Hyplochlorite Method (Worm Bleaching)

- Make fresh hypochlorite solution

30 ml	40ml
- 2.4 ml 5N NaOH	- 3.2 ml 5N NaOH
- 24.6 ml dH_2O	- 32.8 ml dH_2O
- 3 ml Bleach	- 4 ml Bleach

- Wash worms off plates with M9 buffer
- I usually wash off 2 or 4 plates per 15 ml conical tube, depending on amount of worms on plates
- Let worms gravitate to bottom of tube and remove volume off the top until 4 ml total volume left
- Add 2 ml of hypochlorite solution to each tube and mix well
- Incubate on roller for 20 minutes at 25°C (30 minutes at 20°C)
- Centrifuge worms for one minute at 4K with a clinical centrifuge
- Discard supernatant and wash worms with 4 ml of M9 buffer
- Centrifuge again for one minute at 4K and wash again with M9 buffer
- Discard supernatant and resuspend worms in 1 ml M9 buffer and put into a 1.5 ml microcentrifuge tube
- Incubate worms and M9 overnight on roller at 20°C
- The next day plate L1s on seeded plates

M9 Buffer (1 L)
6 g sodium phosphate dibasic (22mM Na_2HPO_4) or 12 g 7-hydrate
3 g potassium phosphate monobasic (22mM KH_2PO_4)
0.25 g magnesium sulfate 7-hydrate (1mM $MgSO_4$) or 0.122 anhydrous
5 g sodium chloride (85mM NaCl)
pH to 6.5 with HCl and autoclave