Meiotic time course protocol

Lui, D., & Burgess, S. M. (2009). Measurement of spatial proximity and accessibility of chromosomal loci in Saccharomyces cerevisiae using Cre/loxP site-specific recombination. Meiosis: Volume 1, Molecular and Genetic Methods, 55–63.

Dresser, M. E. (2009). Time-lapse fluorescence microscopy of Saccharomyces cerevisiae in meiosis. In Meiosis (pp. 65–79). Springer.

Day 1

Retrieve SBY strain of interest (eg. SBY#5909) from box in the -80 ºC freezer.

Patch cells from glycerol stocks onto YPG plates. If mating two strains, patch the second strain on top of the first.

Incubate at 30 ºC overnight, start in the evening as you should not leave for longer than 15 hours.

Day 2

Retrieve YPG plates and leave at RT ºC until the afternoon, approximately 1 pm.

Streak the patches of cells out onto YPD plates, to get single colonies, and incubate at 30 ºC for two days.

Day 4

At approximately 1 pm, single colonies (smooth diploid colonies) from each strain are used to inoculate 5 mL cultures of room temperature YPD, in 15 cm glass test tubes.

Incubate liquid cultures at 30 ºC on roller drum at full speed for 30 hours.

Day 5

At approximately 5 pm, retrieve YPD cultures and check for GFP spots under microscope; vortex culture, take 5 µL, add to a slide, and coverslip.

Determine the OD_{600} of the culture on a spectrophotometer in a cuvette using 20 µL sample and 980 µL of water.

At 7 pm, inoculate 10 mL YPA, in 15 cm glass test tubes, to a final OD of 0.23 (typically around 100 µL, e.g. if OD_{600} = 20, then 2.3/20 * 1000 = 115 µL), and incubate on roller drum at 30 ºC for 14 hours. Also leave the bottle of SPM at 30 ºC.

Prepared 1.5% agarose pads in PBS, by melting agarose and placing approximately 400 µL onto glass slide with coverslips at each end, and place a long (24x50 mm) coverslip onto to spead agarose.

After waiting for agarose to harden (approximately 10 min), remove the coverslips from the slide, and cut the agarose into squares approximately 4x4 mm in size, using a scalpel.

Transfer the agarose pads, using forceps or small metal spatula, into a petri dish containing SPM or YPA (for the t_a time point), and leave the dish at 4 ºC overnight.

Day 6

In the morning, aim for 7 am (12 hours after the transfer to YPA), go to the 3i SDC microscope, turn Oko unit to 30 ºC, and start scope according to instructions (e.g. *SDC_startup-shutdown.pdf*).

Prepare 25x25 mm coverslips for imaging by placing on a paper towel taped to a platform, e.g. the top of a styrofoam box.

Charge the coverslips by adding 10 µL 0.5% polyethyleimine (PEI) to the center of each coverslip and spreading in a small circle.

Wash off the PEI with a squeezy bottle of MQ H_2O and let the slides dry at 37 ºC.

Transfer 750 µL of YPA culture to a 1.5 mL tube and spin at 10,000 g for 15 s. Remove almost all the supernatant and resuspend the pellet in the remaining liquid.

Add 2 uL of cells to a PEI-treated coverslip. Transfer an agarose pad on top of the cells, using a piece of Whatman paper to remove excess liquid.

Position a 25x25 mm silicone gasket in the center of a glass slide and add a small amount of culture media, e.g. 30 uL, and draw up excess to leave a thin liquid film for humidity.

Place the coverslip upside down on top of the gasket and press down on the edges to seal.

Go to the scope and use according to instructions (*definite_focus.pdf*).

After the pre-meiotic timepoint, transfer YPA cultures to 15 mL falcon tubes.

As the time approached 9 am (14 hours after transfer to YPA), pellet the cells at 1,000 g for 3 min, resuspend in 5 mL SPM, and pellet again at 1,500 g for 3 min.

Resuspend in 10 mL warm SPM, transfer to a new, 25 cm, glass test tube, place SPM cultures back on the roller from at 30 ºC (this is designated as t_0).

Sample each timepoint for imaging as above.

For DAPI timepoints, take 50 uL of culture to a tube containing 50 uL 100% ethanol, vortex and store at -20 uL ºC.

To stain timepoints, add 2.5 uL sample to a slide, add 2.5 uL 1:1000 DAPI stock, and place coverslip on top.

Score DAPI timepoints on fluorescent microscope with DAPI filter, counting the number of cells with 1, 2, or 4 nuclei; count until 200 cells have been scored.