Method Sheet 03

Using a multichannel pipette to dispense bacterial cell cultures into 96-well plates

Overview

This method sheet explains how to accurately dispense liquids into a 96-well microplate using a multichannel pipette when setting up bacterial cell cultures for use in screening assays.

Equipment

- Sterile, clear plastic 96-well microplate(s) with lids
- Multichannel pipette (8- or 12-channel) capable of dispensing 100 - 200 µl
- Sterile pipette tips (compatible with the multichannel pipette)
- Reservoir(s) for media containing bacterial cells to set up the plate
- Waste container for used tips

Reagents

- Sterile Luria broth (LB, at least 11 ml per plate)

Method

1) Prepare a sufficient volume (typically 11 ml per plate) of a suspension of bacterial cells (follow Method Sheet 02) in appropriate media.
2) Wipe the multichannel pipette with tissue sprayed with 70% isopropanol, or similar disinfectant, to clean.
3) Pour the bacterial cell suspension to be aliquoted into a suitable sterile reservoir (if unavailable, the lid of a separate, sterile 96-well plate can be used).
4) Set the multi-channel pipette to dispense a volume of 99 µl (standard for most assays, but can change for your own experiment).
5) Firmly press the pipette into a row of sterile pipette tips (8 or 12, depending on the type of pipette), ensuring all are securely attached.
6) If the tips are loose or fall off during use, use gloved finger and thumb to pull up and seat firmly each tip individually, being very careful to touch only the upper part of the tip, and not the lower part of the tip which must remain sterile before use.
7) Push the plunger on the pipette down to the first stop (not all the way to the second stop).
8) Insert the tips into the liquid in the reservoir, ensuring all tips are below the surface of the liquid.
9) Slowly release your thumb to allow the plunger to return to the top position.
10) Look carefully across all the tips to ensure the level of liquid is the same in each tip, if not, dispense the suspension back into the reservoir and try again.

11) Likewise, if there are any large air bubbles in any of the tips, dispense back into the reservoir and try again.
12) Move the pipette to the open sterile plate and dispense all the liquid into the wells of an empty column, pipetting past the first stop all the way to the second stop of the plunger this time.
13) If the tips become loose during this process, you can repeat the tightening of the tips to the pipette as above.
14) Because the same liquid is being pipetted into every well, there is no requirement to change tips between rows or columns when preparing the plate initially (note, you must change tips each time when it comes to the challenge step).
15) If any tips are lost, it may be easier to discard the tips and replace with fresh tips.
16) Repeat this process until every well of the plate contains the cell suspension.
17) Discard the tips in the waste tips container.
18) Repeat for the number of plates you intend to prepare for your experiment.
19) The bacterial cell culture plates are ready for challenging as soon as pipetting is complete.

Notes

- If possible, the plates should be prepared and challenged in a sterile environment, such as within a laminar flow cabinet, however good results can still be obtained from pipetting on the open bench if the lid is removed briefly.
- Technically, only 9.6 ml of cell suspension is necessary to set up a 96-well plate containing 100 µl of suspension per well, but it is standard practise to always make ~10% more than the minimum since some liquid always remains unrecoverable from the reservoir.
- A similar protocol can be followed if pipetting enzyme or substrate mix for an enzyme assay in the absence of cells.