96 parallel DNA isolations from *C. elegans* using Qiagen Gentra Kit
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**Growth of worms:**

1. Chunk a 1 cm square chunk from a recently starved plate of *C. elegans* to a 6 cm NGMA plate spotted with 100uL of overnight OP50 culture and dried overnight at 37ºC.

2. Wait 2 days until the plate has plenty of L4 animals. Pick five L4's to two plates. Store plates at 20ºC for four days. We want lots of gravid animals after four days, so be sure to watch their growth and move them to room temperature if needed.

3. Follow the procedure to bleach a 96-well plate of worms found in HighThroughputBroodSizePumpingAssay v2.0 protocol (steps 7-33).
   a. For each 96-well plate of worms you want to prep, make 10-12 96-well growth plates that can be combined.

4. After aliquoting embryos, seal the plates with Rayon strips and put in a humidity chamber overnight in the Excella shaker at 20ºC, 180rpm.

5. The following morning, make a 100 mg/mL solution of food from HB101 bacterial lysate (include Kanamycin at a final concentration of 50 $\mu$M) in K Medium (recipe below). Using the electric repeat pipettor, add 5 $\mu$L of food solution to all wells containing worms in the 96-well plates.

6. Incubate the plates in a humidity chamber at 20ºC and 180 rpm until starvation.
   a. Check the humidity chamber every other day to make sure there is still moisture.
   b. It takes about a week for the majority of wells to starve

7. After the wells are starved, check each well. Mark the well if any of the following are an issue: mold contamination, bacterial contamination, empty, dehydration, or low numbers of worms. If you choose, record this information in a spreadsheet.
   a. For mold contamination, we remove the largest mold puffs with a sterile pipette tip (making sure to change the pipette tip for each well).
   b. For bacterial contamination, we simply remove all the contents from the well by aspiration and then wash the well three times with $dH_2O$.

8. Combine all growth plates for a single 96-well plate prep into a single deep well plate (#P9636, Denville).

9. Spin down worms at 1100 rpm and remove supernatant (see SetUp96WellAspirator protocol).
   a. If you are prepping more than one plate at a time, make sure to clean aspirator between plates!

10. Proceed to DNA isolation.

**DNA isolation:**

11. For the DNA isolation, we use the Gentra Puregene protocol from Qiagen (cat #158689, Qiagen). For a single DNA prep, add 600 $\mu$L Cell Lysis Solution (cat. # 158908, Qiagen) and 3 $\mu$L of Proteinase K (20 mg/mL; Proteinase K is provided with the kit, but you can also use cat. #03115852001, Roche) to your

sample. Seal the sample well with foil (cat #FS100, Cryogenic Storage Systems and Supplies) and mix by inverting 10 times.

- For a 96-well plate, make enough for 115 samples: 69 mL of Cell Lysis Solution with 345 $\mu$L of 20 mg/mL Proteinase K. Add 600 $\mu$L to each well. Cover with foil sealing tape and mix by inverting 10 times.

10. Incubate worm lysis at 55°C for two hours. Resuspend the cultures every 30-45 minutes using a microtiter plate mixer for 15 sec at 1200 rpm.

11. Incubate on ice for 15 minutes to cool the sample.
    Note: Make sure samples are cold before continuing; it will help with precipitation.

12. Add 200 µL Protein Precipitation Solution (cat. #158912, Qiagen) to each well. Seal well with foil as before. Mix by inverting 10 times.

13. Incubate the samples on ice for 10 minutes.

14. Centrifuge at 2000 x g for 10 minutes. The precipitated proteins should form a tight pellet. If the protein pellet is not tight, mix by inversion and then incubate on ice for 5 minutes. Centrifuge at 2000 x g for 10 minutes.

15. While the plate is spinning, aliquot 750 µL isopropanol into each of the wells of a new 2 mL deep 96-well plate (cat. #P9636, Denville).

16. Transfer 750 µL of the supernatant from the previous step into the new deep-well plate with isopropanol.

18. Seal the plate with foil and mix by inverting gently 10 times.

19. Centrifuge at 2000 x g for 15 minutes. The DNA pellet is difficult (or impossible) to see in the well. Mark the side of the deep-well plate oriented to the outside of the centrifugation step to know where the DNA density should be.

20. Remove supernatant by inverting the plate into the sink. Gently blot the plate onto paper towels to remove excess isopropanol before turning the plate over.

21. Add 800 $\mu$L of 80% ethanol and seal with foil.

22. Centrifuge for 15 minutes at 2000 x g.

23. Remove 80% ethanol by inverting the plate into the sink. Gently blot the plate onto paper towels to remove excess 80% ethanol before turning the plate over.

24. Evaporate off the residual ethanol by incubating at 65ºC for at least two hours.

25. During the drying of the pellet, make 12.5 mL of DNA Hydration Solution (cat. #158916, Qiagen) with 250 $\mu$L of 1 mg/ml RNase (cat. #158924, Qiagen).

26. Add 100 µL DNA Hydration Solution to each well. Seal and vortex on microtiter plate mixer at 2500 rpm for 10 minutes.

27. Incubate at 65°C for 1 hour to dissolve the DNA.

28. Incubate at room temperature (15–25°C) overnight with occasional 1200 rpm vortexes on the microtiter plate mixer.

29. Quantify DNA
    a. To quantify the entire plate, see the protocol “96-wellDNAQuantification_Synergy4_Qubit_v2”.
    b. You can also quantify 12 random wells and use the average. This is not recommended unless you are confident that you are getting approximately the same amount of DNA per well.

<u>K Medium Recipe (volumes in parentheses indicate the amount to add for a final volume of 500 ml)</u>
51mM NaC (5.1 ml of 5M NaCl)
32 mM KCl (16 ml of 1M KCl)
3mM CaCl2 (1.5 ml of 1M CaCl2)
3mM MgSO4 (1.5 ml of 1M MgSO4)

- Mix the four salts with increasing water, then fill to 500 mL of dH2O.

- Filter sterilize with ThermoScientific Filter Unit (Cat #566-0020).

- Add 1.25 ug/mL filtered cholesterol (125 uL of 5 mg/mL cholesterol) (see recipe below).

- Mix, label, and store for up to two weeks. Be sure to check the K-medium prior to use for any floating particulate, which is a sign of contamination. If K-medium is contaminated, pour it down the drain and make new K-medium.

<u>Cholesterol</u>
- To make 10 ml, dissolve 50 mg of cholesterol in 100% ethanol.
- Filter the material using a 0.2 $\mu$m filter (Cat. # SLLG025SS, Millipore)