

# JETFLEX Extended Troubleshooting Guide

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<b>Problem: Low yield of DNA</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
DNA yield is lower than expected.	<p>Determine the number of DNA-containing white blood cells in a whole blood sample (e.g. by a hemacytometer). Blood from a healthy donor contains <math>5 \times 10^6 - 1 \times 10^7</math> white blood cells per ml.</p> <p>As a rule of thumb, 5 – 10 µg of genomic DNA can be expected from 200 µl of whole blood.</p> <p>If the number of white blood cells in the blood sample is low, use higher blood volumes and concentrate the white blood cells with RBC buffer.</p> <p>As an alternative, prepare buffy coat from the blood sample and use only the buffy coat for DNA preparation.</p> <p>If DNA is prepared from tissues, the number of DNA-containing cells in the starting material may be too low. Check the protocol for optimal amounts and weigh in the appropriate amount of tissue prior to CLB buffer treatment.</p>

	<p>Make sure, that cells or tissue samples are lysed efficiently by treatment with CLB buffer and/or proteinase K.</p> <p>Mixing with CLB buffer is best carried out by bump- or pulse-vortexing for 10 – 20 sec. It is essential, that the white blood cells are mixed very thoroughly with CLB buffer to yield a clear lysate. It is mandatory, that no cell clumps are visible after lysis.</p> <p>Ideally, tissue samples are cut or ground to smaller pieces before treatment with CLB buffer and proteinase K.</p> <p>If mixing with CLB buffer was insufficient, subsequent DNA yields may be low as not all DNA was released from the nuclei. In such a case repeat the DNA purification with new samples.</p>
Too high amounts of cells or tissue used	<p>If too many cells or too much tissue are processed, the chemistry of the system is overloaded and CLB buffer will not be able to carry out an efficient lysis. This will result in a low DNA yield.</p> <p>Count cells or weigh tissue prior to the treatment with CLB buffer.</p>
Still cell clumps present after treatment with CLB buffer	<p>Cell clumps may occur when cells were not completely resuspended prior to addition of CLB buffer. To lyse cells that are still present in clumps, incubate the sample at 37°C or room temperature with periodic mixing until the solution is clear and homogeneous.</p> <p>Cell clumps can be dissolved more quickly by adding proteinase K solution to a final concentration of 100 µg/ml. Incubation in CLB and proteinase K can be extended from 1 hour to overnight.</p>
Low amount of DNA containing cells in sample	<p>If the DNA yield from a particular sample is expected to be low, use a carrier (e.g. Glycogen) to enhance DNA precipitation.</p> <p>We recommend adding 0.5 µl Glycogen solution (20 mg/mL) per 300 µl isopropanol. Final concentration of glycogen in the assay should be 33,3 µg glycogen per ml.</p>
Mixing with isopropanol	<p>Use absolute 100% isopropanol for DNA precipitation.</p> <p>As isopropanol has a lower density than the supernatant after protein precipitation, make sure, that isopropanol and supernatant are mixed <b>thoroughly</b> by multiple inverting (up to 50 times).</p>
Was DNA re-dissolved completely?	<p>Genomic DNA prepared with the JETFLEX kit is of quite high molecular weight. Therefore it may take longer to re-dissolve completely in DNA hydration buffer.</p> <p>Continue to hydrate the sample by either incubating overnight at room temperature or by incubating at 65°C for 1-2 hours. An incubation at 65°C for 2 hours doesn't affect the quality of the DNA.</p> <p>Be careful when drying the DNA after the 70% ethanol wash. Using vacuum may lead to over-drying of the DNA. Over-dried DNA is much more difficult to dissolve.</p>

<b>Problem: RNA contamination</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
Precipitated nucleic acids contain not only DNA but also RNA	<p>During the JETFLEX procedure cellular and nuclear structures are disrupted, thus setting free all cellular nucleic acids. Therefore RNA and DNA will be prepared simultaneously.</p> <p>If RNA-free DNA is required, include the optional RNase digestion steps as outlined in the respective protocol.</p> <p>RNase is provided with each JETFLEX kit.</p>

<b>Problem: Red Blood Cells (RBC) do not lyse efficiently</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
After treatment with RBC buffer and the following centrifugation there are still significant amounts of „reddish“ or „brownish“ solid matter	<p>For blood samples the following points should be considered in general:</p> <ol style="list-style-type: none"> <li>Don't use chilled blood or chilled RBC buffer, as incubation in the cold (0-4°C) may prevent complete RBC lysis.</li> <li>Mix blood samples well after addition of 1 vol. RBC buffer by multiple inverting.</li> <li>Thaw frozen blood samples <b>quickly</b> at 37°C to minimize clotting, white blood cell lysis and DNase activity.</li> <li>If the blood sample contains clots or is coagulated, it requires more severe forces to get the red blood cells completely removed. Mince or mechanically remove the blood clot with a suitable pestle fitting into the reaction tube or another suitable device (e.g. a pasteur pipet). Refer to section „Treatment of clotted blood“.</li> </ol>
Higher than average number of red blood cells	Repeat the incubation with RBC Lysis Buffer to lyse remaining red blood cells. Add 1 part of RBC buffer for 1 part of sample, mix well by multiple inverting and incubate for 10 min with occasional inverting.
Blood sample contains clots or is coagulated	For clotted blood please refer to the next section.

<b>Problem: Treatment of clotted blood</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
Blood sample contains clots or is coagulated	<p>Purify DNA from the unclotted portion of the sample. Leave clots behind in the collection tube.</p>
	<p>Try to dissolve red blood cells with RBC buffer. If after spinning down the white blood cells blood clots are still present, try to remove them with pipet tips or forceps. To facilitate clot removal, resuspend cells in PBS (phosphate-buffered saline) or an equivalent salt solution and resuspend the cells by vortexing. Remove any larger clots carefully with either forceps or a pipet tip.</p> <p>After removal of the clots centrifuge to collect the white blood cells at the bottom of the tube, discard the supernatant and proceed with the next step of the purification protocol (addition of CLB buffer).</p>
	<p>Small blood clots can be digested with proteinase K (provided with the kit). Remove unclotted red blood cells with RBC buffer as described in the protocol. Spin down white blood cells and remaining small blood clots. Resuspend the pellet in CLB buffer and add proteinase K to a final concentration of 100 µg/ml. Incubate at 56°C with periodic mixing, until all clots are lysed completely.</p> <p>It is very important, that all blood clots are lysed completely before proceeding to the protein precipitation step to ensure maximum DNA yield with minimum protein contamination.</p>

**Problem: White blood cells or tissue specimen do not lyse efficiently**Question / ProblemHelp and comments

Sample doesn't lyse efficiently in CLB buffer

White blood cells derived from blood samples should dissolve readily in CLB buffer without further enzymatic treatment.

It is however **very important** to pulse-vortex the pelleted cells prior to the addition of CLB buffer. An effective pellet resuspension is key to effective cell lysis.

After having added CLB buffer to the white blood cells, mix the sample thoroughly to facilitate efficient lysis.

DO NOT USE too many cells. Follow the guidelines given in the respective protocol sections. If too many cells were treated with a too low amount of CLB buffer, the system will be overloaded. As a consequence efficient cell lysis will be inhibited; the CLB buffer will become very viscous and cells may clump. In such a case add more CLB buffer to completely lyse the cells.

To prevent incomplete cell lysis, either count the cells present in the sample with a hemacytometer or other cell counter.

If cell clumps persist, incubate the sample at 37°C until it is completely clear. However, avoid too long incubation times at 37°C – the incubation at 37°C should not be carried out longer than 1 hour. If longer incubation times are required, let stand the sample at room temperature overnight.

Incubation in the cold (0-4°C) should be avoided, as the detergent present in CLB buffer precipitates at cold temperatures. As a consequence, cells may not be lysed efficiently and/or DNA may degrade.

Solid tissues processed

Solid tissues don't desintegrate readily in CLB buffer. They require enzymatic digestion with proteinase K.

For solid tissues refer to the respective protocol section and digest the samples with proteinase K as described. Proteinase K is supplied with all JETFLEX kits.

Mincing or cutting tissue samples down to small pieces will enhance proteinase K digestion.

Treatment of fixed cells	<p>Cells that were fixed in a fixative (e.g. methanol/acetic acid or ethanol) should NOT be washed with PBS or another equivalent wash buffer. Treatment with PBS will lyse fixed cells, thereby greatly reducing DNA yield.</p> <p>Remove fixative completely after the centrifugation to pellet the cells and treat directly with CLB buffer. If cell clumps are present after adding CLB buffer, incubate samples at 65 °C until cells are completely lysed (lysate appears fully clear) or enhance dissolving by treatment with proteinase K. Add proteinase K to the lysis mix to a final concentration of 100 µg/ml and incubate at 56 °C until all cells are completely lysed (1 hour to overnight).</p>
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<b>Problem: Protein pellet appears „liquid“ and tends to be co-transferred</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
Protein pellet appears soft and instable after precipitation	<p>Depending on the sample source, the protein pellet may have a different shape and appearance after treatment with protein precipitation buffer (PPT) buffer (PPT).</p> <p>While i.e. the removal of red blood stain and subsequent transfer of supernatant can easily be monitored optically, this may be more difficult for other samples.</p> <p>The following general suggestions can be given if the supernatant is difficult to be transferred from the protein pellet:</p> <ol style="list-style-type: none"> <li>1.) Re-vortex the sample for 20 seconds, ice for 5 min and re-centrifuge for 5 min at either room temperature or in a chilled centrifuge. Then try again to take off the supernatant without carrying with you any trace from the pellet.</li> <li>2.) Use ‚Pellet Compactor‘ according to the description in the respective protocol section. This should yield a very compact pellet in any case.</li> </ol>
	<p>Be sure, that the sample is at <b>room temperature or below</b> when adding protein precipitation buffer (PPT). Elevated temperatures &gt;25 °C may lead to small or loose protein pellets.</p>
	<p>Make sure, that the sample was <b>mixed thoroughly and vigorously</b> after addition of protein precipitation buffer (PPT) in order to remove contaminants quantitatively.</p>

Make sure, that centrifugation is carried out at the recommended speed. Centrifugation at slower speed may result in loose protein pellets.
<b><u>Don't use</u></b> chilled protein precipitation buffer (PPT) as this may lead to loose protein pellets or disks floating on top of the supernatant after centrifugation.

<b>Problem: DNA pellet appears colored or optically impure</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
DNA pellet appears still stained or impure after precipitation	<p>This is often a result of exceeding the recommended amount of sample material, thus overloading the system's chemistry. Follow precisely the respective protocol sections regarding recommended sample amounts.</p> <p>If DNA purification is still problematic, further reduce the amount of starting material.</p>
	<p>Dissolve the still impure DNA completely in DNA Hydration buffer and re-purify the sample according to JETFLEX protocol section „C) Re-purification protocol for still impure DNA's“.</p> <p>If after treatment with protein precipitation buffer (PPT) no precipitate is generated, use ‚Pellet Compactor‘ as recommended in the protocol.</p>

<b>Problem: Low or high <math>A_{260/280}</math> ratio</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
$A_{260}/A_{280}$ ratio of the prepared DNA is too low (<1.70)	<p>A low <math>A_{260}/A_{280}</math> ratio indicates contamination of the purified DNA with protein. This is often a result of exceeding the recommended amount of sample material, thus overloading the system's chemistry. Follow precisely the respective protocol sections regarding recommended sample amounts.</p> <p>If DNA purification is still problematic, further reduce the amount of starting material.</p>

	<p>Dissolve the still impure DNA completely in DNA Hydration buffer and re-purify the sample according to JETFLEX protocol section „C) Re-purification protocol for still impure DNA's“.</p> <p>If after treatment with protein precipitation buffer (PPT) no precipitate is generated, use ‚Pellet Compactor‘ as recommended in the protocol.</p>
	<p>If treatment with protein precipitation buffer (PPT) leads to a protein pellet, that is loose or easily co-transferred with the supernatant, make the pellet more tight and compact by using ‚Pellet Compactor‘ as explained in the respective protocol sections.</p>
<p><math>A_{260}/A_{280}</math> ratio of the prepared DNA is too high (&gt;2.0)</p>	<p>A high <math>A_{260}/A_{280}</math> ratio indicates contamination of the prepared DNA with RNA. If RNA-free DNA is required, use RNase (supplied with each kit) as outlined in the respective protocol section.</p> <p>If after RNase treatment there is still contamination with residual RNA, either double the amount of RNase used and/or extend the incubation time to up to 30 min at 37°C.</p>

<b>Problem: Poor performance of the DNA in subsequent applications</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
<p>DNA doesn't perform well in subsequent applications (e.g. PCR)</p>	<p>Purified DNA still contains contaminants. This is often a result of exceeding the recommended amount of sample material, thus overloading the system's chemistry. Follow precisely the respective protocol sections regarding recommended sample amounts.</p> <p>If DNA purification is still problematic, further reduce the amount of starting material.</p>
	<p>Dissolve the still impure DNA completely in DNA Hydration buffer and re-purify the sample according to JETFLEX protocol section „C) Re-purification protocol for still impure DNA's“.</p> <p>If after treatment with protein precipitation buffer (PPT) no precipitate is generated, use ‚Pellet Compactor‘ as recommended in the protocol.</p>

Buffer used for dissolution of DNA	<p>Don't use standard TE buffers for dissolving purified DNA as a couple of applications are sensitive against EDTA. GENOMED's TE buffer contains only 0.1 mM EDTA which was found to not inhibit any of the applications investigated.</p> <p>If you want to omit EDTA completely, prepare your own 10 mM Tris-HCl buffer with a pH in the range of 8.0 – 8.5 and sterilize it by autoclaving in order to inactivate potential nucleases.</p>
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<b>Problem: Degraded DNA</b>	
<u>Question / Problem</u>	<u>Help and comments</u>
Prepared DNA appears degraded on an analytical agarose gel	Competitor's kit used....
	<p>DNA is degraded due to improper sample collection or storage of starting material. Collect and store samples using methods that preserve DNA integrity.</p> <p>For long-term storage (&gt;5 days) blood and tissue specimens should be stored at –70°C to –80°C. As an alternative, the sample can be lysed in CLB buffer and stored at <b>room temperature</b>.</p>
	Tissue samples were not homogenized to a sufficient extent before CLB buffer and proteinase K were added. Therefore endogeneous DNases were not inactivated quick enough, thus being able to degrade cellular DNA.
	Cell sample wasn't lysed quick enough to a sufficient extent before CLB buffer and proteinase K were added. Therefore endogeneous DNases were not inactivated immediately, thus being able to degrade cellular DNA.
	Cold CLB buffer used; in the cold (0 – 4°C) detergent precipitates from CLB buffer. This may lead to insufficient cell lysis and therefore insufficient inhibition of cellular DNases.