Transfection and Expression of a Full-Length Canine Circovirus Molecular Clone

Steven Hsu, Rie Watanabe, Steven Kubiski, Patricia Pesavento

Why Study Circoviruses?

- Family: Circoviridae
- Genus: Circovirus
- Species:
  - Chicken: Chicken anemia virus.
  - Psittacine: Beak and feather disease virus.
  - Pig: Porcine circovirus 1, porcine circovirus 2.
    - “Post-weaning multi-systemic wasting syndrome”
  - Human...
  - Fish...
  - Others...

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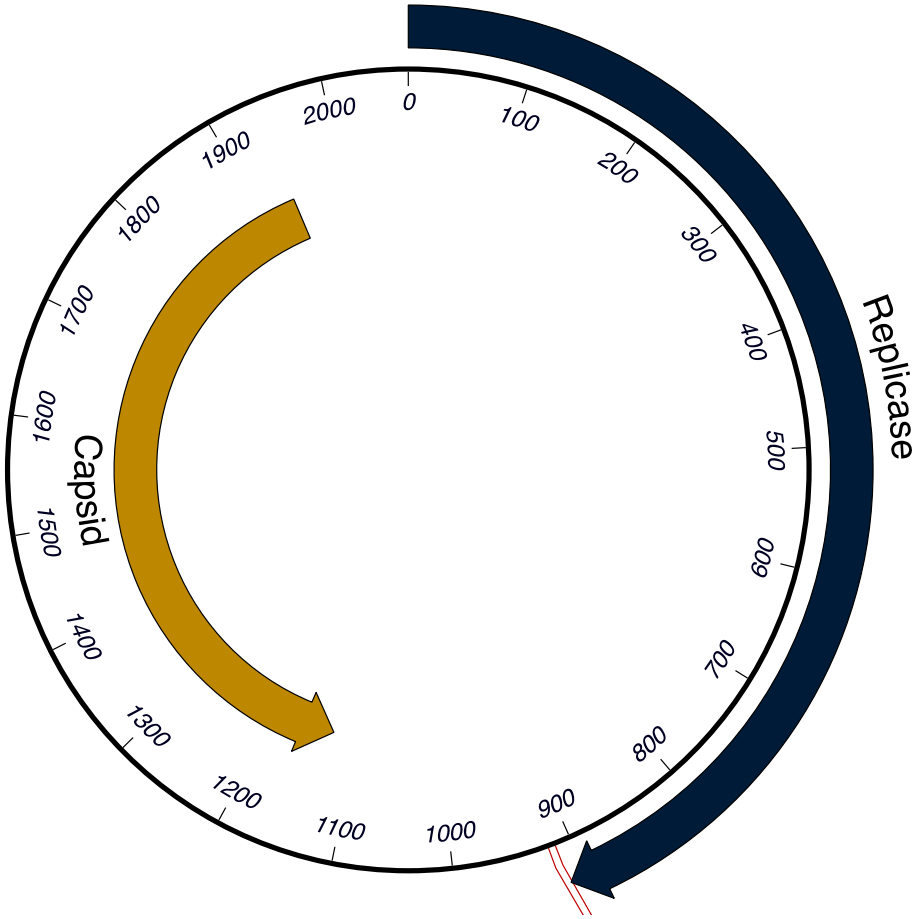
- Newly discovered virus.
  - 2012
- Clinical presentation is variable
  - Asymptomatic to enteric and systemic disease
- Detection of virus is not useful in determining the outcome of disease.

Circoviruses

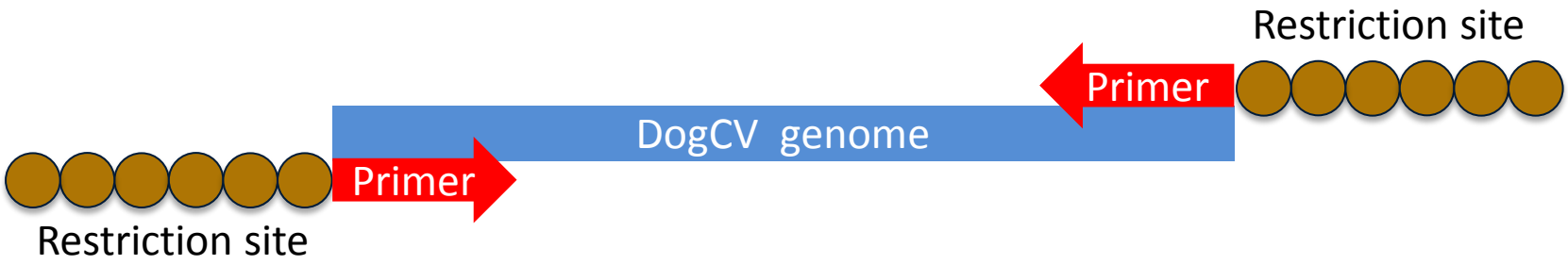
- Circular genome
- Ambisense, single strand DNA.
- Two genes:
  - Replicase
  - Capsid



Summer Objectives

- Construct a full-length molecular clone of canine circovirus.
- Transfect clone into Madin-Darby canine kidney cells (MDCK).
- Confirm successful transfection by detection of viral genome and localizing viral protein.
  - PCR = DNA
  - Immunofluorescence = viral protein.

Clone Construct



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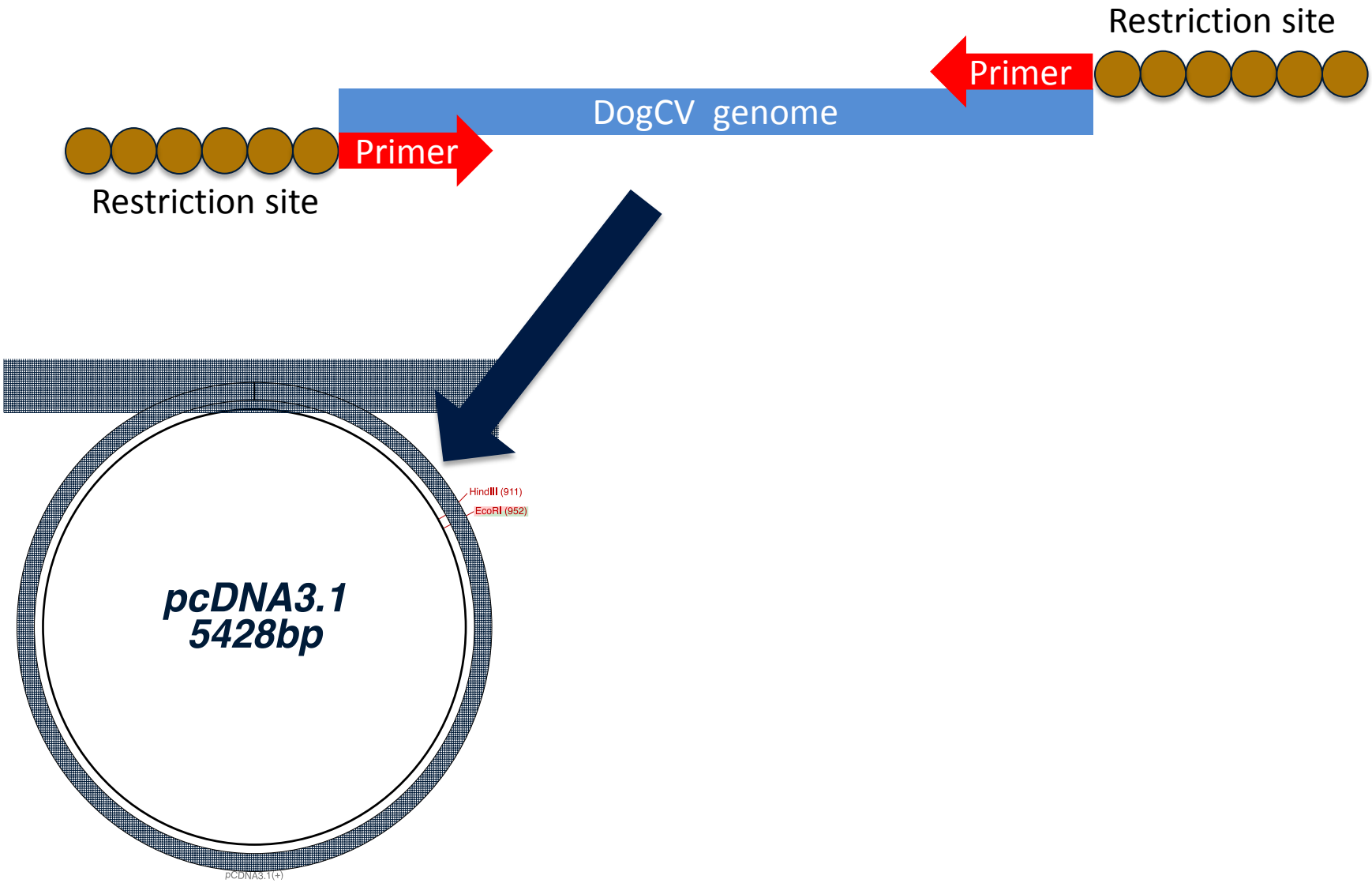

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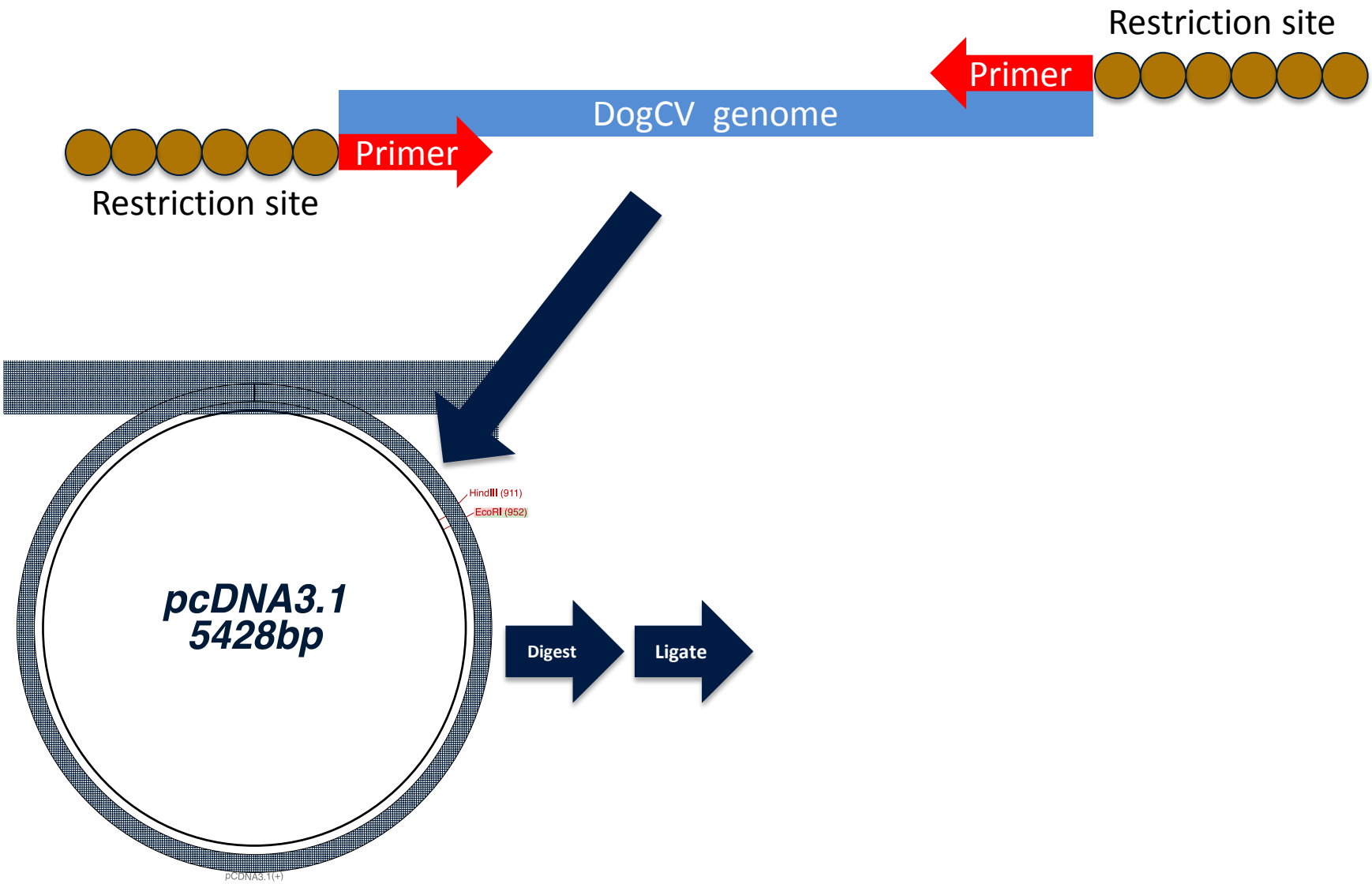

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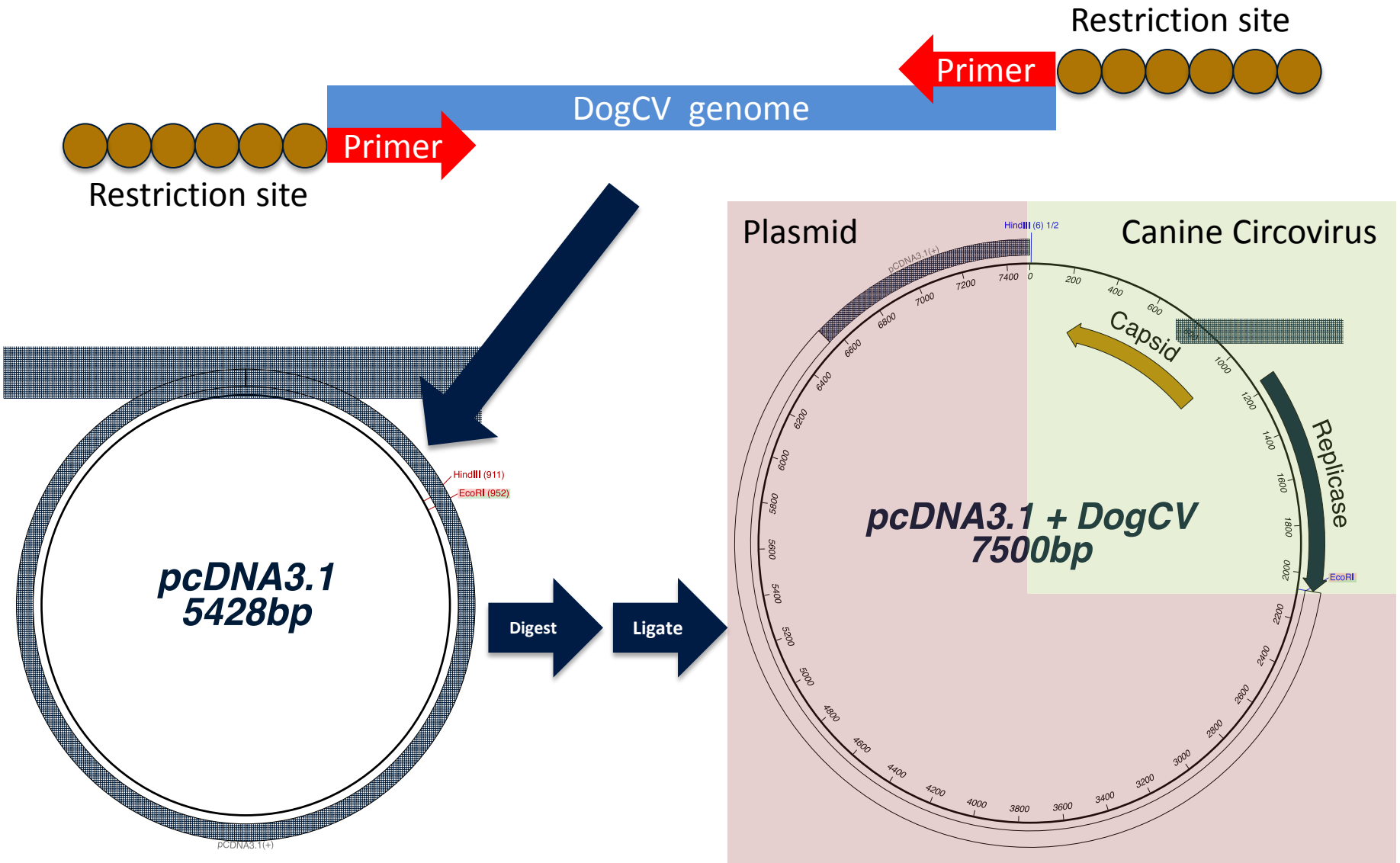

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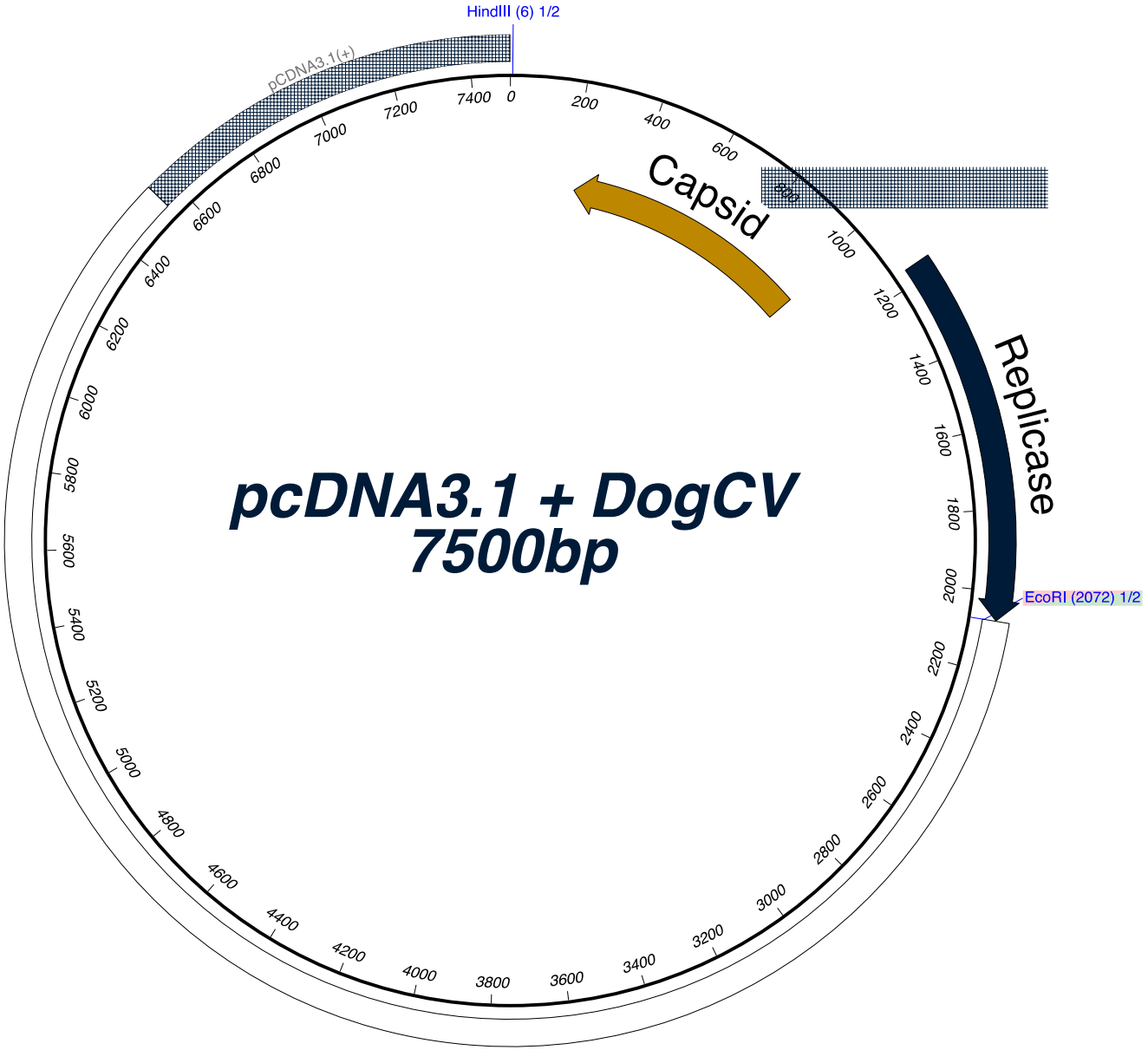
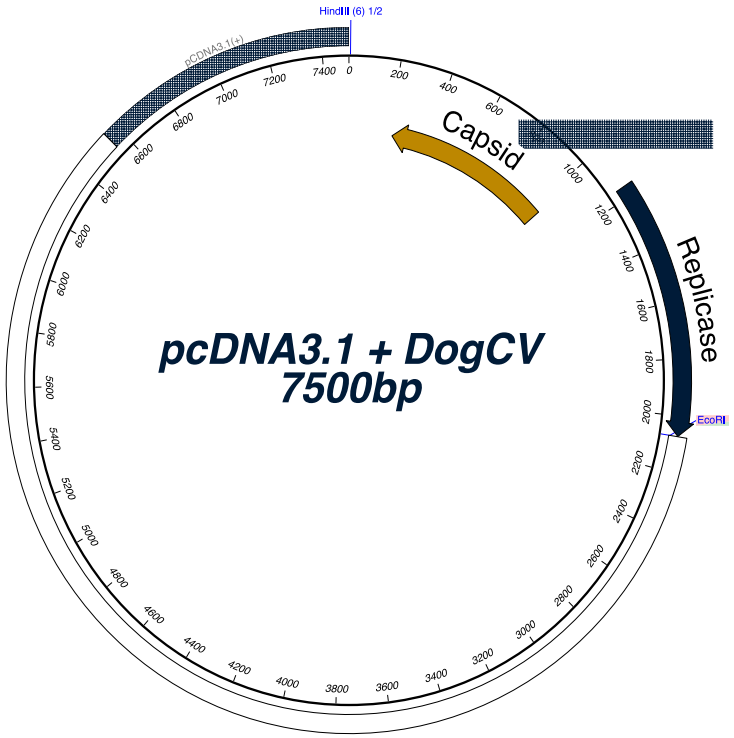


Transfection

- Lipofectamine 3000 transfection reagent into 80% confluent MDCK cells.



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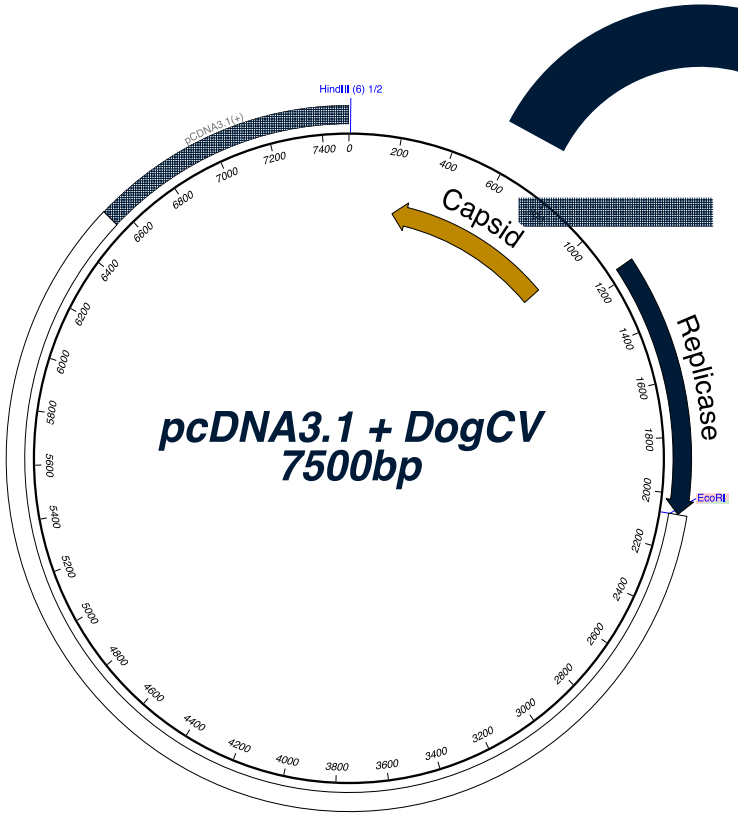
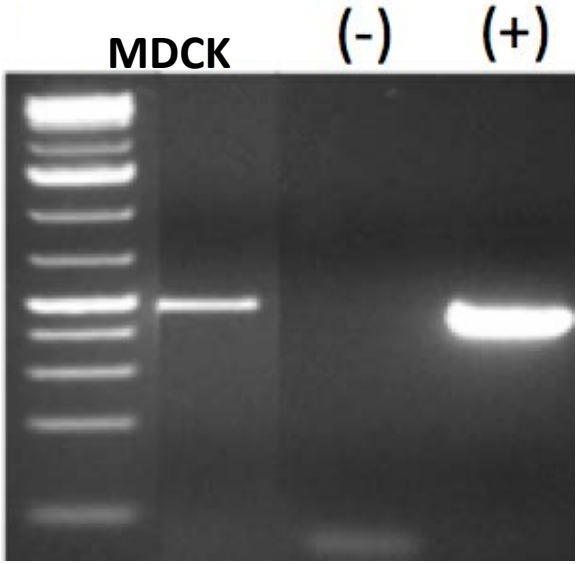


MDCK cell line

Confirm Transfection – DNA Detection

- Passage 1:



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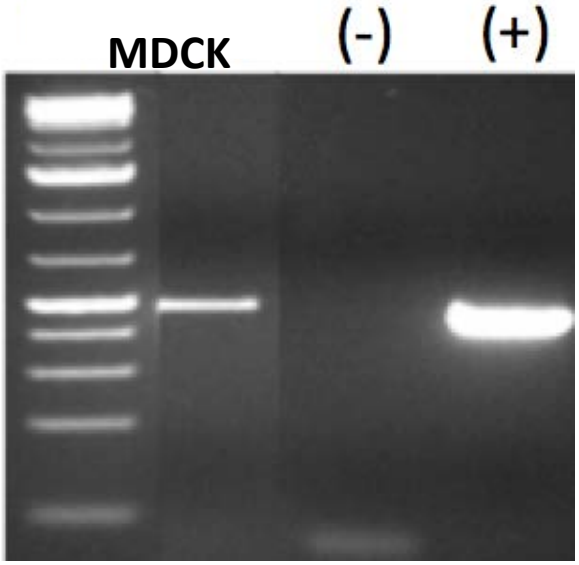

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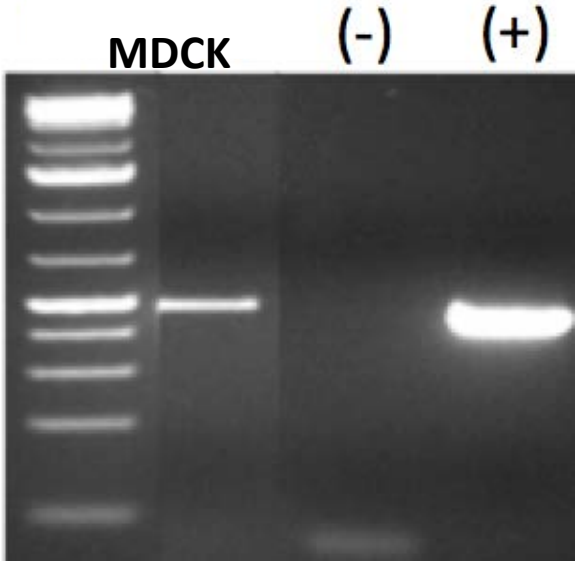
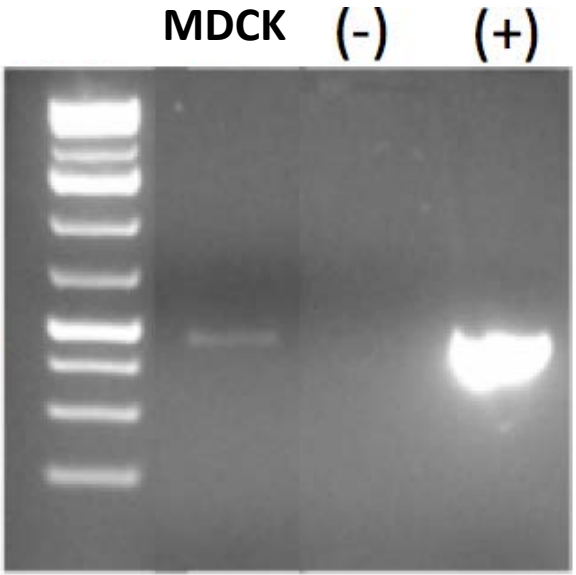


- Passage 5:



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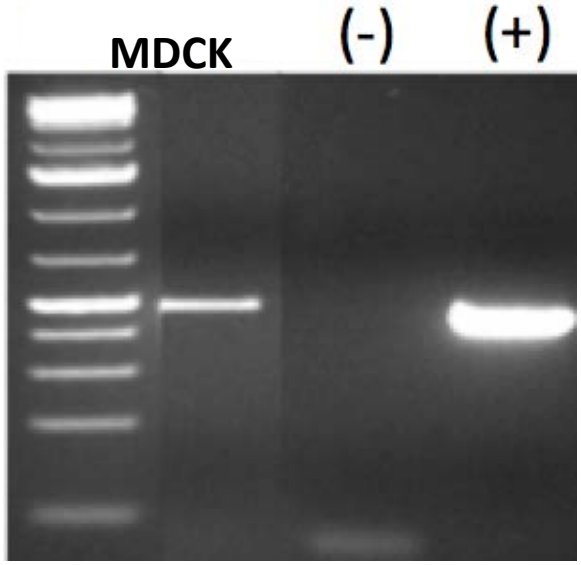
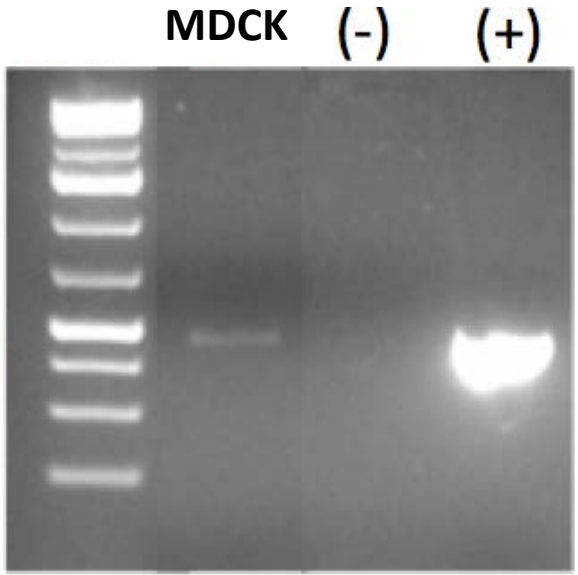


- Passage 5:



Recircularizing?

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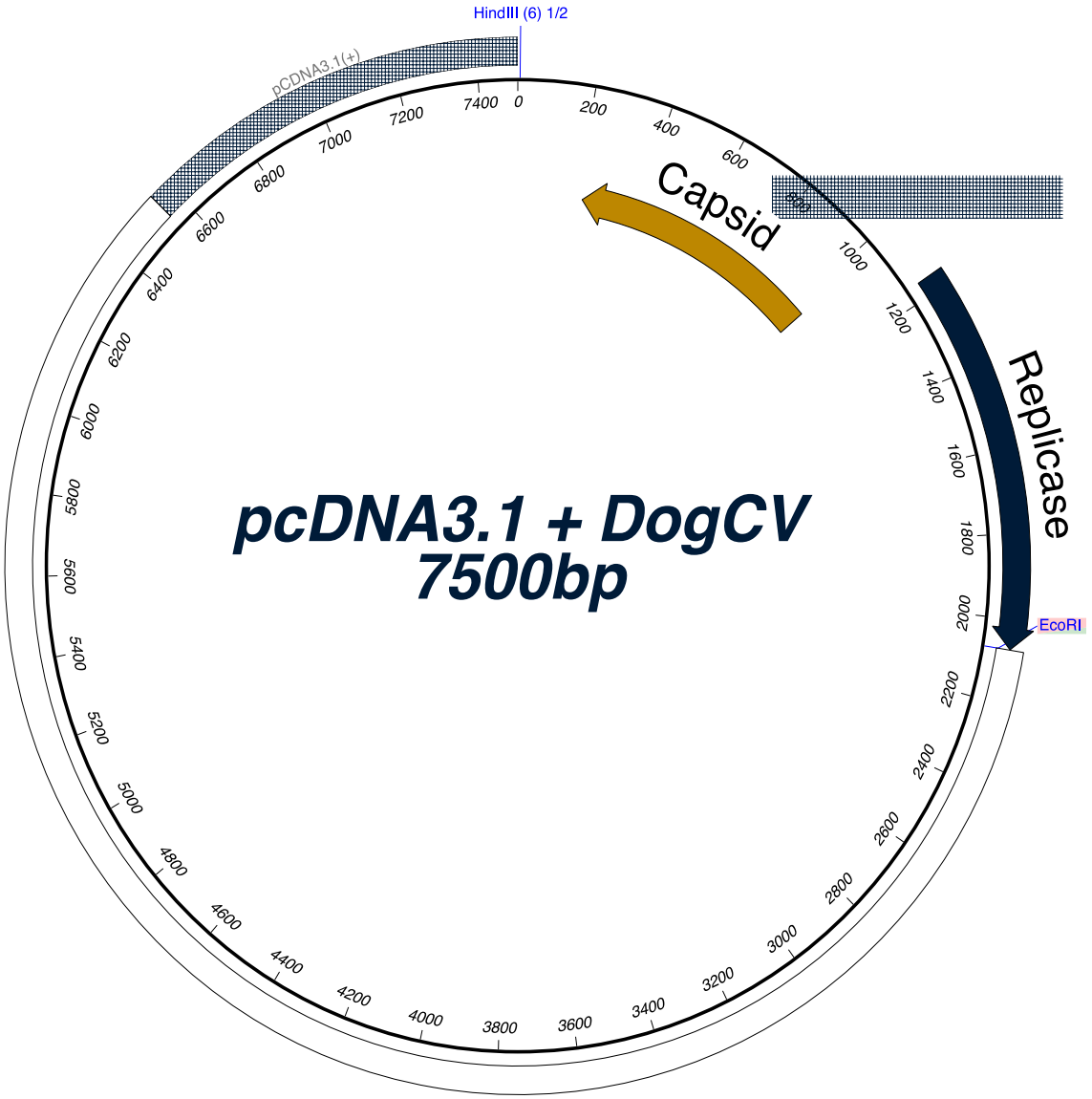
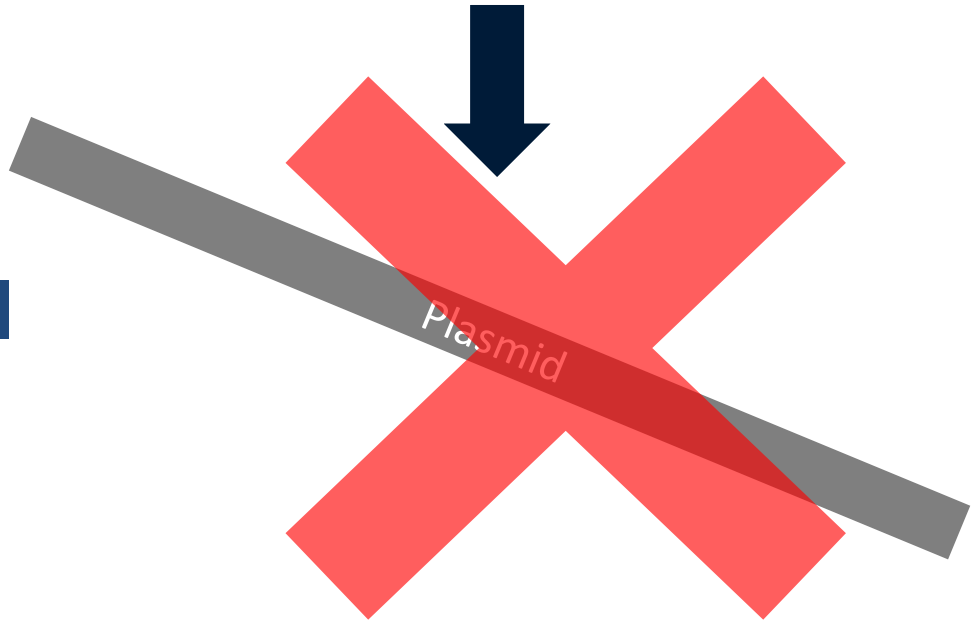



Capsid
3’ region
Plasmid
3’ region
Replicase

Capsid | 3’ region | Plasmid | 3’ region | Replicase



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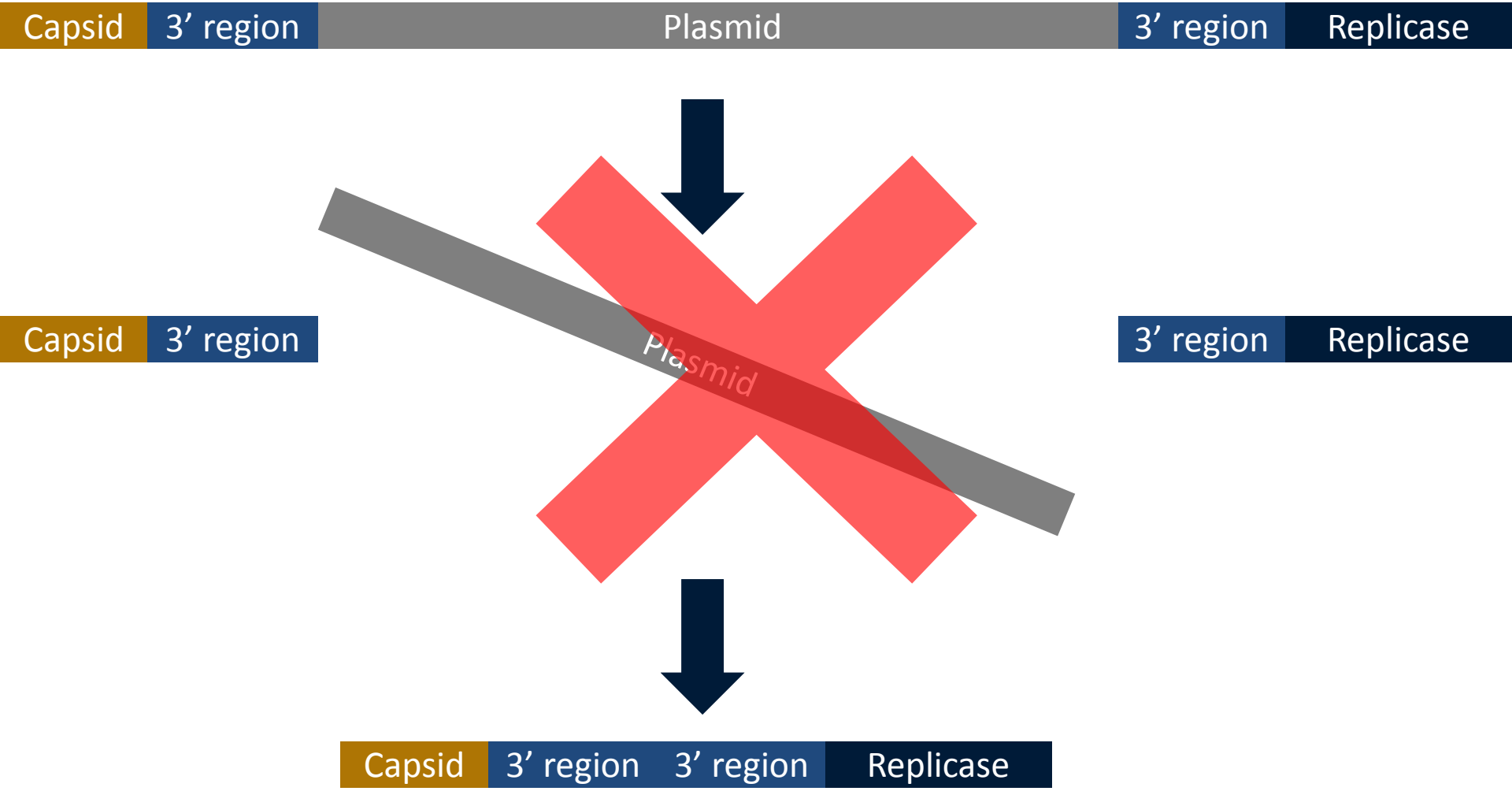
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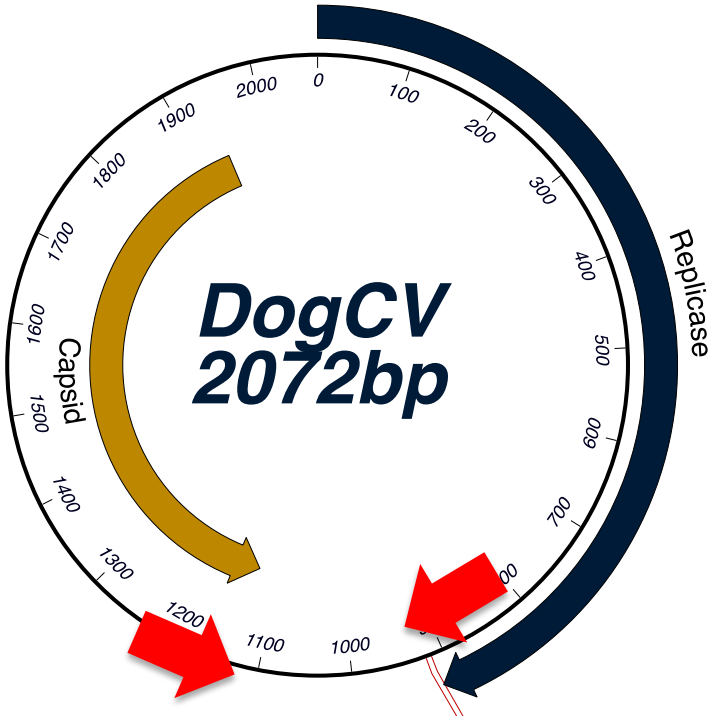

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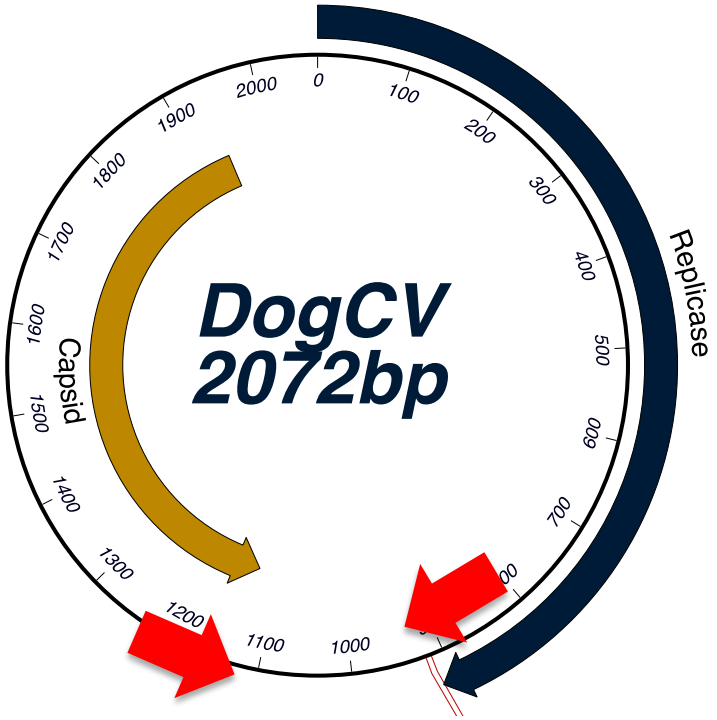

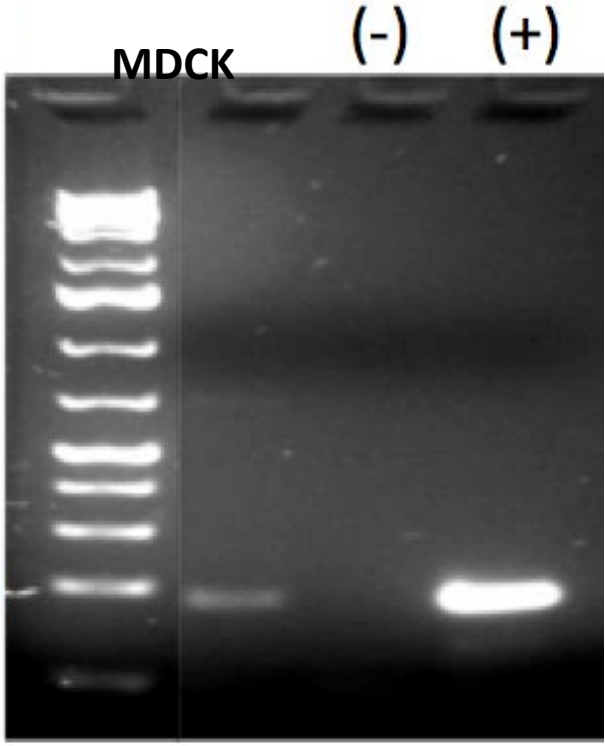

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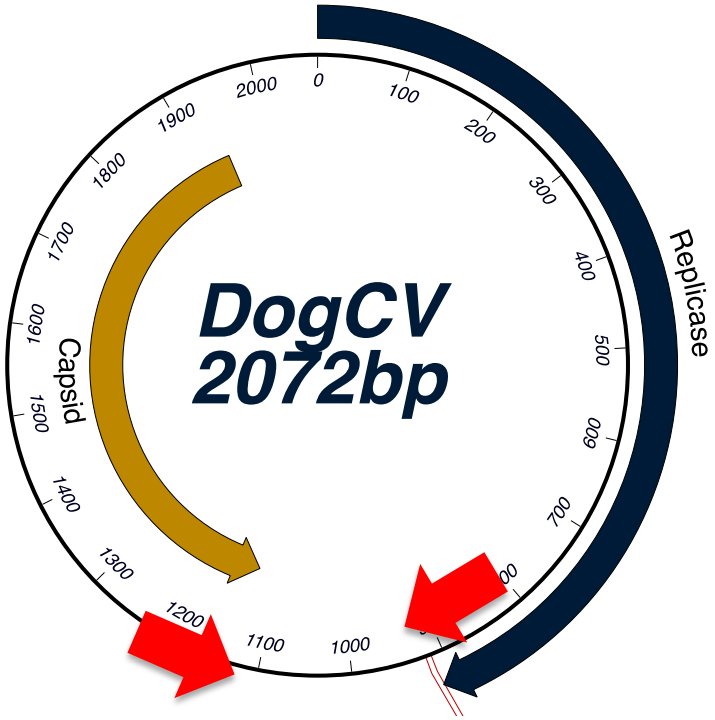
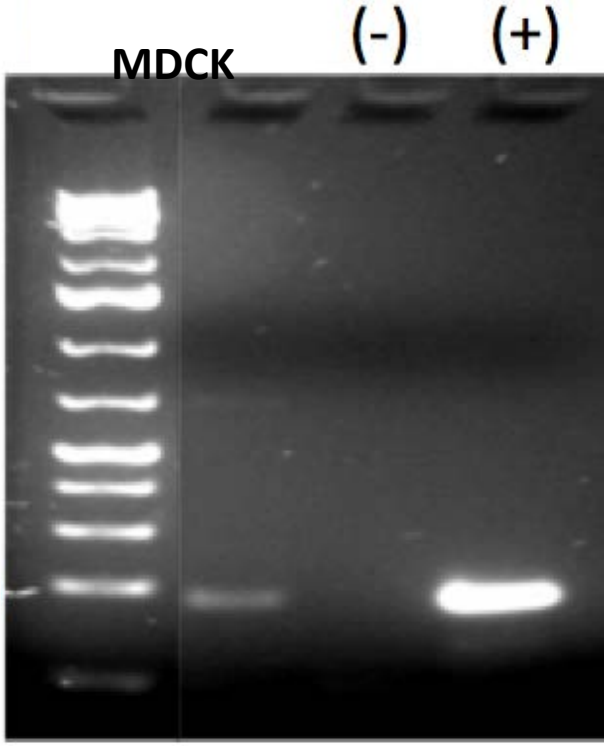
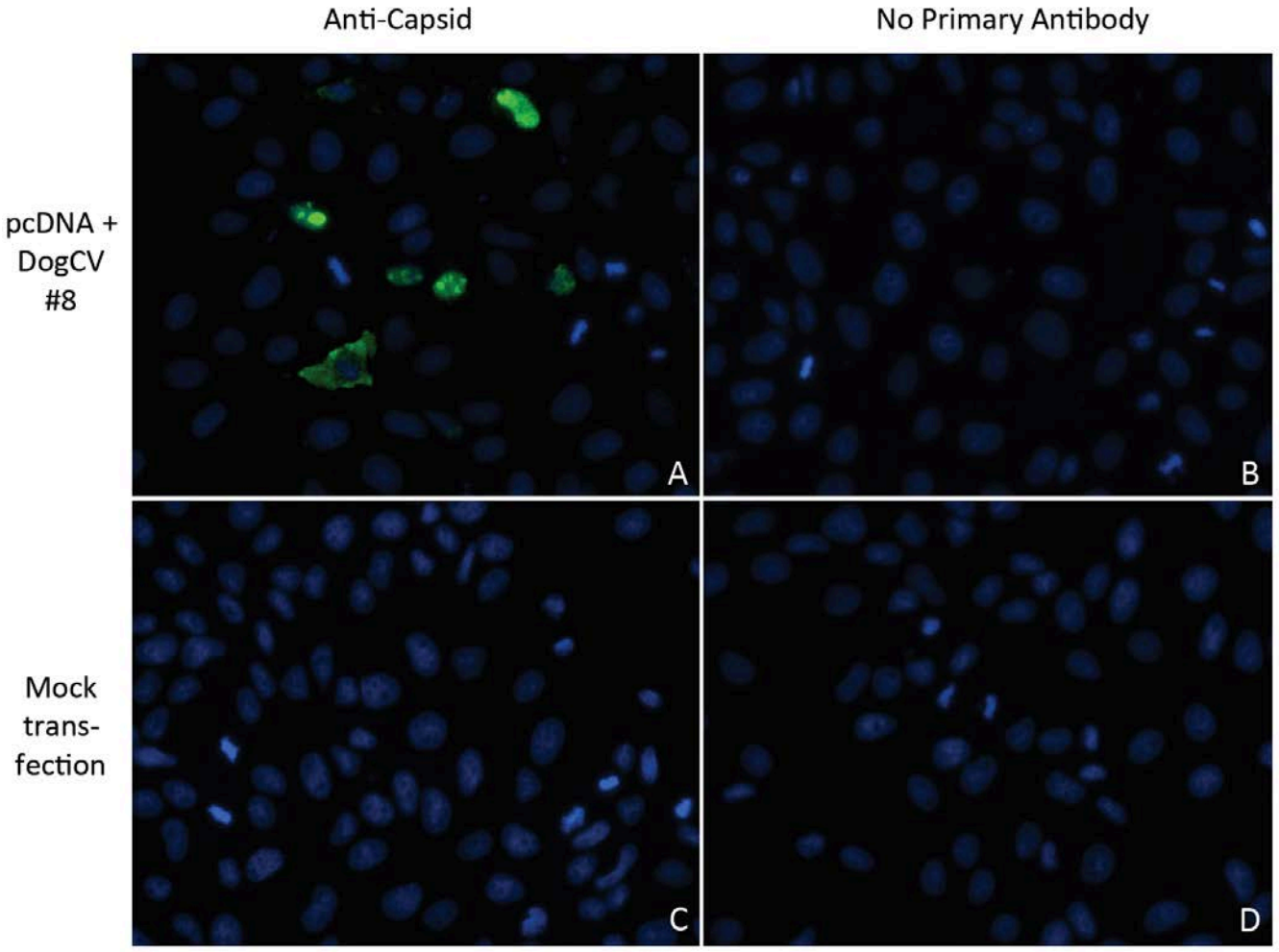




Confirm Transfection – Protein Detection

- Viral capsid detected by immunofluorescence.



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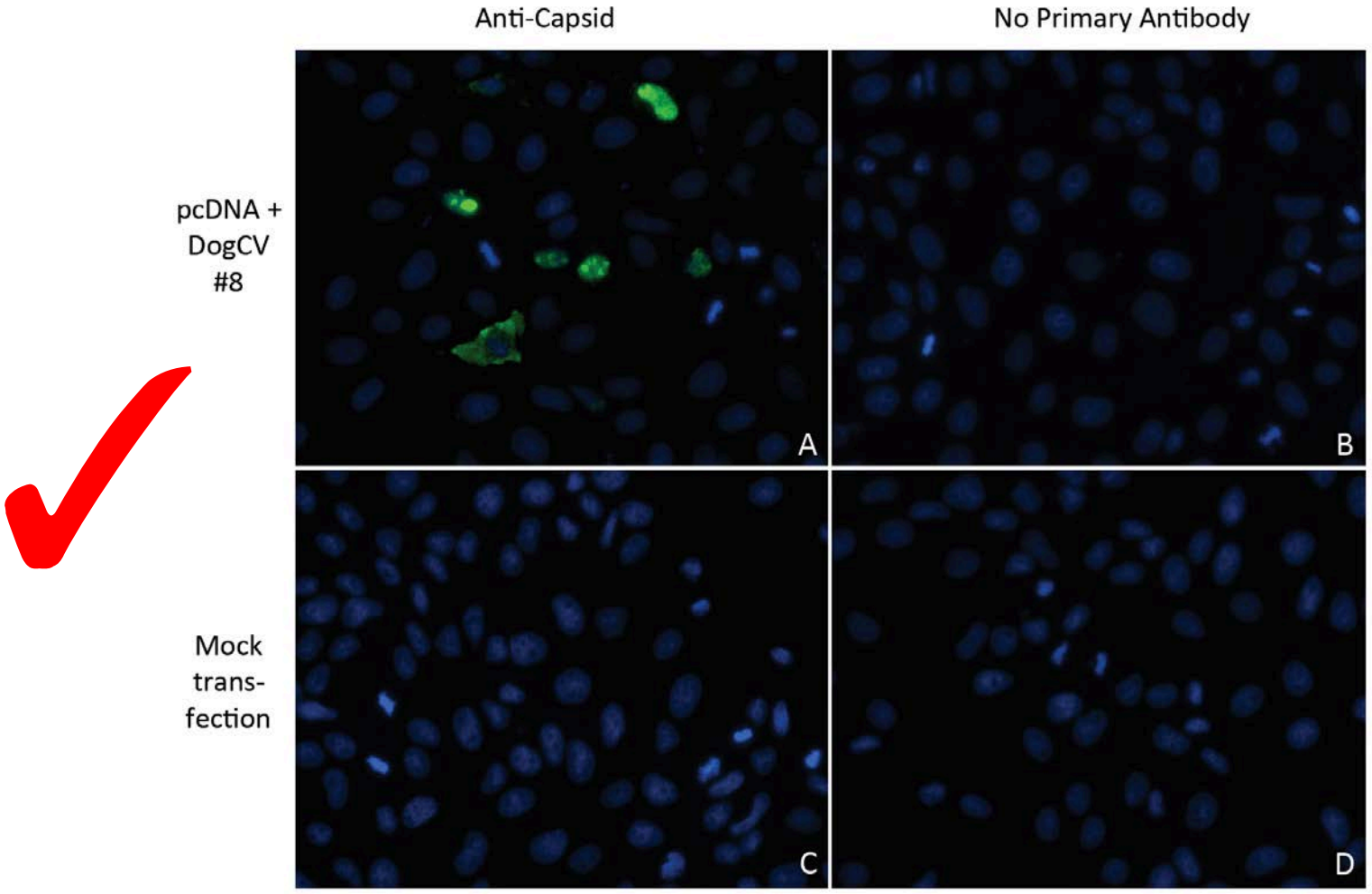

Results

- The full-length genome of DogCV was cloned into pcDNA3.1 vector and transfected into MDCK cells.
- The canine circovirus persistently replicated over at least a month in cell culture.
- Recircularization of DogCV genome was confirmed by PCR.
- Viral capsid protein was produced and confirmed with immunofluorescence assay.

Conclusion

- The result offers us an *in vitro* model to study the canine circovirus.

Question?