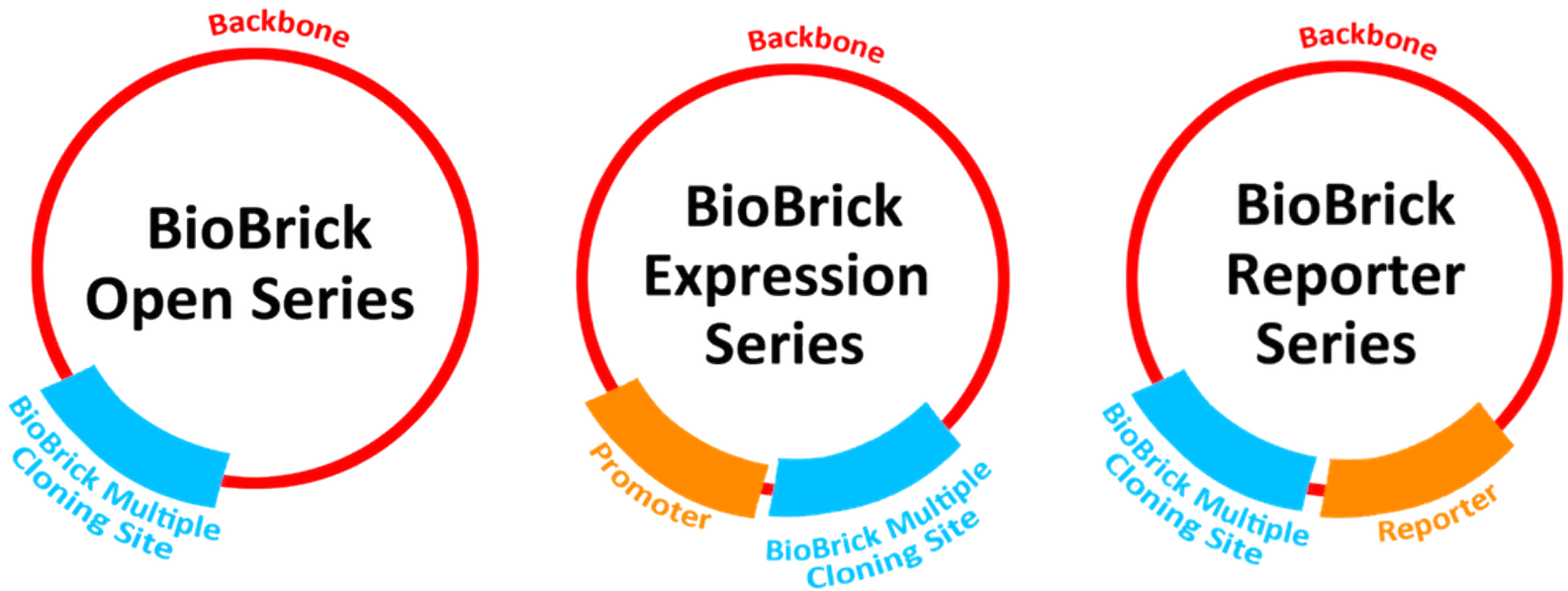
Team Vector

We now have 6 biobrick vectors confirmed by sequencing

- 2 x Open Series (V7,V8) - Biobrick multiple cloning site
- 2 x Expression Series (V9,V10) - Biobrick mcs + promoter
- 2 x Reporter Series - Biobrick mcs (V11,V12) + reporter

Maxiprep of plamid + glycerol stock of E. coli



Team Vector

Attempted to transform vector V10 into agrobacterium

- Electroporated to insert plasmid into agrobacterium
- Grew on LB + Rif + Gent + Kan plates
- Colonies on V10 plate but not on negative controls - success!

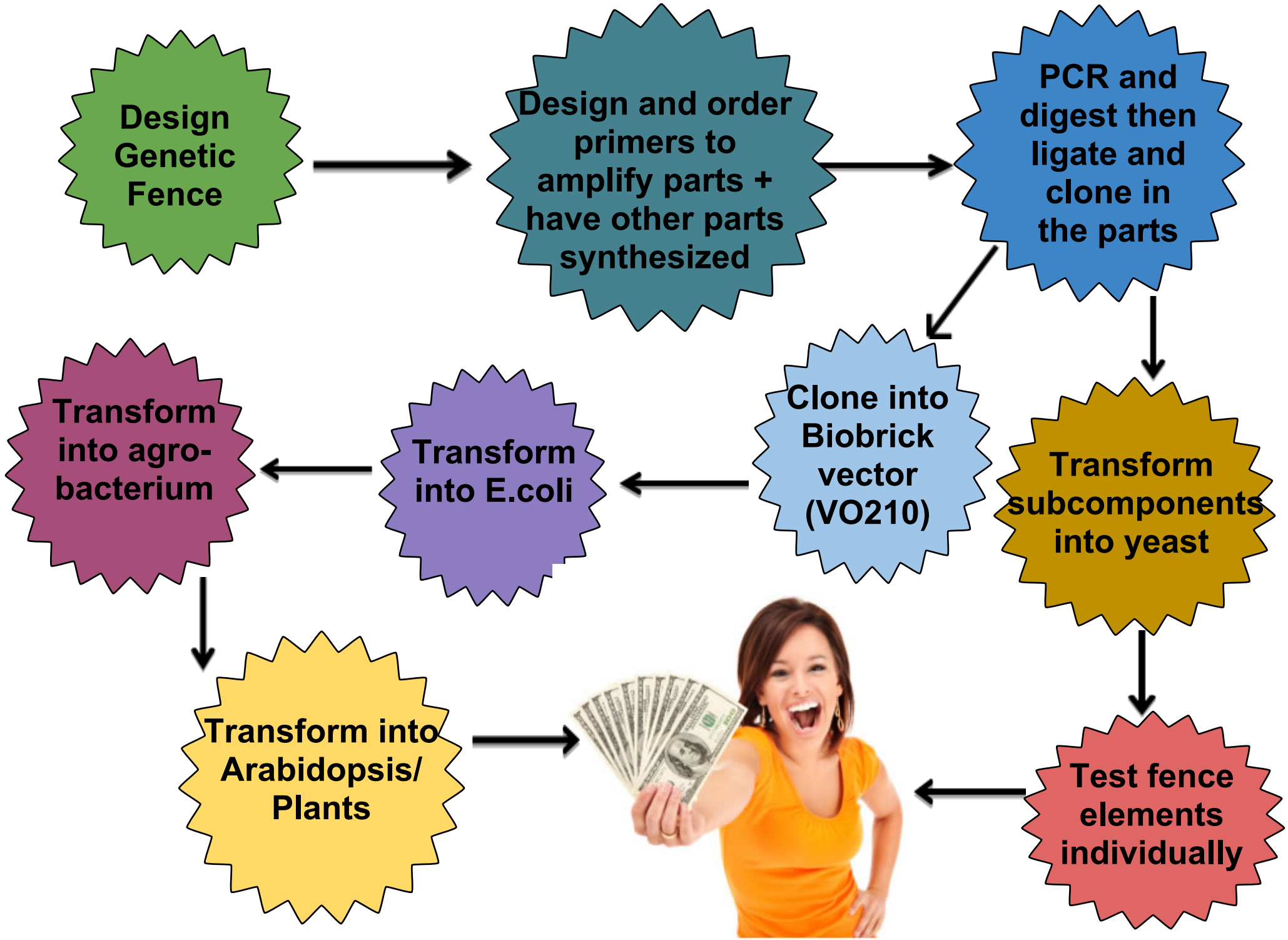
Ready to transform into plants- successful transformation would give Kan resistant seeds

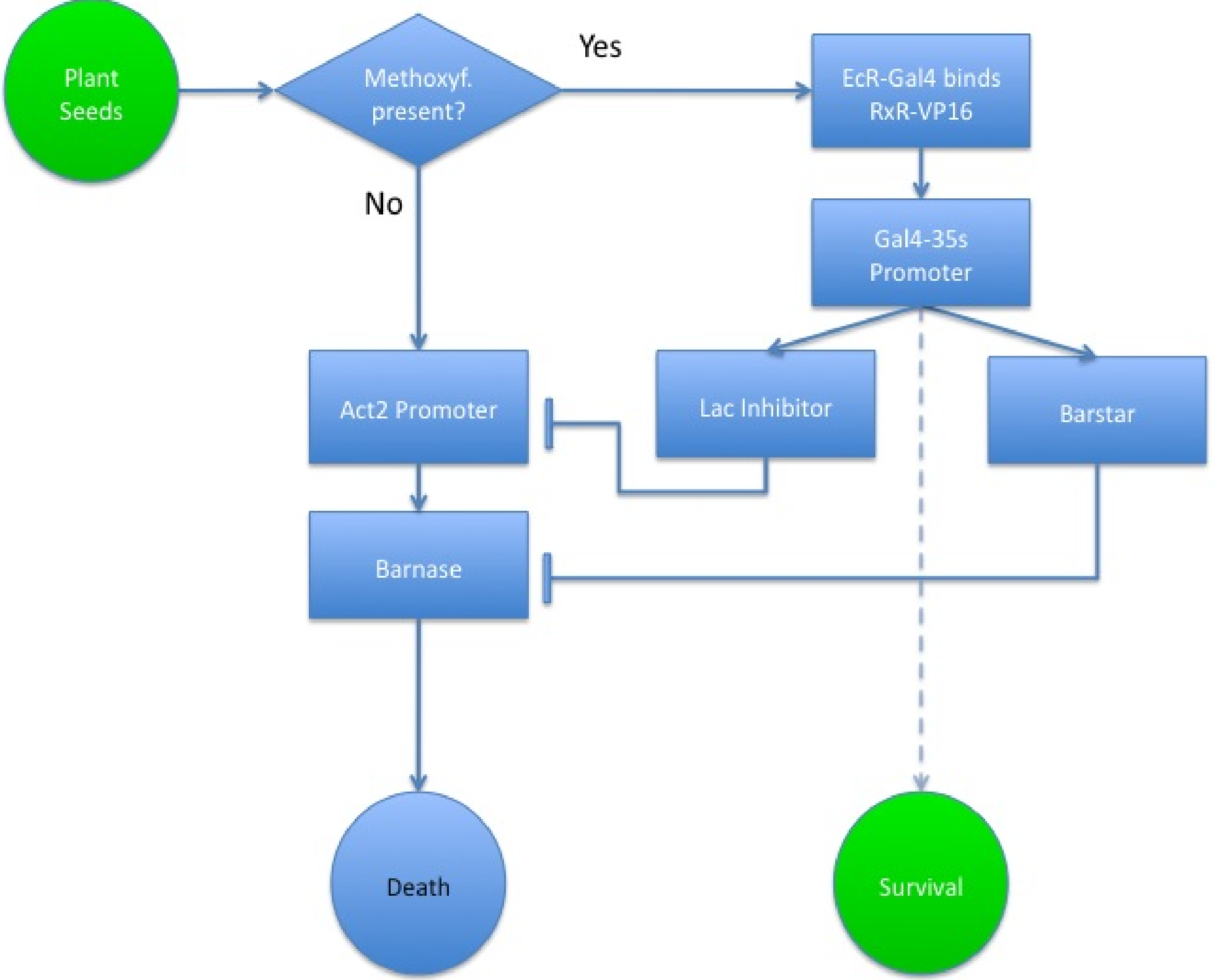
Team Vector

Plants

- Fungal outbreak in incubator!
- Some still alive and might be possible to save them
- Check back in next few days - some possibly ready to try transformation


Team Fence


Design Genetic Fence
Design and order primers to amplify parts + have other parts synthesized
PCR and digest then ligate and clone in the parts
Clone into Biobrick vector (VO210)
Transform subcomponents into yeast
Transform into E.coli
Transform into agro-bacterium
Transform into Arabidopsis/ Plants
Test fence elements individually


Plant Seeds
Methoxyf. present?
Yes
No
EcR-Gal4 binds RxR-VP16
Gal4-35s Promoter
Act2 Promoter
Lac Inhibitor
Barstar
Barnase
Death
Survival

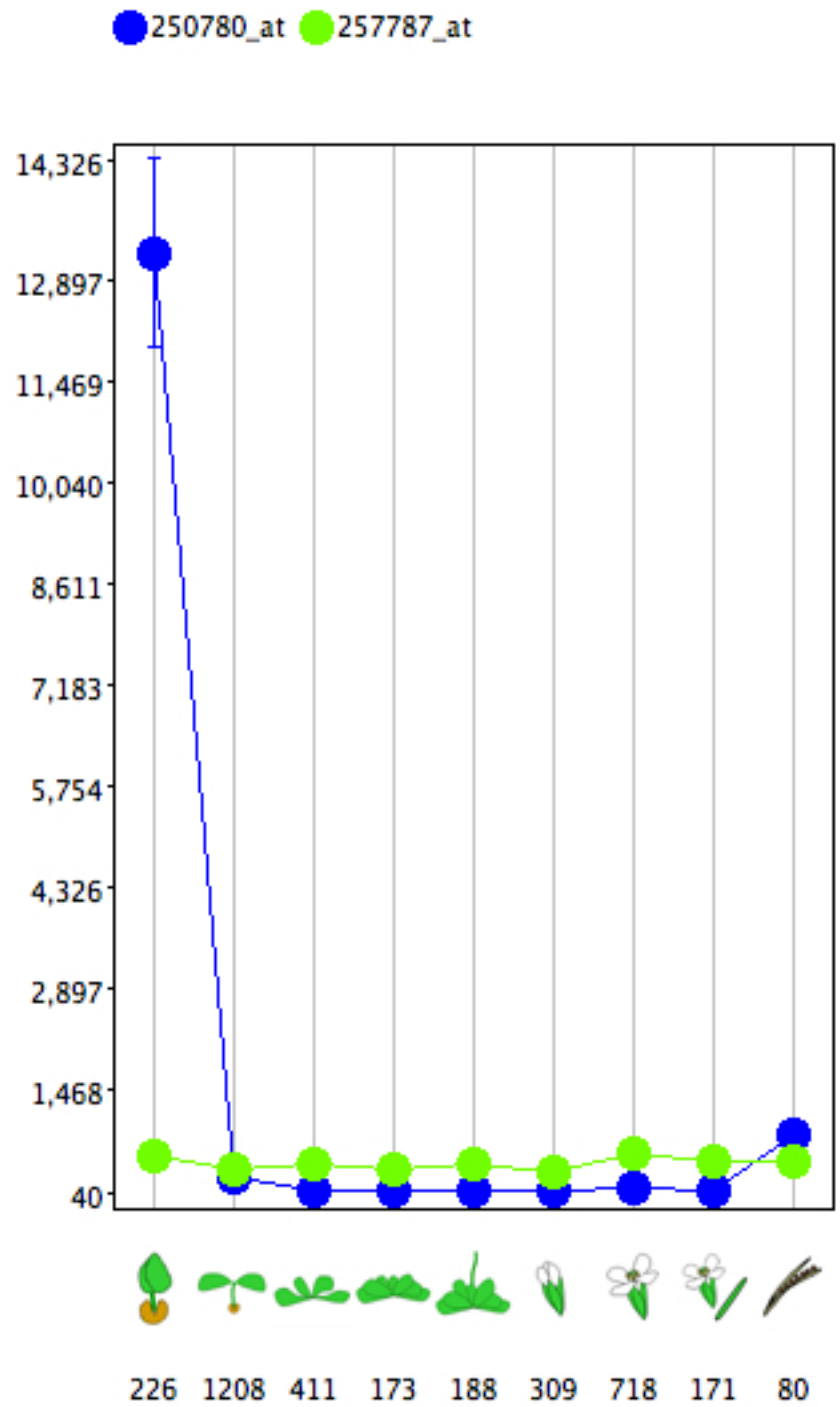
Things Accomplished

- finalized design of alternate genetic fence and designed and ordered primers for arabidopsis promoters
- B21 backbone isolation and purification
- ligation and cloning of NLS, Barstar and LacIN
- troubleshooting and perfection of Barnase + LacIN PCRs
- ligation of Barnase, GAL4 and LacIN (again)
- LVA degradation tail

Arabidopsis Promoters

Blue: Expansin At-EXP2
expressing Barnase

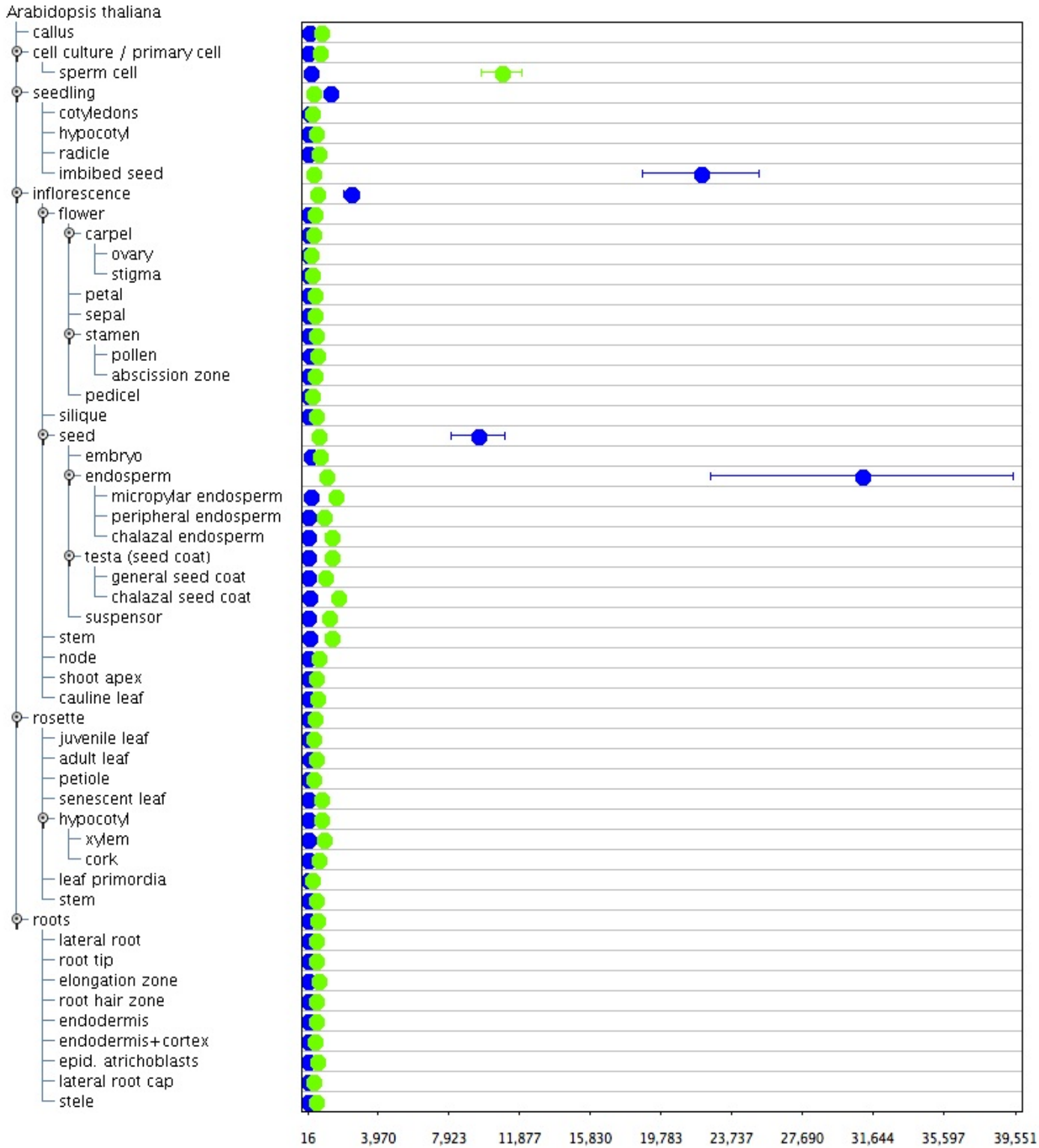
Green: At-ARP2
expressing Barstar



Anatomy Expression Map

Blue: Expansin At-EXP2 expressing Barnase

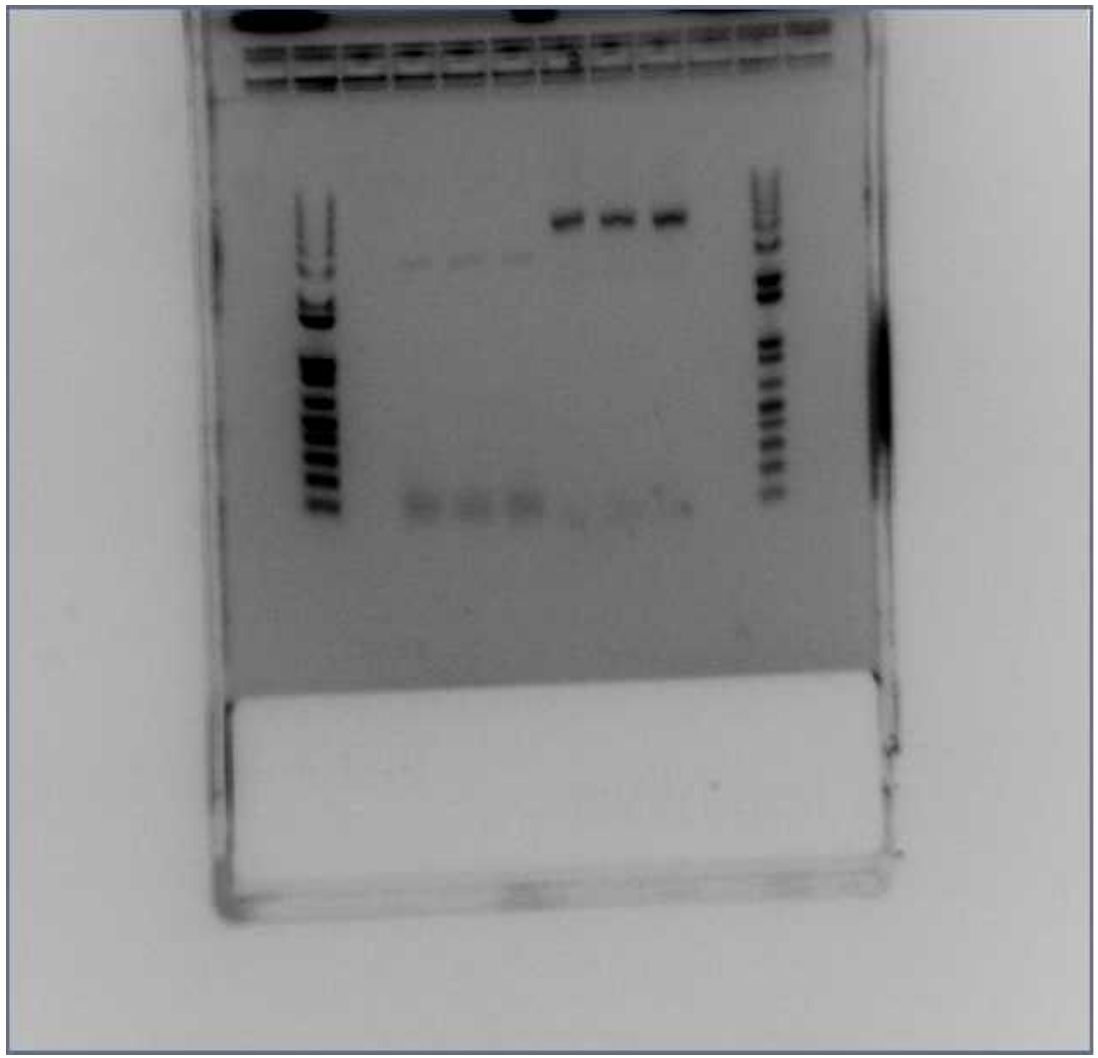
Green: At-ARP2 expressing Barstar



Preparing for Ligation

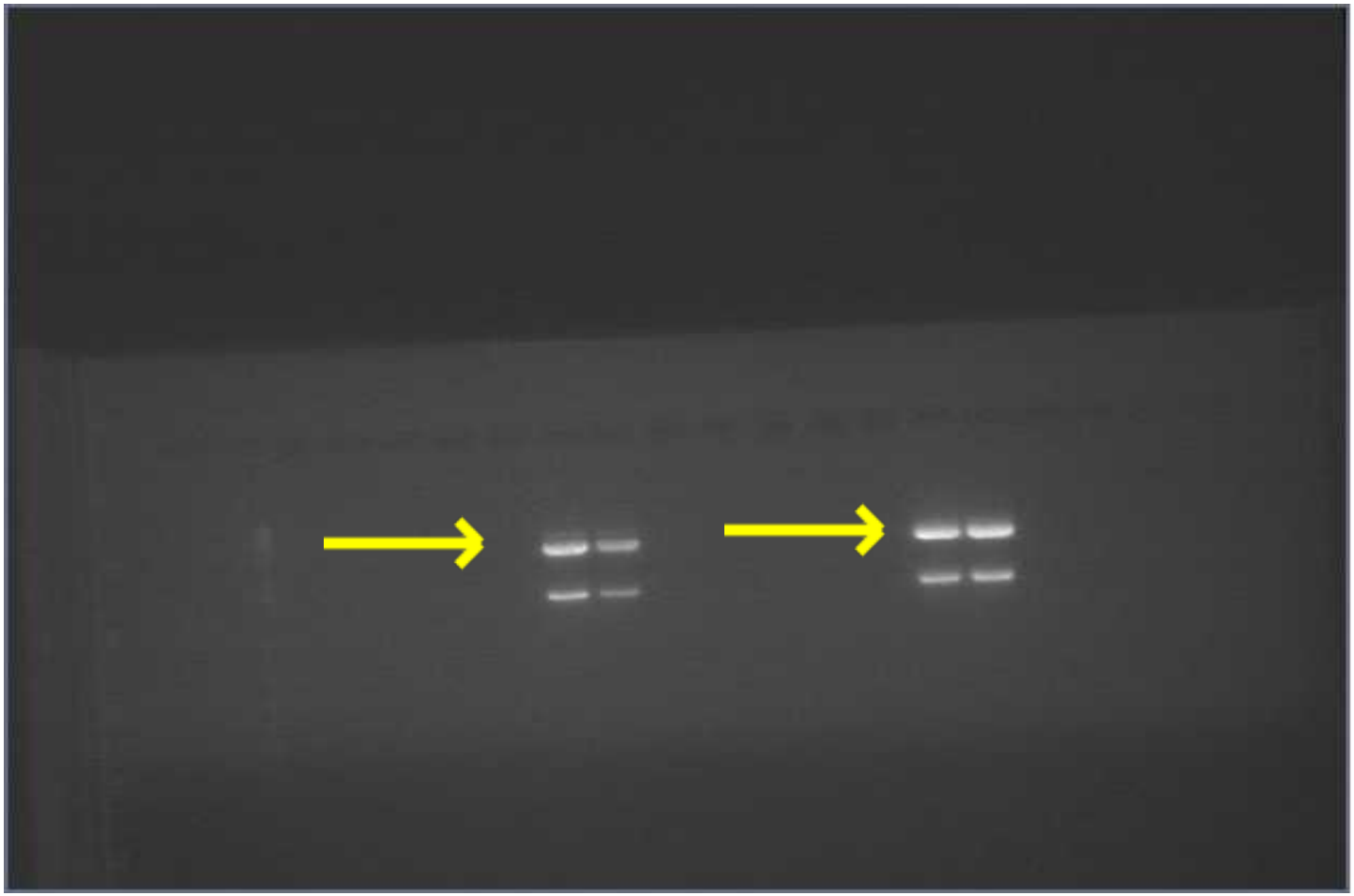
- Maxipreped and digested B21 vector (V0120 with YFP insert) with Xba1 and Pst1

- PCR of Barnase and Gal4DBD (again)
  - Lowered annealing temp to 50 from 56 degrees
  - Unsuccessful (again)



Preparing for Ligation- cont.

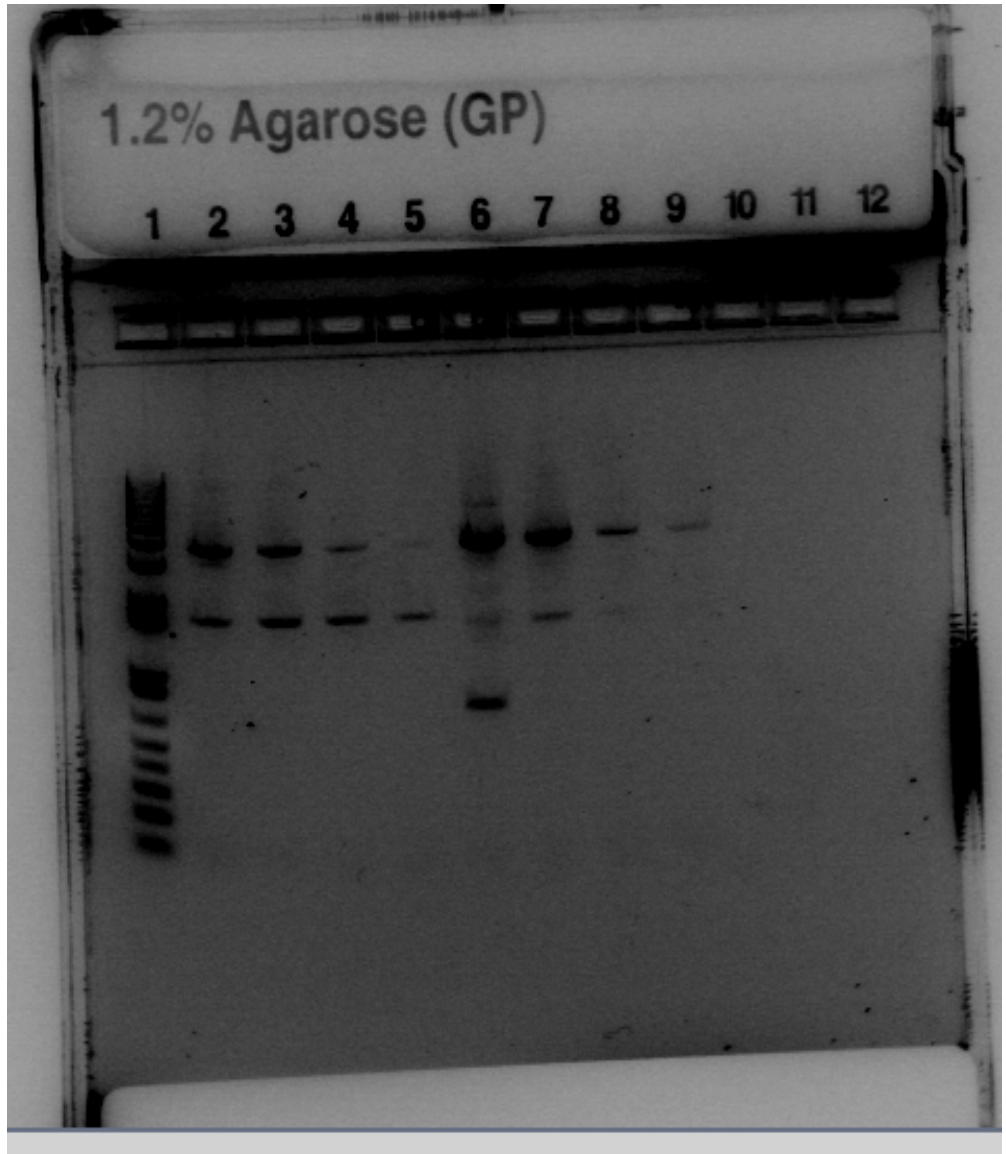
- Gel separated and purified backbone



Further Ligation Preparation

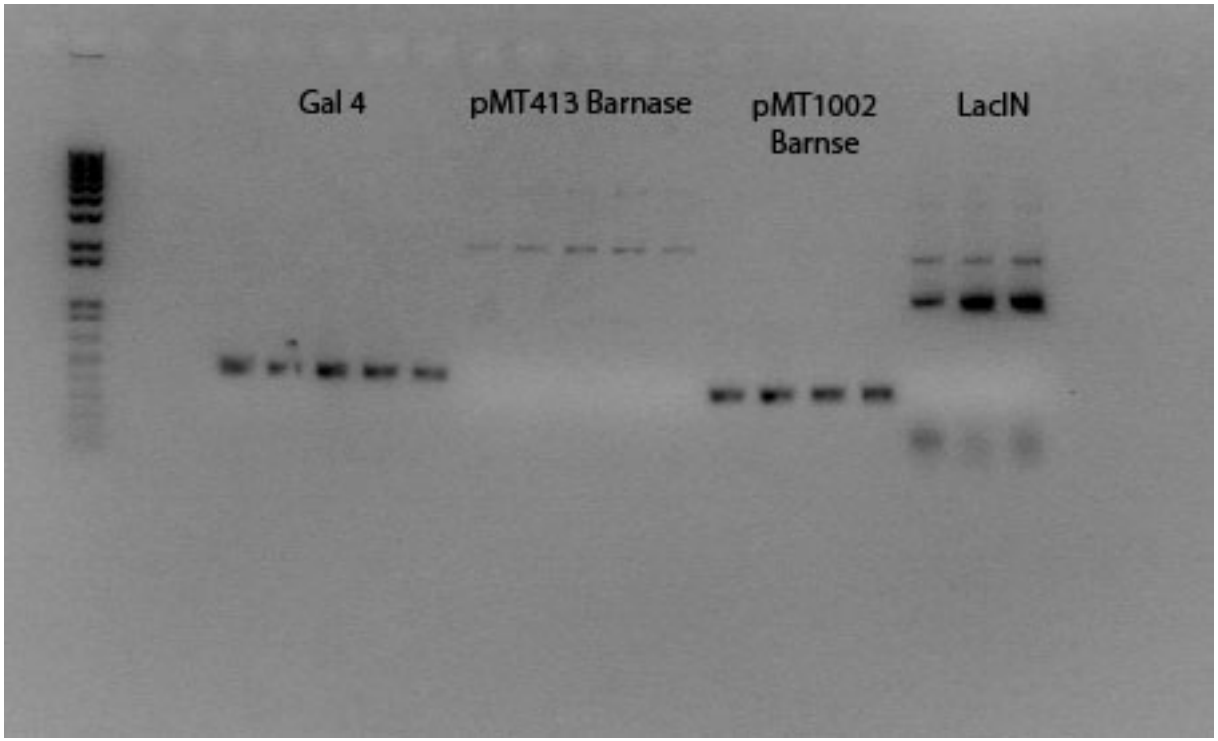
- Barstar PCR was successful from previously, we performed PCR cleanup

- Minipreped pMT1002 (containing Barnase and Barstar) to attempt to amplify Barnase again
  - 3 failed Barnase PCR attempts
  - One of the primers was redesigned, at which point PCR worked

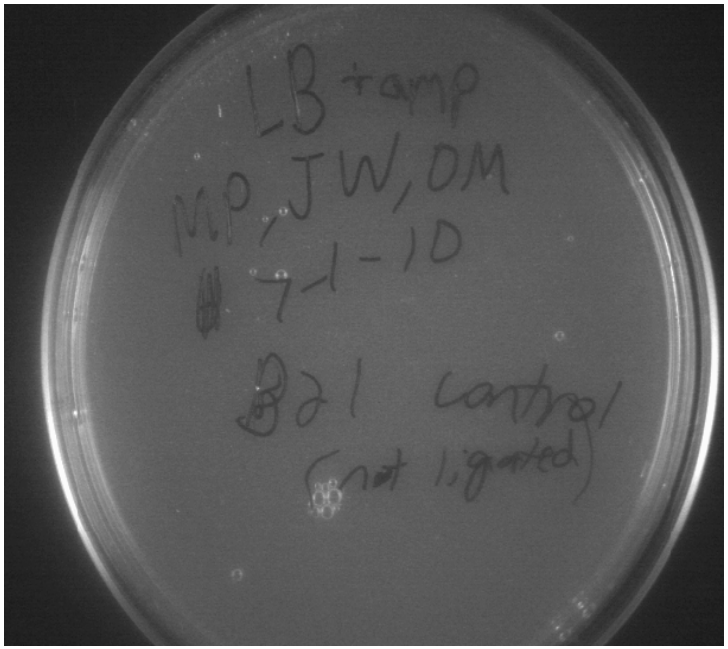


Success!

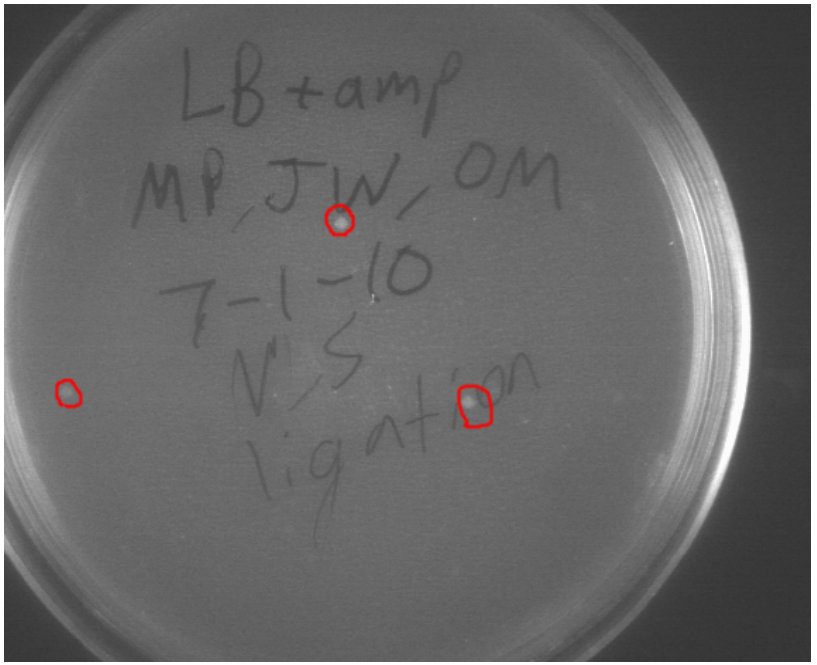
- PCR products from Gal4, pMT1002 Barnase, and LacIN

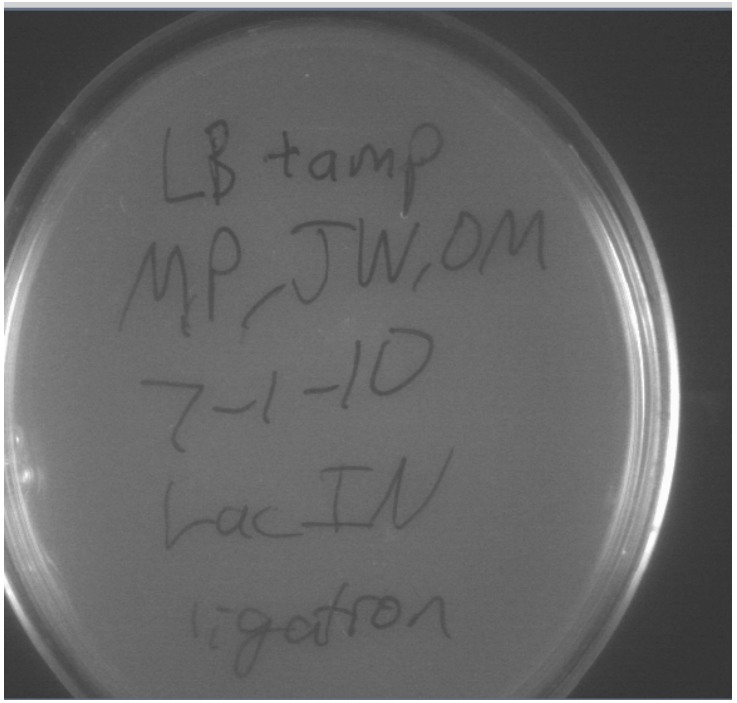


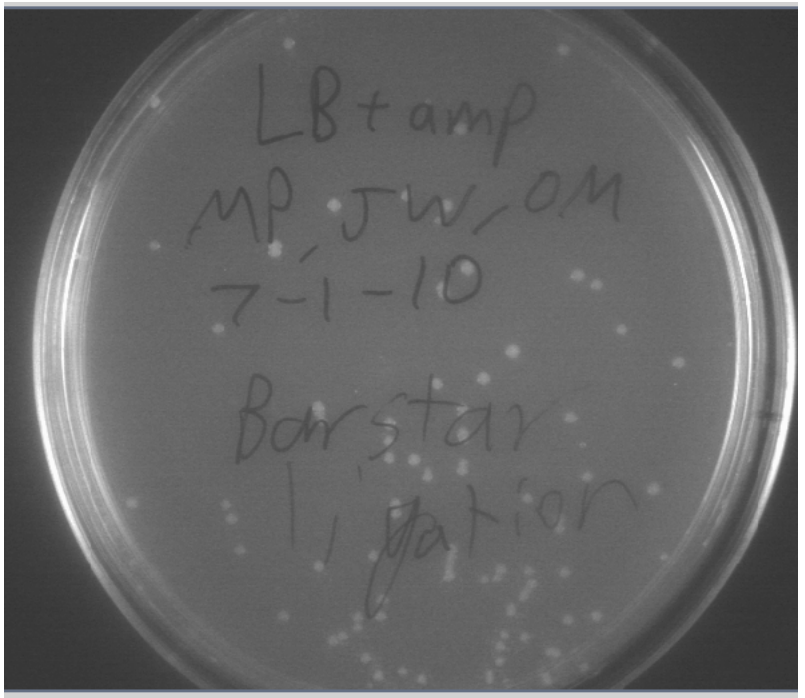
Ligations



- Barstar successful, NLS with only 3 colonies

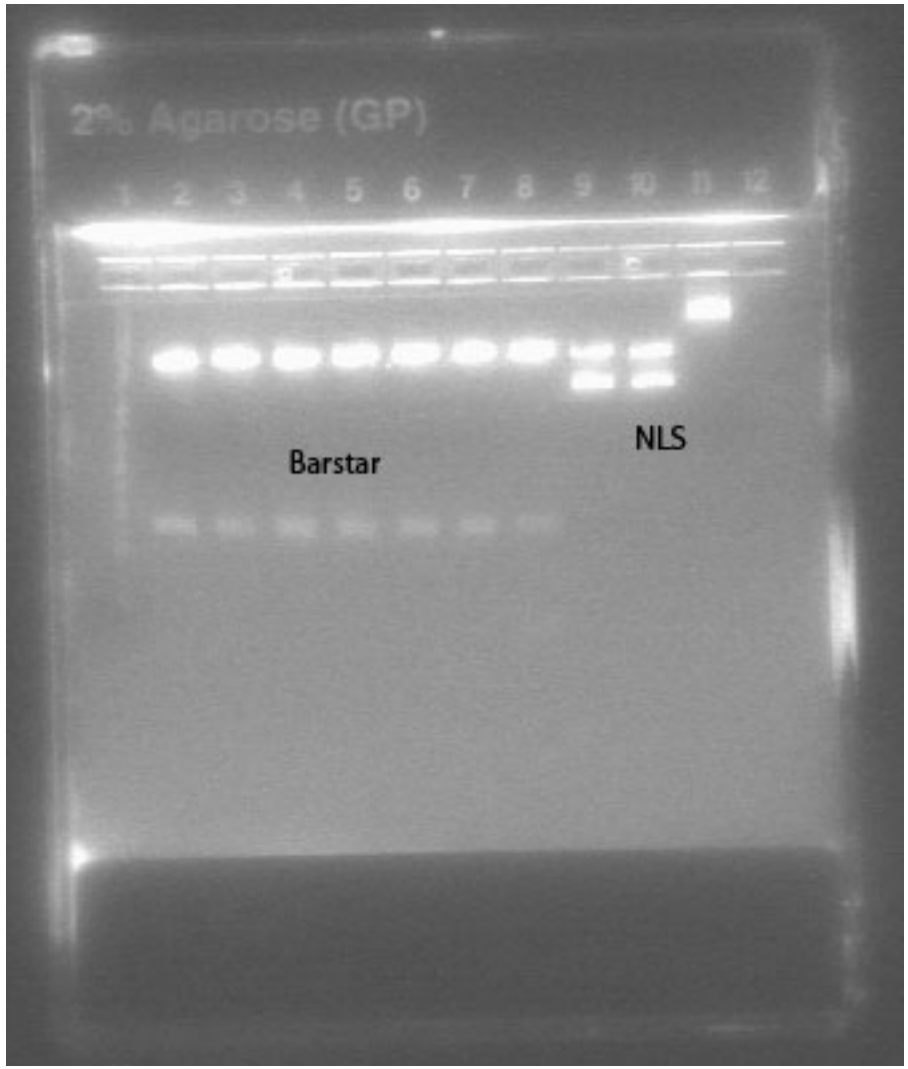






Checking Barstar and NLS

- There is an insert of roughly the correct size in the Barstar ligation plasmids, although because of a problem with the ladder, we can't be sure it is Barstar
- NLS either did not ligate, or ran off the gel
- Barstar submitted for sequencing

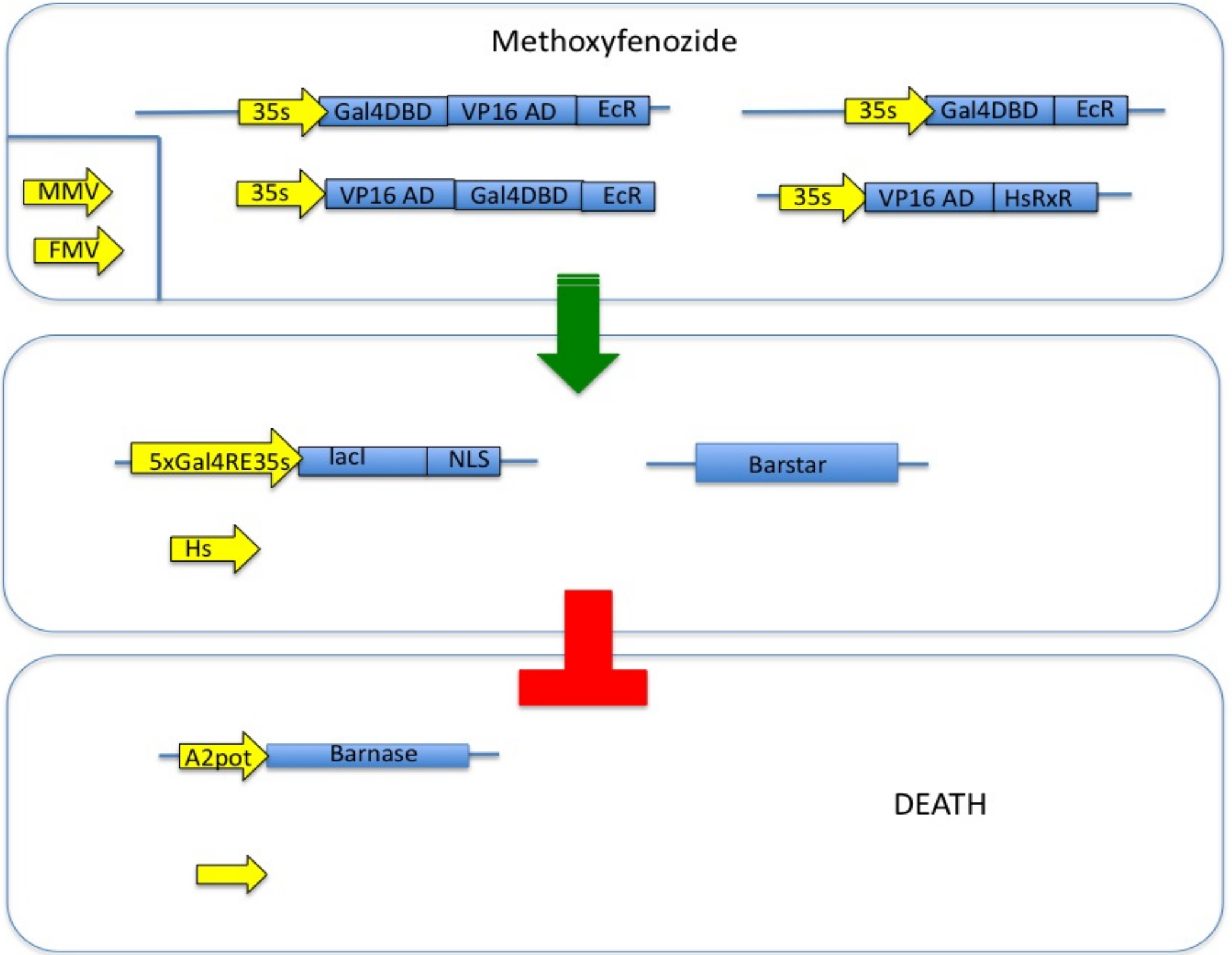


More Ligations

- Performed ligations of Barnase, LacIN, and Gal4
- The resultant plasmids are minipreped but not yet checked

Parts Ordered for Synthesis

- Ecdyzone Receptor (EcR)
- Retinoic Acid Receptor (RxR)
- Act2 promoter with LacO sites
- LVA degradation tail

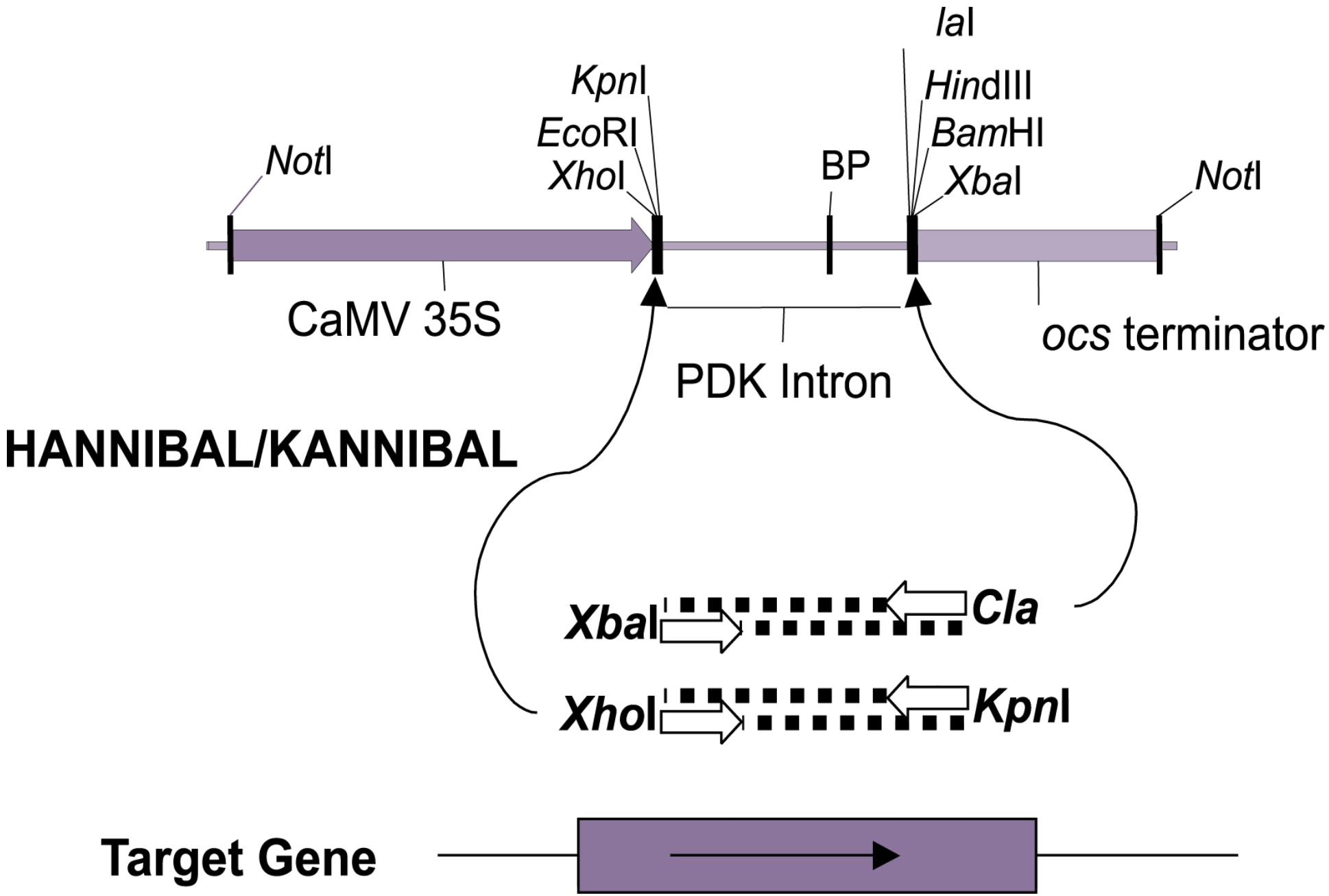

Methoxyfenozide
35s
Gal4DBD
VP16 AD
EcR
35s
Gal4DBD
EcR
MMV
35s
VP16 AD
Gal4DBD
EcR
35s
VP16 AD
HsRxR
FMV
5xGal4RE35s
lacI
NLS
Barstar
Hs
A2pot
Barnase
DEATH

Team Allergy

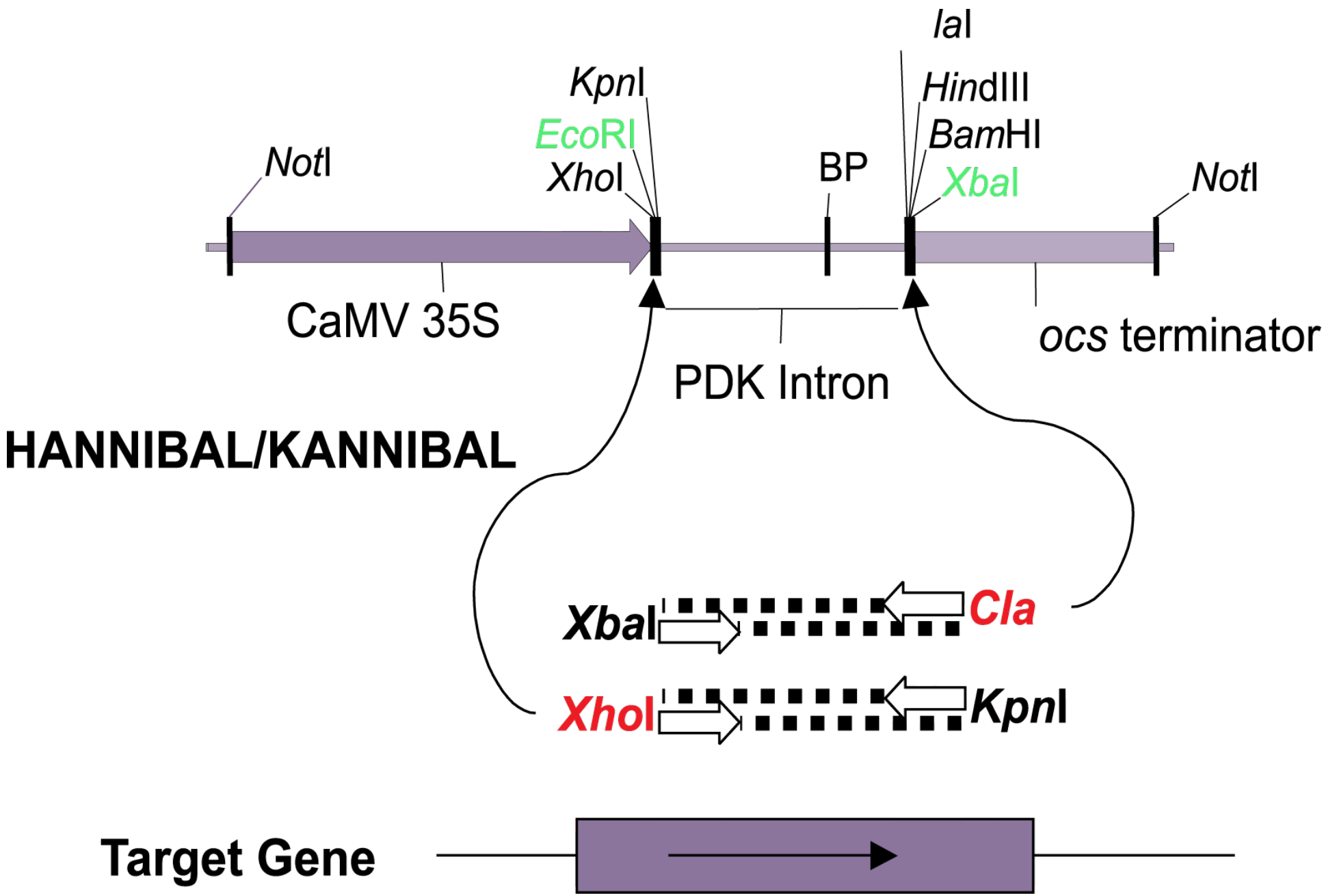
Harnessing the adaptability of biobricks and the power of RNA interference…

Giving you the power to be **allergy free!**

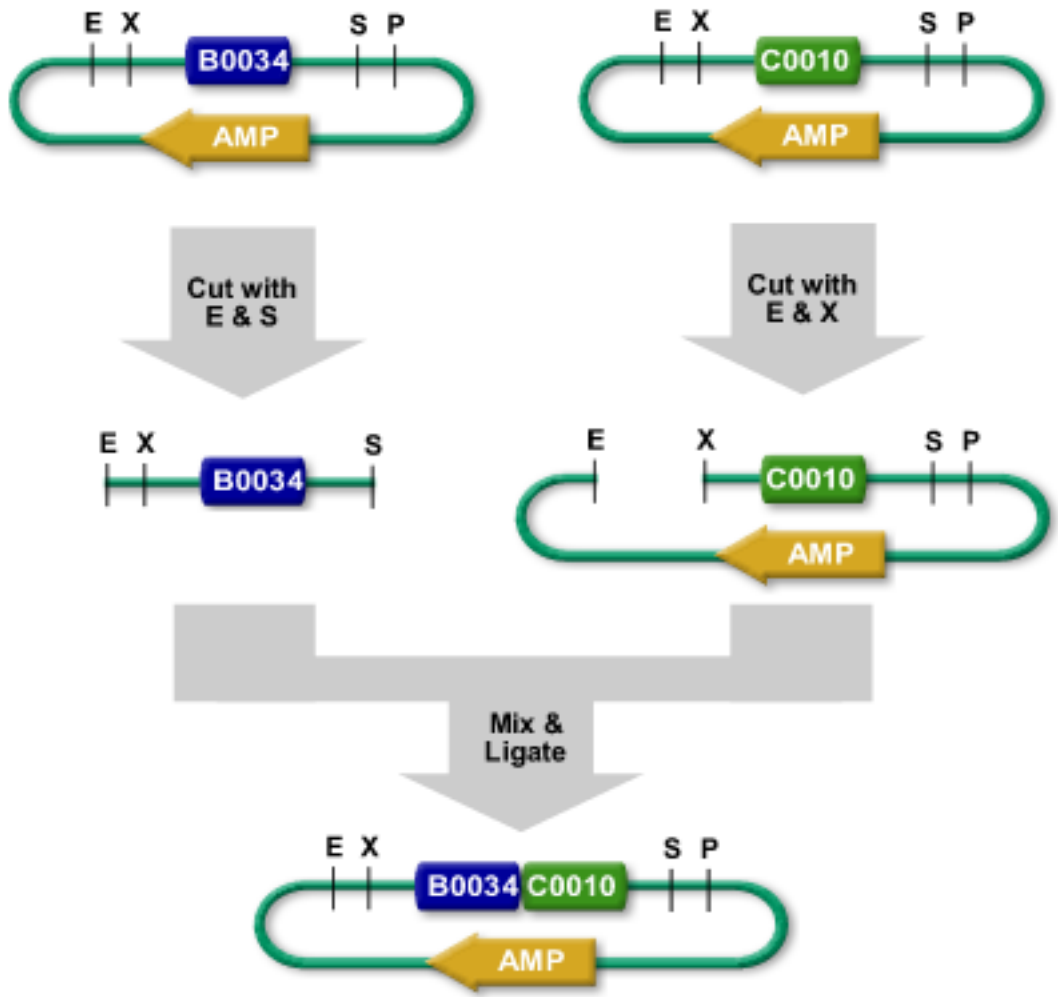
Clarifications: hpRNA



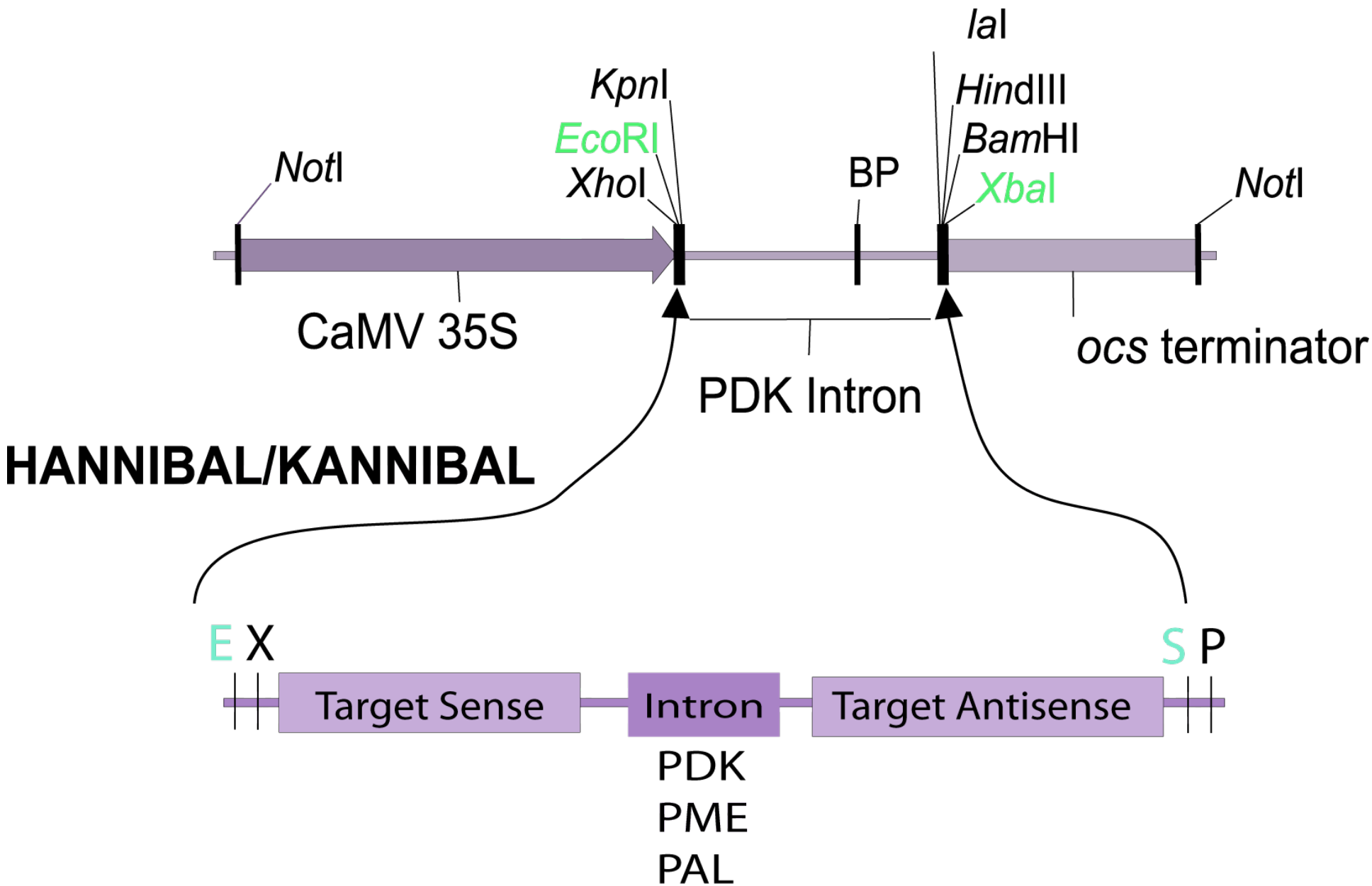
Clarifications: hpRNA



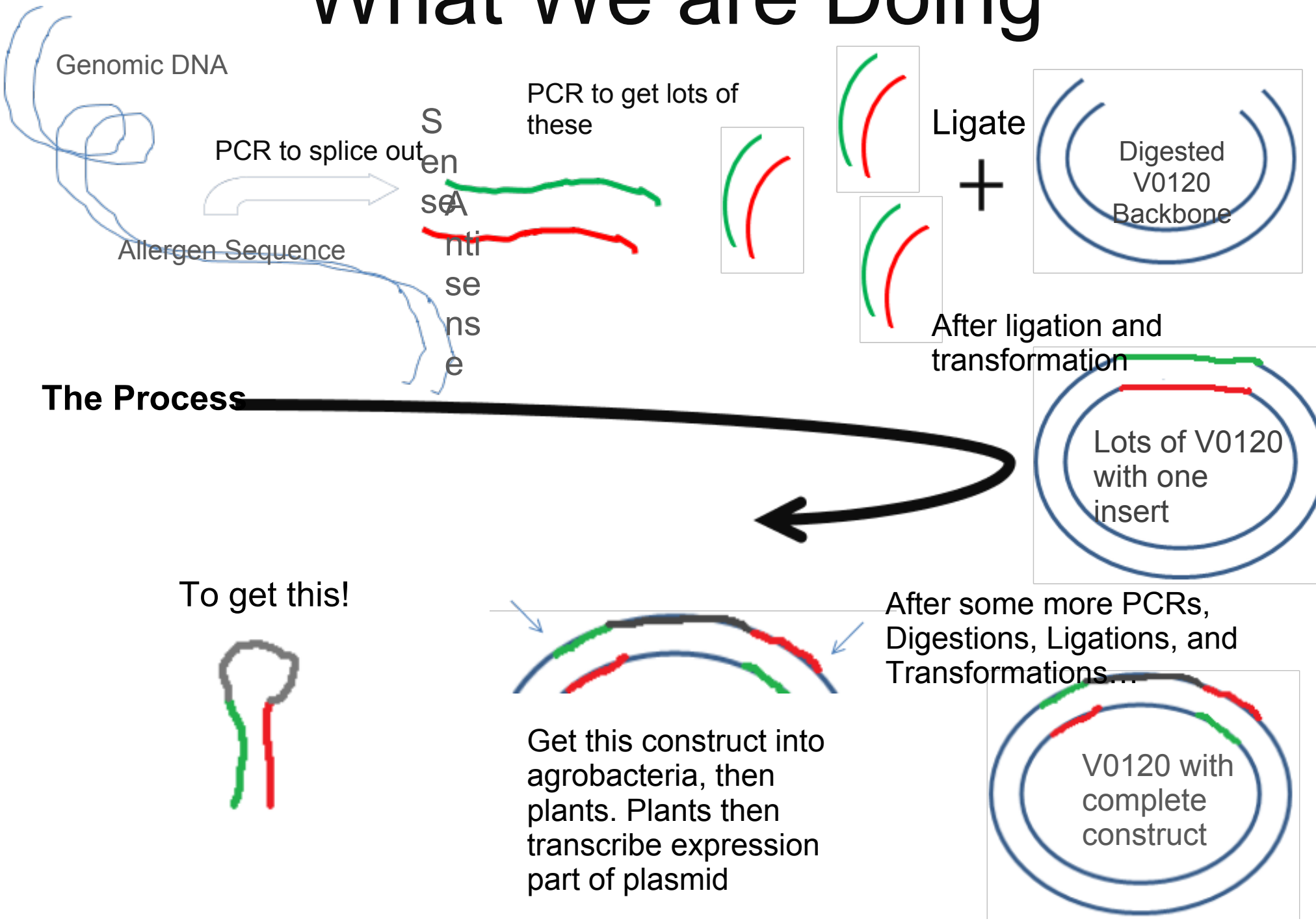
Clarifications: hpRNA Plan



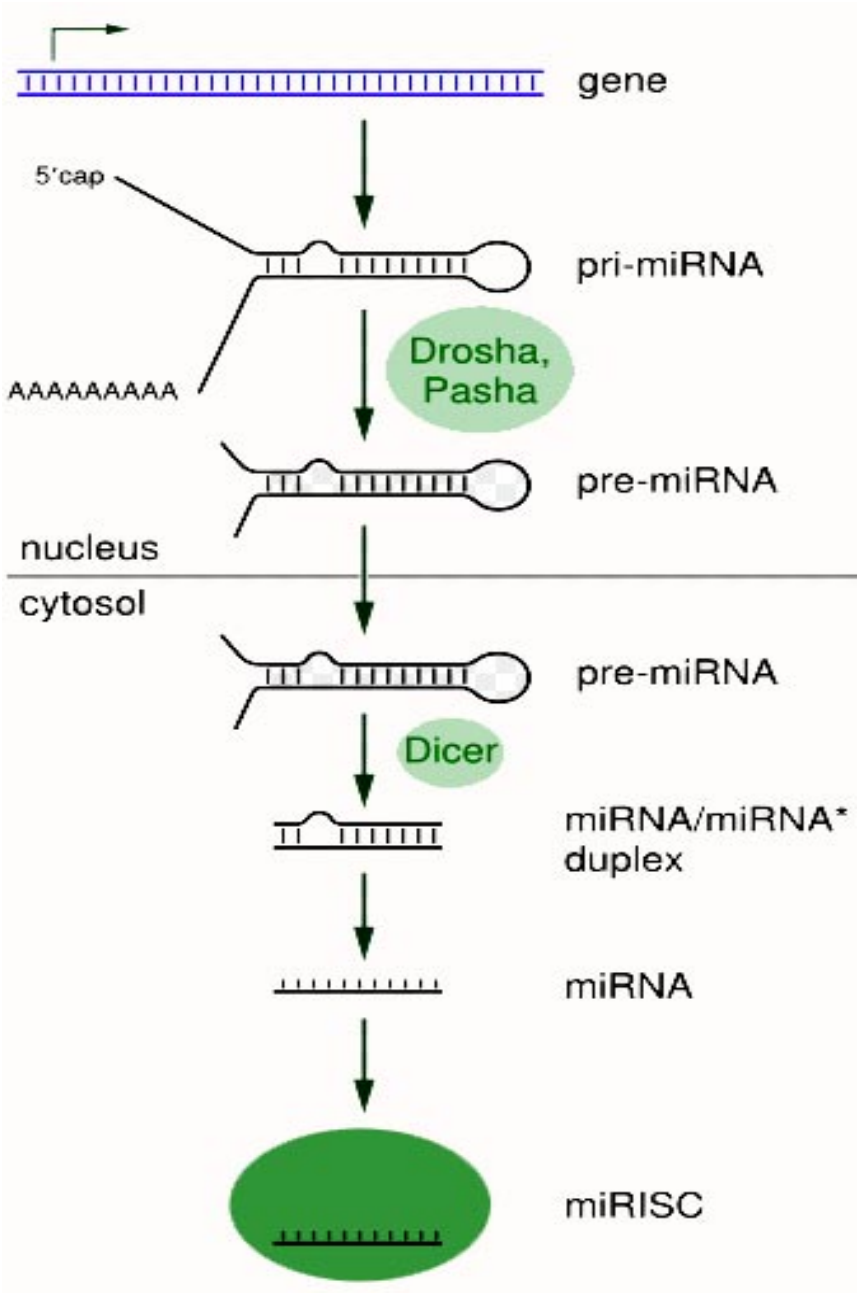
Clarifications: hpRNA Plan



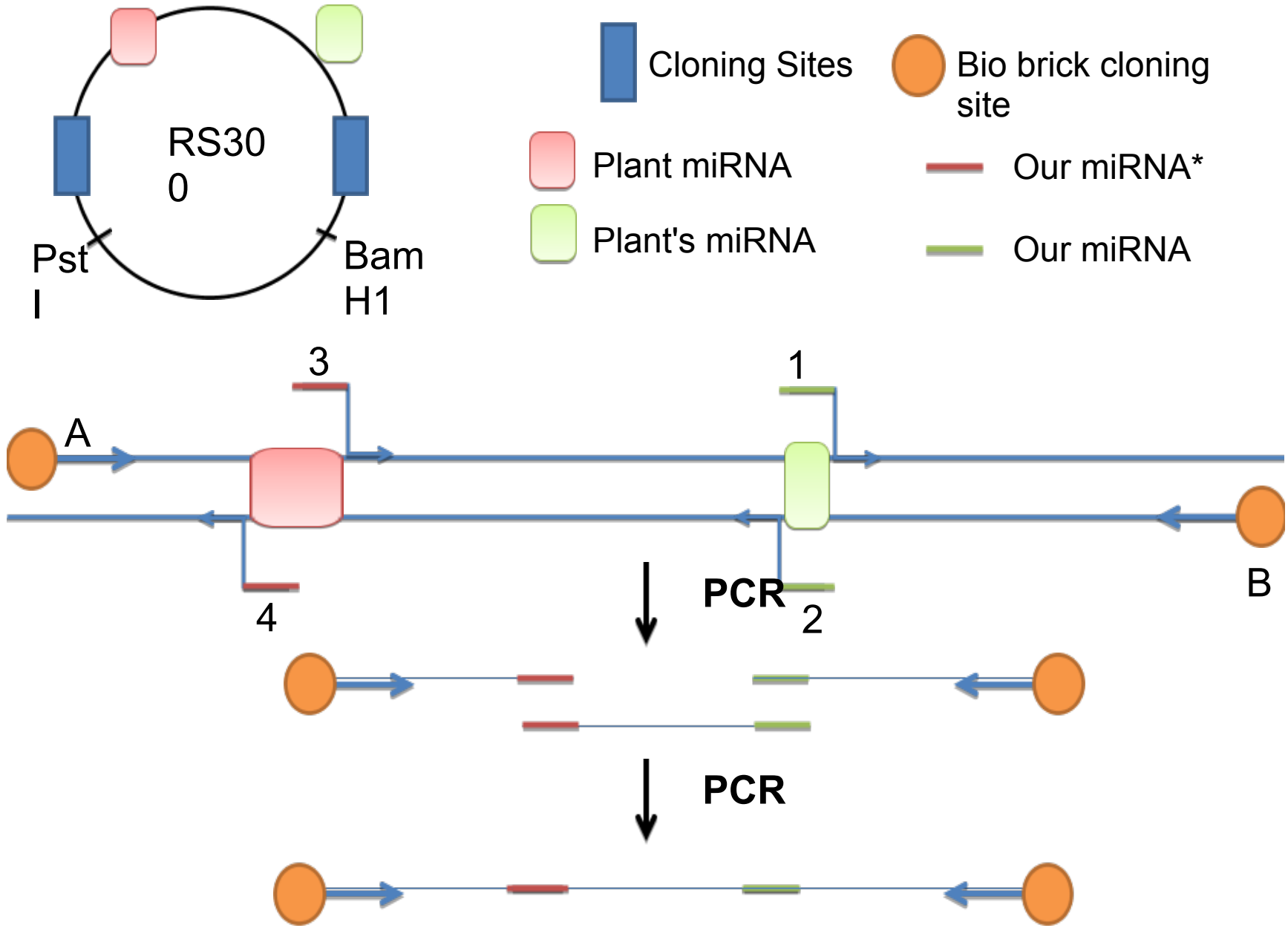
What We are Doing



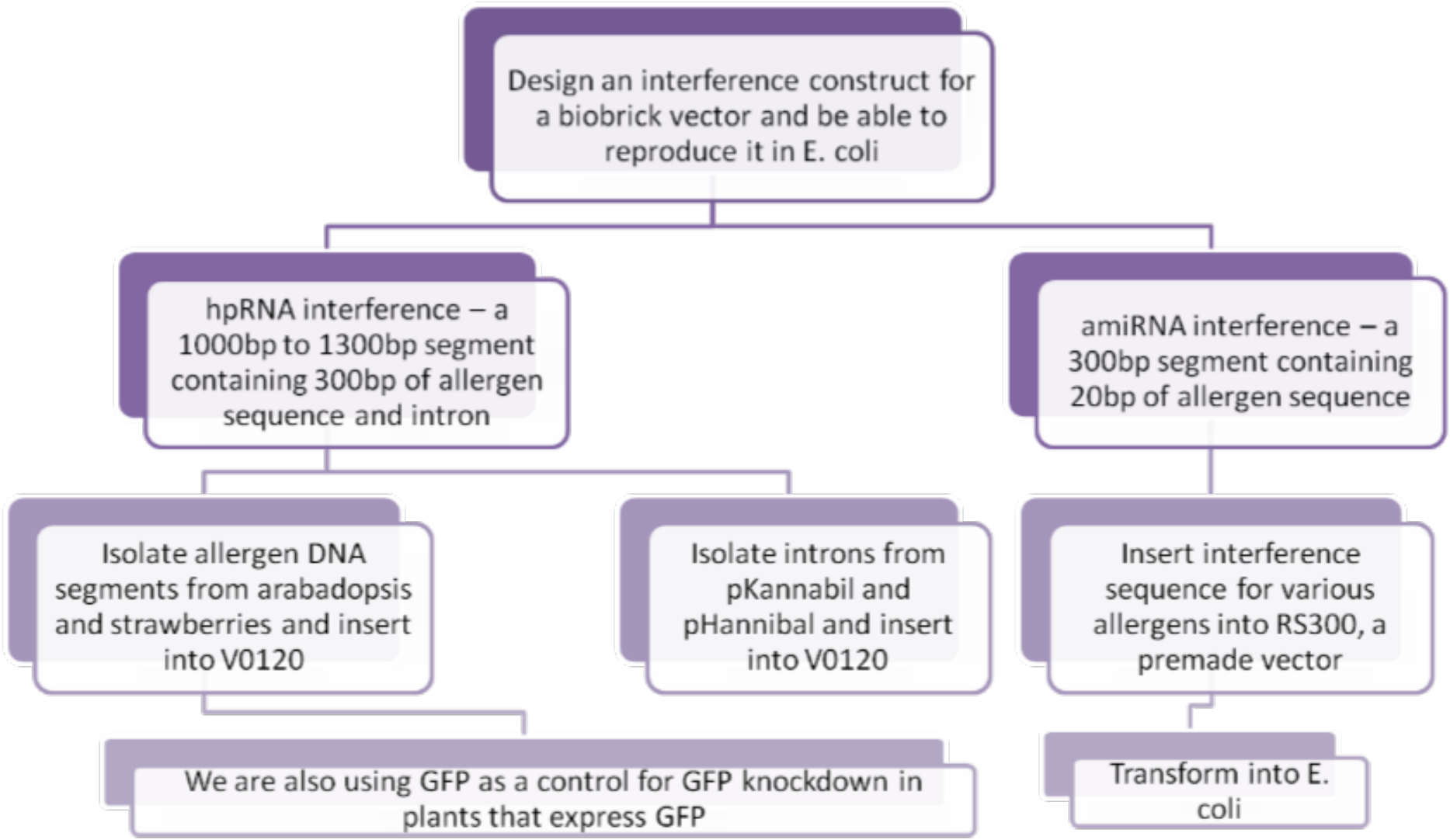
Clarifications: amiRNA



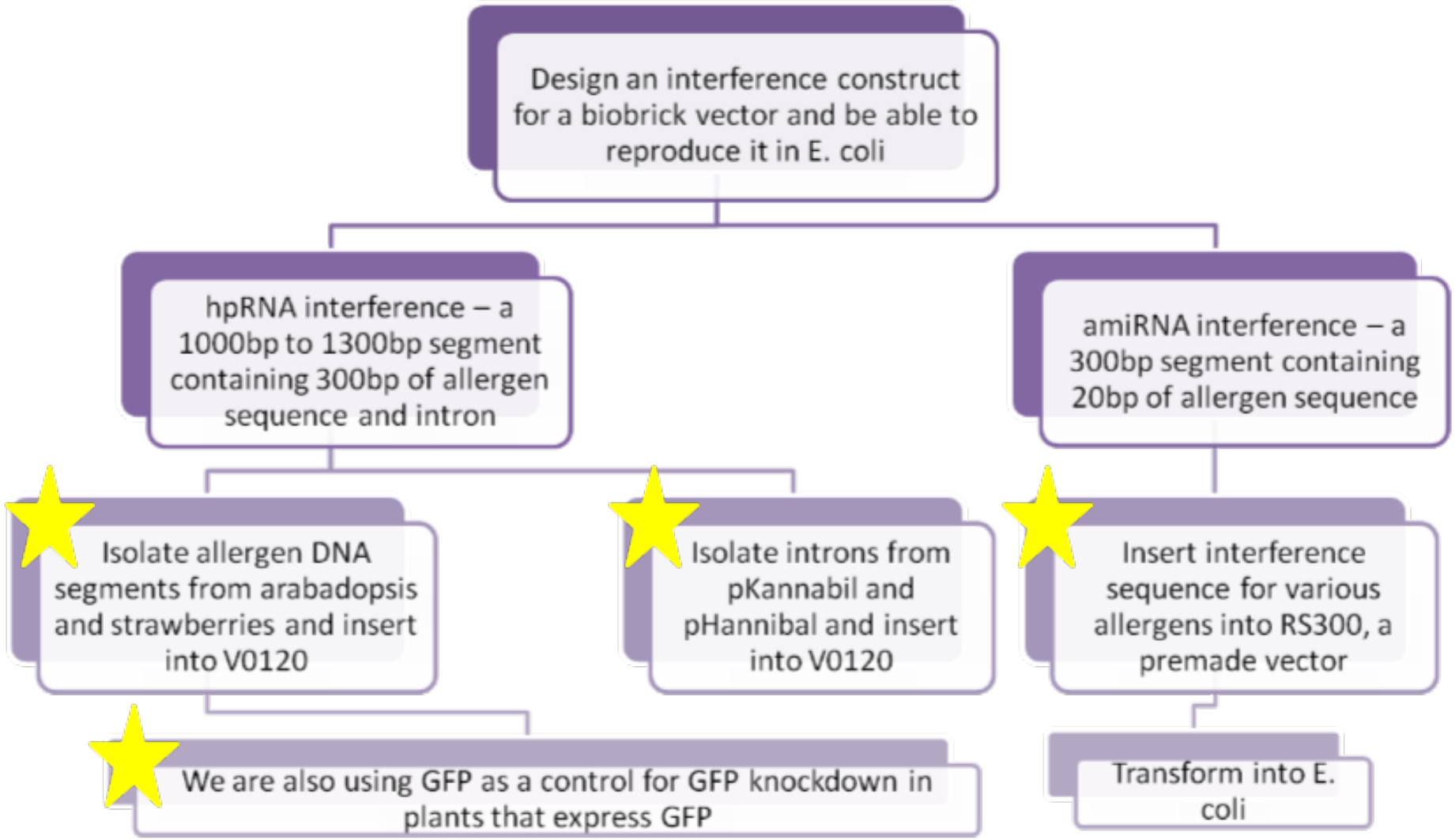
Clarifications: amiRNA



Team Allergy Short Term Goals



Where we are


Design an interference construct for a biobrick vector and be able to reproduce it in E. coli
hpRNA interference – a 1000bp to 1300bp segment containing 300bp of allergen sequence and intron
amiRNA interference – a 300bp segment containing 20bp of allergen sequence
Isolate allergen DNA segments from arabadopsis and strawberries and insert into V0120
Isolate introns from pKannabil and pHannibal and insert into V0120
Insert interference sequence for various allergens into RS300, a premade vector
We are also using GFP as a control for GFP knockdown in plants that express GFP
Transform into E. coli

Team Allergy Long Term Goals

hpRNA

- Combine intron, sense, and antisense parts for hpRNA together on one plasmid and reproducing using E. coli
- Transforming into agrobacteria

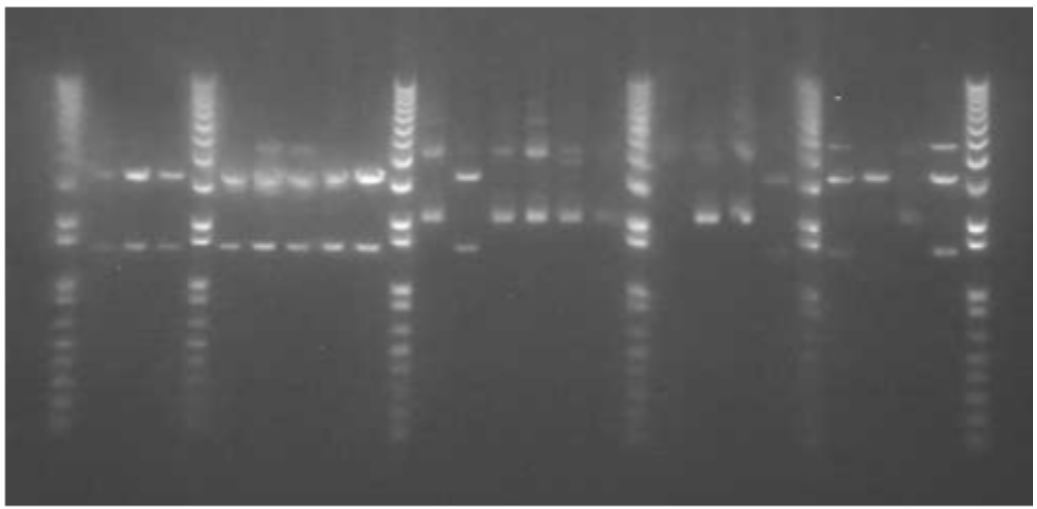
amiRNA

- Take desired sequence from E. coli and transform into agrobacteria

Both

- Introduce to plants and observe knockdown

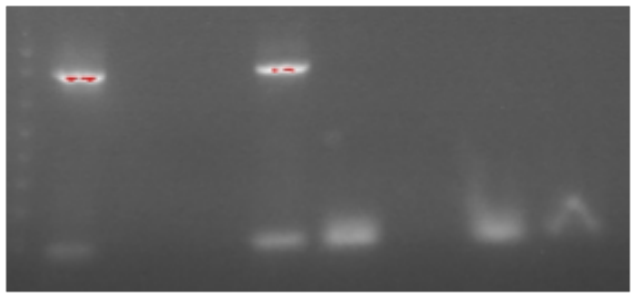
- amiRNA
  - Sent constructs for our allergens (Bet, LTP) and for (GFP) to sequencing
- ihpRNA
  - Sent some constructs for allergen parts (LTP sense, Betv1 sense, and Ger sense)
  - Re-picked colonies for the other allergen parts (LTP antisense, Betv1 antisense, Ger antisense)



Ladder; 2-4 (LTP A); 6-10 Bet v1 A; 12-17 (Betv 1.2 S); 19-22 (Bet v 1.2 A); 24-27 (GerA)

pHannibal/Kannibal; PAL/PME

- pHannibal/Kannibal arrived
  - Able to isolate pdk intron



- Our plates from transformations of PAL and PME had ~ the same number of colonies as our negative control
  - Waiting on PAL sequencing results
  - Unable to grow up colonies of PME
- Plant DNA extraction kit arrived
  - Hoping to use this to redo PCR for PAL/PME & Allergen parts

Progress/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

Sequence

Transform into Arabidopsis/ Respective Plants

Observe Allergen Knockdown


LooM Flavor

Main Goals

- Create BioBrick Parts: Ongoing
  - pORE vector parts being sequenced
- Extract Valencene from Oranges: On Hold
- Express Miraculin, Brazzein: In Progress
  - Express in *E. Coli*: Current Project
    - Tag with *StrepII, YFP-2x*
  - Express in Plants
- Express Wintergreen, Banana Scent Pathways: On Hold
  - Extract pieces of the pathways from BioBrick parts
  - Expressing in *E. Coli*, Plants

Since last meeting...

- Trouble ligating as of last meeting
    - All ligations since have been successful!

1. Plant promoter, terminator, and terminator with stop into v0120 backbone

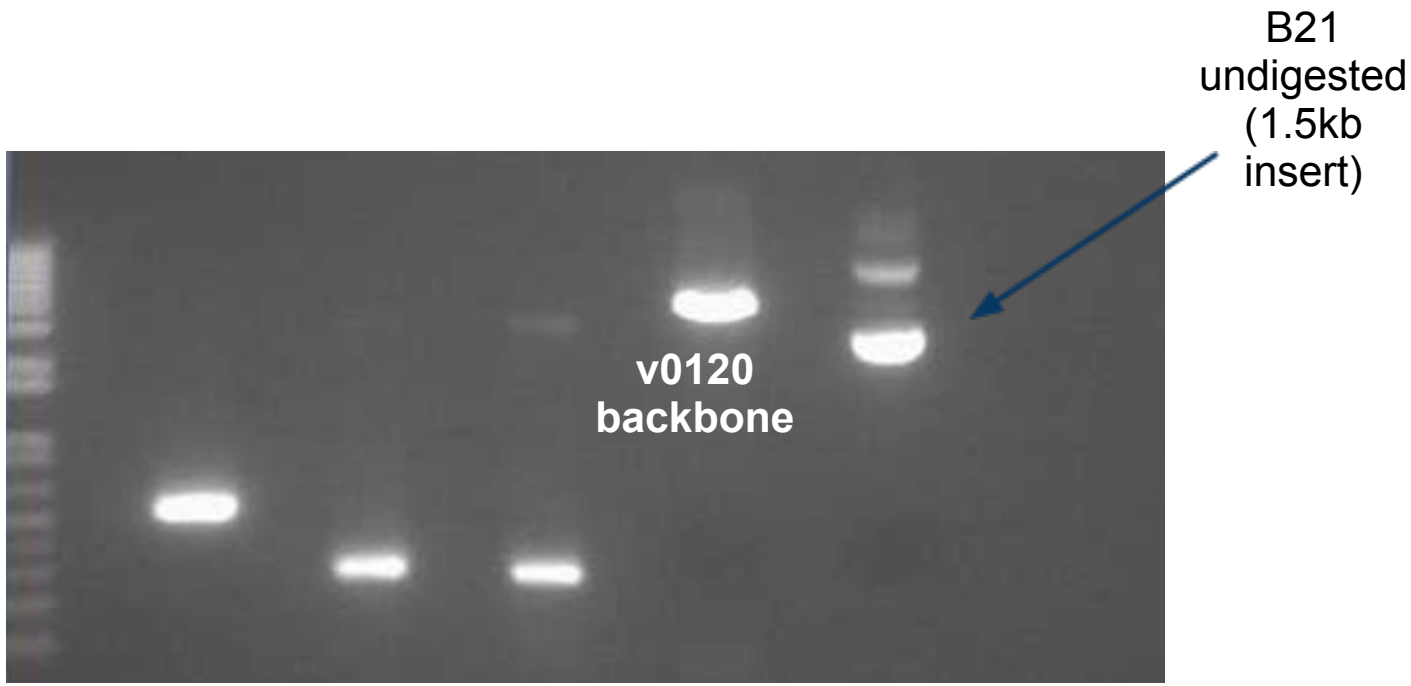
2. Miraculin and Brazzein into v0120 backbone

3. YFP and StrepII tags

4. Valencene

pENTCUP2, NosT, and NosT & Stop

Used slow digest XbaI and PstI



Appears to be successful digestion

pENTCUP2, NosT, and NosT & Stop

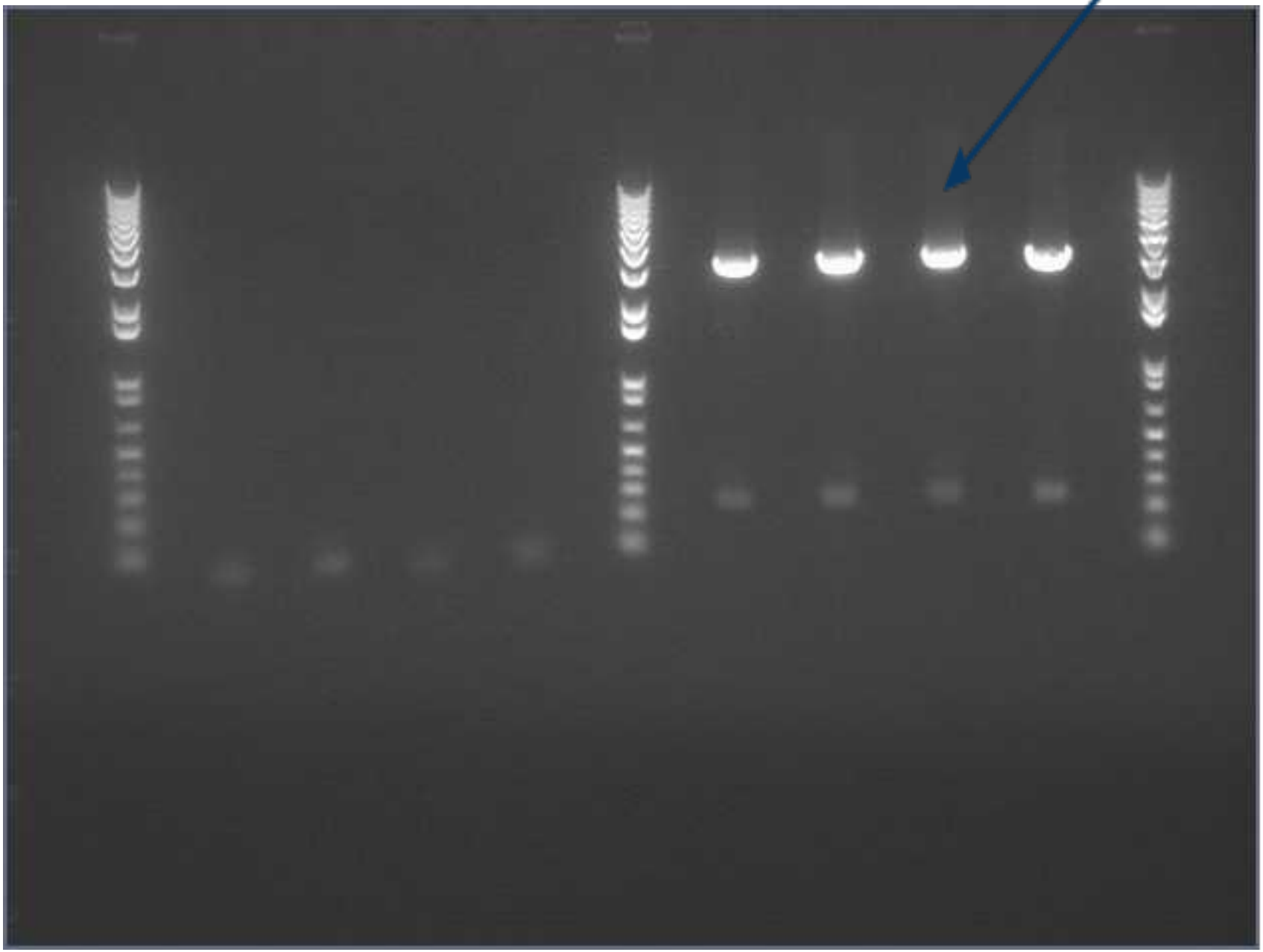
ODs on gel purified DNA

pENTCUP2: 16.5 ng/µL
NOSt: 10.2 ng/µL
NOSt+STOP: 0.4 ng/µL
B15 (backbone vector): 10.9 ng/µL

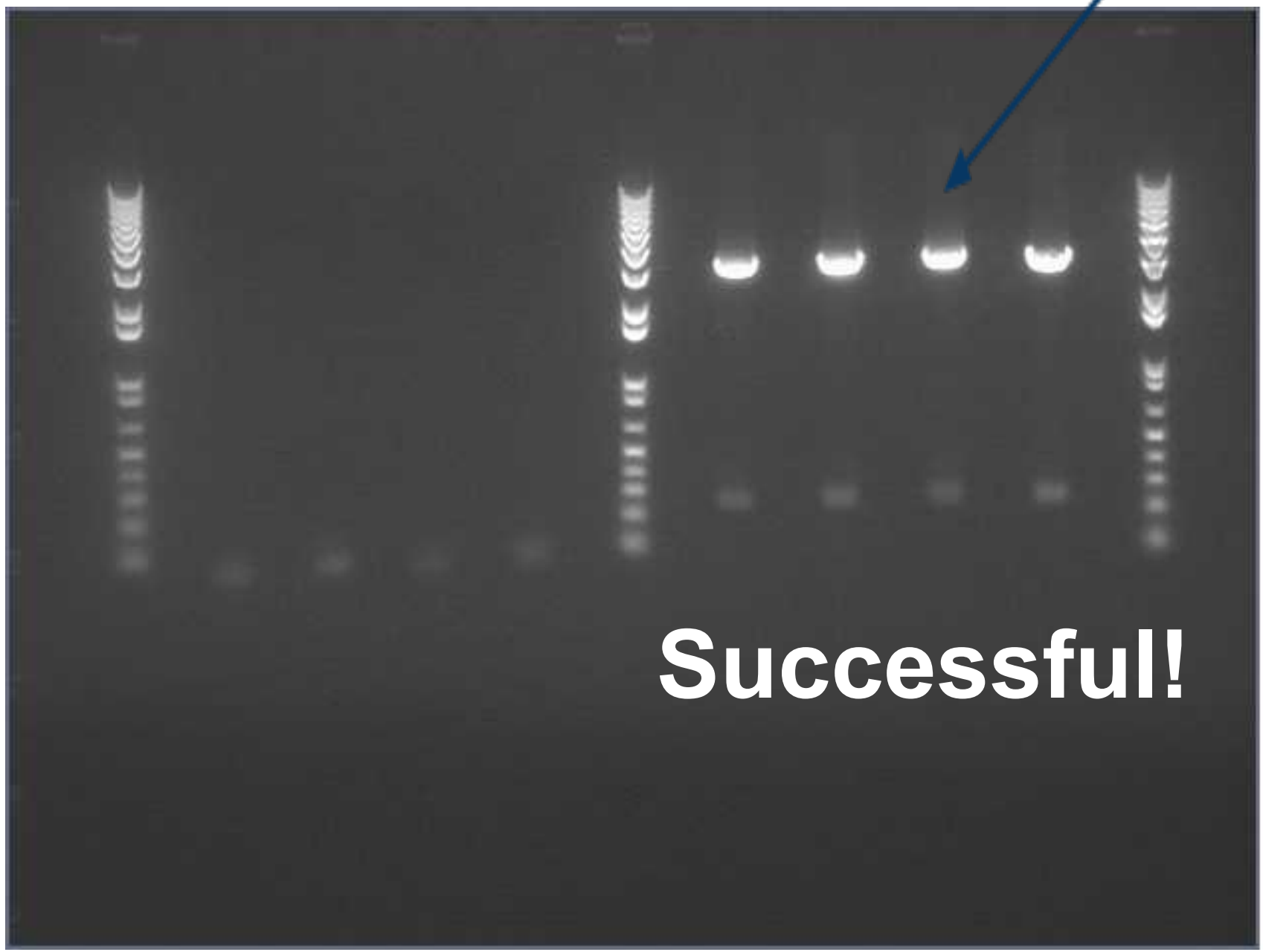
- Ligations worked for pENTCUP2 and NOSt+STOP!
    - DNA concentration insufficient for NOSt

- Fast Digest XbaI came in so we began digestion and ligation attempt with NosT & Stop

NosT and v0120 Ligation

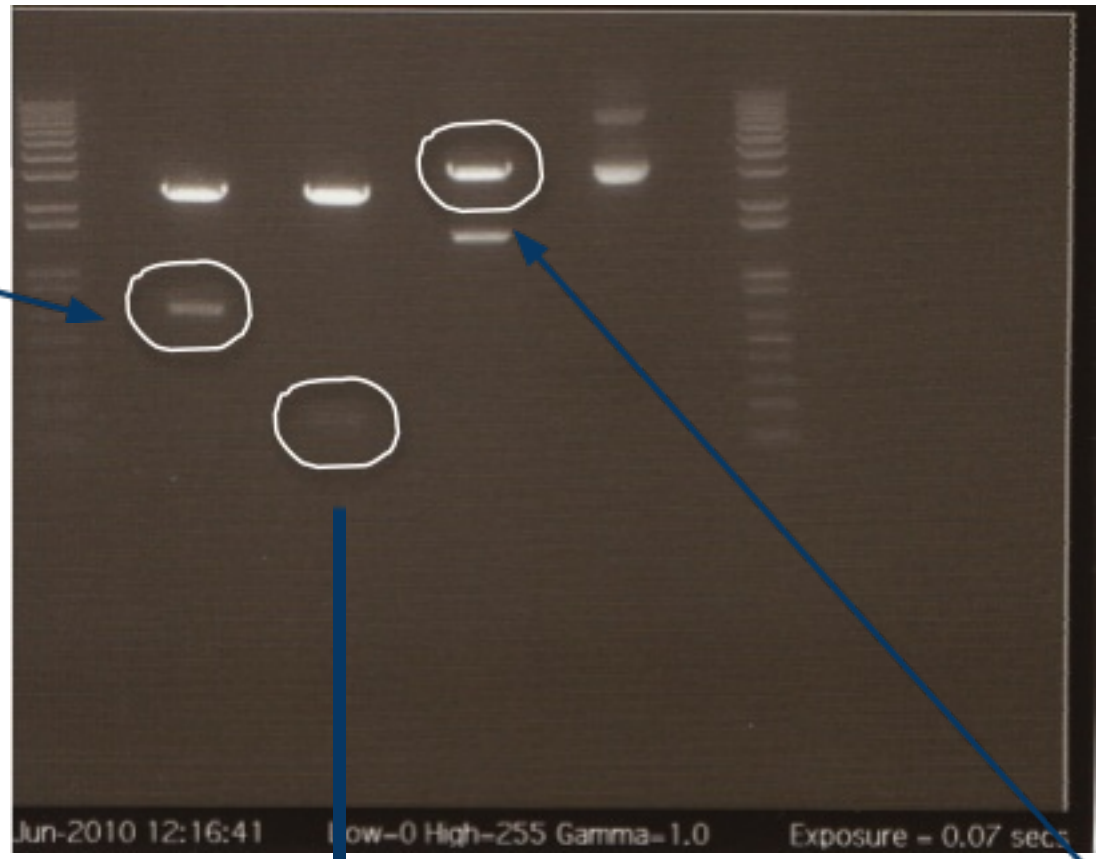


NosT and v0120 Ligation



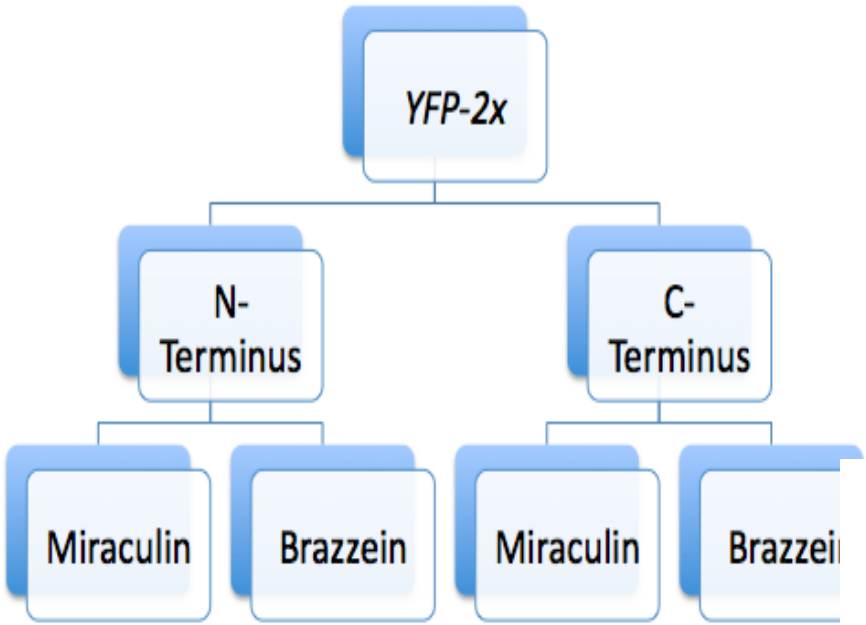
Miraculin and Brazzein

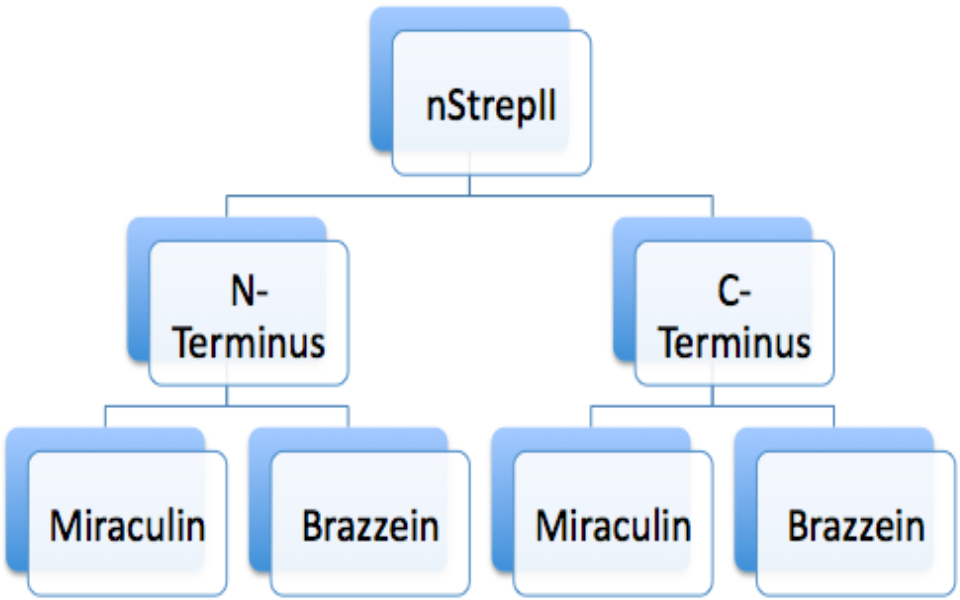
- Digested Miraculin and Brazzein with EcoRI and SpeI



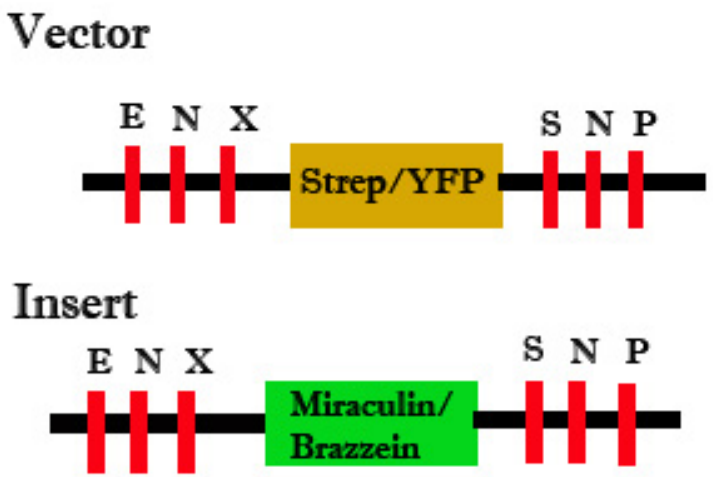
Ligations Successful!

YFP and StrepII tag Miraculin and Brazzein



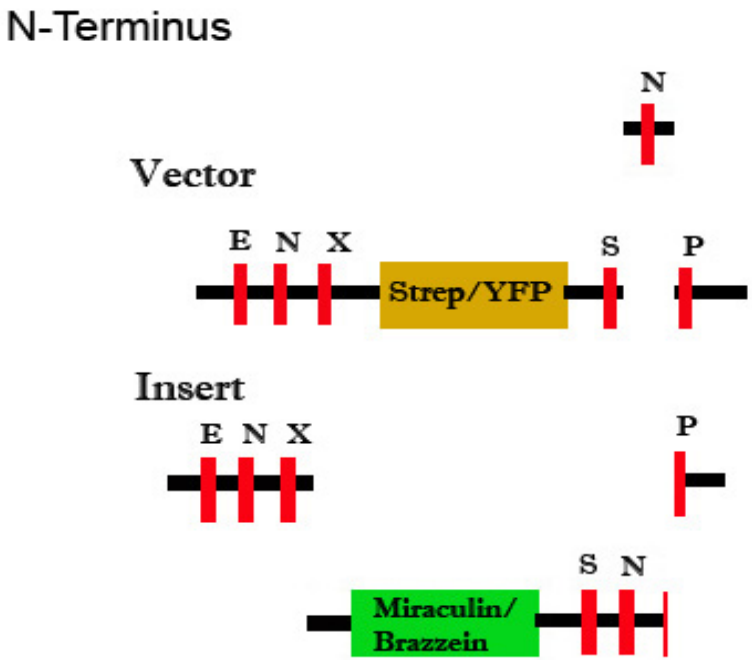


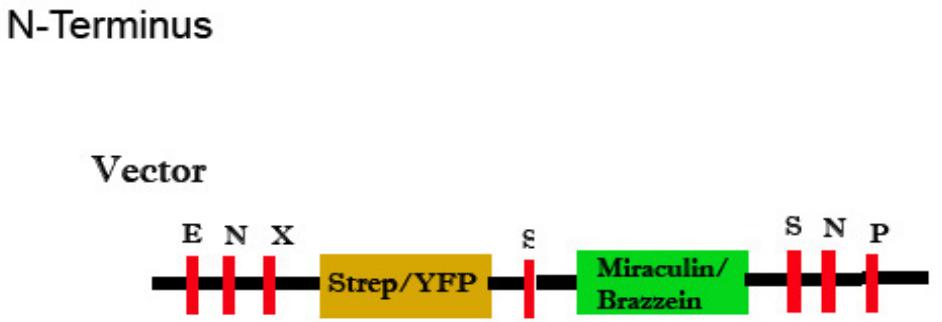
YFP and StrepII tag Miraculin and Brazzein



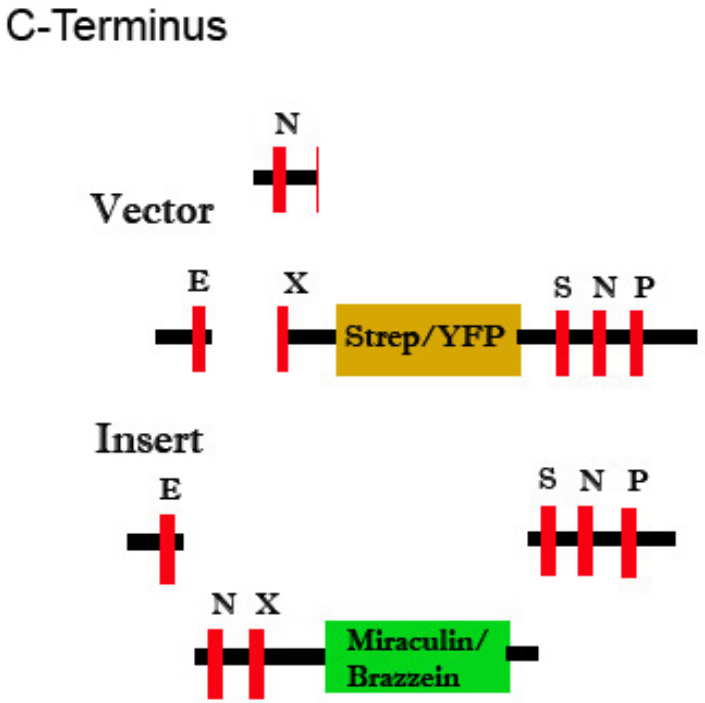
	Vector	Insert
N-Terminus	Spe1/Pst1	Xba1/Pst1
C-Terminus	EcoR1/Xba1	EcoR1/Spe1

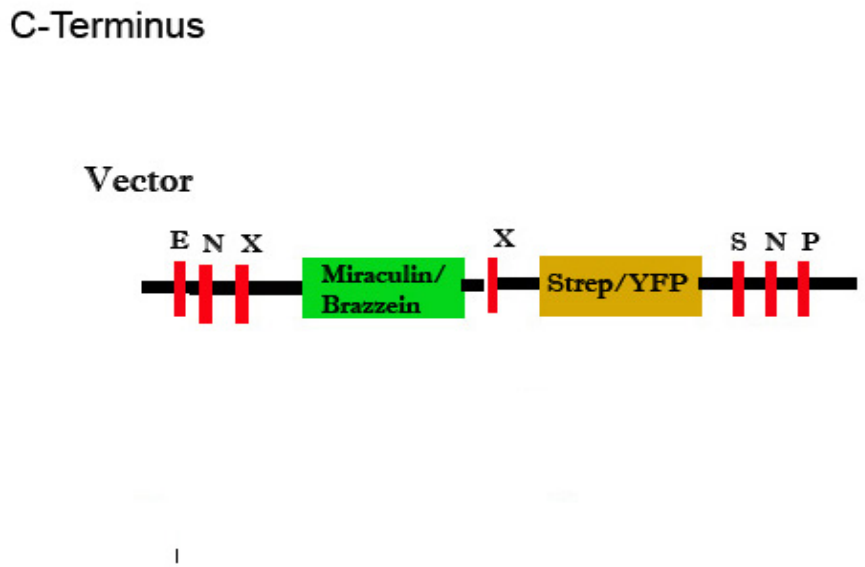
YFP and StrepII tag Miraculin and Brazzein





YFP and StrepII tag Miraculin and Brazzein

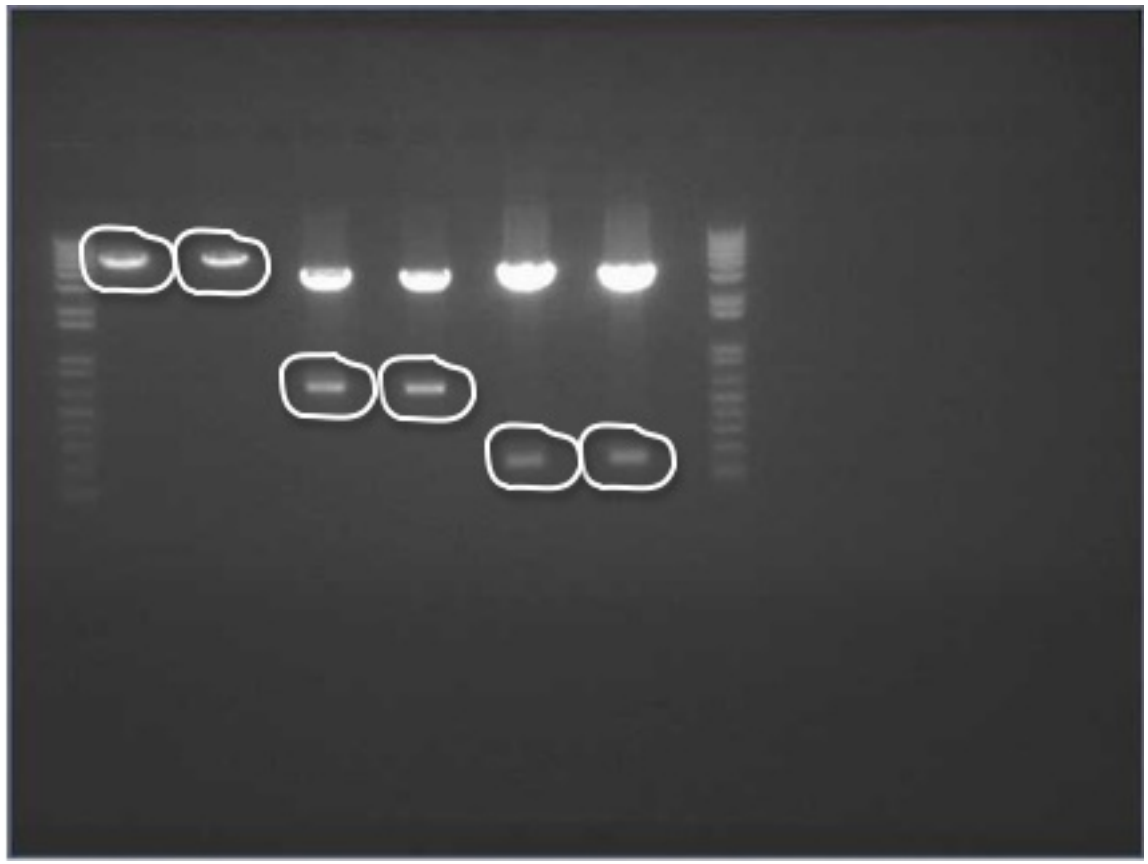




YFP and StrepII tag Miraculin and Brazzein

Digestion Gel YFP tag

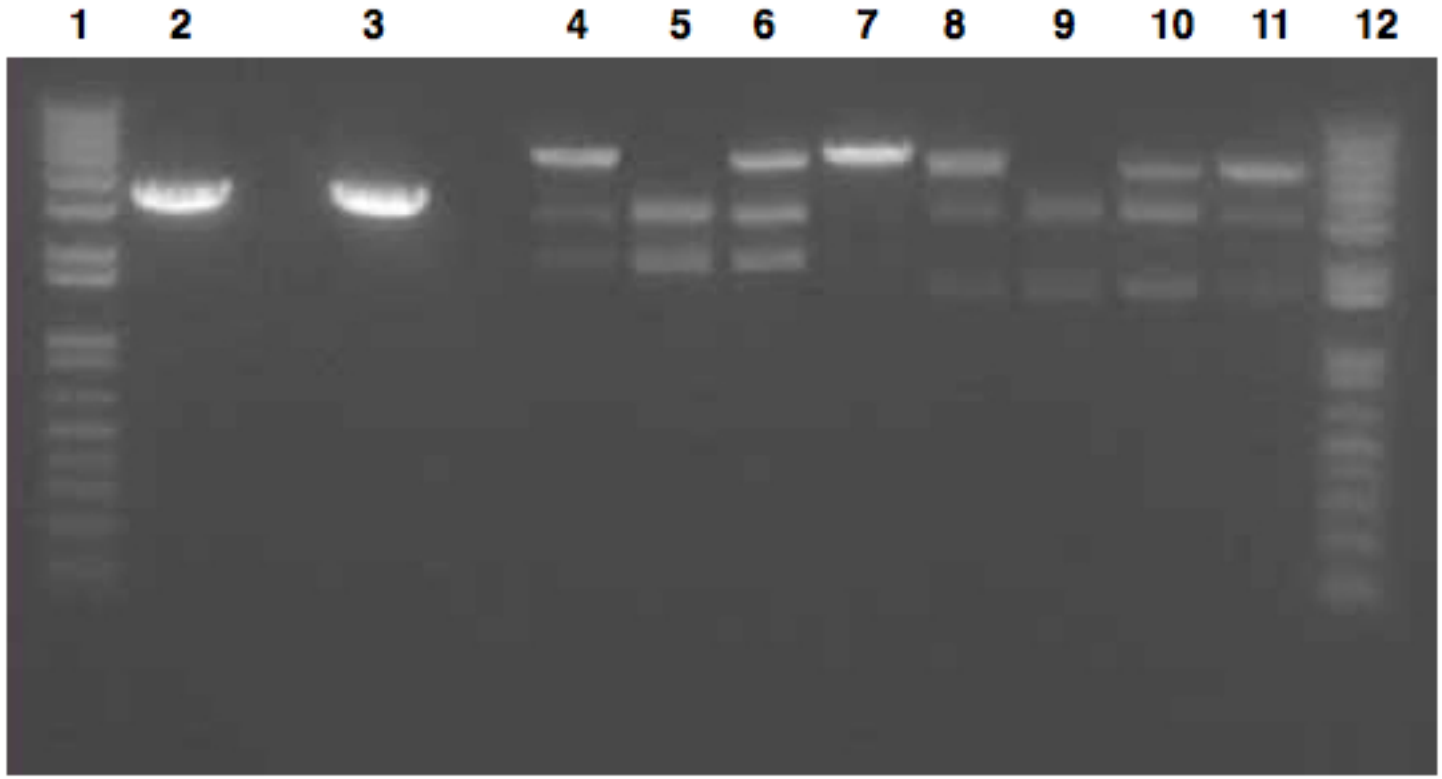
StrepII Miraculin Brazzein



YFP and StrepII tag Miraculin and Brazzein

Gel to confirm YFP and Miraculin/Brazzein ligation

1. Ladder
2. B15 E/X
3. B15 S/P
4. Mira+YFP N1
5. Mira+YFP N2
6. Mira+YFP C1
7. Mira+YFP C2
8. Brazz+YFP N1
9. Brazz+YFP N2
10. Brazz+YFP C1
11. Brazz+YFP C2
12. Ladder



YFP and StrepII tag Miraculin and Brazzein

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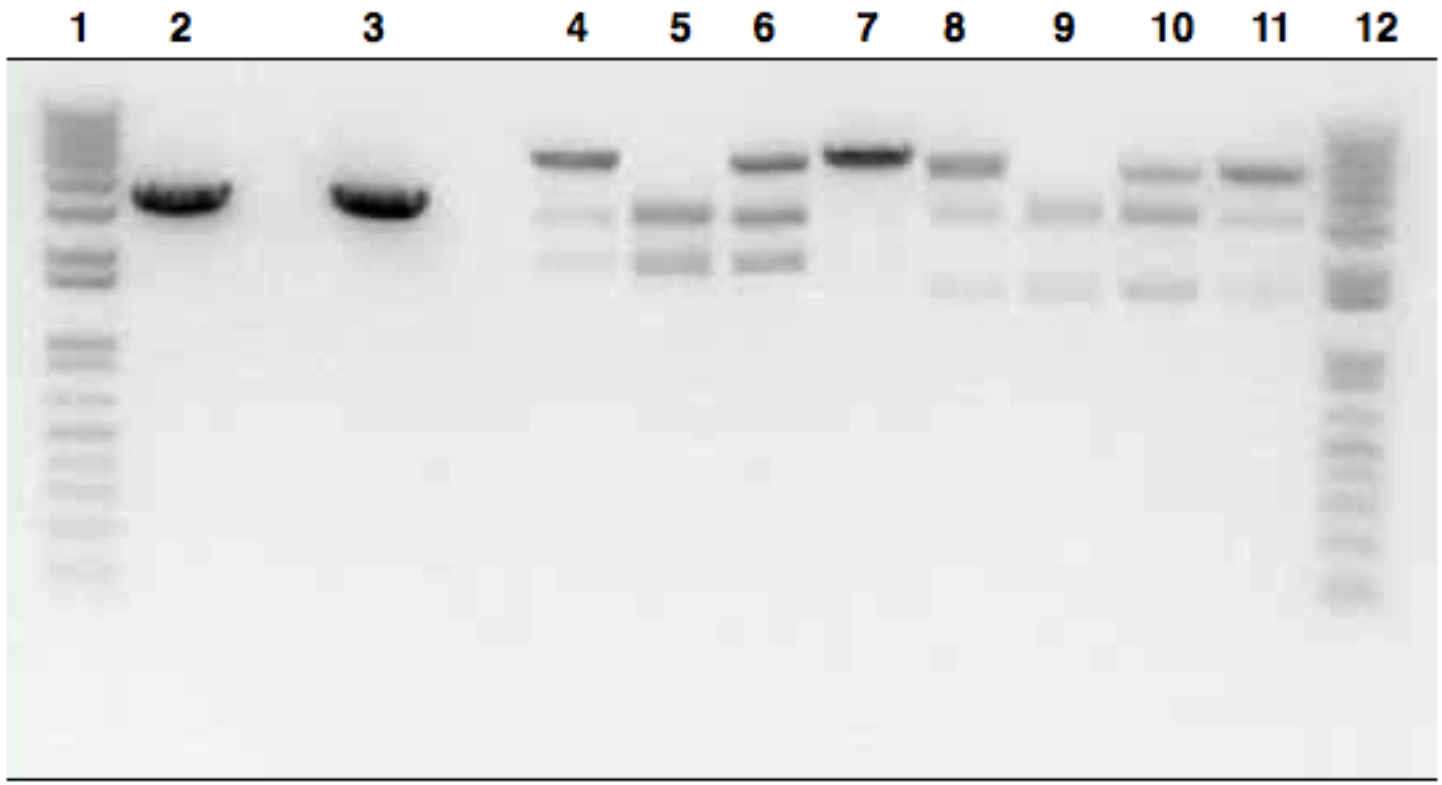
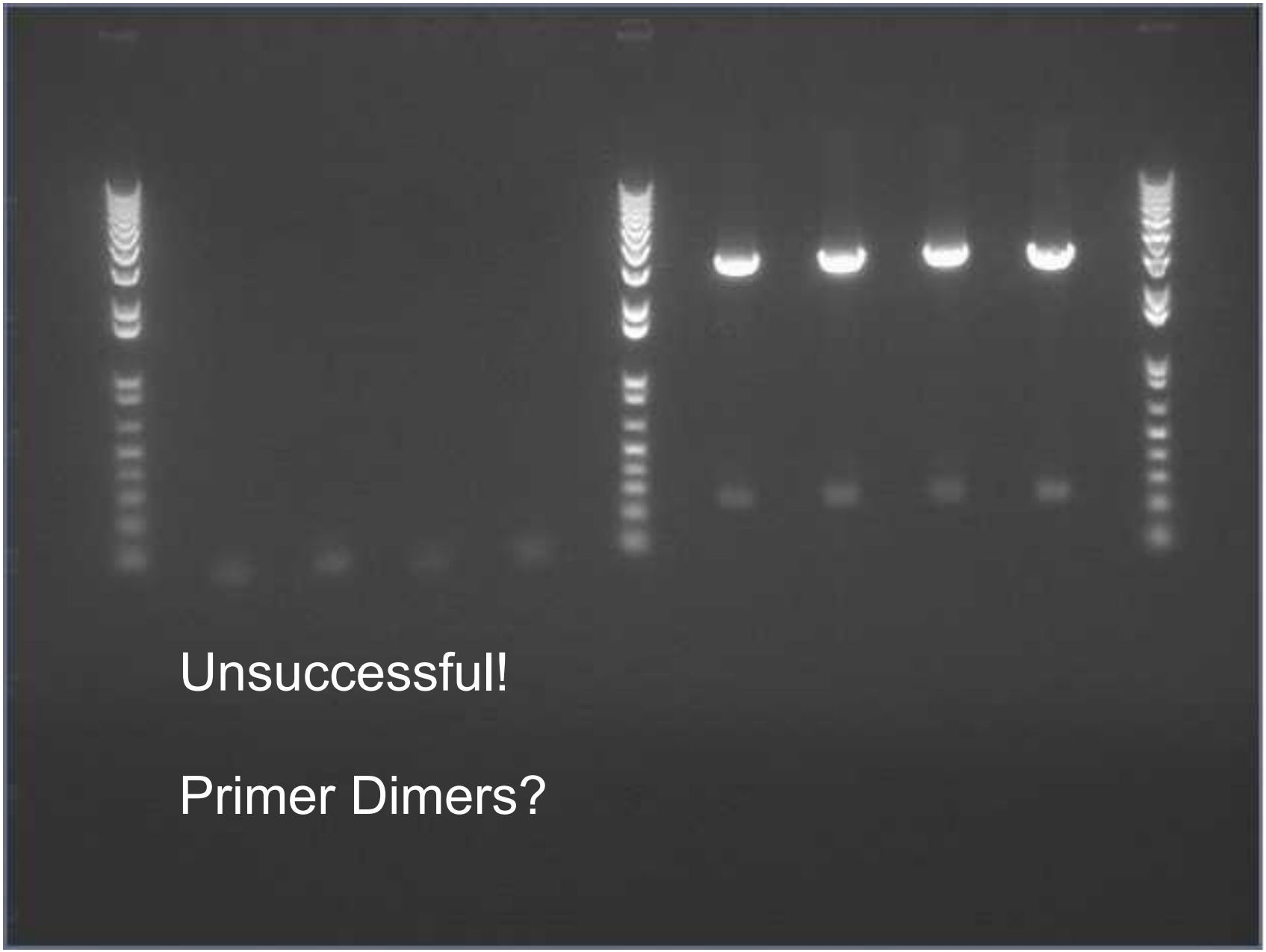
6. Mira+YFP C1
7. Mira+YFP C2
8. Brazz+YFP N1
9. Brazz+YFP N2
10. Brazz+YFP C1
11. Brazz+YFP C2
12. Ladder



RNA extraction from Valencia oranges



RNA extraction from Valencia oranges

RNA extraction from Valencia oranges

New Plan of Attack

- Genomic DNA
  - Not sequenced
    Introns?

- Try RNA extraction again

This Week:

- Insert our Miraculin/Brazzein-YFP-NOSt-STOP constructs in pDUET vectors.
  - Create Miraculin/Brazzein constructs with attached *StrepII* tag
- Transform into expression bacteria (from Patrick)
- Visualize proteins

- Disassemble and re-assemble Wintergreen pathway
- Obtain Banana pathway
- Re-attempt Valencene extraction with DNA extraction kit