steps: a. centrifuge for 2 minutes at top speed; b. remove supernatant immediately after the centrifuge has stopped; c. avoid processing too many samples in parallel. Alternatively, the eluate can be filtered through a micro-spin device with a 0.45 µm membrane (e.g. Millipore Ultrafree-MC Filter Unit, Durapore membrane, 0.45 um) to get rid of any residual beads.

Problem:	**Subsequent enzymatic reactions with the JETNICK-purified DNA are inhibited**
Question / Problem	Hints and suggestions
Did you **resuspend** the pelleted JETNICK resin between each washing step?	**May sound basic, but this is obviously vital!!!** The washing efficiency depends on: 1. complete resuspension of the beads so that adhering contaminations can be washed away. 2. complete removal of the supernatant so that the diluting-out effect for the initially present agarose and the high-salt buffer B2 is maximal.
Were the supernatants after the binding and washing steps simply **poured out** of the reaction tube or were they removed with a pipet tip?	***This isn't recommend for the JETNICK kit!!!*** **The supernatants after the binding and washing steps must be removed *quantitatively* with a pipet tip immediately after centrifugation. *Take care of residual liquid backdraining from the wall of the tube***. Incomplete removal of the supernatants will lead to poor removal of perchlorate and radioactive nucleotides, thus compromising the quality of the purified DNA. These contaminants can only be removed if the JETNICK procedure is repeated on the eluate (adjust the volume of the ' dirty eluate' to 100 µl) and proceed as described in the protocol.
General Suggestions	***see below***
Contamination with JETNICK particles	As JETNICK beads may interfere with enzymatic reactions, downstream applications with JETNICK-purified DNA may be affected. To avoid this, remove the supernatant after the elution very carefully: - Centrifuge at least 30 sec at full speed in a table top centrifuge. - Remove supernatant immediately. - Don´t process too many samples at a time. The JETNICK pellet, although being quite stable directly after the centrifugation, may become unstable during extended standing on the bench. - Allow 2 - 4 µl of eluate to remain on the pellet. - Alternatively spin the eluate with the JETNICK beads through a micro-spin device with a 0.45 µm membrane (see above). This will remove all residual beads and give you nearly 100% recovery.

<table>
<tr><td>Reactions, which are sensitive to $NaClO_4$</td><td>If the washing steps with buffer B3 are not carried out properly, the final concentration of $NaClO_4$ in the eluate may reach concentrations higher than 50 mM. This may compromise the activity of certain enzymes. To avoid this:
1. remove the supernatants of the washing steps quantitatively with a pipet tip.
2. Optionally, wash 3 times with buffer B3.</td></tr>
<tr><td>Reactions, which are sensitive to ethanol</td><td>Dry the pelleted JETNICK resin until all traces of ethanol have evaporated. This can be enhanced by removing the supernatant quantitatively with a small pipet tip after the final washing step.
With DNAs of less than 5 kb in size the JETNICK pellet can be dried under vaccum. Avoid overdrying (see suggestions above)!
A good alternative for the use of vacuum is a 10 min-incubation of the JETNICK pellet at 50-60°C in a thermo bloc or water bath with the cap open after removing the buffer B3 supernatant of the final washing step.</td></tr>
<tr><td>Reactions, which are sensitive to NaCl and EDTA</td><td>If the supernatant is not completely removed after the final washing step the salt concentrations in the eluate for sodium chloride and/or EDTA may reach concentrations, that may compromise the activity of certain enzymes.
To avoid this:
1. remove the supernatants during all washing steps quantitatively with a pipet tip.
2. Use 10 mM Tris/HCl, pH 8.0 <u>without</u> EDTA for elution</td></tr>
</table>

Inquiries from customers

What are the minimal, what the maximal sizes of labelled DNA I can isolate?

Lower limit: 25 bp, upper limit: 45 kbp. Below 50 bp and above 15 kbp reduced recovery rates (up to 50 %) have been taken into account.

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

How much DNA can I isolate ?

Per 10 µl JETNICK resin about 3 µg of DNA can be isolated.

Can I isolate riboprobes?

We didn‘t test JETNICK extensively for the use with riboprobes. But a few customers have successfully tried the isolation of labelled RNAs, so binding of RNA or degradation by RNases should not be a problem.

But one should keep in mind that large single-stranded nucleic acids (>1 kb) may be degraded mechanically by the JETNICK beads and that the components are not certified to be RNase-free.