RNAi by soaking for S. ratti Protocol from Dulovic and Streit, 2019, Plos Pathogens

Materials

- DMEM (stored in fridge)
- Octopamine (100mM)
- siRNA (100µM, stored in freezer)
- 5x Universal siMAX siRNA Buffer (comes with siRNA, stored in fridge)
- RNase OUT (5000U, 40U/µl, stored in freezer)
- RNase-free Water
- Dry V12 (Dulovic et al, 2016, Exp Parasitology) or NGM plates at room temperature, no bacteria
- Sterile 1.5ml Eppendorfs
- Falcon Tubes
- Incubator preset to 19°C

"Late-Stage" Method

- Start worm funnel
- Bring DMEM upto room temperature
- Clean and wash worms 3x until water is completely clean
- Add to sterile Eppendorf and allow to sink to bottom
- Pipette out into small aliquots (i.e. 20µl) to dry plate and allow to crawl out

- Make up soaking master mix under sterile hood – Into sterile 1.5ml Eppendorf (enough for 6 reaction tubes): 448.5µl DMEM, 130µl Octopamine, 6.5µl RNase OUT. Mix by pipetting up and down.
- Thaw siRNAs and 5X Universal siMAX siRNA Buffer to room temperature under hood. Dilute siMAX Buffer to 1x in RNase free H_2O
- Pipette 90µl of soaking master mix into 6 separate sterile 1.5ml Eppendorfs.
- Add 10µl of siRNA or 1x siMAX Buffer to each Eppendorf (final concentration 10µM siRNA)
- Mix gently by pipetting up and down.
- Transfer worms by picking into tubes (ideally around 30 per tube)
- When finished, place all tubes inside a large falcon tube and surround with H_2O.
- Cap Falcon Tube and place in incubator at 19°C. Check every 12 hours for contamination by DMEM colour change.

"Early-stage" Method

- Start worm funnel
- Bring DMEM upto room temperature
- Clean and wash worms 6x until water is completely clean. For final wash use 5ml RNase-free water
- Add to sterile Eppendorf and allow to sink to bottom. Concentrate into a smaller sample (i.e. <50µl)

- Make up soaking master mix under sterile hood – Into sterile 1.5ml Eppendorf (enough for 6 reaction tubes): 416µl DMEM, 130µl Octopamine, 6.5µl RNase OUT. Mix by pipetting up and down.
- Thaw siRNAs and 5x Universal siMAX siRNA Buffer to room temperature under hood. Dilute siMAX Buffer to 1x in RNase free H_2O
- Pipette 85µl of soaking master mix into 6 separate sterile 1.5ml Eppendorfs.
- Add 10µl of siRNA or 1x siMAX Buffer to each Eppendorf.
- Mix gently by pipetting up and down.
- Check worm sample for contamination under microscope. If contaminated, rewash again. If not, pipette 5µl of worms into each tube (should be around 500-150 L1s inside this 5µl)
- Mix by pipetting up and down.
- Place all tubes inside a large falcon tube and surround with H_2O.
- Cap Falcon Tube and place in incubator at 19°C. Check every 12 hours for contamination by colour change of DMEM.