Genomic DNA extraction

- 2 mL of microalgae cultivation broth were centrifuged at 6,000 ×g for 4 min. in a 2 mL reaction tube.
- The supernatants were separated from the biomass pellets, which were re-suspended in 1.5 mL of lysis buffer, 4 ceramic beads were added to the test tube.
- Samples were freeze in liquid nitrogen to then milled the frozen sample at 30 Hz for 2 min in a mixer mill (Retsch®).
- The cell suspension from each test tube were separated from the beads to be again freeze in liquid nitrogen and then thawed at 60°C for 3 min in a safe lock reaction tube, this process was repeated three times to enhance cell disruption.
- 0.5 mL of phenol-chloroform-isoamyl alcohol (25:24:1) were added and vortex for 1 min. The samples were centrifuged at 12,000 ×g for 10 min to separate the samples in layers. 1.3 mL of the top layer of each test tube was transferred to a new test tube. 0.5 mL of chloroform-isoamyl alcohol (24:1) was added to the top layer and vortexed for 1 min.
- The samples were centrifuged ones again at 12,000 ×g for 10 min. 0.5 mL of the top layer were aliquoted into new safe lock test tube to be mixed by inversion 5 times with 1 mL of 2-propanol (previously filtrated with a 0.2 µm cellulose filter and stored at –20°C.
- To enhance DNA precipitation, the alcoholic mixture was stored at –20°C overnight. Samples were centrifuged: 12,000 ×g for 10 min at 4 °C to pellet the DNA.
- The supernatant was separated and 0.5 mL ethanol (–20 °C) were added to the DNA pellet. The DNA samples were pelleted by centrifuging (12,000 ×g for 10 min at 4 °C) the samples to eliminate the alcoholic fraction.
- The ethanol traces left was eliminated by drying the samples for 24 hours at RT. The DNA pellets were re-suspended in 50 µL of autoclaved ultrapure water.