

Purification of genomic DNA from Yeast with JETQUICK (Preliminary Protocol)

I have adapted this protocol which is based on a publication for *Saccharomyces* to the JETQUICK procedure. Although being published for *Saccharomyces* I have no practical result with it for this species, but Australian researchers were successful with it for *Candida*. The literature citation is as follows: *J. D. Boeke, D. J. Garfinkel, C. A. Styles, and G. R. Fink, Cell* **40**, 491 [1985].

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 occurring 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 partial 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 an amount of **2 x 10⁹ cells (approx. 4 – 10 mls of culture)** as the starting material for yeast cells:

The culture volume is only an approximate value. Better calculate with the number of cells. For the growth of cells usually YPD or YEPD are the recommended culture media.

Part A: Processing of the yeast sample

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

- 1.) Grow the *Saccharomyces* culture to saturation in YPD or YEPD. Harvest the cells of the culture by centrifugation (3000 - 5000 x g for 5 - 10 min [4 °C]), discard the supernatant and wash the cells by resuspending them in **2 ml of buffer Y1**.

Buffer Y1: 0.9 M sorbitol
 0.1 M Na₂EDTA (pH 7.5)
 14 mM 2-mercaptoethanol.

This is for the removal of remaining media components and to ensure optimal enzymatic performance with the Zymolyase in step 3.

- 2.) Spin down the cells at 3000 - 5000 x g for 5 - 10 min (4 °C) and discard the supernatant. Resuspend the cells in **1 ml of buffer Y1**. The cell suspension must be homogeneous; no cell clumps must be visible!
- 2.) Add 100 µl of Zymolyase 60,000 (Miles; 2 mg/ml) and incubate at 37 °C for 20 - 30 min.

Zymolyase will break down the cell wall of the yeast cells enzymatically during incubation. Equivalent enzymes to Zymolyase are Lyticase (Sigma, Cat.-No. L8137) or Zymolase (ICI, Cat.-No. 32-093-2). These enzymes should be diluted from their respective stock solutions in distilled water to a final concentration of 1000 U/ml and the incubation with them be performed for at least 30 min at 30 °C. Stock solutions of these enzymes should be stored in aliquots at -20 °C and each aliquot only used once.

Monitor spheroplast formation by examination to detergent sensitivity: a small sample of cells is diluted into 1% SDS, and spheroplasting is sufficient when greater than 90% of the cells burst when examined under the microscope.

- 4.) Spin down the spheroplasts at 5000 x g for 10 min at 4 °C.
- 5.) Resuspend the spheroplasts in **200 µl of buffer T1** 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!.

The spheroplasts should be resuspended as thoroughly as possible. A homogeneous suspension is vital for the next steps.

- 6.) Add **20 µl of proteinase K** (Merck; 20 mg/ml) to the mixture from step 5. Mix thoroughly by inverting the tube several times and incubate for at least *1-2 h at 55 °C* (alternatively overnight at 37 °C). During this incubation mix several times thoroughly by inverting to get the spheroplast 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 sample was initially homogenized during step 5. If there is still particular material visible after the 1-2 h incubation at 55 °C (or the overnight incubation at 37 °C), either prolong the incubation time until all material has dissolved or spin down the residual material for 10 min at $\geq 10.000 \times g$ (4 °C). Buffer T1 lyses the nuclei and denatures 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.

- 7.) **OPTIONAL** (if you want to remove the RNA): Add **20 µl RNase A** (DNase-free, 20 mg/ml) to the cleared lysate from step 6 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 bottle's 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.*

- 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).

- 4.) 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).

- 5.) Discard the flowthrough and re-combine the micro-spin column with the used receiver tube. Pipette **500 µl of reconstituted buffer T3** into the reservoir of the micro-spin column and centrifuge for 1 min at 10.000 x g (approx. 10.600 rpm).

- 6.) 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 and centrifuge for **2 min** at **13.000 rpm (,max. speed')**.

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%.

- 8.) The eluted DNA is ready-to-use. The DNA yield is determined spectrophotometrically, where 1 A_{260} unit means 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.