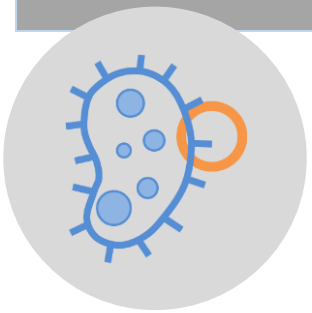

Transformation of plasmid-DNA

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Transformation of bacteria is a fundamental technique for introducing foreign DNA, typically in the form of plasmids. This process utilizes chemo- or electrocompetent cells, enabling the successful uptake of the foreign DNA. The transformed cells can then be employed for various applications, including recombinant protein expression or plasmid production.

For this protocol, chemo-competent *E. coli* cells are utilized for the transformation. It is crucial to select an *E. coli* strain that is compatible with the desired promoter when aiming to perform recombinant protein expression. This ensures optimal expression of the desired protein in the transformed cells.

If the objective is plasmid production, we recommend using the *E. coli* strain DH5alpha. This particular strain is well-suited for efficient plasmid replication and production.

Material	
Cell Strains	*E. coli* strain that is compatible with the promotor *E. coli* DH5α
10 x SOC (Super Optimal Broth)	0.2% KCl 2% $MgCl_2$ 2% $MgSO_4$ 4% Glucose
LB (Lysogeny Broth)	0.5% Yeast Extract 1% Tryptone 1% NaCl 1.5% Agar for LB Agar
Wash Buffer	100 mM RbCl 50 mM $MnCl_2$ 30 mM KOAc 10 mM $CaCl_2$ 15 % Glycerol
Storage Buffer	10 mM RbCl 75 mM $CaCl_2$ 10 mM MOPS 15 % Glycerol

- 2 - Method

Transformation

- Thaw an aliquot of competent *E. coli* cells on ice for 5 minutes
- Add 1 – 50 ng of DNA to the thawed cells
- Mix gently and incubate the mixture on ice for 30 minutes
- Transfer the tube containing the mixture into a 42°C water bath for 30 - 45 seconds
- Place the tube immediately back on ice and chill the cells for 2 minutes.

Cultivation

- Add 900 µl of 1x SOC media to the cell solution
- Incubate the cells at 37°C and 200 rpm for 60 minutes
- Transfer the cells onto an LB agar plate containing the desired selection antibiotic
  You may want to plate several agar plates with different volumes of the culture to achieve varying colony densities
- Incubate the agar plates at 37°C overnight

- 3 - Generation of chemo-competent *E.coli*

Sample Preparation

- Inoculate 5 – 15 ml of LB medium with your desired *E. coli* strain
  Add antibiotics to the medium if necessary
- Incubate the culture at 37°C overnight

Cultivation

- Inoculate 100 ml of LB media with 1 ml of your overnight culture
- Cultivate the cells at 37°C and 200 rpm until reaching an optical density at 600 nm (OD_{600}) of 0.4
- Chill the culture on ice for 10 minutes
- Centrifuge the chilled cells at 4,000 x g and 4°C
- Discard the supernatant

Washing

- Resuspend the cell pellet with 20 ml of Wash Buffer
- Incubate the cells on ice for 15 minutes
- Centrifuge the cold cells at 4,000 x g and 4°C
- Discard the supernatant

Storage

- Resuspend the cell pellet with 4 ml of Storage Buffer
- Incubate the cells on ice for 15 minutes
- Divide the cell solution into 100 µl aliquots
- Immediately shock freeze the cells using liquid nitrogen
  Alternatively, you can shock freeze the cells using a NaCl-Dry Ice mixture
- Store the cells at -80°C until ready for use

- 4 - Trouble Shooting

Reason	Solution
Overgrown Agar Plate	Using a smaller volume of transformed cells for the agar plate helps to prevent an excessive number of colonies and promotes better colony isolation. Reducing the incubation time of the agar plate to less than 16 hours prevents excessive growth and spreading of colonies. Higher antibiotic levels help ensure selective pressure and prevent the growth of non-transformed cells.
Colonies with Empty Vector	Different plasmid copy numbers, like low or high copy number plasmids, can influence the likelihood of successful transformation and reduce the occurrence of empty vector colonies. Lowering the incubation temperature to 20 – 22°C can help alleviate any toxic effects caused by the vector, increasing the chances of successful transformation.
Viability of Cells	Providing a warm environment to the cells during recovery by utilizing preheated SOC media can enhance their viability. Optimizing the heat shock duration between 15 – 45 seconds can improve the transformation efficiency and increase the number of viable cells. The presence of antibiotics during recovery can inhibit the growth and viability of transformed cells.
Controls	Including a positive control to ensure transformation success. The positive control should be a known transformable strain. Including a negative control without DNA. This control ensures that any observed growth is specifically due to successful transformation and not due to contamination or spontaneous resistance.
Competent Cells	Ensure proper storage conditions of -80°C or less to maintain the competency of the cells. It's best to aliquot the competent cells in appropriate volume to avoid repeated freeze-thaw cycles.
Transformation Method	Electroporation, as an alternative to heat-shock, can offer higher transformation efficiency and may be more suitable for certain applications. Optimizing the DNA concentration between 1 pg to 100 ng can improve the chances of successful transformation.
Antibiotic	Verify the appropriate selection antibiotic is used. Different antibiotic resistance genes may require specific antibiotics for effective selection. Allow the LB-agar to cool down before adding the selection antibiotic. Adding the antibiotic to hot agar can degrade its effectiveness.
Incubation	Shaking the culture during incubation helps ensure proper nutrient distribution, which is crucial for growth and expression of resistance genes.