Note

Monday, October 25, 2010
7:21 PM

Miao's note book

7.26:
PbrR MBP construction plan
8.1:
Ⅰ.Use Tag PCR "PbrR MBP(≈500bp)" for commercial plasmid(PET-21a) and standard plasmid (PSB1K3)
Ⅱ.Digestion:The products of PCR(PbrR MBP)[backbone 4100bp;digest site PstⅠ&NdeⅠ]
Ⅲ.Gel for identification & Retrieve the gel
Ⅳ.Miniprep pbrR-mbp-commercial for backups

8.2:
Lpp-OmpA-MBP construction plan
Ⅰ.Use Pfu Mix PCR Nde1+Lpp-OmpA(437bp)+Sal1 & E(EcoR1)NX(Xba1)+Lpp-OmpA+Sal1
Ⅱ.Digest the PCR products by NdeI,SalI&EcoR,SalI ,respectively
Ⅲ.if the products above got right ,Retrieve the gel ,if not ,back to Ⅰ use gel retrieve kit then digestion for 2h,after that, go to purification.
8.3:
MBP construction plan
Ⅰ.Pfu PCR SalI+N-MBP+BspeI
BspeI+C-MBP+SNP
BspeI+C-MBP+XhoI
Ⅱ.Identify them using agarose gel electrophoresis.if right ,go to retrieve the gel, if not ,back to Ⅰ
8.4:
Lpp-OmpA,N-MBP,+C-MBP with backbone PET21a and PSBIK3
Ⅰ.Ligation,Nde1+Lpp-OmpA(437bp)+Sal1 with SalI+N-MBP+BspeI & BspeI+C-MBP+XhoI & PET21a
Ⅱ.Ligation, E(EcoR1)NX(Xba1)+Lpp-OmpA+Sal1 with SalI+N-MBP+BspeI & BspeI+C-MBP+SNP & PSBIK3
Ⅲ.Learn to do the western blotting. Write the protocols.
8.5:
Transformation the products of 4 fragments above.

8.6:
There is something wrong with the primer ,so it doesn't work, back to the work from 8.2
8.7:
Ⅰ.Use Pfu Mix PCR Nde1+Lpp-OmpA(437bp)+Sal1 & E(EcoR1)NX(Xba1)+Lpp-OmpA+Sal1
Ⅱ.Digest the PCR products by NdeI,SalI&EcoR,SalI ,respectively
8.8:
Ⅰ.Pfu PCR SalI+N-MBP+BspeI
BspeI+C-MBP+SNP
BspeI+C-MBP+XhoI
Ⅱ.GeI to identification
8.9:
Ⅰ.Ligation,Nde1+Lpp-OmpA(437bp)+Sal1 with SalI+N-MBP+BspeI & BspeI+C-MBP+XhoI & PET21a
Ⅱ.Ligation, E(EcoR1)NX(Xba1)+Lpp-OmpA+Sal1 with SalI+N-MBP+BspeI & BspeI+C-MBP+SNP & PSBIK3
8.10:
Transform the ligation product into Trans5α strain.
8.11:
Help a teammate transform P(RBS+T_{3pol} 1)&P_{merR}-PSB1C3 Plasmid to OmniMAX2-T_1 Competent cell
Digestion:
Ⅰ.RBS+T_{3pol} 1 with XbaI and pstI(insert)
Ⅱ.P_{merR}-PSB1C3 with SpeI and PstI (vector)
Retrieve the gel
Ligation the parts above.
8.12:
Digestion MBP construct parts
Ligation 4 fragments
Nde1+Lpp-OmpA(437bp)+Sal1 with SalI+N-MBP+BspeI & BspeI+C-MBP+XhoI & PET21a//E(EcoR1)NX(Xba1)+Lpp-OmpA+Sal1 with SalI+N-MBP+BspeI & BspeI+C-MBP+SNP & PSBIK3 overnight
8.13:
Digestion: PET21a 20 μl with NdeI and XhoI
Ligation again
Ⅰ.RBS+T_{3pol} 1 with XbaI and pstI(insert)
Ⅱ.P_{merR}-PSB1C3 with SpeI and PstI (vector)
Insert:vector=1:7&2:6

Transform the ligation product
Finally got clone ,2:6 better than 1:7
Ⅲ.transformation 4 pieces fragments
8.14:
Ⅰ.Plate PCR for identification 4 pieces fragments ligation products
Ⅱ.Miniprep
21a-(1~3) 1K3(1~3) 26-(1~2) 17-(3~4)
Ⅲ.Sent the plasmids for sequencing
8.15:
Ⅰ.Got the right sequence 1K3(1~3) 26-(1~2) 17-(3~4) while 21a-(1~3) without MBP but have Lpp-OmpA inside
Ⅱ.transformation
1K3(1~3) 26-(1~2) 17-(3~4)
Ⅲ.Pick 20 single clones on 21a plate
Ⅳ.PCR for identification
Got the right size from agarose gel electrophoresis (Lpp-OmpA+MBP=735bp)
Ⅴ.Digestion with NdeI and XhoI,then use PCR purification kit to retrieve products.
Ⅵ.Ligation 1(PET-21a):7
Ⅶ.Transformation
9.5:
Assembly:T_7p+RBS(B0034)+DsbA+MBP+TER(B0015)+T_7p+RBS(B0034)+MBP+TER(B0015)+T_7p+RBS(B0034)+Lpp-OmpA+MBP+TER(B0015)
Ⅰ.Digestion:RBS with SpeI and PstI
1-23L terminator with EcoRI and XbaI
Ⅱ.Identify them using agarose gel electrophoresis.if right ,go to retrieve the gel
9.9:
Ligation: RBS+MBP
DsbA+MBP
FORGET DIGESTION!!!!
9.10:
Digestion:MBD with XbaI and PstI
DsbA-MBP with EcoRI and SpeI
Identify them using agarose gel electrophoresis.if right ,go to retrieve the gel
9.11：
Transformation to BL21 for western blotting
Omni for preserve
9.13：

Digestion :with XbaI and PstI (for ligation)
Miniprep T_7p+RBS(B0034)+DsbA+MBP+Ter(1~3)&RBS+MBP（1~3）
9.15：
Ⅰ.Sent the plasmids for sequencing【T_7p+RBS(B0034)+DsbA+MBP+Ter(1~3)&RBS+MBP（1~3）】
T_7p+RBS(B0034)+DsbA+MBP+Ter 1 got right sequence
Ⅱ.Digestion:
T_7p+RBS(B0034)+DsbA+MBP+Ter with EcoRI and SpeI
Identify them using agarose gel electrophoresis.Then retrieve the gel(got the right size)
Ⅲ.Digestion:
RBS+MBP with XbaI and PstI
After Identify them by using agarose gel electrophoresis,the results turns wrong.
Digestion AGAIN!:
RBS+MBP with XbaI and PstI
Meanwhile: Pick 10 single clone for plate PCR by using tagmix
Digest the PCR products with XbaI and PstI ,10 clones all got the right size,so minipret them.
9.16:
Ligation :RBS-MBP(XP)+Ter(SP)
Transformation it.
Culture the plates.
9.17:
Pick the single clone to PCR, but failed
Meanwhile: Digest the RBS-MBP1 for backup
9.18:
Digest the RBS-MBP(2&3)
Ligation: RBS-MBP(2&3) +T_7p
Transformation the Ligation products
9.19:
Use RBS-MBP(2&3) as templete,PCR(Fast pfu),got a mass of RBS-MBP
Identify them using agarose gel electrophoresis.Then go to retrieve the gel
9.20:
Transformation:RBS-MBP-T_7p
Culture the plates
9.21：
Pick 3 single clone T_7p-RBS-MBP(1~3)
Some for PCR some for Miniprep

9.22:
Miniprep T_7p-RBS-MBP(1~3)
Digestion:T_7p-RBS-MBP(1~3) with EcoRI and SpeI
9.23:
Wiki writing
Retrieve the digestion products T_7p-RBS-MBP(1~3)ES
Ligation:
T_7p-RBS-MBP(1~3)+Ter
Transformation:T_7p-RBS-MBP-Ter(1~3)
Culture the plates
9.24:
Pick 3 single clone from each plate(except1)
Miniprep T_7p-RBS-MBP-Ter(2-1~3&3-1~3)
10μl for sequencing
10μl for digestion
Left 20μl
9.25:
Digestion:T_7p-RBS-MBP-Ter with SpeI and PstI
Gel for identification ,the size of T_7p-RBS-MBP-Ter(2-1~3) are right
9.26:
Digestion:PSB1C3 with EcoRI and PstI
Ligation:PSB1C3 + T_7p-RBS-MBP-Ter+T_7p+RBS+Lpp-OmpA+MBP+Ter
Gel retrieving
9.27:
Transformation:Positive cloning
T_7p+RBS+Lpp-OmpA+MBP+Ter&T_7p+RBS(B0034)+DsbA+MBP+Ter
Culture the plates
9.28:
The sequence of T_7p-RBS-MBP-Ter(2-1~3&3-1~3) came out ,but without T_7p inside.
10.2:
Digestion:T_7p+RBS+Lpp-OmpA+MBP+Ter with XbaI and PstI
10.3:
There is something wrong with PSBIC3(RFP inside)
Ligation Again!:
PSB1C3 + T_7p-RBS-MBP-Ter+T_7p+RBS+Lpp-OmpA+MBP+Ter
10.4:
Pick 3 single clone from the plate
Miniprep them.
10.5:

Transformation:Positive cloning:T_7p+RBS(B0034)+DsbA+MBP+Ter
Digestion:RBS-MBP-Ter+T_7p with XbaI and PstI
Identify them using agarose gel electrophoresis.Then go to retrieve the gel
10.6：
Pick 3 single clone from the plate（T_7p+RBS(B0034)+DsbA+MBP+Ter）
Digestion:PSB1C3 with XbaI and PstI （Change backbone）
Identify them using agarose gel electrophoresis.Then go to retrieve the gel
10.7:
Miniprep T_7p+RBS(B0034)+DsbA+MBP+Ter(1~3)
Digestion：RBS-MBP-Ter with XbaI and PstI
Ligation:RBS-MBP-Ter+T_7p(sp) then transformation
10.8:
Ligation again
T_7p+RBS-MBP-Ter-+T_7p+RBS+Lpp-OmpA+MBP+Ter
Transformation it
10.9:
Transformation T_7p+RBS+Lpp-OmpA+MBP+Ter(xp)-PSB1C3(xp)
PCR(tag mix) for identification T_7p+RBS(B0034)+DsbA+MBP+Ter
Culture the plates, but it did not grow up
10.10:
Ligation:T_7p+RBS+Lpp-OmpA+MBP+Ter(EP)+PSB1C3
T_7p+RBS+DsbA+MBP+Ter(EP)+PSB1C3
Transformation
Easy Mix PCR.
Culture the plates
10.11:
Ⅰ Digestion:T_7p+RBS+Lpp-OmpA+MBP+Ter(EP)
T_7p+RBS+DsbA+MBP+Ter(EP)
Identify them using agarose gel electrophoresis.Then go to retrieve the gel
10.16:
Ⅰ.Ligation: T_7p-RBS-MBP-Ter
Ⅱ.Transformation :T_7p-RBS-MBP-Ter
Ⅲ.Culture the plates
Pick 3 single clones
10.17:
T_7p-RBS-MBP-Ter for sequencing
10.20:
Ⅰ.Liagation:T_7p+RBS+DsbA+MBP+TER+T_7p+RBS+MBP+TER+T_7p+RBS+Lpp-OmpA+MBP+TER+PSBIC3

Ⅱ.Transformation

Ⅲ.Culture the plates

Pick 6 Single clones

Ⅳ.T_7p+RBS+DsbA+MBP+TER+T_7p+RBS+MBP+TER+T_7p+RBS+Lpp-OmpA+MBP+TER+PSBIC3 for sequencing