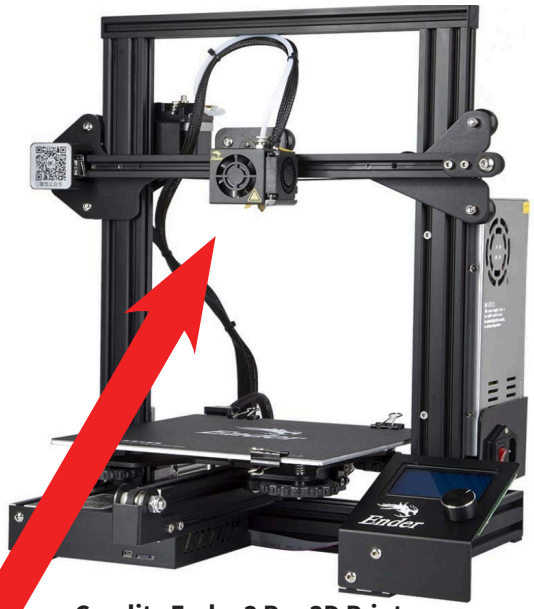
3D Printer PCR

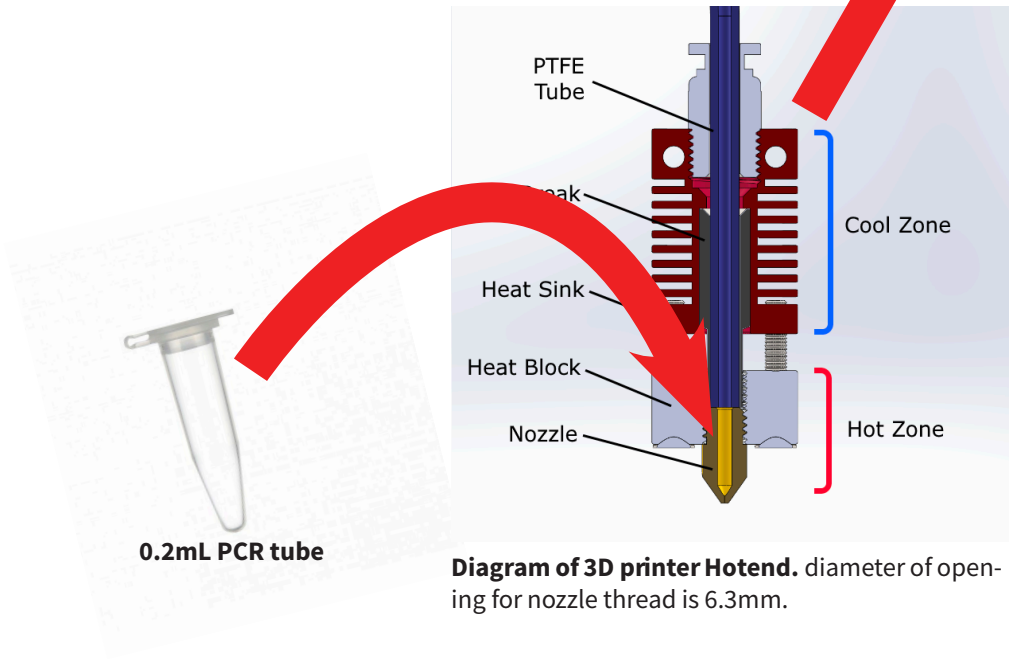
Abstract

Polymerase Chain Reaction (PCR) has revolutionized modern molecular biology, becoming a standard tool for laboratory research and diagnostics. However, it's cost remains prohibitive for low resource settings or for hobbyist use.

Here, I demonstrate an innovative method for transforming a desktop FDM 3D Printer (Creality Ender 3) into a functioning PCR thermocycler (3D-PCR). I accompish this by the the removal of the brass nozzle from the printer's hot end assembly, which allows for an exact fit of a standard 0.2mL PCR tube into the hot end aluminum heat block. A simple gcode program was written to control the cycling of temperatures between denaturation(94°C), annealing (48°C), and extension(58°C) ranges. The 3D-PCR results were comparable to a standard molecular biology PCR machine, yet are about 1/100th the cost.

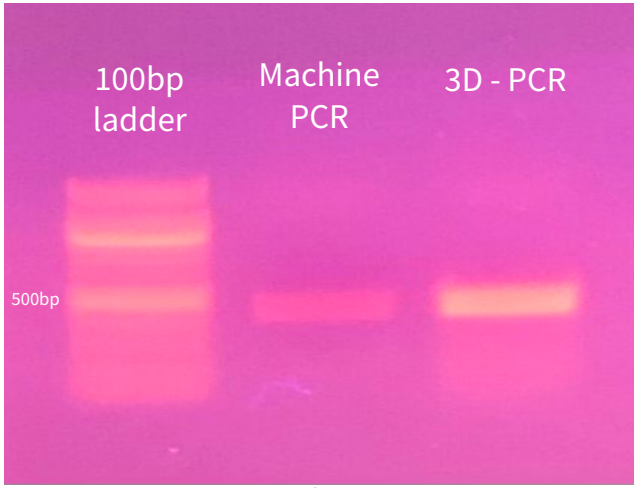


Creality Ender 3 Pro 3D Printer



0.2mL PCR tube

Diagram of 3D printer Hotend. diameter of opening for nozzle thread is 6.3mm.



Agarose Gel Electrophoresis of PCR product from PCR machine and 3D printer PCR using HVR primers. Bands seen at ~500bp, suggesting successful PCR amplification of genomic DNA amplicon

Background

Polymerase Chain Reaction (PCR) has revolutionized modern molecular biology and diagnostics, yet it prohibitive costs have limited its use in low resource settings or in community spaces. Some groups have developed low cost, open-source thermocyclers (Open PCR , PocketPCR) geared towards home DIY use. However, these thermocyclers are all dedicated devices that one would need to go out of thier way to obtain. The advantage to my method is that anyone with a 3D printer can run convert to

3D printing technology has grown significantly in the recent years, with large growth in the hobbyist/home users. Fused-Filement-Fabrication (FFF) 3D printers such as the Creality Ender 3 Pro retail for as little as $199 USD, allowing the easy entry into the hobby for many people around the world. The growing trend of accessable 3D printers presents an opportunity to leverage the technology to affordably improve molecular biology and diagnostics.

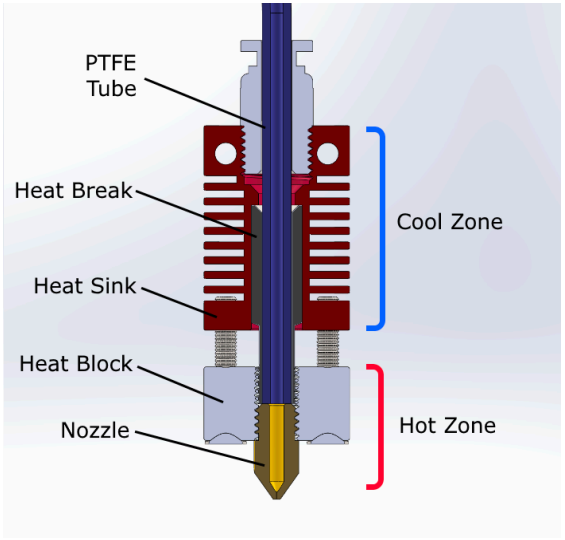
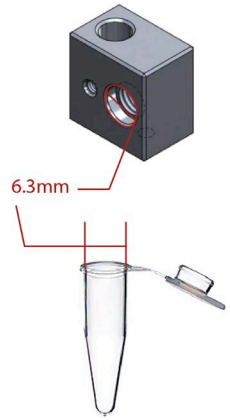


Diagram of 3D printer Hotend. diameter of opening for nozzle thread is 6.3mm.



3D printer aluminum heat-block and 0.2mL PCR tube.

Ender 3 Pro (Creality - $199)

ProFlex™ PCR System
(Applied Biosystems™ - $16,640)

I have developed a method that uses the hotend of an FDM 3D printer as a thermal cycler to run a PCR reaction. 3D printer hotends are designed to rapidly reach the melting temperature of PLA plastics (200C) and effectively dissipate heat to prevent heat creep/clogs from melted plastic in other areas of the printer's hot end. PCR typically cycles temperatures within the to 40C - 94C range, falling within the range of a 3D printers capability. 3D printers use an aluminum heat block as the heating source, and contain an internal thermometer for live temperature readings. Serendipitously, the inner diameter of the 3D printer's heat block and the outer diameter of a standard 0.2mL PCR reaction tube are both 6.3mm. Thus, the conversion of a 3D printer hot-end to a PCR thermocycler requires little re-engineering or modification.
3D printers are controlled with a user-friendly code known as GCODE consisting of a command type (G or M), a command number, and a parameter. A simple program can then be written to cycle the hot end through all the PCR cycle temperatures.

Methods

3D Printer based PCR

For the validation experiment, existing protocols for rapid cheek cell DNA extraction and amplification of human hypervariable region of mtDNA were used from the Biohacker Bootcamp course at Genspace. The PCR mix consisted of genomic DNA obtained from a cheek cell rapid extraction protocol using 0.9% saline and Chelex 100 chelating resin. Supernatant was collected and 5uL gDNA was added to PCR reaction tube with 2X OneTaq Master Mix, and HVR Primers (TTAACTCCACCATTAGCAAC , GAGGATGGTGGTCAAGGGAC). After mixing 30uL mineral oil was added to PCR tube.

Thermocycler Settings

The PCR settings for a 500bp amplicon were set to the following parameters:

Initial Denature 94C - 60s
Denature 94C - 15s
Anneal 48C - 30s
Extending 68C - 30s
*repeat for 30 cycles
Final Extension 68C 120s
Hold 25C

The PCR machine program was set using the machine interface and 3D Printer was set using a custom gcode program.
The following gcode was written using the text editor Sublime Text and saved in the .gcode format
The lines of code comprising of the denature-anneal-extend cycle were entered into a web based text repeater https://pinetools.com/repeat-text and pasted back into the text editor after repeating 30 times. There is no loop function native to Marlin based gcode.

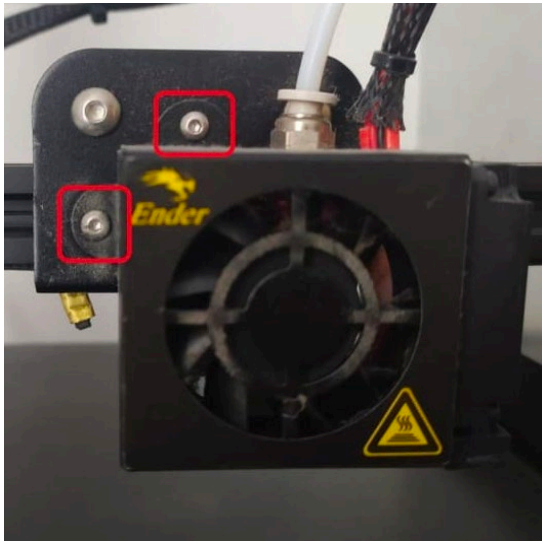
```
//Initial Denaturation
M109 S94; //set hotend temperature to 94C
G4 S60; //dwell for 60 seconds

//Begin Cycle

//Denature
M109 S94; //set hotend temperature to 94C
G4 S30; //dwell for 30 seconds

//Anneal
M109 R48; //set hotend temperature to 48C
G4 S30; //dwell for 30 seconds

//Extend
M109 S68; //set hotend temperature to 68C
G4 S30; //dwell for 30 seconds

//End Cycle

//Final extension
M109 S68; //set hotend temperature to 68C
G4 S120; //dwell for 120 seconds

M109 R25; //set hotend temperature to 94C
```

Gcode program for 3D-PCR thermocycler

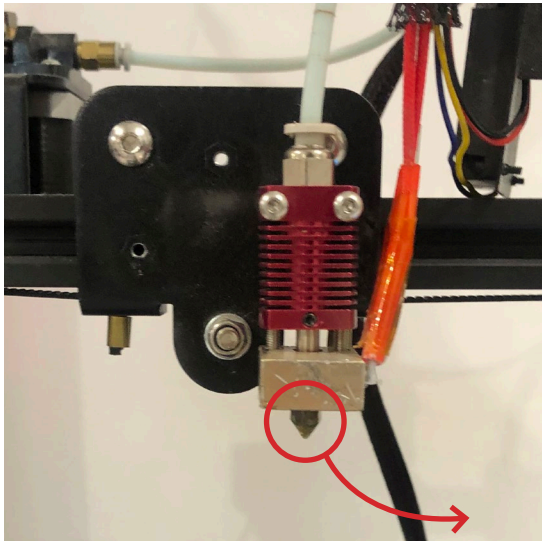
Gcode was uploaded to the Ender 3 Pro 3D printer using a Raspberry pi running OctoPrint - a web interface to allow for live monitoring of temperatures.

Preparation of 3D printer

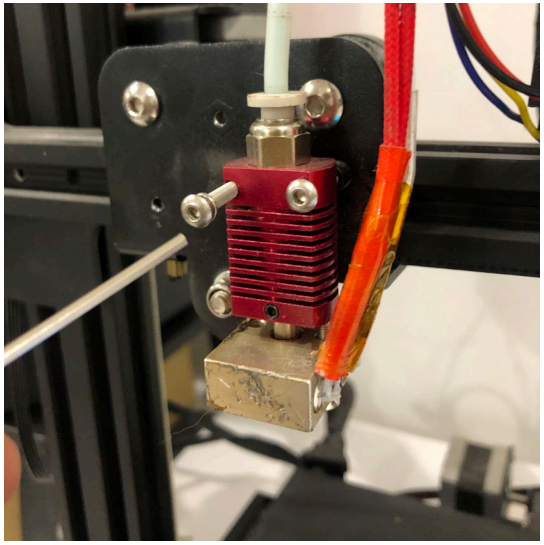
1. The two screws securing the fan shroud on to the hot end were removed and the fan shroud was moved aside.
2. A wrench was used to remove the brass nozzle from the heatblock and set aside.
3. The leftmost screw securing the red heatsink was removed and rightmost screw was loosened to allow free rotation of heatsink about the remaining right screw.
4. The heatsink was rotated counterclockwise to have the aluminum heatblock facing upwards.
5. The PCR reaction tube was wrapped in a small peice of foil and inserted into the heatblock opening.
6.The fan shroud was secured to the inverted hot end with a rubber band to provide cooling of the system.



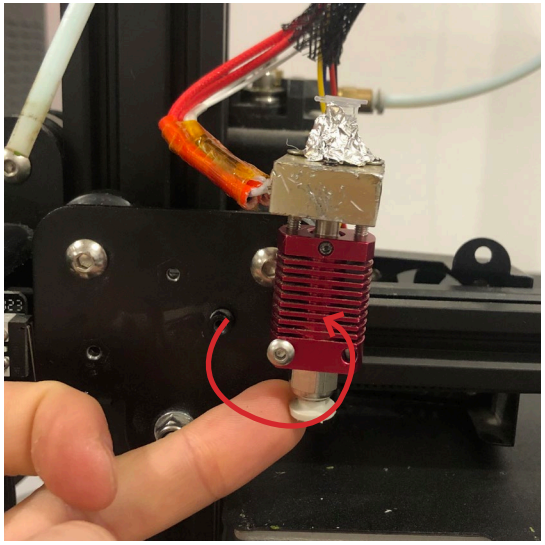
remove fan shroud



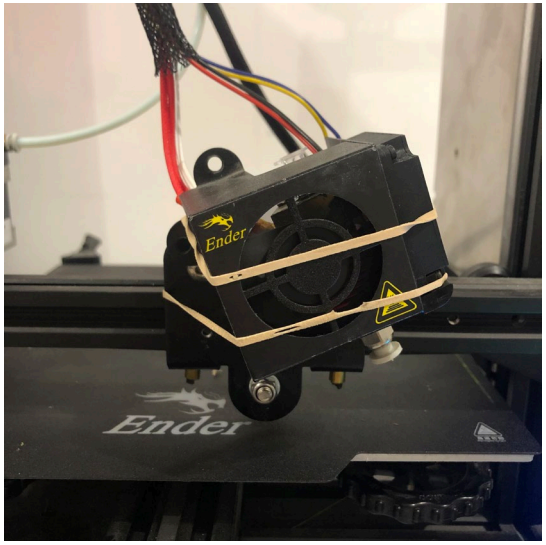
unscrew brass nozzle



remove left screw on heatsink



rotate hotend counter-clockwise

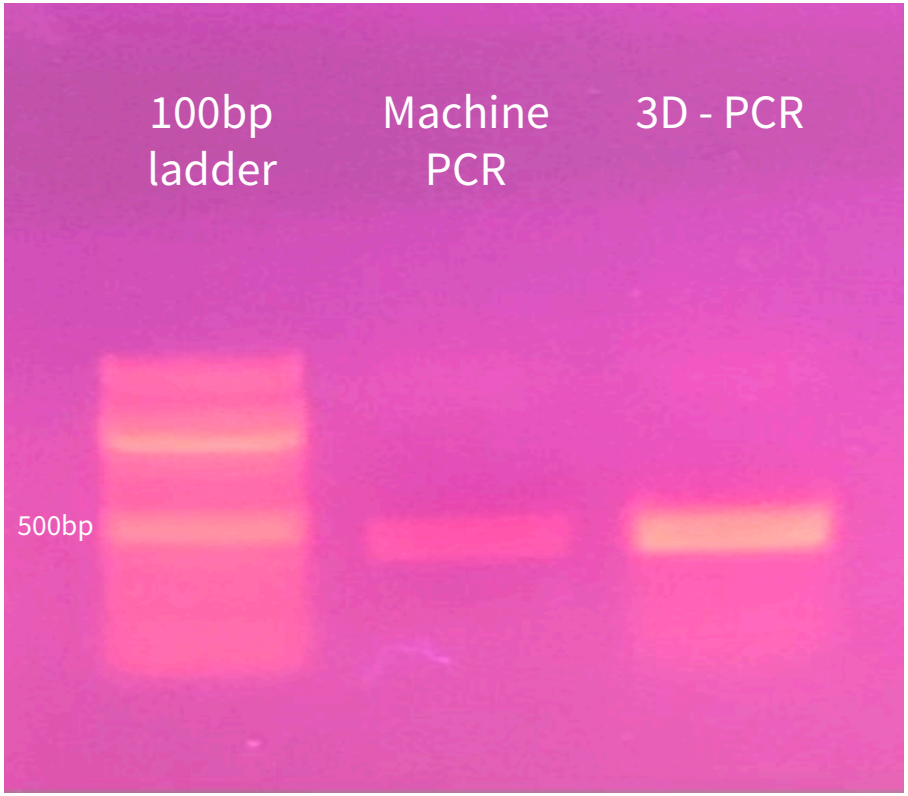


secure fan shroud to cool reaction

Results & Discussion

Gel Electrophoresis shows expected 500bp fragment of DNA

10uL PCR product was run on a 1% agarose gel. 500bp bands appeared in both the PCR Machine and 3DPCR lanes, confirming that there was successful PCR amplification in the 3D printer.



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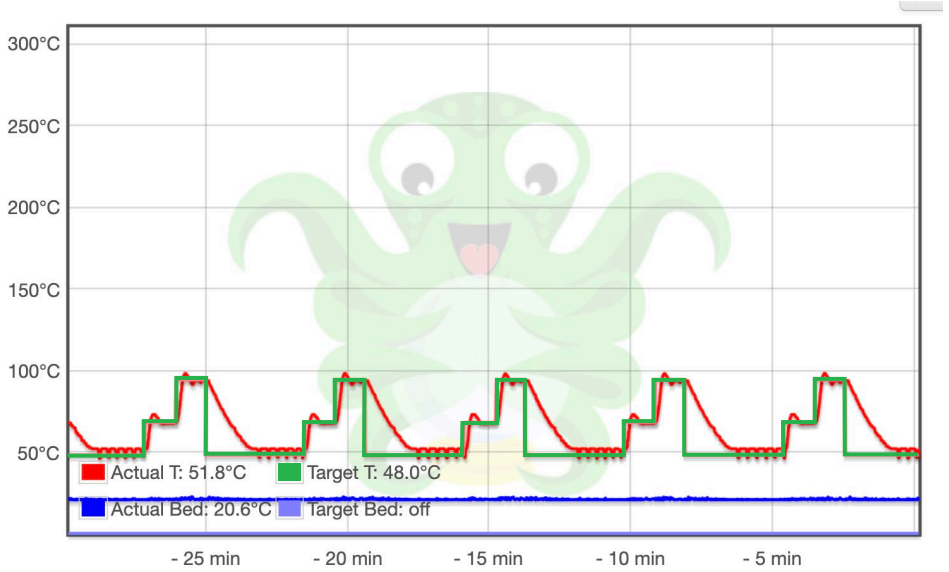

Live Monitoring of 3D printer temperatures with OctoPrint Web Control on Creality Ender 3 Pro printer. The green line shows the Target temperature and the red line shows the measured temperature by the 3D Printer hotend thermometer.

Slower cooling time compared to PCR machine, but no effect on reaction success

The 3D printer does not have an active cooling system, leading to longer overall run times. Its main cooling comes from the 12V shroud fan that was placed over the reaction-containing hot end, rather than an active peltier cooling system seen in commercial PCR machines. The 3D printer cooling rate from denaturation to annealing (94-48C) was ~0.36C/s, compared to the cooling rate of the Machine PCR of ~1.5C/s. Other groups have reported successful PCR with similar cooling rates to the 3D printer (1). Overall, the total reaction time was 2hrs 25min for the 3DPCR and 1hr 10mins for the Machine PCR.

Despite the drawbacks, the 3D-PCR system described here is a functioning and efficient way to amplify DNA in a low resource or home laboratory setting. Further experiments will be attempted to turn this device into a quantitive DNA detection system or use for reaction incubation/heat-shock treatments. This could add further value to the technology as a low cost diagnostic tool.