

Confirming Presence and Mobilization of Partner Quality Genes in *Sinorhizobium meliloti*

Ethan Perez^{1,2}, Ivan Sosa Marquez², and Katy D. Heath²

Bakersfield College, Bakersfield, California¹
Department of Microbiology and Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign²

Background

- Mutualism is an interaction in which two organisms benefit from each other.
- The symbiotic relationship between the bacterium *Sinorhizobium meliloti* and the plant *Medicago truncatula* is dictated by the bacterial plasmids.
- The Heath lab has a collection of 191 *Sinorhizobium meliloti* strains with different symbiotic plasmids that vary across populations (Riley et al., 2022).
- Each strain can be a better or worse partner for its plant host in symbiosis with the variation in fitness being called partner quality (Fig. 1)
- These traits can be traced back to the genetic elements that underlie this effect (Batstone et al., 2022).
- These genetic elements can be found in gene clusters that are gained or lost across strains (Fig. 1; Riaz, Sosa-Marquez, in prep.).

We need assurances of these important and variable clusters presence to understand their mechanism of gene movement

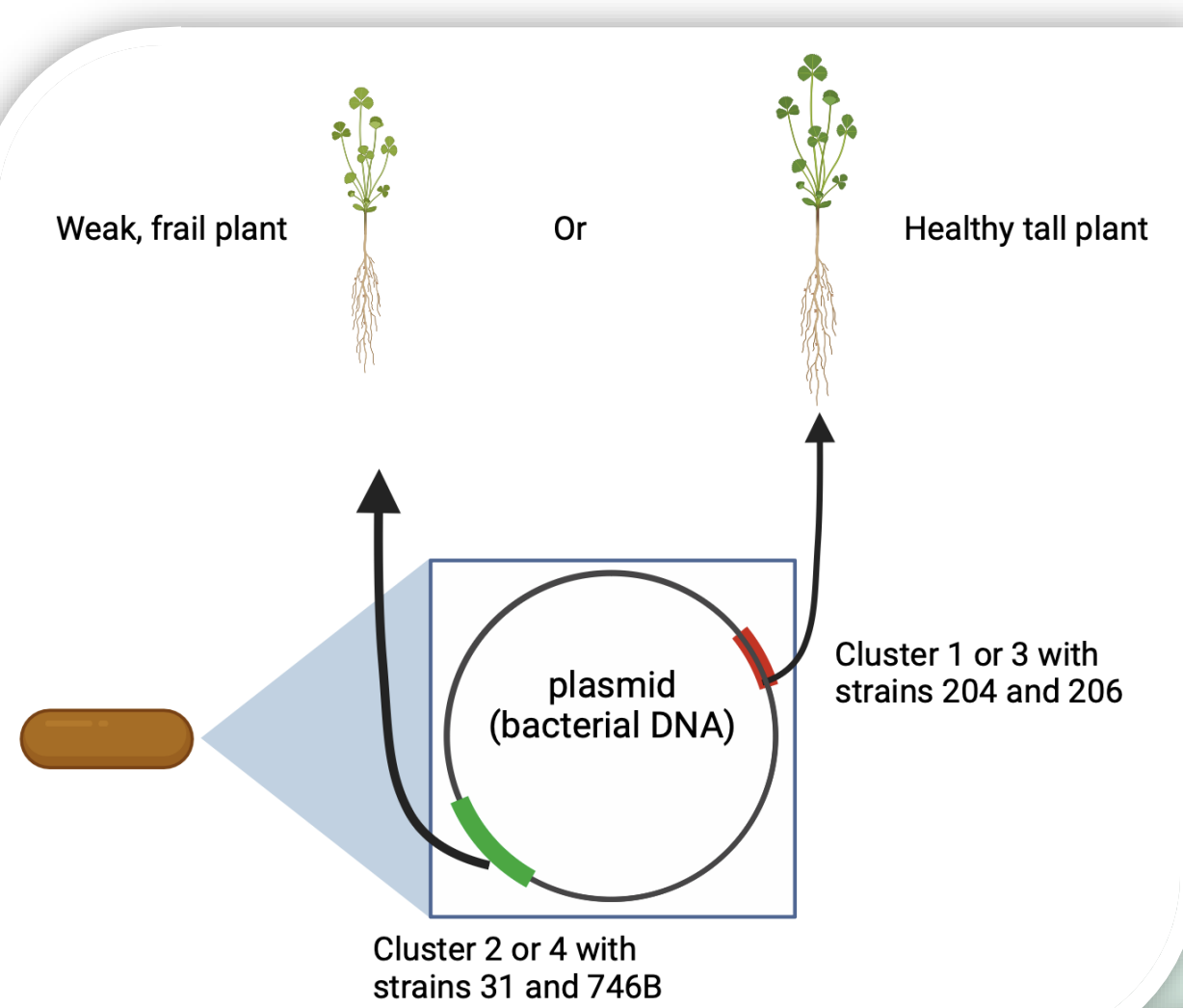


Figure 1: The effect of gene clusters on partner quality



Medicago truncatula

Project Aims

- To confirm that the gene clusters are present or absent in the best and worst partner quality strains.
- Explore possible mobility or circularization of the partner quality related clusters in pSymA

Methods

- Extract bacterial DNA from liquid media via genomic and plasmid DNA extraction at different time intervals (Fig. 3)
 - Design unique inward primers specific for the cluster of genes in question (Fig. 2)
- Amplify using PCR (Fig. 4), and run gel electrophoresis
- If it matches the expected band size on the gel, we would be confirming the P/A pattern of this cluster
- Repeat the same process with outward set of primers to test for circularization (Fig. 2)
 - Results will determine how long the circularizable fragment of DNA is

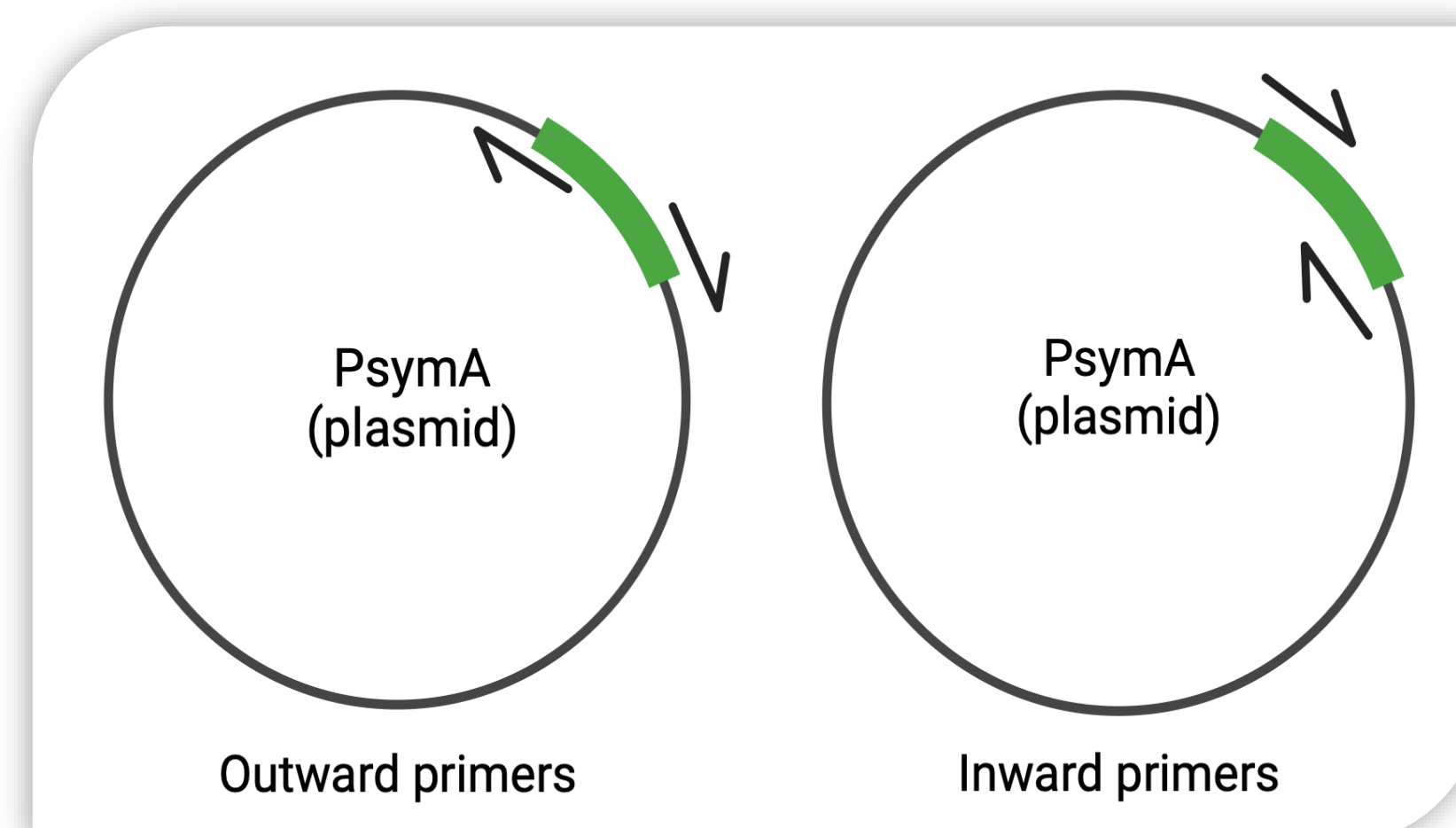


Figure 2: inward and outward primers example

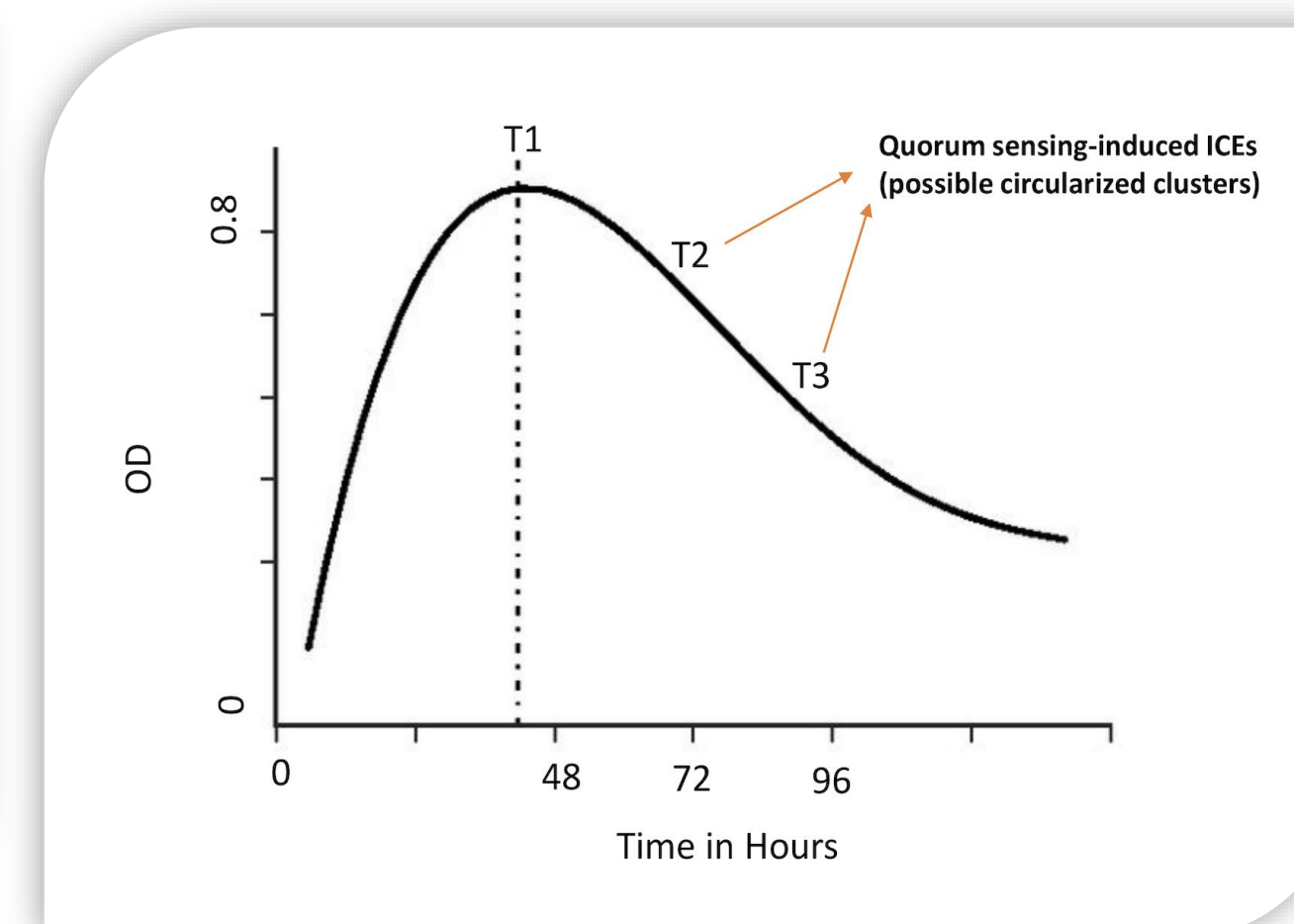


Figure 3: Time points at which samples were taken

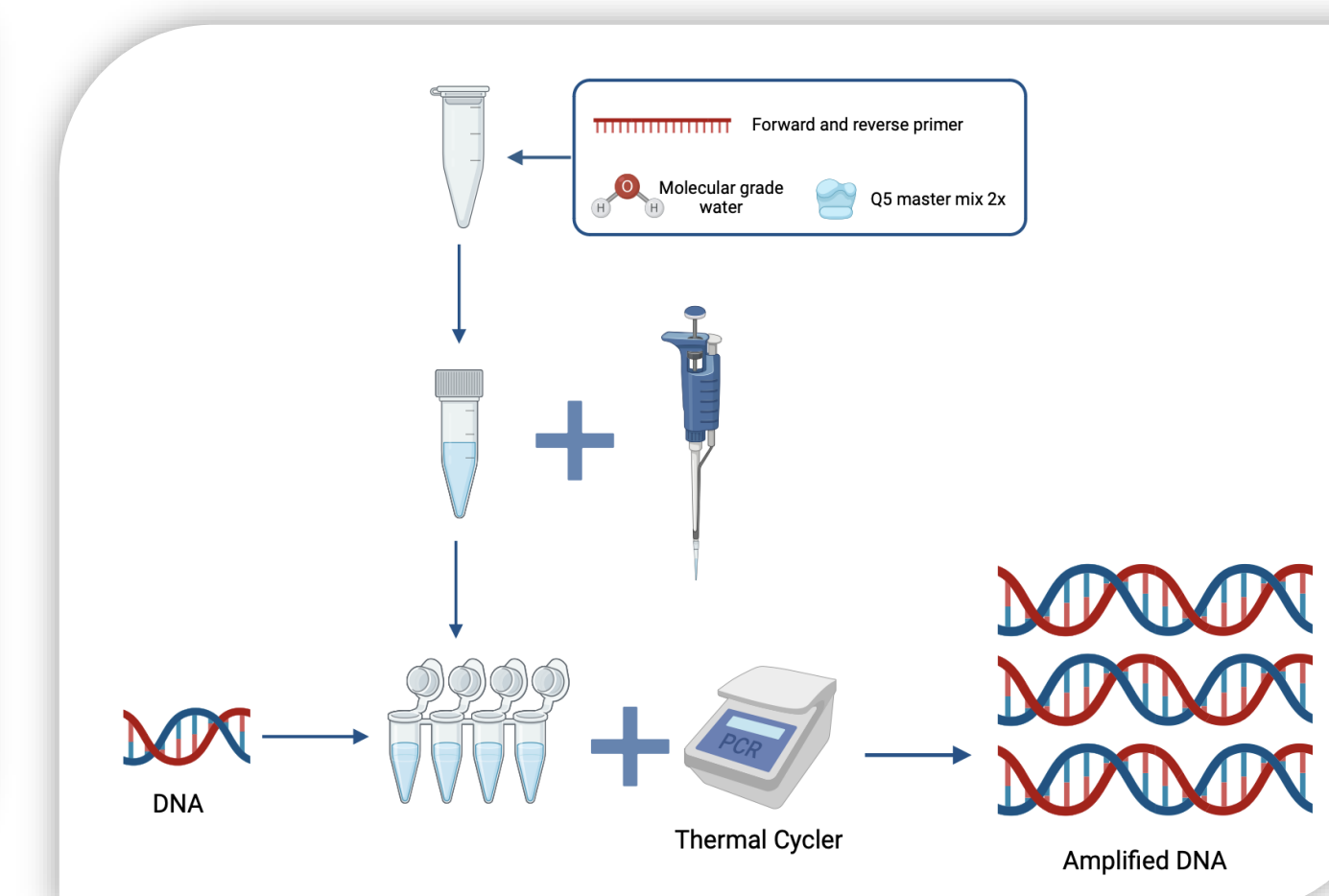


Figure 4: PCR procedure

Results

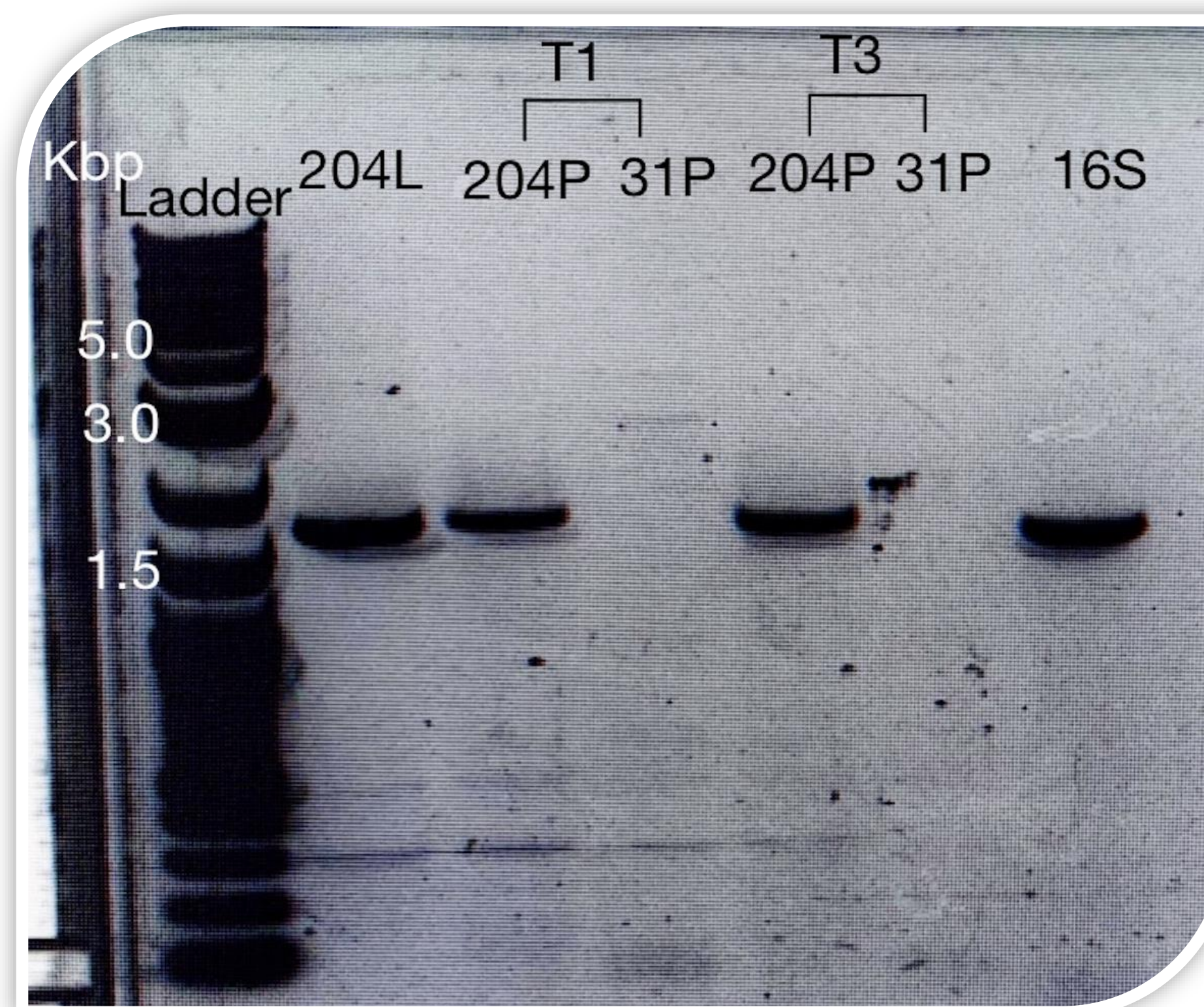


Figure 5: Gel electrophoresis results with plasmid and genomic DNA extractions of strain 31 and 204 at time intervals T1 and T3 using inward primers to test for circularization and confirmation of cluster 1

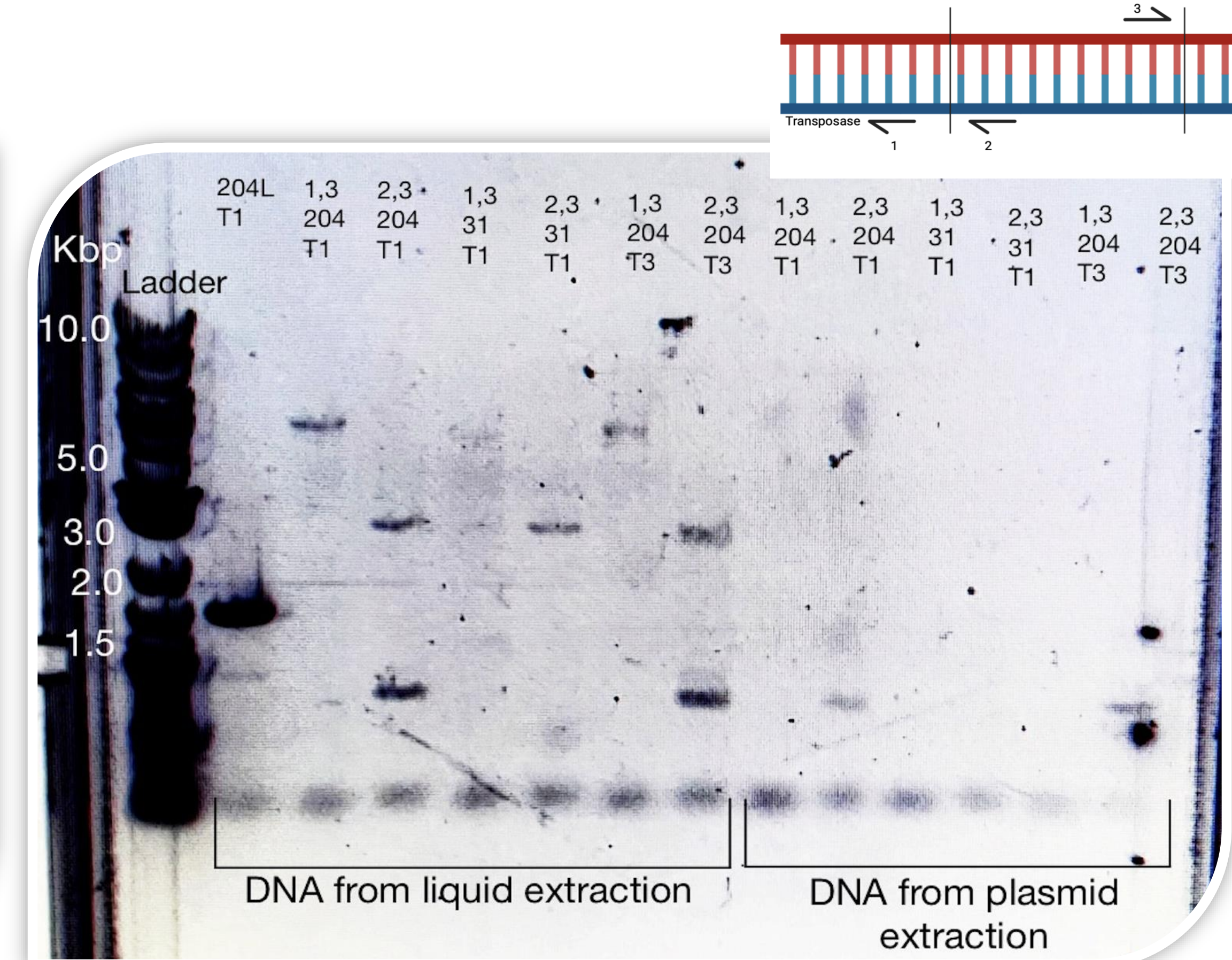


Figure 6: Gel electrophoresis results with plasmid and genomic DNA extractions of strain 31 and 204 at time intervals T1 and T3 using outward primers testing for size of circularizable element containing cluster 1

	C1_204	C3_204	C1_206	C3_206	C2_31	C4_31	C2_746B	C4_746B
T1								
T2								
T3								

Table 1: Which clusters are completely present in plasmid extract at allotted time

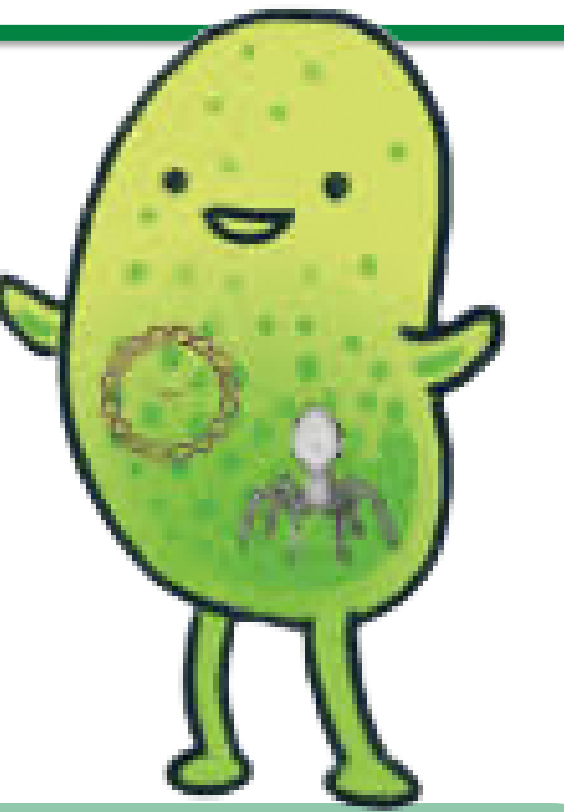


Figure 7: Transposase found before PQ related cluster 1



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Discussion

- Cluster 1, related with positive partner quality was successfully amplified in the strain 204, hence allowing to test for circularization
- Evidence was found that the elements are excised and possibly circularized at different cell densities

Future Work

- We will explore the integrative conjugative elements of our strains
- Keep exploring clusters

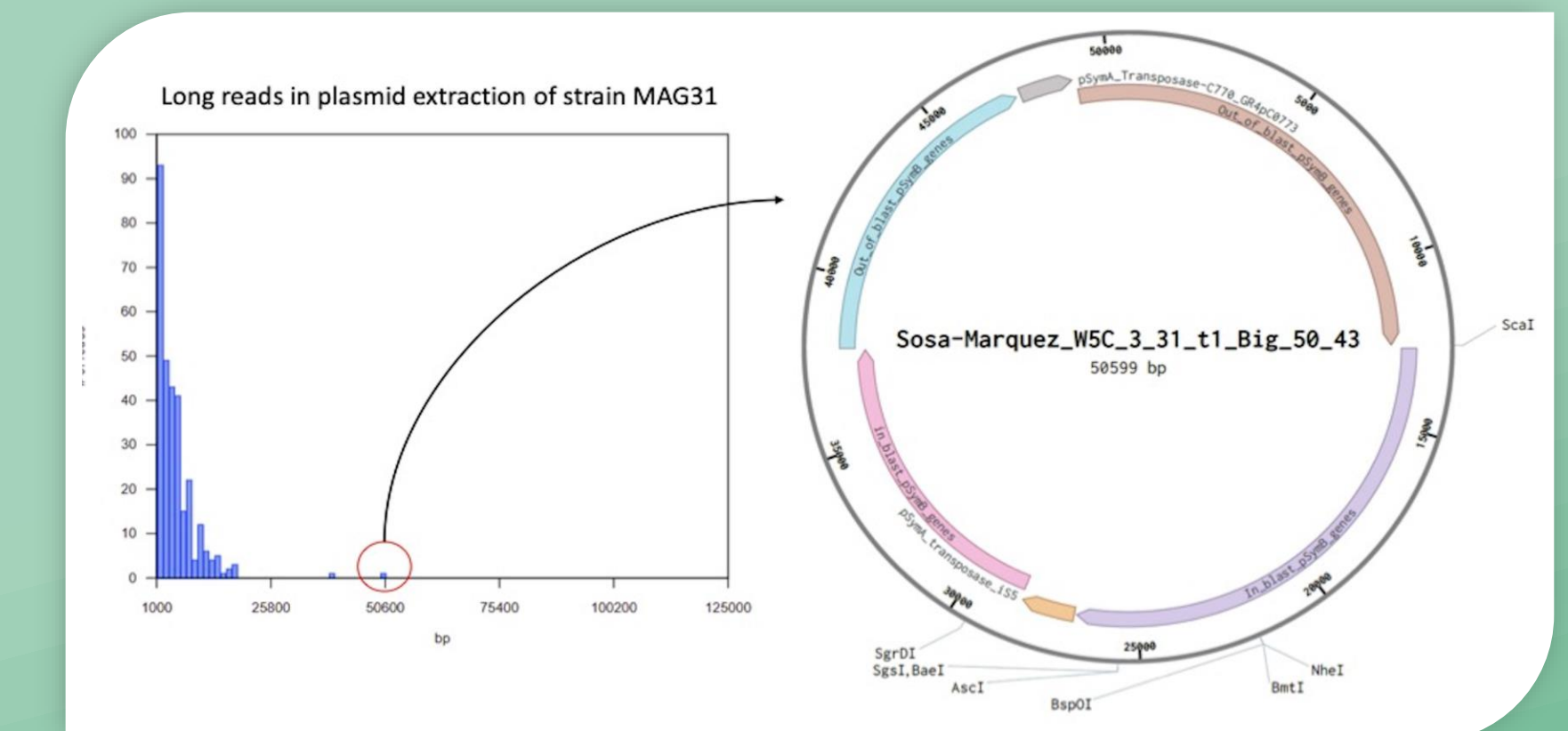


Figure 8: Theory of transposases of psymA moving psymB gene

References

- Batstone, R. T., Lindgren, H., Allsup, C. M., Goralka, L. A., Riley, A. B., Grillo, M. A., ... & Heath, K. D. (2022). Genome-wide association studies across environmental and genetic contexts reveal complex genetic architecture of symbiotic extended phenotypes. *Mbio*, 13(6), e01823-22.
- Riley, A. B., Grillo, M. A., Epstein, B., Tiffin, P., & Heath, K. D. (2022). Discordant population structure among rhizobium divided genomes and their legume hosts. *Molecular ecology*.

Acknowledgments

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Britt Carlson
Nathan Schroeder