Chemotaxis Practical, Backstage Preparation

Preparation of *D. discoideum*

Use AX2 wild type and Gß- strain.

1. Vegetative Cells

1.1 Grow AX2 cells in suspension in HL5c medium at 22^0C, shaking at 180rpm. Grow to a density of 3-4 x 10^6cells/ml for the purpose of the experiment. When growing cells up to the desired volume, always keep within the log phase ie. between $5x10^5$ to $5x10^6$ cells/ml and never dilute the cell density below this range.
1.2 Having achieved the desired cell density, pellet cells for 5 minutes at 700 xg (1700rpm, Beckmann Allegra 6R), wash in Soerensen Buffer once then resuspend at a concentration of 2.5 x 10^8 cells/ml in Soerensen Buffer.
1.3 Keep cells on ice until required (about up to one hour).

2. Starved Cells

2.1 Grow cells as described in 1.1 above
2.2 Pellet cells and wash once in Soerensen Buffer. Resuspend cells in Soerensen Buffer at a concentration of 1 x 10^7 cells/ml, but prepare two to three times the volume that would normally be required for the experiment as the cell number could deplete as much as 40% during the starvation period (starvation of AX2 wild type only resulted in a 10% loss of cells but Gß- cells lost almost 40% of their original cell mass!).
2.3 In theory, a 6-8 hour starvation period is required at 22^0C, but for this particular experiment, cells were starved overnight at 16^0C, 180rpm from 7pm the night before, until 8am the following morning when the cells were transferred to 22^0C, 180rpm for another 3 hours (practical was at 11.30am).
2.4 Pellet cells and resuspend at a concentration of 2.5 x 10^8 cells/ml in Soerensen Buffer.
2.5 Keep cells on ice until required (about up to one hour).

Preparation of Chemoattractants

Stock solutions of 10mM cAMP or Folate were prepared in 100mM NaOH.
Working concentrations were 10µM, 50µM and 250µM. Keep on ice until use.

Preparation of Starvation Plates

16g/L Bacto Agar (Beckton Dickinson, Cat no. 214010) in 1x Soerensen Buffer. Prepared plates that were 4mm depth ie. 20.25ml per 10cm Petri dish. Stored in the cold room until required.

Summary of Chemotaxis Practical

1. Fully rehydrate plates by overlaying with a little Soerensen Buffer.
2. Make troughs using 2mm drinking straws.
3. Add 20-30µl chemoattractant to troughs.
4. Allow surface of agar plates to dry (about 10 minutes) before spotting 1µl cells 5mm from edge of trough.
5. Allow cell spots to dry.
6. Incubate plates in humid chamber for 3-4 hours.
7. Score chemotaxis.

Dicty Development Practical

AX2 cells are grown as described in 1.1 above (see Chemotaxis Practical). Cells are then washed once, pelleted and resuspended to a concentration of 6 x 10^7 cells/ml in Soerensen Buffer. 0.5ml of the cell solution is then plated onto a 6cm Petri dish which contains Soerensen/Agar (see 'Preparation of Starvation Plates' above) at a depth of 1cm. Agar plates are allowed to dry in a hood for 15 minutes prior to plating of cells to get rid of excess moisture and so that the cell solutions will absorb relatively quickly. The plated cells are allowed to "dry" and then incubated upside down under the following conditions to achieve the various stages of development.

The 4 stages of development

A. Homogenous lawn of cells – 1st stage of development

- Plate cells the day before they are required
- Place in cold room after half an hour of plating cells
- Place at room temperature 2 hours before they need to be observed

B. Streaming – 2nd stage of development

- Plate cells the day before they are required
- Place in the cold room after 2 hours of plating cells
- Place at room temperature 4 hours before they need to be observed (we didn't do this, we left it for 5-6 hours at room temperature and streaming was almost over, but previous experience showed that 3-4 hours will lead exactly to streaming)

C. Tipped Aggregates – 3rd stage of development

- Same as **'B'** above but leave plates at room temperature for 8 hours

D. Fruiting bodies Formation – final stage of development

- Plate cells the day before they are required
- Leave at room temperature for 24 hours and observe at 24 hours.