Prepare the following reactions in a PCR tube:

If you are using the 2x Taq Master Mix:

|  |  |  |  |
| --- | --- | --- | --- |
| ***Component*** | ***25 μl reaction*** | ***50 μl reaction*** | ***Final Concentration*** |
| *2X Taq Master Mix(add last)* | *12.5 µl* | *25 μl* | *1X* |
| *10 µM Forward Primer* | *0.5 µl* | *1 μl* | *0.2 µM (0.05–1 µM)* |
| *10 µM Reverse Primer* | *0.5 µl* | *1 μl* | *0.2 µM (0.05–1 µM)* |
| *Template DNA* | *~50ng* | *~100ng* | *<1,000 ng* |
| *Nuclease-free water* | *~10 µl* | *~20 µl* |  |

If you are using another enzyme not in a Master MIx:

|  |  |  |  |
| --- | --- | --- | --- |
| ***Component*** | ***25 μl reaction*** | ***50 μl reaction*** | ***Final Concentration*** |
| *10X Polymerase/PCR Buffer* | *2.5 µl* | *5 μl* | *1X* |
| *10 mM dNTPs* | *0.5 µl* | *1 μl* | *200 µM* |
| *10 µM Forward Primer* | *0.5 µl* | *1 μl* | *0.2 µM (0.05–1 µM)* |
| *10 µM Reverse Primer* | *0.5 µl* | *1 μl* | *0.2 µM (0.05–1 µM)* |
| *Template DNA* | *~50ng* | *~100ng* | *<1,000 ng* |
| *Taq DNA Polymerase\** | *0.5 µl* | *1 µl* | *1.25 units/50 µl PCR* |
| *Nuclease-free water* | *to 25 µl* | *to 50 µl* |  |

Gently mix the reaction and spin down in microcentrifuge.

Cycling Conditions for a Routine PCR:

|  |  |  |
| --- | --- | --- |
| **STEP** | **TEMP** | **TIME** |
| Initial Denaturation | 95°C | 30 seconds |
| 30 Cycles Melting Temp.  Annealing Temp.  Extension Temp. | 95°C  56°C  68°C | 30 seconds  30 seconds  1 minute/kb |
| Final Extension | 68°C | 8 minutes |
| Hold | 4-10°C |  |

I always use 56 or 56.5C for my Annealing Temperature unless the primer has a large region of random nucleotides, then I might drop it down to 52-54C. This has worked on 99.99% of all of my PCR reactions.

Modified from NEB: <https://www.neb.com/protocols/1/01/01/protocol-for-a-routine-taq-pcr-reaction>