

Feb 17, 2022

Version 1

Freezenza Box - Production and Assembly V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.byqmpvu6>



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Protocol Citation: Matthew Meyer, Katarzyna Dobaczewska, Zbigniew Mikulski 2022. Freezenza Box - Production and Assembly. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.byqmpvu6>



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Protocol status: Working

We use this protocol and it's working

Created: October 01, 2021

Last Modified: June 06, 2024

Protocol Integer ID: 53741

Keywords: histology, immunohistochemistry, microscope slide, slide staining, 3D-printing, freequenza box, shandon plastic coverplate, slide immunostaining, time to 3d, assembly, works with shandon plastic coverplate, histology aid, 3d

Abstract

A histology aid for slide immunostaining. Improves staining consistency, reduces reagent use, and saves time. Works with [Shandon Plastic Coverplates](#). For technical specifications, check out this [link](#).

Total hands-on time to 3D-print and assemble a 5×3 model is approximately 5 hours, and 4 days including printing and gluing time

Materials

MATERIALS

- ✕ FDM 3D-Printer
- ✕ Chemical Resistant Filament
- ✕ Super Glue
- ✕ Rubber Bands
- ✕ Thermo Scientific™ Shandon™ Plastic Coverplates
- ✕ 3D-Printable Files
- ✕ 3D-Printing Slicing Software

Safety warnings

- ⚠ Be very careful when cleaning the nozzle, use multiple layers of cloth and appropriate PPE to minimize the chances of burning your fingers.

3D printers generate ultrafine particles. Use them only in well-ventilated areas.

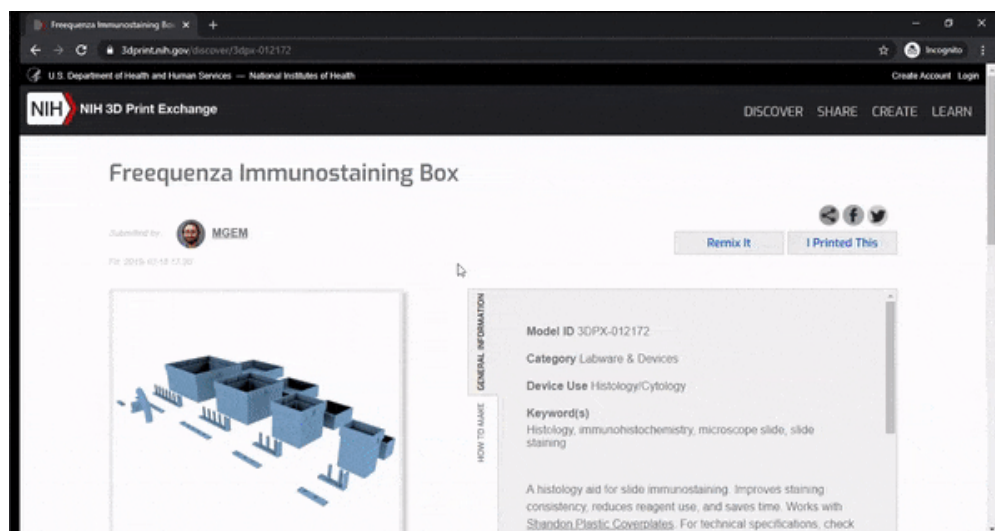
Acetone is highly flammable; use appropriate PPE, and work under the fume hood away from the 3D printer or any potential sources of electrical sparks.

Before start

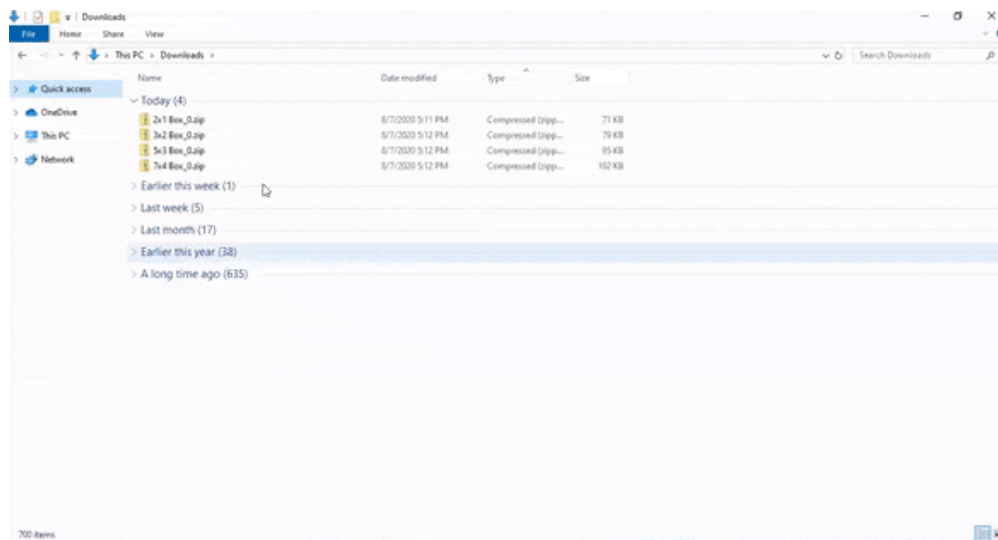
Make sure you understand and follow the safety guidelines for your 3D printer. Test the compatibility of the filament with the solutions you will be using in the future. **[See here for ideas.](#)**

3D-Printing

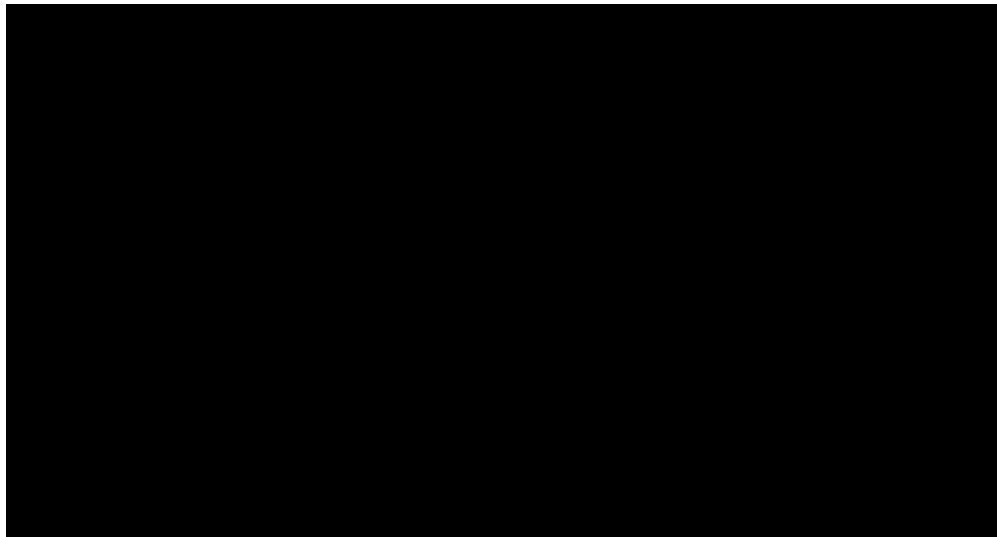
- 1 Navigate to the required **3D-printable files** from the NIH 3D Print Exchange
- 2 Select the desired model type from the below options and download the file in the "Other Files" section
 - **2×1 Box.zip** - (2 slide capacity)
 - **3×2 Box.zip** - (6 slide capacity)
 - **5×3 Box.zip** - (15 slide capacity)
 - **7×4 Box.zip** - (28 slide capacity)



- 3 Extract the .zip file to retrieve the required files
 - *Mac: Double-click .zip file*
 - *PC: Right-click .zip file and click "Extract All..."*
 - *Linux: Follow **video tutorial***

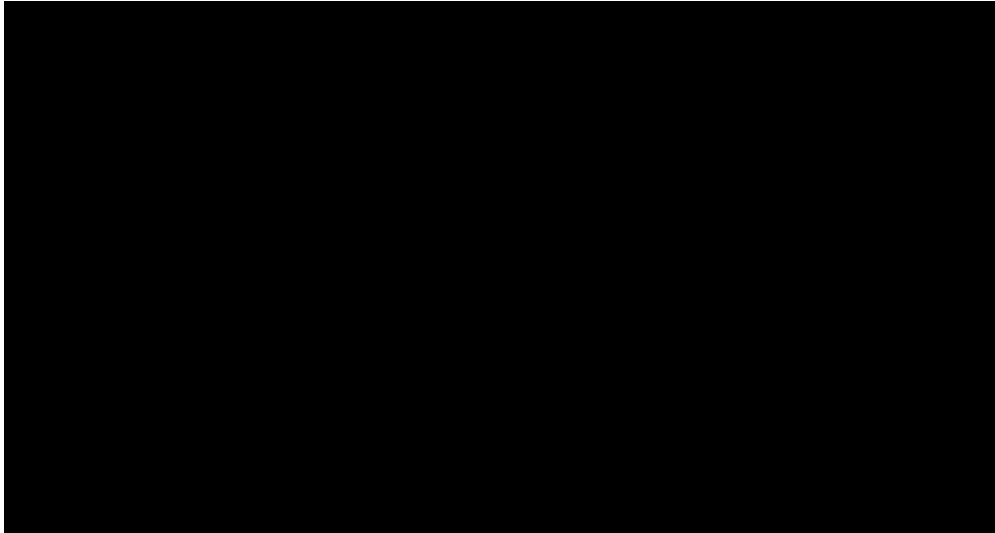


- 4 Launch the **3D-printing slicing software**. If you are experiencing a very poor performance of the software on a computer bound to a domain (often the case in large academic institutions) **see this post** for a potential remedy



We use CURA Lulzbot edition for our Lulzbot TAZ6 printer with nGen ColorFabb filament

- 5 Open the .stl files *+Rack**, *-Rack**, *End*, and *Insert* in the slicing software

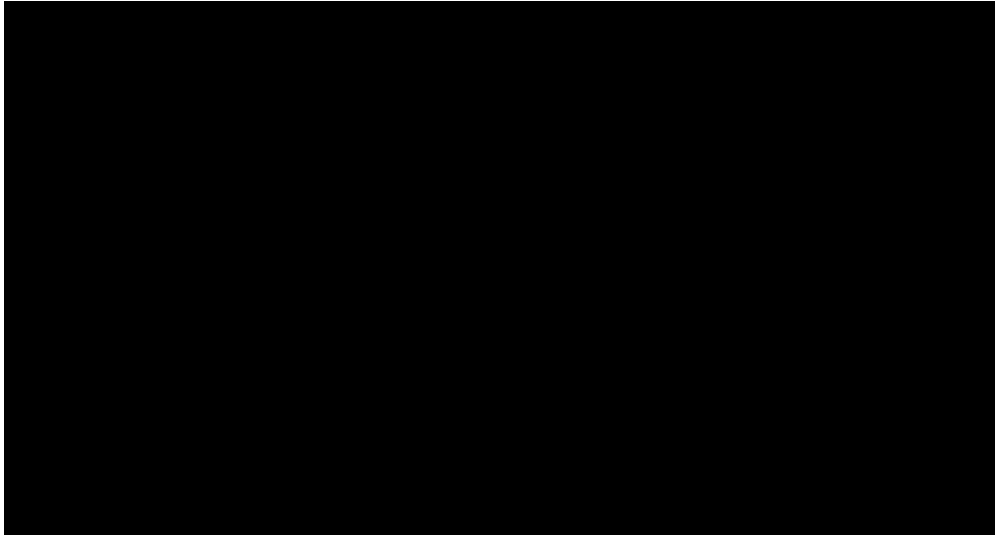


* suffix will differ depending on the model type

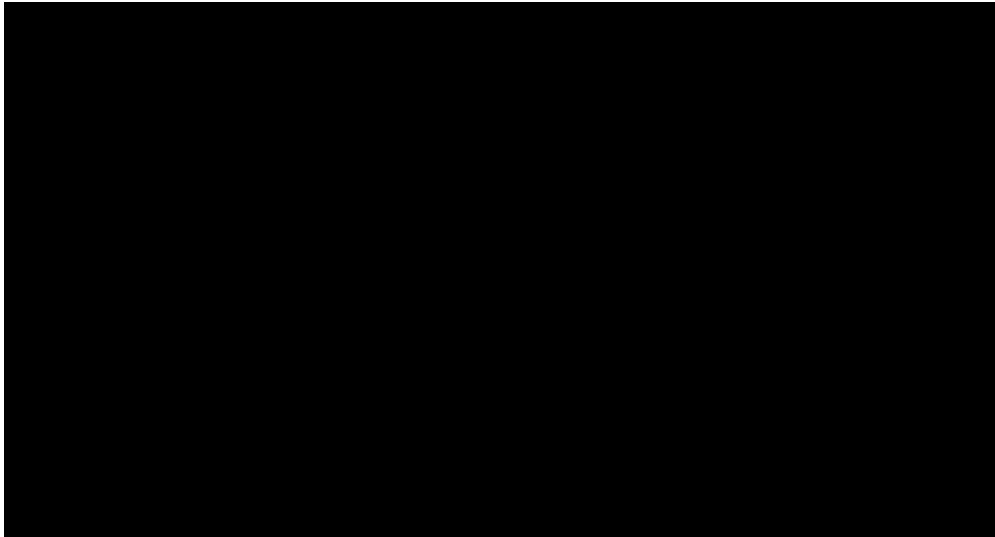
6 Multiply the above .stl files to the appropriate number based on the model type

		2×1	3×2	5×3	7×4
	<i>Box</i>	1	1	1	1
	<i>Top</i>	1	1	1	1
	<i>+Rack</i>	1	2	3	4
	<i>-Rack</i>	1	2	3	4
	<i>End</i>	1	2	3	4
	<i>Insert</i>	2	6	15	28
	<i>Handle*</i>	0-2	0-2	0-2	0-2

* *handles* are optional

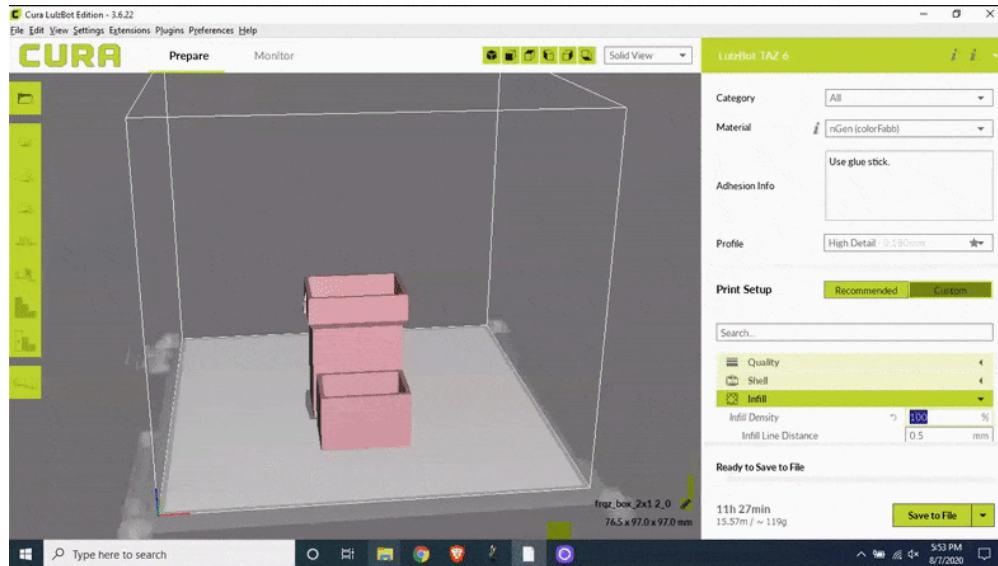


- 7 Use the default setting in a "High Detail" profile (i.e. ≤ 0.18 mm layer height) and prepare/slice the design into a .gcode file



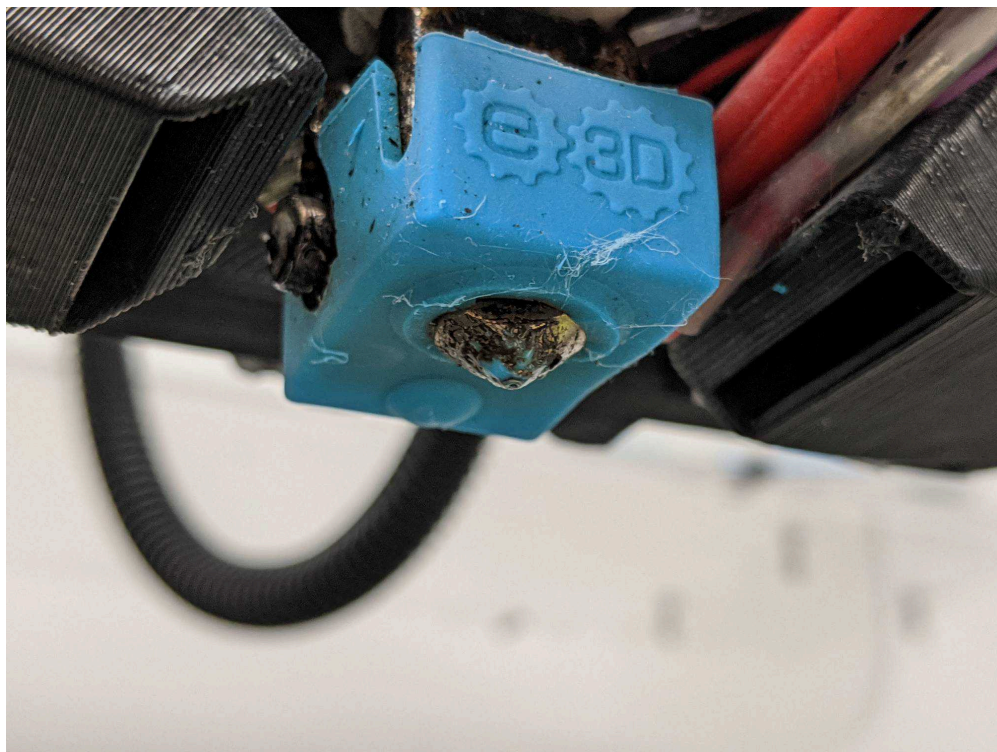
If your printed parts break easily try to inspect your sliced model in the Layer view to see if the infill creates a strong, interconnected lattice in the vulnerable part. Try increasing the wall size to 2 mm, changing the Infill Density to 80% density, and Infill Pattern to Cubic or Gyroid.

- 8 Upload the .gcode file onto an SD card



While some printers offer the option to print directly from the computer, it is highly recommended to print from an SD card due to the increased reliability

- 9 Insert the SD card into the 3D-printer
- 10 Heat the 3D-printer nozzle to approximately 170°C
- 11 Using a clean cloth, wipe the heated nozzle to remove debris



Excess material on the nozzle can stick to the print and cause problems

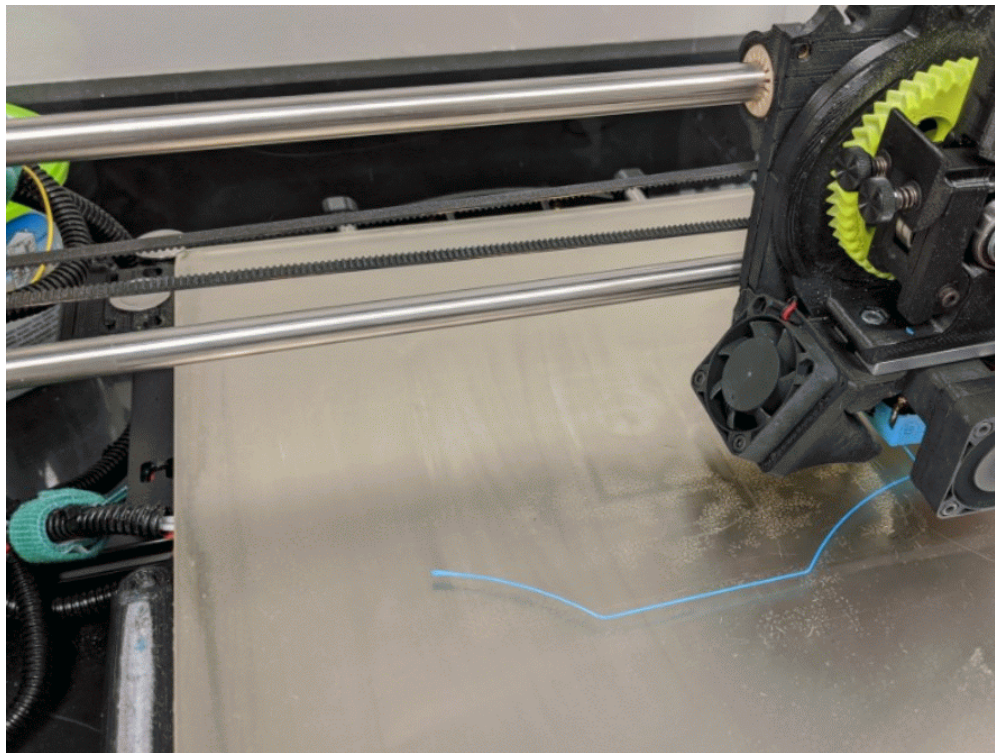


The cloth is usually sufficient to remove excess filament on the nozzle but sometimes a wire brush may be necessary (be very careful not to short the printer with the wire brush)

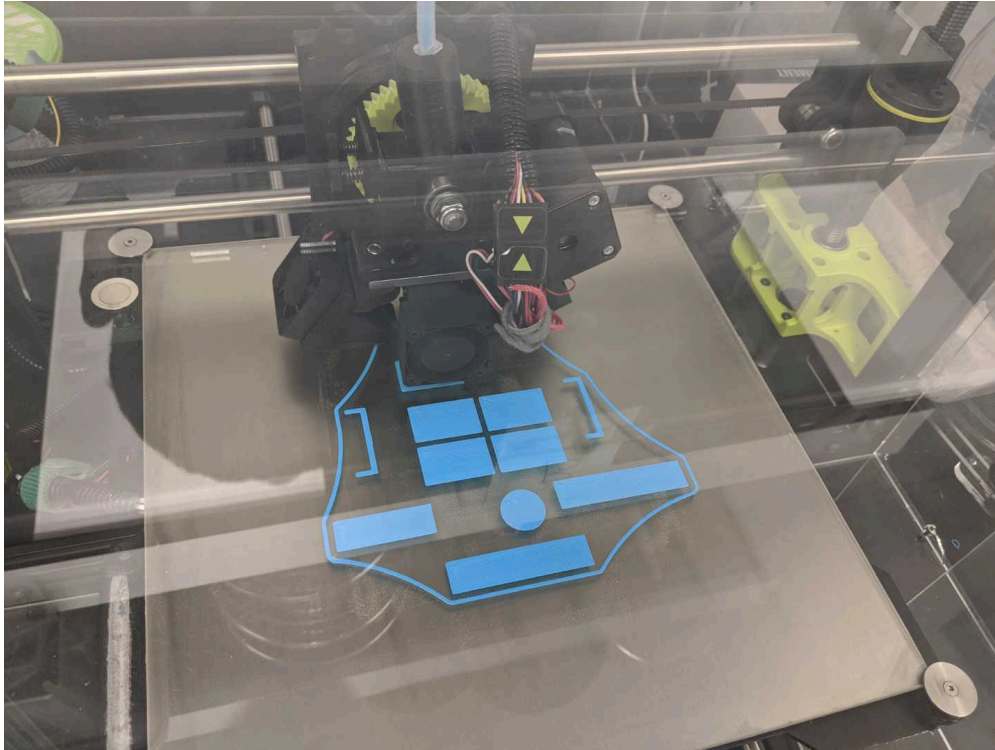


Clean nozzle is key to success. e3D silicone sock V6 can further improve print quality

- 12 Prepare printbed for maximum adhesion of filament (e.g. using a glue stick to cover the area of the printbed which objects will be printed on)
- 13 Select the .gcode file and print

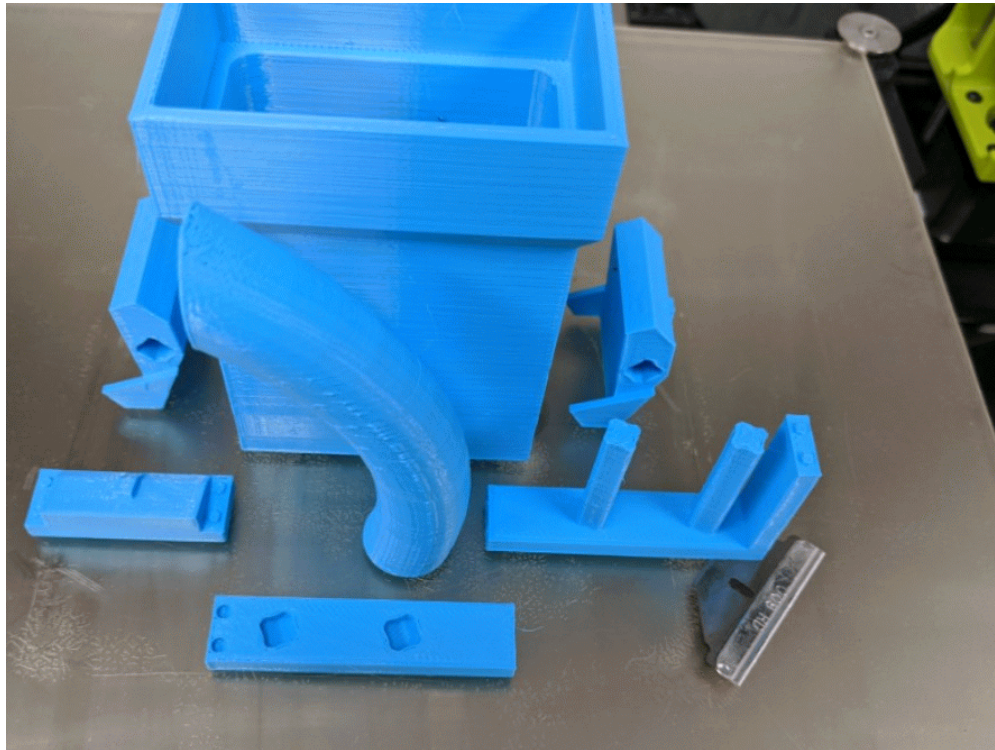


Make sure there is adhesion on the first layer and restart the process if adhesion is not good

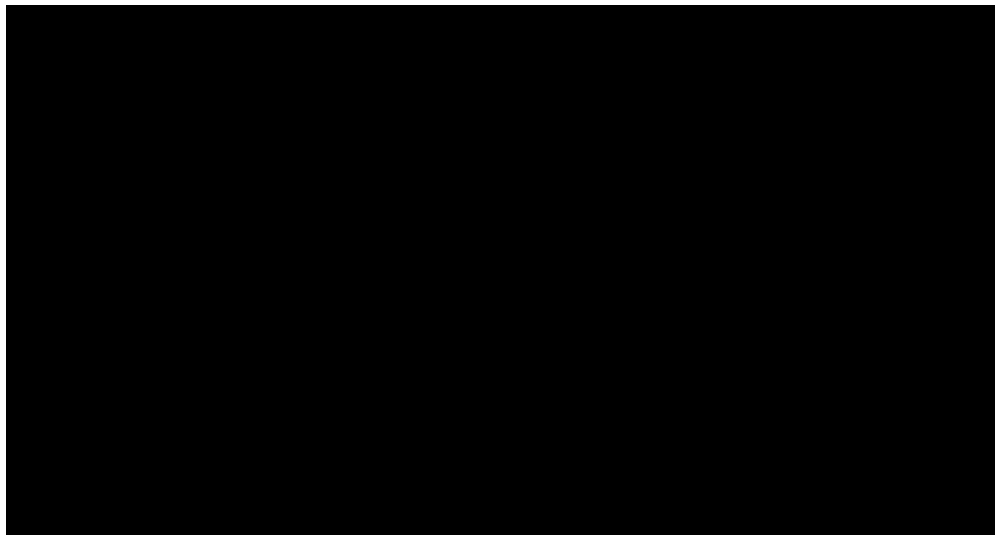


Most problems happen during the first layers of the print

- 14 Carefully remove parts from the 3D-printer printbed using a razor blade



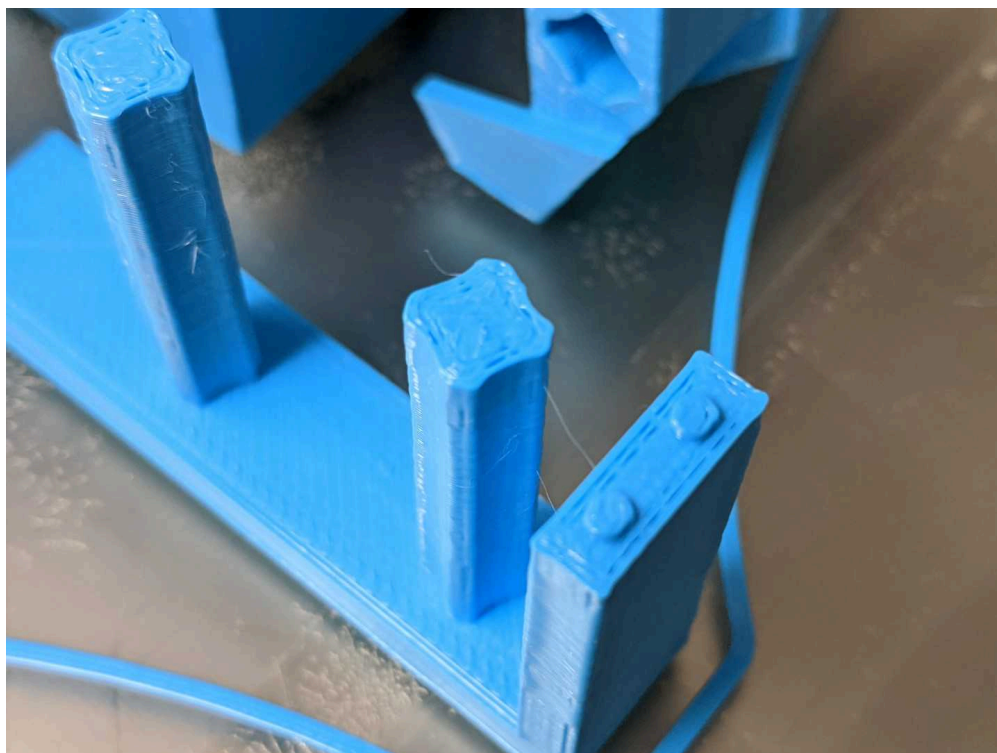
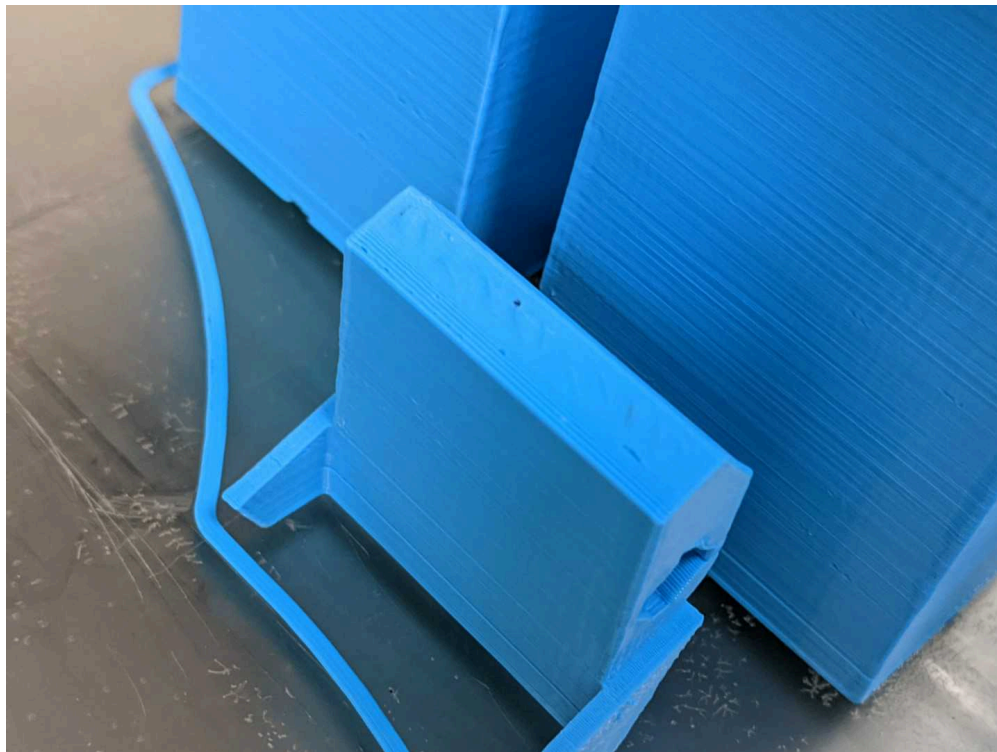
- 15 Repeat steps 4-14 with the *Box* and *Top* .stl files with the "Infill" setting at 100%



- 16 Inspect parts for defects

- 16.1 Acceptable defects (e.g. small blemishes, strings, indentations, etc.) can be cleaned with razor blades, sand paper, or metal brushes



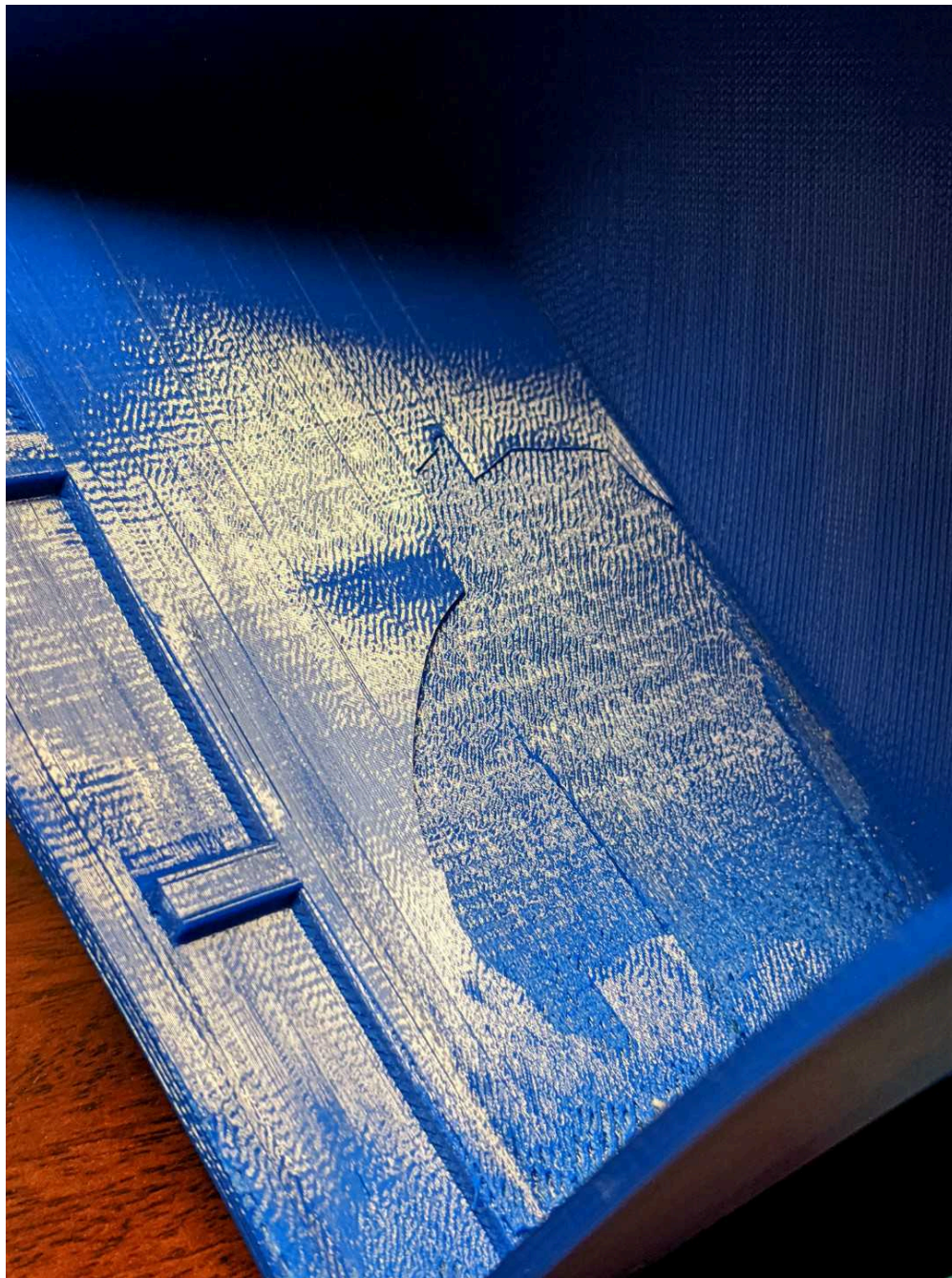


- 16.2 Unacceptable defects (e.g. large holes, wall flaking, warping, etc.) cannot be repaired and will need to be reprinted

Many issues come from the moisture in the filament (it should be stored in a desiccated container with up to 20% relative humidity). Printer issues can be diagnosed with the help of this guide.







17 Fill the *Box* and *Top* with water to check if they leak

17.1 If the part leaks, place the part in a larger container and fill the part with acetone*

* use caution and work under a chemical hood as acetone is flammable and produces hazardous fumes

17.2 Let the part sit until the acetone stops leaking through the previous leak hole

17.3 If the part never stops leaking, the part will need to be reprinted

Assembly

18 Connect *Inserts* and *+Rack*



Assembly of the Inserts, + - Racks, and the End parts

18.1 Lay the long edge of the *+Rack* flat on a counter (rods facing upward) with the short edge oriented leftward

18.2 Lay the *Inserts* on their sides (holes facing upward) with the outward bump oriented leftward

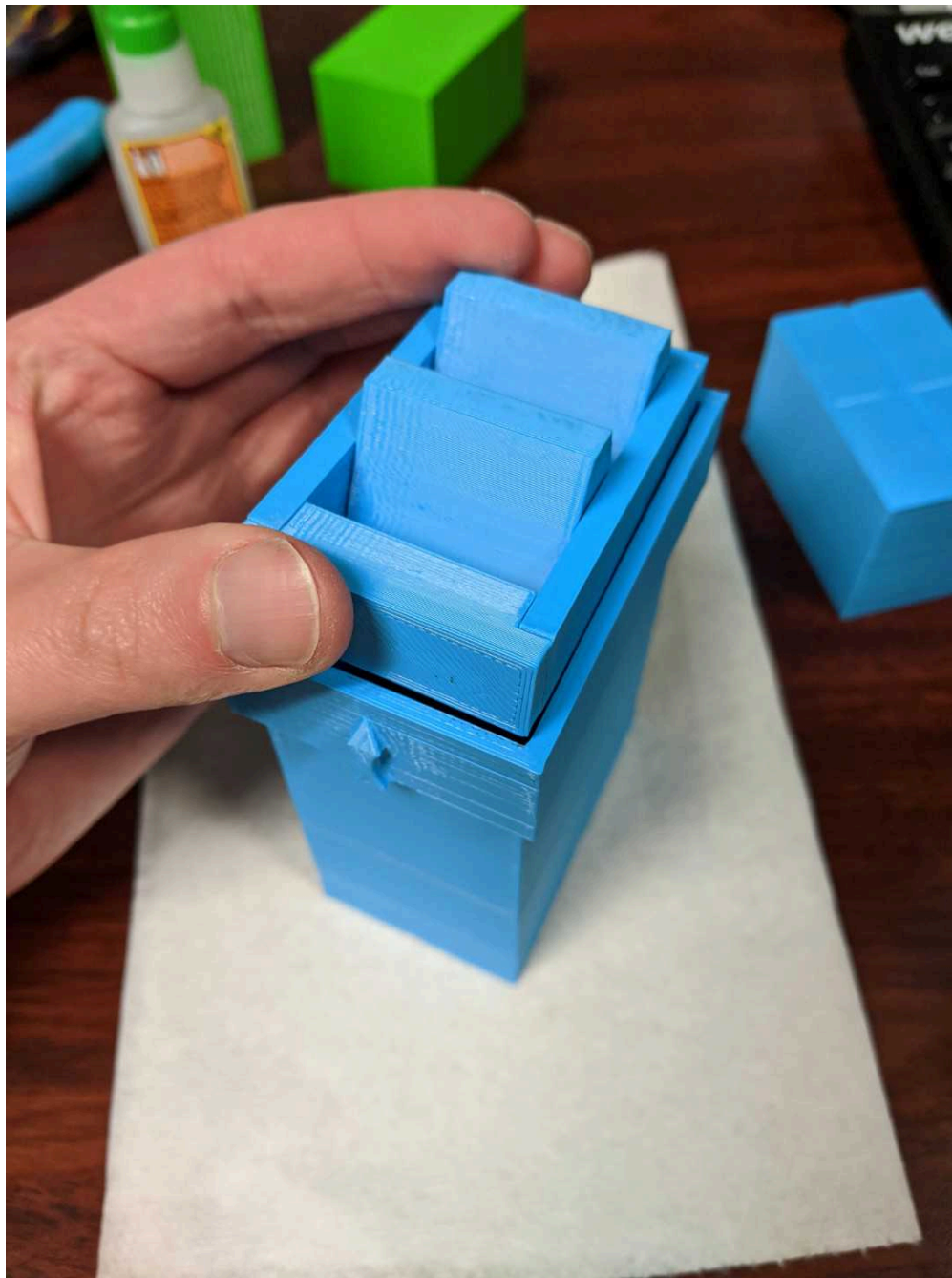
18.3 Maintaining the above orientations, place the holes of the *Inserts* through the rods of the *+Rack*

19 Glue *+Rack*, *-Rack*, and *End* together



3M Scotch-Weld Plastic & Rubber Instant Adhesive PR40 offers stronger bonding than most other glues we tried

- 19.1 Apply 3M Scotch-Weld PR40 or superglue to all holes of the *+Rack* and *-Rack*
- 19.2 Align and connect the holes of the *-Rack* with the rods and studs of the *+Rack*
- 19.3 Orient the outward bump on the *End* the same way as the *Inserts*
- 19.4 Connect the *End* studs into the holes of the *+Rack* and *-Rack*
- 19.5 Wipe off excess glue with a paper towel
- 20 Rubberband the assembled rack together or use woodworking clamps
- 21 Let the glue dry until the parts are completely set
- 22 Place the assembled rack into the *Box* with the outward bumps facing upward



23 Optional: Glue *Handles* to Box



