Transformation of Bacteria and Spreading onto 24-well Agar Deepwell Plates with Eppendorf epMotion® 5075

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Abstract
Transformation of bacteria with DNA and spreading onto agar deepwell plates has been automated using the Eppendorf epMotion® 5075. After overnight incubation, positive colonies could be observed.
The described method is very effective and can be fully automated. It provides significant advantages for all steps of the process of distributing DNA in small aliquots onto agar plates.
The method is inexpensive and simple, as we can routinely generate 24-well agar deepwell plates to obtain desired colonies.

Introduction
Many experiments can today be automated; however, methods like DNA cloning or mutagenesis, where spreading of bacteria is needed, are still quite difficult to automate, in particular the transformation and spreading steps.
For DNA cloning, some steps like PCR product purification or DNA digestion can be automated. However, some steps are still critical.
In this Application Note, we show that casting selective media, transforming bacteria with DNA and spreading on selective media are possible with an automated pipetting system like the epMotion 5075. This system can work contamination-free as described in a recent Userguide [1]. In addition, cell-seeding methods have been described in an Application Note [2] recently, but not the combination of cell seeding, transformation and spreading of bacteria as we demonstrate it here.
The presence of 2 thermomodules on epMotion 5075 allows the transfer of selective LB agar into deepwell plates. Once the solid growth medium is prepared, the transformation reaction can be done on the epMotion 5075. Finally, in this program, the epMotion 5075 can transfer the transformation products to the agar deepwell plates. This way, we could obtain isolated colonies on a 24-well holder. In this Application Note we present a comprehensive solution for complete lab automation.
Materials and Methods

**epMotion 5075 LH or epMotion 5075 TMX** equipped with:
- Dispensing tools TS 1000 and TM 1000-8
- Reservoir rack
- Eppendorf Consumables
- epTIPS® Motion Filtertips 1,000 µl
- 30 ml Reservoirs
- Eppendorf Deepwell plates 96/2,000 µl

Consumables and reagents from other vendors:
- 24-well Deepwell plate, 10 ml
- LB agar
- Suitable Antibiotics
- Bacteria strains (MH1, DH5α, XL1blue or Top10)
- DNA for cloning

**Cell seeding with epMotion**
Set thermomodule 1 at 4 °C and thermomodule 2 at 55 °C, wait for temperature to be reached and add LB agar with appropriate antibiotics into tub 300 ml. 750 µl of LB agar with antibiotics are transferred from tub 300 ml to the 24-well deepwell plate using an 8-channel tool TM-1000-8 (Reagent Transfer). As the labware indicates “deepwell 96 plate”, the number of samples is four over each well and every well receives four times 750 µl, i.e. 3 ml per well. Working with an indicated 96 well plate has the advantage to work with 8-channel dispensing tools instead of single-channel dispensing tools. We can also use two different selective media in a 24-well deepwell plate without a 4 °C position. In this case, solidification just takes longer.

<table>
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<th>Worktable for Cell seeding without Transformation</th>
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</table>

Table 1: Detailed listing of the labware required for the casting of 24-well deepwell plate.

**Transformation of bacteria with DNA and spreading on selective media**
If we run the casting and transformation steps in the same program, we have to move the LB agar plate (thermomodule position 1) and Tub 300 ml (thermomodule position 2) onto other positions. The initial worktable of cast selective media program must be completed.

Keep thermomodule at position 1 at 4 °C and modify temperature of thermomodule 2 to 42 °C.
1 to 2 µl of DNA are transferred from thermorack with 1.5 ml tubes to deepwell plate 96, using a single-channel dispensing tool 50 µl, changing tips before next aspiration (Sample Transfer). Add 50 µl of competent cells from thermorack to deepwell plate, using a single-channel dispensing tool 1000 µl (Reagent Transfer), mix before aspirating and then dispense from top.
Wait 20 minutes at 4 °C and move the transformation plate from thermomodule 1 to thermomodule 2 (42 °C) (Gripper or manually) for 90 seconds and go back to thermomodule 1 for 2 minutes, set up the temperature of thermomodule 2 to 37 °C. 1 ml of regenerated media is transferred from reservoir rack to transformation plate with a Reagent Transfer using a single- or multi-channel tool (TS or TM 1000), according to the number of samples. Move the transformation plate from thermomodule 1 to thermomodule 2 (37 °C) for 1 hour. Then, use a Sample Transfer to dispense from top 100 µl of each transformation sample to LB agar deepwell plate. Please note that 24-well deepwell plate is indicated by a deepwell 96 in the labware.

At the end of method, cover transformation plate with a breath sealer (Greiner bio-one) and incubate over night at 37°C.

**Results and Discussion**

Successful transformation of bacteria is shown in Fig. 3. In the described method, all three steps, i.e., cell seeding, transformation and spreading of bacteria, have been fully automated on the epMotion 5075. The epMotion 5075 with integrated thermomodules allows to perform all steps in one run.
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References


Operation Manual for epMotion 5075

Ordering Information Eppendorf

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