SYNTHETIC BIOLOGY FOR ARTISTS & DESIGNERS

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Contents

Introduction.....2
Step 1 - Streaking and Spreading 3
Step 2 - Taking Dry DNA From Wells.....5
Step 3 - Gel Eclectrophoresis.....6
Step 4 - Transformation of Bacteria.....8
Step 5 - Mini Prep.....10
Step 6 - Elution.....11
Step 7 - Digestion.....13
Step 8 - Ligation.....14
References.....15

INTRODUCTION

Synthetic biology is a combination of biology and engineering in order to create bacteria with different uses and functions. It is fairly easy to learn the theory behind synthetic biology. Just look in the reference for more information.This manual describes a few basic lab protocols that will help you get started with hacking bacteria.

STEP 1

STREAKING AND SPREADING

In order for us to transform bacteria it is essential to have sufficient colonies of bacteria. For this we need to grow the bacteria. Bacteria can be grown either through streaking or by spreading.

You will need:

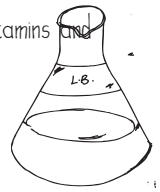
70% alcohol, agar agar, lysogeny broth, Petri dish, ampicillin, inoculation needle, spirit lamp, stirrer.

Process

Before starting wash your hands with 70% alcohol to prevent bacterial contamination.

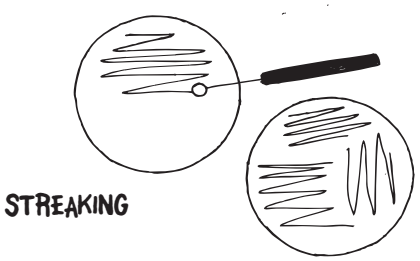
The agar plate is prepared by mixing agar agar with Lysogeny broth (also: Luria or Luria-Bertani broth) to form a nutrient-rich medium for bacterial growth.

The LB broth is made of tryptone - for peptides and peptones, yeast extract - for vitamins and trace elements, and NaCl - for ions for transport and osmotic balance.
We can add ampicillin to prevent of growth of non-antibiotic resistant bacteria.
This is poured onto a Petri dish.



First, a sterilized tungsten loop or inoculation needle is used to collect the E. Coil from the previous sample.

The cells are spread on a new agar plate in a Petri dish by streaking from the loop onto the surface, flaming it, collecting from the sample and streaking again with a little overlap or placing the loop gently on the surface and slowly bringing it inwards while the plate is spun on a wheel so as to get radial streaking.



STREAKING

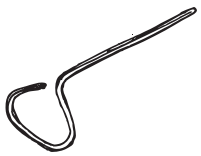
SPREADING

Alternatively, the sample can be spread using a clean stirrer instead of the inoculation needle. This is called spreading. In this case the bacteria sample that grows will occur in large colonies, where as in streaking there is a gradation and a single bacteria can be grown.

The Petri dish is incubated overnight at 37 C.

The bacteria that is resistant to the antibiotic grows.

Spirit Lamp



Stirrer

STEP 2

TAKING DRY DNA FROM WELLS

In order to construct the required bacteria, you may need to extract dry DNA from a DNA kit that contains the parts that we will need to use. There is a specific procedure for 'taking out' the dry DNA from the kit. The DNA is stored in these pits in the box called 'wells'.

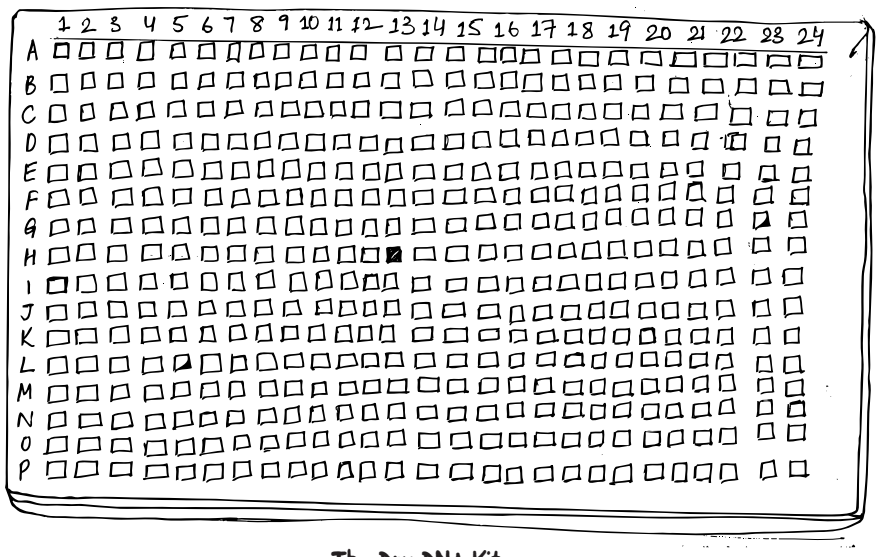
You will need:

Dry DNA kit, deionized water

Process

The first step is to make sure that the plates are properly oriented. Then a hole is punched through the foil cover using a pipette - into the well you want the DNA from. Don't remove foil cover since it might pose the threat of cross-contamination between wells.

Add 15uL of deionized water



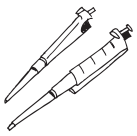
The Dry DNA Kit

STEP 3

GEL ELECTROPHORESIS

Gel electrophoresis is done to be able to view the DNA bands under ultra violet light.

You will need:



beaker, pipette, casting plate, electricity generator, comb and comb stand, nitrile gloves, UV light box,

Agarose, Buffer solution- TAE(Tris-Cl, Acetic Acid, Ethylene diamene tetra acetic acid)1.0x pH 8, Ethidium Bromide (5.25 mg/ml in H20), bromophenol blue, Antibiotic resitant gene(from ampicilin)

Preparation of gel

Make a 1% agarose solution in 100ml TAE, for typical DNA fragments. For 3-4 kb solution of DNA a solution as low as 0.7% is used.

Heat this mixtue to 37 degree C and let it cool.

Now add EtBr of 0.5 ug/ml concentration.

Stir the solution and pour it into the the casting plate.

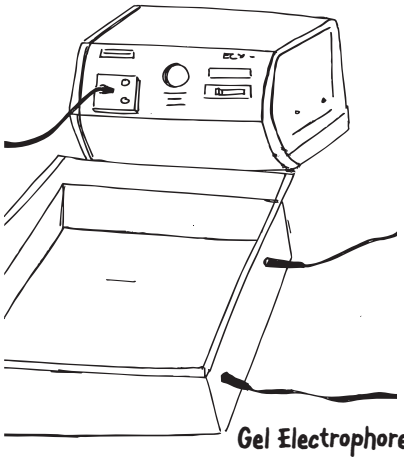
Now insert the comb at one side of the gel about 10mm away from the side.

Once the gel has cooled down and become solid, pull the comb out.

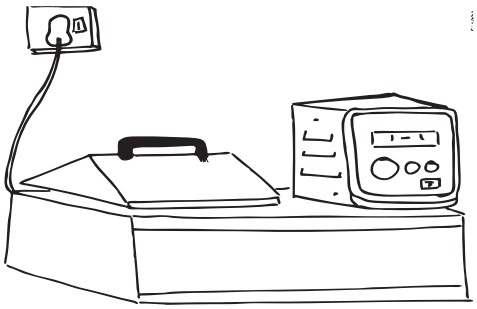
The holes that remain are the wells. The DNA samples travel ahead through these wells.

Put the gel along with the casting plate in a tank with TAE, EtBr at the same concentration can be added.

The gel must be completely covered by TAE and placed such that the wells are at the end electrode passing negative charge.



Gel Electrophoresis Chamber



Heater

Procedure

Using a micropippette inject 2.5 l solution with the DNA ladder into the first well along with the low molecular weight blue dye.

In the same way now inject the DNA samples mixed with the blue dye into the other wells.

Now a current is applied, about 100V for 30 minutes.

Lastly place the slab of gel on a UV light box and observe.

One can also capture a digital image of the same

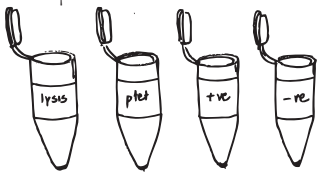
STEP 4

TRANSFORMATION OF BACTERIA

We are attempting to alter the genetic composition of bacteria by adding foreign genetic material to it. For this, the bacteria need to be conditioned to take foreign material and make it its own. Now it is this process of 'conditioning' that forms the scientific method described below. This, in scientific terms is called 'Transformation'.

You will need:

Eppendorf tubes, pipette, water, DH5 alpha E. Coli cells, ice, L.B. centrifuge machine, Petri dish, agar plates, ampicillin.



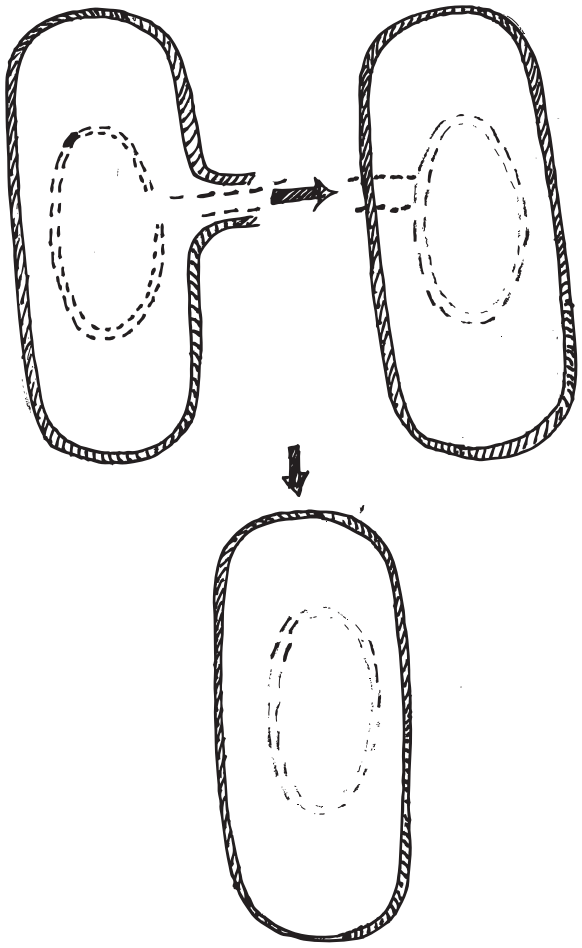
The Process:

Four eppendorf tubes were taken and marked lysis, p-tet, +ve and -ve. The positive control e and the negative control did not. The dry DNA was extracted by mixing each part with 15 micro ml of water using a micropipette.

2-3 micro ml of this plasmid solution was then added to 100 micro ml of DH 5-alpha Escherichia Coli cells.

The bacteria were incubated in ice for 40 minutes, then given a heat shock for 30 seconds @ 42 degrees Celsius and then put in ice for 2-5 minutes. The heat shock causes tiny pores in the walls of the bacteria to expand, allowing the plasmids to go through.

The Bacteria were then mixed into 330 micro ml Luria Bertani the bacteria were then left to cultivate for approximately an hour at 37 degrees Celsius in the shaker.



This was then centrifuged at room temperature for 3 minutes @ 8000 rpm.

Discard 900 micro ml of the supernatant and dissolve pellets in remaining 100 micro ml. Spreading helps ensure that you will be able to pick out a single colony.

These were then spread on LB Agar plates containing 100 micro grams per ml Ampicillin.

The plates were incubated at 37 C for 12-14 hours, making sure the agar side of the plate is

up. If incubated for too long the antibiotics, especially ampicillin, start to break down and un-transformed cells will begin to grow..

STEP 5

MINI-PREP

The mini-prep is done in order to isolate and purify the bacteria containing the required plasmid.

You will need:

Eppendorf tubes, bacteria culture, centrifuge machine, pipette, ALS I, ALS II, ALS III, vortex machine, chloroform, Iso-propanol, 70% ethanol, absolute ethanol, deionized water.

Process

5ul of the culture is taken in an Eppendorf tube.
The culture is centrifuged at 13200 rpm for 3 minutes, making sure the centrifuge is balanced. The centrifugal force created in the centrifuge forces the bacteria in the solution to collect in a pellet at the bottom of the Eppendorf tube.
The process is repeated to get required quantity of the cultures i.e. (1.5 ul twice)3.0 ul and centrifuge again.

The liquid around the collected pellet, called the supernatant is discarded.

The pellets are dissolved in 200ul of ALS I previously stored at 4 degrees Celsius.
This is incubated at room temperature for 3-5 minutes. Incubating at room temperature enables the DNA to get denatured.
200ul of ALS II is added and mixed using a Vortex Mixer and incubated at room temperature for 5 minutes.
200ul of chilled ALS III is added, mixed and incubated in the tubes in ice for 10 minutes.
The tubes are centrifuged at 13.2k rpm for 15 minutes.
The aqueous phase is transferred into a fresh Eppendorf tube.

500 l of Chloroform is added; the solution is mixed well and then left to incubate at room temperature for 3 minutes.
The tubes are centrifuged at 13.2k rpm for 3 minutes.
The upper aqueous phase is take out and transferred into fresh Eppendorf tubes.
0.6 volume of Iso-propanol is added, this is incubated at room temperature for 15 minutes and then centrifuge at 13.2 rpm for 20 minutes.
The supernatant is discarded and 500 l of 70% ethanol is added.
This is centrifuged at 13.2k rpm for 3 minutes at room temperature and the supernatant is discarded.
Now 200 l of absolute Ethanol is added and this is centrifuged again for 3 minutes and the supernatant is discarded.
The pellets are dried at 37-50 degrees Celsius and dissolved in 20 M1/MQ deionized water.
The concentrations are tested using a Nanodrop device.

STEP 6

ELUTION

Elution is a term used in analytical chemistry to describe the emergence of chemicals from the column of a chromatograph. As they elute, the chemicals typically flow into a detector. Predicting and controlling the order of elution is a key aspect of column chromatographic methods.

You will need:

DNA fragment from gel, buffer QG, Vortex machine, 3M sodium acetate, iso-propanol, QIAQuick column, Buffer PE, Buffer EB.

Process

Remove the DNA fragments from the agarose gel with a clean scalpel. Try to get as small a piece of gel as possible, without touching the DNA.
Weigh the gel slice in a colorless tube. Add 3 times the volume of Buffer QG to 1 volume of gel.

Incubate at 50 C for 10 minutes, or until all the gel has dissolved. Mix the tube using the vortex mixer every 2 or 3 minutes to make sure it dissolves.

Check if the mixture is yellow in color. If it is, it means that its pH is around 7.5. This is the pH at which the adsorbtion (stickiness)of the QIAquick membrane is most efficient. If it is orange or violet, it is too acidic. Add 10 l of 3 M sodium acetate (pH 5.0) and mix, if that is the case.

Add isopropanol of a volume equal to the volume of the gel to the sample and mix. The isopropanol precipitates the DNA.

Place QIAquick spin column in a 2ml collection tube.

To collect the DNA, pour the sample into the QIAquick column, and centrifuge for 1 min.

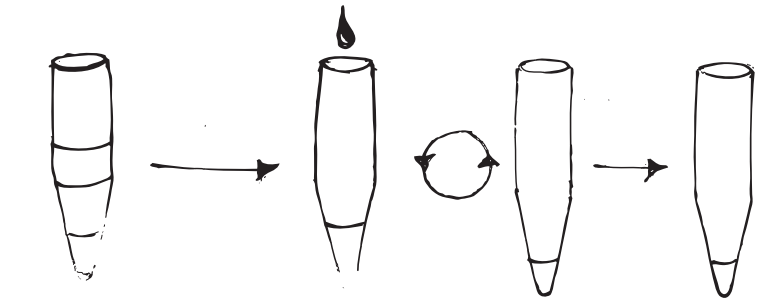
Discard flow-through and place QIAquick column back in the same collection tube.
Collection tubes are re-used to reduce plastic waste.

Add 0.75 ml of Buffer PE to QIAquick column, let it stand for 2 to 5 minutes and then centrifuge for 1 min at 13,000 rpm.

Discard the flow-through and centrifuge the QIAquick column for another minute at 13,000 rpm.

The QIAquick column should then be placed in a fresh eppendorff tube.

To elute (extract) the DNA, add 50 l of Buffer EB (10 mM Tris Cl, pH 8.5) or H2O to the center of the QIAquick membrane (a small disc of silica gel) and centrifuge the column for 1 min.
For stronger concentration of DNA, add 30 l elution buffer to the center of the QIAquick membrane, let it stand for 1 minute, and then centrifuge for 1 minute.



STEP 7

DIGESTION

Digestion of DNA is a process carried out just to be able to look at DNA or, like in this case, cut a band out of the gel for further treatment. Digestion can be done in 2 ways:

Double Digestion:

When you are digesting your DNA with two or more enzymes it is called double digestion. In such cases, you have to make sure to use the buffer that will be most compatible with all the enzymes. Enzyme buffers are specifically formulated to provide the salt concentration for optimal enzyme activity. There should not be too much salt in the digestion because too salt will inhibit enzyme activity.

Single Digestion:

Digesting the DNA with a single enzyme is called single digestion.

You will need:

DH5 alpha E.coli cells, autoclave MilliQ water.

Process

First we isolated the plasmids from E. coli Dh5alfa cells, which was dissolved in 20 micro liter of autoclaved Milli Q water (pH 7.0).

Digestion mixture was prepared as
Plasmid DNA = 3ul
EcoRI = .5ul
PstI = .5ul
Buffer = 2ul
BSA =3ul
MQ =11ul

This mixture was then mixed gently and incubated at 37 degree Celsius for 4 hours in a water bath.
The mixture was run on the 0.7% agarose gel.

One 1kb ladder was used to characterize the specific bands. Bands were detected on gel-doc system.

STEP 8

LIGATION

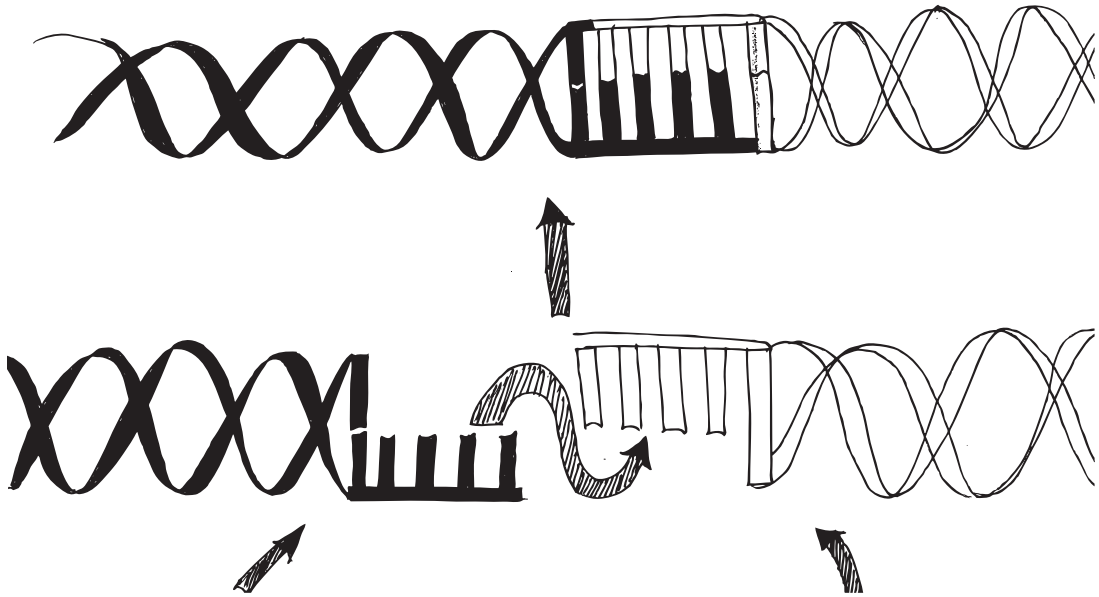
Ligation is a process by which two DNA molecules are joined together in the presence of a catalyst - DNA ligase, an enzyme.

You will need:

vector DNA, insert DNA, Milli Q water, ligation buffer-1X, ligase enzyme

Process:

Mix it properly and do ligation at 16 degree for overnight
The sticky ends from both parts join to form a new part.



REFERENCES

Student Book from IGEM
http://openwetware.org/images/f/f9/IGEM_Student_Book.pdf

Gel Electrophoresis
http://en.wikipedia.org/wiki/Gel_electrophoresis

Extracting DNA at home
http://nature.ca/genome/05/051/pdfs/DNAextract_e.pdf

Harvard 2006: Explaining their process
http://parts2.mit.edu/wiki/index.php/Harvard_2006

The Synthetic Biology Comic
http://www.nature.com/nature/comics/syntheticbiologycomic/

Introduction to Biological Engineering Design
http://openwetware.org/wiki/20.20

Introduction to Synthetic Biology
http://openwetware.org/wiki/Intertech:iSB2008:Materials

Ibio Seminars
http://www.ibioseminars.org/

Gel electrphoresis process
http://web.utk.edu/~khughes/GEL/sld008.htm

Primer on Synthetic Biology
http://openwetware.org/images/3/3d/SB_Primer_100707.pdf

DIY Synthetic Biology
http://www.scribd.com/doc/14663730/DIY-Synthetic-Biology

Drinking straw gel electrophoresis
http://maradydd.livejournal.com/417631.html

Open Gel Box
http://openwetware.org/wiki/DIYbio:Notebook/Open_Gel_Box_2.0

BioBricks on Youtube
http://www.youtube.com/user/BioBricksFoundation

Ginko BioWorks Guide to Synthetic Biology
http://diybio.org/wp-content/uploads/2009/04/CodeCon09_SynthBio_tutorial_handout/appendix3%20-%20GinkgoBioworks%20guide%20to%20engineering%20biology.pdf

DIY Biology Webcast
http://techtv.mit.edu/collections/mitmuseum/videos/1684-soap-box-do-it-yourself-biology

DIYBio Model Organisms
http://groups.google.com/group/diybio/web/diybio-model-organisms

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