

Tips and tricks of the trade

Breaking the Cloning Bottleneck

Any steps in high throughput (HTS) cloning of genes and expression of the corresponding proteins are usually done by robots or liquid handling stations. Well, almost any, since there is still a bottleneck at the plating step in HTS-cloning protocols.

Lab Hint

In contrast to the early HTS-cloning steps compromising PCR, vector construction and cell transformation that are compatible with robots, the plating of transformed cells for clone selection is usually done manually. As this cloning bottleneck leads to a considerable reduction of throughput and rising costs, several HTS-cloning systems have been proposed, which enable researchers to screen whole blocks of clones at one time. All these strategies, however, rely on custom-built 'cloning grills' or plates.

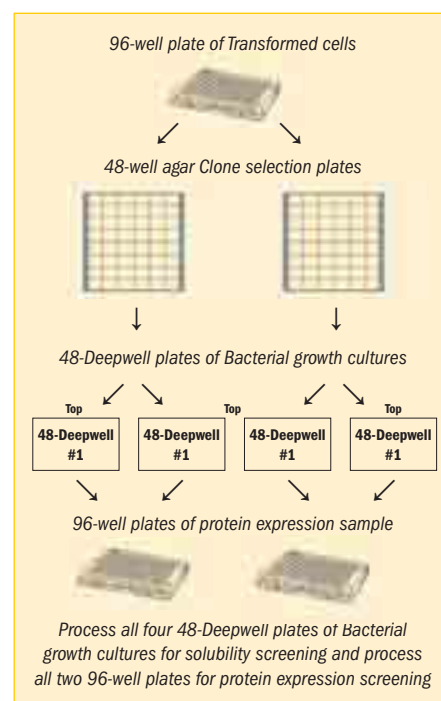
To overcome this shortfall, James Abdullah and Andrzej Joachimiak of Frank Collart's group at the Argonne National Laboratories in Chicago, USA, developed a new plating scheme based on commercially available 48-grid plates. The team from Chicago hence dubbed their new HTS-cloning method 'System-48' (*Methods in Molecular Biology: High Throughput Protein Expression and Purification*, 2009, Vol. 498, 117). According to Abdullah *et al.*, System-48 has

worked successfully in the production of several thousand clones at the Argonne institute.

The System-48 protocol starts with the robot assisted ligation independent cloning (LIC) of PCR fragments into proper vectors, followed by transformation of recombinant vectors into *E. coli* cells. Both initial cloning steps are performed in standard 96-well plates (you may find a detailed protocol of the System-48 procedure in the paper cited above). Instead of specially constructed cloning grills or plates, however, Abdullah and his colleagues use 48-grid agar plates in the following plating step.

First of all, the bottom half of two empty 48-grid agar plates is labelled to map the 96 samples of the transformation plate. Subsequently, LB/Amp agar is poured into the wells of the 48-grid plates with the help of automatic liquid handlers or multichannel pipettes. To be able to spread the transformation solution inside the individual wells of the prepared grid agar plates, Collart's team employs a smart trick. They use sterile tweezers to place a sterilised 3.18 centimetre (1.25 inch) long glass rod roller into each grid location. After pipetting an aliquot (50 to 100 μ l) of the transformation plate solution into each corresponding grid location on the 48-grid agar plate, the mixture is spread by one to two rolls of the glass rod using a pipette tip to manipulate the rod. The glass rod rollers are removed before incubation by inverting the covered plate and tapping the bottom to release the rod onto the inside of the lid (the rods may be washed for further use). After placing a new cover on the plate, it is incubated overnight in inverted (bottom side up) orientation at 37° C.

In the next step, the selected colonies are transferred to four 48-deepwell plates that have been labelled to correspond to the two colonies selected from the 48-grid agar plates. The inoculation is done by scraping part of a selected colony with a pipette tip from the grid plate and dipping it into the corresponding deepwell filled with 2 ml of LB broth. To induce protein expression, IPTG is added to the wells using a liquid



Basic workflow of System-48.

handler or a multichannel pipette and, after incubation at 37° C for two hours, an aliquot from each of the four deepwell plates is pipetted into the corresponding wells of two 96-microwell plates.

At this point we are done with the better part of the System-48 procedure and the remaining work is routine. For analysis of total expressed proteins an aliquot from each sample of the four 48-deepwell plates is transferred to two corresponding 96-deepwell plates. The latter are centrifuged, supernatants are discarded and the pellets are resuspended in SDS-PAGE buffer and loaded onto SDS-PAGE gels. The remaining cultures in the four 48-deepwell plates may be used in a slightly modified protocol to prepare a cell lysate for analysis of soluble protein.

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Do you have any useful tips?

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