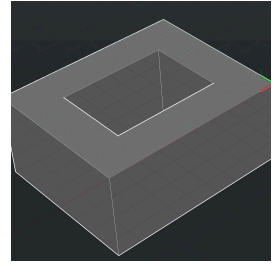


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How to Make Inverted Inserts

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Kristy L Kounovsky-Shafer¹, Charles Polen¹, Esmeralda Mendez-Ortiz¹

¹University of Nebraska - Kearney



Kristy L Kounovsky-Shafer

University of Nebraska - Kearney

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Protocol status: Working

We use this protocol, and it's working.

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Abstract

This protocol details the steps to make an inverted insert. An inverted insert has agarose on the outside and the DNA or cell solution in the middle; the cells can be lysed inside the insert. The agarose protects the DNA during cell lysis. Once completed, the top of the insert can be cut off, and the DNA solution can be used removed from the inverted insert and used for applications that need chromosomal length DNA.

Image Attribution

Inverted insert holder, author: Kristy Kounovsky-Shafer, source: screenshot AutoCAD image

Guidelines

Buffer:

1X TE (10 mM Tris-Cl and 1 mM EDTA) pH 8

Recipe for 1X TE:

1 mL 1M Tris-Cl pH 8.0

0.2 mL 0.5M EDTA pH 8.0

Fill to 100 mL with distilled water

Autoclave the solution.

Glass rod with hook

A glass pasture pipet end is heated in a bunsen burner flame until the end is closed. Then, the part near the end is heated until a hook forms.



Materials

3D printed Inverted insert holder
3D printed Inverted insert mold
3D printed inverted insert top
3D printer Inverted insert plunger
3D printed cross
UltraPure low melting point agarose
0.5 M EDTA pH 9.5
1X TE buffer
6X Loading dye
Corning CoolRack M15
Scotch general purpose masking tape
Hot plate
Distilled water

Protocol materials

 UltraPure™ Low Melting Point Agarose **Catalog #16520-100**

 6X loading dye

Safety warnings

 Don't touch the hotplate when it is hot.

Ethics statement

N/A

Before start

Before you start, make sure the 3D-printed molds are dry. If you need the 3D-printed molds, please contact Kristy Kounovsky-Shafer (kounovskykl@unk.edu).



Procedure

25m

- 1 Tape the bottom of the mold well. Place 3 pieces of tape on the bottom. Place the mold on ice or on a Corning CoolRack M15, which is on ice, so it sits on ice for at least 00:05:00 . I put it on the CoolRack, as it makes it easier and flatter. 5m
- 2 Make 50 mL of [M] 1.5 Mass / % volume UltraPure™ Low Melting Point Agarose **Catalog #16520-100** solution in an 125 mL erlenmeyer flask, and place an empty beaker on the top of the flask. Boil the solution on a hotplate while waiting for the mold to cool down.
- 3 Add the liquid agarose solution to each section of the insert mold until it reaches the top. Mold should be on ice.
- 4 Add the top to the bottom mold for the formation of the wells. Make sure the top and bottom are aligned. Keep the mold on ice.
- 5 The remaining agarose solution was then cooled to 42 °C in a water bath.
- 6 Once the agarose in the mold solidified (00:15:00), the mold top was removed carefully to prevent damage to the integrity of the wells. Wiggling the top while pulling it out helps to keep the inserts in the mold. 15m
- 7 170 µL of DNA or cell solution was added to the wells.
- 7.1 17 µL DNA (lambda, assuming [M] 0.5 µg/µL), 119 µL 1X TE, and 34 µL 6X loading dye with glycerol were mixed with a wide-bore pipet top and added to the wells.
- 7.2 170 µL cell solution was added to the wells.
- 8 Wait 00:05:00 before adding the agarose. 5m



- 9 Place the 3D-printed cross with ends on the device.
- 10 Finally, the agarose from the water bath ( 42 °C) was added to the top of each insert to fully encase the well and finish the inverted insert. It should look like a mushroom.
- 11 Allow to solidify.
- 12 Remove tape from the bottom of the device.
- 13 Flip the mold upside down. Take the 3D-printed pusher and push the insert out of the mold. Be careful when you push them out into a 50 mL conical tube with your storage buffer (ex. 0.5 M EDTA pH 9.5) or lysis buffer. If you see a gap in the top of the insert or the insert broke, throw it away. The insert didn't work so it wouldn't have any DNA left. 
- 14 Rinse at least three times with 1X TE before using the inserts. At least one rinse should go overnight in the fridge. 
- 15 Inserts should be stored in the fridge ( 4 °C) until ready to use.
- 16 Clean a glass rod with a hook with ethanol. To access the DNA, take the insert out with a glass rod with a hook and place it into the holder on a glass slide. Use a glass coverslip (any size bigger than the inverted insert will work) to slice off the top of the device. Use a wide-bore pipet tip to remove the solution and place it into an autoclaved Eppendorf tube for future use. 