Extracting high molecular weight genomic DNA from Saccharomyces cerevisiae

Submitted 12 June 2018 • DOI: https://doi.org/10.1038/protex.2018.076

ABSTRACT

Long read sequencing (Pacific Bioscience, Oxford Nanopore Technologies) and synthetic long read sequencing (chromium , 10x genomics) as well as technologies like next-generation mapping (Bionano Genomics) or Dovetail Chicago method need DNA molecules as long as possible. Even if traditional DNA extraction methods using commercials kits can lead to a maximum of 50-100kb long High Molecular Weight DNA (called HMW DNA), such fragment lengths may not be sufficient for some technologies. Isolation of high quality ultra-High Molecular Weight (uHMW) DNA, defined as a fragment of more than 50-100kb, can be a real challenge. The classical uHMW DNA isolation method is based on the isolation of cells or nuclei in agarose gel plugs. Nowadays, despite the fact that this is time consuming and requires handling expertise, this method is the only one recommended for DNA preparation for optical mapping. The impact of DNA molecule length for long read sequencing, optical mapping and Chicago method appears to be crucial. Here we present the development of a quick and inexpensive method to purify and isolate high yield of high quality uHMW from *Saccharomyces cerevisiae*.

FIGURES

Figure 1

	Qubit		nanodrop ratios	
volume (µl)	ng/µl	quantity (µg)	260/280	260/230
200	400	80	1,91	1,95

Figure 2

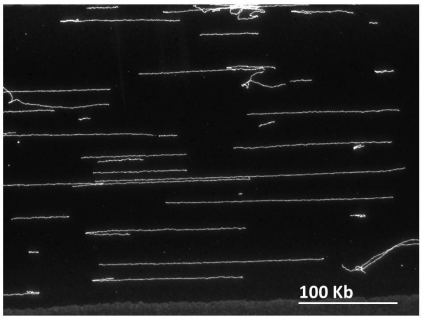

Figure 3

Nb of reads	137 109	
Average length (bp)	14 854	
Maximum length (bp)	461 529	
molecules N50 (bp)	50 800	
Nb of reads > 10kb	42 275	30,8%
Nb of reads > 30kb	19 456	14,2%
Nb of reads > 50kb	11 695	8,5%
Nb of reads > 100kb	3 275	2,4%
% mapping reads	94	
Maximum mapping length	254 104	

INTRODUCTION

This protocol is used for uHMW DNA extraction from *Saccharomyces cerevisiae* and suitable for long read sequencing. First, lysis of the cell wall is performed through a zymolyase treatment. Second, a gentle lysis of the spheroplasts is performed during a

30 min incubation at 50°C in presence of a SDS buffer. Third, proteins are precipitated with Potassium Acetate and centrifugation. Fourth, DNA is precipitated with isopropanol, then washed with ethanol and eluted in TE (10mM TrisHCl, 1mM EDTA).

Our protocol enables high recovery yields of large molecules greater than 200kb in length whilst maintaining high quality nanodrop ratios.

REAGENTS

TrisHCl 1M pH8 – General lab supplier

EDTA 0.5M pH8 – General lab supplier

Sodium chloride – General lab supplier

PVP40 – Sigma cat. no. PVP40-500g

Sorbitol 1M – General lab supplier

SDS (Sodium Dodecyl Sulfate) 10% – Sigma cat. no. 71736

Potassium acetate – General lab supplier

Isopropanol – General lab supplier

Ethanol– General lab supplier

Ultrapure nuclease-free water – Invitrogen cat. no. 10977-035

RNAse A – Qiagen cat. no. 19101

Zymolyase 1000U/ml – MP Biomedicals Europe cat. No.08320932

EQUIPMENT

1.5ml microtubes – General lab supplier

15ml tubes – General lab supplier

50ml tubes– General lab supplier

0.2µm filter unit – General lab supplier

10ml Syringe – General lab supplier

Swinging buckets Centrifuge –Sigma 4K15

Heat block – Eppendorf ThermoMixer C

General lab equipment (pipettors, pipets, barrier tips, 200µl wide bore tips, lab vessels) – General lab supplier

PROCEDURE

General considerations:

From step1, never use vortex. Until step 5, pipetting is not required. Then, use wide bore tips for pipetting. Always gently invert tubes for mixing. Minimize the movement when transferring from a tube to another one.

Prepare lysis buffer:

Always use fresh buffer: TrisHCl 114mM, EDTA 115mM, NaCl 571mM, 1.14% PVP40.

Add 0.5ml TrisHCl 1M, 0.5ml EDTA 0.5M, 0.5ml NaCl 5M, 0.05g PVP40 and 2.875ml Ultrapure nuclease-free water. Mix by vortexing.

Incubate 30min at 65°C. Filter sterilize the buffer.

Saccharomyces cerevisiae culture

Culture media is YPDA. Use only fresh (<4 weeks old) colonies. For pre-culture, one colony on an YPDA-Agar plate is dissolved in 5ml YPDA and grown for 5h at 30°C with shaking at 200rpm. Then 200ml YPDA is inoculated at a theoretical DO of 0,005 and grown for 16 hours (O/N) at 30°C 200rpm. Cultures are centrifuged in 50ml tubes for 5min at 1500g at 4°C. Pellet is dissolved in 20ml TE (10mM TrisHCl, 1mM EDTA) and centrifuged for 5min at 1500g at 4°C. Pellets are used fresh or can be flash frozen in liquid N2 and conserved at -80°C. One pellet contains approximately 1010 cells.

STEP1: Cell wall lysis

Resuspend a yeast pellet in 4ml Sorbitol 1M.

Add 250µl Zymolyase 1000U/ml. Invert gently 5x.

Incubate 30min at 30°C (Thermomixer C: program 10sec at 300rpm each 10min).

Centrifuge 2min30 at 3000g.

Discard supernatant, let the tube upside down to remove most of the liquid.

STEP2: Spheroplasts lysis

Resuspend the pellet in 3.5ml lysis buffer.

Add 500µl SDS 10%

Add 4µl RNAse A 100mg/ml

Invert gently 10-15x.

Incubate 30min at 50°C (Thermomixer C: program 10sec at 300rpm each 10min).

STEP3: Protein precipitation

Add 10ml TE

Add 5ml KAc 5M pH7.5

Invert gently 15x

Place on ice for 5min

Centrifuge 10min at 5000g at 4°C (acceleration 9, deceleration 9)

Transfer the supernatant in two 15ml tubes and repeat the last centrifugation.

STEP4: DNA precipitation

Transfer the two supernatants in one 50ml tube and add 1V Isopropanol at RT.

Invert gently about 15x. You shall see some DNA filaments at this stage.

Centrifuge 5min at 500g at 4°C (acceleration 9, deceleration 1)

Discard supernatant and dislodge pellet with 20ml ethanol 70%

Place on ice for at least 5min

Centrifuge 5min at 500g at 4°C (acceleration 9, deceleration 1)

Discard supernatant, remove as much ethanol as possible

STEP5: DNA elution

Let the pellet air dry for 5min

Add 200µl TE at 50°C

Incubate 10min at 50°C without cap in order to evaporate the remaining ethanol

Transfer carefully with wide bore tip in a 1.5ml tube.

TIMING

The whole procedure, from step1 to step5, takes about 3 to 4 hours.

TROUBLESHOOTING

STEP3: you could observe some white precipitate floating after centrifugation. It should not impact the DNA purity due to its solubility in isopropanol.

STEP4: If no DNA filaments are observed in the tube, increase the first centrifugation speed (500g to 2000g).

ANTICIPATED RESULTS

Quality control was performed on nanodrop, Qubit and Qcard (Opgen) and shows high yield, purity and molecule length (Table 1 and Figure 1). The method has been validated by sequencing on MinIon (Oxford Nanopore technologies) with molecule N50 metrics above 50kb and with high quality reads over 200kb and 94% of total reads that map to the reference genome (Table 2).

ACKNOWLEDGEMENTS

We thank Joseph Schachrer for providing the yeast *Saccharomyces cerevisiae* strain S288C. We thank Stéphanie Fouteau, Stefan Engelen, Julie Poulain and Adriana Alberti for helpful discussions.

Funding was provided by France Génomique (ANR-10-INBS-0009-08) as part of Investissements d'Avenir Program.

CONFLICTING FINANCIAL INTERESTS

The authors declare no competing financial interests. Genoscope Institute was part of the Oxford Nanopore Technologies MinION Access Programme (MAP).