<u>Capitella agarose plates</u> Seaver Lab

Juvenile and adult worms can burrow through these 'sloppy plates'. Use of agarose plates is a great way to observe burrowing behavior or track individuals for short term culturing.

Note: use all 'E' glassware, stir bar and weighing spatula.

- -Weigh out 0.6g corn meal agarose (Sigma # 1176) and mix with 100 ml FSW.
- -Dissolve by heating agarose on a hot plate stirring with a stir bar. Cover with tinfoil. Corn meal agarose should dissolve about the time the solution comes to a boil. Try not to over boil and if you lose volume due to evaporation, add FSW back to original volume. Swirl mixture and make sure all granules have dissolved.
- -Rinse 60 mm petri dishes (or whatever size you want) 3x each with FSW prior to pouring in agarose.
- -Pour plates and allow to solidify overnight at room temperature. 100 ml makes 6 7 60 mm plates.
- -Prior to adding worms, pour off sea water and score surface of plate to allow worms to penetrate into the substrate.
- *Note*: Can also make 'relaxing plates' by dissolving 0.6 gm agarose in 50 ml $FSW + 50\ 0.37\ M\ MgCl_2$. The worms take 20 30 min to stop animals from moving through the substrate. This is also a good way to remove sand and mud grains from a worm by letting them crawl through the agarose and clean themselves off.
- -For longer term culture (e. g. weeks), plates can be conditioned with **unfiltered** sea water: pour thin layer of unfiltered sea water over top of solidifed agarose and leave for a few days to allow colonization with marine bacteria. Not necessary if worms will only be in plates for a few days.