APPENDIX. Purification of genomic DNA from River water sample

1. Filter the water samples using a filter paper. After filtration, add the shredded* "filter paper" toPowerbead™ tube. (*e.g., with scissors)
2. Add 550 μl of Buffer SL to the tube.
3. Homogenize the sample in the Precellys (or similar) equipment twice for 23 seconds, each at 6,500 rpm. Alternatively, secure tubes horizontally on the flat or vortex with tape and vortex at maximum speed for 10 minutes.
4. Centrifuge at 10,000 x g for 10 minutes at room temperature and carefully transfer the supernatant to a 1.5 ml tube (provided).
5. Add 50 μl of Buffer RH.
6. Add 300 μl of Buffer PD and mix well by vortexing.
7. Centrifuge at 10,000 x g for 5 minutes at room temperature and carefully transfer the supernatant to a 2 ml tube (provided).
   Small pellet containing humic acid, cell debris, and protein can formed in the collection tube after centrifugation. Be careful not to disturb this pellet.
8. Add 900 μl of Buffer TB and mix well by vortexing.
   If Buffer TB shows signs of precipitation, pre-heat in a 56°C water bath to dissolve completely.
9. Transfer up to 700 μl of the mixture to a mini spin column.
10. Centrifuge at 10,000 x g for 30 seconds at room temperature.
11. Repeat steps 9-10 two more times using the remainder of the sample.
12. Add 500 μl of Buffer NW to the mini spin column.
13. Centrifuge at 10,000 x g for 30 seconds at room temperature.
    Discard the pass-through and reinsert the mini spin column back into the same tube.
14. Centrifuge at maximum speed for 1 minute at room temperature to remove residual wash buffer.
15. Transfer the mini spin column to a new 1.5 ml tube (provided).
16. Residual ethanol may interfere with downstream reactions. Care must be taken at this step to eliminate the carryover of Buffer NW.
17. Add 50 μl of Buffer EB to the centre of the membrane in the mini spin column.
18. Incubate for 1 minute at room temperature. Centrifuge at 10,000 x g for 1 minute at room temperature.
    Elution volume can be decreased to 30 μl for high concentration of DNA, but this will slightly decrease in overall DNA yield.
    If maximum recovery of DNA is preferred or the starting materials contain large amount of DNA, elution can be done in 200 μl of Buffer EB.