

V. cholerae enrichment in APW (Alkaline Peptone Water):

Prepare APW: 40g (20g of peptone and 20g of sodium chloride) to 1 litre of distilled water. Dissolve, then autoclave.

From faecal sample, mix no more than 10% faeces to APW. E.g., in 20 ml APW maximum 2 gr faeces. Mix the suspension well, then incubate with the tube cap loosened at 37°C for 8 hours. This will select for Vibrio species along with other halophilic bacteria that like salt (e.g., Acinetobacter).

After this step you may leave the culture in the fridge overnight if needed. Otherwise, please continue to preparation for sequencing.

DNA extraction and Sequencing:

Centrifuge at least 4 ml of the enriched APW with faeces at 5000 x g for 10 minutes. Discard the liquid and keep the pelleted bacteria.

Perform DNA extraction following your DNA extraction kit in the lab. Please consult with Saria on saot@food.dtu.dk to check the kit adjustments if needed.

After DNA extractions, follow ONT library preparation kit SQK-LSK114.24, attached (or other version of LSK – consult with Saria if in doubt).

Run the flowcell on MinKNOW following the manufacturer's instructions.