Bi 321 Molecular Genetics Laboratory Winter 2009

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Laboratory: Room 229, Klamath Hall (only open during class hours and by appointment)

Class meetings: one hour lecture/discussion and 8+ hours lab/week during class hours (M, W, F 3-6 PM); ~4 hours/week additional time outside of class
Grading: lab work/notebook (30%), quizzes (20%), final report (40%), effort/other (10%).

General Structure of Course
-students will work mostly independently but for convenience can "collaborate" with a partner for some procedures
-students will keep individual lab notebooks, which will be examined periodically (~weekly); carbon copy notebooks should be purchased before first meeting (bookstore).

OVERALL PLAN OF QUARTER (please see endnotes for some additional information; supplemental information/protocols will be provided)

Week 1

Discussion: Scientific background, laboratory orientation, use of lab notebooks; sterile technique, making solutions and media, use of microscope for determining cell density; planning; technical resources
Laboratory session 1: Make media for mutagenesis[1] and conidia filter beaker[2]; plan mutagenesis and platings to generate mutants (these plates will get "Basta" to select mutants) and to determine survival (these will be non-selective so we'll want to plate many fewer spores).
Laboratory session 2: Harvest and count conidia[3] of strain N2977[4] using hemocytometer[1]; UV-mutagenesis (plates to select mutants and others to calculate survivorship); plan & prepare materials to next part
Laboratory session 3[5]: Add selective top-agar 18 hours after plating; plan & prepare materials

Week 2

Discussion: Sexual crosses; complementation testing; meiotic silencing
Laboratory session 1: score plates and "pick" (transfer) 40-50 potential mutants[6]; grow 2-4 days at 32 C in Basta slants (~1.5 ml of 1X Vogel's, 2% sucrose, 1.5% agar plus supplements[7] in "baby tubes"); prepare media for next week and solutions needed for DNA isolation[8].
Laboratory session 2: If they look ready, suspend conidia and spot-test on hyg FGS medium (save suspensions @ 4C); inoculate 12 mating plates with *Mauriceville* strain (N32) and 12 mating plates with *Sad-1 his-3* strain[9]
Laboratory session 3: Check spot tests; prepare for next week

Week 3

Discussion: Heterokaryon incompatibility; RFLP mapping; DNA isolation
Laboratory session 1: score potential mutants; using stored conidial suspension, inoculate 12 presumptive mutants into liquid medium (for DNA isolation)[10], into baby agar slants (to store for future use) and use the conidia to fertilize both sets of cross plates.

Laboratory session 2: Harvest and lyophilize cultures grown to isolate DNA.
Laboratory session 3: Carry through DNA isolation to step 9 (TCA-ethanol precipitation).

Week 4

Discussion: Gel electrophoresis and Southern blotting
Laboratory session 1: finish DNA isolation; plan restriction digests
Laboratory session 2: do restriction digests & gel electrophoresis on DNA of starting strain and presumptive mutants; set up Southern transfer
Laboratory session 3: Start Southern hybridization; plan & prepare materials

Week 5

Discussion: Genotyping sexual progeny
Laboratory session 1: Wash Southern blot, expose and interpret data
Laboratory session 2: Select one striking (null?) mutant for RFLP mapping (using progeny of cross with Mauriceville) and one for complementation tests (using progeny of cross with *Sad-1* strain) against previously identified methylation mutants[11]; plate and heat-activate ascospores of suitable crosses to select mutants (Hyg, Basta media)
Laboratory session 3: Pick 40 progeny of the Mauriceville cross to properly supplemented Basta slants; similarly, pick 10 progeny of the *Sad-1*cross to check mating type and to test for transmission of the methylation defect

Week 6

Discussion: Bulked Segregant Analysis; Mating Type tests
Laboratory session 1: Prepare conidial suspensions for Mating Type spot tests (*Sad-1* progeny) and to inoculate 5 ml cultures to isolate DNA (Mauriceville progeny)
Laboratory session 2: Examine MT tester plates; Harvest cultures for DNA isolation; lyophilize
Laboratory session 3: Examine MT tester plates; Carry DNA isolation to step 9 (TCA-ethanol precipitation); set up heterokaryon tests (discuss)

Week 7

Discussion: PCR
Laboratory session 1: Finish DNA isolations; examine aliquots (5μl) on gel
Laboratory session 2: Set up PCR on pooled progeny of Mauriceville cross and controls (discuss)
Laboratory session 3: Start restriction digests and gel electrophoresis to score linkage; if heterokaryons appear fully grown, harvest and lyophilize them

Week 8

Laboratory session 1: Harvest heterokayons, lyophilize
Laboratory session 2: Carry DNA isolation to step 9 (TCA-ethanol precipitation) on heterokaryons; run more gels if necessary for mapping
Laboratory session 3: Finish DNA isolations; set up restriction digests

Week 9

Laboratory session 1: gel electrophoresis; set up Southerns
Laboratory session 2: Start Southern hybridization; work on mapping
Laboratory session 3: Wash Southern blot and expose; interpret data

Week 10

Laboratory session 1: Initiate follow-up mapping procedures if desirable (genotyping individual progeny)
Laboratory session 2: Miscellaneous follow-up.
Laboratory session 3: Miscellaneous follow-up.

[1] See note below about requirements for growing the strain. Consider that for each "treatment" (untreated and three UV treatments, e.g. 0, 3, 6, 9 seconds) you should plate about 20,000 conidia/plate on four plates for selection of mutants. But you don't include the selective agent (Basta) right away because this would probably kill nearly all the cells, including your mutants (why?). So, instead, 18 hours after the treatments, 10 ml of Basta-containing agar medium (use 3X Basta because it will be diluted by the bottom agar, which would be ~20 ml). To calculate total viability, you will want to put a smaller number of conidia on plates that won't receive Basta. A 1/50 dilution of what you put on the other selection plates (i.e. 400 rather than 20,000 cells) might be good. Thus you will need at least 20 plates, which you should be able to make from 0.5 liters of medium (use 20 ml/plate). Be sure to wrap plates in foil immediately after UV treatment to avoid photo-activated repair.

[2] Use rubber band or tape to attach double layer of cheesecloth to top of small (~100 ml) beaker making a funnel shape by pushing cheesecloth in middle; cover with foil and autoclave.

[3] Examine the hemocytometer under the compound microscope (use total magnification of 100X or less) and note that in the middle there are a set of 25 squares (5x5), each divided into 16 smaller squares. If you were to count all the spores in the 25 squares, this would correspond to 10^{-4} ml. But you don't really need to count that much. I usually count the 4 corners and the middle square and then multiple by 5 x 10^4 to get the concentration of spores in the suspension. Note that you will need to dilute your stock so that you get a reasonable number of spores to count. It's sometimes necessary to do serial dilutions. Typically, dense conidial suspensions contain 10^8-10^9/ml. You might want to first try counting conidia in a 100 X dilution (dilute 10 μl of your conidial suspension into 1 ml) but it there are too many conidia to easily count, you might then want to do another 10 X dilution (e.g. mix 100 μl of the diluted conidia with 900 μl water). If you were to count conidia in this second dilution and found a total of 40 conidia in 5 of the 25 big squares, you'd calculate the concentration in your original stock to be: 40 x 5 x 10^4 x 10^2 (first dilution) x 10^1 (2^{nd} dilution) = $2x10^9$ conidia/ml.

[4] The genotype of strain 2977 is: *a*; *his-3; am; inl; hph^m bar^m*. Note that growth media must be supplemented with histidine, alanine and inositol for this strain. However, the Basta selection works best with minimal alanine supplementation and with a modified "Vogel's" (basic salts for medium; normally one just needs Vogel's plus sugars and whatever supplements are required for a particular genotype) that lacks ammonium nitrate ("Vogel's w/o NH_4"). Thus, for your UV-mutagenesis, in preparation for the Basta selection, plate on non-selective medium containing Vogel's w/o NH_4; 0.5% proline; 1X FGS (0.5 mg/ml fructose, 0.5 mg/ml glucose, 20 mg/ml sorbose); 1X histidine (0.5 mg/ml); 0.25X alanine (0.25 mg/ml); 1X inositol (50μg/ml) solidified with 1.5% agar. This is the "BOTTOM AGAR". Use 20 ml per plate. 18 hours later, you should add 10 ml of "TOP AGAR" which has the same composition as the bottom agar except that it will contain "3X" Basta when you need to select for mutants and will only have 1.0% agar. When you just want to know how many viable cells you have you don't want to select for Basta and therefore will not include this drug in the top agar.

[4] Examine the hemocytometer under the compound microscope (use total magnification of 100X or less) and note that in the middle there are a set of 25 squares (5x5) each divided into 16 smaller squares. If you were to count all the spores in the 25 squares, this would correspond to 10^{-4} mls. But you don't really need to count that much. I usually count the 4 corners and the middle square and then multiple by 5 x 10^4 to get the concentration of spores in the suspension. Note that you will need to dilute your stock so that you get a reasonable number of spores to count.

[5] Note that the need to add the Basta top-agar 18 hours after the original plating will require an extra (but short) visit to the lab on Thursday.
[6] note in lab book which dose each came from; if you didn't obtain 40-50, see if others have extra candidate mutants you can have.
[7] supplemented with alanine, inositol and histidine because of genetic defects of host strain
[8] To grow tissue for DNA isolation, strains will be grown in 5ml medium in 6" tubes; plan for 12/person, hoping to get at least 10 each that grow OK
[9] Mauriceville is a highly polymorphic wildtype strain that we will use for RFLP mapping; the other strain, which is the standard laboratory "Oak Ridge" background, has a mutation (*Sad-1*) to prevent meiotic silencing (increasing the likelihood of getting deletions and rearrangements through crosses) and will be used to make strains for complementation tests.
[10] Also, grow and isolate DNA from your starting strain, as a control. The medium for this tube should not contain Basta whereas the others should (why?).
[11] We'll confine ourselves to mutants that have no detectable methylation: *dim-2, dim-5, dim-7, dim-8, dim-9, hpo*. To generate a heterokaryon, the two strains have to be "compatible" (same genetic background and same mating type gene) and it is helpful to have "forcing markers". We can complement *inl* or *his* defects of our new mutants and *trp* defects in the previously identified mutants.