

Amplification (Step C)

1. While incubation is occurring, thaw the amplification mix and 10 mM dNTP mix and prepare a master mix with 75.25 ul of nuclease-free water, 9.375 ul of amplification mix, 1.875 ul of dNTP mix, and .9375 ul of Amplification enzyme per sample in a separate tube. Store in ice.
2. Remove samples from thermocycler (step B) and spin them down in the picocentrifuge. Remove the cap and pipette 87.4 ul of sample for a total reaction volume of 91.7 ul. Pipette up and down to mix.
3. Place a new cap on the tubes and spin them down again. Place in the thermocycler and proceed to the next step of incubation. Incubate the samples at 94 degrees for 2 minutes then run 17 cycles of incubation of 94 degrees for 30 seconds and 70 degrees for 5 minutes.
4. After cycling is complete, you can store the samples at -20 degrees or keep them on ice at 4 degrees Celsius until purification can be done.
(If you need to store the samples in -20, it is preferable you purify the PCR products and then store them).