iGEM Bordeaux 2015

Lab Notebook of August

03/08/2015

Again we have some problem with Petri Dishes !!!!
Test pUC-CrdS+p92 with electro competent cells + Petri dishes LB-Amp of Camilla

+ Récupération tapis boite pUC-CrdS+p92 pour réensemencer et avoir bactéries isolées.

- Clonage of pUC-CrdS + p92

(same protocol used that before)

04/08/2015

- Preparation of M63 1X medium

Recovery of M63 5X medium (1L)
(Medium has to be diluted with high quality sterile distilled water)

Stock solution prepared:

Thiamine	**MgSO4**
0,40g weighed in 40mL of H2O => Concentration = 1mg/L (stored at -20°C)	9,87g weighed in 40mL of H2O => Concentration = 1M/L

- 200 mL of M63 5X medium
- 789 mL of H2O
- 10 mL of glucose
- 1 mL ofMgSO4
- 0,1mL of thiamine

=> 1L of M63 1X medium

- Curdlan Production with E.coli (BL21):

=> Take colonies and inoculate 50 mL of M63 (1X) + Antibiotic at 37°C overnight

- Next morning, inoculate 5% (v/v) inoculum for 150 mL of the M63 complete medium with 2% glucose (w/v) in a 500 mL Erlenmeyer flask (in order to have A600: 0.2.)
- Grow cells until A600: 0.7-0.9
- Take out 10 mL of culture, centrifuge 5min at 14,000 rpm and 4°C, discard supernatant and store pellet at -20°C (this is the uninduced time point).
- During stationary phase, re-incubate remaining cultures at 25°C shaking with 180rpm during 21h.
- Collect 1mL fraction for the A600 (at t=1h, t=2h, t=3h, t=4h, t=5h and t=21h after induction)

- Miniprep of Pick selected colony

Recovery of :
- psB1C3+CrdS n°1, n°2, n°3 and n°4

- psB3C5+CrdS n°1, n°2, n°3 and n°4

- Nanodrop quantification:

psB1C3+CrdS	psB3C5+CrdS
1 : 204,1ng/µL	1 : 57,8ng/µL
2 : 221,5ng/µL	2 : 47,6ng/µL
3 : 185 ,8ng/µL	3 : 74,0ng/µL
4 : 345,1ng/µL	4 : 153,6ng/µL

=> Recovery of psB1C3+CrdS n°1 and psB3C5+CrdS n°4 for sequencing

No internet connection, so none possibility to analyse sequencing results => Normally 06/08

- Petrie Dishes ?
=> Recovery of lawyer = Nothing = problems with Petri Dishes !!!!
=> Petri Dishes of Camilla = little bacteria (normal) = Succes !!!

Remade LB+Ampicilline medium
Remade LB+Chloramphenicol medium

05/08/2015

- Transformation

Take out of 3mL culture medium for plasmid verification
- 3mL
- Miniprep
- Digestion with E et P
- Gel 1%
=> Plasmid is always here !!!

- Yeast Curdlan Purification (YEAST TEAM)

Curdlan purification according to protocol 1 modified. 50 mL of chemical lysis yeast culture.
30 mg of precipitate but only 15 µg of purified curdlan.

06/08/2015

- Production

=> Recovery of 50mL flask containing bacteria culture

30mL of bacteria culture
8,3mL of glucose
150µL of Ampicillin
110mL of M63 1X

in a 500 mL Erlenmeyer flask

=> Put the flask at 37°C, 180 rpm

- Grow cells until A600: 0.7-0.9

- Take out 10 mL of culture, centrifuge 5min at 14,000 rpm and 4°C, discard supernatant and store pellet at -20°C (this is the uninduced time point).
- During stationary phase, re-incubate remaining cultures at 25°C shaking with 180rpm during 21h.

- Tube preparation for sequencing

psB1C3+CrdS n°1	psB3C5+CrdS n°4	pUC-CrdS+OsmY n°2
2,4µL of DNA 2,5µL of VF2 primer 5,1µL of H2O	3,2µL of DNA 2,5µL of VF2 primer 4,3µL of H2O	3µL of DNA 2,5µL of VF2 primer 4,5µL of H2O

Protocol

1) 5µL of template DNA with either of following concentrations:
- Plasmid DNA (purified), 80-100ng/µL
- PCR product, 20-80ng/µL
2) Add 5µL of primer 5µM (5pmol/µL)

=> In 1,5mL tubes

07/08/2015

- Miniprep of Pick selected colony

- Recovery of 4 tubes containing pUC-CrdS+OsmY

- Miniprep verification on agarose gel

- Digestion

=> 2h at 37°C

- 1% Agarose Gel

- Dosage Curdlan

Standard range preparation

DYE MIX preparation:
40 volumes of 0.1% aniline blue in water
21 volumes of 1 N HCl
59 volumes of 1 M glycine/NaOH buffer
pH 9.5

Sample preparation:

- Dilute sample with 1N NaOH to a final volume of 300µL in a 1.5 mL microcentrifuge tube. (150µL of sample and 150µL of 1N NaOH)
- Add 30µL of 6N NaOH
- Incubate at 80°C for 30min
- The tube is immediately put on an ice bath.
- Add 630µL of DYE MIX into the tube and mix
- Incubate at 50 °C for 30 min
- The unbound fluorescent dye is decolorized at room temperature for 30 min
- Mesure the fluorescence intensity with a fluorescence spectrophotometer
= emission wavelength of 502 nm (20 nm slit width)
= excitation wavelength of 398 nm (20 nm slit width)

RESULTATS => WE HAVE SOME CURDLAN !!!

10/08/2015

- Transformation of BL21 with pUC-CrdS

Culture of bacteria without plasmid in 5mL of LB
(Use as control for Curdlan production)

- Curdlan purification

- Centrifuge culture
- Add to 1N NaOH and then centrifuge at 10,000 rpm for 10min
- Neutralize the resulting supernatant by adding 3N HCl
- The precipitate formed is collected by centrifugation at 10,000rpm for 10min
- Wash three times with water by centrifugation
- Dehydrate pellets with acetone and dry them in vacuo

Purification of S**T !

- Curdlan production

- in M63 medium
- in LB medium

(Same protocol used than 04/08)

11/08/2015

Prélevement de 3mL des 2 milieux de culture pour vérification plasmide bien présent dans bactéries.

- 3mL
- Miniprep
- Digestion avec E et P
- Gel 1%

 Il est là!!

- End of Curdlan purification

Wash pellets in H2O 3 times

- End of Curdlan production

Production in 150mL

- Pick selected colony

- 2 tubes of bacteria containing pUC-CrdS

=> In 5mL of LB+Ampicillin

=> Put at 37°C, 180 rpm

- Dosage Curdlan

(Same protocol than 07/08)

12/08/2015

CrdA and CrdC 50°C waterbath + vortex !!!

PCR of CrdA and CrdC with more DNA

- Jean : 0,5 ng of DNA in pre-made dilution tube (1µL)
- Jean : 1ng of DNA in pre-made dilution tube 2µL)
- New : 1ng of ADN in new dilution tube dilution (1ng/µL)
- Water Reference !!!!!!!!
- VORTEX !
- 7 tubes at the end

TRANSFO pUC CrdS + p92

Dosage Curdlan LB

13/08/2015

Dosage Curdlan M63

PCR again

Repiquage transfo

14/08/2015

- Transformation:

- Recovery of miniprep tube corresponding to pUC-CrdS+OsmY n°2

Transformation protocol

- Transformed cells spread on 10 petri dishes containing LB+Ampicillin

=> Put on the incubator at 37°C

- Culture

- Recovery of µL of BL21 and put on 5mL of LB

- Miniprep of Pick selected colony

Recovery of 10 tubes containing pUC-CrdS+OsmY

pUC-CrdS+OsmY n°1: 299,6ng/µL	pUC-CrdS+OsmY n°3: 313,9ng/µL	pUC-CrdS+OsmY n°5:544,5 ng/µL	pUC-CrdS+OsmY n°7: 277,6ng/µL	pUC-CrdS+OsmY n°9: 537,8ng/µL
pUC-CrdS+OsmY n°2: 516ng/µL	pUC-CrdS+OsmY n°4: 521,7ng/µL	pUC-CrdS+OsmY n°6: 539,9ng/µL	pUC-CrdS+OsmY n°8: 521,7ng/µL	pUC-CrdS+OsmY n°10: 333,1ng/µL

- Miniprep verification on agarose gel

Digestion :

- 1h at 37°C

1% Agarose Gel :

- Curdlan purification

- Centrifuge culture samples at 12000 x g for 30 min.

(150mL of culture separated in 3 falcon tubes of 50mL)

- Resuspend pellets in 1N sodium hydroxide.

(Each pellet is resuspended in 5mL of NaCl, then the solution is separated in 5 eppendorf tubes with 1mL)

- Mix during10 min at 4°C.
- Stand for 3 h at 4°C.
- Centrifuge samples at 12000 x g for 40 min.
- Curdlan in the clear supernatant was precipitated by neutralization with acetic acid.
- Repeately washed with Water, acetone and ether.

- Cloning

- Digest of purified PCR product of OsmY with EcoRI and SpeI
- Digest of miniprep psB1C3 with EcoRI and Pst1
- Digest of CrdA with XbaI and Pst1
- Digest of CrdC with XbaI and Pst1

=> 3h at 37°C

Dephosphorylation of pUC CrdS :

- Add 5µL Antartica Buffer 10X and 1µL Antartica phosphatase
=> 1h at 37°C
=> Deactivation 10min at 80°C

Ligation :

T4 buffer 10X: 1µL T4 ligase: 0,5µL OsmY: 7,25µL psB1C3: 1,25µL	T4 buffer 10X: 1µL T4 ligase: 0,5µL CrdA: 7,25µL psB1C3: 1,25µL	T4 buffer 10X: 1µL T4 ligase: 0,5µL CrdC: 7,25µL psB1C3: 1,25µL	T4 buffer 10X: 1µL T4 ligase: 0,5µL pCat: 7,25µL psB1C3: 1,25µL
	T4 buffer 10X: 1µL T4 ligase: 0,5µL CrdA: 7,25µL OsmY: 7,25µL psB1C3: 1,25µL	T4 buffer 10X: 1µL T4 ligase: 0,5µL CrdC: 7,25µL OsmY: 7,25µL psB1C3: 1,25µL	T4 buffer 10X: 1µL T4 ligase: 0,5µL pCat: 7,25µL pUC-CrdS: 1,25µL

=> Put at 4°C overnight

17/08/2015

- Curdlan production in M63

- Recovery of petri dishes containing transformed bacteria done the 14/08/2015 (bacteria with pUC-CrdS + OsmY)

Conditions:
- M63 Control with bacteria non-transformed
- M63 (same that always)
- M63 (with a shoot of glucose 4h after the start of the stationary phase)
- M63 (with an add of yeast extract at the start of the stationary phase)

Quantitative analysis at some points of the culture:
- T=3h30
Optical Density at 0.05 in each condition
-T=6h30
Optical Density at 0.07 in M63 Control
Optical Density at 0.05 in other conditions

- Transformations

Same protocol used that before on:
- Bacteria containing psB1C3 + OsmY
- Bacteria containing psB1C3 + CrdA

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- Bacteria containing psB1C3 + CrdC
- Bacteria containing psB1C3 + pcat
- Bacteria containing psB1C3 + OsmY + CrdA
- Bacteria containing psB1C3 + OsmY + CrdC

- Pick selected colonies

- Recovery of petri dishes containing transformed bacteria done the 14/08/2015 (bacteria with pUC-CrdS + OsmY)

- Cloning of B3T5

Digest protocol

- Yeast Curdlan Purification (YEAST TEAM)

Optimization of curdlan purification by more lysis time (4h) and resuspension of base in 0,5 N NaOH and spin down it again. We have a better yield : 40 % vs 20 % of total yeast culture curdlan.

Next step : curdlan purification in yeast overexpressing FKS1.

18/08/2015

- Curdlan production in M63 (started the 17/08/2015)

Quantitative analysis of Curdlan production in M63 medium at T=22h
Optical Density at 1.45 in M63 Control
Optical Density at 0.18 in other conditions

Wait 1h to start the production in the 150mL flask (because we wait the OD at 0.2)

Quantitative analysis
- At T=5h30
Optical Density at 0.78 in M63 Control
Optical Density at 0.11 in G-
Optical Density at 0.11 in G+
Optical Density at 0.10 in Y+
=> Flask containing M63 control put at 25°C

- Curdlan production in LB

Conditions :
- LB Control with bacteria non-transformed
- LB (same that always)
- LB (with a shoot of glucose 4h after the start of the stationary phase)
- LB (with an add of yeast extract at the start of the stationary phase)

Quantitative analysis at some points of the culture :
-T=2h15
Optical Density at 0.15 in LB Control

Optical Density at 0.25 in other conditions

At T=3h, start the production in the 150mL flask (same protocol)

Quantitative analysis :
- At T=2h
Optical Density at 0.1 in LB Control
Optical Density at 0.57 in G-
Optical Density at 0.54 in G+
Optical Density at 0.58 in Y+

- Transformations

Same that yesterday because => petri dishes without colonies

Same protocol used that before on:
- Bacteria containing psB1C3 + OsmY
- Bacteria containing psB1C3 + CrdA
- Bacteria containing psB1C3 + CrdC
- Bacteria containing psB1C3 + pcat
- Bacteria containing psB1C3 + OsmY + CrdA
- Bacteria containing psB1C3 + OsmY + CrdC

- Bacteria containing psB3T5 (in liquid medium with tetracycline)
- Bacteria containing pUC-CrdS + OsmY

- Cloning

Digest
- on psB3T5 (1 tube)

Dephosphorylation
- of psB3T5 (3tubes = 1of today and 2 of yesterday)

Ligation
- psB3T5 + OsmY + CrdA (3 times)
-psB3T5 + OsmY + CrdC (3 times)
=> put at 4°C overnight

- SEQUENCING (again)

- MAKE LIQUID AND SOLID MEDIUMS

19/08/2015

- Verification of digestion of pSB1C3 for sequencing

Gels

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Quantification M63 at T=21h in the 150mL
- Reference condition had passed the stationary phase yesterday
- G- = 0.42
- G+ = 0.68
- Y+ = 0.71
We decided to wait one hour before the passage at 27°C
After 4h at 27°C => shoot og glucose = 8mL = le G+

End of production in LB medium at 15h20 (started Tuesday)
Quantification of no-purify for the 3 conditions = Reference, Normal et Y+

- Picking of positive colony

- Miniprep of pSB3T5

Preparation of different medium at infinite !!!!!!!!
Cast to the infinite à l'infinite

- Starting of 2 production :
M63 and LB
No references et classic production without yeast extract and glucose shoot
Pre-growing at 15h30 (50mL)
M63 = scale up in 150mL at 18h30
LB = scale up in 50mL at17h30

LB transfered to 27°C at 21°C

20/08/2015

At 9h, M63 started yesterday transferred at 27°C

Purification of curdlan for LB production of tuesday

Miniprep
Quantification
Digestion
Gel 1%

Sequencing of pSB1C3 OsmY with VR and Vf2 primers

Transformation :
- B3T5 CrdA OsmY
- B3T5 CrDC OsmY
- B1C3 CrdA
- B1C3 OsmY CrdC
- B1C3 OsmY CrdA
- B1C3 CrdC

=> Stop production of M63 of Monday for quantification of no-purify

21/08/2015

- Purification of production(4 conditions) in M63 (Tuesday)

- Stop production in M63 of Wednesday for quantification of no-purify and purify (so purify)

- Transformation of pSB1C3-OsmY-CrdS (Old et New)

- Production in LB and M63

References and classic conditions all the week-end at 37°C
(50mL during 2h30 , after add 100mL of fresh medium (+glucose for M63) (+Antibiotics)

24/08/2015

- Quantitative analysis for Curdlan production

- Transformation

- With pUC plasmid containing OmsY and CrdS (used for Curdlan production)

Cloning :

Digest :
- pUC-CrdS + OsmY with EcoRI and PstI
- psB1C3-RFP with EcoRI and PstI
(To create Biobrick)

Dephosphorylation :

Ligation :
- OsmY+CrdS with psB1C3

Transformation :

- With psB1C3 plasmid containing OmsY and CrdS

- End of Curdlan production started the 2015/08/21

=> Recovery of flask at 37°C
Conditions :
- M63 Control (with non transformed bacteria)
- M63 (with transformed bacteria via pUC-CrdS + OmsY)
- LB Control (with non transformed bacteria)
- LB (with transformed bacteria via pUC-CrdS + OmsY)

- Quantitative analysis of samples purified and non purified

- Curdlan purification

Centrifugation: 20 min at 4700g
Resuspension of pellets with NaOH 1N
Stand for 3 h at 4°C.
Centrifugation samples at 12000 x g for 40 min (twice to loose less as possible)
Curdlan in the clear supernatant was precipitated by neutralization with acetic acid.
Repeately washed with Water, acetone and ether.

- Curdlan production in M63

- Control
- M63
- M63 + shoot of glucose in stationary phase (T=4h)

15/09/2015

- BIOBRICK PROTOCOL

250ng minimum of purified plasmid DNA (your BioBrick compatible part in pSB1C3)
DNA must be dried down before shipping
We recommend drying down the plate in your lab's laminar flow hood.
- Sterilize the hood surface with 70% Ethanol
- Cover the 96 well plate with the provided plate lid (not with the adhesive foil)
- Place the plate in the hood.
- With sufficient airflow, drying down 10ul (25ng/ul) of purified plasmid DNA in the plate should take four hours.

Once all wells have dried, cover the plate with the provided adhesive foil