

# Phusion HotStart II Hifi PCR

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## Abstract

PCR using Phusion HotStart II Hifi Mastermix (based on ThermoFisher Scientific manufacturer protocol).

**Citation:** Snehadri Sinha Phusion HotStart II Hifi PCR. **protocols.io**

[dx.doi.org/10.17504/protocols.io.gzjbx4n](https://dx.doi.org/10.17504/protocols.io.gzjbx4n)

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## Protocol

### Step 1.

In a PCR tube, combine following components for a 50 uL reaction. Ensure all components are mixed and thawed.

Mastermix (2X) - 25 uL

Template DNA - 1 pg -10 ng

Forward primer - to final concentration of 0.2-0.5 uM

Reverse primer - to final concentration of 0.2-0.5 uM

Water - to 50 uL

### Step 2.

Set up the reaction:

1. 98 C - 2 min
2. 98 C - 10 sec
3. Tm + 3 - 30 sec
4. 72 C - 15-30 sec/kb -> cycle back to 2, 25-35 cycles
5. 72 C - 10 min 6. 4 C - hold

### **Step 3.**

Visualize results of PCR, conduct gel electrophoresis.