# Troubleshooting PCR

Building block assigned \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Expected size of BB (bp) \_\_\_\_\_\_\_\_\_\_\_

BB assigned \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Expected size of BB (bp) \_\_\_\_\_\_\_\_\_\_\_

BB assigned \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Expected size of BB (bp) \_\_\_\_\_\_\_\_\_\_\_

What was the problem with your original finish PCR reaction for each building block (no DNA product, multiple distinct DNA bands, smeared DNA band)?

What is your hypothesis for why your PCR did not work?

What did you change to try to get your finish PCR to work (annealing temp changes, extension temp changes, additional rounds of PCR, etc.)? Remember, each of these BBs must be synthesized eventually, so as much information as you can give us about what has already been tried to troubleshoot each specific BB will be very helpful.

What was the result and how would you interpret it (attach gel images of your original PCR and you troubleshooting gel)? Is this PCR product ready to be ligated into the vector? If not, why not?