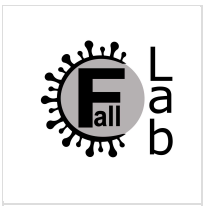

Sep 19, 2022

Double stranded RNA extraction by cellulose

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dx.doi.org/10.17504/protocols.io.4r3l2odrjv1y/v1

Nanovirseq

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ABSTRACT

In this protocol, the viral dsRNA extraction from infected-grapevine plants is explained.

DOI

dx.doi.org/10.17504/protocols.io.4r3l2odrjv1y/v1

PROTOCOL CITATION

Vahid Jalali Javaran 2022. Double stranded RNA extraction by cellulose. **protocols.io** https://protocols.io/view/double-stranded-rna-extraction-by-cellulose-ce3btgin

KEYWORDS

dsRNA extraction, cellulose, viral dsRNAs, grapevine

CREATED

Aug 09, 2022

LAST MODIFIED

Sep 19, 2022

PROTOCOL INTEGER ID

68419

MATERIALS TEXT

Extraction buffer (add ingredients following the order of the list. Do not add another before the added ones completely dissolved):

- 700 ml liter ultra pure water
- 200 ml 1 M Tris buffer(pH 8.3),
- 20 ml 0.5 M EDTA solution,
- 12.7 g Lithium chloride
- 15 g lithium dodecyl sulfate,
- 10 g deoxycholic acid
- 20 g PVP 40000,
- 10 ml Nonidet P-40
- Up to 1 liter of ultra-pure water.
- Mix well

Potassium acetate buffer (5.8 M):

- 500 ml ultra pure water
- 104 ml glacial acetic acid
- 384 g potassium acetate
- up to 1 liter of ultra-pure water.

3 M sodium acetate buffer (NaOAc) (pH 5.2)

- 60 ml ultra-pure water
- 24.6 g sodium acetate
- pH was adjusted to 5.2 with glacial acetic acid and the final volume was brought up to 100 ml by ultra-pure water.

10× STE buffer

- 500 ml ultra pure water
- 100 ml 1 M Tris(pH 8.0)
- 20 ml 0.5 M EDTA (pH 8.0)
- 58.44 g sodium chloride
- Up to 1 liter of ultra-pure water.

1× STE

- 900 ml ultra pure water
- 100 ml 10X STE buffer

1X STE-18 buffer

- 500 ml ultra pure water
- 100 ml of 10× STE buffer
- 180 ml of 100% ethanol
- final volume 1 liter with ultra-pure water.

- **Cellulose-1X STE-18**
- 3 g Sigmacell cellulose type 101 powder (S6790)
- 20 ml 1X STE-18

Total nucleic acid extraction 2h

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1 Weigh ~ 1.5 g of fresh or frozen leaves. Put the leaves in a 50 ml capped centrifuge tube containing 8X 8-mm stainless balls (sterilized). Immerse the tube in the liquid nitrogen for 5 mins. Rapidly mount the tubes in a foam holder and move to the MiniG chamber.
Fix the tubes correctly according to the instructions. Run at 1,500 rpm for 1 min. In the same way, prepare ~1 g of bean leaves (*Phaseolus vulgaris*) in a 50-ml capped centrifuge tube containing 8X 8-mm stainless balls and homogenize in the MiniG as mentioned above.

2 Add 12 ml of extraction buffer and 120 µl of 2-mercaptoethanol to each sample, and mix well. Add 8 ml of extraction buffer and 80 µl of 2-mercaptoethanol to the bean tube, and mix well.

3 Move 120 µl from bean tissue suspension in each sample. shake for 40 mins at 300 rpm and centrifuge at 1000 x g for 1 min at 10◦C to remove bubbles and a large amount of debris. Decant supernatant to a new 50-ml tube.

4 Add 12 ml of 5.8 M potassium acetate to the supernatant, mix thoroughly and centrifuge at 14,000 x g for 15 mins at 10◦C.

5 Decant the supernatant through 3 layers of sterilized cheesecloth (**optional**) into a clean 50 ml centrifuge tube, and add 16 ml of 100% isopropanol. Mix. Leave at -20C for 20 min. (**safe pause point**)

6 Centrifuge at 11,000 x g for 16 mins at 4◦C. Carefully discard the supernatant.

dsRNA purification by cellulose 1h 30m

7 Resuspend the pellet in 20 ml STE-18, vortex. Centrifuge at 14,000 x g for 15 min at 4◦C. Decent to a new 50-ml centrifuge tube.

8 Add 2 ml Sigmacell cellulose suspension (0.3 g) and vortex. Shake at 300 rpm for 15 min at room temperature. Centrifuge tubes at 14,000 x g for 5 min at 20◦C and discard the supernatant.

9 Resuspend the pellet in 40 ml STE-18. Centrifuge at 14,000 x g for 5 min at 20◦C and discard the supernatant. Repeat this step once by adding 20 ml STE-18 to suspend the pellet and centrifuge at 14,000 x g for 5 min at 20◦C. Discard the supernatant.

10 Evaporate ethanol from cellulose pellet at 40◦C for 15 min. Resuspend the pellet in 6 ml 1×STE. Shake at 300 rpm for 15 min at RT (room temperature). Centrifuge at 14,000 x g for 8

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min at 20◦C. Pour the supernatant into a new 50-ml centrifuge tube (it is still ok even with some cellulose particles).

dsRNA precipitation 1h

11 Add 0.6 ml 3M NaOAc and 12 ml anhydrous alcohol. Mix well and leave at -20◦C for 20 min. (**safe pause point**)

12 Centrifuge at 11000 x g for 15 min at 4◦C. Carefully discard the supernatant. Rinse the pellet with cold 70% Ethanol twice (if the pellet detached, centrifuge again). Air dry. Resuspend the pellet in 300 µl TE or sterile water, and transfer the suspension in a 0.85 um PES filter column (Sartorius Stedim Biotech), centrifuge at 13,000 x g for 30 seconds at RT. Use 100 µl TE or water to rinse again the tube and transfer the liquid to the same filter column and centrifuge for 2 mins. The total volume is 400 µl. Discards the filter. Store a -80◦C (**safe pause point**) or proceed to digestion.

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