Applications

Note 217 | October 2009

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

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Abstract

Transformation of bacteria with DNA and spreading onto agar deepwell plates has been automated using the Eppendorf ep*Motion*® 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 ep*Motion* 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 ep*Motion* 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 ep*Motion* 5075. Finally, in this program, the ep*Motion* 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.

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Materials and Methods

ep*Motion* 5075 LH or ep*Motion* 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 ep*Motion*
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.

Worktable for Cell seeding without Transformation		
Position	**Labware**	**Comment**
A3	TIPS 1000	
Temp1	DWP96	MTP24_DWP_10mL
Temp2	Tub	Tub 300mL LB agar with antibiotic

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

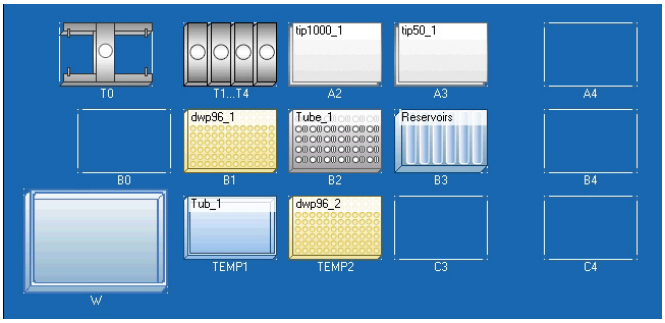

Figure 1: Worktable of epMotion 5075 LH for Cell seeding and DNA transformation

Position	Labware	Comment
A2	*TIPS 1000*	
Temp1	*DWP96*	*MTP24_DWP_10mL*
Temp2	*Tub*	*Reserve 300mL*
A3	TIPS 50	
B1	DWP96	Transformation plate
B2	Rack 1.5mL Thermorack	pre cooled it at 4°C before using : competent cells and DNA
B3	Reservoir rack	Regenerated media (LB or 2YT)

Table 2: Detailed listing of the labware required for the transformation and spreading on 24-well deepwell agar plates.

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 position1) 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.

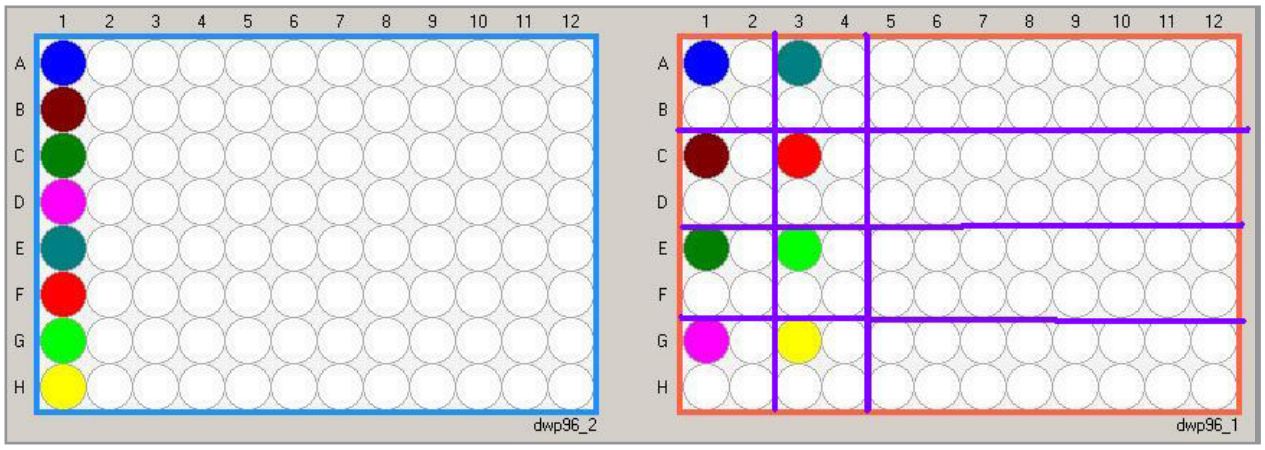

Fig. 2: Example of loading pattern for transformation from thermomodule to LB agar plate.

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 ep*Motion* 5075. The ep*Motion* 5075 with integrated thermomodules allows to perform all steps in one run.

Fig. 3: Results of transformation experiments.
Top: 24-deepwell plate partially filled with agar.
Bottom: Transformed bacteria were spreaded on 24-well deepwell plate and colonies could be observed after overnight incubation (blue circle).

References

[1] Userguide 29: Contamination-free handling of cell cultures. www.eppendorf.com
[2] Application Note 205: Automated Cell seeding in 96-well cell culture plates using epMotion. www.eppendorf.com
Operation Manual for ep*Motion* 5075

Ordering Information Eppendorf

Product	Order no. international	Order no. North America
ep*Motion*® 5075 LH	5075 000.008	960020006
Dispensing tool TM 1000-8	5280 000.258	960001061
Dispensing tool TS 1000	5280 000.053	960001036
Reservoir rack	5075 754.002	960002148
epMotion Reservoir 30 ml	0030 126.505	960051009
Eppendorf Deepwell Plate 96/2,000 µl	0030 501.306	951033405
epTIPS® Motion 1000 µl, Filter	0030 003.993	960050100

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