Feb 19, 2019

Food preference assay of C Elegans

DOI
dx.doi.org/10.17504/protocols.io.yb5fsq6

Priota Islam[1]

[1]Imperial College London

Behavioural Genomics

Priota Islam
Imperial College London

DOI: https://dx.doi.org/10.17504/protocols.io.yb5fsq6

Protocol Citation: Priota Islam 2019. Food preference assay of C Elegans. **protocols.io https://dx.doi.org/10.17504/protocols.io.yb5fsq6**

Protocol status: Working
We use this protocol and it's working

Created: February 19, 2019

Last Modified: February 19, 2019

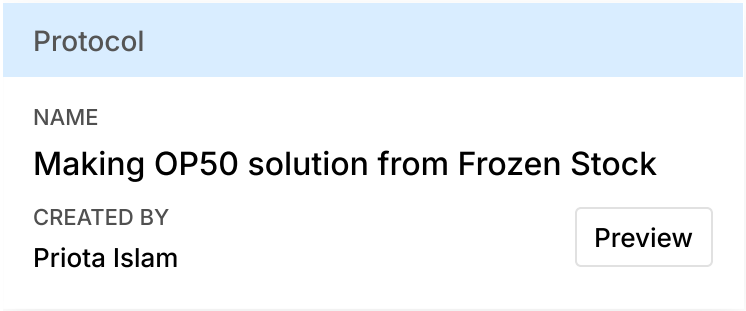
Protocol Integer ID: 20573

Keywords: food preference assay, food, assay

Troubleshooting

Preparing Bacterial solution of choice (Worm food)

1 Fresh bacterial culture of OP50 and HB101 were prepared following the protocol ***Making OP50 solution from Frozen Stock***



1.1 # Day: 1

- Get LB Agar from the media kitchen and autoclave it for 2hrs
- Post autoclave, pour the agar on large petri dishes (60mm) and leave to dry overnight to be transferred to the cold room the next day

1.2 # Day: 2

- Get the frozen tube out of the freezer (Freezer 12, SD Box)
- Take a sterile pipette tip and get some frozen sample
- Streak the pipette on the LB agar plate
- Take another new pipette and streak across the old streak and then at a separate spot streak again
- Repeat this step for one more time
- Keep the plate at 37°C incubator overnight for the bacteria to grow
- Transfer the plate to the fridge the next day (This plate can be used for about 1 month to inoculate bacterial culture)

1.3 # Day:3

- Purchase LB Broth from the Media kitchen (Don't use less than 200ml)
- Get flat bottomed conical flasks from the glassware kitchen (For 200ml LB Broth take 400/500ml flask to allow enough headspace for the bacteria)
- From the culture plate, select and circle a single colony
- Using aseptic technique, carefully scoop the single colony and mix it with the LB Broth that is already poured into the conical flask
- Label the flask and put it on the 37°C shaker for overnight incubation

1.4 # Day:4

- Following overnight incubation, take the flask out of the incubator and measure the OD using a spectrophotometer
- Record the average of the OD600, use LB Broth as Blank
- Aliquot the solution into labelled 15ml falcon tubes and keep them in the 4°C Fridge

Preparing worms

2

- Maintain N2s on NGM plates seeded with either OP50 or HB101 or a mixture of both depending on experiment plan
- Chunk each set of worms on 3 maintenance plates
- Bleach the worms after 2 days following the protocol for ***Bleach synchronisation of C. elegans***

Note

https://www.protocols.io/view/bleach-synchronisation-of-c-elegans-stbeein

- Refeed the starved L1s on 2 different plates that are seeded with either OP50 or HB101 or a mixture of both respectively, 72hr prior to the experiment day

Preparing Imaging plates

3

- Prepare and pour low peptone NGM onto 35mm imaging plates
- Put them in the cold room for at least 2 days before use
- OP50 and HB101 were diluted in M9 to the desired dilution factor (1:1, 1:2, 1:4, 1:8 or 1:10)
- Label and mark the imaging plates according to the experiment plan (Use two dots to mark OP50 and one dot for HB101 making sure they are about 1.5cm apart)
- Seed imaging plates with two 10ul drops of the diluted bacterial solutions the day before recording (the control plates will either have two drops of OP50 or two drops of HB101, the rest of the plates will have one drop of OP50 and one drop of HB101)

- Leave on bench to dry with lid on overnight

Day of recording

4

- Using a hair pick – pick 3 young adults onto the seeded imaging plates
- Put the plates on the tracker – agar side up with the lids off
- Wait 30min for the worms to acclimatise
- Record for 2 hours (See protocol ***Imaging on the multiworm tracker*** for detailed tracking instructions)

Protocol

NAME

Imaging on the multiworm tracker

CREATED BY

Priota Islam

Preview

4.1

- Clean the rectangular glass plate with ethanol and lint free tissue
- Switch ON the rig
- Align the movable part till desired position and lock it by pressing ON (If you want to move it again press EMO, to lock again twist the EMO knob and press ON again)
- Log in to the 3 PCs (Password: BehavGenom709 (BehavGenom710 for PC 3)
- Change the screen lock time to 20 mins
- Set up destination folder (PC → Data Part 1 → Run script (init_exp.ps1) → Name the folder with the date as (year month date e.g. 20181011)
- Turn on the software GECKO on each pc and check the following:
  i) Recording mode: 15 mins
  ii) Video format: hdf5
  iii) Output folder: Select the folder created earlier
  iv) Press the drop down arrow beside the RECORD icon to make sure ALL CAMERA option is selected if you want both the cameras in use otherwise select CURRENT CAMERA option

4.2

- After picking the desired number of worms on the imaging plates, place the plates on the rectangular glass plate underneath the cameras
- Place the worm plates agar side up with the lids off

- Check the focus on each camera of each PC and make sure the ring of food is at the centre of the focus
- Place the worms on the rig for about 15mins before recording so they acclimatize to the surroundings
- When the time comes, and all checks have been done press RECORD

4.3

- Turn OFF the rig and close GECKO
- Run the script to copy the data to the network (PC → Data Part 1 → Run script (move_files_anyPC.ps1)
- Change back the lock screen time to 1 minute
- Discard the plates
- See the protocol for video analysis to proceed with the analysis