Tips for Having a Successful PCR Reaction

1) **Planning the reaction:**

- Sign up for a thermocycler in advance. Include your name, the time and date of use, and the length of the profile.
- Calculate reagent amounts for each reaction independently but use others as a guide. After computing the amount of reagent needed for the total number of reactions, check the value obtained. Does the value make sense?
- Be sure to use the correct protocol for the PCR reaction. Be cautious of the primers and the different Taq kits. 10x PCR buffer varies between companies and may or may not contain magnesium.
- Understand the final concentrations of reagents that are present in each reaction. This knowledge will assist you when writing recipes for new reagents.
- Assemble all reagents before using a pipette. Place DNA templates and taq polymerase on ice. Taq polymerase can also be stored in the freezer until needed.

2) **Prevent reagent contamination**:

- Consider making aliquots of reagents to prevent contamination of stocks.
- Always use a fresh pipette tip when drawing from a bottle of reagents.

3) **Pipettes:**

- Ensure that each pipette used is measuring accurate volumes. PCR reactions can be very sensitive. A pipette should appear to draw the same volume every use. Do not use a pipette if the action feels sticky.
- Use the correct sized pipette for the desired volume, and stay away from the high and low thresholds. Never adjust pipettes beyond their thresholds, as that will necessitate recalibration.
- Each pipette motion should be deliberate. Do not rush while preparing a reaction.

4) **Tubes:**

- Pick the appropriate sized tube for the thermocycler. The MJ takes 0.5 mL tubes only, the Eppendorf in lab takes 0.2 mL tubes or 0.2 mL 96-well plates, and the Eppendorf in room 127 takes 96-well plates and 0.2 and 0.5 mL tubes.
- Label all tubes clearly and consider additional markings that will distinguish your tubes from others. Frequently, marker on tubes becomes faded or distorted from the thermocycler or from handling. If tubes are initially clearly labeled, then they will likely remain legible throughout handling.
- Write on tubes in the same orientation each time. This method will allow faster reading of tubes. Be especially cautious of the numbers ‘6’ and ‘9’ and consider underlining (e.g. <u>6</u>) to differentiate between numbers.

- Arrange tubes into a logical order in your rack. Try to line up reagents in the order you add them. When finished with a reagent, move it to a new position to prevent confusion.

5) **Mixing and centrifugation:**

- Be sure all frozen reagents are fully melted and mixed. All reagents can be mixed on a vortex except for Taq polymerase and template DNA. Mix DNA by flicking the base of the tube with a finger. Do a quick spin down of DNA samples if liquid is present on the sides of the tube.
- Pipette DNA directly into master mix. Touch-spin sample tubes after addition of DNA template to drive all liquid to the bottom of tubes.

6) **Controls when using specific primers**:

- *Negative controls*:
  water in place of template – detects DNA contamination in reagents
  DNA template without target sequence – detects nonspecific amplification
- *Positive control*:
  isolated target sequence – DNA template known to work with primer set.