High molecular weight gDNA extracted from agarose plugs

June 2019

This info sheet describes a phenol-chloroform based method to purify high molecular weight genomic DNA embedded and stored in a 1%, low-melting point agarose plug. We tested this protocol using both Lambda DNA that we embedded in 1%, low-melting point agarose and *S. cerevisiae* DNA embedded in 1%, low-melting point agarose.

Materials

- 1 x agarose plug
- 2 ml Eppendorf tubes
- Incubator, water bath or equivalent (set to 70°C)
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- NaCl 5 M
- Phenol
- Chloroform
- Centrifuge (capable of 9400 x g)
- HulaMixer or equivalent
- Ethanol
- Ammonium acetate 5 M
- Freezer (-20°C)

Method

Step 1

Transfer an agarose plug (1% low-melt point) containing ~3-6 µg of DNA, to a 2 ml Eppendorf tube and add 200-400 µl TE to cover the agarose. If the agarose is on the tube wall, briefly centrifuge the tube. Add NaCl to a final concentration of ~200 mM.

Step 2

Melt the agarose at 70°C. The solution will become transparent and homogeneous, which will take approximately 5 minutes.

Step 3

In a fume hood, add 1 volume of phenol and gently rotate in a HulaMixer for 2 hours at room temperature.

Step 4

Centrifuge the tube for 5 minutes at 9400x g.

Step 5

In a fume hood, retain and transfer the supernatant to a new 2 ml tube and add 1x volume of chloroform.

Step 6

Thoroughly but gently invert to mix. We recommend ~25 inversions.

Step 7

Centrifuge the tube for 5 minutes at 9400x g.

Step 8

In a fume hood, retain and transfer the supernatant to a new 2 ml tube and add 2.5x volumes of 100% ethanol and 1/100 volume of 5 M ammonium acetate.

Step 9

Invert 10 times and incubate overnight at -20°C.

Step 10

Centrifuge the tube for 5 minutes at 9400x g .

Step 11

Discard the supernatant and retain the pellet.

Step 12

Add 1 ml of ice-cold 70% ethanol and invert.

Step 13

Centrifuge the tube for 5 minutes at 9400x g.

Step 14

Repeat Steps 11-13.

Step 15

Discard the supernatant and retain the pellet. Allow the pellet to air-dry for 1 minute.

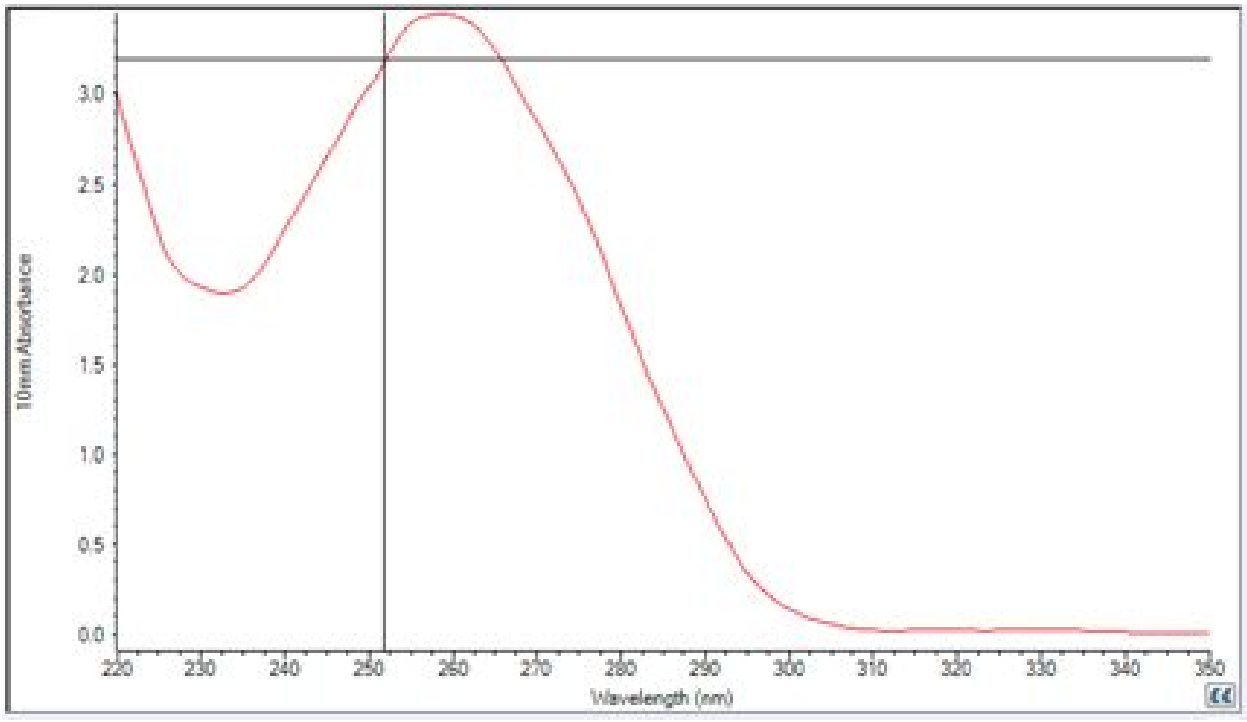
Step 16

Resuspend the pellet in 25 µl TE and incubate at room temperature for 2 hours.

Lambda DNA

Results

- **Yield:** 50-80% of initial DNA amount
- **OD 260/280:** 1.88
- **OD 260/230:** 1.79

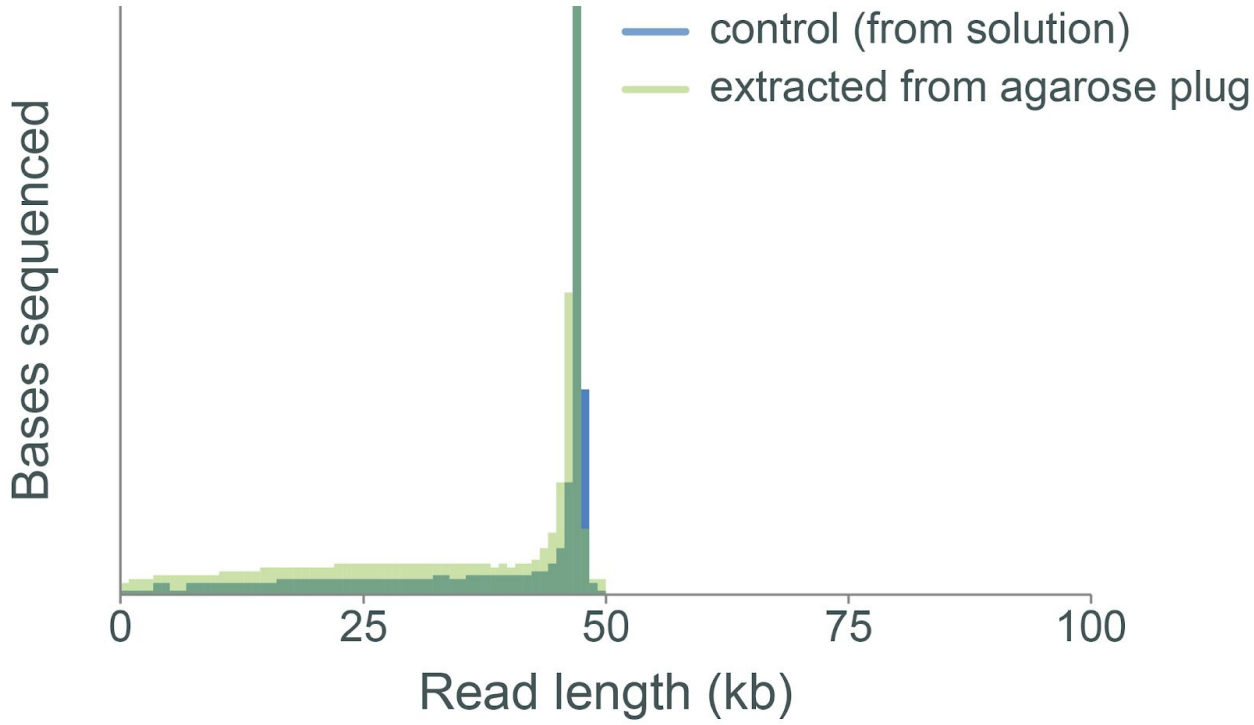


Sequencing performance

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

- Typical throughput: ⋆⋆⋆

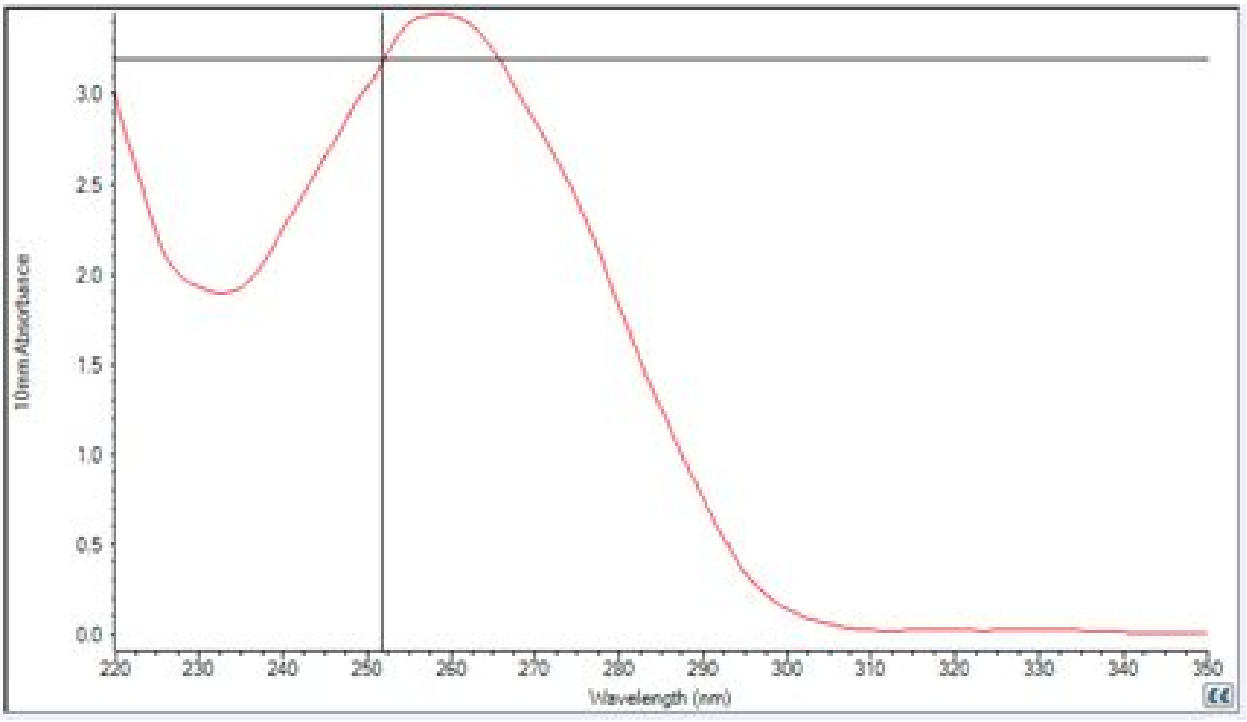
  (8+ Gb in 48 h on FLO-MIN106D) for the Ligation Sequencing Kit, equivalent to the Lambda DNA supplied with the Control Expansion pack (EXP-CTL001).
- Read length profile:



S. cerevisiae DNA

Results

- **Yield:** 50-80% of initial DNA amount
- **OD 260/280:** 2.11
- **OD 260/230:** 1.85



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