Aug 28, 2018

Total DNA "Midi" CTAB prep. for Zymoseptoria tritici

DOI
dx.doi.org/10.17504/protocols.io.s2wegfe

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Zymoseptoria communit...

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.s2wegfe

Protocol Citation: Bruce Mcdonald 2018. Total DNA "Midi" CTAB prep. for Zymoseptoria tritici. **protocols.io https://dx.doi.org/10.17504/protocols.io.s2wegfe**

Protocol status: Working

Created: August 28, 2018

Last Modified: August 28, 2018

Protocol Integer ID: 15158

Abstract

Bruce McDonald 1990 modified from Murray and Thompson

Prepare spores

1 Grow single spore isolate in 40-50mL **YSB** for 5-8 days at room temperature in shaker at 120-160rpm. Harvest spores by centrifuging in 50mL Falcon tube at approx. 3500rpm for 10-15min. Pour off supernanatant.

If not planning to use tissue immediately, transfer tissue to a 5 ml Falcon tube or leave in 50 ml Falcon tube. Spin in centrifuge at full speed to pack tissue and store at -80 degrees Celcius. Lyophilize tissue on lyophilizer overnight or until completely dry.

Prepare CTAB buffer

2 CTAB Extraction Buffer 100 ml

14.0 ml	5 M NaCl	= 700 mM
5.0 ml	1.0 M Tris (pH 8.0)	= 50 mM
2.0 ml	0.5 M EDTA	= 10 mM
10.0 ml	10% CTAB	= 1 %
1.0 ml	*B* Mercapto EtOH	= 1 %
68.0 ml	Distilled water	to 100 ml

10% CTAB stock is good for six months at 4 C

Disrupt the cells

3 Place tissue in mortar and add liquid nitrogen (only if tissue is wet). Grind into a fine powder with pestle. Alternatively, grind lyophilized tissue with a glass rod directly in tube. Transfer powder to a 15 ml centrifuge tube (not more than 1/2 of tube) and immediately add 5 ml CTAB extraction buffer. Mix well by agitating and inverting tube several times.

Warning: CTAB extraction buffer is caustic. Gloves must be worn at all times when handling.

Reminder: Always make a fresh batch of CTAB extraction buffer each day. CTAB extraction buffer goes bad after 48 hours.

Extraction

4 Place tube in 60oC H2O bath for 45-60 min., agitating tube every 10-15 minutes.

Caution: Occassionally the writing on the tube will begin to rub off or will be erased by chloroform or EtOH, therefore it is important in the following steps to frequently check the labeling on the tubes.

5 Add 5 ml chloroform:isoamyl alcohol (24:1, v:v) and emulsify mixture by gently inverting for 10-15 seconds. Do this step in the fume hood to minimize exposure to chloroform vapors. Centrifuge at 10,000 rpm (12,900 X g) for 15min to produce three layers. Transfer upper, aqueous phase to fresh 15 ml tube and do another chloroform extraction.

6 Add equal volume of isopropanol in the 15 ml tube and gently invert tube 15-20 times to precipitate DNA. Let tube sit at room temperature for 10 minutes before centrifuging at 9,000 rpm for 6 min.

7 Pour off supernatent and let pellet drain well on paper towels. Resuspend pellet in 2.0 ml TE.

8 Precipitate DNA by adding 0.5 volume (1.0 ml) 7.5 M NH4OAc and 2 volumes (4.0 ml) EtOH. Mix by inverting tube 15-20 times and let sit for 10 minutes at room temperature. Spin at 9,000 rpm (10,400 X g) for 6 min.

9 Pour off supernatent and let pellet drain well on paper towels. Resuspend pellet in 400 µl of TE. Spin down DNA in the centrifuge at top speed for three minutes to collect all liquid in the bottom of the tube. Transfer the DNA solution to a 1.5 ml microfuge tube.

10 Precipitate DNA by adding 0.5 volume (200 µl) 7.5 M NH4 OAc and 2 volumes (800 µl) EtOH. Mix by inverting tube 15-20 times and let sit for 10 minutes at room temperatures. Spin at top speed in microfuge for 10 minutes.

11 Dry down pellet in vacuum chamber and resuspend in 50-500 µl TE. Store in 4 degreesn Celcius refrigerator overnight and quantify [DNA] on fluorometer. Store at 4 degrees Celcius until ready to digest.