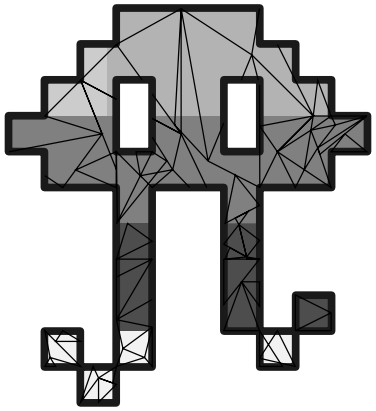


MOLECULAR BIOLOGY 101

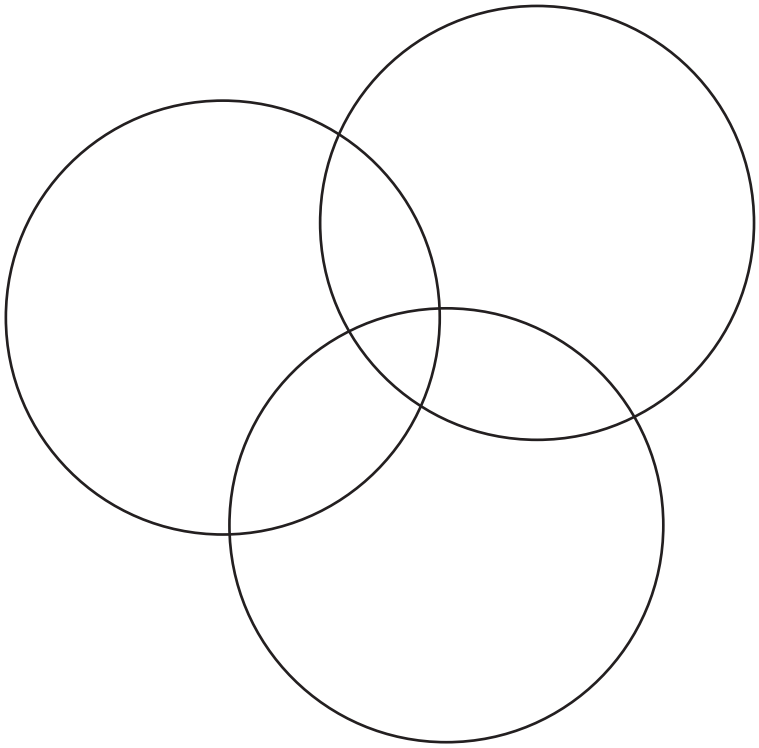
- time to insert DNA into a living organism -

BOSLAB MANUALS

BIOLOGY CLASSES

Transformation is an essential laboratory procedure for incorporating foreign DNA into microbes. Not only will you learn the underlying biology of how this method works, but you will use it to engineer *E. coli* bacteria that express green fluorescent protein, the GFP!

This course will provide you with an introduction to molecular biology techniques, a plate full of glowing bacteria, and the opportunity to start your own project at Bos|Lab!



Transformation
introducing foreign DNA into *E. coli*

1

What you will do

"Transform *E. coli*" - This means you will use a famous scientific technique to insert circular DNA, called a plasmid, into the bacterium *E. coli*. The plasmid you will insert by introducing it through the membrane of the bacteria carries the "glowing function", the GFP learned from our jellyfish friends. If everything goes well, it will enable the transformed bacteria to glow green when exposed to light in the blue to ultraviolet range.

2

How you will do it

Using plates of *E. coli*, microcentrifuge tubes, Petri dishes and pipets, you will put your *E. coli* through a "heat shock". This will make its membrane porous, in particular to plasmids. Allowing your GFP function to be incorporated into the genome of your bacteria.

3

What you will learn

While understanding by practice the basics of molecular biology, you will become a master of pipetting, using Petri dishes and understanding how accessible transformation is. You will also have the opportunity to join our next class and learn more about synthetic biology or conduct your own project at Bos|Lab.

DNA molecular structure identified in 1953

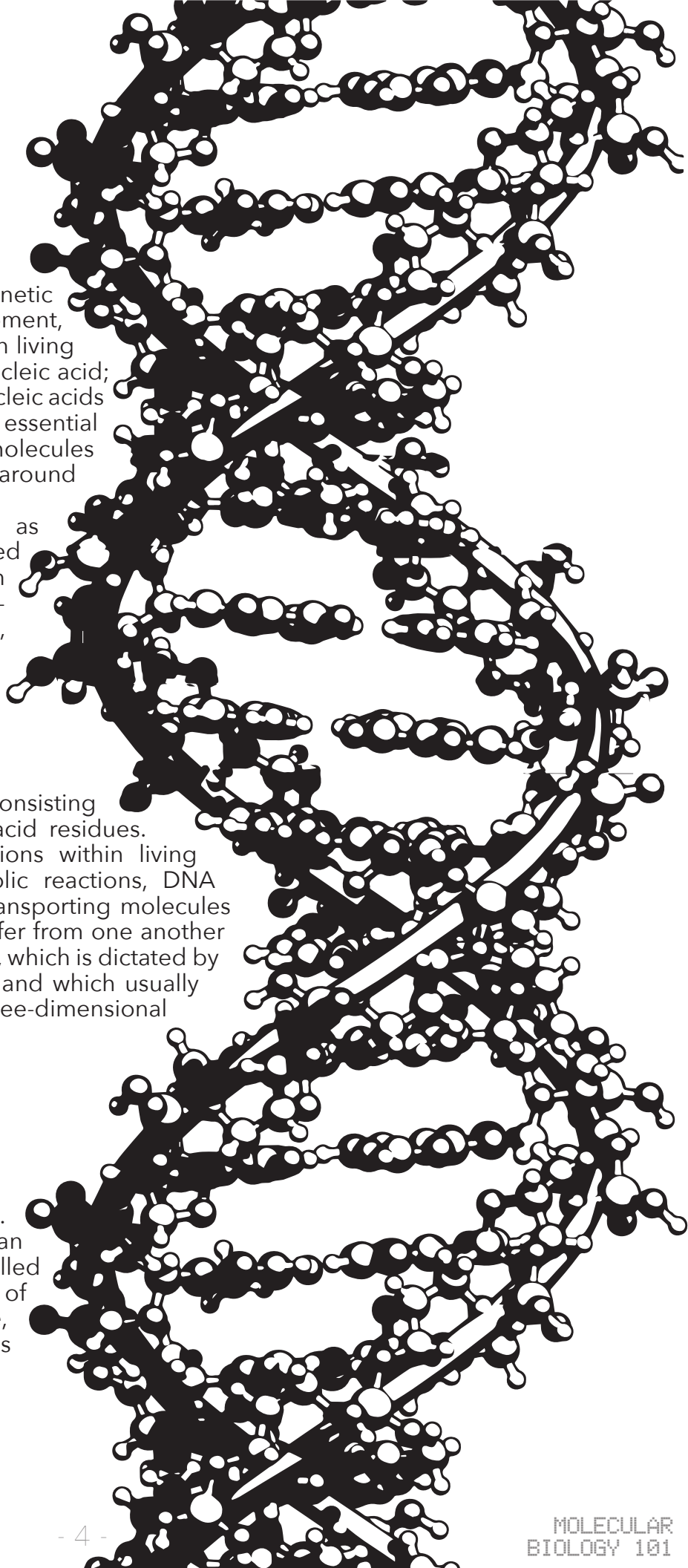
Molecule that carries all of the genetic instructions used in the development, functioning and reproduction of all known living organisms and many viruses. DNA is a nucleic acid; alongside proteins and carbohydrates, nucleic acids compose the three major macromolecules essential for all known forms of life. Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix.
The two DNA strands are known as polynucleotides since they are composed of simpler units called nucleotides. Each nucleotide is composed of a nitrogen-containing nucleobase: cytosine (C), guanine (G), adenine (A), thymine (T).

Protein first described in 1838

Large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. Proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in protein folding into a specific three-dimensional structure that determines its activity.

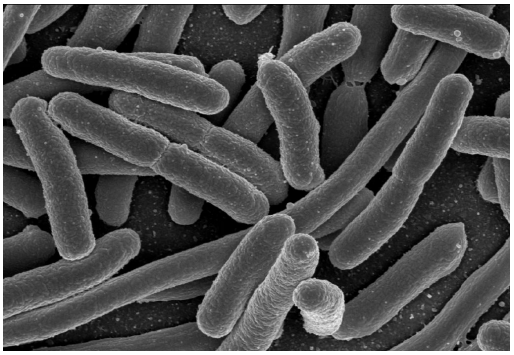
Cell discovered in 1665

The basic structural, functional, and biological unit of all known living organisms. Cells are the smallest unit of life that can replicate independently, and are often called the "building blocks of life". Cells consist of cytoplasm enclosed within a membrane, which contains many biomolecules such as proteins and nucleic acids.



Sources: Wikipedia DNA, Protein, Cell (biology)

E. coli



A Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).
The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy. Organic growth factors included in chemically defined medium used to grow *E. coli* includes glucose, ammonium phosphate, mono basic, sodium chloride, magnesium sulfate, potassium phosphate, dibasic, and water. The exact chemical composition is known for media that is considered chemically defined medium. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes only 20 minutes to reproduce.

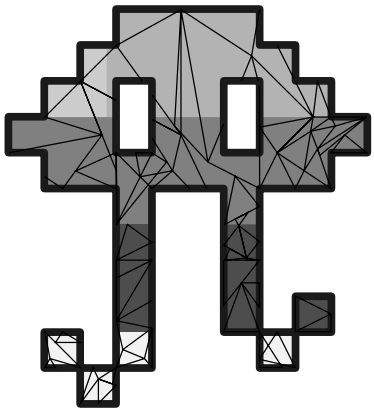
Transformation

In molecular biology, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s). Transformation occurs naturally in some species of bacteria, but it can also be effected by artificial means in other cells. For transformation to happen, bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density.

Competence

In microbiology, genetics, cell biology, and molecular biology, competence is the ability of a cell to take up extracellular ("naked") DNA from its environment. Competence may be differentiated between natural competence, a genetically specified ability of bacteria which is thought to occur under natural conditions as well as in the laboratory, and induced or artificial competence, which arises when cells in laboratory cultures are treated to make them transiently permeable to DNA.

About the experiment | 1



growing a plate of *E. coli* on LB agar media

Use calcium ion and heat shock

Plate the trans-formants on Petri dishes

About the experiment | 2

Materials

Micropippets	P1000,P20	To pipette small amounts of liquid reagents.
Plates	2	For identifying your transformed colonies.
Microcentrifuge tubes	2	For spinning down your bacterial cultures.
Sterilized spreaders/loops	2	To spread bacteria on a plate.
Nitrile gloves	2	To protect your skin from chemicals and bacteria

Chemicals

$CaCl_2$ - 50mM	2x µl	To make your bacteria competent.
LB+Ampicillin	20 ml	To select for bacteria containing pGreen plasmid.
LB+Ampicillin+ X-gal	20 ml	To select for bacteria containing pBlue plasmid.
LB broth	500 µl	To grow your transformed bacteria.

Biologicals

Plasmid DNA (pDNA)	1-2 µl	You will use both the pGreen and pBlue plasmids.
E. coli	1 ml	A prokaryotic cell used for molecular cloning.

Preparation

You will need to prepare some things ahead of time.

The first thing will be growing an overnight culture of E. coli in Luria broth (LB) liquid media, but don't worry, your instructors will prepare this for you.

You will need to make the ice and hot water baths.

The ampicillin plate should be made the day before or the day of and stored at 4°C for longer term storage.

You will also want to pre-chill your calcium chloride solution.

Protocole at a glance | 1

1

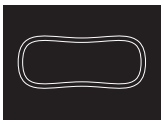
Pipette 1ml of E. coli to each microcentrifuge tube. Then centrifuge each tube for 1 minute at 5000xg. Carefully pipette off the supernatant.

The calcium ions will coat the negatively charged outside of the cell, neutralizing the charge

The bacteria can be sticky. Try twirling or whisking it around in the calcium chloride.
An alternative method is to put log-phase liquid cells into a microcentrifuge tube and spin them down for 30s -1 min, pour off the supernatant, and then resuspend them in ice cold calcium chloride.

2

Gently resuspend the bacteria in 200 µl of ice cold Calcium Chloride by pipetting up and down a few times.



By breaking up the clumps, you are increasing the surface area of the cells, rising the likelihood of causing a transformation.

Do not vortex or violently pippette up and down.
The cells are fragile at this point. Keep as cold as possible.

3

Place on ice for 10 minutes.

Allow Ca^{2+} ions to coat cells

Put in ice bath.
BE SURE THE LID IS CLOSED and BE GENTLE WITH THE CELLS.
As soon as you resuspend the cells in calcium chloride, be gentle with them and try to keep them on ice as much as possible.

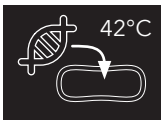
4

Add 1-2 µl of plasmid DNA.

You will add either pGreen or pBlue plasmid.

5

hold your tubes in the 42°C water for 90 seconds, aka “heat shock”.



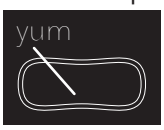
Increase temperature and chance that DNA can slip into the bacteria.

Put in Hot water bath. BE SURE LID IS CLOSED.

Protocole at a glance | 2

6

Add 250µl of LB broth.



This is to help the bacteria survive. You have just put them from a very cold to a very hot, and unusually salty place. They tend to die when you do that, but enough will survive so that you will get some transformants.

Add broth directly into the tube.

7

Incubate at 30-37°C for 5 minutes.

This is to help the bacteria survive.

8

Plate 100-200µl onto your prepared antibiotic plates.

Two points to make here:
1. ampicillin is pretty easy to destroy. So if you let the bacteria recover for very long, and you have an ampicillin resistance plasmid, they will make a lot of enzyme to break it down. This means that you will destroy some of the ampicillin on the plate, allowing non- transformed "satellite" colonies to survive/overgrow your transformants.

2. Spread them out over the plate as much as possible so you get individual colonies(not big groupings which are prone to kicking out plasmid/satellite colonies) when the transformants grow up.

To plate, pippete 100-200 ul (older, dryer plates can absorb more liquid) of liquid from the microcentrifuge tube onto the antibiotic plate. Then spread it around with a sterile spreder, or sterile loop. Try to get it spread evenly over the plate.

9

Let plates incubate overnight at 30-37°C.

If you successfully transformed your bacteria with the plasmid DNA you will see colonies on your plates the next day!

NOTES