Influenza Outbreak Investigation
PCR Teacher Preparation

Reagent Preparation for Influenza Outbreak Investigation Kit PCR

- **Assumes 32 patient samples**
- **Assumes students work in 8 groups of 4 – each group examining 4 patient samples**

2X OneTaq Master Mix

Students will use 12.5ul of 2X OneTaq Master Mix per reaction for a total of 6 reactions per group: 4 patient samples, 1 positive control reaction and 1 negative control reaction. Note: 2X OneTaq Master Mix from New England BioLabs (NEB) comes in two formats: with and without loading dye. This protocol assumes that students are using 2X OneTaq Master Mix *without* loading dye.

Example:

- Each student group will need 12.5ul x 6 = 75 ul. Give each group an aliquot of 80-85 ul.
- 8 groups x 85ul = 680 ul 2X OneTaq Master Mix is needed.

Primer Mix

The primer mixture contains both the forward and reverse primers, each at a concentration of 5uM in the mixture. Students will use 2.5 ul of the primer mix for each PCR reaction.

Example:

- Each student group will need 2.5 ul x 6 = 15 ul. Give each group an aliquot of 20ul.
- 8 groups x 20 ul = 160 ul of Primer Mix is needed.

DNA Samples

PCR Positive Patient Samples

The patient samples that are positive in the PCR reaction contain plasmid DNA at approximately 2.5 ng/ul. Students will use 10 ul of this DNA mixture for each PCR reaction. Of the 32 patient samples, there are 16 samples that are positive. Consult the 'Patient Key' to determine which samples should be positive by PCR and how to label these tubes. Add 15 ul of the DNA solution to each of these sample tubes.

Example:

- 16 positive samples x 15 ul = 240 ul of the "PCR Positive" DNA sample

PCR Negative Patient Samples

The patient samples that are negative in the PCR reaction will not contain any DNA. Use sterile distilled water for these patient samples. Students will use 10 ul of sterile water for each PCR negative patient sample reaction. Consult the 'Patient Key' to determine which samples should

be negative by PCR and how to label these tubes. Add 15 ul of sterile distilled water to each of these sample tubes.

Example:

- 16 negative samples x 15 ul = 240 ul sterile water

PCR Positive and Negative Controls

Each student group will set up a positive control PCR reaction and a negative control PCR reaction.

Positive control (labeled "Pos"): Each student group will use 10 ul of the plasmid DNA mixture in their positive control reaction. Add 15 ul of the DNA solution to each PCR positive control sample tube.

Example:

- 8 PCR positive control samples x 15 ul = 120 ul of the DNA mixture.

Negative control (labeled "Neg"): Each student group will use 10 ul of sterile distilled water in their negative control reaction. Add 15 ul of sterile distilled water to each PCR negative control sample tube.

Example:

- 8 PCR negative samples x 15 ul = 120 ul of sterile distilled water.

<u>6X Loading Dye</u>

Each student group will add 2.5 ul of 6X Loading Dye to each of their 6 PCR reactions before loading them on a gel.

Example:

- Each student group will need 6 ul x 6 = 36 ul of 6X loading dye. Give each group an aliquot of 40 ul.
- 8 groups x 40 ul = 320 ul of 6X Loading Dye is needed

<u>1kb DNA Ladder</u>

Each student group will need 12 ul of the 1kb DNA ladder to load on their gel.

Example:

- Each student group will need 12 ul. Give each group an aliquot of 15ul.
- 8 groups x 15 ul = 120 ul of the 1kb DNA Ladder is needed

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1 X TAE

The TAE is supplied as a 50X solution (50 times more concentrated than what it should be used at). It must be diluted to 1X before use.

Example:

- To make 1 liter of 1X TAE, add 20 ml of the 50X TAE to 980 ml of distilled water.
- Mix well before using.

Ordering information/amounts from New England Biolabs (NEB) for the Influenza Outbreak Investigation PCR Lab. All other reagents are obtained from Shoreline Biotech Experience Staff as part of the Influenza Outbreak Investigation Kit.

1. 2X OneTaq Master Mix – This reagent (M0484S) comes in a volume of 1.25 mL. It is used at 85 ul (75 ul plus 10 ul extra)/student groups (8 student groups). So, one vial can support 1.8 classes investigating 32 patient samples (4 samples + 2 controls / 8 student groups, each group containing 4 students).
2. Quick Load 1kb DNA ladder – This reagent (N0468S) comes in a volume of 1.25 ml. Students load 10 ul on each gel. One vial can support 125 gels. Each student group runs one gel.
3. Gel Loading Dye, Purple (6X), no SDS – This reagent (B7025S) comes in a volume of 4 ml (4,000 ul). Student groups use 2.5ul x 6 reactions = 15 ul. One vial can support 200 student groups.

Note: All of the above reagents last for at least 2 years if properly stored at –20^{o}C degrees (i.e., frozen).

To order these items from NEB, you must:

1. Register for an educators account by completing the form at: https://www.neb.com/promoting-science-education/course-support-and-reagent-donation
2. Email you order to the email address on the Educational Course Support Form and include the Influenza Outbreak Investigation PCR forms already completed for you for this kit, but please double check the quantities requested for the number of students you have using the information above. These forms include:
    a. Educational Course Support Form (add your contact information)
    b. Annotated protocol showing how much of each reagent is used per student
    c. NEB Summary Table (Excel Sheet)
    d. Quantity Order Sheet