Jun 27, 2017 Version 1

High quality DNA from Fusarium oxysporum conidia suitable for library preparation and long read sequencing with PacBio V.1

DOI
dx.doi.org/10.17504/protocols.io.inccdaw

Sukhwinder Kaur[1], Quyen Anh Pham[1], and Lynn Epstein[1]

[1]Dept. of Plant Pathology, University of California, Davis, CA, 95616 USA

High molecular weight DNA extraction from all kingdoms
Tech. support email: **See@each.protocol**

Lynn Epstein
University of California at Davis

DOI: https://dx.doi.org/10.17504/protocols.io.inccdaw

Protocol Citation: Sukhwinder Kaur, Quyen Anh Pham, and Lynn Epstein 2017. High quality DNA from Fusarium oxysporum conidia suitable for library preparation and long read sequencing with PacBio. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.inccdaw

Protocol status: Working

Created: June 27, 2017

Last Modified: March 28, 2018

Protocol Integer ID: 6564

Keywords: Fusarium oxysporum, PacBio, DNA isolation, DNA purification, high quality dna from fusarium oxysporum conidia, fusarium oxysporum conidia, fusarium oxysporum, conidia from multiple isolate, lyophilized conidia, suitable for pacbio library preparation, pacbio library preparation, high quality dna, high molecular weight dna, pacbio, pacbio this protocol

Abstract

This protocol is for isolation of high quality, high molecular weight DNA (20 kb and larger) that is suitable for PacBio library preparation. This protocol has been tested on lyophilized conidia from multiple isolates of *Fusarium oxysporum,* including f. sp. *apii*, f. sp. *ciceris*, and f. sp. *lycopersici*.

Guidelines

- When pipetting solutions with high molecular weight DNA, use low retention pipet tips with ca. 2 mm of the ends removed, i.e., wide-bore tips.
- Use low DNA-binding microfuge tubes.

Mixing is critical for purification, but avoid vigorous vortexing (which shears DNA)

We thank Martijn Rep, Benjamin Schwessinger, and Oanh Nguyen for advice. This protocol is modified from protocols from Dr. Martijn Rep, University of Amsterdam, and Dr. Benjamin Schwessinger, Australian National University.

Materials

MATERIALS

- Sodium 4-aminosalicylate dehydrate **Catalog #** 09415
- Triisopropylnaphthalenesulfonic acid sodium **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8324
- Autoclaved, acid-washed sand
- Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH 8.0 and 1 mM EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Sigma P2069
- Sterile 4M NaCl
- Isopropanol (IPA) 100%
- Ethanol 70% [Note: freshly prepared]
- Water refers to sterilized deionized water
- Proteinase K, Molecular Biology Grade - 2 ml **New England Biolabs Catalog #**P8107S
- RNase A 10mg/ml, DNase and Protease-free **Thermo Scientific Catalog #**EN0531
- A vortexer with low speed (200 rpm) and a holder for holding microfuge tubes horizontally.
- Magna Rack **Catalog #**15000

Troubleshooting

Before start

Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH 8.0 and 1 mM EDTA) (Sigma P2069) [**Note: use stocks that are less than 3 months old]**

1 Sterilize all dry supplies by autoclaving

Preparation of conidia for DNA extraction. Note: The goal is to obtain ca. 0.5 ml packed conidial volume, which yields ca. 250 mg lyophilized conidia.

2 Spread a Fusarium oxysporum conidial suspension onto 10 to 20, 100-mm-diam Petri dishes with potato dextrose agar.
Produce conidia by incubating dishes under fluorescent lights at ca. 25ºC for 6 to 7 days.
To harvest the conidia, from each dish, pour 5 ml water on the culture, and gently rub the culture with a metal “hockey stick.”
Pour the dislodged conidia through two layers of Miracloth into 50 ml tubes.
Repeat the conidial collection from each dish with 5 ml water.
Centrifuge the conidia at 4C at 3620 g for 15 min.
Remove the supernatant (SN), resuspend and re-pellet.
Suspend the pellet in 3 to 5 ml water.
Transfer the conidial suspensions in 1 ml aliquots, each in a 2 ml microfuge tube. (Note: The goal is ca. 50 mg conidia dry wt/tube).
Centrifuge at 3620 g for 15 min.
Remove water, freeze the conidial pellet at -70C overnight (ON) and lyophilize it dry.
[Note: Lyophilized conidia can be stored at -70 C before grinding and extraction]

DNA extraction buffer for approx. 250 mg of lyophilized conidia.

3 Prepare Buffer -5X RNB
1M Tris-HCl pH 8.0
1.25 M NaCl
0.25 M EDTA
Autoclave and store at 4C

Use 1 ml of freshly prepared extraction buffer with RNase per 50 mg of lyophilized *Fusarium oxysporum* conidia.
Prepare fresh just before extraction.
For 5 ml extraction buffer add sequentially:
4 ml Milli-Q nuclease-free water;
1 ml 5xRNB;
and 240 mg PAS (Sodium 4-aminosalicylate dihydrate).
Mix and then vortex until dissolved.
Then add 40 mg TIPS (Triisopropylnaphthalenesulfonic acid sodium)
Mix and then vortex until dissolved.

Wait until the foam disappears.
Add 5µl (250 µg) of RNaseA (10mg/ml stock)

Conidial grinding.

4

1. Cool a mortar (9 cm upper diam) with liquid N_2.
2. After the N_2 evaporates, add 250 mg sand.
3. Add additional liquid N_2 to cool the sand.
4. Then add 50 mg lyophilized conidia.
5. Add 20 to 25 ml liquid N_2 and grind quickly with force for 15 to 20 sec.
6. Then add more liquid N_2 and grind again. Repeat for a total of 3 grinding periods.

   Transfer the ground conidia and sand mixture to a 2 ml microfuge tube (T#1) containing 1 ml of extraction buffer with RNase A. Note: to optimize your grinding procedure, examine ground lyophilized conidia microscopically (Fig. 1); you need to grind sufficiently so that most of the conidia are cracked open. Any more grinding than that only shears the DNA.]
7. We typically prepare 4 tubes, each with 50 mg lyophilized conidia per preparation. The tubes are pooled at the end of the preparation.

DNA extraction and purification.

5

1. Label 3 additional tubes (T#2, T#3, & T#4) per 50 mg conidia.
2. Mix the T#1 tube from step 4 on a horizontal low speed (200 rpm) vortex mixer for 2 min. Incubate at room temperature (RT) for 30 min, with mixing by inverting tubes every 5 min.
3. Add 6 ul (120 ug) of proteinase K to T#1. Mix well on the low speed horizontal vortex mixer for 20 min at RT.
4. Cool tubes on ice for 5 min.
5. Add an equal volume of ice-cold, buffered P:C:I (25:24:1) and mix on the horizontal vortex mixer for 15 sec. Centrifuge at 15000*g* for 6 min at 4C.(Note: the buffer is on top. Only use the lower PCI, here and in the subsequent step.)
6. Transfer supernatant (SN) to a new 2 ml microfuge tube (T#2). Add an equal volume of ice-cold, buffered P:C:I. Mix on the horizontal vortex mixer for 2 min at 4C.[Note: always avoid transferring any of the interface.]
7. Centrifuge at 15000*g* for 10 min at 4C.
8. Transfer SN (Vol = 800 to 900ul) to a new 2 ml tube (T#3). Add an equal volume of ice-cold chloroform:isoamyl alcohol (C:I) (24:1). Mix on the horizontal vortex mixer for 15 sec at 4C.
9. Centrifuge at 15000*g* for 5 min at 4C.
10. Very carefully, remove the SN without disturbing the interface to a new 2 ml tube (T#4).

11. Add 0.1 volume of ice-cold 4M NaCl to T#4 and mix by inverting the tube several times. DNA strings should be visible.
12. Add 1 volume of ice-cold isopropyl alcohol (IPA) and mix well by inverting tubes gently at least 20 times. Incubate on ice for 10 min.
13. Centrifuge at 10,000*g* for 30 min at 4C.
14. Discard the SN. Wash the pellet with 1.5 ml freshly prepared, ice-cold 70% ethanol. Centrifuge for 5 min at 13000*g* at RT.
15. Discard the SN. Wash the pellet with 1.5 ml ice-cold 70% ethanol. Centrifuge at 13000*g* for 5 min at RT.
16. Discard SN and centrifuge for 1 min at 13000*g* at RT. Remove residual ethanol with a fine tip P10 or P20.
17. Air dry the pellet for 15 to 20 min until there is no visible ethanol residue. Add 100 ul of EB (10mM Tris pH 8.5, Qiagen). Incubate at RT overnight. Next morning, incubate at 28 C on a platform orbit shaker at 150 rpm for 1 h.
18. Determine the DNA concentration with a Nanodrop (ND) and by Qubit. The Nanodrop 260/280 ratio should be 1.8-1.9 and the 260/230 ratio should be >2. Based on Qubit, the DNA quantity should be >200ng/ul. At this point, the concentrations of Qubit /ND is often ca. 0.1. [Note: further cleanup is required until the Qubit/ND ratio $\geq$7.]
19. Perform an AMpure bead cleanup using either a 0.45X or 0.5X volume of beads as indicated below. Elute in 50 to100 ul EB.
20. Repeat Nanodrop and Qubit determinations. Nanodrop 260/280 and 260/230 ratios should be as indicated above. The Qubit/ND concentration ratio should be $\geq$7. Based on Qubit, the DNA concentration should be >100ng/ul.
21. The size of the DNA should be checked by gel electrophoresis. We typically yield 80 ug DNA with mol wt. >48 kb per 250 mg lyophilized conidia.

AMpure Bead Cleanup.

6

1. Notes: all steps are at RT. Prepare fresh 70% EtOH.
2. Bring AMpure beads suspension to RT. Pipet out the beads slowly. Add 0.45X or 0.5X volume of the AMpure beads to the DNA.
3. Mix the beads in the DNA solution thoroughly by flicking the tube. Do not vortex.
4. Collect but do not pellet the beads at 7,000 *g* for 2 to 3 sec.
5. Allow the DNA to bind to the beads by shaking at 200 rpm on a horizontal vortex mixer for 10 min.
6. Incubate the tubes on a rotating wheel at 22 rpm for 20 min.
7. Centrifuge tubes at 7000 g for 10 sec to pellet the beads.
8. Place the tubes in a magnetic rack to hold the pellet on the side of the tube.
9. Slowly pipette off the SN. As a precautionary measure, save the SN in a new tube, but only as a backup–your DNA should be bound to the beads. Avoid disturbing the bead pellet.
10. With the beads in the magnetic rack, add 70% ethanol to the side of tube, i.e., avoid the pellet. Fill the 70% ethanol to the rim of the tube.

11. Pipette off the ethanol and wash again with 70% ethanol as above. Remove all the ethanol without disturbing the bead pellet.
12. Remove tubes from the magnetic rack and centrifuge briefly at 7000 g for 30 sec to collect the residual ethanol. Place tubes on the magnetic rack and remove the ethanol with a fine tip Pipette (P20 or P10).
13. Check for any ethanol droplets. If present, repeat the previous step.
14. Remove the tubes from the magnetic rack. Open the caps. Add the amount of EB for >100ng/ul DNA (by Qubit). Mix the EB and beads gently by flicking until the suspension is homogenous. Centrifuge the tubes briefly at 7000 *g* for 30 sec. Place the tubes in the magnetic rack and let the DNA elute off the beads for 5 to 10 minutes. When all the brown beads are on the side with the magnet, carefully and slowly remove the gDNA into a clean 1.5 ml microfuge tube. Do not pipet out any of the magnetic beads.
15. Quantify DNA concentration and quality on Nanodrop and Qubit. For sufficient quality, with Nanodrop, the 260/280 ratio should be between 1.7 to 1.9 and the 260/230 ratio should be higher than 2.0. The Qubit/Nanodrop concentration ratio should be > 0.7X.

Fragments should be size-selected with Blue Pippen before library preparation for PacBio.