

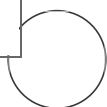


# Automation Protocol for High-Efficiency and High-Quality Genomic DNA Extraction from *Saccharomyces Cerevisiae* V.3

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## ABSTRACT

Here, we describe a protocol for automated extraction of genomic DNA (gDNA) from yeast liquid culture and colonies. The protocol uses a Hamilton NGS-STAR automated liquid handler equipped with thermal-regulated microplate shakers. We have tested this automated protocol with 1.5 mL aliquots of liquid culture ( $OD_{600} = 1$ ) to demonstrate that it can provide high-quality gDNA at high efficiency. The protocol can also be implemented with manual pipetting, and/or to extract gDNA from larger volumes of culture by proportionally increasing the volumes of reagents and using an appropriate magnet separation rack.

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**Protocol status:** Working MATERIALS

We use this protocol and it's working

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#### Reagents:

- PBS, pH 7.4 (Invitrogen, cat. no. AM9625)
- SDS, 20% (Quality Biological, cat. no. 351-066-101)
- EDTA, 0.5 mol/L, pH 8.0 (Ambion, cat. no. AM9262)
- TRIS-HCl, 1 mol/L, pH 8.0 (Invitrogen, cat. no. 15568)
- 1x TE Buffer, pH 8.0 (ThermoFisher Scientific, cat. no. 12090015)
- Polyethylene glycol (PEG-8000, Sigma, cat. no. 89510)
- Sodium Chloride, 5 mol/L (Invitrogen, cat. no. AM9760G)
- Zymolyase 20T (US Biological, Cas. No. 37340-57-1)
- 2-Mercaptoethanol (ThermoFisher Scientific, Cas. no. 60-24-2)
- RNase A/T1 Mix (ThermoFisher Scientific, cat. no. EN0551)
- Speed-Bead Magnetic Carboxylate Modified Particle (Cytiva, cat. no. 65152105050250)
- TWEEN-20 (Life technologies, cat. no. 003005)
- Ethanol, 200 proof (100%) (Fisher Scientific, cat. # 2716TR)
- Nuclease-Free Water (ThermoFisher Scientific, cat. no. AM9938)
- Elution Buffer: 10 mmol/L TRIS-HCl, pH 8.0 (Invitrogen, cat. no. 15568)
- Optional (for step 43): Proteinase K, recombinant, PCR grade, 20mg/mL, (ThermoFisher Scientific cat. no. E00491)

#### Additional labware:

- Spin Plate: 96-well deep-well plate (Eppendorf, cat. no. 951033405)
- Reaction Plate: 96-well midi plate (Abgene, AB-0765)
- Elution plate: Low-binding 96-well (Eppendorf, cat. no. 30603303)
- Universal Lid: (Agilent, cat. no. 202497-100)
- 12-Tube Magnetic Separation Rack (New England Biolabs, cat. no. S1509)
- Aluminum Foil Lids (Beckman Coulter, cat. no. 538619)
- Rubber Roller (Beckman Coulter, cat. no. 538619)
- 50 mL Conical Tubes (FALCON, cat. no. 352098)
- Microcentrifuge Tubes, 1.7 mL, RNase/DNase free (Costar, cat. no. 3207/3620)

#### Equipment:

- Automated liquid handler (Hamilton Robotics, NGS-STAR).
- Centrifuge for 96-well plates.

## Preparation of Zymolyase Stock

- 1 Prepare stock of Zymolyase 20T by dissolving to a concentration of 1 U/ $\mu$ L in 1x PBS.
- 2 Divide Zymolyase solution into 800  $\mu$ L aliquots and store at -20 °C until use.

## Preparation of 2-Mercaptoethanol Stock

- 3 Prepare 0.001 % stock of 2-Mercaptoethanol by diluting 1000-fold into 1x PBS.
- 4 Store at 4 °C until use.

## Preparation of Digestion Master Mix

- 5 Add 22.72 mL 1x PBS
- 6 Add one aliquot (800 µL) of Zymolyase Stock, 1 U/µL
- 7 Add 160 µL 0.001 % 2-Mercaptoethanol
- 8 Add 320 µL RNase A/T1 mix
- 9 Prepare fresh before each use

## Preparation of 4x Lysate Buffer

- 10 Add 30 mL Nuclease-Free Water.
- 11 Add 60 mL 20% SDS
- 12 Add 4 mL 0.5 mol/L EDTA.
- 13 Add 6 mL 1 mol/L TRIS-HCL, pH 8.0
- 14 Store at 4 °C until use.

## Preparation of Magnetic Beads Stock

- 15 Vortex Speed-Bead Magnetic beads to resuspend
- 16 Divide Speed-Bead Magnetic beads into 1 mL aliquots, each in in a 1.7 mL Microcentrifuge Tube.

- 17 Place micro-centrifuge tubes containing magnetic beads solutions on a 12-Tube Magnetic Separation Rack until beads are drawn to the magnet and solutions are clear.
- 18 Remove supernatant.
- 19 Add 1 mL 1x TE Buffer to each micro-centrifuge tube containing beads, remove from magnetic stand, and mix by vortex.
- 20 Repeated steps 17-19 two additional times.
- 21 Store bead stock in the dark at 4 °C. Bring bead stock to room temperature and mix well by vortexing before use.

### **Preparation of Magnetic Bead/PEG-NaCl Binding Buffer**

- 22 Add 9 g PEG-8000 to a new 50 mL Conical Tube.
- 23 Add 10 mL 5 mol/L Sodium Chloride.
- 24 Add 500 µL 1 mol/L Tris-HCl, pH8.

- 25 Add 500  $\mu$ L 0.5 mol/L EDTA.
- 26 Fill Conical Tube to the 40 mL mark with Nuclease-Free Water, then vortex to mix every 1-2 minutes until PEG goes into solution.
- 27 Add 27.5  $\mu$ L Tween-20 and mix gently by inverting 4-6 times.
- 28 Vortex Magnetic Beads Stock (from previous section) to mix thoroughly.
- 29 Add Magnetic Bead Stock:
  - 29.1 For extraction of gDNA from liquid culture, add 3 mL Magnetic Beads Stock to the 50 mL Conical Tube containing the PEG/NaCl solution.
  - 29.2 For extraction of gDNA from yeast colonies, add 1 mL Magnetic Beads Stock to the 50 mL Conical Tube containing the PEG/NaCl solution.
- 30 Fill Conical Tube to the 50 mL mark with Nuclease-Free Water, and gently mix until solution is a uniform brown color.
- 31 Wrap in foil (or place in dark container) and store at 4 °C, mix well by vortexing before use.

## Preparation of yeast liquid culture for extraction

- 32 Aliquot 1.5 mL (OD600=1) in 1-3 columns of 96-well Spin Plate using automated multichannel pipettes.
- 33 Centrifuge at 3700 g for 10 min at 10 °C.
- 34 Remove supernatant from each well using automated liquid handler.
  - 34.1 Use modified labware definition or other constraints on automated pipetting to set a minimum distance of 2.5 mm between the bottom of each well and the tip of each pipette.
  - 34.2 The resulting volume of the remaining cell pellet and residual liquid should be approximately 50 µL.
- 35 Seal Spin Plate using Aluminum Foil Lid and Rubber Roller.
- 36 Store at -20 °C.
- 37 Defrost at 4 °C for 30-60 minutes before continuing with extraction.

## Preparation of yeast colonies for extraction

- 38 Pick up colonies from agar plate and resuspend each in 50  $\mu\text{L}$  of PBS in 1-3 columns of 96-well Spin Plate.
- 39 Proceed with DNA extraction from resuspended colonies as described below.

## Yeast genomic DNA extraction. The following steps are imple...

- 40 Pipette 315  $\mu\text{L}$  of Digestion Master Mix into each well containing a cell pellet or resuspended colony; mix 30 times by repeated aspiration and dispense.
- 41 Transfer 315  $\mu\text{L}$  from each well containing resuspended cell sample to a well in a new Reaction Plate located on a thermal-regulated microplate shaker.
- 42 Incubate at 37  $^{\circ}\text{C}$  for 25 minutes while shaking at 400 rpm.
- 43 Optional (if protein contamination is problematic for down-stream application): Pipette 10  $\mu\text{L}$  of Proteinase K solution to each well.
- 44 Pipette 100  $\mu\text{L}$  of 4x Lysate Buffer to each well and mix 3 times by repeated aspiration and dispense.



- 45** Optional (if Proteinase K was added in step 43): Incubate at 50°C for 25 minutes.
- 46** Incubate at 75°C for 10 minutes.
- 47** Move the Reaction Plate to a microplate shaker at room temperature.
- 48** Pipette 200 µL Magnetic Bead/PEG-NaCl Binding Buffer into each well.
- 48.1** Mix Magnetic Bead/PEG-NaCl Binding Buffer well by repeated aspiration and dispense before each transfer.
- 48.2** After dispensing beads into each well, mix beads with cell lysate with one aspiration and dispense.
- 48.3** During this step, the ratio of Magnetic Bead/PEG-NaCl Binding Buffer to the lysed sample volume is 0.48 (200 µL : 415 µL). Consequently, because of the relatively low PEG concentration in the mixture, only high molecular weight chromosomal DNA will bind to the beads.
- 49** Incubate at room temperature for 7 minutes while shaking at 300 rpm.

- 50 Move the Reaction Plate to a 96-well magnet base to pull the magnetic beads out of suspension
- 51 Wait for 5 minutes.
- 52 Remove and discard supernatant from each sample (approximately 615  $\mu$ L).
- 53 Add 600  $\mu$ L 80 % Ethanol to each well.
- 54 Wait for 1 minute.
- 55 Remove and discard the ethanol supernatant.
- 56 Add 300  $\mu$ L 80 % Ethanol to each well.
- 57 Wait for 1 minute.
- 58 Remove and discard the ethanol supernatant.

- 58.1** After removing most of the supernatant with large-volume tips, use smaller-volume tips (e.g., 50 µL) to remove any residual supernatant from the bottom of each well.
- 59** Allow magnetic bead pellets to dry for 5 minutes at room temperature.
- 60** Move the Reaction Plate from magnet base to an automated thermal-regulated microplate shaker with temperature set to 60 °C.
- 61** Add 100 µL Nuclease-Free Water to each well (note: can also use EB)
- 62** Resuspend beads by repeated aspiration and dispense (12 times).
- 63** Incubate at 60 °C for 10 s while shaking at 1000 rpm.
- 64** Incubate at 60 °C for 7 minutes while shaking at 400 rpm.
- 65** Move the Reaction Plate back to the magnet base.

- 66** Wait for 5 minutes.
- 67** Transfer the supernatant from each well to a new, clean well in the same plate (still on the magnet base).
- 68** Wait for 5 minutes.
- 69** Transfer the supernatant with extracted DNA from each well to a well in the Elution plate.
- 70** Cover the Elution plate containing the extracted DNA samples with a Universal Lid.