

Colony PCR

Introduction

Quickly test a transformation with confirmation primers.

Materials



Procedure

PCR

Label at least 8 colonies on a plate with a marker (circle them and assign a number)

Pick and dissolve each of the colony in 20 ul of mQH2O

Heat at 80°C for 10' then set the following PCR reaction

This protocol is for Taq polymerase, which is cheaper than high fidelity polymerase like Phusion. No fidelity in sequence is required here, just an active enzyme that can polymerize a linear PCR product in presence of the proper primers. In case you use another polymerase, then follow its specific protocol. Here it's used the HF buffer for Phusion which was proven to be functional also for Taq polymerase.

	A	B	C
1			25
2	mQH2O	15.4	385
3	5X HF buffer	5	125
4	10 mM dNTPs	0.5	12.5
5	10 uM F	0.5	12.5
6	10 uM R	0.5	12.5
7	Colony (in 20 ul H2O)	3	
8	Taq DNA pol	0.1	2.5
9		25	

Set the following protocol on a thermocycler

1. 95°C 2'
2. 95 °C 30"
3. Tm 30"
4. 68 °C 1'/kb

5. Go to step 2 for 24 additional times

6. 68 °C 5'

7. 16°C ∞

Run the PCR product on an agarose gel and check the result

Pick the good colony from the plate and let them grow for minipreps

Electroporation

Introduction

Protocol used for fast transformation of a strain with a desired plasmid. No chemical competent cells needed.

Materials

- › o/n culture of desired strain
- › 10% glycerol
- › ice
- › ddH₂O
- › Benchtop centrifuge
- › Electroporation cuvette
- › Electroporator
- › desired Plasmid
- › Plates with appropriate antibiotics
- › LB medium (no AB)

Procedure

Day 1

1. 1. Set up an overnight culture. Pick one or two colonies and inoculate them in microfuge tubes containing 1 mL LB medium and punctured a hole in the lid for air. Incubate at 37°C overnight with shaking.

Day 2

Before starting:

Chill 10% glycerol (ddH₂O) on ice for an hour

Chill electroporation cuvettes (1 mm/2mm gap).

Cool benchtop centrifuge to 2°C.

1. Set up microfuge tubes (with punctured hole in the lid for air) and add 1.4mL fresh LB medium, add 30 µl of overnight culture.

2. Culture for 1-3h at 37°C, shaking at 1000 rpm.

3. Prepare the cells for electroporation -

Centrifuge for 30 sec at 11,000 rpm in a cooled microfuge benchtop centrifuge (at 2°C). Discard the supernatant by quickly tipping it out, and place the pellet on ice.

Resuspend the pellet with 1 ml chilled 10% Glycerol, pipetting up and down a few times to mix the suspension.

Repeat the centrifugation and resuspension of the cells again. Centrifuge and tip out the supernatant once more leaving 100-150 µl left in the tube with the pellet.

4. Take DNA of your choice, add 40ng Plasmid / 400-800ng Linear DNA to your cell pellet. Mix briefly pelleted cells with added DNA.

Transfer the cell suspension from the tube to the chilled electroporation cuvette. If volume does not reach the top of the electrode add remaining with 10% glycerol

5. Electroporate at 1800V for 1mM and 2500 for 2mM, (outputted 4-5 ms pulses are the norm).**6. Resuspend the electroporated cells in 1 ml LB medium without antibiotics and return them to the microfuge tube.****7. Incubate at 37C 1h, shaking at 1000 rpm.****8. Plate on LB plates with proper Antibiotics + supplementations needed.****Electroporator Instructions**

Turn on - hit "on-Standby"

Click ^ v same time to get 1800 or 2500 Volt

Load cuvette in (dry its exterior)

X2 fast clicks on "pulse" will start the shock, wait until machine beeps to know its finished.

Error Prone PCR (Derr et al. 2005)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

>

Procedure

Error-prone dNTPs mix

	A	B	C
1	dNTPs mix	mM in 50 uL	10x stock
2	dATP	0.2	2
3	dGTP	0.2	2
4	dCTP	1	10
5	dTTP	1	10

PCR mix

	A	B	C	D	E	F
1		50	Final concentration (mM)			
2	MgCl ₂ (100 mM)**	1.65	3.5	2.5		
3	MnCl ₂ (10 mM)	1.25	0.25	25 uM		
4	dNTPs error mix (10x stock)	5		0.2 uM	add only 1 ul	
5	Primer FW (10 mM)	10	2		1 ul	
6	Primer RW (10 mM)	10			1 ul	
7	Template (10 ng/ul)	5	50 ng			
8	Taq (5000 U/mL)	0.5	2.5 units		1 ul	
9	Taq buffer	5				
10	H ₂ O	11.6				

**Note, the Taq buffer already contains 1.5 mM MgCl₂. So this calculation is already accounting for that amount. See below:

Table3

	A	B	C	D	E
1	$Ca \cdot Va + Cb \cdot Vb = Cf \cdot Vf$				
2	Ca= mM	100	Va=	1.675	
3	Cb= mM	1.5	Vb=	5	
4	Cf= mM	3.5	Vf=	50	

PCR program

1. 94 °C for 45 s
2. 94°C for 45s
3. 65°C for 45s
4. 72°C for 2min
5. repeat step 2-4 29 times
6. 72 °C for 10 min

Error Prone PCR (Luzi protocols)

Introduction

In this protocol there is a summary of the conditions to play around with and how to do so to get the most mutations.

Materials

>

Procedure

1.

	A	B	C	D	E	F
1		50	Final concentration (mM)			
2	MgCl ₂ (100 mM)**	1.175	2.5			
3	MnCl ₂ (10 mM)	0.125	0.025			
4	dNTPs error mix (10x stock)	1	0.2 uM			
5	Primer FW (10 mM)	1				
6	Primer RW (10 mM)	1				
7	Template (10 ng/ul)	5	50 ng			
8	Taq (5000 U/mL)	1				
9	Taq buffer	5				
10	H ₂ O	34.7				

2. **Note, the Taq buffer already contains 1.5 mM MgCl₂. So this calculation is already accounting for that amount. See below:

3.

	A	B	C	D	E
1	MgCl ₂				
2	$Ca \cdot Va + Cb \cdot Vb = Cf \cdot Vf$				
3	Ca= mM	100	Va=	1.175	
4	Cb= mM	1.5	Vb=	5	
5	Cf= mM	2.5	Vf=	50	

	A	B	C	D
1	MnCl ₂			
2	C _i V _i =C _f V _f			
3	C _i	10	V _i	0.125
4	C _f	0.025	V _f	50

4. The first condition to test: gradient of manganese. Add different concentration and observe the smear. Then pick the condition that has the highest concentration of Mn but with a decent quantity of DNA.
5. Second parameter to play around with: temperature. Try either a gradient of temperature or a touch down.

Error Prone PCR (Tobias)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

>

>

Procedure

1. **This protocol is a general procedure for random mutagenesis onto any gene of interest. Error rate can best be optimized by adjusting the content of MnCl₂ or of the template (here 10 ng template concentration is used).**

2. PCR mix:

	A	B
1	5 µl	10x Taq DNA polymerase buffer
2	5 µl	25 mM MgCl ₂
3	0.5/1/2 ul	1 mM MnCl ₂ (end conc. 0.01/0.02/0.04 mM)
4	5 µl	10x error-prone dNTPs (2 mM GTP and ATP, 10 mM CTP and TTP)
5	1 ul	Template 10 ng/ul (10 ng end amount)
6	5 ul	Forward primer (10 µM)
7	5 ul	Reverse primer (10 µM)
8	0.5 ul	Taq polymerase
9	23 ul	ddH ₂ O

3. Cycling (20 cycles):

4. 95°C 1 min

5. 95°C 20 sec

6. T_A 20 sec

7. 72°C 2 min

8. 72°C 10 min

9.

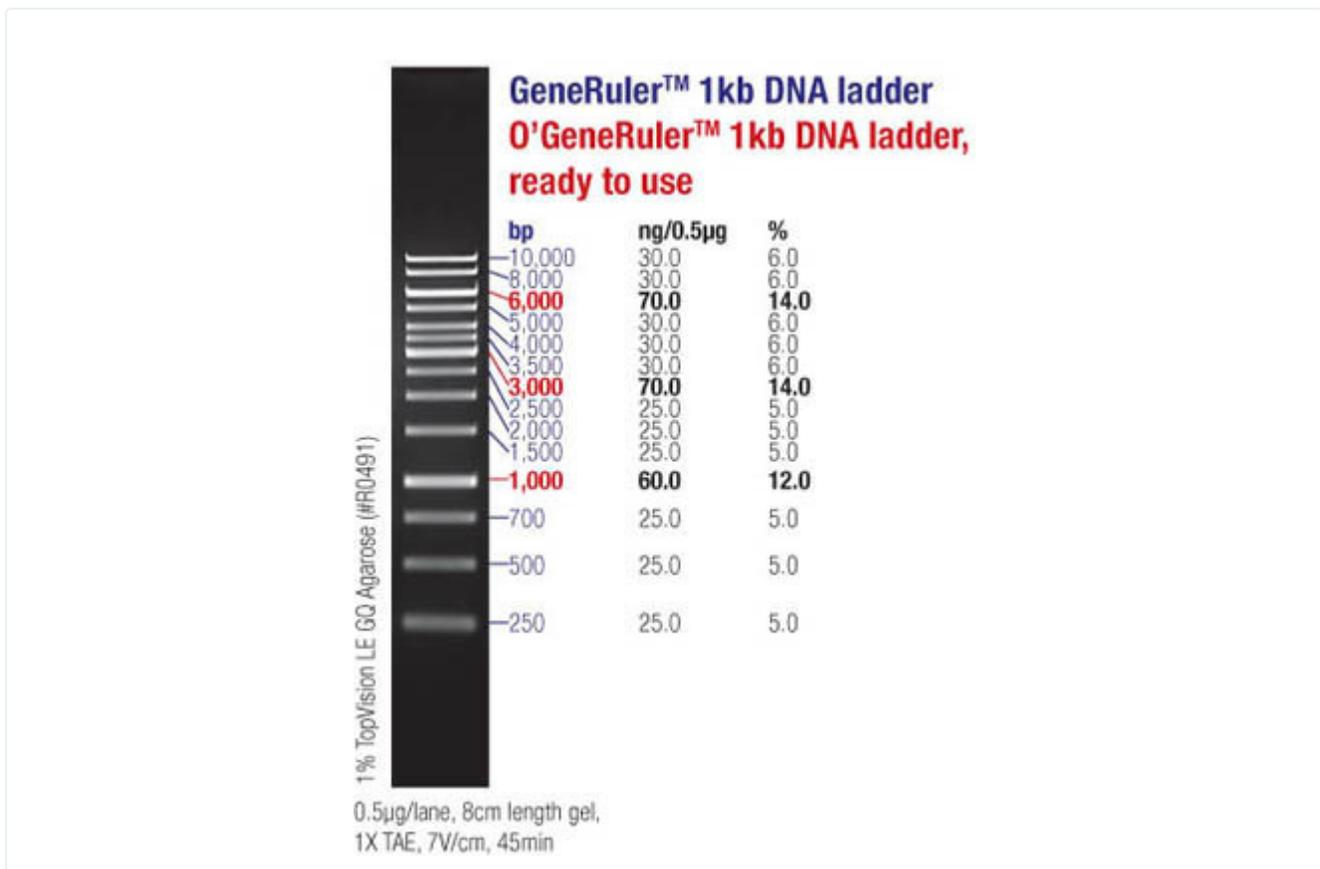
GeneRuler 1 kb band sizes

Introduction

Materials

>

Procedure



Gibson Assembly® Protocol (E5510)

Introduction

This is the protocol for Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510). More information from NEB can be found [here](#).

Materials

› Gibson Assembly Cloning Kit

- › Gibson Assembly® Master Mix
- › NEBuilder® Positive Control
- › NEB® 5-alpha Competent *E. coli* (High Efficiency)
- › SOC Outgrowth Medium
- › pUC19 Transformation Control Plasmid

› DNA Polymerases (for generating PCR products)

- › **Recommended:** [Q5® High-Fidelity DNA Polymerase](#), [Q5 Hot Start High-Fidelity DNA Polymerase](#), or [Q5 Hot Start High-Fidelity 2X Master Mix](#)

› LB (Luria-Bertani) plates with appropriate antibiotic

Procedure

Set up the following reaction on ice:

1. Reaction volumes: *Use this table to calculate reaction volumes and set up the reaction. Remember to **input your total DNA fragment volume in cells B3 and C3** for assemblies with 2-3 fragments and 4-6 fragments, respectively.*
2. *Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess insert*
- 3.

	A	B	C	D	E
1		Insert ng/ul	Insert	Backbone ng/ul	Backbone
2	EG102C	15	15 ul	20	5 ul
3	EG103C	15	15 ul	18	5 ul
4	EG104C	14	15 ul	11	5 ul

Table1		
↖	A	B
1		2-3 Fragments Assembly
2	Concentration Range of DNA fragments	02 - .5 pmols*
3	Total Volume of Fragments (µl)	20
4	Gibson Assembly Master Mix (2x) (µl)	30
5	Deionized Water (µl)	10
6	Total Volume (µl) ***	60

4. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled.

Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see [FAQ section](#)).

**Select the pencil icon and change the timer to 60 minutes if working with 4-6 fragments*

00:15:00



5. Store samples on ice or at –20°C for subsequent transformation.
6. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the [chemical transformation protocol](#) or [electro competent cells transformation protocol](#)
- 7.

Gibson assembly: One-step isothermal DNA assembly protocol

Introduction

Tobias: You'll probably have to do a lot of Gibson assemblies in the coming weeks. As the master mix is really expensive, it makes sense to prepare it yourself. Here's the protocol and recipe we use in our lab. We have the necessary chemicals and as you only need very little T5 exonuclease, you can also steal it from us ;) However, you should get Taq DNA ligase and a "dNTP Set 100 mM Sol. (4 x 0.25ml)" from the shop, they should have it in stock.

If you haven't done so already, I would also recommend to take advantage of the NEB offer to iGEM teams and get a free BioBrick Assembly Kit or something like that.

Materials

- › 6ml of 5x isothermal reaction buffer, prepared by combining:
 - › 3 ml of 1 M Tris-HCl pH 7.5
 - › 150 µl of 2 M MgCl₂
 - › 60 µl of 100 mM dGTP
 - › 60 µl of 100 mM dATP
 - › 60 µl of 100 mM dTTP
 - › 60 µl of 100 mM dCTP
 - › 300 µl of 1 M DTT
 - › 1.5 g PEG-8000
 - › 300 µl of 100 mM NAD
- › 10 U/µl T5 exonuclease
- › 2 U/µl Phusion DNA polymerase
- › 40 U/µl Taq DNA ligase
- › ddH₂O

Procedure

Assembly master mixture

1. An assembly master mixture was prepared by combining:

- 320 µl 5x isothermal reaction buffer
- 0.64 µl of 10 U/µl T5 exonuclease
- 20 µl of 2 U/µl Phusion DNA polymerase
- 160 µl of 40 U/µl Taq DNA ligase
- water up to a final volume of 1.2 ml

2. This reagent-enzyme mix is aliquoted (15 μ L aliquots) and stored at -20 $^{\circ}$ C.

This mixture can tolerate numerous freeze-thaw cycles and remains stable even after one year.

The exonuclease amount is ideal for the assembly of DNA molecules with 20–150 bp overlaps.

For DNA molecules overlapping by greater than 150 bp, 3.2 μ l of 10 U/ μ l T5 exonuclease was used to prepare the assembly master mixture above.

Frozen 15 μ l assembly mixture aliquots were thawed and then kept on ice until ready to be used.

Five microliters of the DNA to be assembled were added to the master mixture in equimolar amounts. Between 10 and 100 ng of each 6 kb DNA fragment was added.

For larger DNA segments, proportional amounts of DNA were added (for example, 250 ng of each 150 kb DNA segment).

Incubations were performed at 50 $^{\circ}$ C for 15 to 60 min (60 min was optimal).

One-step isothermal DNA assembly protocol

3. PCR up the DNA fragments to be cloned (each primer overhang is min 20 nt).
4. Cut open the backbone.
5. Heat inactivate the enzymes if cut open an empty vector **OR** Gel purify the cut backbone if cut open a full vector.
6. Gel purify the PCR fragments.
7. Mix the backbone and PCR fragments in max 5 μ l total volume, in molar ratio 1:1.
8. Thaw reaction mixture on ice.
9. Add DNA mixture (5 μ l) to the reaction mixture (15 μ l).
10. Heat the PCR machine at 50 $^{\circ}$ C.
11. Run the reaction for 1 hour at 50 $^{\circ}$ C.
12. Transform into bacteria (1-5ul or more if necessary).

M9 Protocol

Introduction

obtained from: <https://www.protocolsonline.com/recipes/media/m9-medium-5x/>

M9 is a minimal, low osmolarity media for *E. Coli*, resulting in slower growth rate of these cells (~60 min doubling time)

Materials

› 5X Stock

- › Na₂HPO₄
- › KH₂PO₄
- › NH₄Cl
- › NaCl
- › CaCl₂ (optional)

› Additional Components for 1X Working Solution

- › MgSO₄·7H₂O
- › Carbon source (sugar or glycerol) --> glucose
- › 0.5% vitamin B1 (thiamine)
- › 20% for L amino acids to 40 ug/ml or DL amino acids to 80 ug/ml
- › [Antibiotic](#)

Procedure

Preparing the 5X stock

1. Add the following reagents to a 2-liter flask:

- 30 g Na₂HPO₄
- 15 g KH₂PO₄
- 5 g NH₄Cl
- 2.5 g NaCl
- 15 mg CaCl₂ (optional) --> take 1.351 mL from the 0.1 mM CaCl solution
- 1 liter of high quality distilled water

2. Once the ingredients are added, heat with stirring until the components are completely dissolved. Pour the solution into smaller bottles

split up into 5 x 200mL into 5 1L flasks

- loosen caps and then autoclave at 15 lb/in² for 15 min. If you wish to add antibiotics or nutritional supplements, do this only after the autoclave cycle is complete, as the high temperature may destroy these components. Wait until the bottle is less than 50°C (it should be warm to touch), and then add the components. After the bottles cool to below 40°C, the caps can be tightened and the concentrated medium stored indefinitely at room temperature.

Preparing the 1X Working Solution

- To make 1X working solution, the 5X media should be diluted to 1× with high quality sterile distilled water. Hence, add the following sterile solutions **to 200mL of M9 salts**

1 ml 1 M MgSO₄·7H₂O --> 0.246 g in 1ml
10 ml 20% carbon source (sugar or glycerol) --> glucose
789 ml distilled water

- Typically, several additional components are also added to make a complete medium.

~~0.1 ml 0.5% vitamin B1 (thiamine)~~

~~5 ml 20% Casamino Acids or L amino acids to 40 ug/ml or DL amino acids to 80 ug/ml~~

Antibiotic: add kanamycin to a final concentration of 50 µg/mL

M9 Swimming agar

Introduction

This protocol describes the swimming agar, where colonies are given around a source of molecules (Asp) to see their reaction.

Protocol taken from iGEM Göttingen 2012.

Materials

- › 1.25 % (v/v) glycerol
- › 20 % (v/v) 5x M9 salt stock solution
- › 0.1 % (v/v) of $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ stock solution (20 mg/mL)
- › 0.1 % (v/v) MgSO_4 stock solution (0.12 g/mL)
- › 0.3 % (w/v) of agar

›

Procedure

M9 Swimming Agar

1. Preparation of 500 ml of M9 swimming agar

	A	B	C	D
1	Material	Percentage	Volume/weight	volume from already existing stocks
2	Glycerol	0.0125	6.25	
3	M9 Salts	0.2	100	
4	$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$	0.001	0.5	0.9
5	MgSO_4	0.001	0.5	
6	Agar	0.003	1.5	
7	Leucine	0.0042	0.21	
8	Total volume	500		
9				
10				

M9 swimming medium v2

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

>

>

Procedure

1.

	A	B	C
1			
2	M9	500	ml
3	Glycerol	1	ml
4	thiamine HCl	500	ug
5	methionine	10	mg
6	leucine	10	mg
7	histidine	10	mg
8	agar	1.25	g

Miniprep with GenElute Plasmid Miniprep kit

Introduction

Protocol from the GenElute Plasmid Miniprep kit.

Materials

>

>

Procedure

1.

Motility Buffer

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- › 10 mM potassium phosphate KH_2PO_4 (136 g/mol)
- › 0.1 mM EDTA (292.24 g/mol)
- › 1 μM methionine (149.21 g/mol)
- › 0.4% glucose
- ›

Procedure

Mix the components in 1L dH_2O and ensure a final $\text{pH}=7$

1. add 1.36 g KH_2PO_4
2. make a 100mM solution of EDTA
 - add 292 mg in 10 mL dH_2O --> 0.1 M
 - add 1 mL of this 100mM solution to the mix
3. make a 100 mM solution of methionine
 - add 149 mg in 10 mL dH_2O
 - dilute 1:100 by adding 100 μL in 9.9 mL dH_2O (--> 1mM solution)
 - add 1 mL of this 1 mM solution to the mix
4. add 20 mL of 20% glucose
5. fill up with 978 mL of dH_2O

PCR Amplification (Phusion)

Introduction

PCR amplification of fragments or backbones

Materials

>

>

Procedure

Mix

	A	B	C	D	E	F
1						
2	mQH2O	32.5	48.75			
3	5X HF buffer	10	15			
4	10 mM dNTPs	1	1.5			
5	10 uM F	2.5	3.75			
6	10 uM R	2.5	3.75			
7	(~10 ng/ul) DNA	1	1.5			
8	Phusion	0.5	0.75			
9		50	75			

Set the following protocol on a thermocycler:

1. 98°C 30"
2. 98 °C 10"
3. Tm 10"
4. 72 °C 15-30" /Kb
5. Go to step 2 for 29 additional times
6. 72 °C 10'
7. 10°C ∞

8.

Preparation Chemically Competent Cells

Project: iGEM ETHZ 2018

Authors: Jan Krüsemann

Date: 2018-07-26

THURSDAY, 26/7/18

Preparation of CaCl₂ Chemically Competent Cells

- Grow strain of choice in 50mL Erlenmeyer until it reaches 0.3-0.5 O.D.
- Transfer to 50mL centrifuge tube and place on ice for 15 min
- Pellet cells in 4C pre-cooled centrifuge (4,000 rpm for 10 min)
- Resuspend in 25mL (half volume) ice-cold 0.1M CaCl₂
- Incubate on ice for 1h
- Pellet at 4K,000 rpm for 10 min
- Remove supernatant
- Resuspend with 4mL of ice cold 0.1M CaCl₂ +15% Glycerol
- Aliquot 50/100uL in sterile 1.5mL tubes
- Store in -80°

To Prepare:

- 0.1M CaCl₂ – 250mL

25mL 1M cacl2 and 225 ddH2O

- Send to autoclave
- 0.1M CaCl₂ 15% Glycerol 50mL

5mL 1M cacl2, 7.5mL glycerol in 37.5 ddH2O

- Filter Sterilize

Split Luciferase Complementation Assay

Introduction

Testing for split luciferase complementation via sucrose induction

Materials

- › o/n cultures
- › 20% sucrose in M9
- › **Luciferin Substrate Buffer**: 500 μ M Luciferin in 0.1 M citrate-phosphate buffer
- ›
- ›

Procedure

Dilution of o/n cultures

1. 40 μ L in 4 mL

Growth in incubator at 37 °C until ~ O.D. 1

Spin down cells (if culture not dense enough, spin down several times to increase pellet size)

2. 3000 rpm for 3 min

Resuspend in 1 mL M9 + 20% sucrose

Dilute to equal amounts

Transfer 100 μ L of resuspended culture to 96 luminescence well plate and mix with 100 μ L Luciferin Substrate Buffer or control

Read out luminescence

Tethering Medium

Introduction

As described in *Transient response to chemotactic stimuli in Escherichia coli* (Berg, Tedesco, 1975; sourijk, 2002)

Materials

- › 0.067 M Sodium Chloride NaCl (58.44 g/mol)
- › 0.01 M Potassium Phosphate KH_2PO_4 (136.084 g/mol)
- › 0.1 mM EDTA (292.24 g/mol)
- › 1 μM Methionine
- › ~~10 mM lactic acid~~
- ›

Procedure

Add these to 1L of ddH₂O

1. 3.915 g of NaCl
2. 1.36 g of KH_2PO_4
3. make a 100mM solution of EDTA
 - add 292 mg in 10 mL dH₂O --> 0.1 M
 - add 1 mL of this 100mM solution to the mix

Transformation

Introduction

This protocol is a variant of the Hanahan protocol Hanahan91 using CCMB80 buffer for DH10B, TOP10 and Mach1 strains. It builds on Example 2 of the [Bloom05 patent](#) as well. This protocol has been tested on NEB10, TOP10, Mach1 and [BL21\(DE3\)](#) cells. See [OWW Bacterial Transformation page](#) for a more general discussion of other techniques. The [Jesse '464 patent](#) describes using this buffer for DH5 α cells. The [Bloom04 patent](#) describes the use of essentially the same protocol for the Invitrogen Mach 1 cells.

This is the chemical transformation protocol used by [Tom Knight](#) and the [Registry of Standard Biological Parts](#).

Before you start:

Estimated bench time: 30 min

Estimated total time: 2 hours (plus 2x 14-16 hour incubations)

You can prepare stocks of your bacteria of interest and store at -80°C to seed future batches of competent cells.

Detergent is a major inhibitor of competent cell growth and transformation. Glass and plastic must be detergent free for these protocols. The easiest way to do this is to avoid washing glassware, and simply rinse it out. Autoclaving glassware filled 3/4 with DI water is an effective way to remove most detergent residue. Media and buffers should be prepared in detergent free glassware and cultures grown up in detergent free glassware.

Materials

- › Petri plates with [SOB](#) agar
- › Sterile loop
- › Glycerol
- › Cryotubes
- ›

Procedure

1.

Transformation Chemically Comp. Cells

Introduction

Jan's protocol for transformation of chemically competent cells

Materials

- › Chemically competent cells
- › Ice
- › Heat bath
- › LB
- › Agar plates with resistance
- › Incubator
- ›

Procedure

Transformation procedure

1. Thaw Bacteria from -80° on ice (10 min)
2. Add X μ l Plasmid (up to 10% of total volume) and mix gently with pipette, let it sit on ice for 30min

00:30:00



3. Heat-shock tube in 42° for 45sec

00:00:45



4. Put tube on ice for 2min

00:02:00



5. Add 900 μ l LB

6. Shake in incubator at 37° for 1 hour

01:00:00



7. **Meanwhile:** Prepare appropriate agar plates with antibiotics and pre-heat in 37° (dry) incubator
8. **After an Hour** - Take 50-200µl of the culture and spread on agar plate.
9. (If all bacteria are needed, spin down bacteria to pellet in 4000g for 3 min, and discard of most of media, leaving only a-bit of liquid (~100ul), re-suspend pellet with the remained liquid and spread on plate)
10. Incubate plates in 37° over-night

Transformation Chemically Comp. Cells

Project: iGEM ETHZ 2018

Authors: Elisa Garulli

Date: 2018-07-26

THURSDAY, 26/7/18

Thaw Bacteria from -80° on ice (10 min)

- Add X µl Plasmid (up to 10% of total volume) and mix gently with pipette, let it sit on ice for 30min
- Heat-shock tube in 42° for 45sec
- Put tube on ice for 2min
- Add 900µl LB
- Shake in incubator at 37° for 1 hour
- **Meanwhile:** Prepare appropriate agar plates with antibiotics and pre-heat in 37° (dry) incubator
- **After an Hour** - Take 50-200µl of the culture and spread on agar plate.

(If all bacteria are needed, spin down bacteria to pellet in 4000g for 3 min, and discard of most of media, leaving only a-bit of liquid (~100ul), re-suspend pellet with the remained liquid and spread on plate)

- Incubate plates in 37° over-night

Tryptone Swimming Plate

Introduction

Plates for swimming assay

Materials

- › **Tryptone swimming agar**
- › 1 % (w/v) bacto tryptone
- › 0.5 % (w/v) NaCl
- › 0.3 % (w/v) agar

Procedure

Recipe

	A	B	C	D
1	ddH ₂ O	500	mL	
2	Bacto tryptone	5	g	
3	NaCl	2.5	g	
4	Agar	1.5	g	

Untitled

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

>

>

Procedure

1.

Untitled

Project: iGEM ETHZ 2018

Authors: Elisa Garulli

Date: 2018-07-27

FRIDAY, 27/7/18

Untitled

Project: iGEM ETHZ 2018

Authors: Eline Bijman

Date: 2018-08-05

SUNDAY, 5/8/18

Agarose Plug Assay

Introduction

This assay is used to test whether the bacteria respond to the ligand (aspartate) at all. The bacteria are given into the middle of an agarose covered slide containing Asp. They will use up the Asp and move outwards (if they respond), forming a halo visible by the naked eye.

Materials

- › 1X TAE buffer
- › Agarose
- › Motility Buffer
- › Microscopy slide
- › Cover slip

Procedure

Make the 2% Agarose gel

1. Add 0.2 g of agarose into 10 mL of TAE buffer (use heat-proof container)
2. Microwave in pulses (total of 1-3 min) until agarose is completely dissolved.
3. Keep the agarose at 60°C

Prepare Bacteria

4. From an overnight culture in LB (or M9) dilute to an OD_{600} of 0.05
5. Incubate them at 30°C until an OD_{600} of 0.6 - 0.8 is reached (mid exponential phase)
6. Harvest 10 mL of the culture and centrifuge at 800x g for 5 min
7. Wash the bacteria with 10 mL motility buffer
8. Spin them down again and resuspend in 10 mL motility buffer

Plug Assay

9. Add Aspartate to reach a final concentration of 1mM
take 100 μ L of 10mM Asp and mix with 0.9 mL of 2% agarose

10. Prepare a microscopy slide by putting 2 tapes next to each other with a distance of 1 cm between them
the tape is there to get an appropriate plug thickness
11. Place a drop of 5 μ L onto a microscopy slide and immediately cover it with a cover slip
12. Remove the cover slip, once the plug is hard
13. Pipette bacteria around the plug and wait for 30 - 60 min

Blunt end ligation

Introduction

For introducing a deletion (fragment of multiple bp) into a plasmid.

Adapted from ThermoFisher (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0013192_Selof_Linear_DNA_UG.pdf)

Materials

- › Linear DNA
- › 10x T4 DNA Ligase Buffer
- › T4 DNA Ligase
- › nuclease free Water
- › Polyethylene glycol (PEG) greatly increases the ligation efficiency of blunt-end DNA ligation. The recommended concentration of PEG 4000 in the ligation reaction mixture is 5% (w/v). --> **need 0.05mg/uL -- > prepare 0.5mg/uL = 0.5g/mL and dilute 1:10 in nuclease free water**
- ›

Procedure

Mixing of components

	A	B	C	D
1	Component	Concentration	Mass	Volume
2	DNA		40	#VALUE!
3	Buffer			5
4	Ligase			5
5	PEG 4000	0.5mg/uL	2.5mg	5
6	Water			#VALUE!

Incubation

1. Mix thoroughly, spin down shortly
2. Incubate for 1h at 22°C
3. Heat inactivate ligase at 65°C for 10 minutes

4. . Use up to 5 μl of the mixture for transformation of 50 μl chemically competent cells and 1-2 μl per 50 μl of electrocompetent cells

Bubbling Experiment

Introduction

Materials

- > Vanillin: Sigma-Aldrich, V1104-100G, Lot. BCBV5242
- > O-toluidine
- > N, N-dimethylformamide
- > Acetic acid buffer 0.1 M, 4,5 pH

Procedure

Dilutions

1. Stock solutions

1 mg/mL vanillin

- Weight 0.0100 g of Vanillin add 10 mL of distilled water

1 µg/mL vanillin

- Add 9990µL of ddH2O

- Add 10 µL of 1 mg/mL vanillin stock solution

1ng/mL vanillin

- Add 9990µL of ddH2O

- Add 10 µL of 1 µg/mL vanillin stock solution

1pg/mL vanillin

- Add 9990µL of ddH2O

- Add 10 µL of 1 ng/mL vanillin stock solution

2. Arithmetic serial dilutions:

Read the table as: how much to add from each stock and how much of water, each column is a sample.

	A	B	C	D	E	F	G	H	I	J
1		0	1	2	3	4	5	6	7	8
2	Vanillin µg/mL	1000	500	400	300	200	100	75	50	25
3	1 mg/mL stock solution [mL]	1	0.5	0.4	0.3	0.2	0.1	0.075	0.05	0.025
4	1 µg/mL stock solution [mL]	0	0	0	0	0	0	0	0	0
5	1 ng/mL stock solution [mL]	0	0	0	0	0	0	0	0	0
6	1 pg/mL stock solution [mL]	0	0	0	0	0	0	0	0	0
7	ddH2O [mL]	0	0.5	0.6	0.7	0.8	0.9	0.925	0.95	0.975

3. Prepare solutions for the reaction:

- Mix o-toluidine with N, N-dimethylformamide at 1:3 (v/v) [toxic-substances cupboard third floor]
- Acetic acid buffer 0.1 mM at 4.5 pH

4. Prepare bubbled samples:

- Add three spoons of vanillin in a 1L flask.
- Close the cap and incubate for some time (tbd) so the air gets saturated.
- Change the cap to the one with the tubing, connected to the pump
- Bubble for the desired time in 7.5 mL in a 50 mL Falcon tube (not more or the water will be sprayed in the flask containing vanillin). *
- Immediately transfer 1 mL from each sample to a 1.5 eppendorf tube and close the cap to minimize the evaporation of the vanillin.

*Previously tested times were 3s, 10s, 1m, 5m, 10m and 30m. However, we noticed that the concentration in the water seems to decrease as we increase the bubbling time. This might be because the air gets less and less saturated as we pump it out. We should try to reverse the order and do the longer times before (or the central ones, so to get the maximum of vanillin in).

5. Reaction

Add 1 mL of vanillin solution and 200 µL of o-toluidine solution to a 1.5 mL Eppendorf tube, and shake the mixture was for 5 min at room temperature, take care of preparing also a blank sample with water and the o-toluidine solution. Add acetic acid solution (200 µL) to the mixture, and place the tube in a boiling water bath for 15 min at 100°C. (or thermoblock at 99°C). Be careful as the pressure that builds in the eppendorf tend to make them pop open. Try to keep the caps sealed.

6. Measurement

Depends on the experiment performed. Either plate reader, UV-Vis spectrometer or MS.

Cell Tethering Assay

Introduction

With this method one can observe the CCW and CW rotation of bacteria, which are coated to the surface using anti-flagellin Abs.

Protocol from

Bacterial Tethering Analysis Reveals a "Run-Reverse-Turn" Mechanism for Pseudomonas Species Motility

When we coat proteins to a glass coverslip, we use Cronic-sulphuric acid overnight to degrease. After that we dry the glasses at 60 degrees celsius. Then you can place a drop of protein solution on parafilm. Slide the glass on the protein solution and incubate at RT (1-2 hours). Block in 1% BSA or HSA overnight at 4°C. Store at 4°C, max 2-3 days. You can also freeze the glasses in the blocking solution at -20°C for longer storage

Materials

- › bBSA (0.05 mg/mL (probably less)) in PBS (pH7.4) (*from practical course script*)
- › BSA (40mg/mL) in PBS (pH7.4)
- › Avidin (0.01 mg/mL) in PBS
- › Anti-Flagellin (Anti-FliC) Antibody for E. coli **conjugated to biotin** (AB193301), Abcam
- › PBS

Procedure

Surface Functionalization (*Kuhn & Dittrich 2012*)

1. Fill channels with PBS by centrifugation: 10 mins at 800g
2. Do everything with 5µL/min
3. Prepare the 40 mg/mL BSA and bBSA, vortex it, let it settle for 5 mins and filter through a 0.45 µm filter
4. !! Do this directly after plasma treatment/bonding: Fill the bonded chip with bBSA and incubate for 2h at RT
5. *Wash* with PBS by flushing for 5 min with 5µL/min
6. Fill the chip with BSA for 2-3 h at RT (blocking)
7. *Wash*
8. Fill the chip with avidin and incubate for 2h
9. *Wash*

Antibody Coating

10. Dilute some of the Ab to 0.025 mg/mL in **PBS?** (Huang 2002)
11. Fill the chip with the Ab solution and incubate at RT for 1h
12. *Wash*

Bacteria Immobilization (*Berg 1975, Qian 2013*)

13. Spin down at 3000 rpm for 3 mins (6?) and resuspend cells in tethering medium at a concentration of $\sim 3 \times 10^8$ cells/mL
14. Pass the cells through a 34-gauge blunt-end needle 4 times to shear off flagella
or 60 times through 29-gauge needle (26-gauge (*Block 1982*))
15. Fill the chip with bacterial solution and incubate at RT for 1 h
16. Flush out unbound bacteria with tethering medium at **50 μ L/min**

Chip fabrication

Introduction

Microfluidic chip fabrication protocol

Materials

- › PDMS
- › Curing agent
- › Puncher
- › Blades
- › Cutter
- › Holder
- › Paper towel
- › Glass slides

Procedure

Chip fabrication

1. Pour 40 g of PDMS + 4 g of curing agent in a weighting boat (note the weight needs to be calibrated, the 0 need to be in the middle)
2. Mix really really well, also from bottom up
3. Degas (open and close the inlet to make the bubbles pop)
4. Pour on the wafer in the holder
5. put in the oven for 2-3 hours
6. take out and separate from the white holder with the back part of a cutter
7. separate the chips with the blade, looking at the lines with the light, use the paper towel as a surface
8. punch the holes with a new puncher
9. clean with tape
10. close the chips with tape

11. clean the glasses with distilled water

12. Seal the glass on the chip:

put on the plastic slid both the glass and the chip

put in the plasma lamp, turn the pump on for 45 s with the inlet closed

open the inlet on the small opening until the pressure is between 7 and 8 mBar

turn on the plasma for 45s

turn off, open the inlet on the big opening and turn off the pump

seal the chip on the glass pressing delicately

put on the heater plate