Lab Session 10-08 to 17-08

13 September 2015 08:17

Lab Session 17.08.2015

Digestion was done for NrfA, CysI and NosZ in the following strategy:

1. Digestion with XbaI
    - Plasmids of these part : in 300 ng/uL
    - Digested with XbaI
    - Purified with elution kit
    - Concentrated after elution:
        - 15 ng/uL
        - 21 ng/uL
        - 33 ng/uL

2. Filling Reaction:
    - We have linearised PSB1C3 with XbaI digestion.
    - Filled the reaction using Vent Polymerase.

    (100 uL) in 40 uL of product.

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Experiments were performed to troubleshoot Transformation.

Nanodropic readings:

3O 3 :

- 149.6 ng/uL
- 122.5 ng/uL

Conclusion:

1. DNA is very less.
2. DNA is degraded.

Transformation:

Parts to be transformed:

- Kit plate 5 1A : 113.3 ng/uL > 4.4 kb
- Kit plate 5 1B : 108.6 ng/uL > 759 bp

The following 8 transformations were performed:

	Result
Kit plate 5 1B : Desired Plasmid 1 < x < 2 uL	No
Pbcks : + Control 1 uL on LA.	Yes
Empty : - Control	No
Comp Cell Kit : 1	No
Comp Cell Kit : 3	No
Comp Cell Kit : 4	2 Red
Comp Cell Kit : 5	No

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PCR was used to standardise the buffer. Template dosage was minimised to the +ve of the primer band.

The following compositions were used in the different cases:

1. Same as last one.
2. Template 2X. Primer.
3. Primer 0.5 X Template
4. Template 2X Primer 0.5 X

- Template: K1491005 > 759 bp.
- RBS Super 4FP2 > His

Setup:

Temperature : 323.5 K

	1	2	3	4
W	2	1	3	2
FP	1	1	0.5	0.5
RP	1	1	0.5	0.5
T	1	2	1	2

M	5	5	5	5

Results:

- There is only Primer band.
  - -ve control and others are same.
- No bond of plasmid, i.e. DNA is degraded.
- But nanodropic reading shows: 108.6 ng/uL

Conclusion:

DNA is very less.

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Amplification was done for:

1. J23104 + RBS
2. J23100 + RBS
3. RBS + GFP

Digestion was done for NrfA with EcoRI and SpeI (*Control was put as well).

Vectors were digested with EcoRI and SpeI.

Finally, Cys I was digested with XbaI and SpeI.

Results:

NrfA > 6280 ng/uL

Vector > 1000ng/uL

Digestion set up:

Vector:

Template (1000 ng/mL) : 2 uL

Buffer (Cut smart) : 2 uL

EcoRI (20 U/uL) : 0.5uL

SpeI (10 U/uL) : 1 uL

MQ water : 14.5 uL

Total : 20 uL

NrfA:

Template (6280 ng/mL) : 5 uL

Buffer (Cut smart) : 2 uL

EcoRI (20 U/uL) : 0.5uL

SpeI (10 U/uL) : 1 uL

MQ water : 11.5 uL

Total : 20 uL

Finally, gel run was done.

Result:

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The following parts were revived:

- K608002 : J23104 + RBS

Plate 1 - 3O ; PsB1C3 > 3.4 sec.

- Part J23100 + RBS

Plate 2 - 3F; PSB1C3 > 3.4 sec.

- P > RBS > NrfA > RBS > GFP
- Plate 1 > 13 L.

741006. RBS cro GFP. 3.9 sec.

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Transformation Protocol (as per iGEM):

- 10 uL of water > pipet up and down several times.
- 1 uL of sample : Centrifugation for 30 sec, 10,000 rpm.
- 50 uL of competent cells.
- Labelling of tubes.
- Pre chilling of 2 ml tubes (for 5 min).
- Competent cells are kept aside on ice for 10-15 mins.
- Water Bath at 315 K.
- 200 uL of SOC media per transformation.

Set up Protocol (as per iGEM):

- The competent cells are kept on ice for 10-15 mins.
- Comp. cells are always kept on ice.
- 1 uL of resuspended DNA is pipetted out.
- 1 ml of control DNA is pipetted into the comp. cells.
- It is agitated gently.
- G}Followed by incubation in ice for 30 min.
- Heat shock at 315 K for 1 min.
- It is incubated in ice for 5 min.
- 200 uL of solution in LB.
- Incubated in the shaker for 310 K.
- 200 uL of this is plated finally.

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At this point, the scenario related to CysI was such:

- We didn't have any clones.
- It has sites of EcoRI and PstI.
- Sulphite reductase (NADPH) hemo protein beta component.
- We found out that DSR Pantry NYMU is not valid.

We decided upon the following two cloning strategies:

Strategy 1:
Aim: TS Lac I Cys I Ps

- Problems:
  - What we needed was K741006
    RBS > Signal.
  - In single digestion : Chances of self ligation and foreign gene insertion, with differentiation of bonds.

Strategy 2:
Aim: 3A assembly.

- Synthesise CysI with NheI site at the end.
- Perform standard assembly.