**PREPARING FOR EXAM I**

**For lab –** review the lab manual and associated readings referred to in the lab manual & those handed out in the lab. Review last year’s exam. Ask yourself – what did I do, how did I do it, what did I observe, how can I explain the observations? You will not be asked to details such as the concentration of reagents used in a reaction, but you should know the purpose of reagents (e.g., what is a buffer & why is it used?) What is BSA and why is it in restriction digestions? What is the enzymatic activity encoded by the bla gene *of E. coli*?).

**Experiment 1. Cloning and characterization of a eukaryotic gene. Goal – to test the hypothesis that a segment of *Saccharomyces cerevisiae* DNA encodes a yeast gene.**

1. *Clone a segment of yeast DNA*
* Gel fractionate and purify a piece of yeast DNA (“insert DNA”)
* Cut pTZ18u/19u cloning vectors with EcoRI and HindIII
* Prepare competent TG1 *E. coli*
* Ligate PTZ vector with insert DNA and transform into the competent *E. coli*
* Select transformants on ampicillin plates and score for insertional inactivation of the lacZ gene using X-gal/IPTG for blue/white screen
* Isolate DNA from the presumed recombinant and non-recombinant transformants and determine whether your cloning experiment was successful (i.e., that you generated the desired vector plus insert construct)
1. *Determine whether the cloned yeast DNA contains a gene naturally expressed in yeast*
* Isolate total RNA from yeast. Quantify and fractionate this RNA by denaturing gel electrophoresis
* Transfer the gel-fractionated RNA to a charged nylon membrane and pre-hybridize the membrane.
* Prepare + and – strand “riboprobes” by in vitro transcription of your recombinant DNA with T7 RNA polymerase and NTPs (including 32-P UTP). Quantify radiolabel incorporation and add probe to membrane transfer of yeast RNA in the hybridization step
* Wash off excess probe and expose the membrane to a phosphorimager screen. Use the Typhoon phosphoimager to detect the radioactivity signatures stored on the phosphoimager screen. Based on the signals obtained with the two hybridization probes determine whether the cloned DNA segment contains at gene and if so, determine the direction of transcription in yeast of the natural transcript with respect to the EcoRI and HindIII restriction sites (assume that these same sites are present in the genome of yeast at this locus).
* Use of phosphoimager
1. *Identify the specific gene encoded by your “insert DNA”*
* Purify the double stranded DNA on a Qiagen column and direct it to the sequencing center at the University of Cincinnati
* IN PROGRESS: Do a “Blast” search to identify the gene encoded on your recombinant DNA

**Experiment 2. Isolation of a gene by complementation.** Goal, identify the wildtype allele of a mutation in a gene identified based on a desired characteristic. In this case, we were interested in knowing which genes in yeast are needed for pre-mRNA splicing. The *prp38-1* mutation was identified as encoding a temperature sensitive splicing factor. But what is the structure of the *PRP38* gene or Prp38 protein?

* Transform yeast with three coded plasmids, one of which was an “empty vector” (YCplac33), one the same vector containing a yeast genomic DNA insert that includes *PRP38* (YCplac33-PRP38), and one the simple cloning vector, pTZ18u
* Basics of yeast transformation, yeast/E. coli shuttle vectors, and temperature sensitive mutations

**Experiment 3. Use of the Polymerase Chain Reaction to characterize nucleic acids. Goal to use PCR to 1) amplify a genetic locus to detect a genomic polymorphism and 2) to make a site-specific mutation in a plasmid DNA**

* Isolate yeast genomic DNA from a haploid mutant (*sqs1::KAN),* a haploid wildtype (*SQS1*) and heterozygous mutant/wildtype diploid strain (*sqs1::KAN/SQS1*
* Conduct standard PCR using oligonucleotides (i.e., primers) that flank either side of the *SQS1* locus to amplify the intervening DNA. Compare the lengths of the amplified DNAs to determine which strains correspond to the wildtype, mutant, and heterozygote.
* Create a site-directed deletion by inverse PCR using the circular pTZ18u plasmid as a template. Confirm deletion by agarose gel electrophoresis using a linearized (but unmutagenized) plasmid as a control.

**Experiment 4. Use of the yeast two-hybrid system to study protein-protein interaction in vivo.** Goal: Use the Y2H approach to localize the Prp43 binding domain on the Pxr1 protein.Use plasmids containing full-length Prp43-Gal4 DNA binding domain fusion on the pAS2 plasmid and a series of Pxr1 peptide-Gal4 transactivation domain fusions on pACT2. Introduce into a yeast strain bearing a Gal4-activated *HIS3* reporter gene plus *trp1* and *leu2* mutations. Select the double transformants based on the *TRP1* and *LEU2* plasmid markers then score for reporter gene transactivation on medium lacking histidine (and containing 3 aminotriazole). Overview of technique.

**For Lectures -** The first set of lectures mostly review the “tools of the trade” – enzymes (e.g., nucleases, polymerases, kinase, ligase, phosphatase) & common techniques used in molecular biology research. Review the PowerPoint slides and the assigned reading in the NEB catalog & textbook. Start off by writing down the name of each enzyme mentioned. For each enzyme presented, you should know its 1) substrate preference (e.g., RNA or DNA, single strand or double strand), 2) what the products of the reaction are and 3) applications of the enzyme to molecular biological research.

In addition to the enzyme list, we discussed general features of bacteria (genotype features in TG1) and a few other topics such as restriction/modification systems & DNA topology, use of radioisotopes, basic genetic screens.

**Play it smart - read last year’s exam – some questions on will be very similar this year.**

**My quiz and exam questions derive from our discussions and the assigned readings. *I do not seek out questions from assigned readings that were never discussed in lab/class or covered in the lab manual.*** However, the assigned readings provide background and context and can clarify discussions we have in the lecture and lab. If you do not complete the assigned readings in the textbook and on the class web site, you may not have a full understanding of the material and consequently score less well on the quizzes and exams.

In addition, the NEB catalog will be used for the open book portion of your first exam – you will need an understanding of the information & charts in this catalog to answer questions on the exam.