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## Double stranded RNA extraction by cellulose

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Nanovirseq



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**We use this protocol and it's working**

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## Abstract

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

## Materials

**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

## Troubleshooting

## Total nucleic acid extraction

2h

- 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  $\mu$ l of 2-mercaptoethanol to each sample, and mix well. Add 8 ml of extraction buffer and 80  $\mu$ l of 2-mercaptoethanol to the bean tube, and mix well.
- 3 Move 120  $\mu$ l 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. Decant 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 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 µm 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. Discard the filter. Store at -80°C (**safe pause point**) or proceed to digestion.