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Cloning Type IV-B CRISPR System into a Plasmid

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CRISPR Systems As Adaptive Immune Systems in Microorganisms

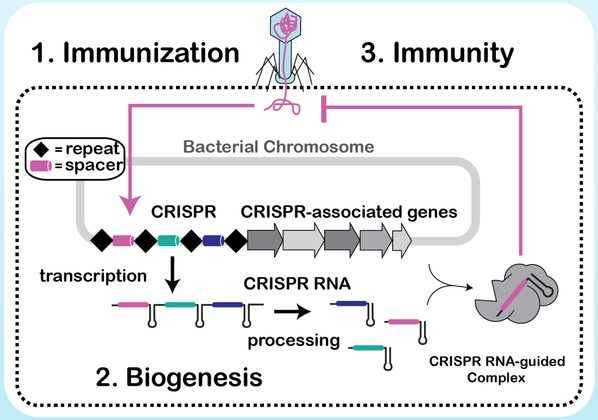

Figure 1: CRISPR Immunity

- Viruses outnumber the bacteria they infect 10 to 1
- Bacteria have evolved highly specialized immune systems known as CRISPR-Cas systems.
- These diverse systems encode as memories (spacers) copies of the nucleic acids which previously infected the cell.
- The transcription of the CRISPR locus creates a complementary RNA (crRNA) which can bind to new invaders, marking them for termination by Cas proteins.

Type IV--B System

- Type IV systems are newly discovered and have only recently been categorized as Class I systems (using multi subunits to bind crRNA)
- There are two types of Type IV systems
- Type IV - B systems are especially unique because they do not encode the CRISPR locus that characteristically is found on any system. Instead it encodes only the Cas proteins.
- Characterizing the Type IV-B system could lead to understanding it's function and role in the cell.

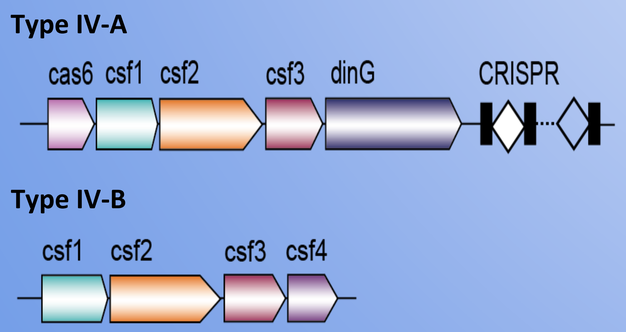

Figure 2: Type IV CRISPR Systems

Methods

To transplant the Type IV-B system into the target plasmid we performed restriction enzyme cloning, as shown in *Figure 3*. The success of our project is dependent on the sequencing results.

PCR Cleavage & Amplification of Type IV–B System

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Double Digest of Target Plasmid

↓

Ligation & Amplification of Open Plasmid & Type IV–B

↓

Colony PCR & Sequencing

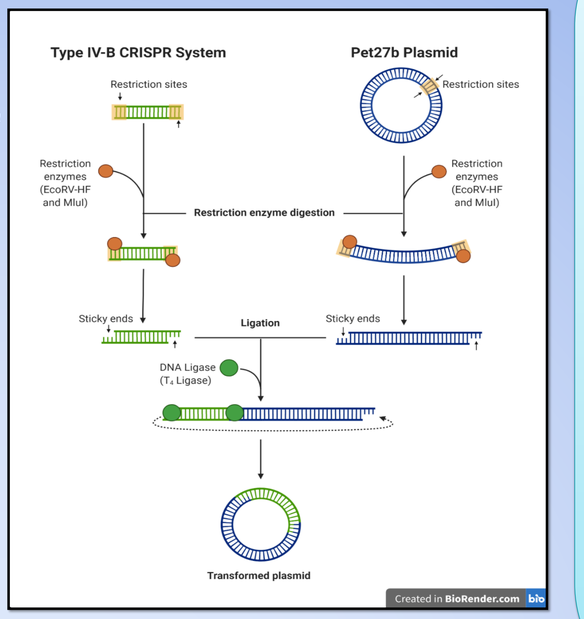

Figure 3: Restriction Enzyme Cloning

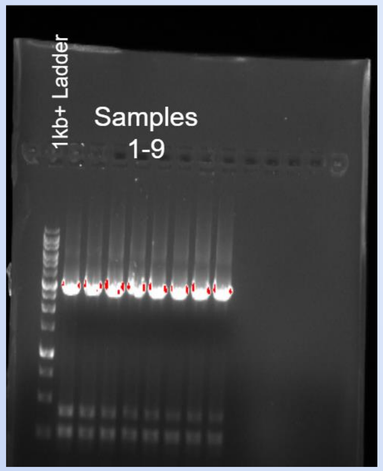

Figure 4: PCR Amplification of Type IV-B System (18 February 2020)

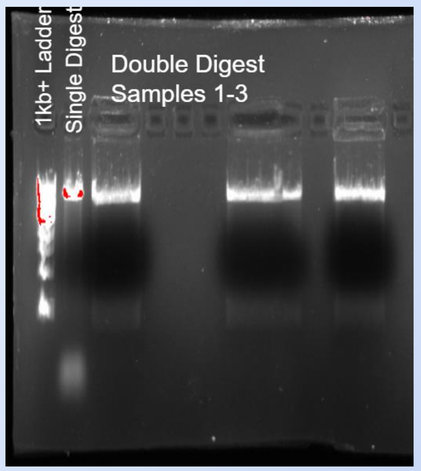

Figure 5: Vector (Target Plasmid) Digest (30 April 2020)

Results

- A successful colony PCR showed the *E. coli* which had taken up the transformed plasmid and so those colonies were chosen for sequencing
- Sequencing results (*Figure 6*) show the cloning was successful. The top line is the Type IV-B template with the several sequencing reactions below. Black color indicates the sequence matches the template. Red zones show sequences that didn't match. However, all red zones overlap with black zones and so we know the entire system was cloned successfully.

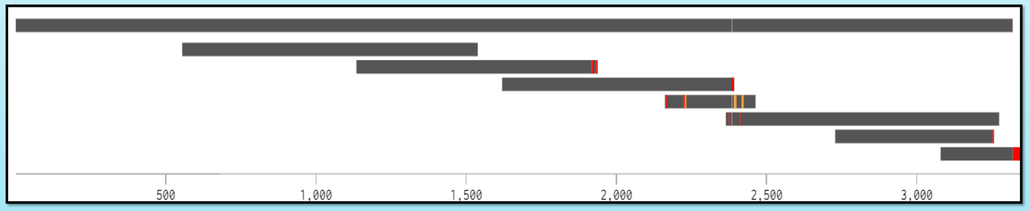

Figure 6: Sequencing results for Type IV-B in target plasmid

Type IV-B as an Anti-CRISPR

- Our hypothesis is that the Type IV-B system will act as an anti-CRISPR, a system that will act to block the function of another CRISPR function, to allow the bacteria to "choose" if an invasive plasmid should be degraded or kept..
- A plasmid curing assay will be performed using our cloned plasmid to determine if this is the case.
- The bacteria grown with the transformed Type IV-B sequence on the plasmid is predicted to have growth because the Type IV-B will block the Type IV-A from destroying the plasmid and therefore retaining the antibiotic resistance gene and allow colonies to grow.

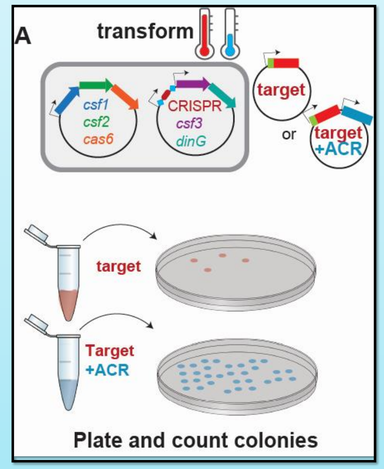

Figure 7: Plasmid Curing Assay with and without ACR (Anti-CRISPR) gene

Future Directions

- The above-described plasmid curing assay is underway and we hope to see results from that, shortly.
- However, if we do not see the activity we are expecting to see, this could indicate that ancillary proteins integral to the Type IV-B system.
- Ancillary proteins found close to the system in native plasmids may be integral to its function. We can test this by cloning the ancillary proteins onto our new plasmid
- This has been our current project for the past couple of months.

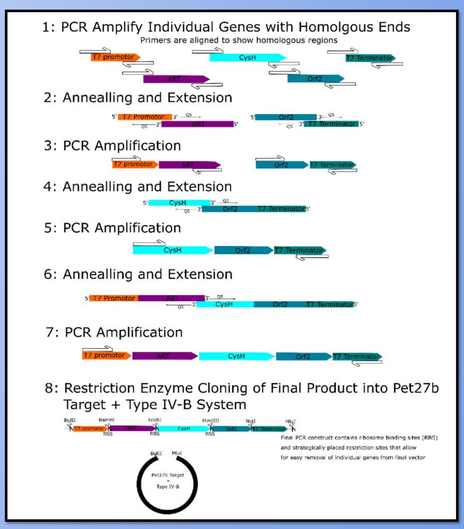

Figure 8: Cloning process for ancillary proteins

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