

Protocol

The preparation of NGM plates

1、 Materials

NaCl, Agar, Peptone, 5mg/ml cholesterol in ethanol (do not autoclave), 1M KPO₄ buffer Ph 6.0 (108.3 g KH₂PO₄, 35.6 g K₂HPO₄, H₂O to 1 litre), 1M CaCl₂, 1M MgSO₄, ddH₂O, petri plate (9cm),

2、 Methods:

- (1) Mix 3 g NaCl, 17 g agar, and 2.5 g peptone in a 1 litre Erlenmeyer flask. Add 975 ml ddH₂O. Cover mouth of flask with aluminium foil. Autoclave for about 70 min until the temperature decrease to 70 °C.
- (2) Add 1 ml 1 M CaCl₂, 1 ml 5 mg/ml cholesterol in ethanol, 1 ml 1 M MgSO₄ and 25 ml 1 M KPO₄ buffer. Swirl to mix well
- (3) Use sterile procedures, and dispense the NGM solution into petri plates. Fill plates 1/2 full of agar.
- (4) Leave plates at room temperature for 1 hours, and spread 300ul op50 *E. coli* to each plate by glass rod.
- (5) Put plates into 37 °C incubator for more than 12 hours.
- (6) Get them out of incubator and store plates in 4 °C freezer to avoid contamination.

Seed ATR NGM plates

- (1) Add 12.5ul 400uM ATR into 1ml *E. coli* op50.
- (2) 1ml liquid can seed 10 6cm NGM plates.

The preparation of M9 buffer (500ml)

1、 Materials:

KH₂PO₄, NaHPO₄·12H₂O, NaCl, 1M MgSO₄, ddH₂O

2、 Methods:

- (1) Weigh 1.5g KH₂PO₄, 8.14g NaHPO₄·12H₂O, 2.5g NaCl
- (2) Add 500ul 1M MgSO₄
- (3) Add ddH₂O up to 500ml
- (4) Swing well and autoclave.

How to inject the *C.elegans* into microfluidics

- (1) Open the injection pump (Longer Pump LSP10-18).
- (2) Suck M9 buffer by injector (BD REF301942)
- (3) Connect the chip and injector with pipes.
- (4) Fix the injector on the pump and start the pump to inject the buffer into the chips (speed:100µL/min)
- (5) After some buffer washing the chip, put the worms into pipes as following steps:

- a) Wash the NGM culture plate of *C.elegans* with M9 buffer.
- b) Suck the buffer filled with worms by an 1ml injector (injector1)
- c) Wait for worms precipitating at the bottom of injector.
- d) Wash the modified petite tip with M9 buffer, and make buffer full of pipe and tip.
- e) Remain little space to contain precipitated worms, and add worms in the petite tip.
- f) Suck worms into pipe by 5ml injector (injector2)
- g) Connect one end of the same pipe to microfluidics, and put injector2 on the pump. Fix injectors.
- h) Start the pump to inject.

How to active worms by light under a specific wavelength

- (1) Inject worms with ATR into microfluidics.
- (2) Wait worms for losing vitalities.
- (3) Use blue light by projector to activate worms and then wait them for losing vitalities.
- (4) Use light of 640nm by microscope to be a control and observe their behavior.
- (5) Wait for losing vitalities, use light of 51% 395nm, 440nm, 470nm, 508nm and 640nm wavelength in turn to be a control and observe their behavior.

The way to attract *C.elegans* by light

- (1) Select one or two worms in NGM plate with ATR, and culture them for at least 8 hours.
- (2) Choose one lively worms to observe
- (3) Connect the light source produced by mercury lamp to an optical fiber with about 1mm diameter.
- (4) Fix a blue optical filter in front of the light source and turn on the mercury lamp
- (5) Use blue light emitted from optical fiber to attract *C.elegans* in NGM plate by using stereoscopic microscope under a dark condition
- (6) Attract *C. elegans* to crawl as any pathway whatever you want, especially the circle.

The way to make *C. elegans* alcohol addictive

- (1) Culture worms in 4 NGM plates with ATR for at least 8 hours
- (2) Add 70ul 25%, 50%, 75%, 100% alcohol in each plate
- (3) Stack up these 4 plates and culture them under blue light in a dark environment for 2 hours
- (4) Put the plate in the bottom of plates up to the top of them every 30min
- (5) Wash worms out of plates by using M9 buffer
- (6) Suck them into tubes, and make worms precipitate
- (7) Throw out supernatants, and put precipitations into one side of new plates
- (8) Put 70ul alcohol with the same concentration as marker into another side of plates. Stand them still for about 30min, and observe worms' collective behavior and distribution.
- (9) Use water instead of alcohol as a control to do the same steps