Introduction

This protocol describes a method to extract high molecular weight genomic DNA from yeast (*Saccharomyces cerevisiae*, ATCC®9763™). The recommended method is based on work previously described by Denis *et al*, 2018. Sequencing performance was determined using a MinION. Prior to library preparation, 3 µg of extracted DNA was size selected using the size selection of HMW DNA by semi-selective DNA precipitation protocol. The Ligation Sequencing Kit was used to generate sequencing libraries from both 1 µg of the extracted DNA and 1 µg of size-selected DNA.

Materials

- *Saccharomyces cerevisiae* ATCC™ 9763™ Culti-Loops™
- Refrigerated centrifuge (capacity for 15 ml and 50 ml Falcon tubes)
- Incubator or water bath (capacity for 30°C and 50°C)
- 0.2 µm filter and syringe
- Ice and ice bucket
- 1.5 ml Eppendorf tubes
- 15 ml Falcon tubes
- 50 ml Falcon tubes
- 1x phosphate buffer saline (PBS)
- Ultra-pure nuclease free water
- Tris-HCl (1M, pH8.0)
- EDTA (0.5 M, pH 8.0)
- NaCl 5 M
- PVP-40
- Sorbitol
- SDS
- Potassium acetate 5 M
- Isopropanol
- Ethanol
- RNase A
- Zymolyase
- TE buffer (1 mM EDTA, pH 8.0)

Method

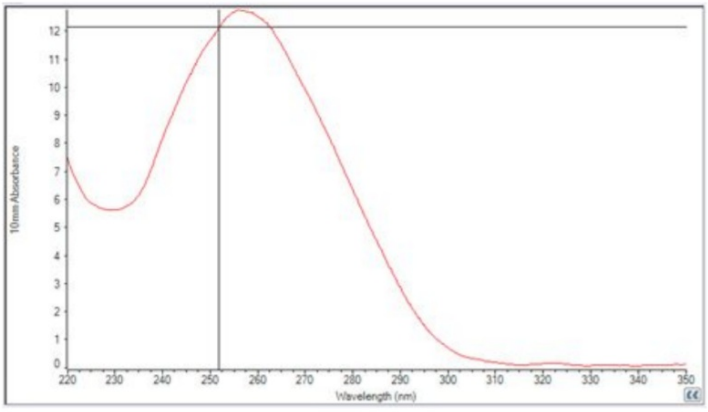
1. Grow 1 Culti-Loop™ of yeast in 150 ml of YPD media, overnight at 30°C with agitation (150 rpm). Measure the OD600 and dilute in YPD media to an OD600 of 0.2. Continue the growth until an OD600 of 0.7 is reached at around 3-4 hours.
2. When the yeast culture reaches an OD600 of 0.7, collect 50 ml of the yeast culture in a 50 ml Falcon tube and centrifuge for 10 minutes at 1500 x g at 4°C to harvest cells.
3. Discard supernatant and add 15 ml of PBS 1x and invert the tube to resuspend the pellet.
4. Centrifuge the resuspended cell pellet for 10 minutes at 1500 x g at 4°C.
5. Discard the supernatant and retain the pellet.
6. *Optional step:* At this stage, the protocol can be paused, and the pellet stored at -80°C.
7. Resuspend the pellet in 4 ml of 1 M sorbitol, either by inversion or using wide-bore tips**Note:** If the pellet has been stored frozen before extracting, add 4 ml of 1 M sorbitol to the frozen pellet. Pipette mixing or inversion will thaw and resuspend the pellet.
8. Add 250 µl of zymolyase (1000 U/ml) and gently invert until the solution is homogeneous.
9. Incubate for 1 hour at 30°C, gently inverting the tube every 15 minutes.
10. Prepare the lysis buffer (0.5 ml of Tris-HCl 1 M, 0.5 ml of EDTA 0.5 M, 0.5 ml of NaCl 5 M, 0.05 g of PVP-40, and 2.875 ml of ultra-pure nuclease free water). Mix it thoroughly by vortexing. Incubate the lysis buffer for 30 minutes at 65°C and then pass through

a 0.2 µm filter to sterilise.

11. Centrifuge the zymolyase-treated cells for 5 minutes at 2000 x g at 4°C.
12. Discard the supernatant and remove as much liquid as possible by aspiration.
13. Add 3.5 ml of prepared, filtered lysis buffer and resuspend the pelleted cells by inverting the tube 10–15 times, until the solution is mixed with the cells.
14. Add 500 µl of 10% SDS and 4 µl of RNase A.
15. Gently invert 15 times.
16. Incubate for 1 hour at 50°C, gently inverting the tubes every 15 minutes.
17. Place the tube on ice for 2 minutes.
18. Add 10 ml of TE and 5 ml of 5 M potassium acetate.
19. Gently invert 15 times.
20. Centrifuge for 15 minutes at 2000 x g at 4°C.
21. Discard the pellet and retain the supernatant; separate the supernatant equally into two 15 ml Falcon tubes and repeat step 20.
22. Discard the pellet and retain the supernatant; pool the two supernatants into one 50 ml Falcon tube.
23. Add 1 volume of room temperature isopropanol, and gently invert 15 times.
24. Centrifuge for 5 minutes at 500 x g at 4°C.
25. Discard supernatant and add 20 ml of ice-cold 70% ethanol to the pellet.
26. Incubate on ice for 5 minutes.
27. Centrifuge for 5 minutes at 500 x g at 4°C.
28. Discard the supernatant. Use a piece of sterile paper to remove the ethanol leftover on the tube walls, being careful not to touch the pellet.
29. Add 200 µl of TE and allow the pellet to resuspend overnight at room temperature.
30. Transfer the resuspended DNA to a 1.5 ml Eppendorf tube. If the DNA looks heterogenous, incubate at 50°C for two hours.

Results

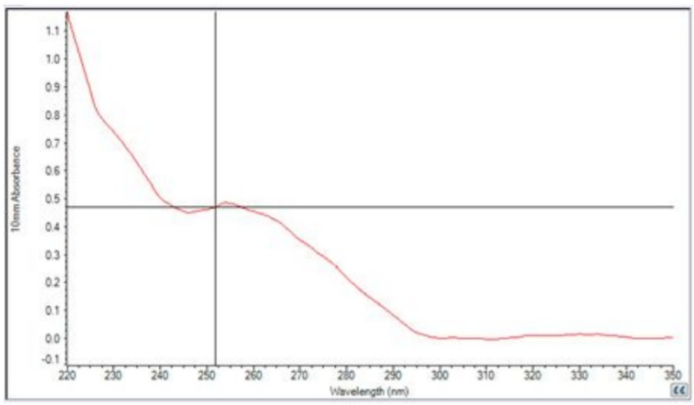
- **Yield:** 20-40 µg
- **OD 260/280:** 1.97
- **OD 260/230:** 2.25



After size selection:

- **Recovery:** ~60%
- **OD 260/280:** 2.11
- **OD 260/230:** 0.61

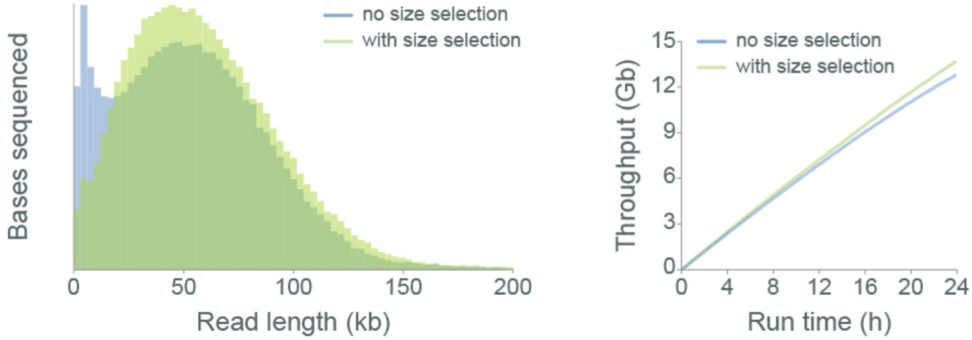
Note: The lower than expected A260/230 ratio is indicative of contamination with the size selection buffer. The performance of the Ligation Sequencing Kit was not adversely affected.



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v3, December 2022	Updated step 10 to the correct weight of PVP-40 in the lysis buffer
v2, September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol
v1, March 2019	Initial protocol publication