High molecular weight gDNA extraction from yeast

28th March 2019

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 by 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

Step 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.

Step 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.

Step 3

Discard supernatant and add 15 ml of PBS 1x and invert the tube to resuspend the pellet.

Step 4

Centrifuge the resuspended cell pellet for 10 minutes at 1500 x g at 4°C.

Step 5

Discard the supernatant and retain the pellet.

Optional step

At this stage, the protocol can be paused, and the pellet stored i.e. freeze at -80°C.

Step 6

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.

Step 7

Add 250 µl of zymolyase (1000 U/ml) and gently invert until the solution is homogeneous.

Step 8

Incubate for 1 hour at 30°C, gently inverting the tube every 15 minutes.

Step 9

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 sterilize.

Step 10

Centrifuge the zymolyase-treated cells for 5 minutes at 2000 x g at 4°C.

Step 11

Discard the supernatant and remove as much liquid as possible by aspiration.

Step 12

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.

Step 13

Add 500 µl of 10% SDS and 4 µl of RNase A.

Step 14

Gently invert 15 times.

Step 15

Incubate for 1 hour at 50°C, gently inverting the tubes every 15 minutes.

Step 16

Place the tube on ice for 2 minutes.

Step 17

Add 10 ml of TE and 5 ml of 5 M potassium acetate.

Step 18

Gently invert 15 times.

Step 19

Centrifuge for 15 minutes at 2000 x g at 4°C.

Step 20

Discard the pellet and retain the supernatant; separate the supernatant equally into two 15 ml Falcon tubes and repeat step 19.

Step 21

Discard the pellet and retain the supernatant; pool the two supernatants into one 50 ml Falcon tube.

Step 22

Add 1 volume of room temperature isopropanol, and gently invert 15 times.

Step 23

Centrifuge for 5 minutes at 500 x g at 4°C.

Step 24

Discard supernatant and add 20 ml of ice-cold 70% ethanol to the pellet.

Step 25

Incubate on ice for 5 minutes.

Step 26

Centrifuge for 5 minutes at 500 x g at 4°C.

Step 27

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.

Step 28

Add 200 µl of TE and allow the pellet to resuspend overnight at room-temperature.

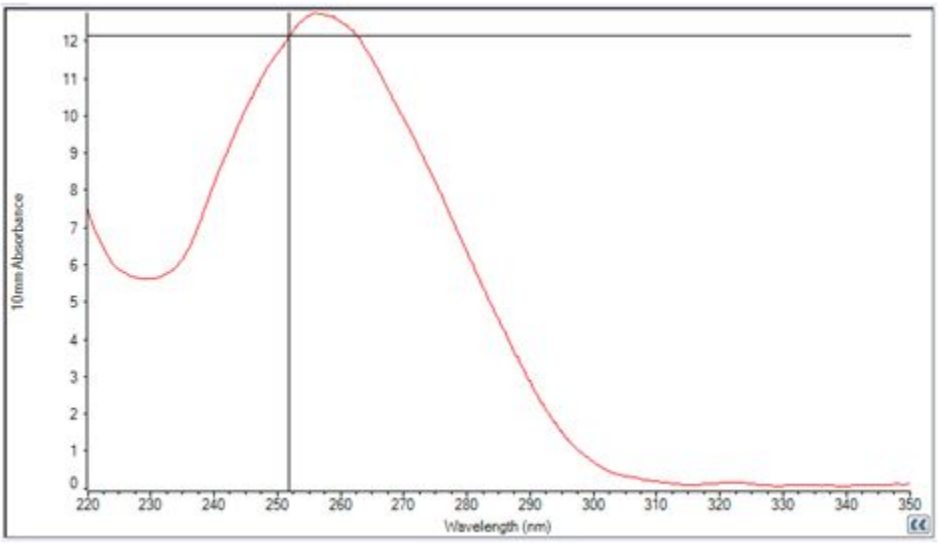
Step 29

Transfer the resuspended DNA to a 1.5 ml Eppendorf tube. If the DNA looks heterogenous, incubate at 50°C for two hours.

Results

Quality Control: PVP extraction method

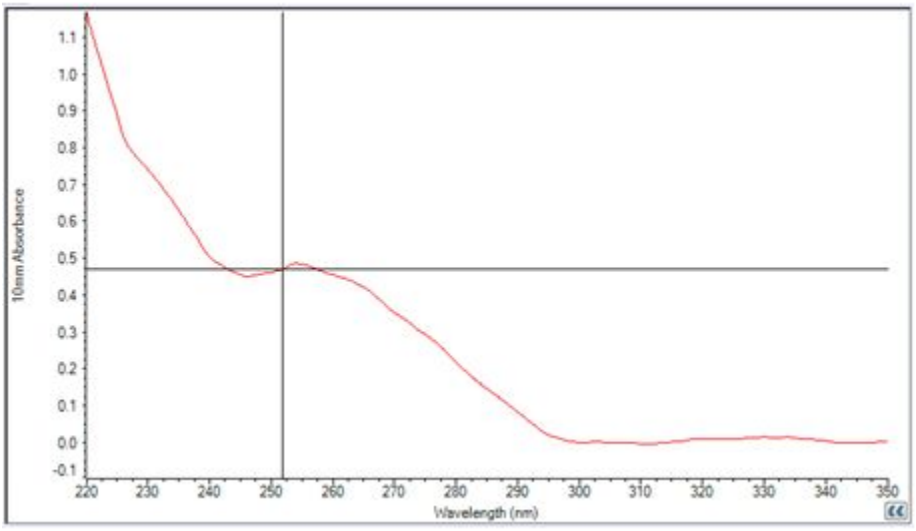
- **Yield:** 20-40 µg
- **OD 260/280:** 1.97
- **OD 260/230:** 2.25



After size selection:

- **Recovery:** ~60%
- **A260/280:** 2.11
- **A260/230:** 0.61

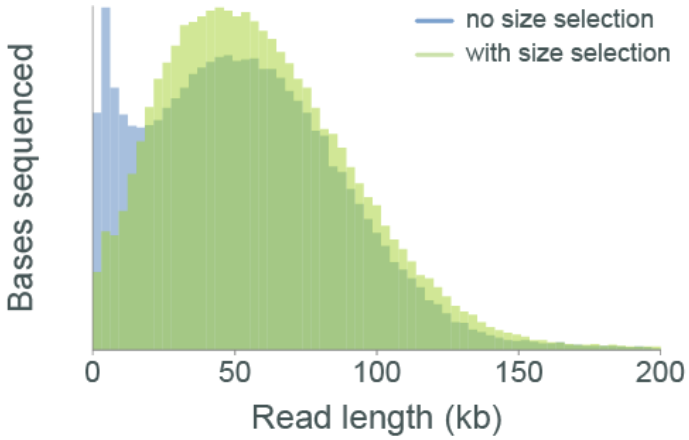
Note: The lower than expected A260/230 ratio is indicative of contamination with the size selection buffer. The performance of the Ligation Sequencing Kit was not adversely affected.

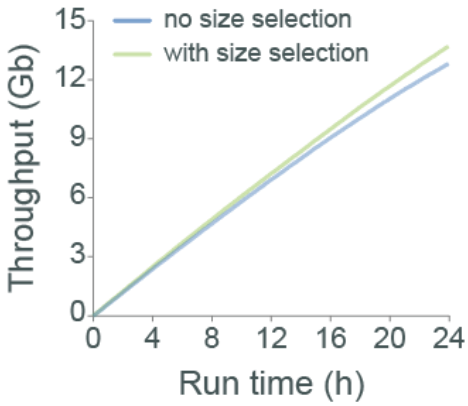


Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109):

- Typical throughput: ★★★ (8+ Gb in 48 h on FLO-MIN106D) for the Ligation Sequencing Kit, equivalent to the Lambda DNA supplied in the Control Expansion pack (EXP-CTL001).
- Read length profile:





Date	Change note
September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol.