COMMUNITY PROTOCOL

High molecular weight gDNA extraction from yeast culture

September 04, 2019

The protocol was used for genomic DNA sequencing of yeast species including *Saprochaete saccharophila, Dipodascus albidus, Magnusiomyces capitatus, Magnusiomyces magnusii, Magnusiomyces ovetensis, Magnusiomyces starmeri, Saccharomyces cerevisiae*, *Saprochaete fungicola, Saprochaete suaveolens* and *Yarrowia lipolytica*.

- Brejova B, Lichancova H, Hodorova V, Nebohacova M, Tomaska L, Vinar T, and Nosek J. (2019) Genome Sequence of an Arthroconidial Yeast *Saprochaete fungicola* CBS 625.85. *Microbiology Resource Announcements* 8(15): e00092–19
- Hodorova V, Lichancova H, Bujna D, Nebohacova M, Tomaska L, Brejova B, Vinar T, and Nosek J. *De novo* sequencing and high-quality assembly of yeast genomes using a MinION device. Poster presented at London Calling, 24th–25th May 2018, London, UK.
- Lichancova H, Hodorova V, Sienkiewicz K, Penir SMU, Afanasyev P, Boceck D, Bonnin S, Hakobyan S, Krawczyk PS, Smyczynska U, Zhivkoplias E, Zlatohurska M, Odrzywolski A, Tralle E, Frolova A, Pryszcz LP, Brejova B, Vinar T, and Nosek J. (2019) Genome Sequence of Flavor-Producing Yeast *Saprochaete suaveolens* NRRL Y-17571. *Microbiology Resource Announcements* 8(9): e00094–19

Materials

- 100 ml overnight yeast culture grown in complex medium (YPD with yeast extract, peptone and glucose)
- QIAGEN Genomic-tip 100/G and QIAGEN Genomic DNA Buffer Set (alternatively, QIAGEN Genomic-tip 20/G can be used)
- 2-mercaptoethanol
- 1 M sorbitol
- 0.5 M EDTA (pH 8.0)
- Zymolyase 20T
- 0.15 M NaCl
- 10% SDS
- Phenol buffered with 10 mM Tris-HCl and 1 mM EDTA (pH 8.0)
- Chloroform : isoamylalcohol (24:1 ratio)
- 10 mg/ml RNase A
- 96% and 70% ethanol
- TE buffer (pH 7.5)
- Agencourt AMPure XP beads
- Nuclease-free water
- 1.5 ml Eppendorf DNA LoBind tubes
- Falcon tubes to harvest the yeast cells

- Vortex mixer
- Magnetic stand for Eppendorf tubes
- Hula mixer
- Microfuge (e.g. Eppendorf 5415R)
- Centrifuge capable of taking Falcon tubes (e.g. Eppendorf 5804R)

Method

Step 1

Harvest the yeast cells from 100 ml of culture by centrifuging in an Eppendorf 5804R for 5 min at 2200 g, 4° C.

Note: In filamenting strains, the hyphae can be harvested from the cultivation medium by filtration.

Step 2

Resuspend the cell pellet in 20 ml 2% 2-mercaptoethanol. Incubate for 15 min at room temperature.

Step 3

Harvest the cells by centrifuging in an Eppendorf 5804R for 5 min at 2200 g. Resuspend the pellet in 10 ml of 1 M sorbitol with 10 mM EDTA (pH 8.0), and 0.125 mg/ml of Zymolyase 20T. Incubate the pellet for 90 min at 30° C with occasional shaking.

Step 4

Centrifuge the resulting spheroplasts for 5 min at 2200 g. Discard the supernatant, and resuspend the spheroplasts in 5 ml of 0.15 M NaCl with 0.1 M EDTA (pH 8.0) and 0.1% SDS.

Step 5

Perform three extractions with phenol (buffered with 10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and one extraction with chloroform : isoamylalcohol (24:1 ratio). In all cases, the ratio of sample-to-phenol or sample-to-(chloroform : isoamylalcohol) should be 1:1. Precipitate the nucleic acids with 0.1 M NaCl and 2 volumes of 96% ethanol for 20 min at −20° C. Centrifuge the sample in an Eppendorf 5415R for 10 min at 16,200 g at 4° C. Wash the pellet with 70% ethanol, and air-dry.

Step 6

Dissolve the pellet from each tube in 1 ml of TE buffer (pH 7.5) containing 0.1 mg/ml RNase A. Incubate the DNA for 30 min at 37° C.

Step 7

Perform one extraction with phenol (buffered with 10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and one extraction with chloroform : isoamylalcohol (24:1 ratio). Precipitate the nucleic acids with 0.1 M NaCl and 2 volumes of 96% ethanol for 15 min at −20° C. Centrifuge the sample in an Eppendorf 5415R for 10 min at 16,200 g at 4° C. Wash the pellet with 70% ethanol, and air-dry.

Step 8

Dissolve the DNA pellet in 150 µl of TE buffer (pH 7.5).

Step 9

Add 1.5 ml buffer QBT to the DNA, and mix by inverting the tube.

Step 10

Purify the DNA using the QIAGEN Genomic-tip 100/G or the QIAGEN Genomic-tip 20/G according to the standard protocol (steps 1−6, pages 49−52). Elute the purified DNA in 100 µl TE buffer (pH 7.5). Analyse the DNA sample using the NanoDrop for concentration, yield, and purity.

Note 1: At this step, genomic DNA can be stored for several weeks at +4° C.

Note 2: Although it is possible to prepare the sequencing library using DNA from Step 10, further purification (Step 11) substantially improves both the pore occupancy and throughput.

Step 11

Mix ~3−6 µg of prepared DNA (adjust the volume to 30−50 µl with TE buffer, pH 7.5) and an equal volume of resuspended Agencourt AMPure XP beads. Incubate on a Hula mixer

for 5 min at room temperature. Spin down the tube and pellet the sample on a magnet. Remove the supernatant and wash the beads twice with 70% ethanol. Spin down the sample and pellet on a magnet. Remove residual ethanol, and briefly air-dry. Remove the tube from the magnetic stand and resuspend the beads in 60 µl of nuclease-free water. Incubate for 5 min at room temperature. Spin down and pellet the beads on a magnet. Remove the supernatant and quantify the DNA concentration using a Qubit fluorometer. Proceed immediately with library preparation.

Results

- **Yield:** 10–50 µg
- **OD 260/280:** 1.6–1.9
- **OD 260/230:** 1.9–2.5
- **Fragment size:** >20 kb based on a gel electrophoresis in 0.6% agarose (in Step 9)

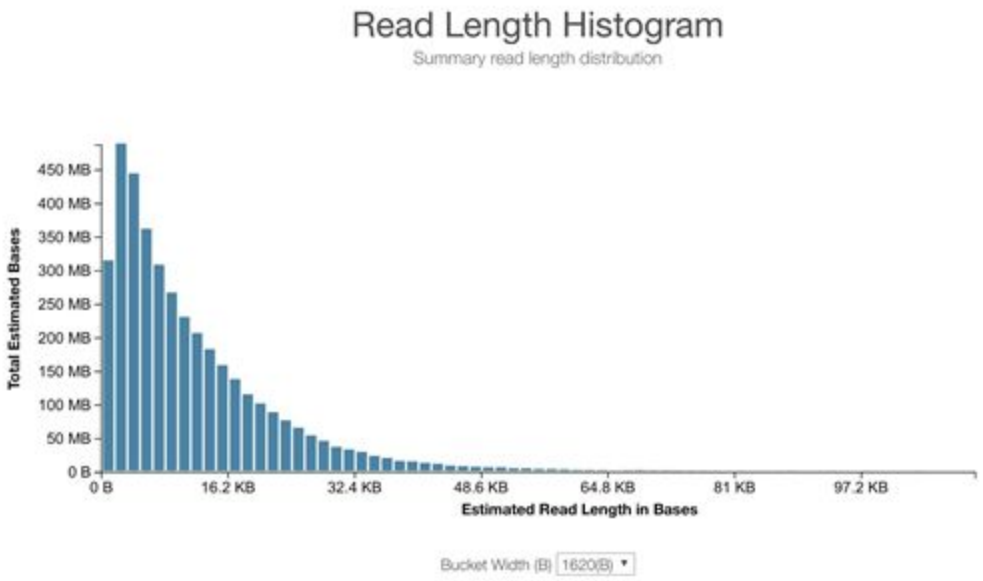
Left lane: MW marker (GeneRuler 1 kb Plus DNA Ladder, upper band 20 kb)

Right lane: 1 µl (200 ng/µl) of the gDNA prep

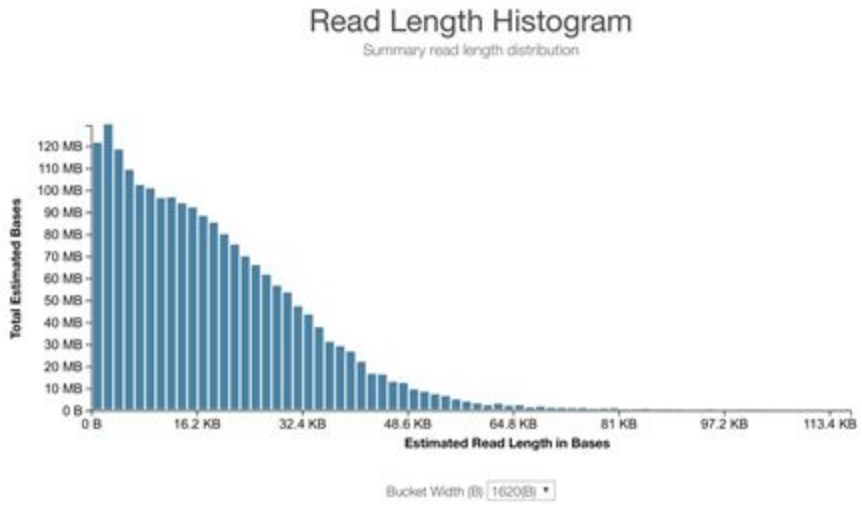
Sequencing performance

Libraries were prepared using either the Ligation Sequencing Kit (SQK-LSK109) or the Rapid Barcoding Kit (SQK-RBK004). Note that DNA fragmentation step using the Covaris g-TUBE was omitted.

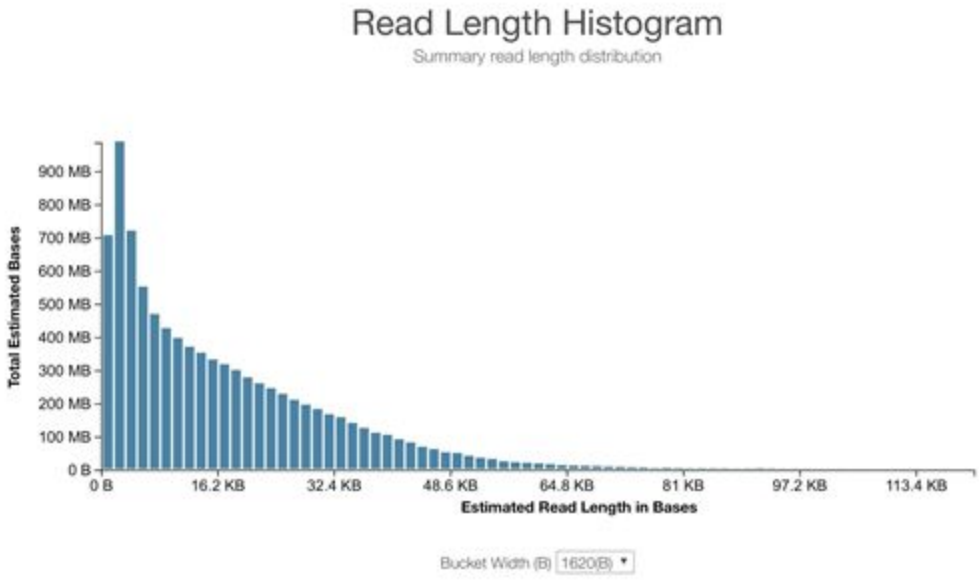
- Typical throughput: ★★★ (8+ Gb in 48 h on FLO-MIN106D), equivalent to the Lambda DNA supplied with the Control Expansion pack (EXP-CTL001).
- Read length profile:
    - *Saprochaete saccharophila* (Ligation Sequencing Kit, SQK-LSK109)



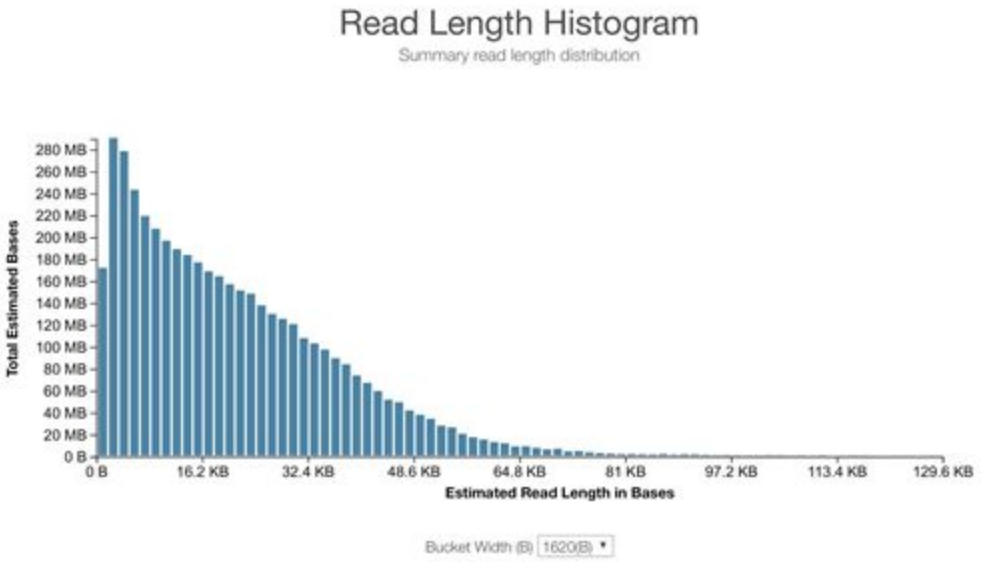
    - *Dipodascus albidus* (Ligation Sequencing Kit, SQK-LSK109)



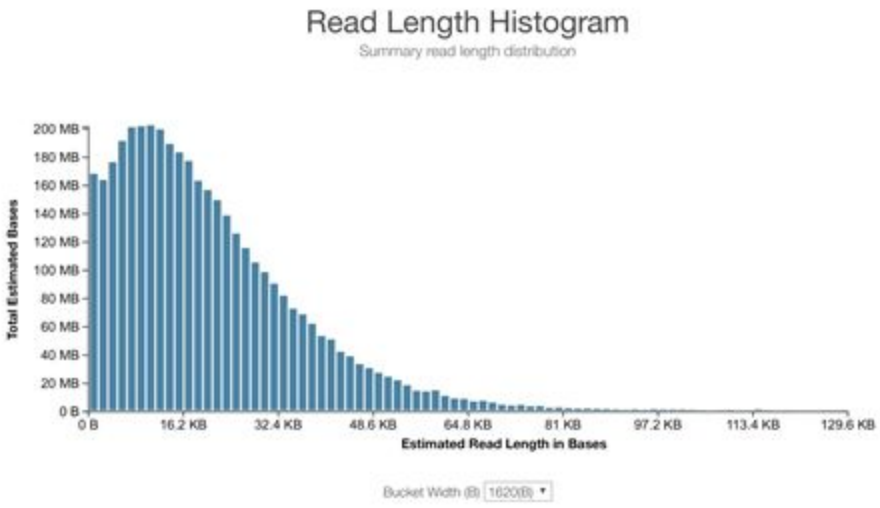
- *Magnusiomyces capitatus* (Ligation Sequencing Kit, SQK-LSK109)



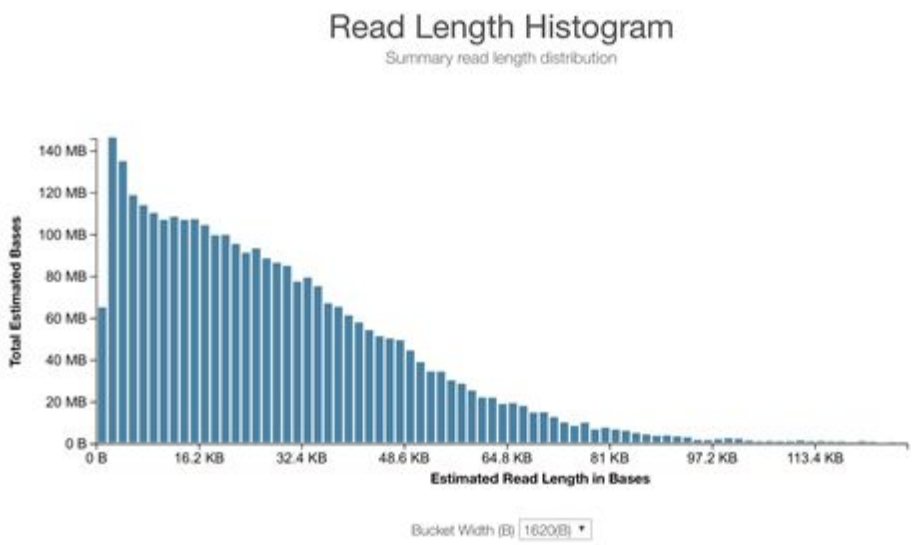
- *Magnusiomyces magnusii* (Ligation Sequencing Kit, SQK-LSK109)



- *Magnusiomyces starmeri* and *Magnusiomyces ovetensis* (Rapid Barcoding Kit, SQK-RBK004)



- *Saprochaete fungicola* (Ligation Sequencing Kit, SQK-LSK109)



- *Yarrowia lipolytica* (Ligation Sequencing Kit, SQK-LSK109)