***Vibrio fischeri* culturing protocol.**
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Plates A and B containing colonies of *Vibrio fischeri* were used to clone further colonies of the same species in both agar and liquid broth media. Aseptic technique was undertaken where possible.

***V. fischeri* AGAR CULTURE**

Media used

Bacto Agar 1% w/v (or 0.5g per 50ml).
LB Miller Broth Powder by Merck 2.5% w/v (or 2.5g per 100ml).

Method

Agar and Broth powder was mixed with deionised water and stirred thoroughly before being boiled three times to ensure even distribution of nutrients and remove air bubbles. The media was then poured onto a beaker and a saucer (ideally a petri dish) and left to cool and set into a solid state.

Then a streak of *V.fischeri* were inoculated from plates A and B into the beaker and the saucer containing cooled media. A sterilised and bent teaspoon was used to transfer the colonies. The area in which the colonies were taken from plates A and B were clearly marked with a pen on the plate.

A grease paper cover was put on top of the beaker and the saucer and left at room temperature for *V.fischeri* to grow.

***V. fischeri* LIQUID CULTURE (100-200ml)**

Media used

LB Miller Broth Powder by Merck 2.5% w/v (or 2.5g per 100ml)

Method

LB broth powder was dissolved in deionised water and stirred with heat to ensure even distribution of nutrients. The media was then poured onto conical flasks.

Once cooled to room temperature colonies of *V.fischeri* from plates A and B were transferred to the liquid media using sterile pipette tips. The conical flasks were covered and left at room temperature to let the bacteria grow.

In addition, three small aliquots (5ml approx) of the liquid culture were made for each plates A and B. Colonies were transferred to 15ml test tubes and also left at room temperature.