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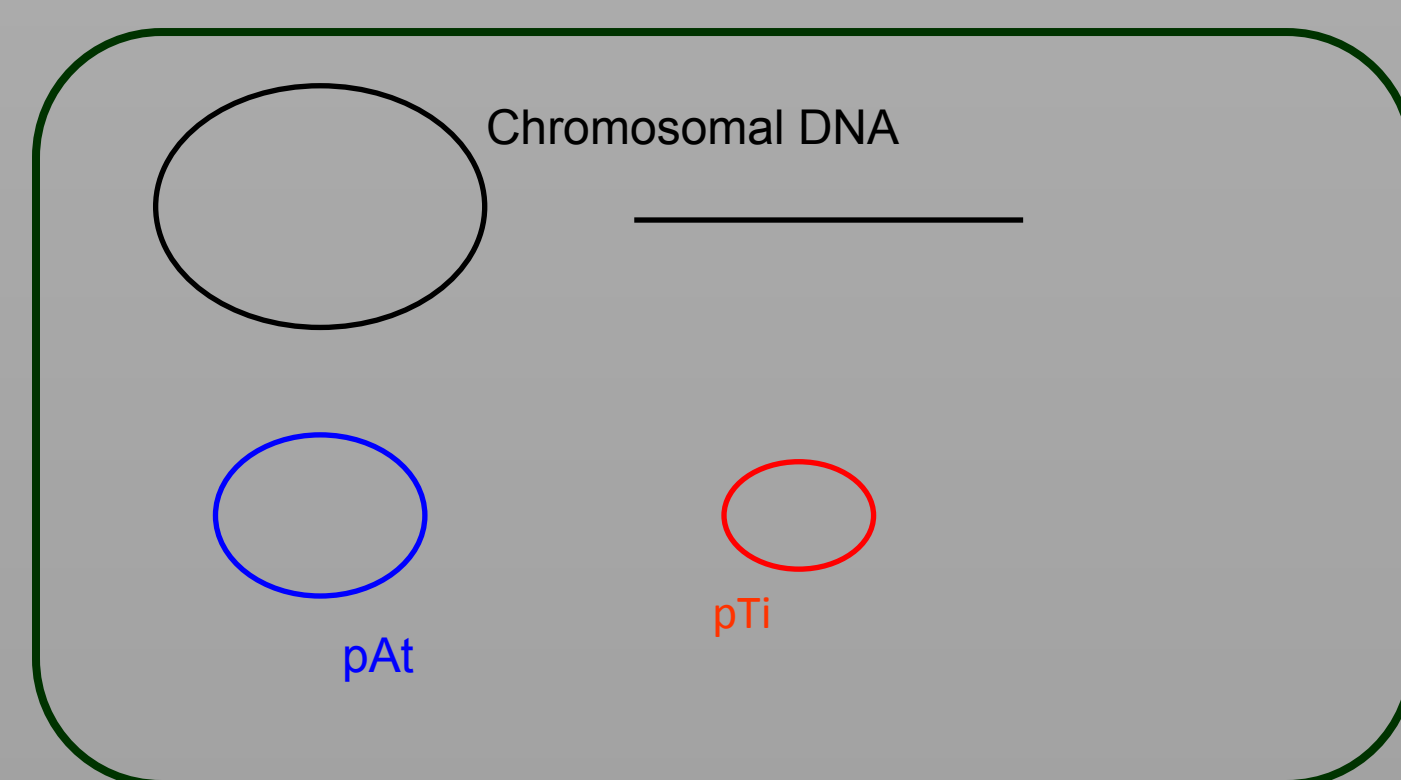
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Does quorum sensing regulate the conjugation of the two co-resident megaplasמידs of *Agrobacterium tumefaciens* 15955

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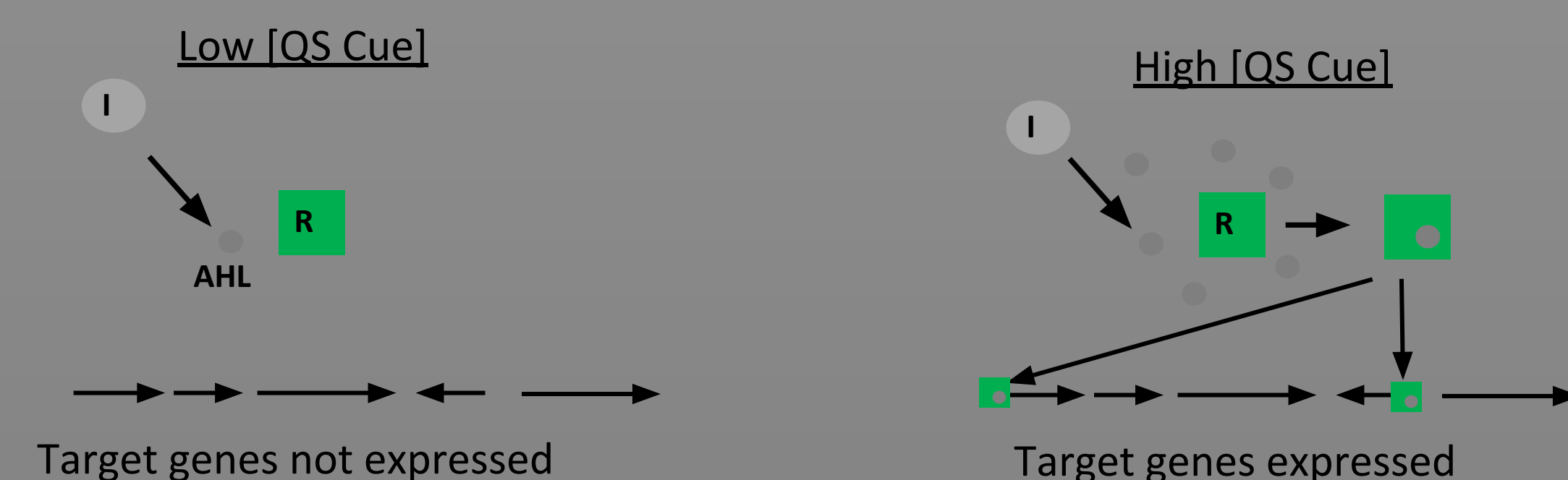
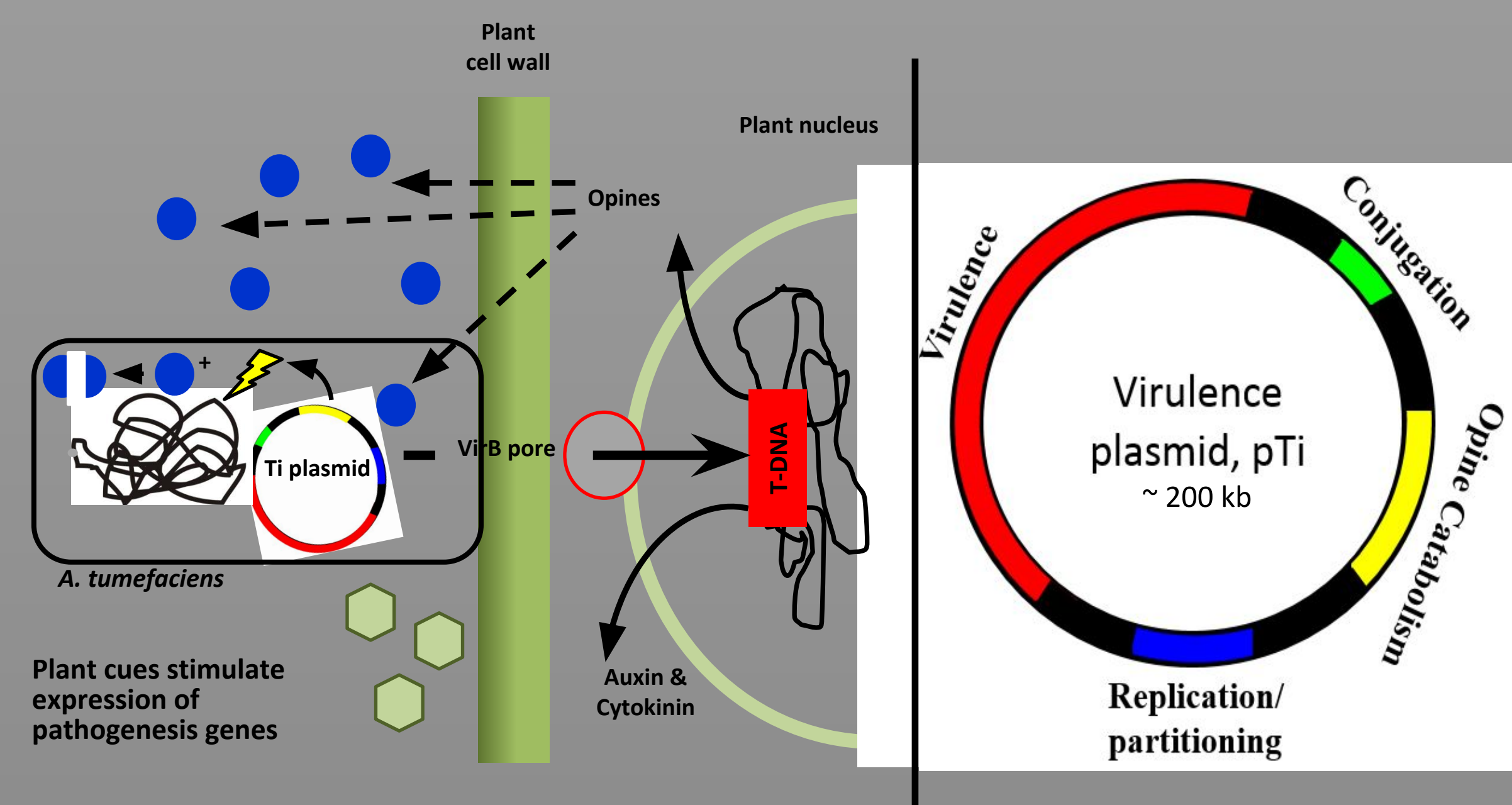
Background



A. tumefaciens genome structure

- Circular chromosome
- Linear chromosome
- Tumor inducing plasmid (pTi)
- *Agrobacterium tumefaciens* plasmid (pAt)

A. tumefaciens infects plants through expression of virulence genes on pTi. The infection causes tumors to form and the release of opine metabolites.



At high cell densities, a system of gene regulation called quorum sensing (QS) is activated. Homologs of *traR* encode QS transcriptional activators. Both pTi and pAt carry *traR* homologs, *traR1* on pTi and *traR2* on pAt.

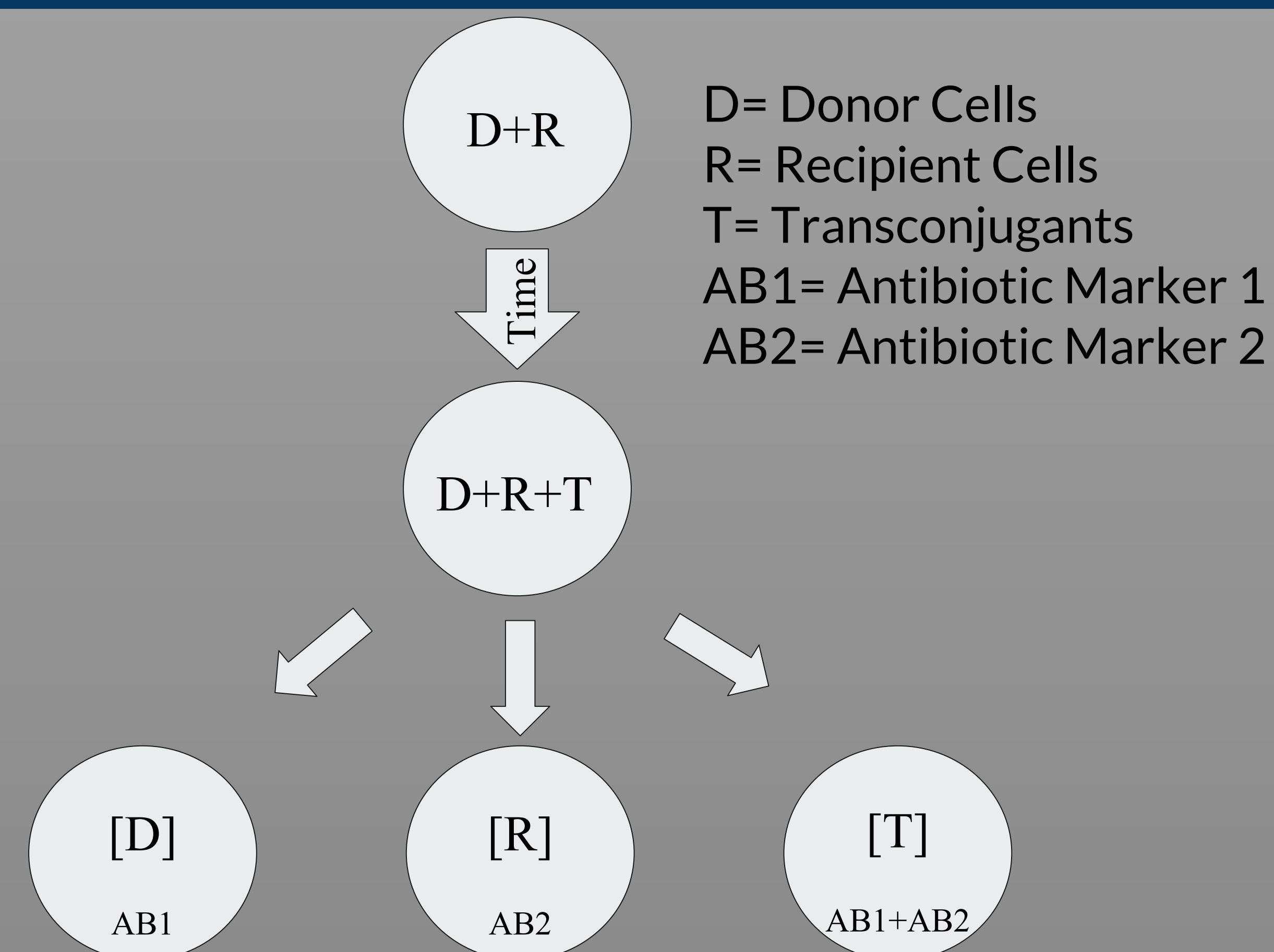
Hypotheses

1. pTi QS system regulates conjugation of pTi
2. pAt QS system regulates conjugation of pAt

Experimental Questions

1. Do pTi *traR1* or pAt *traR2* impact pTi conjugation?
2. Does overexpression of *traR1* and/or *traR2* influence pTi conjugation?
3. Do pTi *traR1* or pAt *traR2* impact pAt conjugation?
4. Does overexpression of *traR1* and/or *traR2* influence pAt conjugation?

Methods



Four mutant strains were created using allelic replacement or megaplasמיד curing.

- 15955 $\Delta traR1$ (clean deletion of *traR1* from pTi::*gent^R*)
- 15955 $\Delta traR2$ (clean deletion of *traR2* from pAt::*tet^R*)
- 15955 $\Delta traR1 \Delta traR2$ (clean deletion of *traR1* and *traR2*)
- C58 pTi⁻ and pAt⁻ (plasmidless recipient with chromosomal *strep^R*/ *spec^R*)

We also created IPTG inducible *traR1* and *traR2* expression plasmids

- pR1 = pSRKKm::*traR1*
- pR2 = pSRKKm::*traR2*
- pEV = pSRKKm (empty vector)

Anticipated Results

Question 1	Donor	Recipient	Anticipated Result
	$\Delta traR1$	C58 pTi ⁻ and pAt ⁻	Reduced pTi conjugation
	$\Delta traR2$	C58 pTi ⁻ and pAt ⁻	Wild-type pTi conjugation
	$\Delta traR1 \Delta traR2$	C58 pTi ⁻ and pAt ⁻	Reduced pTi conjugation
	15955	C58 pTi ⁻ and pAt ⁻	Wild-type pTi conjugation
Question 2	Donor	Recipient	Anticipated Result
	15955 pR1	C58 pTi ⁻ and pAt ⁻	Increased pTi conjugation
	15955 pR2	C58 pTi ⁻ and pAt ⁻	Wild-type pTi conjugation
	$\Delta traR1$ pR1	C58 pTi ⁻ and pAt ⁻	Increased pTi conjugation
	$\Delta traR1$ pEV	C58 pTi ⁻ and pAt ⁻	Reduced pTi conjugation
	15955 pEV	C58 pTi ⁻ and pAt ⁻	Wild-type pTi conjugation
Question 3	Donor	Recipient	Anticipated Result
	$\Delta traR1$	C58 pTi ⁻ and pAt ⁻	Wild-type pAt conjugation
	$\Delta traR2$	C58 pTi ⁻ and pAt ⁻	Reduced pAt conjugation
	$\Delta traR1 \Delta traR2$	C58 pTi ⁻ and pAt ⁻	Reduced pAt conjugation
	15955	C58 pTi ⁻ and pAt ⁻	Wild-type pAt conjugation
Question 4	Donor	Recipient	Anticipated Result
	15955 pR1	C58 pTi ⁻ and pAt ⁻	Wild-type pAt conjugation
	15955 pR2	C58 pTi ⁻ and pAt ⁻	Increased pAt conjugation
	$\Delta traR2$ pR2	C58 pTi ⁻ and pAt ⁻	Increased pAt conjugation
	$\Delta traR2$ pEV	C58 pTi ⁻ and pAt ⁻	Reduced pAt conjugation
	15955 pEV	C58 pTi ⁻ and pAt ⁻	Wild-type pAt conjugation

Summary and Conclusions

- These trials are ongoing and incomplete, however, based on previous literature and the genomic location of *traR2*, we hypothesize that there will be a relationship between plasmid conjugation and the QS regulator they encode.
- The results of the experiments will add to the growing scientific knowledge of QS and its role in bacterial cell-cell signaling.
- Insights into the conjugation of the virulence plasmid relate to the origin of pathogenic strains.