MIC_1v1

Pombe Microscopy Sample Preparation (agarose pad).

by Ramón Ramos as of 15/10/13

Day 1: Prepare pre-culture in YES-EMM (if plasmid selection needed) medium

Day 2: Inoculate o/n culture in appropriate culture vol (e.g. 5 mL-10 mL) (Usually a OD of 0.02 in the afternoon (around 18:00 pm) give you a right OD next morning).

Day 3: Measure OD_{600} – culture should be beginning log-phase (>0.2 and <0.6).

-Pellet 1mL of cells at low rpm (0,5 min at 2000 rpm).

-Resuspend pellet in 10-20 uL of EMM.

-Prepare agarose pad:

-Melt EMM+aas+adenine 1.2% agarose (adenine reduce auto-fluorescence so be sure the EMM has adenine)

-Add 100uL in the center of one microscope slide and put other on top (not make pressure, just put on top).

-After several second separate the slides trying to keep the agarose pad in one of the slides.

-Add to the center of the agarose pad 10uL of sample.

-Cover with a coverslip (don't worry if agarose pad is bigger than coverslip).

-Seal the coverslip edges with VALAP.

-Use the spatula to take a small amount of valap.

-Hold it over the flame to melt it.

-Put a small bead of valap along the open edge of the coverslip.

-Repeat for the other sides of the coverslip.

-Samples are ready to microscopy, both pictures and movies.

Solutions:

VALAP – Total volume of 50-60mL:

10gr of Parafin
10gr of Vaseline
10gr of Lanolin

Slowly melt at low temperature with occasionally mixing. Once melted, transfer it to a bottle with a wide opening.

EMM 1.2% agarose - Total volume 20mL

20mL of EMM+aas (make sure has adenine)
240mg of Agarose.

Melt in the microwave.