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HOW TO CREATE G-CODE FOR MULTI-WELL PLATES

Overview: This protocol shows users how to create g-code with multi-well plates. The steps below can be used for a 6-well, 12-well, 24-well, 48-well or 96-well plate.

Materials:
Well Plate
CELLINK INKREDIBLE or INKREDIBLE+ Bioprinter
Nozzles
Sublime Text Program
G-code of object you'd like to print

Protocol:

1. Put the well plate onto the printing plate. Locate the bottom left well. For the purposes of this protocol, we'll be using a 24 well plate.
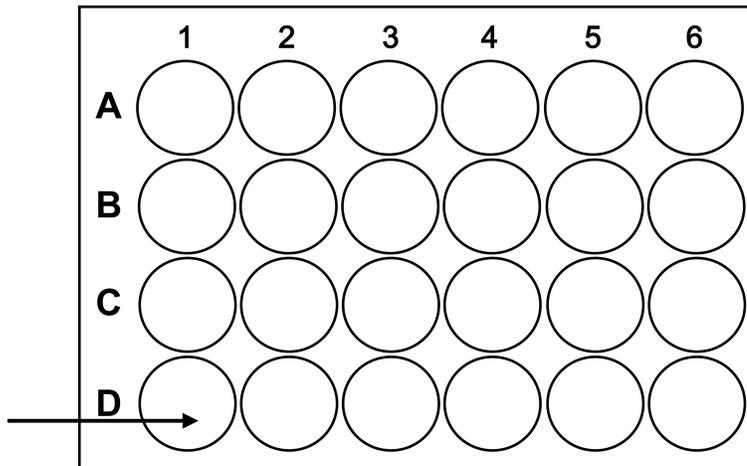
2. Go to the "Utilities" menu, then click "Move Axis". Move the X, Y, and Z axis so that the nozzle you'd like to print with is touching the bottom of the bottom left well (shown in the figure above, D1). Record these X, Y, and Z coordinates.
3. Next, move the nozzle along the X-axis to the center of the next well, D2 in the figure above. Record the new X coordinate and determine the X distance between the two wells.
4. Repeat Step 3 in the Y direction. Move the nozzle along the Y-axis to C1 in the figure above and record the Y-coordinate. Determine the Y distance between the two wells.

5. Open the 24 well g-code template in Sublime Text.
6. On line 4, enter the X and Y coordinates found of the bottom left well. This line is commented with "moving to first well position".
7. In the Sublime Text headings, click "Find" then "Replace". A text editor should pop up on the bottom of the screen. In "Find What", write in G1 Z0.2 . In "Replace With", enter in the Z height found in step 2 plus the layer height of the nozzle using. For example, if you are using a 25G nozzle (layer height 0.3 mm) and the Z coordinate found in step 2 is 0.2, the Z height for printing is 0.3 + 0.2 = 0.5. Click "Replace all" in the bottom right corner.
8. Next, click "Find" then "Replace" from the top menu. In "Find What", write in G0 X19.3 . In "Replace with" write G0 X and the X-distance between the two wells. Write it in the same format as the code in "Find What". There should be no space between the "X" and the coordinate you found.
9. After, click "Find" then "Replace" from the top menu. In "Find What", write in G0 X-19.3. In "Replace with", write G0 X- and then the X-distance you found in step 3. This should be a negative number for this step.
10. Last, click "Find" then "Replace" from the top menu. In "Find What", write in Y19.3 . In "Replace with", write the difference in the distance in the y-direction found in Step 4. Keep it in the same format.
11. The last part of this step is to copy the code that is being printed in each well, and paste it into each well. Find the part in your code where the extrusion begins "M760". Start copying here, and finish copying where the extrusion stops "M761".
12. Paste this code into the template code. This code is pasted between "pre-structure code" and "post-structure code". For the first well, this is lines 8-10. Erase the code for the dots and paste your code. Paste your code in each area between pre-structure code and post-structure code. The dot code is pasted below so you know what to delete and replace.

```
M760
G4 P400
M761
```

13. These steps can be applied to any size well plate, but gets more tedious when there are more wells. To make the process easier and faster, you can also Find and Replace the dot code above with your code. Make sure to include a start and stop extrusion command at the beginning and end of your code (M760 and M761).
14. To modify this protocol for other well-plate template codes, find the "moving to next well in X" to find the specific values to replace for your well plate. This procedure is the same for "moving back one well in X" and "moving back one well in Y". Those are the three values that need replaced, along with the Z coordinate for new well-plates.

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G-codes:

dots_12wellplate.gcode
dots_24wellplate.gcode
dots_48wellplate.gcode
dots_96wellplate.gcode

Further Information:

You are able to paste the same code of your structure for each well because the multi-well codes use relative coordinates. Finding the distance between the centers of adjacent wells in the x and y-directions allows you to manipulate the code without determining the center point of every single well.

When preparing to bioprint, only "Home Axes". Do not set the Z height or Calibrate Z, as this z-height is accounted for in the code when you set the dimensions in Step 2 and 7.