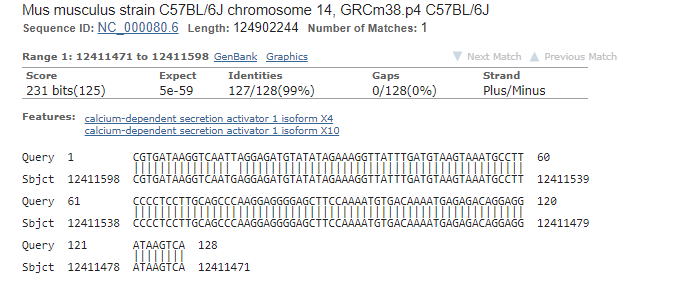
# S2. CRISPR in yeast: Sample Assessments

## Practical Exam I Sample Questions

1. You are provided overnight liquid cultures with bacteria containing the pML104. Briefly list the steps to produce a streaked plate with individual colonies, while maintaining selective pressure. (4 pts)
2. The following list contains reagents that you used throughout the semester. For each reagent, provide a brief description of its function. (6 pts)
   1. Loading Dye
   2. GM119
   3. Silica Column
3. A protocol requests you pour a 2.0% agarose gel. The volume your gel tray holds is 60 ml. How would you make this? (5 pts)
4. What would the running buffer be for the gel you made in Question #3? (2 pts)
5. After isolating pML104 from *E. coli*, you run the plasmid out on an agarose gel along with a 1KB plus DNA ladder. When you image the gel you do not see any plasmid DNA. What is one thing that could have gone wrong in your experiment, and how would you fix that? (4 pts)
6. In the following BLASTN alignment, identify (circle and label on screenshot) and define the following key characteristics. (4 pts)
   1. E value
   2. Hit sequence

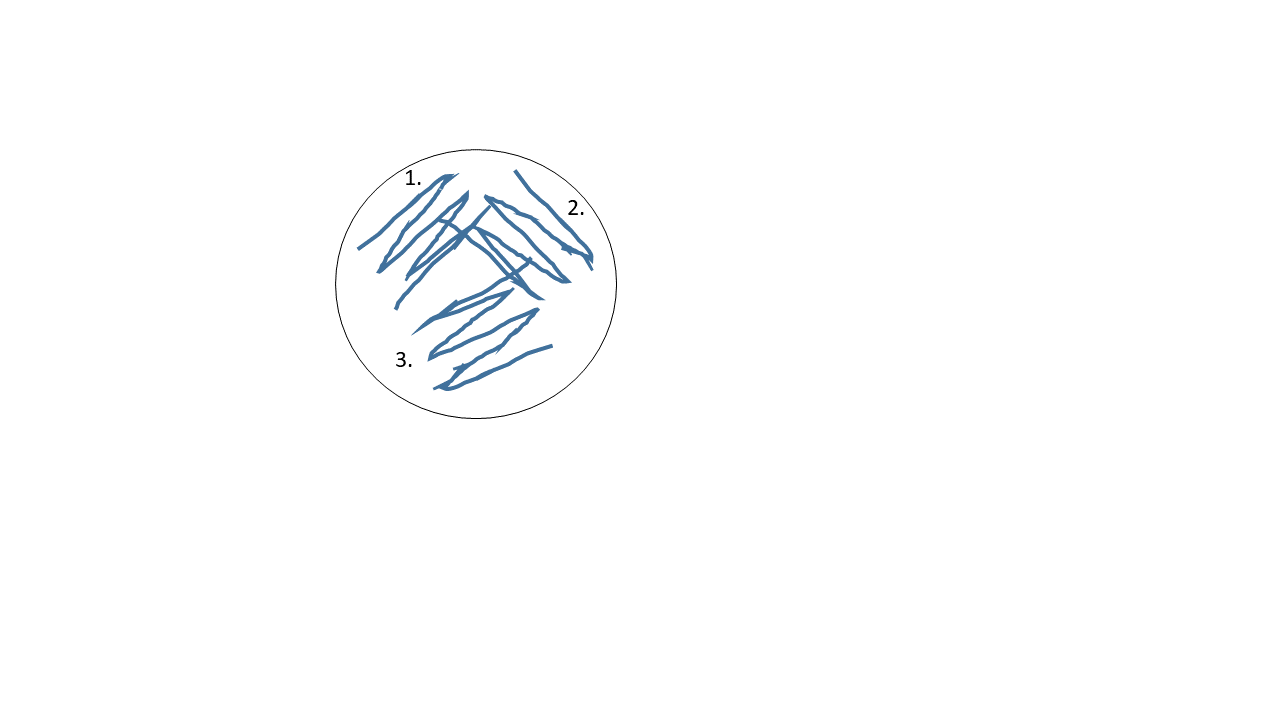


1. Describe three qualities that you considered when designing the template for homology directed repair of CRISPR/Cas9 cute sites. (3 pts)

**You may use a computer in the computer lab to answer the following questions:**

1. What are the first 10 nucleotides of the *HIS3* coding region in yeast? (2 pts)
2. Name the **enzyme** coded for by the *HIS3* gene. (2 pts)
3. What is the first PAM sequence after the start codon in the *HIS3* gene? \_\_\_\_\_\_\_\_\_ About how many basepairs downstream from the start codon is it? \_\_\_\_\_\_\_\_\_ (4 pts)
4. If we were to disrupt the *HIS3* gene in yeast, how would you suggest we phenotype these mutants? (4 pts)

## Practical Exam I Sample Questions – KEY

1. *(1 pt mentioning the plate contents, 2 pts streaking pattern, 1 pt flaming loop between each streak)*
   * *Flame and sterilize an inoculating loop*
   * *Dip loop in bacterial culture*
   * *Spread bacteria over one section of an LB plate with antibiotic*
   * *Re-flame the loop*
   * *Drag loop through the spread area then streak onto a new section of the plate*
   * *Re-flame the loop*
   * *Drag loop through the second streak and streak into another new section*
2. *(2 pts each)*
3. Loading Dye: *allows you to see the sample and weigh it down so that it sinks into well and stays there*
4. GM119: *Strain of E. coli that lacks Dam methylases.*
5. Silica Column: *Binds DNA for purification*
6. *How to make a 2% gel:* 
   1. *Weigh out 1.2 g agarose and add to flask*
   2. *Add 60ml of 1X running buffer (SB in our case)*
   3. *Heat in microwave until boiling and clear*
   4. *Cool slightly then add Ethidium bromide*
   5. *Pour solution into gel mold and add comb*
7. *1X SB or 1X TAE. (Need to be the same as used to make the gel above)*
8. (*1pt for a problem, 2pts for a solution to the problem) Some examples include:* 
   * *Incomplete cell lysis during plasmid purification. Repeat Miniprep, ensuring enough time for lysis*
   * *Forgot to add ethidium bromide to gel. Remake a gel with Ethidium bromide and re-run the sample.*
   * *No selection in bacterial culture. Start a new culture with antibiotic. Repeat miniprep and electrophoresis.*
9. *(2 pts for definition. 2 pts for identifying it)*
10. E value: *Probability that a match (or hit) was found at random*
11. Hit: *The sequence found by blast to have similarity to the input sequence*



1. (3 pts: 1 pt each)

* *Arms of homology*
* *Desired mutation*
* *Mutant or disrupted PAM sequence*

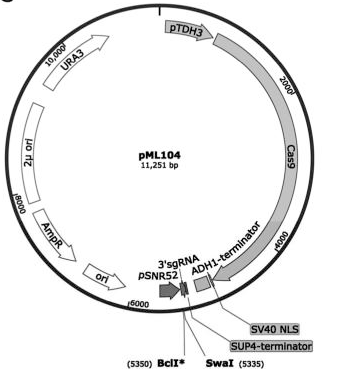
**You may use a computer in the computer lab to answer the following questions:**

1. *ATGACAGAGC AGAAAGCCCT* (4 pts)
2. *imidazoleglycerol-phosphate dehydratase* (2 pts)
3. *GGG. (On the opposing strand;* 2 pts) *12-14nt from the A of the ATG* (2 pts)
4. *Mutants would not be able to grow on media lacking histidine (2 pts), but would be able to grow on complete or rich media (2 pts for control).*

## Practical Exam II Sample Questions

1. Below is the plasmid map of pML104. Label the following (b-d) on the map by drawing a labeled arrow. The first (a) is answered for you, as an example. (4 pts)
   * *Gene encoding Cas9 (Sample. Answered below)*
   * Gene that allows selection in bacteria
   * Gene that allows selection in yeast
   * Yeast origin of replication
   * Restriction sites for gRNA cloning

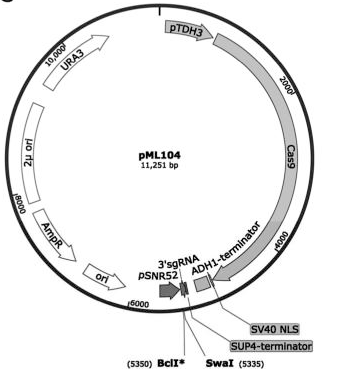
*a*



1. The following list contains reagents that you used throughout the semester. Choose 3 reagents (and only 3) and provide a brief description of its function. (6 pts)
   * Ampicillin
   * Agarose
   * Loading Dye
   * Ethidium Bromide
   * DH5alpha
   * DNA ligase
   * Single stranded DNA template
   * SB Buffer
2. At what temperature do *E.coli* grow? (2 pts)
3. Describe the process of oligo annealing. Include necessary information on the how and why we performed this process. (8 pts)
4. Outline the steps taken to perform CRISPR/Cas9 gene editing on the yeast *TRP1* gene. Start with a yeast that has been transformed with pML104 containing a gRNA gene with a template for repair. Outline the process through phenotyping. Include the materials and purpose in your description (conceptual only; don’t need reaction conditions and volumes, just the purpose/principle). Include expected outcomes of the experiment. Feel free to supplement your descriptions with illustrations or complete your answer in the form of a flow chart (must be complete). (8 pts)
5. Design a gRNA to target CRISPR/Cas9 gene editing for disruption of the *HIS3* gene in *S. cerevisiae*. (You may use a computer and the website used in class to answer this question) Provide the sequence of the gRNA. Also, tell me how many nucleotides downstream from the beginning of the HIS3 start codon Cas9 will cleave the gene. (6 pts)

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## Practical Exam II Sample Questions - KEY

1. *(1 pt each)*

*e*

*d*

*c*

*b*

1. *(2 pts each)*
   * Ampicillin – *antibiotic for selection of E. coli containing the plasmid, pML104*
   * Agarose – *separation of DNA molecules, based on size*
   * Loading Dye – *Adding color and density to DNA samples for ease loading an agarose gel*
   * Ethidium Bromide – *Allows visualizing DNA an an agarose gel, with UV light*
   * DH5alpha – *E. coli strain used to produce cloned pML104-gRNA*
   * DNA ligase – S*ealed the backbones of the gRNA gene to pML104*
   * Single stranded DNA template – *to template HR repair of the CAs9-mediated double stranded break, introducing the desired mutation*
   * SB Buffer – *Buffer for agarose gel electrophoresis*
2. *E. coli grow at 37°C* *(2 pts)*
3. *(2 pts each)*
   * *A pair of synthetic DNA oligonucleotides (Reverse complements) are obtained (ordered)*
   * *Mix the oligos together*
   * *Heat to denature (or boil)*
   * *Slow cool to room temperature*
4. *(8 pts)*

* *Select for yeast that have been transformed with gRNA gene in pML104, or empty pML104 on media lacking uracil (optional)*
* *Pick colonies from both pML104-gRNA plate and pML104 alone (control) and resuspend in water (1 pt)*
* *Make sure that each suspension contain approximately equal concentration of yeast cells by reading the A600 and adjusting them to equal A600 with water (1 pt)*
* *Perform serial dilutions of yeast suspensions (1 pt)*
* *Spot dilutions on a plate lacking tryptophan and on a plate containing tryptophan (1 pt)*
* *After several days of growth, expect to see similar growth of the control (pML104 only) on both plates. Successful mutants will grow on media containing tryptophan but not on media lacking tryptophan. (4 pt)*

1. *gRNA sequence should be: 20 nt guide long (1 pt), not include PAM sequence (1 pt), gRNA is adjacent to PAM sequence (1pt), upstream from the PAM sequence & on the correct strand (2 pt). Cut site is 3 nt upstream of PAM (1 pt)*
   * *Option 1: TAGTAAAGCGTATTACAAAT. Cleaves 12 nt from the start of the coding region*
   * *Option 2: TCAGATTGCGATCTCTTTAA. Cleaves 67 nt from the start of the coding region.*