

Appendix B: PCR Worksheets

Primer Design Worksheet instructions (pg. 1-2)

Student Primer Worksheets (pg. 3-6)

Answers for Primer Worksheets (pg. 7-8)

Primer Design Worksheet

This worksheet will help you select the primers your group will need to perform nested PCR to detect the deletion in your assigned gene.

You will be given the following sequences as Microsoft Word documents:

--Sequence for the **genomic region** that includes your gene of interest

--Sequence for the **deletion allele**

--Sequences for ten **potential primers** to choose from

Instructions are included below for getting these sequences into Geneious files. Please follow the step listed below to complete this exercise and answer the questions at the end.

STEP #1: Create a folder for your group in Geneious

- Open Geneious
- Click on the Nucleotide Documents folder on the left side.
- Choose File → New Folder
- Put the name of your group on your folder.
- Please keep all of your files in your group folder.

STEP #2: Load your sequences into Geneious

- Click on your group's folder on the left side
- Choose File → New Sequence
- Open one of the Microsoft Word documents with one of your sequences
- Copy and paste the sequence into the "New Sequence" window
- Make sure the "nucleotide" is chosen at the bottom
- Click OK at the bottom right
- Your sequence file will appear in the main window
- You will probably want to rename your sequence files. To do this, select it by clicking on it, then click once more. You should be able to rename the file. (Double clicking quickly on the sequence file will open the file.)
- Repeat until all your sequences are entered

STEP #3: Figure out the size of your deletion and where it occurs.

- Select the wild-type and deletion sequence files. Holding the command key will let you select more than one file at a time.
- Choose "Alignment" on the menu bar for the main window. The default settings are fine.
- A new file will appear with a green arrow icon on the left: this file contains the alignment.

- Examine the alignment to figure out where the deletion is located and how many base pairs are deleted.

STEP #4: Select primers for nested PCR.

- Select the DNA sequence and the primers that you wish to test. Holding the command key will let you select more than one file at a time. Choose “Primers” from the menu bar on the main window.
- Select the “Test Primers” options from the very top of the Primer Design dialog box.
- The program will ask you to specify the target sequence and the primers to test.
- Click the OK button to begin testing.
- A dialog box will appear with the results.

Question #1: Which primers should be used for the external PCR reaction to detect the presence of your deletion? Indicate the forward primer and the reverse primer and show how they align to the gene sequence. Draw both strands for the gene sequence. Label the 5' and 3' ends of all sequences.

Question #2: Which primers should be used for the internal PCR reaction to detect the presence of your deletion? Indicate the forward primer and the reverse primer and show how they align to the gene sequence. Draw both strands for the gene sequence. Label the 5' and 3' ends of all sequences.

Question #3: What size band do you expect to see for the internal reactions (when you run them out on an agarose gel) for both the wild-type allele and the deletion allele?

brc-1

The *C. elegans brc-1* gene encodes a homolog of human BRCA1, a ubiquitin ligase subunit that is proposed to play a role in cell proliferation and DNA repair.

BRCA1 stands for breast cancer 1 gene; it is mutated in early onset breast and ovarian cancer (OMIM: 113705).

Deletion allele: *brc-1(tm1145)*

Strain: DW102

Primers to choose from for the primer design exercise:
(all are written in the 5' → 3' direction)

- 1) TCCATGTGGCGCCACCGAAA
- 2) CTGCAGAACCGGTAGAACCG
- 3) TGTCGCATCGTCGGCATTAA
- 4) AATATAGGCACCGGCGGGGA
- 5) CTCCGTAGCTTGAAGTCTCA
- 6) TGAGACTTCAAGCTACGGAG
- 7) CGGTCTACCGTTCTGCAG
- 8) TCCCCGCCGGTGCCTATATT
- 9) TTTCGGTGGCGCCACATGGA
- 10) TTAATGCCGACGATGCGACA

cua-1

The *C. elegans cua-1* gene encodes a putative copper transporting P-type ATPase.

cua-1 is homologous to the human genes KIAA1347 (mutated in Hailey-Hailey disease, OMIM: 604384), MNK (mutated in Menkes syndrome, OMIM: 309400), and WND (mutated in Wilson disease, OMIM: 277900).

Deletion allele: *cua-1(gk107)*

Strain: VC194

Primers to choose from for the primer design exercise:
(all are written in the 5' → 3' direction)

- 1) AGTTCCAACCATAACAGCGG
- 2) CCGCTGTTATGGTTGGAAGT
- 3) CAACGACTGGCACCATTAAA
- 4) ACTCTCACAAATCGTCCGTC
- 5) CGGTTATGTCTCTTCTATTTG
- 6) AACGGTATTTTCAGATGGCG
- 7) CGCCATCTGAAAATACCGTT
- 8) TTTAATGGTGCCAGTCGTTG
- 9) CAAATAGAAGAGACATAACCG
- 10) GACGGACGATTTGTGAGAGT

fshr-1

The *C. elegans fshr-1* gene encodes a putative neuropeptide receptor required for normal acetylcholine secretion by synapses.

fshr-1 is homologous to human genes for follicle-stimulating hormone receptor (mutated in ovarian dysgenesis, OMIM: 136435), thyrotropin receptor (mutated in hyperthyroidism, OMIM: 603372), and luteinizing hormone receptor (mutated in precocious puberty, OMIM: 152790).

Deletion allele: *fshr-1(ok778)*

Strain: RB911

** this deletion also has a small insertion associated with it. This will not affect your primer design exercise (and the insertion is not included in your sequence file). However, this will affect the size of your internal PCR product (it will be 393 bp larger, due to the insertion).*

Primers to choose from for the primer design exercise:
(all are written in the 5' → 3' direction)

- 1) TGAATGTTTTGTCTGGGCAA
- 2) GGTCCGCAAATTTGCGTAAC
- 3) TG TTCAGGGTTAAGTTCGGG
- 4) TTTCCAACCCTGTTCTTTGG
- 5) AATCCAATTGTGGCCGTAAA
- 6) CCCGAACTTAACCCTGAACA
- 7) CCAAAGAACAGGGTTGGAAA
- 8) TTGCCCAGACAAAACATTCA
- 9) TTTACGGCCACAATTGGATT
- 10) GTTACGCAAATTTGCGGACC

mlh-1

The *C. elegans mlh-1* gene encodes a DNA mismatch repair protein that is orthologous to human MLH1.

Mutation of the human MLH1 gene leads to hereditary non-polyposis colon cancer (OMIM: 120436).

Deletion allele: *mlh-1(gk516)*

Strain: VC1121

Primers to choose from for the primer design exercise:
(all are written in the 5' → 3' direction)

- 1) GACGAATATCATAGAATTCT
- 2) AGAATTCTATGATATTCGTC
- 3) CCAATCCATCACCACCTCTC
- 4) GCGCTACTGGACCATTTTCAT
- 5) GAGAGGTGGTGATGGATTGG
- 6) GAATAGATCGGTGGCGGTAA
- 7) TTACCGCCACCGATCTATTC
- 8) AAATTCTTCGCAGTTGTGGC
- 9) GCCACAACCTGCGAAGAATTT
- 10) ATGAAATGGTCCAGTAGCGC

Answers to Questions on Primer Selection Worksheets

Listed below are the primers used in the Primer Selection Handouts. Incorrect primers are indicated in red, with a brief explanation of what they are. The primers are listed in the same order as they appear on the worksheets. Also indicated is the answer to Question 3: What size band do you expect to see for the internal reactions (when you run them out on an agarose gel) for both the wild-type allele and the deletion allele?

brc-1

- 1) TCCATGTGGCGCCACCGAAA (rev. comp of external forward)
- 2) CTGCAGAACCGGTAGAACCG (rev.comp of primer in deleted region)
- 3) TGTCGCATCGTCGGCATTAA (internal forward)
- 4) AATATAGGCACCGGCGGGGA (internal reverse)
- 5) CTCCGTAGCTTGAAGTCTCA (external reverse)
- 6) TGAGACTTCAAGCTACGGAG (rev. comp of external reverse)
- 7) CGGTTCTACCGTTCTGCAG (primer in deleted region)
- 8) TCCCCGCCGGTGCCTATATT (rev. comp of internal reverse)
- 9) TTTCGGTGGCGCCACATGGA (external forward)
- 10) TTAATGCCGACGATGCGACA (rev. comp of internal forward)

Band sizes expected for internal PCR reactions
wild-type: 1446 bp; deletion: 797 bp

cua-1

- 1) AGTTCCAACCATAACAGCGG (internal forward)
- 2) CCGCTGTTATGGTTGGAAC (rev. comp of internal forward)
- 3) CAACGACTGGCACCATTAAA (rev. comp of external reverse)
- 4) ACTCTCACAAATCGTCCGTC (primer in deleted region)
- 5) CGGTTATGTCTCTTCTATTTG (external forward)
- 6) AACGGTATTTTCAGATGGCG (internal reverse)
- 7) CGCCATCTGAAAATACCGTT (rev. comp of internal reverse)
- 8) TTTAATGGTGCCAGTCGTTG (external reverse)
- 9) CAAATAGAAGAGACATAACCG (rev. comp of external forward)
- 10) GACGGACGATTTGTGAGAGT (rev.comp of primer in deleted region)

Band sizes expected for internal PCR reactions
wild-type: 2905 bp; deletion: 1266 bp

fshr-1

- 1) TGAATGTTTTGTCTGGGCAA (rev. comp of external forward)
- 2) GGTCCGCAAATTTGCGTAAC (primer in deleted region)
- 3) TGTTTCAGGGTTAAGTTCGGG (internal forward)
- 4) TTTCCAACCCTGTTCTTTGG (rev. comp of internal reverse)

- 5) AATCCAATTGTGGCCGTAAA (external reverse)
- 6) CCCGAACTTAACCCTGAACA (rev. comp of internal forward)
- 7) CCAAAGAACAGGGTTGGAAA (internal reverse)
- 8) TTGCCAGACAAAACATTCA (external forward)
- 9) TTTACGGCCACAATTGGATT (rev. comp of external reverse)
- 10) GTTACGCAAATTTGCGGACC (rev. comp of primer in deleted region)

Band sizes expected for internal PCR reactions
wild-type: 3351 bp; deletion: 518 bp

mlh-1

- 1) GACGAATATCATAGAATTCT (primer in deleted region)
- 2) AGAATTCTATGATATTCGTC (rev.comp of primer in deleted region)
- 3) CCAATCCATCACCACCTCTC (internal reverse)
- 4) GCGCTACTGGACCATTTTCAT (external reverse)
- 5) GAGAGGTGGTGGTGGATTGG (rev. comp of internal reverse)
- 6) GAATAGATCGGTGGCGGTAA (external forward)
- 7) TTACCGCCACCGATCTATTC (rev. comp of external forward)
- 8) AAATTCTTCGCAGTTGTGGC (internal forward)
- 9) GCCACAACCTGCGAAGAATTT (rev. comp of internal forward)
- 10) ATGAAATGGTCCAGTAGCGC (rev. comp of external reverse)

Band sizes expected for internal PCR reactions
wild-type: 1898 bp; deletion: 486 bp