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1.1 Isolation of gDNA from Streptomyces albus

Protocol adapted from Qiagen DNA blood and Tissue kit for gDNA extraction.

Procedure

- 1. Harvest cells (maximum 2 x 10⁹ cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.
- 2. Resuspend bacterial pellet in 180 µl enzymatic lysis buffer.

Enzymatic lysis buffer:

20 mM Tris·Cl, pH 8.0

2 mM sodium EDTA

1.2% Triton® X-100

Immediately before use, add lysozyme to 20 mg/ml

- 3. Incubate for at least 30 min at 37°C. After incubation, heat the heating block or water bath to 56°C if it is to be used for the incubation in step 5.
- 4. Add 25 µl Proteinase K and 200 µl Buffer AL (without ethanol). Mix by vortexing.

Note: Do not add Proteinase K directly to Buffer AL. Ensure that ethanol has not been added to Buffer AL.

5. Incubate at 56°C for 30 min.

Optional: If required, incubate at 95°C for 15 min to inactivate pathogens. Note that incubation at 95°C can lead to some DNA degradation.

 Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is

- essential to apply all of the precipitate to the DNeasy Mini spin column. This precipitate does not interfere with the DNeasy procedure.
- 7. Pipet the mixture (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.(Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.)
- 8. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x g (8000 rpm). Discard flow-through and collection tube.
- 9. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube. It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x g (14,000rpm).
- 10. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥ 6000 xg (8000 rpm) to elute. Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield.
- 11. Recommended: For maximum DNA yield, repeat elution once as described in previous step. This step leads to increased overall DNA yield. A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from previous step can be reused for the second elution step. Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate