PCR AMPLIFICATION AND GEL ELECTROPHORESIS

Polymerase Chain Reaction (Using Taq DNA Polymerase - Invitrogen)

1. Add the following to the PCR tube (for every reaction)

   - 5 µL 10x PCR Buffer
   - 1.5 µL 50 mM $MgCl_2$
   - 1 µL 10 mM dNTP mix
   - 1 µL 10 mM Forward and Reverse primer mix
   - 0.5 µL Taq DNA Polymerase (~5 units/µL)
   - 2 µL cDNA product from RT reaction
   - to 50 µL autoclaved, distilled water

2. Enter the Following program on the thermocycler:

   - 95°C 5 min
   - 95°C 30 sec
   - 50-56°C 30 sec
   - 72°C 45 sec

   Repeat 35-40 times

   - 72°C 7 min
   - 4°C pause

3. Perform 15-40 cycles of PCR. Use the recommended annealing (may vary with specific primers) and extension conditions for your Taq polymerase.

Gel Electrophoresis

Note: for a 1.5% agarose

1. Add 1.5g agarose per 100 mL of TAE buffer.

2. Heat the solution for ~2 minutes in the microwave, stopping halfway through to mix by swirling. After the agarose has fully dissolved, let the solution cool.

3. Assemble the gel apparatus in the holder with combs.

4. Add 20 µL of EtBr per 100 mL of agarose solution in the hood and mix by swirling.

5. Pour the gel into the mold and let sit for ~15 minutes.

6. Remove the comb from the gel and then the gel from the mold. Place the gel into the gel electrophoresis chamber, filled with TAE buffer so that it is fully submerged.

7. Pipette the ladder and samples into the wells.

8. Run at 120 V for around 30 minutes.

   Note: if run time is unknown, make sure to check on the dye front of the gel to determine how far the samples have run.

9. Visualize gel.