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# Cloning grills: High throughput cloning for structural genomics

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## Abstract

Cloning grills are aluminum grids designed to divide an agar plate into segments, thereby multiplying the number of *E. coli* cultures which can be streaked out on a single plate. The grills are autoclaved and placed in square petri dishes immediately after hot agar is poured. When the agar solidifies, the grill remains embedded in the media, and each of the 12 lanes accommodates the streaking out of a single culture. As the spacing of the grill lanes is the same as that of a 96-well plate, 12 cultures can be streaked at a time using a 12-channel pipette. This allows a plate of 96 cultures to be rapidly and accurately plated for colony isolation on only eight agar plates.

## Introduction

The drive to increase throughput in structural genomics protein production labs has pushed many to embrace a 96-well format for almost every step in the production pipeline (Dieckman *et al.,* 2002; Knaust *et al.*, 2001). PCR, ligation, transformations and expression screening are now routinely performed in 96-well plates or blocks. Unfortunately, not every step is amenable to this format, and specifically, plating out transformants to obtain single colonies remains a step which is typically done one well and one petri dish at a time. This is time-consuming and error-prone. The cloning of transformants is done at least twice for every clone; the first cloning is to propagate plasmid DNA (e.g. DH5$\alpha$ *E. coli*), and the second is to select an expressing clone (e.g. BL–21 *E. coli*). Thus, for a single 96-well plate one ends up with a minimum of 184 petri dishes, a burdensome load.

In an effort to develop an alternative to the large number of plates required by standard cloning techniques, we developed cloning grills. These are simple, aluminum grills which, when embedded in agar, serve to divide the agar into segments which can then be used for plating 12 colonies on a single plate (Figure 1). The 9 mm spacing of the channels, the same as that for 96-well plates (Morozov, 1999), allows for the use of 12 channel pipettes to make the transfers.

## Materials and methods

### *Media and labware*

All agar plates were made with Luria-Bertani (LB) media prepared as per manufacturer's instructions (Difco). Antibiotics were 50 µg/mL ampicillin and 50 µg/mL carbenicillin (Sigma). Square petri dishes (120 × 120 × 50 mm, Greiner) were employed and 45 mL of media was used for each plate.

### *Grills*

Cloning grills were milled in the University of Washington Instrument Services shop out of 0.953 cm 6061-T6 aluminum using a computer-driven CNC milling machine. The grills were fashioned with overall dimensions of 11.8 × 11.8 cm, slot-to-slot spacing of 9 mm, and a barrier thickness of 1.07 mm between slots. Prior to use, the grills were cleaned to remove cutting oil, the channels labeled with a per-

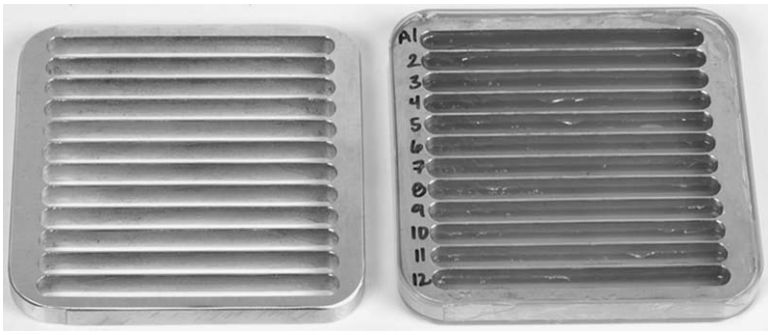


*Figure 1.* An unused cloning grill is shown on the left and a cloning grill labeled and embedded in agar is shown on the right. The lid is removed from the petri dish for clarity.

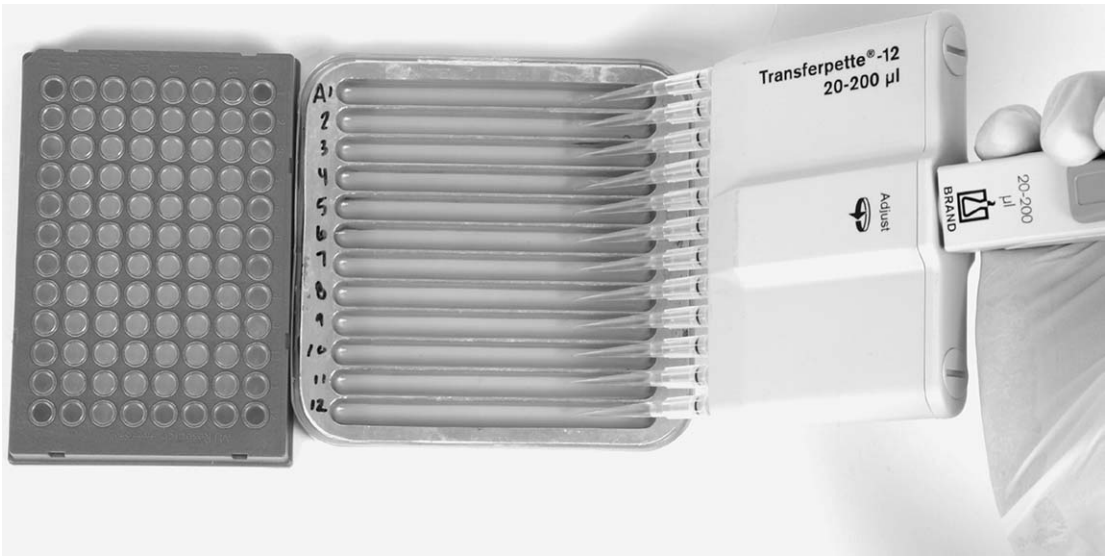


*Figure 2.* The 9 mm spacing of the cloning grill lanes is designed to allow for facile transfer of cultures from 96-well plates using a 12-channel pipette.

manent marker, and the grills were wrapped in aluminum foil and autoclaved.

*Plating cultures*

A 96-well plate containing cultures was lined up next to an agar-embedded cloning grill such that the wells of the plate lined up with the lanes of the grill. The cultures all had antibiotics added to them prior to plating. A 12-channel pipette was used to first deposit 100 µL of LB media with antibiotics in each of the channels at one end; the cultures were then transferred into the other side of the channels and the pipettor was used to simultaneously streak the cultures into the fresh media (Figure 2). The plates were left open at room temperature to dry for about 20 min, covered, and transferred to an incubator for overnight incubation at 37 ºC. Plates were not inverted for incubation.

## Results

The technique provided here allowed for the growth of single colonies from cultures which were very dense (OD about 10) to cultures which were very sparse. Streaking the cultures into a pool of fresh media was important for dense cultures, as these cultures tended to provide a thick mat of bacteria if they were streaked without extra media. In an effort to determine if there was any chance of getting lane-to-lane contamination, a number of cultures were plated using every other lane of the grill, and no cross-contamination has been observed (data not shown).

The use of cloning grills does not allow for good quantitation of the total number of transformants, as each lane typically contains a mat of colonies at one end with a gradient to individual colonies at the other. The dense side of the lane is impossible to count.

It is important to keep antibiotics in all media used with cloning grills, including the media the transformants are in. If transformants are plated out in media without antibiotics, it is possible that some *E. coli* grows on this media even if they lack the proper antibiotic resistance marker.

If cloning grill plates are made with insufficient agar, the agar will tend to dry and split in the lanes. The 45 mL of agar given in the Materials and methods section we have found to be a good trade-off between drying out and agar conservation.

After use, the cloning grills can be easily removed from the agar by twisting the petri dish. The grills can then be cleaned and autoclaved for repeated use.

## Discussion

Cloning grills have become an integral part of our protein production pipeline for the Structural Genomics of Pathogenic Protozoa (SGPP). They are used for routine subcloning procedures, such as when we want to move a plasmid into an expression strain of *E. coli*. We continue to employ a large number of individual agar plates when plating out initial clones of our PCR products, as in this step it is desirable to determine the number of colonies vs. background. An advantage of using the grills is that it is much more difficult to accidentally swap samples because one is plating an entire row of colonies at the same time. As the channels in the grills have the same 9 mm spacing as the wells of a 96-well block, picking colonies to transfer to a block is also more straightforward and less prone to mistakes.

Although we currently use cloning grills for plating cells manually, there is no reason why this kind of system could not be used by robotic liquid handling equipment. The manual approach is so rapid, however, that one would have to weigh very carefully the merits of automating this step versus the costs of setting up, maintaining, and tying up a robotic liquid handler for this simple transfer.

In contrast, picking of the resultant colonies is a step which could benefit from automation. However, colony picking robots are designed to pick many colonies from just a few source plates, a design which works well for making genomic libraries, etc., but which runs opposite to the design required for structural genomics. High-throughput cloning and expression typically calls for picking just one or two colonies from a wide array of sources (such as the spread-out wells from a 96-well plate). Cloning grills could be useful as source plates for colony picking instruments which have a programmable source plate setup.

It has been suggested that certain plasticware may be used instead of cloning grills to achieve the same end. Items such as 12-channel reservoir liners (Costar) may be employed (F.W. Studier, personal communication), the downside being that each of these channels must be individually filled with agar. Ideally, the cloning grills themselves could be fabricated from disposable plastic.

Cloning grills have been repeatedly autoclaved and reused without any noticeable pitting or oxidation. If a higher degree of environmental resistance is required, though, the grills could be anodized.

Devices similar in nature to cloning grills have been suggested in the past (Morozov, 1999), but we are not aware of any commercially available product which provides all the attributes of the one we have fabricated. Cloning grills are an efficient tool for obtaining single colonies from 96-well plates of cultures. Although this method does not allow for quantitation of the number of colonies, it does significantly decrease the time required for plating, the potential for error, and the space required for plate storage.

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