Before 1st August, 2014

- Devised practical experiments.
- Amplified parts(T7 promoter, Terminator).
- Got AxyPrep Plasmid MiniprepKit(50-prep) and GBclonart Seamless Assembly Kit(GB2002-24).
- 100 times dilution of linear plasmid backbones, designed the primers.
- Placed an order for standard primers used for amplifying standard parts.
- Prepared competent Escherichia coli cells successfully.

2nd August, 2014 Sat

- Read papers about data and protocol.
- Determined detailed protocol of lambda red.
- Extracted plasmids of terminator.
- The bacteria had been transformed plasmids of T7 promoter did not form any colony.
- Amplified parts(T7 promoter， GFP,RFP,blue chromoprotein).

3rd August, 2014 Sun

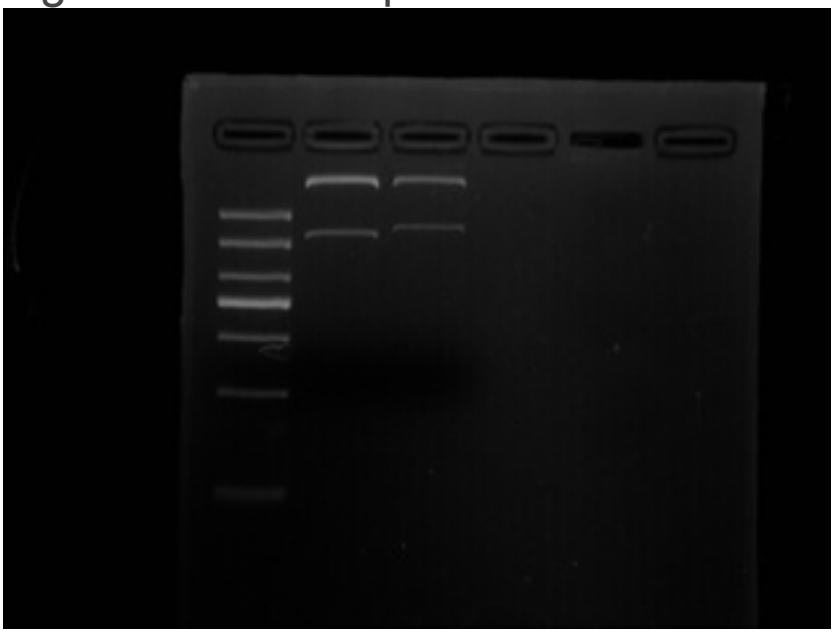
- Prepared 4L Luria-Bertani medium.
- Prepared solid medium (25 withchloromycetin, 10 with ampicillin, 10 without any antibiotic).
- Prepared materials of growth curve experiment, include 8*300ml Luria-Bertani medium.
- Shook and extracted several parts(GFP, RFP, blue chromoprotein, T7 promoter).
- Tested T7 promoter by endonuclease digest and electrophoresis.
- Read papers about recombinase.

- Amplified several parts(BBa_K137058，BBa_K880005，BBa_K404113，BBa_E0420，BBa_K592101).
- Placed an order for PkD46, got a good bargain relatively.
- Determined the homologous sequences of lacZ.

total：3075bp
A：42bp：
CGGCAGTAAGGCGGTCGGGATAGTTTTCTTGCGGCCCTAATC
B：42bp：
ATGTCGTTATCCAGCGGTGCACGGGTGAACTGATCGCGCAGC
inter：317bp

10. Designed primers of the firststep

4th August, 2014 Mon

- Placed an order of genome extraction kit and primers.
- Determined T7 promoter, GFP,RFP by endonuclease digest and electrophoresis.
-  Proved that T7 promoter have to be redone. It is not your fault @Sophie.

- Extracted several parts.
- Transformed several parts(BBa_K137058，BBa_K880005，BBa_K404113，BBa_E0420，BBa_K592101)without activation, but failed. Proved that parts with chloromycetin resistance have to be activated before transformation.
- Activated BL21(A+ and A-)
- VR, VF2，pSB1K3-F，pSB1K3-R had been delivered, diluted to 100μmol and 10μmol before use, stored at -20℃.
- PCR: linear plasmid backbones(pSB1K3，pSB1A3，pSB1C3，pSB1T3), but failed.
- Determined the PCR procedure of PrimerSTAR HS polymerase.
  *The procedure named 20140804, username is CF. If you want to amplify other sequence, you have to change the annealing temperature and time of elongation (elongating speed of PrimerSTAR is about 1K/min)
- Prepared 12 boxes of pipette tips, autoclave at 121℃ for 20 minutes.
- A thorough cleanup of the super clean bench.

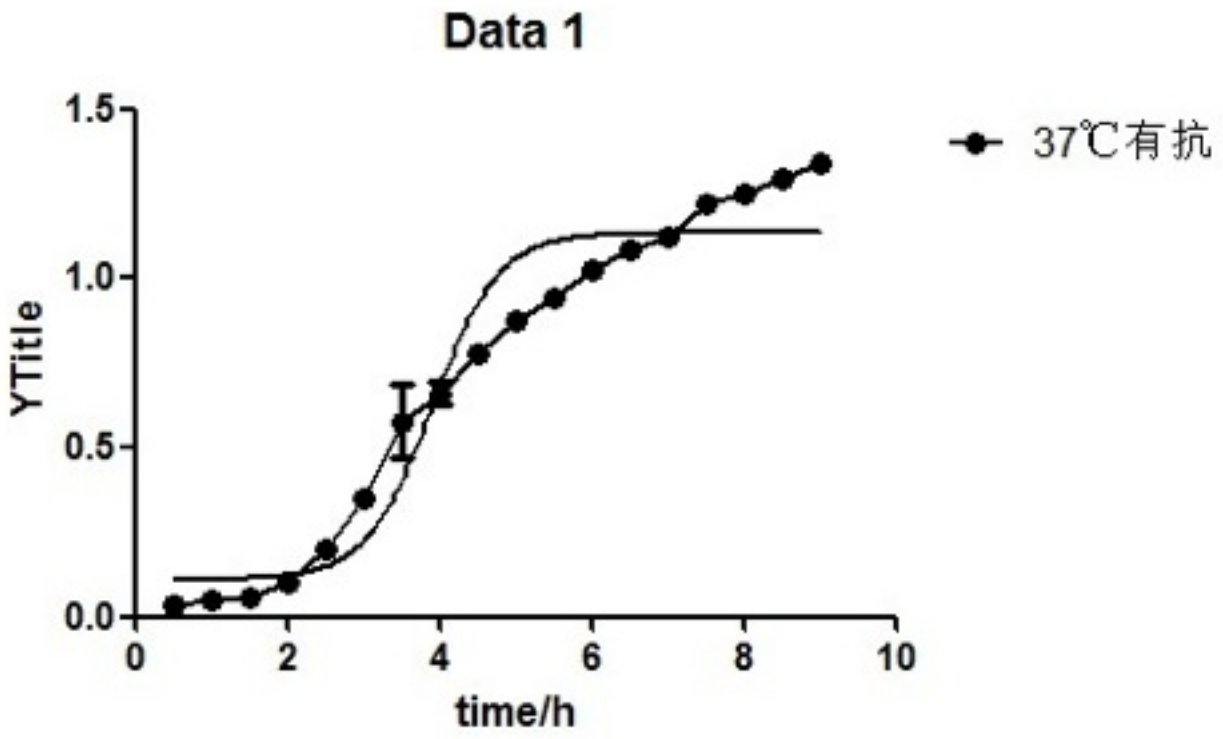
5th August, 2014 Tue

- Prepared 14 boxes of pipette tips, including 2 boxes of 5ml specification and 2*150ml conical flask. Autoclave at 121℃ for 20 minutes.
- Prepared 8*250ml of Luria-Bertani medium. Autoclave at 121℃ for 20minutes.
- Shook and extracted several parts（BBa_K137058, BBa_K880005, BBa_K404113, BBa_E0420, BBa_K592101）

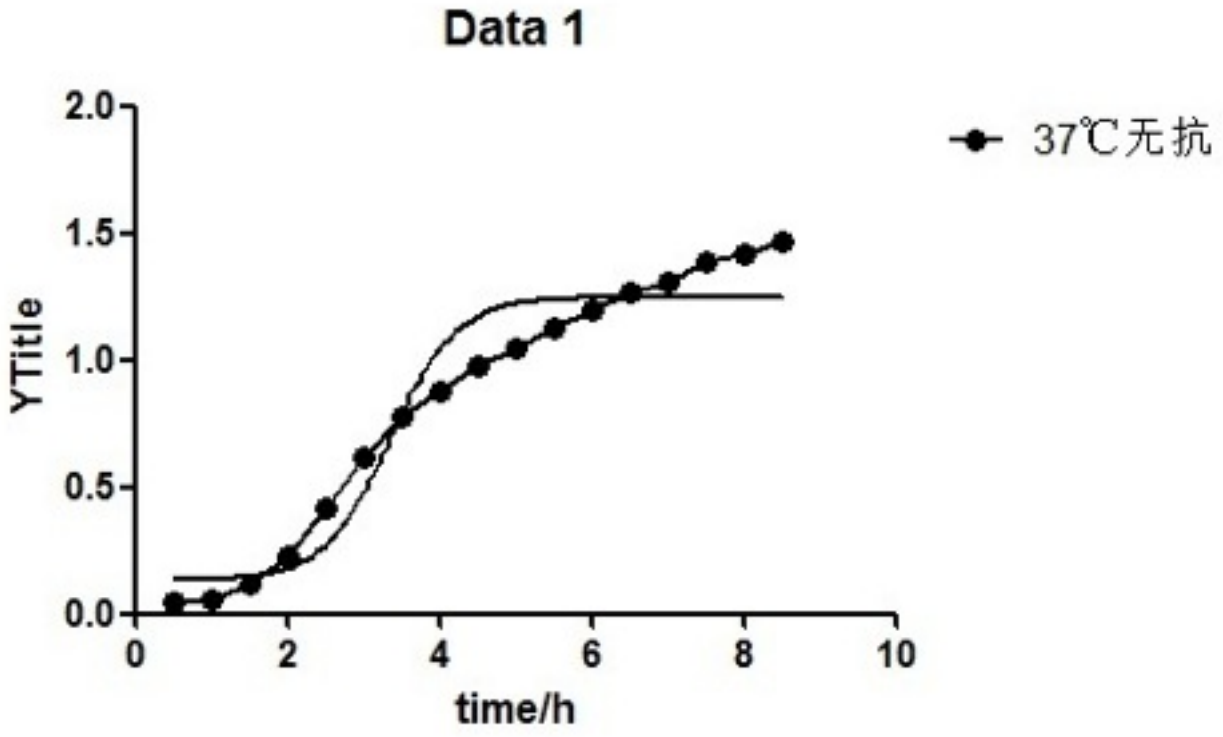
- Determined the PCR procedure of TaKaRa LA Taq polymerase.
  *The procedure named 2014 taq, username is CF. If you want to amplify other sequence, you have to change the annealing temperature and time for elongation(elongating speed of LA Taq is about 1K/min)
- Amplified four linear plasmid backbones (pSB1K3, pSB1A3, pSB1C3, pSB1T3), but failed.
- To investigate why, we change the polymerase(borrowed two kinds of enzymes from 411) and template(plasmid of 2012, 2014, 2013), but still failed. This investigation will be continued.
- Bought 2 bottles(can be used insuper clean bench), a packet of tubes used in shaking bacteria, a packet of 1ml pipette tips and 3 lab coats.
- Prepared 75% ethanol solution, 1*TAE solution. Added rayon balls in the bottle of super clean bench.
- A thorough cleanup of 413.

6th August, 2014 Wed

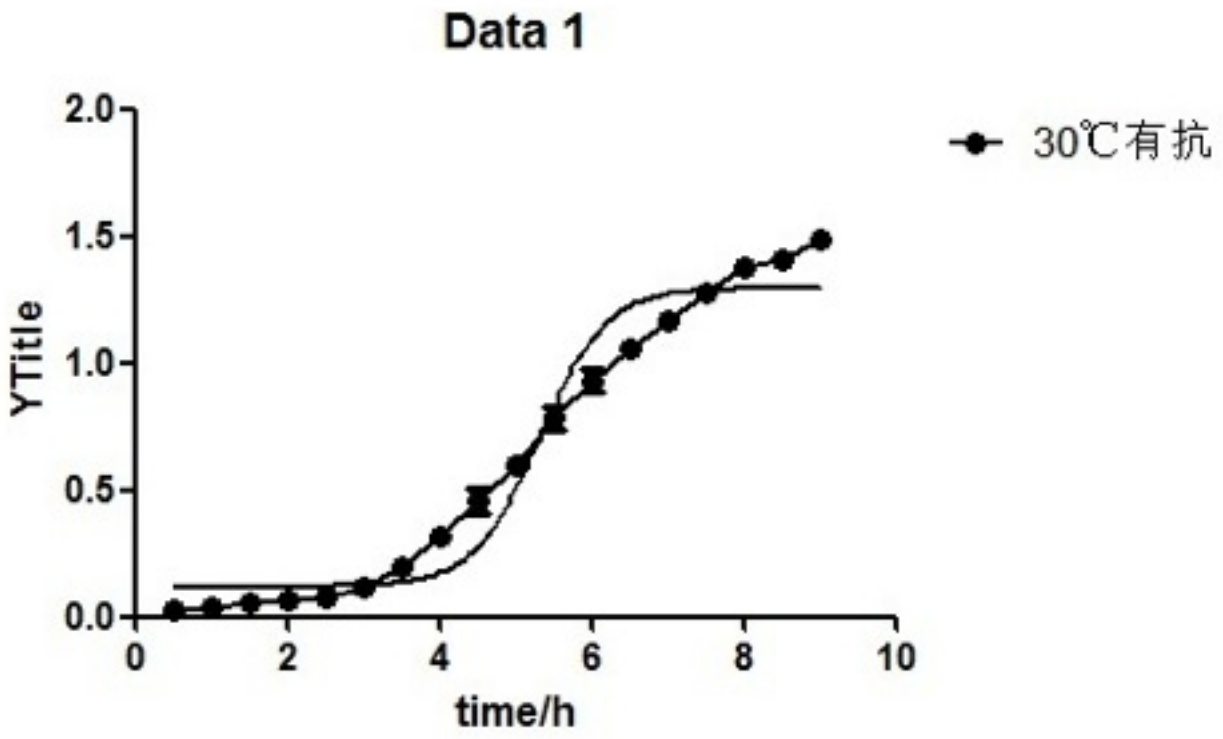
- Explored growth curves ofE.coli in different environments. (37℃ with ampicillin, 37℃ without ampicillin,30℃ with ampicillin, 30℃ without ampicillin)
  *8 bottles in total. Measure the OD value of every bottle inevery 30 minutes. This experiment will last about 16 hours, we will start at 8am.
  37℃ with ampicillin



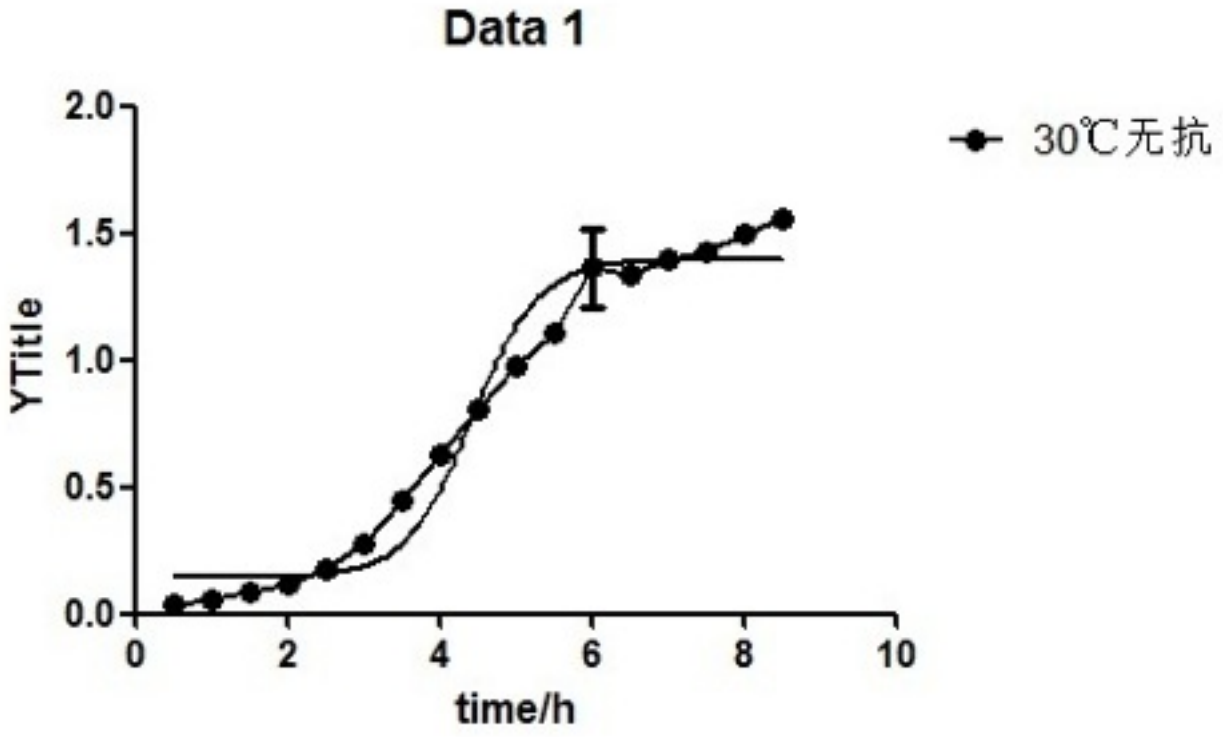
37℃ without ampicillin



30℃ with ampicillin



30℃ without ampicillin

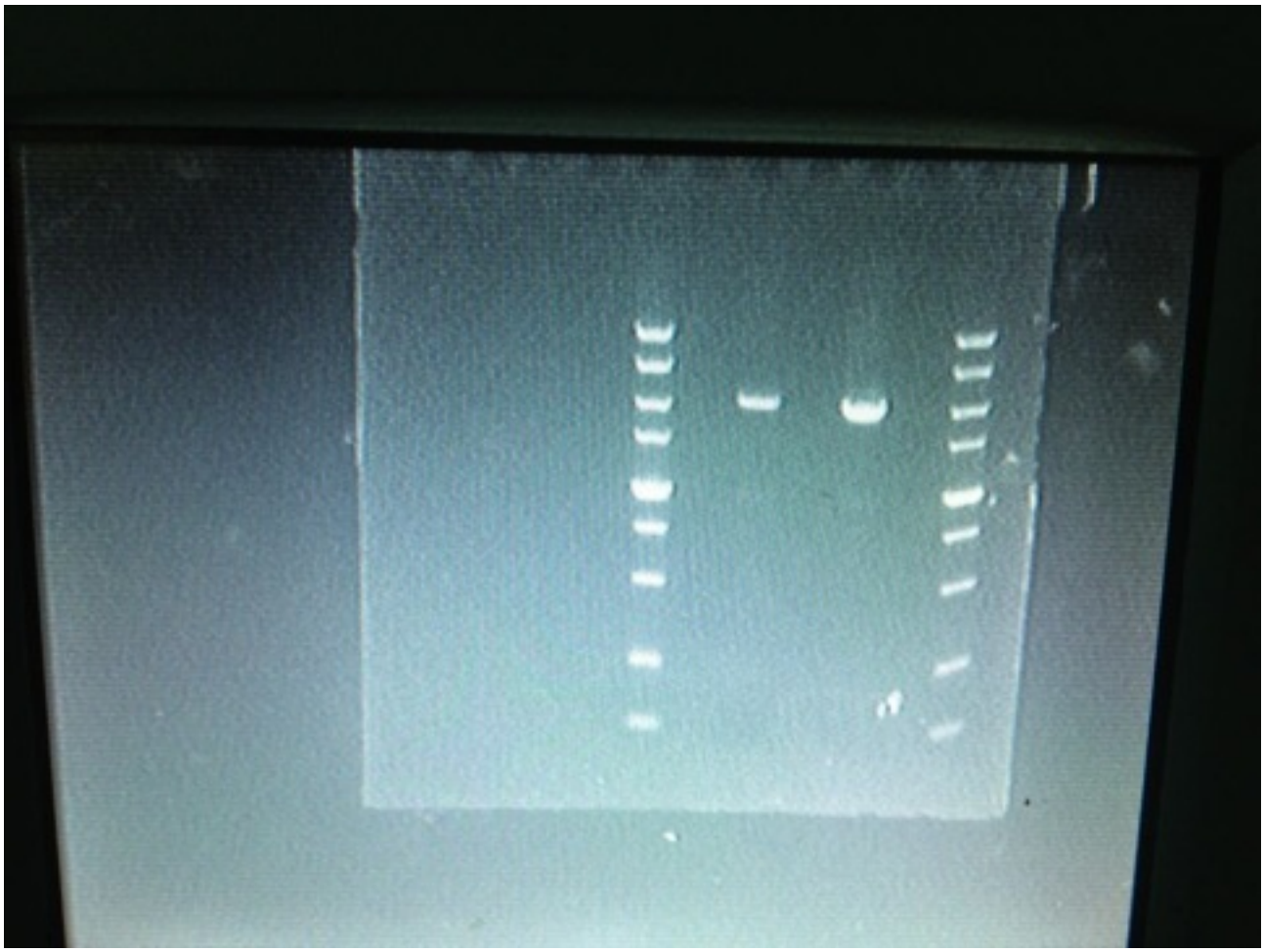


- Prepared materials for competent cell.
-
-
-

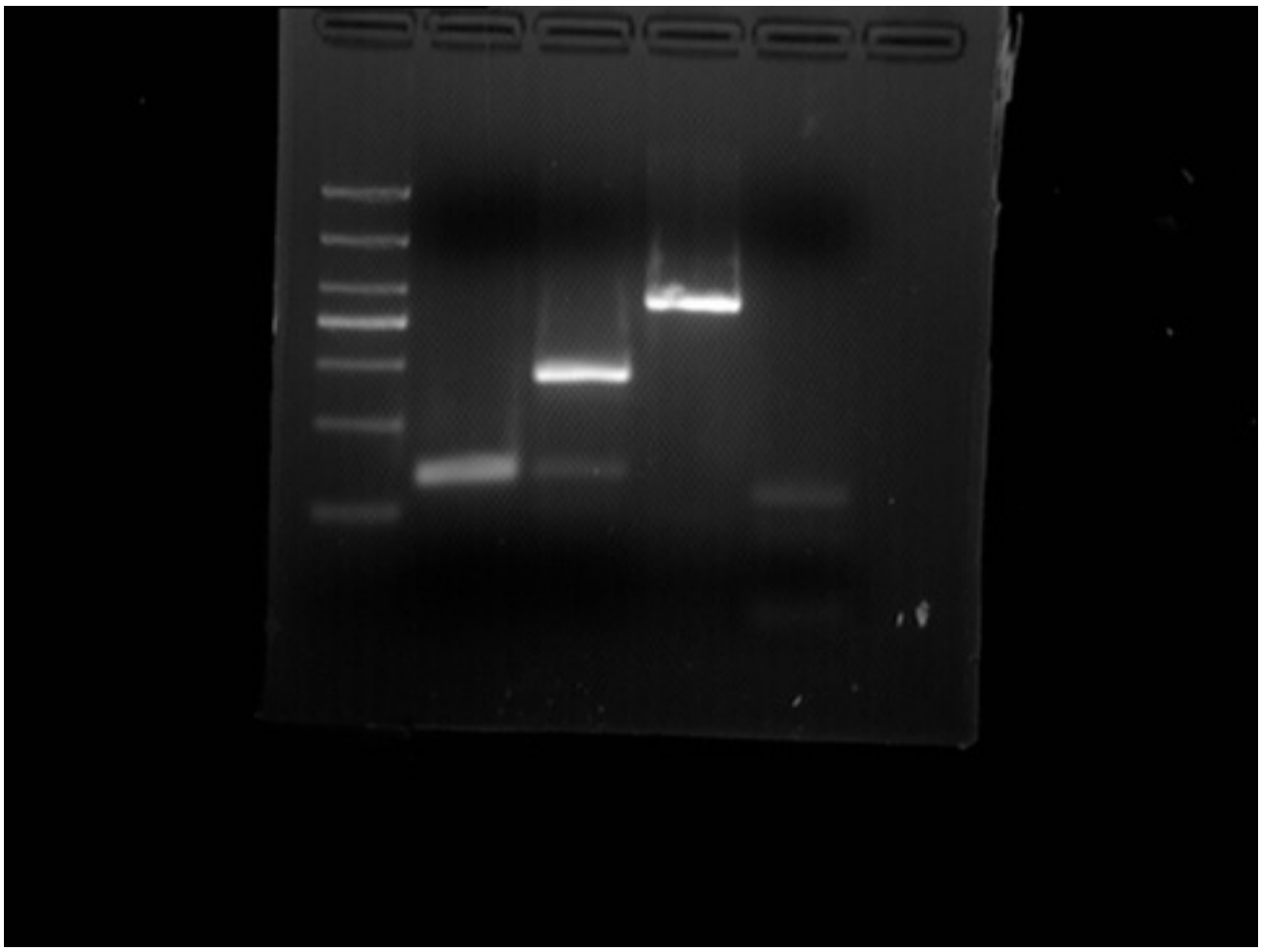
-

7th August, 2014 Thu

- Prepared competent cell(BL21,DH5α).
- Got the reason that why PCRalways fail.
  Plasmid backbone of RFB



Sequences with 84bp homologous arms(50bp, 150bp, 300bp)



- PKD46 delivered, Pick 8 colonyand shook.
- Primers delivered, diluted to100μmol and 10μmol before use, stored at -20℃.

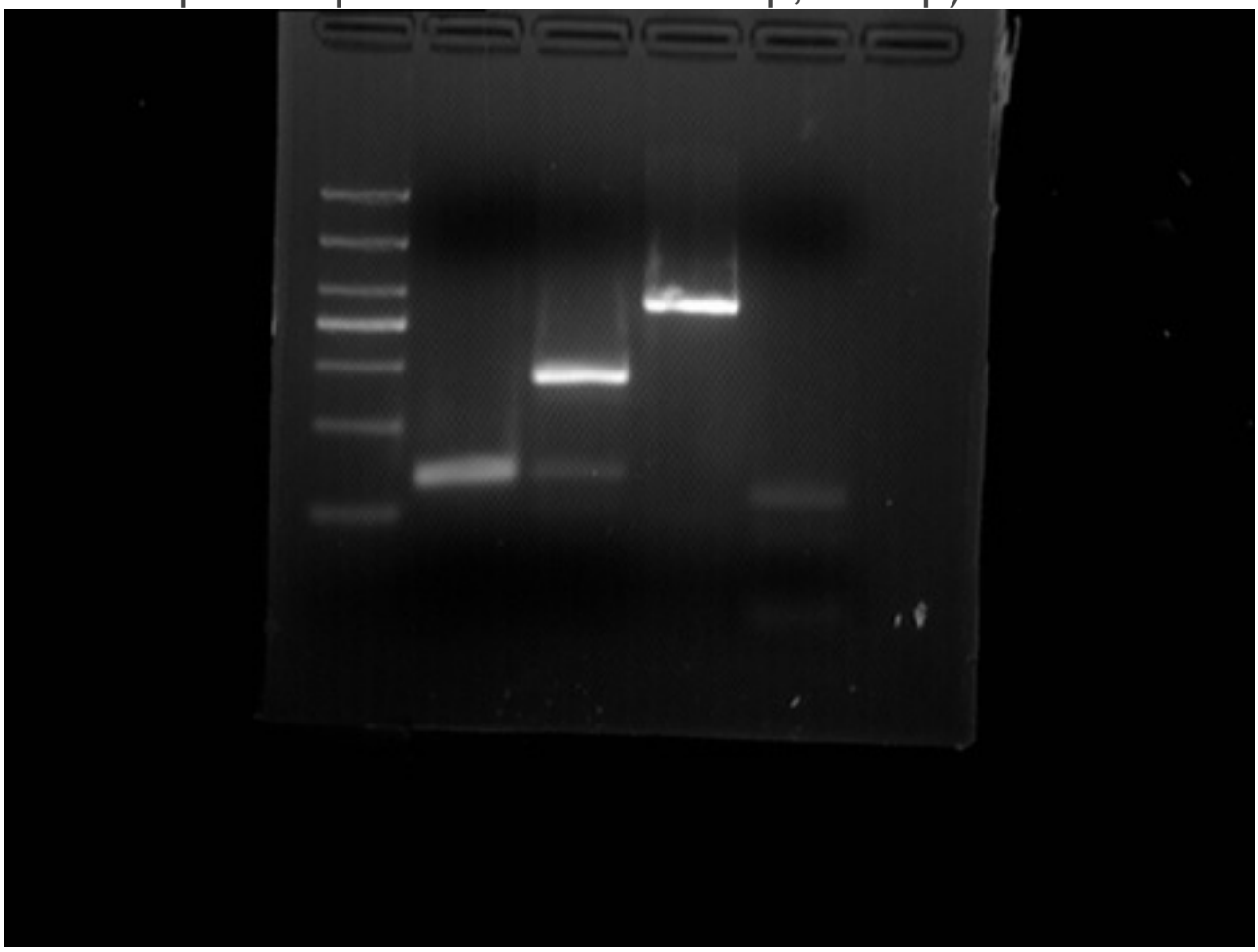
8th August, 2014 Fri

- Extracted plasmids of PKD46.
- Transformed plasmids of PKD46.
- PrimerSTAR HS polymerasedelivered.

9th August, 2014 Sat

1. new competent E.coli DH5α prepared
2. PCR & electrophoresi

3. BL21/pKD46 plate incubation150bp, 300bp)



- PKD46 delivered, Pick 8 colonyand shook.
- Primers delivered, diluted to100μmol and 10μmol before use, stored at -20℃.

8th August, 2014 Fri

- Extracted plasmids of PKD46.
- Transformed plasmids of PKD46.
- PrimerSTAR HS polymerasedelivered.

9th August, 2014 Sat

1. new competent E.coli DH5α prepared
2. PCR & electrophoresi
3. BL21/pKD46 plate incubation

10th August, 2014 Sun

- PCR& electrophoresis(1500~5000bp).
- Prepared competent cell of DH5α.
- Transformed DH5α(Test) andPKD46.
- Shook PKD46.

11th August, 2014 Mon

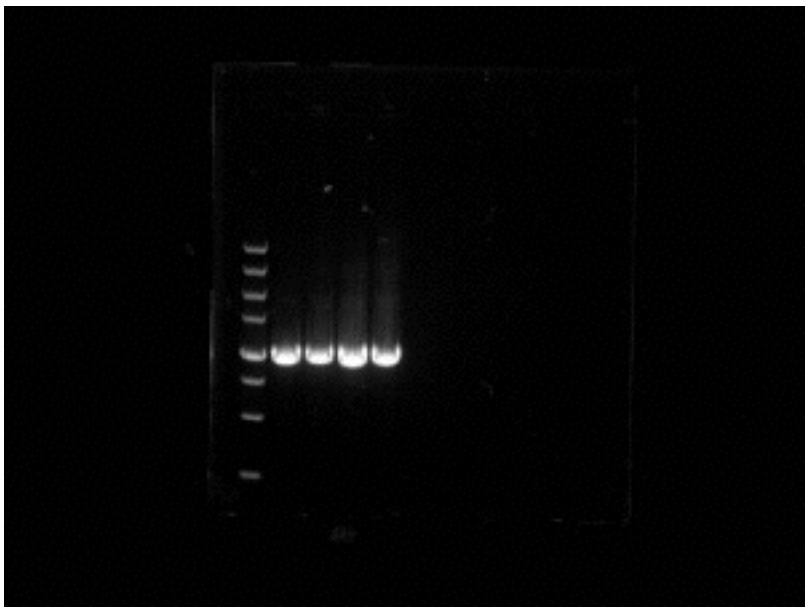
- Designed primers of circuit.
- Prepared LB culture and solidculture with antibiotic.
- Extracted PKD46.
- Digest PKD46 with endonuclease& electrophoresis.

12th August, 2014 Tue

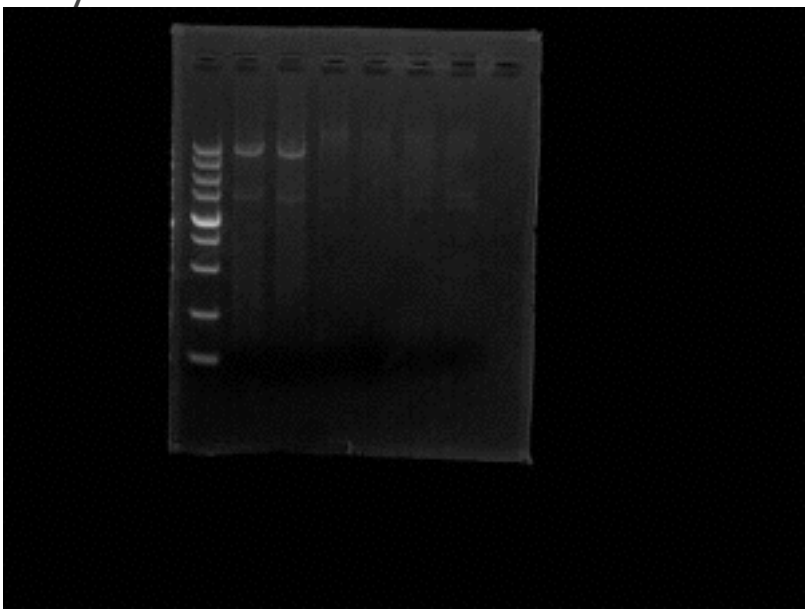
- Prepare competent cell of DH5α: HanqingLiu, Chaofan Zhang.
- Extract, digest with endonuclease & electrophoresis and transform PKD46: Liangxi Wang, Chuanyun Xu.
- PCR of 50bp, 150bp, 300bp.

20th, August

1.BL21/pkD46 confirmation:
colony PCR confirmation: check!

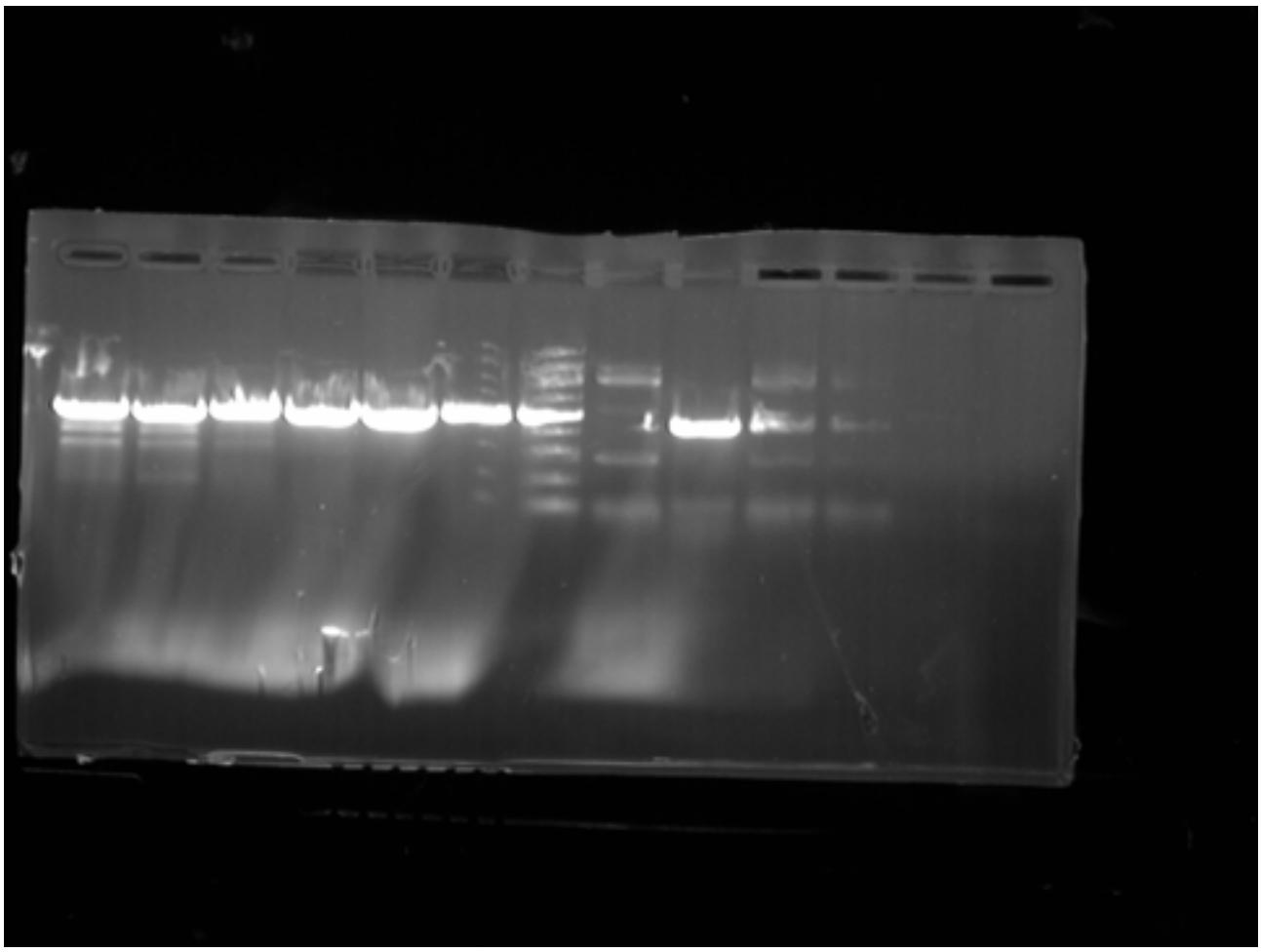


enzyme confirmation: check!

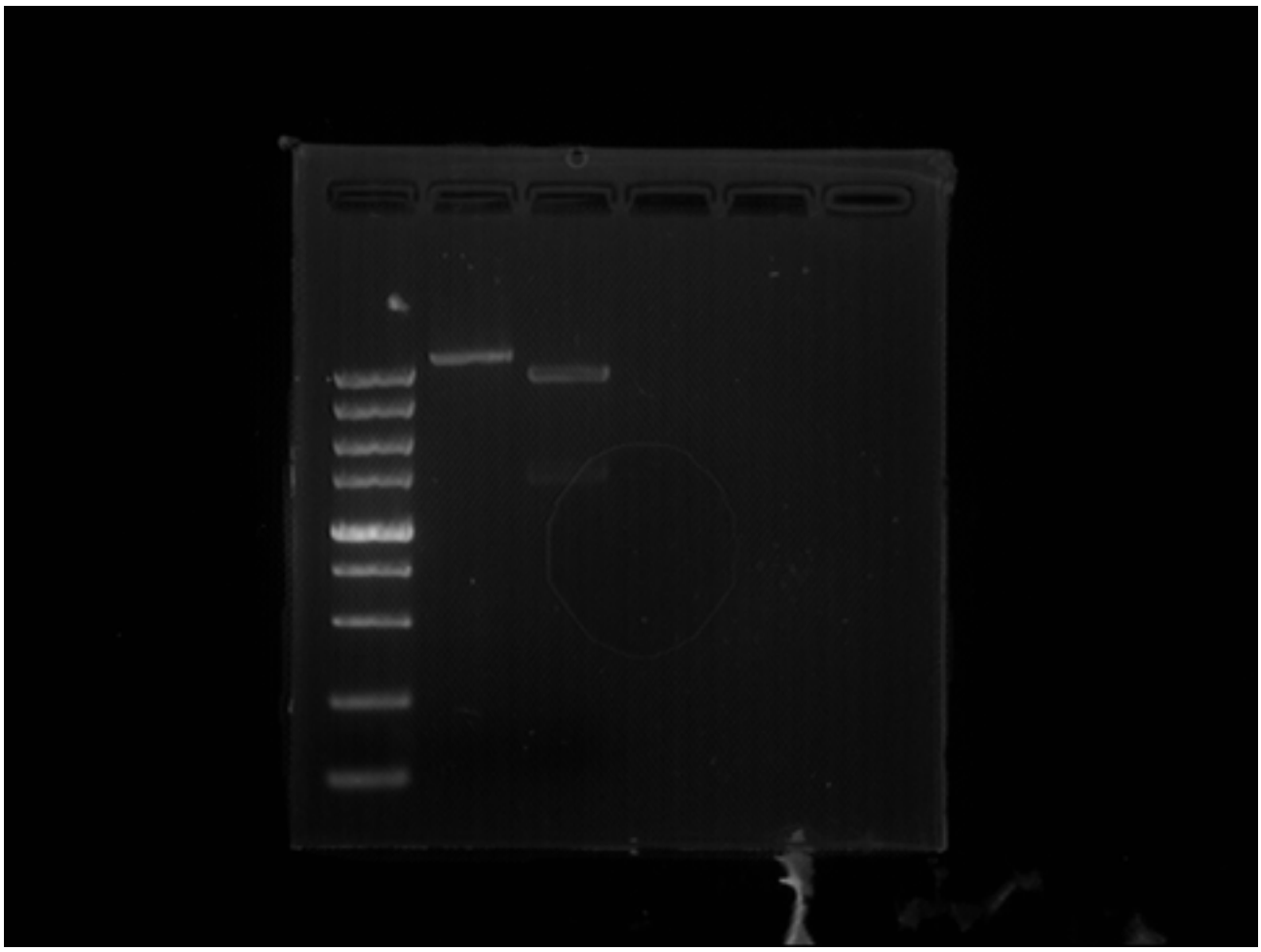


2.pKD46 confirmation:

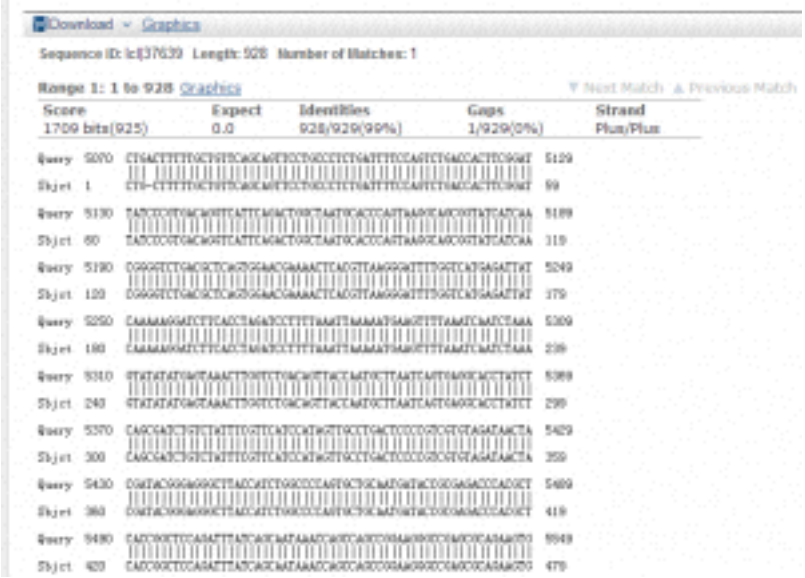
PCR confirmation: check!



restriction enzyme confirmation: check!



sequence analysis confirmation: check!



Download ▾ Graphics

Sequence ID: lcl|37639 Length: 928 Number of Matches: 1

Range 1: 1 to 928 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1709 bits(925)	0.0	928/929(99%)	1/929(0%)	Plus/Plus

```
Query  5070  CTGACTTTTTGCTGTTCAGCAGTCCTGCCCTCTGATTTCCAGTCTGACCACTTCGGAT  5129
             ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     CTG-CTTTTTGCTGTTCAGCAGTCCTGCCCTCTGATTTCCAGTCTGACCACTTCGGAT  59

Query  5130  TATCCCCTGACGGTTCATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAA  5189
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60    TATCCCCTGACGGTTCATTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAA  119

Query  5190  CGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT  5249
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  120   CGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT  179

Query  5250  CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAA  5309
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  180   CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAA  239

Query  5310  GTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT  5369
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Sbjct  240   GTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT  299

Query  5370  CAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTA  5429
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  5430  CGATACGGGAGGGCTTACCATCTGGCCCAGTGCTGCAATGATACCGCGAGACCCACGCT  5489
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Sbjct  360   CGATACGGGAGGGCTTACCATCTGGCCCAGTGCTGCAATGATACCGCGAGACCCACGCT  419

Query  5490  CACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTG  5549
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  420   CACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTG  479
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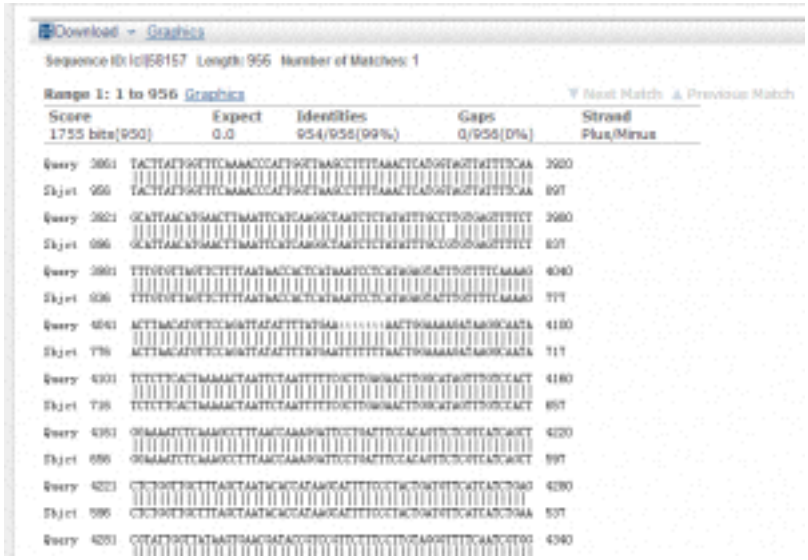
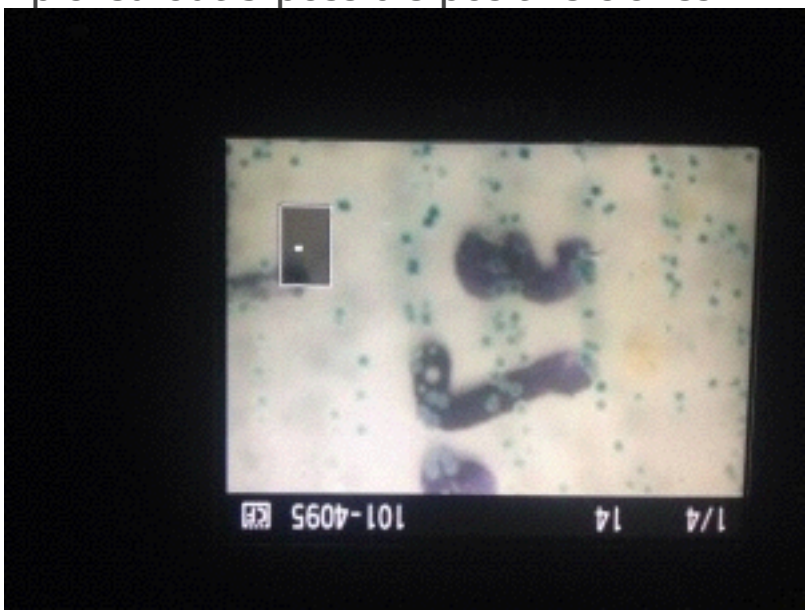
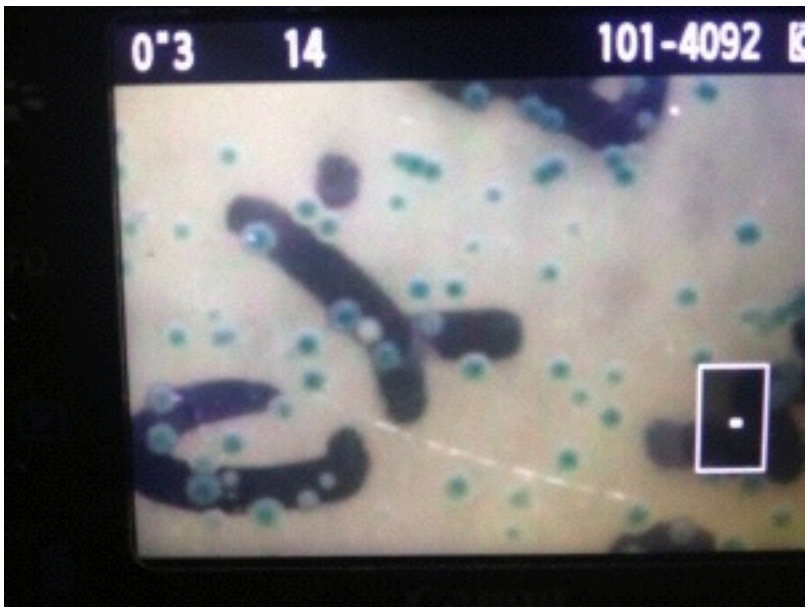


3.blue/white selection--- arabinose induced gradient experiment picked out 3 possible positive clones!





waiting for colony PCR confirmation.....

4.established a practical & appliable colony PCR system