Genomic DNA extraction using the modified CTAB method

(Ausubel *et al*., 1994)

Two types of samples were used in this work: 1) water samples that were filtered to concentrate the cells onto a membrane; and 2) biofilm samples for which around 1 to 2 gr of pellet was used to extract the genomic DNA.

1) Each filter containing immobilized bacterioplankton cells (see pag. 1) was removed from the eppendorfs (the eppendorfs were stored at -20 °C, in 50% ethanol (v/v)), opened, and placed facing down in a glass Petri-dishes previously sterilized (Ø = 6 cm). A lysing solution was then added into each Petri-dish (see Table 1).

2) The remaining sample (50% ethanol cell suspension) was centrifuged at maximum speed on a bench top centrifuge (12 100 g for 2 min) to create a pellet. The ethanol supernatant was discarded. The cell pellet genomic DNA was extracted by adding the lysing solution listed in Table 1 (see Pellet column). Biofilm samples were extracted using the same procedure.

T**able1. S**olutions and volumes added to each sample to obtain the cell lysate.

Solutions (µl)	**Filter**	**Pellet**
TE	900	250 + x*
0,5M EDTA	306	153
25% Chelex 100	200	100
10% SDS	76	38
Proteinase K	10	5

3) The pellet was initially re-suspended in 250 µl of TE and mixed to homogenize before adding the remaining lyse solution. Right before adding proteinase K the TE solution was added until a total volume of 750 µl (x* volume varied depending on biomass volume).

4) All samples were placed at 55 °C, under orbital agitation (100 rev/min), for 1 hr after which additional proteinase K was added to the lysate (5 µl to the pellets an 10 µl to the filters). The samples were left incubating for an extra hour, at 55 °C.

5) The Petri-dish lysate was transferred into a 2,0 ml sterile eppendorf properly labeled (an average of 2 eppendorfs were used per sample). The filter was discarded.

6) All lysates were spun down for approximately 10 to 15 sec, at the max speed and the supernatant was transferred to new 2,0 ml tubes properly labeled. The loosely cell debris pellet was discarded.

7) Depending on the lysate total volume, a 5 M NaCl solution was added to the supernatant (see Table 2) and the tubes were gently inverted. The NaCl addition was followed by the addition of 10% CTAB 0,7 M NaCl solution (see Table 2). The extraction tubes were incubated for 30 min, in a water bath at 65 °C, after adding the CTAB solution.

Table 2. Volumes of 5 M NaCl solution and of 10% CTAB 0,7 M NaCl solution to be added to the cells lysate according to initial lysate volumes.

Lysate (µl)	**5 M NaCl (µl)**	**10% CTAB 0,7 M NaCl solution (µl)**
500	83,3	66,6
600	100,0	80,0
700	117,0	93,0
750	125,3	99,6
800	133,0	107,0

8) An equal volume of 100% chloroform solution was added to each tube after the water bath

incubation and the solution was slowly inverted. All tubes were centrifuged for 5 min, at top speed (12 100 g).

9) The upper layer was removed into a new sterile eppendorf (2,0 ml) properly labeled and an equal volume of Phenol: Chloroform: Isoamylalchool solution (25:24:1) was added. The tubes were slowly inverted, followed by a new centrifugation at top speed (12 100 g) for additional 5 min.

10) Once more the upper layer was transferred to a new eppendorf (2,0 ml), and cold 2-Isopropanol (100%) was added in a 0,62 x ratio (see Table 3) and gently homogenized. The DNA samples were left to precipitate in the fridge, at 4 °C overnight.

Table 3. Volumes of 2-Isopropanol solution added.

Supernatant (µl)	Isopropanol solution (µl)
500	310
550	341
600	372
650	403
700	434
750	465
800	496

11) The overnight precipitated DNAs were centrifuged at 16 100 g, at 4 °C, during 1 hr and 30 min. The supernatant was discarded and the DNA pellets were washed three times with a cold 70% ethanol solution (v/v).

12) The DNA pellets were dried in the Speed-vacuum, under low heat. Each DNA pellet was ressuspended in 25 µl of 10 mM Tris, pH 8. The DNA samples were placed in a 55 °C water bath during 15 to 20 min.

13) The samples were loaded into a 0,8% agarose gel (w/v) with ethidium bromide to visually check the genomic DNA quality. The samples ran for 30 min., in a 0,5X TBE, at 120 V, and 90 mA. The filter genomic's DNA was pooled with the pellet genomic's DNA. The extractions were stored in 2,0 ml eppendorfs, in the fridge, at 4 °C until used.

DNA extraction procedure considerations: Only the 50% ethanol (v/v) preserved samples were used in the present work. For each original filtered sample there were two independent genomic DNA extractions: one from the filter immobilized cells; one from the storage solution cell pellet. The DNA resulting from both extractions was pooled at the end so that the extracted DNA would better represent the genomic DNA of the whole sample.

References: Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1994). *Current Protocols in Molecular Biology*. New York City, NY: John Wiley & Sons Inc.