Introduction

This protocol 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)

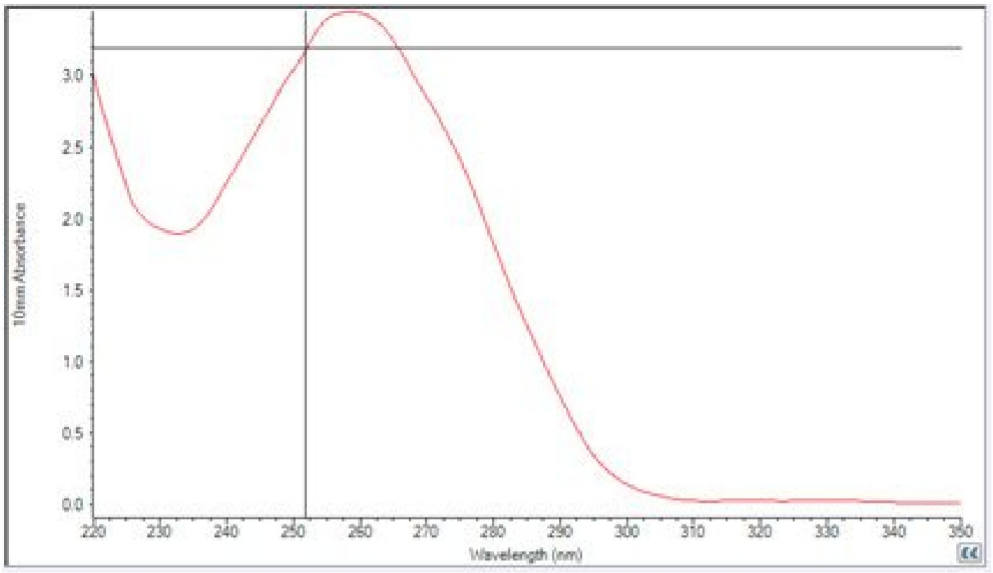
Methods

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.
2. Melt the agarose at 70°C. The solution will become transparent and homogeneous, which will take approximately 5 minutes.
3. In a fume hood, add 1 volume of phenol and gently rotate in a HulaMixer for 2 hours at room temperature.
4. Centrifuge the tube for 5 minutes at 9400 x g.
5. In a fume hood, retain and transfer the supernatant to a new 2 ml tube and add 1x volume of chloroform.
6. Thoroughly but gently invert to mix. We recommend ~25 inversions.
7. Centrifuge the tube for 5 minutes at 9400 x g.
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.
9. Invert 10 times and incubate overnight at -20°C.
10. Centrifuge the tube for 5 minutes at 9400 x g.
11. Discard the supernatant and retain the pellet.
12. Add 1 ml of ice-cold 70% ethanol and invert.
13. Centrifuge the tube for 5 minutes at 9400 x g.
14. Repeat Steps 11-13.
15. Discard the supernatant and retain the pellet. Allow the pellet to air-dry for 1 minute.
16. Resuspend the pellet in 25 µl TE and incubate at room temperature for 2 hours.

Lambda DNA

Results

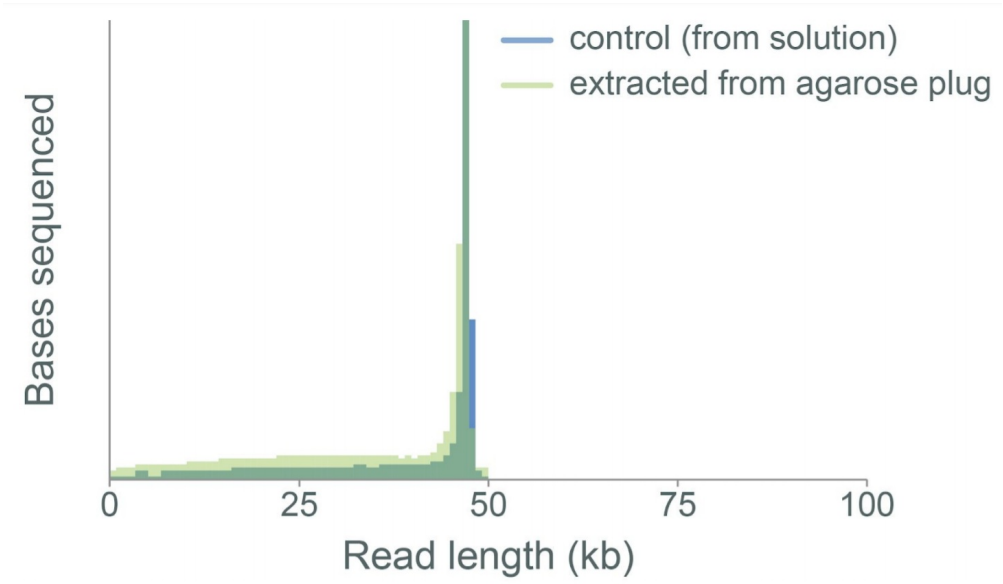
- **Yield:** 50-80% of initial DNA amount
- **OD 260/280:** 1.88
- **OD 260/230:** 1.79



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

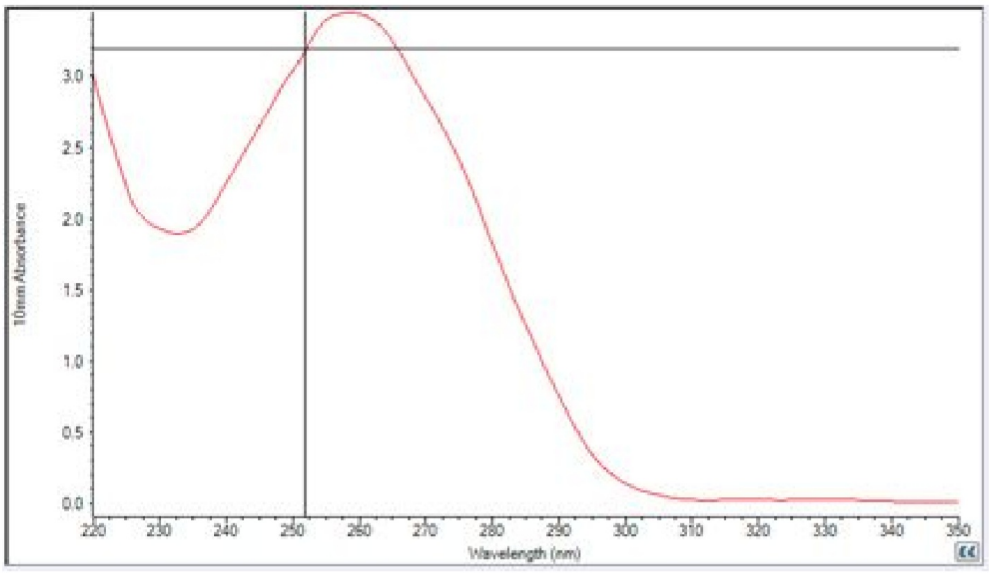
- Read length profile:



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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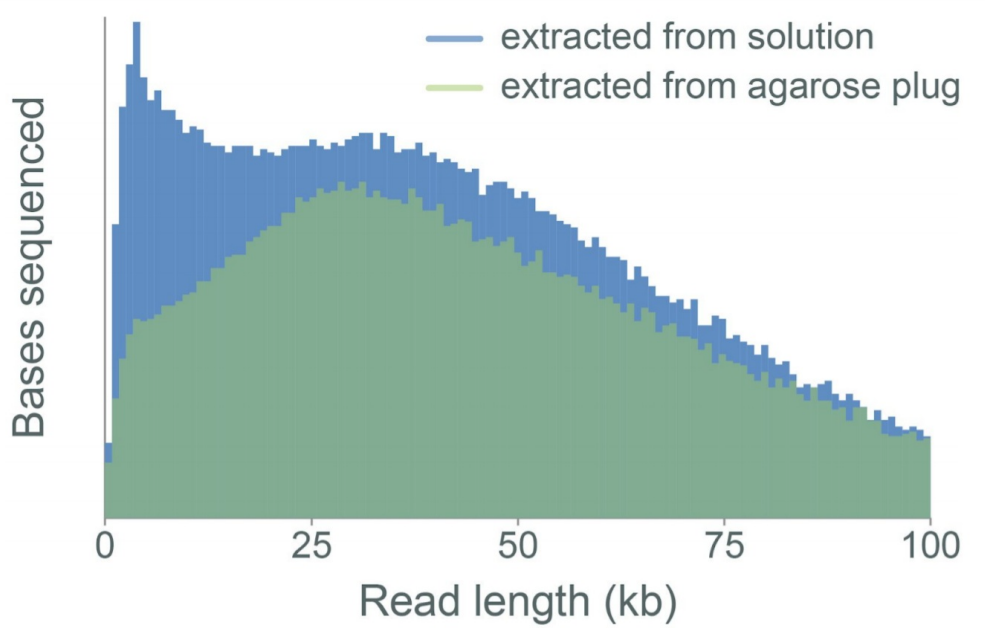

Change log

Version	Change
v1, June 2019	Initial protocol publication