# Quantitative Brood Size Assays

By Erik Andersen (August 2009)

1. Collect all recombinant inbred lines (RILs) and parental strains to score. Randomly select as many as you can do in one assay. Iterate the random selection for as many replicates as you want. **Every time you score RILs, score the parents.** Throw out any assays that deviate from the observed controls. Bristol (N2) should have between 250-300 offspring (allow 10% error); Hawaii (CB4856) should have between 150-200 offspring (allow 10% error). This assay takes ten days. I noted the day each task should be completed in parentheses. In this assay, you will score all of the progeny from six animals. The progeny are spread out over two plates (sets one and two) to make it easier to score.

2. Streak out OP50 from frozen stock onto an LB agar plate (Day 1 – Wed.).

3. Chunk all RILs and parents to new 6 cm NGMA plates spotted with 100 $\mu$L of OP50 (Day 2 – Thurs.).

4. Inoculate OP50 from freshly streaked plate and grow over night at 37°C with shaking (Day 2 – Thurs.).

   a. The next morning, transfer 1 ml of over-night culture to 500 ml of LB (Day 3 – Fri. AM) in a 2 L flask.

   b. Grow the culture at 37°C with shaking until the $OD_{600}$ = 0.5–0.6. This will take about 4 hours. (Day 3 – Fri. PM)

   c. **note** - you can either save the remainder of the culture to spot plates at a later date or scale the amount of LB you inoculate.

5. One day later, pick 5-10 L4 hermaphrodites from each plate to a new NGMA plate (Day 3 – Fri.).

6. Pour 12 2% agarose plates for each strain being scored (Day 3 – Fri. AM).

7. Spot each 2% agarose plate with 100 $\mu$L of OP50 you inoculated that morning (Day 3 – Fri. PM).

8. Dry the plates at room temperature (Days 3 to 6 – over the weekend).

9. Three days after you picked L4 hermaphrodites, setup the assay by picking one L4 hermaphrodite to each of six 2% agarose plates prepared earlier (Day 6 – Mon.). These plates are the first set.

10. Put the plates at 20°C, track the temperature and humidity (Day 6 – Mon.).

11. Two days after setting up the assay, pick each of the two-day-old adults to six new 2% agarose plates (Day 8 – Wed.). These plates are the second set.

12. Count the number of animals on each of the six plates from the first set (Day 9 – Thurs.).

13. Count the number of animals on each of the six plates from the second set (Day 10 – Fri.).

14. Calculate the total number of offspring for each of the six hermaphrodites. Calculate the mean and median. I have found that the median is most reproducible. This assays has a few difficulties. If the bacteria is not totally dry, the animals crawl off the plates and have lower brood (CB4856 especially). Also, if I use plates that are more than a week old, the brood sizes are different. Both of these issues and others are likely due to food quality differences. Try to treat the bacteria the same way each time. Drying the plates reproducibly is still an issue too.