Protocol

The preparation of NGM plates[1]

Large populations of *C. elegans* can be obtained by culturing them either in liquid media or on solid media in plates. They are usually grown on solid NGM (Nematode Growth Media) and fed with *E. coli* bacteria, which is added to the plates either alive or dead (killed by UV[12], by heat[13] or by cold[14]). The most common procedure uses live OP50 *E. coli*, which is defective in the synthesis of uracil and cannot overgrow into a thick layer that would obscure the worms.Methods:

1. Mix 3 g NaCl, 17 g agar, and 2.5 g peptone in a 1 litre Erlenmeyer flask. Add 975 ml ddH_2O. Cover mouth of flask with aluminium foil. Autoclave for about 70 min
2. Cool the flask to 70℃
3. Add 1 ml 1 M $CaCl_2$, 1 ml 5 mg/ml cholesterol in ethanol, 1 ml 1 M $MgSO_4$ and 25 ml 1 M KPO_4 buffer. (all of them but cholesterol previously autoclaved) Swirl to mix well
4. Use sterile procedures, and dispense the NGM solution into petri plates. Fill plates 2/3 full of agar.
5. Once dry, it is advisable to leave plates at room temperature for 2-3 days before use for detection of contaminants. Prepare a streak of OP50 *E. coli* from a glycerol stock (OP50 can be obtained from the Caenorhabditis Genetics Center).
6. Pick a single colony and grow it in LB overnight at 37 °C with agitation.
7. Spread 300ul op50 *E. coli* to each plate by glass rod.
8. Allow the OP50 *E. coli* lawn to grow overnight at room temperature or at 37 °C for 8 hours.
9. Get them out of incubator and store plates in 4 ℃ freezer to avoid contamination.

Synchronize *C. elegans* cultures[1]

The bleaching technique is used for synchronizing *C. elegans*cultures at the first larval stage (L1). The principle of the method lies in the fact that worms are sensitive to bleach while the egg shell protects embryos from it. After treatment with alkaline hypochlorite solution, embryos are incubated in liquid media without food, which allows hatching but prevents further development.

1. Allow worms to grow until adult stage.
2. Recover gravid adults in 2 ml tubes by washing plates with M9 buffer.
3. Pellet worms by centrifuging for 2 minutes at 400xg (~1500 rpm on a standard table centrifuge) at room-temperature and discard supernatant.
4. Perform 1-3 washes until the buffer appears clear of bacteria.
5. Add the desired bleaching solution (375ul bleach and 375ul 1M NaOH) for 6 minutes.
6. Stop reaction by adding 700ul M9 buffer.
7. Quickly centrifuge (since treatment may still be active) for 2 minute at 400 x g and discard supernatant.
8. Wash pellet three more times by filling the tube with M9 buffer.
9. Add 1 ml of M9 buffer to the pellet, or place the eggs to unseeded NGM plates, and incubate at the desired temperature with gentle agitation. Proper aeration should be provided.

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. Weigh 1.5g KH_2PO_4, 8.14g $NaHPO_4 \cdot 12H_2O$, 2.5g NaCl
2. Add 500ul 1M $MgSO_4$
3. Add ddH_2O 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 bufffer 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

Reference:

[1] Portadelariva, M., Fontrodona, L., Villanueva, A., & Cerón, J. (2012). Basic caenorhabditis elegans methods: synchronization and observation. *Journal of Visualized Experiments Jove, 64*(64), e4019.