7.3

Antigen 43 PCR, 20ul system. T3 polymerase PCR.

Identify them by 1% agarose gel electrophoresis.

7.4

Antigen 43 PCR using different annealing temperature, with gradient 1 ℃.

Attend the group seminar.

Retrieve the PCR product of T3 polymerase and digest it with EcoRI and PstI. Digest for the whole night.

7.5

Identify the product by agarose gel electrophoresis. Retrieve the PCR product of Antigen 43.

Retrieve the digested T3 polymerase and ligated it to the plasmid PSB1A2.

Transform the plasmid to Trans 5α strain.

PCR the antigen 43 with much larger annealing temperature gradient. Identify it with electrophoresis.

7.6

Design the primer of antigen 43 again by Primer Premier 5.0.

7.8

PCR antigen 43 using nest-PCR procedure. Using Taq DNA polymerase to do the first step nest PCR.

Identify it by electrophoresis. Retrieve the 3k band and do the second step of nest PCR by using the product of the first step as template.

7.9

Identify the second PCR product by electrophoresis. Retrieve the 3k band and digest it with EcoRI and SpeI to put it into plasmid. Digest it with PstI to test whether this is antigen 43.( antigen 43 have 6 PstI digestion sites.)

Design the six point mutation primer.

7.10

Do the transformation and ligation.

7.12

Prepare plasmid DNA.

Digest the plasmid DNA with PstI to identify it.

PCR the product using Easytaq DNA polymerase to identify the molecule weight of the product.

7.13

Learn to do the western blotting. Write the protocols.

Digest merT, merP and merC with XbaI and PstI. Meanwhile digest B0034(RBS) with SpeI and PstI.

Connect the two digested product together.

Transform the ligation product into Trans5α strain.

Pick the single clone from the plate.

Send the PCR product of Antigen 43 for sequencing.

7.14

Prepare the plasmid DNA of rbs+merT, rbs+merP, rbs+merC.

Do the western blotting.

7.15

Digest the plasmid DNA by EcoRI and PstI to identify it.

Digest rbs+merT with SpeI and PstI and digest rbs+merP with XbaI and PstI.
Identify them using agarose gel electrophoresis.
7.16
Connect rbs with merT, merP and merC again.
Do the first point mutation. First PCR the antigen 43 gene with designed point mutation primers, then retrieve it by electrophoresis. Then do the blunting kination, finally do the ligation and put it into strains by transformation.
7.18
Prepare the plasmid DNA for rbs+merT, rbs+merP and rbs+merC, Antigen 43 mutant 1.
Identify them using electrophoresis.
Connect rbs+merT with rbs+merP.
Do the second step of point mutation for antigen 43. Do the PCR step. Identify it by electrophoresis.
Blunting kination, ligation and transformation.
Send the first point mutation product for sequencing.
The rbs+merT,rbs+merP and rbs+merC sequenced correct.
7.19
Retrieve the product of digested rbs+merT and rbs+merP by electrophoresis. Connect the rbs+merT with rbs+merP,. Do the transformation.
Pick the single clone of antigen 43 second step point mutation strain.
7.20
Prepare the plasmid DNA of antigen 43 second point mutation strain. Send it for sequencing.
Do the third step point mutation.
Identify the plasmid DNA by electrophoresis. Retrieve the third point mutation PCR product by electrophoresis.
Digest rbs+merT+rbs+merP with SpeI and PstI, digest rbs+merC with XbaI and PstI.
7.21
Identify the product of the digestion using electrophoresis.
Pick the single clone of the 3$^{rd}$ point mutation of antigen 43 strain on the plate.
7.22
Connect rbs+merT+rbs+merP with rbs+merC.
Using easyPFu to PCR antigen 43.The forward primer contains a rbs. Digest it with EcoRI and SpeI to put it into PSB1A2 plasmid.
Prepare the plasmid DNA of the product of the third point mutation of antigen 43.digest it with EcoRI and SpeI to identify it.
Do the 4$^{th}$ point mutation PCR step.
7.23
Identify the plasmid using agarose gel electrophoresis.
Send the plasmid DNA for sequencing.
Put the rbs+agn43 into plasmid by ligation.
7.29
Attend the group seminar.
Prepare the plasmid DNA for rbs+merT+rbs+merP+rbs+merC. Digest it with EcoRI and PstI for identification. Digest it with XbaI and PstI and digest the plasmid contains T7 promoter with SpeI

and PstI.

Do the 5th point mutation of antigen 43. Do the PCR step.

Identify the product of the 4th point mutation and the rbs+merT+rbs+merP+rbs+merC by electrophoresis. Collect those correct ones and send them for sequencing.

See the point mutation result in the sequence.

Identify the T7+rbs+merT+rbs+merP+rbs+merC by electrophoresis.

7.30

Digest rbs+agn43 with EcoRI and XbaI. Digest phiR73+Po promoter with EcoRI and SpeI.

Retrieve the digested T7+rbs+merT+rbs+merP+rbs+merC gel.

Do the transformation.

7.31

Digest 2-2E constitutive promoter with EcoRI and SpeI. Digest rbs+agn43 with EcoRI and XbaI.

Connect 2-2E promoter with rbs+agn43.

8.1

Prepare the plasmid DNA of T7+rbs+merT+rbs+merP+rbs+merC and PhiR73+Po promoter+rbs+antigen 43.

Prepare the plasmid DNA of 2-2E promoter+rbs+antigen 43.

Identify PhiR73+Po promoter+rbs+antigen 43 and 2-2E promoter+rbs+antigen 43 using electrophoresis.

8.2

Send the correct ones for sequencing.

Digest rbs+antigen 43 again.

8.4

Pick the clone of PhiR73+Po promoter+rbs+antigen 43 on the plate for autoaggregation assay.

Plan the assay.

Design new primers for antigen 43 which contains different promoters in the forward primer.

8.5

Preliminary autoaggregation assay.

Send PhiR73+Po promoter+rbs+antigen 43 for sequencing.

Transform PhiR73+Po promoter+rbs+antigen 43 into BL21 strains.

8.6

Pick the single clone.

Induce the antigen 43 gene's expression by adding 10^-5 M IPTG for 4 hours.

Do the auto-aggregation assay( for detail, see the inductive aggregation page or antigen 43 part.)

PCR the rbs+agn43 using easyPFU.

Identify it using electrophoresis.

Digest the PCR product using EcoRI and SpeI to put it into PSB1A2.

Do the ligation and transformation.

Send some of the PCR product for sequencing.

8.7

Digest merT+merC with SpeI and PstI, digest merP with XbaI and PstI.

Retrieve the gel. Do the ligation.

Pick three clones of rbs+merP.

8.8

Prepare the plasmid DNA for rbs+merP and merT+merC.
Pick single clones of merT+merP+merC. Do the colony PCR.
Retrieve the rbs+agn43.
Do the ligation and transformation.
8.9
PCR antigen 43.
Nest PCR using nest primers. Do the first step.identify it using electrophoresis.
8.10
PCR antigen 43 using nest primers. Using different template.
Using better template to run PCR.
Do the transformation of antigen 43.
8.11
Retrieve the product of rbs+agn43. Digest it and do the ligation and transformation.
8.12
Pick the single clone of rbs+agn43.
Digest 1-23L with EcoRl and Xbal. Digest T7+merT+merP+merC with EcoRl and Spel.
Digest pmerT+GFP with Spel and Pstl, digest TPC with Xbal and Pstl.
Identify them using agarose gel electrophoresis.
Do the ligation and transformation.
8.13
Identify rbs+agn using electrophoresis.
Digest 1-23L with EcoRl and Xbal. Digest T7+merT+merP+merC with EcoRl and Spel.
Do the ligation and transformation.
8.14
Prepare the plasmid DNA.
Send the correct ones for sequencing.
8.15
See the sequencing result.
8.16
Digest PhiR73+Po promoter with EcoRl and Spel. Digest RBS with EcoRl and Xbal.
Retract the whole genome of K12 strain.
PCR antigen 43 using new template.
Pick single clones of antigen 43.
8.17
T7+TCP+Terminator done.
Transform it to BL21 strain.
Send antigen 43 for sequencing.
PCR antigen 43 using new template and better protocol.
Do the colony PCR of PhiR73+Po promoter+rbs.
Prepare the plasmid DNA and digest it for identification.
Do the ligation and transformation.
Digest pmerT+GFP with Spel and Pstl, digest TPC with Xbal and Pstl.
Digest pPbrA with Spel and Pstl. Digest rbs+T3 pol with Xbal and Pstl.
8.18

Retrieve the digested product .
Identify them using electrophoresis.
Pick the single clone of PCR of PhiR73+Po promoter+rbs, do the colony PCR.
Prepare the plasmid DNA.
Send the correct ones for sequencing.
Connect pPbra with rbs+T3 pol. Connect PhiR73+Po promoter+rbs with antigen 43.
Connect merp+GFP with TCP.
Do the ligation and transformation.
8.19
Identify them using electrophoresis.
Retrieve the gel.
Do the ligation and transformation.
PCR antigen 43. Digest it using EcoRl and Spel.
8.20
Pick the colony of PhiR73+Po promoter+rbs+antigen 43.
Put rbs+antigen 43 into PSB1A2 plasmid. Do the ligation and transformation.
Digest merp+GFP and T3 pol.
8.21
Identify the colony PCR product using electrophoresis.
Do the transformation.
Digest rbs+agn43 with EcoRl and Xbal. Digest PhiR73+Po promoter using EcoRl and Spel.
8.22
Do the autoaggregation assay of antigen 43.
Induce its expression by adding IPTG.
8.23
Do the autoaggregation assay.
8.25
Prepare the plasmid DNA for TPC+Terminator.
Send the correct ones for sequencing.
Construct pPbra using primers annealing.
8.26
Transform antigen 43.
8.27
Pick single clone of antigen 43.
Do the transformation of TPC+terminator.
Do the transformation of pPbrA.
Connect pBAD with rbs. Do the ligation and transformation.
8.30
Do the functional test of antigen 43.
Pick the clone of antigen 43.
Pick the clone of merP+GFP+TCP.
Do the ligation of pPbra+T3 pol again.
Pick the single clone of Pbad+rbs.
Do the transformation.

8.31
Send the correct merp+GFP and Pbad+rbs for sequencing.
Do the transformation.
Induce the expression of antigen 43.
9.1
Connect the antigen 43 into plasmid.
Send merP+GFP+TCP for sequencing.
Connect pPbra+T3 pol using primers annealing.
Do the ligation and transformation.
9.2
Prepare the plasmid DNA for rbs+agn43.
See the sequencing result of merp+GFP+TCP.
Pick the clone of pPbra+T3 pol.
9.3
Send the rbs+agn43 for sequencing.
Digest rbs+agn43 with EcoRl and Xbal. Digest PhiR73+Po promoter with EcoRl and Spel.
Pick the clone of pPbra+T3 pol. Prepare the plasmid DNA.
9.6
Do the PCR of antigen 43 using Hifi Taq DNA polymerase.
Digest merP+GFP+TCP with EcoRl and Spel. Put it into PSB3K3 backbone.
Connect pPbra+T3 pol with 1-23L. pick the single clone. Prepare the plasmid DNA.
Do the ligation and transformation.
Pick the clone of Pbad+T3 pol.
Make competent cell containing pc+merR+merp+rbs+T3pol. Transform T3 promoter into it.
Induce the expression using different concentration of IPTG.
Re-suspend the cell. Measure the GFP intensity by a microplate reader.
9.7
Digest merP+GFP+TCP with EcoRl and Pstl to put it into PSB3K3.
Send pbra+T3 pol+terminator for sequencing.
Identify the pBAD+T3 pol using electrophoresis.
Make competent cells of pc+merR+merp+rbs+T3pol.
Transform T3 promoter into it.
9.8
Re-suspend the cell.
Measure the GFP intensity using microplate reader.
PCR antigen 43 using hifi Taq DNA polymerase.
9.9
PCR Rbs+agn 43 using agn for/rev primer to identify it.
Digest the correct ones using EcoRl and Spel.
Send the correct ones for sequencing.
Transform T3 promoter into cells containing pc+merR+merp+rbs+T3pol.
Send pBAD+T3 pol for sequencing.
pPbra+T3pol+terminator done.
Measure the GFP intensity using a microplate reader.

9.12
Digest rbs+agn43 with EcoRl and Xbal. Digest PhiR73+Po promoter with EcoRl and Spel.
Identify them using electrophoresis.
Retrieve the gel.
Do the ligation and transformation.
Pick the clone.
9.13.
Digest Antigen 43 with EcoRl and Xbal. Digest PhiR73+Po promoter+rbs with EcoRl and Spel.
Identify them using electrophoresis.
9.14
PCR antigen 43.
Get the candidate of rbs+agn43.
Send the correct ones for sequencing.
9.15
Put antigen 43 into PSB1A2.
Do the ligation and transformation.
9.16
Digest PSB1A2 and PSB1A3.
9.17
Digest PSB1C3 with EcoRI and Spel.
Digest antigen 43 with EcoRl and Spel.
Do the ligation and transformation.
9.21
Retract the whole genome of K12 strain.
9.22
Nest PCR of antigen 43 using new template and new primers.
Put antigen 43 into PSB1C3.
Pick the single clone of antigen 43.
9.23
Prepare the plasmid DNA for antigen43.
Digest the plasmid DNA using EcoRl and Spel.
Identify them using electrophoresis.
Make the competent cell contains PBAD+ T3 pol. Transform T3 promoter+GFP into it.
Pick the clone of PMERT+T3 pol.
Digest PSB3C5 using EcoRl and Pstl.
9.24
PSB3K5: EcoRl and Pstl.
T7+TPC+Ter: Xbal and Pstl.
MerP+GFP:EcoRl and Spel.
Do the ligation and transformation.
9.25
Retrieve the digested merp+GFP.
Induce the expression of PBAD+T3 pol using 10^-5 M arabinose.
Do the antigen 43 PCR using touchdown PCR.

Do the ligation and transformation.
9.26
Make the competent cell contains T3 promoter+GFP. Transform pmert+T3pol and 1-18i+merR.
Do the colony PCR to identify it.
9.27
Attend the seminar.
9.28
Connect T7+PhiR73+Po promoter+rbs+antigen 43.
Do the ligation and transformation.
PCR the T3 promoter+PhiR73+Po promoter.
9.29
Retrieve the PCR product.
Digest the product using EcoRl and Spel.
Digest TCP with Xbal and Pstl. Identify it using electrophoresis.
Digest merp+GFP using Spel and Pstl.
Do the transformation.
9.30
Do the ligation and transformation.
Prepare the plasmid DNA and identify it using PCR.
10.1
Digest pTET+T7 pol using EcoRl and Spel.
Retrieve the gel.
Connect T3 promoter+PhiR73+Po promoter+ antigen 43.
Do the ligation and transformation.
10.3
Connect pTET+T7 pol and T7 promoter+PhiR73+Po promoter+ antigen 43.
Do the ligation and transformation.
10.4
Do the auto-aggregation assay of antigen 43.
10.5
Do the auto-aggregation assay.
10.7
Digest merp+GFP using Spel and Pstl.
Digest TCP using Xbal and Pstl.
Do the ligation and transformation.
10.8
Identify the digested product using electrophoresis.
Do the ligation and transformation.
10.9
Prepare the plasmid DNA.
Identify them using PCR.
Send the correct ones for sequencing.
10.10
Antigen 43 clone step done.

Connect ptet+T7 pol with T7 promoter+PhiR73+Po promoter+ antigen 43.
Do the ligation and transformation.
10.11
Do the auto-aggregation assay.
10.12
Do the auto-aggregation assay.
10.13
Measure the result.
10.15
Attend group seminar.
Do the auto-aggregation assay.
10.16-10.21
Connect merp+GFP with TCP.
Connect pc+merR with terminator.
Transform these two plasmid into one single strain.
Antigen 43 auto-aggregation assay.  Analyse the result.
10.21-10.25
Upload and edit parts of our group.