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DNEasy DNA Extraction - Vibrio Gram Negative Broth Bacteria

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Materials

STEP MATERIALS

- Buffer ATL (tissue lysis buffer) **Qiagen Catalog #**19076
- Buffer AL (lysis buffer) **Qiagen**
- Buffer AW1 **Qiagen Catalog #**19081
- Buffer AW2 **Qiagen Catalog #**19072
- Buffer TE 1x
- Buffer TE 1x
- Buffer ATL (tissue lysis buffer) **Qiagen Catalog #**19076
- Buffer AL (lysis buffer) **Qiagen**
- Buffer AW1 **Qiagen Catalog #**19081
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Troubleshooting

1 Put Buffer ATL and AL at 56 C for 5 minutes before using.
00:05:00

2 Transfer 250ul of broth culture to 2ml deep well (autoclaved)
250 µL

3 Harvest cells (maximum 2 × 109 cells) by centrifuging plate for 15 min at 4063 x g. Discard supernatant.
00:15:00

4 Resuspend pellet in 200 µl Buffer ATL by pipetting up and down 15 times.
200 µL
Buffer ATL (tissue lysis buffer) **Qiagen Catalog #**19076

5 Seal with foil and place in oven at 56 C for one hour. Place extra plate on seal so foil doesn't come off.
01:00:00

6 Pipette up and down 15 times and spin down. (May place in -80 C and continue procedure later at this point.)

7 If placed in -80 C, let thaw and spin down @ 1000g for 1 min

8 Add 205 uL Buffer AL and 205 uL molecular grade EtOH.
205 µL
Buffer AL (lysis buffer) **Qiagen**

9 Pipette up and down 15 times and spin down.

10 Place DNeasy 96 plates on top of 2ml deep well plate . Mark the DNeasy 96 plates for later sample identification.

11 Carefully transfer the lysate (approximately 600ul) of each sample from step 7 to each well of the DNeasy 96 plates. Do not transfer more than 900 µl per well.

12 Seal each DNeasy 96 plate with a porous film. Centrifuge for 15 min at 4063g. If lysate remains in any of the wells, centrifuge for a further 10 min.
00:15:00

13 Add 500 µl Buffer AW1 to each sample.
500 µL
Buffer AW1 **Qiagen Catalog #**19081

14 Seal each DNeasy 96 plate with a new AirPore Tape Sheet (provided). Centrifuge for 5 min at 4063g.
00:05:00

15 Remove the tape. Carefully add 500 µl Buffer AW2 to each sample. Centrifuge for 15 min at 4063g. Do not seal the plate with AirPore Tape. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.
500 µL
00:15:00
Buffer AW2 **Qiagen Catalog #**19072

16 Place in new collection rack (2ml deep well plate) and spin again 15 minutes at 4063g.
00:15:00

17 Place each DNeasy 96 plate in the correct orientation on a new rack of VWR 500ul plate.

18 To elute the DNA, add 200 µl Buffer TE to each sample, seal and incubate for 1 minute at room temp. Centrifuge for 4 min at 4063g. 200 µl Buffer TE is sufficient to elute up to 75% of the DNA from each well of the DNeasy 96 plate.
200 µL
00:04:00
Buffer TE 1x

19 Recommended: For maximum DNA yield, repeat step 16 with another 200 µl Buffer TE.
200 µL
00:04:00
Buffer TE 1x

20 Aliquot 20ul of extracted DNA to green 96 microplate. Seal DNeasy 96 plate and 96 microplate with non-sterile foil. Place DNeasy 96 plate in -80 C and 96 microplate in -20 C.
20 µL