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DNA extraction using the Qiagen 67563 MagAttract HMW DNA Kit (48)

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Xanthomonas genomics

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DISCLAIMER

ABSTRACT

This protocol is for extraction of genomic DNA from *Xanthomonas* culture for DNA sequencing.

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1 Heat the mixer (e.g., Eppendorf Thermomixer or an equivalent mixer) to 56°C for the lysis step.

2 If precipitate has formed in Buffer ATL, dissolve by incubating at 37°C with occasional shaking.

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3 Ensure that 96 - 100 % ethanol is added to buffers MW1 and PE.

4 Before proceeding to next step, ensure that the magnetic particles are fully resuspended. Vortex the vessel containing the magnetic particles vigorously for at least 3 min before first use.

5 Gently resuspend the bacterial pellet in 180 µl Buffer ATL using a cut tip as the resuspension will be viscous. Transfer the resuspended sample to a 1.5 ml tubes with a cut tip. Add 20 µl Proteinase K and incubate for 30mins in the Thermomixer at 56°C shaking at 900 rpm.

6 Add 4 µl RNase A to the sample, mix by flicking the tube several times and incubate for 2 mins at room temperature.

7 Resuspend the MagAttract Suspension G by vortexing. Add 15 µl MagAttract Suspension G and 280 µl Buffer MB to the sample. Mix by pulse vortexing.

8 Place the tubes into the Thermomixer and incubate at room temperature for 3 mins at 1400 rpm.

9 Place the tubes into the magnetic rack, wait until bead separation has completed (~1 min) and then remove and discard the supernatant. Avoid disturbing the magnetic bead pellet while aspirating the supernatant. Remove the supernatant completely.

10 Take the tubes out of the magnetic rack and add 700 µl Buffer MW1 to the bead pellet. Mix by flicking the tubes and then place the tubes into the Thermomixer. Incubate at room temperature for 1 min at 1400 rpm.

11 Place the tubes into the magnetic rack, wait until bead separation has completed (~1 min) and then remove and discard the supernatant.

12 Repeat the previous two steps.

13 Take the tubes out of the magnetic rack and add 700 µl Buffer PE to the bead pellet. Mix by

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flicking the tubes and then place the tubes into the Thermomixer. Incubate at room temperature for 1 min at 1400 rpm.

14 Place the tubes into the magnetic rack, wait until bead separation has completed (~1 min) and then remove and discard the supernatant.

15 Repeat the previous two steps.

16 Keeping the tubes in the magnetic rack remove all the supernatant using a small pipette tip to remove any traces of wash buffer (also might be some in the lid).

17 Whilst tubes are still in the magnetic rack, pipette 700 µl nuclease-free water onto the side of the tube opposite to the bead pellet. Incubate for 1 min at room temperature and then remove and discard the supernatant. Do not pipette water directly onto the bead pellet. All pipetting steps must be performed carefully to avoid disturbing the fixed bead pellet.

18 Repeat previous step.

19 Remove the tubes from magnetic rack and add 150 µl of distilled water. Mix the tubes by flicking and then place the tubes into the Thermomixer and incubate at room temperature for 3 mins at 1400 rpm.

20 Place the tubes into the magnetic rack, wait until bead separation has completed (~1 min) and then transfer the supernatant with the cut tip to a new 1.5 ml Eppendorf tube. This contains your high-molecular-weight DNA.

21 For a second elution, take the tubes out of the magnetic rack and add 100 µl of distilled pure water. Mix the tubes by flicking and then incubate at 60 °C for 10 mins.

22 Place the tubes into the Thermomixer and incubate at room temperature for 3 mins at 1400 rpm.

23 Repeat step 20.

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