

# Extracting and analysing plasmid dna from e.coli



**ASSIGN  
BUSTER**

## **Introduction**

Deoxyribonucleic acid (DNA) is a molecule present in all living things, and they carry genetic information which determines every characteristic a person can have. DNA contains 4 chemical units: Adenine, Guanine, Thymine and Cytosine. These 4 letters are organized to make genes which contain information to make proteins.

As scientists have discovered, it is the genome (DNA sequence in a particular arrangement of the 4 letters) that makes every human unique. During the first stages of cell division, the human DNA is organized into 46 tightly coiled structures called chromosomes. As a cell divide, the chromosomes are copied over to the new cells, ensuring they receive a full copy of the genetic blueprint.

## **Objective**

1. Isolate DNA of cheek cells
2. Extract chromosomal DNA from strawberry
3. Extract plasmid DNA from E. coli.

## **General Method**

Collect cells

Split cells open and release contents

Destroy enzymes which break apart DNA

Separate DNA from other cell components

Precipitate DNA

## **General Materials**

Solution I

Solution II

Solution III

Tubes of various sizes according to use

Proteinase K (10mg/ml)

Strawberry

Filter funnel

DNA extraction buffer

Chlorofoam

LB Liquid Medium

5M NaCl

70% Ethanol

95% Ethanol

Centrifuge

Hot water bath

Lysis Buffer

## **DNA of Cheek Cells**

Collect cheek cells by rinsing mouth with saline solution

Saline solution prevents cells from splitting open too soon

Spin solution in a centrifuge to collect cells at the bottom of the tube

Empty out the liquid, leaving the cell pellet at the bottom

Add Lysis Buffer (Contains soap, salts and ions, buffers)

Soap: Destroy fatty membranes that enclose cells

Destroy nuclei membranes in the cells

Salts and ions: Bring up osmotic pressure (pressure applied to solution needed to prevent the inflow of water) outside the cell, which helps break apart membranes

Buffer: To maintain pH

Breaks open cells

DNA released into solution

Add Proteinase K

Digest contaminating proteins

Degrades nucleases which attack nucleic acids

Put the solution in hot water bath

Enables Proteinase K to work efficiently

Kill enzymes in the cytoplasm which can break apart DNA

Add 5M NaCl

Change polarity of solution to differentiate DNA from fats, carbohydrates and proteins

DNA dissolves in ionic solutions, the rest do not

Centrifuge solution

Separates DNA (dissolved in clear liquid) from fats, carbohydrates and proteins (solid pellet)

Transfer clear liquid (containing DNA) to new tube

Add cold 95% ethanol to new tube

Precipitate dissolved DNA from ionic solution since DNA is not soluble in alcohol

The colder it is, the less soluble DNA (Can precipitate more)

Coldness slows down enzymatic reactions which can break DNA apart

Centrifuge new tube

Resulting white pellet is DNA of cheek cells

## **DNA of Strawberry**

Mash strawberry

<https://assignbuster.com/extracting-and-analysing-plasmid-dna-from-ecoli/>

Add DNA extraction buffer (contains shampoo/soap & NaCl) and mash

Shampoo/soap: Dissolves cell membrane which is made up of lipid bilayer

NaCl: Removes proteins that are stuck onto DNA

Prevent proteins from precipitating along with DNA in ethanol

Filter and add cold ethanol

Precipitate DNA

Twirl glass rod at interface between ethanol layer and slurr layer

Resulting sticky mass is the plant DNA

## **Plasmid DNA of E. coli**

Add solution I (contains glucose, Tris, EDTA) to prepared pellet

Glucose: Increase osmotic pressure outside cells

Tris: Maintain constant pH

EDTA (Ethylenediaminetetraacetic acid): Protects DNA from enzymes which will degrade DNA

Add solution II (contains alkali substances & detergent)

Alkali: Breaks open the cells

Break down DNA into single strands

Detergent: Break membrane apart

Add solution III (contains acidic substances)

Neutralizes pH so DNA strands can get back together as double stranded

Precipitates cellular debris

E. coli plasmid DNA remains in solution

Add chloroform

Extract DNA

Centrifuge mixture

Separates plasmid DNA and debris & chromosomal DNA

Transfer some amount of liquid into new tube

Add 95% ethanol

Centrifuge new mixture

Purify plasmid DNA

Pour away liquid and add 70% alcohol

Remove remaining salts

Centrifuge mixture

Pour away liquid and spin the tube

Resulting pellet is plasmid DNA

## **Discussion/Extensions**

Why is DNA extraction important/used for:

Crime and historical identification

Lineage/origin identification

Diagnosis of diseases

Mass produce gene/protein important for treating diseases, using further DNA technology

Genetic engineering

Other DNA extraction methods:

Anion-exchange

Uses chromatography technique

Nucleic acids of DNA are composed of negatively-charged phosphates

Positively-charged substrate used to bind to the negatively-charged phosphates

Proteins and RNA are then removed with medium-salt buffers

Silica Gel

Advantage: Fast, reliable, economical

Uses silica-gel membrane to adsorb nucleic acids of DNA



Catalysts: Chaotropic salts

Buffers used in lysis helps DNA to adsorb on silica-gel membrane, and washes away metabolites and proteins

Salting

Remove proteins and contaminants by using high concentrations of salt

Precipitates removed using centrifuge

DNA recovered with alcohol

Organic extraction

Mix dead cells with phenol, chloroform and alcohol

DNA extracted using alcohol precipitate

Cesium chloride (CsCl)

Mix suspended DNA with CsCl and ethidium bromide

Solution centrifuged

DNA extracted with isopropanol

## **Limitations**

This general method of DNA isolation consists of many limitations:

Inability to remove inhibitors of polymerase chain reaction

Too many steps may be too time-consuming

Multiple tube transfers may increase risk of contaminations by 'outside' DNA

## **Conclusions**

This simple experiment provides an introduction to the procedures that are used in modern microbiological laboratories. Other cases can get much more complex, and will involve more sophisticated methods and equipment. The extraction of DNA is the first step of many other fascinating processes, which includes the manufacturing of medicines as well as genetic engineering which alters the genes of organisms.