Bacterial Transformation Workflow–4 Main Steps

Bacterial transformation is a key step in molecular cloning, the goal of which is to produce multiple copies of a recombinant DNA molecule. In **transformation**, the DNA (usually in the form of a plasmid) is introduced into a competent strain of bacteria, so that the bacteria may then replicate the sequence of interest in amounts suitable for further analysis and/or manipulation.

The four key steps in bacterial transformation are:

- **Preparation of competent cells**
- **Transformation**
- **Cell recovery period**
- **Cell plating**

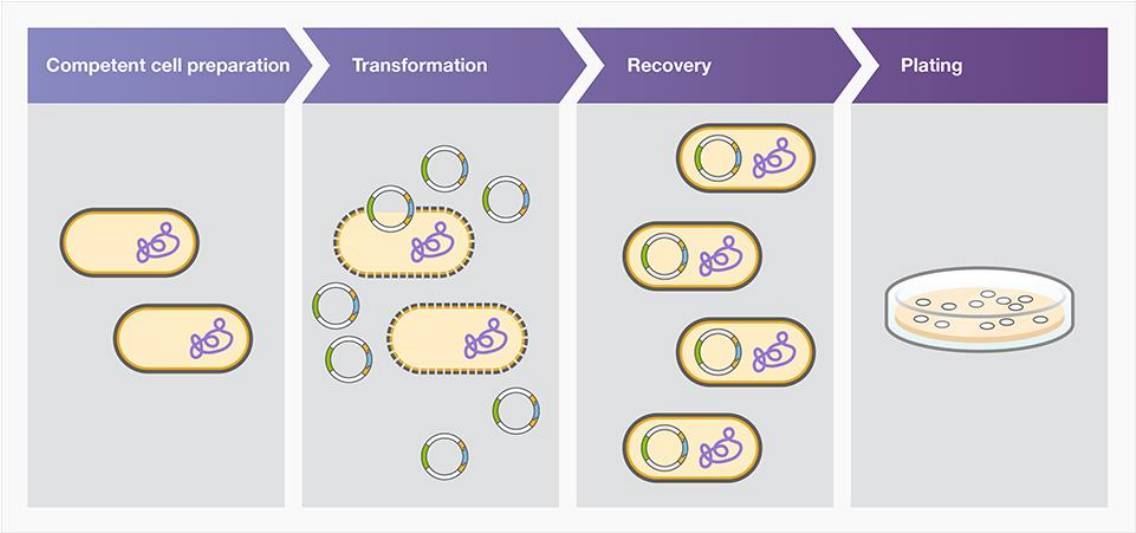

Figure 1. Key steps in the process of bacterial transformation: (1) competent cell preparation, (2) transformation of cells, (3) cell recovery, and (4) cell plating

Competent cell preparation

E. coli is the most common bacterial species used in the transformation step of a cloning workflow. Since the natural competency of *E. coli* is very low or even nonexistent, the cells need to be made competent for transformation by heat shock or by electroporation.

The protocols for preparing competent cells vary by whether transformation is to be achieved via **heat shock** or **electroporation**. In either scenario, a single fresh colony of the desired strain is taken from an agar plate and inoculated into liquid medium for a starter culture (**Figure 2**). This starter culture and the subsequent larger culture are carefully monitored for active growth by continually measuring optical density at 600 nm (OD_{600}). To obtain high transformation efficiency, **it is crucial that cell growth be in the mid-log phase at the time of harvest**—which generally occurs at OD_{600} between 0.4 and 0.9, with the optimal value depending on the culture volume, strain, and protocol. In all steps, care must be taken to use sterile tools and labware, media, and reagents where appropriate or required. Once the cells are harvested for further processing, all samples, reagents, and equipment be kept at 0–4°C in order to improve cell viability and maintain transformation efficiency.

Harvested cells are then processed according to the method of transformation, whether by heat shock or electroporation (**Figure 2**).

- **Heat-shock transformation:** Competent cells are chemically prepared by incubating the cells in calcium chloride ($CaCl_2$) to make the cell membrane more permeable. To further improve competency, Ca^{2+} may be supplemented or substituted with other cations and reagents, such as manganese (Mn^{2+}), potassium (K^+), cobalt ($[Co(NH_3)_6]^{3+}$), rubidium (Rb^+), dimethyl sulfoxide (DMSO), and/or dithiothreitol (DTT), as described by Hannah et al. (1983).
- **Electroporation:** The harvested cells are washed with ice-cold deionized water several times by repeated pelleting and resuspension to remove salts and other components that may interfere with electroporation. After 3 to 4 washes, the cells are finally pelleted and resuspended in 10% glycerol for storage.

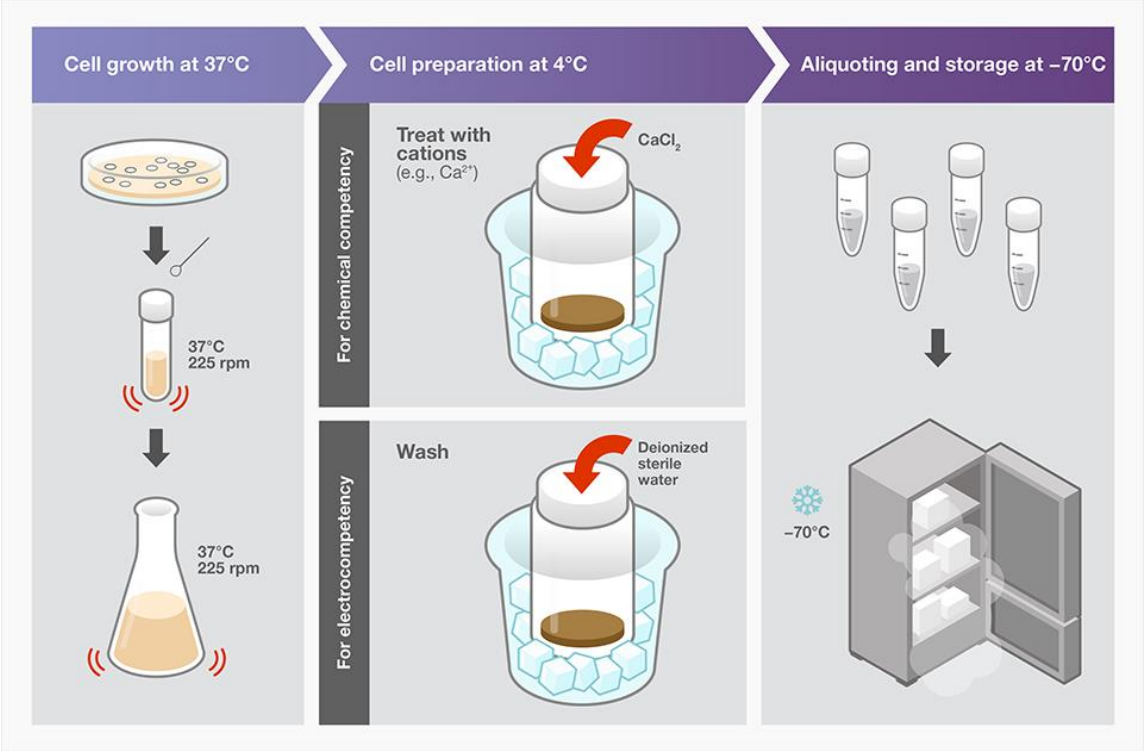

Figure 2. Preparation of chemically competent and electrocompetent cells.

Once prepared, competent cells should be evaluated for transformation efficiency, aliquoted to small volumes to minimize freeze/thaw cycles,and stored at an appropriate temperature to maintain viability. The **transformation efficiency** of competent cells is usually measured by the uptake of subsaturating amounts of a supercoiled intact plasmid (e.g., 10–500 pg of pUC DNA). The results are expressed as the number of colonies formed (transformants), or colony forming units (CFU), per microgram of plasmid DNA used (**CFU/µg**) (see cell plating).

For **storage**, aliquoting prepared cells in single-use volumes in screw-cap microcentrifuge tubes is recommended since each freeze/thaw cycle lowers transformation efficiency by about half. Competent cells should remain stable for approximately **6–12 months** when stored at –70°C with minimal temperature fluctuations. Cells should *not* be frozen or stored in liquid nitrogen, as this practice drastically reduces viability.

For consistency and to save time, **premade** competent cells are available in ready-to-use formats from commercial sources. These competent cells are quality-controlled and tested to meet specifications for transformation efficiency and genotypes. These preparations minimize batch-to-batch variability and significantly simplify the efficient propagation of cloned DNA.

Transformation

The two most popular methods of bacterial transformation are: (1) **heat shock** of chemically prepared competent cells (chemical transformation), and (2) **electroporation** of electrocompetent cells. The choice depends on the transformation efficiency required, experimental goals, and available resources (see competent cell selection). When ready for the transformation step, competent cells should be thawed on ice and handled gently to retain viability. Cells can be mixed by gentle shaking, tapping, or pipetting, but vortexing should be avoided.

With **chemical transformation**, chemically competent cells are mixed with plasmid DNA and briefly exposed to an elevated temperature, a process known as heat shock (**Figure 3A**). First, cells are incubated with DNA on ice for 5–30 minutes in a polypropylene tube. Polystyrene tubes should be avoided, as DNA can adhere to the surface, reducing transformation efficiency. Traditionally, 17 x 100 mm round-bottom tubes have been used for best results. Using 1.5 mL microcentrifuge tubes may result in poor heat distribution due to smaller surface-to-volume ratios of cell suspension, which can reduce transformation efficiency by as much as 60–90%, especially for the higher-efficiency cells.

For successful chemical transformation, 50–100 µL of competent cells and 1–10 ng of DNA are recommended. When a ligation mixture is used as the transforming DNA (often 1–5 µL is sufficient), purification prior to chemical transformation is generally not required. It is important to note that ligation mixtures may result in transformation efficiencies as low as 1–10%, compared to transformation with a supercoiled intact plasmid DNA.

Heat shock is performed at 37–42°C for 25–45 seconds as appropriate for the bacterial strain and DNA used. For smaller volumes of cells in smaller tubes, the heat-shock interval, which depends on the surface-to-volume ratio of the cell suspension, should be reduced. Heat-shocked cells are then returned to ice for ≥2 minutes before the next step (**Figure 3A**).

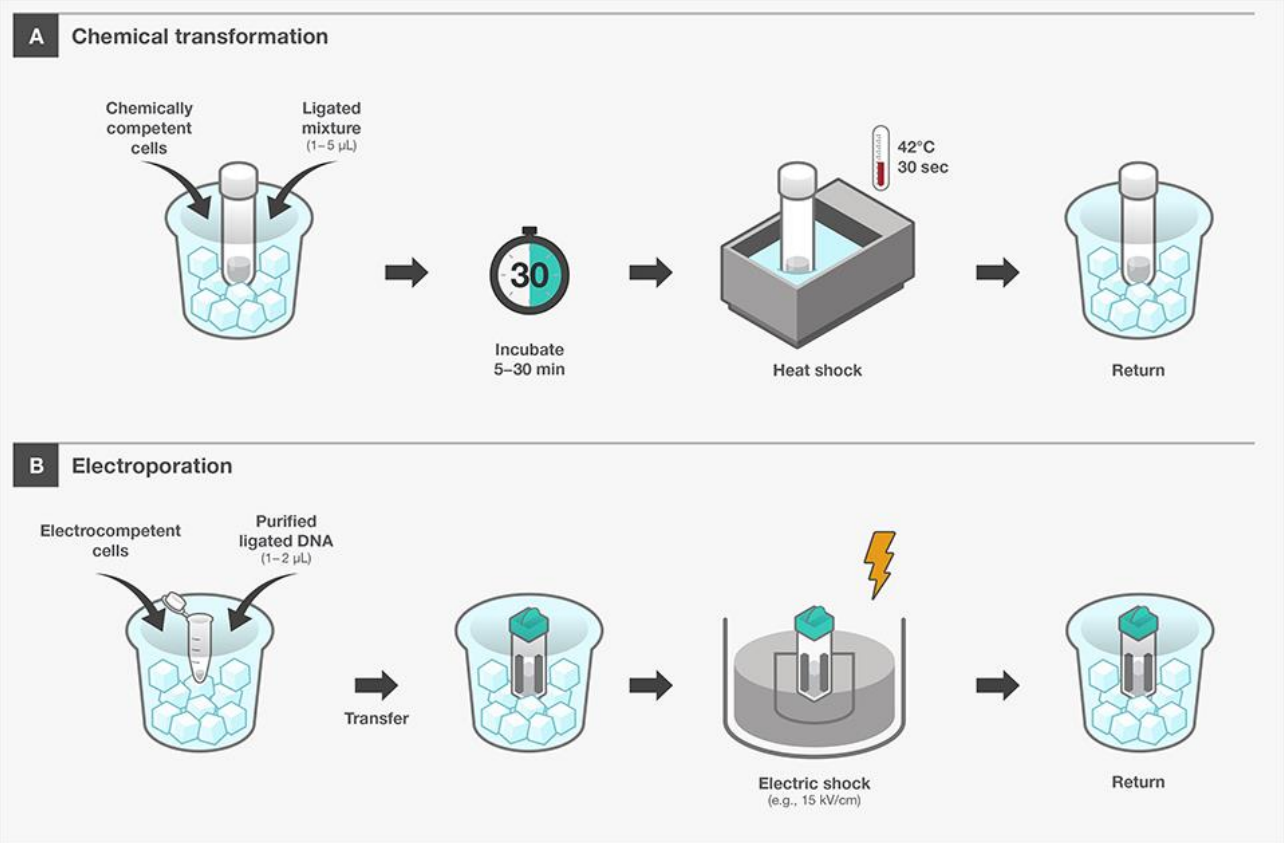

Figure 3. Bacterial transformation using (A) chemically competent cells and heat shock, and (B) electrocompetent cells and electroporation.

Electroporation involves using an electroporator to expose competent cells and DNA to a brief pulse of a high-voltage electric field (**Figure 3B**). This treatment is believed to induce transient pores in cell membranes, which permit DNA entry into the cells (**Figure 4**). The most common type of electric pulse in bacterial transformation is exponential decay, where a set voltage is applied and allowed to decay over a few milliseconds, called the **time constant** (**Figure 4A**). The applied voltage is determined by field strength (V/cm), where V is the initial peak voltage and cm is the measurement of the gap between the electrodes of the cuvette used. Typically, electroporation of bacteria utilizes 0.1 cm cuvettes (20–80 µL volume) and requires a field strength of >15 kV/cm.

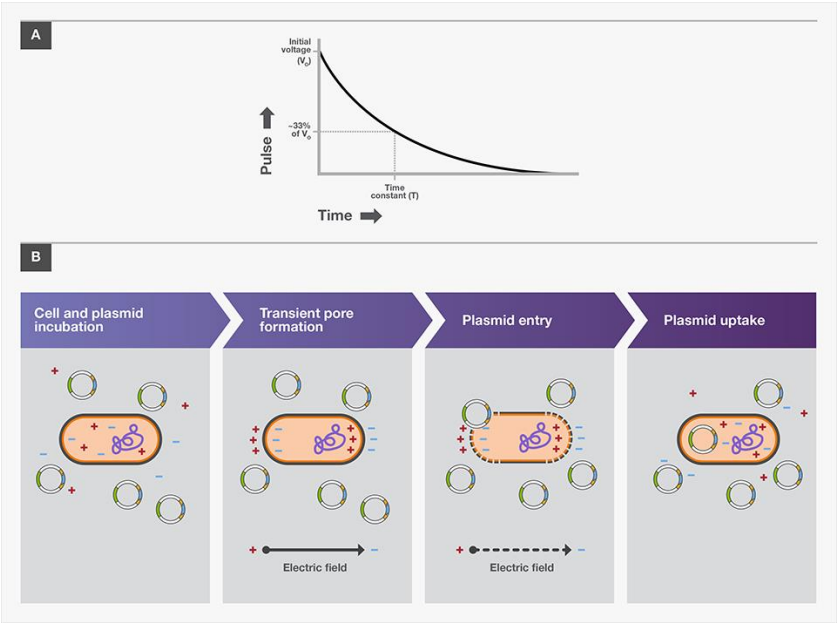

Figure 4. (A) Exponential decay of electric pulse. (B) Electroporation process.

One of the main issues with electroporation is **arcing**, or electric discharge, which may lower cell viability and transformation efficiency. Arcing often results from electroporation in conductive buffers, such as those containing $MgCl_2$and phosphates.

Strategies to prevent arcing include the following:

- Minimize the ionic strength of DNA solutions and electroporation buffers. Ligation DNA mixtures should be column-purified and resuspended in water or TE buffer to remove proteins and salts prior to electroporation.
- Keep the volume of the DNA solution at no more than 5% of the total cell suspension volume (e.g., 2 µL DNA per 40 µL of cells).
- Avoid carryover of agar during preparation of electrocompetent cells.
- Make sure no air bubbles are present in the electroporation cuvette.
- Dispense the cells directly to the bottom of the cuvette.

After transformation, unused competent cells (prepared for either method) may be refrozen. However, this will lower transformation efficiencies by about 50% for each freeze/thaw cycle. For best results, aliquot the cells after initial preparation into single-use volumes to minimize freezing and thawing. To refreeze unused cells, quickly freeze them in a dry ice/ethanol bath for 5 minutes, and store at –70°C. Avoid freezing or storing the cells in liquid nitrogen, which drastically reduces viability.

Cell recovery period

Following heat shock or electroporation, transformed cells are cultured in antibiotic-free liquid medium for a short period to allow expression of antibiotic resistance gene(s) from the acquired plasmid to begin (**Figure 5**). This step improves cell viability and cloning efficiency. For electroporated cells, growing the cells as soon as possible is recommended, since electroporation buffers are not formulated for long-term cell survival.

In the recovery step, transformed cells are cultured in 1 mL of prewarmed S.O.C. medium at 37°C with shaking at 225 rpm for 1 hour. S.O.C. medium, which contains glucose and $MgCl_2$, is recommended to maximize transformation efficiency. Use of S.O.C. medium, instead of Lennox L Broth (LB Broth), can increase formation of transformed colonies 2- to 3-fold. Strains for propagating bacteriophage M13 vectors do not require this step.

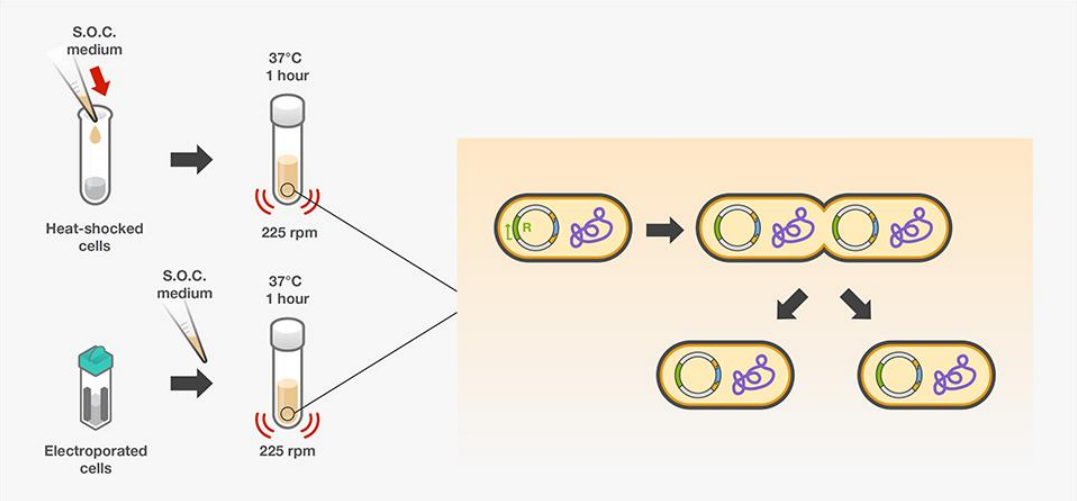

Figure 5. Cell growth during the recovery step.

Cell plating tips

After growing in S.O.C. medium, the cells are plated on LB agar with appropriate antibiotic(s) or other agents for identification and recovery of successful transformants. For example, if blue/white screening is to be performed, X-Gal and IPTG must be included in the agar plate. Avoid using agar plates more than a few weeks old (or days in some cases), to ensure the antibiotic is active. Before cell plating, the plates should be prewarmed to a favorable growth temperature and be free of condensation to prevent contamination and mixed colonies.

The amount of cells plated should produce a sufficient (and also not too numerous) number of individual, distinct colonies for further screening. Cells cultured in S.O.C. medium may be pelleted by centrifugation for 5 minutes at 600–800 x *g* and resuspended in a smaller volume for plating. For plating to a 100 mm plate, 100–200 µL of cell suspension generally works well. If very few colonies are anticipated, the entire cell suspension may be plated. However, if a very high number of colonies is expected, the cell suspension may be diluted up to 1:100 in S.O.C. medium before plating to avoid the formation of a bacterial lawn. The culture plates are examined the next day for colony formation. Prolonged incubation should be avoided, as it often results in fusion of large colonies and the appearance of smaller, antibiotic-sensitive surrounding colonies (called **satellite colonies**) due to antibiotic breakdown around large colonies.

To calculate the transformation efficiency, divide the number of transformants by the amount of DNA added, and factor in cell dilution (if performed), using the following formula:

$$\text{Transformation efficiency (CFU/µg)} = \frac{\text{Number of transformants (CFU)}}{\text{DNA added to the cells (µg)}} \times \frac{\text{Volume of transformation (µL)}}{\text{Volume of cells plated (µL)}} \times \text{Cell dilution factor (in plating)}$$