iGEM 2010 Progress Report

June 28th, 2010

Team Vector

Previously:

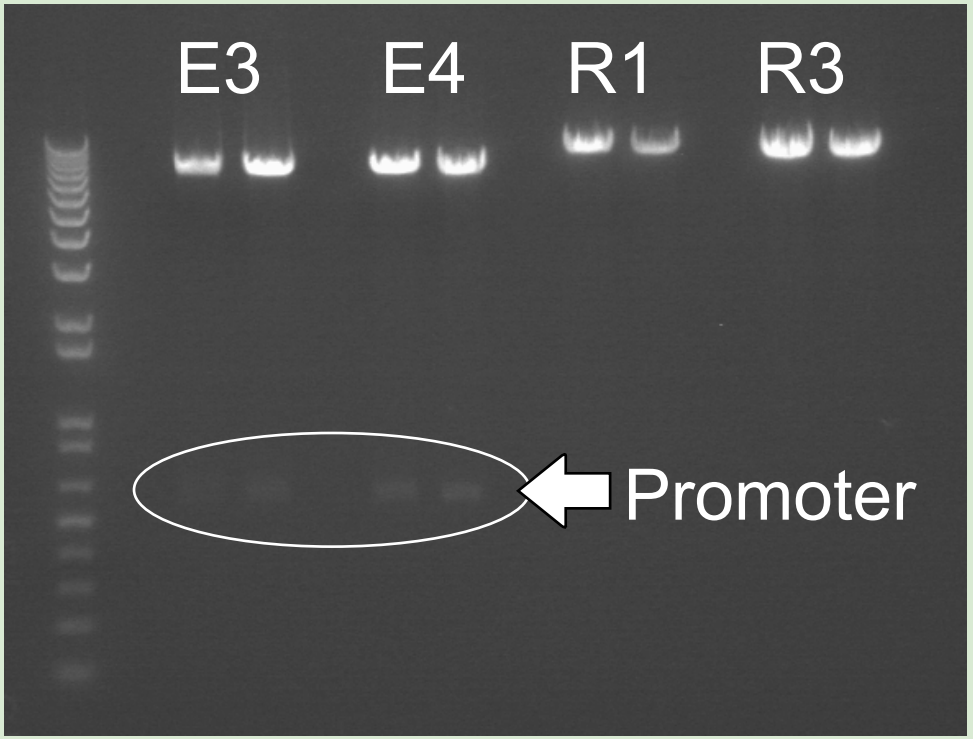
- Biobricked E3, E4, R1 and R3 vectors but not confirmed by sequencing.

Progress:

- Digested with PstI and HindIII -- bands matched DNA fragments we expected to obtain

Next Step:

- Sequencing!
- We retransformed the constructs to grow up enough DNA for sequencing by GeneWiz



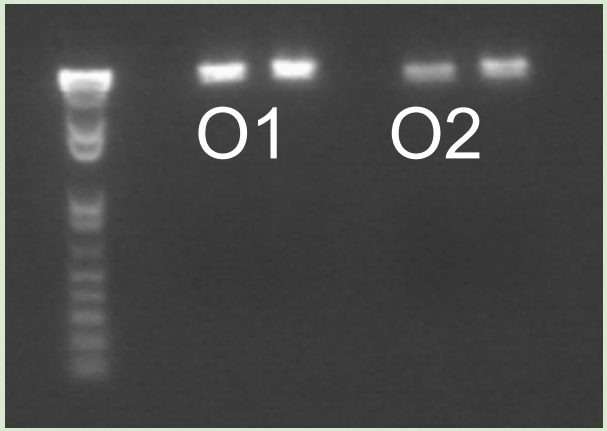
Team Vector

Previously:

- Digested Open Series pORE Vectors with SacII and SpeI to create vector backbones

Progress:

- Annealed oligos synthesized by Mr. Gene to create insert
- Ligated backbones and inserts and transformed into E. Coli
- Got colonies for O1 but not for O2, so repeated digest, ligation and transformation
- Ended the week with miniprepped DNA for both constructs



Next Steps:

- Sequencing!

Team Vector

In the downtime we had while waiting for primers to arrive, we started creating diagrams for the wiki and thinking about human practices.

Team Allergy

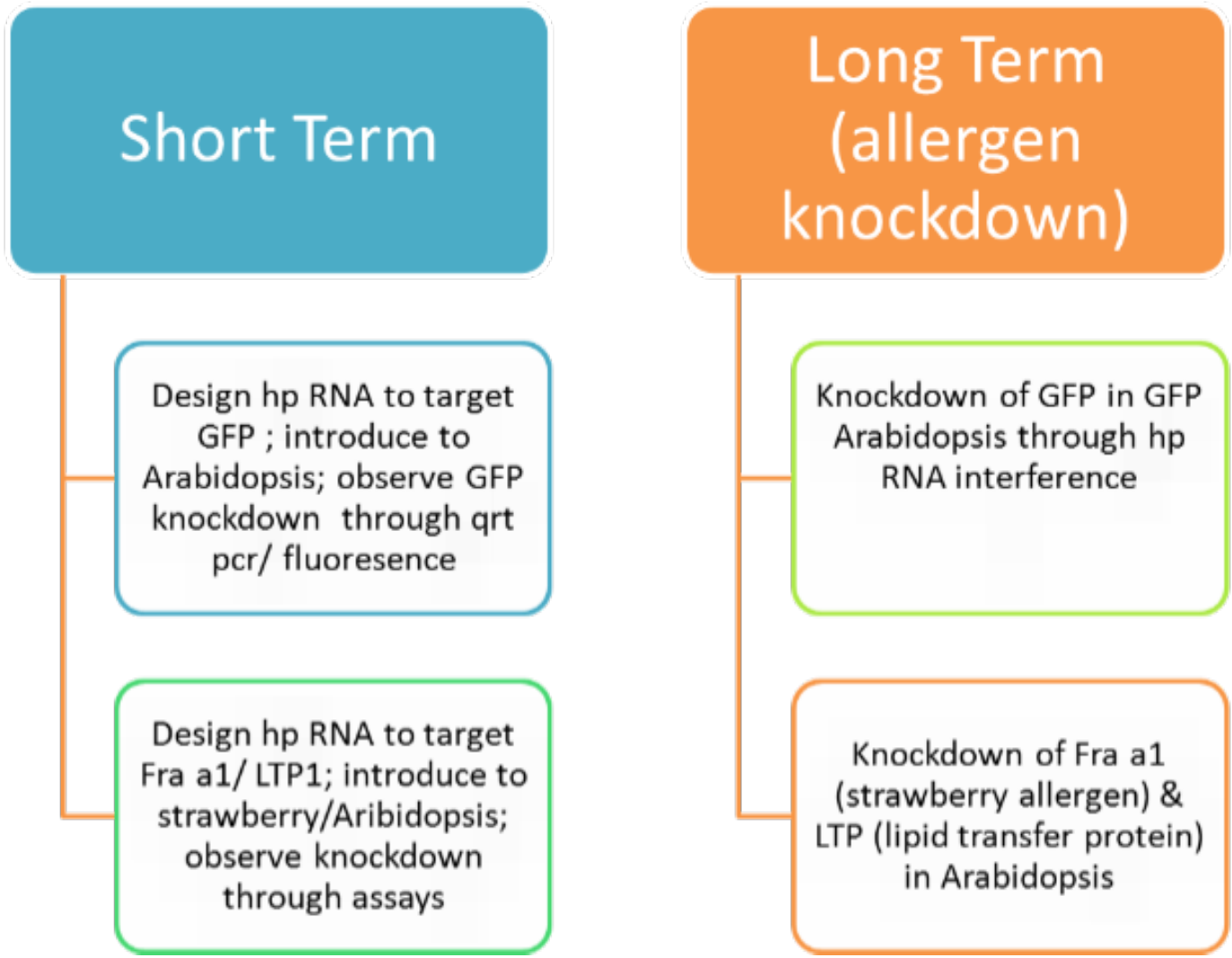
Identify Allergens → Design Primers for shRNA → PCR out our intron, antisense, sense sequences

PCR out our intron, antisense, sense sequences → Clone into Biobrick vector (VO210)

Clone into Biobrick vector (VO210) → Transform into E. coli → Transform into agrobacterium

Transform into agrobacterium → Transform into Arabidopsis/ Plants → Observe Allergen Knockdown

Team Allergy Goals



Our Goals Last Week

Create plasmids that can deliver a form of RNA interference

Artificial Mini RNA interference

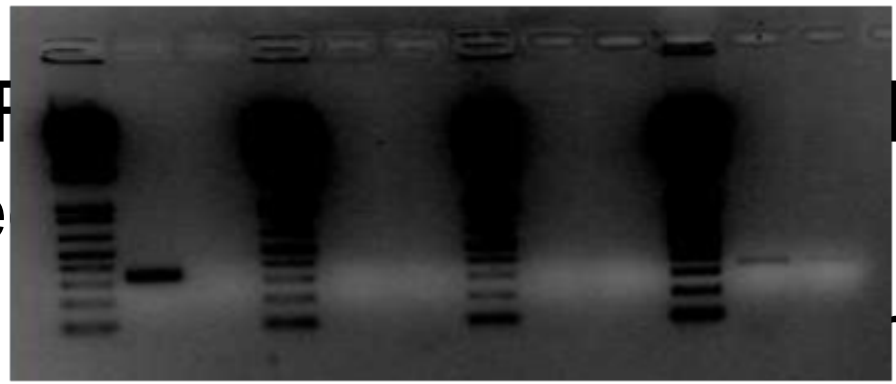
- Further downstream in interference process
- Template is already available (RS300 vector)
- Need to replace existing interference sequence with our own using primers

Hairpin RNA interference

- We had been working on hpRNA since two weeks ago
- Vector was available to us earlier (V0120)
- Requires genomic DNA

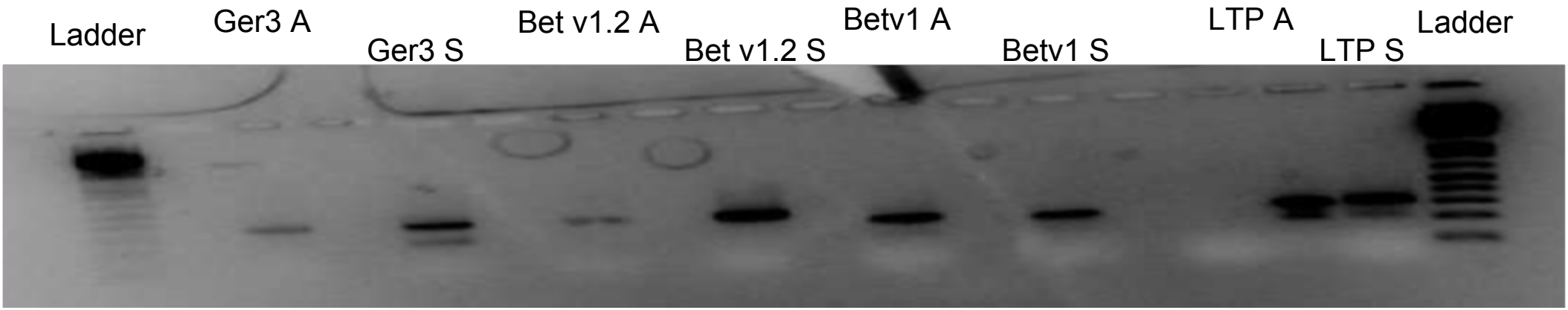
Team Allergy Progress - hpRNA

- PCR of LTP sense, Ger 3 sense/antisense was successful (~ 300 bp bands below)



- Digested (P l w/ V0120, transforme colonies
- Attempted nomic DNA but w/ lower annealing temp

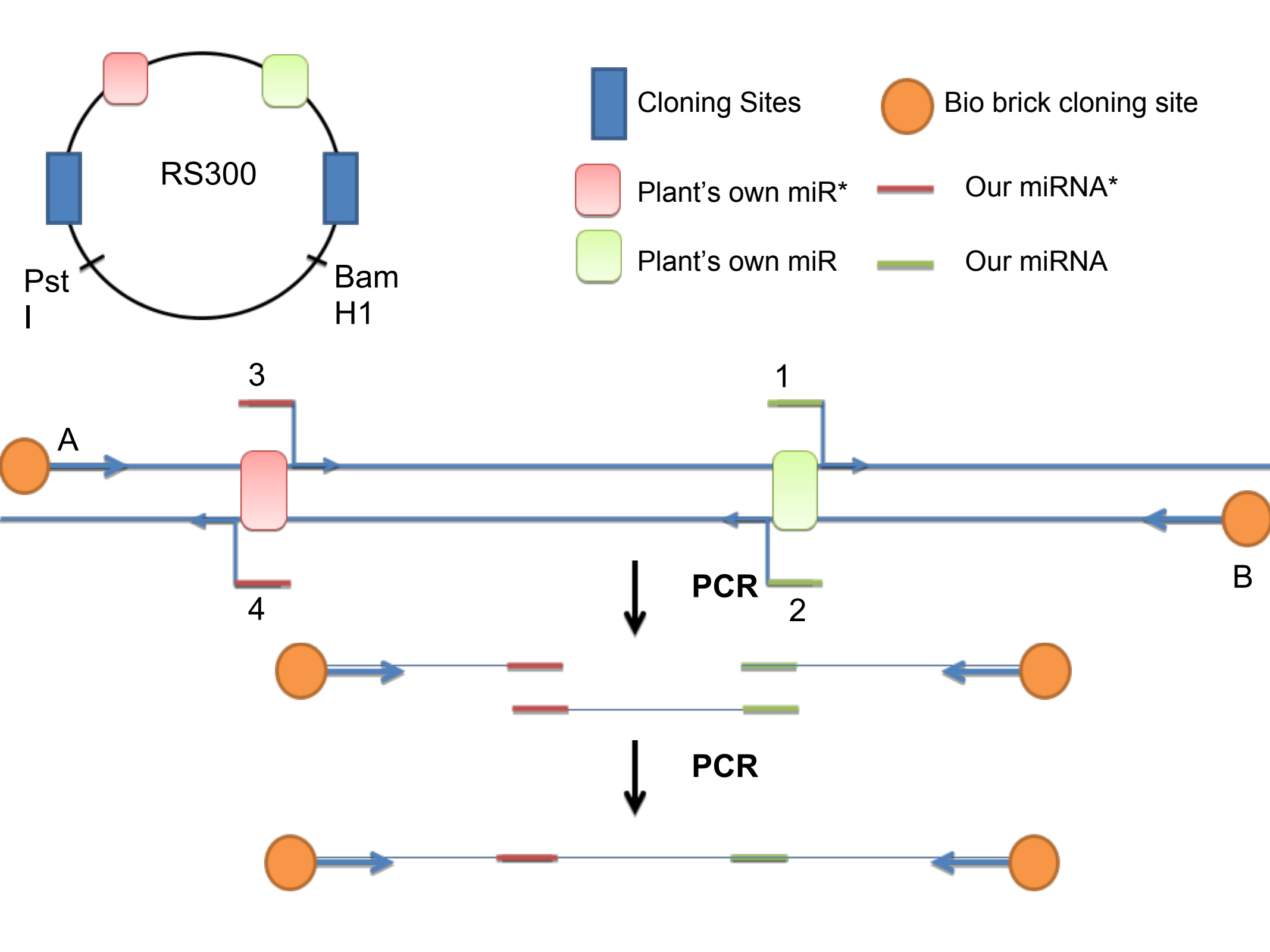
PCR



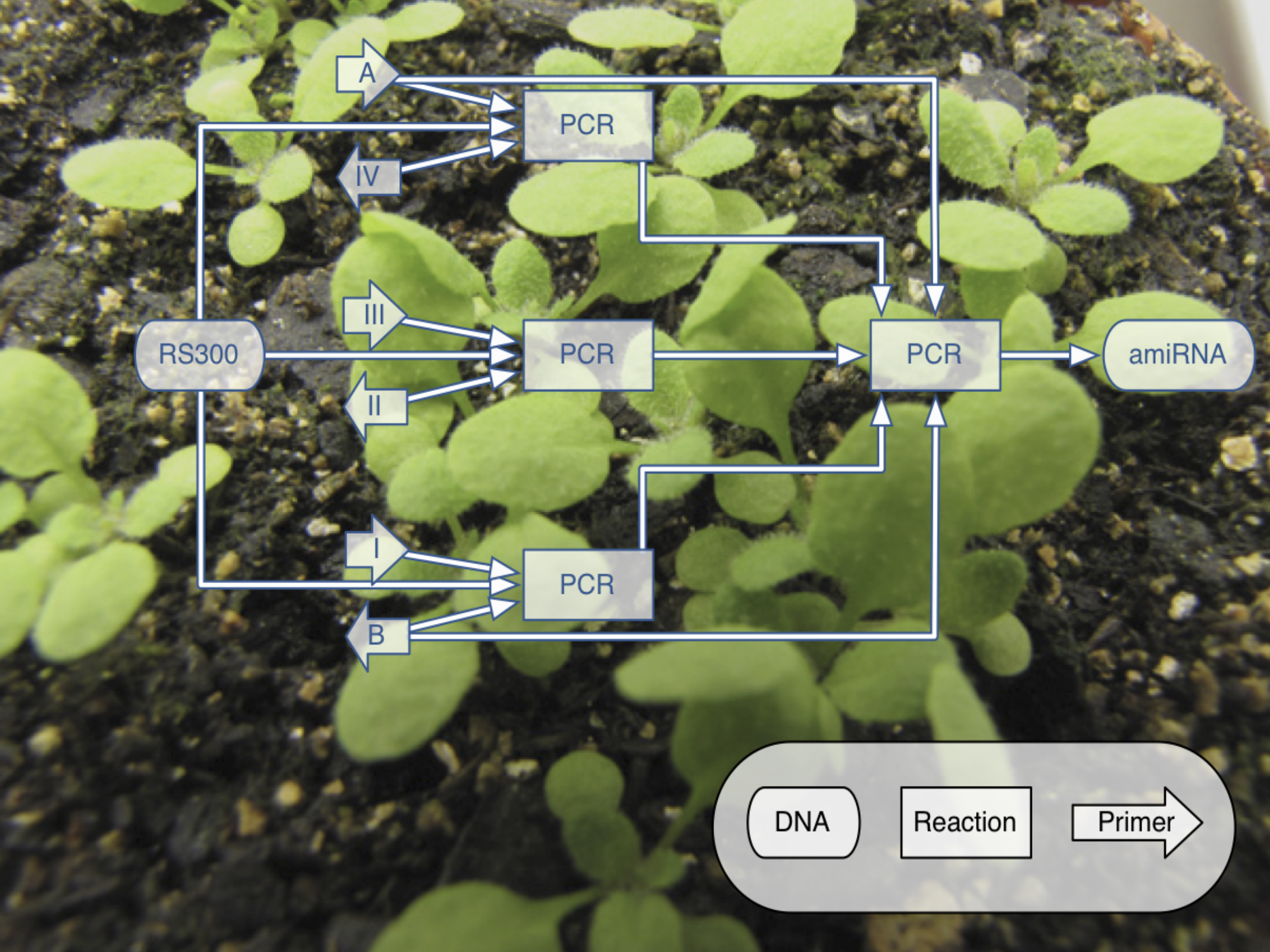
- Allergen PCRs were successful
- In the process of ligating digested sequences (slow digest Xba1 & Pst 1) w/ V0120

Team Allergy Progress - ami RNA

- Ordered and received primers with interference sequence
- Ran 3 step PCR for amiRNA w/ miniprepped RS300 (planning to do “part 2” of the PCR)


RS300
Pst I
Bam H1
Cloning Sites
Bio brick cloning site
Plant's own miR*
Our miRNA*
Plant's own miR
Our miRNA
3
1
A
B
4
2
PCR
PCR

Sequence selection for miRNA involves many factors. Luckily, there's a design tool!


A
PCR
IV
III
RS300
PCR
PCR
amiRNA
II
I
PCR
B
DNA
Reaction
Primer

Future Directions

Identify Allergens

Design Primers for shRNA

PCR out our intron, antisense, sense sequences

amiRNA ✓

Clone into Biobrick vector (VO210)

Transform into E. coli

Insert into agrobacterium

Transform into Arabidopsis/Respective Plants

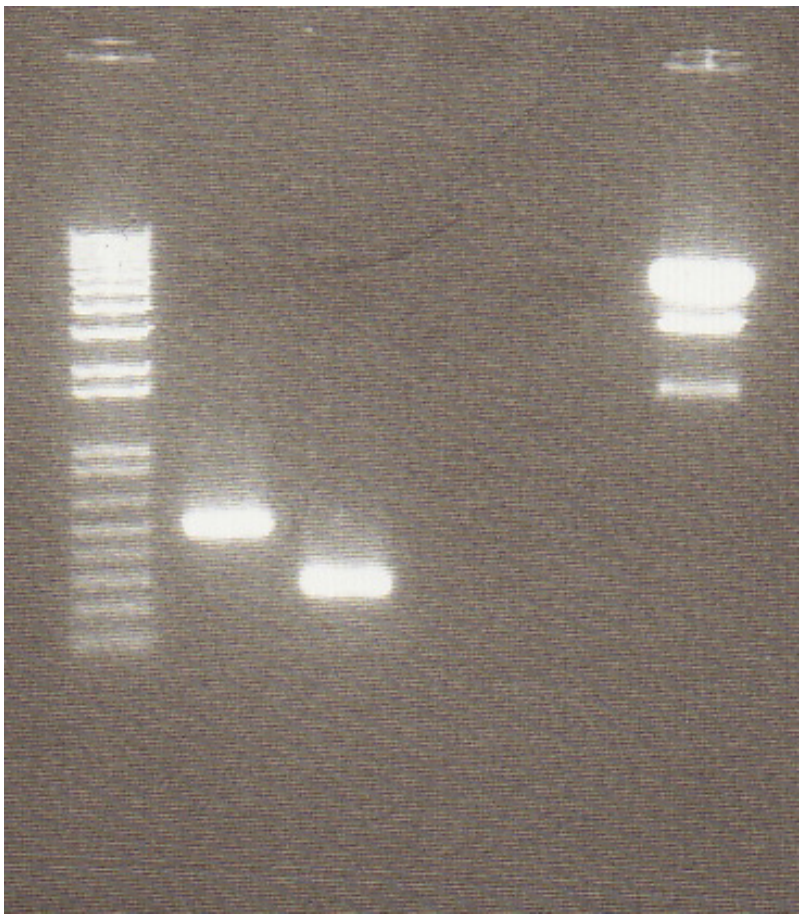
Observe Allergen Knockdown

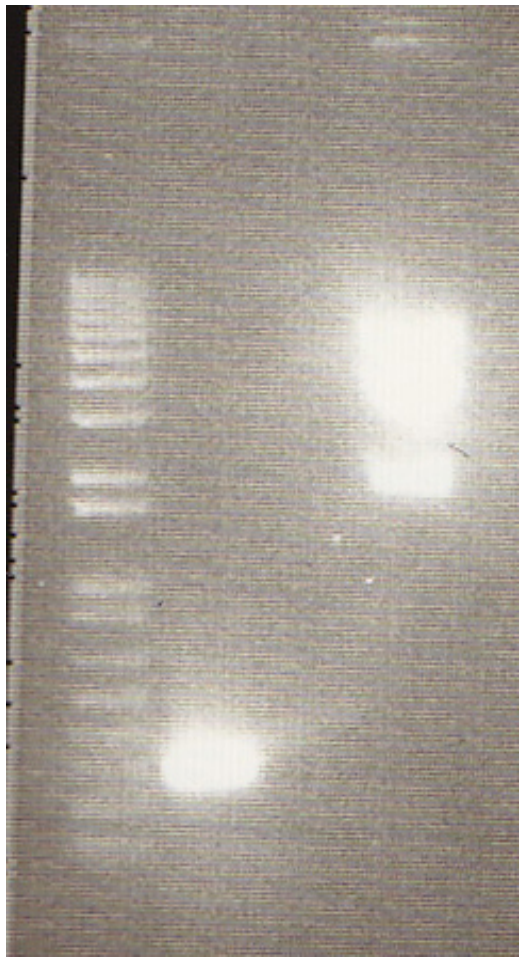
Team Flavor

Things to do:

- BioBrick plant promoter (pENTCUP2), plant terminator (NosT), and plant terminator with stop (NosT + stop)
- BioBrick Miraculin and Brazzein
- BioBrick Valencene
- Work on previous flavors in Registry (Banana and Wintergreen)

1. Ligation of pORE parts into V0120 vector.

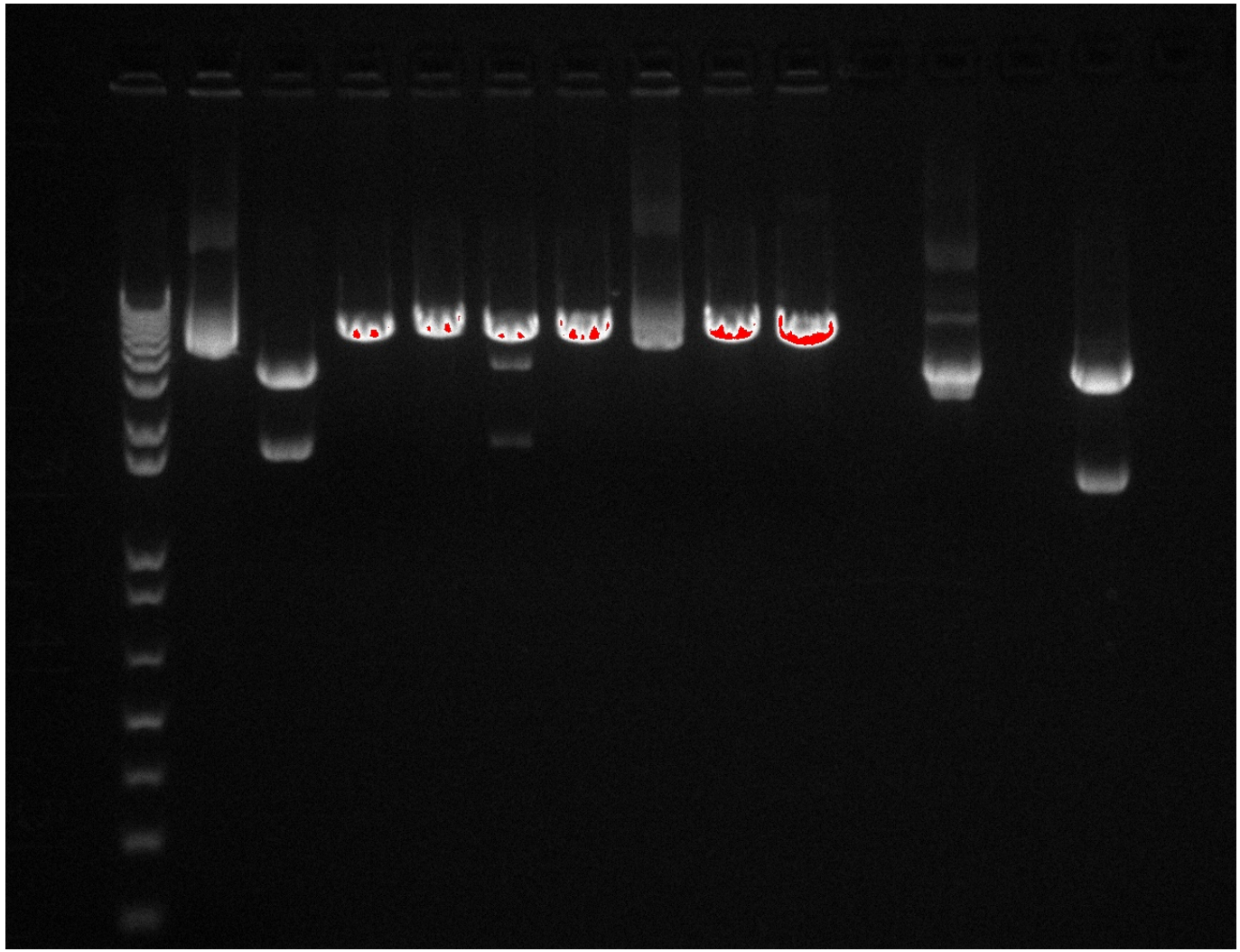




All transformations have been unsuccessful.

2. Troubleshooting

lane order:
1. 1 kb plus ladder
2. v0120 undigested
3. v0120 eco/spe
4. v0120 eco/xba
5. v0120 spe/pstI
6. v0120 xba/pst
7. v0120 ecoRI
8. v0120 xba1
9. v0120 pstI
10. v0120 spe
11. blank
12. B15 (StrepII tag) xba/pstI
13. B21 (YFP) xba/pstI



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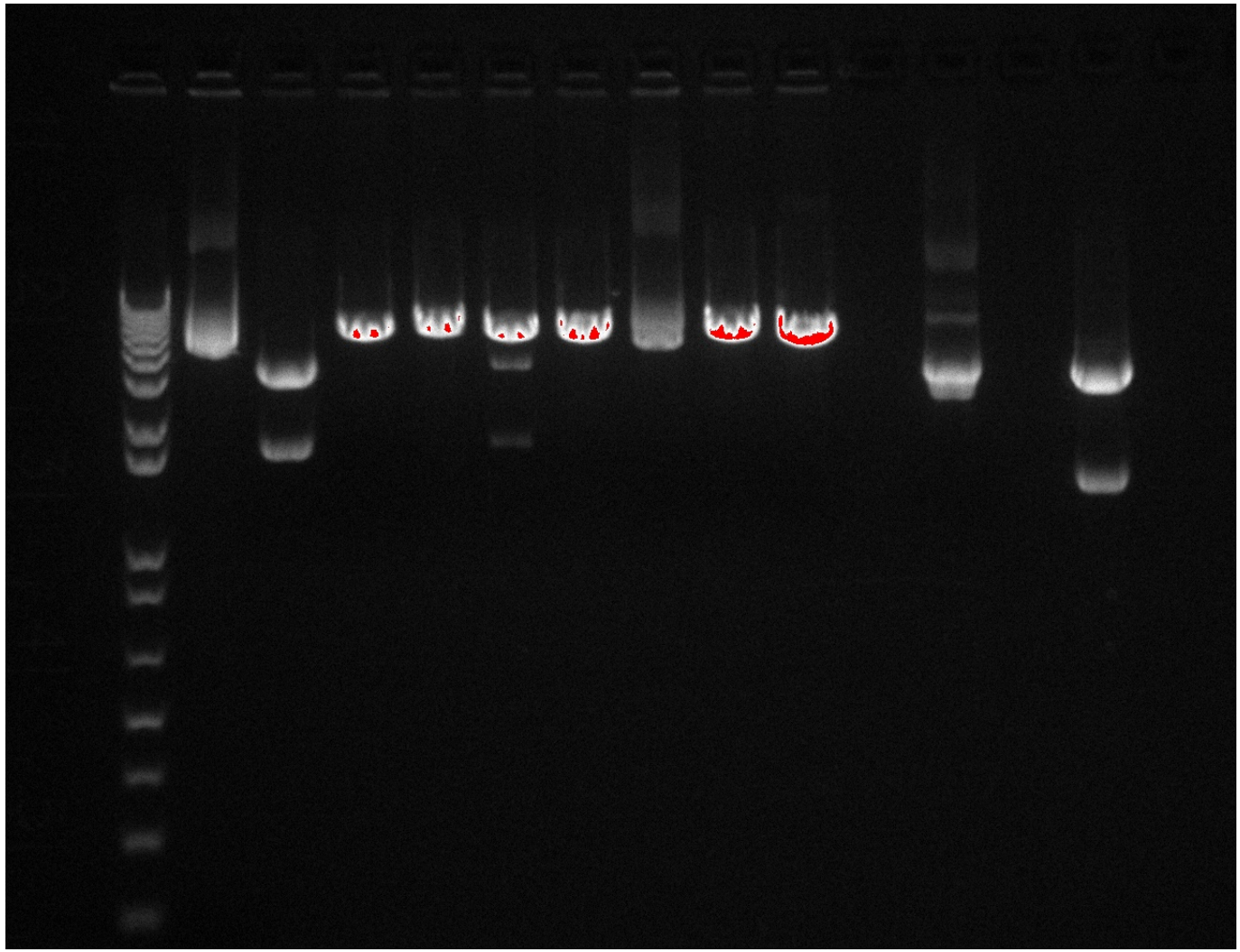
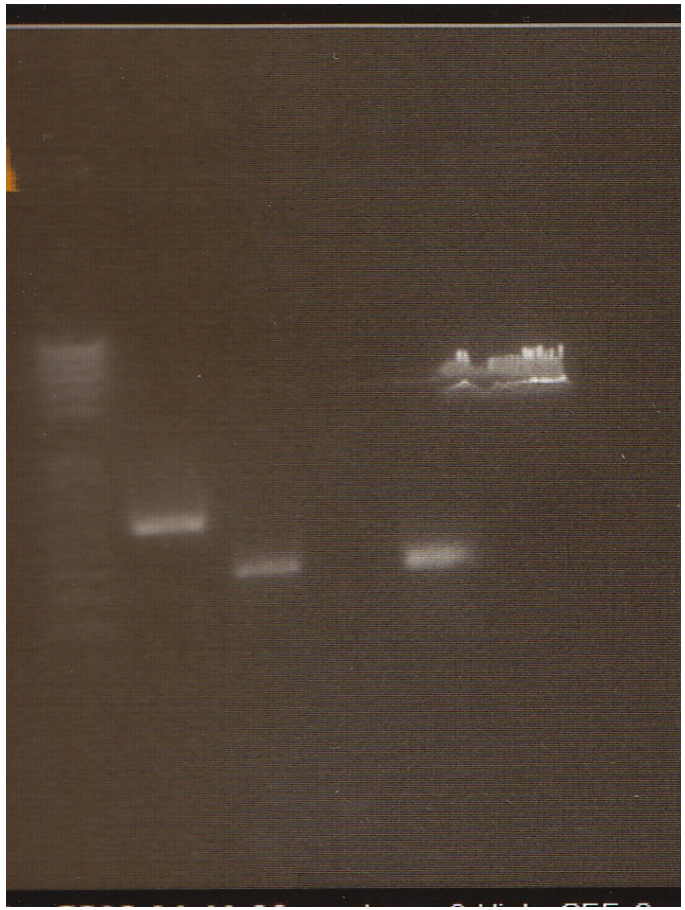


Conclusion: broken Xba1

2. Troubleshooting (cont'd)

Slow Enzyme Digest

pENT NOSt NOSt+ STOP V0120



2. Troubleshooting (cont'd)

Slow Enzyme Digest

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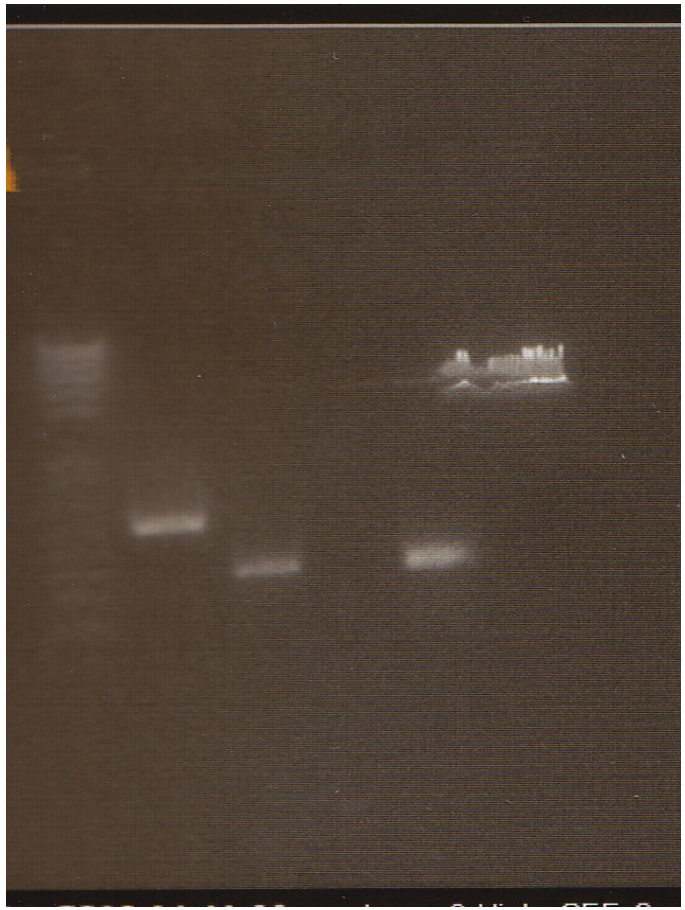


Conclusion: Good news!

3. Extraction of Valencene

Steps:

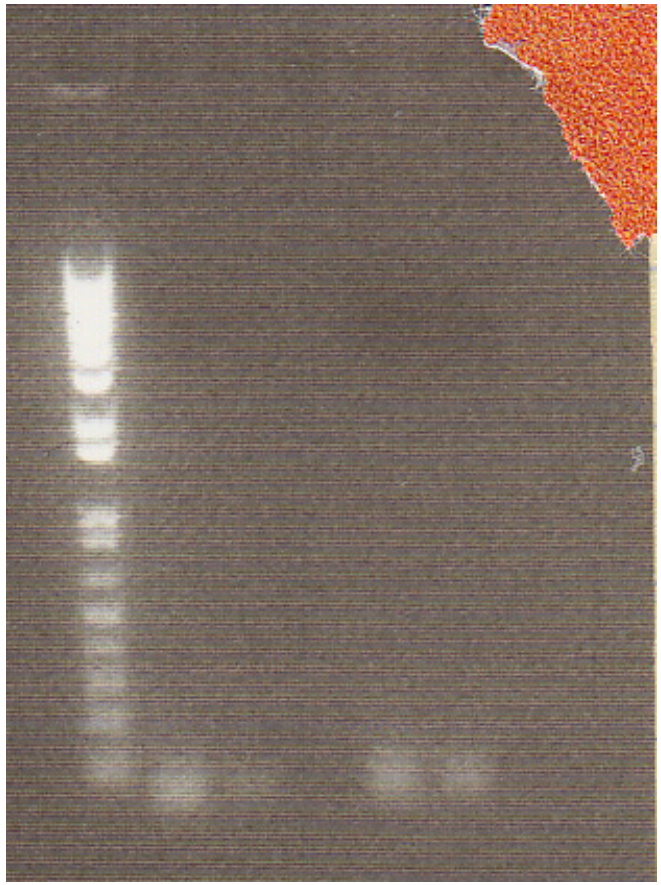
1. Extract RNA from Oranges
2. RT Reaction to obtain DNA
3. PCR of Valencene
4. Site directed mutagenesis
5. BioBricking, Plant expression

3. Extraction of Valencene (cont'd)

Lanes:

1. Ladder
2. Flavedo EcoRI/SpeI
3. Flavedo PstI
4. -empty-
5. Fruit EcoRI/SpeI
6. Fruit PstI

PCR of Valecene



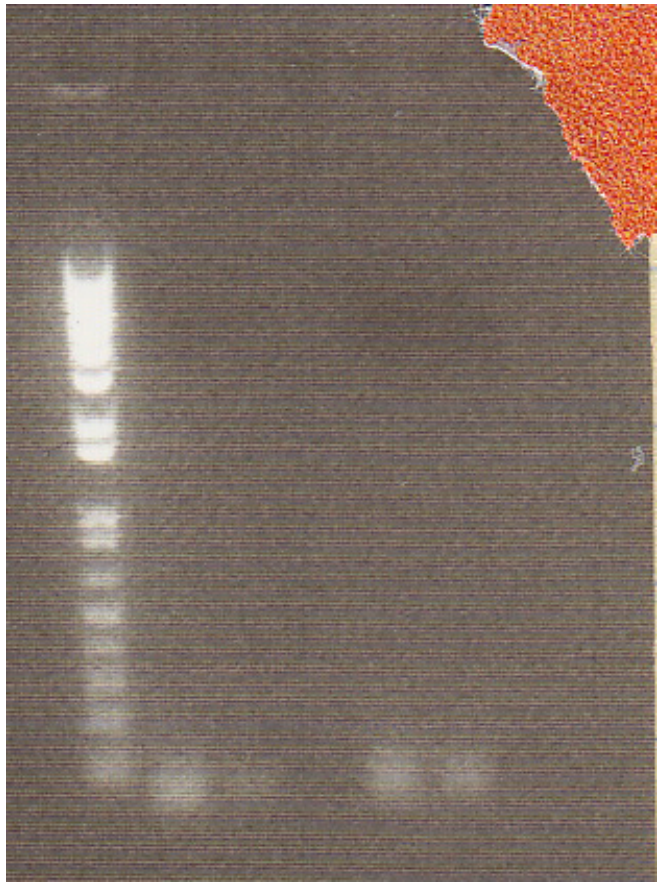
3. Extraction of Valencene (cont'd)

Lanes:

1. Ladder
2. Flavedo EcoRI/SpeI
3. Flavedo PstI
4. -empty-
5. Fruit EcoRI/SpeI
6. Fruit PstI

Conclusion: Primer Dimers?

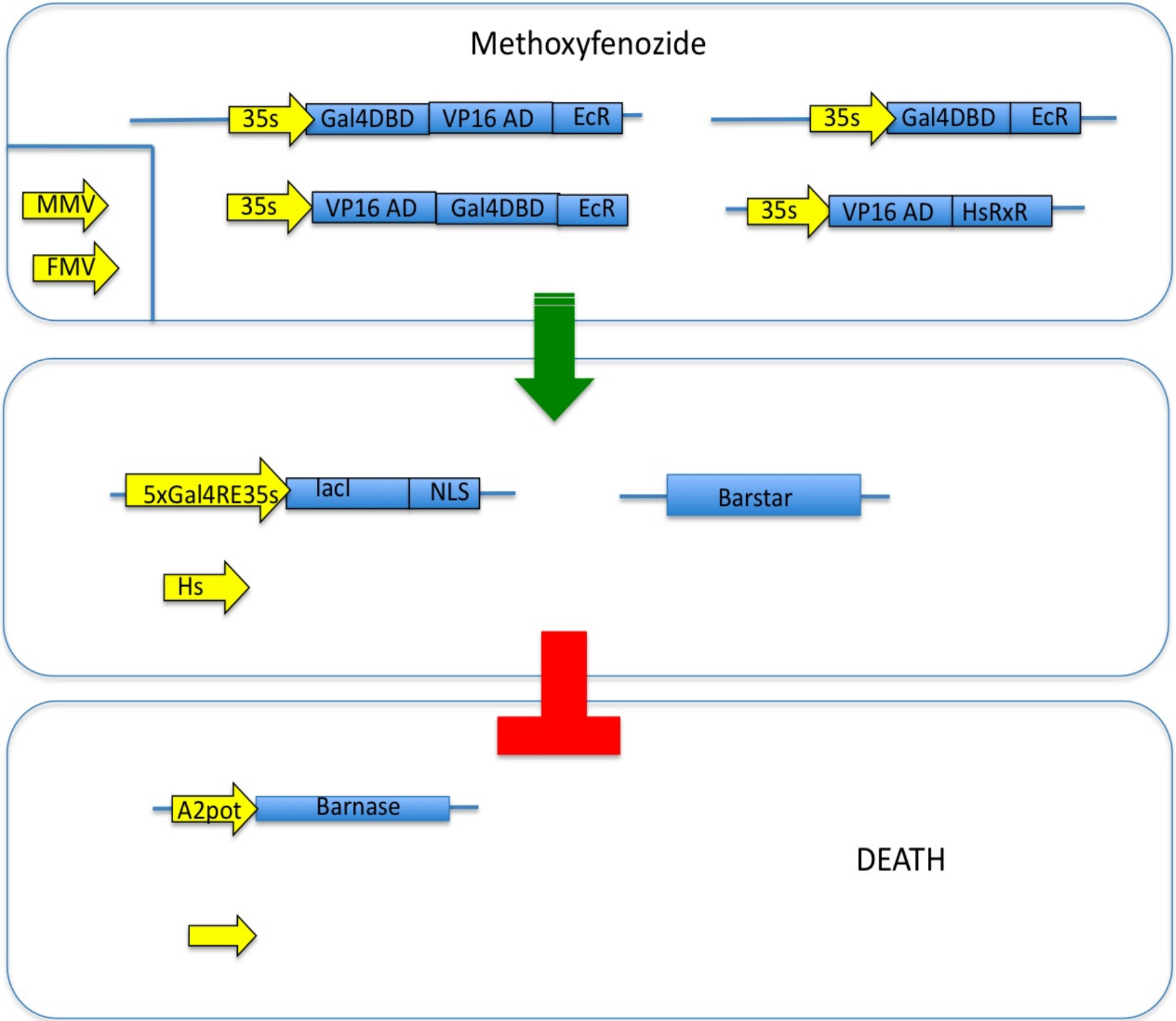
PCR of Valencene



What's Next (the immediate future):

1. Obtain new V0120 (we're out)
    - Could also use backbone of B15, B21 to ligate into - have not attempted yet
2. Obtain new Fast-Digest Xba1
    - In the meantime, it looks promising to use the slow-digest enzymes, though not yet confirmed
3. Re-do the RNA extraction from Valencia Oranges
    - worried about contamination of RNases
4. Start getting results!

Team Fence


Methoxyfenozide
35s
Gal4DBD
VP16 AD
EcR
35s
Gal4DBD
EcR
MMV
35s
VP16 AD
Gal4DBD
EcR
35s
VP16 AD
HsRxR
FMV
5xGal4RE35s
lacI
NLS
Barstar
Hs
A2pot
Barnase
DEATH

Gal4 DNA Binding Domain

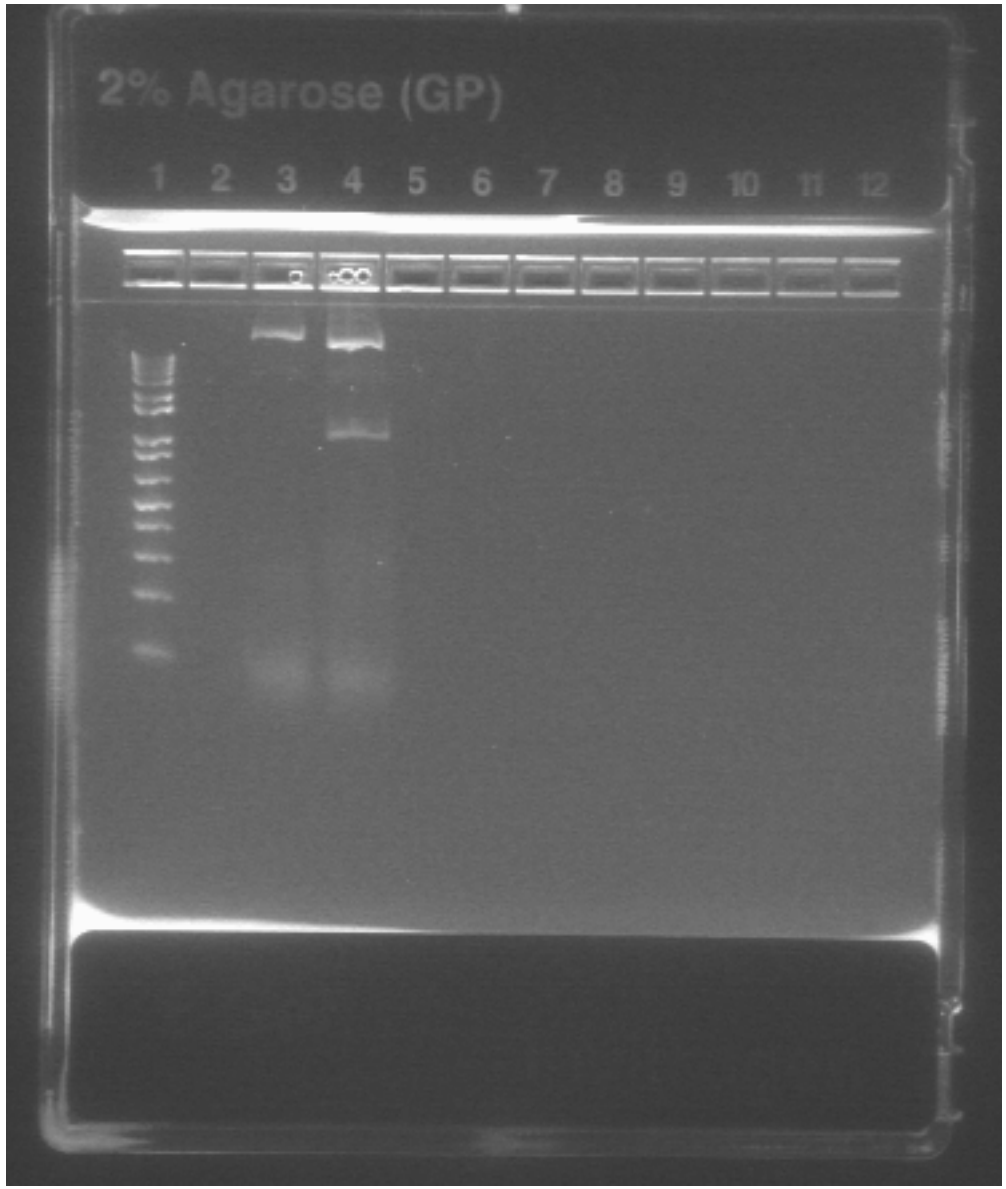
- Innoculated and minipreped GAL4 plasmid
- Ran PCR to amplify the DNA Binding Domain

Barstar, Barnase

- Barstar, Barnase plasmids arrived from ADDGENE, as did the correspoding PCR primers
  - Barstar and Barnase PCR amplified from the plasmids

LacI

- Second LacI NLS PCR attempt was successful
- O2 (lane 3) failed to amplify
- E10 was successful (lane 4)
- Isolated LacI fragment through Gel cutout



Yeast Growth Assay

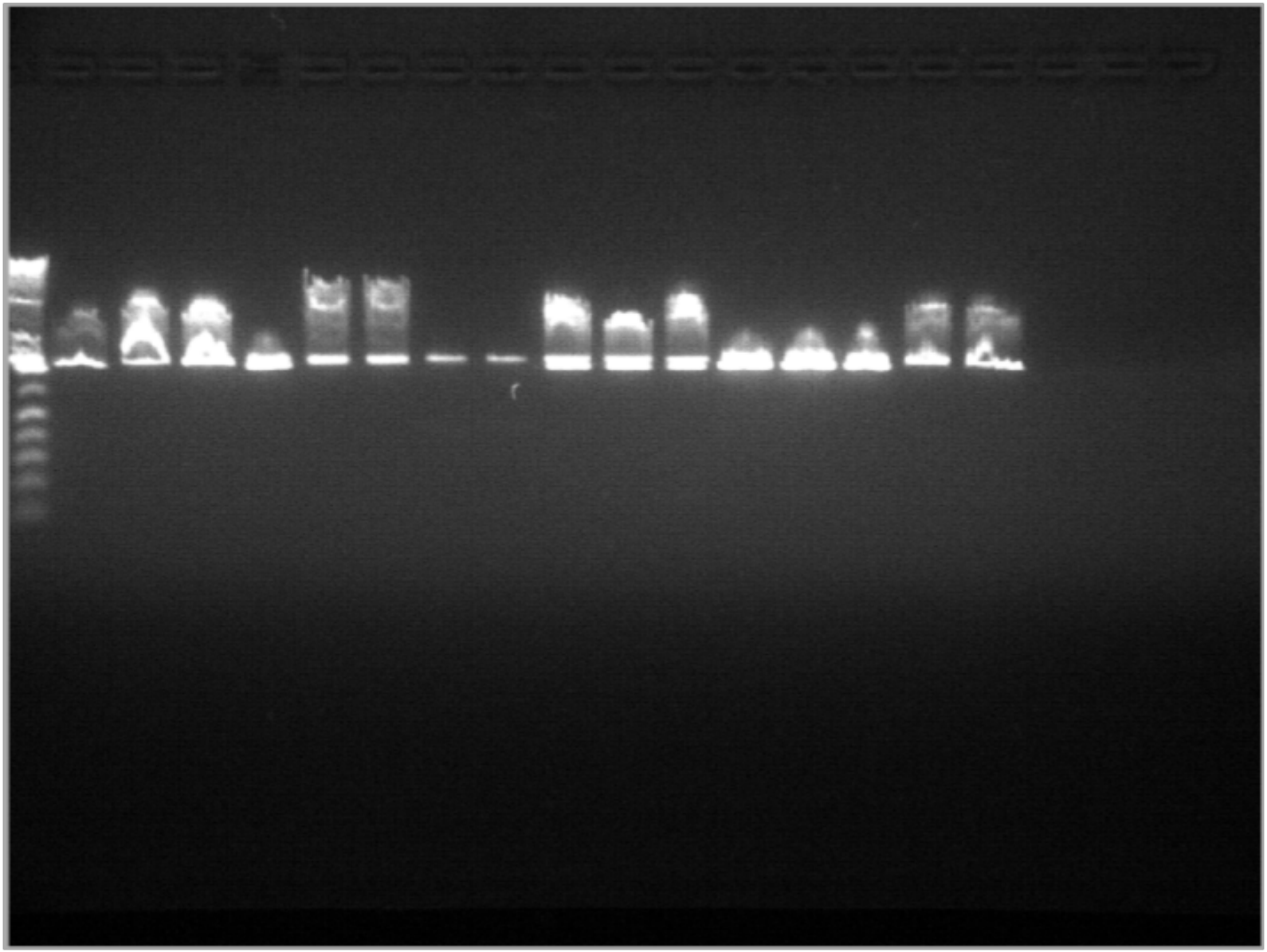
- DMSO, 1 micromolar, 10 micromolar methoxyfenozide conditions
- After 3 hours, all groups, including control failed to grow, so the assay was aborted

Cre, Lox, GFP, etc

- Transformed, innoculated and minipreped:
  - pMT413 (Barstar + Barnase), pMT316 (Barstar), Firefly luciferase, GFP, Cre recombinase, and two lox site plasmids, lox66 and lox71

Plasmid	Quantity (ng/µL)	260/280
Lox 71 #1	41.4	1.82
Lox 71 #2	59.6	1.89
Lox 71 #3	17.4/80.2	1.98
GFP #1	21.2	1.83
GFP #2	110.0	1.90
GFP #3	60.9/124	1.88
pMT316 #1	11.6	1.76
pMT316 #3	16.5	1.81
Cre #1	84.9	1.93
Cre #2	107.7	1.88
Lox66 #2	51.3	1.87
Firefly Luciferase #2	309.3	1.91
Firefly Luciferase #3	325	1.9
pMT413 #1	461.7	1.89
pMT413 #2	362.2	1.91
pMT413 #3	381.8	1.92

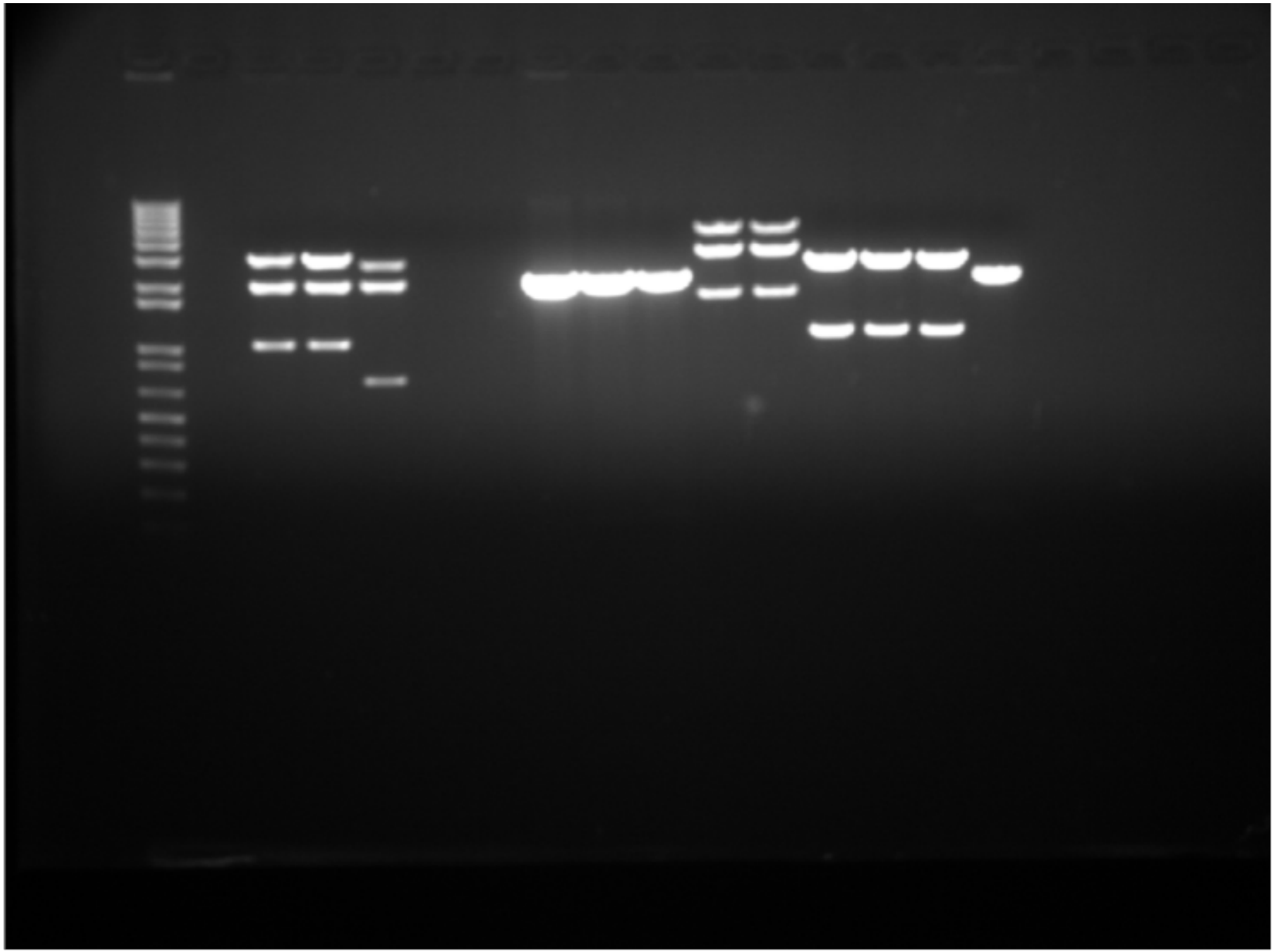
June 24



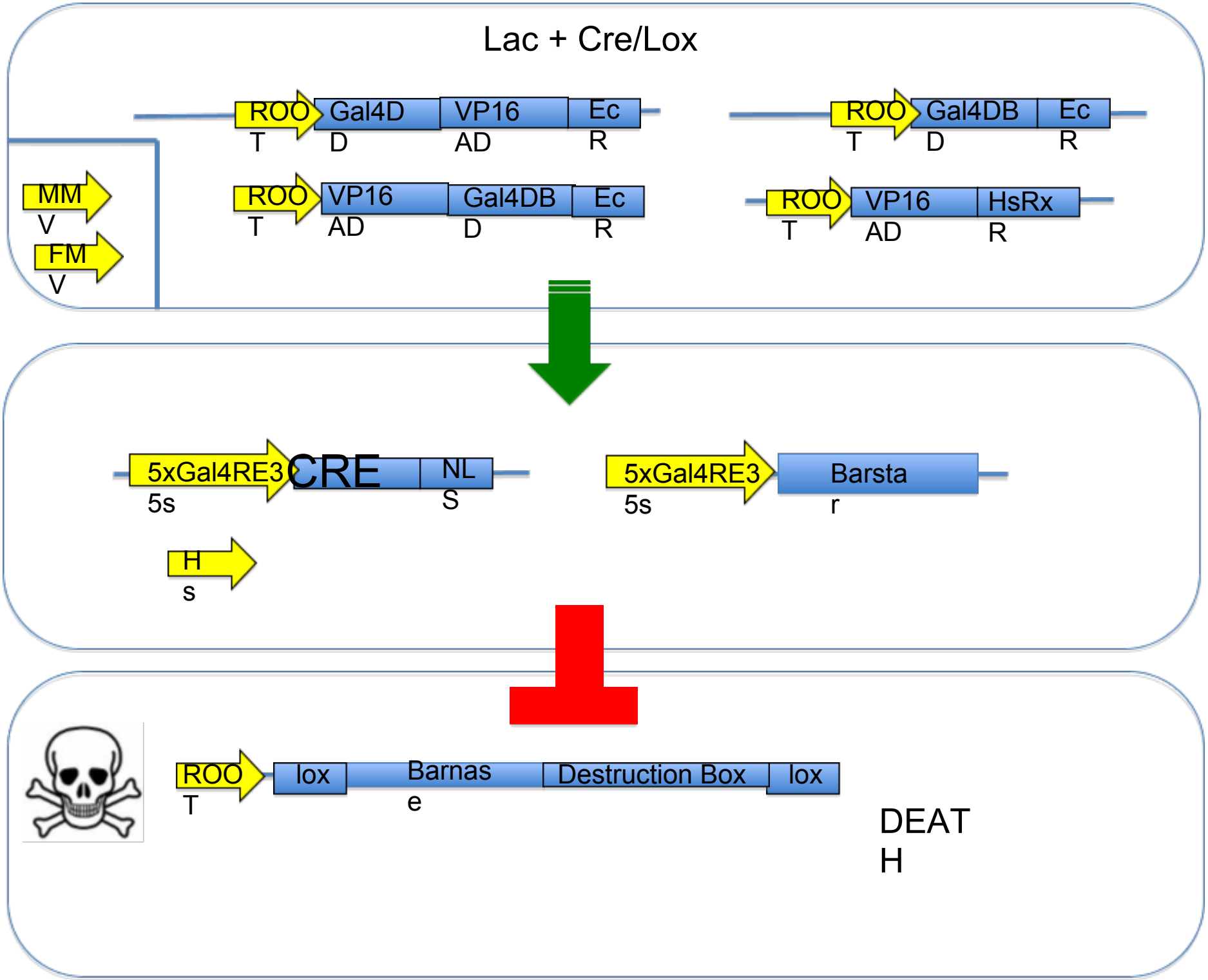
First gel:

8. Barstar
9. Barstar

Re-do



1. ladder
2. blank
3. cre
4. cre
5. gfp
6. gfp
7. gfp
8. lox71
9. lox71
10. lox71
11. firefly
12. firefly
13. barnase
14. barnase
15. barnase
16. lox66


Lac + Cre/Lox
ROO
T
Gal4D
D
VP16
AD
Ec
R
ROO
T
Gal4DB
D
Ec
R
MM
V
FM
V
ROO
T
VP16
AD
Gal4DB
D
Ec
R
ROO
T
VP16
AD
HsRx
R
5xGal4RE3
5s
CRE
NL
S
5xGal4RE3
5s
Barsta
r
H
s
ROO
T
lox
Barnas
e
Destruction Box
lox
DEAT
H