Protocol for the isolation of plasmid DNA from fungi with JETSTAR 2.0

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

Depending on the size of preparation (Mini, Midi, Maxi) the following amounts of cells should be used:

-Mini (up to 20 µg): 2×10^9 (4 - 10 ml of culture),
-Midi (up to 100 µg): 1×10^{10} (20 - 30 ml of culture),
-Maxi (up to 500 µg): 5×10^{10} (100 - 150 ml of culture).

The culture volumes are only approximate values. Better calculate with the number of cells. For the growth of cells usually YPD or YEPD are the recommended culture media.

Protocol (for Mini, Midi and Maxi):

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 (Mini) / 4 ml (Midi) / 12 ml (Maxi) of buffer YS1.

Buffer YS1: 0.9 M sorbitol
0.1 M Na_2EDTA (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 (Mini) / 5 ml (Midi) / 12 ml (Maxi) of buffer YS1. The cell suspension must be homogeneous; no cell clumps must be visible!

3.) Add 100 µl / 300 µl / 1000 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. During this centrifugation equilibrate the JETSTAR column as described in the protocol.

5.) Resuspend the spheroplasts in 500 µl (Mini) / 5 ml (Midi) / 12 ml (Maxi) of buffer E1 from the JETSTAR kit with 100 µg/ml RNase.
The spheroplasts should be resuspended as thoroughly as possible. A homogeneous suspension is vital for the next steps.

6.) Lyse the spheroplasts with 500 µl (Mini) / 5 ml (Midi) / 12 ml (Maxi) of buffer E2. Incubate the lysate for 30 min at 65°C.
The paper mentioned above is stating that long incubation times. Maybe the time can be reduced to 5 - 10 min.

7.) Add 500 µl (Mini) / 5 ml (Midi) / 12 ml (Maxi) of buffer E3 and mix thoroughly until a homogeneous suspension is obtained. <u>Incubate on ice for at least 60 min</u>.
Again, the paper mentioned above is stating a that long incubation time. Maybe the incubation on ice can either be reduced to 5 - 10 min or omitted. Each user must find out this for himself.

8.) Centrifuge for 10-15 min with ≥13,000 x g at 4°C to pellet cellular debris. Transfer the supernatant to a fresh tube and incubate at 25°C in a water bath until the lysate has reached approximately room temperature.

9.) Apply the lysate to the previously equilibrated JETSTAR column (see step 4) and let the column run by gravity flow.

10.) Continue with washing, elution and plasmid DNA precipitation as described in the JETSTAR protocol.

Again, because not extensively tested, this protocol cannot be more than a 'sophisticated guess'. It has been assembled from protocols described to be functional - therefore I'm expecting good results. Nevertheless this can't be taken for optimal - perhaps yeast culture volumes have to be adapted (increased/decreased) or some buffer volumes and/or incubation times (see comments to steps 6 and 7 of the above protocol) can or even must be adjusted.