Bombardment:

You must read background information on bombardment before attempting this protocol; it takes a lot of time and money. See http://www.wormbook.org/toc_wormmethods.html Section 7, for example. Then arrange to do your first bombardment experiment with someone in the lab helping you thru the day-of-bombardment steps. This protocol was adapted from the protocol from John White's lab.

Growing up the worms you will need for bombardment:

Strain DP38 [*unc119(ed3)*] worms should be maintained on NGM/OP50 plates. Always keep a few worms at 15 °C as backup. DP38 will not make dauers, so don't let your plates starve or you may lose the strain, or it may revert. DP38 grows slowly, so plan ahead.

To grow up **large** quantities of worms for bombardment prepare peptone plates:

For 1 liter:
1.2 g NaCl
20 g BactoPeptone
25 g agar
autoclave, then add (sterile):
1 ml 5 mg/ml cholesterol
1 ml 1 M MgSO4
25 ml 1 M KH2PO4 pH 6.0
1 liter is sufficient for 30 plates, around 20 shots

Seed peptone plates with saturated C600 *E. coli* culture grown up at 37 °C in LB. The bacterial lawn should cover almost all of the plate but not touch the plastic edge.

Allow bacterial lawn to grow for 1-2 days. The bacteria must grow and also the liquid must soak in and/or dry out. The dryness of the plates is important. They can't be too wet or too dry. If the agar has cracks around the outside rim by the time your worms are ready to use, then you dried the plates too much at this step.

Seed the plates with DP38 worms. One small NGM/OP50 60 mm plate can be split among 6 peptone/C600 plates. You can seed by simple "chunking", but it is better to more evenly distribute the worms across the plate. If you chunk, use a scalpel to divide the portion of the plate with the worms on it into 6 equal "chunks". Dap the chunks (worm side down) onto the C600 plates ~6x to distribute little patches of worms over the plate and put the plates in the 20 °C incubator. I usually use worms in M9 rather than chunking. Don't use too much M9 or your plates will be too wet. If you seed with a younger plate (not yet starved/lots of L4s—basically with an age distribution similar to what you want to bombard) it will take about 8 days grow up. If you seed with a slightly older semi-starved plate the worms will grow up about ~ 1 day faster. For each individual bombardment shot you will need all the DP38 worms from at least 1 large (100 mm) peptone/C600 plate. (So for 20 bombardments grow 25 plates.)

Place plates in big tub, cover, and leave at RT for ~8 days. Plates can be moved to 15 °C or 25 °C to slow or speed growth if needed. Plates are ready when most of the plates are starved but a small amount of the C600 bacteria is left on each plate.

Reagents:

For each 10 shot experiment, you will need 10 **seeded** 6 cm (regular) NGM OP50 plates (bring to 15°C before experiment for best results) and 30 **seeded** 9 cm (large) NGM OP50 plates. You **must** seed them at least one day before bombarding.
pAZ-based plasmid for transforming, such as pFJ1 or pIC26, uncut, at least 1 mg/ml (1 μl/bombardment). Do 5 minipreps or one maxiprep. You must quantify your DNA with the spectrophotometer. Remember also to freeze down both *E. coli* and DNA of plasmids you bombard for both the lab's and your own freezer stocks.
BioRad 1.0 μm gold beads (catalog no. 1652263)
Stopping screens Cat#1652336 (look like small pieces of window screen)
Macrocarriers Cat# 9202964 120502
Rupture discs Cat # 1652330
50% glycerol (sterile filtered)
2.5 M CaCl2 (sterile filtered)
0.1 M spermidine (stock at -20 °C, dilute with 492 ul sterile water right before use) (buy free base Tissue Culture grade spermidine, Sigma S-4139, in future)
70% ETOH
100% ethanol, 200 proof, water-free
sterile water
macrocarrier holders (these look like steel rings)

Preparing the worms:

For each bombardment you will need DP38 worms from at least 1 large (100 mm) peptone/C600 plate. (So for 20 bombardments grow 25 plates.)

- Before starting to prepare the gold beads, wash the worms off the C600 plates with sterile M9 and collect in two 50 ml falcon tubes. Use a glass pipet or cut off (sterile) pipet tips so you don't squish the worms.
- Allow worms to settle for 5 minutes. Remove supernatant, leaving behind as little supernatant as possible, although you'll never be able to remove it all without disturbing the worm pellet. Combine the worms, and allow them to settle for 5 minutes and remove the supernatant again. Store the worm pellet at 15 °C at this point and don't spot the worms onto the next set of plates until 15 minutes before bombarding.
- Use a wide-bore sterile 200 μl tip to aliquot 100 μl (110 μl if you have a lot of worms) μl of concentrated worm suspension onto the center of round OP50 lawns on 60 mm NGM plates, one per bombardment. Allow excess liquid to soak into the plate (10 minutes). When the worm blob has a matte finish but has not yet begun to crack then they are ready to be bombarded. If they take more than 15 minutes to dry, your NGM plates started out too wet.

Preparing the gold beads on the day of bombardment:

1. For 20 bombardments, weigh out 12 mg of gold particles into a siliconized microfuge tube. (Use Anderson lab balance in room 4302. Gold should be enough to almost fill the concave volume at the bottom of a 1.5 ml tube.)
2. Add 1 ml of 70% ethanol.
3. Vortex for 5 minutes at maximum speed.
4. Spin 5 sec then remove supernatant.
5. Wash the beads 3 times with water. (The pellets will be loose and smeary. If the gold is clumpy, some labs sonicate to loosen it up.)

    Add 1 ml sterile water
    Vortex 1 min
    Allow beads to settle 1 min
    Spin 5 seconds
    Remove supernatant

6. Resuspend beads in 205 μl of 50% glycerol. (You can pause here if needed, although the beads tend to clump. We usually bombard two constructs, 10 plates each, every time we bombard, so place 100 μl into two siliconized tubes now.)
7. Vortex beads for 5 minutes to disperse clumps.
8. Continue vortexing (but at speed 2-3 so the tubes don't fly out and so that you don't shear the DNA) and add the following in order to each 100 μl of gold in 50% glycerol:

    10 μl DNA (0.5-2.0 μg/μl)
    100 μl 2.5 M CaCl2
    40 μl 0.1 M spermidine (diluted right before use)
    close the lids and vortex 2 minutes (not longer) at speed 3

9. Allow beads to settle 1 minute.
10. Spin 2 seconds.
11. Remove supernatant.
12. Wash in 70% Ethanol and then in 100% Ethanol.

    Add 300 μl of 70% without disturbing pellet.
    Wait 1 minute.
    Remove supernatant without disturbing pellet. No need to spin.
    Add 300 μl of 100% EtOH without disturbing pellet.
    Wait 1 minute.
    Remove supernatant without disturbing pellet.

13. Finally resuspend beads in 110 μl of 100% EtOH (per 10 bombardments), or 260 μl (for 20 bombardments). Do **not** use old ethanol that will have absorbed a lot of water from the air.
14. Gently vortex briefly to resuspend.
15. Since the beads tend to form clumps at this point, get the microparticles onto the macrocarriers as soon as possible. Place macrocarriers in a 100 mm Petri dish with the lid off. The first five should be in the macrocarrier holders, pressed all the way in with the plastic cap. **While vortexing** (at speed 0-1), pipet 10 μl of beads from the tube using a cut off 200 μl tip onto the center of the macrocarrier. Do **not** attempt to move or cover these until they are dry (5-10 minutes), unless they are already in one of the macrocarrier holders. Be careful of static. Use within 2 hours.

Bombarding the worms (Using PDS-1000 in room 2475, Genetics/Biotech Center):
Make sure to sign-up ahead of time at http://loci.wisc.edu/sign-up/whitecalendar.html
The portion of this experiment done in room 2475 generally takes about one hour. Remember that you will need at least an hour and a half in our lab before you will be ready to use the bombardment machine. The torque wrench for the PDS-1000 is on the side of the PDS-1000.

Turning on the bombardment machine:

1. Turn on the PDS-1000 (red 0/- switch).
2. Make sure the chamber door is open.
3. Vent the chamber. (Move to the middle position of the vac/vent/hold button.)
4. Turn on the vacuum pump
5. Open the valve on the top of the helium tank.
6. Spray down the inside of the chamber with 70% ETOH to minimize contamination.

Bombardment:

1. Using forceps, place a rupture disc into the rupture disk holder (upper assembly--darker of the two rings you removed). Screw the holder into place tightly with the torque wrench. This is very important. After you have bombarded the worms, make sure that there is an even round hole in the middle of the rupture disk—this means that it has not slipped out of the holder under pressure.
2. Place a stopping screen in to the bottom of the macrocarrier holder assembly (bottom assembly--the white squarish thing).
3. Invert macrocarrier holder (with macrocarrier with beads) into the assembly and screw the holding ring (silver ring) on to the assembly. (The macrocarriers need to be pushed into the macrocarrier holder with a plastic cap, so that they are all the way in, before you do this.)
4. Slide the assembly into the upper-most slot in the chamber.
5. Ensure that the specimen platform is in the second position from the bottom in the chamber. Place the worms in the center of the specimen platform. Remember NOT to leave the lid on the worm plate.
6. Close and latch the chamber door.
7. Draw a vacuum by pressing the vacuum button into the upper position.
8. When the vacuum reaches approximately 28 mm/Hg (with our pump the vacuum tends to only go to about 26.5 mm/Hg) depress and hold the fire button. When the pressure gauge reaches approx 1350 psi, the rupture disk should break (a loud pop). If you are seeing a pop below 1350 psi—make sure that you are tightening the rupture disk holder sufficiently—see note above.
9. Release the fire button and immediately vent the chamber by returning the vacuum button to the middle position.
10. Remove the worms from the chamber and save. The rupture disc, stopping screen, and macrocarrier should be discarded in the trash. Do **not** discard the macrocarrier holder!
11. Repeat steps 1-10 for all of your prepared microcarrier.

Shutting down the bombardment machine:

1. Close the valve on the top of the helium tank.
2. Close and latch the chamber door and draw a vacuum.
3. Depress and hold the fire button; the PDS-1000 pressure gauge should remain at 0 and both the tank and regulator pressures should reach 0.
4. Vent the chamber (move to the middle position of the vac/vent/hold button) and wait until the vacuum is gone.
5. Turn off the vacuum pump.
6. Turn off the PDS-1000 (red 0/- switch).

Make sure you have all 5 macrocarrier holders before you leave. Return the torque wrench to the holder on the side of the PDS-1000!

Post-bombardment:

Let the worms recover for 0.5-1 hour at room temperature. Then wash the worms from each small bombardment plate onto 3 large NGM OP50 seeded plates and place in the 23 °C incubator. (I use around 900 μl M9 per plate to wash, and dispense around 225 μl per plate with a cut off 1000 μl tip.) Also save the original bombardment plates; these will sometimes yield transformants. After the first day or two, place the lid on the plastic container to avoid the plates drying out too much. Wait 2 weeks.

Check your plates for movers. It is worth rechecking your plates twice later. You are looking for plates with multiple young moving worms indicating that there is a rescued worm on the plate that is transmitting properly. (It tends to be useless to pick individual older moving worms—since these can be transformed worms that do not transmit.) Because *unc-119* mutants do not form dauers, the untransformed Unc worms starve and die, making it easy to find non-Unc, wild type transformants. For each plate with moving worms, pick three healthy-looking moving worms onto individual small plates. Since these worms are likely (but not guaranteed) to be siblings you name them to reflect this (i.e.—7A, 7B, 7C). Be aware that a single plate may contain both extrachromosomal arrays as well as integrated lines. Letting these non-Unc worms grow up and starve and then examining their descendents may help you to focus in on stable integrant lines rather than lines carrying extrachromosomal arrays.

Look at progeny under a 60x objective to see if there is fluorescence in the embryos. You will not see fluorescence for most things at 20x (with the exception of histones). For live cell imaging, it doesn't make much of a difference whether the strain is homozygous or obligate heterozygous (integration occurred in an essential gene). However, for biochemistry, you really need a homozygous strain since you need to grow the worms for several generations without any type of selection. Iain has tried purification from heterozygous strains, and they are generally crappy.

Note: Ed in the Meyer lab floods the plates with a small amount of M9 and looks for thrashing worms. You can save the non-Unc worms by pipetting them off. This is worth trying as it may seem easier to some, and starved worms have an inconsistent harsh touch response.