Supplemental Appendix 1: Primer sequences

Supp. Table 1. Primer sequences used to amplify portions of *S. cerevisiae* genes (and melting temperatures, T_m)

***S. cerevisiae* gene**	**Forward Primer (5' to 3') (T_m)**	**Reverse Primer (5' to 3') (T_m)**
LYS2	CACTAAATTCCGACAAGTCCC (53.0°C)	CACCTGCACGAACATAAATC (51.6°C)
MET5	AAAGGATACTTCTTCAGGTGG (51.2°C)	GTGGTTTAACCCAGTTTCATC (51.4°C)
HIS5	TGTTTCCTGCTAAACCAGC (52.3°C)	ACTTCTTTGCCAAGACATTG (50.8°C)
LEU4	AACACTTGATTAAGAGAACGG (50.2°C)	ACGTTCAACAAGTTTGATAGG (50.8°C)
PUT4	CCTTCGCCTTCATTCTTGG (52.9°C)	AGAACTTGTCTTTGAAACCGG (53.0°C)

Notes:

a. All primers bind within the indicated gene's coding region, except for the *HIS5* forward primer, which binds 53 bp upstream of the *HIS5* start codon (in the gene's 'flanking region').

b. The melting temperatures (T_m) of all primers were designed to be approximately the same, so that all primer sets can be used to amplify products in a thermocycler reaction with an annealing temperature of 50°C. The melting temperatures indicated in the table were determined by Benchling.

Supplemental Appendix 2: Student Worksheet - Benchling Predictions

Note: The following worksheet questions could also be adapted for an online quiz to be automatically graded by the course learning management system.

'**Part A'** of the worksheet is directly related to the PCR that students perform in the lab, using the primer sequences in Supp. Table 1. '**Part B'** of the worksheet asks students to apply their knowledge of PCR and their Benchling skills to answer hypothetical questions (unrelated to the PCR that they performed in the lab).

The answers are shown in red.

PART A

Question 1
***LYS2* (7 points)**

- Length of the gene (in bp): **4,179**
- Length of PCR product (in bp): **1,101**
- Do both of the primers bind within the gene? Or does one bind in the flanking region? If so, which one? **Both bind within the gene**
- Number of BanI cut sites within the *LYS2* PCR product: **3**
- Number of expected restriction fragments after digesting the *LYS2* PCR product with BanI: **4**
- Sizes of the restriction fragments you'd expect after digesting the *LYS2* PCR product with BanI: (in bp): **201, 246, 294, 360**

Question 2
***MET5* (7 points)**

- Length of the gene (in bp): **4,329**
- Length of PCR product (in bp): **1,051**
- Do both of the primers bind within the gene? Or does one bind in the flanking region? If so, which one? **Both bind within the gene**
- Number of BanI cut sites within the *MET5* PCR product: **2**
- Number of expected restriction fragments after digesting the *MET5* PCR product with BanI: **3**
- Sizes of the restriction fragments you'd expect after digesting the *MET5* PCR product with BanI: (in bp): **218, 299, 534**

Question 3
***HIS5* (7 points)**

- Length of the gene (in bp): **1,158**
- Length of PCR product (in bp): **1,050**
- Do both of the primers bind within the gene? Or does one bind in the flanking region? If so, which one? **The forward primer binds within the flanking region**
- Number of BanI cut sites within the *HIS5* PCR product: **1**
- Number of expected restriction fragments after digesting the *HIS5* PCR product with BanI: **2**
- Sizes of the restriction fragments you'd expect after digesting the *HIS5* PCR product with BanI: (in bp): **229, 821**

Question 4
***LEU4* (7 points)**

- Length of the gene (in bp): **1,860**
- Length of PCR product (in bp): **1,189**
- Do both of the primers bind within the gene? Or does one bind in the flanking region? If so, which one? **Both bind within the gene**

- Number of BanI cut sites within the *LEU4* PCR product: **2**
- Number of expected restriction fragments after digesting the *LEU4* PCR product with BanI: **3**
- Sizes of the restriction fragments you'd expect after digesting the *LEU4* PCR product with BanI: (in bp): **331, 381, 477**

Question 5
***PUT4* (7 points)**

- Length of the gene (in bp): **1,884**
- Length of PCR product (in bp): **909**
- Do both of the primers bind within the gene? Or does one bind in the flanking region? If so, which one? **Both bind within the gene**
- Number of BanI cut sites within the *PUT4* PCR product: **1**
- Number of expected restriction fragments after digesting the *PUT5* PCR product with BanI: **2**
- Sizes of the restriction fragments you'd expect after digesting the *PUT5* PCR product with BanI: (in bp): **362, 547**

PART B

Referring to your Benchling file for each of the genes below, identify the following:

Question 6 (2 points)
*Using the same **LYS2** reverse primer as before*, what 20 bp-long forward primer sequence would give a *LYS2* PCR product exactly 50 bp longer than the one you identified above? (Don't worry about matching the melting temperatures; just focus on the size of the PCR product).

Write your primer in the 5' to 3' direction.

HINT: Create an annotation for this new primer and check your answer

GGACAATGCTGAAGCCTTCC

Question 7 (2 points)
*Using the same **MET5** forward primer as before*, what 20 bp-long reverse primer sequence would give a *MET5* PCR product exactly 50 bp shorter than the one you identified above? (Don't worry about matching the melting temperatures; just focus on the size of the PCR product).

Write your primer in the 5' to 3' direction.

HINTS:

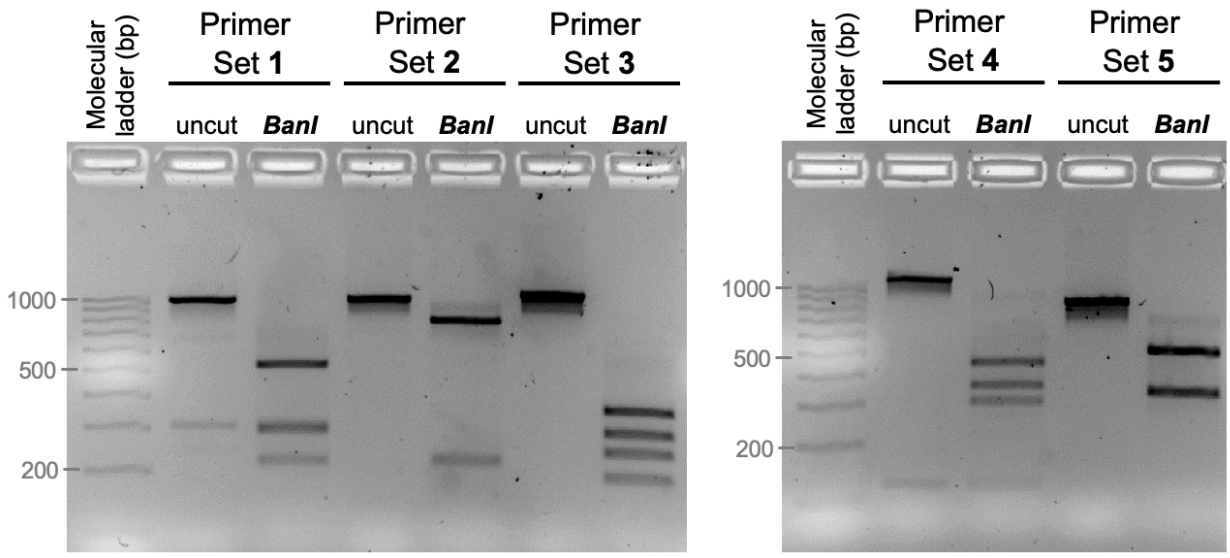
- Create an annotation for this new primer and check your answer
- When you highlight a sequence to copy, you can click 'Copy' near the top tool bar. It will give you options of what version of the sequence you want to copy (eg: DNA Reverse Complement)

ATTTAAACTCAAATGGTCTC

Question 8 (4 points)
Imagine you digest your ***HIS5*** PCR product with EcoRI (and not BanI).

- Number of EcoRI cut sites *within the HIS5 PCR product*: **1**
- Number of expected restriction fragments: **2**
- Sizes of the restriction fragments you'd expect after digesting the *HIS5* PCR product with EcoRI: (in bp): **234, 816**

Supplemental Appendix 3: Sample gel results and relevant experimental conditions



Supp. Figure 1. Sample data. Portions of genes from *S. cerevisiae* were PCR-amplified using the indicated primer pairs. Analysis of the uncut and *BanI*-digested fragments was performed on a 3% agarose gel in 0.25X TAE buffer with a voltage of 200V for 20 minutes. The gel was stained with Sybr-Safe.

Primer Set **1**: ***MET5***
- Uncut PCR product (bp): 1,051
- BanI restriction fragment sizes (bp): 218, 299, 534

Primer Set **2**: ***HIS5***
- Uncut PCR product (bp): 1,158
- BanI restriction fragment sizes (bp): 229, 821

Primer Set **3**: ***LYS2***
- Uncut PCR product (bp): 1,101
- BanI restriction fragment sizes (bp): 201, 246, 294, 360

Primer Set **4**: ***LEU4***
- Uncut PCR product (bp): 1,189
- BanI restriction fragment sizes (bp): 331, 381, 477

Primer Set **5**: ***PUT4***
- Uncut PCR product (bp): 909
- BanI restriction fragment sizes (bp): 362, 547

1X TAE buffer: 40 mM Tris, 20 mM acetic acid, 1 mM EDTA
- **Note:** The agarose gel was made with 1X TAE buffer, but the buffer in the gel running tank was 0.25X TAE.

Molecular ladder: BIORAD EZ LOAD RULER 100BP; Catalog No. 1708352EDU
- Band sizes (bp): 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000

PCR bead: Fisher Scientific PCR BEADS; Catalog No. 46001014: Cytiva PuReTaq Ready-To-Go™ PCR Beads
- **Note:** bead contains polymerase

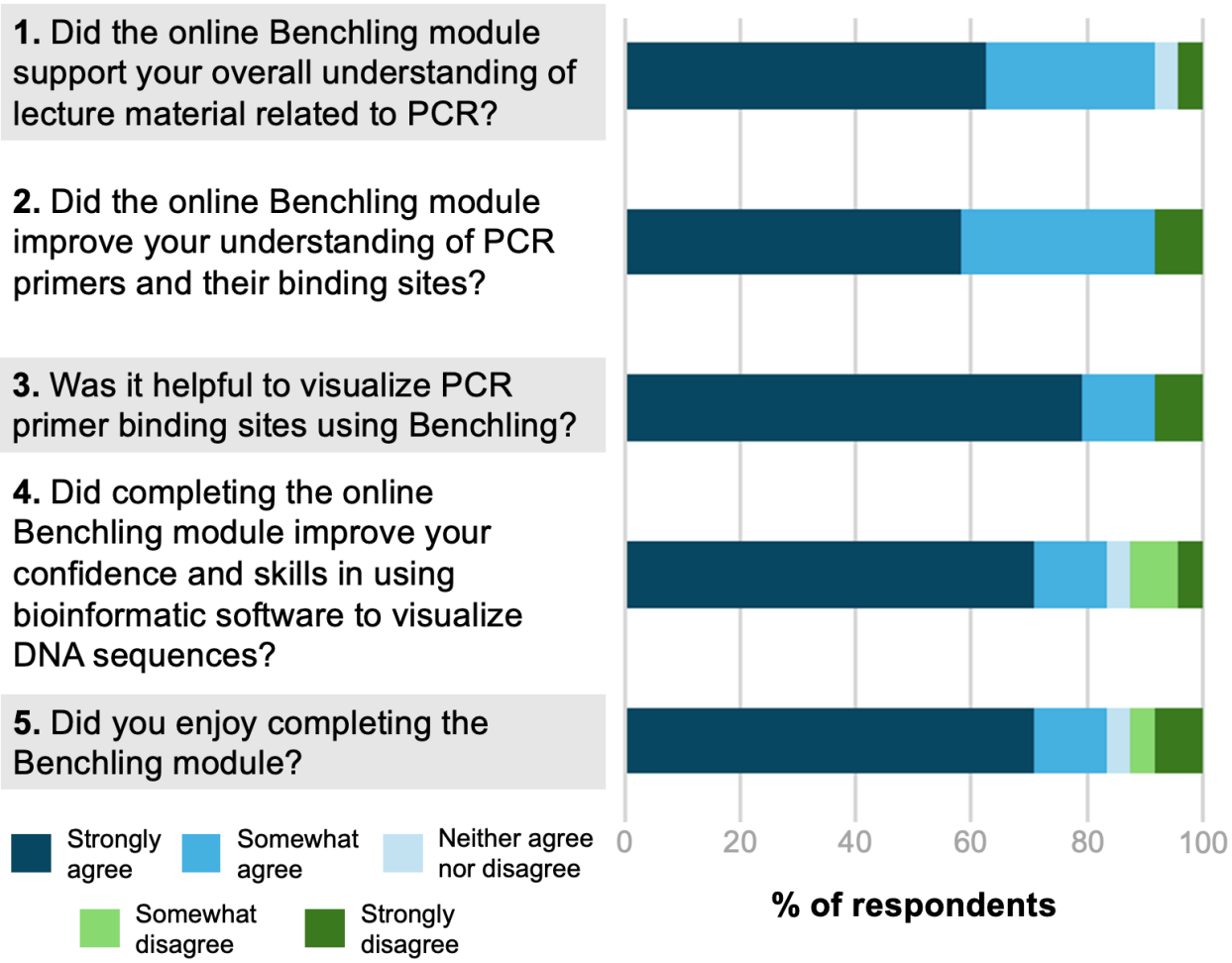
PCR parameters (same for all primer sets):

1. Warm up: 98°C for 5 mins
2. Denature: 98°C for 30 secs
3. Anneal: 50°C for 30 secs
4. Elongation: 72°C for 1 min

[repeat steps 2-4 total of 40 cycles]

5. Final elongation: 72°C 2 mins
6. Hold: 4°C infinite

Supplemental Appendix 4: Student feedback about using Benchling



Supp. Figure 2. Student survey administered during the last week of class. n=24 (of 80 students enrolled in BIOL 311 Spring 2024). The University of Calgary Conjoint Faculties Research Ethics Board has approved this study (REB24-0817).