PREPARATION OF COMPETENT ELECTRICAL BACTERIA

The day before, inoculate the desired strain in 10 mL of LB medium. Incubate in a conical flask at 37 ° C;
Dilute the inoculum overnight to 0.1 OD in 200 ml LB medium and incubate at 37 ° C for about 2 hours;
Measure the OD, which should ideally be at 0.5;
Distribute the contents into 4 50 mL tubes (previously immersed in ice) and incubate for about 15 minutes;
Centrifuge at 4000g, 12 minutes, 4 ° C;
Discard the supernatant. Immediately return the tubes to ice;
Gently resuspend the bacterial pellet in 10 mL of sterile ice cold MilliQ water and make up to 50 mL of water. Join the pellets of two tubes into one, leaving two tubes;
Centrifuge at 4000g, 12 minutes, 4 ° C;
Discard the supernatant. Immediately return the tubes to ice;
Gently resuspend the bacterial pellet in 10 mL sterile ice-cold 10% glycerol. Combine the pellets of the two tubes into one;
Centrifuge at 4000g, 12 minutes, 4 ° C;
Discard the supernatant. Immediately return the tubes to ice;
Gently resuspend the bacterial pellet in 1 mL sterile ice-cold 10% glycerol;
Aliquot into eppendorff tubes (previously immersed in ice) and store at -80 ° C.

ELECTROPORATION PROTOCOL

Pre-immerse (sterile) cuvettes in ice;
Place 50 µl competent bacteria + 1 µl DNA in ependorff;
Distribute the contents of eppendorff into the cuvette by tapping it on the countertop to settle the contents at the bottom and remove the bubbles;
Shock the cuvette (1.8 KV, 25 µF, 200Ω) and immediately add 1 mL LB medium;
Incubate for 1h at 37 ° C;
Plating

Digestion Protocol

NEB	Digestion temperature	Heat Inactivation (°C)
ECORI	37°C	65°C
XBAL	37°C	65°C
SPEI	37°C	80°C

Restriction Enzyme Double Digestion

Steps

1. Set up reaction as follows:

COMPONENT	50 µl REACTION
DNA	1 µg
10X NEBuffer 3.1	5 µl (1X)
XbaI	1.0 µl (or 10 units)
EcoRI	1.0 µl (or 10 units)
Nuclease-free Water	to 50 µl

2. Incubate at 37°C for 5-15 minutes as both enzymes are Time-Saver qualified.

Restriction Enzyme Double Digestion

Steps

1. Set up reaction as follows:

COMPONENT	50 µl REACTION
DNA	1 µg
10X CutSmart Buffer	5 µl (1X)
SpeI	1.0 µl (or 10 units)
XbaI	1.0 µl (or 10 units)
Nuclease-free Water	to 50 µl

2. Incubate at 37°C for 5-15 minutes as both enzymes are Time-Saver qualified.

Protocol
Comparison efficiency test of pBAD and pLac promoters.

Materials:
1 96-well fluorescence culture dish
2 Falcon 50 ml
Half minimum

Arabinose
IPTG
Glucose
Glycerol
PBAD, pLac and PBrasilia overnight culture
Leucine

Procedure:

Prepare the medium:
Half of the plate will be grown with Glucose (0.2%) and half Glycerol (0.2%).
Make 35 ml each, and add Leucine solution as described below.

How to make leucine solution:
100x Solution (5g / l)
weigh 0.25g of Leucine
complete with 50ml H2OmiliQ
(use concentration 0,05g / l)
Filter! Use 0.22 filter
From here, everything must be done in FLOW or fire (it has to be sterile)
Prepare the means with carbon sources:
Glucose:
34.612 ml of minimum medium
388 ul 1M glucose

Glycerol:
82 µl glycerol (85%)
34.918 ml of minimum medium

Place 350ul of leucine solution in 35 ml of glucose and glycerol media. meios

Centrifuge the cultures, and resuspend (in flow) in the medium prepared for OD 0.1 (can calculate using c1v1 = c2v2) on 10 ml with two cultures of each plasmid (pBad pLAc and pBrasilia), one with glycerol and one with glucose, ie 6 dilutions.

Incubate at 37 ° C and shake until OD 0,3

Distribute on plate according to map, placing 200ul in each well
Remove the volume from the plate equal to the treatment well in the wells.
Each plasmid will have 2 treatment concentrations. Attention: put medium in all wells of the margin, wells blank.
Gray wells are from pBrasilia, pBAD red and pLAC blue, the last column is white, and should be placed in it middle. The upper half is glucose and the lower glycerol.

	1	2	3	4	5	6	7	8	9	10	11	12
A		pBAD					pLAC			pBRA		
B		ARA 0,2%	ARA 0,2%	ARA 1%	ARA 1%	IPTG 0,1 mM	IPTG 0,1 mM	IPTG 1 mM	IPTG 1 mM		blank	
C		ARA 0,2%	ARA 0,2%	ARA 1%	ARA 1%	IPTG 0,1 mM	IPTG 0,1 mM	IPTG 1 mM	IPTG 1 mM		blank	
D	glicose 0,2%	ARA 0%	ARA 0%	ARA 0%	ARA 0%	IPTG 0mM	IPTG 0mM	IPTG 0mM	IPTG 0mM		blank	
E	glycerol 0,2%	ARA 0,2%	ARA 0,2%	ARA 1%	ARA 1%	IPTG 0,1 mM	IPTG 0,1 mM	IPTG 1 mM	IPTG 1 mM		blank	
F		ARA 0,2%	ARA 0,2%	ARA 1%	ARA 1%	IPTG 0,1 mM	IPTG 0,1 mM	IPTG 1 mM	IPTG 1 mM		blank	
G		ARA 0%	ARA 0%	ARA 0%	ARA 0%	IPTG 0mM	IPTG 0mM	IPTG 0mM	IPTG 0mM		blank	
H												

for wells with ARA 0.2%:
1ul of ARA (if it is 40% stock)
0.8 ul ARA (if stock is 50%)

for wells with ARA 1%:
5ul of ARA (40% stock)
4ul of ARA (if stock is 50%)

for wells with 0.1 mM IPTG:
0.2 µl IPTG (100mM stock)
for wells with 1 mM IPTG:
2 ul IPTG (100mM stock)

Put the card in the reader
Schedule to take 1 hour readings (Need to shake) and stay until tomorrow morning.
Reading wavelengths: lm1 434, 477 and lm2 503, 543

Antibiotic concentration

	antibiotico	[c] uso	resistência
PJFR1	Chloramphenicol	35 ug/ml	camR

PJFR2	Spectinomycin and Streptomycin	100 ug/ml spect	specR
PJFR3	trimethoprim	10 ug/ml	tnmR
PJFR4	ampicilina	100 ug/ml	ampR
PJFR5	ampicilina	100 ug/ml	ampR
PJFR6	ampicilina	100 ug/ml	ampR