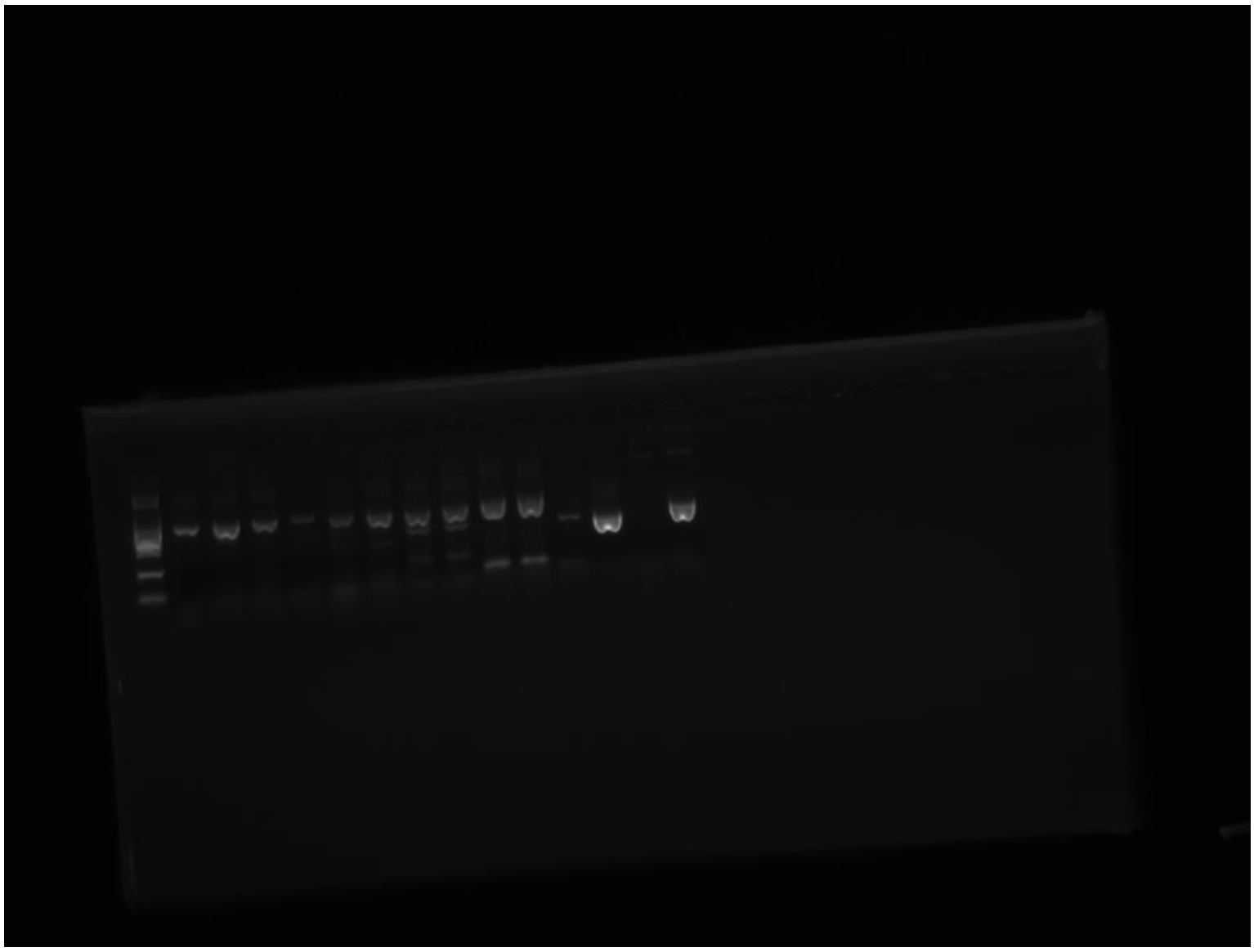
Notebook

2017/10/17

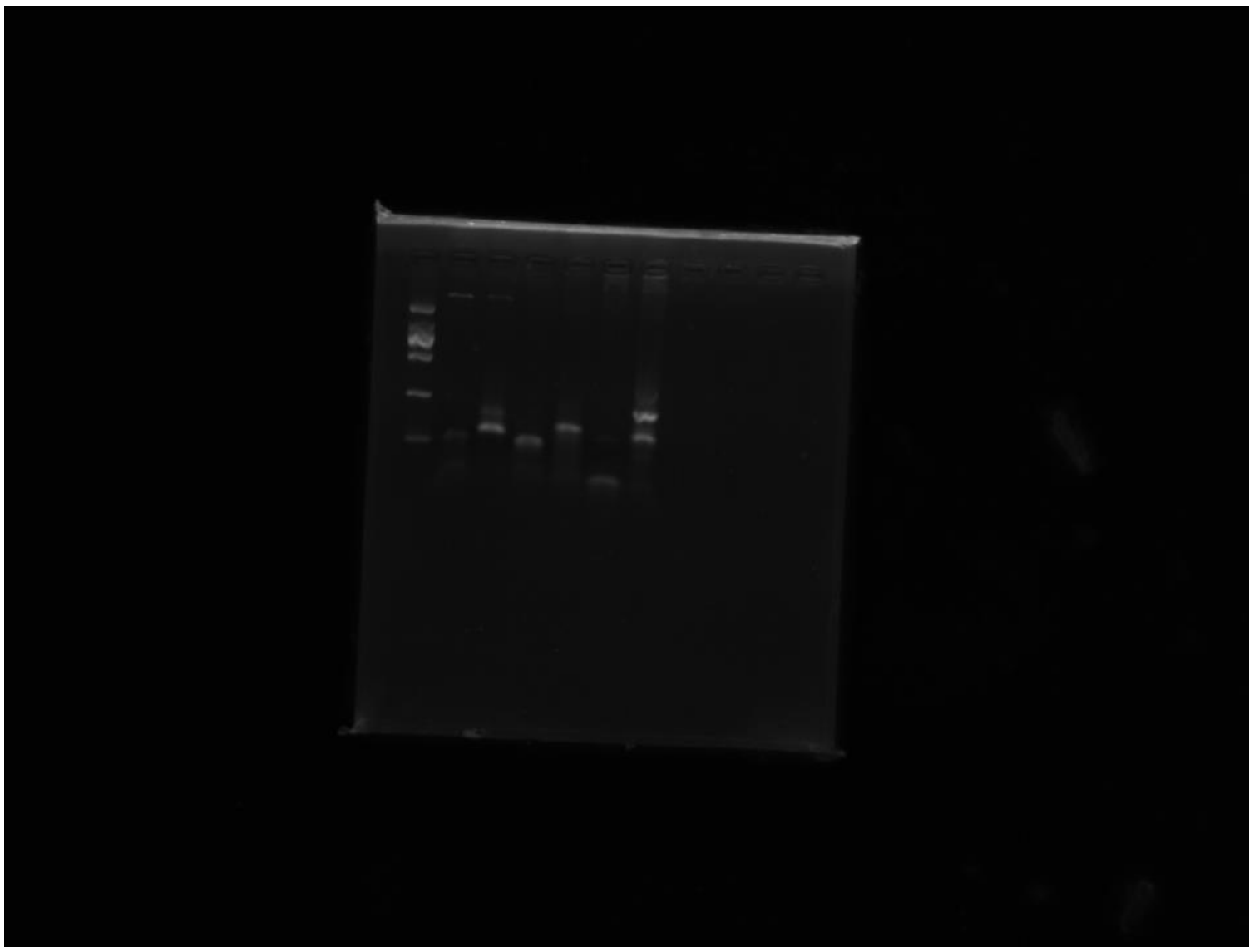
Construct plasmid: As+A3+T3+sfGFP+pETDuet-1.
Extract the plasmids that we are going to submission.
Package the part plasmids.



——Zhuoyang Chen

2017/10/13

Get several parts that we are going to submission.



——Zhuoyang Chen

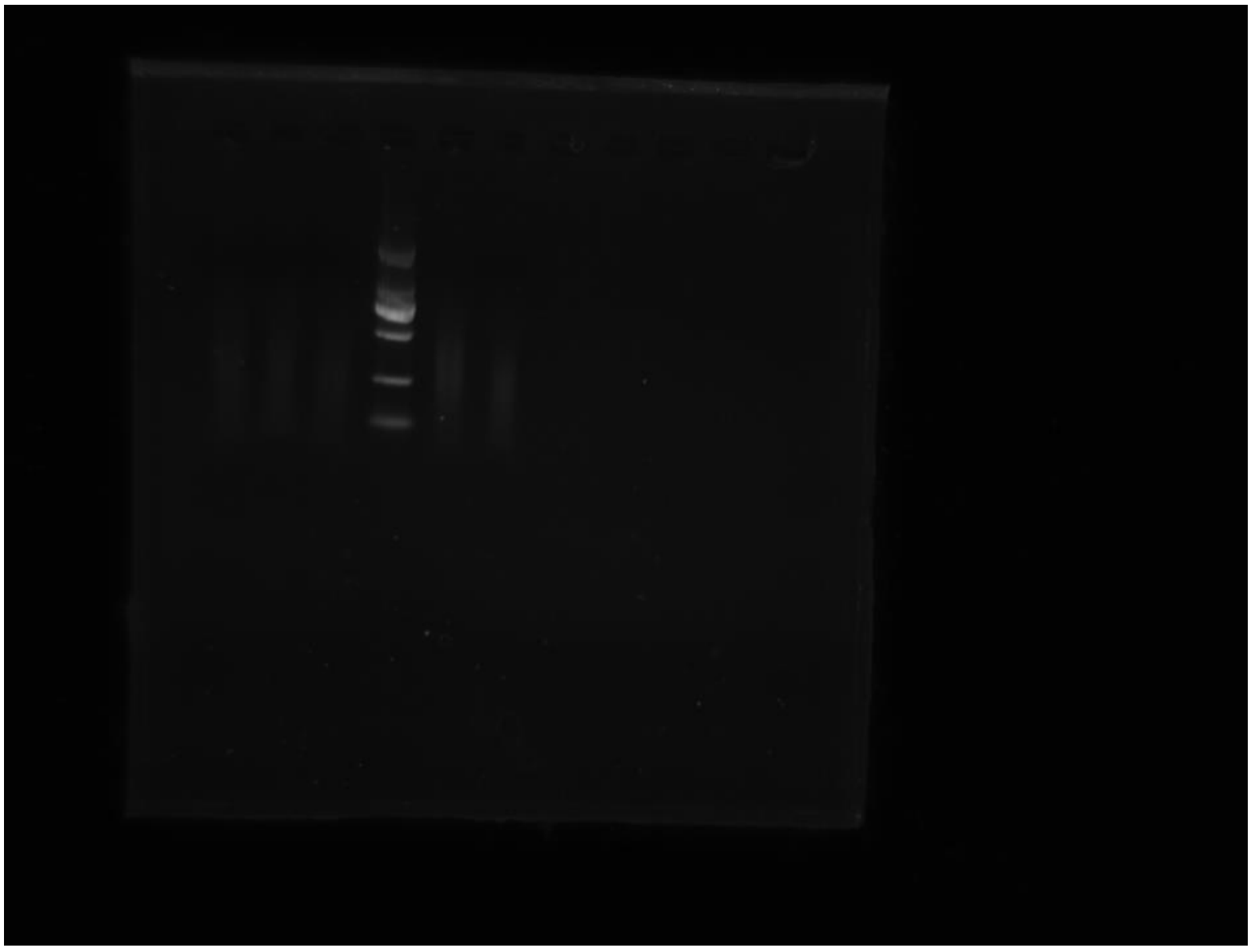
2017/10/12

Design and make the primer of making parts. Tried to get them completely, but didn’t succeed with 3 Tm, then decided to get them separately and then ligate.

——Zhuoyang Chen

2017/10/10

Characterization of Co promoter, but didn’t get a good result.
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. The results show that we failed to construct them.
Construct plasmid: pCDF+T3+sfGFP+Co+A3.



——Zhuoyang Chen

2017/9/30

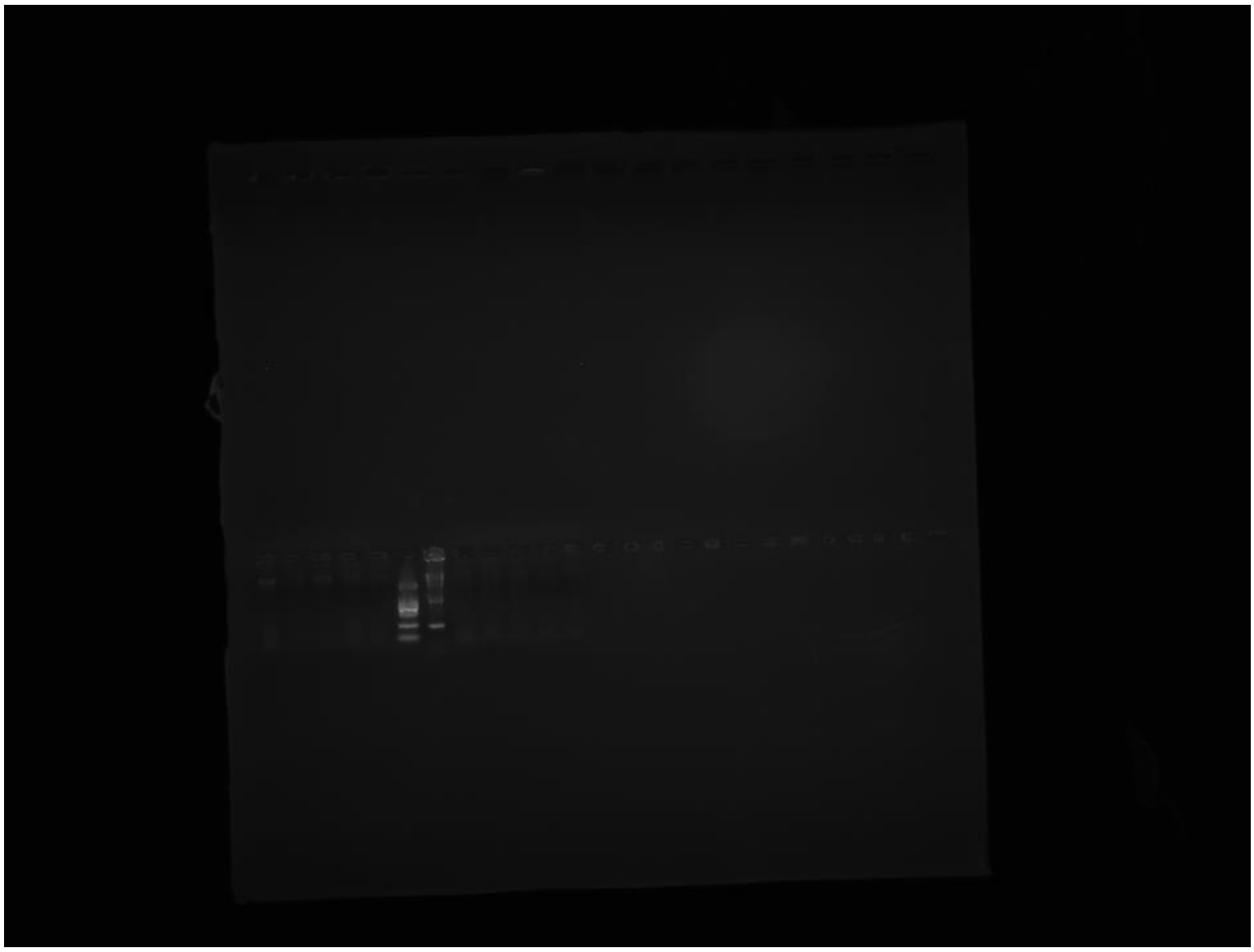
Pick the single colonies of merR+pET，T3+cjBlue+pET and those plasmids we construct for a second time.

Construct plasmids: As+sfGFP+pET.

——Haoyu Liu

2017/9/29

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. The results show that we failed to construct them.
Construct plasmids that we failed to construct for a second time.



——Haoyu Liu

2017/9/28

Pick single colonies of merR+pET，T3+cjBlue+pET.

——Haoyu Liu

2017/9/27

Construct plasmids: merR+pET，T3+cjBlue+pET.

——Haoyu Liu

2017/9/26

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.

——Haoyu Liu

2017/9/25

Pick single colonies of Co[MluI、BamHI]+sfGFPpET、As[EcoRI、BamHI]+sfGFP+pET、T1+cp+pCDF、T3+cp+pET、T3+Red+pET、T3+amilGFP+pET。

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.

——Haoyu Liu

2017/9/24

Construct plasmids: Co+sfGFP+pET，As+sfGFP+pET，T1+amilCP+pCDF，T3+amilCP+pET，T3+eforRed+pET，T3+amilGFP+pET.

Pick single colonies of A3+pET，T1+amilCP+pCDF.

——Haoyu Liu

2017/9/23

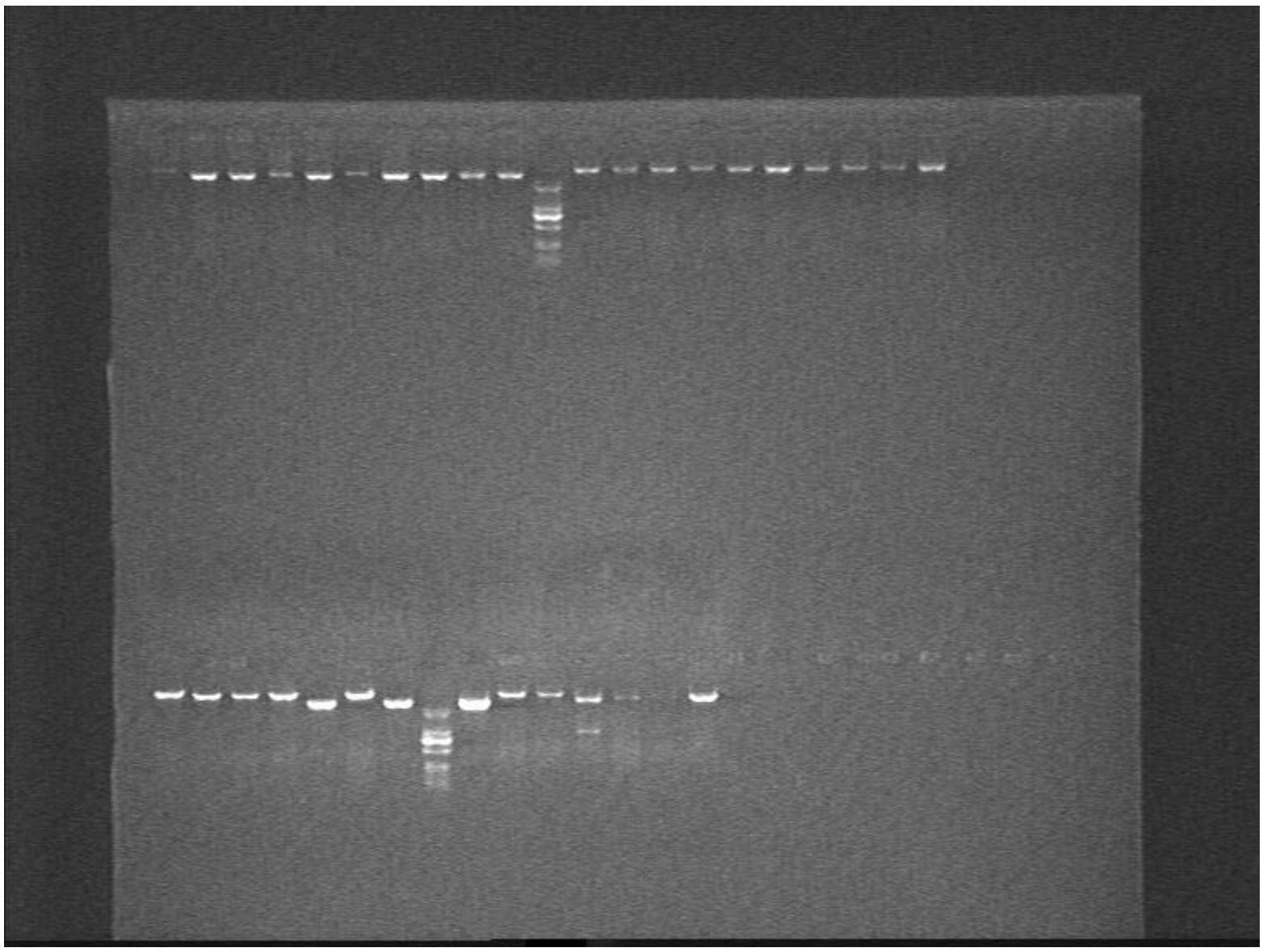
Construct plasmids: A_3+pET，T_1+amilCP+pCDF. Then transform.

——Haoyu Liu

2017/9/15

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.

Construct plasmids: Co+A1+T1+ cjBlue +pCDF，Co+A1+T1+ cjBlue +pET.

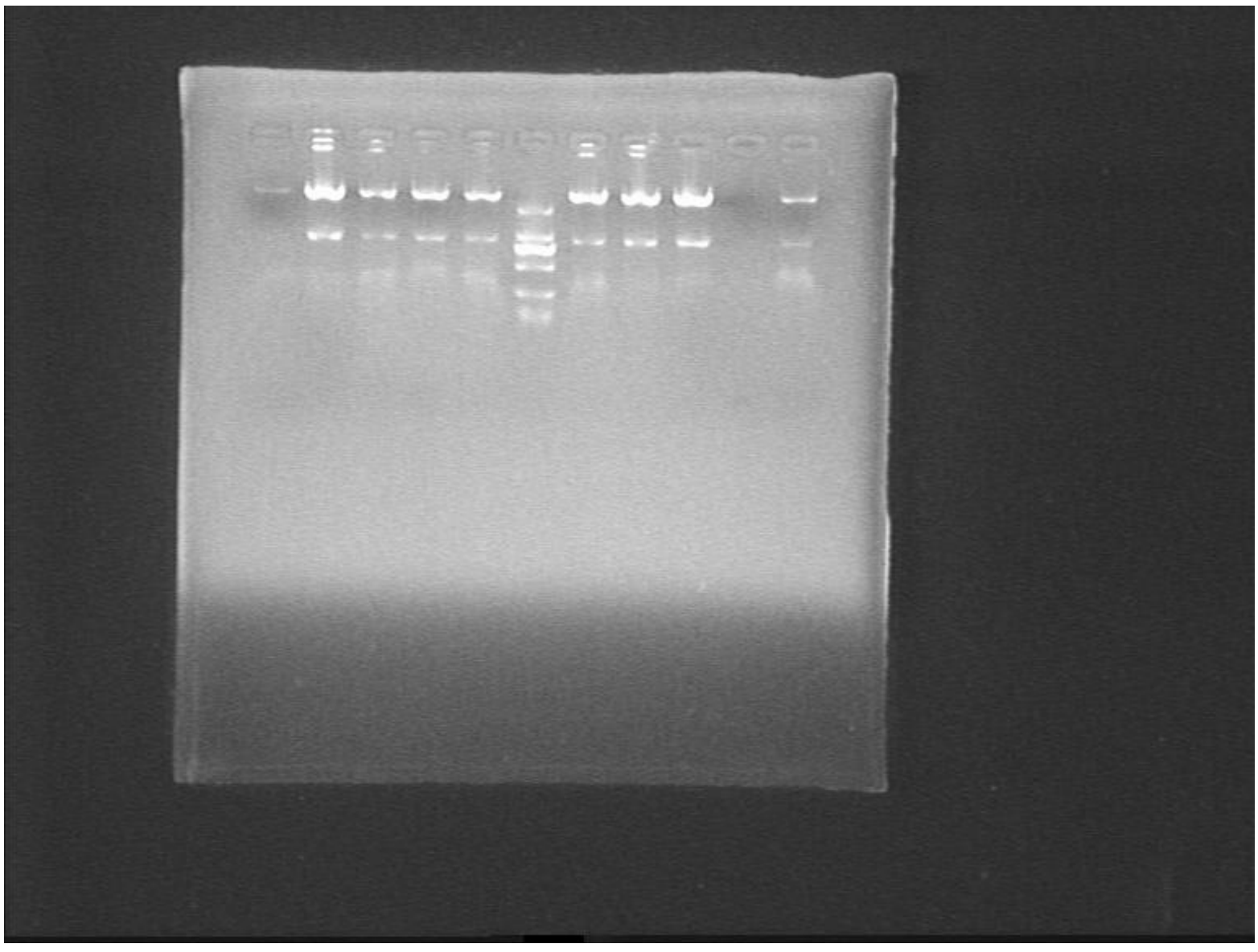


——Haowan Chang

2017/9/14

Extract the plasmid A1+T1+amilGFP+pCDF，A1+T1+eforRed+pCDF, that we constructed before, then restricted digestion and cataphroresis.

Construct plasmids: Co+A1+T1+ sfGFP +pCDF，Co+A1+T1+ sfGFP +pET，Co+A1+T1+ amilGFP +pCDF.



——Haowan Chang

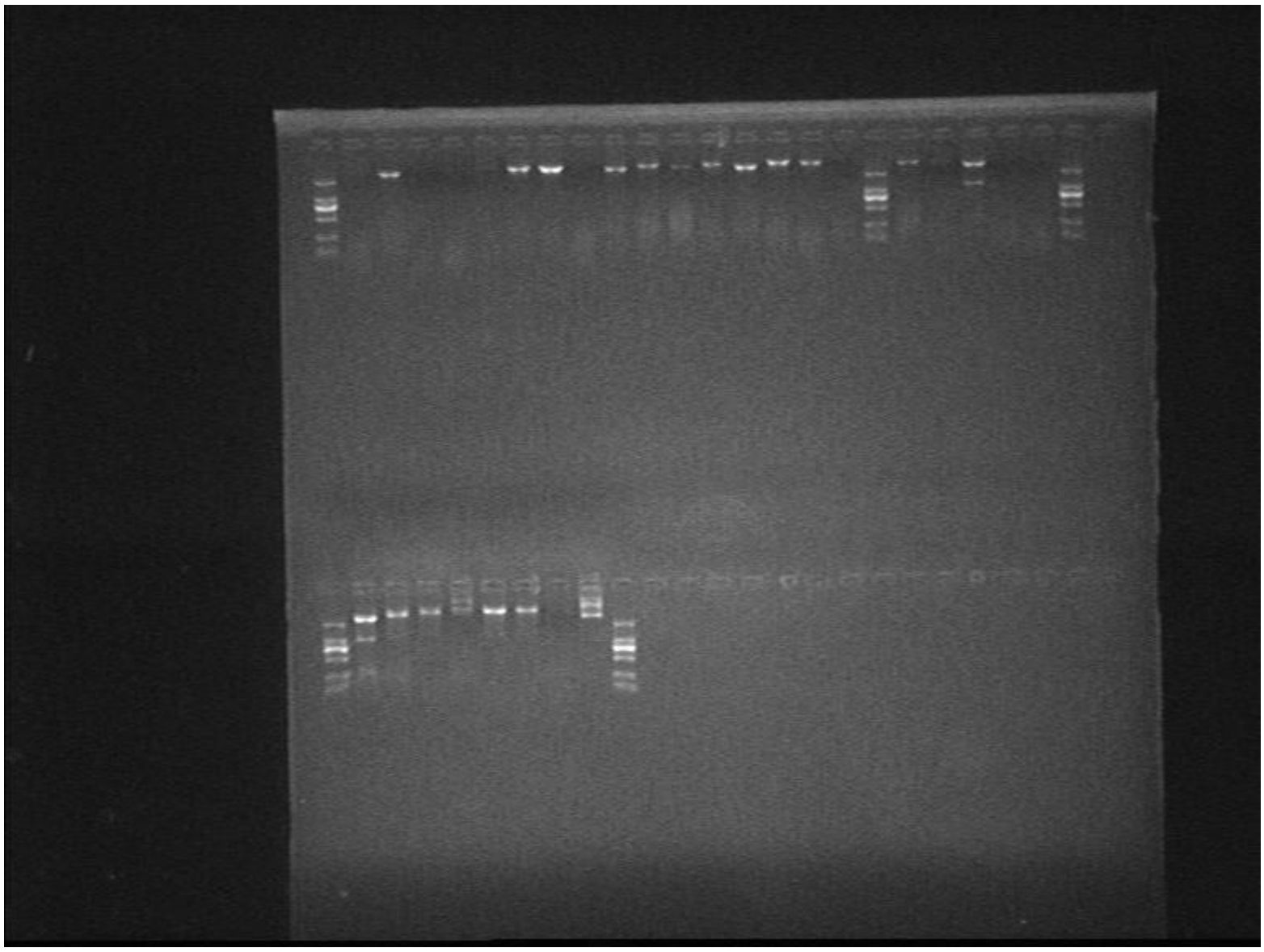
2017/9/13

Construct plasmids: Co+A1+T1+cjBlue+pET，Co+A1+T1+amilCP+pET，Co+A1+T1+amilGFP+pET，Co+A1+T1+ eforRed +pCDF，Co+A1+T1+ amilGFP +pCDF，A2+T2+amilGFP+pET，A2+T2+cjBlue+pET，A2+T2+sfGFP+pET，T1+A1+amilCP+pET，A1+PCDF.

——Haowan Chang

2017/9/12

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.



——Haowan Chang

2017/9/11

Construct plasmids: A1+T1+eforRed+pCDF，A1+T1+amilGFP+pCDF.

——Haowan Chang

2017/9/10

Construct plasmids: Co+A1+T1+sfGFP+pCDF，Co+A1+T1+sfGFP+pET，Co+A1+T1+amilGFP+pCDF，Co+A1+T1+amilGFP+pET，Co+A1+T1+eforRed+pCDF，Hg merR+ pET，Hg merR+pCDF. Then transform.

Transform A1+pET.

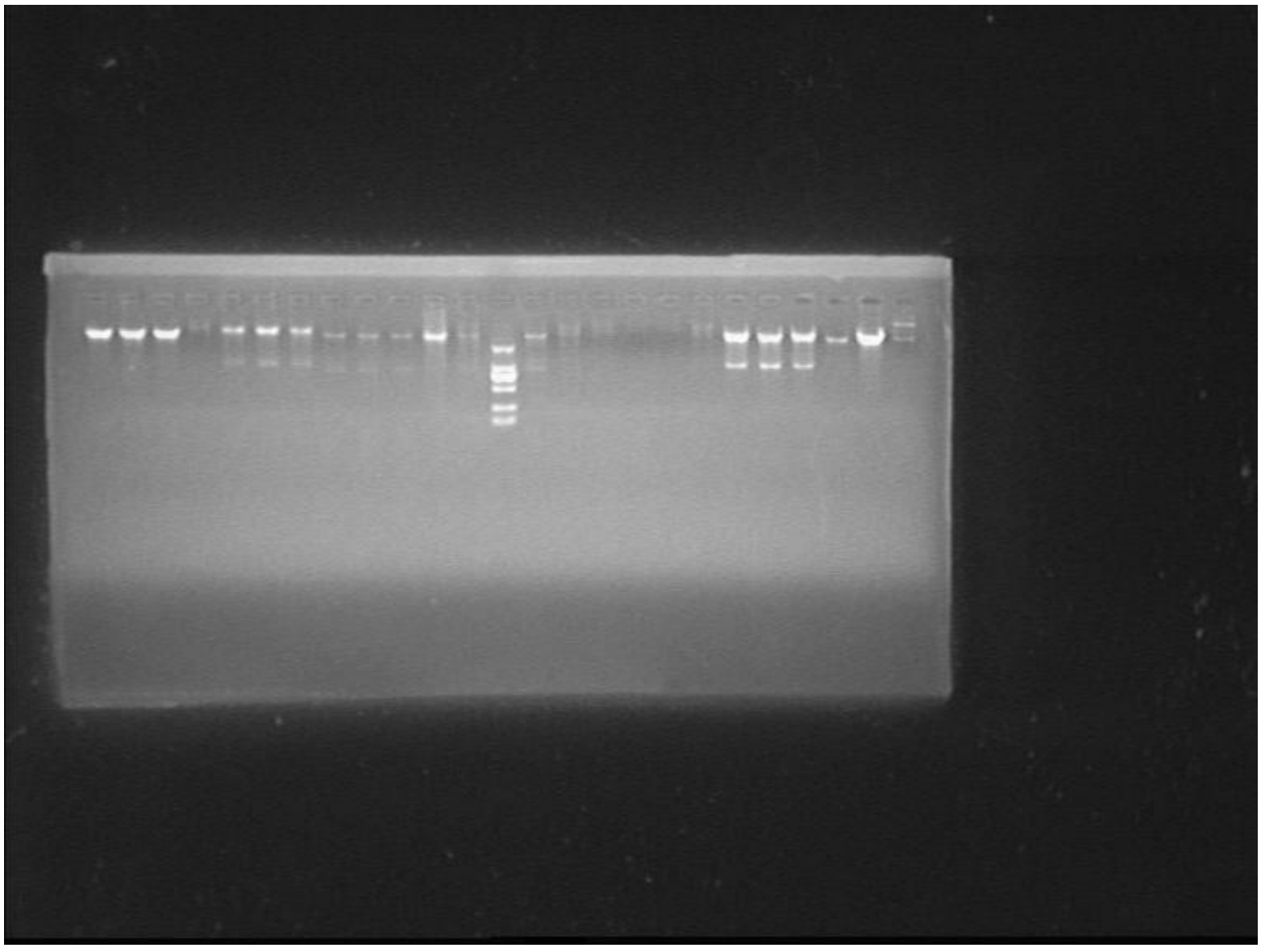
——Haowan Chang

2017/9/9

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.

Construct plasmids: A1+T1+eforRed+pCDF，A1+T1+amilGFP+pCDF，A1+T1+sfGFP+pCDF，Co+A1+T1+sfGFP+pCDF，Hg merR+pET ，Hg merR+pCDF，A1+T1+eforRed+pCDF，A1+T1+amilGFP+pCDF. Then transform.

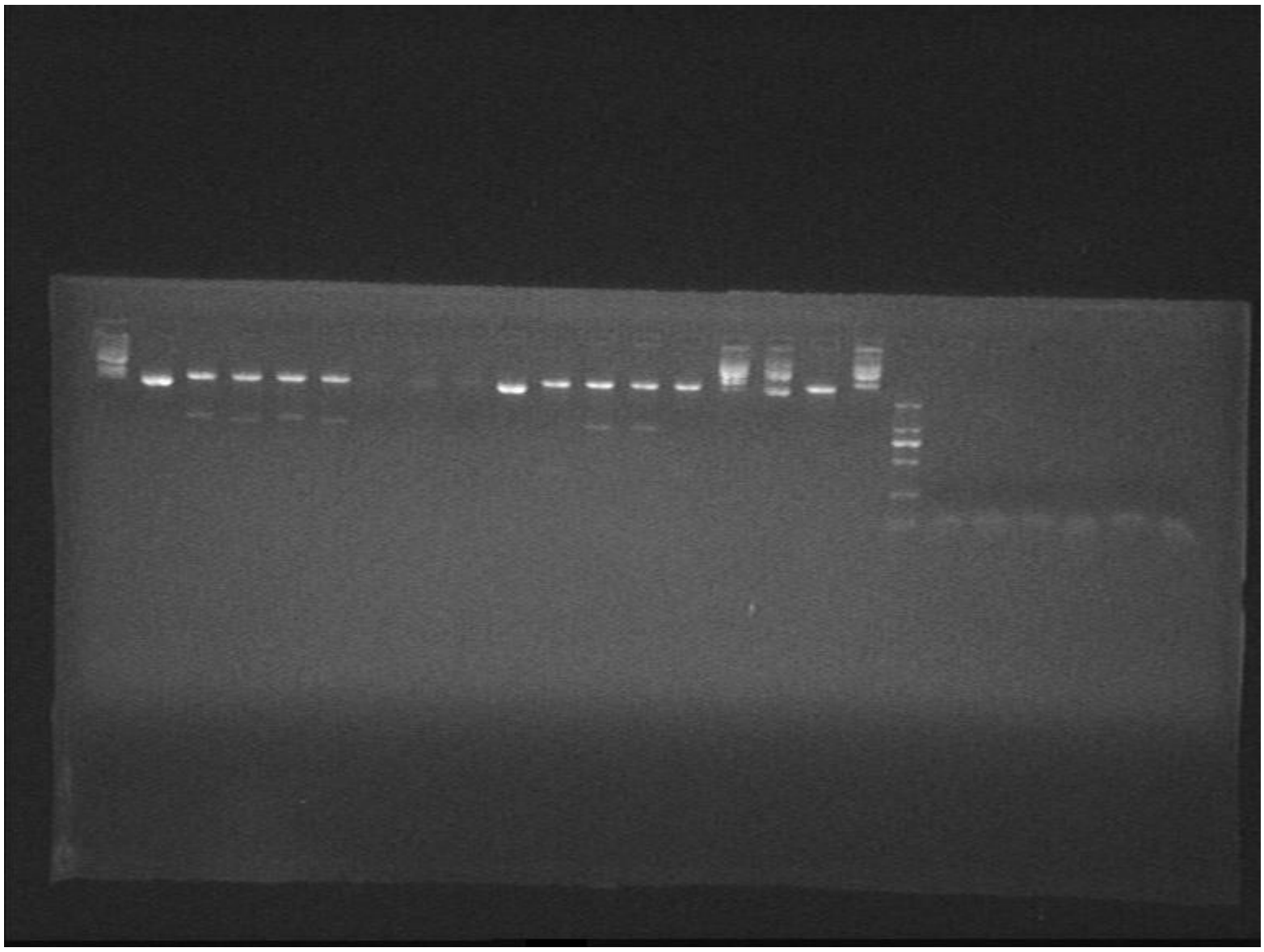
Transform T1+sfGFP+pET.



——Haowan Chang

2017/9/8

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.

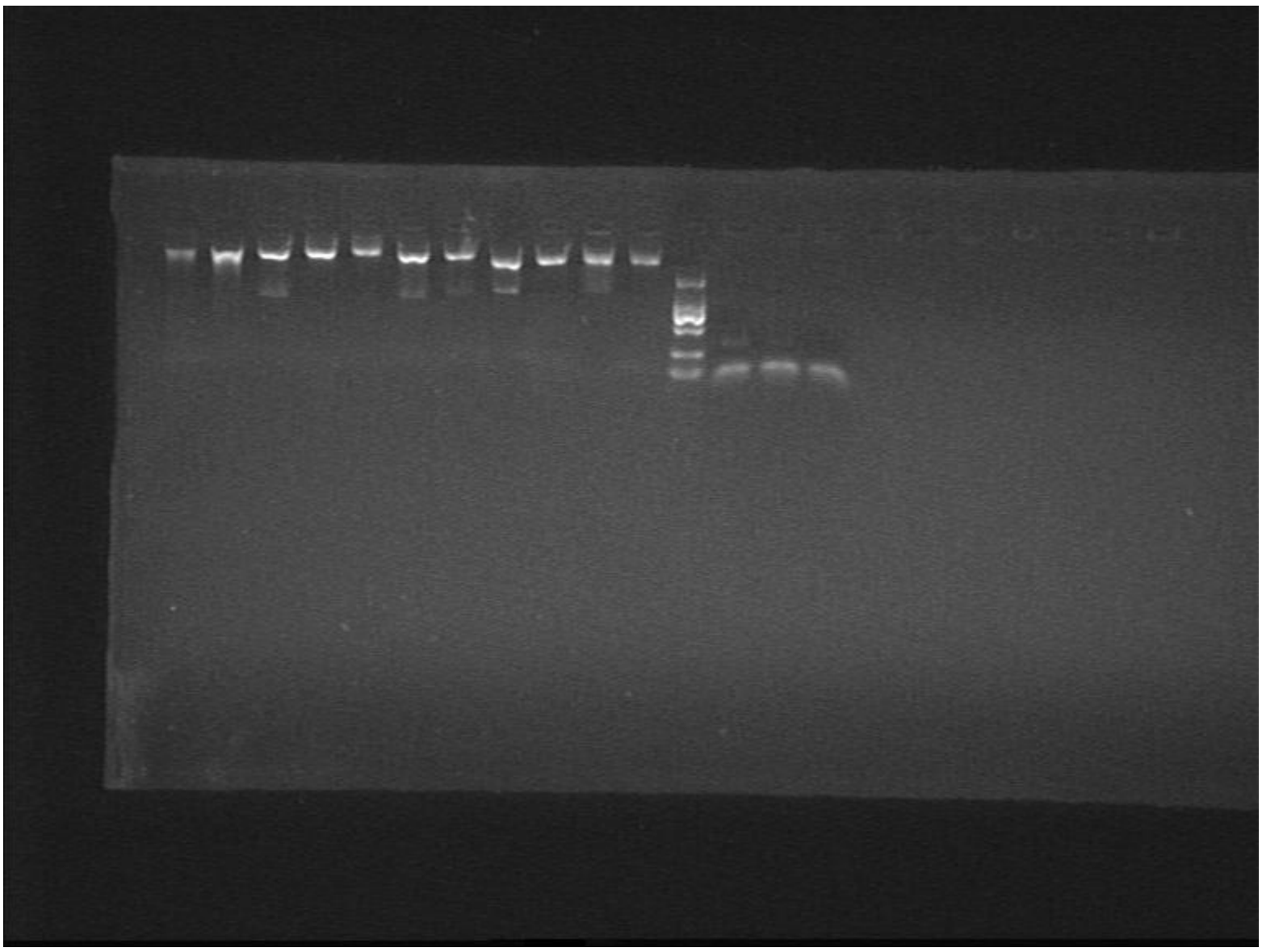


——Haowan Chang

2017/9/7

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. Results show that we failed to construct them.

Construct plasmids: merR+pET，merR+pCDF，Co+A1+T1+sfGFP+pCDF.



——Haowan Chang

2017/9/6

Construct plasmids: A1+T1+amilGFP+pCDF，A1+T1+sfGFP+pCDF，A1+T1+eforRed+pCDF.

——Haowan Chang

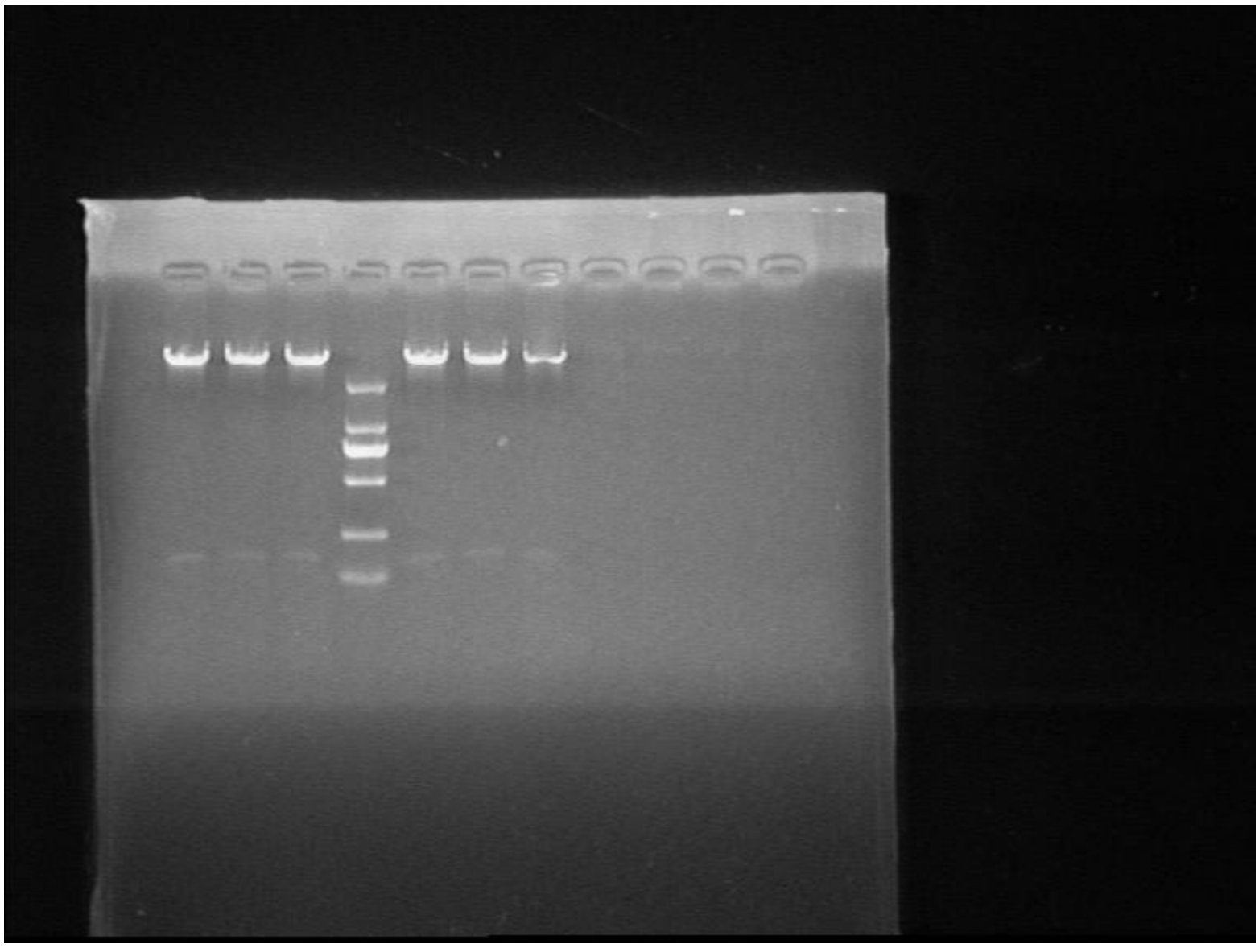
2017/9/5

Construct plasmids: merR+pET，merR+pCDF，A2+pCDF.

——Haowan Chang

2017/9/4

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.

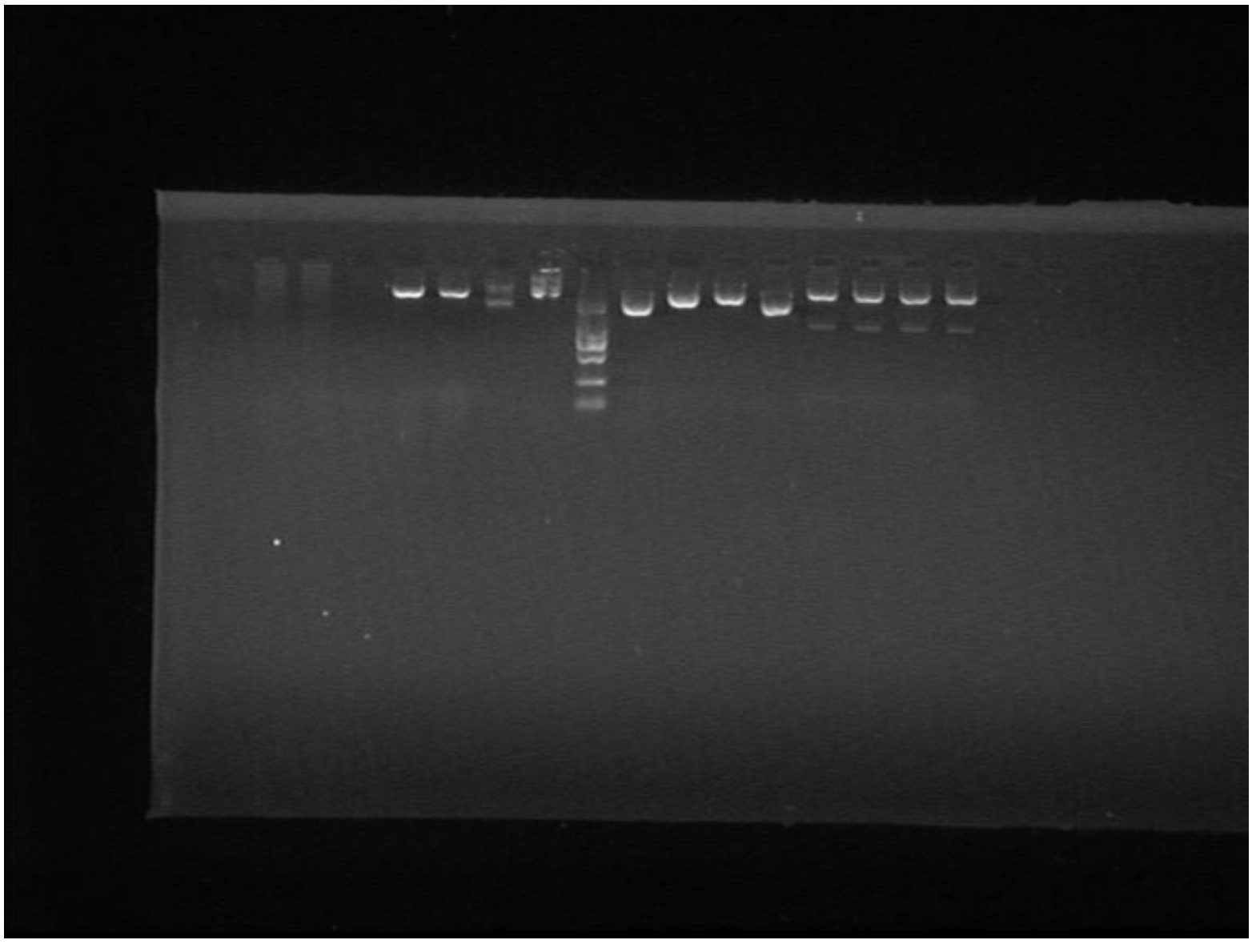


——Yi Yang

2017/9/3

Pick single colonies of pET+A1.

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.



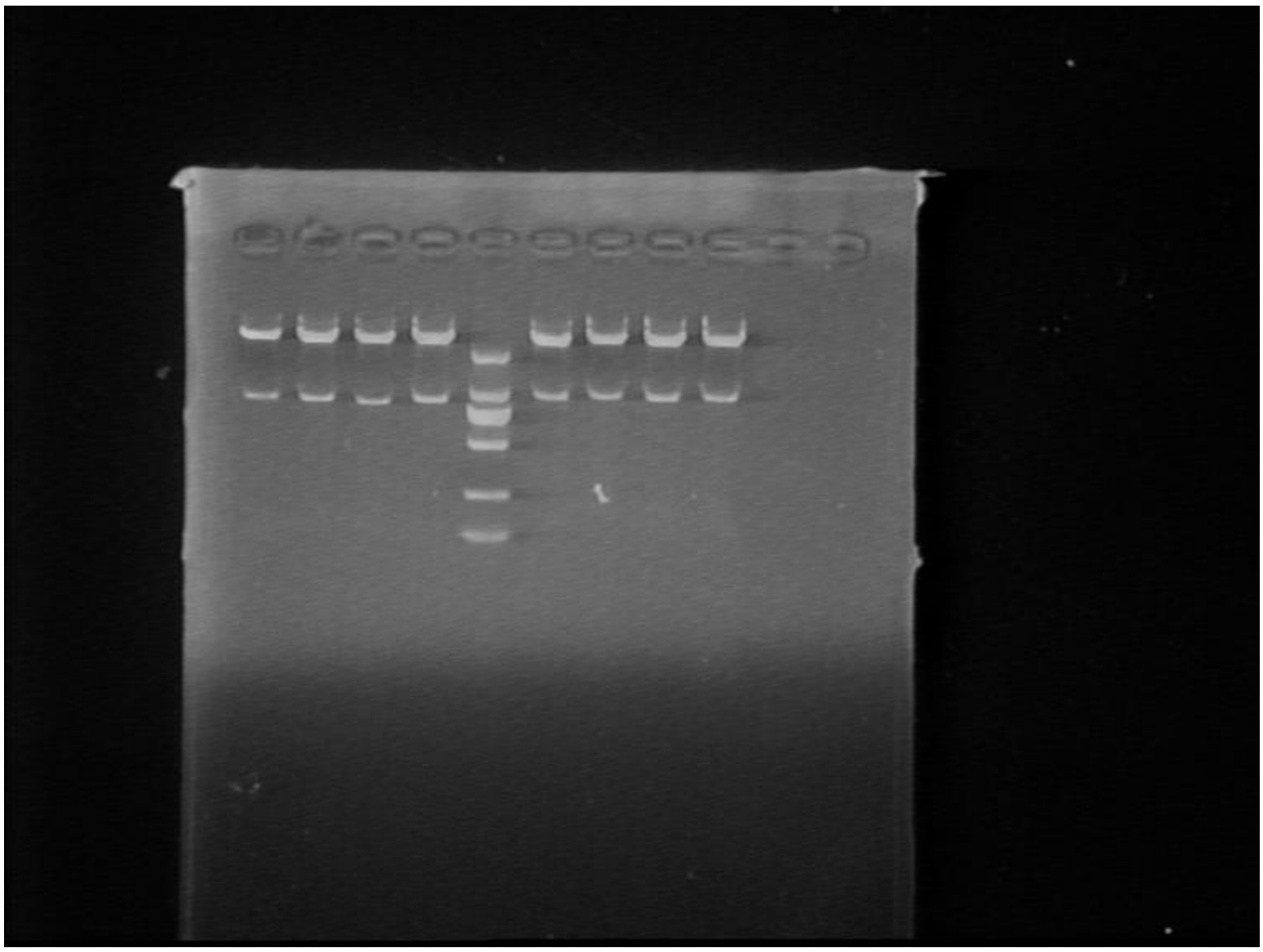
——Yi Yang

2017/9/2

Construct plasmids: pET+A1.

Pick single colonies of pET+A2，pCDF+A2，pET+Co promoter+amilCP，pET+T1+amilCP.

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.



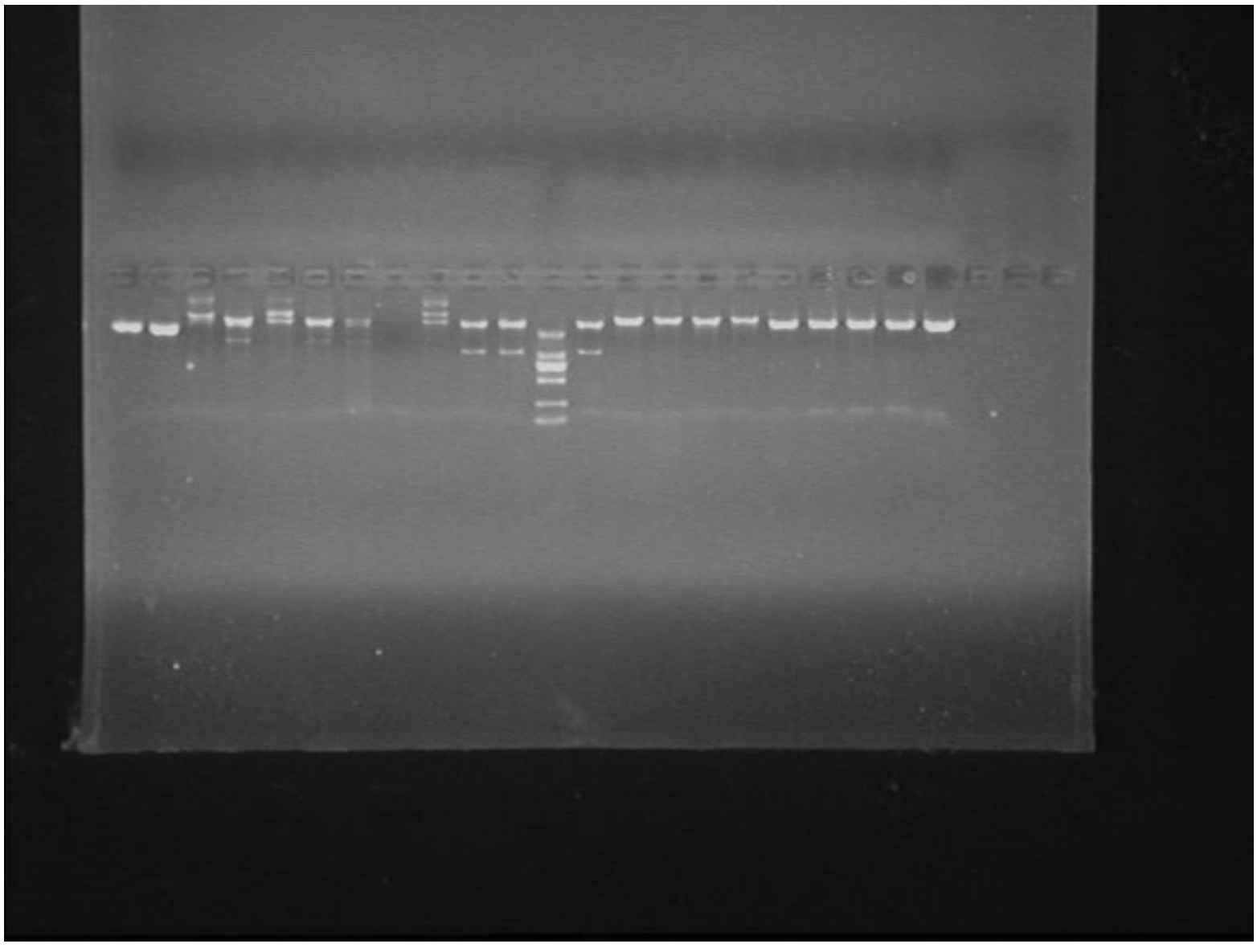
——Yi Yang

2017/9/1

Construct plasmids: pET+A2，pCDF+A2，pET+Co promoter+amilCP，pET+T1+amilCP.

Pick single colonies of pET+Co promoter+amilCP，pET+T1+eforRed，pCDF+T1+eforRed，pET+T2+eforRed，pCDF+T2+eforRed.

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. Results show that we failed to construct pET+A1.



——Yi Yang

2017/8/31

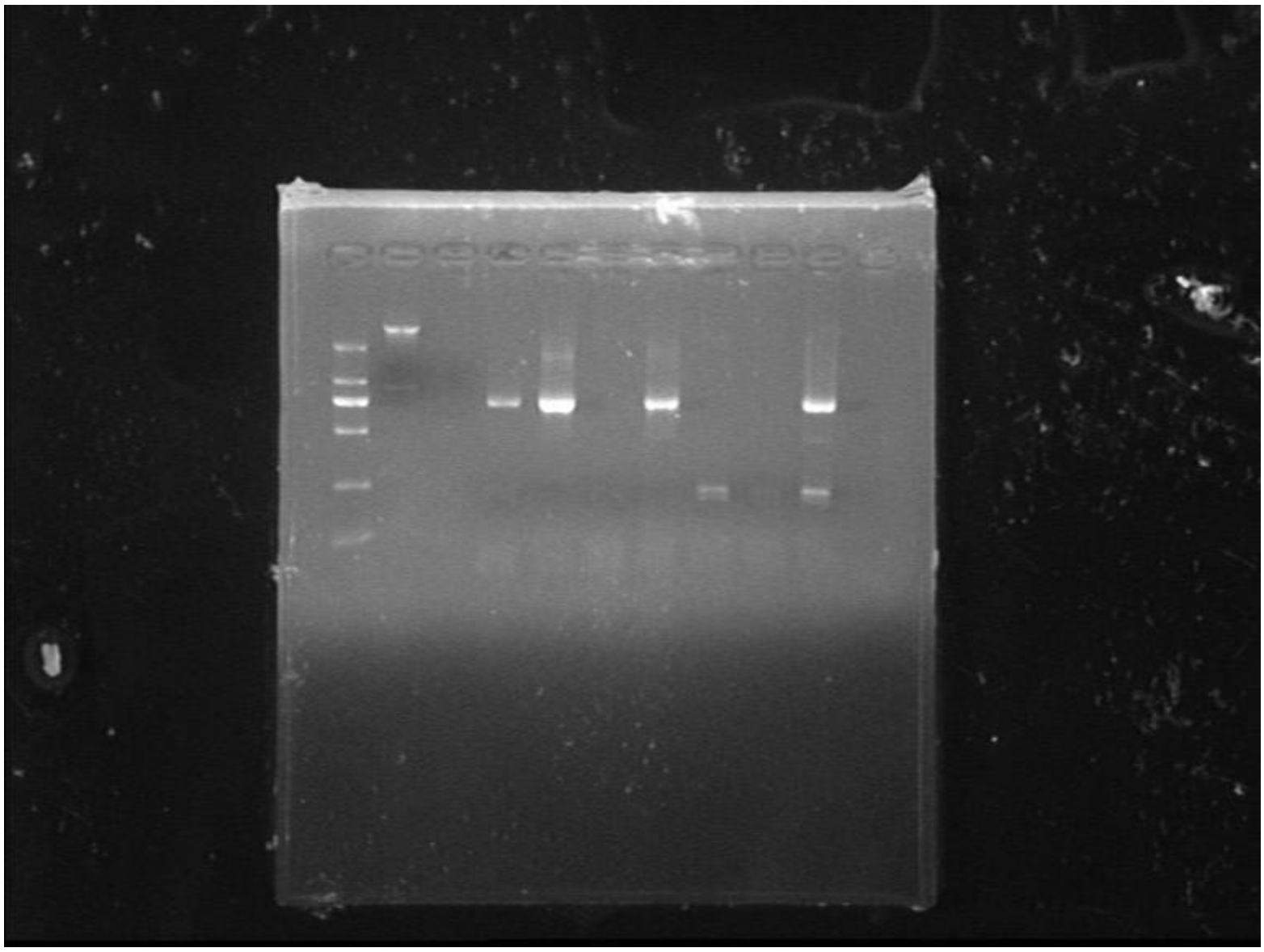
Construct plasmid: pET+Co promoter+amilCP，pET+T1+eforRed，pCDF+T1+eforRed，pET+T2+eforRed，pCDF+T2+eforRed.

Pick the single colonies of pET+T2+amilCP，pCDF+T2+amilCP，pET+A1，pCDF+A1.

——Yi Yang

2017/8/30

Construct plasmid: pET+T2+amilCP，pCDF+T2+amilCP，pET+A1，pCDF+A1.



——Yi Yang

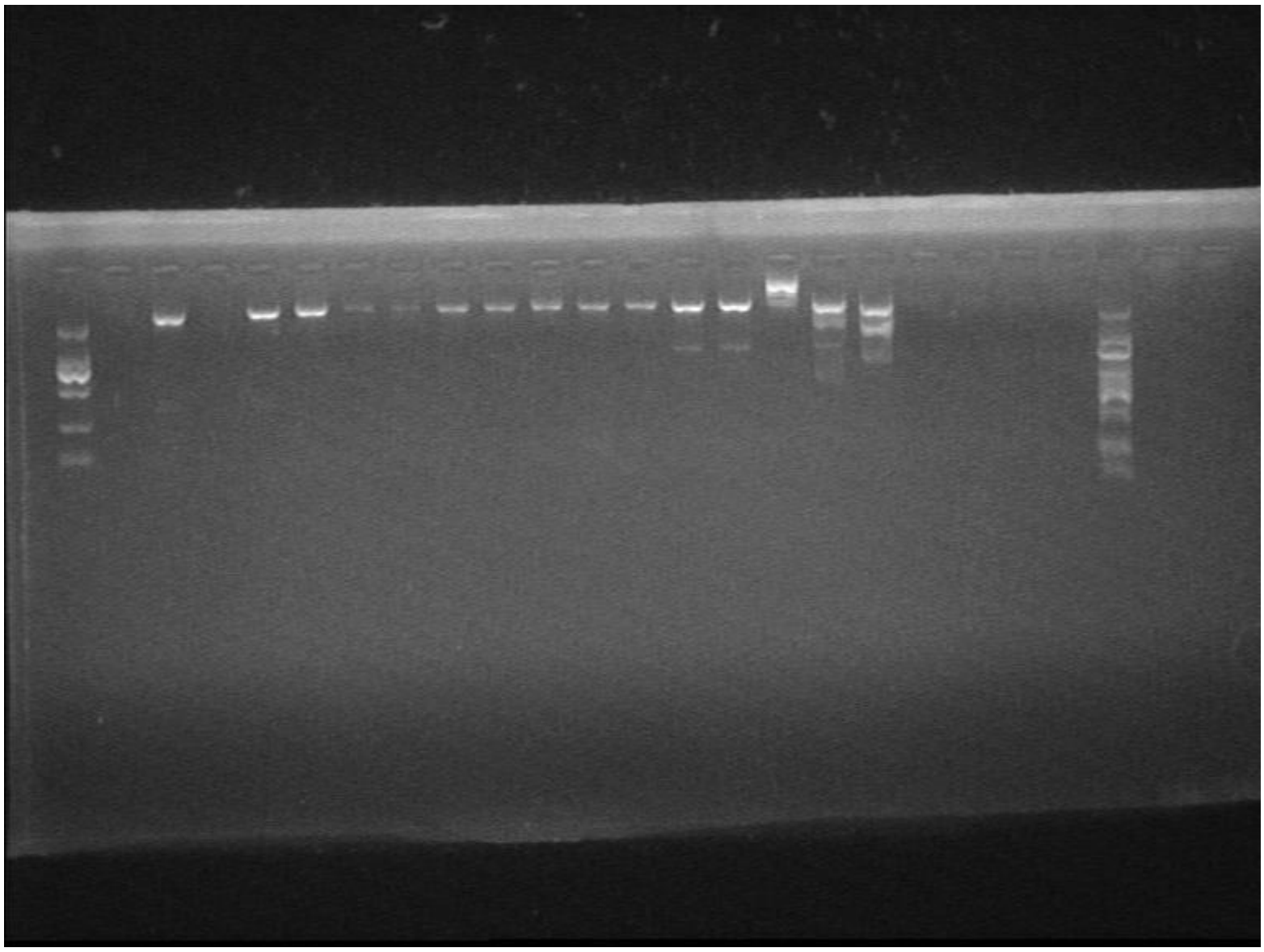
2017/8/29

Pick the single colonies of pET+T1+cjBlue；pCDF+T1+cjBlue.

Transform the vectors constructed yesterday: pET+amilCP，pCDF+eforRed，pCDF+J23100 T2+sfGFP，pCDF+J23150 T2+sfGFP.

Construct plasmids: pET+amilCP，pCDF+eforRed，pCDF+T1+sfGFP，pCDF+J23100 T2+sfGFP，pCDF+J23150 T2+sfGFP，pET+T1+cjBlue，pCDF+T1+cjBlue.

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.



——Yi Yang

2017/8/28

Construct plasmids: pET+T1+cjBlue；pCDF+T1+cjBlue.

The recombinant plasmids was constructed by digestion of the PCR product and plasmid backbone. pET + amilCP; pCDF + eforRed; pCDF + J23100 T2 + sfGFP; pCDF + J23150 T2 + sfGFP.

Pick the single colonies of pET+T1+amilGFP；pCDF+T1+amilGFP；pET+T1+Red；pCDF+T1+ Red；pET+merR.

——Yi Yang

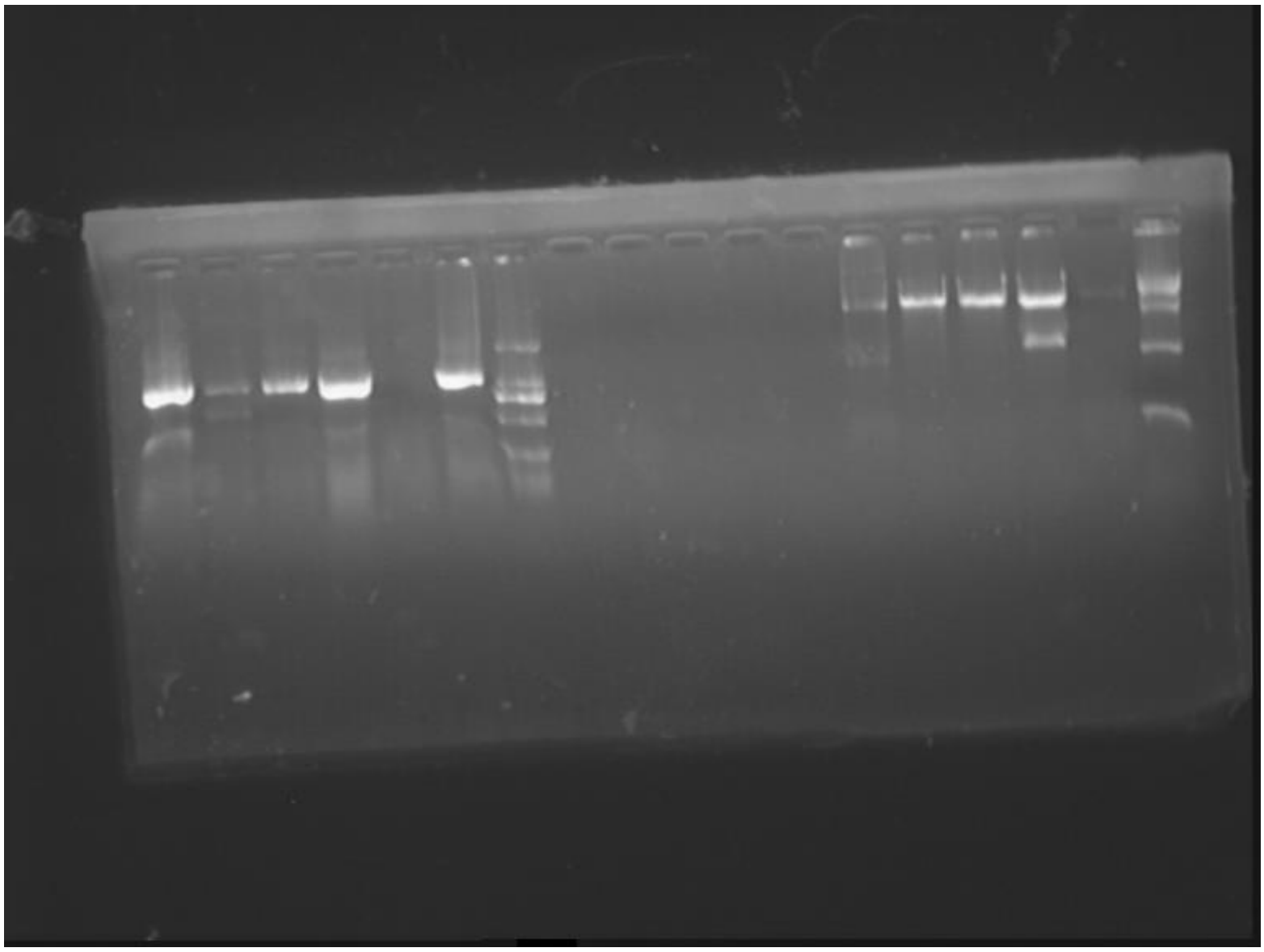
2017/8/27

Construct plasmids: pET+T1+amilGFP；pCDF+T1+amilGFP；pET+T1+eforRed；pCDF+T1+efroRed；pET+merR.

——Yi Yang

2017/8/26

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. Results show that we failed to construct merR+pET.

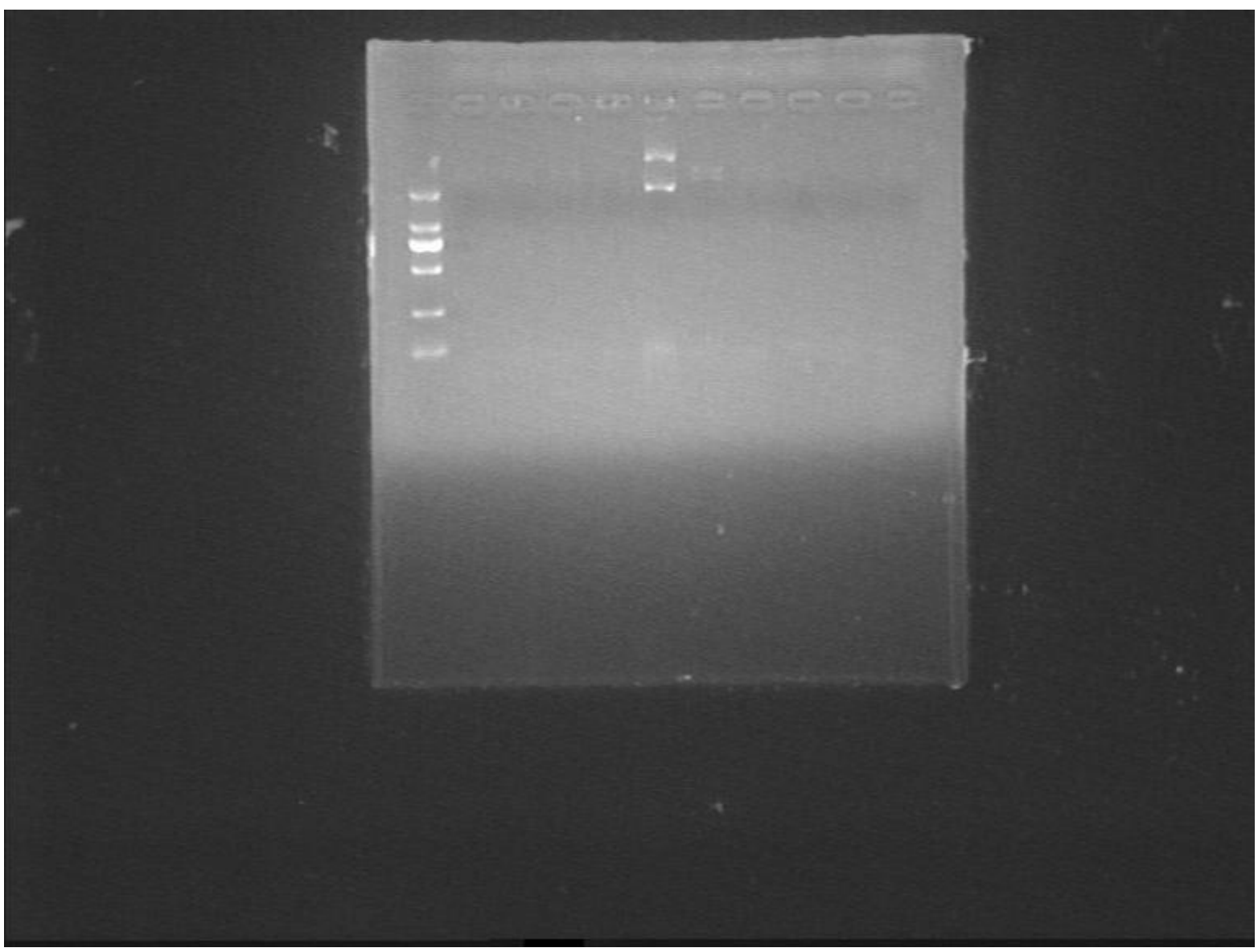


——Yi Yang

2017/8/25

Pick the single colonies of pCDF+T2+cjBlue；pET+T2+amilGFP；pET+T2+amilCP；pCDF+T2+amilCP；merR+pET.

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. Results show that we failed to construct pET+T1+amilGFP.



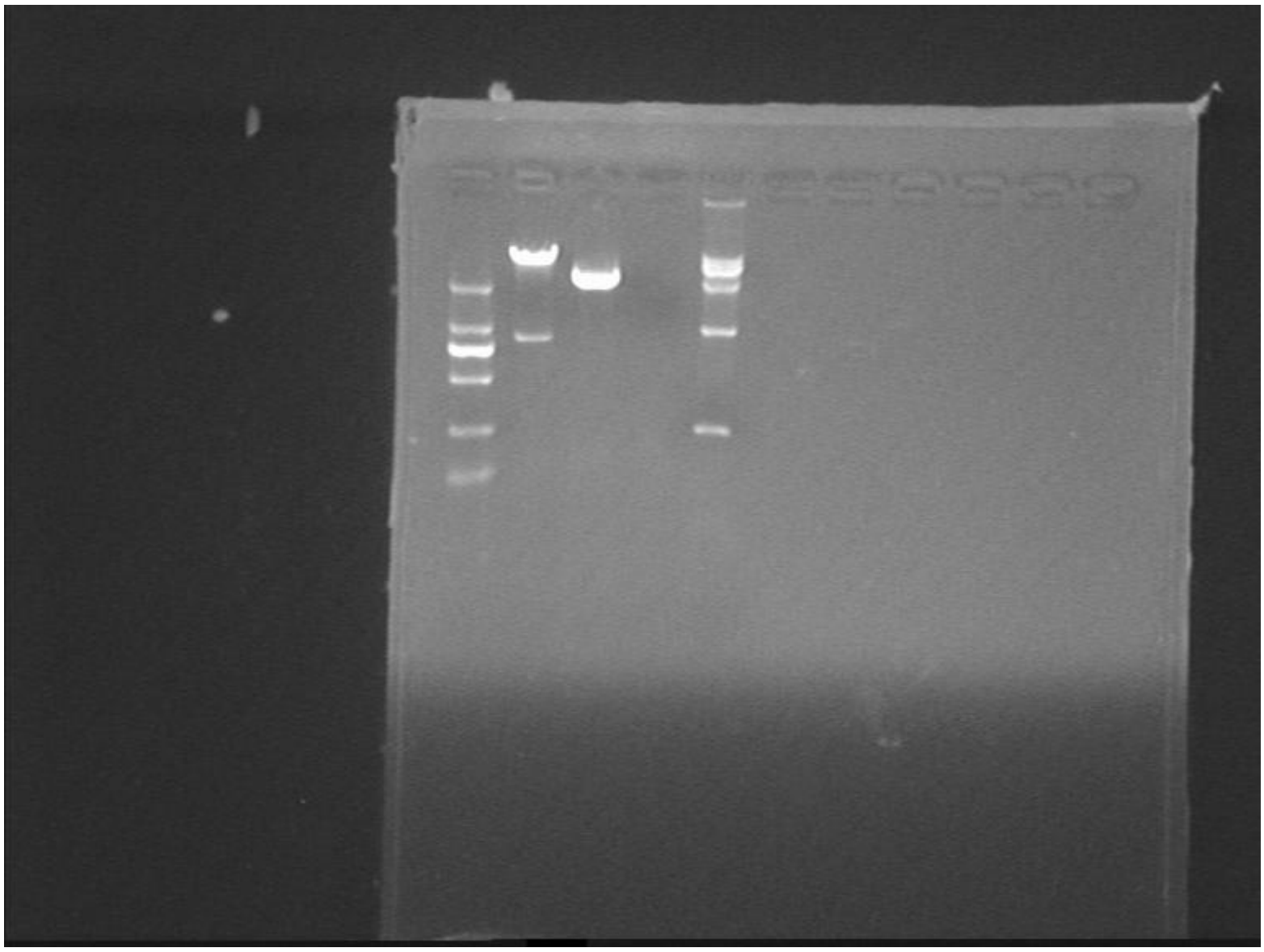
——Yi Yang

2017/8/24

Construct plasmids: pCDF+T2+cjBlue；pET+T2+amilGFP；pET+T2+amilCP；pCDF+T2+amilCP；merR+pET.

Pick the single colonies of pCDF+T1+eforRed；pET+T1+amilGFP；pET+T1+cjBlue；pCDF+T1+cjBlue.

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.



——Yi Yang

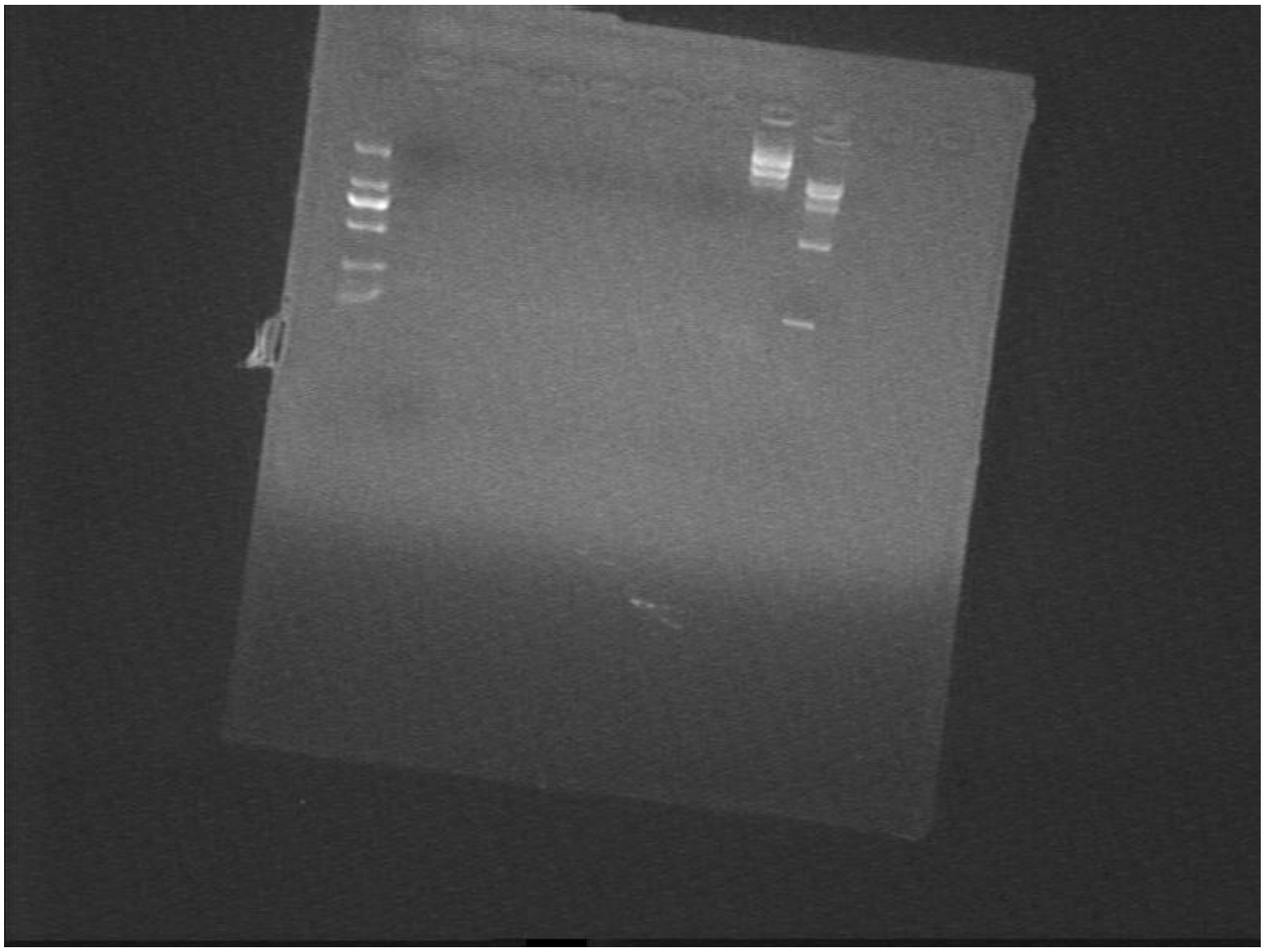
2017/8/23

Pick the single colonies of pET + T1 + eforRed, pET + T1 + amilCP, pCDF + T1 + amilCP.
Construct plasmids: pCDF + T1 + eforRed; pET + T1 + amilGFP; pET + T1 + cjBlue; pCDF + T1 + cjBlue.

——Yi Yang

2017/8/22

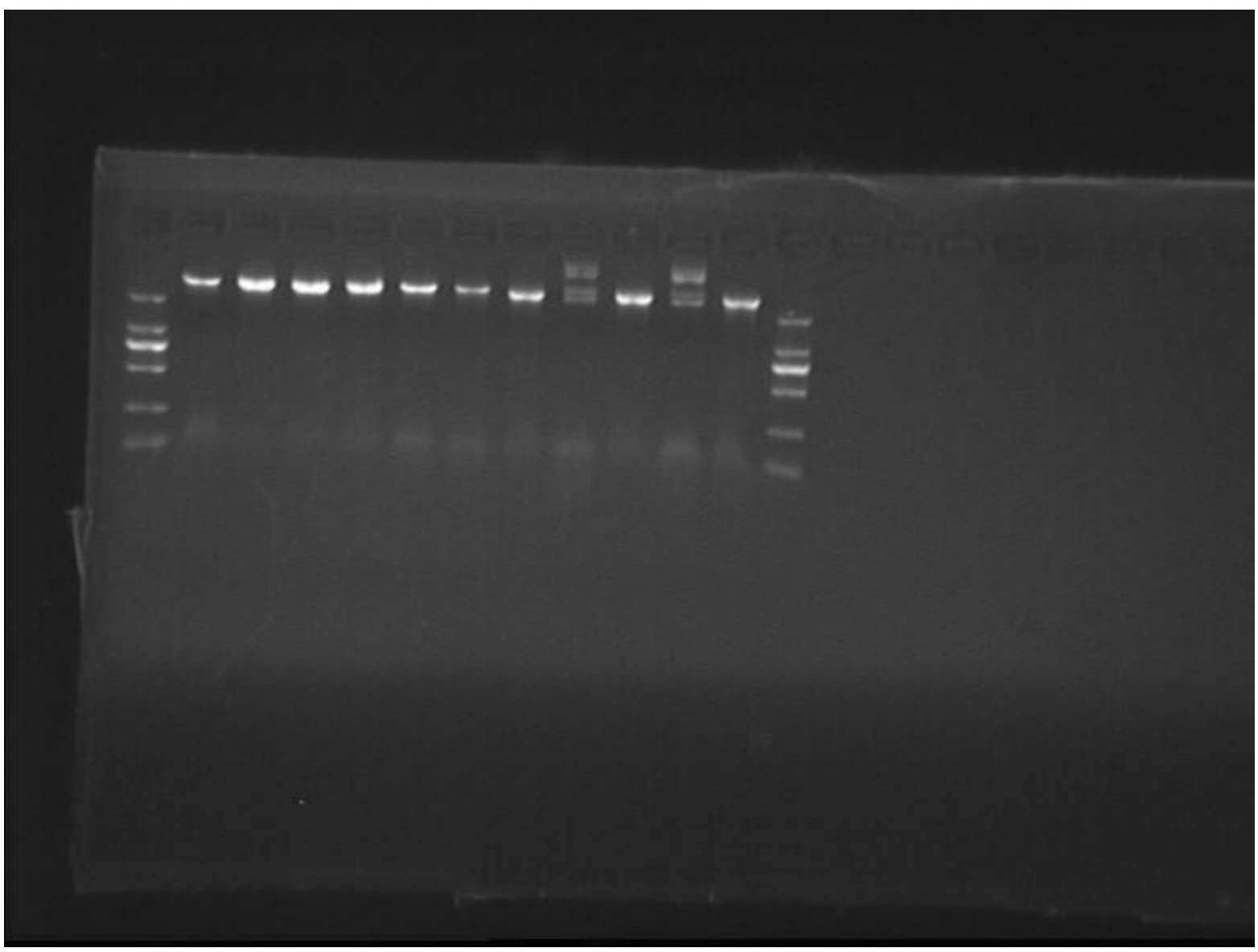
The PCR products of T1, eforRed and amilCP were ligated with the pCDF and pET backbone. Construct four recombinant plasmids: pET + T1 + eforRed; pET + T1 + amilCP; pCDF + T1 + eforRed; pCDF + T1 + amilCP. Then transformed.

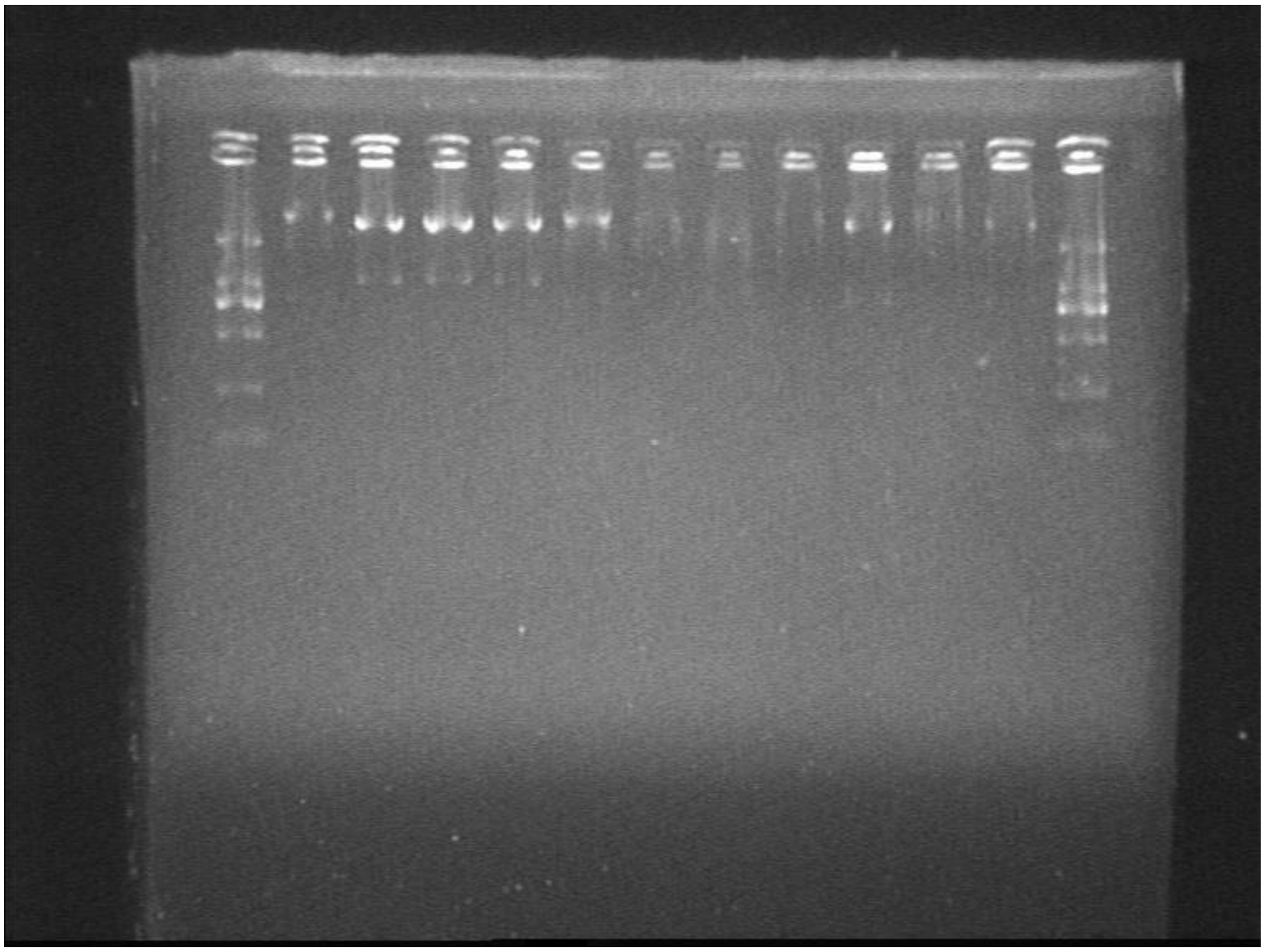


——Yi Yang

2017/8/15

Construct plasmids: amilCP+pETDuet-1, amilCP+pCDFDuet-1, eforRed+pETDuet-1, eforRed+pCDFDuet-1.
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.
PCR to get the part with Hg promoter and sfGFP.





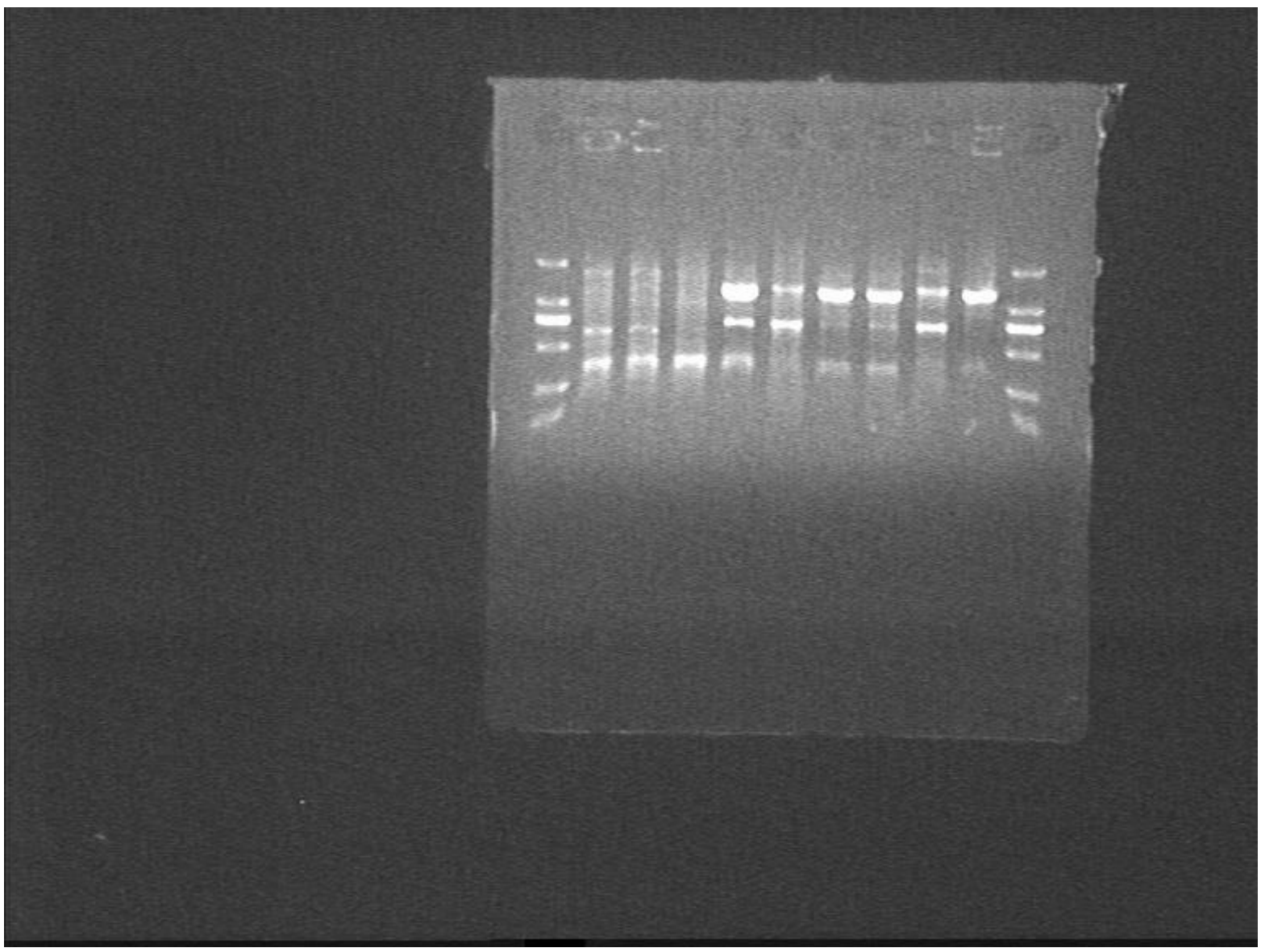
——Chang Gao

2017/8/14

Construct plasmid: T1+sfGFP+pETDuet-1.

PCR to get the parts of A1 and A2 with promoter.

Pick the single colonies of cjBlue+pETDuet-1, cjBlue+pCDFDuet-1, amilGFP+pETDuet-1, amilGFP+pCDFDuet-1. Then PCR in situ, the results support us to continue.

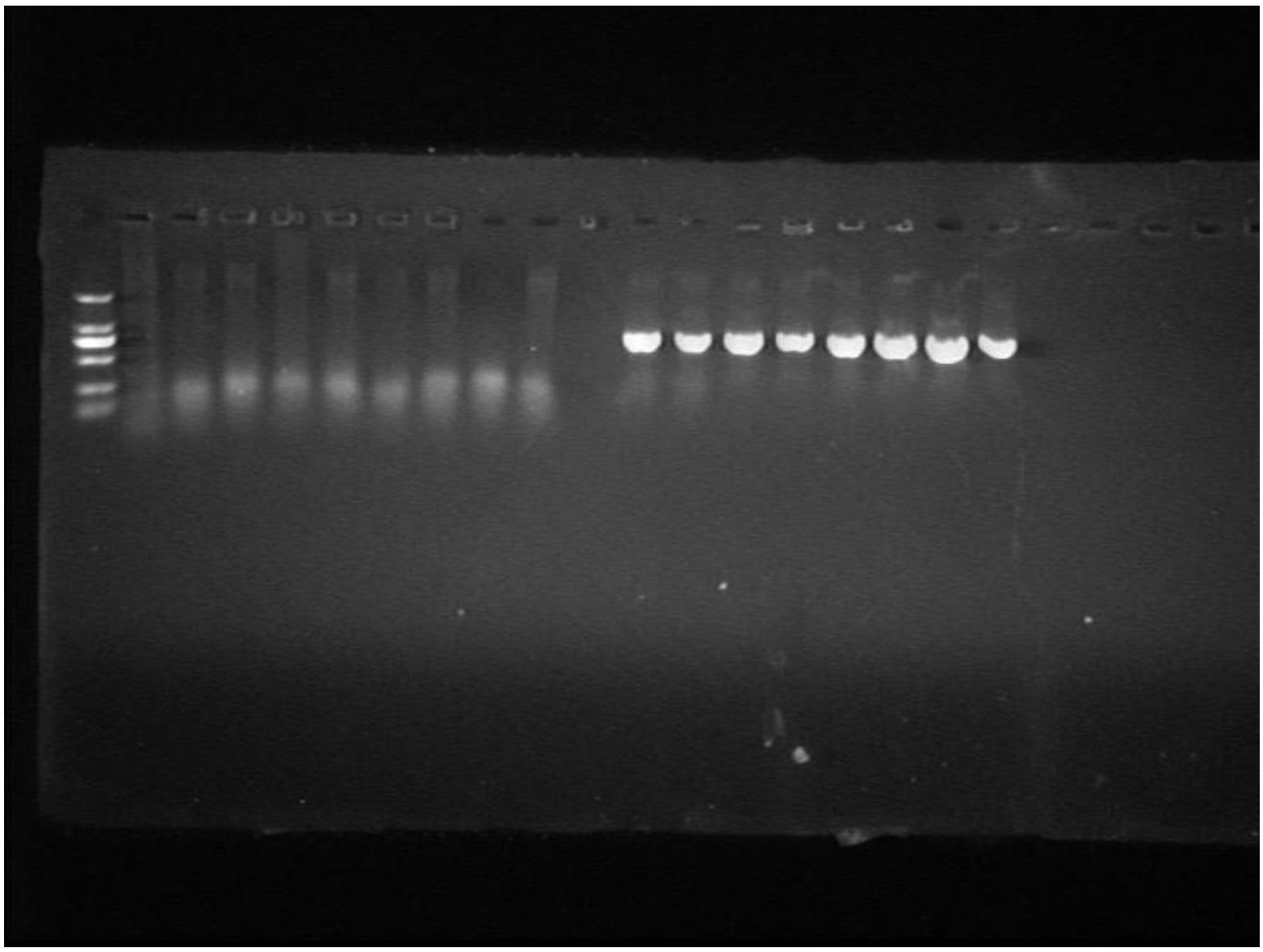


——Chang Gao

2017/8/13

Construct plasmids: cjBlue+pETDuet-1, cjBlue+pCDFDuet-1, amilGFP+pETDuet-1, amilGFP+pCDFDuet-1.

PCR to get the part of amilCP and eforRed with RBS and terminator.
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. The results are same as yesterday.



——Chang Gao

2017/8/12

Construct plasmids: T1+sfGFP+pETDuet-1，Co+sfGFP+pETDuet-1.
PCR to get the part of amilGFP and cjBlue with RBS and terminator.
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. The results show that we failed to construct Co+sfGFP+pSB1C3
Pick the single colonies for a second time.

——Chang Gao

2017/8/10

Extract the plasmids of pET+T2+sfGFP.
Pick the single colonies of cjBlue, then add IPTG in it to induce expression.

——Yi Yang

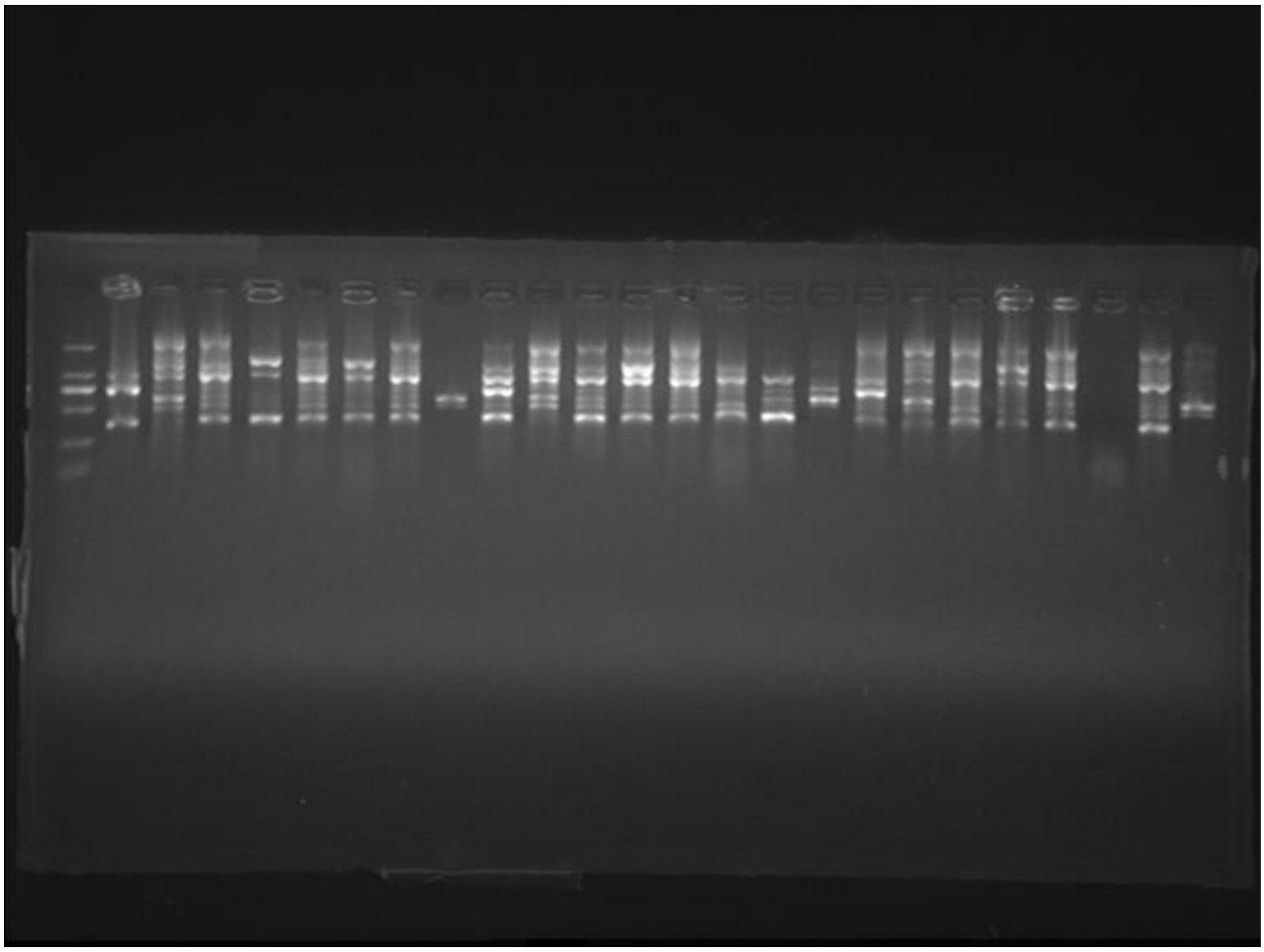
2017/8/9

Pick the single colonies of pET+T2+sfGFP.

——Yi Yang

2017/8/7

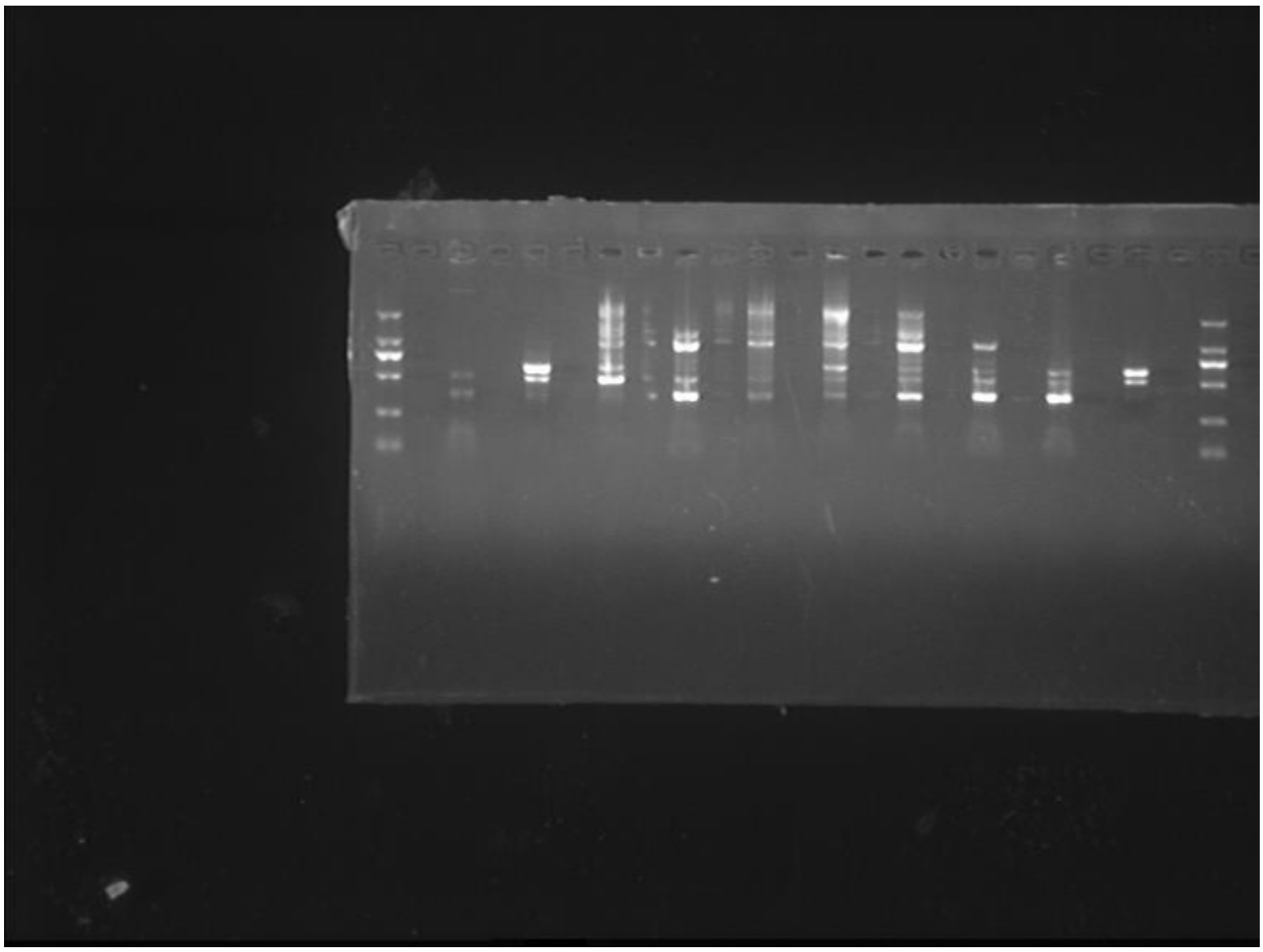
PCR in situ for a third time, and get the suitable Tm.
Construct plasmid: pET+T2+sfGFP.
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.



——Yi Yang

2017/8/6

PCR pET+eforRed, pET+amilGFP and pET+sfGFP in situ for a second time for getting a suitable Tm.
Extract the plasmids of pET+cjBlue，Hg+ pET

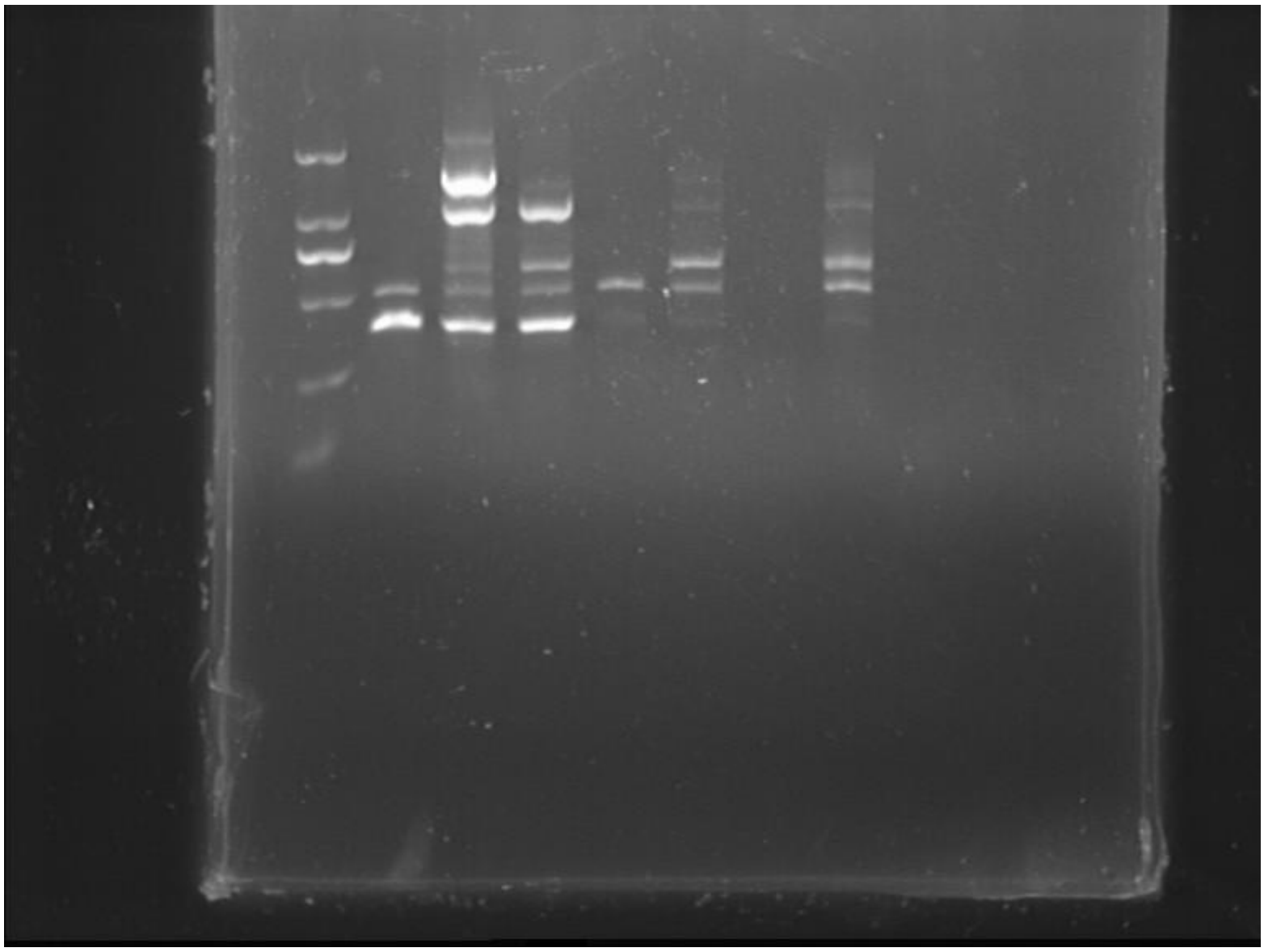


——Yi Yang

2017/8/5

PCR pET+eforRed, pET+amilGFP and pET+sfGFP in situ.
Extract the plasmids of pET+eforRed；pET+amilGFP；pET+sfGFP.

Pick the single colonies of pET+cjBlue， Hg+ pET.



——Yi Yang

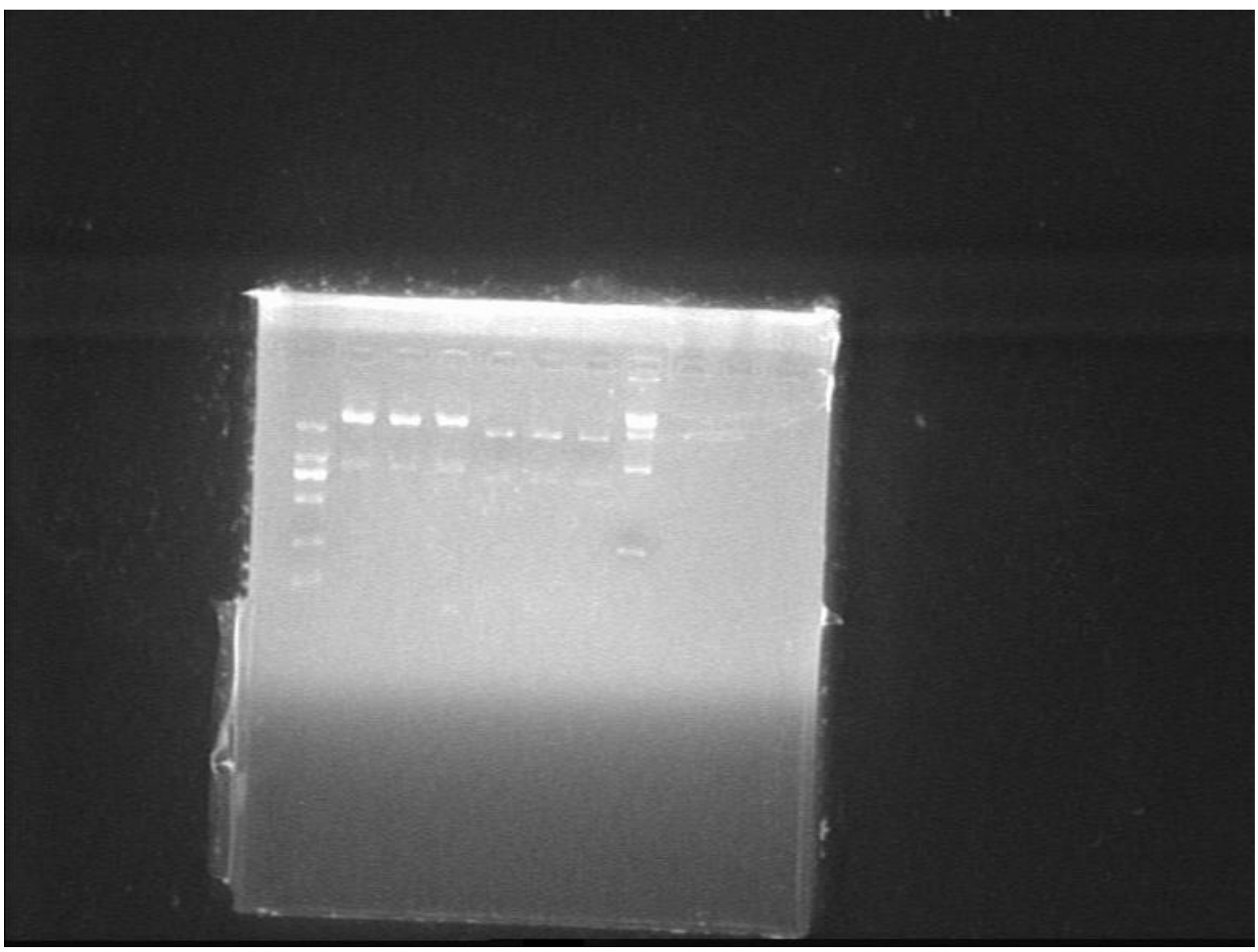
2017/8/4

Pick the single colonies of pET+eforRed, pET+amilGFP and pET+sfGFP.
Construct expression vectors: pET+cjBlue， Hg+ pET. Then transform.

——Yi Yang

2017/8/3

Extract the plasmids of pET and YFP.
Construct expression vectors: pET+cjBlue; pET+eforRed；pET+amilGFP；pET+sfGFP. Then transform.



——Yi Yang

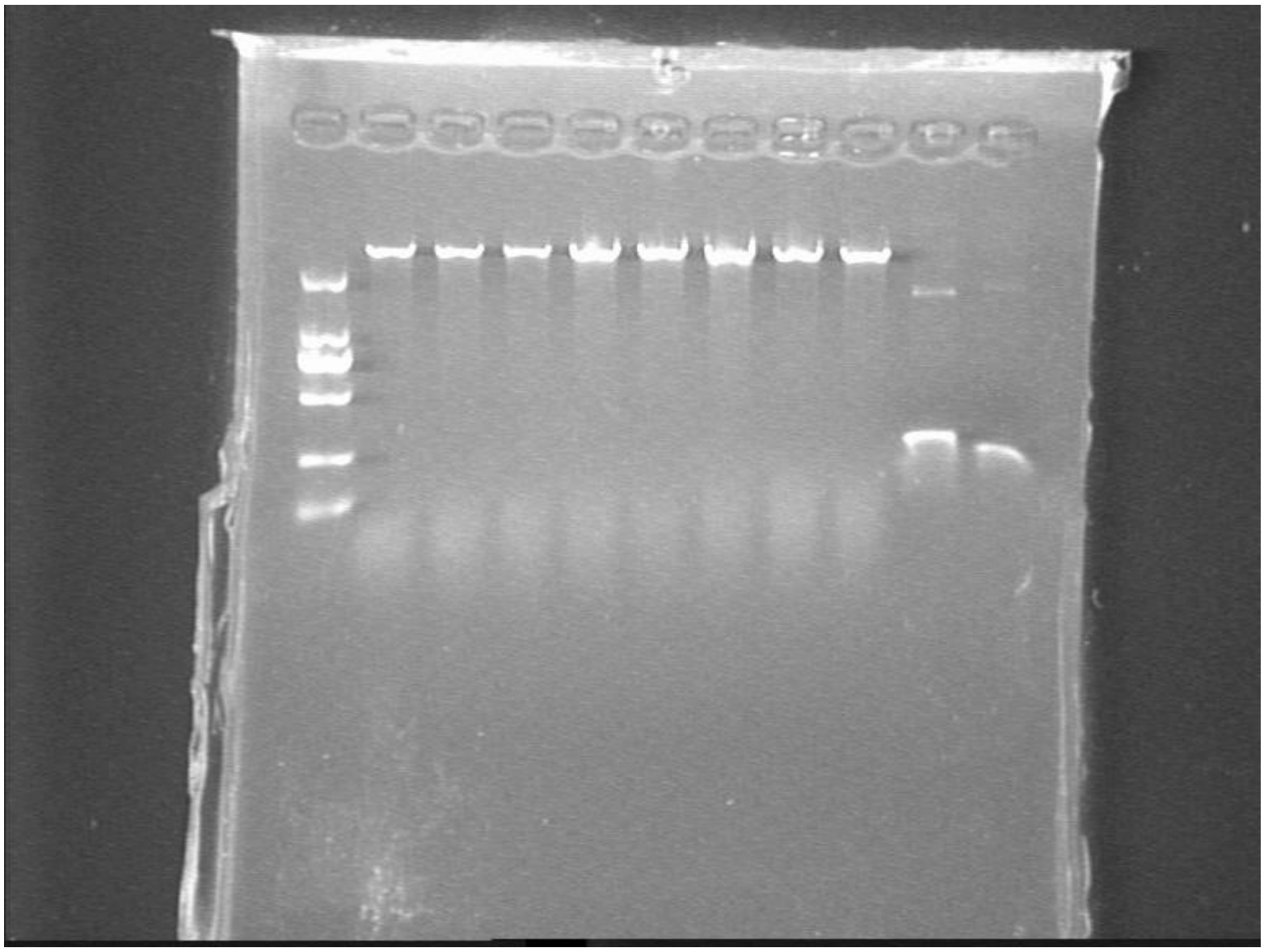
2017/8/2

Pick the single colonies of pET and SYFP.
Measure OD600 of the bacteria with amilCP and RFP, and analyze the color.
Extract the plasmids of pCDF and amilCP.

——Yi Yang

2017/8/1

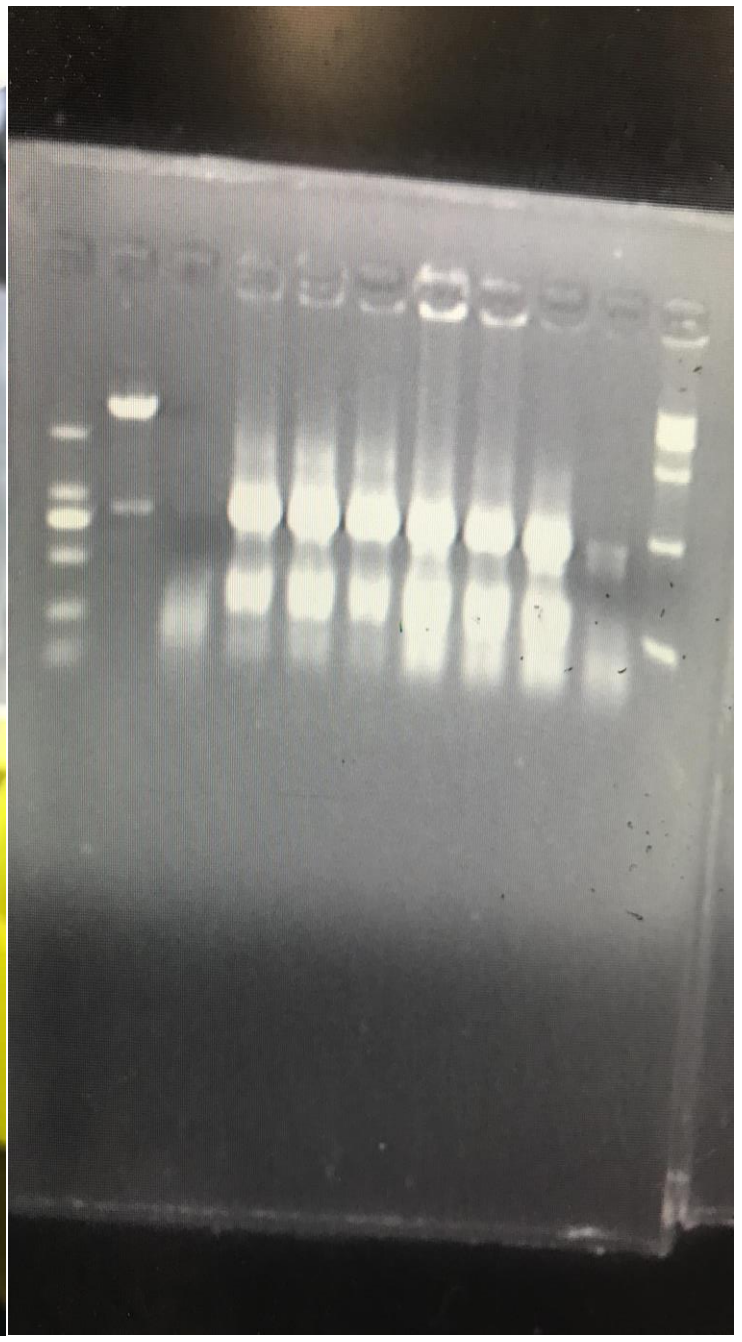
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis.
PCR for a second time, to get part of target2 and antisense2.
Pick the single colonies of amilCP and pCDF. And induce by IPTG at 9 o’clock.
Purify the PCR product of sfGFP that we got yesterday.



——Yi Yang

2017/7/31

1. Extract the plasmid that we constructed before, then restricted digestion and cataphroresis. Before extracting plasmids, we found only one colony has changed its color, and the result show that only this amilCP+pETDuet-1 has the positive result.
2. Pick the single colonies of cjBlue part.



——Haowan Chang

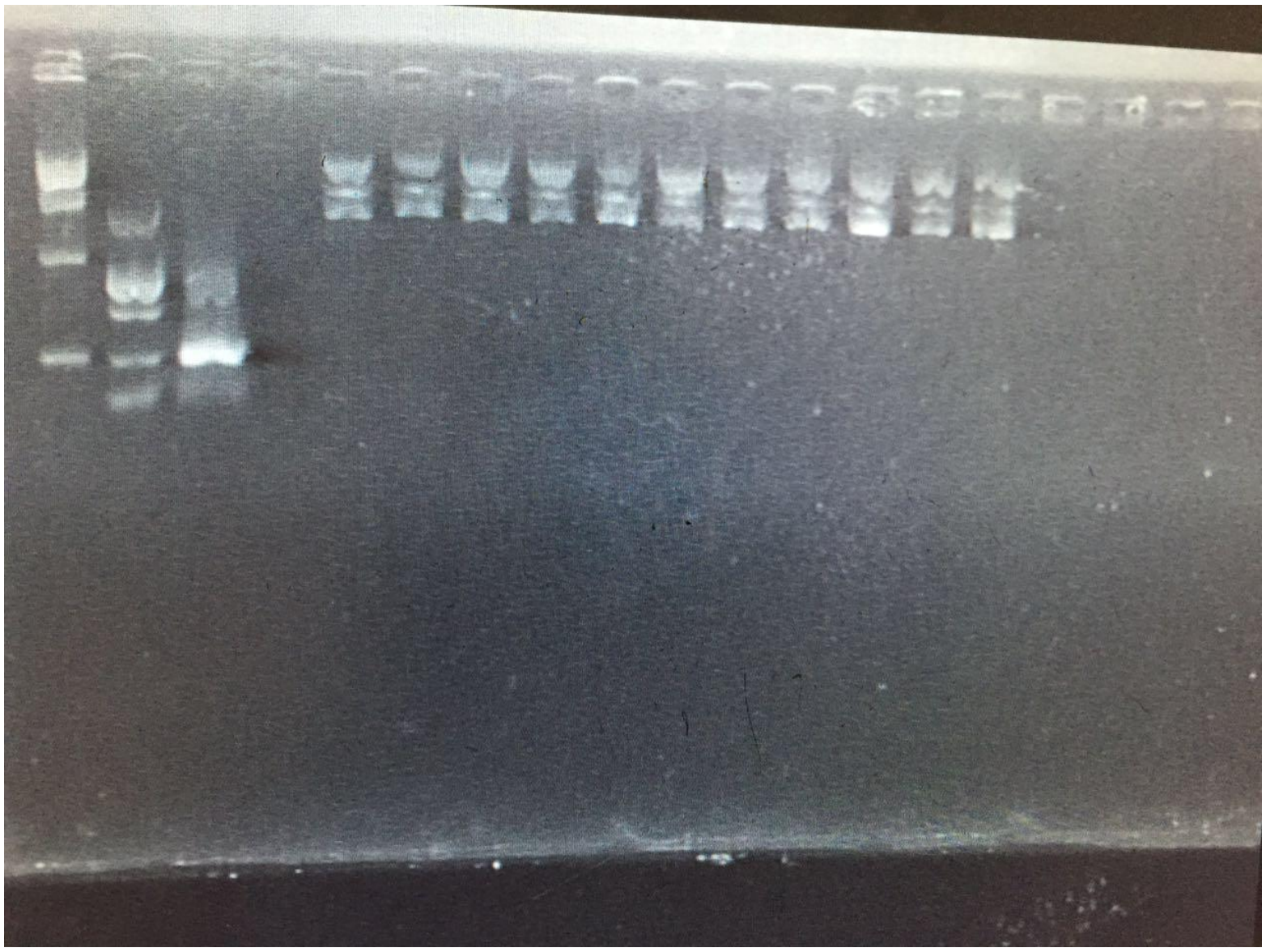
2017/7/30

1. Pick the single colonies of Co, amilCP+pETDuet-1 and cjBlue+pETDuet-1.
2. PCR for antisense2, target2 and sfGFP with RBS and terminator. We got a good result on the second time.

——Haowan Chang

2017/7/29

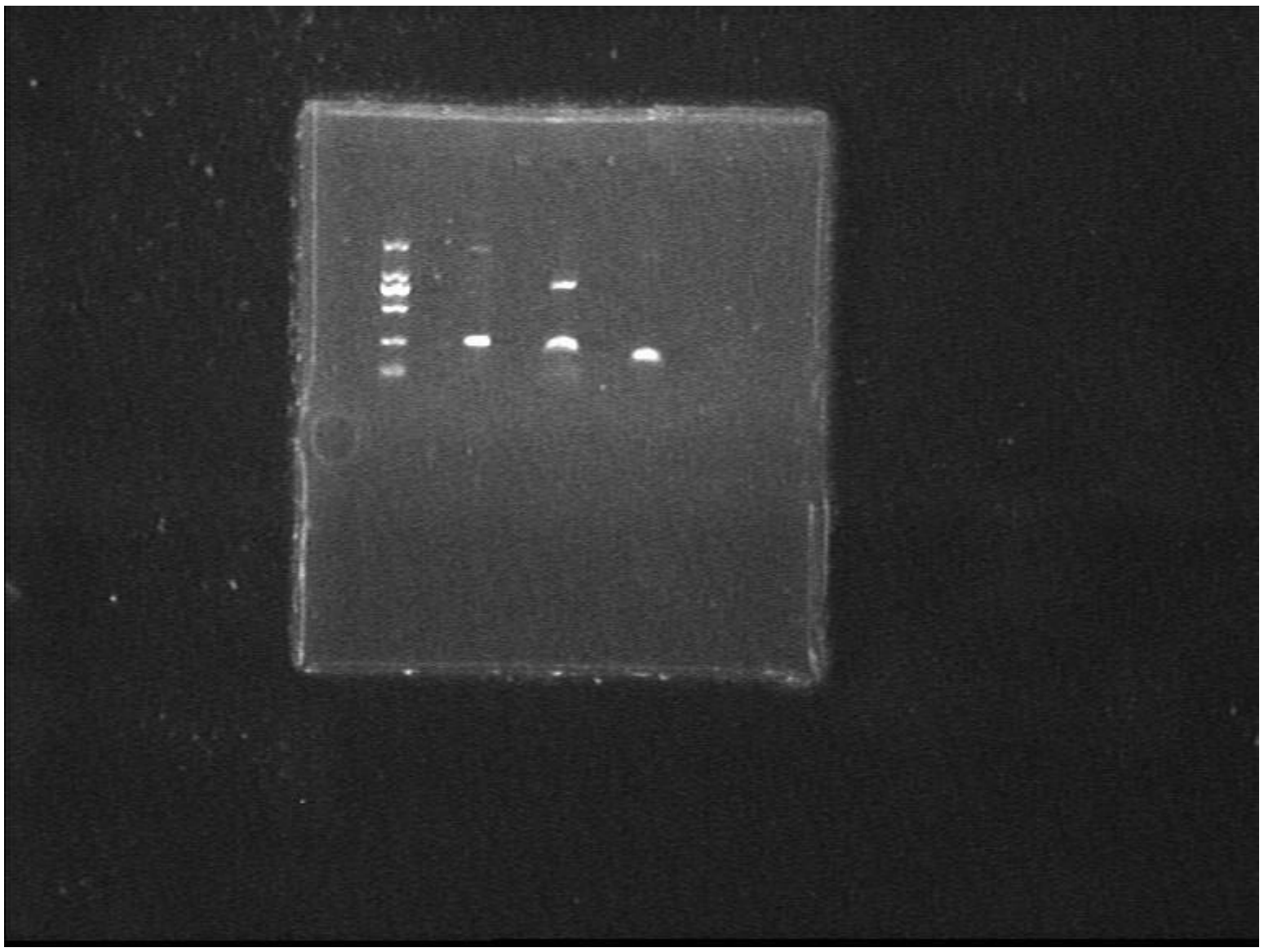
1. Extract the plasmid that we constructed before, then restricted digestion and cataphroresis, but no positive results found.
2. Construct plasmids for characteriazing chromoproteins: amilCP+pETDuet-1, cjBlue+pETDuet-1. Then transform.
3. PCR. We use the program that we explored before to get antisense2 and target2. PCR sfGFP for 2 times, but failed get it.
4. Transform the parts of YFP, which will be used for another teem, and Co.



——Haowan Chang

2017/7/28

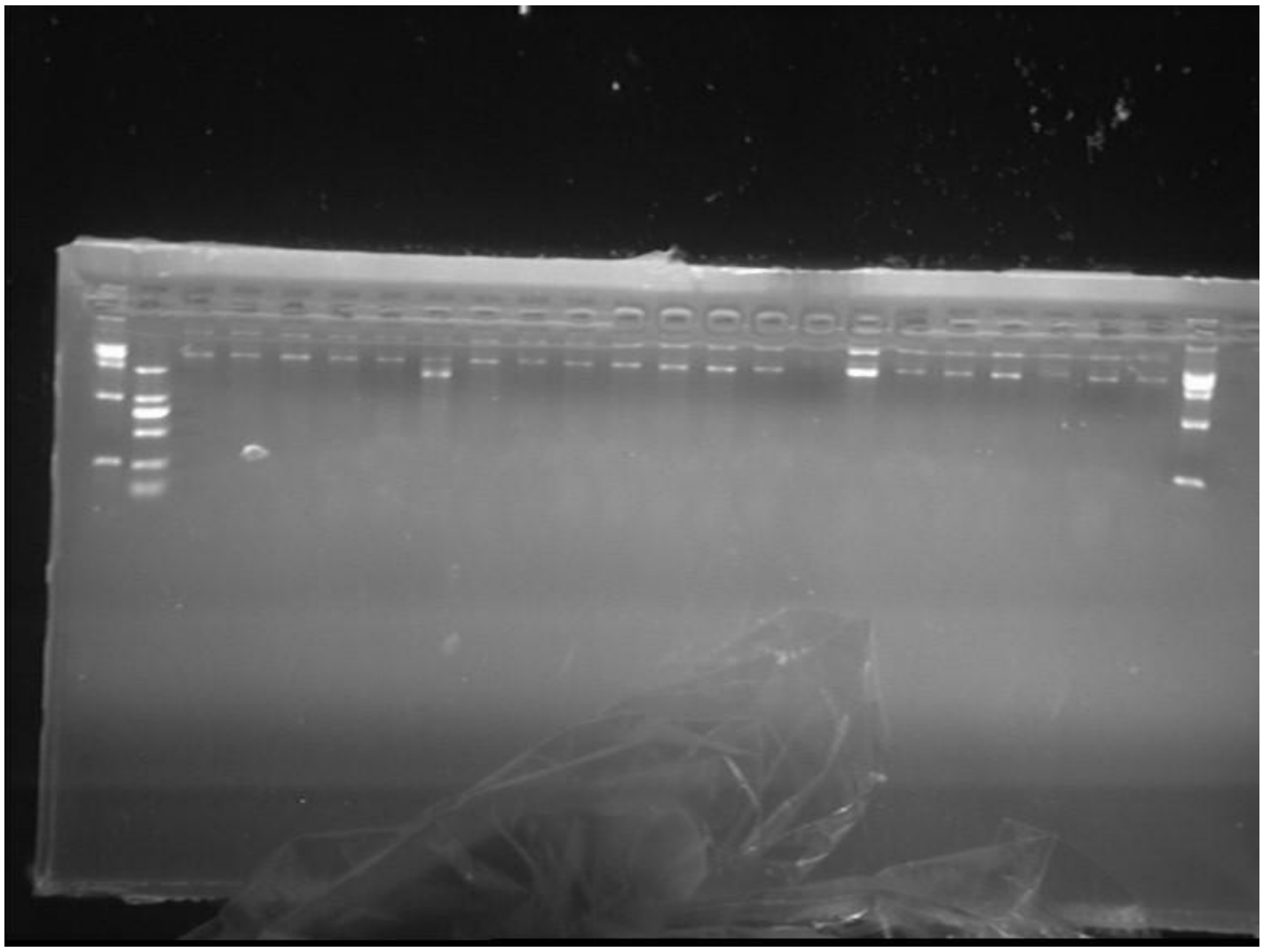
PCR for a fourth time, hoping to get the sfGFP with terminator and rbs, target2 and antisense2. We changed the program but didn't satisfy our need.
Pick the single colonies of the chromoprotein expression vectors.
Extract the plasmid that we constructed before, then restricted digestion and cataphroresis, but no positive results found.

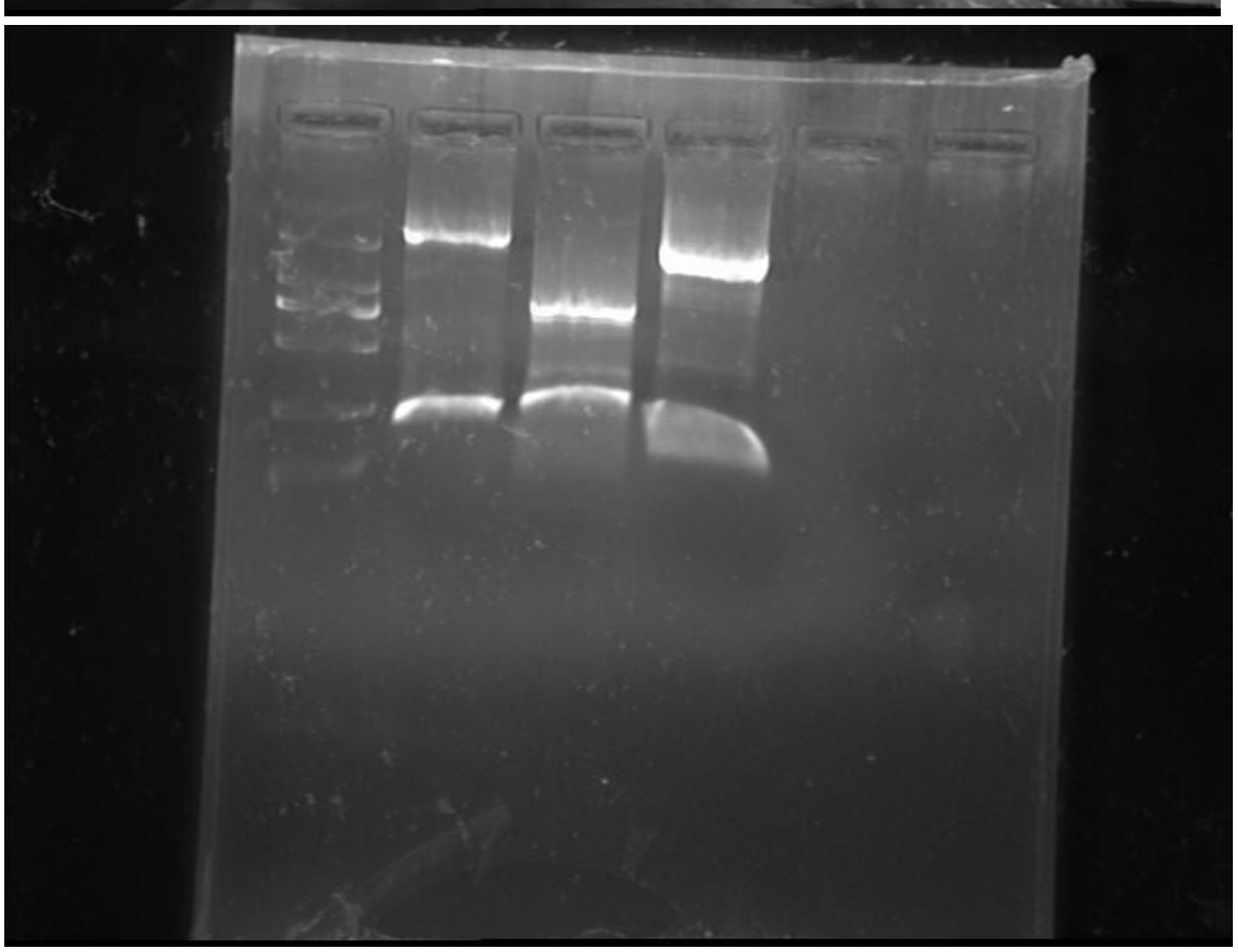


——Chang Gao

2017/7/27

Extract the plasmid that we constructed before, then restricted digestion and cataphroresis, but no positive results found.
Construct the expression vector of amilGFP.
PCR for a third time, hoping to get the sfGFP with terminator and rbs, target2 and antisense2. We changed the program but didn't satisfy our need.

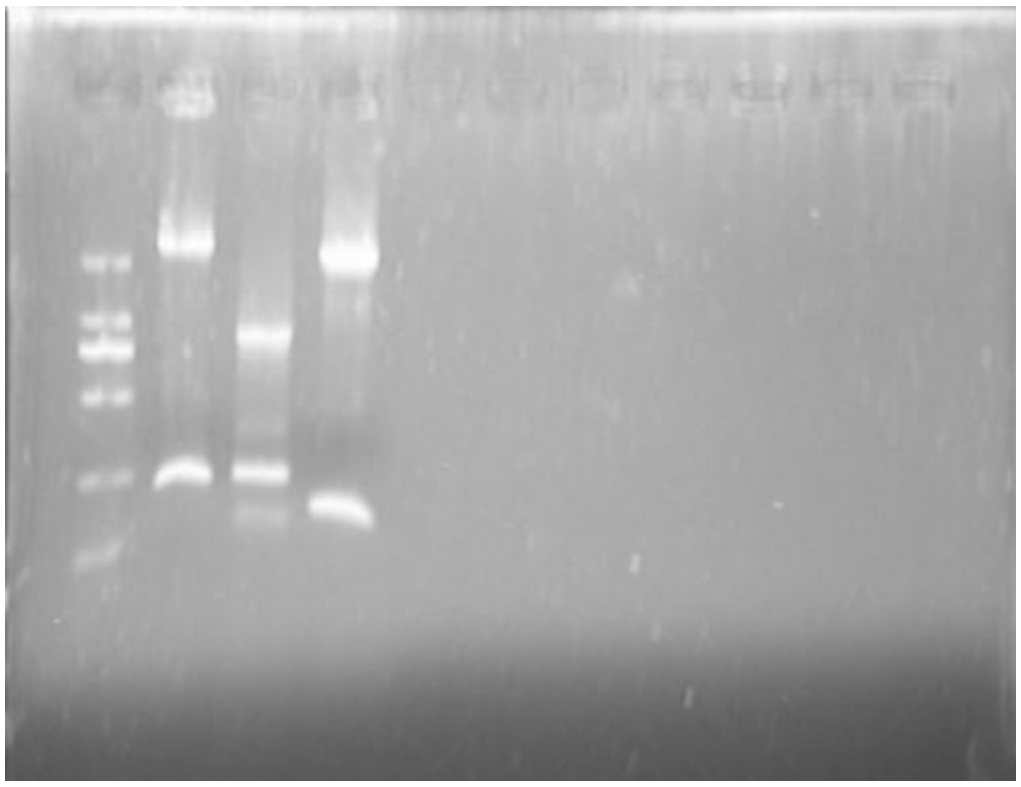




——Chang Gao

2017/7/26

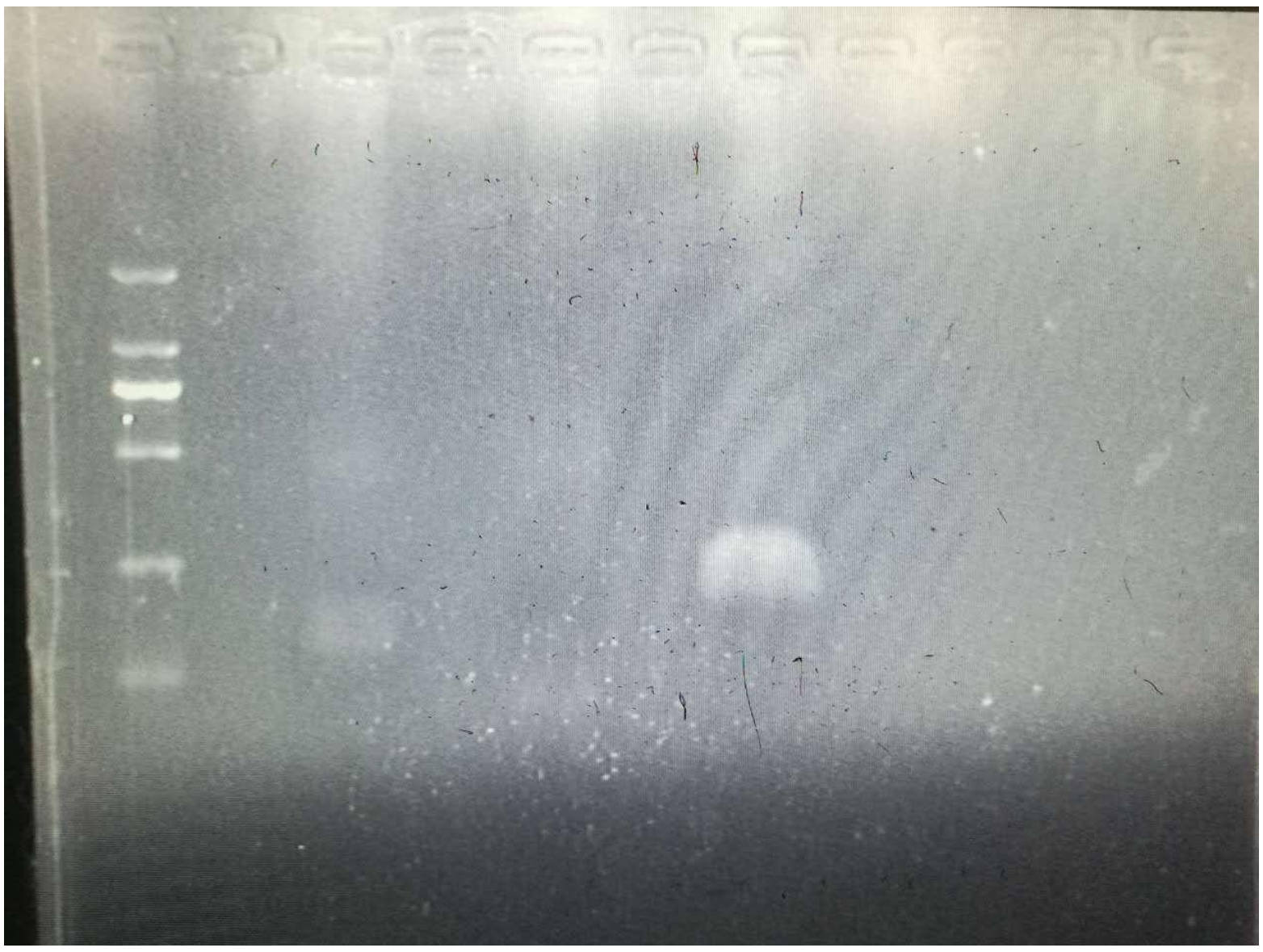
PCR for a second time, hoping to get the sfGFP with terminator and rbs, target2 and antisense2. We changed the program and got a better result.
Pick single colonies of eforRed, amilCP, cjBlue, RFP and pETDuet-1.
Extract plasmid of eforRed, amilGFP, cjBlue, pACYC and pCDFDuet-1.



——Chang Gao

2017/7/25

Construct expression vector of amilCP, eforRed, cjBlue using pETDuet-1.
Pick single colonies of pACYC184 and pCDFDuet-1.
Transform pETDuet-1.
PCR for the 1st time, hoping can find the best Tm and get sfGFP with RBS and terminater, target2 and antisense2. The cataphoresis results couldn't meet our satisfaction.



——Chang Gao

2017/7/24

Extract plasmid. Transform pACYC184 and pCDFDuet-1 for a second time. We found that the reason why we failed before is that the antibiotic we used was false.
Pick single colonies of eforRed, cjBlue, amilGFP. Then we pick single colonies of amilCP expression vector for a second time.

——Chang Gao

2017/7/23

pigment and chromoprotein

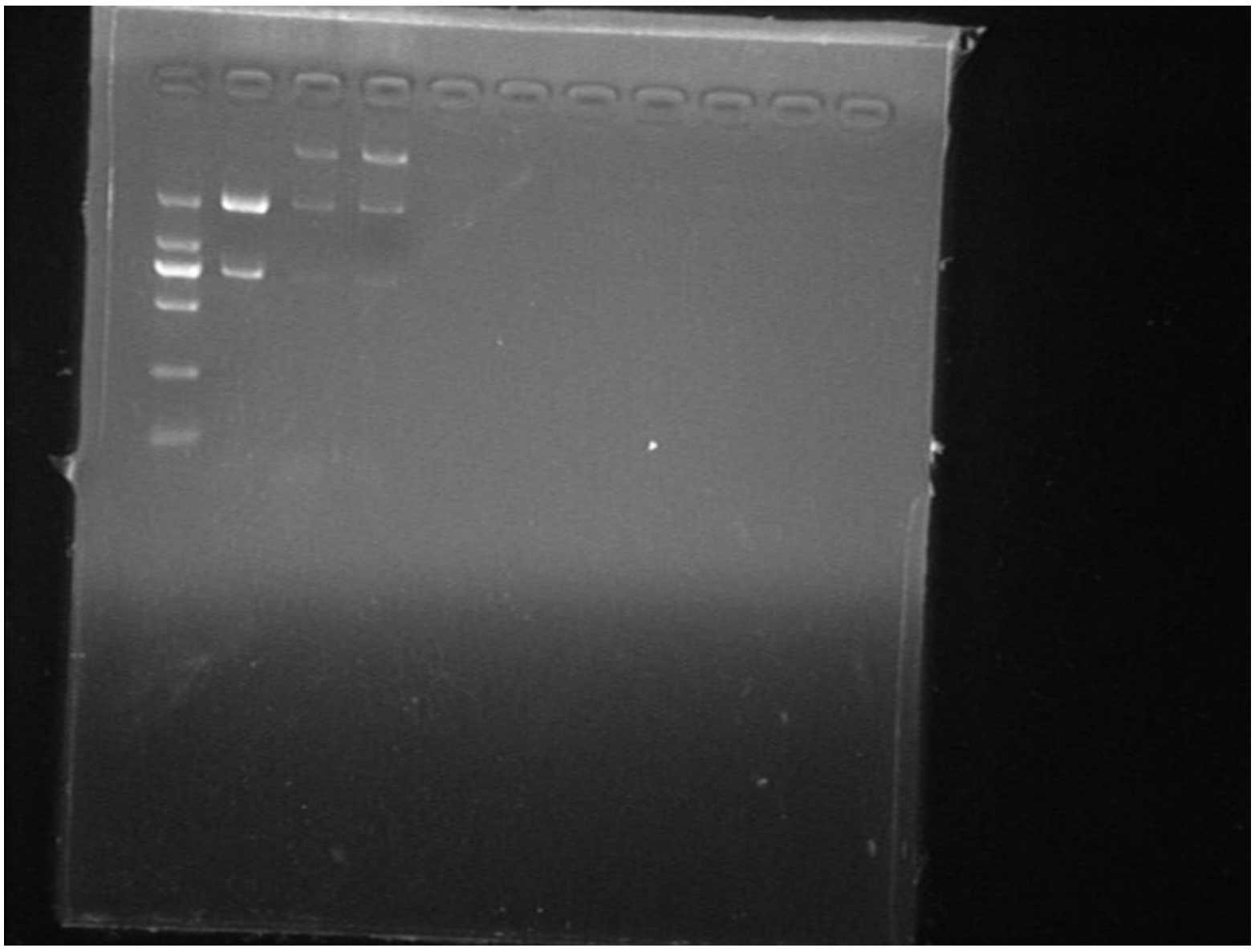
Using pET28a to construct the expression vector of fwYellow and amajLime. Extract plasmid for cjBlue, amilCP, eforRed and amilGFP. Pick single colonies of As promoter, Co promoter, amilCP, amilGFP, eforRed, lead repressor and mercury promoter. Transform pACYC184 and pCDFDuet-1.

——Chang Gao

2017/7/22

pigment and chromoprotein

Extract plasmid, then restricted digestion and cataphroresis. Transform As promoter, Co promoter, lead promoter, Hg promoter. Pick single colonies of cjBlue, amilCP, eforRed and amilGFP.
Add IPTG into the bacteria that transformed expression vector.



——Chang Gao

2017/7/21

pigment and chromoprotein

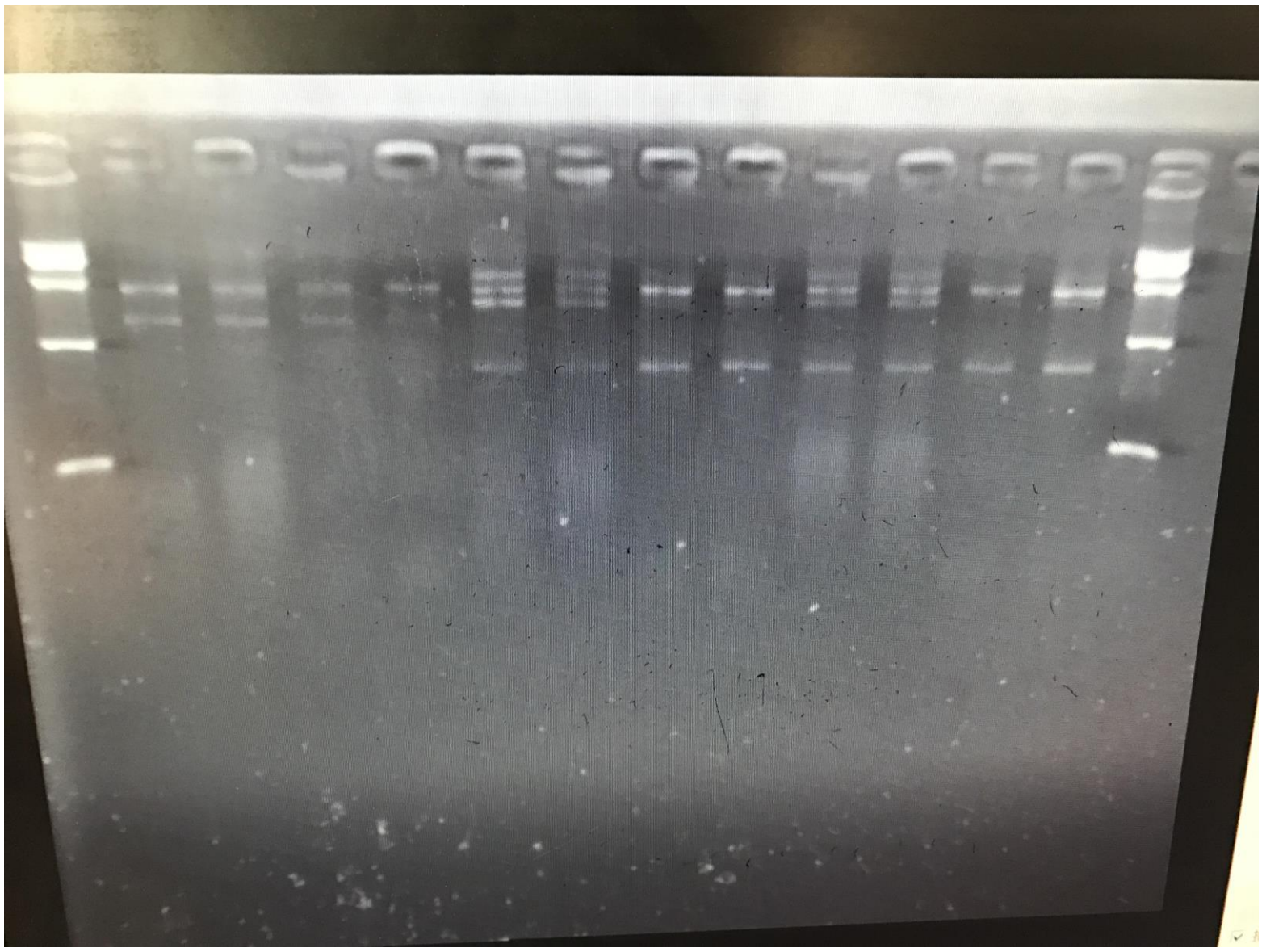
Pick single colonies of pETDuet-1 and expression vector which constructed by ourselves. Transform cjBlue, amilCP, eforRed and amilGFP.

——Chang Gao

2017/7/20

pigment and chromoprotein

Extract plasmid then restricted digestion, cataphoresis. The result is same as our expectation except one part of lead repressor.
Transform pCDFDuet-1, pACYC184, pETDuet-1.



——Chang Gao

2017/7/19

pigment and chromoprotein

Because of some problem found by sequencing, transform spisPink again. Besides, transform eforRed, cjBlue and amilCP.

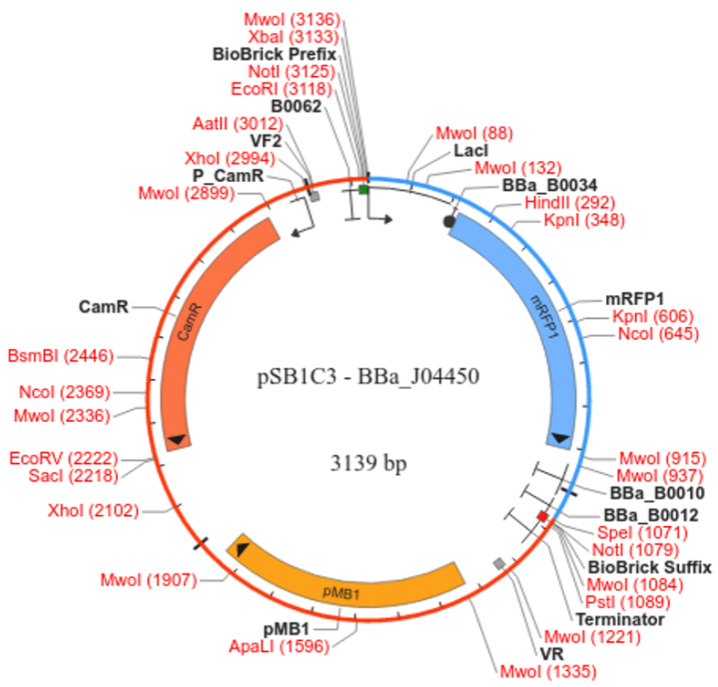
Pick single colonies of fwYellow, amajLime and one part of lead repressor.

To express amilCP, we begin to construct expression vector using pET28a.

——Chang Gao

Somthing wrong also appeared when it comed to the sequencing of crtE. At first we choose the prefix and the suffix as the start and end of the crtE part for sequencing primer design only to find that the result did't correspond to the expected one well. We knew that sequencing performed poorly at the first and last few bases, so we got the sequence of plasmid pSB1C3 which were commonly uesd in igerm parts in order to design primers from the upstream and down stream of the crtE part.

——Zhuoyang Chen



2017/7/18

pigment and chromoprotein

The result of last experiment show that we succeeded culturing competent cells, which, however, a little worse than the competent cells bought from companies.
Transform fwYellow, amajLime, one part of lead repressor.

——Chang Gao

2017/7/17

pigment and chromoprotein

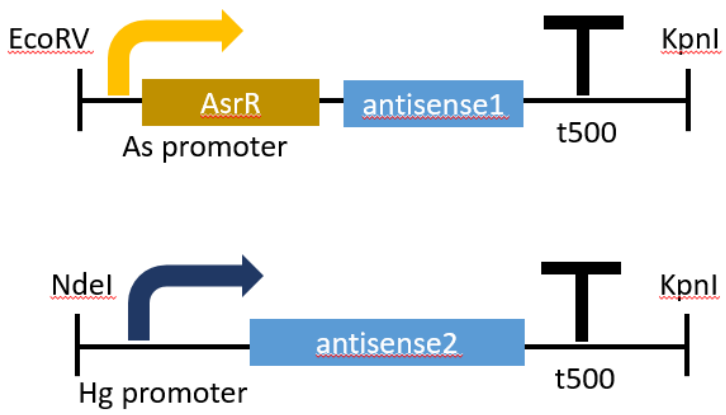
Test competent cells cultured by ourselves using RFP.

——Chang Gao

Primer design

At first, we were going to PCR antisense + t500 from the synthetic sequence J23119+antisense+t500. However, we were told that sequence less than 100bp had a low efficiency when doing PCR and the latter restrction sites operations. So we decided to design two primers to add antisense and t500 at the 3' terminus of As promoter in turn. And because Hg promoter didn't include a coeffector gene, we hand on the whole sequence of Hg promoter+antisense+t500 for direct synthesis. In this sense, we would get a complete device without any restrictions sites. But since we still need to build a device to combine the As promoter and RFP without Star system, extra primers for As promoter specificly also in need.
Besides, each primer should less than 99bp so the two primers strategy was also applied to the addition of terminators B0010 and B0012 to the sfGFP coding part.
Note: compared to the original design, we don't have NotI between promoter and antisense anymore!

——Zhuoyang Chen



2017/7/16

pigment and chromoprotein

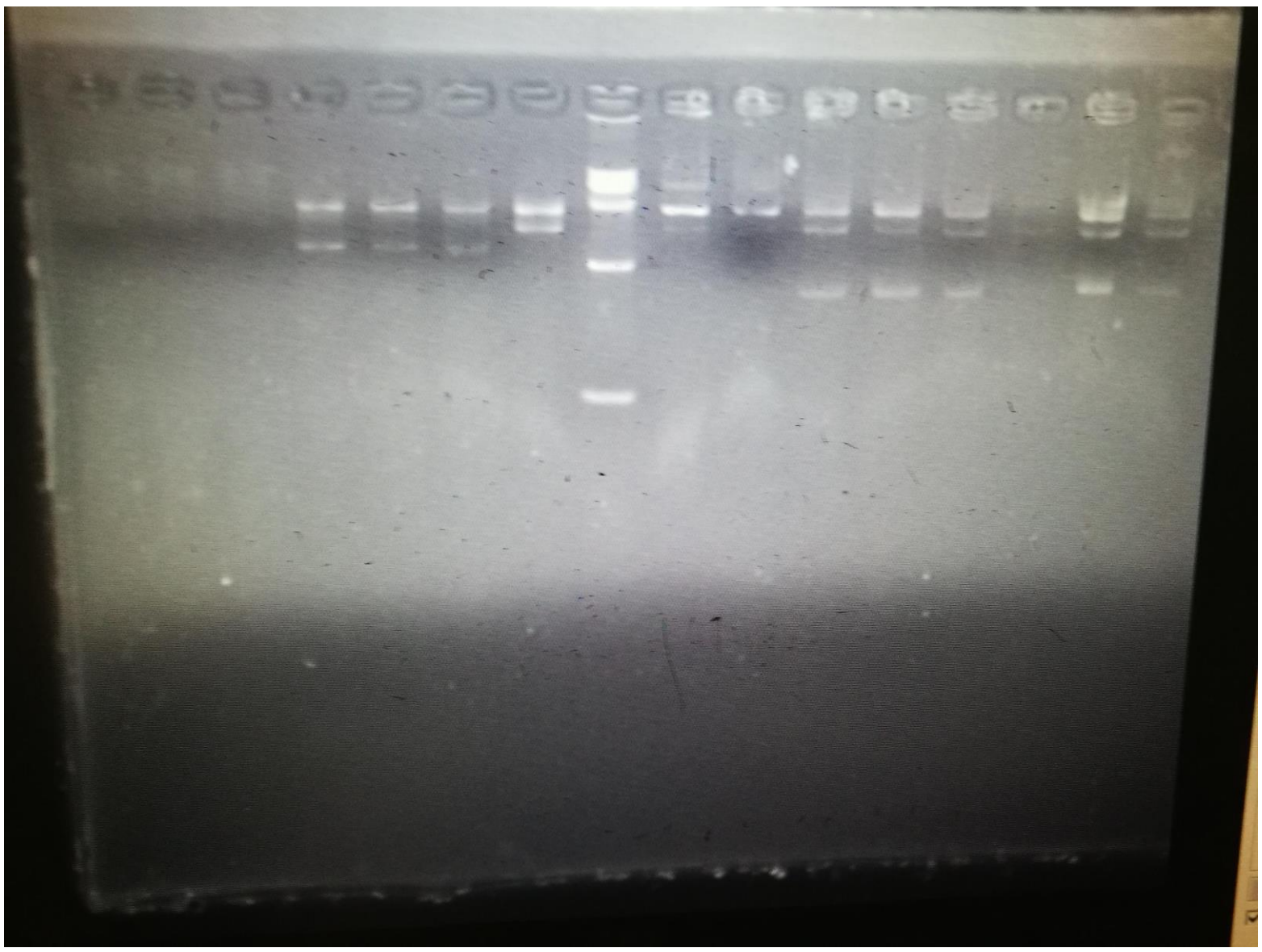
Extract plasmid then restricted digestion, cataphoresis. The result is same as our expectation except one part of lead repressor.
Culture competent cell.

——Chang Gao

2017/7/15

pigment and chromoprotein

Extract plasmid then restricted digestion, cataphoresis. Only crtI with rbs show the result same as our expectation.
Pick single colonies of amilCP, cjBlue, eforRed, Co promoter and two parts of Pb promoter.
Begin to culture competent cell.



——Chang Gao

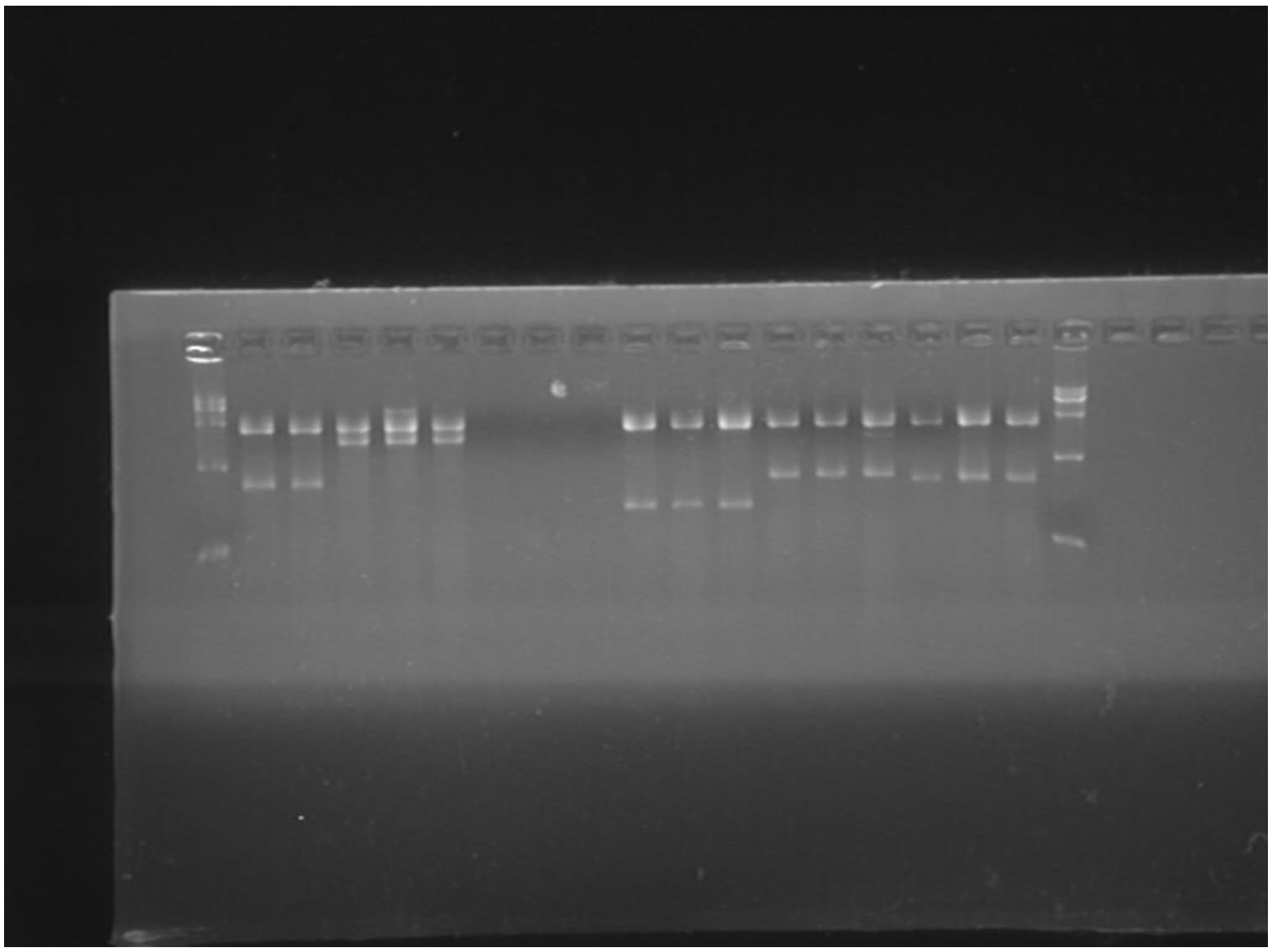
2017/7/14

pigment and chromoprotein

Extract plasmid then restricted digestion, cataphoresis except crtB with rbs because of its low concentration of plasmid. The result is same as our expectation.

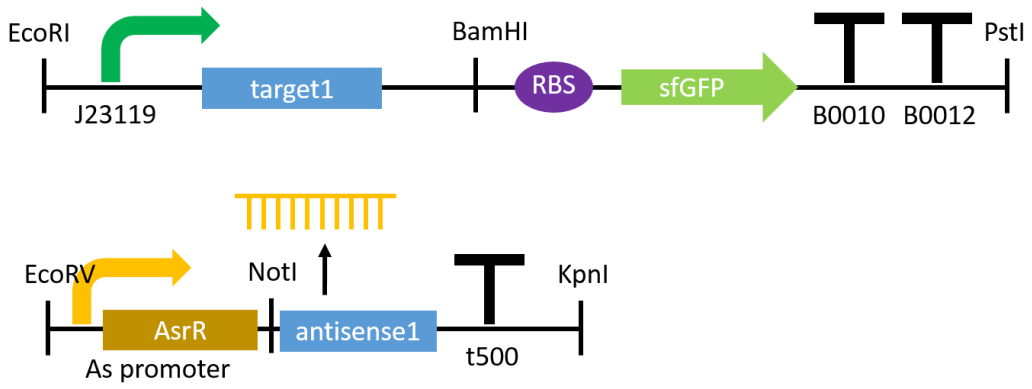
Transform amilCP, cjBlue, eforRed, Co promoter and two parts of Pb promoter.

Pick single colonies of merR, Lime, YFP, crtI with rbs and crtB with rbs.



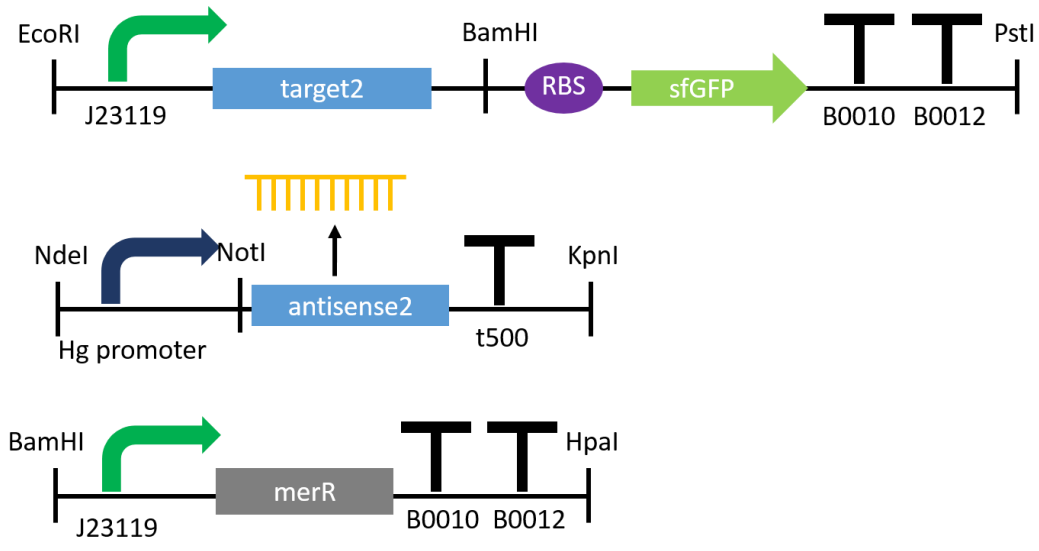
——Chang Gao

As promoter characterization with Star system1 in pCDFDuet-1



Note: notice that we didn't choose the NdeI at the upstream of As promoter because the same restriction sequence was also found in AsrR gene!

Hg promoter characterization with Star system2 in pETDuet-1

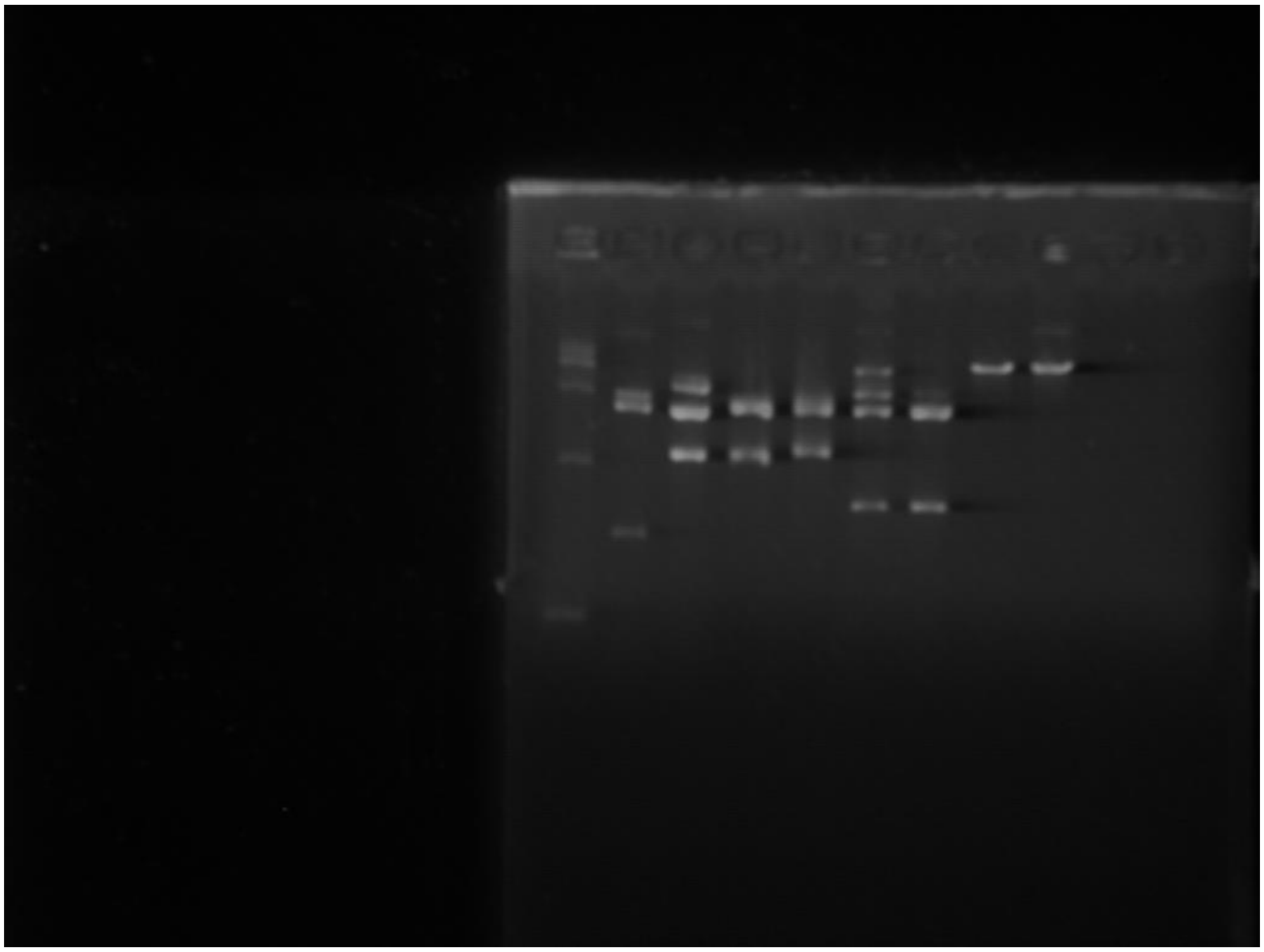


——Zhuoyang Chen

2017/7/13

pigment and chromoprotein

Extract plasmid then restricted digestion, cataphoresis. One tube of bacteria with crtE are red after culturing, we suspect that it had been polluted. Results of cataphoresis are same as our expectation.
Pick single colonies of fwYellow, crtI, amilGFP, sfGFP, crtB with rbs and one part of Hg promoter, culture for 18 hours.
Transform crtI with rbs, amajLime, one part of Hg promoter and YFP.



——Chang Gao

Promoter for Hg

here is the information of the Hg promoter and its activator:
BBa_K346001: RBS + merR
BBa_K346002: Hg promoter with merR binding region

——Zhuoyang Chen

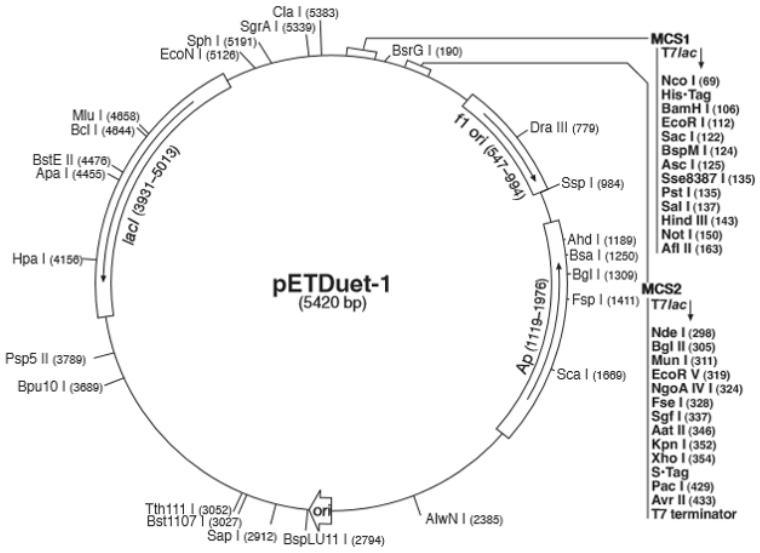
2017/7/12

pigment and chromoprotein

Pick single colonies of crtE, spisPink, As promoter and pET28a.
Sequencing results show that fwYellow and amajLime are normal.
Transform crtB, crtI, amilGFP, Hg promoter and sfGFP for a second time.
Transform two parts, one has crtB and rbs, the other has crtI and rbs.
Use RFP to test the other package of competent cells made years ago.

——Chang Gao

Some of the heavy metal ions promoters must work with its coeffectors binding at operator regions so genes of the corresponding activators or repressors should be inserted in the plasmid of our Star system.



We were going to choose restriction site BamHI in the CMS1 and HpaI in the lacI region to cut down part of the lacI gene in order to reduce the length of the plasmid and to leave enough space for us to insert our genes for coeffectors.

——Zhuoyang Chen

2017/7/11

pigment and chromoprotein

Using competent cell which cultured by ourselves to transform RFP.
Transform crtE, crtB, crtI, spisPink, As promoter, amilGFP, pET28a, sfGFP and 2 parts for Hg promoter.

——Chang Gao

Plasmid pETDuet-1

Because we lacked of plasmid pRSFDuet-1 in our lab, we decided to use pETDuet-1 instead. It was also a plasmid for coexpression with two CMS for genes insertion.

——Zhuoyang Chen

2017/7/10

pigment and chromoprotein

To test the competent cells made years ago, transform RFP. Besides, we used the RFP both from the teacher and those we extracted before.

——Chang Gao

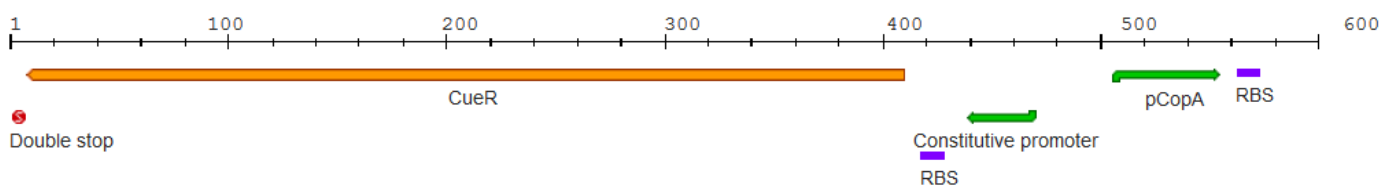
2017/7/9

STAR

Promoters for Cu and As were chosen:

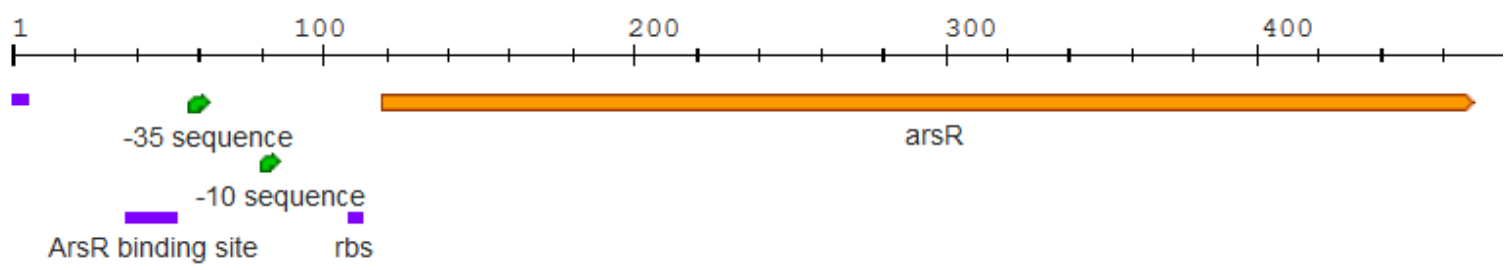
Since we have chosen five heavy metal ions promoters from 2017 kit, we finally decided to use promoters for Cu and As (BBa_K1980006 and BBa_J33201 respectively). As we know, operon is composed of repressor or activator genes and promoter region. We didn't find compositive parts of Pb, Hg promoter, which means that they need a addition repressor gene from other parts. So for convenience's sake, we didn't choose promoterf for Hg and Pb. Besides, promoter for Co isn't so Co-specific for it is said on the website that it also works in the presence of Nickle. That's why we were going to use Cu and As promoter.

BBa_K1980006 (promoter for Cu):



Note: pCopA already has its own RBS! Unfortunately, we later found that this part was not included in our 2017 kit, so we used promoter for Hg instead.

BBa_J33201 (promoter for As):



Note: this part doesn't have its own RBS so the gene inserted must have a RBS!

——Zhuoyang Chen

pigment and chromoprotein

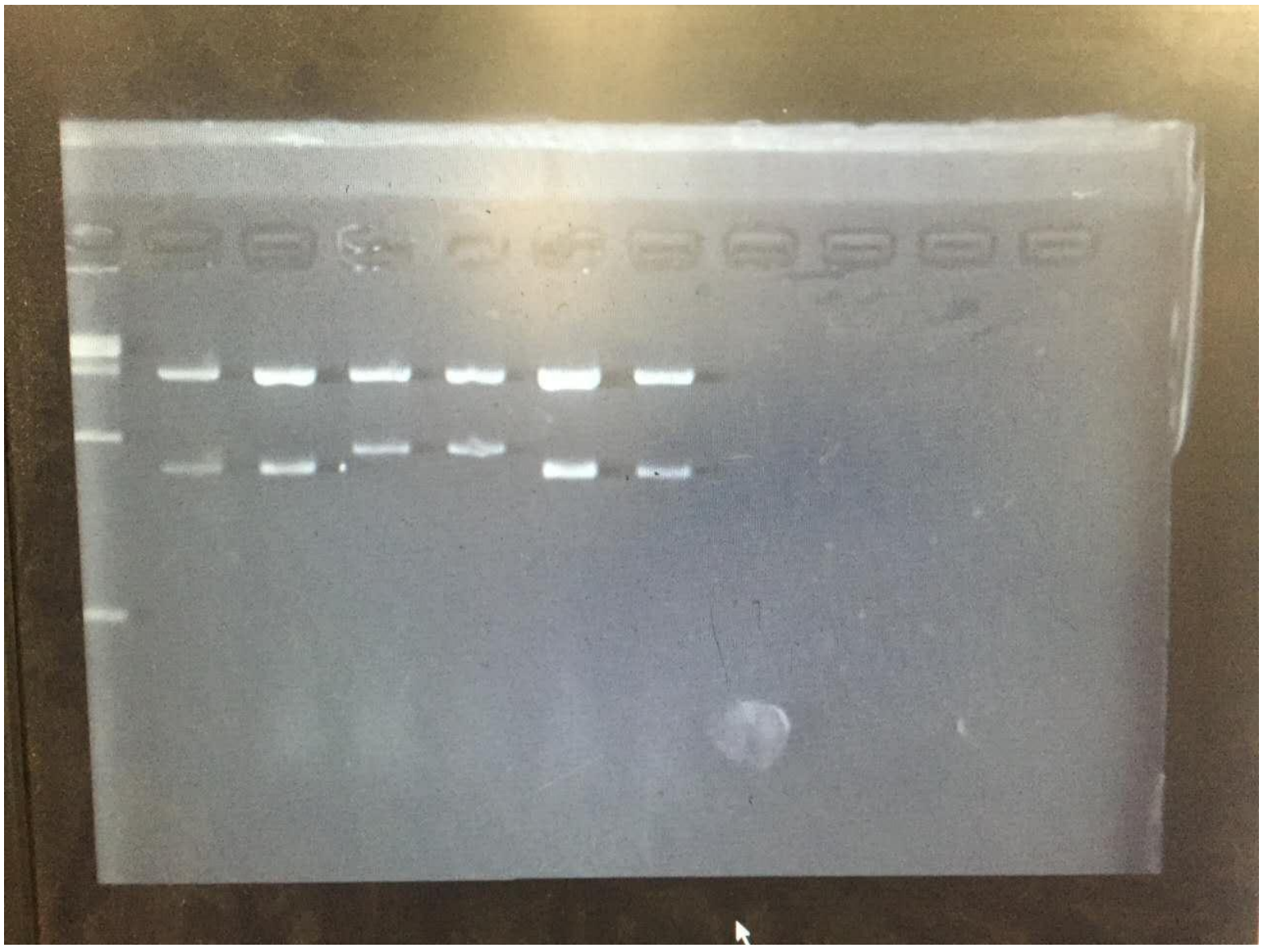
Culture competent cells. Before adding CaCl2 solution, OD=0.13. Add 1mL CaCl2 finally, and divided them into 10 EP tubes to preserve.

——Chang Gao

2017/7/8

pigment and chromoprotein

Extract plasmids with parts of cjBlue, amilCP and eforRed. The concentration of these plasmids are lower than we expected execpt amilCP.Then restricted digestion, cataphoresis. The result is same as our expectation.



——Chang Gao

2017/7/7

pigment and chromoprotein

Pick single colonies of cjBlue, amilCP and eforRed. Cultured for 20 hours. We cannot pick single colonies on plate of amilGFP, which seems to have been contaminated.
Cryopreserve bacteria with RFP of glycerol.
Culture competent cells for a second time. Streak inoculation.

——Chang Gao

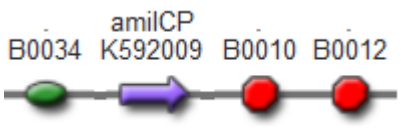
2017/7/6

STAR

Two chromoproteins were selected in our project: amilCP and cjBlue.

BBa_K1357009:

compositive part for amilCP:



BBa_K592011:
coding part for cjBlue: without RBS and its own terminator.

Note: because cjBlue only has the coding part (start with start codon atg and end with stop codon taa), its PCR primer for sense strand need to add RBS B0034 at the 5' terminus, and primer for antisense strand need to add two terminators at the 5' terminus.

Two vevtors for chromoprotein expression:

pCDFDuet-1: J23119 + Target1 + amilCP　　J23119 + antisense1 + t500
pRSFDuet-1: J23119 + Target2 + cjBlue　　J23119 + antisense2 + t500

——Zhuoyang Chen

pigment and chromoprotein

Since we did not find any colony of cjBlue or amilCP, we suspect that the concentration of competent cells we made by ourselves was too low.
Transform for a second time. Using the comprtent cells we bought to transform cjBlue, amilCP and eforRed, while using our own competent cells to transform amilGFP. As we suspect the concentration of cells made the experiments failed, we used all bacteria to spread the plate.

——Chang Gao

2017/7/5

STAR

We got three plasmids for expression from Miss Wang. They are pACYCDuet-1(CamR), pCDFDuet-1 and pRSFDuet-1. All of them have two T7 promoters and their MCS respectively, which means two gene clusters can be inserted into the same vector and accomplish the co-expression of our Star system.

To establish the second Star system that hasn't been used in Imperial College in 2016, we also need to fisinish the characterization of the T181.S7 and the T181.A6. Report gene sfGFP also be chosen here.

We need to build up a vector below for Star2 characterization:

J23119 + T181.S7(Target2) + sfGFP + TrrnB
J23119 + T181.A6(Antisense2) + t500

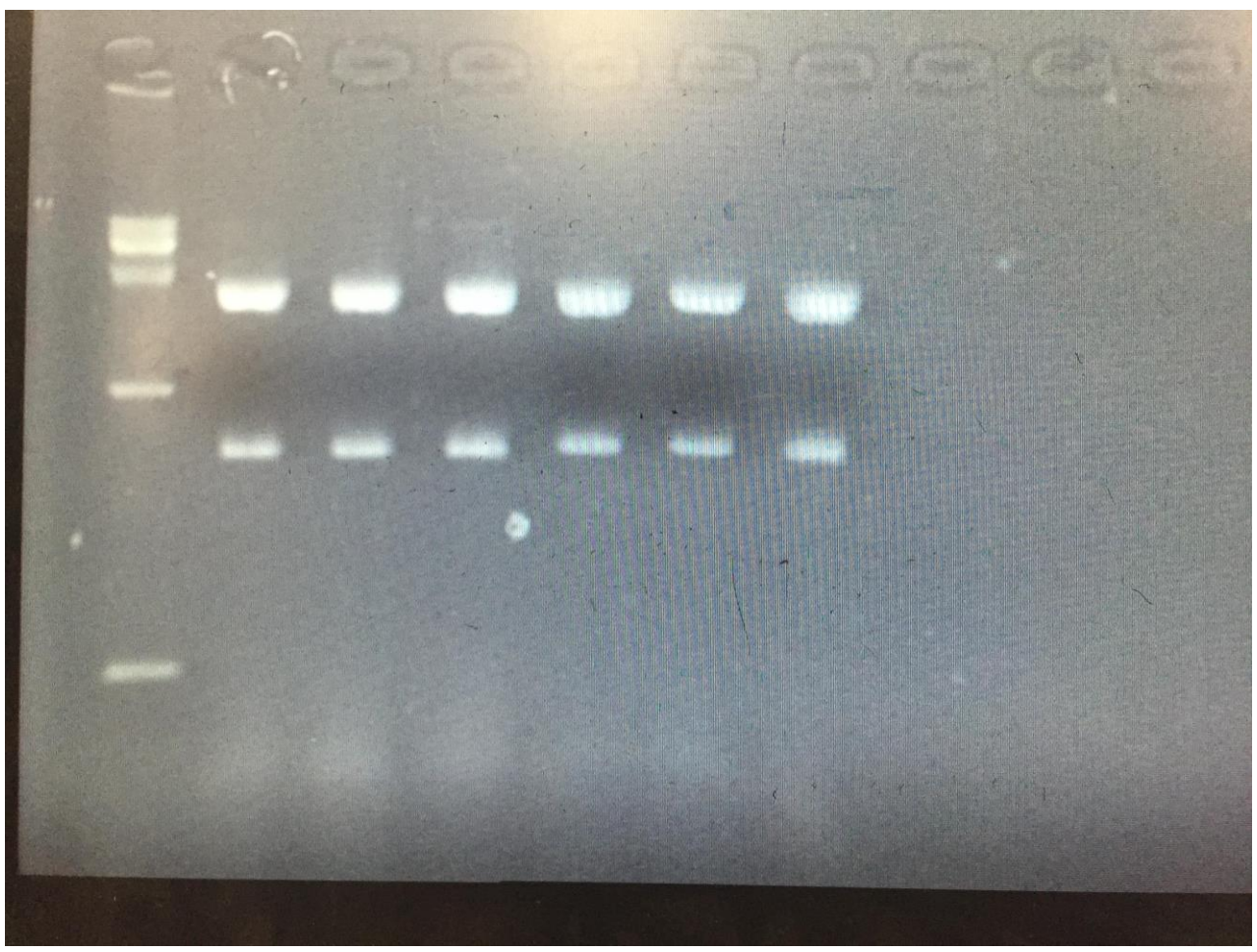
experiments (simillar to the one in Imperial College 2016):

three types of E.coli should be included and here are results we expect:
background (No expression): baterial without any plasmid transformation
Few expression: sfGFP without Star2
high expression: sfGFP with Star2

——Zhuoyang Chen

pigment and chromoprotein

Culture competent cells. Before adding CaCl2 solution, OD=0.054.
Parts of fwYellow & amajLime were extracted, then measured concentration, restricted digestion, cataphoresis. Results of these experiments are normal.
Pick single colonies of RFP for a second time with ampicillin.
Transform 2 parts with cjBlue and amilCP using competent cells we made before. Set control groups without ampicilin.



——Chang Gao

2017/7/4

pigment and chromoprotein

Pick single colonies of chromoproteins and competent cells.

——Chang Gao

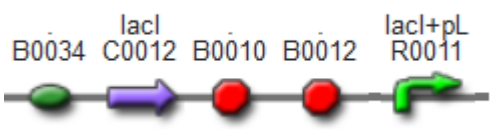
2017/7/3

STAR

At first we tried to pick up the Lac and Ara promoter sequences from the plasmids on www.addgene.org directly but only to found that they lacked the repressors genes. To solve this problem, we used the 2017igem kit information to help us find the composive part of the above operons (those includes the represser genes on the upstream). At last, we chose BBa_Q04121 as our Lac operon of Star system1 and BBa_K228009 as the Ara operon of Star system2.

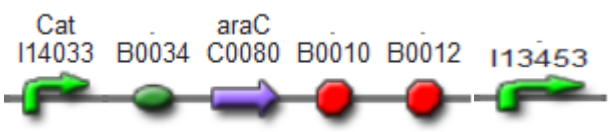
Note: since part BBa_Q04121 didn't have its own promoter for the LacI represssor, we chose the Anderson promoter J23119 we referred in the report part before!

Star system1: **J23119+**



+ Star1 + t500

Star system2:



+ Star2 + t500

Besides, we also selected some useful promoters for the further tests of heavy metal ions.

Promoters available：

BBa_K346002
merR: BBa_K346001 ;
BBa_K540001
BBa_J33201
BBa_K1758330
BBa_K1980006

——Zhuoyang Chen

pigment and chromoprotein

Because we used too much bacteria to culture, they grew on the plate densely. After 12 hours, they still did not show red color. Then 3 hours later they became red. We picked 3 single colonies to culture 16 hours without ampicillin. Culture competent cells. Medium preparation. Streak inoculation after adding 500uL liquid LB to 50 uL cells then cultured for 1 hour.
Transform the parts of chromoprotein. Pick parts of fwYellow and amajLime to transform.

——Chang Gao

2017/7/2

STAR

We got the DNA sequences of lactose operon and Arabinose operon and used them as the Star system promoters.

We chose this two promoters to testify if Star system can work well on preventing leakage and try to establish the linear relationship between brightness of the color and the concentration of certain factors. These promoters are well-studied and well-known for their convenience and efficience to ues.

Our ultimate goal is to detect heavy metal ions in the environment or those can be used as an index for health.

——Zhuoyang Chen

pigment and chromoprotein

Transform RFP for a second time, use ampicillin instead of chloramphenicol. We suspected the reason why transformation of crtEIB failed so many times is that the time of heat shock was too short to make the transform, so that we design an experiment to confirm it. Some bacteria were heat shocked for 45s, others were for 90s. To make sure there will exist enough bacteria, we used all 500uL to spread the plates instead of 200uL. Then after spreading 2 plates we cultured them for about 12 hours.

——Chang Gao

2017/7/1

pigment and chromoprotein

We found the reason why we failed this time, and drew a conclusion that the failure of RFP is because we added chloramphenicol instead of Ampicillin. Medium preparation.

——Chang Gao

2017/6/30

STAR

The Star system conludes a sense part and a report part:

Report part (RFP, three kinds of chromoprotein, iycopene are used):

J23119(promoter) + AD1.S5(target1) + RFP + B0010 + B0015(terminator)
J23119 + AD1.S5 + chromoprotein1/2/3
J23119 + AD1.S5 + Crt EIB

Note: T181.S7(target2) is alternative here!

sense part (promoters for heavy metal ions):

PLac + AD1.A5(antisense1) + t500(terminator)
PAra + T181.A6(antisense2) + t500

note: Plac and PAra are used only for test. They are supposed to be replaced by promoters for heavy metal ions later!

Chappell J, Takahashi M K, Lucks J B. Creating small transcription activating RNAs[J]. Nature chemical biology, 2015, 11(3): 214-220.

——Zhuoyang Chen

pigment and chromoprotein

Transform crtEIB and RFP for a second time, neither succeeded.

——Chang Gao

2017/6/29

STAR

We decided to contain terminator B0010 and B0012 in the RFP as it was shown in Part BBa_K516132.

It is not necessary to have the two terminators above in our RFP gene for every plasmid has its own promoter and terminator so . However, the primers designed via primer premier 5 got a poor score with proper Tm and GC ratio. So we considered to contain the two terminators in the original part.

——Zhuoyang Chen

pigment and chromoprotein

Pick single colony: choose 4 pionts, cultrue for 6 hours without chloramphenicol, then add 2.5uL, 1.5mol/L IPTG solution, cultrue next 6 hours.
Transform for a second time: we cultured bacteria on the solid LB for 10 hours then found no colony.

——Chang Gao

2017/6/28

pigment and chromoprotein

——Chang Gao

2017/6/27

pigment and chromoprotein

After medium preparation, we transformed a part including crtEIB genes from 2017 kits, finally did not find any colony after adding chloramphenicol to LB.
The concentration of chloramphenicol that we used was 25mg/ml, and we added to LB in 1:1000.

2017/5/31

pigment and chromoprotein

Extract plasmid for a second time: the concentration of plasmids is between 30ng/ul to 50ng/ul, which is close to our prediction.

2017/5/30

pigment and chromoprotein

Extract plasmid: the concentration of plasmids is between 10ng/ul to 20ng/ul, which is far lower than we thought.
Transform for a second time: we did not find any colony with either RFP or scaffold.

2017/5/29

pigment and chromoprotein

Results of the transformation: colonies with mutant GFP did not become green visibly, those with RFP became red, and we did not find any colony with scaffold.
Pick single colony: choose 9 pionts from GFP, 4 points from RFP.

2017/5/28

pigment and chromoprotein

Transform plasmids: GFP mutation, RFP mutation and scaffold.

2017/5/27

pigment and chromoprotein

Medium preparation and other preparations for experiments, as well as learning the usage of autoclave.
Lab safety training.