Effects of capsid and delta Orsay virus proteins on the Intracellular Pathogen Response of *C. elegans*

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Background

Research Question: How do pathogens evade host immunity?

Selective pressure → Pathogens evolve to evade detection by host → Host's detection & resistance against pathogen weakened

Significance: Immune system failure → infection

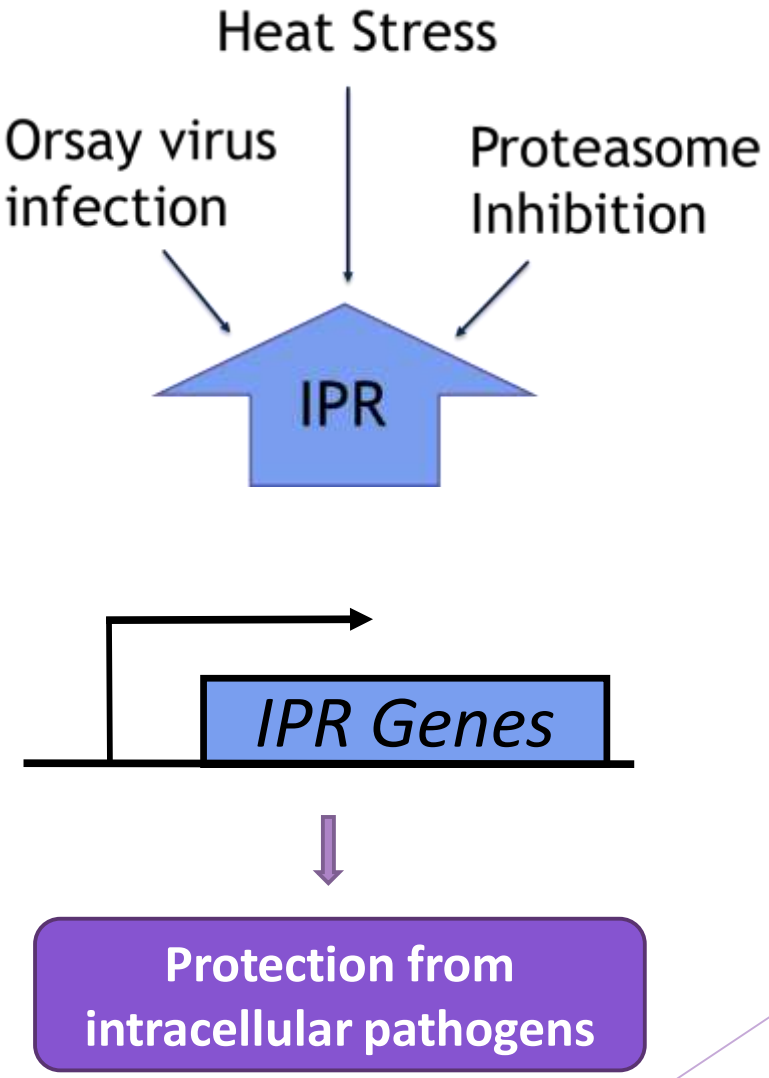
- Innate immunity: first line of defense
- Predict and modulate responses in patients

C. elegans as a model system

- Roundworm
- Transparent, genetic modification
- Exclusively innate immunity
- Signaling mechanisms that regulate immune responses are well conserved

C. elegans immune system

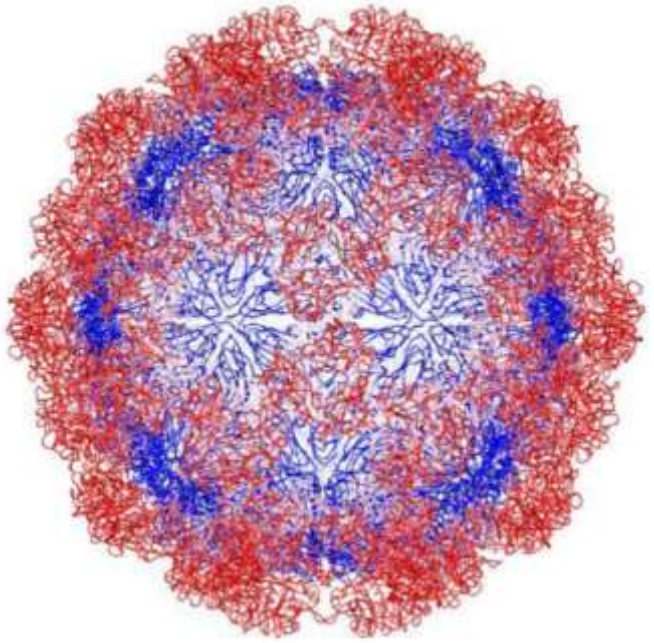
- **Intracellular Pathogen Response (IPR)**
  - Innate immune response
  - Set of ~80 genes upregulated by diverse stimuli
  - Activated by Orsay virus
  - Provides protection from intracellular pathogen infections



Bakowski, M. A., *et al* (2014)
Reddy, K. C., *et al* (2017)

Orsay virus is a natural pathogen of *C. elegans*

- (+) ssRNA
- 4 proteins
- Capsid and delta



- **Preliminary evidence:** One or a combination of Orsay proteins can suppress IPR

Felix, M. A. et al (2011)
Bakowski, KM A. et al (2014)
Jiang, H. et al (2014)
Yuan, W. et al (2018)
Guo, Y. R. et al (2014)

Orsay virus genome contains 4 proteins

Orsay Protein	Function
Capsid	Mediates viral packaging
Delta	Mediates viral exit from cell
Delta-fusion	Mediates viral entry into cell
RNA-dependent RNA polymerase (RdRP)	Viral transcription and replication

- **Orsay virus can suppress *C. elegans* IPR**
- Which proteins are responsible for this suppression?
  - Individual effects of Orsay proteins on IPR unknown

Yuan, W. et al (2018)
Jiang, H. et al (2014)
Sowa, J. N. et al (2020)

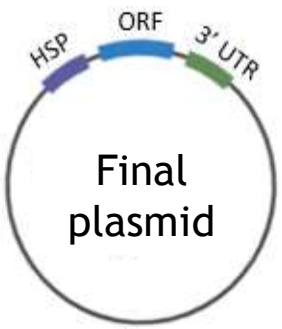
Experimental Overview

Project aim: Determine whether **individual expression** of the Orsay virus **capsid** and **delta** proteins can suppress the IPR

Create transgenic *C. elegans* that overexpress each of the two Orsay proteins

1. **Molecular cloning** to generate plasmids
2. **Microinject plasmids** into *C. elegans*
3. **Test the animals** to observe individual effects of Orsay proteins on IPR

1. Gibson Cloning



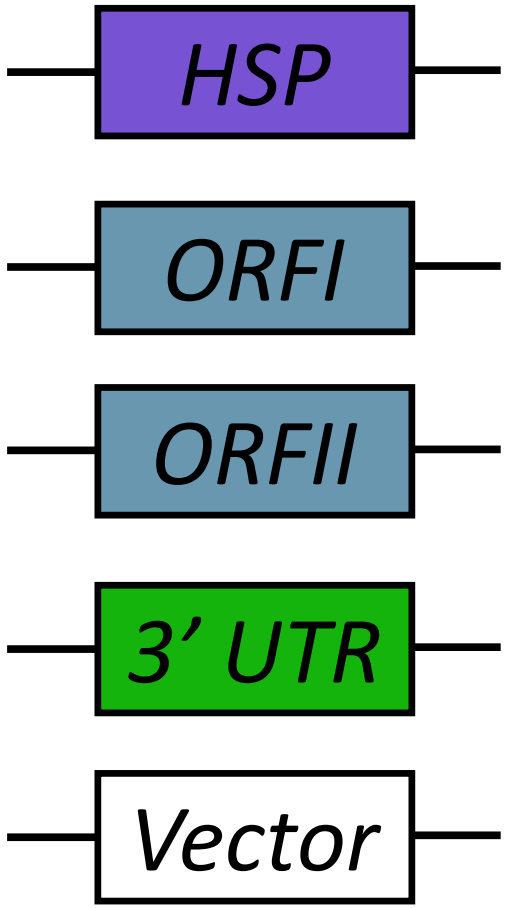
Transform plasmids into bacteria

Isolate plasmids via miniprep

2. Microinject plasmids into *C. elegans*

3. Test the animals

DNA fragments to generate capsid and delta plasmids

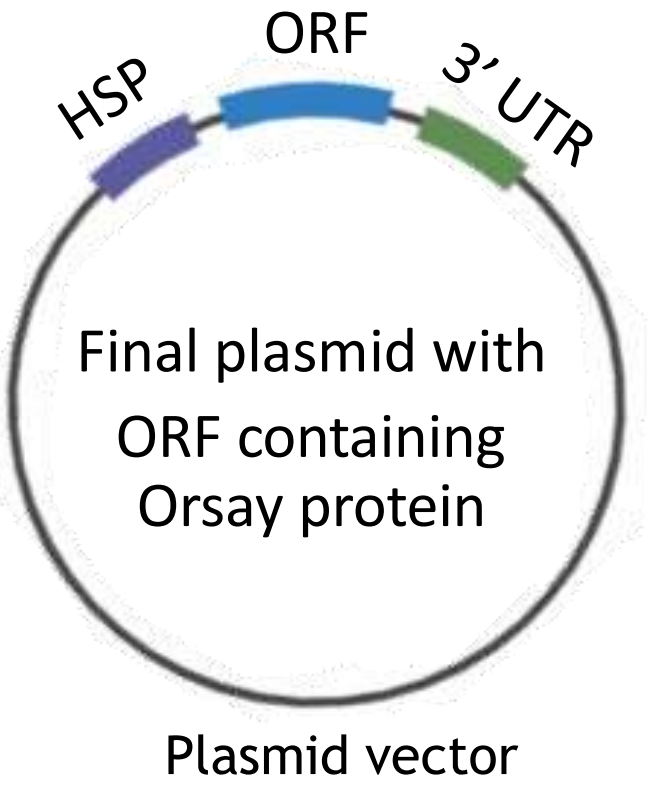


DNA Fragment	Template
Heat-Shock Promoter	High temp: Induce expression of gene (capsid or delta) downstream Normal temp: Gene silent
Open reading frame	Express capsid (ORFI) or delta (ORFII) protein
3’ Untranslated Region	Regulate mRNA-based processes (mRNA localization, stability, and translation)
Plasmid Vector	Plasmid backbone

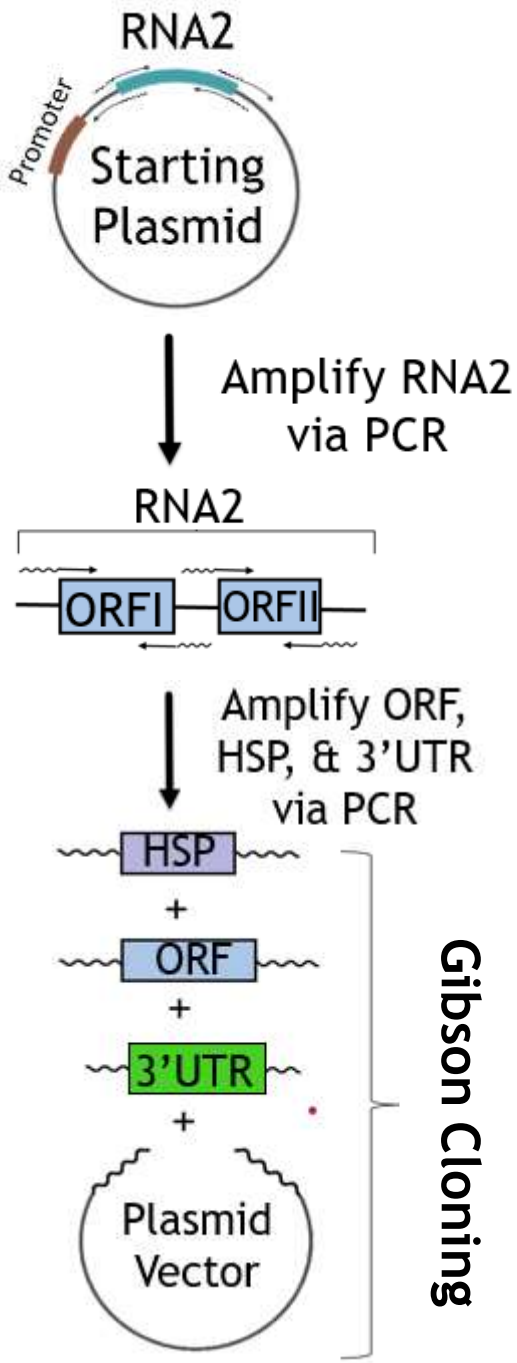
Jiang, H. et al (2014)

1. Perform **molecular cloning** reaction to create desired plasmids containing capsid and delta proteins

Desired DNA fragments:



- **Gibson cloning**
  - Multiple overlapping DNA fragments are joined to form a new plasmid



1. Perform **molecular cloning** to create plasmids containing capsid and delta proteins

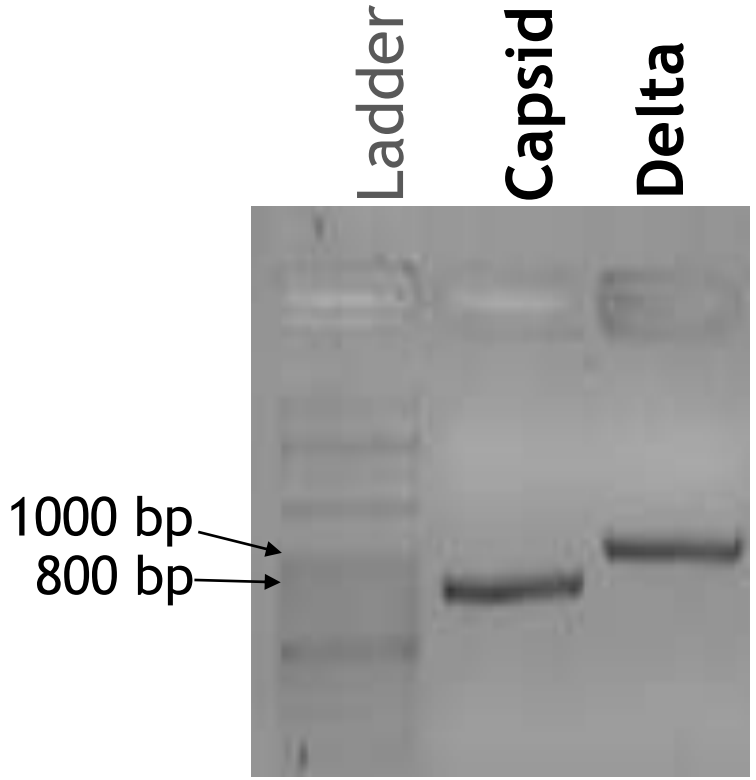
- Starting point: Premade plasmids from collaborator
- Isolate fragments via **PCR** or restriction digestion
- DNA products analyzed via **gel electrophoresis**

DNA Component	Template
Capsid & delta proteins	pET714 plasmid
Vector backbone	pCFJ150 plasmid
3'UTR	Genomic DNA
HSP promoters	Genomic DNA

- Performed Gibson Cloning reaction

Successful gel electrophoresis of capsid and delta

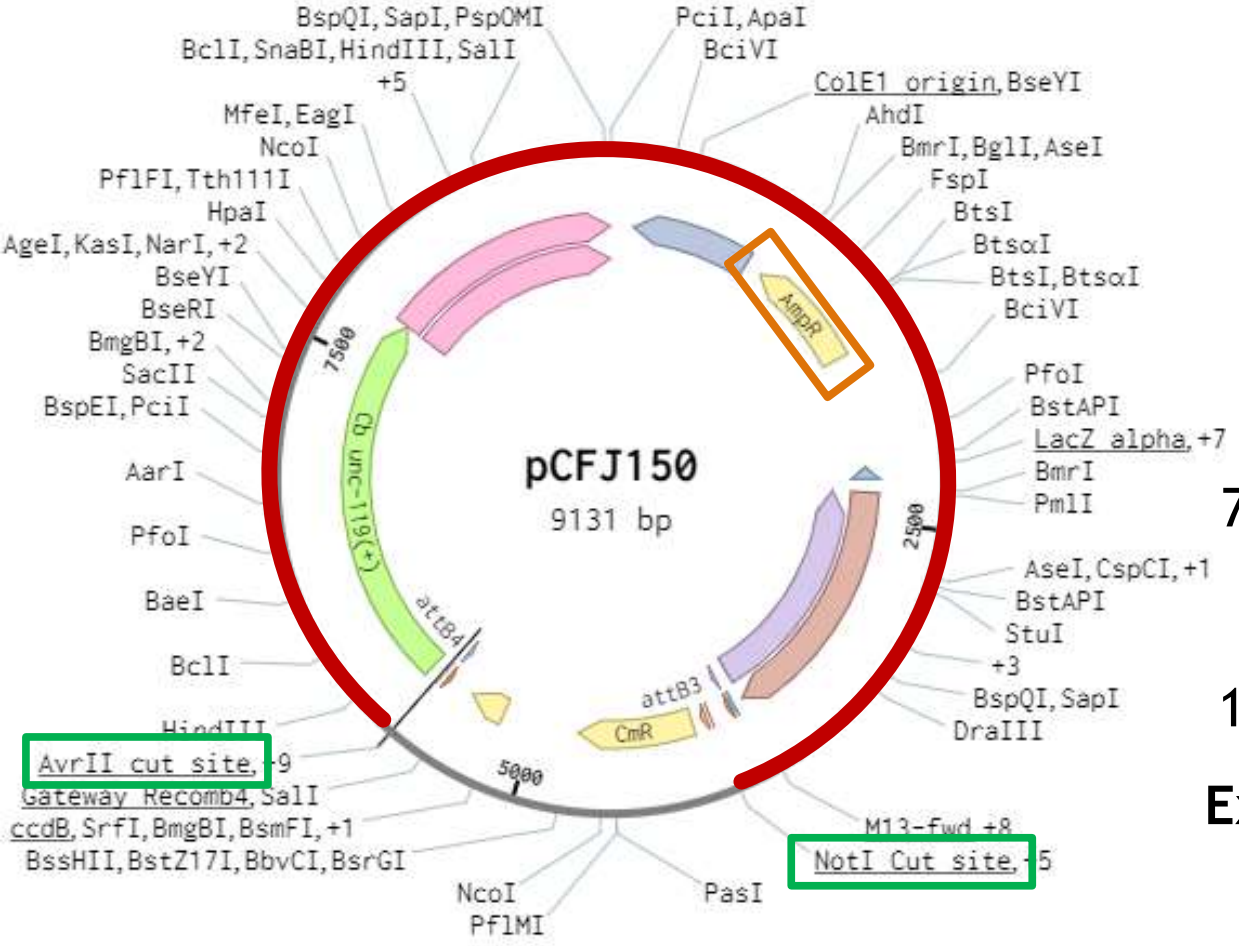
1. **Isolate desired DNA fragments** (**capsid & delta**) via PCR

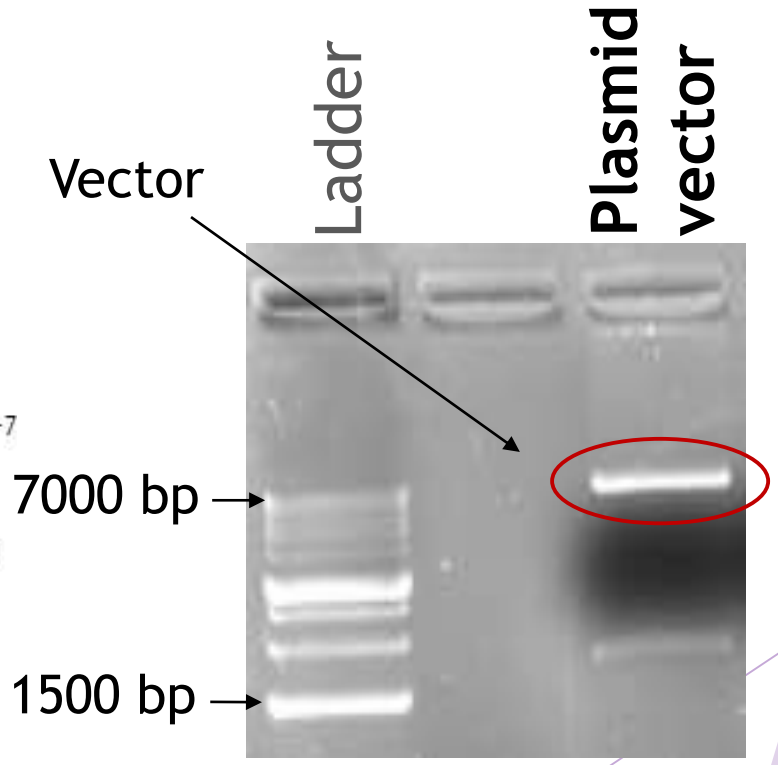


Expected band sizes (bp): 762 1041

Successful gel electrophoresis of plasmid vector backbone

1. **Isolate desired DNA fragment** (**vector**) via restriction digestion

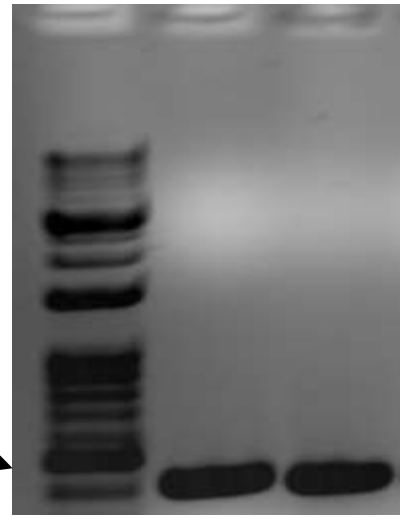




Expected band sizes (bp): Top: 7513 Bottom: 1618

Successful gel electrophoresis of capsid and delta heat-shock promoters (HSP)

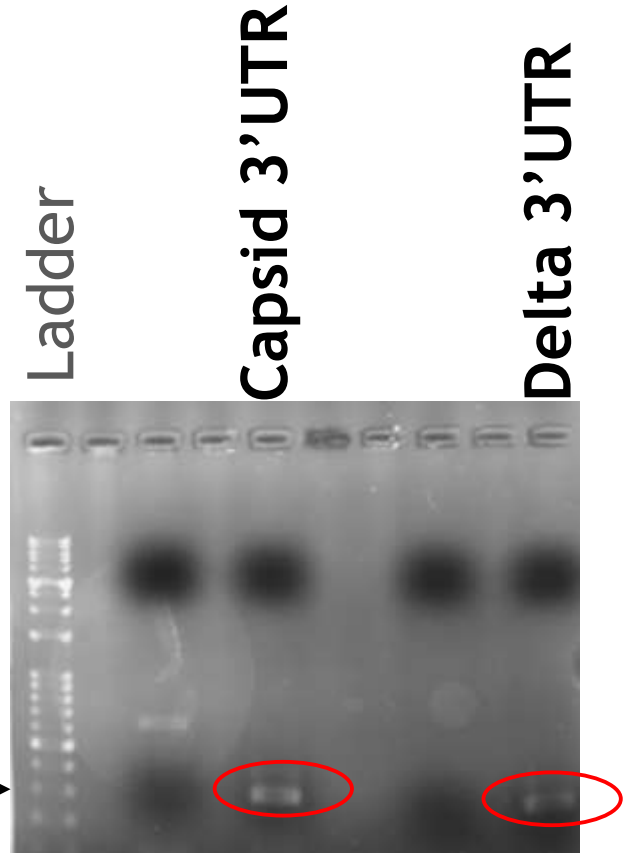
1. **Isolate desired DNA fragments** (capsid & delta **HSP**) via PCR



Expected band sizes (bp): 392 392

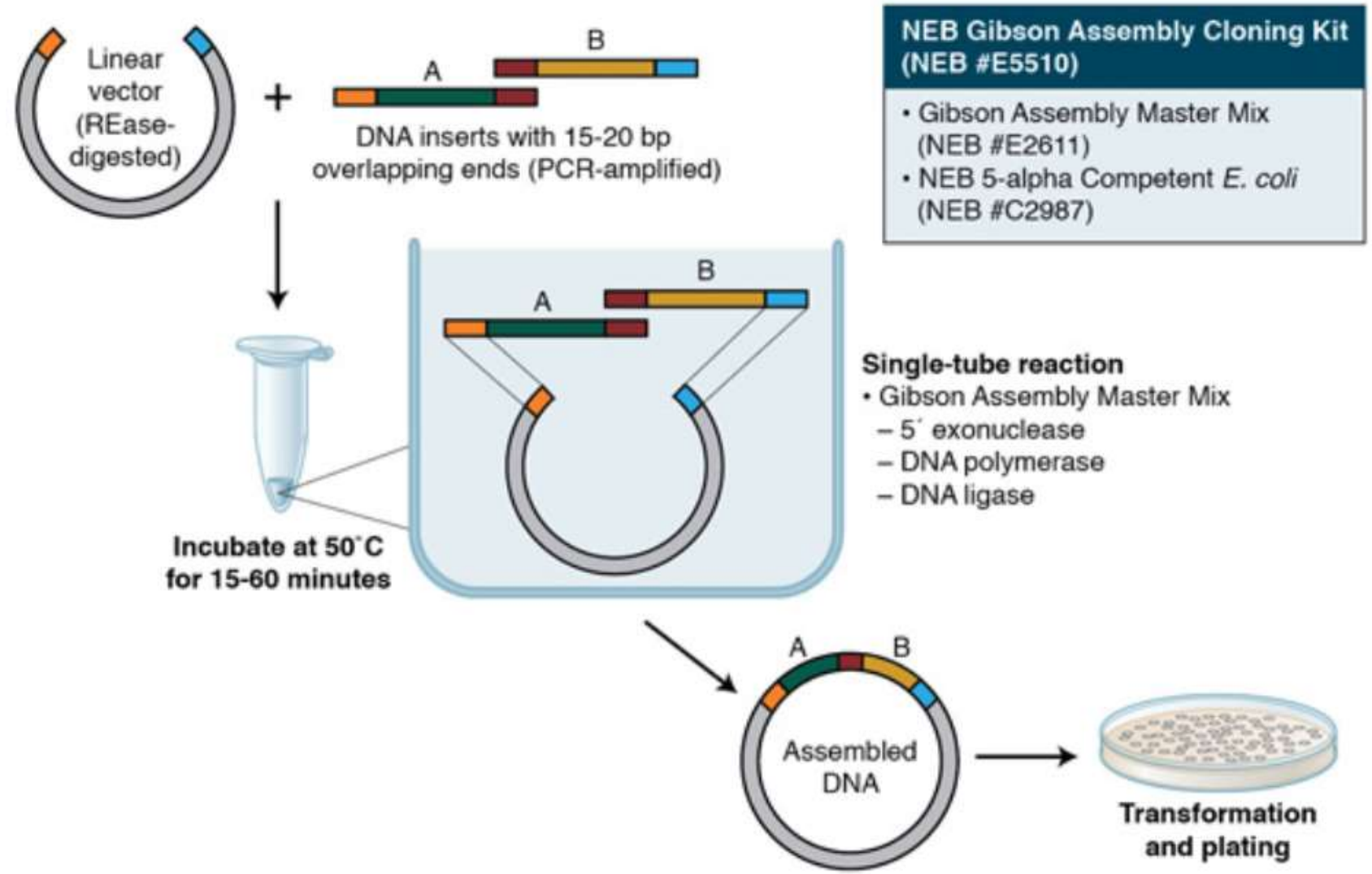
Successful gel electrophoresis of capsid and delta 3’ untranslated region (3’ UTR)

1. **Isolate desired DNA fragments** (capsid & delta **3’UTR**) via PCR



Expected band sizes (bp): 280 280

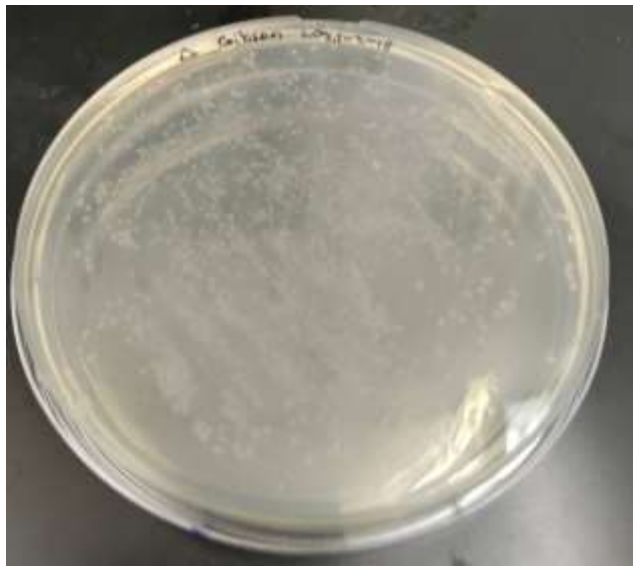
2. Perform **Gibson cloning** reactions to generate capsid and delta plasmids



Successful transformation of constructs after Gibson assembly

3. **Transform** capsid and delta plasmids into bacteria onto ampicillin-resistant plates

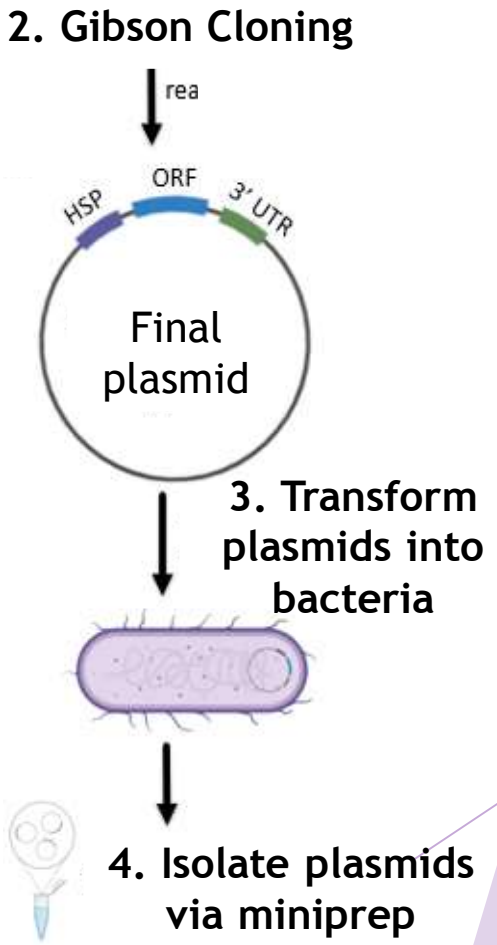
Capsid Gibson
Bacterial Transformation



Delta Gibson
Bacterial Transformation

Current Progress

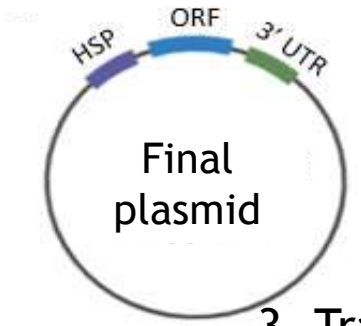
1. Isolate desired DNA fragments via PCR or restriction digestion
2. Perform Gibson cloning to generate capsid and delta plasmids
3. Transform plasmids into bacteria
4. Isolate plasmids from bacteria via miniprep



Future Plans

1. Isolate DNA fragments via PCR

2. Gibson Cloning



3. Transform plasmids into bacteria

4. Isolate plasmids via miniprep

5. Verify plasmid via sequencing

6. Microinject plasmids into *C. elegans*

7. Test the animals

5. Verify capsid & delta plasmids via **sequencing**

6. **Microinject** plasmids into *C. elegans*

7. **Test** the animals to observe individual effects of capsid & delta protein overexpression on IPR

Significance

Research Question: How to pathogens evade host immunity?

- Investigate individual effects of Orsay virus capsid and delta proteins on *C. elegans* innate immune response (IPR)

Significance: Immune system failure → infection

- Humans possess innate immunity
- Predict and modulate responses in patients

Acknowledgements

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