



Challenge

Automation of bacterial transformation processes in molecular biology laboratories

Solution

Fully automated method for the high-throughput transformation and growth of bacteria cells using the CyBio FeliX

Automated High-Throughput Transformation of Bacteria Cells

Lorna Suckling¹, David McClymont¹, Chris Hirst²

¹London DNA Foundry, SynbiCITE, ²Analytik Jena

Abstract

The transformation of bacteria cells typically remains a manual, low-throughput activity in molecular biology laboratories. The ability to transform bacteria transformation in a high-throughput, automated manner would greatly aid laboratories looking to increase their throughput and improve the robustness and reproducibility of their experiments.

SynbiCITE, in collaboration with Analytik Jena, have used the CyBio FeliX pipetting platform to develop a fully automated method for the high-throughput transformation and growth of bacteria cells. This method is used routinely in SynbiCITE's London DNA Foundry and is described in detail here.

Introduction

Bacterial transformation is the process by which foreign plasmid DNA is introduced into a bacteria cell. The transformation of DNA into bacteria cells, such as *Escherichia coli* (*E. coli*), is an essential molecular biology technique used for both the study of bacteria itself and also for the storage and/or replication of plasmids. To enable bacterial transformation, DNA plasmids are typically designed to contain a bacterial origin of replication (ORI) and an antibiotic resistance gene, for use as a selection marker.

One simple method for the transformation of plasmid DNA into bacteria cells is to use heat shock. Plasmid DNA is added to competent bacteria cells and incubated in the cold. The cells are then heated to a high temperature for a short amount of time before being returned to the cold. This 'heat shock' enables the transfer of the plasmid DNA inside the bacteria cell. After recovery in media, cells containing the plasmid DNA of interest are ready to be grown, typically on solid agar. Through the addition of antibiotics to the agar, antibiotic selection can be used to select for those cells which have been successfully transformed with DNA.

The transformation of bacteria cells typically remains a manual, low-throughput activity in molecular biology laboratories. The ability to transform bacteria transformation in a high-throughput, automated manner would greatly aid laboratories looking to increase their throughput and improve the robustness and reproducibility of their experiments. The CyBio FeliX offers a compact pipetting platform for performing the liquid handling steps required for a high-throughput transformation method. When used on an automation platform alongside a thermal cycler, a heating/cooling block, a shaking microplate-compatible incubator, and a robotic arm the transformation method can be fully automated (Figure 1). The use of the CyBio FeliX has enabled SynbiCITE to develop a fully automated, multi-well plate based method for the simultaneous transformation of 96 DNA plasmids into bacteria cells by heat shock, and the subsequent plating and growth of these transformed cells on solid agar. The method can also be easily implemented to process multiple plates at a time with the use of additional heating/cooling blocks.

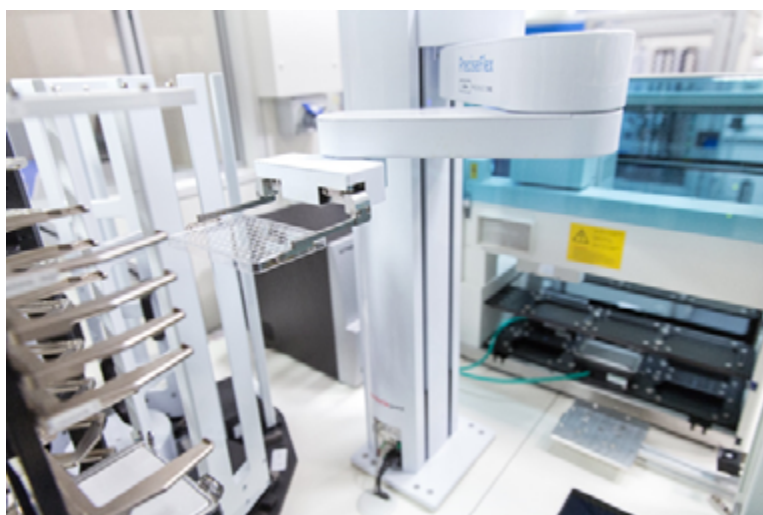


Figure 1: Automation platform, including a CyBio FeliX pipetting platform, in SynbiCITE's London DNA Foundry laboratory at Imperial College London.

Materials and Methods

Reagents and Instrumentation

Reagent	Manufacturer	Part number
Super Optimal broth with Catabolite repression (SOC)	Formedium	SOC0202
Lysogeny broth (LB) agar	Merck	1102850500

Table 1: Reagents required for method

Instrument	Manufacturer
CyBio FeliX	Analytik Jena
Pipetting Head R 96/250 μ L	Analytik Jena
CyBio RoboTipTray 96-250 μ L DW	Analytik Jena
TRobot Thermocycler	Analytik Jena
CPAC Ultraflat heating/cooling block	Inheco
CoolRack® XT PCR96	BioCision
StoreX shaking incubator	LiCONiC Instruments
PreciseFlex Robot	Precise Automation

Table 2: Instrumentation used in method

Consumables	Manufacturer	Part number
96-well, F-bottom plate	Greiner	781201
96-well, PCR plate	4titude	4ti-0960
384-well, PCR plate	4titude	4ti-0384
OmniTray	Nunc	242811

Table 3: Consumables used in method

Sample preparation

- Prepare a 384-well PCR plate containing plasmid DNA samples (>10 $\mu\text{L}/\text{well}$). Ensure a positive control plasmid is included which contains the appropriate antibiotic resistance gene(s). N.b. DNA will be transferred from the 384-well source PCR plate to a 96-well destination plate, therefore samples should be added to the 384-well plate accordingly (Figure 2)
- Plate 20 $\mu\text{L}/\text{well}$ competent *E. coli* cells in a hard shell 96-well, PCR plate. Store at -80°C prior to use
- Prepare Lysogeny broth (LB) solid agar, containing the appropriate antibiotics for selection, and set in an OmniTray, 30 mL/plate

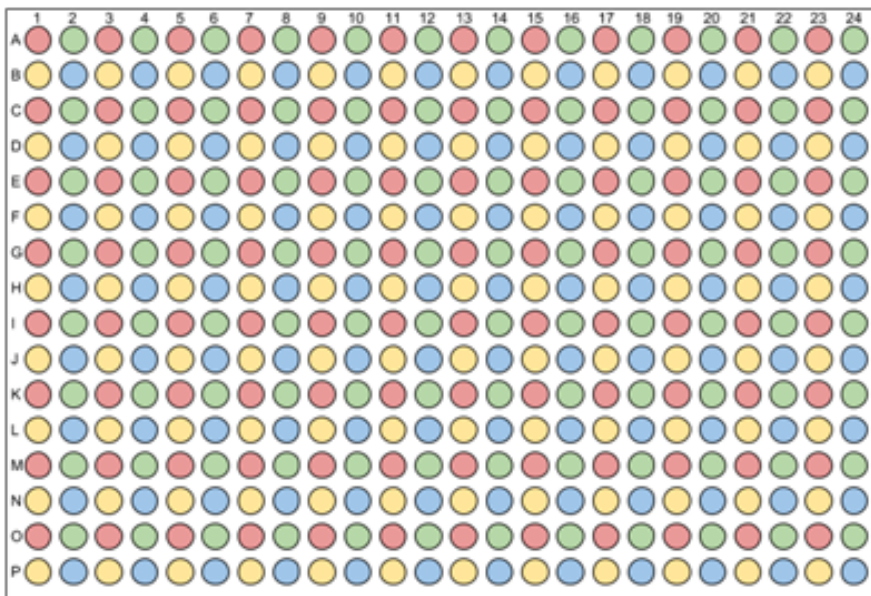


Figure 2: 384-well PCR DNA source plate. DNA will be transferred from a 384-well PCR source plate to a 96-well destination plate. One 384-well PCR DNA source plate can be used to store DNA for up to four 96-well transformations. To enable transfer to a 96-well plate, DNA should be added to the 384-well plate in quadrants: red = quadrant A1; green = quadrant A2; yellow = quadrant B1; blue = quadrant B2.

Method

- Warm a 96-well, flat-bottom plate, containing 120 $\mu\text{L}/\text{well}$ Super Optimal broth with Catabolite repression (SOC), to 37°C
- Add 2 x CyBio RoboTipTray 96-250 μL DW, to the deck of the CyBio Felix (Figure 3)
- Add the 384-well plasmid DNA source plate to the deck of the CyBio Felix (Figure 3)
- Remove a 96-well PCR plate containing 20 $\mu\text{L}/\text{well}$ competent *E. coli* cells from -80°C and place immediately onto a CoolRack[®] XT PCR96, which has been cooled to 4°C
- Put the CoolRack[®] (with the cell plate) on to the heating/cooling block set to 4°C and thaw cells for 10 minutes
Important: ensure there is no moisture on the surface of the heating/cooling block and/or the base of the CoolRack[®], prior to use. Any moisture will prevent robot from being able to pick up CoolRack[®] in subsequent steps
- Using the CyBio Felix, aspirate 5 $\mu\text{L}/\text{well}$ plasmid DNA from the source plate and hold the volume in the tips
- Transfer the cell plate, still in the CoolRack[®], from the heating/cooling block to the deck of the CyBio Felix robot (Figure 3) and immediately dispense the 5 μL plasmid DNA
- Quickly return the CoolRack[®] (with the cell plate) to the heating/cooling block, set at 4°C

- Incubate the cells, with DNA, for 30 minutes at 4 °C
- Heat shock the cells for 45 seconds at 43 °C using a thermal cycler. N.b temperature and time should be optimized independently for each automation platform
- Immediately replace the cells on the CoolRack® and incubate for 2 minutes at 4 °C
- Transfer the cells to the deck of the CyBio FeliX (Figure 3)
- Transfer the warmed 96-well, F-bottom plate containing SOC to the deck of the CyBio FeliX (Figure 3)
- Aspirate 80 µL/well SOC and add to the cell plate
- Resuspend the cells in the warmed SOC by mixing once
- Transfer the entire volume of SOC/cells to the 96-well, F-bottom SOC plate
- Add a lid to the 96-well, F-bottom SOC/cells plate and incubate at 37 °C, for 60 minutes, shaking
- During the incubation, warm the OmniTray plate, containing the solid LB agar, to 37 °C, without a lid, to dry the agar prior to the plating of the cells. N.b. ensure the incubator has low humidity to allow for drying, drying time will vary depending on incubator but may take the full 60 minutes
- Return the 96-well plate, containing the SOC recovered cells, and the LB agar plate from the incubator to the deck of the CyBio FeliX (Figure 3)
- Using a new set of tips, mix the SOC cell samples and aspirate 3 µL/well
- Dispense the cell solution onto the surface of the LB agar. N.b. set the height so that the tips make contact with the agar surface but do not penetrate
- Allow the samples to dry on the surface of the agar (approximately 2–3 minutes)
- Repeat the transfer of the samples to the surface of the LB agar as many times as required, leaving sufficient drying time between 'spotting' events
N.b. The CyBio FeliX method can be set up to enable these parameters to be specified by the user at the beginning of the protocol
- Add lids to the LB agar plate and incubate overnight in a humidified incubator at 37 °C, to allow for colony growth.
N.b. if required, add a reservoir containing H₂O to the incubator to maintain humidity and prevent splitting of agar

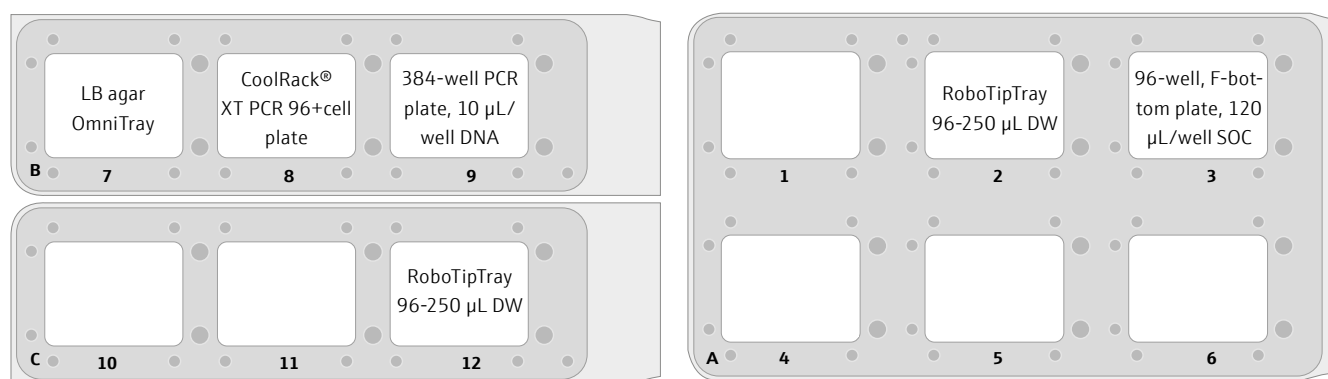


Figure 3: CyBio FeliX deck layout for the automated transformation method. Here we show an example of the FeliX deck layout, as used in the London DNA Foundry. After the DNA addition step, the 384-well PCR plate containing the DNA is transferred to an accessible position on the automation platform, allowing for the safe removal of the plate from the platform and its subsequent storage for potential future use. After the transfer of the cells from the 96-well PCR plate to the 96-well, F-bottom SOC plate the CoolRack® (with cell plate) can be removed from the FeliX deck to free up position 8. The empty spaces (blank) represent available positions which allows for the simple expansion of the method to process multiple DNA/cell/LB agar plates at a time.

Results and Discussion

The transformation efficiency of *E. coli* cells was found to be highly sensitive to the temperature of the thermal cycler, used for the heat shock. The optimal temperature will likely vary between different machines and laboratories so should be determined independently by any group using this method. Using the TRobot Thermocycler in the London DNA Foundry, a heat shock for 45 seconds at 43 °C gave the optimal transformation of DNA into *E. coli* cells. For lower concentrations of plasmid DNA, the transformation efficiency was significantly better at 43 °C than when cells were heated to 42 °C for the same amount of time (Figure 4).

We demonstrate the importance of keeping the competent *E. coli* cells at 4 °C at all times after removing them from the -80 °C freezer, prior to the heat shock step. Removing the cells from 4 °C for less than 2 minutes for the DNA addition step causes a significant reduction in the transformation efficiency of the cells (Figure 5). To keep the cells at 4 °C during the automated addition of DNA on the CyBio FeliX, we chose to use a CoolRack® XT PCR96 (BioCision). The CoolRack® is first cooled to 4 °C in the fridge, prior to use. Competent *E. coli* cells are immediately transferred to the CoolRack® on removal from -80 °C and the CoolRack® temperature is maintained at 4 °C by placing it on a heating/cooling block, integrated on the automation platform and set to 4 °C. The CoolRack®, with the cell plate on top, is transferred to the deck of the CyBio FeliX for the addition of the DNA, before being immediately replaced on the heating/cooling block. This maintains the cells at a low enough temperature for a good transformation efficiency. In order to minimize the time the CoolRack® and cells are off the heating/cooling block, the CyBio FeliX is programmed to aspirate the DNA before the robot moves the CoolRack® and cells from the heating/cooling block to the deck of the CyBio FeliX.

The efficiency of this automated transformation method is sufficient for use with plasmids with a low copy number, such as those with the p15a ORI (Figure 4). However, the use of a plasmid vector containing an ORI with a high copy number, such as pUC19, gives rise to a greater number of colony forming units, as compared to a plasmid with a lower copy number, such as pBBR1 (Figure 6) or p15a (Figure 4). The plasmid copy number should therefore be taken into account when determining the concentration of DNA to transform into cells and also when deciding the amount of transformed sample to plate onto solid agar for the overnight growth step. This method can also be used to effectively transform plasmid DNA into different strains of competent *E. coli* cells, which are amenable to heat shock transformation (Figure 7).

The use of transformation controls is recommended when running this method. As a positive control, a plasmid DNA with the same antibiotic resistance gene as the test plasmids should be used. Replicates are recommended to monitor the variability of the transformation efficiency between wells (Figure 8). As a negative control, a number of wells of the cell plate should have no DNA added. When these cells are grown on solid agar containing antibiotics, there should be no bacterial growth in these positions (Figure 8). The automated method described here, makes use of the CyBio FeliX robot to enable the simultaneous transformation of 96 different DNA plasmids into *E. coli* cells by heat shock and their subsequent plating onto agar. The result is an agar plate containing bacterial colonies grown in specific distinct regions, according to a standard 96-well reference, from which individual clones can be picked and grown for further analysis (Figure 8).

The method described here is for the full automation of bacterial transformation and colony growth. However, the protocol can be easily adapted for use on a standalone CyBio FeliX pipetting platform. In combination with the manual completion of some steps, the use of a standalone CyBio FeliX would still provide a high-throughput and robust method for bacterial transformation and cell plating, in laboratories where a fully automated platform is not available.

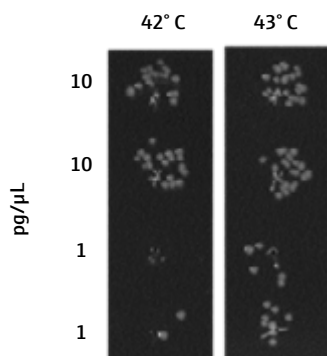


Figure 4: Temperature of the Biometra TRobot thermocycler for the heat shock step. The optimal temperature for the heat shock of bacterial cells will be dependent on the equipment being used and should be optimized for each laboratory independently. A difference in 1° C can have a great effect on transformation efficiency, as seen here where cells were either heated to 42° C or 43° C. In the London DNA Foundry, 43° C was the optimal temperature for a 45 second heat shock, showing greater transformation efficiency of lower concentrations of DNA (1 pg/μL), as compared to 42° C. Data is shown for DH5α *E. coli* cells transformed with a plasmid containing Chloramphenicol and Kanamycin resistance genes (p15a ORI).

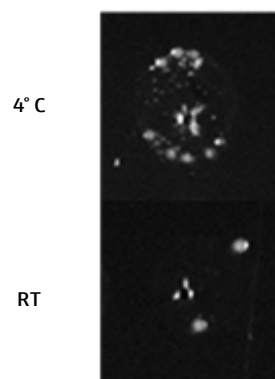


Figure 5: Temperature of cells for DNA addition step. It is very important for competent *E. coli* cells to be kept at 4° C at all times prior to the heat shock step. Shown here are DH5α cells which were either maintained at 4° C or moved temporarily to the CyBio FeliX deck at room temperature (RT; <2 minutes), for the DNA addition step. The transformation efficiency becomes very low when the DNA addition is carried out at room temperature. Therefore, during the DNA addition step on the CyBio FeliX, *E. coli* cells must be kept at 4° C. This can be achieved by placing the cell plate on a CoolRack® XT PCR96, cooled to 4° C, and transferring the CoolRack® (containing the cell plate) to the CyBio FeliX deck for the addition of the DNA.

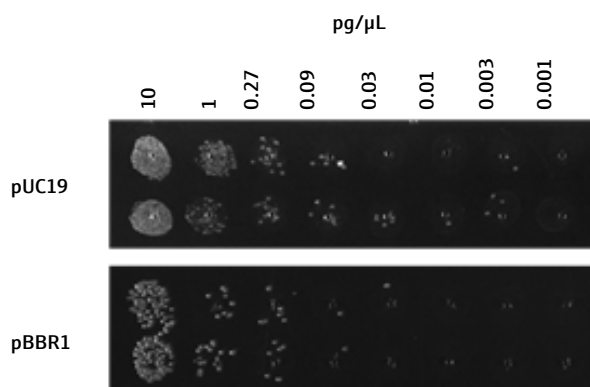


Figure 6: Transformation of *E. coli* cells with plasmids of different copy number. As expected, the copy number of a plasmid affects the number of colony forming units observed after overnight growth. A higher copy number plasmid, pUC19, gives rise to a larger number of colonies as compared to a medium copy number plasmid, pBBR1, when the same concentration of DNA is added to the cells.

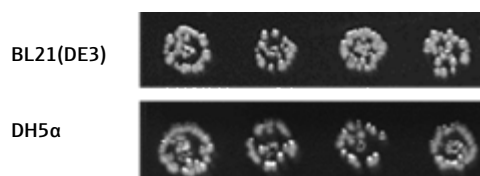


Figure 7: Different *E. coli* strains transformed with a positive control plasmid. The transformation protocol can be used to effectively transform different strains of *E. coli* cells with plasmid DNA. Here, two *E. coli* strains, BL21(DE3) and DH5α, have been transformed with the same plasmid DNA, containing Chloramphenicol and Kanamycin resistance genes (p15a ORI).

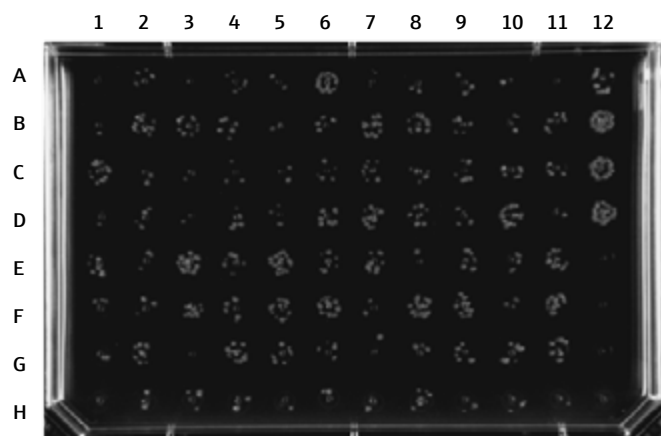


Figure 8: DH5α *E. coli* clones, grown in a 96-well layout on a single LB agar plate after an automated 96-well transformation with plasmid DNA. The automated transformation method can be used to transform 96 plasmid DNA samples simultaneously. Controls should be included in each transformation experiment. Here, column 12 is used for the controls: A-D = positive control plasmid (with appropriate antibiotic resistance genes); E-H = negative control (no DNA added).

Conclusion

The ability to perform high-throughput, automated bacterial transformations would greatly benefit laboratories wanting to increase their throughput and improve the robustness and reproducibility of their experiments. Here, we have developed a fully automated method for the transformation of plasmid DNA into *E. coli* cells by heat shock, using the CyBio FeliX robot pipetting platform. We demonstrate that this method can be used to effectively transform 96 DNA plasmids simultaneously into different *E. coli* strains and is suitable for plasmids with ORIs ranging from low- to high-copy number. The use of the compact CyBio FeliX as part of an integrated robotic platform provides an option for full automation of the method. Alternatively, the method used on a standalone CyBio FeliX pipetting platform, in combination with manual completion of some steps, would still provide a high-throughput and robust method for bacterial transformation and cell plating.

This document is true and correct at the time of publication; the information within is subject to change. Other documents may supersede this document, including technical modifications and corrections. Printout and further use permitted with reference to the source.

Acknowledgements

In cooperation with SynbiCITE



Headquarters

Analytik Jena GmbH
Konrad-Zuse-Strasse 1
07745 Jena - Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

Version: 1.2 | Author: Lorna Suckling
en - 12/2020

© Analytik Jena GmbH | Pictures: Analytik Jena GmbH