# Purification of genomic DNA from tissue samples with JETQUICK (Revised Version)

This protocol is a modification of the method published by Bowtell (1987).[1] The procedure was adapted to the JETQUICK system. The major advantages are high speed and the opportunity to process many samples in parallel. A disadvantage is maybe the rather "rough" treatment of the high-molecular weight DNA because of shearing forces occuring during this procedure. Having this in mind, one cannot expect DNA's with a major size of > 200 kb. The average size of the JETQUICK-prepared DNA will be approximately 50 kb. But because a main application for the JETQUICK-purified DNA is its subsequent use in PCR, the partially degradation will be even an advantage, because the DNA will act better as a template.

During the development of the JETQUICK system it became clear that the spin columns are susceptible against overloading. For this reason it is of utmost importance **not to overload** the JETQUICK spin columns. We recommend the following amounts of starting material for different types of tissue:

- Brain, Lung, Heart, Kidney: 25 - 30 mg,
- Liver, Spleen: 10 - 20 mg,
- Mouse tail: 0,8 - 1,2 cm.

This protocol purifies **all** types of DNA from the respective tissue samples. This includes mitochondrial DNA.

It should be easily possible to adapt this protocol to the isolation of DNA from other animal tissue samples, blood or other body fluids, bacteria, fungi, insects or virus particles.

## Part A: Processing of the tissue sample

### *Prepare the proteinase K and RNase A as stated on the respective labels!*

1.) Cut the tissue sample into small pieces with a scalpel or freeze the tissue in liquid nitrogen and grind it to a fine powder with a mortar and pestle.
The tissue powder should be as fine as possible. Be careful not to spill pieces of your tissue sample on the bench.
*The material should be as fine as possible to ensure an optimal lysis during the next step.*

2.) Add **200 µl** of **Buffer T1** to the homogenized sample from step 1 in a 1.5 ml- or 2 ml reaction vessel (i.e. Eppendorf).

**Buffer T1:** Contains a detergent. Handle with care! Wear protective labwear (lab coat, gloves, safety goggles).

Mix buffer and sample thoroughly by inverting the reaction vessel several times. The mixture should be as homogeneous as possible!

[1] Bowtell, D.L.L. 1987. Rapid isolation of eukaryotic DNA. *Anal. Biochem.* **162**: 463

3.) Add **20 µl** of **proteinase K (20 mg/ml)** to the mixture from step 2. Mix thoroughly by inverting the tube several times and incubate for at least *1-2 h at 56°C*(if the tissue will not dissolve readily, this incubation can be extended to overnight at 56°C). During the incubation mix several times thoroughly by inverting to get the tissue material dissolved as good as possible. After the proteinase K digest the mixture should appear clear.

*The length of the incubation depends on how well the tissue sample was initially homogenized during step 1. If there is still particular material visible after the 1-2 h incubation at 56°C (or the overnight incubation), either extend the incubation time until all material has dissolved or spin down residual material for 5 min at ≥10.000 x g (4°C). Buffer T1 lyses the nuclei and denaturates proteins (i.e. nucleases, histones). The proteinase K digests the denatured proteins into smaller fragments. Buffer T1 and proteinase K, in combination, strip the genomic DNA of all bound proteins, thus facilitating efficient removal during purification.*

4.) **OPTIONAL** (if you want to remove cellular RNA): Add **20 µl RNase A** (DNase-free, 20 mg/ml) to the cleared lysate from step 3 and incubate for a further **5 min at 37°C**.

*The RNase will degrade the cellular RNA. Residual RNA fragments will be removed during the subsequent JETQUICK spin column procedure.*

## Part B: JETQUICK Spin Column Procedure

***Reconstitute buffers TX and T3 with absolute ethanol as stated on the bottles label!***

1.) Add **200 µl Buffer T2** to the cleared lysate of the last step and mix thoroughly until you have obtained a homogeneous mixture. Incubate for **10 min** at **70°C**.

**Buffer T2**: Contains guanidine hydrochloride and a detergent. These substances are irritants. Use with proper precaution! Wear gloves and safety goggles!

2.) Let the mixture cool down for approximately 1 min. Then add **200 µl** of **absolute ethanol**.

*Mix quickly and very thoroughly in order to prevent a precipitation of DNA due to too high local alcohol concentrations.*
*Despite of proper mixing there may occur a precipitate with some tissue types at this step. If there is a visible precipitate, be sure that this is transferred together with the liquid onto the micro-spin column in the next step.*

3.) Assemble a spin unit by fitting a JETQUICK micro-spin column into the suitable receiver tube (supplied). Transfer the mixture from step 2 into the reservoir of the micro-spin column and centrifuge for 1 min at 10.000 x g (approx. 10.600 rpm).

5.) Discard the flowthrough and re-combine the micro-spin column with the used receiver tube. Pipette **500 µl** of **reconstituted buffer TX** into the reservoir of the micro-spin column and centrifuge for 1 min at 10.000 x g (approx. 10.600 rpm).

*Buffer TX is a new high-salt buffer being capable of removing residual contaminations which may affect downstream applications. This high-salt wash replaces one of the two low-salt washes in the old protocol and will lead to an improved DNA purity.*

6.) Discard the flowthrough, re-combine the micro-spin tube and the used receiver tube.
Repeat step 4 with **500 µl** of **reconstituted buffer T3** as described above.

*Buffer T3 is a low-salt buffer that changes the high-salt conditions on the silica membrane after step 4 to low-salt conditions, thereby ensuring a low salt content in the final eluate.*

7.) Discard the flowthrough and again combine the micro-spin tube and the used receiver tube.
Centrifuge for **1 min** at **13.000 rpm** (‚max. speed') to remove residual buffer T3.

*It is vital to remove the ethanol-containing wash buffer as good as possible, because residual ethanol can affect subsequent enzymatic reactions (i.e. Taq DNA polymerase).*

7.) Discard the receiver tube and insert the JETQUICK micro-spin column into a clean, sterile 1.5 ml reaction tube. To elute the DNA, pipet **200 µl prewarmed (65-70°C) elution buffer** (10 mM Tris-HCl [pH 9,0] oder bidistilled water) directly onto the surface of the silica membrane, **incubate for 5 min at room temperature**, and centrifuge subsequently for **2 min at 13,000 rpm**.

***OPTIONAL**: In order to increase the elution efficiency, repeat this elution step with another **200 µl** of **elution buffer** as described. Pool both eluates.*
*One can alternatively try to improve the yield by re-using the first eluate for a second elution. After spinning through the first 200 µl of elution buffer, re-heat the eluate to 65-70°C and apply these prewarmed 200 µl onto the silica membrane in the micro-spin tube. Then proceed as described before. This will improve the final yield by about 15%.*
***IMPORTANT NOTE:** Take your time and let the eluate incubate for 5 min after having applied it onto the silica membrane. This will significantly improve DNA yield.*

8.) The eluted DNA is ready-to-use. The DNA yield is determined spectrophotometrically, where 1 $A_{260}$ unit corresponds to a concentration of 50 µg DNA/ml. The $A_{260}/A_{280}$ ratio of pure DNA is within a range of 1.7 and 1.9.