Detailed Protocol for sDR 11/6/07

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0. Prepare *ad libitum* (AL) plates

- Grow one colony of OP50-1 bacteria (from a streaked out plate) in LB medium + 300 μg/ml streptomycin O/N at 37 degree C.
- The next morning, take the OD @ 600 nm. A 1:10 dilution of an O/N culture of OP50-1 usually gives an OD of ~0.15.
- Calculate the bacterial concentration corresponding to this OD value (this needs to be done 1 day before the experiment is started)
    - Plate 50λ of serial dilutions of 10 on LB plates.
    - Count the number of colonies on 2 plates where the colonies number is between 20-300.
    - From this value, calculate the CFU corresponding to the OD value
- Take $7.5x10^{11}$ bacteria in an eppendorf tube
- Centrifuge at 16,000 rcf for 1 min at room temperature.
- Resuspend the pellet in 1.5 ml of S Medium (S Medium inhibits bacterial growth) so that the concentration is $5x10^{11}$ bacteria/ml.
- Add 150 μl of this bacterial suspension ($7.5x10^{10}$ bacteria) on a 60 mm NGM plate with 300 μg/ml streptomycin.
- Let it dry for 2 hours at room temperature with cover open near a bunzen burner flame.
- Use the same day.

-**Note #1**: OP50-1 cultures can be stored for a little over a week at 4 degrees but if this is done the cultures should be left at room temperature for an hour and ODed before use.

-Note #2: OD values may correspond to different concentrations of bacteria depending on the lab stock of OP50.

-**Note #3**: We use OP50-1, which is a strain of OP50 that contain a streptomycin resistance plasmid and hence add streptomycin to all our plates and cultures. We have performed sDR with OP50 without this resistance and observed similar effects.

S Medium: Autoclave S basal: 5.85 g NaCl, 1g K_2HPO_4, 6g KH_2PO_4, 1ml cholesterol (5mg/ml in ethanol) in 1 liter of dH_20. Add 10 ml 1M potassium citrate pH 6 (20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate in 1 litre of dH_20 sterilized by autoclaving), 10 ml trace metal solution (1.86 g disodium EDTA, 0.69 g $FeSo_4•7H_20$, 0.2 g $MnCl_2•4H_20$, 0.29 g $ZnSO_4•7H_20$, 0.025 g $CuSO_4•5H_20$ in 1 liter of dH_20 sterilized by autoclaving and stored in the dark), 3ml 1 M $CaCl_2$, 3ml 1m $MgSO_4$ all added with sterile technique and not autoclaved.

1. Day 1: Get a synchronized population of worms.
- Add 20 N2 adults to several AL plates at 20 degree C
- Remove the adults after 4-6 hours.
- If there are developmental differences between worm strains, set up several staggered plates of young adult worms to get developmentally synchronized L4.

Note #4: We tested four different types of N2 strains (from Man-Wah Tan, Andy Fire, Cynthia Kenyon, and Andy Dillin's labs) and they all displayed a ~30% increase in response to sDR.

2. Day 3: change worms onto fresh plate

- Put 30 adult worms per 60 mm AL plate (see point 0 on how to make AL plates).
- Do three plates per condition (90 worms/condition total).

3. Day 4 (and all even days until the worms lay no more eggs)
- Remove the new progeny (for example by burning them with a pick)

Note #5: In this assay, no FUdR is used to keep worms from laying progeny so it is necessary to remove the progeny on the even days. We have performed these assays with FUdR and seen similar lifespan extension of N2 worms.

4. Day 5
- Transfer worms onto a fresh 60 mm AL plate.

5. Day 6
Kill off new progeny as on day 4.

6. Day 7 initiate AL versus sDR.
-Take 150μl of $5x10^{11}$ bacteria/ml resuspended in S medium and perform serial dilutions in S medium (1:10, 1:100, 1:1000 . . .) so that the concentrations are $5x10^{10}$, $5x10^{9}$, $5x10^{8}$ . . . Plate 150μl of these dilutions on 60 mm NGM plates with 300 μg/ml streptomycin.

-**Note #1**: In our experience, 150μl of $5x10^{11}$ bacteria/ml (AL) versus 150μl of $5x10^{8}$ bacteria/ml (sDR) are the concentrations that give the optimal lifespan extension (~30%).

-**Note #2**: It is important to do the entire gradient (from $5x10^{12}$ to $5x10^{6}$), as small variations in OP50 growth, temperature, or worm strains could affect the results

- **Note #3:** It is important to seed the plates no more than 2 hours before

switching the worms because the bacteria grow despite the resuspension in S medium.

- Note #4: Bacteria will grow over these two days at 20degC both in AL and in sDR plates.

7. From Day 7 on
- Switch worms every two days to freshly made AL or sDR plates
- Mark worms as dead if they don't respond to repeated prodings (5-8 times).