Bleaching to synchronize worms-microfuge protocol

1) Wash the worms into a 15-ml Falcon tube with 1x M9. Centrifuge at 650g for 1 min in a Beckman Coulter Allegra 6KR or similar at RT to pellet the worms. **Throughout protocol use M9 with 0.05%-0.1% Tween20, gelatin, or PEG to prevent worm sticking.**

2) Aspirate media down to ~1 ml and transfer to a 1.5 ml Eppendorf tube.

3) Spin worms 1 min at 650 g. Aspirate off supernant and wash with 1 ml M9. Repeat wash 1-2 times, depending on how much bacteria was washed off of the plate.

4) After the final wash, aspirate down to a volume of ~500 µl. Add 500 µl of bleaching solution:

Per bleaching:
100 µl 5M KOH
200 µl bleach
200 µl dH20

Incubate for 3-7 min at RT and vortex vigorously for 30 s every minute. Proceed to the next step as soon as the worms dissolve and the eggs are released (monitor the Falcon tube under a dissecting microscope).

4) Pellet the eggs by centrifugation at 650g for 1 min. Discard the supernatant.

5) Wash the pellet four times with 1 ml of 1x M9. Centrifuge at 650g for 1 min between washes to pellet the eggs.

6) After final wash resuspend in **EITHER** 500 µl M9 and plate on unseeded 10 cm plate OR 200 µl M9 and plate on unseeded 6cm plate