

## PCR Troubleshooting

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### **If you PCR sample does NOT amplify (or amplifies very weakly) try these steps in order:**

- If you sample does not work at all the first time, try again with the exact same parameters and protocol.
- If you sample still does not work at all or amplified very weakly, next lower your annealing temperature by 2-5°C (i.e. If you originally amplified at 54°C try re-amplifying at 52°C), but keep all else the same.
- If lowering by 2°C does not work, try lowering the annealing temperature again by another 2-3°C.
- If lowering the temperature does not work, try adding more MgCl<sub>2</sub>. Add 1µL more MgCl<sub>2</sub> per reaction.
- In some rare cases with nuclear genes, raising (not lowering) the annealing temperature actually helps. Try raising by 2-5°C from the original annealing temperature.
- You may also consider including an additive in your PCR (or changing the additive if you are currently already using one). Examples include BSA, DMSO, etc.

### **If a sample is not amplifying for multiple genes using the steps above:**

- First try adding additional DNA template to your PCR reaction (i.e. if you originally were using 1µL per reaction, try adding 2-4µL per reaction).
- If you have reason to believe there is too much DNA in that particular extraction (i.e. this was the largest of your ants, beetles, muscle tissue, etc.), dilute a subsample of your DNA extraction by half with PCR water (i.e. 20µL original DNA extraction and 20µL PCR water) and use this as the template for the PCR.
- If the steps above do not work, re-extract that sample if you have additional material available.

### **If your PCR sample amplifies but sequences poorly in both directions:**

- Re-PCR with a higher annealing temperature. If the samples still amplifies well (bright band on gel) try sequencing this product.
- If after amplifying with a higher annealing temperature this does not improve your sequence quality but your samples still amplify, then try raising the temperature again.
- If you still have low quality sequences, try reamplifying with less MgCl<sub>2</sub>.

### **Special notes:**

- You should always use barrier/filter tips for all PCRs.
- To get good sequences you must start with good PCR product.
- Remember lowering the annealing temperature and adding MgCl<sub>2</sub> both decrease the specificity of your reactions, so this can lead to amplifying non-target regions of the genome or contaminates.
- Never amplify your PCRs below an annealing temperature of 45°C.
- **Remember there is no such thing as being too careful in the lab.**