### PCR assignment

**Please be sure to attach a printout of your gel with each lane labeled.**

|  |  |  |  |
| --- | --- | --- | --- |
| **PCR product**  | **Are you expecting a PCR product?** | **If so, what is the expected size (bp)?** | **Actual size (bp; approximate)** |
| TPCR PC |  |  |  |
| TPCR NC |  |  |  |
| FPCR PC |  |  |  |
| FPCR NC |  |  |  |
| BB |  |  |  |

The positive control (PC) consists of a plasmid and two primers. Why do you include this control when running a templateless or finish PCR reaction?

What would expect to see if you were to run the templateless PCR product on a gel? Therefore, why is there little point in running a templateless PCR product on a gel?

After DNA fragments have been separated by gel electrophoresis, how do we visualize the DNA (which is normally clear and colorless)?

What is the difference between template-dependent and template-independent PCR? Is the Finish PCR a template-dependent or template-independent PCR?

The templateless PCR begins at an annealing temperature of 69 degrees and then uses annealing temperatures of 65 and 61 degrees. The finish PCR uses an annealing temperature of 55 degrees. Why would the annealing temperatures for these PCR reactions differ?

If your PCR is not sensitive enough and you get no PCR product, what are two things that you could change (in terms of the reaction conditions) to improve your results to get a PCR product?

When performing PCR, you want to maximize the yield of specific product and minimize the amount of nonspecific product that can result from primers binding to non-identical regions of DNA. If your PCR is not specific enough and you get more than one PCR product, what are two things that you could change (in terms of reaction conditions) to improve your results to get only one PCR product?

### Ligation and Transformation Assignment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Number of blue colonies | Number of white colonies | Total number of colonies  | Percent white colonies |
| DNA building block # : |  |  |  |  |
| Control insert |  |  |  |  |

1. What is the role of X-Gal in the bacterial plates? On plates with X-Gal and IPTG, what color cells contain plasmids with your insert and why?
2. Based on the colonies on your bacterial plate, how would you be able to tell if your **ligation** didn’t work (what differences would you expect in the number or color of bacterial colonies)?
3. Based on the colonies on your bacterial plate, how would you be able to tell if your **transformatio**n didn’t work (what differences would you expect in the number or color of bacterial colonies)?
4. The next step in our project is to pick colonies of the correct color and screen them by PCR to verify the presence of an insert of the correct size. If a bacterial colony is the correct color, why is this next screening step necessary?
5. It is easy to sequence the mixture of PCR products directly without first cloning them into a plasmid. Why is it important to clone the PCR products first before screening for perfect DNA molecules?
6. When you pick bacterial colonies for PCR screening, it is crucial to pick only one colony and not to accidentally combine two adjacent colonies together. Why would it be a problem if you did accidentally pick two colonies together (think about your answer to question 5 above)?
7. Bacteria containing a plasmid with a DNA insert in the LacZ gene will usually produce a white colony; however, sometimes, bacteria containing a plasmid with an insert in the LacZ gene will produce light blue colonies. How does cloning an insert into the LacZ gene disrupt function of the LacZ protein and lead to white colonies? Why might an insert cloned into the LacZ gene occasionally produce blue bacterial cells (hint: think about the reading frame of the LacZ gene)?
8. When cloning using restriction enzymes, we must very carefully pick which enzymes we use based on the sequence of our insert DNA. When cloning using topoisomerase, why can we clone an insert into the vector regardless of the sequence of each building block?
9. What is the difference between a selection and a screen? Is ampicillin used in a selection or a screen? Is X-gal used in a selection or a screen?

### Colony Screening PCR Assignment

1. Why is it important to screen the bacterial cultures prior to sending them for sequencing? What would be the problem if we just picked 12 white colonies from the bacterial plate, grew cultures, and sent them for sequencing?

2. When we perform colony-screening PCR we often get three types of products: those that contain a building block of the correct size, those that contain an insert that is shorter than the building block is supposed to be, and PCR products that are about 200 bp long. How could we get a PCR product that was approximately 200 bp and why might a PCR product of this size be frequently produced when we do not get proper ligation of a building block into the vector (Hint: look at where the primers bind on the vector map and the size product that is produced)?

3. When troubleshooting your finish PCR, you make some modifications that decrease the specificity of the reaction. In other words, you get more finish PCR product of the correct size, but also more PCR products of the incorrect size. Why would this pose a major problem when you get to the colony-screening PCR step?

4. Based on the map of the multiple cloning site on page 4, design T7 and T3 primers to amplify your building block insert. The primers should be 20-22 bp long and should have melting temps that are within 2 degrees of each other (use the formula Tm=2(A+T) + 4(G+C)). Make sure to indicate the 5’ and 3’ ends of your primers.

5. Results. **Attach a printout of your colony screening PCR gels**. For each building block, indicate how many bacterial clones gave PCR products of the correct size. Those bacterial clones are now ready for sequencing.

Building block name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# clones screened by colony-screening PCR\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Expected size of PCR products\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# clones with PCR products of the correct size \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

% of screened clones with PCR products of the correct size\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

### Yeast Transformation Assignment

1. Fill in the table below with the results of your yeast transformation:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Reaction | Positive control | Negative control | Chunk plate 1 | Chunk plate 2 |
| Number of colonies |  |  |  |  |

1. Have you updated the database with the results of your yeast transformation and yeast DNA preparation?
2. The yeast strain we are using has the genotype his3delta1 leu2delta0 met15delta0 ura3delta0. Go to: <http://wiki.yeastgenome.org/index.php/Commonly_used_auxotrophic_markers> and look up each of the four alleles and then complete the table below (you need to scroll all the way to the bottom of the page for the last 3 markers)

|  |  |  |
| --- | --- | --- |
| Allele | Is this marker a complete deletion of the gene? | If not, what portion of the gene is missing? |
| his3delta1 |  |  |
| leu2delta0 |  |  |
| met15delta0 |  |  |
| ura3delta0 |  |  |

1. Each allele contains a hyperlink to the Saccharomyces Genome Database. Each of these alleles is an auxotrophic marker, meaning that yeast containing the allele requires a particular nutrient. Under “Name Description”, determine what nutrient yeast containing each of the alleles requires.

|  |  |  |
| --- | --- | --- |
| Allele | Name of gene | Nutrient Required |
| his3delta1 |  |  |
| leu2delta0 |  |  |
| met15delta0 |  |  |
| ura3delta0 |  |  |

1. For this experiment, our chunk DNA was cloned into a plasmid containing the HIS3 gene as its selectable marker, transformed into yeast strain BY4741, and selected on SC-His medium. Based on your results above, what three other genes (selectable markers) could have been used instead of *HIS3* and what medium would you use for selection?
2. Why is it harder to introduce DNA into yeast cells, causing the heat shock step of transformation take 20 minutes for yeast cells but only 45 seconds for *E. coli* bacteria?
3. When we wanted to combine our RFP gene with a plasmid vector we used Gibson assembly in E. coli. When we want to combine 3-4 minichunks plus two linker DNAs, we used assembly in yeast. Why do we use yeast for chunk assembly rather than bacteria?
4. Following transformation of yeast, we will break open the yeast cells, remove the plasmid and transfer it into *E. coli* to use blue-white screening to check for the presence of a DNA (assembled chunk) insert. Why is this step necessary? Is it possible for yeast colonies to form without a DNA insert and if so, how?
5. Yeast share many advantages with bacteria for lab work. What are three advantages to using yeast as a eukaryotic system? The section on “Why Study Yeast?” At this site may be helpful: <http://wiki.yeastgenome.org/index.php/What_are_yeast%3F>
6. After transformation we incubated our bacterial plates at 37 degrees and our yeast plates at 30 degrees. Why have yeast and bacteria evolved to have different optimal growth temperatures (think about the natural habitat of yeast, which is plant leaves, fruits, and soil, versus the natural habitat of *E. coli* bacteria, which is the human body)?