Worm lifespan protocol
Bacterial preparation
Depending on whether you are performing lifespan experiments on regular OP50-1 bacteria or RNAi the protocols are slightly different.

-If using OP50-1 you should set up a flask of ~100mls of OP50 and allow this to shake overnight at 37°. This bacteria can be stored at 4° and reused (I typically do not like to reuse a bacterial stock for longer than 2 weeks).
I then will seed NGM plates with 200μl of OP50 and allow the plates to dry with the cover half off near an open flame to avoid contamination (this usually takes 1-2hours. Can also dry overnight with plate closed if contamination is a problem).

-If using RNAi you should pick the appropriate RNAi construct and empty vector (E.V.) control from the Ahringer or Open-biosystems libraries stored at -80°. This bacteria should be streaked out on LB +Amp plates and single colonies should be picked and grown in 300mls of LB +Amp overnight. Cultures should be spun at ~5000 rpm for 15 minutes and the suppernatent should be discarded. The pellet should then be resuspended so that it is at a 50x concentration. (If it is the first time I am using an RNAi construct I will also take 1ml of the overnight culture and do a miniprep on it and send the construct for sequencing as ~5-10% of the RNAi constructs in the library are supposed to be incorrect). These 50x stocks of RNAi bacteria can also be stored at 4° but again you don't want to do that for too long.
I then will seed NGM containing Ampicillin (100 $mg \cdot ml^{-1}$) and IPTG (0.4 mM) with 150μl of E.V. control and RNAi's of interest bacteria.

Lifespan assays
Birthing worms
There are two accepted methods for beginning lifespan assays, you can either bleach the parents and get a synchronized population of eggs or you can perform an egg laying for 4-6 hours. I have always done the egg laying as I am afraid that bleaching the parents could unduly affect the progeny. (If a specific mutant is more or less susceptible to stress then putting the egg in bleach before it hatches might very well amplify or dampen the real longevity phenotype.)

It is necessary to begin experiments with worms which have been clean of bacterial contamination. I don't like to take parent hermaphrodites which were bleached as eggs but prefer to allow them to grow for at least a generation off of the bleach.

I take 20-30 young adult hermaphrodites and place them on a freshly seeded OP50 NGM plate. These worms are allowed to lay eggs for 4-6 hours and then removed. I will typically set up 3 plates of 20-30 worms per strain.

If starting with a new strain and you are unsure as to whether it has a different developmental growth period from the N2 (WT) strain, it is a good idea to set up several staggered egg lays. This can be done by simply moving the hermaphrodites

from the initial plate onto a new plate and allowing them to lay eggs for 4-6 hours again.

With RNAi lifespan experiments depending on what you want to do you can birth the worms on the RNAi itself or you can birth the worms on OP50 plates and then move them to the RNAi at different stages (L1, young adult, post egg lay).

Switching worms

I will typically allow the newly hatched worms to get to the stage where they have begun to lay some eggs of their own (~day 3 at 20°) and then switch 3 different plates of 30 worms each per condition. If you are a novice to worm lifespan assays it is a good idea to switch the worms to new plates every day until the egg laying period is over (~day 7of life). When you become more experienced you can switch the worms every other day while picking off the progeny which have grown a lot on the off days. When you gain further confidence you can switch the worms every other day and not worry about removing the progeny.

Worms should be switched to a new seeded plate every other day and the worms which are dead or censored should be counted. There is a handy excel macro which allows you to stamp your excel sheets with the date and time that you switch the worms.

Statistical analysis

To analyze the different lifespan assays and create Kaplan-Meier curves I prefer to use StatView 5.0.01 using a Logrank (mantel-cox) test. This program only runs on Mac OS9 which can be installed and run on any computer. I prefer this program as it gives good looking graphs and also allows you to perform basic statistical comparisons. For comparing the interaction between two different treatments (RNAi and genotype for instance) two-way ANOVA tests can be done using Prism. If you have multiple independent replicates of lifespan assays (which I think is a must if you want to make any conclusions) you can use Fisher's combined probability tests to combine the p values of independent replicates.

Independent experiments should never be combined into one graph as this is very misleading and there are slight differences from experiment to experiment. It is therefore necessary to always carry the appropriate controls in your experiments so you can draw real conclusions.