

SUCROSE FLOATING

- Make sure have COLD 100mM NaCl AND 60% sucrose ready
- Wash worms off plates with M9 buffer (3-4 plates per conical tube)
- Centrifuge worms for one minute at 4K
- Discard supernatant (with glass pipets not plastic) and resuspend worms in 7 ml cold 100mM NaCl and mix tubes
- Centrifuge worms for one minute at 4K
- Discard supernatant and resuspend again in 7 ml cold 100mM NaCl
- Mix well and incubate on ice for 10 minutes
- During downtime get tubes of 10 ml 100mM NaCl ready and leave on ice
- After incubation on ice, add 7 ml 60% sucrose to each tube and mix well
- Centrifuge for 5 minutes at 4K
- DO NOT expose worms to sucrose for too long because it will kill them
- After spin, you will see a brown pellet at the bottom of the tube which is the debris and a light brown layer at the top of the tube which are your floated worms
- Using a glass pipet, remove the floating worms and pipet into the tubes filled with 10 ml of 100mM NaCl sitting on ice
- Mix the tubes well and spin 4K for 1 minute
- Discard supernatant and add 5 ml 100mM NaCl to each tube
- Wash the worms 2 more times with 5 ml 100mM NaCl to get rid of all sucrose
- After last wash, resuspend worms in approximately 1 ml of 100mM NaCl and put into 1.5 ml microcentrifuge tubes
- Freeze instantly at -80°C for future lysis