Quick protocol: Obtaining synchronized *C. elegans* for imaging in the *vivo*Chip™

Newormics

The protocol below is our recommended method of obtaining synchronized *C. elegans* **(a more detailed guide for L4 stages is featured in the manual – Tip #5)**. Please reach out to us if you prefer to use a different method so we can help you optimize your protocol for best results with the *vivo*Chip.

1. Place L4 worms (suggested number: 3-4) on a 10 cm NGM plate with food. Grow at 20°C for 5 days to obtain a large number of gravid worms.
2. Wash down with M9 and collect all the worms and eggs into a 1.5 mL centrifuge tube.
3. Centrifuge at 1.3 ×g for 30 s to pellet the worms, and remove the supernatant fluid.
4. Add 500 µL bleach solution (0.5 mL 10 M potassium hydroxide, 1.65% final concentration of sodium hypochlorite (e.g. 2 mL of 8.25% bleach or 2.75 mL of 6% bleach) and H_20 up to a final volume of 10 mL). Incubate for 4 minutes, shaking tube every minute.
5. Add 1 mL M9 to the tube (neutralizes bleach), shake to mix, and immediately centrifuge at 1.3 ×g for 30 s. Remove supernatant leaving egg pellet in the tube.
6. Repeat this wash step (step 5) 4× to completely remove bleach. Add 1 mL of fresh M9 to the tube.
7. Incubate the tube containing the eggs at 20°C with gentle rotation for 16-24 hr.
8. Filter the suspension using the supplied blue 40 µm filter to help remove adult cuticles/large debris.
9. Plate the appropriate number of L1 worms for your experiment (**Note:** For adult stages reduce numbers or be prepared to: a. switch to a new food plate; b. add food to the 24-well plate).
    a. 200-400 on a 10 cm NGM plate for L4 imaging.
    b. 100 per well of 24-well plate (with 500 µL S media/bacteria) for L4 imaging.

Incubate the plate for the appropriate length of time at 20°C to allow the worms to develop to your stage of interest before imaging in the *vivo*Chip. Check food levels after a few days.

L2	L3	L4	D1	D2
8-12 hr	24-30 hr	48 hr	72 hr	96 hr

Note: The above times are just a guide, exact times may vary depending on strain, treatment, temperature, liquid vs agar culture etc., so observe growth carefully before imaging.

For L1 stages: use arrested L1 larvae straight away after 16-24 hr incubation in M9. Alternatively, filter suspension ~6 hr after bleaching through a 20 µm filter. The filtrate contains freshly hatched L1 larvae before growth arrest.

For D2+: After D2, progeny of the synchronized parent generation will appear in the culture at a mixture of life-cycle stages. It will become difficult to distinguish generations and the increased population will rapidly eat all available food. Filter the synchronized adult worms out at D2 (follow Tip #3 in manual up to step 7) and transfer adults to a fresh plate to obtain these older animals.