

PCR 2 Protocol: Adding Adaptors

Read this protocol in its entirety, and program your thermocycler before setting up your PCR

PCR2 Thermocycling conditions:

Program your miniPCR thermocycler

- 1) 95°C 3 minutes Denature template
- 2) 98°C 20 seconds Denature template
- 3) 60°C 15 seconds Primers Anneal to template
- 4) 72°C 15 seconds This is the optimal temperature for polymerase activity, so new DNA is synthesized
- 5) go to step 2 and repeat 7 times (total of 8 cycles) Synthesize new copies of DNA
- 6) 72°C 1 minute This allows any strands that may not have been synthesized to completion to finish extension

Set these out in your workspace:

- ☐ bench paper
- ☐ thermocycler
- ☐ centrifuge (round rotor and adaptors, and rotor with tiny holes for PCR tubes)
- ☐ Extra fine tip sharpie
- ☐ A 10µl or 20µl pipettor and tips
- ☐ Freezer
 - ☐ A tube of PCR grade water (2ul/rxn)
 - ☐ **Keep cold:** 2 PCR2 tubes
 - [These are tiny PCR tubes in the PCR2 freezer bag labeled with letter and numbers, like 1-A1, 1-B2, etc]
 - [each tube contains Kapa HiFi Ready mix(10µl)+indexed primer mix (2ul)]
- ☐ Fridge
 - ☐ Cleaned up product from PCR1

☐ If PCR1 for both samples, use the positive control that you cleaned up in the last lab. If at least one of your PCR1 reactions was successful, use your own PCR1. You only need to get data from one supplement.

☐ **Record** which PCR2 tube you will use for each supplement or positive control (record the letters and numbers that are on top of the tube: 1-A1, 1-B2, etc) on the [spreadsheet](#)

☐ **Number** each PCR2 tube with the sample number (or + for the positive control) that will go into it.

- ☐ **Flick** your clean PCR1 and your PCR2 tubes to mix the liquid, then centrifuge briefly to get liquid to the bottom of the tubes. PCR2 tubes need the centrifuge rotor with tiny holes
- ☐ **Add to each PCR2 tube:**
Pipette the liquid directly into the liquid already in the tube
2 μ l PCR grade water
6 μ l of your **cleaned up** PCR1 or positive control (make sure each PCR1 goes into the appropriately labeled PCR2 tube)
- ☐ **Close the lid** of the PCR2 tube. Make sure it is **totally closed**. If not, your sample will evaporate in the thermocycler.
- ☐ Put your PCR2 tubes in the miniPCR **thermocycler** and take a **picture** of your tubes in the thermocycler.
- ☐ Run the PCR2 program. Take a **picture** of the miniPCR app as it is running your PCR2 program.

Cleanup

- ☐ While the PCR is running
 - ☐ Return your cleaned up PCR1 and water to the refrigerator
 - ☐ Used tubes and tips can be disposed of in the trash
 - ☐ Wash hands
- ☐ This PCR reaction only takes about 20 minutes. When the PCR is done
 - ☐ label the tubes PCR2
 - ☐ Put them in the fridge for storage. You will run a gel to see if PCR2 worked, and then send these tubes to UAF for sequencing.
 - ☐ Return the thermocycler to its original box
 - ☐ Wash the counters/table where you were working
 - ☐ Wash hands