Applications

Note 219 | October 2009

Eppendorf Eporator®: Efficient transformation of the soil bacterium *Agrobacterium tumefaciens*

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Abstract

The Eppendorf Eporator® achieves efficient transformation of *Agrobacterium tumefaciens*. When using electro-competent cells, the Eporator yields reproducibly high transformation rates.

Introduction

Agrobacterium tumefaciens mediated transformation is a routine method used to introduce foreign genes into plants. The soil bacterium *Agrobacterium tumefaciens* is able to infect dicotyledons via wounds and generate the well known tuberous tumors (galls, see figure 1).

Figure 1: Crown gall on euonymus stems.
This picture was kindly provided by Simeon Wright, Plant Diagnostic Lab Coordinator, UMC, Columbia, USA.

The information for tumor growth lies on the ti plasmid (tumor-inducing plasmid). This t-DNA (transferred-DNA) may be transferred into the cell during an infection, and it subsequently integrates into the plant genome in a random fashion [1]. Gene technology takes advantage of the possibilities of this naturally occurring gene transfer, since transformation also occurs when the tumor gene has been replaced with foreign DNA. We have demonstrated earlier that the Eppendorf Electroporator 2510 is ideally suited for transformation of this bacterium with plasmid DNA [2].

This Application Note will show that the transformation efficiency (TE) of *A. tumefaciens* using the Eppendorf Eporator®, the successor model to the renowned Eppendorf Electroporator 2510, is not only comparable, but even higher in a parallel experiment.

For optimum comparability, commercially available competent cells and vector were used in strict adherence to the manufacturer's instructions.

Materials and Methods

Materials

Media and bacteria

pBI121 DNA (1 ng/µl) (ElectroMAX™ Kit, Invitrogen, Karlsruhe, Germany)

ElectroMAX™ LBA4404 electrocompetent cells *A. tumefaciens* (Invitrogen)

YM Medium: yeast extract 0.04 %, mannitol 1.0 %, 1.7 mM NaCl, 0.8 mM $MgSO_4$x $7H_2O$, 2.2 mM K_2HPO_4x $3H_2O$, pH 7, autoclaved

100 µg/ml streptomycin and 50 µg/ml kanamycin were added to the YM Medium for agar plates.

Instruments and consumables

Eppendorf Eporator®
Eppendorf Electroporator 2510
Electroporation cuvettes, gap width 1 mm, 100 µl, sterile
Eppendorf Thermomixer® comfort
Eppendorf Biopur® Safe-Lock reaction tubes 0.5 ml
Incubator Heraeus type VT 5042 EK (Heraeus, Hanau, Germany)
Falcon® tubes 15 ml (Becton Dickinson, Heidelberg, Germany)

Methods

Electroporation using the Eppendorf Eporator and the Electroporator 2510

The electrocompetent *A. tumefaciens* were thawed on ice. 20 µl cell suspension from one aliquot were mixed with 1 µl DNA (1 ng/µl), respectively, transferred to a pre-cooled Eppendorf cuvette with a gap width of 1 mm, and electroporation was performed in parallel in both instruments at 1440 V. The Eppendorf electroporation instruments for bacteria perform an exponentially declining pulse with a defined time constant of 5 ms. Immediately following the pulse, 1 ml YM medium (room temperature) was added directly to the cuvette, and the thus diluted cells were transferred to a 15 ml Falcon tube. For recovering, the cells were subsequently incubated for 3 hours at 30 °C and 300 rpm in the Thermomixer comfort. The transformed cells were then diluted 1:10 in YM medium, and 100, 200 and 300 µl were plated on pre-warmed selection plates. The plates were incubated for 56 hours at 30 °C in the incubator. The colonies grown were counted, and the transformation rate (TE) was calculated.

	Eppendorf Eporator		Electroporator 2510	
TE	1	2	1	2
100 µl	2.500.000	2.900.000	2.000.000	2.000.000
200 µl	2.600.000	3.800.000	1.700.000	1.700.000
300 µl	3.400.000	3.100.000	1.700.000	2.400.000
Av	2.833.333	3.266.667	1.800.000	2.033.333
Av combined	**3.050.000**		**1.916.667**	

Table 1: Results from the individual experiments. Per reaction, three plates were plated with 100 µl, 200 µl or 300 µl bacterial suspension, respectively. The TE was derived from the number of colonies per microgram DNA.

Results and Discussion

All experiments were performed in duplicate, and the results are listed in table 1. The TE is defined as the number of colonies (transformants) per microgram of DNA used. The total amount of pBI121 DNA used was 1 ng. This was diluted 1:10 to a volume of 1 ml with YM medium, of which 100, 200 and 300 µl were plated. Thus, the equivalent of 0.01 ng, 0.02 ng, und 0.03 ng DNA was plated per plate. The results show that both experiments were able to yield a TE in the 10^6 range. These results are in accordance with the manufacturer's data. Furthermore, it could be demonstrated that both duplicate reactions performed in the optimized successor Eppendorf Eporator® yielded a TE which was approximately 50 % above the value achieved with the Electroporator 2510 (see figure 2). Since all experiments were performed with competent cells from the same aliquot, this dataset not only verifies the compatibility of Electroporator protocols with its successor, but it also shows that efficiencies may even be increased by using the Eppendorf Eporator®.

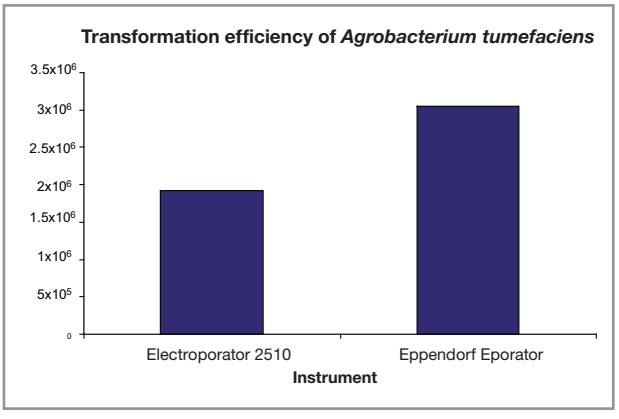

Fig. 2: Average values of transformation efficiencies following electroporation using the Eppendorf Eporator® and the Electroporator 2510, respectively.

References

[1] Savka MA, and Binns AN. Introduction of DNA into Plants. Gene Transfer Methods. Edited by Nortan PA and Steel LF Eaton Publishing, Natick, MA Publishing 2000; Chapter 8:159-192.

[2] Agrobacterium tumefaciens – transformation protocol for Electroporator/Multiporator, Protocol No. 4308 915.502 – 12/2001; www.eppendorf.com/literature

Ordering Information Eppendorf

Product	Description	Order no. international	Order no. North America
Eppendorf Eporator®	Electroporation instrument for transformation of bacteria, yeast and other microorganisms	4309 000.019	4309 000.027
Electroporation cuvettes Gap width 1 mm	Sterile, 50 each, total volume 100 µl	4307 000.569	940001005
Electroporation cuvettes Gap width 2 mm	Sterile, 50 each, total volume 400 µl	4307 000.593	940001013
Cuvette stand	For 16 electroporation cuvettes	4308 078.006	940001102
Thermomixer® comfort Thermomixer® R	Mixer with adjustable temperature range of 1 °C - 99 °C, without exchangeable thermoblock	5355 000.011	022670107
Exchange block for 15 ml Falcon tubes	for 8 times 15 ml Falcon tubes	5366 000.013	022670531

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