Method Sheet 04

Challenging bacterial cell cultures with extracts from the *Phytotitre* natural product library

Overview

This method sheet explains how to challenge bacterial cell cultures in 96-well plate format with extracts from the *Phytotitre* natural product library using a multichannel pipette, with the aim of exploring the impact of individual extracts on bacterial growth.

Reagents

- Defrosted *Phytotitre* or *Puretitre* library

Equipment

- Multichannel pipette (8- or 12-channel) capable of dispensing 1 - 10 µl
- Sterile pipette tips (compatible with each pipette)
- Static (non-shaking) 37°C incubator
- Waste container for used tips

Method

1) Fully defrost a *Phytotitre* or *Puretitre* library plate in a 37°C incubator (follow Method Sheet 01).
2) Prepare a 96-well plate containing bacterial cell cultures at 99 µl per well (follow Method Sheet 03, should be done on the same day as challenge).
3) Carefully remove the cap mat from the library plate and place with the round domes facing upwards (do not discard the cap mat).
4) Column 1 of the library plate is left empty to allow space for negative controls - you can make use of this by pipetting 100 µl of sterile DMSO into each well of this column in the library plate to use as a vehicle only control.
5) Place both library and culture plates side by side and ensure both are in the correct orientation (i.e. check that well A1 is in the top left position).
6) Set a small volume multichannel pipette to dispense 1 µl (this is a common starting point, but can vary depending on your own experiment, see notes below).
7) Attach tips to the pipette and ensure all are firmly seated as in the previous method.
8) Work from left to right, starting at column 1 and moving across, one pipetting cycle at a time, to column 11.
9) It can be difficult to keep track of which column is being pipetted from and to when challenging plates for screening, so you can cover the wells that have already been completed with a sterile 96-well plate lid to help keep your place as you move along the plate from column to column.
10) Push the plunger down to the first stop (not all the way to the second stop).

11) Place the tips below the surface of the liquid in each well of the respective column of the library plate (containing stock extracts).
12) Slowly allow the plunger to return to the top position.
13) Look carefully across all the tips to ensure the level of liquid in each tip is the same, if not, dispense the suspension back into the correct wells of the library plate and try again.
14) Likewise, if there are any large air bubbles in any of the tips, dispense and try again.
15) Move the pipette to the plate containing the bacterial cell culture and dispense all the liquid into the respective wells, being careful to pipette into the liquid and not onto the side of the well.
16) When dispensing, push the plunger past the first stop all the way to the second stop.
17) Discard the tips after pipetting into each column (this is essential to prevent cross-contamination of the library contents).
18) Place fresh tips on the pipette before moving onto the next column.
19) Move the plate lid marker one column to the right to make it easier to see which column is next to work from.
20) Continue until every well of the plate between columns 1 and 11 have been challenged.
21) Column 12 is available for you to pipette positive control compounds (if desired, these will be specific to each type of experiment).
22) Move the culture plate backwards and forwards, then side to side, gently several times to mix the compound into the suspension.
23) Avoid jarring movements that may splash liquid between wells and cause cross-contamination.
24) Measure the absorbance of the plate straight after challenge (to obtain the baseline, time = 0 h readings) using a microplate reader (follow Method Sheet 05).
25) Place the culture plate in a static 37°C incubator to allow growth to begin (does not need to be a shaking incubator).
26) Reseal the *Phytotitre* library plate with the cap mat in the correct orientation and return it to the freezer for storage.

Notes

- Column 1 of the library plate is left empty to allow space for negative controls - you can make use of this by pipetting 100 µl of sterile DMSO into each well of this column in the library plate to use as a vehicle only control.
- Column 12 of the library plate is also left empty to allow space for customers to insert their own positive controls of interest (if available, for examples antibiotics with established toxicity for the strain of interest).
- When dispensing compound, ensure the liquid goes directly into the larger culture volume, and does not touch and then adhere to the plastic on the side of the well.
- If using the *Phytotitre* library, the stock plant extracts are at 10 mg/ml, so transfering 1 µl of stock extract into 99 µl of culture represents a 1:100 dilution, which means the final concentration of plant extract in each culture will be 100 µg/ml.

- Accordingly, the final concentration of DMSO (the solvent, also called vehicle, used to dissolve the plant extracts) will be 1% (wt:vol) when following this protocol.
- If using the *Puretitre* collection instead, the stock compounds are at 10 mM, which means the final concentration of each compound in each culture will be 100 µM.
- *E. coli* and *M. luteus* cultures can typically tolerate up to 2% DMSO with limited impact on growth, so it is possible to double the concentration of both vehicle control and plant extracts if preferred (i.e. 2 µl extract into 98 µl culture for 200 µg/ml).
- While a shaking incubator is essential for efficient bacterial growth in large culture volumes, it is not necessary for culture of the small, 100 µl, culture volumes in the present experiment, and in our experience a static incubator tends to give more reproducible results than a shaking incubator when using microplates for screening.