Applications

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Automated seeding of HeLa cells in various cell densities in 24-well cell culture plates using the ep*Motion*® 5070 CB

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Abstract

To date, very few automated processes are available which would facilitate the labor-intensive manual steps involved in cell culture work. With the ep*Motion*® 5070 CB, seeding of HeLa cells in various cell densities into a 24-well cell culture plate via serial dilution steps is now possible, thus providing an opportunity to automate certain aspects of cell culture.

Introduction

Despite increasing significance of cell culture in the pharmaceutical and biotechnology industries, as well as in basic research, there are few possibilities to perform simple or more complex work in an automated fashion. For these cell culture applications a sterile environment is critical. To meet this requirement, the ep*Motion* 5070 CB was developed specifically for use in the sterile laminar flow hood, hence enabling the automation of diverse work processes under sterile conditions.

Materials and Methods

Eppendorf ep*Motion* 5070 CB
Reservoir Rack
Reservoirs, 100 ml
Height adapter 85 mm (optional)
24-well cell culture plates
epT.I.P.S Motion, 1000 µl
HeLa cells
Medium (RPMI 1640) + 10% FCS (fetal calf serum)
Trypsin
PBS (phosphate buffered saline)
Neubauer hemocytometer
Cell culture flasks, 75 cm2

HeLa cells grown in a cell culture flask (75 cm^2) were detached with trypsin, washed in PBS and resuspended in fresh medium. The number of HeLa cells was determined using a Neubauer hemocytometer and the cell concentration was adjusted to 1 million cells/ml. Fresh medium and the cell suspension were each filled into a 100 ml reservoir, respectively, and placed into the reservoir rack alongside a third, empty, 100 ml reservoir. Alternatively, 50 or 15 ml falcon tubes may be used. However, in this case the automatic liquid level detection function is not available [1].

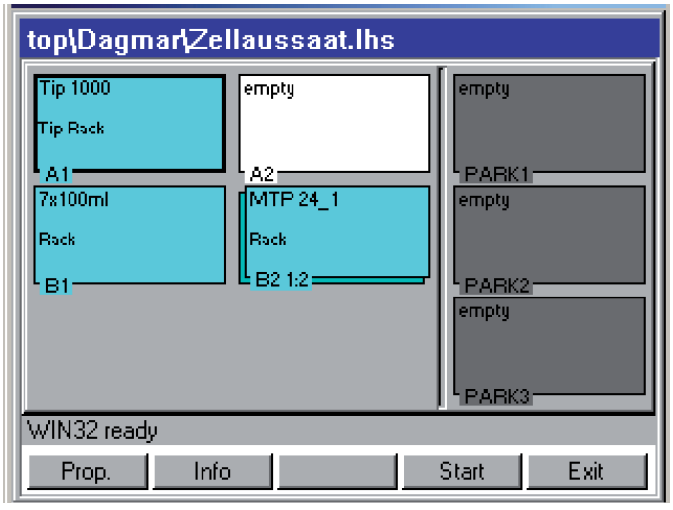

Fig. 1: Screenshot of the Control Panel of the ep*Motion* 5070 CB showing the placement of lab ware for the method “Cell Seeding” in 24-well cell culture plates.

The first row of the 24-well cell culture plate was to be seeded with 50,000 cells per well. Subsequently, for each well a serial dilution of half the previous cell number was to be prepared. To this end, 1 ml of fresh medium was placed into each of the 24-wells using a “Reagent Transfer“ [1]. The liquid class “protein” was chosen. The cells were seeded as follows: 100 µl each of the cell suspension was transferred into the first 6 wells of the plate, also using a “Reagent Transfer“ [1]. Subsequently the well volume was filled to a total of 2 ml (using a “Reagent Transfer”). The dilutions were subsequently performed using the command “Dilute“ [1].

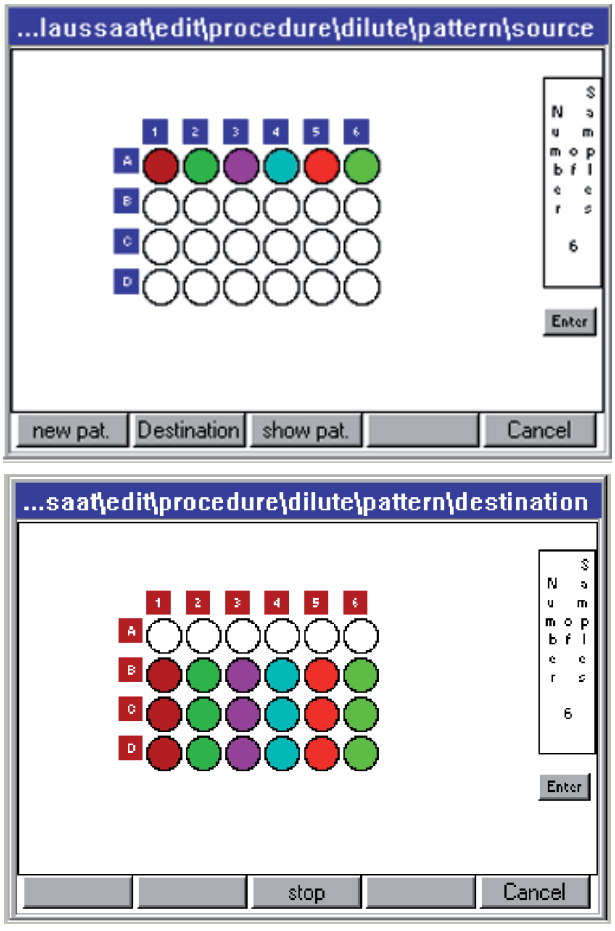

Fig. 2: Screenshots of the Control Panel of the ep*Motion* 5070 CB showing the pattern of the “Dilute” command [1]. The wells in the first row are the source (A). From these, 1 ml of cell suspension is removed and transferred into the wells below, from which yet another 1 ml is removed and distributed further, etc. This way each column of this plate contains one serial dilution. This results in the “Destination Position” pattern for the “Dilute” command, as illustrated above [1] (B).

1 ml each was used for the serial dilution, where mixing occurred prior to aspiration as well as following dispensing of the cell suspension. Mixing parameters were chosen as follows: 2 cycles of 5 mm/s. Therefore the last row of the plate contained 2 ml of cell suspension. Whereas the number of cells per ml contained in each well of this last row was one half of the previous row, the total number of cells per well is the critical value, and thus one half of the cell suspension had to be removed from the wells in the last row. This was achieved with the command “PoolOneDest“ [1]. The empty 100 ml reservoir was used for waste disposal.

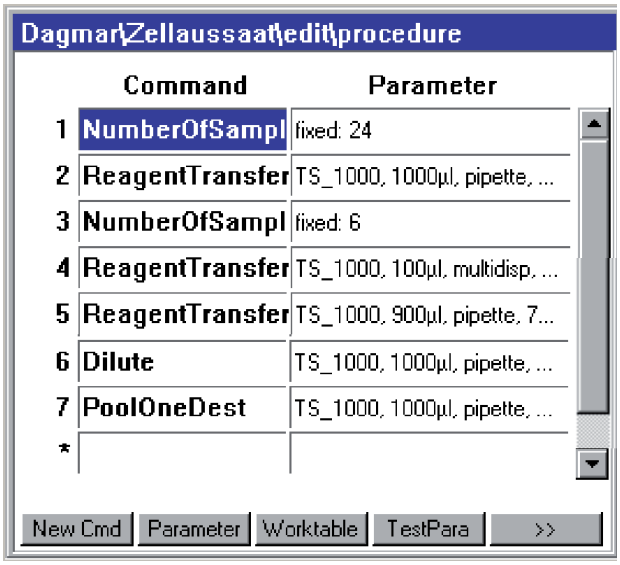

Fig. 3: Screenshot of the Control Panel of the ep*Motion* 5070 CB listing the commands required for the method “Cell Seeding in a 24-well Cell Culture Plate”.

The cells were subsequently incubated for 24 h at 37 °C in a 5 % carbon dioxide atmosphere with 100 % humidity.

Result

The results (Figure 4) show that cells can be seeded using the ep*Motion* 5070 CB in an automatic fashion. The HeLa cells were seeded into 24-well cell culture plates in different cell densities.
Cell density was determined using the Neubauer hemocytometer prior to begin of each procedure.

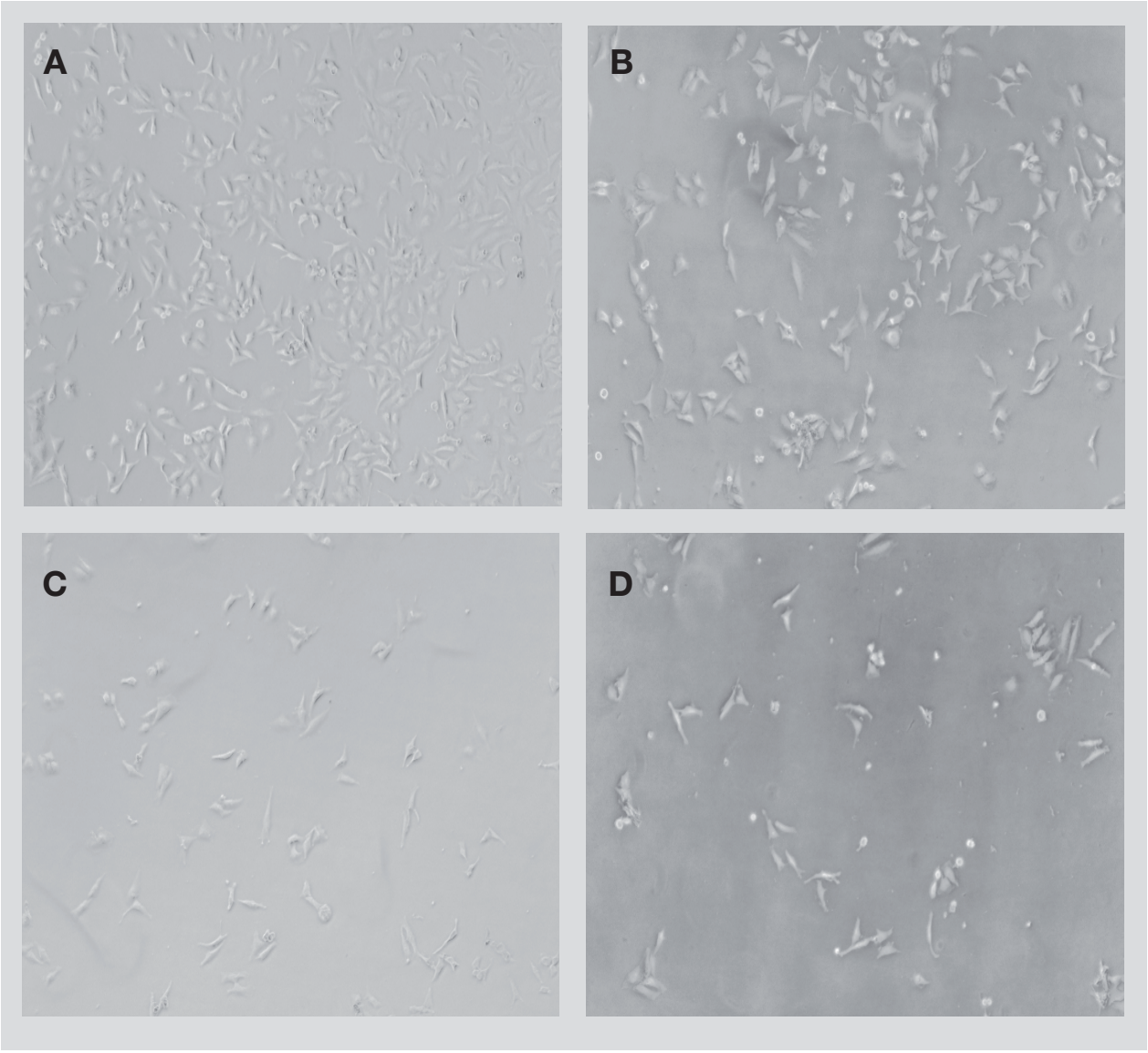

Fig. 4: HeLa cells in a 24-well cell culture plate one day after automatic seeding using the ep*Motion* 5070 CB. Cell densities seeded were (A) 50,000 cells/ml, (B) 25,000 cells/ml, (C) 12,500 cells/ml, (D) 6,250 cells/ml. The cells shown were photographed with 5x magnification.

Conclusions

The ep*Motion* 5070 CB is suited for automatic cell seeding of adherent cells. In combination with automated medium change [2] this system offers a comfortable setup for the cultivation of adherent cells in 24-well cell culture plates.

References

[1] Eppendorf manual for ep*Motion* 5070 CB
[2] Eppendorf Application Note 185

Ordering Information

Product	Order no. International	Order no. North America
ep*Motion* 5070® CB	5070 000.700	960000021
Single channel dispensing tool TS_1000	5280 000.053	960001036
Reservoir Rack	5075 754.002	960002148
ep*Motion* Reservoir 100 ml, set of 50 each	0030 126.513	960051017
Height adapter 85mm (optional)	5075 751.003	960002105

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