Protocol on Delivery System

Wash buffer

This buffer is used during the preparation of bacteria for chemotaxis and can also be used as chemotaxis buffer during capillary assays. This is recipe for 100 ml:

- 0.136 g of KH_2PO_4
- 0.174 g of K_2HPO_4
- 3 mg of EDTA
- Control pH and adjust to be in between 7.0 and 7.4 pH.
- Autoclave

Some notes:

- Addition of EDTA is absolutely necessary as it removes any heavy metal ions, which can inhibit chemotactic behavior and motility altogether.

Attractant

We used three kinds of attractant: malate, succinate and citrate (0.1M), using wash buffer to dissolve them, and then autoclave. When you need them, use wash buffer to dilute them in several gradient concentrations.

M9 minimal medium semi-solid agar

In chemotaxis assays semi-solid agar is used as it allows greater diffusion of molecules and allows movement of bacteria within agar. This is a recipe for a total volume of 1 L (dissolved in distilled water):

- 17.1 g of $(Na_2HPO_4)12H_2O$
- 3 g of KH_2HPO_4
- 0.5 g of NaCl
- 1 g of NH_4Cl
- Adjust pH to 7.0 - 7.4
- Add 4 ml of glycerol
- 2 g agar
- stirred by a magnetic stirrer for 30min
- Autoclave.

- Cool down to 50°C and add required antibiotics and the following separately sterilized solutions:
- 2 ml of 1 M filter sterilized $MgSO_4$
- 100 µl of 1 M filter sterilized $CaCl_2$
- Pour plates, not too thin or too thick.

Preparation before chemotaxis experiments

This is a procedure required to achieve optimum growth of flagellated bacteria that will move towards a source and their preparation for chemotaxis assays:

- Add required amount of antibiotic into LB broth (20 ml) before inoculation of bacteria.
- Inoculate cells into LB (20 ml) and grow them at 30℃ at shaking 100 rpm overnight.
- Centrifuge overnight culture at 5000 rpm for 10 minutes, and resuspend in 2 ml LB.
- Inoculate 1 ml of resuspended cells into a conical flask with 100 ml LB.
- Grow at 30°C and shaking 150 rpm 1-3h, until middle of exponential phase is reached ($O.D._{600}$ 0.3 - 0.6,not over 0.6 absolutely).
- Take 50 ml of mid-exponential phase cell culture and centrifuge it down at 3000 rpm for 20 min.
- Resuspend the centrifuged cells in 10 ml of Wash buffer.
- Centrifuge resuspended cells at 3000 rpm for 20 minutes.
- Resuspend the centrifuged cells in 2 ml of Wash buffer.

Agar plug assay

- Take small circles of filter papers and soak it in the bacterial suspension obtained from the preparation before the experiment and insert into the semi–solid agar plate. Make sure not to insert bacteria too deep into the semi-solid agar since they might start to move using twitching motility on the surface and that is not the desired movement we require during chemotaxis assays.
- Add 20 µl of attractant on to another set of filter paper circles. Position these 2 cm away from the bacterial circle on each of the semi-solid agar plates.
- Leave bacteria to grow in the plates 2 days at 37°C, record them each day.

Capillary assay

- Prepare bacteria for chemotaxis.

- Dilute attractant to desired concentrations in wash buffer in the 96 - well plates, and each well contains 200 µl of attractant. We used 5 concentration gradients:0.0001mM、0.01mM、1mM、10mM、100mM.

- Set up a structure above the 96 well plates to hold all the tubes uniformly, add the tubes into it and position them, so they relate to the 96 well plate .We used the shelf of the 10µl and 200µl pipette tips.

- Insert a number of capillary tubes into the attractant (this number depends on the number of attractant concentrations and a number of replicates that is going to be measured).Wait for 1 minute ,then seal the top of the capillary tubes by wax to make sure that the volume inside the tubes does not change. Once sealed, the tubes can be taken out of the wells without losing the attractant from the tubes.

-Dilute the bacteria prepared for chemotaxis to a suitable concentration (we used 20000) and add 200 µL of bacterial culture into separate wells of 96 well plate, filling a number of wells that corresponds to a number of capillary tubes and incubated for 40min.

- After 40 min, put the capillary out of the bacterial suspension, and break the top of the tubes , then use bulb for pipet to blow the solution in the tubes on the LB solid medium .CFU (colony forming unit) count can be performed to obtain data.

Some notes

- If the bacteria with fluorescence，you can use FACScan to count the number the cells. Or if you have a microplate reader, you can read the OD representing the concentration of the bacteria. Both of the methods can ease the work, but we haven't tried them.