Lab Note

7/22

Transformation

- Transformation of biobrick 2μL to DH5α competent cell 33μL.
- Sample
  - lacI C0012 C^r
  - RFP E1010 C^r
  - P_{lac} R0010 C^r
  - P_{UV} I765001 A^r

7/23

PCR

- PCR of transformed products(7/22) to amplify the insert gene.
- Sample
  - lacI C0012 C^r 1-3
  - RFP E1010 C^r 3-8
  - P_{lac} R0010 C^r 1-8

Electrophoresis

- Electrophoresis of PCR products(7/23) to check the insert gene base pair.
- Sample
  - lacI C0012 C^r 1-3
  - RFP E1010 C^r 3-8
  - P_{lac} R0010 C^r 1-8

7/24

Cultivation

- Cultivation of transformed products(7/22).
- Sample
  - lacI C0012 C^r 1-3

- RFP E1010 C^r 3-8
- P_{lac} R0010 C^r 1-8

Miniprep

- Purify plasmid which have insert gene.(7/24)
- Sample
  - LacI C0012
  - RFP E1010
  - PI_{ac} R0010
  - P_{UV} I765001

Transformation

- Transformation of biobrick 2μL to DH5α competent cell 33μL.
- Sample
  - Promoter+RBS J23101+B0034 A^r
  - GFP E0040 A^r
  - P_{UV} I765001 A^r

7/25

Digestion

- Digest the plasmid (7/24) with EcoRI, XbaI, SpeI and PstI.
- Sample
  - LacI C0012 (XP)
  - RFP E1010 (XP)
  - PI_{ac} R0010 (ES)

PCR

- PCR of transformed products(7/24) to amplify the insert gene.
- Sample
  - Promoter+RBS J23101+B0034 1-6
  - GFP E0040 7-12

Electrophoresis

- Electrophoresis of PCR products(7/25) to check the insert gene base pair.
- Sample
  - Promoter+RBS J23101+B0034 1-6
  - GFP E0040 7-12

Electrophoresis

- Electrophoresis of digested products(7/25) to check the pasmid digestion.
- Sample
  - LacI C0012 (XP) 1-4
  - RFP E1010 (XP) 5-10
  - PI_{ac} R0010 (ES)11-18

Transformation

- Transformation of biobrick 2μL to DH5α competent cell 33μL.
- Sample
  - GFP E0040 A^r I
  - GFP E0040 A^r II
  - RBS B0030 A^r
  - P_{UV} I765001 A^r I
  - P_{UV} I765001 A^r II

7/26

Cultivation

- Cultivation of ligased products.
- Sample
  - Promoter+RBS J23101+B0034 A^r 1-6
  - GFP E0040 A^r 7-12

Resuspend the biobrick

- Resuspend the biobrick by adding ddH_2O.
- Sample
  - Terminator B0015 C^r

- RBS B0034 A^r
- Promoter J23101 A^r
- GFP E0040 A^r
- 37°C RBS K115002 C^r

Transformation

- Transformation of biobrick 2μL to DH5α competent cell 33μL.
- Sample
  - Terminator B0015 C^r
  - RBS B0034 A^r
  - Promoter J23101 A^r
  - GFP E0040 A^r
  - 37°C RBS K115002 C^r

PCR

- PCR of transformed products(7/25) to amplify the insert gene.
- Sample
  - GFP E0040 A^r I 1-4
  - GFP E0040 A^r II 5-12
  - RBS B0030 A^r 13-20

Electrophoresis

- Electrophoresis of PCR products(7/25) to check the insert gene base pair.
- Sample
  - GFP E0040 A^r I 1-4
  - GFP E0040 A^r II 5-12
  - RBS B0030 A^r 13-20

7/27

PCR

- PCR of transformed products(7/26) to amplify the insert gene.
- Sample
  - B0015 C^r 1-4

- K115002 C^r 5-10
- B0034 A^r 11-16
- E0040 A^r 17-22
- J23101 A^r 23-28

Electrophoresis

- Electrophoresis of PCR products(7/27) to check the insert gene base pair.
- Sample
  - B0015 C^r 1-4
  - K115002 C^r 5-10
  - B0034 A^r 11-16
  - E0040 A^r 17-22
  - J23101 A^r 23-28

Cultivation

- Cultivation of transformed products(7/26).
- Sample
  - B0015 C^r 1-4
  - K115002 C^r 5-10
  - B0034 A^r 11-16
  - E0040 A^r 17-22
  - J23101 A^r 23-28

8/22

PCR

- PCR of transformed products to amplify the insert gene.
- Sample
  - J23101+B0034 K^r 1-8
  - E0010 C^r 9-16
  - cjBlue C^r 2014 I 17-19
  - cjBlue C^r 2014 K 20-22
  - -cons 23-30
  - Test1 31

- Test2 32-34
- Test3 35-40

Electrophoresis

- Electrophoresis of PCR products(7/25) to check the insert gene base pair.
- Sample
  - J23101+B0034 K^r 1-8
  - E0010 C^r 9-16
  - cjBlue C^r 2014 I 17-19
  - cjBlue C^r 2014 K 20-22
  - -cons 23-30
  - Test1 31
  - Test2 32-34
  - Test3 35-40

Cultivation

- Cultivation of transformed products.
- Sample
  - cjBlue C^r 2014 I 1-3
  - cjBlue C^r 2014 K 4-6

8/25

Resuspend the biobrick

- Resuspend the biobrick by adding ddH_2O.
- Sample
  - +cons
  - -cons
  - Test1
  - Test2
  - Test3
  - Test4
  - Test5

- Test6

Transformation

- Transformation of biobrick 2μL to DH5α competent cell 33μL.
- Sample
  - +cons
  - -cons
  - Test1
  - Test2
  - Test3
  - Test4
  - Test5
  - Test6
  - BFP 2017
  - BFP 2014
  - cjBlue 2017 I
  - cjBlue 2017 K
  - cjBlue 2014 I
  - cjBlue 2014 K

Digestion

- Digest the plasmid with EcoRI, XbaI, SpeI and PstI.
- Sample
  - cjBlue 2014 IA (XP)
  - cjBlue 2014 IB (XP)
  - cjBlue 2014 IC (XP)
  - cjBlue 2014 KA (XP)
  - cjBlue 2014 KB (XP)
  - cjBlue 2014 KC (XP)
  - J23101+B0034 I (ES)
  - J23101+B0034 II (ES)
  - J23101+B0034 III (ES)
  - J23101+B0034 IV (ES)

- J23101+B0034 V (ES)
- J23101+B0034 VI (ES)
- pSB1A3 (EP)

10/2

Cultivation

- Cultivation of pSB1K3.
- Sample
  - pSB1K3

10/3

PCR of IDT gblock fragment

- Amplify the DNA to revise the mistake.
- Sample
  - Taq3 1-1
  - TAq3 1-2
  - Taq3 2
  - Taq3 3
  - Taq3 4
  - Taq3 5

Electrophoresis

- Check IDT PCR products(10/3).
- Sample
  - Taq3 1-1
  - TAq3 1-2
  - Taq3 2
  - Taq3 3
  - Taq3 4
  - Taq3 5

Miniprep

- Purify the plasmid of pSB1K3 (10/2).
- Sample
    - pSB1K3

PCR of IDT gblock fragment

- Amplify the DNA to revise the mistake.
- Sample
    - Taq4 1-1
    - Taq4 1-2
    - Taq4 2
    - Taq4 3
    - Taq4 4

Digestion

- Digest pSB1A3 and pSB1K3(10/3) with EcoRI and PstI to ligase the RFP of pSB1A3 into pSB1K3.
- Sample
    - pSB1A3(EP)
    - pSB1K3(EP)

10/4

Electrophoresis

- Check IDT PCR products(10/3).
- Sample
    - Taq4 1-1
    - Taq4 1-2
    - Taq4 2
    - Taq4 3
    - Taq4 4

Electrophoresis

- Electrophoresis of digested products(10/3) to check the pSB1K3 digestion.
- Sample

- pSB1A3 (EP)
- pSB1K3(EP)

Ligation

- Ligase RFP into the pSB1K3(10/3).
- Sample
  - pSB1K3 I
  - pSB1K3 II

PCR of IDT gblock fragment

- Amplify the DNA to revise the mistake.
- Sample
  - Taq5 1-1
  - Taq5 1-2
  - Taq5 2
  - Taq5 3
  - Taq5 4

Electrophoresis

- Check IDT PCR products(10/4).
- Sample
  - Taq5 1-1
  - Taq5 1-2
  - Taq5 2
  - Taq5 3
  - Taq5 4

Transformation

- Transformation of ligased products 10μL to DH5α competent cell 33μL.
- Sample
  - pSB1K3 I (10/4)
  - pSB1K3 II (10/4)

PCR of IDT gblock fragment

- Amplify the DNA to revise the mistake.
- Sample
    - Taq6 1-1
    - Taq6 1-2
    - Taq6 2
    - Taq6 3
    - Taq6 4

Electrophoresis

- Check IDT PCR products
- Sample(10/4 PCR products)
    - Taq6 1-1
    - Taq6 1-2
    - Taq6 2
    - Taq6 3
    - Taq6 4

PCR of IDT gblock fragment

- Amplify the DNA to revise the mistake.
- Sample
    - Taq7 1-1
    - Taq7 1-2
    - Taq7 2
    - Taq7 3
    - Taq7 4

Electrophoresis

- Check IDT PCR products
- Sample(10/4 PCR products)
    - Taq7 1-1
    - Taq7 1-2
    - Taq7 2

- Taq7 3
- Taq7 4

10/8

Transformation

- Transformation of pSB1K3 10μL to DH5α competent cell 33μL.
- Sample
  - pSB1K3

Digestion

- Digest the plasmid with EcoRI, XbaI, SpeI and PstI.
- Sample
  - IDT 1-1 (ES)
  - IDT 1-2 (XP)
  - IDT 2 (XP)
  - IDT 3 (EP)
  - IDT 4 (EP)
  - IDT 5 (ES)
  - pSB1A3(EP)
  - pSB1C3(EP)
  - pSB1K3(EP)

Electrophoresis

- Electrophoresis of digested products to check.
- Sample(10/8 digested products)
  - IDT 1-1 (ES)
  - IDT 1-2 (XP)
  - IDT 2 (XP)
  - IDT 3 (EP)
  - IDT 4 (EP)
  - IDT 5 (ES)
  - pSB1A3 (EP)

- pSB1C3(EP)
- pSB1K3 (EP)

PCR of IDT gblock fragment

- Amplify the DNA to revise the mistake.
- Sample
  - 1-1
  - 1-2
  - 2
  - 3
  - 4

Electrophoresis

- Check IDT PCR products
- Sample(10/8 PCR products)
  - 1-1
  - 1-2
  - 2
  - 3
  - 4

Ligation

- Ligase IDT gene into different backbone(10/8 digested products).
- Sample
  - 1-1+1-2+K^r
  - 3+A^r
  - 5+2+C^r
  - 4+C^r

10/9

Transformation

- Transformation of ligased products(10/8) 10μL to DH5α competent cell 33μL.

- Sample
  - 1-1+1-2+K^r
  - 3+A^r
  - 5+2+C^r
  - 4+C^r

PCR

- PCR of transformed products to amplify the insert gene.
- Sample
  - cjBlue 2014 IA
  - cjBlue 2014 IB
  - cjBlue 2014 IC
  - cjBlue 2014 KA
  - cjBlue 2014 KB
  - cjBlue 2014 KC
  - cjBlue 2014 I
  - cjBlue 2014 K
  - BFP 2014 A
  - BFP 2014 B
  - BFP 2014 C
  - BFP 2014 D
  - BFP 2014 E

Electrophoresis

- Electrophoresis of PCR products(10/9) to check the insert gene base pair.
- Sample
  - cjBlue 2014 IA
  - cjBlue 2014 IB
  - cjBlue 2014 IC
  - cjBlue 2014 KA
  - cjBlue 2014 KB
  - cjBlue 2014 KC
  - cjBlue 2014 I

- cjBlue 2014 K
- BFP 2014 A
- BFP 2014 B
- BFP 2014 C
- BFP 2014 D
- BFP 2014 E

10/10

Miniprep

- Purify plasmid(10/9) which have insert gene.
- Sample
  - 3+A^r 1-8

10/16

Transformation

- Transformation of ligased products and the plasmid 10μL to DH5α competent cell 33μL.
- Sample
  - 1-1+1-2+K^r ligation
  - 3+A^r ligation
  - 5+2+C^r ligation
  - 4+C^r ligation
  - 3+A^r mini

Restore IDT in gblock fragment

- Save DNA
- Sample
  -

PCR of IDT gblock fragment

- The IDT PCR amplify the insert gene and use phusion DNA polymerase (PFU) to improve the PCR accuracy rate.

- Sample
    -

Electrophoresis

- Check IDT PCR products
- Sample(7/9 PCR products)
    -

Sequencing

- determine insert gene’s Sequence.
- Sample
    - Hv1a-his 3(The result is correct)
    - Hv1a-lectin-his 2