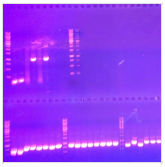

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BSCI:414 Lab 3 Analyze Agarose Gel Results and Create RNAse P Primers

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ABSTRACT

BSCI:414 Lab 3

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Review Lab 2 Experiment; Analyze Data

1 Review Lab 2 hypothesis and experimental setup.

2 Watch experiment process video, pausing to discuss.
https://youtu.be/SJoLg-GCeOY

3
1. Analyze PCR fragment lengths on agarose gel. You can find a copy of the gel in our ELMS course: Files>Lab Results>Lab 2 Agarose Gel>"Lab2.agarose.gel.50.samples.jpg"
2. Which lanes have bands? Which lanes do not? Why?
3. Discuss cases in which F and R primers point in same direction or away from each other.

4 Copy results to lab notebook. Is your band size in agreement with your hypothesis?

Find CDC Primers in RNAse P Gene

5 In the "BSCI:414 Plasmids" under the class root folder, find gene "RNAseP_gene_NM_006413". Copy this gene to a new folder you create under "BSCI:414 Lab 3" using your name e.g. "F20_HarleyKing_Lab 3". Also give the plasmid a new

name like "F20_HarleyKing_RNAseP_gene_NM_006413".

6 Navigate to the "CDC Coronavirus Real-Time RT-PCR Primers and Probes" site:
https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

7 Make primers in Benchling using the primer and probe sequences from "RNAse P". Make sure to save the primers underneath your Lab 3 folder. The primers should have a memorable name like, "F20_HarleyKing_Lab3_RNAseP-FWD".

RP-F	RNAse P Forward Primer	AGA TTT GGA CCT GCG AGC G	None	500nM
RP-R	RNAse P Reverse Primer	GAG CGG CTG TCT CCA CAA GT	None	500nM
RP-P	RNAse P Probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1	FAM, BHQ-1	125nM
RP-P	RNAse P Probe	FAM-TTC TGA CCT /ZEN/ GAA GGC TCT GCG CG-3IABkFQ	FAM, ZEN, 3IABkFQ	125nM

Find these sequences in Benchling and make primers. Use the "Manual" method to make primers.

8 Answer the following questions in your lab notebook with a table:
1. What is the Tm of the forward primer? How many bp is it?
2. What is the Tm of the reverse primer? How many bp is it?
3. What is the Tm of the probe quenched with BHQ1? How many bp is it?
4. What is the Tm of the probe quenced with 3IABkFQ? How many bp is it?

9 Copy and paste the emission spectra of the FAM probe in your lab notebook. What is its excitation/emission wavelength?