Autoclaving Media

Used to collect sterile eggs from adult *C. elegans* worms.

- Ages of adult worms when performing egg prep are important. You do not want to do the egg prep too early, as not all the worms may be pregnant or laying eggs. This means you will get a very low yield of eggs.
- Use as many plates as you can, to ensure you get enough eggs. Around 8-10 plates should give more than enough eggs.

1. Wash/rinse adult worm plates using 10ml of M9 media and transfer to a 15ml Falcon tube.
2. Allow the worms and eggs to settle to the bottom of the falcon tube.
3. Aspirate all the excess M9 media- if you disturb the worm pellet, allow it to settle again before continuing.
4. Make a 7:8 solution of bleach to 4M NaOH.
5. Add 350µl of the bleach solution to the egg pellet.
6. Vortex the mixture to destroy any adult worm bodies and release the eggs. This should take around 3 minutes. **Note**: do not bleach the mixture for too long as this will also kill any viable eggs. The mixture should turn a yellowish colour when the eggs have been released.
7. Immediately dilute the mixture down to ~10ml using sterile M9 media.
8. Centrifuge down the eggs in the mixture. 5000rpm for 1 minute, or 4000rpm for 3 minutes.
9. Remove excess M9 and replace with 10ml of fresh M9 media. Resuspend the pelleted eggs (bang the falcon tube on the benchtop- need to be quite aggressive!).
10. Repeat the centrifuge and wash steps a further 3 times.
11. After final wash, resuspend egg pellet and transfer solution to an empty petri dish.
12. Incubate the solution at 20^{o}C, eggs should hatch in 24 hours.

[**Note**: you can check your solution for eggs when you have transferred it to the petri dish. If eggs have been collected you should be able to see floating black specks under the dissection microscope].