Lab Note

7/22

Transformation

* Transformation of biobrick 2µL to DH5α competent cell 33µL.
* Sample
  + lacI C0012 Cr
  + RFP E1010 Cr
  + Plac R0010 Cr
  + PUV I765001 Ar

7/23

PCR

* PCR of transformed products(7/22) to amplify the insert gene.
* Sample
  + lacI C0012 Cr 1-3
  + RFP E1010 Cr 3-8
  + Plac R0010 Cr 1-8

Electrophoresis

* Electrophoresis of PCR products(7/23) to check the insert gene base pair.
* Sample
  + lacI C0012 Cr 1-3
  + RFP E1010 Cr 3-8
  + Plac R0010 Cr 1-8

7/24

Cultivation

* Cultivation of transformed products(7/22).
* Sample
  + lacI C0012 Cr 1-3
  + RFP E1010 Cr 3-8
  + Plac R0010 Cr 1-8

Miniprep

* Purify plasmid which have insert gene.(7/24)
* Sample
  + LacI C0012
  + RFP E1010
  + Plac R0010
  + PUV I765001

Transformation

* Transformation of biobrick 2µL to DH5α competent cell 33µL.
* Sample
  + Promoter+RBS J23101+B0034 Ar
  + GFP E0040 Ar
  + PUV I765001 Ar

7/25

Digestion

* Digest the plasmid (7/24) with EcoRI, XbaI, Spel and PstI.
* Sample
  + LacI C0012 (XP)
  + RFP E1010 (XP)
  + Plac 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
  + Plac R0010 (ES)11-18

Transformation

* Transformation of biobrick 2µL to DH5α competent cell 33µL.
* Sample
  + GFP E0040 Ar I
  + GFP E0040 Ar II
  + RBS B0030 Ar
  + PUV I765001 Ar I
  + PUV I765001 Ar II

7/26

Cultivation

* Cultivation of ligased products.
* Sample
  + Promoter+RBS J23101+B0034 Ar 1-6
  + GFP E0040 Ar 7-12

Resuspend the biobrick

* Resuspend the biobrick by adding ddH2O.
* Sample
  + Terminator B0015 Cr
  + RBS B0034 Ar
  + Promoter J23101 Ar
  + GFP E0040 Ar
  + 37°C RBS K115002 Cr

Transformation

* Transformation of biobrick 2µL to DH5α competent cell 33µL.
* Sample
  + Terminator B0015 Cr
  + RBS B0034 Ar
  + Promoter J23101 Ar
  + GFP E0040 Ar
  + 37°C RBS K115002 Cr

PCR

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

Electrophoresis

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

7/27

PCR

* PCR of transformed products(7/26) to amplify the insert gene.
* Sample
  + B0015 Cr 1-4
  + K115002 Cr 5-10
  + B0034 Ar 11-16
  + E0040 Ar 17-22
  + J23101 Ar 23-28

Electrophoresis

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

Cultivation

* Cultivation of transformed products(7/26).
* Sample
  + B0015 Cr 1-4
  + K115002 Cr 5-10
  + B0034 Ar 11-16
  + E0040 Ar 17-22
  + J23101 Ar 23-28

8/22

PCR

* PCR of transformed products to amplify the insert gene.
* Sample
  + J23101+B0034 Kr 1-8
  + E0010 Cr 9-16
  + cjBlue Cr 2014 I17-19
  + cjBlue Cr 2014 K20-22
  + -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 Kr 1-8
  + E0010 Cr 9-16
  + cjBlue Cr 2014 I17-19
  + cjBlue Cr 2014 K20-22
  + -cons 23-30
  + Test1 31
  + Test2 32-34
  + Test3 35-40

Cultivation

* Cultivation of transformed products.
* Sample
  + cjBlue Cr 2014 I1-3
  + cjBlue Cr 2014 K4-6

8/25

Resuspend the biobrick

* Resuspend the biobrick by adding ddH2O.
* 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, Spel 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, Spel 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+Kr
  + 3+Ar
  + 5+2+Cr
  + 4+Cr

10/9

Transformation

* Transformation of ligased products(10/8) 10µL to DH5α competent cell 33µL.
* Sample
  + 1-1+1-2+Kr
  + 3+Ar
  + 5+2+Cr
  + 4+Cr

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+Ar 1-8

10/16

Transformation

* Transformation of ligased products and the plasmid 10µL to DH5α competent cell 33µL.
* Sample
  + 1-1+1-2+Kr ligation
  + 3+Ar ligation
  + 5+2+Cr ligation
  + 4+Cr ligation
  + 3+Ar 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