April 20th, 2011

Inoculum and Xylene addition

Materials:

- LB broth 500ml
- 24 Falcon tubes
- 10ml pipette
- Xylene solutions (0.3-0.4Mm)
- Micropipette
- Tips
- Gloves

Procedure:

1. Drop 10ml of LB broth in each falcon tube
2. The tubes are prepared with the following concentrations (Replications were labeled as A1 and A2)

Label	Containing	[Xylene+ Ethanol]	Test
A1, A2	10 ml LB	.3mM	1 y 2
B1, B2	10 ml LB	.4mM	1 y 2
C1,C2	10 ml LB	.5mM	1 y 2
D1,D2	10 ml LB	.6mM	1 y 2
E1,E2	10 ml LB	.7mM	1 y 2
F1,F2	10 ml LB	.8mM	1 y 2

3. Also were made 6 negative controls

Label	Containing	[Xylene+ Etanol]	Test
A-	10 ml LB	0m M	1 y 2
B-	10 ml LB	0m M	1 y 2
C-	10 ml LB	0m M	1 y 2
D-	10 ml LB	0m M	1 y 2
E-	10 ml LB	0m M	1 y 2
F-	10 ml LB	0m M	1 y 2

4. Note that all the xylene-ethanol concentrations were added after incubation 24h, 37°c at 100rpm
5. In order to have a spectrophotometer reference to measure the turbidity of the cultures we also prepared A-F tubes with 10ml LB + Xylene-Ethanol in the accurate concentration (.3mM-. 8Mm)

April 26, 2011

Materials

- 12 Falcon tubes
- 60ml of LB Broth
- 2.4 ml Xylene

Procedure

1. The concentrations from the yesterday experiment were read at 600nm
2. We found a new reference that points that we could neglect the xylene volatility and use it without the ethanol (Martha Eugenion Audiffred Mayorsonl, 2008), therefore we prepare the following tubes:

	Control	1%	5%	10%
LB	5mL	4.95mL	4.75mL	4.5ml
Xylene	0mL	.05mL	.25mL	.5ml

3. The medium were inoculated and incubated 37°C at 100rpm overnight.

April 27th, 2011

1. We read at 600nm in the spectrophotometer.

May 4th , 2011

1.- Growth of E. coli at new concentrations (5,6 and 7%). There were used 250 ml of LB Broth and 200 ml of LB Agar.
2.- After autoclaving the media, we proposed adding xylene to lb Agar

June 20, 2011

E. coli culture

1.- There were prepared 500 ml of LB Broth

- 5 tubes with 5 mL LB Broth (Falcon).
- 3 tubes with E.C media.

2.- LB tubes and two of E.C presented growth. Only one tube of E.C didn't have turbidity.

3.- Preparation method and culture of E.C.

- Disolve 14.8 g of EC (powder) in 200 ml of water.
- Autoclave 121 ºC for 15 minutes. Incubate 44.5 ºC 24-36 hours. Formation of gases must be shown.

June 23, 2011

1.- 2 colonies were re-sowed in tubes with LB Broth os strain BW2778 → 11:30 am.

2.- 2 colonies were re-sowed in tubes with LB Broth of strain DH5α → 11:30 am

3.- 3 tubes of strains were re-sowed, 5 ml of LB Broth+ isolated colony.

K-12	1
DH5α	3
BW2778	2

Incubation is needed for 32 h at 37 ºC, 112 RPM.

Solution of $CaCL_2$* $2H_2O$

Preparation of 500 mL 0.1 M (1x)

- 7.3505 g $CaCl_2$ * $2H_2O$
- 500 mL H_2O

June 24, 2011

Tubes that were re-sowed yesterday presented turbidity at 20 hrs after being sowed.

Buffers' preparation

1.- TRIS Buffer: 0.1 M

For 500 mL: 6.1 g Tris base+ 2.21 mL Concentrated HCl.

2.- TE: Tris-EDTA

For 500 mL 10X: 6.05 g Tris base+10 mL 0.5 M EDTA (pH 8).

3.- TAE: Tris-Acetate-EDTA

For 500 mL 10x: 500 mL 10x+24.2 g Tris base+10 mL 0.5 M EDTA (pH 8)

4.- TBE: Tris-Borate-EDTA

For 500 mL 5x: 27 g Tris base+10 mL 0.5 M EDTA (pH 8).

Preparation of competent cells:

1.-Tubes that were inoculated yesterday presented turbidity and sedimentation.

2.- From each of the 3 tubes (AV1,AV2 and AV3), 0.3 mL were extracted and 3 tubes were inoculated with 3 mL of LB.

11:30 AM→ Incubate at 37 ªC with agitation.

1.- OD_{600} was 0.361

2.- Tubes with 3mL was moved in an ice bath for 10 minutes.

3.- We centrifuged at 6krpm for 3 minutes. We discarded supernatant and resuspended in cold $CaCl_2$ (10 mL to each tube) and left in rest with ice for 20 minutes. We repeated the centrifugation and resuspended pellet with 5 mL of $CaCl_2$ 0.1 H/15% Glycerol.

June 25, 2011

1.- Competent E. coli (tubes of June 24) that wre frozen at -73 ºC formed crystals.

2.- New samples of DH5α , K12 and BW were prepared.

3.- 0.3 mL of each bacteria and LB Broth was added to new tubes.

4.- There were incubated for 2:30 hours y OD_{600} was measured.

Results

DH	0.311
K12	0.037
BW	0.424

5.- 7 Petri plates with LB Agar+Ampicillin+Arabinose for evaluation of competent cells

LB Agar → 120 mL

Amp → 600 μ

Ara → 1.98 mL

Also, it was prepared a Buffer 60% glycerol (50 mL)

Glycerol → 30 mL

$CaCl_2$ (0.1 M) → 2 mL

Asses to 50 mL

Eppendorf tubes were labeled with numbers C= frozen with glycerol 15%

1	BW27783 (24/6)	C
2	DH5α (24/6)	C
3	DH5α (24/6)	
4	K12 (24/6)	C
5	K12 (25/6)	
6	BW27783 (25/6)	

In incubator: 6 eppendorf tubes, 6 petri plates (LB/Amp/ Ara), 1 petri plates.

June 29, 2011

1.- Competent cells were prepared

1 colony BW27783 + 5 mL LB Broth.

1 colony k12 + 5 mL LB Broth

1 colony DH5α + 25 mL Broth.

1 colony BW27783 + 25 mL LB Broth.

1 colony k12 + 25 mL LB Broth

1 colony DH5α + 25 mL Broth.

Those with 25 mL were incubated for 3 hours, those with 5 mL , 24 hours.

They were incubated at 37 ºC

June 29, 2011

Bacteria (BW27783, K12, DH5α) were placed in refrigerator after being inoculated.

Spectrophotometer was used to measure OD_{600}:

BW27783	0.671
K12	0.502
DH5α	0.414

K-12 was incubated two extra hours because it didn't reach the minimum value of 0.3 at $OD_{600.}$

Competent cells' protocol

1.- Ice → 10 minutes

2.- Centrifugation

3.- Discard supernatant and add 10 mL $CaCl_2$ 0.1 M.

4.- Incubation in ice.

5.- Discard supernatant, add 0.1 M $CaCl_2$/ 15 % glycerol and freeze at -80 ºC.

June 30, 2011

For 24 h-culture the same protocol for competent cells was followed.

1.- 0.3 mL was taken of each culture and were transferred to different Falcon tubes.

2.- 3 mL of LB Broth was added to each tube.

3- They were incubated at 37 ºC for 3 hours at 100 rpm.

Labels:

IGEM E. coli BW27783 30/06/11	IGEM E. coli K12 30/06/11	IGEM E. coli DH5α 30/06/11

Absorbance at OD_{600} was measured for each bacteria:

BW27783 → 0.467
K12 → 0.375
DH5α → 0.321

Transformation of cells to competent cells:

1.- Incubation in ice (10 minutes).

2.- Centrifugation at 3500 rpm (6 minutes).

3.- Discard supernatant and add of $CaCl_2$ 0.1 M.

4.- Incubation in ice for 20 minutes.

5.- Discard supernatant and add 5 mL of $CaCl_2$ 0.1 M/ 15 % Glycerol

Note: Adaptation of original protocol. It suggests inoculation of 1 mL of E. Coli in 100 mL LB Broth. We adapted to 1 mL of E. Coli in 10 mL of LB (1/10).

June 30, 2011

LB Agar was prepared (200 mL), only 3 plates with ampicillin 1mL

The rest (200-(3x-20)= 140 mL was prepared with arabinose (2.31 mL for 140 mL LB).

10μl of plasmid were prepared and they were left in rest for 5 minutes in order to achieve a proper hydration.

6 eppendorf were labeled.

July 6, 2011

Miniprep

LB Broth preparation

For each liter of LB Broth 20 g of LB powder are needed.

1 lt LB → 20 g LBP For 50 mL LB → 1 g LBP is needed

5 mL of LB Broth were prepared in a flask. It was autoclaved in wet cycle for 30 minutes.

<u>GTE Buffer Preparation</u>

1.8g glucose 50 mM

0.6067 g Tris CL 25 mM

0.744 g EDTA 10 mM

200 ml dH_2O

<u>EDTA</u>

0.744 for 1000 mL

NaOH

1.6 g NaOH 0.2 M

2 g SDS

198 H_2O mlQ

July 8, 2011

Re-incubation of bacteria

Preparation of 6 tubes with 10 mL of LB/Amp (50 mg/mL), 6 tubes with 10 mL LB/Amp/Kan and 6 tubes with 10 mL LB/Kan.

Today only 6 tubes were prepared with LB/Amp (50 mL) and ampicillin (50 mL)

Note: Amp: Ampicillin

Kan: Kanamicin

50 μl antibiotic/ 10 ml LB broth

Bacteria used were:

- E. coli: BW27783 (pSBIA3) from 01/07/11 LB/Amp.
- E. coli: k12 (psBIA3) from 01/07/11 LB/Amp.
- E. coli DH5-α (PSBIA3) from 01/07/11

Plate 3: A17 with IGEM part BBa_ k123009

- E. coli K12 pGLO LB/Amp/Ara
- E. coli: BW27783 pGLO LB/Amp/Ara
- E. coli: DH5-α pGLO LB/Amp/Ara

pGLO

Of the groups of 6 plates, one colony was taken and was transferred to one tube. Bothe experiments (psBIA3 and PGLO) have resistance to ampicillin, which will be needed for MINIPREP

3 tubes were made with Amp/ Ara/LB Broth 165 μl/10 ml (0.2 g Arabinose/ml).

Procedure:

Re-inoculation 14:30 pm 08/07/11 for miniprep

One colony was added.

Tubes were rotulated.

E. coli strain	Experiment	Ampicilina (μl)	Lb Broth (ml)
BW27783	pGLO*	50	10
DH5-α	pGLO *	50	10
K12	pGLO*	50	10
BW27783	pSBIA3	50	10
DH5-α	pSBIA3	50	10
K12	pSBIA3	50	10

*0.165 μl arabinose was added. One green colony and one cream colony were taken.

Tubes were incubated at 37 ºC and 140 rpm for 36 hours.

Agar preparation

3 LB Agar flasks with 100 ml of dH_2O dissolving 3.5 g of agar to each one.

They were autoclaved.

Labels:

A: 500 μl of ampicillin.

K: 500 μl of kanamycin

AK: 250 μl ampicillin + 250 μl kanamycin

Nelson, Laura

Plate 1 Parts:

AK-2B-pSBIAK3-BBa_J52028_GFP

K-5A-pSBIK3-BBa_J04450-RFP

3 tubes containing competent cells were taken. They were maintained in ice in order to defrost (aprox 1 h 30 min)

From plate 1 of Biobrick we used wells 2B and 5A. 100 μl of competent cells in two different eppendorf tubes labeled as:

K-LC-08/08/11-BBa_J04450

AK-LC-08/07/11-BBa_J52028

After 5 minutes 3 μl of resuspended DNA to the corresponding tube. After 30 minutes, competent cells were placed in refrigerator (-68 ºC).

Bath was prepared at 41.5 ºC and incubator was programmed at 37 ºC, 140 rpm and 2 hours. We placed both tubes to the bath, they were maintained for 2 minutes and immediately returned to ice.

Incubation

N.C-08/07/11	BBa-J04450	200 μl
N.C-08/07/11	BBa_J04450	20μl
N.C-08/07/11	BB_J052028	200μl
N.C-08/07/11	BB_J052028	20μl

Nelson/Laura

Incubation of six tubes 37 º C, 140 rpm, 36 hours → No growth after 12 hours.

10/07/11

Laura, Aldo, David, Prashant

Bacteria from 08/07/11 that presented growth: K-12 PGLO, DH5-α PGLO, BW27783 PGLO

Miniprep: New protocol obtained biotech.biology.arizona.edu/labs/DNA/isolate_plasmid.html. The procedure was made twice.

Transformation using competent cells, incubation in plates with LB/Amp/Ara.

11/07/11

Mariana, Israel

Plasmid extraction

MP1=X1

MP2=X2

MP3=X3

No DNA was extracted, maybe Potassium Acetate diluted in miliQ was the reason of this result.

Plamids (AK1, AK2, AK3) were obtained. BBa_J5208 LB/AK 10/07: P2A, P2B, P2C.

Electrophoresis→ Use of 0.8% agarose/100 mL TAE 1X; use of 15µl DNA sample+ 5µl orange loading dye 5x.

12/07/11

Miniprep (BBa_J04450) and PGLO

Electrophoresis Gel

Plamids BBa_j5208 and BBa_J04450 and PGLO, Plasmid stock at -22 ºC.

Centrifugation 8500 rpm.

Nelson, Laura

Transformation 10 µl of each plasmid

pGLO_BW	K1 I	AK1	P2A
pGLO_K12	K2 D	AK2	P2B
	K3 P		

100 µl of competent cells BW for pGlo and all of them with K12; 200 µl of LB.

13/07/11

Nelson, Laura

Photograph of plates inoculated 12/07/11 showed that BBa_J04450 worked. For pGLO the inductor arabinose was left, for BBa_J52028 only showed fluorescence 2 hours, it is believed that ipt6 is the inductor.

Four Falcon tubes were prepared:

LB+Amp+Ara	pGLO BW	10 ml LB/165 μl Ara/50 μl Amp 37ºC, 140 rpm
LB+Amp+Ara	pGLO K12	
LB+Amp+Ara	BBa_J52028	
LB+Amp	BBa_J52028	

Incubation for 2 hours and were observed with transiluminator. No growth was observed.

pGLO of plates BW and K12 only contained LB/Amp. Incubation for 20 hours→ Plasmid extraction.

In order to know if there was fluorescence in the culture, part of the Falcon tubes was centrifugated and observed in the transiluminator were the pellet showed fluorescence.

A transformation was made (6 pieces).

22/07/11

Piece resistant to kanamycin (Bba_K091190→ 9B)

Transformation protocol:

1. Plamisd was rehydrated with dH_2O → 5 minutes.
2. 2.3 μl plasmid for each 100 μl of bacteria.
3. Strains (DH5-α, BW27183) were used.
4. Ice for 30 minutes.
5. Hot water bath 2 minutes at 42 ºC.
6. Ice for 5 minutes.
7. 200 μl of LB and it wa sincubated for 2 hours at 37 ºC.

The goal of this experiment is to prove that bacteria were resistant to kanamycyn because of transformation.

03/08/11

Minipreps (ampicillin pieces): I, II, XVII, XI, XII, XIII, XIV, XV, XVI, XVII.

Glycerol solution preparation

60 ml- Glycerol

36 ml-dH_2O

4 ml-$CaCl_2$ 0.1 M

08/08/11

TB (1L)

- Bacto tryptone 12 g/l
- Yeast extract 24 g/l
- KH_2PO_4 0.17 M (For 500 mL dH_2O, 11.567 g)
- K_2HPO_4 0.72 M (For 500 mL dH_2O, 67.705 g)
- Glycerol 4 mL

1. Mix of tryoptone, yeast ans glycerol.
2. Autoclave liquid cycle
3. Add 100 mL phosphate solutions.

SOB (1L):

Final concentration:

20 g Bacto tryptone	0.5 %
5 g Yeast Extract	2%
0.584 g NaCl	10mM
0.186 g Kcl	2.5mM
2.4 g $MgSO_4$	20mM
25 mL NaOH pH= 7.5	

SOC (1L):

- 1 L SOB
- 20 mL glucose solution (20%).

$CaCl_2$ (500 mL 0.1 M):

7.35 g

500 mL dH_2O

08/08/11

- 500 mL $CaCl_2$ 0.1 M
- 100 mL $CaCl_2$ 0.1 M/Glycerol 15 %
- 500 mL SOB
- 500 mL SOC

- 100 mL Potassium phosphate solution
- 100 mL NaOH 1 M
- 100 mL glucose 20%

12/08/11

Four endonuclease digestions

26/08/11

QIAquick PCR Purification Kit Protocol

220 ml of ethanol (96%) was added to PE Buffer.

600 µl of pH indicator was added to PB buffer.

Procedure:

- 600 µl PB Buffer was added to 120 µl of DNA sample.
- Supernatant discarded.
- They were transferred to the columns → Centrifugation 13 krpm for 1 minute.
- 750 µl was added to the column and again, it was centrifuged 1 minute at 13 krpm.
- Supernatant was discarded → Centrifugation 13 krom 1 minute
- The column was placed inside microcentrifuge tube→ 50 µl of EB buffer was added; centrifugation 1 minute at 13 krpm.

27/08/11

Twelve samples were prepared

Two buffers: EcoR1, Bpe1, NEB2

Three DNA concentrations: 5µl, 10µl, 20µl.

5µl DNA	10 µl DNA	20 µl DNA
1µl EcoR1	1.5 µl EcoR1	2 µl EcoR1
1µl Spe1	1.5 µl Spe1	2 µl Spe1
5µl Buffer	5 µl Buffer	7.5 µl Buffer
38µl NFH_2O	32 µl NFH_2O	18.5 µl NFH_2O

DNA/RNA assay

09/09/11

BW27787 and DH5α were acclimated and cultivated in LB agar plates.

10/09/11

The plates showed growth.

Transformation:

- Miniprep XX
- Kit

0.5 µl of plasmid solution were diluted in 50 µl of miliQ water. 50 µl of that solution were taken for transformation.

09/09/11

Digestion in BSA

Pieces Bba_J04450 (XX, XXI, kanamycyn and ampicillin resistance).

Pieces were digested with EcoR1 and SpeI using NEB_2 Buffer ; backbones with EcoR1 and SpeI using $REACT_2$. BSA will be added to all the samples, final volume: 25 µl .

- 10 µl DNA
- 1 µl DNA
- 1 µl sPEI or PstI
- 5µl NEB_2 or $REACT_2$.
- 0.5 µl BSA
- 32.5 µl NF-H_2O.

Tubes

0) XXI
1) XXI
2) XXI
3) XXI
4) XXI
5) pSBIA3
6) pSBIA3
7) pSBIK3
8) pSBIK3

15/09/11

Results (Growth on plates and tranformation tubes)

Growth	No growth
Agar	BXXIII and DXXIII
DXIX and BXIX	DV
BXX and DXX, BX and DX	BV
BXXII and DXXII	DXXI
BXXI	DXXX and BXXX
DXXIV and BXXIVBXVIII and DXVIV	
Tubes	
D-XXII D-XXIV	D-XXX
D-XVIII D-XX	B-XXX
D-V D-XXI	
D-XXIII D-XIX	
D-X	

19/09/11

For digestion:

EcoRI and SpeI	**EcoR1**	**Master Mix**
10 µl DNA	10 µl DNA	10 µl React 2 Buffer
1 µl EcoR1	1 µl EcoR1	1.25 µl EcoR1
1 µl SpeI	1 µl SpeI	1.25 µl SpeI
5 µl Neb2 Buffer	5 µl React2 Buffer	2.5 µl BSA
0.5 µl BSA	0.5 µl BSA	35 µl NF H_2O
32.5 µl NF+H_2O	32.5 µl NF+H_2O	
50 µl	**50 µl**	**50 µl** (10 µl for each tube with 5 µl of DNA)

I-Const O-D, original glycerol 1409 stock
II-RBS-D, refrigerator 1409
X-ORS-D 1609, glycerol
XI-λ-D, 1409, glycerol
XIV-GFP-B 1409, glycerol
XV-Stop D 2009 incubator
XVIII-RecA D 2009 incubator
XX-Crx-st D 1609 glycerol
XXI Ta-st D 1609, glycerol
XXIV ITAST D 1909 incubator
XXX- Bba_J04450

Tb media

Corresponding antibiotic (5 μl)

100 μl of original culture were taken.

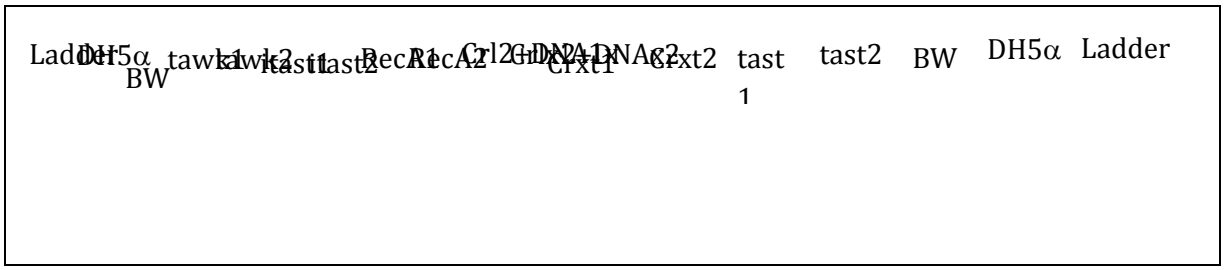
26/09/11

Miniprep (PureYield ™ Plasmid Miniprep System Protocol)

1) tawk	7) Cr12+DNAx
2) itast	8) Cr12+DNAx ‘
3) RecA	9) Crx-st
4) DH5α	10) Crx-st ‘
5) itast’	11) ta-st
6) tawl ‘	12) Rec A

Electrophoresis Gel (1% agarose; lithium acetate 1X prepared 23.09, 27.09)

- 4 μL ladder Fermentas.
- Sample 15 μL + Dye 0.5 μL



27/09/11

Ligation were nada with the following concentrations

- 3μl digested DNA insert
- 3μl digested backbone
- 2μl10x T4 DNA ligase buffer
- 1μl T4 DNA ligase
- 11μl dd H_2o

Final volume per tube: 20μl

6 different DNA insert were used:

1. Ta st
2. Ita st
3. Ta wk
4. Crx st
5. Cr12 DNAα
6. Rec A

Incubation 10minutes @ Room temperature; heat incubation at 80°C for 20min in water bath.