Preparation of embryos for eFACS

Cultivate at least 4 Mio Synchronized Worms until they are very young adults which just start to produce eggs!

- Cool down centrifuge to 2°C **(pre-cooling takes ~30 minutes!!)**
- Cool down the M9 Buffer on ice!
- Cool all tubes on ice!

- Allow worms to settle in tubes, pool worms and wash with 0.1M NaCl/H_2O
- If worms were cultivated in liquid or highly contaminated clean on Ficoll
- Distribute worm pellets to have around **5mL worm pellet in 15mL**
- Bleach worms (in 20mL):

  15mL H_2O and worm pellet

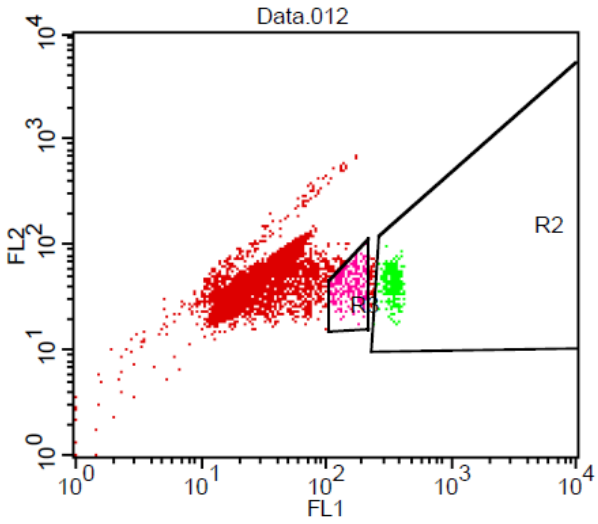
  2.5 mL Bleach (12% Bleach solution)

  2.5 mL NaOH 5N

- Bleach for 7 minutes!! Observe dissolved worms under the Stereo.
- While bleaching prepare ice bucket, with tubes and filters
- Vortex shortly and add **ice cold (!)** M9 buffer up to 50 mL
- Spin down at 280*xg* (1,200 rpm) for 30 seconds
- Discard supernatant
- Resuspend in ice cold M9 and vortex to rupture all remaining worms
- Wash 2x with ice cold M9
- Filter through 40µm cell strainer with a pipette
- Pool embryos in a 15mL Falcon tube
- Spin down (280*xg* 30'')
- Discard supernatant to 1mL
- Slowly add 8mL of 80% MeOH -20°C while vortexing (!) with mid. speed
- Fix on overhead shaker at 4°C for at least 1 hour
- Spin down at 280*xg* for 30''
- Resuspend in PBS containing 1% BSA and 0.05%Tween (filtered!)
  - optionally add 10mM Ribonucleoside-vanadyl Complex (NEB S1402S).
- Filter through 40µm cell strainer into FACS tube shortly before sorting.
- Always keep tubes on ice!
- eFACS

***Sorting embryos* eFACS**

- **Sorting parameters:**
  Drop delay ~10 (take the highest drop possible for sorting)
  Sorting speed ~500-800 events/sec
- **Select channels**, GFP(FL1) and one other channel (e.g. FL2) for visualizing autofluorescence.
- **Select gates** as shown in scatter plot:
  - **R2**(high GFP positive) ~ 1-cell stage
  - **R3** (intermediate GFP positive) ~ 2-4 cell stage.
    - This can be even further resolved up to 8-cell stage might become more contaminated with older embryos.
- Sequencially sort all fixed embryos. Always keep embryos on ice. Adjust concentration/density to be able to sort at not more than 1,000 events/second
- Sort into vessel containing 1% BSA/0.05%Tw/PBS on ice.



- Resort population once to increase purity.
- During the resort drop ~200 embryos onto a slide to check for sorting purity under a fluorescence microscope.