Protocol used for PrepGEM digestion tubes:

1. Label the tops and sides of 200 uL PCR tubes with the appropriate individual nematode number

2. Prepare 300 uL of 1X PrepGEM Gold buffer using PCR grade water. Use a 1.7ml tube for this solution. This is the solution used for cutting the nematodes and transferring them to the digestion tubes.

3. Prepare a digestion mix consisting of: 21.25 uL PCR grade water and 2.5uL PrepGEM Gold 10X Buffer per reaction.

4. Aliquot 23.75 uL of digestion mix into each 0.5 ml PCR tube. Keep these tubes refrigerator prior to use.

5. Transfer the nematode (following microscopy) to a drop of buffer prepared in step 2 above. Cut the nematode in two. Use a P2 pipetter to draw up the nematode pieces in about 1 uL and place in the appropriate labeled tube containing digestion mix as aliquoted in step 4.

6. Use the dissecting microscope to visually confirm the presence of nematodes in each tube following transfer. Use a microcentrifuge to collect all fluid into the bottom of tubes as needed.

7. Store the tubes from step 6 in the -20C freezer until ready to treat a group of individual samples.

8. When treating a group of samples: Add 0.25 uL of PrepGEM enzyme to each tube using a P-2 pipettor and separate tips for each tube. Gently mix tube using your finger to "vortex" the contents. Spin the tube down briefly in a microcentrifuge to collect all fluid into the bottom as needed.

9. Incubate tubes @75C for 1 hour in PCR machine. Set thermal cycler to use heated lid.

10. Remove the tubes from the thermal cycler. Freeze/thaw the plate 3 times using dry ice. Samples will freeze in ~4 min. Spin the tube down briefly in a microcentrifuge to collect all fluid into the bottom as needed.

11. Return tubes to the thermal cycler and incubate again @75C for 1 hour. Set thermal cycler to use heated lid.

12. Heat kill the PrepGEM enzyme @95C for 10 min. Set thermal cycler to use heated lid.

13. Freeze the tubes at -20C for future use.