Experiment 4 Mitochondria Tracking

Mitochondria are the cell's little energy factories generating ATP from glucose. These organelles are transported by molecular motors on microtubules tracks - the cells' highway and transport system. However, only little is known about how motors attach to mitochondria and how mitochondria motility is regulated. It is thought that two types of plus-end directed motors, kinesin-1 and KIF1B, are involved in mitochondria movement. Today, we will use MitoTracker green as an example to measure the rate of mitochondria transport in N2 wild type and mutant strain.

Strain: FF41, N2

1. Wash the plates with M9 Buffer, collect liquid in a 1.5 ml eppendorf. Centrifuge for 3 minutes 1,300rpm at 22°C.
2. Wash worms for another 2 times. (M9 Buffer, 1,300rpm, 3 minutes, 22°C)
3. Suspend worms in 1 ml of M9. Worms were incubated 30 min in M9 to digest bacteria in their guts. After 30 min, using M9 Buffer wash again.
4. Centrifuge the eppendorf. Remain 100 µl pellet of worms.
5. Add another 699µl M9 Buffer into the eppendorf.
6. Add 1µl MitoTracker green dye into the eppendorf.
7. Incubate worm suspensions at room temperature for 1 h with constant rotation. Keep the eppendorf cap from light.
8. After staining, the worms were washed 3 times with M9 buffer.
9. Use confocal microscope to record the mitochondria movement.

Reagents

M9 Buffer

MitoTracker green

Dissolve MitoTracker green in dimethyl sulfoxide as stock solution (concentration: 4 $\times 10^{-3}$M). **(DMSO is toxic. Wear gloves, mask and protective glasses)**. Store the stock at -20°C in a foil wrapped tube. Dilute the stock 1:800 in M9 for mitochondria tracking experiment.

Homework

1. Use "wormbase.org" to find out the meaning of the strain FF41. Why are we using the strain for the experiment? What is the mammalian analogue of the gene mutated in FF41.
2. Write down your experiment outcomes and calculate transport rate by using Kymograph analysis.