Mated reproductive span protocol

1. Generate a population of WT C. elegans hermaphrodites and males via heat shock and propagation.

2. Sync a population of the strain to be tested (usually done via bleaching, although one can pick from a mixed population if necessary.) When utilizing the bleaching method, allowances for developmental variation should be considered when bleaching multiple strains. For example, bleach slower-developing strains earlier so that they are at the same stage of development at the onset of testing. Care should be taken to avoid overly dense populations per plate; utilizing HG plates can help to avoid this issue.

3. On the next day, seed 35mm NGM plates with 30µl of OP50. For each experimental condition, 25-50 plates will be required.

4. At ~48 hours from the initial bleaching, pick L4 worms of each strain to the seeded 35mm plates, one worm per plate. Also pick five males from the propogated WT stock to each plate. A labeling mechanism should be utilized to distinguish each condition, such as striping the plates. In addition, label the plates with the day of the experiment and an individual number for each worm per strain. For example, the first worm of a strain picked at this time would be '1.1'

5. On the same day, seed an equal number of 35mm NGM plates as in (3). This procedure is repeated daily throughout the span of the experiment.

6. On the next day, move the adult worm to a fresh seeded plate. **Do not dispose of the previous day's plates.** These will be kept to check for progeny at a future date. Take care to avoid transferring any progeny when picking. Also take care to distinguish between the strain in question and the WT males. Remove and dispose of the males from each plate. Some worms may need to be censored from the experimental pool. If the worm has crawled off the plate, note this worm as 'lost' and include the day of the event. If progeny have hatched within the worm, note this as 'bagged' and include the day as well. If the integrity of the worm has been compromised, usually due to vulval protrusion, note this as 'exploded.' Finally, if the worm has died prior to the cessation of its measured reproductive span, note this as 'dead.'

7. Label these plates with the day of the experiment, as well as the number of the worm. For example, the new plate containing the worm from (4) would now be labeled '2.1'

8. Continue daily to move the worms to freshly seeded plates, taking care to differentiate the adult from any progeny throughout. Keep the plates from all the previous days

9. On Day 4 of the experiment, check the plates from Day 1 for the presence of progeny. Log which plates contain progeny, referring to the strain, day, and number of each individual worm. A spreadsheet of some type should be utilized. Worms that have been censored for any reason (lost, bagged, exploded, or dead) should be included in the tally, noting the day at which said event happened.

10. Continue moving the worms to fresh plates, and checking the previous plates for progeny, utilizing the three-day lag as previously explained. When a non-censored worm has produced no progeny for two **consecutive** days, this is considered the end of that worm's reproductive

span. These worms no longer need to be moved.

11. Move the remaining worms and continue checking the plates until the entire experimental population has either ceased production or been censored.

12. Upon completion, calculate the reproductive span of each individual worm, as the number of days from the onset to cessation of progeny production. Tabulate these results for each strain individually, and for the range of documented reproductive spans, count the number of worms that continued for the same span in days. Do the same for the censored worms, and note this separately.

13. Visualize this data in graphical form. A useful tool that produces extensive statistical depth can be found at http://sbi.postech.ac.kr/oasis.