

## Steps

1. Pick old L4 larvae and transfer them in a small NGM plate (without bacteria)
2. Rinse the plate with M9 buffer (using less liquid as possible) Remove M9 buffer containing L4 larvae with a pipette into a 2 ml tube; fill the tube up 2/3 with M9 buffer.
3. Mix carefully by inverting 2-3 times Incubate for 3-5 minutes at RT
4. Spin at 1000/2000 rpm for few seconds (10s)
5. Pipette the pellet and transfer it in a small NGM plate (without bacteria) Check the amount of L4 obtained; centrifuge again for few seconds if necessary.
6. With a mouth pipette remove carefully the liquid Transfer with a pick or a pipette using oil 3 clean L4 larvae in a 200µl tube containing 4 µl of RNAi solution
7. Incubate at 20°C for 24 h
8. Remove L4 larvae by pipeting and transfer them in a small seeded NGM plate (this transfer is necessary because after 24h it could have already L1 larvae)
9. Transfer 3 worms (1 per well) in a row of a seeded 6-well plate Incubate 48h at 20°C
10. Progeny test