	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Bacterial Transformation **Module Hours: 3.5**	**Effective Date: 03/11/2024** **PRQs: Autoclave, Pipette use and calibration**	**Revision 1.0** **Author: Z. Hazlett-Klein** **Checked by:** **M. Stowell**

Background

Bacteria have evolved the ability to transfer genetic information between organisms, a process known as horizontal gene transfer.[1] Researchers have taken advantage of this characteristic for the purpose of genetic manipulation. One method commonly used is DNA cloning. Using versatile circular DNA vectors called plasmids, researchers can insert pieces of DNA into plasmid vectors, and insert those plasmids into a bacterial host, a process known as *transformation*. This newly inserted plasmid DNA can then be amplified as the host cells multiply, generating large copy numbers of the plasmid of interest. These amplified plasmids can then be harvested and purified from bacterial cells to obtain the desired intact cloned DNA (Fig. 1).

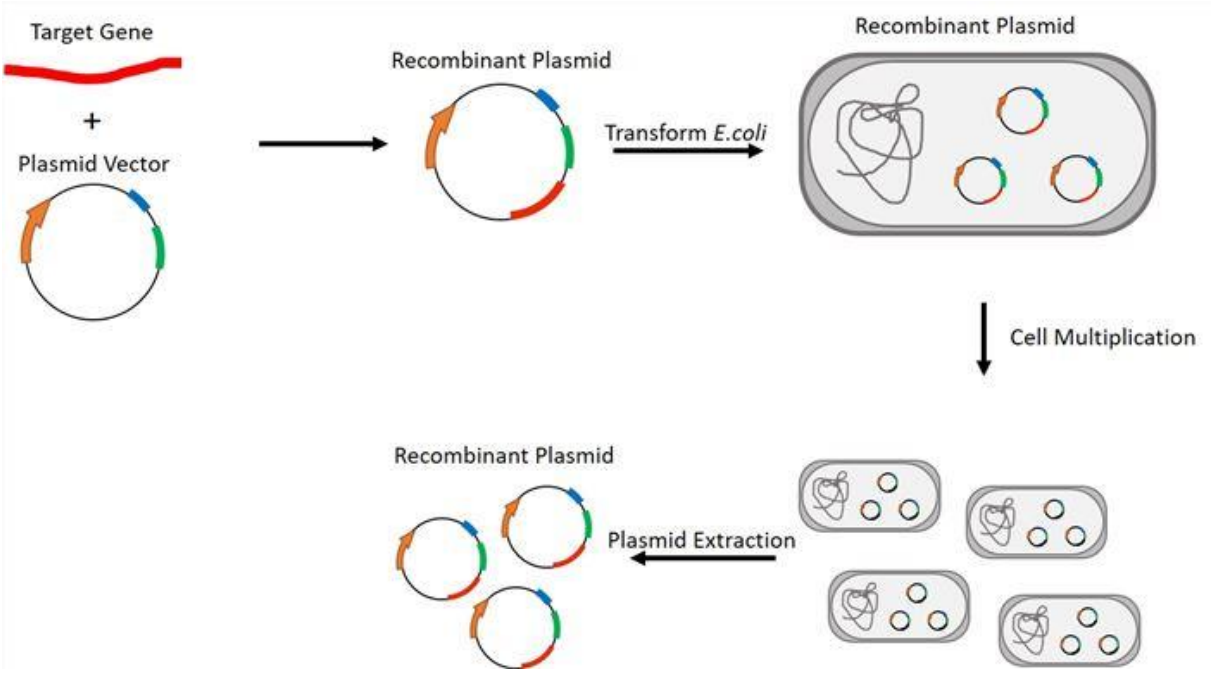
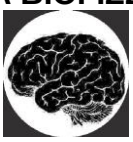


Figure 1. Target genes can be amplified by insertion into plasmid vectors, and those vectors transformed into competent *E.coli* strains, grown, harvested and purified from the host cell.[2]

	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
Bacterial Transformation **Module Hours: 3.5**	**Effective Date: 03/11/2024** **PRQs: Autoclave, Pipette use and calibration**	**Revision 1.0** **Author: Z. Hazlett-Klein** **Checked by:** **M. Stowell**

Transformation of DNA plasmids can be beneficial for both cloning and protein expression. After the initial steps of DNA cloning involving restriction digest of plasmid and gene insert, followed by ligation of insert onto the plasmid, there are still single stranded DNA knicks that must be repaired by the host cell's DNA repair machinery prior to the cell's replication of the plasmid. Bacterial strains such as the common cloning strain, DH5α, have been developed with characteristics specific for cloning applications.[3] Other bacterial strains such as the BL21 strain have been generated to facilitate controlled protein expression of target genes on pure, intact, transformed plasmids.[4] Examples of these cell strain modifications include knocking out non-essential proteases to maximize protein expression of your target gene. Keep in mind that there are many different types of bacterial strains that can be used in transformation, all of which have different modifications for different applications.

As the transformation technique takes advantage of bacteria's ability to accept extra-genomic DNA, specific methods have been established to maximize efficiency of gene transfer. In general, these techniques involve some form of stimulus that renders bacterial outer membranes more permeable for a brief period of time, allowing uptake of DNA. The two most common transformation techniques currently used are heat shock of chemically competent bacterial strains and electroporation (electric shock) of electrocompetent bacterial strains. In the former, cells are treated with calcium chloride to make the cell membrane more permeable and to facilitate attachment of the plasmid DNA to bacterial cell membranes. Heat shock of these cells opens pores in the cell membrane allowing entry of the plasmid DNA.[5] Electroporation on the other hand, generates pores in the bacterial cell wall and entry of plasmid DNA through electrical pulses of the cells in solution.[6] On average, electroporation yields higher efficiency in plasmid uptake relative to heat-shock transformation and does not require any prior chemical treatment of the cells. However, electroporation is more expensive as it uses an electroporator and specialized cuvettes to deliver the charge to the cells in solution. The choice of method must be made depending on the resources available and the desired transformation efficiencies of one's experiment.

After transformation, cells must recover from the shock with a brief period of growth in nutrient rich media (SOC media is commonly used), then the cells can be plated on LB

agar plates containing the proper antibiotic to select for cells that successfully accepted the transformed plasmid. Depending on the type of plasmid transformed (ligation product or pure intact plasmid) and the cell line used, the resulting bacterial colonies can be grown up in media again for either purification of the plasmid by DNA mini-prep or controlled protein expression of the gene target.

1. Purpose

The purpose of this SOP is to instruct students on the background and procedures necessary for bacterial DNA transformations.

2. Scope

This procedure applies to qualified skills center users.

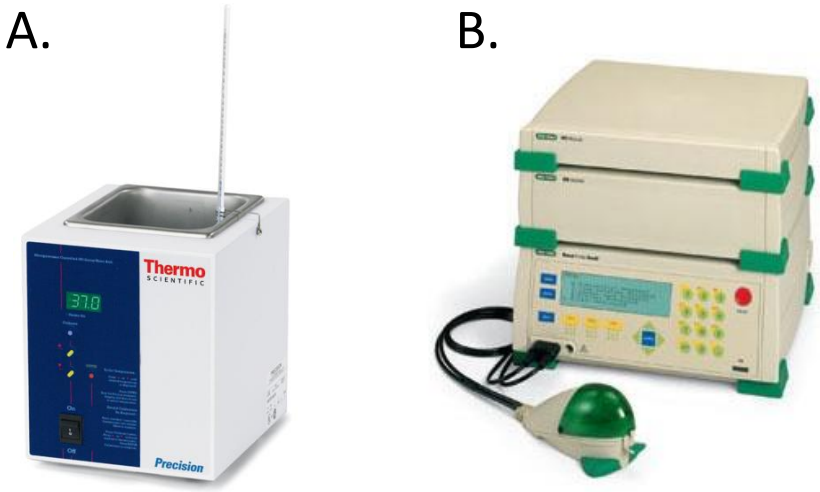


Figure 2. Equipment used in bacterial transformation either for A) Heat shock of chemically competent cells or B) Electroporation of electrocompetent cells. Image Sources: A) FisherSci. B) Harvard Medical School.

3. Responsibility

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure.

4. Definitions

4.1 DNA Cloning – The process of making many copies of a sequence of DNA. In the case of transformation that is achieved using a plasmid vector and a bacterial host to replicate that DNA.

4.2 Transformation – The process of inserting extra-genomic DNA into bacterial, yeast, or plant cells. (Note: Transfection is defined as the process of inserting DNA into mammalian cells.)

4.3 Plamids – Circular DNA with distinct genetic components that allow for gene insertion, replication, selection, and in some cases controlled gene expression.

5. Materials/Equipment

5.1 Plasmid DNA
- 5.1.1 Will use the pET His6 GFP TEV LIC cloning vector (1GFP; Plasmid #29663)

5.2 Ice in insulated bucket

5.3 Chemically or electrically competent bacterial strain
- 5.3.1 Take note of the antibiotic resistance gene in plasmid that is used.

5.4 LB Agar plates (Made with selection antibiotic or with antibiotic added to plate)
- 5.4.1 Plates can come made with selective antibiotic or without. If antibiotic is not already present on plate, one must sterilely add antibiotic to plate. Consider dilution necessary for antibiotic on plate.
- 5.4.2 pET 1GFP plasmid contains gene for Kanamycin resistance.

5.5 Sterile plating equipment
- 5.5.1 Pre-sterilized wand or
- 5.5.2 Metal wand with Bunsen burner for sterilization or
- 5.5.3 Metal wand with Bactizapper Sterilizer (currently using these in skills lab)
- 5.5.4 Sterilized beads

5.6 42º water bath (Figure 2A)

5.7 37º ThermoMixer
5.8 37º Incubator
5.9 Polypropylene pcr and microcentrifuge tubes
NOTE: Polystyrene tubes should be avoided as DNA can adhere to the surface, reducing transformation efficiency.[7]
5.10 Electroporator (Figure 2B)
5.12 Electroporation Cuvettes
5.13 SOC media

6. Procedures (Adapted from NEB – Chemical Transformation and Electroporation Protocols[8,9])

In both of the following bacterial transformation procedures, it is important to have a *negative control* to observe the expected results from post-procedure growth of cells that don't contain the transformed plasmid. In this case, these cells will not have the antibiotic selection marker provided for by the plasmid and should not grow in the presence of that antibiotic. When transforming plasmids that have newly inserted gene sequences, recall these plasmids still require repair of single stranded knicks from restriction digested and ligated plasmid and insert. In this case, transformation is lower than when transforming a fully intact plasmid. It is important here to have a *positive control*, where one transforms the fully intact plasmid containing the selection marker but without the gene insert. This allows for observing the expected results of what the growth should look like given proper transformation and antibiotic resistant bacterial growth under the right conditions.

Chemical Transformation[8]

6.1 Thaw competent bacterial cells on ice for 10 minutes.
6.2 Prepare LB-agar plates (two for each sample or control).
6.2.1 If plates were not made containing antibiotic, plate the appropriate antibiotic. Consider stock and working concentrations of antibiotic and estimated volume of LB agar on plate.

6.2.2 If small volumes of antibiotic are needed (5-20 µl) this amount can be added to 100 µl ddH20, mixed well, and added to plate for a more uniform distribution.

6.2.3 Using sterile wand or beads spread plated antibiotic across the entire surface of the LB Agar plate.

6.2.4 Place plates to dry in 37ºC incubator.

6.3 Add 1 pg-100 ng of plasmid DNA (1-5 µl) to a pcr transformation tube (test sample)

6.4 Mix cells gently by pipetting and carefully pipette 50 µl of cells into the transformation tube containing plasmid DNA (the test sample), and the same volume of cells into tube without plasmid DNA (the negative control). Mix gently without vortexing.

6.5 Place on ice for 30 minutes.

6.6 Heat shock at 42°C for 30 seconds.

6.7 Place on ice for 5 minutes.

6.8 Add 950 ul of room temperature SOC.

6.9 Shake on 37ºC ThermoMixer at 250 rpm for 60 minutes. Alternatively, place on rotator in 37º incubator for 60 minutes.

6.10 Mix cells without vortexing and perform two 10-fold serial dilutions in SOC (100 µl total).

6.11 Spread 50-100 µl of each dilution onto separate pre-warmed selection plates with sterile wand or beads and incubate at 37°C overnight.

6.11.1 Sterilize wand with Bactizapper benchtop sterilizer.

Electroporation[9]

6.12 Thaw cells, and prepare plates as directed in 6.1 - 6.2. Place electroporation cuvettes on ice. Prewarm the SOC media in 37º incubator or water bath.

6.13 Mix DNA and cells as appropriate for test and controls as directed in 6.3-6.4.

6.14 Transfer full amount of cell (control) and cell/DNA (test) mixtures into separate chilled electroporation cuvettes without introducing bubbles. Make sure cells deposit across the bottom of the cuvette.

6.15 Electroporate using the following conditions for BTX ECM 630 and Bio-Rad GenePulser electroporators: 2.1 kV, 100 Ω, and 25 µF. The typical time constant is ~2.6 milliseconds.
6.16 Immediately add 975 µl of 37°C SOC to the cuvette, gently mix up and down twice, then transfer to a polypropylene microcentrifuge tube.
6.17 Shake vigorously (250 rpm) or rotate at 37°C for 1 hour.
6.18 Mix cells without vortexing and perform two 10-fold serial dilutions in SOC (100 µl total).
6.19 Spread 50-100 µl of each dilution onto separate pre-warmed selection plates with sterile wand or beads and incubate at 37°C overnight.
6.19.1 Sterilize wand with Bactizapper benchtop sterilizer.

7. Troubleshooting

8. References

1. Keeling, P., Palmer, J. Horizontal gene transfer in eukaryotic evolution. Nat Rev Genet 9, 605–618 (2008). https://doi.org/10.1038/nrg2386
2. Plasmids. *Creative-Biolabs*. Retrieved from: https://www.creative-biolabs.com/drug-discovery/therapeutics/plasmid-vectors.htm
3. DH5α Competent Cells. *ThermoFisher Scientific*. Retrieved from: https://www.thermofisher.com/us/en/home/life-science/cloning/competent-cells-for-transformation/competent-cells-strains/dh5a-competent-cells.html.
4. Competent Cell Essentials—10 Molecular Cloning Strategies. *ThermoFisher Scientific*. Retrieved from: https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/transformation/competent-cell-selection-applications.html
5. Chemically Competent Cells. *ThermoFisher Scientific*. Retrieved from: https://www.thermofisher.com/us/en/home/life-science/cloning/competent-cells-for-transformation/chemically-competent.html

6. Electrocompetent Cells. *ThermoFisher Scientific*. Retrieved from: https://www.thermofisher.com/us/en/home/life-science/cloning/competent-cells-for-transformation/electrocompetent-cells.html
7. Bacterial Transformation Workflow – 4 Main Steps. *ThermoFisher Scientific*. Retrieved from: https://www.thermofisher.com/us/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/transformation/bacterial-transformation-workflow.html#transformation
8. Chemical Transformation Tips. New England BioLabs. Retrieved from: https://www.neb.com/tools-and-resources/usage-guidelines/chemical-transformation-tips
9. Electroporation Protocol. New England BioLabs. Retrieved from: https://www.neb.com/protocols/0001/01/01/electroporation-protocol-c2986

9. MODULE METHODS TASK

9.1 Obtain pET 1GFP plasmid for transformation. Look up documentation of plasmid and provide the following information:
- 9.1.1 What is the plasmid used for (purpose)?
- 9.1.2 How many base pairs is the plasmid?
- 9.1.3 What is the antibiotic resistance/selection marker?
- 9.1.4 Which gene/insert does it contain if any?
- 9.1.5 Which tag/fusion protein does it contain if any?

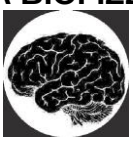
9.2 Transform this plasmid into chemically competent bacterial cells using the protocol outlined above.
- 9.2.1 Plate on the appropriate antibiotic containing LB Agar plates alongside proper controls for each method.

9.3 Take pictures of results and attach them to this file.

9.4 Compare your expected results to the results you obtained and draw conclusions based on those results.

9.5 What would change if this protocol used the ligation products of a plasmid gene insertion to transform instead of an intact plasmid?

9.6 Submit to a proctor for assessment.

	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
Bacterial Transformation **Module Hours: 3.5**	**Effective Date: 03/11/2024** **PRQs: Autoclave, Pipette use and calibration**	**Revision 1.0** **Author: Z. Hazlett-Klein** **Checked by:** **M. Stowell**