Designing Life
with AI

EPFL

Wet lab protocols (short version)

DLAI

October 29, 2024

Contents

1 PCR **3**
1.1 Preparing the reagents 3
1.2 PCR steps: 3

2 Gibson assembly **4**

3 Gel electrophoresis **5**

4 Bacteria transformation **5**

5 Incubation **6**

6 Digestion **6**

7 Ligation **7**

1 PCR

Materials :

- Forward primer 100mM
- Reverse primer 100mM
- Phusion or another PCR polymerase (for example Taq polymerase)
- Nuclease-free H_2O
- dNTP mix
- Phusion buffer (Taq buffer)
- $MgCl_2$ *Optional*
- DNA

1.1 Preparing the reagents

Component	Final concentration/amount
Phusion buffer 5X (Standard Taq buffer 10X)	10μl (5μl)
Phusion (Taq polymerase)	0.5μl (0.25μl)
dNTP mix	10 mM 1μl
Forward Primer	0.25μl
Reverse Primer	0.25μl
$MgCl_2$ *Optional*	1.5μl
Plasmid DNA *Optional*	12.5-50 ng
Water	to 50μl

1.2 PCR steps:

The following are the PCR steps for the Phusion polymerase:

Steps	Temp	Time	nb of cycles
Initial denaturation	98°C	30 sec	
Primer annealing	[T_m-5,T_m]°C	45 sec	30-35
Extension	72°C	30s/kb	30-35
Final extension	72°C	5 min	

And these are the PCR steps for the Taq polymerase:

Steps	Temp	Time	nb of cycles
Initial denaturation	95°C	30 sec	
Primer annealing	[T_m-5,T_m]°C	45 sec	30-35
Extension	68°C	1min/kb	30-35
Final extension	68°C	5 min	

2 Gibson assembly

Materials :

1. PCR cleanup product
2. Nuclease-free H_2O
3. Digested vector
4. Gibson assembly mastermix (2X)

Computation of doses

This website provides a convenient tool for calculating gibson doses (which are the same as ligation doses).
Neb gibson calculator

Note that for the vector mass it is standard to put 50ng and the standard ratio of insert to vector is 3:1 but the bigger the ratio the more likely the ligation is to work so if your first Gibson assembly doesnt work you should increase the ratio.

Component	Gene A
Nuclease free water	to 10 μl
Gibson MM	5μl
Vector	varies
Insert	varies

Then incubate the samples at 50°C for 15 minutes.

Source:NEB gibson assembly protocol

3 Gel electrophoresis

Materials :

- 1X TAE buffer
- Agarose powder
- 6X loading buffer
- 1kb ladder
- GelRed

Preparing the gel

1. Prepare a mix of 1X TAE buffer and agarose powder, containing 0.6-1% of agarose.
2. Add 1/10000 of GelRed (for 120ml you'll add 12μl).
3. Prepare the gel-casting chamber and let it cool for 1 hour.
4. Cover the gel with 1X TAE buffer and load samples.
5. Run the gel at 120V for 30-60 minutes.

4 Bacteria transformation

Materials :

- Plasmid DNA
- suspended competent bacteria
- SOC media
- LB+ Ampicillin plate

Protocol:

1. Pipet up and down slowly 10ng of plasmid DNA into 50 μl of suspended bacteria (If you cant isolate 10ng because your concentration of plasmid DNA is too high just pipet up and down 1μl of you plasmid DNA solution no matter the concentration).
2. Put the tube 30 min on ice.
3. Put the tube at 42°C for 30 sec (hot water bath).
4. Put the tube 2-3 min on ice.

5. Transfer in 1 ml of SOC media in a 15 ml falcon. tube and put at 37°C for 30-60 min.
6. Transfer 1 ml into a 1.5 ml eppendorf tube.
7. Centrifuge for 20 sec at max speed (shortspin).

   After this step you should see a little brown stain at the bottom of the tube.
8. Remove the supernatant and leave 50μl - 100μl.
9. Pipet up and down.
10. Plate on LB+ Ampicillin plate with a pipette pasteur also plate your negative control to compare the amount of colonies.
11. Put the plates at 37°C overnight.
12. The day after there should be colonies.

5 Incubation

Materials :

- Transformed bacteria
- LB + Ampicillin media

Protocol:

1. Put 6 ml of LB+ Ampicillin media into a falcon tube.
2. Dip the tip of a pipette into a colony (gently) and dip it into the faclon tube then swirl.
3. Close the tube loosely (air needs to be able to flow in and out of the tube).
4. Put in a shaking incubator at 37°C overnight.

6 Digestion

Materials :

- DNA plasmid
- 10X rCutSmart Buffer

- Restriction enzyme 1
- Restriction enzyme 2
- Nuclease-free water

Protocol

1. Set up the reaction as follows:

Component	50μl reaction
DNA plasmid	1μg
10X rCutSmart Buffer	5μl (1X)
Restricti]on enzyme 1	1μl
Restriction enzyme 2	1μl
Nuclease-free water	to 50μl

2. Incubate at 37°C for 3 hours
3. When running the gel to check digestion also run the undigested plasmid as negative control.

7 Ligation

- T4 DNA ligase buffer 10X
- Vector DNA
- Insert DNA
- Nuclease-free water
- T4 DNA ligase

Computation of doses

This website is pretty handy when it comes to compute ligation doses.
Neb ligation calculator

Protocol

1. Set up all the given materials in a micro-centrifuge tube on ice. T4 DNA ligase should be added last. In parallel set up a negative control which consists of the exact same mix but lacking the insert.

Component	20μl reaction
vector DNA	50ng (can vary)
insert DNA	varies
T4 DNA ligase buffer	2μl (10X)
Nuclease-free water	to 20μl
T4 DNA ligase	1μl

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
4. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours
5. Heat inactivate at 65°C for 10 minutes.
6. Chill on ice and transform 1-5μl of the reaction into 50μl competent cells.